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Review Article

Spray Drying Encapsulation of Probiotics for Functional Food Formulation-A Review

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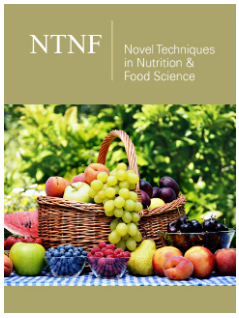

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Abstract

Health consciousness coupled with enhanced health care cost has led consumer's inclination towards functional foods. Documented health benefits have projected probiotics as a new functional ingredient in the current era of self-care and complementary medicine. It has been established that the probiotic viability is necessary for the exhibition of health benefits but is lost during processing, storage and gastrointestinal transit. Probiotic viability can be retained with the adoption of spray drying encapsulation technique and better survival of the encapsulated probiotics than the free cells in the food matrix have been reported. Major challenges faced during the encapsulation of probiotic by spray drying are modulation of processing parameter, selection of probiotic strains and coating materials and stability during gastric transit. Proper selection of probiotic strains and cell wall materials, modulation of technological parameters of spray drying and storage conditions would yield spray dried encapsulated probiotics with greater viability. Spray dried encapsulated probiotics had higher viability than free cells and its supplementation may be a practical alternative for better retention of viability and functionality of probiotics during the formulation of functional foods.

Keywords: Functional food; Health benefits; Microencapsulation; Probiotic; Spray drying encapsulation

Introduction

Globally, consumer's inclination towards health promoting foods has led to development of functional foods due to health deterioration, busy lifestyles, low consumption of convenience foods and insufficient exercise, increased incidence of self-medication, increased awareness of the link between diet and health [1]. Although functional foods lack a standard definition [2], but the production and consumption of functional foods has increased due to their capabilities of providing health benefits beyond basic nutrition [1], meeting the needs of the aging population and to cope with the rising costs of health care [3]. Documented health benefits of probiotics [4] and their capability to colonize gut [5] have projected probiotics as a new functional ingredient. Functional foods containing probiotic bacteria are gaining popularity in the global market [6,7], especially in Japan, Europe and the United States [8]. It has been established that survival of probiotics in the harsh acidic conditions during gastric transit and reaching the large intestine in an adequate amount for colonization and proliferation are prerequisites for exhibiting health benefits [9]. Therefore, retention of probiotic viability in the food matrix during processing, subsequent storage as well as during gastric transit are key issues during formulation of functional foods.

Microencapsulation is defined as "the technology of packaging of solid, liquid or gaseous materials miniaturized in capsules that can release their contents in a controlled manner and only under certain conditions". Microencapsulation has emerged as an alternative technology for the protection of probiotic bacteria from adverse environments [10], resisting processing and packaging conditions, improving taste, aroma, stability, nutritional value and product appearance [11]. Furtherer, microencapsulation also allows controlled release of functional components at targeted sites and masks unpleasant taste and odour of the substances [12]. Desai et al. [13] reported the following reasons for adoption of microencapsulation process in the food industry:

a) Protection of the core material from degradation by reducing its reactivity to its outside environment

b) Reduction of the evaporation or transfer rate of the core material to the outside environment

c) Modification of the physical characteristics of the original material to allow easier handling

d) Tailoring the release of the core material slowly over time or at a particular time

e) To mask an unwanted flavors or taste of the core material

f) Dilution of the core material when only small amounts are required, while achieving uniform dispersion in the host material

g) To help separate the components of the mixture that would otherwise react with one another.

Microencapsulation of probiotics can be done by employing various encapsulation techniques, but spray drying is widely adopted by the food industries during the development of functional foods. In the present paper an attempt has been made to highlight potentiality of spray drying technique for probiotic encapsulation and their subsequent application for functional food formulation.

