Review

Alginate-based and protein-based materials for probiotics encapsulation: a review

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Summary

Owing to their considerable beneficial effects on human health, probiotics have been increasingly incorporated into food products. However, many findings have demonstrated that their survival and stability are very sensitive to processing and host gastrointestinal tract. To solve these problems, encapsulation techniques have been received considerable attention these days. So, in this review paper, methods for probiotics encapsulation, alginate-based and protein-based materials for probiotics encapsulation and application of encapsulated probiotics in food industry were discussed.

Keywords

Alginate, encapsulation, prebiotics, probiotics, protein.

Introduction

Probiotics are considered as being ‘live microorganism which administered in adequate amounts, can confer a beneficial physiological effect on the host’ (Rokka & Rantamaki, 2010). The potential health benefits of probiotics (most popular Lactobacillus acidophilus and Bifidobacteria) are such as antimutagenic and anticarcinogenic properties, anti-infection properties, immune system stimulation, serum cholesterol reduction, alleviation of lactose intolerance and nutritional enhancement (Macfarlane & Cummings, 1999; Mombelli & Gismondo, 2000). To satisfy health requirement of human, probiotics industry has developed a variety of new products. Most probiotics relevant products in food industry are dairy products, with yogurts, kefir and cultured drinks representing the major categories.

The global market for probiotic ingredients, supplements and foods was worth $14.9 billion in 2007 and US$16 billion in 2008 respectively. Estimated sales target in 2013 will reach to US$19.6 billion. Presently, two largest probiotics markets are North America and Europe. Analysis of the North American Probiotics markets found that the probiotics sector earned revenues of US$ 698 million in 2006. It is expected to reach US$1.70 billion in 2013, with compound annual growth rate (CAGR) of 13.7% (Granato et al., 2010a). European food and beverage probiotics market is expected to rise from US$ 61.7 million in 2006 to US$ 163.5 million by 2013 (Granato et al., 2010b).

To exert their biological effects on the host, one of most important consideration is viability and activity of probiotic bacteria, even though non-viable probiotics can still have biological activity. Another concern for consumer and food producer is minimum beneficial dose per day or per gram of product. The International Dairy Federation has recommended a minimum number of 10⁷ CFU g⁻¹ of the product consumed. However, most probiotics are very sensitive to environmental conditions, such as processing including oxygen stress, freezing, temperature and drying, harsh action of gastrointestinal tract such as low pH and bile salt. Therefore, development of enhancing probiotic viability techniques is highly necessary (Siuta-Cruce & Goulet, 2001; Mattila-Sandholm et al., 2002).

Until now, several ways to enhance probiotic viability have been studies, such as selection of bile and acid tolerant strains, inclusion of protective compounds, manipulation of starter cultures, selection of appropriate packaging materials, two-stage fermentation, stress adaptability, inclusion of oxygen scavengers and encapsulation (Sarkar, 2010). Of all methods investigated now, encapsulation is regarded as one of most useful methods for protecting viability of probiotics. From microbiological point of view, encapsulation is a technology used to ‘package’ microorganisms cells in miniature capsule, which release it at controlled rates. Encapsulation of probiotics has been shown to protect probiotics from detrimental environmental factors such

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Bio-materials for probiotics encapsulation Q.-Y. Dong et al.

Encapsulation methods for probiotics

Spray drying and spray cooling drying

Spray drying commonly used in food industry involves atomisation of an aqueous or oily suspension of probiotics and encapsulation materials in a vessel with a nozzle or spinning wheel into hot gas, resulting in rapid evaporation of water (Chen & Chen, 2007; Kailasapathy, 2009; De Vos et al., 2010). Spray drying is the relatively economic and effective method for probiotics encapsulation. The disadvantage of this method is that high temperature during this process is not favourable for the survival of probiotics. Thus, to our best knowledge, spray drying has not been developed commercially for probiotics in food industry due to low survival rate during this process. Several parameters can affect the viability of encapsulated probiotics, such as type of strain, drying temperature, drying time, type of atomisation, carrier material et al. To minimise osmotic, oxidative and mechanical stress subjected to probiotics during spray drying, protectants should be added to the media before drying, for example, granular starch, soluble fibre, trehalose, non-fat milk solids, adnitol, et al. (Manojlovic et al., 2010). To improve protection ability further, spray-dried beads can be coated by an additional layer (Semyonov et al., 2011; Nedovic et al., 2011; Gbassi & Vandamme, 2012; Sousa et al., 2012). And, increase in sensory properties stability of food products also can be achieved using encapsulation method (Augustin, 2003; Parada & Aguilera, 2007).

