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MICROENCAPSULATION-THE FUTURE OF PROBIOTIC CULTURES

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ABSTRACT

In the recent past, there has been an explosion of probiotic cultures based health products in Indian markets. The survival of the probiotic bacteria in gastro-intestinal gut is questionable, because of the poor survival of probiotic bacteria in these products. Basically the viability of probiotic cultures is very weak in these food products. Probiotic based products are health potentiators and are associated with many health benefits. Microencapsulation of the probiotic cultures is one of the recent, demanded and highly efficient techniques. Among the different approaches proposed to improve the survival of probiotics during food manufacturing process and passage in the upper part of gastrointestinal tratct (GI tract), microencapsulation has received considerable attention. Encapsulated probiotic cultures have longer shelf life of the products. This microencapsulation technology is used to maintain the viability of probiotic bacteria during food product processing and storage. This article reviews the principles, techniques and need for microencapsulation of probiotic cultures.

Keywords: Microencapsulation, probiotics, viability, health potentiators

INTRODUCTION

Probiotics means '*for life',* contains microorganisms which are beneficial to the host organism. According to the currently adopted definition by FAO/WHO, probiotics are 'live microorganisms which when administered in adequate amounts confer health benefit on the host animal by improving the intestinal microbial balance' **FAO (2001)**. **Fuller (1989)** suggested a definition of probiotics as, "*a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance*". Probiotics are living, health-promoting microorganisms that are incorporated to flourish into various kinds of foods and are rapidly becoming a popular and important tool for preserving our natural health. **Heller (2001)** suggested various microbes considered to be beneficial to the human body and include the genus names *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, and *Saccharomyces* and more specifically the microbes *are Lactobacillus bulgaricus*, *L. acidophilus*, *L. casei, L. rueteri*, *Streptococcus lactis*, *S. citrovorus*, *Bifobacterium bifidium*, *Saccharomyces boulardii* and others.

MECHANISM OF PROBIOTIC ACTION

Probiotics employ different mechanisms for affecting human health by normalizing the intestinal microbiota. **Abott (2004)** suggested that a healthy human gastro intestinal (GI) tract contains about 1.2 kg of bacteria and large numbers of yeasts. These native microbes in the GI tract play an important role in the health and well-being of the host. The favourable effects of these bacteria may comprise inhibition of pathogens, stimulation of the immune system, help in digestion, synthesis of vitamins and drug metabolism. Probiotic bacteria also reinforce the intestinal walls by crowding out pathogenic organisms, thereby helping to prevent their attachment to where they can cause disease. Probiotic bacteria also stimulate antigen-specific and nonspecific immune responses. The ingestion of Lactobacilli is known to result in the reduction of faecal enzymes such as β-glucuronidase, azoreductase and nitroreductase in humans, which are capable of converting pro-carcinogens to carcinogens in the digestive tract. Thus, they lower the chances for tumour development. However, the number of viable probiotic bacteria, delivering their targeted beneficial effect is too low. Many factors such as acidity, oxygen content, and concentration of lactic and acetic acids affect the survival of probiotics in food and in the gastrointestinal tract of the host. Several methods have been used to enhance the viability of probiotics, including selection of resistant strains, stress adaptation, incorporation of micronutrients, and microencapsulation.

PROBIOTICS AS HEALTH POTENTIATORS

Probiotics have become an important part of nutrition because our microbial populations have been altered by the use of antibiotics and other substances that are designed to kill germs and disease. The beneficial bacteria that make up our gut flora have many functions in the body and are essential to our health. Various reported health benefits of probiotics include boosting of the immune system, inhibition of the growth of pathogenic organisms, prevention of diarrhoea from various causes, prevention of cancer, reduction of the risk of inflammatory bowel movements, improvement of digestion of proteins and fats, synthesis of vitamins, detoxification and protection from toxins. Some potential benefits have been demonstrated as; managing lactose intolerance, prevention of colon cancer, lowering cholesterol, lowering blood pressure, improving immune function and preventing infections, *Helicobacter pylori*, antibiotic-associated diarrhoea (AAD), reducing inflammation, improving mineral absorption, preventing harmful bacterial growth under stress, irritable bowel syndrome and colitis, managing urogenital health, anti-microbial activities (**Hobbs, 2000; Brady *et al.*, 2000**; **Sanders, 2000; Reid *et al.*, 2005; Ouwehand *et al.*, 2002**; **Hamilton-Miller, 2003; Szajewske and Mrukwicz, 2005**; **Mcfarland, 2006**; **Braat *et al.*, 2004; Hitti and Mirand, 2006).**
Unluckily, most of the probiotics lack the ability to survive the harsh conditions of acidity and bile concentrations commonly encountered in the gastrointestinal tracts of humans (**Ouwehand & Salminen, 1998**) which prompted the need for microencapsulation. The consumption of probiotics at a level of 10^8–10^9 cfu/ml per day is a commonly quoted figure for adequate probiotic consumption, equating to 100g of a food product with 10^6 to 10^7cfu/ml (**Kebary *et al.*, 1998; Lee & Heo, 2000; Dave & Shah, 1997).**

MICROENCAPSULATION

Microencapsulation is a method defined as the "entrapment of a compound or a system inside a dispersed material for its immobilization, protection, controlled release, structuration and functionalization". 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. Beads without coating can also be considered as microencapsules in a broad sense. Coating protects the active content from environmental stresses such as acidity, oxygen and gastric conditions, and can be used, for example, to help the content pass through the stomach. Besides enhancing the viability of bacteria, microencapsulation facilitates handling of cells and allows a controlled dosage.

The use of probiotics, as dietary adjuncts is currently a subject of growing interest. One of the most important prerequisites for use of probiotics is that they survive and keep their health-promoting properties throughout the production process or during technological food treatment and storage until the end of shelf life. Moreover, because viable and biologically active microorganisms are usually required at target site in the host, it is essential that probiotics withstand the host's natural barriers against ingested bacteria.