Modulation of gut flora by probiotics

Human intestine is sterile at birth and is subsequently seeded with microorganisms due to swallowing of colonized amniotic fluid [14] and from various sources such as environment, maternal vagina and faeces [15,16]. Early colonization and balance between commensal and pathogenic bacteria is of utmost importance for normal function, immunology and homeostasis in the healthy intestine and any disruption of this balance may lead to disease conditions [17] such as allergies, obesity and diabetes [18]. Beneficial microbes capable of colonizing the gut regulate overall health of humans by restructuring the gut microbial balance [19]. Diversity in microflora of gastrointestinal tract of humans during different stages of life have been noted (Table 1) and modulation of the gut flora with suitable probiotic formulations may be a practical solution for maintaining good health.

Table 1: Diversity in microflora of GIT.

Stages of life/ Human conditions	Constituting Predominant Microflora	References
Normal infants	*Bifidobacterium, Lactobacillus, Bacteroides*	Penders et al. [112] Stenger et al. [116] Patel & Denning [111] Jakaitis & Denning [110]
Preterm infants	*Enterobacteriaceae, Clostridium*	
	Staphylococcus aureus, Klebsiella	Gewold et al. [106]
Vaginally delivered infants	*Bifidobacterium longum,* Bifidobacterium *catenulatum*	Biasucci et al. [105]
Cesarean delivered infants	No *bifidobacteria*	Biasucci et al. [105]
	Low *Bacteroides fragilis*	Gronlund et al. [107]
After first week	*Bifidobacterium, Lactobacillus*	Balamurugan et al. [103]
Upper portion of the colon	*Enterobacter, Streptococci, Staphylococci, Lactobacilli, Propionibacterium, Bacilli*	Steer et al. [115]
Lower portion of colon	*Bacteroides, Bifidobacterium, Eubacteria,* Peptococci, *Fusobacterium, Clostridium*	
Centre of lumen and epithelial surface	*Bacteroides*, Bifidobacterium	Sullivan et al. [117]
Breast-fed infants	*Lactobacilli, Bifidobacteria*	Harmsen et al. [109]
Breast-fed infants (After 5 days)	*B.catenulatum*	Guemonde et al. [108]
Breast-fed infants (After 3 weeks)	*B. longum*	Guemonde et al. [108]
	Bifidobacterium bifidum	Yuhara et al. [122]
	Bifidobacterium breve, B. longum	Benno et al. [104]
Formula-fed infant	*Bacteroides, Clostridium, Enterobacteriaceae*	Harmsen et al. [109]
	Enterobacteriaceae, Enterococci, Bacteroides	Rodriguez et al. [113]

Formula-fed infant (After 7 days)	*Bacteroides fragilis*	Yoshioka et al. [121]
After weaning	*Bacteroides*, *Prevotella*, *Ruminococcus*, *Clostridium*, *Veillonella*	Valles et al. [119]
Adult	*Bifidobacterium*, *Clostridium*, *Bacteroides*, *Eubacterium*, *Escherichia*, *Enterococcus*, *Streptococcus*, *Klebsiella*	Shanahan [114] Vasiljevic & Shah [120]

Probiotics are live microorganisms which when administered in adequate amounts confers health benefits to the host [20]. Reviewed literature indicated link between probiotics and health [4,21-23] and probiotics confer diverse human health benefits such as normalization of the intestinal flora, anti-carcinogenesis, hypocholesterolemic effect, alleviation of lactose malabsorption, allergy [4], enhance the immune system [24] and prevention of antibiotic-associated diarrhoea [25]. Recently, [26] declared that probiotics can affect the gut composition and their metabolic functions through gastrointestinal pathways or modulation of the gut bacterial community. Rolfe [27] declared following mechanisms by which probiotics exert health benefits.

