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Preparation of alginate/chitosan/carboxymethyl chitosan complex microcapsules and application in *Lactobacillus casei* ATCC 393

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ABSTRACT

Lactobacillus casei ATCC 393 was encapsulated with alginate, chitosan and carboxymethyl chitosan by extrusion method and the product could increase the cell numbers of *L. casei* to be 10^8 cfu/g in the dry state after storage at 4 °C for 4 weeks. After incubation in simulated gastric (pH 2.0, 2 h) and bile juices (1%, 6 h), the encapsulated *L. casei* cell numbers were 7.91 and 7.42 log cfu/g, respectively. Results indicated that alginate–chitosan–carboxymethyl chitosan microcapsules could successfully protect *L. casei* against adverse conditions and this approach might be useful in the delivery of probiotic cultures as a functional food.

1. Introduction

Probiotics, defined as "living microorganisms which upon ingestion in certain numbers, exert health benefits beyond inherent general nutrition" (Guarner & Schaafsma, 1998) have become increasingly popular during the last decade. The maintenance of the viability and functionality of the probiotics until they reach their destination in the human gut is one of the key requirements (Heidebach, Forst, & Kulozik, 2009; Mattila-Sandholm et al., 2002; Ross, Desmond, Fitzgerald, & Stanton, 2005). Ingested probiotics must survive transit through the gastric environment and reach the colon in large quantities to facilitate colonization and thus to exert a beneficial effect on host. It has been recommended that food containing probiotic bacteria should contain at least 10^7 live microorganisms per g or ml, at the time of consumption in order to produce therapeutic benefits (Kim et al., 2008; Li, Chen, Cha, Park, & Liu, 2009). Unluckily most of the probiotics including LAB lack the ability to survive in a high proportion the harsh conditions of acidity and bile concentration commonly encountered in the gastrointestinal tract of humans (Krasaekoopt, Bhandari, & Deeth, 2003; Nazzaro, Fratianni, Coppola, Sada, & Orlando, 2009).

The delivery of active probiotic cells in microencapsulated form has received reasonable attention during the last 10 years, since it can reduce losses of sensitive bacteria induced by detrimental external factors such as oxidative or acid stress during storage and digestion (Donthidi, Tester, & Aidoo, 2010; Heidebach, Först, & Kulozik, 2010; Sandoval-Castilla, Lobato-Calleros, García-Galindo, Alvarez-Ramírez, & Vernon-Carter, 2010).

Carbohydrate polymers such as alginate have been used in various food applications (Allan-Wojtas, Truelstrup Hansen, & Paulson, 2008; Fabian, Huynh, & Ju, 2010). The reversibility of encapsulation, i.e. solubilizing alginate gel by sequestering calcium ions, and the possible release of entrapped cells in the human intestine is another advantage (Chandramouli, Kailasapathy, Peiris, & Jones, 2004; Prakash & Jones, 2005). However, the gel is susceptible to disintegration in the presence of excess monovalent ions, Ca^{2+} chelating agents and harsh chemical environments (Krasaekoopt, Bhandari, & Deeth, 2004). A cross-linked alginate matrix system at very low pH is reported to undergo a reduction in alginate molecular weight causing a faster degradation and release of active ingredients (Gombotz & Wee, 1998; Krasaekoopt, Bhandari, & Deeth, 2006).

Cationic chitosan can form gels with non-toxic multivalent anionic counterions such as polyphosphate and sodium alginate (Anal & Stevens, 2005; Lucinda-Silva, Salgado, & Evangelista, 2010) by ionic cross-linking. N, O-carboxymethyl chitosan (NOCC) is a chitosan derivative having carboxymethyl substituents on some

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of both the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure (Chen & Park, 2003; Tavakol, Vasheghani-Farahani, Dolatabadi-Farahani, & Hashemi-Najafabadi, 2009). NOCC is non-toxic and has already been used extensively in a wide range of biomedical applications due to its unique properties especially its good solubility in water and excellent biocompatibility (Dolatabadi-Farahani, Vasheghani-Farahani, & Mirzadeh, 2006; Fan et al., 2006; Liu, Jiao, & Zhang, 2007).

The purpose of the present work was to encapsulate *Lactobacillus casei* ATCC 393 with sodium alginate, chitosan and carboxymethyl chitosan by the extrusion method. In addition, the effect of microencapsulation on the survival of *L. casei* after drying and exposure to simulated gastro-intestinal conditions were also investigated.

