Preparation and properties of milk proteins-based encapsulated probiotics: a review

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REVIEW PAPER

Preparation and properties of milk proteins-based encapsulated probiotics: a review

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Abstract The potential health and nutritional benefits of probiotics have boosted the demand for functional probiotic foods. The efficacy of probiotics depends on providing a specific number of viable cells on their consumption. Microencapsulation (ME) has been used to provide protection for probiotics all through food processing and marketing until they reach the target site in the gastrointestinal (GI) tract. The biomaterials and techniques used in ME are the main factors affecting the viability of encapsulated probiotics. Milk proteins offer several advantages in comparison to other biomaterials widely used in ME of probiotics. Several techniques have been developed for the use of whey proteins and casein in ME of several *Lactobacillus* and *Bifidobacterium* probiotic strains. Also, the survival of probiotics encapsulated in milk proteins during preparation, storage, and in simulated GI environment has been studied. The present review gives an overview on the use of milk proteins in ME of probiotics with emphasis on the efficiency of the developed techniques.

Keywords Milk proteins · Whey proteins · Casein · Microencapsulation · Probiotics

1 Introduction

Probiotics are "Live microorganisms which when administered in adequate amounts confer a health benefit on the host live microorganisms" (FAO/WHO 2002). They are recognized as very potential bacteria, believed to play a beneficial role in the ecosystem of the human intestinal tract in such way that they can remove the harmful bacteria from the intestine and reinforce the body's natural defense mechanisms (Jia et al. 2008). One of the primary benefits associated with probiotic bacterial cultures is that they can

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reduce pathogenic bacteria from the small and large intestine (Kos et al. 2008). The potential health benefits have led to an increased incorporation of probiotics in foods and the development of probiotic ingredients and supplements. *Lactobacillus* and *Bifidobacteria* are the two most common genera of microbes which are extensively used as probiotics.

The demand of probiotics has grown markedly all over the world during the last decade. A steady increase in the global expenditure on probiotic ingredients, supplements, and foods amounted to 21.6 and 24.32 billion US $ in 2010 and 2011, respectively, and is expected to reach 31.1 billion US $ in 2015 with an annual growth percentage of 7.8 (Pedretti 2013). The rapid growth of probiotic market has been driven by several factors:

1. The consumer awareness of the potential health and nutritional benefits of probiotics.
2. The rigorous evaluation of the health claims of newly discovered probiotics (Donovan et al. 2012) and safety of probiotics (Vankerckhaven et al. 2008). The health and therapeutic benefits of several probiotics is now well documented (Solanki et al. 2013).
3. The large number of newly developed probiotic foods and beverages. More than 500 products have been introduced in the past decade mostly in the form of milk, fermented dairy products, cheese, and fermented soy products.
4. The regulatory guidelines and specification (FAO/WHO 2002) established a frame for the development and quality control of probiotic foods.
5. The emerging trend for the inclusion of probiotic in animal feed industry.

The efficacy of probiotics is dose dependent, and in order to achieve its beneficial effects on human health, probiotic foods must contain at least 10^6–10^7 cfu of viable probiotics per gram at the time of its consumption. In Canada, the Food Inspection Agency (2014) requires that a serving of stated size of a product should contain a minimum of 1×10^9 cfu per food portion of one or more of eligible microorganisms that are the subject of the claim. In addition, probiotics must retain viability throughout storage and products' shelf-life, and they must survive adverse environment during gastrointestinal (GI) passage. However, for some applications, it may not be necessary for probiotics to be viable, as non-viable forms have also been proved to provide some health effects (Ouwehand and Salminen 1998; Salminen et al. 1999). In addition to non-viable bacteria, probioactives, i.e., bacterial derived products and/or bacterial metabolites, could provide some health benefit (Farnworth and Champagne 2010).

During food processing and storage, probiotic bacteria are exposed to several challenges such as oxidative stress, temperature, acid–base changes, and molecular entrapments that compromise their viability. The viability of probiotics decreases rapidly in fermented dairy products stored at temperatures above refrigeration. Also, during their passage through GI, probiotics are adversely affected by enzymatic action of pepsin and low pH of the stomach with further antagonism associated with antimicrobial activity of bile salts and protease-rich conditions of the intestine (Farnworth and Champagne 2010; Tripathi and Giri 2014).

Several technologies have been proposed to improve the survival and viability of probiotics throughout food processing, storage, and consumption including appropriate selection of acid- and bile-resistant strains, use of oxygen-impermeable containers or oxygen scavengers, two-step fermentation, stress adaptation, microencapsulation (ME), and addition of micro-nutrients such as peptides and amino acids (Corona-Hernandez et al. 2013; Tripathi and Giri 2014). Also, the food matrix affects greatly the viability of probiotics (Farnworth and Champagne 2010). The buffering capacity of the food matrix is arguably a critical factor. Therefore, the delivery of probiotics via cheese has several advantages as the cheese creates a buffer against the high acidity encountered in the stomach, forms a dense protein matrix, and protects the cells by the possible presence of fat (Burgain et al. 2013a). However, ME is nevertheless considered as a powerful method to protect probiotics and to improve their survival and viability.

Encapsulation is a technology used mainly to "package" sensitive bioactive materials and microorganisms in miniature capsule. The microbial cells have typical sizes that range from 1 to 5 μm in diameter. Therefore, they can be entrapped by ME. The purpose of ME of probiotics is to provide a protective barrier between them and the destructive factors prevalent in the surrounding environment such as heat, oxygen, and moisture. The main elements that must be considered with respect to encapsulation of probiotics are (a) keeping them alive until they reach the target site and (b) effective release of the entrapped microorganisms. Many areas in the GI could be targeted by probiotics, and it is important that cells all be released at those points (Maldonado Galdeano et al. 2009).

Milk proteins are among the favorable materials for use singly or in combination with other biomaterials as capsule materials for probiotics. Compared to soy protein isolate, milk proteins offered better protection for *Bifidobacterium longum* 1941 after freeze drying and during exposure to acid and bile environment (Dianawati et al. 2013).

Several reviews have covered the ME of probiotics from several aspects including biomaterials and methods used for microencapsulation (Anal and Singh 2007; Heidebach et al. 2012; Huq et al. 2013; Tavares et al. 2014), but no cited work gave a special emphasis on ME of probiotics using milk proteins. The present review has been devoted to the use of milk proteins for encapsulation of probiotics and their use in food products.

2 Microencapsulation

Microencapsulation consists of coating or entrapment of a core material into capsules of sizes ranging from a few micrometers up to a few millimeters (Burgain et al. 2011). Encapsulation of probiotics involves random imbedding and immobilizing of the living cells as the core material in a continuous matrix (the shell), which is often a hydrogel (Anal and Singh 2007). Several methods have been suggested to measure the efficiency of ME of probiotics (Heidebach et al. 2012), but the most important criteria addressed in these methods are the viability (number of viable cells/total cell count) of the entrapped cells and the

cell load (cell count/capsule) in the microcapsules. The following factors were found to affect the efficiency of ME of probiotics:

2.1 Biopolymer used

The type, concentration, and properties of the capsular material markedly affect the viability of the encapsulated probiotics. Numerous biomaterials including polysaccharides (such as alginate, carrageenan, xanthan, and gellan gums, pectin, and chitosan) and proteins (such as milk proteins, soy proteins, zein, and gelatin) have been used or have the potentials to be used for encapsulation of probiotics as long as they satisfy the requirements of the generally recognized as safe (GRAS) materials. The choice of the capsule materials is a major element for successful ME of probiotics and the use of ME probiotics in functional foods (Huq et al. 2013). Generally, capsular materials must be resistant to the acidic conditions. In addition, they should fulfill the requirements for the release of entrapped probiotics in the targeted part in the GI. Probiotics targeted for the small intestine should be decomposed after subjecting them to the pH of the small intestine or pancreatic enzymes, but for those targeting the large intestine, capsules should be tolerant to these conditions. However, no single biomaterial can satisfy the requirements for optimum ME of probiotics; each has its advantages and limitations. For example, the most widely used material in encapsulation of probiotics, i.e., Na-alginate, has been preferred as low-cost, non-toxic, and biocompatible material which can be used simply and efficiently for ME of probiotics. However, the gelling properties of Na-alginate is source dependent, and the formed beads may offer weak protection of the entrapped cells (Mortazavian et al. 2007). New biomaterials of favorable ME characteristics and/or use of combinations of biomaterials for efficient ME of probiotics is an area of growing interest.