a) Production of inhibitory substances against pathogens

b) Blocking of pathogenic bacteria adhesion sites

c) Nutrient competition and production

d) Degradation of toxins and toxin receptors

e) Modulation of immune responses

For exerting health benefits, viable probiotics must be consumed at a higher concentration and must be stable during its transit through the gastrointestinal tract. According to International Dairy Foods Association, viable probiotic cells in any probiotic product should be more than 10^7cfu/g up to the date of minimum durability [28] and daily intake (cfu/g) dosages should be10^8 [29], >10^8-10^{10} [30] or 10^8-10^9 [31]. Karimi et al. [32] suggested daily intake of an approximately100g of probiotic products to ensure 10^9 viable cells into the intestine. It can thus be concluded that probiotics must survive in sufficient quantities in the functional foods and must be ingested at sufficient quantity for exhibition of its functional properties.

Microencapsulation techniques

Encapsulation can be done by different techniques such as extrusion, emulsion, fluid bed, rennet-gelled protein encapsulation, freeze drying and spray drying. Tyagi et al. [33] classified microencapsulation techniques into three groups.

a) Physical methods such as spray drying, lyophilization, supercritical fluid precipitation and solvent evaporation

b) Physico-chemical methods including coacervation, liposomes and ionic gelation

c) Chemical methods such as interfacial polymerization and molecular inclusion complexation.

Amongst various encapsulating techniques, freeze drying, and spray drying are commercially widely adopted [34]. Application of encapsulated probiotics obtained with the freeze drying or spray drying extend certain advantages in terms of a relatively long shelf life, stable quality control and convenience in handling, transportation and storage [35,36]. Spray-drying method is considered better than extrusion or emulsion methods due to production of stable micro-particles with low diameters and homogenous size distribution [37-39]. Encapsulation by Freeze drying may be the preferred over spray drying due to less viability losses (4-27.5% vs. 19-40%) of Bacillus coagulans [40].

In freeze-drying, initially water in the foodstuffs is frozen [41], followed by the removal of frozen water through the sublimation process. It has been noted that crystal formation and stress condition by high osmolarity during freezing causes damage of cell membrane to leak the intracellular substances and requires addition of cryoprotectants prior to fermentation to assist in the adaptation of probiotics to the environment [42].

Spray drying process involves passage of foodstuffs through a nozzle to form droplets in the drying chamber, where the droplets are dried immediately when they come in contact with hot air to form powder [43]. Spray drying method is preferred over freeze drying as the former method is faster (30 minutes) than the latter method (72 hours). Dehydration has adverse effects on cell membranes and secondary protein structures and freeze-drying generally has less impact on the viability than the harsher processing conditions of spray-drying [44,45]. However, encapsulation by spray drying is the predominant technique adopted by dairy industries as the cost of spray drying is six times less than freeze-drying for removal of per kilogram of water [46] and can be stored for longer period at lower cost [47]. Zuidam & Shimoni [48] denoted that selection of encapsulation technologies should be based on certain considerations such as effect of processing on probiotics viability, processing conditions employed during food production or processing, storage conditions of the food product containing the encapsulated bacteria prior to consumer use, particle size and density required for incorporation encapsulated bacteria into the food product, mechanisms of release and the cost constraints.

Factors affecting efficacy of encapsulation

Microencapsulation of bacteria in polymeric and biodegradable matrix is done for retention of the viability of the probiotic strains under the harsh conditions of food product preparations coupled with efficient delivery of probiotic strains to the intestine [49]. Diverse factors affecting efficacy of microencapsulation are enumerated below.

