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SCIENTIFIC PAPER

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PROTECTION OF PROBIOTIC MICROORGANISMS BY MICROENCAPSULATION

Probiotic bacteria are used in the production of fermented dairy foods, pharmaceutical products and health supplements. They play an important role in promoting and maintaining human health. In order, to produce health benefits probiotic strains should be present in a viable form at a suitable level during the product's shelf life until consumption and maintain high viability throughout the gastrointestinal tract. Despite the importance of these beneficial microorganisms many investigations have shown their poor viability and stability, especially for bifidobacteria in fermented products. The introduction of microencapsulation techniques for protection of probiotic strains has resulted in greatly enhanced viability of these microorganisms in food products as well as in the gastrointestinal tract.

This paper gives an overview of available microencapsulation techniques and materials for probiotic protection and stabilization. Several methods of microencapsulation for probiotic bacteria, including extrusion, emulsification, drying (fluidized bed, spray, freeze) and spray coating techniques, are presented. The commonly used supporting materials like alginate, starch, chitosan, gelatin, waxes, biogums, and some others are also discussed.

Key words: Probiotics, Stabilization, Microencapsulation, Techniques, Carriers.

The application of microorganisms called probiotics has been a fast growing trend in production of functional foods and pharmaceutical products. Probiotics are defined as live microbes which can act beneficially in host by improving its gastrointestinal microbial balance [1]. Several positive effects of probiotics have been proposed including antagonism towards intestinal pathogens [2], lactose maldigestion, treatment of acute rotavirus diarrhea [3,4] constipation, inflammatory bowel disease [5] as well as food allergy. The majority of probiotic strains belong to two genera: *Lactobacillus* and *Bifidobacterium*. Good selection criteria, and molecular and functional characterisation of probiotics are required in order to achieve consistent and positive probiotic effects [6,7,8].

There is general agreement that foods containing probiotic bacteria should contain at least 10^6 live microorganisms per g or ml at the time of consumption, in order to produce therapeutic benefits [9]. Microencapsulation has previously been used as a technology that can provide protection to sensitive probiotic cultures, improving their stability and viability in food products and performing target delivery in the gastrointestinal tract. The viability of *Bifidobacterium pseudolongum* and *B. longum* in simulated gastric environment was improved using encapsulation techniques [10,11]. Microencapsulated probiotic bacteria showed protection from freezing and freeze–drying. A higher stability was reported for *Lactobacillus acidophilus* and *B. longum* entrapped in alginate beads compared with free cells during storage of dairy desserts [9]. Immobilization in alginate also improved the survival of *L. bulgaricus* in a milk–based dessert [12]. Cell encapsulation also extended the storage life of probiotic in fermented dairy products. The survival of *B. breve* entrapped in whey proteins were significantly higher then those of free cells after 28 days in yoghurt stored at 4°C, but no effects was achieved for *B. longum* [13]. The encapsulation was effective in the protection of *B. bifidum* and *B. infantis* cells immobilized in alginate and incorporated into mayonnaise [14]. In addition, the preliminary investigation showed protective role of microencapsulation against oxygen toxicity during storage of yoghurt [15]. Furthermore, it was found that encapsulated lactobacilli in calcium–alginate beads improved their heat tolerance [16] as well as prolong the viability of a spray–dried *B. ruminantium* during storage [17].

Several methods of microencapsulation of probiotic bacteria have been reported including extrusion, emulsion, drying (fluidized bed, spray, freeze) and spray coating techniques. The most commonly used carrier materials are alginate, kappa–carragenan, starch, hitosan, gelatin, gellan gum, lipids, whey protein, waxes, such as carnauba, and biogums, including xanthan, guar gum and locust bean gum. This paper is discussing the state of the art of the encapsulation techniques and materials used for probiotic protection. Additional information can be found in recent reviews on this topic (18,19)

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ENCAPSULATION TECHNIQUES

Microencapsulation is defined as a process for packaging sensitive material in specialized semipermeable polymer membranes by producing capsules or particles, which can release their content at controlled rates under specific conditions [20].