2.2 Method of encapsulation

The viability of encapsulated probiotics is affected greatly by the used encapsulation process. The characteristics of the formed capsules particularly the particle size and shape of the formed capsules from the same biopolymer are determined by the ME process. The viability of the entrapped probiotics is affected by the particle size and shape of the microcapsule (Mortazavian, et al. 2007). Microcapsules can be produced either as soft gel beads or as dried powder in different shapes characterized by smooth or irregular surfaces with or without the presence of pores (Mortazavian et al. 2007). The presence of pores reduces the encapsulation efficiency. Capsules of different shapes and sizes can be prepared from the same capsular material depending on the used ME technique (Burgain et al. 2011).

The size of the microcapsule can be an important factor for the stability and efficacy of the entrapped probiotics (Zhao et al. 2008). Generally, large microcapsules offer better protection for probiotics than small capsules, but they are poorly dispersed and impart sandiness in foods. Capsules of sizes ranging from 1 to 3 mm are preferred (Heidebach et al. 2012) to satisfy the requirements of cell growth and mechanical strength of the capsule. Below 1 mm size, gel beads may result in mechanical instability during long continuous fermentation (Heidebach et al. 2012). Therefore, it is necessary to control the conditions leading to microcapsules of optimum size with respect to

efficiency of entrapment of the viable cells and use of prepared capsules in food. The sizes of the micro-beads have a crucial effect on the viability of probiotics and their metabolic rate. Increasing the particle size up to a particular limit (depending on the type of the capsule and entrapped microorganism) generally improves the resistance of the entrapped probiotic to environmental factors, but further increase of the bead diameter had no effect on the viability of the cells (Burgain et al. 2011). Also, the capsule size affects their distribution and dispersion in the food matrix and sensory properties of the final product (Krasaekoopt et al. 2003; Picot and Lacroix 2003). Micronization using a spiral jet mill as a grinding system was reported to be an effective way for reducing powder particle size of freeze-dried probiotics at low heat treatment for subsequent cell microencapsulation (Picot and Lacroix 2003).

2.3 Interaction between probiotics and capsular materials

Knowledge about the interaction between probiotics and the capsular materials is important in order to understand the mechanism of encapsulation and to improve the encapsulation yield and survival rate, and release of entrapped microorganisms. The cell/capsular material interactions depend mainly on their surface properties.

The microbial surface properties have been widely studied in order to understand the interactions between bacteria and interfaces resulting in the formation of biofilms, a phenomenon important in many fields such as biomedical and food safety, corrosion, and environment. Several macromolecules are located on the bacterial surfaces which enable bacteria to interact specifically or non-specifically with other compounds through electrostatic, hydrophobic interactions. Addition of bacteria to oil/water emulsions stabilized by milk proteins (sodium caseinate, whey protein concentrate or whey protein isolate) at different pH (from 3 to 7.5) affected the emulsions' stability depending on the surface properties of strains and also on the characteristics of emulsions (Ly et al. 2008). The surface charge and hydrophobicity of the bacteria were suggested to be involved in its interaction with proteins (Ly et al. 2008). A study revealed that the interaction of probiotics with milk proteins is strain specific and that the type of milk protein and pH affect these interactions being non-specific in case of casein and specific in case of whey proteins (Burgain et al. 2013b). The efficiency of encapsulation of the *Lactobacillus rhamnosus* GG (LGG) wild type and mutants lacking exopolysaccharide, with modified surface charge and lacking pili, was compared. The mutant lacking the SpaCBA pili was the least to interact with casein and whey protein matrices (Burgain et al. 2014). Also, the pilus appeared to be crucial for location of the LGG inside the microcapsule (Burgain et al. 2014).

2.4 Density of gel network

The density of the gel network determines greatly the available space for cell growth in the capsules (Heidebach et al. 2012). Limited space for cell growth may lead to mechanical stresses on the matrix and subsequent cell leakage from fully occupied gel matrix in the surrounding media. A low-density gel network which provides

sufficient space for the production of concentrated biomass within the polymer gel is a possible way to ameliorate the problem of cell leakage.

3 The use of milk proteins in microencapsulation

Protein hydrogels are generally accepted as suitable materials for ME in food applications particularly in liquid and semi-solid foods. They have the advantage to develop matrixes of controlled sizes without any adverse effect on the sensory properties of the prepared food (Chen et al. 2006). Also, proteins possess the ability to interact, protect, and reverse binding with a wide range of active compounds through their functional groups (Chen et al. 2006). In addition, they might have desirable stabilizing effects on food texture (Chen et al. 2006).

The unique functional and nutritional properties of milk proteins are well documented (Fox and McSweeney 2013) and they are universally valued as food ingredient. Therefore, milk proteins have been considered as good choices for nano- and microencapsulation of nutriceuticals and probiotics (Abd El-Salam and El-Shibiny 2012; Augustin et al. 2012; Heidebach et al. 2012; Tavares et al. 2014). In addition to the general advantages of protein hydrogels, milk proteins have several specific advantages in food microencapsulation:

1. A large number of diversified milk protein products are available commercially. These products have different composition, protein content, and functional properties such as whole milk protein, acid and rennet caseins, caseinate salts, β-casein, whey protein concentrates (WPC) and isolates (WPI), and β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) rich fractions. This offers a wide range of choices for encapsulation of probiotics in milk proteins.
2. Milk proteins are flexible to encapsulate any type of hydrophilic, hydrophobic, or viable probiotic cells
3. The nature and conditions of gel formation are key factors for the successful ME of the living bacteria. Milk proteins can form gels through different mechanisms under mild conditions. This offers different choices for the successful uses of milk proteins in ME of living bacteria (Heidebach et al. 2009a, b). Gelation of casein can be induced with milk clotting enzymes or in the presence of acids. Acid gelation of casein occurs near its isoelectric point where the hydrophobic interactions act as the main forces for aggregation and gelation. Also, formation of new covalent bonds in milk proteins by the action of transglutaminase results in the formation of hydrogels. Gels can be prepared from whey proteins through heat denaturation and subsequent cold gelation by acids, salts, and polyelectrolytes.
4. Concentrated solutions of milk proteins have moderate viscosities for easy dispersion of the bacterial cells and when used in capsulation give gels of high density and better protection for the entrapped probiotics.
5. Milk proteins are rich sources for bioactive peptides of different physiological effects. Such bioactive peptides are generated by the action of the digestive enzymes to exert their beneficial effects. The liberated bioactive peptides may offer a synergistic effect on the probiotic action.

3.1 Whey proteins as capsular materials for probiotics

Whey proteins (WP) are considered as favorable biomaterial for encapsulation of probiotics. They have been found to increase the resistance of probiotic *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* against acid and bile salts (López 2013). A study showed that the addition of 1 or 2% of alginate, WPC (50% protein), chitosan, L-carrageenan, and xanthan gum to the medium affected variably the growth and the viability of five strains of *Lactobacillus* and *Bifidobacterium* (Rodrigues et al. 2011a). Alginate and WPC were found to be the most efficient materials to maintain the viability of all strains during incubation except for alginate at 2% in case of *Lactobacillus acidophilus* Ki and *Lactobacillus casei* 01 where slight decreases in their count were found. Whey proteins have been used singly or in combination of several polysaccharides in microencapsulation of probiotics.