Many materials, such as polysaccharide (alginate, chitosan, gellan gum, xanthan gum, pullulan gum, κ-Carrageenan et al.), protein (whey protein, soy protein, pea protein et al.) and lipid, have been used to encapsulate probiotics (Heidebach et al., 2012). Most adopted encapsulation materials are alginate-based and protein-based materials. Therefore, alginate-based and protein-based materials for probiotics encapsulation will be reviewed in this article. Methods for probiotics encapsulation and application of encapsulated probiotics in food area also will be discussed.

Emulsion method

Emulsion (O/W, W/O, W/O/W) technique has been successfully applied for probiotics encapsulation. The advantage of emulsion technique is easy to scale up, flexible adjustment of the resulting capsule size and give a high survival rate of probiotics (Chen & Chen, 2007). The main parameters to control the size of the microbeads are including the energy input during emulsion, the addition of emulsifiers and the viscosity ratio between the dispersed and the continuous phase. To improve viability of probiotics further, the microbeads can be coated using a second polymer (Kailasapathy, 2009).

Papagianni & Anastasiadou (2009) developed three O/W emulsions systems. Pediococcus acidilactici cells were enclosed in the oil phase. The first emulsion contained corn oil micro-droplets (mean diameter 1.5 μm) emulsified with peptides and stabilised with SDS. The other two were food grade systems with micro-droplets of corn or olive oil (2.1 and 2.2 μm respectively) emulsified with peptides and stabilised with rather than. Encapsulation preserved 85% viability rates of encapsulated cells. As much as 92% of the initially encapsulated cells could be released at the target point.

Ding & Shah (2007) investigated eight strains of probiotic bacteria were encapsulated in alginate matrix by W/O method. When free probiotic bacteria were exposed to oxgall, viability was reduced by 6.51 log CFU mL⁻¹, whereas only 3.36 log CFU mL⁻¹ was lost in encapsulated strains. After 30 min of heat treatment, encapsulated probiotic bacteria survived with an average loss of only 4.17 log CFU mL⁻¹, compared with 6.74 log CFU mL⁻¹ loss with free probiotics. However, after 1 h of heating, both free and encapsulated probiotic strains showed similar losses in viability.

Sabikhi et al. (2010) encapsulated L. acidophilus using sodium alginate by W/O method. The organism survived better in the protected form at high temperatures (72, 85 and 90 °C) and at high salt concentrations containing the bioactive compound is atomised through a nozzle into a vessel. The advantage of spray cooling is low temperature that is used to solid the beads, compared with spray drying that uses hot air to dry the droplet. Spray cooling is considered as the least expensive encapsulation technology. However, so far, it has been rarely used for probiotics encapsulation, as other technologies are easier to be established in laboratories. Rutherford et al. (1993) studied freeze-dried probiotics that was encapsulated using molten lipids (e.g. 60–75% stearic acids at 60 °C) by spray cooling. Encapsulates with size of 75–300 μm were obtained. The contact time of freeze-dried probiotics during encapsulation process should remain very short, but no details about the survival rate of freeze-dried probiotics at 60 °C were given in their studies.
(1, 1.5 and 2%). The free cells were completely destroyed at 90 °C, whereas the encapsulated cells reduced by 4.14 log reduction. The free and protected cells registered 5.47 and 2.16 log reduction, respectively, after 3 h incubation at 2% bile salt.

W/O/W emulsion for L. acidophilus encapsulation was prepared by Shima et al. (2006). The relative viability of the bacteria included in the W/O/W emulsion was 49% at 2 h in the model gastric juice, whereas the viability of the bacteria directly dispersed in the juice declined to 1.3% even at 0.67 h.

The emulsion systems may have a large number of applications in the food sector. These studies demonstrated that emulsion technology for probiotics encapsulation is an effective technique for protection against extreme processing conditions and simulated gastrointestinal environment.

**Extrusion method**

The extrusion technique involves adding concentrated probiotics into an aqueous hydrocolloid solution, and extruding the hydrocolloid–cell mixture through a nozzle that forms droplets. Extrusion is a simple and cheap method that causes no damage to probiotic cells and gives high probiotic viability. The disadvantage of this method is that it is difficult to be scaled up (Kra-saeKooyt et al., 2003). Sodium-alginate used widely in food material can form gel beads by dropping it to Ca²⁺ solution. The size of the resulting beads depends on the diameter of the nozzle, the distance between the outlet, the hardening solution and the viscosity of the hydrocolloid–cell mixture.

Musikasang et al. (2009) investigated the survival rate of free and encapsulated Enterococcus durans KT3L20 in alginate beads under the conditions of simulated small intestine juice after sequential use of gastric intestine. In this study, encapsulated probiotics obtained by the extrusion technique exhibited higher survival rates than those from the emulsion technique and free cell respectively. Borges et al. (2012) evaluated the viability of L. casei, L. paracasei, L. acidophilus Kt and B. animalis BB-12 encapsulated in alginate beads through extrusion method during exposure to lethal conditions (25% NaCl, pH 3.0 and 55–60 °C). Results demonstrated that survival of probiotic strains under the imposed lethal stress conditions was strain dependent. Todorov et al. (2012) evaluated the effect of encapsulation in alginate beads on survival in simulated gastric and intestinal fluids. Results also showed that encapsulation could protect the cells from bile and simulated gastric fluid.

