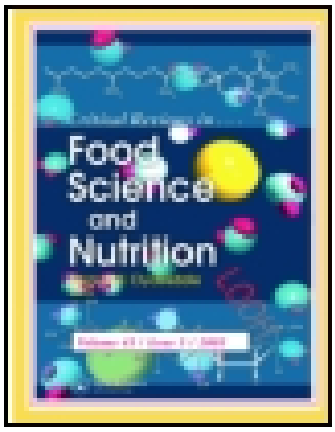

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Issues Deserve Attention in Encapsulating Probiotics: Critical Review of Existing Literature

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ABSTRACT

Probiotic bacteria are being increasingly added to food for developing products with health-promoting properties. However, the efficacy of probiotics in commercial products is often questioned due to the loss of their viability during shelf storage and in human gastrointestinal tracts. Encapsulation of probiotics has been expected to provide protection to probiotics, but not many commercial products contain encapsulated and viable probiotic cells owing to various reasons. To promote the development and application of encapsulation technologies, this article

has critically reviewed previous publications with a focus on the areas where studies have fallen short, including insufficient consideration of structural effects of encapsulating material, general defects in encapsulating methods and issues in evaluation methodologies and risk assessments for application. Corresponding key issues that require further studies are highlighted. Some emerging trends in the field, such as current treads in encapsulating material and recently advanced encapsulation techniques, have also been discussed.

Keywords: Probiotics; Encapsulation; Gastrointestinal tracts; Storage; Food

INTRODUCTION

Probiotics are live microorganisms (bacteria or yeasts) that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002). Evidence from scientific studies to date suggests that probiotics may beneficially affect human health through modulating immunity, metabolic activities, and functions of the digestive system thus reducing incidences of certain diseases (Aureli et al., 2011). Incorporation of probiotics into human diets has led to the prevailing of probiotic-containing products as functional foods. The probiotic-containing functional foods represent approximately 65% of the world functional food market (Agrawal, 2005) and continue to expand with a predicted global market value of $32.6 billion by 2014 (Cook et al., 2012). However, many reports have indicated poor survival of some probiotic bacteria in these products (Micanel et al., 1997; Shah et al., 1995). Furthermore, survival of these bacteria during transition through the gastrointestinal (GI) tracts is often questionable, since low pH and high bile salts in the GI tracts can damage the bacterial cells. Therefore, encapsulation of probiotics has been proposed to protect probiotics in order to confer their promised health benefits (de Vos et al., 2010; McClements et al., 2009).

There are several excellent review articles published recently on the topic of encapsulation of probiotics, which mainly reviewed encapsulation technologies, materials and their applications in the food industry (Anal and Singh, 2007; Cook et al., 2012; Gbassi and Vandamme, 2012; Rokka and Rantamäki, 2010; Vidhyalakshmi et al., 2009). The current paper

attempted to address some issues beyond the scope of the previous articles with a focus on the areas where previous studies had fallen short and the key areas that deserve further studies, e.g. emerging encapsulation technologies and wall materials.

ENCAPSULATING MATERIALS

Selection of encapsulation materials is paramount to ensure the desired protection and production efficiency. In the past, there have been different criteria for the selection. A balance between protection of probiotic cells from environmental factors (during the storage and passing the stomach) and the release of the cells into the GI tracts should be identified and the selection of encapsulating materials should be optimized. Some other factors, such as physicochemical properties, potential toxicity, manufacturing and sterilization processes (Gbassi and Vandamme, 2012), and targeted release properties should also be considered when selecting biomaterials for probiotic encapsulation.

Selection of encapsulation materials

Many materials such as polysaccharides (alginate, xanthan gum, *k*-carrageenan, starch and its derivatives, etc.), proteins (gelatin and milk proteins, etc.) and lipid (milk fat, cocoa butter, and hydrogenated fat, etc.) have been used to encapsulate probiotics. Most adopted encapsulation materials are polysaccharide- and protein-based materials, which have intensively been discussed

in recent review articles (Burgain et al., 2011; Gbassi and Vandamme, 2012; Rokka and Rantamäki, 2010). Lipid-based encapsulation can be achieved by mixing probiotic with molten fat and subsequent cooling. Although fat and other hydrophobic substances have been proposed to be used as potential cell immobilization matrices because diffusion of acids, water and oxygen across membranes of lipid capsules is limited (Kim and Olson, 1985; Lahtinen et al., 2007), lipid-based materials have received less research attention. Picot and Lacroix (2004) reported a large mortality of probiotic cells after microencapsulation in milk fat by spray-drying due to thermal and dehydration inactivation. Recently, Okuro et al. (2013) demonstrated the potential of solid lipid microparticles generated from spray chilling as the vectors for co-encapsulating probiotics with prebiotics. It appears that choosing a suitable processing technique is critical when using lipids as encapsulating materials. There are also other technical obstacles when using fat as encapsulating material. For instance, dispersion of probiotic cell concentrates in oil was reported to be difficult (Picot and Lacroix, 2004). Premature melting of the capsules at elevated temperatures during transportation, storage, food processing and consumption (i.e. body temperature) also needs to be considered. Storage of lipid-based capsules at low temperature is preferred to reduce the rates of detrimental chemical reactions such as fatty acid oxidation. In addition, such capsules are more suitable for solid foods or foods with lower water-content (Lahtinen et al., 2007). Using lipid-based capsules in water-based products requires proper

emulsification to avoid separation of lipids. Hence, these technical obstacles need to be taken into account when one chooses lipid-based materials for encapsulating probiotics.

Importance of structural information on encapsulation materials

In the literature, there is a lack of consistency in published results on the protective efficacy of various encapsulating materials against adverse gastrointestinal conditions or in products' shelf life. Calcium-alginate matrix, one of the most commonly used encapsulating material in food and pharmaceutical industries (Kanmani et al., 2011a), is a good example to illustrate this issue. Numerous studies have reported that micro-immobilization of probiotics in calcium alginate beads can significantly increase the survival rate of probiotic bacteria in products over an extended shelf life (Homayouni et al., 2008; Sousa et al., 2012) or when microcapsules were exposed to low pH and high bile salts (Ding and Shah, 2009; Chandramouli et al., 2004). In contrast, there are also some reports showing that alginate microcapsules failed to protect the microorganisms from the adverse conditions (Gbassi et al., 2009; Krasaekoopt et al., 2004). The failure was attibuted to the porous structure of alginate matrix that is susceptible to disintegration in the presence of excess monovalent ions, Ca^{2+} chelating agents and harsh chemical environments. These discrepancies in performance of alginate based encapsulation products could have resulted not only from different strains and encapsulating methods that had been used, but also from different structure and molecular weight of alginates used. It is known that the

physical properties of alginate hydrogels or beads depend largely on the composition, sequential structure and molecular size of the polymer. However, in the past, insufficient attention has been given to the influences of structure differences (molecular weight, ratio of L-guluronic acids to D-mannuronic acids, block of guluronic acid) of alginate on encapsulation efficiency. Among many previous studies, only a few reports provided information on the structural features of alginate that had been used (Sandoval-Castilla et al., 2010; Cook et al., 2011). In fact, contradictory results and a lack of structural information have also been identified with other materials used for encapsulation. More attention is, therefore, required to the relationship between molecular properties of wall materials and production and performance of encapsulation probiotic products.

Current trends in selection of encapsulating materials

Since one type of hydrocolloids may not be able to protect probiotics effectively, there is an increased interest in using a mixture of different types of biopolymers in a formulation. The combination of two or more types of biopolymers has its own advantages. For example, Sandoval-Castilla et al. (2010) described that the beads made with sodium alginate–amidated low methoxyl pectin blends in 1:4 and 1:6 ratios provided a much better protection to the entrapped probiotic than using alginate or pectin alone under simulated gastrointestinal

conditions. Sometimes the combination is more cost-effective, when an expensive biopolymer can be replaced with a cheap one (Ducel et al., 2004).