3.1.1 Whey protein products used

Several whey protein products of different protein contents have been used in ME of probiotics ranging from whey powder (~15% protein) to whey protein isolates (WPI >90% protein). Studies in vivo and in vitro have shown that the nature of food harboring probiotics affected their viability (Ainsley Reid et al. 2007). In this respect, the nature of the whey proteins was found to affect the entrapment of the probiotic *Lb. rhamnosus* GG (Doherty et al. 2010). *Lb. rhamnosus* GG was immobilized separately in native, denatured, and partially hydrolyzed WPI (degree of hydrolysis, i.e., percentage of hydrolyzed peptide bonds, 6.5±0.4). Microscopic examination revealed phase separation of immobilized cells from the surrounding matrixes of denatured and hydrolyzed WPI. Also, hydrolyzed and denatured WPI enhanced the viability of the immobilized probiotic enhanced survival by 6.1±0.1 and 5.8±0.1 $\log_{10}$ cycles, respectively, following 14-day storage at 37 °C, and both treatments generated thermal protection at 57 °C (7.3±0.1 and 6.5±0.1 $\log_{10}$ cfu.mL^{-1}). Furthermore, denatured WPI enhanced probiotic protection (8.9±0.2 $\log_{10}$ cfu.mL^{-1}) following 3 h gastric incubation at 37 °C, while the native WPI exhibited the weakest protection for the entrapped probiotic (Doherty et al. 2010). An interesting finding is that succinylation of β-LG offered a novel protecting agent for probiotic bacteria. Tablet produced by direct compression of a dry mixture of *Bifidobacterium longum* HA-135 and succinylated β-LG protected the delivered probiotic against the adverse conditions of the GI tract while the native β-LG did not ensure cell survival (Poulin et al. 2011).

3.1.2 Techniques used

Selection of the encapsulation technique depends mainly on the nature of the biomaterial used as the wall material and the ability of the entrapped microorganisms to retain their viability and activity. Probiotic activity differs from probiotic survival in that the activity takes into account the ability of cells to resist the GI environment and to adhere to intestinal mucosa. Several techniques

have been developed for the encapsulation of probiotics in whey proteins and are summarized in Table 1 as follows:

Extrusion Microencapsulation of probiotics by this technique is based on extruding a mixture of concentrated viable probiotic cells and WP or WP-polysaccharide solution through a nozzle to form droplets that fall into a hardening solution. The gelled droplets are usually called micro-beads. Also, extrusion can be done in two steps in which probiotics are first entrapped in polysaccharide beads followed by coating the obtained beads with WP.

The size of the formed capsules depends on the extrusion conditions (Anal and Singh 2007). The method is simple and provides high cell viability but gives capsules of relatively large diameters (Table 1). The obtained beads by this method demonstrated high acid stability and resistance against peptic digestion. However, in the presence of the intestinal enzymes, the beads underwent rapid degradation (Doherty et al. 2011). It has been suggested that the gel structure, rather than proteolysis, modulates probiotic release from whey protein-based capsules (Doherty et al. 2011). Extrusion can be combined with coacervation in order to improve the stability of the obtained capsules (Gerez et al. 2012; Gebara et al. 2013).

The native WP has been used as outer cover for alginate micro-beads, loaded separately with three strains of *Lactobacillus plantarum* (Gbassi et al. 2009). The WP-coated beads showed better survival of the entrapped microorganisms in simulated gastric environment compared to the uncoated beads, and only bacteria in coated beads survived simulated intestinal fluid after simulated gastric treatment (Gbassi et al. 2009). However, the survival of the entrapped microorganisms subjected to simulated gastric environment was found to be strain dependent (Gbassi et al. 2009).

Similarly, probiotic *Lactobacillus bulgaricus* was entrapped in alginate-milk microspheres by extrusion method (Shi et al. 2012). The size and shape of the formed capsules were dependent on the nozzle used and the concentration of the alginate. Using nozzle 0.45 and 0.20 mm gave beads of 830±10 and 381±8 μm, respectively. Increasing the alginate concentration increased the bead diameter and the shape became more spherical. The efficiency of encapsulation was reported to be ~100% and the viability of the entrapped microorganism was almost unchanged in simulated gastric conditions.

Spray drying Microencapsulation of probiotics by the spray drying techniques (Table 1) has the advantage of low-cost operation, better storage stability than frozen or fresh cultures, and the possibility of storage at room temperature (Maciel et al. 2014). However, spray drying causes some viability losses of the encapsulated probiotics mainly due to the physical injury of the microcapsules, release of bacterial cells, and heat generation during the drying process. Ying et al. (2012) found that the addition of glucose in WPI-maltodextrin and WPI-inulin as encapsulants for *Lb. rhamnosus* GG prior to spray drying had no marked influence on the survival of probiotics during drying. However, addition of glucose markedly improved the survival of the entrapped LGG during subsequent long-term storage. Also, Dianawati et al. (2013) reported that polyalcohols were superior to maltodextrin in the protection of *Bifidobacterium longum* encapsulated in milk proteins during spray drying. The viability of *Saccharomyces boulardii* within the matrix of whey protein was largely dependent on the

Table 1 Microencapsulation of probiotics in whey proteins or its mixture with other biopolymers using different encapsulation methods

Encapsulant	Probiotic	Particle size/shape	EE and viability	GI stability	Reference
Extrusion					
DWPI	*Lb. rhamnosus*	Beads (~3 mm)	EE 98%, survival 23%	ND	Ainsley Reid et al. (2005)
WP	*Saccharomyces cerevisiae*	Beads 2.6 mm	No negative effect on the cell viability	Release of 2.2±0.9% of initial entrapped yeasts in SGF	Hébrard et al. (2006)
DWPI/Al (68/32)	*Saccharomyces boulardii*		High EE (95%)	Survival 60% in SGF compared to 10% for free cells	Hébrard et al. (2010)
DWPI HWP	*Lb. rhamnosus* GG	Irregular-shaped gel particles immobilized cells, phase-separated from the surrounding protein matrix	HWP and DWPI enhanced survival by 6.1±0.1 and 5.8±0.1 $\log_{10}$ cycles, respectively	3.2±0.2 and 0.1±0.1 $\log_{10}$cfu.mL^{-1} cell loss in SGF for HWP and DWPI, respectively	Doherty et al. (2010)
DWPI	*Lb. rhamnosus* GG	Beads (~200 μm), high strength	2.7×10^4 cfu.g^{-1} bead, high cell viability	Acid stability-pepsin resistance in SGF	Doherty et al. (2011)
DWP/pectin (1:1)	*Lb. rhamnosus*	Irregular microcapsules (185±20 μm)	High viable cells (9.2 log cfu.g^{-1})	Survived at pH 1.2	Gerez et al. (2012)
M pectin/WP coat	*Lb. acidophilus*	Regular particles	High yield (84.35±0.60%)	Remained intact for 120 min in SGF, disintegrated after 300 min in SIF	Gebara et al. (2013)
Spray drying					
DWPI	*B. breve* or *B. longum*	Capsule 3–75 μm with central void	*B. breve* high yield (25.67%) EE, log 9.2 cfu.g^{-1}, *B. longum* low yield (1.44%) EE, log 7.93 cfu.g^{-1}	Survived exposure to SGF for 30 min, 1.0×10^{-1} to 7.2×10^{-1} cfu.mL^{-1} after 6-h incubation at pH 7.5 in SIF	Picot and Lacroix (2004)
WPC (50%) with/without L-cysteine	*Lb. acidophilus*, *Lb. paracasei* L26, *B. animalis* BB-12	Irregular shaped microspheres (5–50 μm)	Reduced viability of *B. animalis* in the absence of cysteine	ND	Rodrigues et al. (2011b)
Rennet whey	*B. animalis* BB-12	Spherical particles 11.23±4.2 μm	High cell load (9 log.g^{-1}), stable during cold storage	Remained >6 log in high bile salt concentration	De Castro-Cislaghi et al. (2012)
Sweet whey (30% solids)	*Lb. acidophilus*	Particle diameter 12.94±0.78 μm	Viability ~7 log.g^{-1}	No changes after 7 h at simulated GI conditions versus reduction of 3.11 log in free cells	Maciel et al. (2014)
DWPI with/without RS	*Lb. rhamnosus* GG	Spherical (15 μm)	Loss in viability (1 log cfu.g^{-1}) during drying and on hydration	Reduction of 0.8–2.6 $\log_{10}$ cfu.100 mL^{-1} in apple juice	Ying et al. (2013)

