Microencapsulation of a probiotic bacteria with alginate–gelatin and its properties

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Abstract

Lactobacillus casei ATCC 393-loaded microcapsules based on alginate and gelatin had been prepared by extrusion method and the product could increase the cell numbers of L. casei ATCC 393 to be 10⁷ CFU g⁻¹ in the dry state of microcapsules. The microparticles homogeneously distributed with size of 1.1 ± 0.2 mm. Four kinds of microcapsules (S₁, S₂, S₃ and S₄) exhibited swelling in simulated gastric fluid (SGF) while the beads eroded and disintegrated rapidly in simulated intestinal fluid (SIF). Cells of L. casei ATCC 393 could be continuously released from the microcapsules during simulated gastrointestinal tract (GIT) and the release amounts and speeds in SIF were much higher and faster than that in SGF. Encapsulation in alginate–gelatin microcapsules successfully improved the survival of L. casei ATCC 393 and this approach might be useful in delivery of probiotic cultures as a functional food.

Key words: Microcapsule; lactobacillus casei ATCC 393; relative humidity (RH); stability; release

Introduction

Probiotics have been defined as ‘live microorganisms which, administered in adequate amounts, confer a beneficial physiological effect on the host’¹. Lactic acid bacteria (LAB), which are among the most important probiotic microorganisms typically associated with the human gastrointestinal tract (GIT), have many beneficial effects on the human gut flora including immune stimulation, cholesterol reduction, inhibition of pathogen growth, maintenance of a healthy gut microflora, prevention of cancer, improvement in lactose utilization, prevention of diarrhoeal diseases or constipation, absorption of calcium and synthesis of vitamins and predigestion of proteins.

In order to exert positive health effects, viability of probiotic bacteria in a product at the point of consumption is an important consideration for their efficacy, as they have to survive during the processing and shelf-life of food and supplements, transit through high acidic conditions of the stomach and enzymes and bile salts in the small intestine. After the LAB pass through the stomach and upper intestinal tract, LAB should preferably adhere to the epithelium of the intestinal tract and grow⁶. As a guide, the International Dairy Federation has recommended that the bacteria should be active and abundant in the product and be present at least 10⁷ CFU g⁻¹ to the date of minimum durability⁷. Unluckily most of the probiotics including LAB lack the ability to survive in a high proportion of the harsh conditions of acidity and bile concentration commonly encountered in the gastrointestinal tract of humans⁸.

To improve the survival of LAB, different approaches that increase the resistance of these sensitive microorganisms against adverse conditions have been proposed, including appropriate selection of acid- and bile-resistant strains, use of oxygen-impermeable containers, two-step fermentation, stress adaptation, incorporation of micronutrients such as peptides and amino acids and microencapsulation⁹. However, these methods had only a limited success.

Therefore, encapsulation of bacterial cells in alginate gels is currently gaining attention to increase viability of probiotic bacteria in acidic products such as yoghurt¹⁰–¹² and it is the commonly used technique because this method is very mild and is done at room temperature.
in aqueous medium by using physiologically acceptable chemicals. Encapsulation is a process in which the cells are retained within an encapsulating membrane to reduce cell injury or cell loss and it has been widely utilized to protect microorganisms including probiotics during transit through the human gastro-intestinal tract. The microbial cells are entrapped within their own secretions (exopolysaccharides (EPS)) that act as a protective structure or a capsule, reducing the permeability of material through the capsule and therefore less exposed to adverse environmental factors such as gastric acid and bile salts.

Carbohydrate polymers such as alginate have been used in various food applications. Alginate, a natural polysaccharide found in brown algae, is a linear 1, 4 linked copolymer of \( \beta \)-D-mannuronic acid (M) and \( \alpha \)-L-guluronic acid (G) and has the benefits of being non-toxic to the cells being immobilized and it is an accepted food additive. The reversibility of encapsulation, i.e. solubilizing alginate gel by sequestering calcium ions, and the possible release of entrapped cells in the human intestine are other advantages. However, alginate beads are not acid resistant and it has been reported that the beads undergo shrinkage and decreased mechanical strength during lactic fermentation.

Gelatin is a protein derived from denatured collagen that contains high levels of hydroxyproline, proline and glycine and is useful as a thermally reversible gelling agent for encapsulation. Gelatin was selected here because of its excellent membrane-forming ability, biocompatibility and non-toxicity. The applicability of gelatin as a hydrogel matrix is limited because of its low network rigidity. However, its physical properties can be improved through the addition of cross-linking agents. Because of its amphoteric nature, it also is an excellent candidate for cooperation with anionic polysaccharides such as alginate and so on.

