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Review Article

USE OF ENCAPSULATED PROBIOTICS IN DAIRY BASED FOODS

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ABSTRACT
Due to the perceived health benefits of probiotics, there has been an increased use in different health based products. Probiotics have been incorporated into a wider range of dairy products, including yogurts, cheese, ice cream and dairy desserts. The viability of probiotic cells is of paramount importance because to have their beneficial effects on the health, they must stay alive until they reach their site of action. There are some problems pertaining to the survivability of probiotic bacteria in dairy foods due to various product factors. This has encouraged developing different innovative methods to improve the probiotic cells viability in the product incorporated. Microencapsulation of probiotics is one of the approaches which is currently receiving considerable attention. Microencapsulation of probiotic bacteria can be used to enhance and improve the viability during processing and also in gastrointestinal tract. But there are some challenges with respect to microencapsulation process and the conditions prevailing in milk products and in human gut. This review focuses on probiotics microencapsulation, encapsulation methods and coating materials utilised for probiotic encapsulation.

Key Words: *Probiotics, Microencapsulation*

INTRODUCTION
The term probiotic is derived from two Greek words which literally means "for life". Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) defines probiotics as "live micro organisms that when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). The popularity of probiotic has continuously been growing and various food products have been marketed.

Probiotics have been reported to play a therapeutic role by lowering cholesterol, improving lactose tolerance, nutritional enhancement and preventing some cancers and antibiotic associated diarrhoea (Fitton and Thomas, 2009). The probiotic health benefits may be due to the production of acid and/or bacteriocins, competition with pathogens preventing their adhesion to the intestine and enhancement of the immune system (Chen and Chen, 2007). Majority of the probiotics belong to the genera *Lactobacillus* and *Bifidobacterium* and are extensively investigated for their beneficial effects and are incorporated in fermented foods (Rokka and Rantamaki, 2010; Saranya and Hemashenpagam, 2011). The probiotics of genera *Lactobacillus* spp., *Bifidobacterium* spp. and *Lactococcus* spp. have been given the GRAS status (Salminen and von Wright, 1998).

Food, particularly dairy products are considered as an ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract (Ross *et al.,* 2002). The food carriers such as dairy products may enhance microbial survival in gastric juice, most likely due to a buffering or protective effect (Ross *et al.,* 2005). At present probiotic bacteria are mainly incorporated into dairy products such as yogurt, fermented milks, milk powder, ice cream, cheese, frozen desserts and chocolate products (Ranadheera *et al.,* 2010).

The viability of probiotic cells is of paramount importance because to have their beneficial effects on the health, they must stay alive until they reach their site of action. They also should not affect the sensory characteristics of the food in any perceptible way, remain stable throughout processing and storage of the product and be resistant to the gastrointestinal environment. The efficiency of added probiotic bacteria depends on dose level and their viability must be maintained throughout storage, products shelf-life and they must survive the gut environment (Kailasapathy and Chin, 2000). Hence, survivability of probiotic bacteria is critically very important in probiotic-based food products.

Several factors have been reported to affect the viability of probiotics in fermented dairy products, including titratable acidity, pH, hydrogen peroxide, dissolved oxygen content, storage temperature, species and strains of associative culture organisms, concentration of lactic and acetic acids and even whey protein concentration (Kailasapathy, 2006; Anal and Singh, 2007; Sabikhi *et al.*, 2010).

Analysis of probiotic products has confirmed that probiotic strains exhibit poor survival in traditional fermented dairy products (Shah, 2000; Lourens-Hattingh and Viljoen, 2001). Various studies have reported low viability of probiotics in dairy products such as yogurt and frozen dairy desserts due to the concentration of lactic acid and acetic acid, low pH, the presence of hydrogen peroxide, and the high oxygen content (De Vos *et al.,* 2010). In view of the decline, higher levels of viable microorganisms (1×10^7cfu/g) are recommended in probiotic foods for better efficacy in regulating beneficial effects (Gardiner *et al.*, 2002).

Various approaches have been attempted so as to increase the resistance of probiotic bacteria against adverse conditions like use of micronutrients such as peptides and amino acids, and micro-encapsulation. Providing probiotic living cells with a physical barrier against adverse conditions is an approach currently receiving major interest (Kailasapathy, 2009).

