Microencapsulation of Probiotics and Its Applications

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Kumar Satya Prakash, Rupesh Chavan, and Vijendra Mishra

Abstract

Probiotics are the live microorganisms which when administered in adequate amounts confer a health benefit on the host. Research has indicated that probiotics provide various health benefits to consumers such as reduction in different forms of diarrhoea, antimutagenic effect, alleviation of lactose intolerance and immunomodulation. But these effects are considered to be strain dependent and also depend on a number of other technological factors. For any organism to be considered as probiotic, it must survive the upper gastrointestinal (GI) passage tract and must remain viable at the site of its action, and it must be able to function in the gut environment. Various techniques have been applied to enhance viability of probiotics, and encapsulation is a method whereby the organism is entrapped in a matrix so that it can withstand passage of GI tract. The chapter provide various aspects of microencapsulation in probiotics.

Keywords

Probiotics • Health benefits • Microencapsulation • Drying

3.1 Introduction

Hippocrates, a Greek philosopher (and father of medicine), introduced the concept that food could serve as medicine. During the last decade, the demand for such foods has increased remarkably because of changes in the lifestyle, eating habits, urbanisation and globalisation. This has led to the shift in eating habits from ordinary food to the foods which besides providing nutrition also promote health benefits, for which the names *func-*

K.S. Prakash • R. Chavan • V. Mishra (✉)
National Institute of Food Technology Entrepreneurship and Management, Plot 97 Sector 56, HSIIDC, Kundli, Sonipat, Haryana, India
e-mail: vijendramishra.niftem@gmail.com

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tional foods, *probiotics*, *synbiotics*, *etc*. have been given (Sharma and Devi 2014).

The word *probiotic* refers to the single or diverse cultures of the live beneficial microorganisms (usually *bifidobacteria*, *lactobacilli* and *streptococci*) which, when consumed, produce a favourable effect on the host by convalescing the properties of the indigenous microflora (Holzapfel et al. 1998). As per FAO/WHO (2002) guidelines, probiotics are the "live microorganisms which when administered in adequate amounts confer a health benefit on the host". The word "probiotic" is derived from Greek words "pro" and "bios" translated as "for life" and was initially used as an antonym of the word "antibiotic" (Hamilton-Miller et al. 2003). It is believed that the word was first used in 1954 by Vergio (Holzapfel and Schillinger 2002). However, there exists sufficient literature that suggests that fermented milk was consumed in India long before for promoting the health benefits. It was at the beginning of this century a Russian Nobel laureate Elie Metchnikoff, at the Pasteur Institute, linked health and longevity with the consumption of fermented milk products. He postulated that the bacillus present could positively modify the bacterial community structure of the colon, thus contributing to human health status (*monograph reprinted in* Metchnikoff 2004; Vasiljevic and Shah 2008).

3.2 Desired Characteristics of Probiotic

The probiotics ought to have superior technological properties accordingly; it can be contrived and unified into food products without losing its viability and functionality and without creating unpleasant flavours or textures, it also must survive the upper gastrointestinal (GI) passage tract and must remain viable at the site of its action, and it must be able to function in the gut environment. In order to check the characteristics, the following tests are carried out to check its effectiveness:

1. Acid and bile salt tolerance
2. Antimicrobial action
3. Adherence properties

3.3 Health Benefit of Probiotics

Probiotics are a group of good bacteria which gives strength to our intestinal function. King et. al. (2003) reported that probiotic might reduce both the duration of illness and the frequency of stools because probiotic prohibits colonisation by the invader and controls the intestinal pH through the release of acetic acid and lactic acid. These bacteria could effectively prevent the constipation and diarrhoea caused by pathogenic bacteria. Saxelin et al. (2003) concluded that the probiotic foods contain living probiotic microorganism in ample concentration, so that the proposed effect is achieved after their ingestion. Parvez et al. (2006) reported that probiotics can be used as food supplement which provides the protection against the gastrointestinal (GI) disorders, viz. infections and bowel syndromes, by favouring and colonising the healthy gut microflora. Some health benefits of probiotics are summarised in Table 3.1.