Among the different approaches proposed to improve the survival of probiotics during the food manufacturing process and the passage in the upper part of GI tract, microencapsulationhas received considerable attention. Cell immobilization generally tends to increase the viability and the stability of microorganisms during their exploitation. However, efficiency can vary according to the method used and the culture considered. In almost all cases, gel entrapment using natural biopolymers such as calcium alginate and *κ*-carrageenan has been favored by researchers for probiotic applications (**Picot & Lacroix, 2004**). Although promising on a laboratory scale, the technologies developed to produce gel beads present all serious difficulties for large-scale production (**Poncelet, 2001**). In addition, encapsulation in such matrices does not necessarily protect efficiently the cells from the effect of pH, organic acids, or other soluble compounds like oxygen that can easily diffuse in a very hydrated medium. Consequently, the development of cell encapsulation technologies using effective, food-grade, economical coating materials, constitutes a real priority to generalize the use of encapsulated probiotics in the food processing industries.

PREREQUISITES FOR DESIGNING MICROCAPSULES

Various elements must be taken into consideration when designing microcapsules to preserve the viability of probiotics in food products. First, dry microcapsule preparations with low and controlled particle size are desirable for various reasons; viz higher stability, easier handling, storage of cultures, and limited effects on sensorial properties of the final product, especially texture. Second, considering the number of detrimental factors encountered during processing and storage, the development of multiphase microcapsules using coating materials with multiple barrier properties seems to be the most promising way to ensure process effectiveness. Barrier properties of coating materials include resistance to elevated temperatures and pressures, low permeability to moisture and oxygen, low hygroscopicity, low solubility in water, resistance to low pH or gastro-resistance. Among the food grade coating materials available in the market, polysaccharides and proteins form films that are generally permeable to moisture, especially at high relative humidity values (hygroscopic materials). On the other hand, they usually exhibit good barrier properties to gases and lipids. Lipid-based coatings present excellent water barrier properties, retard gas migration, and are relatively heat-stable (compounds with a high melting point). However, their mechanical properties are often weak.

Finally, for encapsulating probiotics, there must be a higher number of viable and metabolically active cells. To this end, the use of bacterial cultures in dried form (easier to handle, less vulnerable and less reactive to their environment) can prove to be a particularly relevant strategy. Among the numerous techniques that can be employed to encapsulate cells, fluidized air bed coating of powder particles of dried microorganisms constitute certainly the most promising technology so far (**Siuta-Cruce & Goult, 2001**).

The selection of suitable coating materials is of crucial importance to ensure efficient protection of probiotics. Unfortunately, the ideal coating material does not exist. Combining barrier properties of several coating materials in multilayered microcapsules seems to be the key for a successful encapsulation of probiotics. Of course, a compromise between process efficacy and cost must be found. The use of suitable coating materials and encapsulation technology should allow probiotics to be formulated into food systems more readily, thus increasing the number of applications. It should also allow manufacturers to place assurances on the viability and quantity of probiotics in finished products, which is not currently the case.

MICROENCAPSULATION TECHNIQUES

There are a number of different methods used to fabricate microcapsules. The main purpose of these techniques is to provide protection to microbial cells from adverse environment conditions and target delivery of viable cells to gastrointestinal tract. The microencapsulation technique employed is determined by the type and size of microcapsules one wants to obtain. The characteristics of the microcapsule must also take into consideration the function that the microcapsule will ultimately undertake. There are generally three main stages to the process of microencapsulation: (i) incorporation of the ingredients into a solution by mixing or dispersion, to make up the core of the microcapsule; (ii) mechanical operations such as spraying or emulsification, to form the droplets; (iii) product stabilization through coating, followed by a number of physical or chemical processes. Each step of microencapsulation can be optimized according to the desired characteristics of the final formulation.

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

The most commonly used encapsulation procedure for food application is based on the capsules formation by entrapment of probiotics within a polymeric matrix, using extrusion or emulsion techniques. The commonly used supporting materials are κ-carageenan, gellan, agarose, gelatin, alginate, chitosan, xanthan, and locust bean gum etc. Many currently available equipments for microencapsulation based on emulsion and extrusion techniques can not generate large quantities of uniform sized micro or nano-capsules. The introduction of spray drying and spray coating methods has resulted in the generation of particles and capsules in large quantities for industrial applications.

(1) Emulsion technique

(i) Emulsification and ionic gelification. Emulsification is a chemical technique to encapsulate probiotic living cells and use hydrocolloids (alginate, carrageenan and pectin) as encapsulating materials (figure. 1) (**Burgain *et al.*, 2011**). 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; Vos *et al.*, 2010)**. The emulsion technique is easy to scale-up and gives a high survival rate of the bacteria **(Chen *et al.*, 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 targeted microcapsule size by variation of agitation speed and the water/oil ratio (**Kailasapathy, 2009**). The gel beads can be introduced into a second polymer solution to create a coating layer that provides added protection to the cell or may be give improved organoleptic properties **(Kailasapathy, 2009)**.

(ii) Emulsification and enzymatic gelification. One problem with classical encapsulation technologies is the use of coatings such as alginate, κ-carrageenan, gellan-gum or xanthan which are not allowed in dairy products in some countries (**Picot and Lacroix, 2004**). The solution can be the use of milk proteins in which probiotics will be encapsulated by means of an enzymatic induced gelation (**Heidebach *et al.*, 2009a**). Milk proteins have excellent gelation properties and they are natural vehicles for probiotics (**Livney, 2010**). This method gives water insoluble and spherical particles (**Heidebach *et al.*, 2009b**) detailed an example of encapsulation by means of rennet gelation (figure 2).