**Fluid bed coating**

In spray-coating, the core material in a solid form is kept in motion in a specially designed vessel. A liquid coating is sprayed through a nozzle over the core material into a hot environment (Champagne & Fustier, 2007; De Vos et al., 2010). The advantage of spray coating is easy to scale up. Success coating can be influenced by the stickiness of the coating material, the wettability of particles by the coating liquid and the operating conditions. In food industry, the material for probiotics coating is mostly lipid based (waxes, fatty acid and specialty oil et al.), proteins or carbohydrate.

Institut Rosell and Lal’food, a Canadian company, developed products containing probiotic products using fluid bed coating technology. The process is based on coating freeze-dried probiotics with fatty acids. The technology allows strains to resist harsh effects of temperature, gastric acidity and compression. A Danish-Korean company developed and patented a dual coating technology for probiotics. The first layer of coating is made of soy peptides and the second layer is made of cellulose and gum. The technology allows an increase in probiotic viability during processing shelf life and during their passage through simulated gastric tract.

Detailed characteristics, advantages and disadvantages of encapsulated methods mentioned above were outlined in Table 1.

**Other new methods**

Most of the methods for probiotics encapsulation typically involve exposure of the probiotics to either water or organic solvent. This may comprise survival of encapsulated cells as they are sensitive to solvents and moisture. Thus, use of solvents should be avoided to improve survival of probiotics. Thantsha et al. (2009) investigated the survival of B. longum Bb-46 encapsulated in interpolymer complexes formed in supercritical carbon dioxide under simulated gastrointestinal fluids. Their results showed that the interpolymer complex displayed pH-responsive release properties, with little to no release in simulated gastric fluid and substantial release in simulated intestinal fluid. There was a limited reduction in viable counts at the end of exposure period due to encapsulation.

Supercritical Emulsion Extraction technology has been recently proposed for preparing biopolymer microspheres. Porta et al. (2012) investigated the possibility of L. acidophilus encapsulation using poly-lactic-co-glycolic acid as biopolymer by Supercritical Emulsion Extraction technology. A double emulsion was used with an internal water phase (W1) composition of L. acidophilus suspended in MRS broth plus the 0.4% of poly-vinyl alcohol (PVA); Other emulsion phases were as follows: Oil phase containing ethyl acetate (EA) and PLGA at 10% (w/w) and W2-phase of water plus 0.6% of PVA (w/w). This emulsion treated by
Supercritical Emulsion Extraction at 90 bar and 37 °C for 30 min allowed the formation of PLGA microbeads. The results showed that Supercritical Emulsion Extraction technology is an innovative and efficient encapsulation technology.

Sohail et al. (2011) adopted a novel impinging aerosol method to encapsulate probiotics. The advantages of dual aerosol technique are the continuous processing capability and scale-up potential. It can offer a technology for an efficient encapsulation of probiotics in very small alginate microbeads. In this study, alginate microbeads (10–40 µm) containing the probiotics B. animalis and L. acidophilus were produced. Microbeads produced by novel aerosols technique offered comparable protection to L. rhamnosus in high acid and bile environment.

Electrospinning is a process that produces continuous polymer fibres with diameters in the submicrometre range through the action of an external electric field imposed on a polymer solution or melt. Recently, a number of bioactive agents, including whole microbial cells, have already been encapsulated in nanofibres using this technology. Lo’pez-Rubio et al. (2009) studied the suitability of the electrospinning method for the encapsulation of the strain B. animalis Bb12 using poly(vinyl-alcohol) (PVOH) as the encapsulating polymer. Incorporation of B. animalis Bb12 led to a decrease in melting point and crystallinity of the PVOH fibres and to an increase in the polymer glass transition temperature. The storage stability tests showed that B. animalis Bb12 encapsulated within the electrospun PVOH fibres remained viable for 40 days at room temperature and for 130 days at refrigeration temperature, whereas a significant viability decrease was observed in both cases for free probiotics. Whey protein concentrate and pullulan were also used for B. animalis Bb12 encapsulation through an electrospaying process (Lo’pez-Rubio et al., 2012). The results showed that encapsulation through electrospaying substantially increased the viability of the bifidobacterial strain.