Table 1 (continued)

Encapsulant	Probiotic	Particle size/shape	EE and viability	GI stability	Reference
Whey (20% solids)	*Lb. reuteri*	Particle size (4.9–5.5 μm)	Viability loss of 2 $\log.g^{-1}$ during drying and 1 $\log.g^{-1}$ during storage	Higher survival rate (35%) than free cells in SIF	Jantzen et al. (2013)
Maltodextrin/WP/glucose	*Lb. acidophilus*	Spherical particles 10.96±0.63 μm	Survival 69.9±4.3% after drying, viability loss 0.011/day at 4 °C, 0.041/day at 25 °C	ND	Behboudi-Jobbehdar et al.(2013)
Emulsification					
Whey/canola oil	*Lb. rhamnosus*	Stable double emulsion (W/O/W) droplet size 8.24±2.56 μm	EE 95.9%	Increased survival (108 and 128%) in low pH and in the presence of bile salt, respectively	Pimentel-González et al. (2009)
Fluidized bed					
WP/LGG on Cellets® (core)	*Lb. rhamnosus* GG	WP/LGG layer (15–20 μm), fat layer (30–40 μm)	Viability reduction 5 log $cfu.g^{-1}$	ND	Weinbreck et al. (2010)
Whey powder/shellac	*Lb. reuteri*	Particle size (~206 to ~235 μm) of coated granules	High viability (42.69±4.84%)	Survival after SGF and SIF 76.74±24.36% compared to 17.75±10.51% for free cells	Schell and Beermann (2014)
Electrospraying					
WPC 80 solution (30–40% solids)	*B. animalis* BB-12	Fused capsule-like structure >1 μm	No negative effect on viability	ND	López-Rubio et al. (2012)
Coacervation					
WPI/κ-carrageenan	*Lb. plantarum*	Particle size 220–230 μm	EE 39.6%	High survival in low pH (9.75 log $cfu.g^{-1}$) and the presence of bile salts (9.66 log cfu) than free cells (9.51 and 5.93 log, respectively)	Hernández-Rodríguez et al. (2014)

WP whey protein, *WPI* whey protein isolates, *DWPI* denatured WPI, *WPC* whey protein concentrates, *Al* alginate, *EE* encapsulation efficiency, *SGF* simulated gastric fluid, *SIF* simulated intestinal fluid, *ND* not determined, *LGG* Lactobacillus rhamnosus GG

physicochemical properties of the protein network (Duongthingoc et al. 2014). Spray drying of WP solution at pH ~5 with addition of 50–100 mM $CaCl_2$ resulted in an efficient encapsulant which provided optimum protection and high viability for the entrapped *S. boulardii*. Early agglomeration of whey proteins by heating at pH 4 before mixing with the yeast and spray drying was found to improve the survival of *S. boulardii* (Duongthingoc et al. 2013). Compared to WPI solution heated at pH 6–7, that heated at pH 4 yielded capsules of larger size and higher survival (38%) of the entrapped *S. boulardii* (Duongthingoc et al. 2013) which was attributed to increased protein-protein interaction. The type of the wall material and the atomization method used during spray drying seem to affect the survival of the encapsulated probiotics in simulated GI tests. The viability of *Bifidobacterium infantis* microencapsulated with WPC and spray dried using two-fluid nozzle was significantly higher than that encapsulated in soy protein concentrate and spray dried using centrifugal atomizer in the simulated GI tests (Lee 2012). *Lb. plantarum* MTCC 5422 was microencapsulated in undenatured and denatured WPI with Na-alginate, respectively, using spray-drying and freeze-drying techniques (Rajam et al. 2012). Cells entrapped in denatured WPI showed better stability in simulated acidic and bile acids conditions than that entrapped in undenatured WPI. The optimum spray-drying conditions (temperature and flow rate) for the production of maximally viable microencapsulated *Lb. acidophilus* in a 60:20:20 (*w*/*w*) maltodextrin/WPC/D-glucose carrier were 133.34 °C and 7.14 $mL.min^{-1}$ (Behboudi-Jobbehdar et al. 2013).

Emulsification Single or double emulsions can be used for the entrapment of probiotics. For the preparation of a single emulsion (water/oil, W/O), a slurry of the viable probiotic cells and whey proteins is emulsified in a vegetable oil with the aid of a surfactant and homogenization. The single emulsion can be used in the preparation of the double emulsion (Table 1) by homogenization in an aqueous phase containing a suitable surfactant. Emulsification generates oily or aqueous droplets commonly named capsules of wide range of sizes. However, the capsules obtained by this technique have sizes much less than that obtained by the extrusion method. The emulsions suffer from poor instability, need for vigorous stirring which can be detrimental to cells survival, and random incorporation of cells into the capsules.

Fluidized bed and spray coating Fluidized bed drying is a technique where the probiotic bacteria are encapsulated first in the supporting material followed by drying of the granulated material in the fluidized bed air drier. It offers the advantage of total control over the drying temperature, but the drying process took a long time. The spray coating consists of two successive coatings: the first is an aqueous-based coating and the second is a lipid-based coating (Table 1).

Electrospinning/electrospraying Electrospinning/electrospraying is a simple and highly versatile method to produce fibers and/or capsules in the sub-micron range, presenting a large surface-to-volume ratio, through the action of an external electric field applied between two electrodes and imposed on a polymer solution or melt. It has the advantage that no temperature is required in the process and that biopolymers can be electrospun from an aqueous solution just by adjusting the process parameters and/or changing the solution properties through the addition of proper additives (López-

Rubio and Lagaron 2012). Electrospraying has been successfully used in the preparation of micro- and nanocapsules from WPC solution containing glycerol and adjusted pH for encapsulation of bioactive compounds (López-Rubio and Lagaron 2012). This technique has been used for encapsulation of *Bifidobacterium animalis* Bb12 (Table 1).

Coacervation This process involves the separation of colloidal particles from a solution and subsequent deposition on a core target. In this process, coacervate nuclei adsorb to the surface of core material and form a uniform layer around the core particles. Finally, solidification of coating material is done by cross-linking using a chemical, thermal, or enzymatic method. It has the advantages of high payload, control over the release of the core material, and it can be carried at room temperature which offers a suitable condition for encapsulation of probiotics (Anal and Singh 2007). A study (Hernández-Rodríguez et al. 2014) has described the use of WPI/κ-carrageenan coacervate for successful microencapsulation of *Lb. plantarum* (Table 1).

Transglutaminase (TGase)-induced gelation The TGase-induced method (Heidebach et al. 2009a) originally developed for encapsulation of probiotics in casein gels (described under native casein) was used for encapsulation of *Bifidobacterium bifidum* F-35 in WP (Table 1). The prepared TGase WP microcapsules loaded with *B. bifidum* F-35 were freeze dried and compared with microcapsules prepared by spray drying of mixture of *B. bifidum* F-35 and WP (Zou et al. 2012). The WP microcapsules prepared by TGase-induced gelation were ten times larger and denser than that prepared by spray drying. The survival rate of *B. bifidum* F-35 in TGase-induced WP gels at pH 2 and in the presence of pepsin and storage stability for 1 month at 4 °C were better than that prepared by spray drying. Addition of sucrose improved the encapsulation efficiency of TGase-induced microcapsules.