Several methods of microencapsulation have been used with probiotic strains: emulsion, extrusion, spray drying and spray coating. The main purpose of these techniques is to provide protection to bacterial cells from adverse environment and target deliver viable cells to the gastrointestinal tract.

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 common used supporting materials are kappa–carrageenan, gellan, agarose, gelatin, alginate, chitosan, xanthan, locust bean gum.

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 generation of particles and capsules in large quantities for industrial applications.

EXTRUSION TECHNIQUES

Extrusion techniques are based on making capsules with hydrocolloids. These methods involve preparing a hydrocolloid solution, inoculating with bacterial cells, and extruding the viscous polymer–bacterial suspension through a gauge needle using syringe pump.The most common extrusion techniques include resonance [21] and jet–cutting technique [22,23], as well as electrostatic droplet generation [24,25] (Figure 1). The main carrier materials used for extrusion is alginate, a linear heteropolysaccaride of D–mannuronic and L–guluronic acid which is present in the cell walls of brown algae.

The main properties of alginates are their ability to increase the viscosity of aqueous solutions as well as to form gels (when calcium salt is added). Ca–alginate was one of the first materials used for production of beads encapsulating probiotics due to miled 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 0.1–5.0 mm (Table 1).

Although, alginate is frequently used for entrapment of probiotics it has undesirable properties such as susceptibility and degradation by acids [31]. It was observed that alginates of 73% α–l–guluronic acid content had less shrinking compared to the 38% guluronic materials [32]. In addition, it was reported that a mixed gel of gellan and xanthan gums has better

Table 1. Microencapsulation of probiotic strains by extrusion technique

Strains	Carrier	Diamether of beads	Refe-rence
B. longum	alginate	2.6 mm	11
B. lactis	0,75% gellan /1% xanthan	20 to 2200 μm	27
L. acidophilus and *B. longum*	alginate	–	9
B. bifidum and *B. infantis*	alginate	–	28
B. infantis	0,75% gellan/ 1% xanthan	3 mm	29
Lactococcus lactis ssp. Cremoris	2% alginate	2 mm	30

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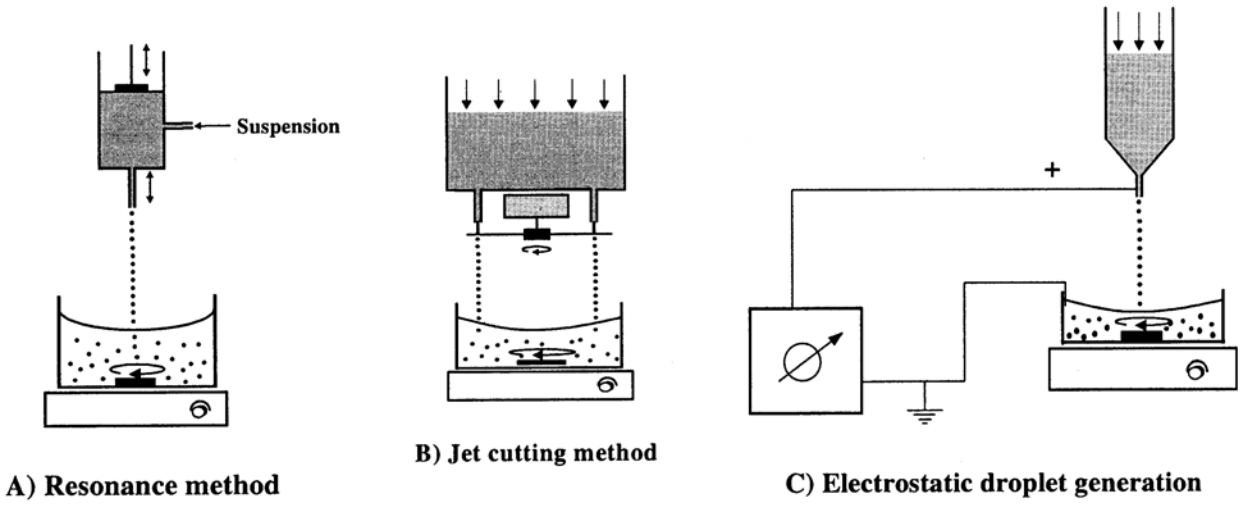

Figure 1. Microsphere preparation by extrusion techniques (Adopted from ref. 26)

technological properties for microencapsulation by extrusion technique than alginate [27,29].