The choice of an appropriate drying method is also very important in the case of LAB, so as to increase their survival rates during dehydration itself and subsequent storage. Freeze-drying and spray-drying have commonly been used for the dehydration. Freeze-drying, in particular, is the most common process for the production of large amounts of concentrated microbiological cultures. However, during this process, bacteria are subjected to adverse conditions, such as low temperature and low water activity, that produce structural and physiological injury to the bacterial cells resulting in the loss of viability of many species. Spray-drying as a method for preparing concentrated cultures of microencapsulated cells has many disadvantages associated with this approach. A major limitation of spray-drying of probiotic cultures is the loss of viability which occurs during processing and storage of the powders. Previous reports have shown, following spray-drying, probiotic \textit{Lactobacillus acidophilus} showed increased sensitivity to lysozyme and NaCl, indicators of cell wall and cell membrane damage.

In this paper, alginate and gelatin were used to immobilize \textit{Lactobacillus casei} ATCC 393 cells and the survival of \textit{L. casei} ATCC 393 after drying (at 4°C) was described since there were only a few mentions in the literature about lactic acid bacteria encapsulated with alginate and gelatin. Other characteristics such as the morphology of beads, effect of cross-linking on the release profile of \textit{L. casei} ATCC 393 cells, the swelling and stability of beads in different media (different pH, different ion intensity) were also investigated.

**Materials and methods**

**Materials**

Sodium alginate, gelatin, calcium chloride and sodium citrate were purchased from Sigma (St. Louis, MO). The de Man Rogosa Sharpe (MRS) broth was purchased from Oxoid (Australia). The simulated gastric juice (SGF) was prepared by suspending pepsin (10 g 1\(^{-1}\), Sigma, St. Louis, MO) in saline (0.5%, v/v) and the pH adjusted to 1.2 with 6 mol\(^{-1}\) HCl. It was then sterile-filtered through a membrane (0.22 \(\mu\)m). The simulated intestinal fluid (SIF) was prepared by dispersing pancreatic enzymes (Sigma, St. Louis, MO) in sterile sodium phosphate buffer (pH 6.8) to a final concentration of 10 g 1\(^{-1}\), with 0.5% bile salts (Oxoid) added. The resulting suspension was sterile-filtered through a membrane (0.22 \(\mu\)m).

**Bacterial strain and culture preparations**

\textit{L. casei} ATCC 393 was used in this study. \textit{L. casei} ATCC 393 was sub-cultured initially in 20 ml MRS broth at 37°C for 18 h. The resulting cultures were transferred into 20 ml MRS broth and incubated under the same conditions. Cultures were harvested by centrifugation at 4500 g at 4°C for 30 min and washed with phosphate buffer saline (pH 7.4) and collected by centrifugation as above. The washed bacterial cells were mixed with a MRS broth for use.

**Microencapsulation of microorganisms**

\textit{L. casei} ATCC 393-loaded microcapsules were prepared with different wall materials (alginate, gelatin) to get samples (S\(_1\), S\(_2\), S\(_3\) and S\(_4\)) (shown in Table 1). \textit{L. casei} ATCC 393 microcapsules were prepared by mixing 5 ml cell suspensions and 30 ml sterilized wall materials (alginate solution, sterilized at 121°C for 15 min; gelatin solution,
sterilized by ultra-high temperature processing (140°C, 5 s)) at 37°C, then the mixture was injected through a syringe needle into 0.2 mol l⁻¹ calcium chloride solution at room temperature (25°C) to get S₁ and S₂ (the distance between the syringe and the calcium chloride collecting solution was 10 cm). The beads were allowed to stand for 1 h for hardening before being aseptically transferred to a sterile flask for storage. S₃ and S₄ were prepared by suspending S₂ in 0.06 mol l⁻¹ sodium citrate for 15 min and 30 min, respectively. Samples were rinsed twice with distilled water and dried without protective ingredients under controlled air flow, temperature (4°C) and relative humidity (52 ± 5%) of the air. The microcapsules could be dried within 24 h.

Surface morphology and bead size determination

The shape and surface characteristics were determined by scanning electron microscopy (SEM) using a gold sputter technique. The microcapsules were vacuum-dried, coated with gold palladium and observed microscopically. Particle sizes of four samples (S₁, S₂, S₃ and S₄) were measured by naked eye with a meter and the size of microcapsules was studied. In all measurements at least 100 particles were examined.

