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Encapsulation of probiotics: past, present and future

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Abstract

Background: *Probiotics* are live microbial supplements known for its health benefits. Consumption of probiotics reported to improve several health benefits including intestinal flora composition, resistance against pathogens. In the recent years, there is an increasing trend of probiotic-based food products in the market.

Main body: Probiotics cells are targeted to reach the large intestine, and the probiotics must survive through the acidic conditions of the gastric environment. It is recommended to formulate the probiotic bacteria in the range of 10^8–10^9 cfu/g for consumption and maintain the therapeutic efficacy of 10^6–10^7 cfu/g in the large intestine. During the gastrointestinal transit, the probiotics will drastically lose its viability in the gastric environment (pH 2). Maintaining cell viability until it reaches the large intestine remains challenging task. Encapsulating the probiotics cells with suitable wall material helps to sustain the survival of probiotics during industrial processing and in gastrointestinal transit. In the encapsulation process, cells are completely enclosed in the wall material, through different techniques including spray drying, freeze drying, extrusion, spray freeze drying, emulsification, etc. However, spray-drying and freeze-drying techniques are successfully used for the commercial formulation; thus, we limited to review those encapsulation techniques.

Short conclusions: The survival rate of spray-dried probiotics during simulated digestion mainly depends on the inlet air temperature, wall material and exposure in the GI condition. And fermentation, pH and freeze-drying time are the important process parameters for maintaining the viability of bacterial cells in the gastric condition. Improving the viability of probiotic cells during industrial processing and extending the cell viability during storage and digestion will be the main concern for successful commercialization.

Keywords: Probiotics, Viability, Encapsulation, Spray drying, Digestion, Storage, Denaturation

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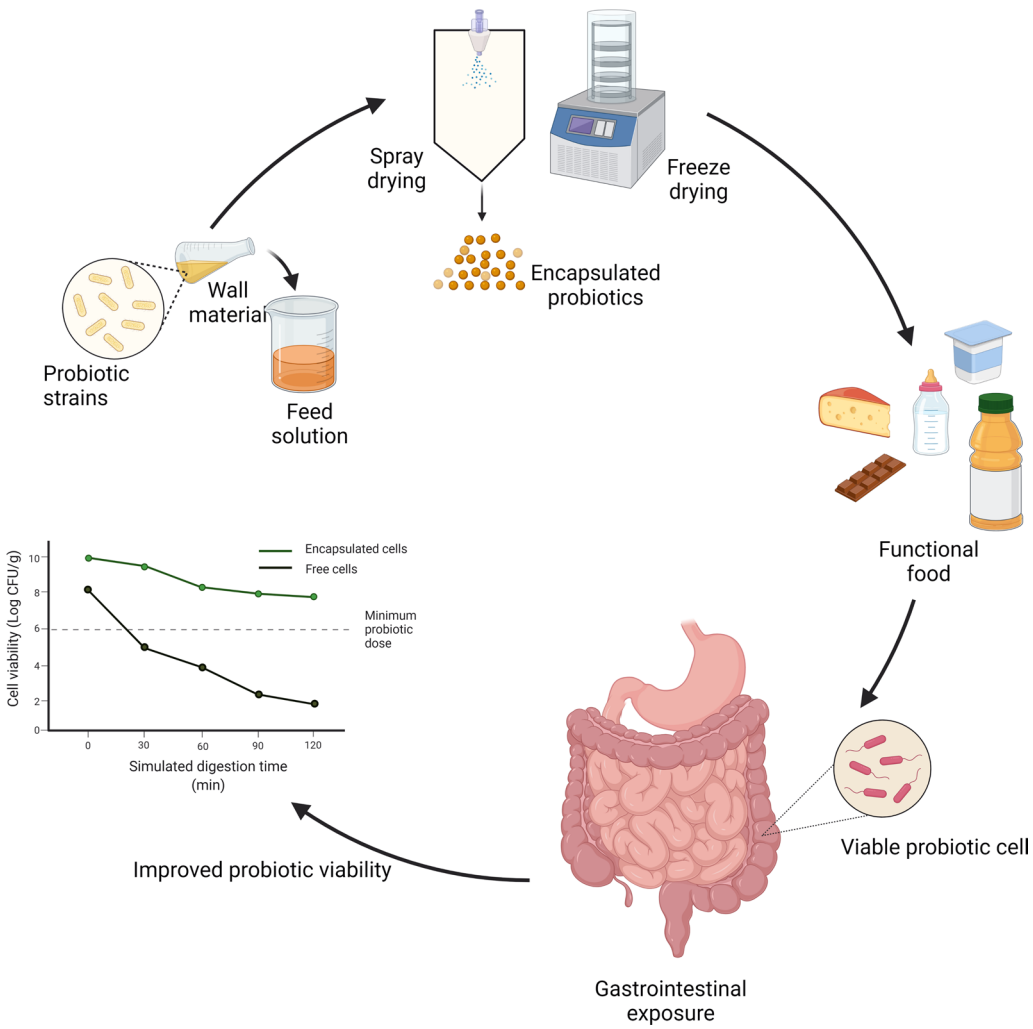
Graphical abstract



1 Background

Probiotics are live microbial supplements known for the health benefits [1]. The genus *Lactobacillus* and *Bifidobacterium* have been used as probiotics over the years and are mainly associated with human gastro intestinal tract [2]. In recent decades, the probiotic strains of *Lactobacillus plantarum* are frequently used in many fermented foods for the following health benefits: respiratory and urogenital tracts, colonization in the intestinal tract, lactose metabolism, inhibition of carcinogenesis, absorption of calcium and vitamin synthesis, etc. [3, 4]. Further, the probiotic efficacy relies on the viability and dose of administration, including the metabolic stability in the food matrix and the ability to survive in the acidic stomach environment, because a significant portion of the ingested probiotics cells lose their viability during the passage through the gastrointestinal (GI) tract and during the storage. Thus, encapsulation of probiotic strains is necessary to protect them from harsh processing conditions and to improve the final sensory property when incorporated in the functional food [5].

The scientific evidence for the concept of probiotic sets with the theories of Russian scientist Elie Metchnikoff in the early twentieth century. Scientist Metchnikoff observed that the Bulgarian farmers were living longer and healthy due to the huge consumption of fermented milk. He postulated that a human body is slowly poisoned by toxic substances produced by pathogens in the gastrointestinal tract, thereby weakening the body's resistance to the proliferation of pathogens. This condition can be prevented by

the consumption of fermented milk that contains bacteria producing lactic acid [6, 7]. Soon after his hypothesize, strains of *Lactobacillus* and *Bifidobacterium* were applied in food products. For instance, in 1905 Prof. Grigoroff used *Lactobacillus bulgaricus* as starter culture for commercial production of the "*kiselomleko*" (sour milk). Subsequently, few strains of *Lactobacillus acidophilus* were identified to colonize in the human intestinal tract. These findings triggered commercial attention in food products fermented by the strain *Lactobacillus acidophilus* [6]. Later, in 1930, scientist Minoru Shirota in Japan isolated and developed a culture of *Lactobacillus* strain which had the potential of survival in the harmful environmental conditions of the gastrointestinal tract. The culture was known as the strain *Lactobacillus casei* and it was successfully utilized for the commercial production of fermented dairy product named "*Yakult*." Since then, the commercial production of probiotics incorporated functional foods and dietary supplements were increasing rapidly.

Nowadays, it is recognized that daily ingestion of the probiotic microorganisms favors to improve and maintain the beneficial intestinal microflora and thus preventing various gastrointestinal infections. Even though probiotic related works initiated in the early days, intensive efforts to determine the health benefits of probiotics largely started in 1980s. The volume of research speeded up in the early 2000s to till now with more than 30,000 research and review manuscripts and more than 2000 probiotic products.

1.1 Definition of probiotics

The word "probiotics" was derived from the Greek word "pro bios" meaning "for life" [8]. In 1965, Lilly and Stillwell defined probiotics are "substances produced by specific microorganism that stimulate the growth of the other microorganism." In 1974, Parker proposed that "probiotics are microorganisms and its substances, which contribute to the intestinal microflora balance." In the late 1980s, following definition was accepted "*live microbial feed supplements, which beneficially affects the host organism by improving their intestinal microbial balance*" [9]. However, this definition relates more to animals than to humans. A decade later, Salminen et al. [10] defined "*probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host*". Today, the most accepted definition is proposed by FAO/WHO as "probiotics are *live microorganisms which when administered in adequate amounts confer a health benefit on the host*" [11].

1.2 Commonly used probiotic microorganisms

A large number of microorganisms from different genera and species could have the potential probiotic properties. However, the genus *Lactobacillus* and *Bifidobacterium* have been used as probiotics over the years [2]. The main reason being, both these species are considered as GRAS (generally recognized as safe) and are the most dominant microorganisms in the human intestinal tract. In particular, the probiotic strain *Lactobacillus* spp. is frequently used in the dairy sector [12]. Lactobacilli are gram-positive, non-spore-forming and rod-shaped organisms usually live in a non-aerobic environment but are acid-tolerant, aero-tolerant, fermentative and fastidious [13]. A few of the known lactic acid bacteria that are used as probiotics are listed in Fig. 1.