### Encapsulation materials for probiotics

#### Alginate-based materials

Alginate, a naturally derived anionic polysaccharide extracted from various species of algae, has two structure units consisting of D-mannuronic and L-guluronic acids. The ratio of D-mannuronic and L-guluronic acids of distribution in alginate molecular determines the functionality of alginate as encapsulation material. Usually, aqueous alginate solution dropped into a calcium-containing bath will form gel beads by rapid crosslinking between alginate guluronic units and calcium ions. Alginate has been widely used for encapsulation of probiotic bacteria (Rowley et al., 1999) due to its simplicity, non-toxicity, biocompatibility and low cost (Krasaekoopt et al., 2003). Factors, such as alginate concentration, calcium concentration, hardening time of beads, probiotics concentration, viscosity of alginate solution, diameter of the orifice, distance between the outlet and coagulation solution et al., affecting on alginate beads preparation have been investigated well. Mandal et al. (2006) reported L. acidophilus encapsulated in alginate beads showed higher survival rate under different conditions than free probiotics. The survival of L. acidophilus increased proportionately with increasing of alginate concentration. The viability of encapsulated probiotics in simulated gastric fluid increased with the increase in beads size. Truelstrup Hansen et al. (2002) found that very larger alginate beads (> 1 mm) can protect probiotics well and that small size beads (< 100 µm) do not significantly protect the bacterial in simulated gastric fluid.

However, some disadvantages of alginate beads, such as easy degradation in the acidic environment,
easy disintegration subjected to monovalent ions or chelating agents, difficulty in scaling-up of the process, have been found (Mortazavian et al., 2008). In addition, obtained alginate beads are very porous (Gouin, 2004). These problems can be overcome by co-encapsulation with other compounds, coating the beads with another polymer or modifying of alginate structure using different additives (Krasaekoopt et al., 2003).

The positively charged amino groups of chitosan and negatively charged carboxylic acid groups of alginate form the membrane on alginate beads surface, which blocked the pore of alginate beads. Various research works on chitosan-coated alginate beads used for encapsulating probiotic bacteria have been reported. Lee et al. (2004) investigated the effect of chitosan with three different molecular weights coated alginate on the survival and viability of L. bulgaricus. The results show that the survival and stability of probiotic bacteria encapsulated in chitosan-coated alginate beads are largely dependent on the molecular weight of chitosan. The beads prepared with high molecular weight chitosan provided a higher survival rate (46%) compared with the beads prepared with low molecular weight chitosan (36%). This is due to the thicker membrane of the beads made with high molecular weight chitosan, which protected probiotics well than other beads made with low and medium molecular weight chitosan and free cells. Urbanska et al. (2007) reported the survival and stability of L. acidophilus encapsulated in chitosan-coated alginate beads in simulated gastrointestinal model. The results showed that the beads successfully maintained their structure in simulated gastric fluid and decreased their integrity while exposed to simulated intestinal fluid, indicating their potentiality in oral administration of probiotic bacteria. Alginate beads coated with or without chitosan were used to encapsulate probiotics (Graff et al., 2008). In vitro, < 1% of the non-encapsulated probiotic survived after 120 min at pH 1.1, whereas the majority viability of encapsulated cells in alginate–chitosan beads remained. Zou et al. (2011) and Chavarri et al. (2010) also found that alginate–chitosan beads can improve the survivability of probiotics in simulated gastrointestinal conditions, and be potential for probiotics oral delivery.

Another common poly-cationic polymer poly-L-lysine has been used for alginate beads coating. Similar to chitosan, poly-L-lysine can form strong complexes with alginate and give it similar characteristics as mentioned for chitosan. Cui et al. (2000), Cui et al. (2006, 2007) investigated the survival and stability of poly-L-lysine-coated alginate loaded with B. bifidum in vitro and in vivo after oral administration. The survival of bifidobacteria loaded in the beads remained highest (2.67 × 10⁹ CFU g⁻¹) at pH 6.8 while the number is reduced at lower pH (1.5, exposure time 2 h) to 5.0 × 10⁷ CFU g⁻¹. The stability of poly-L-lysine-coated alginate beads loaded with bifidobacteria was also improved during storage at 4 °C, compared with free bifidobacteria. Ding & Shah (2009) used poly-L-lysine-coated alginate microbeads to enhance the survival of probiotic bacteria. The results indicated that the addition of Poly-L-lysine to alginate microbeads improved the average viability of probiotic bacteria by > 1 log CFU mL⁻¹ when compared with alginate microbeads at 2 h of exposure to acidic conditions. To improve the stability of probiotics further, poly-L-lysine-coated alginate beads can be coated using alginate. The alginate–poly-L-lysine–alginate beads have been prepared as carriers for probiotics encapsulation (Quong et al., 1999; King et al., 2001). Chen et al. (2005a,b) investigated the potential use of alginate–poly-L-lysine–alginate beads for oral delivery probiotic bacteria. About 80% viability of encapsulated cells was kept after 5 min incubation in simulated gastric fluid (pH 2.0), although viability was considerably decreased to 8.3, 2.6 and 0.2% after 15, 30 and 60 min, respectively, indicating that this beads was effective, but not sufficient to protect the probiotics for oral delivery application. Martoni et al. (2007) obtained similar results with Chen et al. (2005a,b). Cell viability in microbeads was maintained above 10⁹ CFU mL⁻¹ at pH 2.5 and 3.0 after 2 h residence time, whereas viability decreased linearly over time at pH 2.0 although it was maintained above 10⁶ CFU mL⁻¹ under similar conditions. In simulated stomach condition at pH 1.5, microencapsulated cells were not viable after 30 min exposure time. Ouyang et al. (2004) prepared alginate–polylysine–pectinate–polylysine–alginate microbeads with multilayer structure, which were for oral delivery of L. reuteri. The result showed no damage of the microbeads for 12 h at 250 rpm mechanical shaking when exposed to simulated gastric fluids. The stability studies in different pH conditions revealed that 92.8 ± 3.1% of the microbeads loaded with L. reuteri remained intact at pH 1, 3, 5 and 7, and no damages were observed for 24 h.

