International Dairy Journal 21 (2011) 247–253

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Microencapsulation of probiotic bacteria using pH-induced gelation of sodium caseinate and gellan gum

Arup Nag, Kyoung-Sik Han, Harjinder Singh*

Riddet Institute, Massey University, Private Bag 11 222, Palmerston North, New Zealand

ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form
19 August 2010
Accepted 11 November 2010

ABSTRACT

A sodium caseinate and gellan gum mixture was gelled by gradually decreasing pH with glucono-δ-lactone (GDL). *Lactobacillus casei* cells were successfully entrapped into this gel matrix by a water-in-oil emulsion. The optimum ingredient combination, based on elastic modulus and relative gelation time to attain adequate gel strength, was 10% (w/w) sodium caseinate, 0.25% (w/w) gellan gum and 2.5% (w/w) GDL. A very fine, uniform capsule particle size distribution resulted. The surface-weighted and volume-weighted mean capsule diameters were about 287 and 399 μm, respectively. The ratio of the core bacteria to the wall ingredients was optimized to achieve a high encapsulation yield of ~89.5%. The survival of encapsulated cells after 30 min of incubation in simulated gastric fluid was significantly ($P < 0.001$) greater than that of free cells, both with and without the addition of pepsin. The capsules also provided significant protection for *L. casei* against detrimental bile salts.

1. Introduction

Microencapsulation has been defined as the incorporation of food ingredients, enzymes, oils, bacterial cells or other nutraceuticals into small capsules that can release their contents at controlled rates under specific conditions and that protect their contents from degradation by the detrimental factors in their environment (Desai & Park, 2005). The purpose of microencapsulating probiotic bacteria is to stabilize and maintain viability during storage (O'Riordan, Andrews, Buckle, & Conway, 2001), to protect against harsh gastro-intestinal environment (Muthukumarasamy, Allan-Wojtas, & Holley, 2006) and controlled release in the colon (Reid et al., 2005). Among the common techniques of microencapsulation, the widely used methods for probiotic bacteria are spray drying, extrusion, spray coating and coacervation (Kailasapathy, 2002; Mortazavian, Razavi, Ehsani, & Sohrabvandi, 2007).

The use of dairy proteins in microencapsulation has been studied with great interest because of their well-known functional properties (Chen & Subirade, 2005; Rosenberg & Sheu, 1996). Dairy proteins easily meet GRAS (generally recognized as safe) standards and have high nutritional value and excellent gelation, foaming and water-binding capacity, thus allowing them to be highly suitable for encapsulating probiotics or other nutraceutical carrier materials that can be administered orally (Chen & Subirade, 2005). Among dairy proteins, sodium caseinate appears to offer ideal physical and functional properties for microencapsulation because of its amphiphilic character and emulsifying characteristics (Hogan, McNamee, O'Riordan, & O'Sullivan, 2001; Madene, Jacquot, Scher, & Desobry, 2006).

The gelation of protein has traditionally been achieved by heat treatment, during which the polypeptide chains unfold and subsequently self-aggregate to form a three-dimensional network. However, heat treatment is unsuitable for the encapsulation of various heat-sensitive materials, such as probiotic bacteria. The cold-induced gelation of food proteins has been suggested as a potential solution to this problem (Barbut & Foegeding, 1993; Heidebach, Forst, & Kulozik, 2009a, 2009b; Maltais, Remondetto, Gonzalez, & Subirade, 2005).

Heidebach et al. (2009a) recently developed a method in which *Lactobacillus paracasei* and *Bifidobacterium lactis* strains were encapsulated by the enzymatic gelation of sodium caseinate through crosslinking with transglutaminase enzyme. In other work, Heidebach et al. (2009b) took a similar approach to milk protein gelation by encapsulating probiotic cells using rennet as a coagulating agent. Sodium caseinate can also be coagulated and gelled by acidification with glucono-δ-lactone (GDL) (Lucey, van Vliet, Grolle, Geurts, & Walstra, 1997) and the rheological properties of the milk gel have been studied extensively (Cobos, Horne, & Muir, 1995; Lucey et al., 1997; van Vliet & Keetels, 1995).

* Corresponding author. Tel.: +64 6 350 4401; fax: +64 6 350 5655.
E-mail address: h.singh@massey.ac.nz (H. Singh).

0958-6946/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.idairyj.2010.11.002

In our preliminary experiments, we could not achieve a GDL-induced gel matrix with sufficient barrier strength when using sodium caseinate as the sole wall material. However, a protein–polysaccharide mixture of sodium caseinate and gellan gum was found to give better properties for the encapsulation of bacterial cells. The most significant functionality of gellan gum is that it can hold small particles in suspension without the viscosity increasing significantly (Baird & Pettitt, 1991). As gellan gum is not easily degraded by the action of enzymes (Baird & Pettitt, 1991; Lee, 1996) and is resistant to acidic environments (Sun & Griffiths, 2000), a complex of gellan gum with sodium caseinate was hypothesized to be an ideal wall material for encapsulating probiotic bacteria.

In the present study, we encapsulated a strain of the common probiotic bacterium, *Lactobacillus casei*, into a mixture of sodium caseinate and gellan gum using a combination of gelation and emulsification techniques. The idea of GDL-induced gelation of sodium caseinate confined in a water-in-oil emulsion system to encapsulate probiotic bacteria in a low pH matrix has not been attempted previously. A combination of sodium caseinate with gellan gum to provide additional gel strength for the encapsulating matrix under these conditions has not been described before. We optimized the encapsulation process and compared the in vitro survival of encapsulated cells and free cells in simulated gastric fluid and bile salt solution.