Effect of relative humidity (RH) on the moisture resorption of microcapsules

The water resorption experiment was carried out according to the following procedures. The dried microcapsules were kept in a dessicator at a temperature of 25°C and a relative humidity of 33% (magnesium chloride), 52% (magnesium nitrate), 75% (ammonium chloride) and 97% (potassium sulphate), respectively. The weight of microcapsules were determined at the end of 1 week and compared with the data of freshly prepared dried microcapsules.

All experiments were done in triplicate. The water resorption ability of beads was calculated from the formula:

$$R_s (\%) = \frac{W_t - W_0}{W_0} \times 100$$

where W₀ was the initial weight of the beads and Wₜ was the weight of the microcapsules at equilibrium in different relative humidity (RH) after 1 week.

Evaluation of the stability of microcapsules in different media

The stability of four samples (S₁, S₂, S₃ and S₄) was assessed in SGF (pH 1.2), SIF (pH 6.8), respectively, as described in¹⁸. Briefly, dried microcapsules of known weight (10 g) were placed in a glass vial containing 100 ml of solution and incubated at 37°C with 110 rpm of shaking for 4 h. The beads were periodically removed and weighed. The wet weight of the swollen beads was determined by blotting them with filter paper to remove moisture adhering to the surface, immediately followed by weighing on an electronic balance and the mean value was reported. Effect of pH and ion intensity on the stability of beads was checked with the same method.

All experiments were done in triplicate. The percentage of swelling of the beads was calculated from the formula:

$$S_W (\%) = \frac{W_t - W_0}{W_0} \times 100$$

where W₀ was the initial weight of the beads and Wₜ was the weight of the swollen beads at equilibrium swelling in the media.

In vitro release studies (GIT)

To examine the release behaviour of L. casei ATCC 393 from microcapsules in GIT in vitro, samples (S₁, S₂, S₃ and S₄, 10 beads) were added to 50 ml SGF (pH 1.2) and incubated at physiological temperature (37°C) for 2 h and subsequently transferred into SIF (pH 6.8) for another 4 h. At specific time intervals (30 min), 2.0 ml aliquots were removed and assayed OD₆₀₀ for L. casei ATCC 393 in triplicate.

Survival of L. casei ATCC 393-loaded in microcapsules

Non-encapsulated L. casei ATCC 393 were enumerated in the MRS agar. Peptone water was used to prepare the

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wall material</th>
<th>Time (min)</th>
<th>Live cell amount (CFU g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium alginate, Gelatin (3:0, w/w)</td>
<td>–</td>
<td>4.79 × 10⁷</td>
</tr>
<tr>
<td>S₁</td>
<td>Sodium alginate, Gelatin (2:1, w/w)</td>
<td>0</td>
<td>2.83 × 10⁷</td>
</tr>
<tr>
<td>S₂</td>
<td>Sodium alginate, Gelatin (2:1, w/w)</td>
<td>15</td>
<td>3.53 × 10⁸</td>
</tr>
<tr>
<td>S₃</td>
<td>Sodium alginate, Gelatin (2:1, w/w)</td>
<td>30</td>
<td>3.02 × 10⁸</td>
</tr>
</tbody>
</table>

Table 1. The wall material, function time of sodium citrate (0.06 mol l⁻¹) and live amount of L. casei ATCC 393 in different samples during 1 week.
serial dilutions and culture was plated by the pour plate technique. The plates were incubated at 37°C for 48 h. To determine the viable counts of the encapsulated *L. casei* ATCC 393, 0.1 g of microcapsules were resuspended in 10 ml of sodium citrate (0.06 mol l⁻¹) and stirred for 45 min using a magnetic stirrer at 37°C. Complete release of bacteria from the microcapsules in 45 min was previously assured by comparing the released number of cells from the microcapsules. The colony forming units (CFU g⁻¹) were determined by plating on MRS agar plate and incubating at 37°C for 48 h. The plating procedures were carried out in triplicate.

**Statistical analysis**

The assays were performed in triplicate on separate occasions. The data collected in this study were expressed as the mean value ± standard deviation (SD).