Addition of prebiotics is an emerging alternative that can further enhance probiotic activity. In general, prebiotics can be utilised to promote the growth of probiotics. Prebiotics are non-digestible carbohydrates that are not absorbed in the intestine. It can provide the colonic microbiota with a fermentable carbohydrate substrate. Examples of prebiotics are fructooligosaccharides, isomaltooligosaccharides, resistant starch (Sajilata et al., 2006; Anal & Singh, 2007; Mortazavian et al., 2008) and lactose. Encapsulation with both alginate and prebiotics is referred to as co-encapsulation. Chen et al. (2005a,b) concluded that co-encapsulation increases the survival of the active probiotics. Survival rate of the co-encapsulated bacteria was 1000 times higher than for alginate alone. In another report,
Iyer et al. (2005) used an extrusion method to encapsulate probiotic bacteria in an alginate–starch system. The incorporation of Hi-Maize starch improved the encapsulation of viable bacteria compared with the bacteria encapsulated without starch.

Succinylated alginate and N-palmitoylaminoethyl alginate were prepared and investigated for encapsulation of probiotics. For free cells, the initial count was dropped from $1 \times 10^8$ CFU mL$^{-1}$ to uncountable level after 30 min in simulated gastric fluids. Succinylated alginate beads loaded with probiotic bacteria showed better protection in simulated gastric fluids, with a slight decrease in viability. The best protection in simulated gastric fluids was obtained for N-palmitoylaminoethyl alginate with a slight decrease in bacterial cell from $2.5 \times 10^7$ to $2.2 \times 10^7$ CFU mL$^{-1}$. The minor loss of encapsulated cells from N-palmitoylaminoethyl alginate showed a promising formulation to protect the live bacteria from acidic environment and to improve their survival and stability. Recently, Rao et al. (2008) reported that a succinylated alginate to immobilise a Lactobacillus strain for lactic acid production. The encapsulation of L. delbrueckii into succinylated alginate beads provided better stability and durability of the encapsulated live bacterial cells under an acidic environment compared with unmodified alginate. These investigations therefore suggest that modified alginites are able to provide a potentially promising encapsulation system for delivery as well as industrial uses of live probiotic bacteria.

**Protein-based materials**

**Gelatin**

Gelatin, a kind of protein gum, has been used for probiotic encapsulation, alone or in combination with other compounds. Due to its amphoteric nature, it is an excellent candidate for cooperation with anionic polysaccharides such as gellan gum. These hydrocolloids are miscible at a pH higher than 6, because they both carry net negatives charges and repel each other. However, the net charge of gelatin becomes positive when the pH is adjusted below the isoelectric point and this causes the formation of a strong interaction with the negatively charged gellan gum (Krasaekoopt et al., 2003; Anal & Singh, 2007).

Alginate-coated gelatin microspheres were produced to encapsulate probiotic B. adolescentis 15703T (Annan et al., 2008). Gelatin microspheres were cross-linked with the non-cytotoxic genipin and coated with alginate cross-linked by Ca$^{2+}$. The alginate prevented pepsin-induced degradation of the gelatin microspheres in simulated gastric juice (pH 2.0, 2 h), resulting in significantly ($P < 0.05$) higher numbers of survivors. After sequential incubation in simulated gastric (1 h) and intestinal juices (pH 7.4, 4 h), number of surviving cells was 7.6 log CFU g$^{-1}$ for alginate-coated microspheres, while 6.7 and 6.4 log CFU g$^{-1}$ were obtained for cells in uncoated gelatin microspheres and free cells respectively. This is an encapsulation method, which protects bifidobacterium during exposure to adverse environmental conditions.

**Dairy proteins**

Dairy proteins can offer suitable functional properties to be used as shell or matrix materials for encapsulation, such as bland flavour, high solubility, low viscosity in solution, good emulsion and film-forming properties. Consequently, dairy protein-based microbeads have been vehicles for probiotics cells (Picot & Lacroix, 2004; Reid et al., 2005; Livney, 2010; Weibreak et al., 2010).