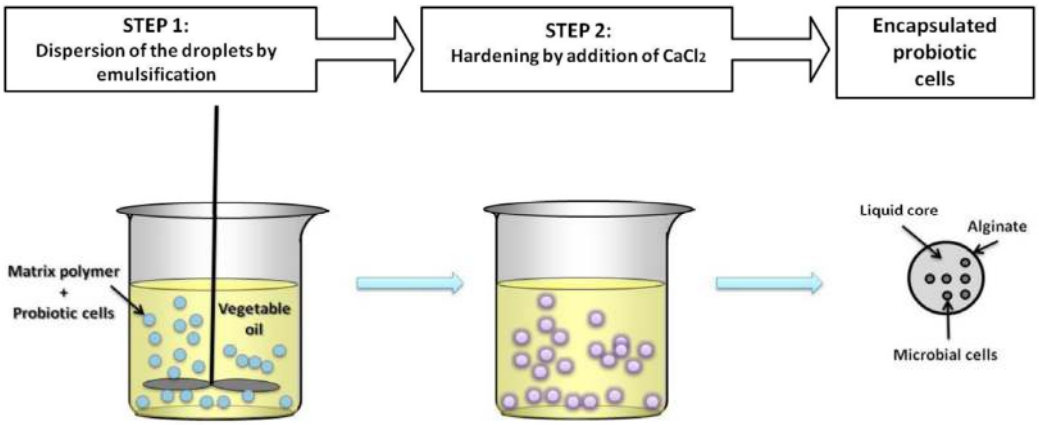

Figure 1 Schematic presentation of the emulsification procedure. A small volume of the cell-polymer suspension (i.e., the discontinuous phase) is added to a large volume of vegetable oil (i.e., the continuous phase). The mixture is then homogenized to form a water-in-oil emulsion. Once the water-in-oil emulsion is formed, the water-soluble polymer must be insolubilized to form tiny gel particles within the oil phase. ***Source*: Burgain *et al.*, 2011**

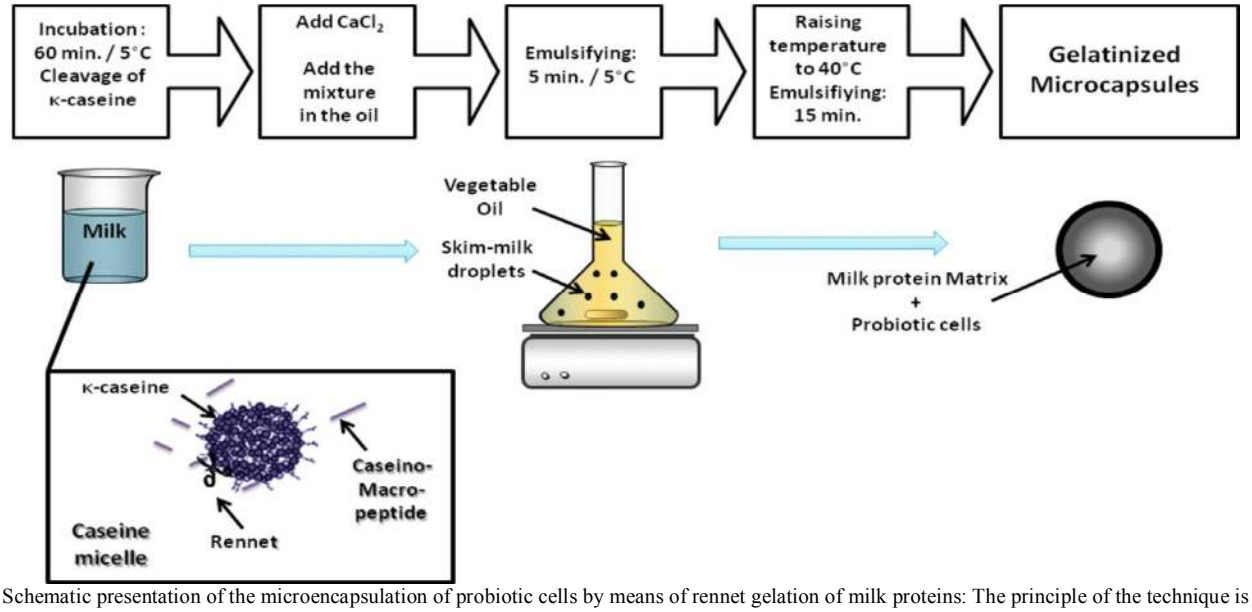

Figure 2 Schematic presentation of the microencapsulation of probiotic cells by means of rennet gelation of milk proteins: The principle of the technique is based on using dairy proteins which have been put into contact with rennet at low temperature. This allows keeping a liquid system where *kappa*-casein is cleaved by the enzyme. After that, dairy proteins have been emulsified in a cold oil to form water in oil emulsion. Thermal induction of enzymatic coagulation allows proteins flocculation and provides microparticles where probiotics are dispersed in coagulated dairy proteins. ***Source*: Burgain *et al.*, 2011**

(iii) Emulsification and interfacial polymerization. Interfacial polymerization is an alternative technique which is performed in a single step. The technique requires the formation of an emulsion: the discontinuous phase contains an aqueous suspension with the probiotic cells and the continuous phase is an organic solvent. To initiate the polymerization reaction, a biocompatible agent which is soluble in the continuous phase, is added. The droplets obtained containing probiotic cells are enveloped in a thin and strong membrane **(Kailasapathy, 2002)**. Interfacial polymerisation is used to encapsulate microorganisms in order to improve their productivity in fermentation (**Yanez-Fernandez *et al.*, 2008**).

The common supporting materials used in emulsion techniques are: κ-carageenan and locust bean gum, alginate, chitosan and gelatin, cellulose acetate phthalate and gellan-xanthan gum **(Prakash *et al.*, 2011)** (Table 1). With emulsion technique, smaller beads can be produced compared with extrusion technique. The size of the beads is controlled by the mixer and the reactor design and speed of agitation and can vary between 25 µm to 2mm. (Table 2), This technique has been used for encapsulation of lactic acid bacteria for batch and continuous fermentation. In addition, the entrapped *Lactobacillus delbrueckii ssp. bulgaricus* in artificial seasame oil emulsions showed a significant increase (approximately 10^4 times) in survival rate when subjected to simulated high acid gastric or bile salt conditions, compared with free cells.

(2) Extrusion technique

Extrusion is a physical technique to encapsulate probiotic living cells and uses hydrocolloids (alginate and carrageenan) as encapsulating materials. The microencapsulation of probiotic cells by extrusion consists in projecting the solution containing cells through a nozzle at high pressure. If the formation of droplets occurs in a controlled environment way (as opposed to spray-drying), the technique is known as prilling. This is preferably done by the pulsation or vibration of the jet nozzle. The use of coaxial flow or an electrostatic field is the other common technique to form droplets (**Kailasapathy, 2002**). The principle of the technique is explained in figure 3 (**Krasaekoopt *et al.*, 2003; Chen *et al.*, 2007; Kailasapathy, 2009; Vos *et al.*, 2010**). Extrusion 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**).