2. Experimental

2.1. Materials and microorganisms

Chitosan, derived from crab shell, molecular weight 540 kDa; deacetylation degree 89.6%, prepared as previously by the method of degradation with acetic acid according to Chen, Zheng, Wang, Lee, and Park (2002). Carboxymethyl-chitosan (CMCS) with different molecular weight were prepared from the reaction of chitosan and chloroactic acid in our laboratory. Sodium alginate, calcium chloride and sodium citrate, were purchased from Sigma (St. Louis, MO, USA). The de Man Rogosa Sharpe (MRS) broth was purchased from Oxoid, Australia.

L. casei ATCC 393 was used in this study. *L. casei* was cultured in MRS medium at 37 °C. Cultures were harvested by centrifugation at 4500 × *g* at 4 °C for 30 min, washed with phosphate buffer saline (pH 7.4) and collected by centrifugation as above. The washed bacterial cells were mixed with peptone water (1 g/l) for use.

2.2. Encapsulation procedure

2.2.1. Coating with alginate

The extrusion technique of microencapsulation was used (Krasaekoopt et al., 2003). Alginate microcapsules were prepared as follows: sodium alginate was dissolved in distilled water (1.5%, w/v) and sterilized at 121 °C for 15 min. After cooling the alginate mixture, the cell suspension (20 ml) and microporous starch (6 g) were mixed with the alginate mixture (5 ml) homogeneously and injected through a syringe into sterilized 0.2 mol/l $CaCl_2$ solution that was stirred continuously to form capsules. The capsules were allowed to harden for about 30 min in $CaCl_2$ solution and then washed twice with 0.1% peptone solution to remove excess calcium ions and untrapped cells. The beads were dried under controlled air flow and temperature (4 °C).

2.2.2. Coating with alginate and chitosan

Alginate–chitosan microcapsules were prepared as follows: the wet state of alginate coated beads (prepared in Section 2.2.1) were immersed in 100 ml of chitosan solution (1%, w/v) and shaken at 100 rpm for 40 min on an orbital shaker for coating followed by rinsing with 0.1% peptone solution to remove the excess chitosan and the beads were dried under controlled air flow and temperature (4 °C).

2.2.3. Coating with alginate, chitosan and CMCS

Alginate–chitosan–CMCS microcapsules were prepared as follows: the wet state of alginate–chitosan coated beads (prepared in Section 2.2.2) were added into 100 ml of CMCS solution (0.5%, w/v) and shaken at 100 rpm on an orbital shaker. After being hardened for 30 min, the micro-gel beads containing *L. casei* were obtained. The beads were washed with 0.1% peptone solution and dried under controlled air flow and temperature (4 °C). Different molecular weight (1771.5 kDa, 720.9 kDa, 490.2 kDa, 172.7 kDa) and different concentration (0.5%, 1.0%, 2.0%, w/v) of CMCS were chosen to prepare the *L. casei*-loaded microcapsules.

2.3. Physical examination of microcapsules

2.3.1. Surface morphology and bead size determination

The shape and surface characteristics were determined by scanning electron microscopy (SEM) using gold sputter technique. The microcapsules were vacuum-dried, coated with gold palladium and observed microscopically. The size of the *L. casei*-loaded microcapsules was measured with Laser Diffraction Particle Size Analyzer SALD-3101 (Shimadzu, Japan).

2.3.2. Moisture content

The residual moisture content of dried microcapsules was determined by oven-drying the particles at 102 °C, determining the difference in weight, and expressing the weight loss as a percentage of the initial powder weight (Sunny-Roberts & Knorr, 2009).

2.3.3. Permeability

Lysozyme (M_W, 13.930 kDa) and bovine serum albumin (BSA, M_W, 67.000 kDa) were selected as the investigated targets in our experiments. Approximately 2 ml of alginate–chitosan microcapsules (Blank) and alginate–chitosan–CMCS microcapsules were put into a test tube containing 15–30 ml protein solution and the concentration of protein was 0.6 mg/ml. The supernatant absorbance of protein solution was measured using an UV spectrometer (UV-260, Shimadzu Corporation, Japan) when the solution was mixed sufficiently on a gyratory shaker at different time periods. Therefore, the protein concentration could be represented by protein absorbance for the facility of calculation. A_0 was the initial absorbance of protein and A_t was the absorbance of protein at time t (Qi et al., 2006).

