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.

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 10^7 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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as high acidity and low pH, bile salts, molecular oxygen and other processing conditions (Favaro-Trindade *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, pullan 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.

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.*, 2010). Until now, spray drying of mixtures of probiotics concentrates with aqueous solutions of various polymers, such as modified starch (O'Riordan *et al.*, 2001), gum Arabic (Rodriguez-Huezo *et al.*, 2007), gelatin (Lian *et al.*, 2003), whey protein isolate (Rodrigues *et al.*, 2011), maltodextrin mixed with gum Arabic (Su *et al.*, 2007) and β-cyclodextrin mixed with gum Arabic (Zhao *et al.*, 2008), has been reported.

Spray cooling is similar to spray drying. In this technology, a molten matrix with low melting point 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.

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

(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 of 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 (Krasaekoopt *et al.*, 2003). Sodium-alginate used widely in food material can form gel beads by dropping it to Ca^{2+} 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 Ki* 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

Table 1 Characteristics, advantages and disadvantages of encapsulation methods

Methods	Characteristics	Advantages	Disadvantages
Spray drying	Cells encapsulated individually in the drying medium Medium Cell load (10^{10}–10^{11} CFU g^{-1})	Many possibilities for coating materials Rapid cell release if ingredients dissolve rapidly	High temperatures in the process kill many strains
Emulsion	Homogenisation of aqueous and lipid phases	Easy to scale up, flexible adjustment of capsule size Lipid-based systems theoretically good for protection against acids and oxygen	Emulsifiers can be detrimental to viability High losses in liquid phase Liquid core may be unfavourable to long-term stability
Extrusion	Cells blended with various polymers and then extruded Low cell load (10^9–10^{10} CFU g^{-1})	Many possibilities for coating materials Particles can be air-dried Mild and simple preparation process	Difficulty to be scaled up
Fluid bed coating	True coating: cells in core powder Coat generally lipid-based High cell load (> 10^{11} CFU g^{-1})	Easy to be scaled up Multiple layers can be added for controlled release or density adjustments	Phase separation in beverages if coating is lipid-based Slow cell release at low temperature

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 *L. rhamnosus* GG and *L. acidophilus* NCFM 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 electrospraying process (Lo′pez-Rubio *et al.*, 2012). The results showed that encapsulation through electrospraying 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 ᴅ-mannuronic and ʟ-guluronic acids. The ratio of ᴅ-mannuronic and ʟ-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 (2.67×10^9 CFU g^{-1}) at pH 6.8 while the number is reduced at lower pH (1.5, exposure time 2 h) to 5.0×10^7 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 10^9 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 10^6 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 $92.8 \pm 3.1\%$ 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 lactlose. 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 1×10^8 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 2.5×10^7 to 2.2×10^7 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 on a succinylated alginate to immobilise a *Lactobacillus* strain for lactic acid production. The encapsulation of *L. delbruckii* 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 alginates 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; Weibreck *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 (Lucey *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 SGF conditions used to simulate the stomach

Gastric fluid	pH	Pepsin content (g L^{-1})	Exposure time (min)	Free cell viability (Log CFU mL^{-1})	Encapsulated cell Viability (Log CFU g^{-1})	References
NaCl (0.2%)	1.5	0	1 h	4.24	8.07	Mandal *et al.* (2006)
			3 h	3.38	7.54	
HCl (0.1 M)	1.1	0	2 h	7.17	7.47	Graff *et al.* (2008)
NaCl (0.2%)	2.0	0	2 h	0	4.25	Zou *et al.* (2011)
NaCl (9 g L^{-1})	2.0	3	2 h	< 1.0	6.95	Chavarri *et al.* (2010)
NaCl (0.2%)	2.0	0.32	2 h	5.90	7.63	Nag *et al.* (2011)
NaCl (0.2%)	2.0	0.3	1 h	7.12	8.00	Annan *et al.* (2008)
NaCl (0.2%)	2.0	0	2 h	1.21	8.01	Klemmer *et al.* (2011)

inulin and oligofructose) under simulated gastrointestinal conditions and heat treatments. The results show that microspheres produced with skim milk and prebiotics showed higher protection for bifidobacteria under stress conditions.