Ying et al. (2011) investigated the effect of various parameters on the survival of probiotics during spray drying with whey protein as carriers. Doherty et al. (2010a,b, 2011) reported that L. rhamnosus GG was encapsulated by extrusion of a pre-heated whey protein. The authors concluded that denatured whey protein was suitable matrices for probiotics encapsulation, while native protein provided the weakest safeguard against thermal and acid stress. Gerez et al. (2012) found that pectin matrix coated with whey protein could increase the microspheres ability to protect L. rhamnosus at pH 1.2.

Lactobacillus paracasei and B. lactis strains were encapsulated by the enzymatic gelation of sodium caseinate through cross-linking with transglutaminase enzyme (Heidebach et al., 2009a). In other work, Heidebach et al. (2009b) took a similar approach to encapsulate probiotic cells using rennet as a coagulating agent. The viable cell numbers of encapsulated L. paracasei and B. lactis were 0.8 and 2.8 log units CFU g$^{-1}$ higher compared with free cells after 90 min incubation at pH 2.5. The improved survival of encapsulated cells can probably be explained by a higher local pH value within the protein matrix of the capsules caused by the protein buffering capacity. Sodium caseinate can also be coagulated and gelled by acidification with glucono-δ-lactone (Lucy et al., 1997). Nag et al. (2011) reported that L. casei was successfully entrapped with caseinate-gellan gum gel induced by glucono-δ-lactone. The survival of encapsulated cells after 30 min of incubation in simulated gastric fluid was significantly greater than that of free cells. The microbeads also provided significant protection for L. casei against detrimental bile salts. All above-mentioned studies indicate that dairy protein-based gelation for the encapsulation of probiotic cells can be a suitable alternative to current available technologies.

Fritzen-Freire et al., 2012, 2013) evaluated the survival of encapsulated B. BB-12 microspheres containing skim milk and prebiotics (inulin, oligofructose-enriched
Table 2 SFG conditions used to simulate the stomach

<table>
<thead>
<tr>
<th>Gastric fluid</th>
<th>pH</th>
<th>Pepsin content (g L(^{-1}))</th>
<th>Exposure time (min)</th>
<th>Free cell viability (Log CFU mL(^{-1}))</th>
<th>Encapsulated cell Viability (Log CFU g(^{-1}))</th>
<th>References</th>
</tr>
</thead>
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<td>NaCl (0.2%)</td>
<td>1.5</td>
<td>0</td>
<td>1</td>
<td>4.24</td>
<td>8.07</td>
<td>Mandal et al. (2006)</td>
</tr>
<tr>
<td>HCl (0.1 M)</td>
<td>2.0</td>
<td>0</td>
<td>3</td>
<td>3.38</td>
<td>7.54</td>
<td>Graff et al. (2008)</td>
</tr>
<tr>
<td>NaCl (0.2%)</td>
<td>2.0</td>
<td>0.32</td>
<td>2</td>
<td>5.90</td>
<td>7.63</td>
<td>Zou et al. (2011)</td>
</tr>
<tr>
<td>NaCl (9 g L(^{-1}))</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>&lt; 1.0</td>
<td>6.95</td>
<td>Chavarrri et al. (2010)</td>
</tr>
<tr>
<td>NaCl (0.2%)</td>
<td>0.32</td>
<td>0</td>
<td>2</td>
<td>7.12</td>
<td>8.00</td>
<td>Nag et al. (2011)</td>
</tr>
<tr>
<td>NaCl (0.2%)</td>
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<td>0</td>
<td>2</td>
<td>1.21</td>
<td>8.01</td>
<td>Annan et al. (2008)</td>
</tr>
</tbody>
</table>

Application of encapsulated probiotics in food area

The incorporation of probiotics into food products is a challenge task due to sensitive properties of probiotics. The technology used to transform the product into a carrier for probiotics must be well understood. And metabolism of the added probiotics in food products should be studied extensively. Also, it should be done, so as to provide sufficient viability of the probiotics in the products during processing and shelf storage life, and without any negative influence on the sensory acceptance of the products (Gruz et al., 2009). Encapsulation technology can provide probiotics in a physical barrier to resist adverse environmental conditions, having been widely used in food industry. The potential of incorporation of encapsulated probiotics into food products has been studied.

Cheese

Many studies have reported the use of encapsulated probiotic cells in cheese (Table 3). Cheddar cheese has a good carrier property for probiotics, such as relative high pH (pH 5.5), good buffering capacity and its relatively high fat content (Gardiner et al., 1998; Stanton et al., 1998).