Another increasing trend is to include some protectants, such as cryoprotectants, antioxidants, prebiotics, and different probiotics in the encapsulation matrix as described below.

(1) Cryoprotectants.

A cryoprotectant is a substance used to protect biological tissue from freezing damage. Different kinds of cryoprotectants have been tested as additives to maintain the viability of probiotic bacteria during freeze-drying. Lactose and trehalose were proved to be good protectants for cells not only during freeze-drying but also when incubated in simulated gastric fluid. In addition, incorporation of these two carbohydrates contributed to a better bile salt tolerance and shelf stability (Nag and Das, 2013). The protective effect of cryoprotectants may be ascribed to an improvement of cold tolerance (Capela, 2006), an reduction of osmotic difference between inside and outside of cells (Meng et al., 2008), as well as additional energy effects of some metabolizable cryoprotectants such as trehalose, glucose and lactose (Corcoran et al., 2005).

(2) Prebiotics.

Prebiotics are defined as “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health” (Roberfroid, 2007). Addition of prebiotic components

("Raftilose P95" and polydextrose) was reported to be a promising technology for protection of probiotics with improved stability (Capela et al., 2006; Rafael et al., 2012). Given that the major functions of prebiotics are achieved mainly through the enrichment of probiotic bacterial populations in the human/animal intestine, the protective effects of prebiotics reported previously may have resulted from the synergy in the interactions between prebiotics and probiotics. Since different prebiotics can vary in their ability to enrich particular groups of probiotic bacteria in particular intestinal regions (Okuro et al., 2013; Sathyabama et al., 2014), screening for "protective" prebiotics appears to be required. Some isolates of *Bifidobacterium* have demonstrated the capacity to synthesize prebiotics, such as galacto-oligosaccharides (Tzortzis et al., 2005; Roy et al., 2002). Co-encapsulation of such a probiotic isolate can be a new trend, which might reduce the cost of using prebiotics.

(3) Antioxidants.

Chemical deteriorative reactions, particularly the oxidation of membrane lipids, have been suggested to be a major cause for the death of lactic acid bacteria and *Bifidobacterium* when exposed to air, high temperature and high humidity. The most common approach to prevent the detrimental effects of oxygen on the probiotic cultures is the addition of antioxidants such as ascorbic acid, L-cysteine-HCl and tocopherol to encapsulation formula. However, the results from previous studies are inconsistent and some of them were unexpected. Sousa et al. (2012) reported that the presence of L-cysteine-HCl in the alginate matrix improved cell viability of *B.*

animalis BB-12, while Rodrigues et al. (2011) found a negative effect of L-cysteine-HCl on *B. animails* BB-12 survival in whey protein-based microcapsules. The inconsistence of results may have resulted from the different matrices used in the two studies, which interacted with L-cysteine-HCl differently. In addition, Ying et al. (2011) reported that an addition of tocopherol improved probiotic viability during the storage, while incorporation of Na-ascorbate alone or in combination with tocopherol had detrimental effects on probiotic survival, possibly due to the production of acetic acids arising from chemical degradation and catabolism of Na-ascorbate by *L. rhamnosus* GG. These studies imply that the compatibility of encapsulating material and/or co-entrapped materials with probiotics should be taken into account when selecting biomaterials for probiotics encapsulation.

ENCAPSULATION METHODS

One significant challenge for encapsulating probiotics is the large size of bacterial cells (typically 1-4 μm) or particles of a freeze-dried culture (more than 100 μm). This characteristic limits cell loading for small beads. Therefore, many encapsulation methods commonly used in food and neutraceutical fields, such as liposome, molecular inclusion in β-cyclodextrin molecules, and centrifugal suspension separation, have hardly been used in encapsulation of probiotics. Conventional methods used for encapsulating probiotics, such as spray-drying, freeze-drying, extrusion, and emulsification, have been extensively reviewed (de Vos et al., 2010;

Krasaekoopt et al., 2003). However, no review article has addressed the use of these conventional encapsulation technologies in probiotic-containing food/nutraceutical. In the present article, this missing point was reviewed, and some emerging encapsulation techniques are discussed. In addition, the shortcomings of the existing encapsulation procedures/equipment have also been pointed out.

Use of encapsulation technology in probiotic-containing food/nutraceutical

Although promising results have been obtained for many existing techniques on a laboratory scale, large-scale production of microencapsulated microorganisms still presents many challenges in food industry. These challenges include low production capacity, large bead diameters for the droplet extrusion methods, broad size distribution for the emulsion techniques, and the difficulty to maintain aseptic conditions (Thantsha, 2007). Beads formed by using different encapsulation technologies have been incorporated into various food matrices both in the laboratory scale or commercially available. For example, probiotics immobilized with emulsification (Dinakar and Mistry, 1994) or spray-drying method (Gardiner et al., 2002) was incorporated into cheese. A successful Danish-Korean company has produced many products based a dual coating technology (Cell Biotech, 2014). Institut Rosell-Lallemand developed probiotic products based on coating freeze-dried lactic acid bacteria with fatty acids (Durand and Panes, 2007). Generally, the features of encapsulation technologies determine the characters of

resulting beads, which influence their final applications in food/nutraceutical products. Several aspects need to be considered when selecting a suitable technology. (1) The size of beads resulting from each encapsulation technology. The addition of microcapsules should not affect the sensory properties of food products. For example, macroparticles produced by extrusion and emulsion technologies would not be suitable for applications in liquid/ semi-solid foods, but it's use in solid foods would be appropriate. Adhikari et al. (2003) encapsulated *bifidobacteria* using emulsification technology and incorporated them into stirred yogurt. However, consumers complained a grainy texture in these yogurts. Compromised sensory quality is a major problem for consumer acceptance. (2) The solubility. Beads obtained from spray drying are usually water soluble and the integrity of the beads can be lost during rehydration (Bhandari, 2008). Therefore, the risk of leaking probiotics when such beads are applied to aqueous food should be considered. (3) The cost and operating efficacy. It is noteworthy that while most of the literature on the encapsulation of probiotics is related to extrusion and emulsification techniques, most commercial products, at least from a nutraceutical perspective (e.g. pills, caplets, supplements), are spray-coated products. Because both the conventional extrusion and emulsification techniques are non-continuous processes, scale up can be problematic. In comparison, spray coating is a flexible, continuous, and more importantly, economical operation.

Emerging encapsulation techniques

The prevailing of probiotic-containing products and limitations of existing techniques have encouraged food scientists to develop new encapsulation techniques. Recently, techniques such as electrospinning (López-Rubio et al., 2012), impinging aerosol (Sohail et al., 2012), interpolymer complex in supercritical CO_2 (Thantsha et al., 2009), and supercritical emulsion extraction technology (Della Porta et al., 2012) were invented to encapsulate probiotics. The principles, advantages and disadvantages of these techniques are summarized in Table 1.

Shortcomings of existing encapsulation methods

No matter which type of encapsulation method is used, the requirement for aseptic conditions is universal for all the methods. In addition, many probiotic bacteria have an intestinal origin and thus anaerobic conditions are often required. The degree of oxygen presence can significantly damage cell viability and activity of these bacteria during the process of encapsulation, especially for *Bifidobacterium* since this group of bacteria are strict anaerobes. In addition to the antioxidant used together with encapsulating materials to prevent the oxygen toxicity, an anaerobic environment should be set up during the entire encapsulation procedures, including deoxygenization of encapsulating solution to establish and maintain an anaerobic environment of encapsulating instruments. In the literature, it is common that no specific deoxygenization procedure has been clearly described except for the addition of antioxidants. An anaerobic glove box/chamber can provide an anaerobic environment during encapsulation.

However, the utilization of an anaerobic glove box/chamber is inconvenient and does not suit a large scale of processing like spray drying and other applications commonly used by the industry. Although the cost can be a major concern, innovations on the systems (including both procedures and devices) that are able to reduce the loss of probiotic viability due to oxygen toxicity should thus be encouraged. Inclusion of oxidation-reduction indicators to exhibit the anaerobic conditions would be a plus to the system.