2. Materials and methods

2.1. Ingredients and chemicals

Sodium caseinate containing about 90% (w/w) protein was obtained from Fonterra Co-operative Group Ltd (Palmerston North, New Zealand). Gellan gum and GDL were purchased from Hawkins Watts (Auckland, New Zealand). Canola oil was purchased from a local supermarket. All chemicals used in this study were of analytical grade and were obtained from Sigma Aldrich (St. Louis, MO, USA).

2.2. Bacterial strain and cell suspension

A commercial strain of *L. casei* 431 was obtained from Christian Hansen (Hørsholm, Denmark) and was grown in MRS broth (Difco Laboratories, Franklin Lakes, NJ, USA) at 37 °C for 18 h under anaerobic conditions (GazPak EZ anaerobe container system; Becton, Dickinson & Company, Franklin Lakes, NJ, USA). The bacteria were subcultured at least three times prior to being used for the preparation of cell suspensions. For each cell suspension, 8 mL of fresh culture was inoculated into 400 mL of MRS broth and was incubated for 18 h. The culture was centrifuged (Thermo Fisher Scientific, MA, USA) at 4600 × *g* for 20 min. The supernatant was discarded and the cell precipitate was washed thoroughly with sterilized 0.2% peptone water (Difco Laboratories). This washing process was repeated three times and the final cell slurry was made up to 10 mL by adding the required amount of peptone water and was mixed thoroughly. For enumeration of bacteria, the number of colony forming units (cfu) was determined on MRS agar using the plate count method at 37 °C for 48 h under anaerobic conditions.

2.3. Gelation of a mixture of sodium caseinate and gellan gum

Gel formation was carried out using different concentrations of the encapsulation ingredients. The gradual development of gel strength along with the elapsed time was important to understand, therefore, the rheological analysis of the gels was performed with a rheometer (Model AR-G2, TA Instruments, Crawley, UK). A time sweep test was performed for a duration of 180 min to monitor the development of the elastic modulus (*G'*) during gel formation in the presence of GDL. The procedure was carried out in oscillatory mode with a flat plate geometry at a fixed strain of 0.02 and a frequency of 1 Hz. The temperature of the samples was maintained at 30 °C. Initially, 10% (w/w) sodium caseinate solution was acidified with various concentrations of GDL (1.0, 1.5, 2.0 and 2.5%, w/w). Then sodium caseinate and gellan gum mixture solutions containing different concentrations of gellan gum (0.10, 0.25 and 0.50%, w/w) were examined at the constant concentration of GDL chosen from the initial experiment. The changes in the pH of solutions were also measured during incubation of 240 min at 20 °C.

2.4. Microencapsulation

The microencapsulation technique used in this study is described by a process flow diagram in Fig. 1. All glassware was autoclaved at 121 °C for 15 min prior to use. The sodium caseinate and the gellan gum were rehydrated into the required quantity of reverse osmosis (RO) water for 16 h at 4 °C under slight agitation with a magnetic stirrer (Biolab, Auckland, New Zealand) and were thereafter completely dissolved at 60 °C. The mixture was then heated to 90 °C for 30 min for sterilization and cooled to room temperature. GDL was added directly into the mixture with continuous stirring at 300 rpm and the pH of the mixture was measured. After 5 min, 5 mL of *L. casei* cell suspension was added to the mixture, followed by continuous stirring for another 5 min; 100 g of this mixture was then added slowly into an Erlenmeyer flask containing 400 mL of pre-sterilized canola oil under constant stirring at 1000 rpm. After holding for 120 min, the particles were allowed to settle out and the oil was decanted off. The microcapsules were washed three times with sterilized RO water to remove any residual oil that adhered to the particle surfaces. The microcapsules were then stored at 4 °C for subsequent analysis.

2.5. Encapsulation efficiency

Various proportions of cell suspension were added into the sodium caseinate and gellan gum mixture of the constant concentrations decided previously. The cell suspension (10 mL) prepared as described previously was divided into four parts (1, 2, 3 and 4 mL), which were made up to the final volume of 5 mL by adding the required quantity of sterilized 0.2% peptone water. Each of these portions was added to 100 g of the sodium caseinate and gellan gum mixture (Table 1). The bacterial counts were measured before and after microencapsulation. The encapsulation efficiency (EE) was calculated using a slightly modified version of the equation devised by Heidebach et al. (2009a), as shown below, where WCM is the wall material and cell suspension mix.

$$\mathrm{EE}(\%) = \frac{\text{Total solids}_{\text{wcm}}}{\text{Total solids}_{\text{slurry}}} \times \frac{\text{cfu(g capsule slurry)}^{-1}}{\text{cfu(g WCM)}^{-1}} \times 100 \quad (1)$$

The dry matter contents of the WCM and the capsule slurry were determined by weighing samples before and after drying in an oven maintained at 105 °C for 24 h and then measuring the difference in weight as a result of the moisture loss. The viable cells of the capsule suspension in aqueous media were counted by breaking the microcapsules (1 g capsule + 9 mL peptone water) with a Colworth 400 laboratory stomacher (Model BA6021, A.J. Seward, London, UK) until a homogenous mass was obtained. The homogenization process was confirmed again with compound