Kailasapathy and Chin (2000) and Sanders et al. (2007) concluded that probiotics possess therapeutic role as they modulate the immunity, prevent lactose intolerance, lower the cholesterol level and prevent some cancers. Weichselbaum (2009), Patel et al. (2010) and Tuomola et al. (1999) also revealed that probiotics have the possible beneficial effects on our health. He classified the potential beneficial effects of probiotics into three-way action, which are listed:

I. The first effect is due to the immunity modulation within the host, which possess importance as it is responsible for the prevention of infectious disease of GI tract. Probiotics modulate the immune system as a result of products like microbial metabolites, components of the cell wall or deoxyribonucleic acids (DNA), which are recognisable to the host cells due to the presence of particular receptors on the epithelial cells of the gut. The possible interaction between these metabolites and host immune cells via adhesion leads to the immunity modulation.
II. The second mechanism suggests that there exists direct interaction between probiotics and other microorganisms (which may be patho-

Table 3.1 Some of the established effects of probiotics

S. no.	Target health benefit	Postulated mechanism
01	Anti-colon cancer	Deactivation of carcinogenic compounds, inhibition of carcinogen-producing enzymes of colonic microbes, immune modulation, mutagen binding
02	Aid in lactose digestion, resistance to enteric pathogens	Bacterial lactase, secretory immune effect, alteration of intestinal conditions to be less favourable for pathogenicity (pH, SCFAs, bacteriocins), alteration of toxin-binding sites, influence on gut flora populations
03	Allergy	Prevention of antigen translocation into bloodstream
04	Blood lipids, heart disease	Assimilation of cholesterol within bacterial cell, increased excretion of bile salts due to deconjugation by bile salt hydrolase
05	Small bowel bacterial overgrowth	Influence on activity of overgrowth flora, decreasing toxic metabolite production, modification of intestinal environment
07	Crohn's disease	Reduced bowel movements
08	*Helicobacter pylori* infections	Lactic acid production

Source: Sanders and Huisin't Veld (1999), Sharma and Devi (2014)

genic). Here the restoration of the good microorganisms in the gut results in the treatment of the infections like inflammation. This idea is supported by the fact that probiotics have the capacity to compete with the pathogens in terms of their adhesion to the intestines.

III. The third mechanism of action has been summarised as the ability of the probiotics to affect the secondary metabolites (toxins) of the pathogenic microbes and also the host products like bile salts and some food ingredients.

3.4 Microencapsulation

Microencapsulation can be defined as a technology in which the liquids, volatiles (gases) or solid materials (including flavours, enzymes, cells, medicine or other active ingredients) are packed within a continuous polymer film, so that the contents are released at predictable and controlled manner under specific conditions (Desai and Park 2005). Microencapsulation dates back to the early 1930s, and this technology has boosted during the past decade due to the advancement in technologies and modifications in the entrapping materials. Champagne and Fustier (2007) found that there are various points to be taken into consideration while microencapsulating probiotic cells such as strain of probiotics, their beneficial effects on host when reached in gastrointestinal (GI) tract, quantity required to give beneficial effects and viability of probiotic cells during processing, storage and sensory properties of product.

3.4.1 Beneficial Effects of Microencapsulation

Microencapsulation has resulted in various advantages towards the probiotics like prevention against the bacteriophages, contamination, etc. Steenson et al. (1987) found that when the probiotics were encapsulated into the matrix of alginate beads, the bacteriophage attack was avoided because of the small pore size. Kearney et al. (1990) demonstrated that the survivability of the *L. plantarum* in alginate beads was enhanced by 30 % after lyophilisation. Similar results were reported by Kim and Yoon (1995); they found that the survivability of the probiotic cultures was enhanced up to 40 % when encapsulated in the calcium-alginate beads after of the milk.