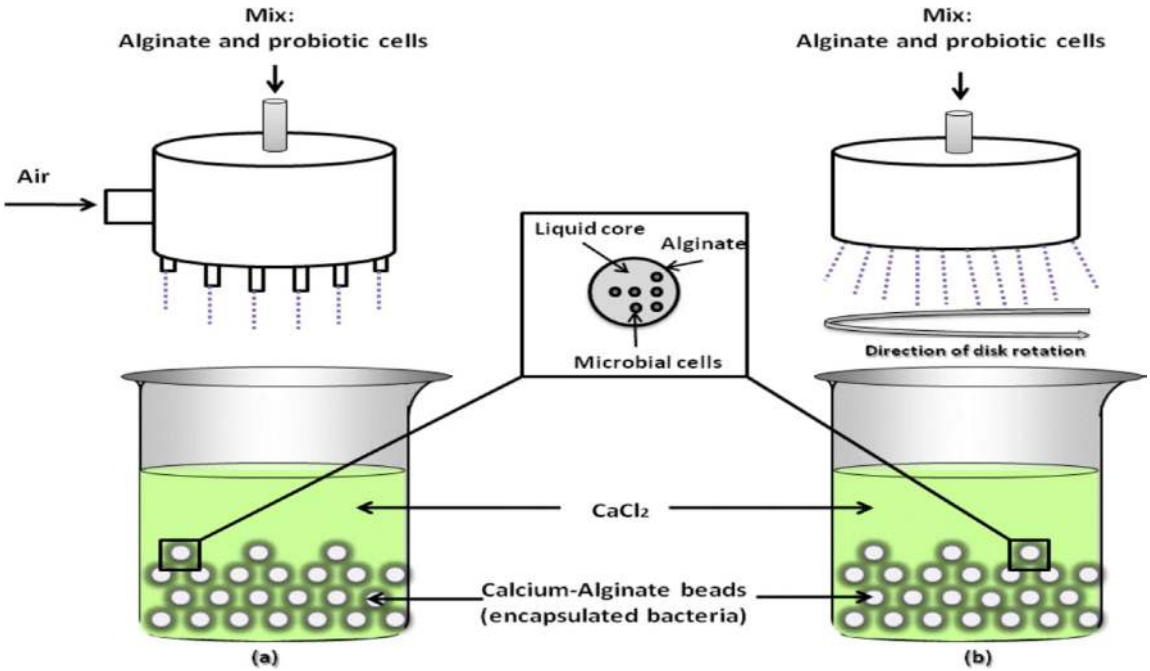

Figure 3 Extrusion technologies: Simple needle droplet-generator that usually is air driven (a) and pinning disk device (b). The probiotic cells are added to the hydrocolloid solution and dripped through a syringe needle or a nozzle spray machine in the form of droplets which are allowed to free-fall into a hardening solution such as calcium chloride. ***Source*: Burgain *et al.*, 2011**

Table 1 Types of microcapsules available for the targeted delivery of probiotic bacteria

Types of Microcapsules	Bacteria	References
Alginate Beads	*Lactobacillus rhamnosus, L. salivarius, L. plantarum, L. acidophilus, L. paracasei, L. casei, L. reuteri, Bifidibacterium longum, B. lactis,*	**Krasaekoopt *et al.*, 2004; Ding & Shah, 2007; Lee & Heo, 2000; Capela *et al.*, 2006; Muthukumarasamy & Holley, 2007; Chandramoul *et al.*, 2004.**
Alginate-cellulose acetate phthalate	*B. lactis, L. acidophilus*	**Favaro-Trindade & Grosso, 2002**
Alginate-chitosan	*B. animalis* subsp. *lactis, L. bulgaricus*	**Liserre *et al.*, 2007; Lee *et al.*, 2004**
Alginate-chitosan Alginate	*B. bifidum, L. casei*	**Krasaekoopt *et al.*, 2004**
Alginate-poly-L-lysinealginate	*B. bifidum, L. reuteri, L. casei*	**Krasaekoopt *et al.*, 2004; Martoni *et al.*, 2008**
Alginate-starch	*L. acidophilus, B. lactis, B. infantis*	**Kailasapathy, 2006; Sultana *et al.*, 2000; Homayouni *et al.*, 2008**
Gelatin-gum arabic-soluble starch	*B. infantis, B. longum*	**Lian *et al.*, 2002; Lian *et al.*, 2003; Hsiao *et al.*, 2004**
Gelatin-toluene-2-4-diisocyanate	*L. lactis*	**Hyndman *et al.*, 1993**
Gellan-alginate	*B. bifidum*	**Chen *et al.*, 2007**
Gellan-xanthan	*B. adolescentis, B. bifidum, B. breve, B. infantis, B. lactis, B. longum*	**Sun & Griffiths, 2000; McMaster *et al.*, 2005)**
Genipin-crosslinked alginate-chitosan	*L. plantarum*	**Chen *et al.*, 2007**
Pectin-casein	*B. lactis, L. acidophilus*	**Oliveira *et al.*, 2007**
Potato starch granules-amylose	*B. longum*	**Lahtinen *et al.*, 2007**
Whey protein	*B. breve, B. longum, L. rhamnosus*	**Picot & Lacroix, 2004; Reid *et al.*, 2005**
κ-carageenan	*B. longum, S. thermophiles, L. bulgaricus S. lactis*	**Adhikari *et al.*, 2002; Audet *et al.*, 1988**

Table (2) Microsphere preparation by emulsion technique

Strains	Carrier	Continuous phase	Diameter of beads
Lactobacillus delbrueckii ssp. bulgaricus	-	Artificial seasame Oil	20-200 µm
Bifidobacterium longum	ƙ-carageenan	Vegetable oil/0.1% Tween 80	-
B. pseudolongum	10% cellulose acetate phthalate	White light paraffin Oil	-
L. casei ssp. casei	3% ƙ-carageenan & locust bean gum	Vegetable oil	1-2 µm
L. delbrueckii ssp. bulgaricus	3% alginate	Vegetable oil/ 0.2% Tween 80	25-35 µm
L. casei, L. acidophilus, B.infantis	2% alginate, 2% starch	Vegetable oil/ 0.2% Tween 80	150-500 µm
B. longum Lactococcus lactis	ƙ-carageenan and/ locust bean gum	Vegetable oil	1-2µm