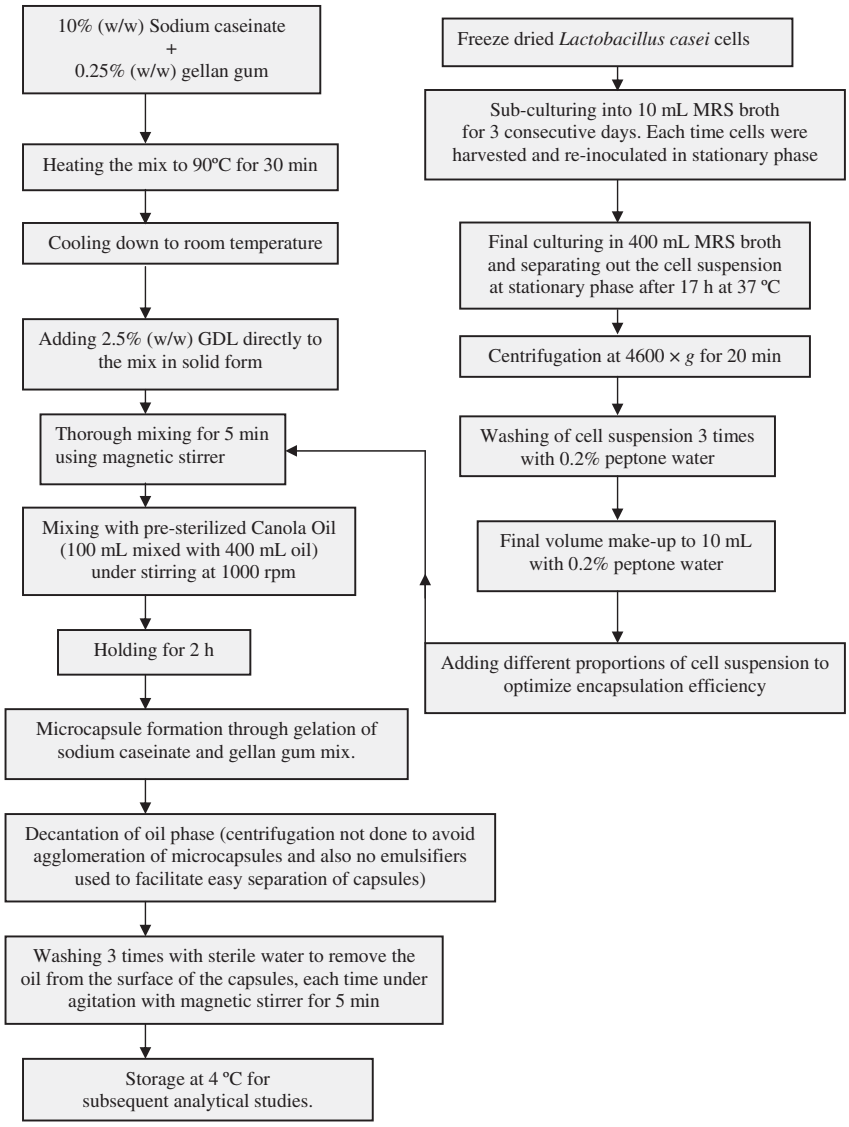

Fig. 1. Optimized process flow diagram of the cell culturing and microencapsulation of *Lactobacillus casei* 431 bacteria.

microscopic examination and the viable cells were enumerated using serial dilution and pour plating on MRS agar.

2.6. Particle size distribution

The surface-weighted and volume-weighted mean diameters and the cumulative size distributions of the microcapsules were measured by a Malvern Mastersizer 2000 Ver. 5.54 (Malvern Instruments Ltd, Malvern, UK) using laser diffraction technology. The standard operating procedure (SOP) was performed with a particle refractive index (RI) of 1.37 and the sample concentration was 0.6563% (v/v). The dispersant used was RO water with an RI of 1.33. Samples were analysed in duplicate.

Table 1
Effects of various concentrations of free cell suspensions on bacterial counts in capsules and the encapsulation efficiency.

Sample	Composition (mL)		$\log_{10}$ cfu $(\text{g capsule})^{-1}$	Encapsulation efficiency (%)
	Cell suspension	Peptone water		
A	1	4	11.0	41.9
B	2	3	11.2	67.3
C	3	2	11.3	89.5
D	4	1	11.3	86.6

2.7. Survival of encapsulated and free cells in simulated gastric fluid

Simulated gastric fluid (SGF) was prepared according to method USP31-NF26 of the US Pharmacopeia (2008) with 0.2% NaCl, and the pH was then adjusted to 2.0 with HCl. Two different tests were performed with and without the addition of 0.32% pepsin (from 800 to 2500 units of pepsin per milligram of protein) to observe the effect of pepsin on the sodium caseinate and gellan gum gel matrix. One gram of microcapsules was added to four pairs of Kimax tubes, each containing 9 mL of pre-warmed SGF, and was incubated in a water bath maintained at 37 °C under orbital agitation at 100 rpm based on the methods of Guerin, Vuillemard, and Subirade (2003). After every 30 min of incubation, one sample was removed and the pH of the sample was immediately raised to 7.0 with 0.1 M NaOH to stop the enzymatic reaction. The capsules were then smashed with the stomacher to release the entrapped bacterial cells, followed by plate counting (as described in Section 2.5 above). Exactly the same approach was taken for free cells; 1 mL of cell suspension was

added into 9 mL of SGF. In each case, negative controls in peptone water (pH 7.0) were analysed to measure the initial counts. Samples were plated in duplicate for each dilution level and all experiments were replicated three times to obtain mean values.