In addition to the oxygen toxicity, prevention of a target probiotic bacterium from microbial contamination is also essential for the quality control of encapsulation. Thus application of aseptic techniques to the encapsulation process of probiotics, including sterilization of encapsulation materials and equipment, should be implemented whenever it is possible. In fact, several types of encapsulators that can be autoclaved have been designed and become commercially available, such as Encapsulator B-395 from BUCHI Laboratory Equipment and VAR B Gen II (Nisco Engineering Inc., Zurich, Switzerland). Yet, devices with the capacity to be both oxygen- and germ-free remain to be developed.

EVALUATION OF ENCAPSULATION PRODUCTS

Evaluation of probiotic survival during digestion

In vitro evaluation of encapsulated probiotics under simulated GI tract conditions mainly assesses the tolerance of probiotics to low pH, bile salt and digestive enzymes. There are two

major approaches commonly used in the field, i.e. the "independent model" and "sequential model". The independent model simulates either the stomach or intestines. It consists of a single reactor containing the simulated gastric fluid or simulated intestinal fluid. The "sequential model" simulates digestion in the GI tracts, where microcapsules are treated firstly with simulated gastric juice followed by the treatment with the simulated intestinal juice (or bile salt solution and simulated pancreatic juice added sequentially) with pH values at 7.4-7.5 (Annan et al., 2008; Picot and Lacroix, 2004). The "sequential model" has also been automated into a dynamic model (Mainville et al., 2005), mimicing the condition of different GI regions such as the stomach, duodenum, jejunum and ileum. Besides the different models, the simulated conditions in evaluating probiotics varied significantly in previous publications (Table 2). The variables include the components of gastric juice (e.g. NaCl, HCl or broth); concentration of bile salt (3 to 45 g/L), addition of enzymes (pepsin, pancreatic enzymes, or none), pH values (1.2 to 4.0), and time for treatments (30 to 180 min). Obviously, there is a lack of a standard in *in vitro* simulating the stomach or GI tracts, which may have been responsible for the controversial results reported previously in evaluating protection efficiency of encapsulated probiotics. Alginate based microcapsules, as we discussed above, is one example. Standardizing the methods for probiotic evaluation appears to be urgent for comparisons among different studies.

It has been well documented that *in vitro* evaluation of encapsulated probiotics cannot replace *in vivo* assessments due to the fact that no *in vitro* system can fully mimic the

physiological conditions in the human GI tracts (Mokarram et al., 2009). On the one hand, the presence of food components *in vivo* may temporarily elevate the gastric pH (Mainville et al., 2005). On the other hand, *in vitro* studies may not be able to take into account some factors such as strong peristaltic movement in the bowel (Corthésy et al., 2007). Mucin production and its functions on the intestinal surface is another factor that is difficult to mimic in an *in vitro* evaluation. As an alternative approach to the *in vitro* evaluation, *ex vivo* studies have been pursued, which can overcome some drawbacks of an *in vitro* evaluation system. The *ex vivo* studies generally refer to the experiments or measurements that are conducted in or on a human/animal living tissue in an artificial environment outside the organism with the minimum alteration of the natural condition inside of human/animal bodies. Intestinal digesta has often been used in the *ex vivo* evaluation of probiotics (Doherty et al., 2012; Iyer et al., 2005). Since it is challenging to keep the physiological conditions of tissues unchanged or close to their natural physiological status, cautions need to be taken when interpreting the results from an *ex vivo* study. There are limited reports and evaluation methods for the *in vivo* study of encapsulated probiotics. Graff et al. (2008) evaluated the protective effect of alginate microspheres and chitosan-coated alginate microspheres by measuring the number of viable yeasts excreted in the feces of Wistar rats over 96 h after administration. In the reports by Kanmani et al. (2011a, b), Wistar male rats were sacrificed every 2 h after the administration of microencapsulated probiotic cells in microcapsules, followed by examination of the morphology of microcapsules

recovered from the stomach and intestines. Further efforts to advance the research and technology should be focused on *in vivo* evaluation of encapsulated probiotics, and correlate the *in vitro* tests to *in vivo* validation. The progress in this area will certainly enhance probiotic research and application.

Evaluation of probiotic stability during shelf storage

While a high survival rate in the passages through the GI tract is critical for the effective delivery of probiotic bacteria, improving survival of probiotics during processing and storage presents another challenge in the development and application of probiotic products. The storage conditions i.e. storage temperature, moisture content of powders, relative humidity, powder composition, oxygen content, exposure to light and storage materials, have significant influences over the survival of probiotics in dried powders (Meng et al., 2008). The stability of probiotics during storage is often measured directly in beads or in the presence of food matrix. When measured in beads only, the major factors to be considered include pH, storage temperature (freeze injury), humidity/water activity, and oxygen content. After a systematic evaluation of these factors, suitable food carrier could be selected based on the characteristics of beads. Beads may also be incorporated directly into a food matrix, such as infant formula powder (Weinbreck et al., 2010), ice cream (Homayouni et al., 2008), yoghurt (Picot and Lacroix, 2004), and milk (Hansen et al., 2002) (Table 3). As such, the assays for evaluation become more goal-oriented,

and the assay conditions are set up according to the environmental conditions under which the food products are to be stored before and during marketing. For example, ice cream is usually stored at -20^{o}C, while Kasar cheese at 10^{o}C. However, the stability studies in either type of the test published previously were often carried out using single-factor analysis, i.e. change one variable (such as humidity) and examine the response of other dependables (such as temperature and oxygen content). This analysis may have not truly evaluated the stability of probiotic products. The application of a more complex orthogonal experiment or response surface methodology can provide a systemic approach in investigating the effect of multiple selected environmental factors on the stability of encapsulated probiotics during the shelf storage.

Another issue in previous studies is the overlooking of leakage of probiotics from beads during storage. Because the release of probiotics may result in undesired growth of probiotics in the food matrix, which may affect the shelf life and sensory properties of the food negatively, considerable attention also needs to be paid to prevent probiotic leakage from encapsulated beads in addition to their stability.

Other probiotic attributes

The number of a viable probiotic bacterium during storage and digestion is an important feature to ensure the quality of the probiotics. Nonetheless, high viable counts and survival rates of probiotics attained after shelf storage and passage through the GI tracts do not fully guarantee

that specific probiotic attributes are remained. Recent studies have further revealed that probiotic effects are often determined by specific bioactive molecules or effector molecules from probiotic cells (Kleerebezem and Vaughan, 2009). Some of these effector molecules have been identified as (glyco)proteins that are preserved in order to achieve functional effects (Konstantinov et al., 2008). Retaining the effector molecules in the product during shelf storage and the passage through the GI tracts is essential for probiotic functions, which is affected by multiple factors and presents more challenges. Recently, we attempted to encapsulate a bacterial isolate (Strain LS-100) from the chicken intestine for effective delivery to the GI tracts, which is capable of transforming deoxynivalenol to non-toxic deepoxy-4-deoxynivalenol (Yu et al., 2010). Its detoxification ability was diminished after immobilization in Ca-alginate microspheres, even though the bacterium was live during the storage and *in vitro* assay (data not shown). It is unclear at present how the activity was lost. One can speculate that immobilisation has altered the physiology of the bacterium responsible for the detoxification activity. Thus evaluation of encapsulated probiotics should not only be focused on the viability of probiotic cells, but also so on retaining probiotic attributes.

Colonization in the human GI tracts generally benefits probiotic bacteria in exerting their functions to promote the host health. However, many previous reports described that orally administrated strains did not become established members of the normal intestinal microbiota, but generally persist only for the period of consumption followed by a relatively short period

thereafter (Corthésy et al., 2007; Jacobsen et al., 1999; Klingberg and Budde, 2006). Encapsulation appears to be particularly useful to foster the colonization of probiotics. Del Piano et al. (2010 and 2011) have reported that microencapsulated probiotic bacteria (*L. plantarum* LP01 and *B. breve* BR 03) were five times more efficient than the same unencapsulated strains in colonizing the host intestine after the administration. This may be due to the protection of encapsulation that prevented the bacterial cells from the damages by human gastric juice and bile salts, and simulated pancreatic solution. Further studies are required to validate the protection offered by the encapsulation for probiotic colonization, which may further increase the merit of encapsulation of probiotics and hence stimulate the development of probiotic-containing products.