Furthermore, to improve the characteristics of alginate, coating beads by cross–linking with a cationic polymer career has been suggested. Coated beads protect cell from release as well as increase mechanical and chemical stability [30]. Furthermore, mixing with starch, and incorporation of additives (cryoprotectants such as manitol and glycerol) can improve stability of encapsulated beads [33].

EMULSION TECHNIQUE

In the emulsion technique a small volume of an aqueous biopolymer solution containing the probiotic cells (discontinous phase) is added to a large volume of vegetable oil (continous phase), like soybean oil, sunflower oil, canola oil or corn oil. This oil phase is then emulsified to form a water–in–oil emulsion (W/O). When the desired droplet size is obtained, the matrix material is stabilized by crosslinking. Then, the oil phase is removed and the beads can be washed with a solution containing stabilizing ions for the gel. Furthermore, the particles can either be dried, or used used in a wet form (Figure 2). The common supporting materials used in emulsion techniques are: κ–carrageenan and locust bean gum (34,35), alginate [12], chitosan and gelatin, cellulose acetate phthalate [10], and gellan–xanthan gum [36]. With emulsion technique smaller beads can be produce compared with extrusion technique. The size of the beads is controlled by the mixer and reactor design and speed of agitation and can vary between 25 μm–2 mm (Table 2). This technique has been used for encapsulation of lactic acid bacteria for batch [35] and continuous fermentation [37]. In addition, the entrapped *L. delbrueckii ssp. bulgaricus* in artificial sesame 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 [38].

Table 2. Microspere preparation by emulsion technique

Strains	Carrier	Continous phase	Diamether of beads	Reference
L. delbrueckii ssp. *bulgaricus*		artificial sesame oil	20 to 200 μm	38
B. longum	κ–carrageenan	Vegetable oil/0.1% Tween 80	–	39
B. pseudolongum	10% cellulose acetate phthalate	White light paraffin oil	–	10
L. casei ssp. *casei*	3% κ–carrageenan and locust bean gum	Vegetable oil	1–2 mm	34
L. delbrueckii ssp *bulgaricus*	3% alginate	Vegetable oil/ 0,2% Tween 80	25–35μm	12
L. casei, L. acidophilis, B. infantis	2% alginate 2% strarch	Vegetable oil/ 0,02% Tween 80	150–500μm	40
B. longum Lactococcus lactis	K–carrageenan and/locust bean gum	Vegetable oil	1–2 mm	19