2.3.4. Effect of relative humidity on the moisture retention of microcapsules

The water retention experiment was carried out according to the following procedures. The dried microcapsules were kept in a desiccator at a temperature of 25 °C and a relative humidity of 33% (magnesium chloride), 52% (magnesium nitrate), 75% (ammonium chloride) and 97% (potassium sulfate), 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 retention ability of beads was calculated from the formula:

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

where W_0 was the initial weight of the beads and W_t was the weight of the microcapsules at equilibrium in different relative humidity (RH) after 1 week.

2.4. Survival of L. casei loaded in microcapsules

To determine the viable counts of the encapsulated *L. casei*, 0.1 g of microcapsules were resuspended in 10 ml of sodium citrate (0.06 mol/l) and stirred for 45 min using a magnetic stirrer. Complete release of bacteria from the microcapsules in 45 min was previously assured by comparing the released number of cells from the capsules. The colony forming units (cfu/g) were determined by anaerobic plating on MRS agar plate and incubating at 37 °C for 48 h. The plating procedures were carried out in triplicates. Non-encapsulated *L. casei* was enumerated in the MRS agar as control.

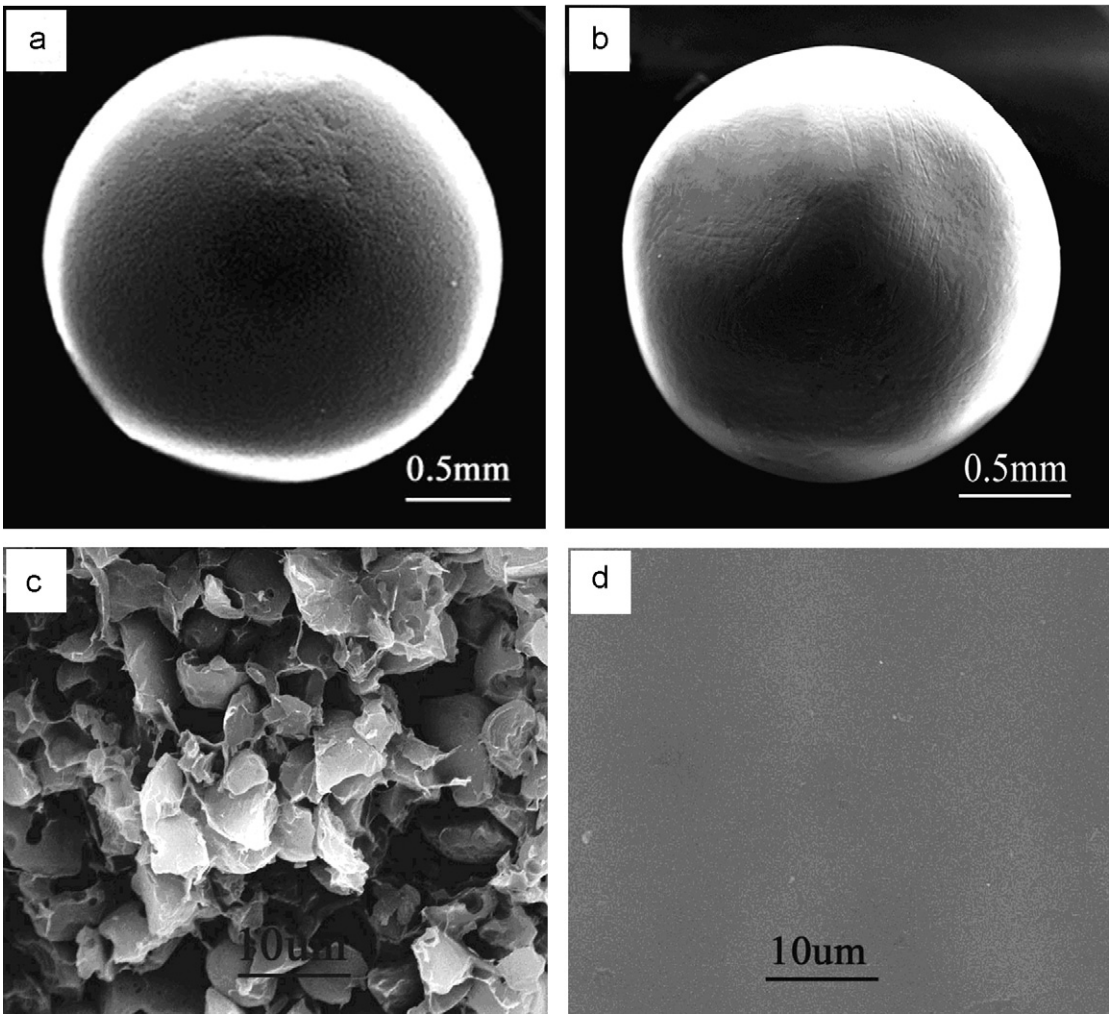

Fig. 1. Scanning electron micrograph and surface morphology of alginate–chitosan microcapsules and alginate–chitosan–CMCS microcapsules. (a, c) Alginate–chitosan microcapsules; (b, d) alginate–chitosan–CMCS microcapsules.

2.5. Effect of relative humidity on the survival of dried L. casei loaded in microcapsules