The technology designed to improve delivery of probiotic bacteria into the human gastrointestinal tract by providing protection to the sensitive bacterial microorganisms may be referred as microencapsulation. It is currently receiving a considerable interest for improving the viability of probiotic bacteria in dairy based probiotic foods. Microencapsulation is a useful tool for improving the delivery of bio-active compounds in foods particularly probiotics (Champagne and Fustier, 2007). Microencapsulation is utilized to protect the cells against an adverse environment more than controlled release.

Microencapsulation is entrapping a substance by a material such that it is released at controlled and required rate under suitable conditions. Microencapsulation is a process by which live cells are packaged within a shell material, which confer them protection by preventing their direct exposure to unfavourable environment, but permits diffusion of nutrients in and out of the matrix, thereby supporting the viability of the cells (Talwalkar and Kailasapathy, 2004b).

Microencapsulation improves the survivability of probiotics and sensory attributes of product due to protective action against adverse conditions in fermented dairy foods like yoghurt. Electron microscopy is an effective technique to provide evidence of the presence of probiotics in capsules or beads and to assess the bacterial loading and size structure of the capsule (Gbassi *et al.*, 2009). The microbeads with diameters more than the special limit (>100 μm) can deteriorate mouth-feel properties of products such as liquid milk, yogurt and sour cream due to the appearance of the special sense of coarseness (Mortazavian *et al.*, 2007).

Various biopolymers have been utilized for coating probiotic cells. Typical biomaterials used for the purpose of probiotic encapsulation include: alginate, carrageenan, gelatin, chitosan and cellulose acetate phthalate (Gbassi and Van Damme, 2012).

Need For Microencapsulation

Microencapsulation of materials is resorted to ensure that the encapsulated material reaches the area of action without adversely affected by the environment through which it passes (Dubey *et al.*, 2008).

Among the principal reasons for encapsulation are:

Increases probiotics viability through passing from acidic-enzymatic-bile conditions of GIT.

Production of bacterial starter cultures with higher viability.

Improve viability of probiotic microorganisms due to its protective effects against detrimental environmental factors such as high acidity, low pH, molecular oxygen, poisoning agents generated during the process (especially heat treatment), digestive enzymes, bacteriophages, hydrogen peroxide, shortchain fatty acids, carbonyl-aromatic compounds and heat processing (e.g. drying) (Mortazavian *et al.*, 2006a).

Application in Fermentor- increasing the tolerance of microorganisms against factors such as bacteriophage infection, chemical poisoning agents, protecting microorganism cells against unwanted

changes such as genetic mutations, reaching good productivity in metabolite production especially at high agitation rates and producing more dense biomass (Champagne *et al.,* 1992b).

Production of food products- increasing viability of probiotics in products till the moment of consumption, achieving new methods in food manufacture, fixing and improving the sensory properties of probiotic products and immobilizing probiotic cells in the products.

Achieving new methods in food manufacture- Yoghurt production with encapsulated yogurt bacteria (*Streptococcus salivarius* ssp. *thermophilus* and *Streptococcus delbrueckii* ssp. *bulgaricus*) with following advantages: product with relatively fixed sensory properties can be produced, viability of bacteria remains very high and the proportion bacteria from initial to final stages of fermentation process and as a result, flavor of the product can be well controllable (Krasaekoopt *et al.,* 2003).

Immobilization of probiotic cells - has been carried out by using the encapsulation process to make homogeneous dispersion of the cells throughout the product.

Fixing and improving the sensory properties of probiotic products: Microencapsulation of probiotics helps to fix and/or improve the sensory properties of the final product.

Superior handling of the active agent (e.g., conversion of liquid active agent into a powder, which might be dust free, free flowing, and might have a more neutral smell)

Improved stability in final product and during processing

Improved safety (e.g., reduced flammability of volatiles like aroma, no concentrated volatile oil handling)

Controlled release (differentiation, release by the right stimulus) (Mortazavian *et al.,* 2007)

Microencapsulation of Probiotics

Microencapsulation is a powerful technology which has been developed for use in the food industry and allows the protection of bacterial cells (Borgogna *et al.,* 2010). A microcapsule consists of a semipermeable, spherical, thin and strong membrane surrounding a solid or liquid core, with a diameter varying from a few microns to 1 mm (Anal and Singh, 2007). The obtained microparticles have to be water-insoluble to maintain their integrity in the food matrix and in the upper part of the GI tract and finally, particle properties should allow progressive liberation of the cells during the intestinal phase (Picot and Lacroix, 2004; Ding and Shah, 2007).

