MICROENCAPSULATION OF PROBIOTIC BACTERIA OF AVAILABLE TECHNIQUES, FOCUSING ON BIOMATERIALS - A REVIEW

Santosh Chopde*, Nilkanth Pawar, Vijay Kele and Sudhakar Changade
College of Dairy Technology,
Udgir, Latur-413 517, India

Received: 16-05-2014
Accepted: 30-08-2014

ABSTRACT

In the recent past, there has been a rapid increase in the popularity of probiotic health-based products. Benefits of probiotics to human and animal health have been proven in many of scientific research. Survival and stability of probiotic organisms in functional foods have been a major concern, because a high number of organisms are needed to confer health benefits to consumers. Unfortunately, many processing (heating, freezing) and storage conditions (redox level, pH) used in dairy technology are detrimental to the viability of the probiotic bacteria. Providing probiotic living cells with a physical barrier against adverse environmental conditions is therefore an approach currently receiving considerable interest. Microencapsulation of the probiotic cells is one of the highly efficient methods, which is now under the special consideration and is being developed by various researchers. This increase has been the result of several factors, but the primary factor has been the increased awareness on the part of the food industry of the real advantages offered by encapsulated ingredients. This review focuses on probiotics microencapsulation, its methods and biomaterials utilized for encapsulation.

Key words: Biomaterial, Microencapsulation, Probiotics

The term probiotic is derived from two Greek words which literally means “for life”. Probiotics are microorganisms which settle in the intestine and render healthful effects on the host (humans or animals), substantially via maintenance and improvement of the microbial balance (between the healthful and harmful microorganisms) of the intestine (Gismondo et al. 1999). The most commonly used definition of probiotics is “probiotics are live microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance” (Fuller 1989). Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) define probiotics as “Live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host” (FAO/WHO, 2001). The probiotic microorganisms consist mostly of the strains of the genera Lactobacillus and Bifidobacterium, but strains of Bacillus, Pediococcus and some yeasts have also been found as suitable candidates.

Because of their perceived health benefits, probiotic bacteria have been increasingly included in fermented dairy products, including yoghurts, soft-, semi-hard and hard cheeses, ice cream and frozen fermented dairy desserts (Stanton et al., 2005). Food, particularly dairy products are considered as an ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract (Ross et al., 2002).

Various health benefits have been attributed to probiotics such as anti-mutagenic and anti-carcinogenic properties, anti-infection properties immune system stimulation, serum cholesterol reduction, alleviation of lactose intolerance and nutritional enhancement (Mombelli et al., 2000). 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 et al., 2000). In order to provide health benefits for probiotic

*Corresponding author e-mail: Santosh.der@gmail.com
bacteria (Shah, 2007) it has been recommended that they must be present at a minimum level of \(10^6\) CFU/g of food product or \(10^7\) CFU/g at point of delivery or be eaten in sufficient amounts to yield a daily intake of \(10^8\) CFU (Lopez-Rubio et al., 2006).

Probiotic survival in products is affected by a range of factors including pH, post-acidification (during storage) in fermented products, hydrogen peroxide production, oxygen toxicity (aerobic conditions), storage temperatures, stability in dried or frozen form, poor growth in milk, lack of proteases to break down milk protein to simpler nitrogenous substances and compatibility with traditional starter culture during fermentation (Shah, 2000). This has encouraged researchers to find new efficient methods of viability improvement. Different approaches that increase the resistance of these sensitive microorganisms against adverse conditions have been proposed, including appropriate selection of acid- and bile-resistant strains, use of oxygen impermeable containers, two-step fermentation, stress adaptation, incorporation of micronutrients such as peptides and amino acids, and microencapsulation (Gismondo et al., 1999).

Microencapsulation is a powerful technology which has been developed for use in the food industry and allows the protection of bacterial cells (Fig. 1) (Borgogna et al., 2010). Microencapsulation is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influences of specific conditions (Anal et al., 2006). From a microbiological approach, microencapsulation can be defined as the process of entrapment/enclosure of microorganisms by means of coating them with proper hydrocolloid(s) in order to isolate the cells from the surrounding environment; in a way that results in appropriate cell release in the intestinal medium (Picot et al., 2003a).

The technology of microencapsulation of probiotic bacterial cells has evolved from the immobilized cell culture technology. Its importance, as an efficient manner for increasing probiotics viability, justifies reviewing the achievements of this technology. The present article reviews principles and methods of probiotics microencapsulation including discussions of microcapsule structure and components used for microencapsulation.

**From immobilization to microencapsulation:** The technology of microencapsulation of probiotic bacterial cells has evolved from the immobilized cell culture technology used in the biotechnological industry to sophisticated and precise microcapsule formation. While encapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained within the capsule wall as a core of encapsulated material, immobilisation refers to the trapping of material within or throughout a matrix. Entrapment of cells in a gel matrix of alginates is the most popular system of immobilisation reported (Champagne et al., 1994). Compared to immobilisation/entrapment techniques, microencapsulation has many advantages.