Bifidobacteria are rod-shaped gram-positive bacteria and grow at the pH range of 4.5—8.5, but they are strictly anaerobic [14]. Bifidobacteria actively ferment carbohydrates and produce acetic acid and lactic acid in a 3:2 (v/v) molar ratio, without producing carbon dioxide, propionic acid, or butyric acid during fermentation. Figure 1 lists the selected species of *Bifidobacterium* that are used as probiotics in food sector. Other than these bacteria, species belong to *Lactococcus, Enterococcus* (e.g., *Ent. faecalis, Ent. Faecium*), some types of yeasts (e.g., *Saccharomyces boulardii* and *Saccharomyces cerevisiae*) and fungi (e.g., *Aspergillusoryzae*) have also been identified as having probiotic effects [14].

1.3 Health benefits

Numerous scientific reports revealed the potential of probiotics in the prevention and treatment of gastrointestinal disorders. The human gut, particularly the large intestine, has a complex bacterial composition comprising of more than 50 genera of bacteria including harmful (toxins) or beneficial (synthesizing vitamins) to the biological system. Administration of probiotic bacteria stimulates the growth of beneficial gut microbiota, crowds out potentially harmful bacteria and reinforces the body's natural defense mechanisms [10]. Broadly, probiotics improve the human health by following mechanisms,

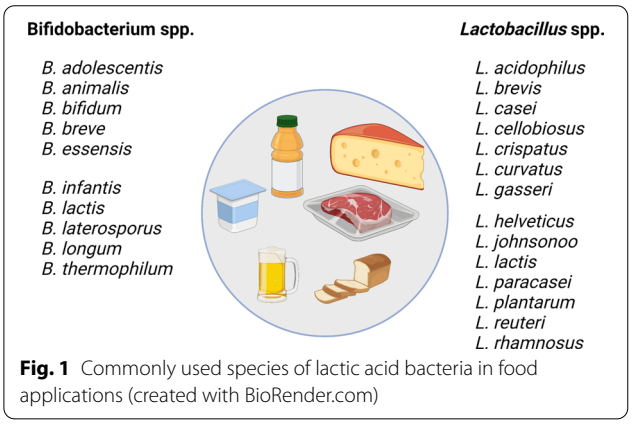


Fig. 1 Commonly used species of lactic acid bacteria in food applications (created with BioRender.com)

balancing the intestinal microbiota composition, boosting the immune system, and metabolic process.

Thus, the consumption of probiotics through functional food products is an ideal approach. Probiotic foods comprise a maximum of up to 70% of the total functional food market. The global functional food market is predicted to reach $ 309 Billion in 2027 and is expected to maintain the compound annual growth rate of 7.5% between 2020 and 2027 [15]. The demand for probiotic-based functional foods increased rapidly due to the awareness of the consumers [16], and most dairy sectors rely on the probiotics cultures for the formulating functional foods. A wide list of food materials has been examined for probiotics recently including yogurt [17], various types of cheese [18], fermented milk, ice cream, milk powder, milk chocolate [19], frozen dairy desserts, sour cream and flavored liquid milk.

2 Main text

2.1 Microencapsulation of probiotics

Microencapsulation is defined as the "technology of packing solids, liquids, or gaseous substances in miniature, sealed capsules that can release them at controlled rates under the influence of specific conditions" [20]. In the encapsulation process, small quantities of core materials containing nutrients/therapeutic compounds are encapsulated within the wall material to form capsules [21]. Microencapsulation is an effective way to protect the bioactive compounds like probiotics from the processing conditions, temperature, transportation and during the intestinal transit [22].

The primary objective of encapsulation is to protect the probiotics (core) from the adverse environmental conditions. In food industries, the microencapsulation process is applied for various reasons including (1) protection of core from degradation by reducing the reactivity to its outside environment (e.g., temperature, moisture content, oxygen and light), (2) reducing the core material evaporation, (3) improve material handling by modifying the physical characteristics, (4) ability to modify the release characteristics, (5) mask the flavor, color, unwanted taste of the core material, (6) to achieve a uniform dispersion upon dilution [20]. Microencapsulation has been investigated for enhancing the viability of probiotic microorganisms. The survival of encapsulated probiotic cells depends on the physicochemical properties of the microcapsules including bacterial strain, microcapsule size, concentration of the wall material, initial cell numbers.

The selection of wall material for microencapsulation of probiotics is very important since the substances used to encapsulate probiotics should be food grade, biodegradable and able to form a physical barrier between the core and its surroundings [23]. The basic criteria for the selection of wall material are mainly based on the ability to improve the viability of bacterial cells during processing and prolonged storage [24] and also based on solubility, molecular weight, film-forming, and emulsifying properties. For instance, microencapsulation by spray drying is usually carried out from aqueous feed formulation; therefore, the wall material must be soluble in water. The wall material should be designed to protect the probiotic cells from the factors that may cause inactivation and release them in the intestinal tract. Microencapsulation of probiotics is often achieved with various biopolymers such as plant extrudates (gum Arabic, acacia gum), marine extracts (alginate, carrageenan), proteins (milk or whey protein, soy protein, gelatin, gluten), dietary fibers (resistant starch, maize starch), microbial and animal polysaccharide (chitosan, xanthan) [5, 23]. Encapsulation efficiency, physio-chemical characteristics and stability of microcapsules highly depend on the wall material compositions.

2.2 Techniques for microencapsulation of probiotics

Microencapsulation is a physiochemical or mechanical process that entraps a potentially sensitive microorganism and provides a protective barrier from an external environment [25]. Encapsulation technique differs from cell immobilization technique. In the encapsulation process, cells are completely enclosed in the wall material, whereas in the immobilization technique, cells may be exposed to the outside environment [26]. The size of the bacterial cell range between 1 and 5 μm and must be maintained viable during the encapsulation process. Thus, it is highly crucial for the selection of appropriate encapsulation technology. Incomplete encapsulation favors the exposure of sensitive probiotics to outside environment during industrial processing and storage, resulting in breakage of cell walls and reduction in nutritional properties.

Encapsulation methods for microencapsulation of probiotics fall into three main categories such as extrusion, emulsion and drying. For industrial production of encapsulated probiotics technique like spray drying, lyophilization, emulsion, lipid-based delivery system, coacervation and extrusion were commonly employed. The possibility of delivering the probiotics through mucoadhesive oral films and lipid is also widely investigated across the food and pharmaceutical industries [27, 28]. The emulsion and extrusion techniques generally encapsulate the probiotics in complex hydrocolloid matrices [29]. Each technique produces microcapsules with different characteristics in terms of moisture content, microcapsule size, encapsulation efficiency and release during digestion. Table 1 summarizes the experimental advantages and disadvantages

Table 1 Advantages and disadvantages of encapsulation technologies

Sl. No	Spray drying	Freeze drying
Advantages		
1	Rapid drying process	Minimum damages to the product
2	Directly convert the dried powder from the liquid feed	Freeze dried powder can be stored in atmospheric conditions
3	Easy to change the process variables and improve the product quality	Retain the aroma, flavor and nutritional content
4	Products in free-flowing powders	Porous structured powder due to sublimation of water
5	High production efficiency	
6	Less operator requirement	
7	Scaleup to large production capacity	
Disadvantages		
8	May not suitable for heat sensitive materials	Lengthy drying time [24 – 36 h]
9	Complex equipment, and requires more area for installation	Complex equipment and difficult to change the process
10	High capital and maintenance cost	High capital and maintenance cost
11	Less thermal efficient	Less thermal efficient

of encapsulation technique. The disadvantage of the extrusion technique is that it is difficult to scale up, and there will be a significant reduction in the viability after the extrusion process [30]. A higher ratio of core to wall material is important in the economic as well as the sensory point of view. On the other hand, the emulsification technique is easy to scale up, but the main disadvantage is with the large bead size and shapes [31].

Production of microcapsules in dried form could be an alternative to the wet microcapsules obtained by emulsion and extrusion techniques. Moreover, spray drying may reduce the size of microcapsules and provide a more favorable anaerobic environment for the probiotic bacterial and improve the storage properties [32]. Further, spray drying is the extensively used technique in dairy, pharmaceutical and food sector for processing bioactive compounds including enzymes, vitamins, etc. [33]. The cost analysis and benefits of microencapsulation of probiotics through spray drying and freeze drying are illustrated in Fig. 2. Microcapsules can be easily handled and stored for a long time if they are in the form of dry powder. The common encapsulation processes to obtain dry formulations with prolonged shelf life are spray drying, freeze drying, spray-freeze-drying and fluidized bed drying. Spray-drying and freeze-drying methods are the most frequently used microencapsulation technique for probiotics, and this review will be limited only to these encapsulation techniques.

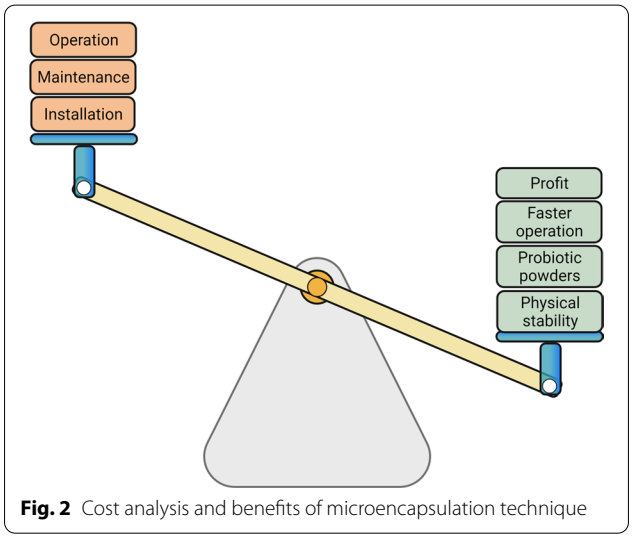


Fig. 2 Cost analysis and benefits of microencapsulation technique

2.3 Spray drying

Spray drying is one of the well-established methods used in the food and pharmaceutical industries for producing large quantities of dried microcapsules in a simple and continuous processing operation. The main advantage of using spray drying includes rapid drying, flowable powders and manipulate particle size [34]. Encapsulation by spray drying has been used in the food sectors for the past 7 decades, to encapsulate food ingredients like flavors, vitamins and probiotics [22, 35, 36]. Spray drying consists of three process stages such as (i) atomization—produce droplets, (ii) mixing—between droplets and hot air, and evaporates water and (iii) separation—dried powders will be collected from the cyclone separator [37]. The process of spray drying is illustrated in Fig. 3.