Type of solvent used

a) Strains of lactic acid bacterial employed

b) Composition of encapsulation material [50]

c) Drying conditions such as inlet and outlet temperatures, flow rate and humidity of drying gas, feed flow rate, pressure and speed of atomizer [51]

d) Storage temperature [52]

e) Viscosity, solids content and surface tension of feed formulation [51]

Basic principle of Spray drying encapsulation

Basic principle involved in the spray drying includes the atomization of cells in a polymer solution into a drying chamber through atomizer, drying of liquid droplets inside the drying chamber [53], which leads to solvent evaporation and formation of the microcapsules [54] and recovery of powder through a cyclone separator [55]. Schafroth et al. [56] declared that spray drying technique comprises of three stages (i) Homogenization of the feed liquid by an atomizer (ii) Drying of the feed solution by a hot gas carrier to achieve the evaporation of the solvent (iii) Collection of the dry particles by cyclones or a filter. Recently, Broeckx et al. [57] divide the spray drying process in four stages.

a) Liquid feed is atomized into a spray of little droplets

b) Atomized spray comes in contact with a heated gas in the drying chamber

c) Drying of the droplets and the particle formation

d) Solid particles are separated from the drying air

Feed samples intended for spray drying encapsulation are prepared by dissolving the core materials in a single or a combination of carrier materials with the objective of forming an emulsion or a suspension. Afterwards mixing of core compounds and carrier materials is performed through high-speed mixing or high-shear emulsification to form a coarse emulsion. Commonly used carrier materials in spray drying are hydrophilic polysaccharides such as maltodextrins, chitosan, alginate and different types of gums and proteins like whey protein), whereas the core materials can be active hydrophobic or hydrophilic molecules [58,59]. Huang et al. [36] declared that for optimization of probiotics encapsulation by spray drying, following stages should be considered.

1) Pre-drying

2) During spray drying

3) Post-drying

Advantages of Spray drying encapsulation

Spray drying is most widely used by industries for encapsulation of food bioactive compounds owing to following reasons.

1) It is an economical technique due to high production rate with minimum operating costs [60,61]

2) Highly suitable for heat sensitive compounds as total drying time is few milliseconds to few seconds [62,63]

3) Higher stability, lower storage and transport costs and easier usage [51]

4) Diameter of the spray dried microcapsules generally ranges between 5 to 100μm [64,65], which facilitates a greater contact surface for the nutrient's availability [66] without affecting the palatability of microencapsulated incorporated food products [67]

5) Protection of microencapsulated cells from bacteriophages and detrimental factors such freeze drying, freezing and storage, thereby retaining higher viability [68]

6) Homogeneous distribution throughout the product due to conversion in powdered form [68]

Challenges of encapsulation of probiotic by Spray drying

Owing to certain advantages of low cost, high productivity and rapid processing, spray drying is one of the most commonly used microencapsulation technologies within the food industry and has emerged as the most promising technique for retention of probiotic viability but also has few challenges.

Modulation of processing parameter

Heat, osmotic, oxidative and desiccation stresses are usually considered to be the main mechanisms which cause the inactivation of bacteria during and after spray-drying [69]. Higher temperature during spray drying results in the formation of cellular pores and leakage of the intracellular substances [42]. Spray drying at higher outlet temperature induced greater viability losses due to more dehydration resulting from greater exposure of micro-particles at higher temperature [70]. On the other hand, lowering of inlet temperature results in higher post-encapsulation viability but greater moisture and water activity adversely affects the prolonged storage [71-73] reported that loss of viability of *L. plantarum* WCFS1 during drying is due to dehydration inactivation, thermal inactivation or a combination of both types of inactivation. Adoption of suitable protectants during spray drying conferred protection to the encapsulated cells [74,75], which can be attributed to mechanisms of enhancing the intrinsic stress tolerance of probiotic cells, providing extracellular protection on cells as physical shield and having favorable drying kinetics [76]. Additional thermo-protection can be achieved through the addition of free radical scavengers or by reducing water mobility through the cell membranes and the cell wall thus modulating dehydration upon heating [77]. Considerations should be given to pre-drying, during spray drying and post-drying [36] for retaining the viability of encapsulated probiotics at recommended level [31].