**Results and discussion**

** Characteristics of *L. casei* ATCC 393 microcapsules**

The *L. casei* ATCC 393-loaded microcapsules were prepared by the extrusion method with alginate and gelatin and dried at 4°C. The shapes and surface morphologies of the microparticles were investigated using SEM and shown in Figure 1. The microparticles homogeneously distributed without evidence of collapsed spheres. The spherical shape of the microcapsules in wet state was lost after drying and the surface of the microcapsules was rough with some visible cracks. The microparticles of S₁ were spherical with a wrinkled surface, probably due to the loss of water content during the drying process (seen in Figure 1(a)). The introduction of gelatin (S₂) resulted in a more dense surface of the microcapsules compared with S₁ (Figure 1(b)). The surface morphology of microcapsules treated with sodium citrate (S₃ and S₄) was different from the untreated ones (S₁ and S₂) and its surface were in the form of sheets. With the time increased from 15 min (S₃) to 30 min (S₄) the surface morphology of the microcapsules exhibited much uniformity (shown in Figures 1(c) and (d)). Microparticles usually had a heterogeneous structure with a dense surface layer and a loose core due to the heterogeneous gelation mechanism, which resulted in the abnormality of microsphere during the drying process.

The size of microspheres prepared in this study was in the range of 1.1 ± 0.2 mm and the results were shown in Figure 2. The diameter of S₂ was ~1.3 ± 0.1 mm and the particle size of other samples (S₁, S₃ and S₄) was slightly smaller than that of S₂. The coating material and the function of sodium citrate (0.06 mol l⁻¹) had no obvious influence on the diameter of four kinds of microcapsules.

Four samples of microencapsulated *L. casei* ATCC 393 (S₁, S₂, S₃ and S₄) were stored at 4°C to check the survival amount of the live cells in the products during a period (1 week). The original live cell number (0 week) of encapsulated *L. casei* ATCC 393 in each sample was 4.79 × 10⁷ CFU g⁻¹, 2.83 × 10⁷ CFU g⁻¹, 3.53 × 10⁸ CFU g⁻¹ and 3.02 × 10⁹ CFU g⁻¹, respectively (shown in Table 1), compared to 10⁶ CFU g⁻¹ of the dried non-encapsulated cells of *L. casei* ATCC 393 (without any protective agent) and 1.2 × 10⁴ CFU g⁻¹ of encapsulated *L. casei* ATCC 393 without protectants with freeze-drying (data not shown). The viable count of microencapsulated cells loaded in S₁ and S₄ was only 0.9 log decrease after storage at 4°C for 7 days while S₁ and S₂ showed nearly 2 log decrease in cell numbers. There was an increased survival of cell numbers of S₂ and S₄ compared with S₁ and S₂, the reason might be that the structure of S₃ and S₄ was more beneficial to the survival of *L. casei* ATCC 393. The presence of citrate in the particle or a higher pH in the citrate-containing particles might also lead to the increased survival of microencapsulated *L. casei* ATCC 393. Additionally, microencapsulated *L. casei* ATCC 393 in S₂ and S₄ had constant characteristics and higher stability than that of S₁ and S₂ during storage at 4°C.

Results indicated that microcapsules dried at 4°C could obviously improve the survival of *L. casei* ATCC 393 in comparison with freeze-drying. The freeze-drying process evokes several environmental fluctuations, subjecting the LAB cells to low temperature-, freeze-, osmotic- and desiccation stress. The major causes of loss cell viability in freeze-drying are probably ice crystal formation, high osmolarity due to high concentrations of internal solutes with membrane damage, macromolecule denaturation and the removal of water, which affect properties of many hydrophilic macromolecules in cells[19] and with consequent decrease in viability, a higher sensitivity to air exposure and loss of reproductive capability.

**Moisture resorption property**

The moisture resorption ability of microcapsules was studied to determine their sensitivity to different kinds of humidity conditions and the results are shown in Figure 3. The moisture uptake rate of the different samples was determined at four storage humidities (33%, 52%, 75% and 97%, RH). It was obvious that the behaviour of four samples was quite similar to each other at the low relative humidity (33%, 52%, 75%, RH) and the values of Rs were much the same. Microcapsules (S₁, S₂, S₃ and S₄) had no water uptake after incubated for 7 days at relative humidity of 33% and 52% and became a little higher but not more
Figure 1. Morphology of alginate and gelatin microspheres. (a) S1; (b) S2; (c) S3; (d) S4.

Figure 2. Size distribution analysis of microcapsules (data shown were the mean ± SD, n = 3).