New technologies for predicting encapsulation performance

Evaluations of protective effects of encapsulation reported to date have mainly been based on the methods we discussed above. There are a large proportion of reports on the encapsulation of probiotics, which described the phenomenon of stability or release of probiotics from beads made of different encapsulating materials but no in-depth studies to reveal the mechanisms underlying the phenomenon. Ding and Shah (2009) encapsulated 10 different probiotics using various alginate, guar gum, xanthan gum, locust bean gum or carrageenan gum, and found that microcapsules made of alginate, xanthan gum and carrageenan gum more efficiently improved

the survival of probiotic cells when exposed to acidic conditions and bile salts. However, the reason why these materials exhibited the best efficiency in protection was not well explored. It is known that the stability and release of probiotics are affected by the integrity and deformation of beads. The formation of beads is closely linked to the flow of material solution during encapsulation, which can significantly impact the properties and quality of the beads. Since rheology largely studies the science of material deformation and flow, it appears that rheology can have significant potential in predicting the behaviour of the beads under particular environmental conditions. One example is the report from McMaster and Kokott (2005) who conducted a rheological study on the solution of mixtures containing gellan and xanthan gum before immobilization. They identified that a mixture containing 0.75% gellan and 1% xanthan gum behaved like a non-Newtonian material with a proper yield stress that was suitable for encapsulation of *B. lactis* to be incorporated into soft foods/beverages. The beads produced by this formulation exhibited a high survival rate during a 21-day survival study. This is a study among very few reports thus far that have used the approach of rheology to predict the properties of beads made from particular encapsulation materials. The approach also represents one of future directions that would assist the development of encapsulated probiotics.

TARGETED RELEASE OF PROBIOTICS

An ideal encapsulation product should not only preserve the stability and functionality of probiotics, but also releases them specifically in the target regions of intestines. Target release of encapsulated probiotics offers an advantage for probiotics to maximize their functions. Most previous studies generally aim for maintaining the viability of encapsulated probiotics in the upper GI tract and releasing them in the lower GI tract, only a few studies thus far pointed out a specific section in the lower GI tract where the release of encapsulated probitotics were measured. Iyer et al. (2005) reported that the chitosan-coated alginate-starch microcapsules were able to release viable probiotic cells completely in ileal and colonic digesta in an *ex vivo* study. Chan and Zhang (2005) predicted from their *in vitro* results that the release of alginate encapsulated probiotics in the human digestive tracts should occur near the end of the ileum and beginning of the colon. Doherty et al. (2009) encapsulated *L. rhamnosus GG* with milk proteins, and these probiotics were released in the digesta from the jejunum in an *ex vivo* assay. While these studies have provided some useful information, *in vivo* studies are certainly required to conclude the location where encapsulated probiotics are released.

Target delivery of probiotics to the positions where the specific strains are originally located would be a logical choice for probiotic delivery. Since the microbiota colonizes mainly in the colon and colon alone is estimated to contain over 70% of all the microbes in the human body, it is reasonable to deliver the probiotics back into the colon for exerting their health benefits. However, the specific site of the body where probiotics ought to be released should be addressed

case by case depending on the physiological functions to be improved by probiotics. de Vos et al. (2010) stated that if probiotics are added to food to stimulate immune response and regulation, the release of the probiotics in the area where the immunological signalling occurs would be preferred. In this case, the favourable positions for bacterial release would be the ileum where the Peyer patches are located or more distal area where other mucosa associated lymphatic tissues can be found. While in the case for the treatment of urogenital infections such as recurrent yeast vaginitis, bacterial vaginosis and urinary tract infections, encapsulated probiotics should be delivered to the vaginal tract by a local or a systemic delivery (Reid et al., 2001; Pliszczak, et al., 2011).

Triggering the release of encapsulated probiotics in a specific intestinal region is another challenge for target delivery of probiotics. In general, there are four basic approaches to achieve the release of encapsulated probiotics. Approach 1 is mechanical breaking of matrix, which bases on the strong peristaltic waves in the colon. Approach 2 employs pH gradient in the GI tracts. Approach 3 designs a time dependent system. The system releases probiotics from encapsulated beads at a predetermined time after administration, so that the probiotic would be delivered at the right site at right time. Approach 4 uses carriers degraded by bacteria/or enzymes produced by the microbiota located essentially in the colon. The location of some bacterial species and their associated enzyme activities in the GI tracts can be region specific, thus allowing for precise delivery of probiotics. This system is receiving an increased attention from the food industry as it

enables the precise release of bioactive food components. A large number of polymers can be rapidly degraded by colonic bacteria or their enzymes, which leads to significant release of probiotics. For instance, pectin can almost be totally degraded by colonic bacteria, but not by gastric or intestinal enzymes (Ashford et al., 1993). Considering the characters of Approach 4, the system appears to be pH and stress resistant, time delayed and microbial triggered. The variations in redox potential induced by the microbiota in the colon may also be used as a specific tool for resolving encapsulated beads and delivery of probiotics. For example, peptide drugs that had been coated with polymers cross-linked by azoaromatic groups could be released in the colon through the reduction of azo bonds by the indigenous microbiota in rats (Saffran et al., 1986). The synthesis and characterization of a series of novel azo hydrogels for colon-targeting drug delivery have been described (Jain et al., 2006; Roldo et al., 2007). The applicability of this method for probiotics remains to be determined.

SAFETY ISSUES

Encapsulated probiotics, in the form of beads, have been incorporated into different food matrices. The interplay between food matrices and probiotic viability/activity has been reviewed by Burgain et al. (2011) and Sanders and Marco (2010). Nevertheless, safety issues with the use of probiotic capsules may deserve more discussions

The safety in producing encapsulated probiotics is an essential requirement for the application of probiotics. A bacterium used as a probiotic should not be an opportunistic pathogen and have no horizontal transferrable plasmids and antibiotic resistance. In general, bacterial isolates from the human GI tracts are preferred in the selection since they have ecological advantages over other bacterial isolates with non-intestinal origin to colonize the GI tracts. However, some intestinal bacterial isolates may produce unfavourable by-products when mixed with food. One example is the production of biogenic amines by *Enterococcus faecium* and *Enterococcus faecalis* (Ladero et al., 2009). Therefore, interactions of probiotics with food matrices during processing, e.g. encapsulation, should also be taken into account when selecting probiotic candidates and food matrices. In addition, the intestinal homeostasis is important for the normal functions of GI tracts. A diverse, balanced, and stable microbiota is generally required to keep the intestinal homeostasis and any disturbance of the ecosystem (including the balance among the microbiota members and between the interactions with their host) may lead to a disorder of intestinal functions (Holzapfel and Schillinger, 2002). Probiotics were early defined by Fuller (1989) as “live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance”. It is obvious from the early definition that a proper delivery and release of probiotics in a particular intestinal region is critical to probiotic functions and also to the homeostasis of the intestinal region. Since the microbiota is an adaptive entirety with intrinsic balance, a change in the proportion of some bacterial groups can alter the

community structures of microbiota (Vaahtovuo et al., 2007). From this viewpoint, slow release of probiotics in a target intestinal region may have fewer chances to disturb the local original population balance. Thus, more attention needs to be paid when probiotics are initially but robustly introduced to the intestinal region from encapsulated beads. Finally, a proper evaluation of a probiotic product (including encapsulated) for its safe use should be required. In the case for human consumption, clinical trials should always be carried out, not only to determine the efficacy of a function that has been claimed but also to assess the risk associated with the use of a probiotic product.