2.8. Survival of encapsulated and free cells under simulated bile conditions

A simulated environment containing bile salts was prepared according to the method described by Muthukumarasamy et al. (2006). Monobasic potassium phosphate (KH_2PO_4, 0.68%, w/w) and porcine bile extract (1.0%, w/w) were added to deionized MilliQ water (Millipore, Molsheim, France) and the pH was adjusted to 6.8 with 0.2 M NaOH. One gram of microcapsules was added into four pairs of Kimax tubes, each containing 9 mL of bile salt solution, pre-warmed and incubated in a water bath maintained at 37 °C with orbital agitation at 100 rpm. The same process was followed for free cells. The viable cells in the samples were counted after 2, 4, 6 and 8 h. Samples were plated in duplicate for each dilution level and all experiments were replicated three times to obtain mean values.

2.9. Statistical analysis

The results were expressed as mean ± standard deviation (SD). The data for survival in SGF and under simulated bile conditions were analysed using SAS/PROC ANOVA (GLM) and TTEST respectively (SAS, version 9.1, SAS Institute Inc., Cary, NC, USA). Means for the survival in SGF at each incubation time were compared using Duncan's multiple range test. The statistical significance for survival under simulated bile conditions was accepted at $P < 0.001$.

3. Results

3.1. Gelation of sodium caseinate and gellan gum mixture

An increase in the concentration of GDL resulted in more rapid formation of the sodium caseinate gel and higher *G'* values (Fig. 2). Gel formation was initiated after about 30 min of incubation with 2.5% GDL; lower concentrations of GDL led to slower gel formation and the addition of 1.0 and 1.5% GDL was not sufficient to produce a gel with adequate strength, even after 180 min of incubation. Three distinct phases of gelation were visible with 2.5% GDL (Fig. 2): the initiation of gel formation; a rapid increase in gel strength; a trend to a plateau when the GDL hydrolysis was possibly complete and no more gluconic acid was available for a further reduction in pH. Based on the elastic modulus and the relative gelation time, a GDL concentration of 2.5% was chosen for subsequent analysis.

Gellan gum at different concentrations (0.10, 0.25 and 0.50%) was added to the combination of 10% sodium caseinate and 2.5% GDL. The interaction between sodium caseinate and gellan gum was evident by a marked increase in the *G'* of the mixture after 180 min of incubation (Fig. 3). As a very high *G'* of the wall material might create difficulty in releasing the core material from the gel network, a gellan gum concentration of 0.25% was chosen. To understand the contribution of gellan gum in the gel formation behavior of the composite mix, we conducted similar rheological analysis of 0.25% gellan gum solution alone acidified with 2.5% GDL. Fig. 2 shows that gellan gum formed a gel with steady increase in *G'* values during the 180 min time sweep test with a final *G'* value of 2053 Pa. Because the *G'* values of caseinate–gellan mix (8339 Pa) were higher than that of caseinate alone (6447 Pa) and of gellan alone (2053 Pa), it is clear that interactions between caseinate and gellan contributed to increased gel strength.

The rate of decrease in pH by the addition of 2.5% GDL was similar in both sodium caseinate solution and the sodium caseinate and gellan mixture solution (Fig. 4).

3.2. Encapsulation efficiency

The EE of various concentrations of free cell suspensions was calculated (Table 1). The EE varied from 41.9 to 89.5% with an increase in the cell loading but showed a slight decrease on further increase in the cell concentration above a certain level (3 mL of cell suspension).

3.3. Particle size distribution

The size distribution for the capsules was found to be uniform and exhibited a diameter range from about 40 to 1100 μm. The surface-weighted and volume-weighted mean diameters of the capsules were found to be about 287 and 399 μm respectively.

3.4. Survival of encapsulated and free cells in SGF and under simulated bile conditions

The initial count of free cells was adjusted with suitable dilution to about 10.7 log cfu, to match with the initial cell population of the capsules. After 30 min of incubation in SGF without pepsin, the

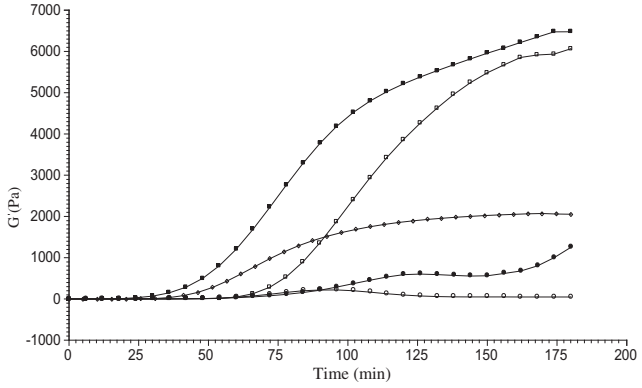

Fig. 2. Changes in the elastic modulus (*G'*) of 10% sodium caseinate solution mixed with 1.0% (○), 1.5% (●), 2.0% (□) and 2.5% (■) glucono-δ-lactone and 0.25% gellan gum solution mixed with 2.5% glucono-δ-lactone (◇).