Several reports have shown that the stability of the probiotic cultures can be increased by encapsulation, for example, the probiotic bacteria when encapsulated in the gel matrix of calcium

alginate showed much stability during the storage (Sheu and Marshall 1993; Kebary et al. 1998). Woo et al. (1999) reported that the survival of the *B. longum* when entrapped into the matrix of 3 % alginate and 0.15 % xanthan gum enhanced. Similarly, the survivability of the bacterium *L. casei* when entrapped in the chitosan-coated alginate beads confirmed higher survivability upon storage (Koo et al. 2001). Microencapsulation also prevents the probiotics from the harsh environment of gastric solution. Several studies have shown that the survival of the probiotic strains like *L. casei*, *B. longum*, *L. gasseri*, *L. acidophilus*, *B. bifidum*, *B. infantis*, *B. breve* and *B. pseudolongum* inside the gastric environment enhanced when entrapped within the wall materials like chitosan and alginates in comparison to the non-entrapped cells (Rao et al. 1989; Urbanska et al. 2007; Chávarri et al. 2010).

Soma et al. (2009) reported an increase in the number of *Lactobacillus acidophilus* ATCC 43121 by just about 3 logs, after they were entrapped within the beads of xanthan–chitosan–xanthan network. Other researchers reported similar results when they encapsulated the strains of *L. rhamnosus* and *B. longum* with a blend of gellan and xanthan gum (Jiménez-Pranteda et al. 2009; Ding and Shah 2007). Kharter et al. (2010) and Zou et al. (2011) carried out research on the some strains of the LAB and bifidobacteria after encapsulating them in chitosan–alginate beads and alginate-maize resistant starch; they concluded that protection against gastric juices increased to a greater extent.

3.4.2 Methods of Microencapsulation

In microencapsulation a thin layer coating is done. Different techniques have been developed so far for the successful entrapment of the probiotic cells which have been classified into three categories, viz. (1) physico-mechanical, (2) physico-chemical and (3) chemical methods.

The different types of encapsulation techniques and the wall materials have been listed in Figs. 3.1 and 3.2, respectively. In physical