CONCLUSION

Probiotic bacteria possess beneficial functions to human health and their encapsulation for effective delivery and protection are becoming important in application. There are still challenges to fully protect and deliver probiotics in an encapsulated form with some issues requiring special attention. These include overlooking of structural effects of encapsulating material, general drawbacks in encapsulating methods, and a lack of proper standards for evaluating encapsulated probiotics and risk assessments in the application of probiotic capsules. Resolution of these issues will no doubt enhance probiotic development and application.

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REFERENCES

Adhikari, K., Mustapha, A., and Grün, I. (2003). Survival and metabolic activity of microencapsulated *Bifidobacterium longum* in stirred yogurt. *Journal of Food Science*. **68**: 275-280.

Agrawal, R. (2005). Probiotics: an emerging food supplement with health benefits. *Food Biotechnology*. **19**: 227-246.

Anal, A. K., and Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science & Technology*. **18**: 240-251.

Annan, N., Borza, A., and Hansen, L. T. (2008). Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. *Food Research International*. **41**: 184-193.

Ashford, M., Fell, J., Attwood, D., Sharma, H., and Woodhead, P. (1993). An evaluation of pectin as a carrier for drug targeting to the colon. *Journal of Controlled Release*. **26**: 213-220.

Aureli, P., Capurso, L., Castellazzi, A. M., Clerici, M., Giovannini, M., Morelli, L., Poli, A., Pregliasco, F., Salvini, F., and Zuccotti, G. V. (2011). Probiotics and health: an evidence-based review. *Pharmacological Research*. **63**: 366-376.

Bhandari, B. (2008). Device and method for preparing microparticles. US Patent Application 20110008293.

Brinques, G. B., and Ayub, M. A. Z. (2011). Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration, and yogurt. *Journal of Food Engineering*. **103**: 123-128.

Burgain, J., Gaiani, C., Linder, M., and Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering*. **104**: 467-483.

Capela, P. (2006). Use of cryoprotectants, prebiotics and microencapsulation of bacterial cells in improving the viability of probiotic organisms in freeze-dried yoghurt. Victoria University.

Capela, P., Hay, T., and Shah, N. (2006). Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. *Food Research International*. **39**: 203-211.

Cell Biotech (2014). Dual-coated Probiotics. Internet: http://www.cellbiotech.com/rnd/rndTechnology.

Chávarri, M., Marañón, I., Ares, R., Ibáñez, F. C., Marzo, F., and Villarán, M. C. (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves

survival in simulated gastro-intestinal conditions. *International Journal of Food Microbiology*. **142**: 185-189.

Chan, E. S., and Zhang, Z. (2005). Bioencapsulation by compression coating of probiotic bacteria for their protection in an acidic medium. *Process Biochemistry*. **40**: 3346-3351.

Chandramouli, V., Kailasapathy, K., Peiris, P., and Jones, M. (2004). An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. *Journal of Microbiological Methods*. **56**: 27-35.

Chen, K. N., Chen, M. J., and Lin, C. W. (2006). Optimal combination of the encapsulating materials for probiotic microcapsules and its experimental verification (R1). *Journal of Food Engineering*. **76**: 313-320.

Cook, M. T., Tzortzis, G., Charalampopoulos, D., and Khutoryanskiy, V. V. (2011). Production and evaluation of dry alginate-chitosan microcapsules as an enteric delivery vehicle for probiotic bacteria. *Biomacromolecules*. **12**: 2834-2840.

Cook, M. T., Tzortzis, G., Charalampopoulos, D., and Khutoryanskiy, V. V. (2012). Microencapsulation of probiotics for gastrointestinal delivery. *Journal of Controlled Release*. **162**: 56-67.

Corcoran, B., Stanton, C., Fitzgerald, G., and Ross, R. (2005). Survival of probiotic *Lactobacilli* in acidic environments is enhanced in the presence of metabolizable sugars. *Applied and Environmental Microbiology*. **71**: 3060-3067.

Corthésy, B., Gaskins, H. R., and Mercenier, A. (2007). Cross-talk between probiotic bacteria and the host immune system. *The Journal of Nutrition*. **137**: 781S-790S.

de Vos, P., Faas, M. M., Spasojevic, M., and Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy Journal*. **20**: 292-302.

Del Piano, M., Carmagnola, S., Andorno, S., Pagliarulo, M., Tari, R., Mogna, L., Strozzi, G. P., Sforza, F., and Capurso, L. (2010). Evaluation of the intestinal colonization by microencapsulated probiotic bacteria in comparison with the same uncoated strains. *Journal of Clinical Gastroenterology*. **44**: S42-S46.

Del Piano, M., Garmagnola, S., Ballarè, M., Sartori, M., Orsello, M., and Balzarini, M. (2011). Is microencapsulation the future of probiotic preparations? *Gut Microbes*. **2**: 120-123.

Della Porta, G., Castaldo, F., Scognamiglio, M., Paciello, L., Parascandola, P., and Reverchon, E. (2012). Bacteria microencapsulation in PLGA microdevices by supercritical emulsion extraction. *The Journal of Supercritical Fluids*. **63**: 1-7.

Dinakar, P., and Mistry, V. (1994). Growth and viability of *Bifidobacterium bifidum* in Cheddar cheese. *Journal of Dairy Science*. **77**: 2854-2864.

Ding, W., and Shah, N. P. (2007). Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. *Journal of Food Science*. **72**: M446-M450.

Ding, W., and Shah, N. P. (2008). Survival of free and microencapsulated probiotic bacteria in orange and apple juices. *International Food Research Journal*. **15**: 219-232.

Ding, W., and Shah, N. P. (2009). Effect of various encapsulating materials on the stability of probiotic bacteria. *Journal of Food Science*. **74**: M100-M107.

Doherty, S., Auty, M., Stanton, C., Ross, R., Fitzgerald, G., and Brodkorb, A. (2012). Survival of entrapped *Lactobacillus rhamnosus* GG in whey protein micro-beads during simulated *ex vivo* gastro-intestinal transit. *International Dairy Journal*. **22**: 31-43.

Doherty, S., Ross, R., Stanton, C., Fitzgerald, G., and Brodkorb, A. (2009). Evaluation of protein matrices as structural safeguards for controlled delivery of probiotic bacteria **In:** XVIIth International conference on bioencapsulation. Groningen, Netherlands.

Ducel, V., Richard, J., Saulnier, P., Popineau, Y., and Boury, F. (2004). Evidence and characterization of complex coacervates containing plant proteins: application to the microencapsulation of oil droplets. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. **232**: 239-247.

Durand, H., and Panes, J. (2007). Particles containing coated living micro-organisms, and method for producing same. US Patent 7157258.

FAO/WHO (2002). Guidelines for the evaluation of probiotics in Food. FAO/WHO Joint Working Group Report.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Microbiology*. **66**: 365-378.

Gardiner, G. E., Bouchier, P., O'Sullivan, E., Kelly, J., Kevin Collins, J., Fitzgerald, G., Paul Ross, R., and Stanton, C. (2002). A spray-dried culture for probiotic Cheddar cheese manufacture. *International Dairy Journal*. **12**: 749-756.

Gbassi, G. K., and Vandamme, T. (2012). Probiotic encapsulation technology: from microencapsulation to release into the gut. *Pharmaceutics*. **4**: 149-163.

Gbassi, G. K., Vandamme, T., Ennahar, S., and Marchioni, E. (2009). Microencapsulation of *Lactobacillus plantarum* spp. in an alginate matrix coated with whey proteins. *International Journal of Food Microbiology*. **129**: 103-105.

Graff, S., Hussain, S., Chaumeil, J.-C., and Charrueau, C. (2008). Increased intestinal delivery of viable *Saccharomyces boulardii* by encapsulation in microspheres. *Pharmaceutical Research*. **25**: 1290-1296.

Guerin, D., Vuillemard, J. C., and Subirade, M. (2003). Protection of *bifidobacteria* encapsulated in polysaccharide-protein gel beads against gastric juice and bile. *Journal of Food Protection*. **66**: 2076-2084.