Source: **Petrovic *et al.*, 2007**

The technology does not involve deleterious solvents and can be done under aerobic and anaerobic conditions. The most important disadvantage of this method is that it is difficult to use in large scale productions due to the slow formation of microbeads. Extrusion techniques are based on making capsules with hydrocolloids. These methods involve preparing a hydrocolloid solution, inoculation with bacterial cells, and extruding the viscous polymer-bacterial suspension through a gauge needle using syringe pump. The droplets are allowed to fall into a hardening solution. In this technique, alginate, κ-carrageenan, *κ*-carrageenan plus locust bean gum, xanthan plus gellan, alginate plus corn starch, and whey proteins have been used as wall materials for microencapsulation of lactobacilli and bifidobacteria.

The main properties of alginates are their ability to increase the viscosity of aqueous solutions as well as form gels (when calcium salt is added). Calcium alginate was one of the first materials used for production of beads encapsulating probiotics due to mild conditions for the cells during the encapsulation process, then their buffering capability, cheapness, simplicity and biocompatibility. By using extrusion techniques, a large range of bead size can be obtained, in the range of 0.1 to 5.0 mm (Table 3).

Table 3 Bead sizes obtained by extrusion technique

Strains	Carrier	Diameter of beads
Bifidobacterium longum	Alginate	2.6 mm
B. lactis	0.75% gellan/1% Xanthan	20-2200 µm
Lactobacillus acidophilus & B.longum	Alginate	2.6 mm
B.bifidum & B.infantis	Alginate	
B. infantis	0.75% gellen/ 1% xanthan	3 mm
Lactococcus lactis ssp. cremoris	2% alginate	2 mm

Source: **Petrovic *et al.*, 2007**

Although alginate is frequently used for entrapment of probiotics it has undesirable properties such as susceptibility and degradation by acids. In addition, it was observed that a mixed gel of gellan and xanthan gums has better technological properties for microencapsulation by extrusion technique than alginate. Furthermore to improve the characteristics of alginate, coating beads by cross-linking with a cationic polymer carrier has been suggested. Coating beads protect cell from release, increase mechanical and chemical stability. Furthermore, mixing with starch and incorporation of additives (cryoprotectants such as mannitol and glycerol) can improve the stability of encapsulated beads.
The size of microcapsules is affected by the nozzle size. Also, diameter of the obtained alginate beads is found to increase as the concentration of sodium alginate increases, but alginate concentration does not significantly influence the numbers of free cells. A mixture of gellan and xanthan has better technological properties than alginate, κ-carrageenan, or locust bean gums, but the shape and size of gellan and xanthan gum capsules has been found to vary.

(3) Spray drying/spray coating

Drying is an encapsulation technique which is used when the active ingredient is dissolved in the encapsulated agent, forming an emulsion or the suspension. The "solvent" is commonly a hydrocolloid such as gelatin, vegetable gum, modified starch, dextrin, or non-gelling protein. The solution that is obtained is dried, providing a barrier to oxygen and aggressive agents. There are several drying techniques for microencapsulation of probiotics as spray-drying, fluid-bed drying and freeze drying.

i. Spray drying: Spray drying is a commonly used method of encapsulation in food industry. 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. The capsules are obtained as dry powder. The spray drying process is controlled by means of the product feed, gas flow and temperature. Yet, despite many advantages of spray-drying method, high temperatures needed to facilitate water evaporation lower the viability of the probiotics and reduce their activity in the final product (Figure 4) (**Burgain *et al.*, 2011**). In spray drying, a solution containing probiotic living cells and the dissolved polymer matrix is prepared. The polymer matrices are generally gum arabic and starches because they tend to form spherical microparticles during the drying process **(Chen *et al.*, 2007; Kailasapathy, 2009; Vos *et al.*, 2010)**. The advantages of spray drying are the rapidity and 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 the fact that this technique has a small field of application but the main problem is the use of high temperature which is not compatible with the survival of microorganisms. In order to improve probiotic survival, protectants can be added to 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. Moreover, spray-dried capsules can be coated by an additional layer in order to give a protection against acidic environment of the stomach or to reduce the deleterious effect of bile salts