Coating protects the active component from environmental stresses such as acidity, oxygen and gastric conditions, and can be used, for example, to help the content pass through the stomach. The major benefit of this technology is the fact that the probiotic bacteria can be delivered directly and continuously to the gut, where it effectively disintegrates leaving viable amount of probiotic bacteria to confer beneficial effects onto the host (Gbassi and Van Damme, 2012). Encapsulation in hydrocolloid beads entraps or immobilizes the cells within the bead matrix, which in turn provides protection in an environment used in fermented, other dairy products and in the gastrointestinal tract (Krasaekoopt *et al.,* 2003; Picot and Lacroix, 2004).

Micro-encapsulation confers protection to sensitive probiotic lactic acid bacteria from oxygen (Sunohara *et al.,* 1995), freezing (Shah and Ravula, 2000) and acidic conditions during manufacture and storage (Adhikari *et al.,* 2000) and gastrointestinal transit (Lee and Heo, 2000) and its efficacy is established by pH, composition and texture of food matrix (Kailasapathy, 2003), strains of culture employed (Lian *et al.,* 2002), initial cell population (Lee and Heo, 2000), method of encapsulation and wall material used (Muthukumarasamy and Holley, 2006). In dairy industry, microencapsulation has been applied to improve survival and delivery of bacterial cultures (Sultana *et al.,* 2000).

MATERIALS AND METHODS

The most common techniques used in microencapsulation of probiotics are extrusion, emulsion and spray drying**.** The size of the obtained microcapsules is important because it influences the sensory properties of foods.

Extrusion Method

Extrusion method is the oldest and most common procedure of producing hydrocolloid capsules (King, 1995).

In extrusion technique (Fig. 1.), the hydrocolloid (alginate) is mixed with probiotics. The resulting mixture is fed into an extruder, typically a syringe. Pressure exerted on the syringe plunger drops the contents of the syringe into a hardening solution, with gentle stirring. Hardening solution consists of multivalent cations (usually calcium in the form of calcium chloride). After dripping, alginate polymers immediately surround the added cells and form three-dimensional lattices by cross linkages of calcium ions (Krasaekoopt *et al.*, 2003).

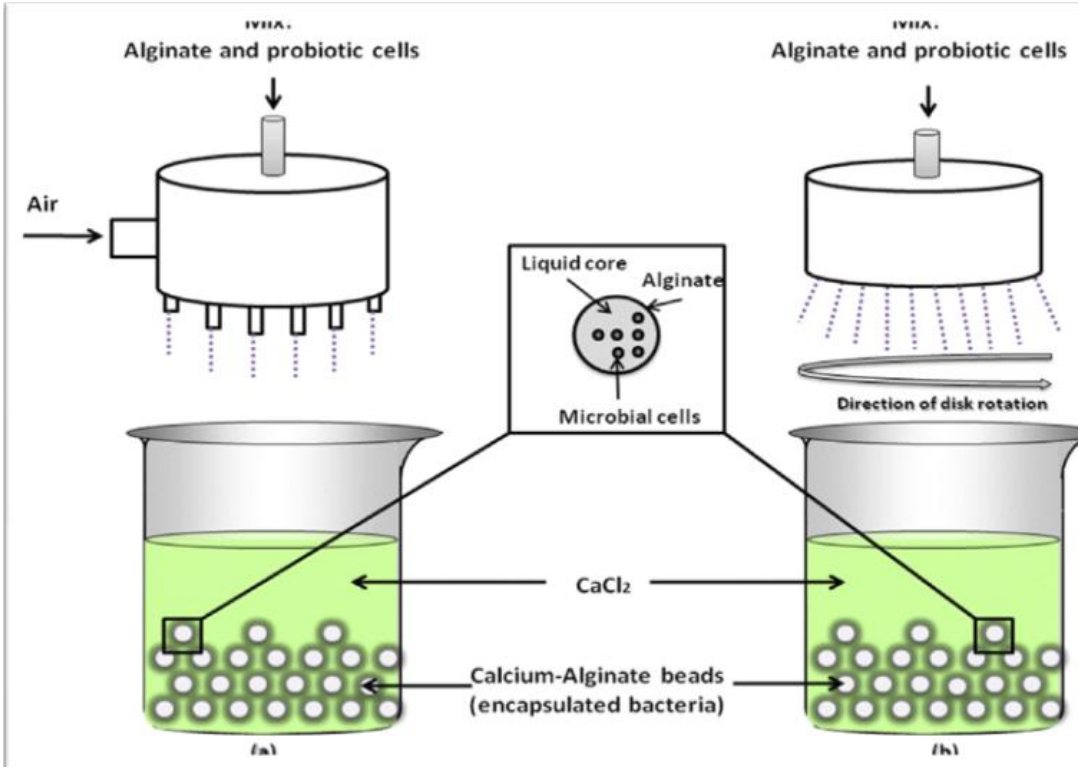

Figure 1: The encapsulation process of probiotics by extrusion technique (Burgain *et al.*, 2011)