Hansen, L. T., Allan-Wojtas, P., Jin, Y. L., and Paulson, A. (2002). Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food Microbiology*. **19**: 35-45.

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

Holzapfel, W. H., and Schillinger, U. (2002). Introduction to pre-and probiotics. *Food Research International*. **35**: 109-116.

Homayouni, A., Azizi, A., Ehsani, M., Yarmand, M., and Razavi, S. (2008). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry*. **111**: 50-55.

Iyer, C., Phillips, M., and Kailasapathy, K. (2005). Release studies of *Lactobacillus casei* strain Shirota from chitosan–coated alginate–starch microcapsules in *ex vivo* porcine gastrointestinal contents. *Letters in Applied Microbiology*. **41**: 493-497.

Jacobsen, C. N., Nielsen, V. R., Hayford, A., Møller, P., Michaelsen, K., Paerregaard, A., Sandström, B., Tvede, M., and Jakobsen, M. (1999). Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. *Applied and Environmental Microbiology*. **65**: 4949-4956.

Jain, A., Gupta, Y., and Jain, S. K. (2006). Azo chemistry and its potential for colonic delivery. *Critical Reviews in Therapeutic Drug Carrier Systems*. **23**: 349-400.

Kanmani, P., Kumar, R. S., Yuvaraj, N., Paari, K., Pattukumar, V., and Arul, V. (2011a). Cryopreservation and microencapsulation of a probiotic in alginate-chitosan capsules improves survival in simulated gastrointestinal conditions. *Biotechnology and Bioprocess Engineering*. **16**: 1106-1114.

Kanmani, P., Kumar, R. S., Yuvaraj, N., Paari, K., Pattukumar, V., and Arul, V. (2011b). Effect of cryopreservation and microencapsulation of lactic acid bacterium *Enterococcus faecium* MC13 for long-term storage. *Biochemical Engineering Journal*. **58**: 140-147.

Kim, S. C., and Olson, N. F. (1985). Characteristics of viable *Brevibacterium linens* cells, methionine and cysteine in milkfat-coated microcapsules. *Journal of Microencapsulation*. **2**: 197-206.

Kleerebezem, M., and Vaughan, E. E. (2009). Probiotic and gut *lactobacilli* and *bifidobacteria*: molecular approaches to study diversity and activity. *Annual Review of Microbiology*. **63**: 269-290.

Klingberg, T. D., and Budde, B. B. (2006). The survival and persistence in the human gastrointestinal tract of five potential probiotic *Lactobacilli* consumed as freeze-dried cultures or as probiotic sausage. *International Journal of Food Microbiology*. **109**: 157-159.

Konstantinov, S. R., Smidt, H., de Vos, W. M., Bruijns, S. C., Singh, S. K., Valence, F., Molle, D., Lortal, S., Altermann, E., and Klaenhammer, T. R. (2008). S layer protein A of

Lactobacillus acidophilus NCFM regulates immature dendritic cell and T cell functions. *Proceedings of the National Academy of Sciences*. **105**: 19474-19479.

Krasaekoopt, W. (2013). Microencapsulation of probiotics in hydrocolloid gel matrices: a review. *Agro Food Industry Hi Tech*. **24**, 76-83.

Krasaekoopt, W., Bhandari, B., and Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*. **13**: 3-13.

Krasaekoopt, W., Bhandari, B., and Deeth, H. (2004). The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. *International Dairy Journal*. **14**: 737-743.

López-Rubio, A., Sanchez, E., Wilkanowicz, S., Sanz, Y., and Lagaron, J. M. (2012). Electrospinning as a useful technique for the encapsulation of living *bifidobacteria* in food hydrocolloids. *Food Hydrocolloids*. **28**: 159-167.

Ladero, V., Fernandez, M., and Alvarez, M. A. (2009). Isolation and identification of tyramine-producing enterococci from human fecal samples. *Canadian Journal of Microbiology*. **55**: 215-218.

Lahtinen, S., Ouwehand, A., Salminen, S., Forssell, P., and Myllärinen, P. (2007). Effect of starch- and lipid- based encapsulation on the culturability of two *Bifidobacterium longum* strains. *Letters in Applied Microbiology*. **44**: 500-505.

Lin, J., Yu, W., Liu, X., Xie, H., Wang, W., and Ma, X. (2008). *In vitro* and *in vivo* characterization of alginate-chitosan-alginate artificial microcapsules for therapeutic oral delivery of live bacterial cells. *Journal of Bioscience and Bioengineering*. **105**: 660-665.

Mainville, I., Arcand, Y., and Farnworth, E. (2005). A dynamic model that simulates the human upper gastrointestinal tract for the study of probiotics. *International Journal of Food Microbiology*. **99**: 287-296.

McClements, D. J., Decker, E. A., Park, Y., and Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. *Critical Reviews in Food Science and Nutrition*. **49**: 577-606.

McMaster, L., and Kokott, S. (2005). Micro-encapsulation of *Bifidobacterium lactis* for incorporation into soft foods. *World Journal of Microbiology and Biotechnology*. **21**: 723-728.

Meng, X., Stanton, C., Fitzgerald, G., Daly, C., and Ross, R. (2008). Anhydrobiotics: The challenges of drying probiotic cultures. *Food Chemistry*. **106**: 1406-1416.

Micanel, N., Haynes, I., and Playne, M. (1997). Viability of probiotic cultures in commercial Australian yogurts. *Australian Journal of Dairy Technology*. **52**: 24-27.

Mokarram, R., Mortazavi, S., Najafi, M., and Shahidi, F. (2009). The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. *Food Research International*. **42**: 1040-1045.

Nag, A., and Das, S. (2013). Effect of trehalose and lactose as cryoprotectant during freeze–drying, *in vitro* gastro–intestinal transit and survival of microencapsulated freeze–dried *Lactobacillus casei* 431 cells. *International Journal of Dairy Technology*. **66**: 162-169.

Okuro, P. K., Thomazini, M., Balieiro, J. C., Liberal, R. D., and Favaro-Trindade, C. S. (2013). Co-encapsulating of *Lactobacillus acidophilus* with inulin or polydextrose in solid lipid microparticles provides protection and improves stability. *Food Research International*. **53**: 96-103.

Oliveira, A., Moretti, T., Boschini, C., Baliero, J., Freitas, O., and Favaro-Trindade, C. (2007). Stability of microencapsulated *B. lactis* (BI 01) and *L. acidophilus* (LAC 4) by complex coacervation followed by spray drying. *Journal of Microencapsulation*. **24**: 685-693.

Ortakci, F., Broadbent, J., McManus, W., and McMahon, D. (2012). Survival of microencapsulated probiotic *Lactobacillus paracasei* LBC-1e during manufacture of Mozzarella cheese and simulated gastric digestion. *Journal of Dairy Science*. **95**: 6274-6281

Özer, B., Kirmaci, H. A., Şenel, E., Atamer, M., and Hayaloğlu, A. (2009). Improving the viability of *Bifidobacterium bifidum* BB-12 and *Lactobacillus acidophilus* LA-5 in white-brined cheese by microencapsulation. *International Dairy Journal*. **19**: 22-29.

Özer, B., Uzun, Y. S., and Kirmaci, H. A. (2008). Effect of microencapsulation on viability of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 during Kasar cheese ripening. *International Journal of Dairy Technology*. **61**: 237-244.

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

Pliszczak, D., Bourgeois, S., Bordes, C., Valour, J. P., Mazoyer, M. A., Orecchioni, A. M., Nakache, E., and Lantéri, P. (2011). Improvement of an encapsulation process for the preparation of pro- and prebiotics-loaded bioadhesive microparticles by using experimental design. *European Journal of Pharmaceutical Sciences*. **44**: 83-92.

Rafael, Z.-V., Luis, M.-S. J., Eduardo, M.-F. H., Rebeca, F.-M., Víctor, M.-R. C., José, V.-G., and Ariza, O. T. D. J. (2012). Effect of incorporating prebiotics in coating materials for the microencapsulation of *Sacharomyces boulardii*. *International Journal of Food Sciences and Nutrition*. **63**: 930-935.