Figure 3. Moisture resorption ability of microcapsules in different relative humidity (RH) (data shown were the mean ± SD, n = 3).
than 21% in 1 week when relative humidity increased to 75%. However the water uptake of S₃ and S₄ increased obviously which attained 106.42%, 97.99% compared with S₁ (Rₛ, 31.91%) and S₂ (Rₛ, 29.75%) in the same time interval when the relative humidity increased to 97%, perhaps due to the looser structure of them (S₃ and S₄). This result indicated that microcapsules treated with sodium citrate had a better ability of absorbing water compared with the untreated ones and the Rₛ value of four samples (S₁, S₂, S₃ and S₄) became much higher with the increase of RH (from 33% to 97%).

**Stability of the L. casei ATCC 393-loaded microcapsules**

**Stability of microcapsules in SGF and SIF.** In order to obtain data on the behaviour of microcapsules during simulated gastrointestinal tract, the stability in SGF (pH 1.2) and SIF (pH 6.8) was investigated respectively and the results are shown in Figure 4. In SGF, the beads showed swelling without any sign of disintegration during 4h. The results presented in Figure 4(a) show that four samples (S₁, S₂, S₃ and S₄) swelled rapidly in the initial 30 min, after 1 h, the swelling of microcapsules (S₁, S₂, S₃ and S₄) reached the equilibrium without any erosion and the maximum values were 87.7%, 111.8%, 137.7% and 120.6%, respectively (Figure 4(a)). In SIF, all the microspheres swelled associating with erosion which resulted from calcium-alginate cross-linking network were ionized and absorbed water. For microcapsules, the maximum water uptake reached after 2.5 h and after shaking of 4 h, S₁ and S₂ maintained their spherical shape with slight erosion, while the treated ones (S₃ and S₄) were damaged or even broken into pieces. Samples (S₃ and S₄) treated with sodium citrate swelled rapidly and reached higher SW (1447.7% and 1303.8%) while S₁ and S₂ exhibited maximum water uptake of 470.7% and 100.0% in SIF. The results meant that microcapsules (S₁ and S₂) without the function of sodium citrate were much more stable than the treated ones (S₃ and S₄) and the introduction of gelatin (S₃) in the system reduced the percentage water uptake of beads while maintaining their stability compared with the alginate microcapsules (S₁) in SIF.

**Effect of pH on the stability of microcapsules.** The stability of microcapsules in disodium hydrogen phosphate-citric acid buffer with pH 2.4, 3.8, 5.2, 6.4, 7.8, 8.0 were studied and shown in Figure 5. The results demonstrated that the beads changed their behaviour when the environmental pH was altered. When the beads were immerged in low pH (2.4) their size exhibited swelling and reached the equilibrium after 1 h (Figure 5(a)). For pH 3.8, the water uptake of S₁, S₂, S₃ and S₄ continued all the process (Figure 5(b)). With the increase of pH (from 5.2 to 8.0), the microcapsules exhibited the same behaviour (first swelled and then began to disintegrate) and carboxyl groups of alginate that were not cross-linked by Ca²⁺ or disrupted from calcium-alginate cross-linking network were ionized and absorbed water, which resulted in the disintegration of the beads. It was reported that the disruption of the calcium-alginate gel matrix occurred fast in phosphate buffer solution with pH above 5.5 due to the chelating action of phosphate ions⁵⁰. The affinity of phosphate for calcium was higher than that of alginate²¹. Moreover, the bonds of alginate-Ca were partially broken during the preparation of S₃, S₄ and the erosion speed was much rapider than that of S₁ and S₂. The results showed that microcapsules became unstable when the environment changed from acidic to neutral conditions.
Figure 5. The stability behaviour of microcapsules in different pH conditions (data shown were the mean ± SD, n = 3): (a) pH 2.4; (b) pH 3.8; (c) pH 5.2; (d) pH 6.4; (e) pH 7.8; (f) pH 8.0.
Figure 6. Effect of ion intensity of aqueous media on the stability of microparticles (data shown were the mean ± SD, n = 3): (a) 0.01 mol l⁻¹; (b) 0.05 mol l⁻¹; (c) 0.1 mol l⁻¹; (d) 0.5 mol l⁻¹; (e) 1 mol l⁻¹.
Effect of ion intensity on the stability of the microcapsules. Different concentrations of NaCl (0.01, 0.05, 0.1, 0.5 and 1 mol l\(^{-1}\)) were chosen to test its influence on the stability of four samples (S\(_1\), S\(_2\), S\(_3\) and S\(_4\)) and the results are shown in Figure 6.