3.1.3 Post-preparation behavior of probiotic loaded WP micro-beads

Storage stability *Lb. acidophilus* La-5 microencapsulated in sweet whey by spray drying retained high viability during storage showing an average decrease of 0.43 log cfu.g^{-1} at the end of 90 days of storage and remaining higher than 6 log cfu.g^{-1} (Maciel et al. 2014). The storage stability of probiotics in WP-based microcapsules was strain dependent; *Lb. paracasei* was the least susceptible to storage conditions (temperature, humidity, and time) followed by *B. animalis* Bb12 while *Lb. acidophilus* underwent higher losses during storage (Rodrigues et al. 2011b). The relative humidity was found to be the most important factor in maintaining the viability during storage of *Lb. rhamnosus* GG microencapsulated in WPI and WPI-maltodextrin and WPI-inulin and that the addition of glucose in the encapsulant material improved the viability of the entrapped probiotic (Ying et al. 2012).

Survival in simulated gastrointestinal (GI) environment Most of the studies have been concerned with the survival of the entrapped probiotics when subjected to in vitro simulated GI environment, and little followed the changes in the micro-beads under such conditions. Doherty et al.(2012) demonstrated that WP micro-beads undergo contraction in simulated gastric juice and weakened micro-bead strength, but no

proteolysis was found which was attributed to the formation of dense protein matrix resisting acid penetration (pH <1.8) and pepsin attack. At pH 7.5 and in the presence of intestinal enzymes, WP micro-beads underwent rapid disintegration with sequential decrease in the molecular weight of the formed peptides. *Bifidobacterium breve* entrapped in WP microcapsules showed significantly higher survival (+2.7 log cycles) than the free cells after sequential exposure to simulated gastric and intestinal environment (Picot and Lacroix 2004). Free cells of *Lb. rhamnosus* GG showed no survival during ex vivo porcine gastro-intestinal incubation while those entrapped in beads of native WP survived by 5.7±0.1, 5.1±0.2, and 2.2±0.2 $\log_{10}$cfu.mL^{-1} following 180 min of incubation at pH 3.4, 2.4, and 2.0, respectively (Doherty et al. 2012). Entrapment of *Lb. rhamnosus* in double emulsion was reported to improve the survival of the microorganisms in acid environment and in the presence of bile salts. Increases of 8 and 28% from the initial count of the entrapped *Lb. rhamnosus* were found after exposure to acid and bile salt environment (Pimentel-González et al. 2009). A high solubility >40% of the protein covering pectin/WP (native and denatured) micro-beads was observed at pH 1.2, indicating that the beads were extremely fragile to acidic pH in the presence of pepsin, and beads were disintegrated at pH 7.0 and in the presence in pancreatin (Souza et al. 2012).

Behavior in food products Yoghurt was made with the use of free and whey protein encapsulated *B. breve* (6–7×10^6 cfu.mL^{-1}) and *B. longum* (5–6×10^7 cfu.mL^{-1}) as adjunct culture. The encapsulation yield and viability differed significantly according to the method and the strain used. *B. breve* gave higher encapsulation yield with a survival rate of 25.77±0.1% compared to 1.47±0.2% for *B. longum* encapsulated with spray-drying technique which was attributed to its thermal tolerance. Viable counts of encapsulated *B. breve* in yoghurt were significantly higher than those of free cells after 28 days of storage at 4 °C (+2.6 log cycles), but no protective effect was found in case of *B. longum* (Picot and Lacroix 2004). The viability of *Lb. rhamnosus* R011 microencapsulated in whey protein beads during the production and storage of biscuits, frozen cranberry juice, and vegetable juice was compared to free cells freeze-dried in the milk-based protective solution and in a denatured whey protein isolate-based solution (ungelled) enriched with lactose and sucrose (Ainsley Reid et al. 2007). During the production and storage of biscuits for 2 weeks at 23 °C, cells micro-entrapped in whey protein isolate gel particles showed the minimum drop in cell counts (from 1.3×10^7 to <10^3 cfu.g^{-1}). However, free cells prepared in the milk-based matrix maintained the highest viability during storage of vegetable juice, as well as during freezing and storage of cranberry juice. *Bifidobacterium* Bb-12 was microencapsulated by spray drying with whey. When the microcapsules were added to a dairy dessert, the probiotic count remained above 7 log cfu.g^{-1} for 6 weeks (De Castro-Cislaghi et al. 2012). Microencapsulation of *Lb. rhamnosus* GG (LGG) in WPI or WPI/resistant starch (RS) provided better protection to LGG in apple juice or citrate buffer than that entrapped in RS alone (Ying et al. 2013) during storage at low or ambient temperature for 5 weeks. The favorable effect of WPI on viability of entrapped LGG has been attributed to the protective effect of the generated buffered micro-environment of cells from the stresses of the external environment.

3.2 Casein as a capsular material for probiotics

Casein and caseinates have the advantages of gel formation at room temperatures by acids or enzymes. This offers suitable condition for microencapsulation of probiotics without any marked effect on their viabilities. Several forms of casein have been used in microencapsulation of probiotics as follows:

3.2.1 Native casein

Simple and versatile techniques have been developed for encapsulation of probiotics on the basis of the enzymatic gel formation of the naturally occurring casein in milk using chymosin and transglutaminase (TGase) (Heidebach et al. 2009a, b, 2010). Although the mechanisms of gel formation of the two enzymes are different, the methods used have similar steps.

For the encapsulation using TGase, 10 U TGase.g^{-1} casein was added to the protein-cell mixture at 40 °C, immediately dispersed in five folds of vegetable oil with continuous stirring until the casein/probiotic droplets were converted to gelatinized beads, separated by centrifugation, washed, and freeze dried. Encapsulation in TGase cross-linked casein beads improved the survival of *Bifidobacterium lactis* Bb12 during storage under different temperatures (5 and 25 °C) and relative humidity (11–33%) for up to 90 days while it had no protective effect on *Lactobacillus paracasei* subsp. paracasei F19 (Heidebach et al. 2010).

For rennet gelation, cold reconstituted milk (35% total solids) was mixed with probiotic *Lb. paracasei* F19 or *B. lactis* Bb12 concentrates (14:1) to give a mixture containing 12.7% protein and 10^9 cfu.g^{-1}. The mixture was cooled (5 °C) and incubated with rennet solution to perform the cleavage of κ-casein. $CaCl_2$ solution (10%) was added to the renneted mixture, mixed with vegetable oil (1:10), and homogenized by stirring while raising the temperature to 18 °C to induce gelation and formation of rigid microcapsules. Microcapsules were separated from the oil phase by centrifugation and washing (Heidebach et al. 2009b). Spherical microcapsules with small (~100 μm) and narrow range of diameters were obtained. The method gave high encapsulation yield and had no adverse effects on the survival of the encapsulated probiotics (Heidebach et al. 2009b).

The casein capsules provided good protection of *B. lactis* during incubation at low pH, but significant losses were found in case of *Lb. paracasei* under similar conditions. The improved survival of encapsulated cells can be attributed to the higher local pH value within the protein matrix of the capsules caused by the protein buffering capacity. Addition of lecithin and changing agitation speed during encapsulation of *B. animalis* subsp. *lactis* Bb12 using the method of Heidebach et al. (2009b) resulted in microcapsules of smaller diameter (Lisová et al. 2013).