Survival of microencapsulated *L. casei* at different relative humidity was carried out according to the following procedures. The dried microcapsules (1 g) were kept in a desiccator at a temperature of 25 °C and a relative humidity of 33% (magnesium chloride), 52% (magnesium nitrate), 75% (ammonium chloride) and 97% (potassium sulfate) for 1 week in order for moisture to equilibrate uniformly. Then the cell numbers of microencapsulated *L. casei* was counted after 1 week of storage as described previously. The plating procedures were carried out in triplicates.

2.6. Survival of encapsulated L. casei in simulated gastric juice and bile solutions

Simulated gastric solution containing 0.2% NaCl was prepared by suspending pepsin (3 g/l) in saline (0.5%, v/v) and adjusting the pH to 2.0, 3.0 or 6.5 (control) with 5 mol/l HCl or 1 mol/l NaOH solution. It was then sterile-filtered through a membrane (0.45 μm, Gelman Science, Ann Arbor, MI, USA). Dried microcapsules (1 g) and 1 ml of the free cell suspension (10^{10} cells/ml) were placed separately in test tubes containing 9 ml simulated gastric pH solution and incubated at 37 °C. At the end of 0.5 h, 1 h and 2 h, beads were harvested, washed and immediately used for enumeration of viable cells (Sandoval-Castilla et al., 2010).

Tolerance of microencapsulated *L. casei* to simulated bile salt was carried out as follows (Lee & Heo, 2000). Similar to low pH tolerance, 1 g of microcapsules and 1 ml of the free cell suspension (10^{10} cells/ml) were transferred in 9 ml of bile salt solution containing 0 (control), 5.0 and 10.0 g/l bile salts (Oxgall, Sigma). Triplicate samples were withdrawn after incubation at 37 °C for 0, 3 and 6 h and cell counts of free and encapsulated bacteria were enumerated on MRS agar.

2.7. 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).

3. Results and discussion

3.1. Characteristics of L. casei-loaded microcapsules

3.1.1. Surface morphology

Morphology of *L. casei*-loaded microcapsules (alginate–chitosan microcapsules and alginate–chitosan–CMCS microcapsules) was shown in Fig. 1. Both types of microcapsules were found to have