It is common to apply concentration ranges of 1-2% and 0.05-1.5 M for alginate and calcium chloride, respectively. Most of the generated beads size is in the range of 2-3 mm in diameter (Krasaekoopt *et al.*, 2003). This parameter is strongly influenced by the factors such as type of alginate, its concentration and as a result, viscosity of alginate solution, distance between the syringe and setting batch and particularly diameter of the extruder orifice (needle) (Smidsrod and Skjak-Braek, 1990). Beads diameter decreases along with increasing concentration and viscosity of the encapsulation solution.

It is a simple and cheap method that uses a gentle operation which causes no damage to probiotic cells and gives high probiotic viability (Krasaekoopt *et al.*, 2003). The technology does not involve deleterious solvents and can be done under aerobic and anaerobic conditions.

Emulsion Method

Emulsification is a chemical technique to encapsulate probiotic living cells and use hydrocolloids (alginate, carrageenan and pectin) as encapsulating materials (Fig. 2). The principle of this technique is based on the relationship between the discontinuous and the continuous phases. For encapsulation in an emulsion, an emulsifier and a surfactant are needed. A solidifying agent (calcium chloride) is then added to the emulsion (Chen and Chen, 2007; Kailasapathy, 2009; De Vos *et al.*, 2010).

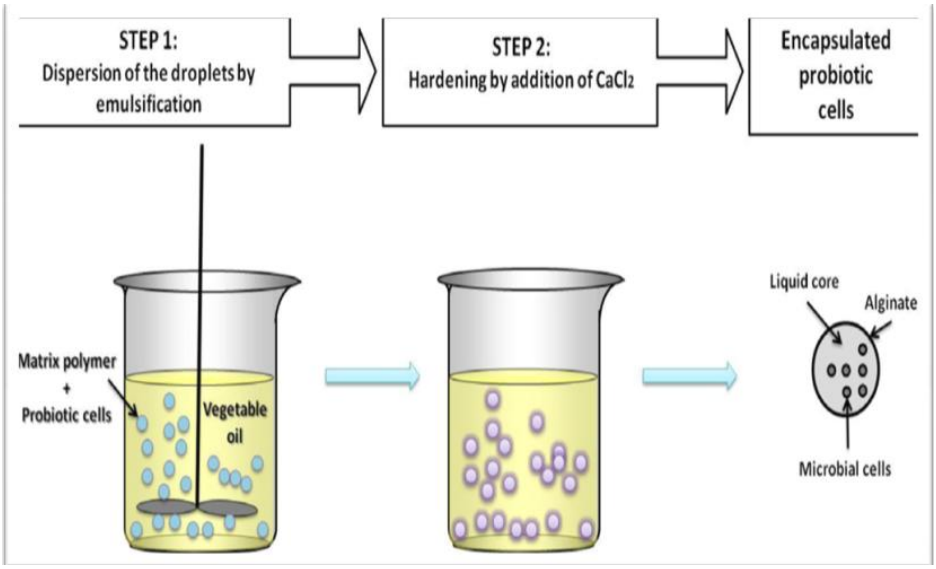

Figure 2: The encapsulation process of probiotics by emulsion technique (Burgain *et al.*, 2011)

The emulsion technique is easy to scale-up and gives a high survival rate of the bacteria (Chen and Chen, 2007). The obtained capsules have a small diameter but the main disadvantage of this method is that it provides large size range and shape. The emulsion procedure enables the production of the targeted microcapsules size by variation of agitation speed and the water/oil ratio (Kailasapathy, 2009).

In contrary with the extrusion technique, it can be easily scaled up and the diameter of produced beads is considerably smaller (25 µm-2 mm). But, this method requires more cost for performance compared with the extrusion method due to need of using vegetable oil for emulsion formation (Krasaekoopt *et al.*, 2003). Microbeads produced by emulsion method are usually recovered by the membrane filtration technique. It has been reported that concentration and viscosity of the encapsulation mix before gelation and its agitation rate are the main parameters that control the diameter of the final formed microbeads (Hansen *et al.*, 2002).