Addition of denatured whey proteins influenced the encapsulation of *Lb. rhamnosus* GG by rennet-induced gelation of micellar casein (Burgain et al. 2013a). A mixture (13.5:1.5) of micellar casein and denatured WP produced micro-beads of average size of 59 μm with low polydispersity, slow changes in their shape during digestion, high elastic modulus after digestion, and offered the best bacterial survival (99%) and encapsulation rate (97%) in comparison with formulations containing either only casein or casein and native WP (Burgain et al. 2013a). Also, addition of emulsifiers and

changing the conditions of emulsification were studied in order to optimize the encapsulation of *Lb. casei* in rennet-induced casein gel (Clemente 2013). Capsules of the best size (~52 μm) and spherical shape were obtained using 0.5% *w*/*w* of lecithin and continuous stirring at 500 rpm during emulsification. The encapsulated probiotic retained high viability up to 6 h in simulated gastric juice, while the free cells disappeared under similar treatment. Also, the encapsulated *Lb. casei* was unaffected by cold storage in yoghurt and in brined cheese up to 4 weeks.

3.2.2 Sodium caseinate

Nag et al. (2011) found that acid gelation of Na caseinate solution with glucono δ-lactone (GDL) could not achieve a matrix with sufficient barrier strength, but acid gelation of a mixture of Na caseinate-gellan gum gave suitable gel for the encapsulation of probiotics. They successfully entrapped *Lb. casei* cells into caseinate-gellan gel matrix by emulsification and the obtained capsules had uniform particle size distribution (average ~287 μm) and high encapsulation yield of 89.5%. The capsules offered better acid and bile salt resistance to the entrapped probiotic.

Emulsification of *Lb. longum* 1941 in mixed Na caseinate (12%) and mannitol (3%) solution and subsequent freeze drying offered high stability for the cells after freeze drying and during exposure to acid and bile environment (Dianawati et al. 2013).

Microcapsules containing *B. lactis* (BI 01) and *Lb. acidophilus* (LAC 4) were prepared by complex coacervation of a casein/pectin mixture as the wall material, followed by spray drying (Oliveira et al. 2007). Spherical shaped microcapsules were obtained which provided efficient protection for the studied microorganisms against the spray-drying process and simulated gastric juice.

Addition of probiotic *Lb. helveticus* M92, either free or microencapsulated in Na caseinate (Pavunc et al. 2011), decreased the fermentation time and significantly enhanced the appearance and consistency of probiotic set yoghurt. However, microencapsulated *Lb. helveticus* showed better survival than free cells in the produced yoghurts during storage and during exposure to simulated gastrointestinal conditions.

The use of prebiotic in the wall material was reported to improve the survival of the encapsulated probiotic (Crittenden et al. 2006). A *B. infantis* strain was efficiently microencapsulated in a film-forming protein-carbohydrate-lipid emulsion containing canola vegetable oil, caseinate, and prebiotic fructo-oligosaccharides (FOS) plus either dried glucose syrup (DGS) or microfluidized resistant starch (RS). The emulsions were heated to 98 °C for 30 min to improve their film-forming and oxygen-scavenging properties, cooled to 10 °C, and probiotic bacteria concentrate was added and the mixture was spray dried. The produced microcapsules were small (15 to 20 μm in diameter), with low water activities (0.2 to 0.3). No free bacterial cells were apparent in the scanning electron micrograph of the microcapsules indicating that the encapsulation efficiency was high. The microencapsulated probiotic retained high viability during storage for 5 weeks at 25 °C and relative humidity of 50%. Microscopic examination showed that the bacteria remained entrapped within the capsule material in simulated gastric fluid but were released when transferred to simulated intestinal fluid (Crittenden et al. 2006). *Lb. plantarum* and *Lb. bulgaricus* were grown MRS to stationary phase, centrifuged, and the bacterial pellets (10^{10}–10^{11} cfu.g^{-1}, a_w 0.99) were mixed with casein powder (a_w 0.75) at the ratio of 1:1.2 (*w*/*w*) for 15 min and then dried in a

fluidized bed dryer at <35 °C and flow rate of 1.33 L.min^{-1} and relative humidity of 1%. Under these conditions *Lb. plantarum* retained 100% viability, while *Lb. bulgaricus* retained 2.1% viability (Mille et al. 2004).

4 Future trends

The area of ME of probiotics is gaining an increasing interest in parallel to the growing demand for probiotic fermented foods. The selection and design of the biomaterial to be used as wall material to encapsulate, protect, and release probiotic bacteria at the required site of action are a crucial step in successful microencapsulation of probiotics. Milk proteins offer several advantages for the entrapment and microencapsulation of probiotics and in most cases increase their survival during digestion. However, optimizing the use of milk proteins in microencapsulation of probiotics requires fundamental studies in several areas.

Limited studies have been done on the interaction between milk proteins and microorganisms which showed the importance of the adhesion of the bacterial surfaces to milk proteins for the successful entrapment of probiotics. Variable structures are found on the surface of the bacterial cells such as pili and exopolysaccharides that can interact with milk proteins through different forces and mechanisms. Further studies along this area are needed for better understanding the mechanisms and factors affecting the interaction of probiotics and milk proteins which in turn would lead for efficient ME of probiotics.

Most of the studies on the ME of probiotics depended on the use of the classical milk protein products. Several new milk protein products are now available commercially such as β-LG and α-LA rich fractions and β-casein which can extend the potential uses of milk proteins as wall materials for encapsulation of probiotics. The functional properties of milk proteins can be variably changed and controlled by several simple technologies. For instance, the heat denaturation of whey proteins at variable pH and heating conditions results in gel formation of different structures (fibril and capsular) and properties. Also, limited proteolysis of both casein and whey proteins results in substantial changes in their functional properties which may lead to better entrapments of the probiotics.

Milk proteins can be variably modified by the addition of several functional groups. A study (Poulin et al. 2011) showed that succinylated β-LG protected the delivered probiotic against the adverse conditions of the gastrointestinal tract while the native β-LG did not ensure cell survival. Along this line, unlimited number of modified milk proteins can be prepared and tested for efficient encapsulation of probiotics.

Few studies have been done on the application of milk protein capsules loaded with probiotics in food products. Milk proteins are characterized by bland flavor without any adverse effect on the sensory properties of fermented food. However, there is a need to evaluate the effect of their capsular forms on the quality of the supplemented fermented foods. Also, the protective effect of milk protein capsules on the survival of the entrapped probiotics during processing and storage of fermented foods needs further studies.

Most of the techniques developed for ME of probiotic in milk proteins have been limited to the laboratory scale. Selection, standardization of the developed ME

techniques, and subsequent pilot- and industrial-scale production are required. Large-scale production and application of ME probiotic would allow for better in vivo assessment of survival of consumed probiotics and their beneficial effects on human health.

References

Abd El-Salam MH, El-Shibiny S (2012) Formation and potential uses of milk proteins as nano delivery vehicles for nutraceuticals: a review. Int J Dairy Technol 65:13–21

Ainsley Reid A, Vuillemard JC, Britten G, Arcand Y, Farnworth E, Champagne CP (2005) Microentrapment of probiotic bacteria in a Ca^{2+}-induced whey protein gel and effects on their viability in a dynamic gastro-intestinal model. J Microencapsul 22:603–619

Ainsley Reid A, Champagne CP, Gardner N, Fustier P, Vuillemard JC (2007) Survival in food systems of *Lactobacillus rhamnosus* R011 microentrapped in whey protein gel particles. J Food Sci 72:M031–M037

Anal AK, Singh H (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci Technol 18:240–251

Augustin MA, Sanguansri L, Shan Z, Weerakkody R, Yung DY (2012) Milk protein-based microencapsulated bioactive for improving the nutritive value of foods. IDF World Dairy Summit, Cape Town, South Africa, 4–8 November 2012

Behboudi-Jobbehdar S, Soukoulis C, Yonekura L, Fisk F (2013) Optimization of spray-drying process conditions for the production of maximally viable microencapsulated *L. acidophilus* NCIMB 701748. Drying Technol Int J 31:1274–1283

Burgain J, Gaiani C, Linder M, Scher J (2011) Encapsulation of probiotic living cells: from laboratory scale to industrial applications. J Food Eng 104:467–483