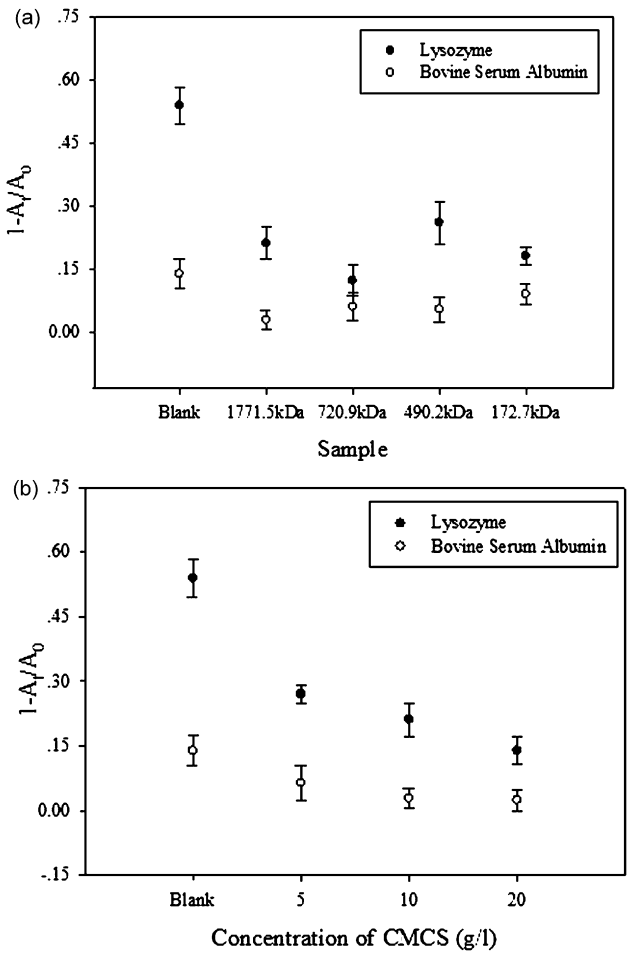

Fig. 2. Effect of carboxymethyl chitosan on the permeability of alginate–chitosan–CMCS microcapsules. (a) Carboxymethyl chitosan molecular weight; (b) carboxymethyl chitosan concentration.

similar appearance such as spherical, uniform, no aggregation and a rather smooth surface (Fig. 1a and b). The size of both types of microcapsules was in the range of 2.2 ± 0.1 mm and the introduction of CMCS had no influence on the size of the capsules in this experiment. However, the coating of CMCS influenced the surface of alginate–chitosan–CMCS microcapsules markedly and made its surface much smoother than that of alginate–chitosan microcapsules (Fig. 1c and d).

3.1.2. Moisture content

The moisture content of probiotic powders is a critical factor influencing shelf-life stability of the live bacteria (Meng, Stanton, Fitzgerald, Daly, & Ross, 2008; Wang, Yu, & Chou, 2004). In general, microorganisms survive better with low-water activity. However, over drying may diminish the viability and stability of microorganisms. The moisture content of the dried capsules prepared in this study ranged from 8.3% to 10.6%.

3.1.3. Permeability

The polymer membrane of microcapsule was of permeability which was related to the component and concentration of the wall material. In this experiment, lysozyme and BSA were chosen to study the permeability of alginate–chitosan microcapsules and alginate–chitosan–CMCS microcapsules.

Effect of CMCS molecular weight on the protein diffusion in alginate–chitosan–CMCS microcapsules was shown in Fig. 2a. The results showed that lysozyme could enter both types of microcapsules much easier and BSA with high molecular weight could hardly enter the gel matrix. Compared to alginate–chitosan microcapsules, the protein diffusivity in alginate–chitosan–CMCS microcapsules was much harder. That could be interpreted as the formation of thicker and denser membrane with the coating of CMCS. However, the effect of CMCS molecular weight on the permeability of microcapsules presented no regularity. As for lysozyme, the highest diffusion rate was 0.27 when the CMCS molecular weight was 490.2 kDa. For BSA, the highest diffusion rate was 0.09 and the molecular weight of CMCS was 172.7 kDa.

The influence of CMCS concentration on the protein diffusion in alginate–chitosan–CMCS microcapsules was shown in Fig. 2b. The protein diffusivity decreased with the increase of CMCS concentration. With the CMCS concentration increased from 5 g/l to 20 g/l, the diffusion rate of lysozyme and BSA decreased from 0.54 to 0.14 and from 0.14 to 0.02, respectively. The results indicated that the introduction of CMCS could change the permeability of microcapsules. The increase of CMCS concentration resulted in a thicker membrane and enhanced the resistance to protein diffusion.

3.2. Survival of microencapsulated *L. casei* in dry state

In this part, effects of carboxymethyl chitosan molecular weight and concentration on the survival of dried *L. casei* were investigated.

3.2.1. Effect of carboxymethyl chitosan molecular weight

Effect of carboxymethyl chitosan molecular weight on the viability of dried *L. casei* was investigated and the result was shown in Fig. 3a. At 0 week, the live amount of encapsulated *L. casei* loaded in alginate–chitosan microcapsules and alginate–chitosan–CMCS microcapsules (the carboxymethyl chitosan molecular weight of 1772 kDa, 721 kDa, 490 kDa and 173 kDa) were 3.6×10^8, 8.3×10^8, 7.4×10^8, 7.9×10^8 and 3.2×10^8 cfu/g, respectively. In the following weeks the amounts of *L. casei* loaded in both types of microcapsules were decreased (shown in Fig. 3a). However, alginate–chitosan–CMCS microcapsules made with 721 kDa was much stable in the live cell amounts and the live cell numbers of *L. casei* still stabilized at 5.6×10^8 cfu/g at the 4th week compared with that of 4.3×10^7 cfu/g loaded in alginate–chitosan microcapsules. The results indicated that the introduction of carboxymethyl chitosan could effectively improve the survival of *L. casei* against drying. The carboxymethyl chitosan molecular weight of 721 kDa was selected in the following studies.