Spray Drying

Spray drying is a commonly used method of encapsulation in the food industry (Picot and Lacroix, 2004). Spray drying involves atomization of an emulsion or a suspension of probiotics and carrier material into a drying gas, resulting in rapid evaporation of water.

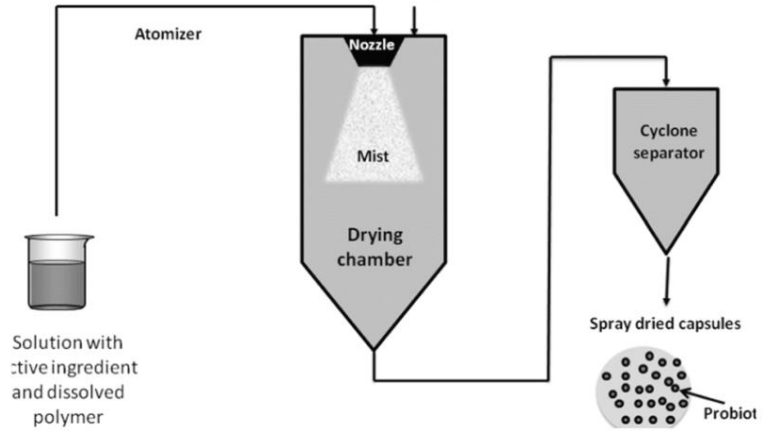

Figure 3: The encapsulation process of probiotics by spray drying (Burgain *et al.*, 2011)

The capsules are obtained as dry powder. The spray drying process is controlled by means of the product feed, gas flow and temperature. Besides polysaccharides, proteins can also be used as carriers; skim milk has proved to be a better wall material than gelatin, soluble starch and gum arabic, for instance (Lian *et al.,* 2002; Hsiao *et al.,* 2004). The advantages of spray drying are the rapidity and the relatively low cost of the procedure. The technique is highly reproducible and the most important is that it is suitable for industrial applications. One disadvantage of spray drying is that the use of high temperature which is not compatible with the survival of bacteria. In order to improve probiotic survival, protectants can be added to the media prior to drying. For example, granular starch improves culture viability during drying and storage, soluble fibre increase probiotic viability during storage and trehalose is a thermoprotectant.

Coating Materials for Probiotic Encapsulation

Probiotic encapsulation is based almost almost entirely on the usage of natural and synthetic polymers (Gbassi and Van Damme, 2012). The encapsulation efficiency and the capsule stability are greatly dependent on the encapsulating material known as wall material. Ideally the wall material should be water soluble since most spray drying suspensions are water based and possess good mechanical strength, compatibility with the core materials, emulsification properties and film forming and low viscous properties (Reineccius, 2004).

The viability of encapsulated probiotic cells depend on the physico-chemical properties of the capsules. In fact, the type and the concentration of the coating material, particle size, initial cell numbers and bacterial strains are some parameters which are important to master (Chen and Chen, 2007). Biopolymers, natural gums (acacia, k-carrageenan, alginates, etc), low molecular weight carbohydrates and proteins (whey protein, gelatin, etc.) are generally considered as good wall materials (Reineccius, 2004).

Encapsulation of Probiotics Using Alginate

Alginate is the biopolymer most used and investigated for encapsulation. Alginate is a naturally derived polysaccharide extracted from various species of algae and composed of β-D-mannuronic and α-L-guluronic acids. The composition of the polymer chain varies in amount and in sequential distribution influences functional properties of alginate as supporting material. Alginate hydrogels are extensively used in cell encapsulation (Rowley *et al.,* 1999) and calcium alginate is preferred in the concentration of 0.5-4% for encapsulating probiotics.