Protection conferred to probiotics is also dependent on the size of encapsulated cells. Larger microcapsules provide better protection to probiotics than smaller capsules but are poorly dispersed and impart sandiness in foods. On the other hand, microencapsules with size below 1mm result in mechanical instability during prolonged fermentation. Optimum size of capsules should range between 1 to 3mm [78] to satisfy the requirements of cell growth and provide

mechanical strength to the capsule. Considerations should be given to control the conditions leading to microcapsules of optimum size

Selection of probiotic strains

All probiotics are not equally capable of withstanding the processing or storage conditions. During spray drying variability in thermal resistance were noted amongst *Bifidobacterium* Bb-12, *Lactobacillus acidophilus* La-5 and *Propionibacterium jansenii* 702 [79,80]. Spray drying leads to increased cell permeability by affecting the cell membrane, causing loss of intracellular components from the cell into the surrounding environment [31]. High or low temperatures and/or the use of organic solvents are detrimental for the viability of the probiotic cultures [78]. Considerations should be given to avoid loss of the internal components of the cell due to increase in the cell permeability of probiotics [81]. Rodrigues et al. [82] noted greater storage stability of whey protein-based microcapsules of *Lb. paracasei* than *B. animalis* Bb12 and *Lb. acidophilus.* Selection of suitable bacterial strains that can withstand osmotic stress, drying conditions and desiccation must be considered [51].

Selection of coating materials

Viability of probiotics during encapsulation by spray drying is also influenced by the type of core material employed. Complete loss of viability of *Lactobacillus zeae* LB1 was encountered during spray drying employing water without any wall material [83]. Scanning electron microscopy of spray dried microparticles of *Lactobacillus acidophilus* La-5(4.85µm) and *Bifidobacterium* Bb-12(8.75µm) revealed high porosity and ruptures in their structure, which may be related to the low solids concentration (12% m/v) used in the formulations [64,84]. Earlier single polymer formula was used to encapsulate probiotic cells using spray drying but adoption of binary systems extended advantages in terms of higher encapsulation efficiencies and/or higher survival rates, superior in vitro gastrointestinal digestion and storage properties [85]. Further, coating material like chitosan is reported to exhibit antimicrobial activity and therefore it becomes necessary to use other coating materials as co-protectors or a two-step microencapsulation [7,86,87]. A combination of different coating materials has been suggested as application of a single material does not satisfy all desired requirements [13]. Selection of the coating material should be based on the physicochemical properties, solubility, viscosity in the prepared solution, the compatibility with the core-material and the intended size and surface of the final microcapsules [88].

Stability during gastric transit

During gastric transit, probiotics are adversely affected by the enzymatic action of pepsin, low pH of the stomach and antagonism associated with antimicrobial activity of bile salts and protease-rich conditions of the intestine [31,89]. Spray dried microcapsules are water soluble, resulting in an early release of the cells at an undesired site or time, thus probiotic bacteria are not protected from adverse conditions of storage and gastrointestinal transit [90]. Stability of microencapsulated probiotics in fermented milks and efficacy of microencapsulation to deliver probiotics in the gastrointestinal tract is challenging [91]. Considerations should be given so that encapsulated probiotics are released in the gut microenvironment due to changes in pH, enzymatic activity or osmotic strength to foster their colonization in the intestine [78,92]. Type of the wall material and atomization method employed during spray drying also influence the stability of encapsulated probiotics in simulated gastrointestinal tests. During simulated gastrointestinal tests, significantly higher viability of microencapsulated *Bifidobacterium infants* was reported with use of whey protein concentrate and two-fluid nozzle than that those employing soy protein concentrate and spray centrifugal atomizer [93].

Functional properties of spray-dried probiotics

Probiotics are incorporated during fermented milk formulations with the objective of enhancing its functional properties. Probiotics are generally introduced in fermented milk in non-capsulated form and viability of probiotics is influenced by the food matrix, associated metabolic behavior with other starter cultures, strains of probiotic cultures employed, processing and storage conditions.