Cheese containing encapsulated bifidobacterium was shown to possess similar flavour, texture and appearance compared to the control (Dinakar & Mistry, 1994; Desmond et al., 2002). Kailasapathy & Mason-dole (2005) have reported that production of feta cheese incorporating encapsulated probiotic bacteria (L. acidophilus and B. lactis) is technologically feasible. Gardiner et al. (2002) reported that survival rate and growth rate of L. paracasei were improved in cheddar cheese after 3 months of ripening. McBrearty et al. (2001), Godward & Kailasapathy (2003) and Darukaradhyra (2005) also obtained similar conclusions.

Ozer et al. (2008) and Gonzalez-Sanchez et al. (2010) concluded that encapsulation can be a good
way to enhance probiotic viability in Kasar cheese and Kefir cheese respectively. No difference was noted between the two encapsulation techniques with regard to bacterial counts, proteolysis and organoleptical properties of the final products. In another study, the same strain encapsulated by the same techniques was incorporated in white-brined cheese (Özer et al., 2009). Cheeses made with encapsulated probiotics contained higher acetaldehyde and diacetyl levels than the control. Experimental cheeses containing encapsulated probiotics were not different from the control cheese in terms of sensory properties.

**Yogurt**

One of the main harmful factors for low probiotics viability is oxygen in the yogurt. Gruz et al. (2010) optimised the processing of probiotic yogurt supplemented with glucose oxidase and determined the levels of glucose and glucose oxidase that minimise the concentration of dissolved oxygen. Low values for dissolved oxygen (0.52 ppm) and high *B. logum* (8.74 log CFU mL$^{-1}$) were observed in their study. The incorporation of encapsulated probiotic living cells in yogurt has been reported (Chen & Chen, 2007; Weichselbaum, 2009). Some examples of encapsulated probiotics were shown in Table 4. The protective effect by encapsulation can be explained by limited diffusion of inhibitory substances such as metabolic products from the starter cultures, lactic acid and bacteriocin into the beads (Sun & Griffiths, 2000; Krasaekoopt et al., 2006). Talwalkar & Kailasapathy (2004) showed that encapsulation in alginate hydrogels offers substantial protection for probiotics under aerobic conditions and could therefore be responsible for higher survival rates of encapsulated cells during storage in yogurt.

Kailasapathy (2006) explained that the incorporation of capsules containing probiotic cells did not significantly alter yogurt’s properties such as appearance, colour, flavour, taste and acidity. Concerning acidity, it was shown that the addition of probiotic cultures slows down the post-acidification during the storage of yogurt. In another study, Kailasapathy et al. (2008) demonstrated a correlation between post-storage pH and the survival of probiotic bacteria, which is negatively affected by the presence of fruit pulp. However, all the obtained yogurts contained the recommended levels of probiotic bacteria even after 35-day shelf life.

In the study of Brinques & Ayub (2011), the survival of free and encapsulated *L. plantarum* in yogurt was investigated. Results showed that probiotics encapsulated in alginate–chitosan beads in yogurt presented good cell viabilities, with losses of only 0.55 log cycles, compared with 6.73 log cycles for free cells. Sandoval-Castilla et al. (2010) also found that survivability of encrapped *L. casei* in alginate-based beads in yogurt is higher compared with free *L. casei*. This suggested the efficiency of encapsulation technique to increase the survival of probiotics in yogurt under refrigerated storage.

**Ice cream**

It is not easy to incorporate probiotics into ice cream because of high acidity in the product, freeze injury and exposure to the incorporated air during freezing.
(Chen & Chen, 2007). Some examples were shown in Table 4. The introduction of probiotic bacteria in an encapsulated form into it may overcome these difficulties. Entrapment of lactobacilli in alginate beads provides a higher survival rate (40%) compared with free cells, when freezing ice cream (Sheu & Marshall, 1993; Sheu et al., 1993). The high total solids in ice-cream mix, including the fat (emulsion), may provide protection for the bacteria (Kailasapathy & Sultana, 2003). Homayouni et al. (2008) manufactured two types of symbiotic ice cream containing 1% of resistant starch with free and encapsulated *L. casei* and *B. lactis*. The results indicated that encapsulation can significantly increase the survival rate of probiotic bacteria in ice cream over an extended shelf-life. The addition of encapsulated probiotics had no significant effect on the sensory properties of non-fermented ice cream.

**Chocolate**

The incorporation of encapsulated probiotic cells in chocolate has been reported (Table 5, Maillard & Landuyt, 2008). According to their work, probiotic viability in the small intestine was three times higher when incorporated in chocolate than in dairy product. Possemiers et al. (2010) also incorporated encapsulated probiotic cells in chocolate. Results have shown that the introduction of encapsulated probiotic strains into chocolate can be an excellent solution to protect them from environmental stress conditions. In chocolate, the lipid fraction of cocoa butter was shown to be protective for bifidobacteria (Lahtinen et al., 2007). In 2007, Company Barry Callebaut developed a process to produce chocolate containing encapsulated probiotic cells. The addition of encapsulated probiotic cells has no influence on chocolate taste, texture and mouth feel. A consumption of 13.5 g per day of probiotic chocolate seems to be sufficient to ensure the balance of the intestinal microflora.