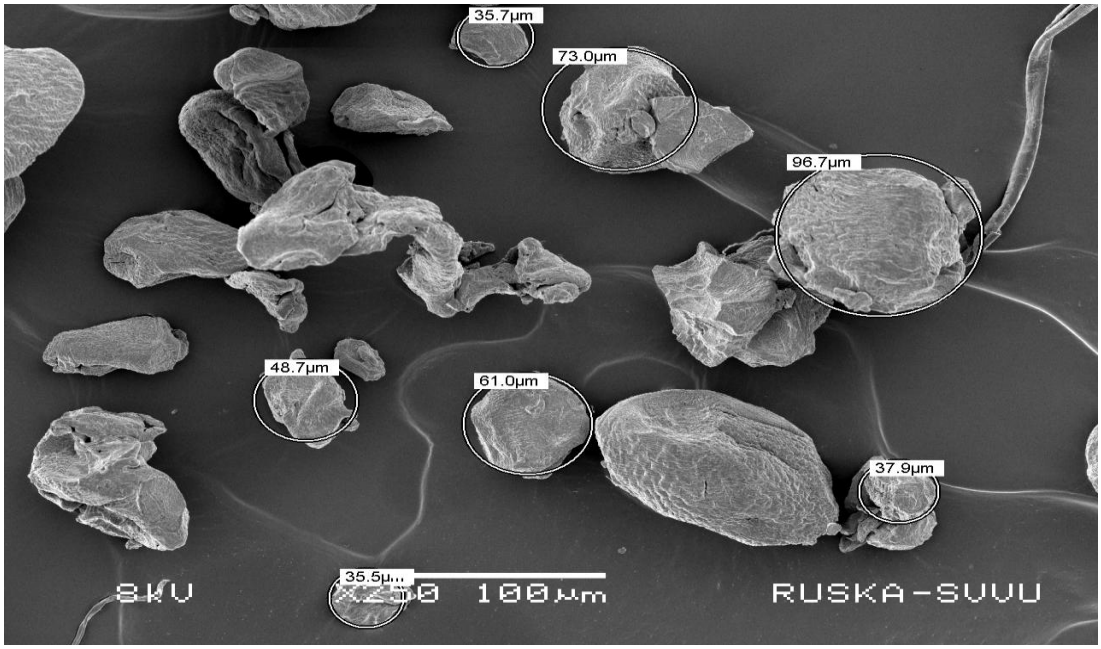

Figure 4: Scanning Electron Microscopy (SEM) showing varying sizes of alginate microcapsule. (Vivek *et al.,* 2013)

Alginate bead gelation occurs by crosslinkage when the Ca^{2+} gelling ions diffuse into the alginate-containing system. because of its simplicity, easily forming gel matrices around bacterial cells, non-toxicity, mild processing conditions needed for their performance, properly resolves in the intestine and releasing entrapped cells, biocompatibility and low cost (Krasaekoopt *et al.,* 2003; Chandramouli *et al.,* 2004). But, has disadvantages concern the scaling-up of the process that is very difficult. In addition, the microparticles obtained are very porous which is a drawback so as to protect the cells from its environment (Gouin, 2004). The defects can be compensated by mixing alginates with other polymer compounds, coating the capsules by another compound or applying structural modification of the alginate by using different additives (Krasaekoopt *et al.,* 2003).

The survivability of micro-encapsulated *Bifidobacterium longum* Bb-46 using alginate as a coating material was improved than free cells during refrigerated storage in milk with 2% fat (Hansen *et al.,* 2002). Encapsulation of bacteria in alginate has been found to improve survival rates by one log when compared to free cell counts when stored in skim milk for 24 h (Talwakar and Kailasapathy, 2003).

Encapsulation of Probiotics Using Gellan Gum and Xanthan Gum

Gellan gum is a microbial polysaccharide derived from *Pseudomonas elodea* which is constituted of a repeating unit of four monomers that are glucose, glucuronic acid, glucose and rhamnose (Chen and Chen, 2007). It is possible to induce a thermo-reversible gelation upon cooling of gellan gum solutions and the gelation temperature will depend on the polymer concentration, ionic strength and type of counterions presents in the medium.

Xanthan is a microbial polysaccharide derived from *Xanthomonas campestris* which is constituted of a repeating unit of pentasaccharide units formed by two glucose units, two mannose units and one glucoronic acid unit. This polymer is soluble in cold water and hydrates rapidly. Although gellan gum is able to generate gel-bead structure for microencapsulation, a disadvantage is that it is not used in this way for this purpose because of having a high gelsetting temperature (80-90°C for about 1 h) which results in heat injuries to the probiotic cells (Sun and Griffiths, 2000).

A mixture of xanthan–gelan gum has been used to encapsulate probiotic cells (Sultana *et al.,* 2000; Sun and Griffiths, 2000) and contrary to alginate, the mixture presents high resistance towards acid conditions.