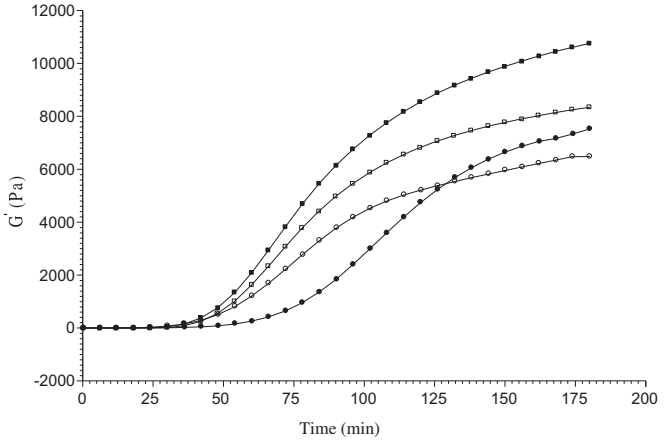

Fig. 3. Changes in the elastic modulus (*G'*) after the addition of 0.10% (●), 0.25% (□) and 0.50% (■) gellan gum into a control sample (○) containing 10% sodium caseinate and 2.5% glucono-δ-lactone.

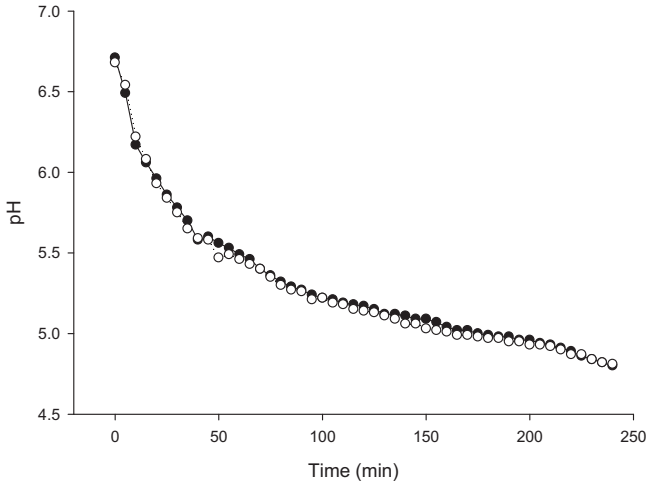

Fig. 4. Changes in the pH by the action of 2.5% glucono-δ-lactone on 10% sodium caseinate (●) and 10% sodium caseinate mixed with 0.25% gellan gum (○) during incubation for 240 min at 20 °C.

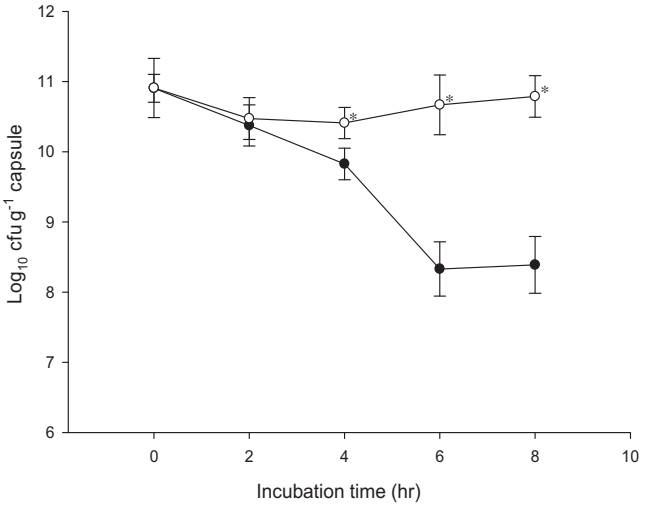

Fig. 6. Survival of encapsulated cells (○) and free cells (●) of *Lactobacillus casei* under simulated bile conditions during 8 h of incubation. The error bars indicate standard deviations from the mean values of three replicated experiments.

viability of free cells declined to 9.4 log cfu and finally reached 4.9 log cfu after 120 min of incubation. In contrast, addition of pepsin to the SGF provided protection to the free cells (Fig. 5) because the viable count in SGF with pepsin decreased only to 5.9 log cfu after 120 min of incubation, which was significantly higher than for the free cells in SGF without pepsin. However, pepsin had almost no effect on the viability of encapsulated cells in SGF. With or without the presence of pepsin in SGF, the viable count of the encapsulated cells was significantly higher than that of the free cells.

The viable count of encapsulated cells in bile salt solution was also significantly higher than that of free cells after 4 h of incubation (Fig. 6); beyond 4 h, the detrimental action of bile salt on the free cells was accelerated. The free cell count had decreased by about 2.6 log cfu at 6 h but then remained almost constant between 6 and 8 h of incubation. For the encapsulated cells, the viable count decreased by only about 0.5 log cfu during the first 4 h of incubation, increased slightly at 6 h and then almost reached the initial cell count level after 8 h of incubation.