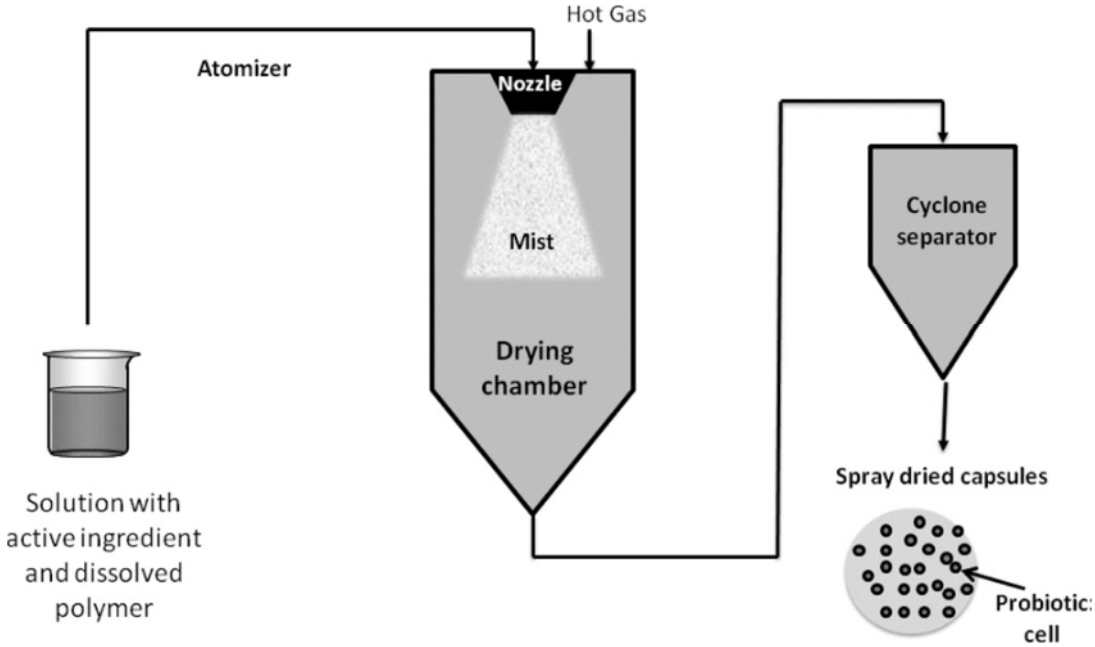

Figure 4 Schematic presentation of the spray-drying procedure. The solution is pressured and then atomized to form a ''mist'' into the drying chamber. The hot gas (air or nitrogen) is blown in the drying chamber too. This hot gas allows the evaporation of the solvent. The capsules are then transported to a cyclone separator for recovery. ***Source***: **Semyonov *et al.*, 2010**.

ii. Spray Freeze drying. Spray freeze drying method combines processing steps that are common to freeze-drying and spray drying. Probiotic cells are in a solution which is atomized into a cold vapour phase of a cryogenic liquid such as liquid nitrogen. This step generates a dispersion of frozen droplets. Frozen droplets are then dried in a freeze dryer (**Wang *et al.*, 2006; Kailasapathy, 2009; Vos *et al.*, 2010; Semyono *et al.*, 2010**). Spray freeze drying presents various advantages, like providing controlled size, larger specific surface area than spray-dried capsules. The technique also has some disadvantages including the use of high energy, long processing time and cost which is 30–50 times more expensive than spray-drying (**Zuidam and Shimoni, 2009**). Capsules can be coated by an additional shell to give protection against adverse environmental conditions (**Semyonov *et al.*, 2010**).

iii. Matrix encapsulation: A group of encapsulation technologies is referred to as matrix encapsulation because the microcapsules lack a core/shell structure but have a number of particles located at their surface. Still, the obtained properties are often sufficient to achieve the desired delayed release of ingredients. Encapsulation by MicroMAX® technology, for instance, using proteins, lipids and carbohydrates provides protection for probiotics during spray drying and storage, as well as during transit through the stomach. In spray chilling, the atomization step is similar to spray drying, but the solidification of gel particles is based on the injection of cold air into the vessel. Spray chilling is a cheap technology that can be used to generate smaller beads. Freeze drying of probiotic bacteria, where the frozen material is dried in a vacuum, is also widely used in industry.

COATING OF MICROCAPSULES

Coating the microcapsules produced by different technologies with an additional film can prevent their exposure to oxygen during storage as well as improve their stability at low pH. Possible coating materials include chitosan, poly- L-lysine, alginate, starch, gum and gelatin. Chitosan-coated alginate beads are reported to provide better protection in simulated gastric conditions than poly- L-lysine or alginate coating. Low-molecular weight chitosan has been found to show better control of cell release than high-molecular weight chitosan and to result in more spherical beads without changing their size. Moreover, in a study on chitosan-coated alginate beads, beads coated with high-molecular weight chitosan partly collapsed. Coating of microcapsules with alginate produces a uniform 1–2 µm thin exterior layer and has been found to improve the survival of bifidobacteria. Coating the beads with poly-L-lysine and alginate is reported to limit *Lactococcus lactis* release but also to reduce the acidifying activity of the culture. In a study by **Reid *et al.* (2005)**, beads produced with a commercial whey protein isolate and soaked in a milk-based solution were big in size, and they were not perfect spheres.

SURVIVAL OF MICROENCAPSULATED PROBIOTICS

The survival of probiotic bacteria during processing, storage, and in gastric conditions is highly dependent on the strain used. Stability of the strain is thus one of the main criteria in selecting suitable probiotics. Further, food matrix environment has to be taken into account when selecting the materials for microencapsulation (**Rokka & Rantamäki, 2010**).

METHODS FOR STUDYING SURVIVAL RATES

Survival rates of probiotic bacteria after various treatments have generally been studied by the plate count method, which is easy to carry out. Plate counting, however, does not always tell the whole truth about the viability of bacteria. A non-cultivable population might still be metabolically active and provide the desired health-promoting effect in its target. The studies of microencapsulation of probiotics, however, do usually not contain any conformation of health effects caused by encapsulated bacteria. Thus, the use of commercial live/dead kits and flow cytometry can provide more information about the metabolic status of processed bacteria. These methods are based on the use of fluorescent staining of nucleic acids, which distinguishes live bacteria with intact cytoplasmic membranes from dead bacteria with compromised membranes **(Rokka & Rantamäki, 2010)**. Moreover, the particle size distribution of the obtained microcapsules can be analyzed by a light microscope, a scanning electron microscope or a laser difractometer (**Rokka & Rantamäki, 2010**).

SURVIVAL OF BACTERIA DURING ENCAPSULATION AND DRYING

Thermal and osmotic resistance of lactic acid bacteria is species dependent (**Lian *et al.*, 2002**). The survival of probiotics after spray drying also depends on the kind and concentration of the carriers used as well as on the outlet temperature of spray dryer (**Ananta *et al.*, 2005**; **Gardiner, *et al.*, 2002**). Bacterial membranes are the main site of injury during spray drying (**Ananta *et al.*, 2005**). As water is important stabilizer in biological molecules, therefore, removal of water may damage cell membranes and associated proteins.

Typical survival rates in the spray-drying and freeze drying processes are in the range of 70–85%. Although a survival rate may be acceptable, the prolonged storage stability of the product is often low. The presence of deoxidant and desiccant has been found to improve cell survival. Sugars are known to protect dehydrated biomaterials, and it has been suggested that they act as water substitutes and replace water molecules around proteins and polar residues of membrane phospholipids. Sugars are also able to form hydrogen bonds with the proteins when water is removed and prevent protein denaturation. It has been reported that disaccharides were effective in protecting both bacterial membranes and proteins during drying. Cells from fresh cultures are reported to survive better than cells from freeze-dried cultures during encapsulation by emulsion and spray drying (**Rokka & Rantamäki, 2010**).