Encapsulation of Probiotics Using Chitosan

Chitosan is a linear polysaccharide composed of glucosamine units which can polymerise by means of a cross-link formation in the presence of anions and polyanions. It can be isolated from crustacean shells, insect cuticles and the membranes of fungi. It is biodegradable and biocompatible. Due the possibility of a negative impact in the viability of bacteria, and due that chitosan has a very good film-forming ability, chitosan is more used as external shell in capsules made with anionic polymers as alginate. This component has not shown a good efficiency for increasing cell viability by encapsulation and it is preferably use as a coat but not as a capsule (Mortazavian *et al.,* 2008). In order to achieve sufficient stability, chitosan gel beads and microspheres can be ionically cross-linked with polyphosphates (Anal and Stevens, 2005) and sodium alginate (Anal *et al.,* 2003). Lee *et al*., (2004) concluded that microencapsulation of LAB with alginate and a chitosan coating offered an effective means of delivering viable bacterial cells to the colon, maintaining their survivability during refrigerated storage. The encapsulation of probiotic bacteria with alginate and a chitosan coating provides protection in simulated GI conditions and therefore, it is a good way of delivery of viable bacterial cells to the colon (Chávarri *et al.,* 2010).

Encapsulation of Probiotics Using K Carrageenan

Carrageenan is a natural polysaccharide that is extracted from marine macroalgae and is commonly used as a food additive. κ -Carrageenan is a natural polymer with sulphated polysaccharides. This polymer is largely used as thickening, gelling agent, texture enhancer or stabilizer on food, pharmaceutical and cosmetic formulations. Gelation of κ -carrageenan is generally dependent on a change in temperature. The technology using the compound requires a temperature comprised between 40 and 50°C at which the cells are added to the polymer solution. By cooling the mixture to room temperature, the gelation occurs and

then, microparticles are stabilised by adding potassium ions (Krasaekoopt *et al.*, 2003). The encapsulation of probiotic cells in κ -carrageenan beads keeps the bacteria in a viable state but the produced gels are brittle and are not able to withstand stresses (Chen and Chen, 2007). The κ -carrageenan beads for probiotic encapsulation can be produced using extrusion as well as emulsion techniques.

Encapsulation of Probiotics Using Gelatin

Gelatin is a heterogeneous mixture of single or multi-stranded polypeptides, protein gum, which makes a thermoreversible gel and was used for probiotic encapsulation, alone or in combination with other compounds. It is an excellent candidate for cooperation with anionic polysaccharides such as gellan gum due to amphoteric nature. This material is useful to obtain beads using extrusion technologies or form a w/o emulsion by cooling, but to stabilize the gel the beads may need to be crosslinked using glutaraldehyde or salts of Chrome. These hydrocolloids are miscible at a pH higher than 6, because they both carry net negatives charges and repel each other. However, the net charge of gelatin becomes positive when the pH is adjusted below the isoelectric point and this causes the formation of a strong interaction with the negatively charged gellan gum (Krasaekoopt *et al.*, 2003; Anal and Singh, 2007).

Encapsulation of Probiotics Using Starch

Starch is a polysaccharide composed by α-D-glucose units linked by glycosidic bonds, produced by all green plants. Resistant starch is the starch which is not digested by pancreatic enzymes (amylases) in the small intestine. Resistant starch can reach the colon where it will be fermented (Sajilata *et al.*, 2006; Anal and Singh, 2007). This specificity provides good enteric delivery characteristic that is a better release of the bacterial cells in the large intestine. By its prebiotic functionality, resistant starch can be used by probiotic bacteria in the large intestine (Mortazavian *et al.*, 2008). Finally, resistant starch is an ideal surface for the adherence of the probiotic cells to the starch granules (Anal and Singh, 2007) and this can enhance probiotic delivery in a viable and a metabolically active state to the intestine (Crittenden *et al.*, 2001). Resistant starch can be used to ensure the viability of probiotic populations from the food to the large intestine. The incorporation of Hi-Maize starch improved the encapsulation of viable bacteria compared with the bacteria encapsulated without starch (Sultana *et al.*, 2000; Iyer and Kailasapathy, 2005).

Encapsulation of Probiotics Using Cellulose Acetate Phthalate

Cellulose acetate phthalate is used for controlling drug release in the intestine due to its safety nature and being physically inert (Mortazavian *et al.*, 2008). The advantage of this component is that it is not soluble at acidic pH (less than 5) but it is soluble at pH higher than 6 because of its ionizable phthalate groups. The encapsulation of probiotic bacteria using cellulose acetate phthalate provides good protection for microorganisms in simulated GI conditions (Fávaro-Trindade and Grosso, 2002).