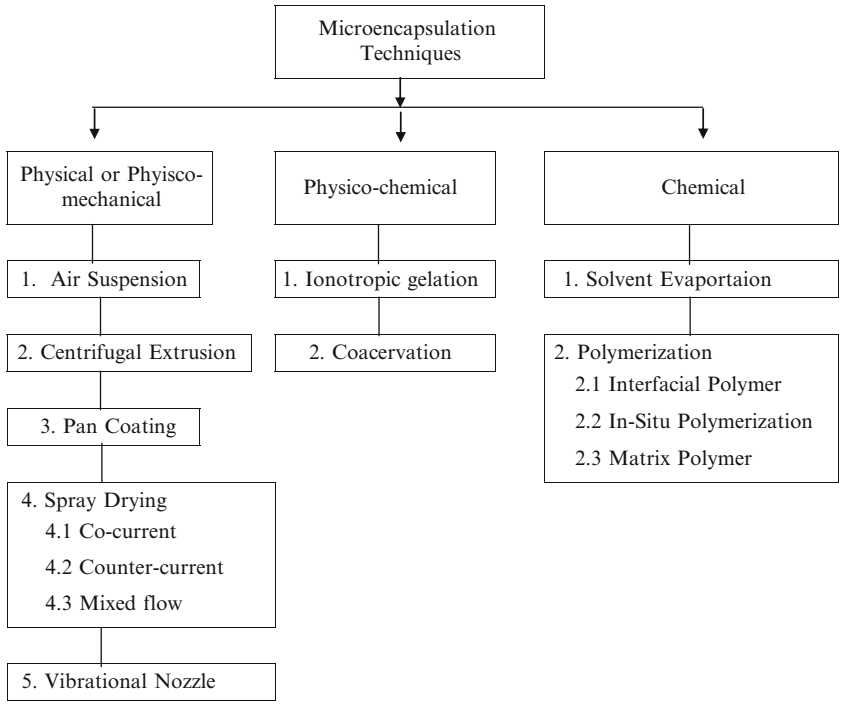

Fig. 3.1 General overview of encapsulation techniques

methods there is conversion of liquid phase into solid immobile phase in the form of powder, in which the probiotic is entrapped. The probiotics encapsulated in this method are released when the powder gains moisture, while in the case of chemical methods, there is the formation of the hydrocolloidal gel phase via cross-linking or chemical reactions. Here the probiotics are released only when there is change in the pH or the ionic conditions of the wall material change.

3.4.2.1 Spray-Drying

It (Fig. 3.3) is the most widely used stabilisation procedure in food/pharma/chemical industries due to high throughput and low costs. During spray-drying, a matrix material is first dissolved in the continuous phase which surrounds the probiotic cells inside the spray droplet. This solution is rapidly atomised in heated air and shrinks surrounding the cells in an envelope. Petrovic et al. (2007) observed that in spray-drying technique, the centrifuged cell mass of probiotic bacteria is dispersed in suitable encapsulating material like any food polymeric solution, gums (hydrocolloids), modified starches, non-gelling proteins and dextrin to form an emulsion or dispersion. These encapsulating materials give the protective coating to the central probiotic bacteria and

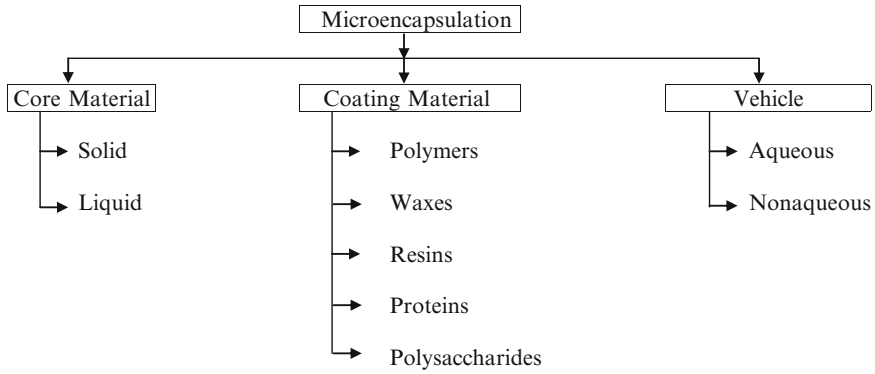

Fig. 3.2 Type of core materials, coating materials and vehicles used in microencapsulation