Atomization is the most important operation in the spray-drying process. During this process, the feed liquid is disintegrated into millions of micron-sized droplets (10–200 μm). Such tiny droplets increased the surface area enormously resulted in rapid evaporation of water from the droplets [38]. The atomizing droplet

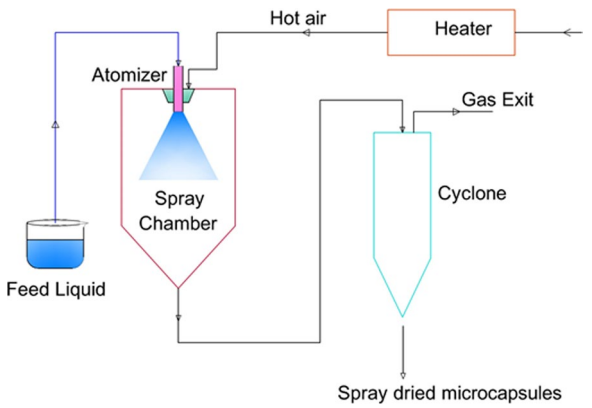


Fig. 3 Schematic diagram for spray drying

size depends on the physical properties of feed solution including surface tension, viscosity and density, pressure drop inside the nozzle and the velocity of the spray. The atomized droplet size distribution and velocity of the droplets also determine the residence time and drying rate [39].

Three types of nozzles are used in spray dryers, namely (1) centrifugal or rotary wheel atomizer, (2) pressure nozzle and (3) twin fluid or pneumatic nozzle.

Centrifugal or rotary wheel atomizer: Feed liquid is fed into the center of the rotating wheel (5 to 50 cm diameter) with a peripheral velocity of 100–200 m/s. Speed of the rotating wheel, feed rate and viscosity strongly determines the size of the droplets emerge from the atomizers. The horizontal particle trajectories of these atomizers require a large diameter spray chambers.

Pressure nozzle: Feed liquid is pressurized by a pump and forced through the nozzle orifice (0.4 to 4 mm diameter). Due to their smaller spray angle, the drying chamber can be narrower and taller.

Pneumatic nozzle: These nozzles use compressed air to atomize the liquid, by mixing the air and feed solution and atomize. Pneumatics nozzles are suitable for sterile or aseptic applications.

Droplet—hot air contact takes place after atomization in the drying chamber, and it initiates the drying. Three types of droplet-air contact systems employed in the spray-drying process are:

Co-current contact: The liquid is atomized in the same direction as the flow of hot air through the spray chamber. Thus, there will be a rapid increase in droplet temperature. The heat and mass transfer rate reduce after the initial droplet-air contact. The final product temperature (50–80 °C) is lower than the inlet air temperature (150–220 °C) and makes suitable for heat-sensitive material.

Counter-current contact: The flow of atomized droplets and hot air is opposite in direction. In this configuration, the final product temperature is higher than that of outlet air that limits the application of this process to heat-sensitive products.

Mixed flow contact: In this method, the droplets experience both counter-current and co-current pattern of fluid movement. The air stream enters at the top, and the atomizer is located at the middle. Mixed flow contact is employed when a coarse product is required.

Water evaporation: At the time of droplet-hot air contact, heat is transferred from hot air to the droplet due to temperature gradient, parallelly moisture transfer is carried out due to the vapor pressure difference in the opposite direction [38]. Thus, the complete evaporation of water from the droplets resulted in a dry powder. The dried particles can be hollow or compact which depends upon the experimental conditions and resulted in the rigid outer shell [40].

Powder recovery/ separation: The dried powder is discharged from the drying chamber to a powder separator by drying air stream. Spray-drying chambers are equipped with cyclone separators to facilitate the collection of dry powder.

2.3.1 Spray-drying process for microencapsulation of probiotic bacteria

Microencapsulation process initiates from the preparation of feed solution by dispersing probiotics cells with wall material. In spray drying, the prepared feed solution will be atomized to evaporate the water molecules and produce dry microcapsules range of 10–100 μm. The moisture content of spray-dried products ranges between 4 and 7% that is optimum for storage stability [3]. Table 2 summarizes the experimental conditions and wall materials that have been recently used for the microencapsulation of different species and strains of probiotic bacteria. The differences in the survival rate of probiotic microorganisms may be related to the natural resistance of the probiotic strain, wall material and operating conditions used for encapsulation by spray drying. However, the major drawback with this techniques is the loss of bacterial cells in the hot drying environment [41].

2.3.2 Mechanism of cell inactivation during spray drying

The cell membrane is the most susceptible target site in bacterial cells to the heat stress associated with spray drying. Further, the heat and mechanical stress can induce cytoplasmic membrane dehydration, cell wall rupture and denaturation of DNA and RNA [59]. Cell membrane, which lines the bacterial cell wall, consists of lipids, proteins and water. The phospholipids arranged in bilayers act as a selective membrane to allow substances

Table 2 Viability of encapsulated probiotic strains during storage and digestion

Microorganism	Wall material	Encapsulation technique	EE (%)	Storage			Digestion		Research findings	Reference
				Temp	Days	Viability (log CFU/g)	Method	Viability (log CFU/g)		
L. acidophilus	Calcium alginate + WPI	Freeze drying	92%	7 °C	90	8.34	Static in vitro	8.13	Probiotics with multilayers of protein and sodium alginate improved the cell viability during storage and digestion	[42]
L. acidophilus	Pectin, hi-maize, inulin, rice bran	Freeze drying	68%	7 °C	120	6.60	Static in vitro	7.4 at Ileum condition	These novel wall materials along with pectin improves the cell viability by internal gelation and protects the probiotics cells	[43]
				25 °C	120	4.20				
L. acidophilus	Whey powder and gum arabic	Spray drying	94%	4 °C	28	8.5	Static in vitro	7.98	Encapsulated probiotics were incorporated in the yogurt, and the samples were stable for 28 days with similar cell count	[44]
L. acidophilus	Skim milk, Sucrose, Maltodextrin (MD), Corn starch	Spray drying, freeze drying	78 – 80.5%	4 °C	30	9.20	–	–	Maltodextrin had better protection of the bacterial cells with higher viability during storage	[45]
L. rhamnosus	WPI, crystalline nanocellulose, inulin	Freeze drying	90%	–	–	–	In vitro static method	1.6	The complex wall material system significantly improved the survivability of the bacteria during the simulated digestion	[46]
L. rhamnosus	WPI + modified starch	Spray drying	–	4 °C	60	No significant change	–	–	Loss of 0.96 log CFU/g after spray drying was observed. Probiotics incorporated functional food (green tea) maintained the cell viability of 7.33 CFU/mL for 5 weeks at 4 °C	[47]

Table 2 (continued)

Microorganism	Wall material	Encapsulation technique	EE (%)	Storage			Digestion		Research findings	Reference
				Temp	Days	Viability (log CFU/g)	Method	Viability (log CFU/g)		
L. rhamnosus	FOS + sodium alginate	Extrusion	91%	4 °C	30	7	In vitro Gastric	~8.5	SA-FOS encapsulated probiotics were stable during the simulated digestion and maintained the cell viability above 8 log CFU/g	[48]
							Bile condition	~9.2		
L. rhamnosus	Eudragit® S100	Spray drying	–	5 °C	56	0.4 log reduction	In vitro Gastric	1.5 log reduction	Eudragit maintained the viability of bacterial cells during simulated gastrointestinal condition and can be an ideal candidate for colon specific delivery of probiotics	[49]
L. rhamnosus	SPI, MTGase	Spray drying, freeze drying, electrospraying technique	93%	4 °C	30	10.8	In vitro Gastric	9.6 (1 h)	Spray drying provided better encapsulation than the freeze drying and electrospraying technique. Cross-linking effect of SPI with MTGase improved the viability during storage and GI digestion	[50]
				25 °C	30	10.8	In vitro Intestine	5.6 (2 h)		
L. plantarum	Soluble starch	Spray drying	–	4 °C	56	~9.6	–	–	Soluble starch has the highest glass transition temperature among the selected carbohydrate-based wall materials. 2.5% soluble starch can provide better protection against heat	[51]

Table 2 (continued)