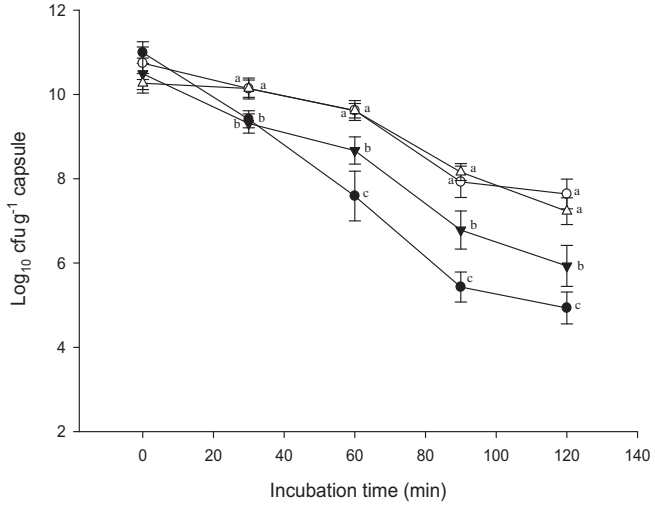

Fig. 5. Survival of encapsulated cells (△, with pepsin; ○, without pepsin) and free cells (▼, with pepsin; ●, without pepsin) of *Lactobacillus casei* in simulated gastric fluid incubated at pH 2.0 for 120 min. The error bars indicate standard deviations from the mean values of three replicated experiments.

4. Discussion

The rheological properties of sodium caseinate gels induced by cold acidification with GDL have been studied extensively (Lucey et al., 1997). In the present study, increasing the concentration of GDL resulted in more rapid formation of the sodium caseinate gel and higher *G'* values, which is in agreement with the findings of Lucey et al. (1997) and Menendez, Schwarzenbolz, Rohm, and Henle (2004). However, the strength of the sodium caseinate gel appeared to be inadequate for forming microcapsules with sufficient rigidity. It was important that the soft gel particles retained their shape and that their size was intact without any coalescence. Therefore, we designed a protein–polysaccharide complex using sodium caseinate and gellan gum. Another reason for including a polysaccharide was the instability of sodium caseinate gels at higher pH.

Gellan gum in combination with sodium caseinate was found to provide a synergistic effect in terms of the gel strength and the stability at higher pH values. It is considered that gellan gum does not simply result into higher viscosity but also forms a complex structure when added into sodium caseinate solution. This was evident from the higher *G'* values obtained than the corresponding *G"* values for any combination of caseinate–gellan solutions (data not shown). Similar rheological analysis of caseinate–gellan mix by Sosa-Herrera, Berli, and Martinez-Padilla (2008) supports this structure formation. They found some intermediate complex formation in a caseinate–gellan mix at pH 5.4 where both polymers were negatively charged, so the possibility of a coacervation was ruled out. It was also concluded that the observed phenomenon could be due to electrostatic interaction as suggested by De Kruif and Tuinier (2001) and more recently by Ye, Flanagan, and Singh (2006) for sodium caseinate/gum arabic mix or intermolecular hydrogen bonding (Dickinson, 2003).

In the dynamic microencapsulation system used in our study, the droplets move around continuously and tend to coalesce, form larger particles and flocculate to form aggregates in the absence of any emulsifier. McClements (1999) explained this phenomenon as electrostatic attractions between the casein molecules near the isoelectric point. To avoid this situation, the entire microencapsulation process was completed within approximately 2 h, when the pH of the mixture had decreased to about 5.2. Although a final pH of around 4.6 was needed to complete the coagulation and to attain

a firmer gel structure, the final pH decrease may have continued after the microcapsules were stored at 4 °C in a distilled water suspension. Our process was capable of preventing further agglomeration of the casein particles.

The high EE can be attributed to the entire process not including any detrimental steps such as heat treatment or high shear force. In addition, gellan gum in combination with sodium caseinate may improve the EE. The EE appeared to be in agreement with the results of Heidebach et al. (2009a), who used a similar process with a sodium caseinate emulsion. As shown in Table 1, the EE improved steadily with an increase in the cell loading but there was a slight decrease after a certain optimum loading, because the cells present on the droplet surface were possibly lost into the oil phase or drained with the washing water.

Microparticle size distributions over a very wide range have been reported by several researchers. Muthukumarasamy et al. (2006) encapsulated *Lactobacillus reuteri* using a variety of gel matrices and concluded that an extrusion process generally produces beads of much larger diameter (average 2–4 mm) than an emulsification process (from 20 μm to 1 mm). Adhikari, Mustapha, and Grun (2003) used a combination of emulsification and coacervation to encapsulate *Bifidobacterium longum* and reported microcapsules in the range 22–350 μm. The optimum microcapsule size is a compromise between the effectiveness of encapsulation and the sensory properties. In general, coarseness in the mouth occurs for a particle size above 1000 μm but is not detectable below 3 μm (Singer & Dunn, 1990). A minimum diameter of 100 μm has been suggested to offer better protection for *Bifidobacterium* in gastric juice (Hansen, Allan-Wojtas, Jin, & Paulson, 2002) and an optimum range of 100–200 μm has been proposed (McMaster, Kokott, & Slatter, 2005). The mean diameter of our capsules was slightly above this suggested range. Although the diameter range showed a wider variation in size (40–1100 μm), 82.5% of the particles were between 100 and 630 μm. The actual impact of this size distribution can be measured only by proper sensory analysis after incorporating the microcapsules in a suitable food formulation. Moreover, this study has been conducted as a proof of principle and the process optimization steps will be carried out in our future work to address this issue.