–, no record

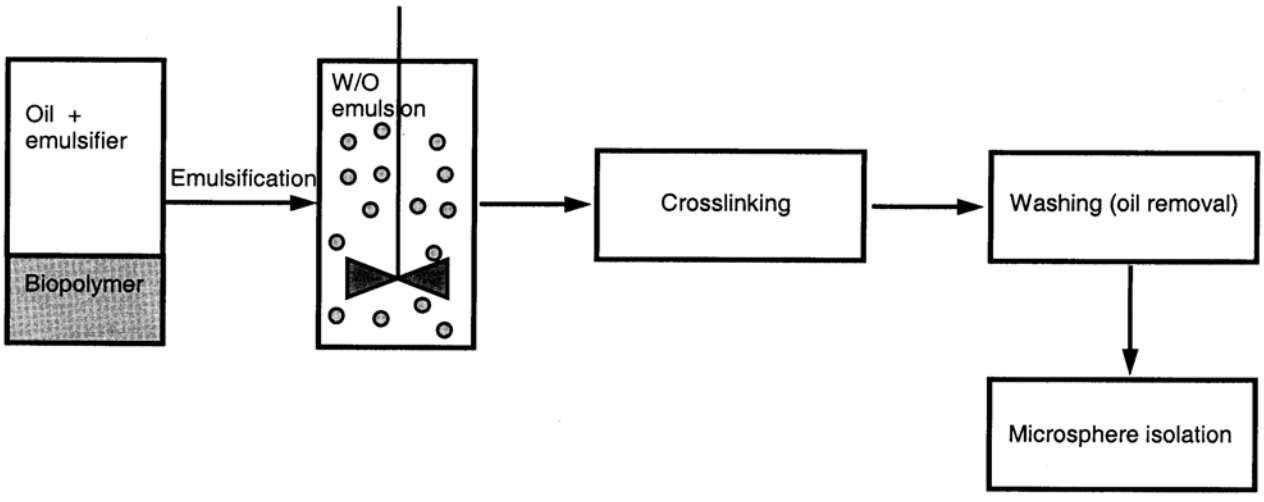

Figure 2. Microsphere preparation by emulsion technique

DRYING AND SPRAY COATING TECHNIQUES

Drying is an encapsulation technique which is used when the active ingridient is disolve in the encapsulating agent, forming an emulsion or a suspension. The "solvent" is commonly a hydrocolloid such as gelatine, 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. The particles obtained with these techniques are in dry powder form.

Spray drying technology offers high production rates at relatively low operating costs and resulting powders are stable and easily applicable. However, most probiotic strains do not survive well the high temperatures and dehydratation during the spray–drying process. Loss of viability is principally caused by cytoplasmatic membrane damage although the cell wall, ribosomes and DNA are also affected at higher temperatures [41]. It was reported that the stationary phase cultures are more resistant to heat compare to cells in exponential growth phase [42]. The introduction of thermoprotectants such as trehalose [43] and prebiotics [44] can improved cell viability.

Spray coating techniques are based on dispersing the molten droplets of a coating material onto a surface, to produce a homogeneous membrane. The sprayed liquid, or coat material, can be a solution, a suspension, an emulsion or a melt. The protective film or coating enable separation of active core ingredient (probiotic) from adverse environment. It was found that microencapsulation in alginate microparticles coating with high molecular weight chitosan improved the survival of acid–sensitive *L. bulgaricus* in simulated gastric juice [45] (Table 3).

Table 3. Particles preparation by spray drying or spray coating methode

Strains	carrier	coating	Reference
L. acidophilus *B. lactis*	cellulose acetate phthalate		46
L. bulgaricus	alginate	Chitosan coating	45
L. paracasei	Milk based medium Gum acacia		44
B. ruminantium		Starch	17
B. breve and *B. longum*	Whey protein	Whey protein	13
B. longum and *B. infantis*	gelatin soluble starch gum arabica skim milk	–	47

–, no record

These techniques can be applied solely or as a last phase of extrusion or emulsion processes. They have high productivity and large potential for the food industry.

CONCLUSION

The application of microencapsulation techniques in protection of probiotic strains has received considerable attention due to the low survival generally observed for these sensitive bacteria in food products. Microencapsulation of probiotics has been applied to the production of fermented dairy products (bio–yoghurt, cheese, cultured cream and frozen dairy desserts), mayonnaise, cereal and health bars, chocolate bars, fruit juices, etc. The microencapsulation techniques and carriers provide protection of probiotics against adverse environment such as oxygen and acidity in food, gastric solution and freezing. In addition, the important benefit for application of microencapsulated probiotics is their easier incorporation into food system, more constant characteristics, higher viability and stability during storage. Application of entrapped probiotic strains allows manufactures to place assurance on the viability and quantity of probiotics in finished products (10^7CFU g^{-1} or ml^{-1}).

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