In freeze drying, drying media can have a greater effect on the stability of probiotics than microencapsulation itself. Cryoprotectants can be added to maintain probiotic viability. **Sultana *et al.* (2000)** reported that glycerol improved probiotic survival in freezing 100-fold. However, in long-term storage, the addition of a cryoprotectant or prebiotic has not been found to enhance the viability of microencapsulated cells. Wheat dextrin and polydextrose have proven promising fibre carriers to protect *Lactobacillus rhamnosus* during freeze drying. Other results show that alginate offers better protection for probiotic bacteria than whey protein during freeze drying **(Kailasapathy & Sureeta, 2004).**

Addition of Hi-Maize starch to alginate has been found to result in a higher number of live bacteria in microcapsules **(Sultana *et al.*, 2000)**. Increasing the alginate concentration and capsule size also increases the survival of probiotics in heat treatment. **Chen *et al.* (2007)** reported that in heat treatment, the best protection for *Bifidobacterium bifidum* was provided by 2% sodium alginate combined with 1% gellan gum. The prebiotic effect of peptides was also confirmed. Another study observed that the use of non-fat milk in extrusion process increases the number of viable cells (**Ross *et al.*, 2008).**

SURVIVAL UNDER SIMULATED PHYSIOLOGICAL CONDITIONS

The general aim of microencapsulation is, firstly, to protect probiotic culture in foods and in the passage through the stomach, since free cells usually do not survive in gastric conditions, and secondly, to release the probiotics in their target, the gastro intestinal gut. The survival of probiotic bacteria depends on the strain, and the type of food ingested in gastric environment. The survival of probiotics is commonly studied under simulated physiological conditions. Simulated gastric juice typically consists of pepsin and sodium chloride adjusted to pH 1–3 with HCl. A simulated intestinal solution consists of bovine or porcine bile and pancreatin at pH 7.4 –7.5 **(Annan *et al.*, 2008; Chen *et al.*, 2007; Picot & Lacroix, 2004; Lian *et al.*, 2003; Mandal *et al.*, 2006).** Several studies have shown that microencapsulation of bacteria with alginate or whey proteins protect them against acid stress, allowing the cells to survive in stomach and be delivered in the intestines. An optimal capsule combination reported in literature for probiotic survival in gastric conditions is 3% sodium alginate, 1% pancreatic digested casein and 3% fructo-oligosaccharides **(Chen *et al.*, 2006)**.Also caseinate and fructo-oligosaccharides with either dried glucose syrup or resistant starch are found to provide protection. It has also been reported that the diameter of alginate microcapsules decreases in simulated stomach exposure **(Martoni *et al.*, 2008)**. Various results indicate that increasing the alginate concentration and capsule size enhances the survival of probiotics, whereas the $CaCl_2$ concentration and the initial cell numbers do not affect bacterial death rates. *L. rhamnosus* and bifidobacteria encapsulated with starch have been shown to survive passage through the human gastrointestinal tract, whereas Hi-Maize starch encapsulation did not protect *Lactobacillus acidophilus* or *Bifidobacterium infantis* from high acid conditions in a study by **Sultana *et al.* (2000)** Bifidobacteria encapsulated with gellan and xanthan, gum arabic and gelatin, and skim milk in simulated gastric juice have been found to survive somewhat better than free cells. Also, the surface characteristics of microporous glass membrane microcapsules protect probiotic cells even in highly acidic conditions **(Song *et al.*, 2003)**.

STABILITY OF MICROENCAPSULATED PROBIOTICS IN FOOD

Probiotics often have low viability in food products due to the high concentration of lactic and acetic acid, low pH, and the presence of hydrogen peroxide and oxygen. The viability of acid-sensitive bifidobacteria in yogurt can be increased by microencapsulation, but this effect depends on the strain **(Talwalkar & Kailasapathy, 2003)**. Studies have reported that in yogurt, a high amount of bifidobacteria encapsulated in carrageenan, gellan-xanthan, or alginate and Hi-maize, and some of the cells encapsulated in whey protein, remain viable, whereas the number of free bacteria decrease significantly.

SENSORY QUALITY OF FOODS WITH MICROENCAPSULATED PROBIOTICS

Microencapsulation has certain consequences for the sensory quality of foods. Particle size influences the texture of foods, but particles with a diameter below 10µm should not affect the mouth feel properties of most foods. The size of probiotic bacteria is typically 14µm. The shape of capsules, on the other hand, determines their flow properties, which is an important factor for industrial processes **(McMaster *et al.*, 2005)**. Moreover, in spray drying, the outlet temperature may affect the color of capsules due to Maillard reaction **(Su *et al.*, 2007).** The organic acid profile of fermented dairy products reflects the metabolic activity of the added bacterial cultures. Additional amounts of acetic and propionic acids produced by the probiotic organisms may cause reduced consumer acceptability of a product. **Adhikari *et al.* (2002)** observed that encapsulation lowered the acetic acid content in yogurt significantly if bifidobacteria were added to the product before fermentation. The lactic acid content was dependent on the strains used. Encapsulated probiotic bacteria have also been reported to lower the pH in yogurt during storage less than free bacteria.

The starch and sodium alginate used in capsular matrix may have an influence on the mouth feel of the product. **Kailasapathy (2006)** observed that while encapsulated bacteria did not affect the color, flavor or aftertaste of a probiotic product, their smoothness showed significant differences.

The probiotic product with encapsulated probiotics was considered more undesirable by a sensory panel. Production of exo-polysaccharide by probiotics and using starch as a filler polymer has been found to help maintain the stability of yogurt gel as well as to increase the water-holding capacity in feta cheese.