The reduction in viability of free cells in SGF without pepsin of about 6.1 log cfu after 120 min of incubation can be compared with the 5 log cfu reduction of *L. paracasei* observed after 90 min at pH 2.5 by Heidebach et al. (2009a). However, Song, Cho, and Park (2003) observed better resistance of *L. casei* YIT 9018 and only 4 log cfu reduction was recorded after 3 h of incubation at pH 1.2. A more pronounced lethal effect was reported by Ding and Shah (2009); when nine strains of lactobacilli and bifidobacteria were tested for acid resistance at pH 2.0, all strains were found to be badly affected, with an average log cfu reduction of 6.5–7.0 after 120 min of incubation. The addition of pepsin, a proteolytic enzyme that is secreted in the stomach, to SGF appeared to have a protective effect on free cells (Fig. 5). In this study, after 120 min of incubation, the reduction in free cells was only about 4.6 log cfu in SGF with pepsin compared with 6.1 log cfu in SGF without pepsin. This observation is similar to the findings of Saarela et al. (2005), who explained that it could be due to the presence of other unknown compounds in commercial pepsin extracts obtained from porcine gastric mucosa and also to the strain-specific action of pepsin.

The viability of encapsulated cells in SGF both with and without pepsin was reduced by only about 3.1 log cfu after 120 min of incubation. When compared with the reduction for free cells, this finding is important for our microencapsulation technique. Heidebach et al. (2009a) used sodium caseinate gelled with transglutaminase enzyme for *L. paracasei* encapsulation and similarly found about 3.0 log cfu reduction after 90 min of incubation at pH 2.5. They also investigated a different gelling technique using rennet but the differences in survival rate for encapsulated cells compared with free cells of *L. paracasei* and *B. lactis* were only 0.8 and 2.8 log cfu higher respectively (Heidebach et al., 2009b).

The better survival rate in an encapsulated environment can be attributed to the absence any direct contact of the cells with the acidic medium, which is common for any kind of encapsulation technique; additionally, the buffering nature of milk protein might provide some enhanced protection (Guerin et al., 2003; Kos, Suskovic, Goreta, & Matosic, 2000; Reid et al., 2005). In this study, the better survivability of encapsulated cells in SGF might also be explained by the synergistic effect of gellan gum as well as by the pre-adaptability of bacterial cells in low pH caseinate gels.

The neutral pH of bile extract solution may cause destabilization of the gel network and the properties of the bile salts could possibly cause emulsification of the entrapped or surface oil to some extent, thereby releasing the *L. casei* cells (Ding & Shah, 2009). In the present study, a very high bile tolerance was observed for encapsulated cells when compared with free cells. As different researchers have used various concentrations and sources of bile salts, it is difficult to make any comparison with our finding. However, a lethal action of bile salts on probiotic bacteria has generally been observed (Ding & Shah, 2009; Guerin et al., 2003; Song et al., 2003). Guerin et al. (2003) and Trindade and Grosso (2000) observed opposite results; free cells and encapsulated bifidobacteria cells showed higher viability after 3 h of incubation in the presence of bile salts.

5. Conclusions

The novel encapsulation technique developed in the present study offers a high density gel network with low viscosity. The system is easy to handle, gives full control over particle sizes and provides adequate protection for probiotic bacteria against harsh acidic environments and the detrimental action of bile salts. However, the study is not complete without further analysis of the produced microcapsules in terms of sensory properties, shelf life of entrapped probiotics and release behavior of the encapsulating matrix in the gastro-intestinal tract. We shall be carrying out further research on these aspects. It can be concluded that this system may be another promising encapsulation technique that can be effectively utilized for the application of probiotic bacteria in various foods.

References

Adhikari, K., Mustapha, A., & Grun, I. U. (2003). Survival and metabolic activity of microencapsulated *Bifidobacterium longum* in stirred yogurt. *Journal of Food Science, 68*, 275–280.

Baird, J. K., & Pettitt, D. J. (1991). Biogums used in food and made by fermentation. In I. Goldberg, & R. Williams (Eds.), *Biotechnology and food ingredients* (pp. 223–264). New York, NY, USA: Van Nostrand Reinhold.

Barbut, S., & Foegeding, E. A. (1993). Ca^{2+}-induced gelation of pre-heated whey protein isolate. *Journal of Food Science, 58*, 867–871.

Chen, L. Y., & Subirade, M. (2005). Chitosan/beta-lactoglobulin core-shell nanoparticles as nutraceutical carriers. *Biomaterials, 26*, 6041–6053.

Cobos, A., Horne, D. S., & Muir, D. D. (1995). Rheological properties of acid milk gels. I. Effect of composition, process and acidification conditions on products from recombined milks. *Milchwissenschaft, 50*, 444–448.

De Kruif, C. G., & Tuinier, R. (2001). Polysaccharide protein intereactions. *Food Hydrocolloids, 15*, 555–563.

Desai, K. G. H., & Park, H. J. (2005). Recent developments in microencapsulation of food ingredients. *Drying Technology, 23*, 1361–1394.

Dickinson, E. (2003). Hydrocolliods at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids, 17*, 25–39.

Ding, W. K., & Shah, N. P. (2009). An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. *Journal of Food Science, 74*, 53–61.

Guerin, D., Vuillemard, J. C., & Subirade, M. (2003). Protection of bifidobacteria encapsulated in polysaccharide-protein gel beads against gastric juice and bile. *Journal of Food Protection, 66*, 2076–2084.

Hansen, L. T., Allan-Wojtas, P. M., Jin, Y. L., & Paulson, A. T. (2002). Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food Microbiology, 19*, 35–45.

Heidebach, T., Forst, P., & Kulozik, U. (2009a). Transglutaminase-induced caseinate gelation for the microencapsulation of probiotic cells. *International Dairy Journal, 19*, 77–84.

Heidebach, T., Forst, P., & Kulozik, U. (2009b). Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. *Food Hydrocolloids, 23*, 1670–1677.