Soy protein
Soy protein isolates have been extensively used in biomedical and pharmaceutical sectors due to their functional properties (Maltais *et al.*, 2009, 2010). However, the gelation of soy proteins is conventionally achieved through heat treatment, thus not applicable for heat-sensitive core materials such as live microorganisms. Probiotic delivery system developed by the use of microbial transglutaminase (MTG) cross-linked soy protein isolate incorporated with agrowastes such as banana peel, banana pulp and pomelo rind has been reported (Yew *et al.*, 2011). Soy protein carriers in simulated gastric fluid did not show degradation of structure, whereas a major collapse of network was observed in simulated intestinal fluid, indicating controlled-release in the intestines. The results showed that soy protein isolate carriers containing agrowastes may be useful transports for living probiotic cells through the stomach prior to delivery in the lower intestines.

Pea protein
Klemmer *et al.* (2011) studied encapsulation of *B. adolescentis* and fructoligosacchardes within a pea protein isolate–alginate matrix by extrusion method. Capsules were accessed based on the levels of entrapped fructoligosacchardes and protein, size, swelling and probiotic survival within simulated gastric juice and release properties within simulated intestinal fluids. All designs offered sufficient protection to *B. adolescentis*. The results showed that encapsulation of probiotics using plant-based materials could play a role as a food supplement.

Parts of SGF conditions used to simulate the stomach were shown in Table 2.

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 & Masondole (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 Darukaradhya (2005) also obtained similar conclusions.

Özer *et al.* (2008) and Gonzalez-Sanchez *et al.* (2010) concluded that encapsulation can be a good

Table 3 The applications of encapsulated probiotics in cheese

	Probiotics	Methods	Materials	References
Cheddar	*Bifidobacterium bifidum*	Emulsion	k-Carrageenan	Dinakar & Mistry (1994)
Cheddar	*L. paracasei*	Spray drying	Skim milk	Gardiner *et al.* (2002)
Cheddar	*Lactobacillus acidophilus* *B. infantis*	Emulsion	Alginate/starch	Godward & Kailasapathy (2003)
Feta	*L. acidophilus* *B. lactis*		Alginate	Kailasapathy & Masondole (2005)
Kasar	*L. acidophilus* *B. bifidum*	Extrusion and emulsion	Alginate	Özer *et al.* (2008)
White brined	*L. acidophilus* *B. bifidum*	Extrusion and emulsion	Alginate	Özer *et al.* (2009)

Table 4 The application of encapsulated probiotics in yogurt and frozen dairy dessert

Probiotics	Methods	Materials	References
Lactobacillus acidophilus	Extrusion	Alginate	Krasaekoopt *et al.* (2004)
L. acidophilus *Bifidobacterium bifidum* *L. casei*	Extrusion	Alginate–chitosan	Krasaekoopt *et al.* (2006)
L. acidophilus *B. lactis*	Extrusion	Alginate–chitosan	Kailasapathy *et al.* (2008)
L. casei	Extrusion	Alginate/pectin	Sandoval-Castilla *et al.* (2010)
L. bulgaricus	Emulsion	Alginate	Sheu & Marshall (1993)
L. casei *B. lactis*	Emulsion	Alginate	Sheu *et al.* (1993)
L. acidophilus *B. lactis*	Emulsion		Kailasapathy & Sultana (2003)
L. casei *B. lactis*	Emulsion	Alginate	Homayouni *et al.* (2008)

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. lognum* (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. rhaninosus* cultures were more stable during storage than free cells in the same

Table 5 The application of encapsulated probiotics in food industries

	Probiotics	Methods	Materials	References
Chocolate	*Lactobacillus helveticus* *Bifidobacterium longum*	Spray coating	Fatty acids	Maillard & Landuyt (2008)
Chocolate	*L. helveticus* *B. longum*	Spray coating		Possemiers *et al.* (2010)
Mayonnasie	*B. bifidum* *B. infantis*	Emulsion	Alginate	Khalil & Mansour (1998)
Tomato juice	*L. acidophilus*	Extrusion	Alginate	An-Erl King *et al.* (2007)
Cranberry and vegetable juice	*L. rhamnosus*	Extrusion	Whey protein	Reid *et al.* (2007)
Tomato juice	*L. acidophilus*	Extrusion	Alginate	Tsen *et al.* (2008)
Carrot juice	*L. acidophilus*	Extrusion	Alginate-inulin-xanthan gum	Nazzaro *et al.* (2009)
Sausages	*L. reuteri*	Extrusion	Alginate	Muthukumarasamy & Holley (2006)
Sausages	*L. reuteri* *B. longum*	Extrusion	Alginate	Muthukumarasamy & Holley (2007)

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 be 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 gastro-intestinal 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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