3.2.2. Effect of carboxymethyl chitosan concentration

Effect of carboxymethyl chitosan concentration on the survival of microencapsulated *L. casei* during storage at 4 °C was studied and the result was shown in Fig. 3b. At 0 week, the live amount of *L. casei* loaded in alginate–chitosan–CMCS microcapsules (the carboxymethyl chitosan concentration of 2.0%) was 1.6×10^9 cfu/g compared with that of 3.6×10^8 cfu/g loaded in alginate–chitosan microcapsules. With the storage time increased from 0 to 4 week the amount of *L. casei* loaded in each sample was decreased (shown in Fig. 3b). Alginate–chitosan–CMCS microcapsules prepared with 1.0% (carboxymethyl chitosan concentration) was much stable and the live cell numbers of *L. casei* was 2.6×10^8 cfu/g at the 4th week. The introduction of carboxymethyl chitosan could effectively improve the survival of *L. casei* against drying.

The results indicated that microcapsules prepared with alginate, chitosan and carboxymethyl chitosan could successfully improve the survival of dried *L. casei* compared to alginate–chitosan microcapsules. The reason might be that the introduction of carboxymethyl chitosan made for a denser surface and effectively protected the dried *L. casei* to death. The encapsulation parameters of carboxymethyl chitosan that given maximum viable cell counts

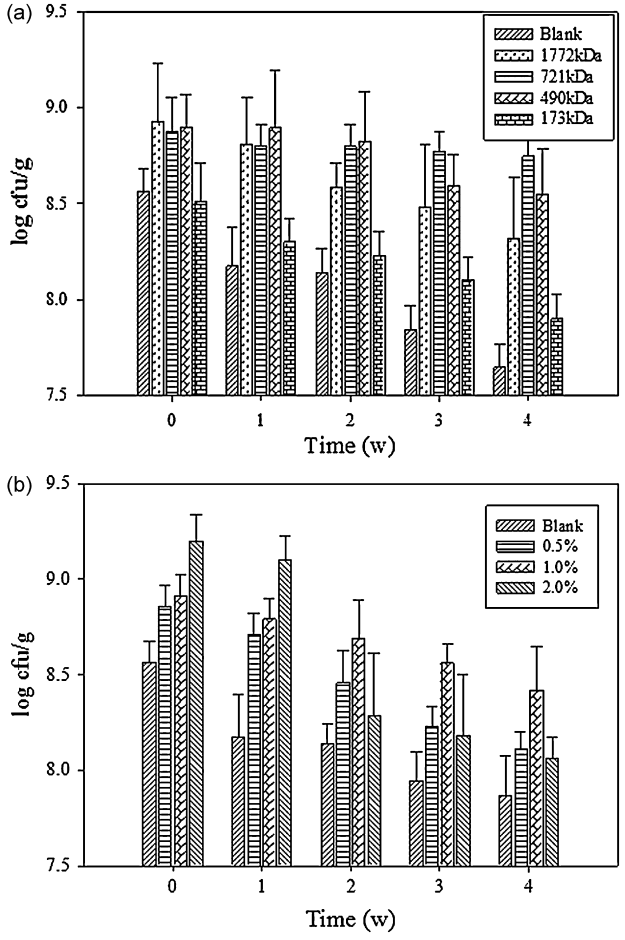

Fig. 3. Effect of carboxymethyl chitosan on the survival of microencapsulated *L. casei* in dry state. (a) Carboxymethyl chitosan molecular weight; (b) carboxymethyl chitosan concentration.

after drying were selected as an optimum condition: molecular weight, 721 kDa; concentration, 1.0% (w/v).

3.3. Moisture absorption

Effect of carboxymethyl chitosan molecular weight on the water retention of alginate–chitosan–CMCS microcapsules under different humidity conditions (33%, 52%, 75% and 97%, RH) was studied and the results were shown in Fig. 4. It was obvious that the behavior of all samples was quite similar when the humidity was lower than 52% and the maximum R_S value was 3.5%. With the humidity increased to 97% the R_S value of alginate–chitosan and alginate–chitosan–CMCS microcapsules increased significantly and reached 74% and 22% (the carboxymethyl chitosan molecular weight was 1771.5 kDa), respectively. The effect of carboxymethyl chitosan molecular weight on the water uptake of alginate–chitosan–CMCS microcapsules was unobvious. This result indicated that alginate–chitosan microcapsules had a better moisture resorption ability compared with alginate–chitosan–CMCS microcapsules.