Reid, G., Bruce, A. W., Fraser, N., Heinemann, C., Owen, J., and Henning, B. (2001). Oral probiotics can resolve urogenital infections. *FEMS Immunology & Medical Microbiology*. **30**: 49-52.

Roberfroid, M. (2007). Prebiotics: the concept revisited. *The Journal of Nutrition*. **137**: 830S-837S.

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Rodrigues, D., Sousa, S., Rocha-Santos, T., Silva, J., Sousa Lobo, J., Costa, P., Amaral, M., Pintado, M., Gomes, A., and Malcata, F. (2011). Influence of L-cysteine, oxygen and relative humidity upon survival throughout storage of probiotic bacteria in whey protein-based microcapsules. *International Dairy Journal*. **21**: 869-876.

Rokka, S., and Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. *European Food Research and Technology*. **231**: 1-12.

Roldo, M., Barbu, E., Brown, J. F., Laight, D. W., Smart, J. D., and Tsibouklis, J. (2007). Azo compounds in colon-specific drug delivery. *Expert Opinion on Drug Delivery*. **4**: 547-560.

Roy, D., Daoudi, L., & Azaola, A. (2002). Optimization of galacto-oligosaccharide production by *Bifidobacterium infantis* RW-8120 using response surface methodology. *Journal of Industrial Microbiology and Biotechnology*, **29**, 281-285.

Saffran, M., Kumar, G. S., Savariar, C., Burnham, J. C., Williams, F., and Neckers, D. C. (1986). A new approach to the oral administration of insulin and other peptide drugs. *Science*. **233**: 1081-1084.

Sanders, M. E., and Marco, M. L. (2010). Food formats for effective delivery of probiotics. *Food Science and Technology*. **1**. 65-85.

Sandoval-Castilla, O., Lobato-Calleros, C., García-Galindo, H., Alvarez-Ramírez, J., and Vernon-Carter, E. (2010). Textural properties of alginate–pectin beads and survivability of entrapped *Lb. casei* in simulated gastrointestinal conditions and in yoghurt. *Food Research International*. **43**: 111-117.

Sathyabama, S., Ranjith kumar, M., Bruntha devi, P., Vijayabharathi, R., and Brindha priyadharisini, V. (2014). Co-encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment. *LWT - Food Science and Technology*. **57**: 419-425.

Shah, N. P., Lankaputhra, W. E., Britz, M. L., and Kyle, W. S. (1995). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated storage. *International Dairy Journal*. **5**: 515-521.

Sohail, A., Turner, M. S., Prabawati, E. K., Coombes, A. G., and Bhandari, B. (2012). Evaluation of *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* NCFM encapsulated using a novel impinging aerosol method in fruit food products. *International Journal of Food Microbiology*. **157**: 162-166.

Sousa, S., Gomes, A. M., Pintado, M. M., Malcata, F. X., Silva, J. P., Sousa, J. M., Costa, P., Amaral, M. H., Rodrigues, D., and Rocha‐Santos, T. A. (2012). Encapsulation of probiotic strains in plain or cysteine‐supplemented alginate improves viability at storage below freezing temperatures. *Engineering in Life Sciences*. **12**: 457-465.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P., and Kailasapathy, K. (2000). Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *International Journal of Food Microbiology*. **62**: 47-55.

Sun, W., and Griffiths, M. W. (2000). Survival of *Bifidobacteria* in yogurt and simulated gastric juice following immobilization in gellan–xanthan beads. *International Journal of Food Microbiology*. **61**: 17-25.

Thantsha, M. S. (2007). Cell immobilization techniques for the preservation of probiotics. Faculty of Natural and Agricultural Sciences, Department of Microbiology and Plant Pathology, University of Pretoria,

Thantsha, M. S., Cloete, T. E., Moolman, F. S., and Labuschagne, P. W. (2009). Supercritical carbon dioxide interpolymer complexes improve survival of *B. longum* Bb-46 in simulated gastrointestinal fluids. *International Journal of Food Microbiology*. **129**: 88-92.

Tzortzis, G., Goulas, A. K., & Gibson, G. R. (2005). Synthesis of prebiotic galactooligosaccharides using whole cells of a novel strain, *Bifidobacterium bifidum* NCIMB 41171. *Applied Microbiology and Biotechnology*. **68**, 412-416.

Vaahtovuo, J., Korkeamäki, M., Munukka, E., Hämeenoja, P., and Vuorenmaa, J. (2007). Microbial balance index - A view on the intestinal microbiota. *Livestock Science*. **109**: 174-178.

Vidhyalakshmi, R., Bhakyaraj, R., and Subhasree, R. (2009). Encapsulation "The future of probiotics" - A review. *Advances in Biological Research.* **3**: 96-103.

Weinbreck, F., Bodnár, I., and Marco, M. (2010). Can encapsulation lengthen the shelf-life of probiotic bacteria in dry products? *International Journal of Food Microbiology*. **136**: 364-367.

Ying, D., Sanguansri, L., Weerakkody, R., Singh, T. K., Leischtfeld, S. F., Gantenbein-Demarchi, C., and Augustin, M. A. (2011). Tocopherol and ascorbate have contrasting effects on the viability of microencapsulated *Lactobacillus rhamnosus GG*. *Journal of Agricultural and Food Chemistry*. **59**: 10556-10563.

Yu, H., Zhou, T., Gong, J., Young, C., Su, X., Li, X. Z., Zhu, H., Tsao, R., and Yang, R. (2010). Isolation of deoxynivalenol-transforming bacteria from the chicken intestines using the approach of PCR-DGGE guided microbial selection. *BMC Microbiology*. **10**: 182.

Table 1. Some recently advanced encapsulation techniques.

Technique	Principle	Advantage	Disadvantage
Electrospinning	Figure 1	Simple and easy to handle; producing microcapsules with small sizes; no temperature is required	Low production efficiency; many synthetic polymers are not acceptable in food applications
Impinging aerosol	Figure 2	Producing spherical microspheres with small size; continuous operation and can be scale-up	Loss of materials by adhesion to walls of the spray chamber; broad particle size distribution due to aggregation of some microcapsules

Interpolymer complex in supercritical CO_2	The bacterial cells were immobilized during the process of interpolymer complex formation in supercritical carbon dioxide. After the reaction, the supercritical carbon dioxide was gasified by depressurizing.	Environmentally superior, organic solvents free and suitable for up-scaling	High investment requirements relating to the high pressure operation; the feasibility of these techniques in food industry needs to be justified by an economic evaluation
Supercritical Emulsion Extraction technology	Emulsion was prepared in a traditional way and sprayed into a vessel that was continuously purged with supercritical CO_2. The fast extraction of the organic solvent and the antisolvent effect of supercritical CO_2 cause the co-precipitation of bacteria and polymers, resulting the formation of microcapsules.		

Table 2. Conditions used for evaluating the viability of encapsulated microorganisms during *in vitro* gastrointestinal digestion.