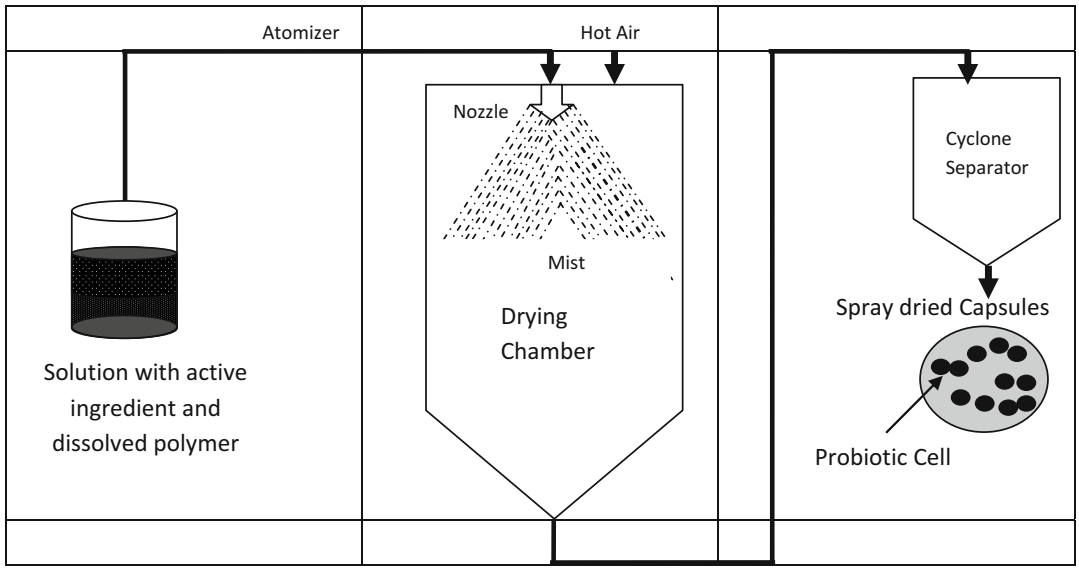

Fig. 3.3 Outline of spray-drying for probiotics

provide oxygen barrier. Fu and Etzel (1995) studied that encapsulation by spray-drying is relatively cheap and can be operated on large scale and on continuous mode, but the disadvantage of spray-drying is that there is huge loss in viability of probiotic bacteria because of dehydration and high inlet temperature, but by adjusting the proper inlet and outlet temperatures, the viable count of encapsulated cells can be obtained (Kailasapathy 2002). Crittenden et al. (2006) used protein–carbohydrate–oil film for the successful encapsulation of the *B. infantis* by spray-drying at an inlet and outlet temperatures of 160 and 65 °C, respectively. Some of the researchers were successful even encapsulating the probiotics at higher temperatures like Zhao et al. (2008) found that strains of *L. acidophilus* when encapsulated with gum acacia and cyclodextrin at inlet and outlet temperatures of 170°C and 90 °C, respectively, the post spray drying survival of the encapsulated *L. acidophilus* was 1.50×10^{9}c.f.u./ml and the survival after 8 weeks of storage at 4°C, was more than 10^{7}c.f.u./ml. Ying et al. (2011) found that encapsulated *L. rhamnosus* by spray-drying in the matrix of resistant starch and whey protein had a superior storage stability in contrast to freeze-drying technique.

Jantzen et al. (2013) observed that the spray-drying of probiotic *L. reuteri* with whey at outlet temperature (55 ± 2 and 65 ± 2 °C) and viability of microencapsulated probiotics has decreased by 2 log cycles after drying. Pereira et al. (2014) found that the spray-dried probiotic cashew apple juice showed levels of *L. casei* NRRL B-442 viable cells higher than the recommended minimum level for probiotic bacteria after the drying process; it is indicating that the spray-drying can be used for producing probiotic cashew apple powder.

3.4.2.2 Freeze-Drying

It is a drying process in which the water is removed from the food system/matrix in the form of vapours via sublimation process. In freeze-drying the food material is subjected to freezing under vacuum. The reduced temperature freezes the free water and low pressure results in the conversion of this ice into vapours directly. The process has been classified into three stages which include:

(a) Freezing
(b) Primary drying
(c) Secondary drying

In case of microencapsulation by freeze-drying, both core and the wall (i.e. probiotics and entrapping material) along with the water present in the matrix are frozen to temperature as low as −20−−90 °C and then dried by direct sublimation process under reduced pressures, i.e. below 4.7 mmHg. As freeze-drying occurs at both reduced temperature and pressure, a porous and unshrinking edifice culture of the probiotics is obtained. Although freeze-drying, being the nonthermal process, has emerged as a most standard technique for the drying process in the microbiological industries, there occurs some loss in cell viability, and also the energy consumption is very high as compared to the spray-drying technique (30–50 times more expensive). In order to prevent the loss during the freezing and to improve the survivability of the probiotics, the demand for the cryoprotectants (like proteins, maltodextrins, etc.) has increased (Gharsallaoui et al. 2007; Morgan et al. 2006). Carvalho et al. (2004) reported that the probiotic cultures of the strains of *L. bulgaricus* survived for over 10 months at −20 °C when sorbitol and fructose were used as cryoprotectants during freeze-drying. The various cryoprotectants used for the freeze-drying of the probiotics are listed in Table 3.2.