3.4. Survival of microencapsulated L. casei under different relative humidity

Cell injury and inactivation occur not only during processing but also during storage of dried cultures. The storage conditions, i.e., storage temperature, moisture content, water activity, relative humidity, powder composition, oxygen content, exposure to light,

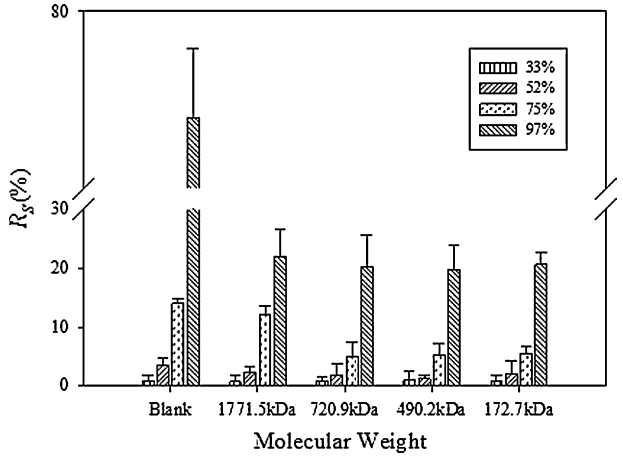

Fig. 4. Effect of carboxymethyl chitosan molecular weight on the water uptake of alginate–chitosan–CMCS microcapsules.

and storage materials, have significant influences on the survival of probiotics in dried powders, and the correct storage conditions are essential to maintain viable populations of dried probiotic bacteria (Meng et al., 2008; Zayed & Roos, 2004).

Effect of relative humidity (33%, 52%, 75% and 97%) on the viability of microencapsulated *L. casei* in dried powders was studied and the results were shown in Fig. 5. All the samples (alginate microcapsules, alginate–chitosan microcapsules and alginate–chitosan–CMCS microcapsules, the carboxymethyl chitosan molecular weight was 1771.5 kDa) showed similar survival profiles at different relative humidities. At relative humidity 33%, the live cell numbers of alginate microcapsules, alginate–chitosan microcapsules and alginate–chitosan–CMCS microcapsules were 2.3×10^7, 3.9×10^8, 1.3×10^9 cfu/g, respectively. With the humidity increased to 97%, the cell numbers decreased to 3.4×10^2, 2.1×10^4, 1.2×10^5 cfu/g, respectively. The results showed that the cell numbers of microencapsulated *L. casei* in dried powders decreased when the relative humidity increased from 33% to 97% and the lower humidity (33% and 52%) was beneficial to the survival of dried *L. casei*.

3.5. Tolerance to simulated gastrointestinal conditions

In order to exert their beneficial effects in the host, probiotics must be able to survive the harsh conditions of acidity and bile

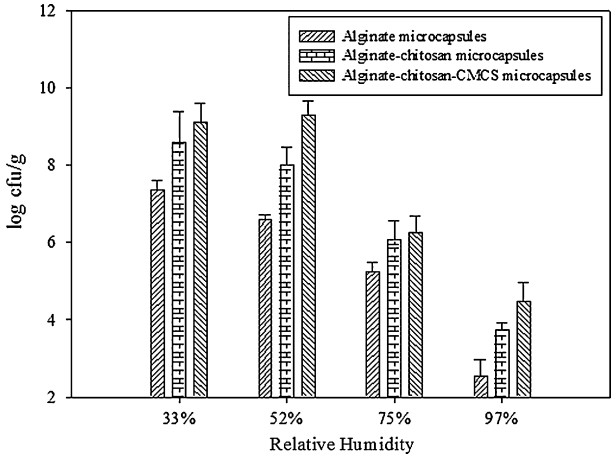

Fig. 5. Effect of relative humidity on the survival of dried *L. casei*-loaded in alginate microcapsules, alginate–chitosan microcapsules and alginate–chitosan–CMCS microcapsules.

Table 1
Survival of microencapsulated *L. casei* after exposure to simulated gastric juice at pH 2.0, 3.0, 6.5.