- The bacterial cells are retained within the microcapsules.
- There is no solid or gelled core in the microcapsule and its small diameter helps to reduce mass transfer limitations.
- The nutrients and metabolites can diffuse through the semi permeable membrane easily.
- The membrane serves as a barrier to cell release and minimizes contamination.

**Benefits of microencapsulation:** Microencapsulation has proven one of the most potent methods for maintaining high viability and stability of probiotic
bacteria. The encapsulated core material is released by several mechanisms such as mechanical rupture of the cell wall, dissolution of the wall, melting of the wall and diffusion through the wall (Franjione et al., 1999). It will be used as tools to encapsulate both prebiotic ingredients and probiotic bacteria within the same capsule to enhance growth and multiplication of these bacteria through symbiotic effects when they are released in the gastro-intestinal tract. Micro-encapsulation confers protection to sensitive probiotic lactic acid bacteria from oxygen (Sunohara et al., 1995), freezing (Shah and Rarula, 2000) and acidic conditions during manufacture and storage (Adhikari et al., 2000) and gastrointestinal transit (Lee and Heo, 2000) 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 et al. 2000), method of encapsulation and wall material used (Muthukumarasamy et al., 2006). In dairy industry, microencapsulation has been applied to improve survival and delivery of bacterial cultures.

**Biomaterial for microencapsulation:** Biomaterial is “any natural material or not, which is in direct contact with a living cells and is intended to act with biological systems”. The biomaterials used for probiotics encapsulation include natural polymers and synthetic polymers (Gentile et al., 1995). Coating material stabilizes core ingredients, they are inert toward active ingredients, they control release under specific condition, they are economical, flexible, non hygroscopic, tasteless, stable and soluble in aqueous media or solvent (Campos et al., 2011). Various biopolymers have been utilized for coating probiotic cells. Issues involved when selecting biomaterials for probiotics encapsulation are: physicochemical properties (chemical composition, morphology, mechanical strength, stability in gastric and intestinal fluids); toxicology assay; manufacturing and sterilization processes. Typical biomaterials used for the purpose of probiotic encapsulation include: alginate, carrageenan, gelatin, chitosan and cellulose acetate phthalate (Gbassi et al., 2012).

**Alginate:** Alginate gels are known to be insoluble in acidic media (Harnsilawat et al., 2006). The success of the use of alginate in microencapsulation of probiotics is due to the basic protection against acidity it provides to the cells (Gbassi et al., 2009). It is a linear polymer of heterogeneous structure composed of two monosaccharide units: acid a-L-guluronic (G) and acid β-D-mannuronic (M) linked by β(1-4) glycosidic bonds (Dong et al., 2006). The M/G ratio determines the technological functionality of alginate. The gel strength is particularly important that the proportion of block G is high.

**Carrageenan:** The use of carrageenan in microencapsulation of probiotics is due to its capacity to form gel that can entrap the cells. Carrageenan is a natural polysaccharide that is extracted from marine macro-algae and is commonly used as a food additive. Three types of carrageenan are known: kappa (κ) carrageenan, iota (i) carrageenan and lambda (λ) carrageenan. κ-carrageenan (monosulfated) and ê carrageenan (bisulfated) have an oxygen bridge between carbons 3 and 6 of the D-galactose. This bridge is responsible for conformational transitions. The ê-carrageenan (trisulfated) that does not have this bridge is unable to gel. Carrageenan gelation is induced by temperature changes. A rise in temperature (60 to 80 °C) is required to dissolve it and gelation occurs by cooling to room temperature (Mangione 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 et al., 2007). The ê-carrageenan beads for probiotic encapsulation can be produced using extrusion as well as emulsion techniques.

**Whey proteins:** Whey proteins are usually used because of their amphoteric character. 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 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).

**Gelatin:** Gelatin is a protein derived by partial hydrolysis of collagen of animal origin. 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 (Anal et al., 2007).

**Chitosan:** Chitosan is a positively charged polysaccharide formed by deacetylation of chitin. Its solubility is pH-dependent. It is water insoluble at a pH higher than 5.4. This insolvibility presents the drawback of preventing the complete release of this biomaterial into the gut which pH is greater than 5.4 (Huguet et al., 1996). However, studies have reported the effectiveness of chitosan as a coating agent of alginate gel beads (Krasaekoopt et al., 2004). 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 (Chavarri et al., 2010).

**Cellulose acetate phthalate (CAP):** CAP is widely used as a coating agent. It 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 CAP provides good protection for microorganisms in simulated GI conditions (Favaro-Trindade et al., 2002).