Burgain J, Gaiani C, Cailliez-Grimal C, Jeandel C, Scher J (2013a) Encapsulation of *Lactobacillus rhamnosus* GG in microparticles: influence of casein to whey protein ratio on bacterial survival during digestion. Innovative Food Sci Emerg Technol 19:233–242

Burgain J, Gaiani C, Francius G, Revol-Junelles AM, Cailliez-Grimal C, Lebeer S, Tytgat HLP, Vanderleyden J, Scher J (2013b) In vitro interactions between probiotic bacteria and milk proteins probed by atomic force microscopy. Colloids Surf B 104:153–162

Burgain J, Scher J, Lebeer S, Vanderleyden J, Cailliez-Grimal C, Corgneau M, Francius G, Gaiani C (2014) Significance of bacterial surface molecules interactions with milk proteins to enhance microencapsulation of *Lactobacillus rhamnosus* GG. Food Hydrocoll 41:60–70

Canada Food Inspection Agency (2014) Health claims. Probiotic claims. Inspection/gc.ca/food/labeling/food labeling for industry/healthclaims/eng/1392834838383/139283488

Chen LY, Remondetto GE, Subirade M (2006) Food protein-based materials as nutraceutical delivery systems. Trends Food Sci Technol 17:272–283

Clemente IMM (2013) Encapsulation of *Lactobacillus casei* and its stability in model foods. M.Sc, Instituto tecnico, Lisbon. Https://fenix.tecnico.ulisboa.pt/…/Extended%20Abstract.pdf

Corona-Hernandez RI, Alvarez-Parrill E, Lizardi-Mendoza J, Islas-Rubio AR, de la Rosa LA, Wall-Medrano A (2013) Structural stability and viability of microencapsulated probiotic bacteria: a review. Compr Rev Food Sci Food 12:614–628

Crittenden R, Weerakkody R, Sanguansri L, Augustin MA (2006) Synbiotic microcapsules that enhance microbial viability during non refrigerated storage and gastrointestinal transit. Appl Environ Microbiol 72: 2280–2282

De Castro-Cislaghi FP, Silva CDRE, Fritzen-Freire CB, Lorenz JG, Sant'Anna ES (2012) Bifidobacterium Bb-12 microencapsulated by spray drying with whey: survival under simulated gastrointestinal conditions, tolerance to NaCl, and viability during storage. J Food Eng 113:186–193

Dianawati D, Vijay Mishra V, Shah NP (2013) Survival of *Bifidobacterium longum* 1941 microencapsulated with proteins and sugars after freezing and freeze drying. Food Res Int 51:503–509

Doherty SB, Gee VL, Ross RP, Stanton C, Fitzgerald GF, Brodkorb A (2010) Efficacy of whey protein gel networks as potential viability-enhancing scaffolds form cell immobilization of *Lactobacillus rhamnosus* GG. J Microbiol Methods 80:231–241

Doherty SB, Gee VL, Ross RP, Stanton C, Fitzgerald GF, Brodkorb A (2011) Development and characterisation of whey protein micro-beads as potential matrices for probiotic protection. Food Hydrocoll 25: 1604–1617

Doherty SB, Auty MA, Stanton C, Ross RP, Fitzgerald GF, Brodkorb A (2012) Survival of entrapped *Lactobacillus rhamnosus* GG in whey protein micro-beads during simulated ex vivo gastro-intestinal transit. Int Dairy J 22:31–43

Donovan SM, Schneeman B, Gibson GR, Sanders ME (2012) Establishing and evaluating health claims for probiotics. Adv Nutr 3:723–725. doi:10.3945/an.112.002592

Duongthingoc D, George P, Gorczyca E, Kasapis S (2013) Effect of whey protein agglomeration on spray dried microcapsules containing *Saccharomyces boulardii*. Food Chem 141:1782–1788

Duongthingoc D, George P, Gorczyca E, Kasapis S (2014) Studies on the viability of *Saccharomyces boulardii* within microcapsules in relation to the thermomechanical properties of whey proteins. Food Hydrocoll 42:232–238

FAO/WHO (2002) Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food, London, Ontario, Canada, April 30 and May 1, 2002. ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf

Farnworth ER, Champagne C (2010) Production of probiotic cultures and their incorporation into foods. In: Watson ER, Preedy VR (eds) Bioactive foods in promoting health. Academic, London, pp 3–17

Fox PF, McSweeney PLM (eds) (2013) Advances in dairy chemistry. Vol 1A. Proteins. Basic aspects. Springer, NY, p 548

Gbassi GK, Thierry Vandamme T, Ennahar S, Marchioni E (2009) Microencapsulation of *Lactobacillus plantarum* spp in an alginate matrix coated with whey proteins. Int J Food Microbiol 129:103–105

Gebara C, Chaves KS, Ribeiro MCE, Souza FN, Grosso CRF, Gigante ML (2013) Viability of *Lactobacillus acidophilus* La5 in pectin–whey protein microparticles during exposure to simulated gastrointestinal conditions. Food Res Int 51:872–878

Gerez CL, Font de Valdez G, Gigante ML, Grosso CRF (2012) Whey protein coating bead improves the survival of the probiotic *Lactobacillus rhamnosus* CRL 1505 to low pH. Lett Appl Microbiol 54:552–556

Hébrard G, Blanquet S, Beyssac E, Remondetto G, Subirade M, Alric M (2006) Use of whey protein beads as a new carrier system for recombinant yeasts in human digestive tract. J Biotechnol 127:151–160

Hébrard G, Hoffart V, Beyssac E, Cardot J-M, Alric M, Subirade M (2010) Coated whey protein/alginate microparticles as oral controlled delivery systems for probiotic yeast. J Microencapsul 27:292–302

Heidebach T, Först P, Kulozik U (2009a) Transglutaminase-induced caseinate gelation for the microencapsulation of probiotic cells. Int Dairy J 19:77–84

Heidebach T, Först P, Kulozik U (2009b) Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. Food Hydrocoll 23:1670–1677

Heidebach T, Först P, Kulozik U (2010) Influence of casein-based microencapsulation on freeze-drying and storage of probiotic cells. J Food Eng 98:309–316

Heidebach T, Först P, Kulozik U (2012) Microencapsulation of probiotic cells for food applications. Crit Rev Food Sci Nutr 52:291–311

Hernández-Rodríguez L, Lobato-Calleros C, Pimentel-González DJ, Vernon-Carter EJ (2014) *Lactobacillus plantarum* protection by entrapment in whey protein isolate: k-carrageenan complex coacervates. Food Hydrocoll 36:181–188

Huq T, Khan A, Khan R, Riedl B, Lacroix M (2013) Encapsulation of probiotic bacteria in biopolymeric system. Crit Rev Food Sci Nutr 53:909–916

Jantzen M, Göpel A, Beermann C (2013) Direct spray drying and microencapsulation of probiotic *Lactobacillus reuteri* from slurry fermentation with whey. J Appl Microbiol 115:1029–1036

Jia W, Li HK, Zhao LP, Nicholson JK (2008) Gut microbiota: a potential new territory for drug targeting. Nat Rev Drug Discov 7:123–129

Kos B, Šušković J, Beganović J, Gjuračić K, Frece J, Iannaccone C, Canganella F (2008) Characterization of the three selected probiotic strains for the application in food industry. World J Microbiol Biotechnol 24: 699–707

Krasaekoopt W, Bhandari B, Deeth H (2003) Evaluation of encapsulation techniques of probiotics for yoghurt. Int Dairy J 13:3–13

Lee E Y (2012) Effect of atomization on viability of microencapsulated probiotics. M.Sc., Univ. Illinois. Http://www.ideals.illinois.edu/bitstreem/Lee_EunYoung.pdf?…1