SELECTION OF APPROPRIATE TECHNIQUES, MATERIALS, AND BACTERIAL STRAINS

The main challenge in applying microencapsulation of probiotics to new foods to meet consumer interests has to do with finding the appropriate microencapsulation technique, safe and effective encapsulating materials, and

potent bacterial strains. Microencapsulation is expected to extend the shelf life of probiotics at room temperature in various food matrices, increase their heat resistance, improve their compression and shear stress resistance, and enhance their acid tolerance**(Siuta-Cruce & Goulet, 2001).** Environmentally conscious consumers also expect the applied technology to be nature-friendly and avoid the use of hazardous chemicals. Also, aqueous coating systems should be preferred to prevent harmful effects from organic compounds.

A wide variety of potential microencapsulation techniques are already available now-a-days. The use of supercritical carbon dioxide is an interesting recent approach **(Thantsha *et al.*, 2009).** As to the basic techniques, the weak points of spray drying, low survival rates and low stability during storage are tried to overcome by seeking strains that tolerate elevated temperatures, optimizing processing parameters, selecting appropriate drying medium, and using a stabilizing precondition treatment. Effective thermo-protectants are known to enhance the survival of probiotics in spray drying **(Ananta *et al.*, 2005; Saarela, 2007).** Extrusion and emulsion techniques avoid using high temperatures during encapsulation process. By both the methods high survival rates of bacteria are achieved. Because the emulsion techniques are easier to scale up and the size of the beads is smaller, these techniques probably have potential to develop into large-scale technology. For instance, the microporous glass membrane emulsification technique is both simple and easy to scale up **(Song *et al.*, 2003).** The costs of emulsion techniques are, however, increased by the use of vegetable oil **(Krasaekoopt *et al.*, 2003)**.

The selected microencapsulation technique determines the materials used. This means, for instance, evaluating the thermal conductivity properties of food-grade biopolymers and lipids. Probiotic/prebiotic combinations may be among the most important interests in the future. New carrier materials of natural origin, such as shellac and fruit polysaccharides, are also being tested. Special attention needs to be paid to their safety to create consumer confidence, and all raw materials must naturally be of food grade quality. The fact that most food companies in Europe do not use ingredients derived from genetically modified organisms may restrict the use of some products, e.g. maize-derived starches. Bovine Spongiform Encephalopathy (BSE) and foot-and-mouth disease epidemics have reduced consumer confidence in the use of materials of animal origin, e.g. gelatin.

Several criteria are proposed for selecting a preferable probiotic strain for use in health foods. Some of these like the probiotic's tolerance to acid, human gastric juice and bile are facilitated by microencapsulation. Other important concerns include the adherence of the strain to epithelial surfaces and persistence in the human gastrointestinal tract, antagonistic activity against pathogens, antimutagenic and anticarcinogenic properties, and immunostimulation.

Often the persistence in the human gastrointestinal tract is tested *in vitro* in the connection of microencapsulation experiments. More research is needed to confirm that the *invivo* health effects of bacteria retain in microencapsulation processes. The technology also has to consider the large size of the microbial cells (typically 1–4 μm) and particles of freeze-dried culture (even exceeding 100 μm), which demands a large capsule size that influences the textural and sensorial properties of foods. On the other hand, larger microcapsules have been proven to give better protection to bacteria.

COSTS OF MICROENCAPSULATION

Microencapsulation technique is expensive one mainly because of two reasons; first novel encapsulation takes both time and financial resources. Secondly, the microencapsulation phase adds costs to food processing. Since the margins in food ingredients are relatively low, encapsulated end products will have higher prices. The effect may vary greatly depending on the used technique and the volume of the product. Spray chilling, rarely reported for probiotics, is considered the least expensive encapsulation technology. Encapsulation of probiotics using natural biopolymers is often difficult to scale up, and the processing costs are high. Polysaccharides, e.g. alginate, and proteins are expensive to use in spray drying because of their low solubility in water **(Gouin, 2004)**.

On the other hand, cost savings can be derived from easier manufacture of products, lower wastage of bacterial material and better health impact of the product. **Brownlie (2007)** estimates that the price of encapsulated probiotic bacteria may be two or three times that of non-encapsulated probiotics. Nevertheless, despite the extra costs, microencapsulation has profit-making potential, e.g., in markets for higher-value products, in products where microencapsulation is absolutely necessary, and in markets where scale economies can be applied.

FUTURE CHALLENGES

The use of probiotic bacteria in novel foods to provide beneficial health effects is today of increasing interest in the food industry. The process stability of probiotics is, however, not always optimal. Microencapsulation technology can be used to maintain the viability of probiotic microorganism during food product processing and storage. Both true microcapsules with coating as well as microspheres where the probiotic microbes are evenly spread in the coating material are discussed. It is important that encapsulation keeps the probiotics active through the gastrointestinal tract and releases them in their target organ. The survival of microencapsulated cells in simulated gastric conditions is therefore also reviewed. Microencapsulation of probiotic bacteria in foods on an industrial scale faces technological, microbiological, and financial challenges, and also questions linked to consumer behavior. More research data on appropriate technologies, carrier matrices, and bacterial strains is still required in order to promote surviving of bacteria under heat, osmotic and oxygen stresses as well as digestive stress. Probiotic containing food supplies are considered as functional food and their market is continuously growing in several countries. The extra costs incurred by microencapsulation have to be realistically estimated so that they can be minimized.

Future development efforts must also take into account the growing consumer interest in healthy food as well as in ecological aspects.

CONCLUSION

The use of probiotics in food industry is currently expanding from dairy products to other food categories such as juices, energy bars, and chocolate products. In these new products, the environment for probiotic survival is even more challenging than in dairy foods. Microencapsulation has proven one of the most potent methods for maintaining high viability and stability of probiotic bacteria, as it protects probiotics both during food processing and storage as well as in gastric conditions. Besides the polysaccharides traditionally used as a matrix in microencapsulation, starches, gelatin, and milk proteins can also be employed as bead material. New materials are being tested for carrier matrices. One of the major interests in future concerns the use of probiotic/prebiotic (synbiotics) combinations. Another question concerns the coating of capsules, which not only enhances the stability of cells but also increases the capsule size. Techniques for encapsulation are developing, and new industrial-scale methods are being made available. Emulsion technology, in particular, shows many promising applications. Consumer health issues and environmental consciousness deserve special attention in the design of future carrier matrices and technology. Further research on these issues will benefit the development of novel functional food products.

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