Encapsulation of Probiotics Using Milk Proteins

Among other proteins, milk proteins are very interesting as an encapsulation material by their physic-chemical properties. Just like the gelatin, milk proteins are able to form gels in the suitable conditions. Milk proteins are natural vehicles for probiotics cells and owing to their structural and physico-chemical properties, they can be used for delivery system (Livney, 2010). For example, the proteins have excellent gelation properties and this specificity has been recently exploited by Heidebach *et al*., (2009a, b) to encapsulate probiotic cells. The results of these studies are promising and using milk proteins is an interesting way because of their biocompatibility (Livney, 2010).

Encapsulation of Probiotics Along With Prebiotics

Probiotic cells can be encapsulated with prebiotic ingredients (e.g. resistant starch) or cryoprotectants (e.g. glycerol) to improve their viability (Sultana *et al.*, 2000). It has been shown that this technique enhances probiotic survival in the product but not under simulated gastro intestinal conditions (Sultana *et al.*, 2000). The co-encapsulation is another way to enhance probiotic viability (Godward and Kailasapathy, 2003c). Encapsulation with both alginate and prebiotics is referred to as co-encapsulation. Incorporating both prebiotics and calcium alginate in coating materials may better protect probiotics in food systems and the gastrointestinal tract due to synbiosis (Chen *et al.*, 2005).

Chen *et al.,* (2005) observed that 1% sodium alginate mixed with 1% peptide and 3% FOS as coating material gives highest survival count of probiotics. The co-encapsulation of different probiotic bacteria with Hi-maize starch (prebiotic) and further coating with chitosan significantly enhanced the survival of encapsulated probiotic bacteria (Iyer and Kailasapathy, 2005). Akhiar, (2010) studied co-encapsulation of *L. acidophilus* CSCC 2400 with alginate and prebiotics and evaluated viability at 0 and 6th weeks and found improved survival rate of co-encapsulated probiotic than compared to free cells. Vivek *et al*., (2013) encapsulated *B. bifidum* 235with prebiotics FOS using extrusion method in dahi and reported improvement in sensory attributes than in free cells in dahi.

CONCLUSION

Amongst the various approaches, microencapsulation has emerged as the best alternative so as to overcome the problem of poor survivability of probiotic cultures in the food matrix as well as in the gastrointestinal environment. The technology of micro-encapsulation has developed just from a simple entrapment to defined, better and absolute micro capsule formation. Even though, the challenges are to select the appropriate microencapsulation technique and encapsulating materials. Till date, the research on encapsulation of probiotics has focused mainly on maintaining the viability of the probiotic bacterial cells at low pH and high bile concentrations.

One important challenge for cell encapsulation is large size of microbial cells (typically 1-4 mm) or particles of freeze-dried culture (more than 100 mm).The larger the capsule size, the greater the negatively impact on textural and sensory attributes of food products in which they are incorporated. Although promising on a laboratory scale, the developed technologies for producing gel beads still present serious difficulties for large-scale production of food-grade microencapsulated microorganisms. Another challenge is the improvement of viability of probiotics during the manufacturing processes, particularly heat processing.

There are no commercially available probiotic products that are stable at high temperatures. Keeping in view of these difficulties, new approaches are required in further research. It can be by recognising naturally heat tolerant probiotics by *in vitro* techniques or by genetic modification, use of fats or lipids as a coating material for capsules due to their high melting point property. One of the major interests in the future concerns will be the use of probiotic/prebiotic combinations. Nanoencapsulation may assume importance in the near future to develop designer probiotic bacterial preparations which could be delivered to specific parts of the gastro-intestinal tract where they interact with specific receptors.

Even food grade thermotolerants may also be helpful in resisting higher temperature along with encapsulation. More human trials are required so as to know the efficacy of microencapsulation in delivering probiotic bacteria and their controlled release in the gastro-intestinal system. In the food processing industry, preservation and storage, micro-encapsulation will continues to play a pivotal role in protecting the viability and enhance the survival of bacteria against adverse environmental conditions. Future research must determine the cost effectiveness of the process, optimum size of encapsulated cells which confers maximum protection to the probiotics without impairing the organoleptic properties of the product.

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