Lisová I, Horáčková Š, Kováčová R, Rada V, Plocková M (2013) Emulsion encapsulation of *Bifidobacterium animalis* subsp. *lactis* BB12 with the addition of lecithin. Czech J Food Sci 31:270–274

López LAV (2013) Influence of "added" whey protein isolates on probiotic properties of yoghurt culture bacteria and yoghurt properties. M.Sc. thesis, Louisiana State Univ. etd.lsu.edu/docs/available/etd-11152013…/LuisVargasThesis.pdf

López-Rubio A, Lagaron JM (2012) Whey protein capsules obtained through electrospraying for the encapsulation of bioactives. Innovative Food Sci Emerg Technol 13:200–206

López-Rubio A, Sanchez E, Wilkanowicz S, Yolanda Sanz Y, Lagaron JM (2012) Electrospinning as a useful technique for the encapsulation of living bifidobacteria in food hydrocolloids. Food Hydrocoll 28:159–167

Ly MH, Aguedoc M, Goudota S, Lea ML, Cayotd P, Teixeirac JA, Leb TM, Belina J-M, Wache Y (2008) Interactions between bacterial surfaces and milk proteins, impact on food emulsions stability. Food Hydrocoll 22:742–751

Maciel GM, Chaves KS, Grosso CRF, Gigante ML (2014) Microencapsulation of *Lactobacillus acidophilus* La-5 by spray-drying using sweet whey and skim milk as encapsulating materials. J Dairy Sci 97:1–8

Maldonado Galdeano C, de Moreno de LeBlanc A, Carmuega E, Weill R, Perdigôn G (2009) Mechanisms involved in the immunostimulation by probiotic fermented milk. J Dairy Res 76:446–454

Mille Y, Obert J-P, Beney L, Gervais P (2004) New drying process for lactic bacteria based on their dehydration behavior in liquid medium. Biotechnol Bioeng 88:71–76

Mortazavian A, Razavi HS, Ehsani RM, Sohrabvandi S (2007) Principles and methods of microencapsulation of probiotic microorganisms. Iran J Biotechnol 5:1–18

Nag A, Han K-S, Singh H (2011) Microencapsulation of probiotic bacteria using pH-induced gelation of sodium caseinate and gellan gum. Int Dairy J 21:247–253

Oliveira AC, Moretti TS, Boschini C, Baliero JCC, Freitas O, Favaro-Trindade CS (2007) Stability of microencapsulated *B. lactis* (BI 01) and *L. acidophilus* (LAC 4) by complex coacervation followed by spray drying. J Microencapsul 27:685–693

Ouwehand AC, Salminen SJ (1998) The health effects of cultured milk products with viable and non-viable bacteria. Int Dairy J 8:749–758

Pavunc AL, Beganovic J, Kos BE, Buneta A, Beluhan SI, Kovic JS (2011) Influence of microencapsulation and transglutaminase on viability of probiotic strain *Lactobacillus helveticus* M92 and consistency of set yoghurt. Int J Dairy Technol 64:254–261

Pedretti S (2013) Probiotic market. Nutrafoods 12:N18–N19. doi:10.1007/s13749-0134006x

Picot A, Lacroix C (2003) Effects of micronization on the viability and thermo tolerance of probiotic freeze-dried culture. Int Dairy J 13:455–462

Picot A, Lacroix C (2004) Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. Int Dairy J 14:505–515

Pimentel-González DJ, Campos-Montiel RG, Lobato-Calleros C, Pedroza-Islas R, Vernon-Carter EJ (2009) Encapsulation of *Lactobacillus rhamnosus* in double emulsions formulated with sweet whey as emulsifier and survival in simulated gastrointestinal conditions. Food Res Int 42:292–297

Poulin JF, Caillard R, Subirade M (2011) β-Lactoglobulin tablets as a suitable vehicle for protection and intestinal delivery of probiotic bacteria. Int J Pharm 405:47–54

Rajam R, Karthik P, Parthasarathi S, Joseph GS, Anandharamakrishnan C (2012) Effect of whey protein–alginate wall systems on survival of microencapsulated *Lactobacillus plantarum* in simulated gastrointestinal conditions. J Funct Foods 4:891–898

Rodrigues D, Rocha-Santos T, Sousa S, Gomes AM, Pintado MM, Malcata FX, Lobo JS, Silva JP, Costa P, Amaral MH, Freitas AC (2011a) On the viability of five probiotic strains when immobilized on various polymers. Int J Dairy Technol 64:137–144

Rodrigues D, Sousa S, Rocha-Santos T, Silva JP, Lobo JS, Costa P, Amaral MH, Pintado MM, Gomes AM, Malcata FX, Freitas AC (2011b) Influence of L-cysteine, oxygen and relative humidity upon survival throughout storage of probiotic bacteria in whey protein-based microcapsules. Int Dairy J 21:869–876

Salminen S, Ouwehand A, Benno Y, Lee YK (1999) Probiotics: how should they be defined? Trends Food Sci Technol 10:107–110

Schell D, Beermann C (2014) Fluidized bed microencapsulation of *Lactobacillus reuteri* with sweet whey and shellac for improved acid resistance and in-vitro gastro-intestinal survival. Food Res Int 62:308–314

Shi L-E, Li Z-H, Li D-T, Xu M, Chen H-Y, Zhang Z-L, Tang Z-X (2012) Encapsulation of probiotic *Lactobacillus bulgaricus* in alginate-milk microspheres and evaluation of the survival in simulated gastrointestinal conditions. J Food Eng 117:99–104

Solanki HM, Pawar DD, Shah DA, Prajapati VD, Jani GK, Mulla AM, Thakar PM (2013) Development of microencapsulation delivery system for long-term preservation of probiotics as biotherapeutics agent. BioMed Res Int, Article ID 620719, doi:10.1155/2013/620719

Souza FN, Gebara C, Ribeiro MCE, Chaves KS, Gigante ML, Grosso CRF (2012) Production and characterization of microparticles containing pectin and whey proteins. Food Res Int 49:560–566

Tavares GM, Croguennec T, Carvalho AF, Bouhallab S (2014) Milk proteins as encapsulation devices and delivery vehicles: applications and trends. Trends Food Sci Technol 37:5–20

Tripathi MK, Giri SK (2014) Probiotic functional foods: survival of probiotics during processing and storage. J Funct Foods 9:225–241

Vankerckhaven V, Huys G, Vancanneyc M, Vael C, Klare I, Romond M-B, Entenza JM, Moreillon P, Wind RD, Knol J, Wiertz E, Pot B, Vaughan EE, Kahlmeter G, Gossens H (2008) Biosafety assessment of probiotics for human consumption recommendations from the EU-PROSAFE project. Trends Food Sci Technol 14:102–114

Weinbreck F, Bodnár I, Marco ML (2010) Can encapsulation lengthen the shelf-life of probiotic bacteria in dry products? Int J Food Microbiol 136:364–367

Ying DY, Sun J, Sanguansri L, Weerakkody R, Augustin MA (2012) Enhanced survival of spray-dried microencapsulated *Lactobacillus rhamnosus* GG in the presence of glucose. J Food Eng 109:597–602

Ying DY, Schwander S, Weerakkody R, Sanguansri L, Gantenbein-Demarchi C, Augustin MA (2013) Microencapsulated *Lactobacillus rhamnosus* GG in whey protein and resistant starch matrices: probiotic survival in fruit juice. J Funct Foods 5:98–105

Zhao RX, Sun JL, Torley P, Wang DH, Niu SY (2008) Measurement of particle diameter of *Lactobacillus acidophilus* microcapsule by spray drying and analysis on its microstructure. World J Microbiol Biotechnol 24:1349–1354

Zou Q, Liu X, Zhao J, Tian F, Zhang HP, Zhang H, Chen W (2012) Microencapsulation of *Bifidobacterium bifidum* F-35 in whey protein-based microcapsules by transglutaminase-induced gelation. J Food Sci 77: M270–M274