Preserving the efficacy of probiotic bacteria represents the most challenging during the development of functional food products. Jantzen et al. [94] noted decline in counts of *Lactobacillus reuteri* after drying (2 log cycles) as well as after 4 weeks of storage (1 log cycle) but survival rate of encapsulated bacteria was 32% higher compared with non-encapsulated when exposed to artificial digestive juice. Spray drying of *L. casei* in presence of using vegetable extracts and maltodextrin induced only one log cycle reduction in viable counts but retained its viability at a level of $>10^7$cfu/g of *L. casei* after 60 days of storage and can be used for the development of functional food [65]. In another investigation, Ivanovska et al. [95] noted that synbiotic spray dried micro-particles of *Lactobacillus casei* obtained employing, sodium alginate, chitosan and fructo-oligosaccharide retained viability above the minimum therapeutic during incubation of 24h in simulated gastrointestinal conditions (7.67±0.4 log cfu/g) as well as after 3 months of cold storage (8.1±0.6 log cfu/g). Maciel et al. [96] also encountered higher storage stability of spray dried encapsulated *Lb. acidophilus* La-5 in sweet whey with an average decrease of only 0.43 log cfu/g at the end of 90 days of storage and retained viability above 6 log cfu/g. Significantly higher viable counts of encapsulated *B. breve* in comparison to free cells were encountered in yoghurt supplemented with whey protein encapsulated *B. breve* ($6\text{-}7\times10^6$cfu/ml) and *B. longum* ($5\text{-}6\times10^7$cfu/ml) after 28 days of storage at 4 °C [38]. Recently, Fazilah et al. [97] also encountered higher viability (cfu/ml) of spray dried microencapsulated *Lactococcus lactis* ($\sim10^7$ vs. $\sim10^5$) in yogurt in comparison to non-microencapsulated cells.

Spray drying has emerged as an effective way to produce encapsulated probiotics with high survival rates and improved resistance during gastric transit [98,99]. Among four stains better survival (log cfu/ml) of spray dried encapsulated *Lactobacillus casei* subsp *L. rhamnosus* TISTR 047 (8.31±0.11 vs. 4.06±0.08) in comparison to non-encapsulated cells was encountered [100]. A double blind, randomized, cross-over study revealed no difference in kinetics of intestinal colonization in healthy volunteers receiving

non-encapsulater or encapsulated probiotic strains of *Lactobacillus* plantarum LP01 and *Bifidobacterium breve* BR03 after 21 days of feeding [101]. Further it was reported that functional properties of probiotics are not affected by spray dried encapsulation. Burns et al. [102] reported that the anti-inflammatory capacities of both isolated *Bifidobacterium animalis* subsp. *lactis* INL1 and commercial strain *B. animalis* subsp. *lactis* BB12 were not affected by spray-drying [103-122].

Conclusion

Potential health and nutritional benefits of probiotics have boosted the demand for functional probiotic foods in the current era of self-care and complementary medicine. It has been established that viability of probiotics is of utmost importance for extending health benefits and must be consumed in sufficient quantity. Probiotic viability may be lost during processing, storage and gastric transit and therefore retention of viability of probiotics during formulation of probiotic functional foods are challenging. Encapsulation of probiotics has emerged as the most promising technique for retention of probiotic viability. Amongst different techniques, spray drying is most commonly commercially adopted encapsulation technique for preservation of probiotic viability. Major challenges faced during encapsulation of probiotics by spray drying are modulation of processing parameters, selection of probiotic strains and coating materials and stability during gastric transit. Heat, osmotic, oxidative and desiccation stresses are usually considered to be the main mechanisms which cause the inactivation of bacteria during and after spray-drying. Higher viability in spray dried encapsulated probiotics could be achieved by proper selection of probiotic cultures, wall materials, controlling pre-drying, drying and post-drying conditions and storage. Spray dried encapsulated probiotics have great potential for formulation of functional foods and its commercial application would benefit both industries and consumers.

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