Table 3.2 List of cryoprotectants used in freeze-drying of probiotics (Krasaekoopt 2012)

Microorganism	Cryoprotectant	References
L. acidophilus, *B. bacterium*	Skim milk	Goderska and Czarnecki (2008)
L. rhamnosus	Glycerine	Savini et al. (2010)
L. casei	High-resistance maize starch and inulin	Babu et al. (2011)

3.4.2.3 Spray-Freeze-Drying

It is a drying technique which involves the spraying of a liquid solution into a freezing medium like liquid nitrogen, resulting in the freezing of water within it. After that the frozen material is subjected to conventional vacuum freeze-drying for the removal of water via sublimation process (Kailasapathy 2009; Semyonov et al. 2010). This process has advantages that the size of the product is uniform and controlled with larger surface area as compared to the spray-dried powder. Dolly et al. (2011) encapsulated *L. plantarum* by spray-freeze-drying using whey protein as an encapsulating agent; they found that the final product has 20 % higher viability as compared to the spray-dried product. They also demonstrated that it had more tolerance to acidic conditions.

3.4.2.4 Extrusion Technique

Extrusion is a more laborious, simple, economic but less hazardous method of encapsulation and consists of projecting a solution consisting of the encapsulation matrix and the core materials through a spout at a reasonably high pressure. The resultant droplets free-fall from the nozzle into a hardening solution (Krasaekoopt et al. 2003; Lakkis 2007). Capsule size is influenced by the nozzle size (De Vos et al. 2010). If droplet formation is performed in a controlled environment, this technique is referred to as prilling. To achieve this control, the jet nozzle is subjected to pulsation (Burgain et al. 2011). Some of the main technologies to ensure fluid dispersion into droplets and subsequent capsules are coaxial airflow, use of an electrostatic field, jet-cutting and spinning disc atomisation (Whelehan and Marison 2011; Cook et al. 2012). Due to the gentle operation of the extrusion process and the fact that deleterious solvents are not required, high cell viability can be maintained (Burgain et al. 2011; Krasaekoopt et al. 2003). Also the viscosity of the fluid used does not limit production of capsules (Lakkis 2007). Extrusion can be performed under both the aerobic and anaerobic conditions, an advantage when using the predominantly oxygen-sensitive LABs and bifidobacteria.

3.4.2.5 Emulsion Method

It is a type of chemical technique, employed for the encapsulation of probiotic cells on the basis of interaction between the continuous phase, i.e. vegetable oil (corn oil, sunflower oil, etc.) or mineral oil, and discontinuous phase, i.e. cell-polymer suspension. Following homogenisation to generate a water-in-oil emulsion, the water-soluble polymer is cross-linked to form small-gel particles within the continuous oil phase. Microencapsulation can be down in three steps: in the first step we disperse droplets with the emulsification, in the second case we add calcium chloride ($CaCl_2$), and in final step we got encapsulated probiotic. To make the emulsification process more effective, some emulsifiers may be added like Tween 80 (Krasaekoopt et al. 2003). Figure 3.4 depicts diagrammatically the process of emulsification. Oliveira et al. (2007) demonstrated that *L. acidophilus* and *B. lactis* when entrapped within the matrix of pectin–casein complex by emulsion technique produced a higher viability at 7 °C storage temperature; however, protection against the low pH conditions was not up to the mark.

3.5 Importance of Microencapsulated Probiotics in the Ready-to-Eat Foods

The incorporation of the probiotics into the ready-to-eat foods is not a new concept, but the concept has advanced to a greater extent in recent years due to the emergence of new techniques and the identification of new probiotic strains. The various foods that can be used as a carrier for probiotics include frozen dairy desserts, yogurt, cheese, certain fruit juices, etc. And as per the WHO guidelines, the minimum level of the probiotic microorganisms at the time of consumption shall be above the minimum level, i.e. 10^7 cfu/g, and also the probiotic should not change the sensory quality of food.