Microorganism	Wall material	Encapsulation technique	EE (%)	Storage			Digestion		Research findings	Reference
				Temp	Days	Viability (log CFU/g)	Method	Viability (log CFU/g)		
L. plantarum	WPI, gum arabic	Spray drying (SD), Freeze drying (FD)	67% (SD) 84 (FD)	25 °C	60	60.52 (SD)	–	–	First time reported the co-encapsulation of phytosterols and probiotics by complex coacervation technique. Developed Ironian white cheese with the coencapsulated probiotics maintained the viability of bacterial cells than the free cells	[52]
						82.03 (FD)				
L. plantarum	Acrycoat S100	Spray drying	81%	–	–	–	–	–	Encapsulated probiotics were incorporated into Milano-type salami. Incorporation of probiotics in salami didn't influence the sensory characteristics	[53]
L. plantarum	Skim milk	Spray drying		4 °C	21	7.6 (Yogurt)	–	–	Successfully developed probiotic cheese and yogurt and both showed excellent sensory characteristics	[54]
					56	8.8 (Cheese)				
L. plantarum	Maltodextrin, gum Arabic, beetroot powder	Spray drying		25 °C	90	3.59	–	–	Reduction in the cell viability after spray drying	[55]
L. plantarum	Soy milk, Soyyogurt	–	–	–	–	–	Dynamic digestion DIVRSD-II plus	–	Change in digestive pattern of soy protein due lactic fermentation and influence of protein digesta on human faecal microbiota	[56]

Table 2 (continued)

Microorganism	Wall material	Encapsulation technique	EE (%)	Storage			Digestion		Research findings	Reference
				Temp	Days	Viability (log CFU/g)	Method	Viability (log CFU/g)		
L. plantarum	–	–	–	–	–	–	Dynamic model. SIMGI	7 – 9 in colon	*L. plantarum* can act as a bio-enhancer of polyphenol metabolisms	[57]
Bacillus coagulans	Incorporated in orange juice, yogurt	–	–	–	–	–	Semidynamic model	7.25 (juice) 7.10 (yogurt)	*B. coagulans* in food matrix (orange juice and yogurt) had highly resistant to simulated gastric and intestinal conditions	[58]

that move in (nutrients) and out (waste excretions) of the cell through specific channels. Lipid bilayers are normally found in the liquid-crystalline phase when the cells are alive. This lipid bilayer might be transformed into a gel phase and re-orients to a hexagonal form due to temperature change during dehydration by drying (Fig. 4). Further, this membrane phase transition affects the cell membrane permeability, cell component synthesis and subsequent transport across the cell membrane. The functions of the cell membrane are important for bacterial cell activity and viability. To preserve the cells from adverse environmental conditions, the cell membrane should be protected in terms of permeability and stability despite the osmotic stresses [60].

2.3.3 Viability of spray-dried probiotic bacteria

It has been reported that the influence of the spray-drying process on survival rate can be related to the air temperature, feed flow rate, type of atomization, the extent of shear during atomization, heat and mass transfer between the droplets and hot air, and drying kinetics [61]. Moreover, spray-drying air temperature and wall material formulations have a direct impact on the survival of the bacterial cells after drying and during storage [62].

Damage in the bacterial cell membrane is very prone for spray-dried probiotics due to simultaneous dehydration and thermal stress. Several studies reported that the outlet temperature plays a key role in maintaining the cell viability during the spray-drying process. In practice, outlet temperature is controlled by the parameters such as airflow rate, inlet temperature [63], feed flow rate, and feed solution concentration [64]. The survival rate of microorganisms can be directly related to the temperature of droplets and the residence time of the droplets that underwent lethal temperature range [65]. Spray-drying operation with higher outlet temperature rapidly brings the maximum temperature of droplets and ensures the maximum residence time of droplet in their lethal temperature range. For mesophilic organisms, operating above 55 °C could become lethal. Though the outlet air temperature does not reflect the entire temperature, it could be used to some extent as an indication of the particle temperature inside the drier [23]. Previous research showed that proper protective wall materials, outlet temperature and residence time inside the spray dryer yielded acceptable cell survival after spray drying [66]. Alternatively, increasing the feed flow rate can improve the cell survival during spray drying; however, there will be an increase in moisture content due to poor incomplete drying of droplets [67].

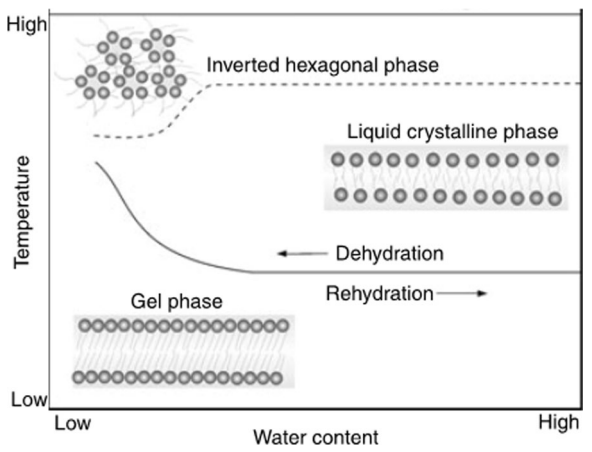


Fig. 4 Schematic diagram of drying and rehydration process of phospholipids [60]

Apart from outlet temperature, another factor associated with the temperature of atomized droplet is the wall material and initial solid concentration. The total solid concentration of the wall material solution can strongly induce the depression on the melting temperature of the microcapsule. This phenomenon affects the structural integrity of cytoplasmic membrane and cell wall rupture [68]. Typically, 10–20% (w/w) wall material concentration is used for microencapsulation of probiotics [69, 70]. It has been reported that the survival rate of spray-dried probiotics decreased with an increase in solid content of the feed solution, due to the lower rate of water evaporation and longer drying time [71]. The complex composition of the wall material is reported for decreasing the heat and osmotic stress during drying and enhances the viability of probiotics [72, 73]. For instance, the sugar as a wall material protects the probiotics against thermal degradation by following two mechanisms: (1) chemical reaction between the sugars and cell membrane and (2) reduction of thermal and oxidative stress during dehydration [23].

2.3.4 Factors affecting the viability of microencapsulated probiotics during storage

Parameters that affect the viability includes temperature, relative humidity, storage environment, the wall material composition, and exposure to light [74, 75]. Different wall materials were employed to enhance the survival rate of probiotics and the cell viability is dependent on the storage temperature and wall matrix composition. Figure 5 illustrates the viability of encapsulated probiotic cells at different storage conditions. In a recent study, Arepally and Reddy (76) reported that encapsulated *L. acidophilus* cells were more viable at 4 and 25 °C than the free cells for a storage period of 12 weeks. However, encapsulated cells stored at 4 °C showed higher viability than

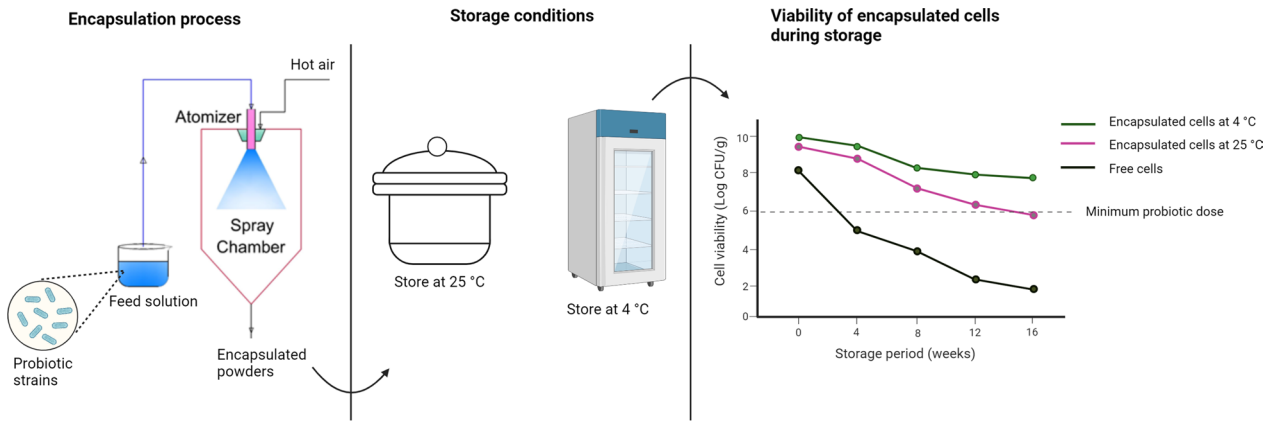


Fig. 5 Viability of encapsulated probiotics during storage [created with BioRendor.com]

25 °C. The reduction in cell viability when increasing the storage temperature is mainly attributed to the oxidation of membrane lipids and protein denaturation leading to the denaturation of macromolecules in the bacterial cells [23, 76]. Thus, the efficiency of the encapsulation process in many research studies was restricted to 4 °C and maintain the cell viability to minimum 10^6 log CFU/g.

In another study, Russo, Abeijón-Mukdsi [77] encapsulated feruloyl esterase (FE) producing *Lactobacillus* strains in different compositions of inulin, sodium alginate and maltodextrin and evaluated the cell viability and feruloyl esterase activity at 4 °C storage condition. Feruloyl esterase (FE) are hydrolytic enzymes that release ferulic acid from its esterified form and the ferulic acids are widely reported for their antioxidant, antidiabetic, antimicrobial, anticancer, and antihypertensive properties [78, 79]. Releasing feruloyl esterase in the gut through probiotics strains is an intelligent way to improve the bioavailability of ferulic acid and improve oxidative status. Authors encapsulated three strains of FE producing Lactobacillus viz., *L. acidophilus*, *L. fermentum,* and *L. johnsonii*. Surprisingly, the encapsulated *L. fermentum,* and *L. johnsonii* maintained the cell viability for 12 months at 4 °C, whereas *L. acidophilus* were showing minimum cell viability of $\geq 10^6$ CFU/g only for 4 months of storage at 4 °C. Thus, temperature, bacterial strains and their level of damage to the intercellular components determine the viability of encapsulated probiotic bacteria during storage.