**Mayonnaise**

Good quality mayonnaise was obtained when encapsulated bifidobacteria was incorporated (Table 5, Khalil & Mansour, 1998). Alginate beads can provide protection for bifidobacteria. Bifidobacteria strains survived only for 2 weeks in mayonnaise at pH 4.4 and 5 °C. However, within alginate beads, they survived for 12 and 8 weeks respectively. Other advantages can be quoted when considering the use of encapsulated probiotic cells such as growth inhibition of yeasts over 10 weeks (probably due to the antibacterial effect of the probiotics) and the improvement of mayonnaise’s sensory properties.

**Fermented plant-based products**

Encapsulation allows probiotic cells to survive against the unfavourable pH encountered. Furthermore, the sensory quality of the product has been improved upon incorporation of encapsulated cells compared with free cells (Table 5, An-Erl King et al., 2007; Tsen et al., 2008). The fermentation of banana media and tomato juices using encapsulated probiotic cells was carried out by Tsen et al. (2004, 2008).

Probiotics encapsulated in whey particles were added to a pasteurised vegetable juice cocktail and the viability of the probiotic cultures was examined during storage. The encapsulated *L. rhamnosus* cultures were more stable during storage than free cells in the same

<table>
<thead>
<tr>
<th>Table 5</th>
<th>The application of encapsulated probiotics in food industries</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probiotics</strong></td>
<td><strong>Methods</strong></td>
</tr>
<tr>
<td>Chocolate</td>
<td>Lactobacillus helveticus</td>
</tr>
<tr>
<td>Chocolate</td>
<td>Bifidobacterium longum</td>
</tr>
<tr>
<td>Mayonnais</td>
<td>B. bifidum</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>L. acidophilus</td>
</tr>
<tr>
<td>Cranberry and vegetable juice</td>
<td>L. rhamnosus</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>L. acidophilus</td>
</tr>
<tr>
<td>Carrot juice</td>
<td>L. acidophilus</td>
</tr>
<tr>
<td>Sausages</td>
<td>L. reuteri</td>
</tr>
<tr>
<td>Sausages</td>
<td>L. reuteri</td>
</tr>
</tbody>
</table>

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whey-based medium. In the same study, it was found that encapsulation also can improve the probiotic stability in a cranberry juice concentrate (Reid et al., 2007). Nazzaro et al. (2009) also found that encapsulation can improve survival ability of L. acidophilus in carrot juice. This shows that encapsulation can benefit to the stability of probiotics cultures in fermented plant products.

Meat

Muthukumarasamy & Holley (2006) showed that encapsulated L. reuteri can be used in dry fermented sausages to ensure that a desirable level of probiotic organisms is maintained in the final product at consumption without altering the sensory quality of these traditional small goods. It has been shown that L. casei cells when encapsulated in alginate beads were more resistant to heat processing at 55–65 °C and pH 5.0. These data suggest that probiotic cells encapsulated in alginate beads could be used in meat processing, which required moderate heat treatments (Table 5, Lemay et al., 2002; Mandal et al., 2006). In addition, it was shown that probiotics could reduce E. coli O157:H7 in number, but encapsulation decreased this potential (Muthukumarasamy & Holley, 2007).

Adding encapsulated probiotics into food products can affect sensory acceptance of products. The size of encapsulated probiotics may be the main factor influencing the sensory acceptance of products. Truelstrup Hansen et al. (2002) suggested that size of encapsulated probiotics below 100 μm would be suitable for food products. However, according to Champagne & Fustier (2007), the effects on sensory properties can become desirable if the consumer expects the presence of the particle. Preliminary studies including different probiotics concentration in encapsulated microspheres, different probiotics and encapsulation method et al. also should be carried out to optimise the sensory properties of the products, mainly with respect to the product acidity and sensory performance.

Conclusions

Encapsulation technology has been explored as a way of enhancing the resistance of probiotic cells in gastrointestinal tract and for prolonging the shelf-life of probiotics in food products. In most cases, alginate-based and protein-based materials have been used to encapsulate probiotic cells. However, the results are only promising in a laboratory scale. Encapsulation still has to face many challenges for its application on an industrial scale. On one hand, technological challenges to obtain microbeads with the best properties must be enhanced. On the other hand, consumer behaviour towards probiotics foods should be taken into account. Even probiotics encapsulation faces so the challenges, it is evident that probiotic market has a strong future as the consumers demand is increasing. Good hopes are also visualised for the microencapsulation of probiotics in the future.

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Bio-materials for probiotics encapsulation Q.-Y. Dong et al. 11


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