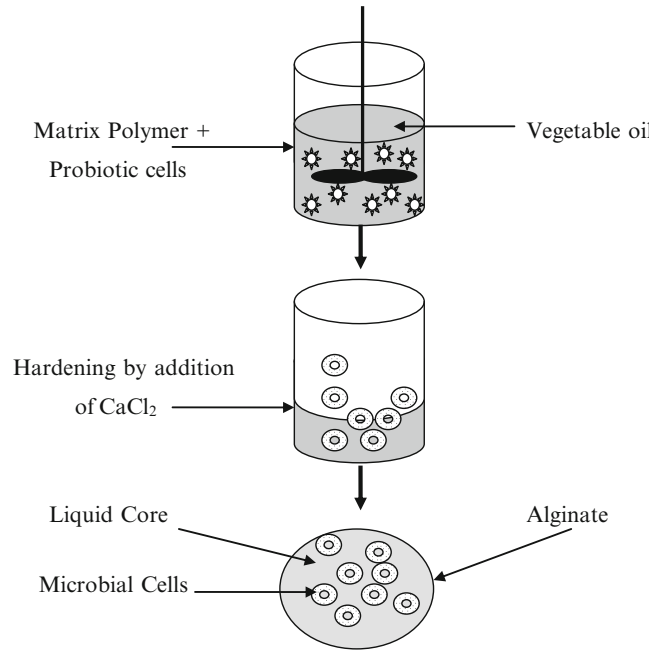

Fig. 3.4 Diagrammatic representation of emulsion method

Table 3.3 List of some strains subjected to encapsulation in yogurt preparation (Krasaekoopt 2012)

Probiotic strain	Microencapsulation technology	Materials	References
L. acidophilus	Emulsification	Alginate–starch	Sultana et al. (2000)
B. longum	Emulsification	k-Carrageenan	Adhikari et al. (2003)
L. acidophilus and B. infantis	Emulsification	Alginate–starch	Godward and Kailasapathy (2003)
B. breve	Emulsification	Milk fat and whey protein	Picot and Lacroix (2003)
L. acidophilus	Extrusion	Ca-alginate and chitosan	Iyer and Kailasapathy (2005)
L. acidophilus and L. casei	Emulsification	Alginate	Capela et al. (2006)
L. acidophilus	Emulsification	Alginate–starch	Kailasapathy (2006)
L. acidophilus	Extrusion	Alginate–chitosan	Urbanska et al. (2007)
L. casei	Extrusion	Alginate–pectin	Sandoval-Castilla et al. (2010)

3.5.1 Yogurt

Yogurt is made from whole or partly defatted milk with small quantity of skim milk powder. The two microorganisms *L. bulgaricus* and *Streptococcus thermophilus* that grow together symbiotically are responsible for conversion of lactose to lactic acid. The therapeutic value of the yogurt can be enhanced by the incorporation of the probiotic cultures into it (Weichselbaum 2009). As yogurt has lactic acid that reduces its pH to around 4.2–4.6, which makes it a poor carrier for probiotics, there has been some study in which encapsulated cultures of probiotics were incorporated into the yogurt (Table 3.3) and their survival was much better (Kailasapathy 2009). But the problem arises in the low acetic acid production which is responsible for the characteristic flavour in yogurt. Adhikari et al. (2003) produced yogurt that had encapsulated *bifidobacteria*, but the problem in the stirred yogurt arose attributed to its poor sensory quality because of

the development of grainy/sandy texture that reduced its consumer acceptance. Sultana et al. (2000) encapsulated the probiotic cells with prebiotic resistant starch and cryoprotectants (glycerol) to improve their viability. Krasaekoopt et al. (2011) demonstrated that encapsulated *L. acidophilus and L. casei* in the matrix of galactooligosaccharides produced the best protection against the simulated gastric (pH 1.55) and intestinal juice (0.6 % bile salt). They also concluded that the viability was higher as much as 0.8 logs in entrapped probiotics. Many other studies have confirmed the use of co-encapsulation (i.e. use of two different probiotic cultures) with a probiotic like raftilose, inulin, etc. (Iyer et al. 2005).