2.4 Freeze drying

Freeze drying is commonly used for drying heat-sensitive biological materials, pharmaceuticals, and foods. Freeze drying is also known as "lyophilization," combines the critical freezing step and sublimation step, by first freezing the water and then convert the frozen water into vapor by sublimation under reduced pressure [80]. The freeze drying process was first developed commercially during World War II to preserve blood plasma and penicillin. Later, French virologist Charles Merieux used freeze drying technology for vaccine preservation. Scientist, Max Mortgenthaler invented freeze-dried coffee in 1938, which led to the development of powdered food products. The low operating temperature in the freeze-drying process minimizes the denaturation of products that usually occur in other drying methods [81]. However, the main disadvantage of this technique is the high capital cost associated with setup, operation, and maintenance. Further, freeze-drying process can cause structural damage to the bacterial cells and leads to a decrease in viability and metabolic activity [82, 83].

Freeze drying process: Three stages are involved in the conventional freeze-drying process: (i) freezing, (ii) primary drying (sublimation) and (iii) secondary drying (desorption).

Freezing: In the first step of freeze drying, freezing initiates the ice crystal growth in the liquid solution and resulted in the separation of water molecules from the solution by ice crystals [84].

Primary drying: During primary drying, the ice crystals are removed from the frozen product by sublimation, by reducing the chamber pressure and initiate the sublimation of ice by increasing the shelf temperature at a controlled rate [85].

Secondary drying: Even after primary drying, considerable amount (15–20%) of unfrozen water retains with the product, which are desorbed in the secondary drying by altering the chamber pressure and temperature. The elevated temperature under vacuum finally allows the desired residual moisture content (2–10%) of the product [84].

2.4.1 Microencapsulation of probiotics by freeze drying

Freeze drying is a popular technique for preserving probiotics in dried form for a long storage period, but using freeze drying for encapsulation is relatively a new concept [61]. Microencapsulation by freeze drying involves dispersion of probiotic cells in an aqueous solution of wall material and freezing at low temperatures, followed by sublimation of the frozen water under vacuum [23, 29]. Although processing conditions are milder, loss of cell viability occurs, particularly during the freezing stage. The cell inactivation during freezing depends on the cooling rate, and maximal survival loss occurs during the slow cooling stage (− 4 °C to − 20 °C) [60]. Freezing stage can induce intense stress and damage to the cell wall due to formation of ice crystals [61]. Carvalho et al. [86, 87] found the following two mechanisms responsible for the survival loss of bacteria during freeze drying: (1) change in the physical state of the membrane lipids and (2) change in the structure of the sensitive proteins in the cell. The main advantage of freeze drying is water phase transition, and oxidation can be avoided.

2.4.2 Cell inactivation and viability during freeze drying

In freeze drying method, cell inactivation mostly occurs during freezing stage [75]. During freezing, the formation of extracellular ice crystals increases extracellular osmolality, and the cell begins to dehydrate. However, the rate of freezing influences the formation of extracellular ice, because slow freezing creates ice formation outside the cell wall and fast freezing leads to excessive cellular shrinkage leading to cellular damage [88]. Cell size also influences the survival during freeze drying, wherein spherical-shaped cells are more resistant than the large rod-shaped cells [75].

The dehydration mechanism of freeze drying is different from spray drying; thus, the cryo-injuries and thermal injuries are different [23]. The wall materials permeate the cell wall during the freeze drying process leading to reduction in viability. To improve the cell viability during freeze drying and also to stabilize them during storage, protective agents like skim milk, whey milk, sucrose, amino acids, dietary fibers, or prebiotics are incorporated in the carrier medium [82]. *Lactobacillus plantarum* NRRL B4496 cells entrapped in the enzymatically extracted purple rice bran fiber resulted in less than one log reduction after freeze drying, whereas unencapsulated cells had greater than 6 log reductions. Casein increased the viability of probiotic cells after freeze drying and during storage [31]. Apart from that, some prebiotic fibers (fructooligosaccharide, mannitol, sorbitol, lactulose, inulin, xylitol, and raffinose) were also found to protect the stability and viability of probiotic bacteria during freeze drying [89]. Probiotic bacteria (*L. acidophilus, L. casei, B. bifidum, and B. longum*) encapsulated in peptide (1% w/w), sodium alginate (1% w/w) and fructo oligosaccharide (3% w/w) had improved cell viability after freeze drying [90]. Capela and Hay (91) used prebiotic (2.5% w/v) as cryoprotectant during freeze drying, and it improved the cell viability up to 7%. Thus, cryoprotectants play an important role in reducing the osmotic pressure difference between the probiotic cells and freeze-drying chamber [92]. Cryoprotectants are also incorporated into the growth medium before fermentation to assist the adaptation of probiotic cells to the environment [91, 93].

Currently, to improve the probiotic functionality, probiotic cells are encapsulated with blends of protein-polysaccharide matrix [31]. The viability of *L. casei* was found to be higher in microcapsules than probiotic microcapsules. Microencapsulated *Lactobacillus plantarum* in alginate matrix and whey protein showed better survival compared to uncoated beads [94].

2.4.3 Cell viability of microencapsulated bacteria during storage

Freeze drying is one of the most frequently used methods to preserve the probiotic cells for long term storage [75]. During the freeze drying process, the decrease of water activity leads to damage of the cellular structure, which can be avoided by cryoprotectants [87]. These cryoprotectants (glycerol, trehalose, sucrose, lactose) are aimed to replace the water loss during dehydration process [95]. Further, these cryoprotectants also form a glassy matrix and restrict the molecular interactions [96]. Thus, the glass transition temperature (Tg) at different water content should be taking into account for long-term storage of freeze-dried microcapsules [97]. In order to understand the influence of glass transition and molecular mobility on the viability of probiotic bacteria upon storage, Tymczyszyn and Gómez-Zavaglia [98] correlated the molecular mobility and Tg with the rate of survival at different temperatures. The rate of survival of probiotic cells during different storage time helps to calculate the cell inactivation rate.

Storage temperature is the critical parameter for cell survival, and the probiotic microcapsules stored above refrigeration temperature may increase the rate of metabolism and hence lead to survival loss [99]. Tymczyszyn and Díaz (83) observed drastic loss of viability of freeze-dried *Lactobacillus delbrueckii* subsp. *Bulgaricus* after 45 days of storage at 32 °C in glassy galactooligosaccharide matrix. In a recent study, freeze dried *Lactobacillus casei*, and *Lactobacillus acidophilus* with the combination of wall materials WPI and FOS were survived for 30 days storage at 4 and 25 °C. Surprisingly, the cell counts of encapsulated *L. casei*, and *L. acidophilus*

samples stored at 4 °C after 30 days were above 8 CFU/mg [100]. Most reports focused on survival during freeze drying, not during storage and exposure in harsh environmental conditions. Carlvalho et al. [86, 87] suggested that the dried microcapsules should be stored under vacuum without light and moisture exposure. Increasing relative humidity and temperature leads to higher loss of cell viability during storage [101].

2.5 Viability of microencapsulated probiotics in the gastrointestinal condition

2.5.1 In vitro digestion models

In the past two decades, there is an increasing trend in the development and application of in vitro digestion models, because in vitro models can allow the researcher to conduct digestion studies at a rapid phase with less labor, less cost, and does not need ethical clearance like in vivo studies. Briefly, in vitro digestion models are classified into three categories as static, semi-dynamic and dynamic in vitro models.

2.5.2 Static digestion model

Static models are also called biochemical models. Wickham and Faulks (102) define the static models as the products of digestion are not removed during the digestion process (i.e., no absorption) and do not mimic the physical processes that occur in vivo (e.g., shear, mixing, hydration, changing conditions over time, etc.). Mostly, static models are a batch process, where the digestion was carried out for the predetermined time and the sample was collected for analysis. Static models are preferred among researchers because it is economical and simple to arrange the digestion setup, and most times, the conical flask with a simple stirring mechanism is highly sufficient to conduct digestion. Working with static models are simple, involving the homogenization of food matrix containing probiotics or encapsulated probiotics, bringing the gastric pH in acidic environment, gradual addition of gastric enzyme and secretions [to simulate the gastric digestion], followed by neutralization of pH and addition of intestinal secretions like pancreatic enzymes, bile salts [intestinal environment]. The entire digestion step should be conducted under constant mixing (through orbital shaker/magnetic stirrer) at 37 °C. Only the limited parameters can be simulated in the static method and not able to mimic the other physiological process like shearing, mixing, changes in conditions over time, peristaltic contraction, absorption, etc. Thus, the static digestion models are useful for the digestion of simple foods/isolated bioactive compounds [102, 103]. Recent probiotics digestion studies on various in vitro models are detailed in Table 2.

2.5.3 Dynamic digestion model

Static models cannot simulate the dynamic process of digestion, especially the change in pH, circumferential contraction of the gastric wall, gastric emptying through the pylorus, intestinal wall motility (segmentation and peristalsis) for mixing of chyme with the digestive enzymes. To overcome these limitations, researchers are interested in developing dynamic digestions models. The dynamic model aims to bring digestion closer to the animal/human physiological conditions, by reproducing the variable gastric conditions like secretion of digestive fluids and enzyme concentration, chyme transit, and mixing due to peristalsis. Interested readers can refer the fabrication of dynamic gastrointestinal simulator [57], multicompartmental dynamic model [104], engineered small intestinal system [105].