At the beginning, four kinds of microcapsules (S\(_1\), S\(_2\), S\(_3\) and S\(_4\)) absorbed water and swelled continuously in different ion intensity mediums. After 3h, the beads attained maximum swelling, subsequently they began to show weight loss and dissolve. Samples treated with sodium citrate (S\(_3\) and S\(_4\)) swelled much more rapidly and reached higher S\(_{10}\) compared with S\(_1\) and S\(_2\) at the same ion intensity. As the quantity of NaCl in the medium increased (from 0.01 to 1 mol l\(^{-1}\)) this kind of difference (the value of S\(_{10}\) between the treated ones and untreated ones) became inconspicuous.

Alginates were hydrophilic and water-soluble anionic polysaccharides, but the Ca\(^{2+}\)-induced cross-linked beads of alginate were sufficiently stable in the aqueous media. When the microcapsules were placed in the buffer system containing Na\(^+\), the Na\(^+\) ions present in the external solution underwent an ion-exchange process with Ca\(^{2+}\) ions which were binding with COO\(^-\) groups mainly in the polyguluronate sequences. As a result, the electrostatic repulsion among negatively charged –COO\(^-\) groups increases which ultimately caused the chain relaxation and enhances the gel swelling. In the later stage of the swelling process, the egg-box structure began to loosen and hence the beads started to disintegrate and lose their weight. It was the ion exchange process between Na\(^+\) and Ca\(^{2+}\) ions which was supposed to be responsible for the swelling and subsequent degradation of the beads. In this way, the ion-exchange process between Na\(^+\) ions binding with carboxylate groups in polyguluronate and polymannuronate blocks was ultimately responsible for the swelling and subsequent degradation of the microcapsules\(^{22}\).

In vitro release studies of L. casei ATCC 393 from the microcapsules

Microcapsule samples (S\(_1\), S\(_2\), S\(_3\) and S\(_4\)) were treated with SGF and then with SIF to check the continuously release characteristics of L. casei ATCC 393 in GIT and the results are shown in Figure 7. In the first step, the release amounts of cells were minor in SGF (pH 1.2) from each sample of the microspheres. After the samples (S\(_1\), S\(_2\), S\(_3\) and S\(_4\)) were transferred from SGF to SIF, the larger amounts and faster release rate of L. casei ATCC 393 cells were found. The release profile of L. casei ATCC 393 between four samples had slight differences in the neutral condition. Compared to the release profile in SIF only (without prior incubation in SGF) the release of L. casei ATCC 393 from microcapsules in GIT was much faster when the microcapsules were transferred into SIF (data not shown). When the beads were treated with SGF, the alginate component underwent acid catalysed hydrolysis and also the conversion of –COO\(^-\) groups into –COOH groups, the electrostatic attraction between Ca\(^{2+}\) and –COO\(^-\) groups in ‘egg-box’ junction almost disappears\(^{23}\) and hence the beads began to disintegrate much more rapidly. This result indicated that cells of L. casei ATCC 393 could continuously be released from the microcapsules in GIT and the release amounts and speeds of L. casei ATCC 393 cells SIF were much higher and faster than that in SGF.

Conclusions

L. casei ATCC 393-loaded microcapsules based on alginate and gelatin had been prepared by extrusion technology and the product could increase the live cell numbers to be 10\(^7\) CFU g\(^{-1}\) in the dry state of microcapsules. The microparticles obtained by the extrusion method homogeneously distributed without evidence of collapsed spheres and non-aggregated with a size of 1.1 ± 0.2 mm. The relative humidity had little effect on the characteristics of microcapsules (S\(_1\), S\(_2\), S\(_3\) and S\(_4\)) when it was not more than 75\%. The pH values and ion intensity of solution affected the swelling behaviour of alginate–gelatin microcapsules and the microparticles became unstable and disintegrated much rapidly with the increase of pH (from 2.4 to 8.0) and ion intensity (from 0.01 to 1 mol l\(^{-1}\)). Cells of L. casei ATCC 393 could be continuously released from the microcapsules during GIT and the release amounts and speeds in SIF (pH 6.8) were much higher and faster than that in SGF (pH 1.2). In summary, the microencapsulation method reported in this paper under optimum conditions proved to be very efficient in increasing the viability of
probiotic bacteria compared to non-encapsulated free cells. Alginate–gelatin microcapsules might be potentially used as a safe and protective delivery vehicle for administering viable probiotic bacteria.

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