3.5.2 Cheese

Cheddar cheese has emerged as an excellent carrier for the probiotics because of its high pH, i.e. 5.5, high fat content (33 %) and excellent buffering activity. These properties protect the probiotics from the harsh environment in the stomach and intestines. Some studies have shown that the survivability of the *bifidobacterium* when used as probiotic in cheese extended up to 24 weeks; however, the cell number reduced significantly, which was attributed to the reduced temperature (around 7 °C) and decreased lactose content. Although the sensory properties remained unaffected (Dinakar and Mistry 1994; Stanton et al. 1998), Ozer et al. in 2009 demonstrated that the sensory quality of the cheese (white brined and kasar) remained unchanged when the entrapped probiotics *L. acidophilus and B. bifidum* were added into the cheese.

3.5.3 Fruit Juice

The consumption of fruit juices is increasing day by day and has already covered a large population already round the globe. So, the idea of using fruit juices as a carrier for encapsulated probiotics has flourished in recent years and has gained attention both for researchers and industries. Tourila and Cardello (2002) suggested that fruit juices could be used as a carrier for the probiotics. Yoon et al. (2004) developed a probiotic drink of tomato juice in which the probiotic cells *L. acidophilus* grow within the fruit juice. They reported this fermented juice could be used by the consumers having allergy to milk and milk products. Although probiotication reduced the sensory properties of the juice, Suomalainen et al. (2006) reported that the survivability of the *L. rhamnosus* was improved to a greater extent in the GI tract after mixing whey in the orange juice. Krasaekoopt et al. (2010) assessed the quality parameters of the fruit (orange and grape) juices containing the encapsulated probiotics within the matrix of the alginate beads with chitosan coating. They reported that the consumer acceptance was about 85 %. Krasaekoopt and Tandhanskul (2008) and Sohail et al. (2011) used alginate and calcium chloride as wall materials for the encapsulation of the probiotic cultures *L. casei*, L. *rhamnosus and L. acidophilus*; they concluded that survivability of the probiotic cells in the commercial fruit juice (orange) at low temperatures (4 °C) was enhanced by about 4 logs. Additionally, there were no changes in the pH of the juice upon storage for around 35 days.

3.5.4 Miscellaneous Foods

There exist many other foods that can be used as a carrier medium for the encapsulated probiotics like chocolates, bakery items and meat products. Muthukumarasamy and Holley (2007) successfully developed the fermented meat sausages containing the encapsulated probiotic bacteria cells. However, it was found that the probiotics were not able to inhibit the activity of the *E. coli*. Maillard and Landuyt (2008) encapsulated the probiotic cells by spray coating to be used in the chocolate. They concluded that the survivability in the chocolate was much higher (approximately three times) as compared to the dairy products. Lahtinen et al. (2007) demonstrated that the fat content in the chocolate acts as a shield for the probiotics towards the harsh conditions.

3.6 Conclusion

Microencapsulation has emerged as a wonderful technique to shield the sensitive food components and increase the survivability of the probiotic cells until they reach the target organ. The encapsulated materials are very easy to handle and their efficiency depends upon the encapsulating technique and wall materials employed. Although various encapsulating techniques, wall materials, and probiotic strains have been identified, most of them have limited usage because of the scaleup problems.

This chapter gives a brief outline of the probiotic cultures commonly employed along with the brief emphasis on the different techniques and foods employed for encapsulation of the probiotics. A lot more needs to be done to develop techniques which are economically feasible, and also the final size of the product is minimised as it is inversely related to the stability of the product.

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