***In vitro* gastric simulation**				
Gastric fluid	**pH**	**Pepsin (g/L)**	**Exposure time (min)**	**Reference**
NaCl (9 g/L)	1.8-2.0	3	120	(Okuro et al., 2013)
NaCl 5 g/L	2.0	3	60	(Chen et al., 2006)
0.08 M HCl 2 g/L NaCl	1.5, 2.0 or 2.5	0	120	(Sun and Griffiths, 2000)
HCl 2 g/L NaCl	1.4	0.3	60	(Ortakci et al., 2012)
HCl	1.9	0.26	30	(Picot and Lacroix, 2004)
HCl	3.0	0	180	(Sandoval-Castilla et al., 2010)
HCl Phosphate buffer NaCl	1.2, 2.0 or 4.0	0.32	120	(Chan and Zhang, 2005)
0.2 M HCl , 0.2 M	1.5	0	120	(Guerin et al., 2003)

KCl, 0.75% $CaCl_2$				
NGYC medium	2.0, 3.0, 4.0 or 6.5	0	180	(Chandramouli et al., 2004)
MRS broth	2.0	0	120	(Ding and Shah, 2007)

***In vitro* intestinal simulation**						
Intestinal fluid	**pH**	**Bile and enzyme**			**Exposure time (min)**	**Reference**
		Bile (g/L)	**Pancreatin (g/L)**	**Trypsin (g/L)**		
9 g/L NaCl	6.5	3	10	10	180	(Gbassi et al., 2009)
5 g/L NaCl	8.0	45	1	0	120	(Brinques and Ayub, 2011)
6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L $CaCl_2$, and 1.386 g/L $NaHCO_3$	7.5	3.0	0	0	120	(Chávarri et al., 2010)
6.8 g/L KH_2PO_4,	6.8	0	10	0	360	(Thantsha et al.,

0.9 g/L NaOH						2009)
6.8 g/L KH_2PO_4, 0.9 g/L NaOH	7.5	0	0	10	1440	(Lin et al., 2008)
Milk-yeast extract medium	6.9	0, 5.0 or 10.0	0	0	360	(Chandramouli et al., 2004)
NGYC broth	4.5	10 or 20	0	0	180	(Sultana et al., 2000)

Table 3. Conditions used when evaluating the viability of encapsulated microorganisms during shelf storage.

Sample	Temperature (^{o}C)	Oxygen	Humidity	Duration(days)	Reference
Beads	-18, 7 or 22	Atmosphere or vacuum	11%	120	(Okuro et al., 2013)
Beads	4 or 25	-	11% or 33%	90	(Heidebach et al., 2010)
Beads	4 or 25	-	32, 57 or 70%	180	(Ying et al., 2011)
Beads	-20, 4 or 35	Airtight container	-	180	(Kanmani et al., 2011b)
Beads	7 or 37	-	64.5%	120	(Oliveira et al., 2007)
Infant formula powder	37	Atmosphere or nitrogen (3 to 4% oxygen)	Water activity (0.15 or 0.7)	42	(Weinbreck et al., 2010)
Ice cream	-20	-	-	180	(Homayouni et

					al., 2008)
Kasar cheese	10	Vacuum	-	90	(Özer et al., 2008)
White-brined cheese	4	-	-	90	(Özer et al., 2009)
Mozzarella chess	4	Vacuum	-	42	(Ortakci et al., 2012)
Yoghurt	4	-	-	28	(Picot and Lacroix, 2004)
Milk	4	-	-	15-16	(Hansen et al., 2002)
Orange juice	4	-	-	42	(Ding and Shah, 2008)

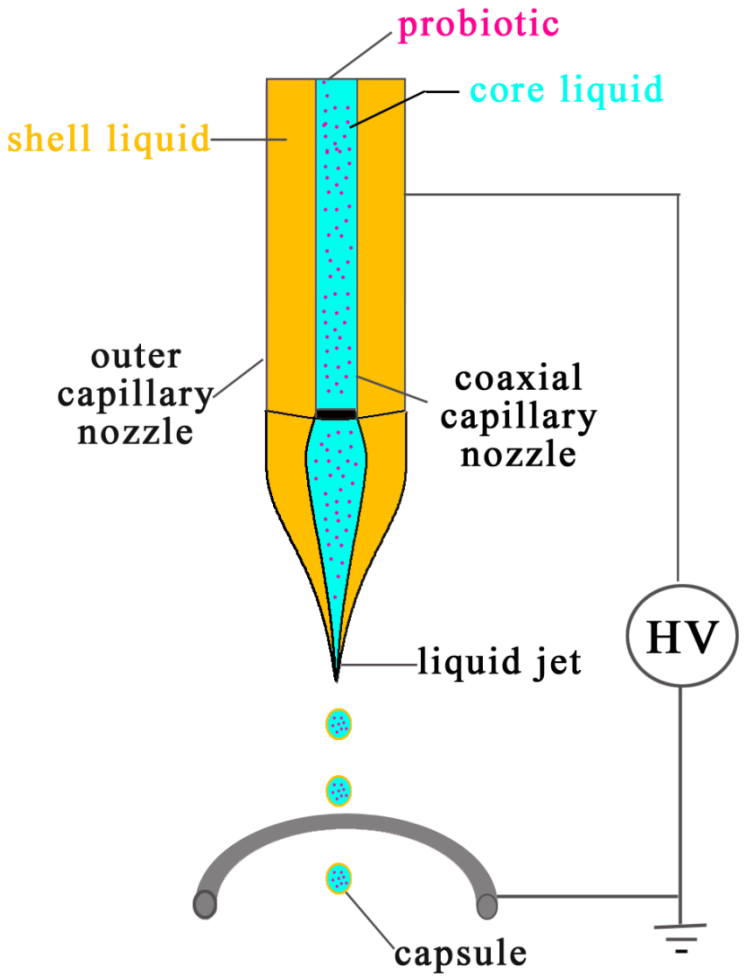

Figure 1. Schematic representation of electrospinning. Probiotics were dispersed in a favorable fluid medium that acts as a core material. Electrospinning produces capsules through the action of an external electric field applied between two electrodes and imposed on a polymer solution. As a result, the probiotics become entrained by the outer polymeric shell.

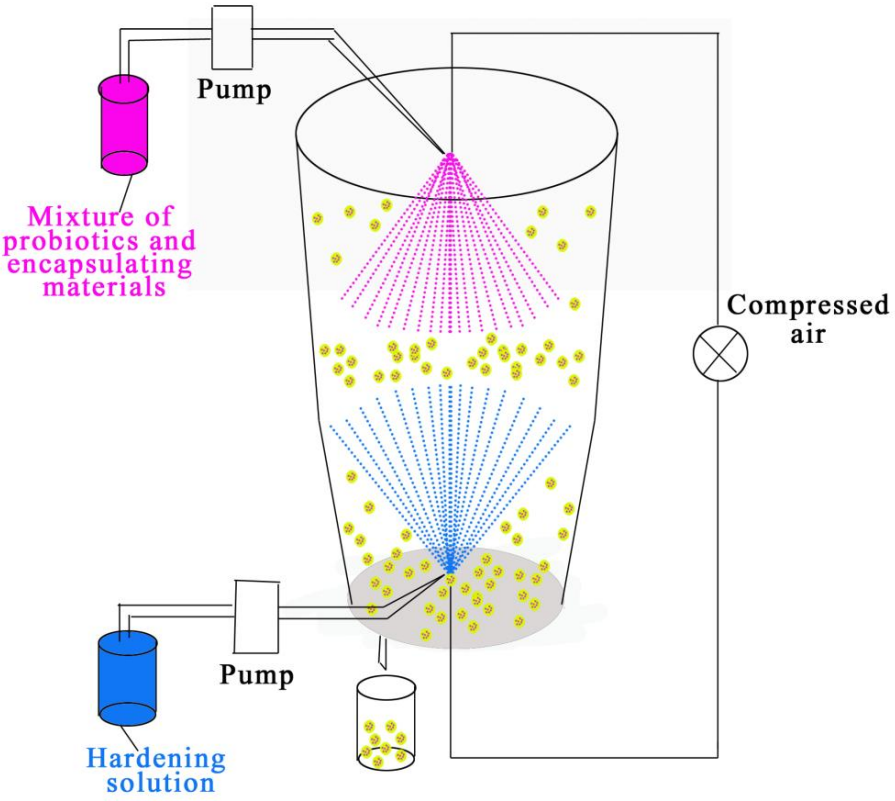

Figure 2. Schematic representation of impinging aerosol, adapted from Krasaekoopt (2013). An aerosol of the mixture of encapsulating material and probiotcs is sprayed at the top of chamber into a fine mist of hardening solution sprayed from the bottom, resulting in an immediate gelation of the microdroplets, which can be collected at the outlet of the chamber base.