Hogan, S. A., McNamee, B. F., O'Riordan, E. D., & O'Sullivan, M. (2001). Micro-encapsulating properties of sodium caseinate. *Journal of Agricultural and Food Chemistry, 49*, 1934–1938.

Kailasapathy, K. (2002). Microencapsulation of probiotic bacteria: technology and potential applications. *Current Issues in Intestinal Microbiology, 3*, 39–48.

Kos, B., Suskovic, J., Goreta, J., & Matosic, S. (2000). Effect of protectors on the viability of *Lactobacillus acidophilus* M92 in simulated gastrointestinal conditions. *Food Technology and Biotechnology, 38*, 121–127.

Lee, B. H. (1996). Bacteria-based processes and products. In B. H. Lee (Ed.), *Fundamentals of food biotechnology* (pp. 219–290). New York, NY, USA: John Wiley and Sons.

Lucey, J. A., van Vliet, T., Grolle, K., Geurts, T., & Walstra, P. (1997). Properties of acid casein gels made by acidification with glucono-delta-lactone. 1. Rheological properties. *International Dairy Journal, 7*, 381–388.

McClements, D. J. (1999). Emulsion formation. In *Food emulsions: Principles, practices, and techniques* (pp. 161–183). Boca Raton, FL, USA: CRC Press.

McMaster, L. D., Kokott, S. A., & Slatter, P. (2005). Micro-encapsulation of Bifidobacterium lactis for incorporation into soft foods. *World Journal of Microbiology and Biotechnology, 21*, 723–728.

Madene, A., Jacquot, M., Scher, J., & Desobry, S. (2006). Flavour encapsulation and controlled release – a review. *International Journal of Food Science and Technology, 41*, 1–21.

Maltais, A., Remondetto, G. E., Gonzalez, R., & Subirade, M. (2005). Formation of soy protein isolate cold-set gels: protein and salt effects. *Journal of Food Science, 70*, 67–73.

Menendez, O., Schwarzenbolz, U., Rohm, H., & Henle, T. (2004). Casein gelation under simultaneous action of transglutaminase and glucono-delta-lactone. *Nahrung, 48*, 165–168.

Mortazavian, A., Razavi, S. H., Ehsani, M. R., & Sohrabvandi, S. (2007). Principles and methods of microencapsulation of probiotic microorganisms. *Iranian Journal of Biotechnology, 5*, 1–18.

Muthukumarasamy, P., Allan-Wojtas, P., & Holley, R. A. (2006). Stability of *Lactobacillus reuteri* in different types of microcapsules. *Journal of Food Science, 71*, 20–24.

O'Riordan, K., Andrews, D., Buckle, K., & Conway, P. (2001). Evaluation of microencapsulation of a Bifidobacterium strain with starch as an approach to prolonging viability during storage. *Journal of Applied Microbiology, 91*, 1059–1066.

Reid, A. A., Vuillemard, J. C., Britten, M., Arcand, Y., Farnworth, E., & Champagne, C. P. (2005). Microentrapment of probiotic bacteria in a Ca2+-induced whey protein gel and effects on their viability in a dynamic gastro-intestinal model. *Journal of Microencapsulation, 22*, 603–619.

Rosenberg, M., & Sheu, T. Y. (1996). Microencapsulation of volatiles by spray-drying in whey protein-based wall systems. *International Dairy Journal, 6*, 273–284.

Saarela, M., Virkajarvi, I., Alakomi, H. L., Mattila-Sandholm, T., Vaari, A., Suomalainen, T., et al. (2005). Influence of fermentation time, cryoprotectant and neutralization of cell concentrate on freeze-drying survival, storage stability, and acid and bile exposure of *Bifidobacterium animalis* ssp *lactis* cells produced without milk-based ingredients. *Journal of Applied Microbiology, 99*, 1330–1339.

Singer, N. S., & Dunn, J. M. (1990). Protein microparticulation – the principle and the process. *Journal of the American College of Nutrition, 9*, 388–397.

Song, S. H., Cho, Y. H., & Park, J. (2003). Microencapsulation of *Lactobacillus casei* YIT 9018 using a microporous glass membrane emulsification system. *Journal of Food Science, 68*, 195–200.

Sosa-Herrera, M. G., Berli, C. L. A., & Martinez-Padilla, L. P. (2008). Physicochemical and rheological properties of oil-in-water emulsions prepared with sodium caseinate/gellan gum mixtures. *Food Hydrocolloids, 22*, 934–942.

Sun, W. R., & Griffiths, M. W. (2000). Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads. *International Journal of Food Microbiology, 61*, 17–25.

Trindade, C. S. F., & Grosso, C. R. F. (2000). The effect of the immobilisation of *Lactobacillus acidophilus* and *Bifidobacterium lactis* in alginate on their tolerance to gastrointestinal secretions. *Milchwissenschaft, 55*, 496–499.

US Pharmacopeia. (2008). USP31-NF26. In *The United States pharmacopeia–national formulary*. Rockville, MA, USA: US Pharmacopeia.

van Vliet, T., & Keetels, C. J. A. M. (1995). Effect of pre-heating of milk on the structure of acidified milk gels. *Netherlands Milk and Dairy Journal, 49*, 27–35.

Ye, A., Flanagan, J., & Singh, H. (2006). Formation of stable nanoparticles via electrostatic complexation between sodium caseinate and gum arabic. *Biopolymers, 82*, 121–133.