2.5.4 protocol

Irrespective of the digestion model, researchers need to follow a digestion protocol for preparing the simulated digestion fluids, enzyme solution for the digestion. Though there are plenty of digestion protocols to prepare simulated digestion fluids, the standardized protocol developed by the COST INFOGEST network simulates the wide range of digestion conditions [106–108]. Digestion study involves three successive stages of exposure: oral, gastric and intestinal phases. And the simulated digestion fluids should be prepared for each stage comprising different enzymes and quantity of electrolytes.

Oral phase Food matrix is first exposed in the oral phase and amylase acts on the food. To mimic this process in the lab, food is mixed with simulated salivary fluid (SSF) at 1:1 (w/w) ratio with or without amylase. The selection of amylase is based on the starch content in the food and the final mixture of the oral phase is called "oral bolus." Brodkorb et al. [107] recommended masticating the solid food into semisolid consistency using an electric mincer, and 2 min of exposure with the SSF is sufficient to mimic the oral phase.

Gastric and intestinal phase Before the gastric phase, the final oral bolus volume should be noted. Then, the gastric phase is initiated by adding equal portion of simulated gastric fluid (SGF) in the oral bolus. SGF contains digestive enzymes like pepsin and gastric lipase, and the concentration of digestive enzymes is calculated for the final volume of each digestion phase. Then, the digestion process is simulated by incubating the mixture at 37 °C for 2 h under agitation/mixing. The variation in the static vs dynamic model begins here. For the static model, constant mixing with a magnetic stirrer or orbital shaker is employed in the digestion mixture. In the case of a dynamic model, gastric wall motility will be replicated by some external force and mimics the mixing process of

digestive content with gastric secretions, and the gastric secretions will be added gradually through a peristaltic pump. Then, the gastric chyme is then diluted with simulated intestinal fluid (SIF) at 1:1 ratio. SIF contains bile salts; pancreatic enzymes and the intestinal phase is simulated by incubating at pH 7 for 2 h.

2.6 Stability of microencapsulated probiotics during gastrointestinal digestion

For the spray-dried probiotics, the ability to survive during simulated digestion mainly depends on the inlet air temperature, wall material and exposure in the GI condition [109]. Further the probiotic cells that underwent sub-lethal heat injuries during spray-drying operation showed poor survival in the simulated GI conditions [110]. Further, protein-based microcapsules have less polar group than polysaccharides, and therefore, the proteins keep the acidic effect out of the microcapsule core [109]. Thus, the bacterial cells encapsulated with whey protein have shown to offer better protection in the acidic environment [111]. *B. longum* encapsulated in gum arabic, gelatin, water-soluble starch by spray drying showed a higher protective effect in simulated gastric solution [64]. O'riordan and Andrews [112] reported that the viability of starch encapsulated bifidobacteria did not showed any significant improvement in the viability during gastric exposure. But when resistant starch was used as a wall material, the encapsulated probiotics were viable even after 8 h residence in the simulated gastrointestinal conditions [113].

For the freeze-dried probiotics, fermentation pH and drying time are the important process parameters for maintaining the viability of *L. rhamnosus* in the gastric condition. Natural hydrocolloids and gums (alginate, carrageenan) can effectively protect the bacterial cells in the gastric condition [7]. Freeze-dried *B. longum* encapsulated in 20% polysaccharide with 20% skim milk matrix enhanced the resistance of the cell in simulated digestive conditions. The viability of cells without protectants decreased from 9.20 to 2.91 log (CFU/g), while there was only a reduced loss in encapsulated cells from 9.26 to 7.83 log (CFU/g). Freeze-dried *B. longum* encapsulated in chitosan and alginate protected the bacterial cells from the harsh acidic condition and bile salt injury and maintained the viability of 6.43 CFU/g after 2 h intestinal phase [114]. Encapsulation with single/multiple biopolymer coatings was suggested as an effective method to protect probiotics from gastric and bile fluids [115]. Similarly, whey protein encapsulated probiotics maintained its viability during the simulated gastrointestinal conditions [116, 117].

3 Conclusions

Microencapsulation is one of the most effective techniques to enhance the stability and viability of live probiotic strains from industrial processing conditions, storage and also protect them from the gastrointestinal environment. In this review, application of drying techniques (spray drying and freeze drying) for encapsulation of probiotics was discussed. Both drying techniques have their own merits and demerits. Spray drying is used to produces fine encapsulates with single unit operation; however, there will be reduction in viability due to higher drying temperature. Alternatively, freeze drying may retain the cell viability due to low drying temperature; however, freeze drying involved high operating cost and it produces uneven flaky structure. Though a handful of marketed products on probiotics is available, however, maintaining cell viability for a longer period is still difficult to achieve. Fortification of probiotics with nutraceuticals, co-encapsulation of probiotics with other bioactive compounds, extending cell viability during the storage, processing, and digestion should be evaluated for successful commercialization.

Abbreviations

FD: Freeze drying; FE: Feruloyl esterase; GI: Gastrointestinal; GRAS: Generally recognized as safe; SD: Spray drying; SGF: Simulated Gastric fluid; SIF: Simulated intestinal fluid; Tg: Glass transition temperature.

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References

1. Rajam R, Anandharamakrishnan C (2015) Spray freeze drying method for microencapsulation of Lactobacillus plantarum. J Food Eng 166:95–103
2. Anal AK, Singh H (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci Technol 18(5):240–251