	Survival amount (log cfu g)								
	Alginate microcapsules			Alginate–chitosan microcapsules			Alginate–chitosan–CMCS microcapsules		
	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
pH 2.0	7.97	7.34	7.11	8.09	8.05	7.38	8.43	8.29	7.91
pH 3.0	8.33	8.25	7.64	8.34	8.17	7.87	8.73	8.52	8.37
pH 6.5	8.44	8.28	8.19	8.67	8.58	8.41	8.93	8.84	8.65

Table 2
Survival of encapsulated *L. casei* after exposure to bile salt at 0.5% and 1%.

	Survival (log cfu g)								
	Alginate microcapsules			Alginate–chitosan microcapsules			Alginate–chitosan–CMCS microcapsules		
	0 h	3 h	6 h	0 h	3 h	6 h	0 h	3 h	6 h
0%	6.13	–	–	8.30	–	–	8.69	–	–
0.5%	–	4.80	4.13	–	7.71	7.30	–	8.28	7.92
1%	–	4.03	3.12	–	7.12	6.42	–	7.90	7.42

concentration commonly encountered in the gastro-intestinal tract of humans. It is generally accepted that probiotic bacteria must be alive in the product at the time of consumption and also capable of reaching the large intestine in high enough quantities to facilitate colonization and proliferation.

3.5.1. Resistance to simulated gastric juice

Viability of immobilized and free cells of *L. casei* in simulated gastric juice was evaluated and the results were shown in Table 1. At pH 6.5 (control), survival of *L. casei* in simulated gastric juice remained above 8 log cfu/g after 2 h incubation at 37 °C whether encapsulated or not. At pH 2.0, none of the free cells survived after 2 h incubation in simulated gastric juice and the results suggested that *L. casei* was sensitive to the acidic environment (pH 2.0). In the same condition (pH 2.0, 30 min), the live cell numbers of *L. casei* in alginate microcapsules, alginate–chitosan microcapsules and alginate–chitosan–CMCS microcapsules were 7.97, 8.09 and 8.43 log cfu/g and with the time increased to 120 min, the decline of three types of microcapsules were 0.86 log, 0.71 log and 0.52 log, respectively. The survival of *L. casei* in acid conditions showed that there was a similar decrease of the bacterial cfu/g at pH 2.0 and 3.0. It was clear that the survival of encapsulated cells was significantly better than that of the free cells after exposure to simulated gastric juice (pH 2.0) and alginate–chitosan–CMCS microcapsules could protect *L. casei* against acid environment effectively.

3.5.2. Resistance to bile salts

Being capable to survive bile concentrations produced in the human small intestines and to take up residence and multiply in human large intestine is another important characteristic of *lactobacillus* to be used as probiotic dietary adjuncts. The bile tolerance of *L. casei* was evaluated by supplement with bile (Oxgall).

Survival of free and microencapsulated *L. casei* exposure to bile solution (0.5% and 1%, w/v) was shown in Table 2. In the presence of 0.5% bile solution, the survival rate of *L. casei* loaded in alginate microcapsules, alginate–chitosan microcapsules, alginate–chitosan–CMCS microcapsules was 67.4%, 87.9%, 91.1% after 6 h of exposure while that of the free cells was 0.1%. After exposure to 1% bile solution for 6 h the survival rates of *L. casei* loaded in alginate–chitosan–CMCS microcapsules was 85.4% while the free cells was 0.03%. This result indicated that the resistances of various microencapsulated *L. casei* to bile solution were higher than those of the free cells.

The formation of a hydrogel around the cell pellet is thought to be the basis for the cell protection. This is because the acidic fluid and the bile salt need to permeate through the gel layer before reaching the cells (Mokarram, Mortazavi, Habibi Najafi, & Shahidi, 2009). The results indicated that the alginate–chitosan–CMCS microcapsules were the most effective in protecting probiotic bacteria from acidic fluid and bile salt and making this approach potentially useful for delivery of probiotic cultures to the gastro-intestinal tract of humans.

4. Conclusions

Encapsulated cells of *L. casei* were extruded and dried at 4 °C. The alginate–chitosan–carboxymethyl chitosan microcapsules extended the survival of *L. casei* after drying. Moreover, these microcapsules increased the tolerance of *L. casei* to strongly acidic media and bile; therefore this technical approach might be useful for the delivery of probiotic cultures to the human gastro-intestinal tract.

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