**Structure of microcapsule:** A microcapsule consists of a semi-permeable, spherical, thin and strong membrane surrounding a solid or liquid core, with a diameter varying from a few microns to 1 mm (Anal et al., 2007). The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called shell, coating, wall material, biomaterial or membrane. Each microcapsule consists of hydrocolloids coated around the bacterial cell(s). The coating serves as a barrier to cell release and minimizes contamination. The coating may also be designed to open in the specific areas of the body. It can therefore be used that is able to withstand acidic conditions in the stomach acids and allows those active ingredients to pass through the stomach (Anal et al., 2005).

**Common techniques for microencapsulation of probiotics:** Probiotic microencapsulation requires techniques that are gentle and non-aggressive towards the cells. Most of the reported literature on probiotic microencapsulation is based on small-scale laboratory procedures. The first encapsulation techniques developed to improve the shelf-life of probiotics were to transform cells cultures into concentrated dry powder. The techniques of spray-drying, freeze-drying or fluidized bed drying have shown their limitations because the cells encapsulated by these techniques are completely released into the product. However, probiotics in dried or freeze-dried form exhibit compatibility with traditional starter culture such as milk or cheese and have a longer shelf-life compared to their cell slurry form (Rokka et al., 2010). In general, three precautions need to be considered for developing microcapsules: formation of the wall around the material, ensuring that leakage does not occur and ensuring that undesired materials are kept out. The most common techniques used in microencapsulation of probiotics are spray drying, emulsion and extrusion.

**Spray drying:** Because of its low cost, rapidity and available equipment spray drying is a commonly used method of encapsulation in the food industry (Picot et al., 2004). Spray-drying of active agent is commonly achieved by dissolving, emulsifying, or dispersing the active in an aqueous solution of carrier material, followed by atomization and spraying of the mixture into a hot chamber (Fig. 2 and Gharsallaoui et al. 2007). During this process a film is formed at the droplet surface, thereby retarding the larger active molecules while the smaller water molecules are evaporated. Finally, a porous, dry particle is formed. The minimum air inlet temperature reported in the literature for probiotic encapsulation is 100 °C while the maximum is 170 °C. Generally the droplet dries at wet bulb temperature of drying.
gas/air. 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 (Hsiao et al., 2004). The spray drying process is controlled by means of the product feed, gas flow and temperature. 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 thermo-protectant. The technique is highly reproducible and the most important is that it is suitable for industrial applications. Conventional spray-dried encapsulates release their active agent immediately upon addition to water (which may also depend on the porosity of the particles). Recent introductions of more hydrophobic and/or crosslinked carrier materials may provide a more gradual release upon dilution in water. Examples of these are denatured proteins, cross-linked proteins or cross-linked biopolymers.

**Emulsion:** Emulsions are kinetically rather than thermodynamically stable two-phase systems and ultimately, both the continuous and dispersed phase will separate. It is a chemical technique to encapsulate probiotic living cells and use hydrocolloids (alginate, carrageenan and pectin) as encapsulating materials (Fig. 3). The principle of this emulsion technique is based on the relationship between the discontinuous and continuous phase. Proper formulation design of both phases and the interface, including choice of ingredients like emulsifiers, might prevent the separation of non-miscible fluids (Appelqvist et al. 2007). The emulsion technique involves the dispersion of an aqueous phase containing the bacterial cells and polymer suspension into an organic phase, such as oil, resulting in water in oil emulsion. The dispersed aqueous droplets are hardened by cooling or by addition of a gelling agent or a crosslinking agent in the case of polyacrylamide gels. Following gelation, the beads are partitioned into water and washed to remove oil. The emulsion technique results in smaller diameter beads, and is better suited to scale up applications. This type of probiotics has been successfully applied to yoghurt cheddar cheese, ice cream (Adhikari et al., 2002).

**Extrusion:** It is a simple and cheap method that uses a gentle operation which causes no damage to probiotic cells and gives high probiotic viability. The technique involves preparing a hydrocolloid solution, adding the probiotics ingredient to the solution and dripping the cell suspension through a nozzle spray machine in the form of droplets which are allowed to fall freely into a hardening solution (Fig. 4). 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. 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 (Krasaeekoopt 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 et al., 1990). Mass production of beads can either be achieved by multi-nozzle systems, rotating disc atomizers or by the jet cutting technique (Heinzen, 2002). Most of the literature reported on the encapsulation of probiotic bacteria has used the emulsion technique to produce small amount of capsules.

**CONCLUSION**

The technology of microencapsulation has developed from a simple immobilization or entrapment to sophisticated and precise micro capsule formation. The use of microencapsulated probiotics for controlled release applications is a promising alternative to solving the major problems of these organisms that are faced by food industries. The delivery of viable micro encapsulated probiotic bacteria will become important in the near future. In the future multiple-delivery technique may be developed, such as co-encapsulating prebiotics and probiotics as well as nutraceuticals, thus a new area of more complex nutritional matrices will need to be explored. New food regulations may specify labelling including the strains and the number of viable probiotic bacteria at the end of shelf life of a food or supplement claimed to be probiotic.

**REFERENCES**