3. Rajam R, Anandharamakrishnan C (2015) Microencapsulation of Lactobacillus plantarum (MTCC 5422) with fructooligosaccharide as wall material by spray drying. LWT-Food Sci Technol 60(2):773–780
4. Zommiti M, Feuilloley MGJ, Connil N (2020) Update of probiotics in human world: a nonstop source of benefactions till the end of time. Microorganisms 8(12):1907
5. Burgain J, Gaiani C, Linder M, Scher J (2011) Encapsulation of probiotic living cells: from laboratory scale to industrial applications. J Food Eng 104(4):467–483
6. Vasiljevic T, Shah NP (2008) Probiotics—from Metchnikoff to bioactives. Int Dairy J 18(7):714–728
7. Holzapfel WH, Schillinger U (2002) Introduction to pre-and probiotics. Food Res Int 35(2–3):109–116
8. Hamilton-Miller J, Gibson GR, Bruck W (2003) Some insights into the derivation and early uses of the word 'probiotic.' Br J Nutrit. 90(4):845
9. Rf AFRC (1989) Probiotics in man and animals. J Appl Bacteriol 66(5):365–378
10. Salminen S, Ouwehand A, Benno Y, Lee YK (1999) Probiotics: how should they be defined? Trends Food Sci Technol 10(3):107–110
11. FAO/WHO. Health and nutritional properties and guidelines for evaluation - Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. 2006.
12. Burgain J, Scher J, Francius G, Borges F, Corgneau M, Revol-Junelles AM et al (2014) Lactic acid bacteria in dairy food: Surface characterization and interactions with food matrix components. Adv Coll Interface Sci 213:21–35
13. Axelsson L (2004) Lactic acid bacteria: classification and physiology. Food Sci Technol N York Marcel Dekker 139:1–66
14. Shah NP (2007) Functional cultures and health benefits. Int Dairy J 17(11):1262–1277
15. Precedence. Functional Food Market Size Worth Around USD 309 Bn by 2027 2020. https://www.globenewswire.com/news-release/2020/11/20/2130656/0/en/Functional-Food-Market-Size-Worth-Around-USD-309-Bn-by-2027.html.
16. Tripathi MK, Giri SK (2014) Probiotic functional foods: Survival of probiotics during processing and storage. J Funct Foods 9:225–241
17. Lim S-M, Lee N-K, Kim K-T, Paik H-D (2020) Probiotic Lactobacillus fermentum KU200060 isolated from watery kimchi and its application in probiotic yogurt for oral health. Microb Pathogene. 147:104430
18. Langa S, Peirotén A, Gaya P, Escudero C, Rodríguez-Mínguez E, Landete J, et al. Development of multi-strain probiotic cheese: Nisin production in food and gut. LWT. 2021;148:111706.
19. Aspri M, Papademas P, Tsaltas D (2020) Review on non-dairy probiotics and their use in non-dairy based products. Fermentation 6(1):30
20. Desai KGH, Jin PH (2005) Recent developments in microencapsulation of food ingredients. Drying Technol 23(7):1361–1394
21. Anandharamakrishnan C, Parthasarathi S (2019) Food nanotechnology: principles and applications. CRC Press, Cambridge
22. Parthasarathi S, Anandharamakrishnan C (2016) Enhancement of oral bioavailability of vitamin E by spray-freeze drying of whey protein microcapsules. Food Bioprod Process 100:469–476
23. Fu N, Chen XD (2011) Towards a maximal cell survival in convective thermal drying processes. Food Res Int 44(5):1127–1149
24. Razavi S, Janfaza S, Tasnim N, Gibson DL, Hoorfar M (2021) Microencapsulating polymers for probiotics delivery systems: Preparation, characterization, and applications. Food Hydrocolloids. 120:106882
25. Kent RM, Doherty SB (2014) Probiotic bacteria in infant formula and follow-up formula: microencapsulation using milk and pea proteins to improve microbiological quality. Food Res Int 64:567–576
26. Mitropoulou G, Nedovic V, Goyal A, Kourkoutas Y (2013) Immobilization technologies in probiotic food production. J Nutrit Metab
27. Subramanian P (2021) Mucoadhesive delivery system: a smart way to improve bioavailability of nutraceuticals. Foods 10(6):1362
28. Subramanian P (2021) Lipid-based nanocarrier system for the effective delivery of nutraceuticals. Molecules 26(18):5510
29. Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV (2012) Microencapsulation of probiotics for gastrointestinal delivery. J Control Release 162(1):56–67
30. Jacobsen NMY, Caglayan I, Caglayan A, Bar-Shalom D, Müllertz A (2020) Achieving delayed release of freeze-dried probiotic strains by extrusion, spheronization and fluid bed coating - evaluated using a three-step in vitro model. Int J Pharmaceut. 591:120022
31. Heidebach T, Först P, Kulozik U (2012) Microencapsulation of probiotic cells for food applications. Crit Rev Food Sci Nutr 52(4):291–311
32. Souza M, Mesquita A, Veríssimo C, Grosso C, Converti A, Maciel MI (2020) Microencapsulation by spray drying of a functional product with mixed juice of acerola and ciriguela fruits containing three probiotic lactobacilli. Drying Technol. 1–11
33. Ghandi A, Powell IB, Chen XD, Adhikari B (2012) The effect of dryer inlet and outlet air temperatures and protectant solids on the survival of Lactococcus lactis during spray drying. Drying Technol 30(14):1649–1657
34. Kuriakose R, Anandharamakrishnan C (2010) Computational fluid dynamics (CFD) applications in spray drying of food products. Trends Food Sci Technol 21(8):383–398
35. Parthasarathi S, Ezhilarasi PN, Jena BS, Anandharamakrishnan C (2013) A comparative study on conventional and microwave-assisted extraction for microencapsulation of Garcinia fruit extract. Food Bioprod Process 91(2):103–110
36. Rajam R, Karthik P, Parthasarathi S, Joseph GS, Anandharamakrishnan C (2012) Effect of whey protein – alginate wall systems on survival of microencapsulated Lactobacillus plantarum in simulated gastrointestinal conditions. J Funct Foods 4(4):891–898
37. Anandharamakrishnan C, Gimbun J, Stapley A, Rielly CD (2009) Application of computational fluid dynamics (CFD) simulations to spray-freezing operations. Dry Technol 28(1):94–102
38. Masters K (1985) Spray drying handbook. Spray drying handbook
39. Poozesh S, Akafuah NK, Campbell HR, Bashiri F, Saito K (2020) Experimental and mathematical tools to predict droplet size and velocity distribution for a two-fluid nozzle. Fluids 5(4):231
40. Gouaou I, Shamaei S, Koutchoukali MS, Bouhelassa M, Tsotsas E, Kharaghani A (2019) Impact of operating conditions on a single droplet and spray drying of hydroxypropylated pea starch: process performance and final powder properties. Asia-Pac J Chem Eng. 14(1):e2268
41. Huang S, Vignolles M-L, Chen XD, Le Loir Y, Jan G, Schuck P et al (2017) Spray drying of probiotics and other food-grade bacteria: A review. Trends Food Sci Technol 63:1–17
42. de Araújo Etchepare M, Nunes GL, Nicoloso BR, Barin JS, Moraes Flores EM, de Oliveira Mello R et al (2020) Improvement of the viability of encapsulated probiotics using whey proteins. LWT. 117:108601
43. Raddatz GC, Poletto G, Deus CD, Codevilla CF, Cichoski AJ, Jacob-Lopes E et al (2020) Use of prebiotic sources to increase probiotic viability in pectin microparticles obtained by emulsification/internal gelation followed by freeze-drying. Food Res Int. 130:108902
44. Leylak C, Özdemir KS, Gurakan GC, Ogel ZB (2021) Optimisation of spray drying parameters for Lactobacillus acidophilus encapsulation in whey and gum Arabic: Its application in yoghurt. Int Dairy J. 112:104865
45. Tang HW, Abbasiliasi S, Murugan P, Tam YJ, Ng HS, Tan JS (2020) Influence of freeze-drying and spray-drying preservation methods on survivability rate of different types of protectants encapsulated Lactobacillus acidophilus FTDC 3081. Biosci Biotechnol Biochem 84(9):1913–1920
46. Maleki O, Khaledabad MA, Amiri S, Asl AK, Makouie S (2020) Microencapsulation of Lactobacillus rhamnosus ATCC 7469 in whey protein isolate-crystalline nanocellulose-inulin composite enhanced gastrointestinal survivability. LWT. 126:109224
47. Hernández-Barrueta T, Martínez-Bustos F, Castaño-Tostado E, Lee Y, Miller MJ, Amaya-Llano SL (2020) Encapsulation of probiotics in whey protein isolate and modified huauzontle's starch: an approach to avoid fermentation and stabilize polyphenol compounds in a ready-to-drink probiotic green tea. LWT. 124:109131
48. Azam M, Saeed M, Pasha I, Shahid M (2020) A prebiotic-based biopolymeric encapsulation system for improved survival of Lactobacillus rhamnosus. Food Bioscience. 37:100679
49. Akanny E, Bourgeois S, Bonhommé A, Commun C, Doleans-Jordheim A, Bessueille F et al (2020) Development of enteric polymer-based microspheres by spray-drying for colonic delivery of Lactobacillus rhamnosus GG. Int J Pharmaceut. 584:119414
50. Azizi S, Rezazadeh-Bari M, Almasi H, Amiri S (2021) Microencapsulation of Lactobacillus rhamnosus using sesame protein isolate: effect of encapsulation method and transglutaminase. Food Bioscience. 41:101012

51. Tantratian S, Pradeamchai M (2020) Select a protective agent for encapsulation of Lactobacillus plantarum. LWT. 123:109075
52. Sharifi S, Rezazad-Bari M, Alizadeh M, Almasi H, Amiri S (2021) Use of whey protein isolate and gum Arabic for the co-encapsulation of probiotic Lactobacillus plantarum and phytosterols by complex coacervation: enhanced viability of probiotic in Iranian white cheese. Food Hydrocolloids. 113:106496
53. MachadoVasconcelos LI, Silva-Buzanello RAD, Kalschne DL, Scremin FR, Stival Bittencourt PR, Gaudêncio Dias JT et al (2021) Functional fermented sausages incorporated with microencapsulated Lactobacillus plantarum BG 112 in Acrycoat S100. LWT. 148:111596
54. Mirković M, Mirković N, Miočinović J, Radulović A, Paunović D, Ilić M, et al. Probiotic yogurt and cheese from ultrafiltered milk: Sensory quality and viability of free-living and spray dried Lactiplantibacillus plantarum 564 and Lactiplantibacillus plantarum 299 v. J Food Process Preserv. e15713.
55. Janiszewska-Turak E, Hornowska Ł, Pobiega K, Gniewosz M, Witrowa-Rajchert D (2021) The influence of Lactobacillus bacteria type and kind of carrier on the properties of spray-dried microencapsules of fermented beetroot powders. Int J Food Sci Technol 56(5):2166–2174
56. Zhang Z, Fu Y, Liu Q, Simpson BK, Azarpazhooh E, Dong M et al (2021) Effect of lactic fermentation on soy protein digestive pattern assessed by an in vitro dynamic gastrointestinal digestion model and the influence on human faecal microbiota. J Sci Food Agric 101(3):871–879
57. Gil-Sánchez I, Cueva C, Tamargo A, Quintela JC, de la Fuente E, Walker AW et al (2020) Application of the dynamic gastrointestinal simulator (simgi®) to assess the impact of probiotic supplementation in the metabolism of grape polyphenols. Food Res Int. 129:108790
58. Almada-Érix CN, Almada CN, Souza Pedrosa GT, Lollo PC, Magnani M, Sant'Ana AS (2021) Development of a semi-dynamic in vitro model and its testing using probiotic Bacillus coagulans GBI-30, 6086 in orange juice and yogurt. J Microbiol Methods. 183:106187
59. Bhagwat A, Bhushette P, Annapure US (2020) Spray drying studies of probiotic Enterococcus strains encapsulated with whey protein and maltodextrin. Beni-Suef Univ J Basic Appl Sci 9(1):33
60. Santivarangkna C, Kulozik U, Foerst P (2008) Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. J Appl Microbiol 105(1):1–13
61. Martín MJ, Lara-Villoslada F, Ruiz MA, Morales ME (2015) Microencapsulation of bacteria: a review of different technologies and their impact on the probiotic effects. Innov Food Sci Emerg Technol 27:15–25
62. Arepally D, Reddy RS, Goswami TK (2020) Encapsulation of Lactobacillus acidophilus NCDC 016 cells by spray drying: characterization, survival after in vitro digestion, and storage stability. Food Funct 11(10):8694–8706
63. Sunny-Roberts E, Knorr D (2009) The protective effect of monosodium glutamate on survival of Lactobacillus rhamnosus GG and Lactobacillus rhamnosus E-97800 (E800) strains during spray-drying and storage in trehalose-containing powders. Int Dairy J 19(4):209–214
64. Lian W-C, Hsiao H-C, Chou C-C (2002) Survival of bifidobacteria after spray-drying. Int J Food Microbiol 74(1):79–86
65. Desmond C, Stanton C, Fitzgerald GF, Collins K, Ross RP (2002) Environmental adaptation of probiotic lactobacilli towards improvement of performance during spray drying. Int Dairy J 12(2–3):183–190
66. Aragón-Rojas S, Quintanilla-Carvajal MX, Hernández-Sánchez H (2018) Multifunctional role of the whey culture medium in the spray drying microencapsulation of lactic acid bacteria. Food Technol Biotechnol 56(3):381–397
67. Schutyser MA, Perdana J, Boom RM (2012) Single droplet drying for optimal spray drying of enzymes and probiotics. Trends Food Sci Technol 27(2):73–82
68. Behboudi-Jobbehdar S, Soukoulis C, Yonekura L, Fisk I (2013) Optimization of spray-drying process conditions for the production of maximally viable microencapsulated L. acidophilus NCIMB 701748. Drying Technol. 31(11):1274–83
69. Farahmandi K, Rajab S, Tabandeh F, Shahraky MK, Maghsoudi A, Ashengroph M (2021) Efficient spray-drying of lactobacillus rhamnosus PTCC 1637 using total CFU yield as the decision factor. Food Biosci. 40:100816
70. Arepally D, Goswami TK (2019) Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying. LWT 99:583–593
71. Santivarangkna C, Kulozik U, Foerst P (2007) Alternative drying processes for the industrial preservation of lactic acid starter cultures. Biotechnol Prog 23(2):302–315
72. Diệp Huy Vũ P, Rodklongtan A, Chitprasert P. Whey protein isolate-lignin complexes as encapsulating agents for enhanced survival during spray drying, storage, and in vitro gastrointestinal passage of Lactobacillus reuteri KUB-AC5. LWT. 2021;148:111725.
73. Jiang N, Dev Kumar G, Chen J, Mishra A, Mis Solval K. Comparison of concurrent and mixed-flow spray drying on viability, growth kinetics and biofilm formation of Lactobacillus rhamnosus GG microencapsulated with fish gelatin and maltodextrin. LWT. 2020;124:109200.
74. Morgan CA, Herman N, White P, Vesey G (2006) Preservation of microorganisms by drying; a review. J Microbiol Methods 66(2):183–193
75. Meng X, Stanton C, Fitzgerald G, Daly C, Ross R (2008) Anhydrobiotics: the challenges of drying probiotic cultures. Food Chem 106(4):1406–1416
76. Arepally D, Reddy RS, Goswami TK (2020) Studies on survivability, storage stability of encapsulated spray dried probiotic powder. Curr Res Food Sci 3:235–242
77. Russo MI, Abeijón-Mukdsi MC, Santacruz A, Ross R, Malo AL, Gauffin-Cano P, et al. Spray dried lactobacilli maintain viability and feruloyl esterase activity during prolonged storage and under gastrointestinal tract conditions. J Food Sci Technol. 2021.
78. Oliveira DM, Mota TR, Oliva B, Segato F, Marchiosi R, Ferrarese-Filho O et al (2019) Feruloyl esterases: biocatalysts to overcome biomass recalcitrance and for the production of bioactive compounds. Biores Technol 278:408–423
79. Palani Swamy SK, Govindaswamy V (2015) Therapeutical properties of ferulic acid and bioavailability enhancement through feruloyl esterase. J Funct Foods. 17:657–66
80. Marcial-Coba MS, Cieplak T, Cahú TB, Blennow A, Knøchel S, Nielsen DS (2018) Viability of microencapsulated Akkermansia muciniphila and Lactobacillus plantarum during freeze-drying, storage and in vitro simulated upper gastrointestinal tract passage. Food Funct 9(11):5868–5879
81. Geankoplis CJ, Hersel AA, Lepek DH (2018) Transport processes and separation process principles. Prentice Hall, Boston, MA
82. Araújo CM, Sampaio KB, Menezes FNDD, Almeida ETDC, Lima MDS, Viera VB et al (2020) Protective effects of tropical fruit processing coproducts on probiotic lactobacillus strains during freeze-drying and storage. Microorganisms. 8(1):96
83. Tymczyszyn EE, Díaz R, Pataro A, Sandonato N, Gómez-Zavaglia A, Disalvo EA (2008) Critical water activity for the preservation of Lactobacillus bulgaricus by vacuum drying. Int J Food Microbiol 128(2):342–347
84. Kasper JC, Friess W (2011) The freezing step in lyophilization: physico-chemical fundamentals, freezing methods and consequences on process performance and quality attributes of biopharmaceuticals. Eur J Pharm Biopharm 78(2):248–263
85. Oikonomopoulou VP, Krokida MK, Karathanos VT (2011) Structural properties of freeze-dried rice. J Food Eng 107(3–4):326–333
86. Carvalho AS, Silva J, Ho P, Teixeira P, Malcata FX, Gibbs P (2004) Relevant factors for the preparation of freeze-dried lactic acid bacteria. Int Dairy J 14(10):835–847
87. Carvalho AS, Silva J, Ho P, Teixeira P, Malcata FX, Gibbs P (2004) Effects of various sugars added to growth and drying media upon thermotolerance and survival throughout storage of freeze-dried Lactobacillus delbrueckii ssp bulgaricus. Biotechnol Progress. 20(1):248–54
88. Fowler A, Toner M (2006) Cryo-injury and biopreservation. Ann N Y Acad Sci 1066(1):119–135
89. Ann EY, Kim Y, Oh S, Imm JY, Park DJ, Han KS et al (2007) Microencapsulation of Lactobacillus acidophilus ATCC 43121 with prebiotic substrates using a hybridisation system. Int J Food Sci Technol 42(4):411–419
90. Chen KN, Chen MJ, Liu JR, Lin CW, Chiu HY (2005) Optimization of incorporated prebiotics as coating materials for probiotic microencapsulation. J Food Sci 70(5):M260–M266
91. Capela P, Hay T, Shah NP (2006) Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. Food Res Int 39(2):203–211
92. Schwab C, Vogel R, Gänzle MG (2007) Influence of oligosaccharides on the viability and membrane properties of Lactobacillus reuteri TMW1. 106 during freeze-drying. Cryobiology. 55(2):108–14

93. Basholli-Salihu M, Mueller M, Salar-Behzadi S, Unger FM, Viernstein H (2014) Effect of lyoprotectants on β-glucosidase activity and viability of Bifidobacterium infantis after freeze-drying and storage in milk and low pH juices. LWT-Food Sci Technol 57(1):276–282
94. Gbassi GK, Vandamme T, Ennahar S, Marchioni E (2009) Microencapsulation of Lactobacillus plantarum spp in an alginate matrix coated with whey proteins. Int J Food Microbiol 129(1):103–105
95. Santivarangkna C, Aschenbrenner M, Kulozik U, Foerst P (2011) Role of glassy state on stabilities of freeze-dried probiotics. J Food Sci 76(8):R152–R156
96. Tymczyszyn EE, Sosa N, Gerbino E, Hugo A, Gómez-Zavaglia A, Schebor C (2012) Effect of physical properties on the stability of Lactobacillus bulgaricus in a freeze-dried galacto-oligosaccharides matrix. Int J Food Microbiol 155(3):217–221
97. Higl B, Kurtmann L, Carlsen CU, Ratjen J, Först P, Skibsted LH et al (2007) Impact of water activity, temperature, and physical state on the storage stability of Lactobacillus paracasei ssp. paracasei freeze-dried in a lactose matrix. Biotechnol Progress. 23(4):794–800
98. Tymczyszyn E, Gómez-Zavaglia A, Disalvo E (2007) Effect of sugars and growth media on the dehydration of Lactobacillus delbrueckii ssp. bulgaricus. J Appl Microbiol. 102(3):845–51
99. Ranadheera R, Baines S, Adams M (2010) Importance of food in probiotic efficacy. Food Res Int 43(1):1–7
100. Massounga Bora AF, Li X, Zhu Y, Du L (2019) Improved viability of microencapsulated probiotics in a freeze-dried banana powder during storage and under simulated gastrointestinal tract. Probiot Antimicrob Proteins 11(4):1330–1339
101. Lapsiri W, Bhandari B, Wanchaitanawong P (2013) Stability and probiotic properties of Lactobacillus plantarum spray-dried with protein and other protectants. Drying Technol 31(13–14):1723–1733
102. Wickham M, Faulks R, Mills C (2009) In vitro digestion methods for assessing the effect of food structure on allergen breakdown. Mol Nutr Food Res 53(8):952–958
103. Alegría A, Garcia-Llatas G, Cilla A. Static digestion models: general introduction. The impact of food bioactives on health2015. p. 3–12.
104. Minekus M, Marteau P, Havenaar R, Veld JHJHIT. A Multicompartmental Dynamic Computer-controlled Model Simulating the Stomach and Small Intestine. Alternatives to Laboratory Animals. 1995;23(2):197–209.
105. Parthasarathi S, Bhushani JA, Anandharamakrishnan C (2018) Engineered small intestinal system as an alternative to in-situ intestinal permeability model. J Food Eng 222:110–114
106. Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C et al (2014) A standardised static in vitro digestion method suitable for food – an international consensus. Food Funct 5(6):1113–1124
107. Brodkorb A, Egger L, Alminger M, Alvito P, Assunção R, Ballance S et al (2019) INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc 14(4):991–1014
108. Mulet-Cabero A-I, Egger L, Portmann R, Ménard O, Marze S, Minekus M et al (2020) A standardised semi-dynamic in vitro digestion method suitable for food – an international consensus. Food Funct 11(2):1702–1720
109. Arslan S, Erbas M, Tontul I, Topuz A (2015) Microencapsulation of probiotic Saccharomyces cerevisiae var. boulardii with different wall materials by spray drying. LWT-Food Sci Technol. 63(1):685–90
110. Golowczyc MA, Gerez CL, Silva J, Abraham AG, De Antoni GL, Teixeira P (2011) Survival of spray-dried Lactobacillus kefir is affected by different protectants and storage conditions. Biotech Lett 33(4):681–686
111. DeCastro-Cislaghi FP, CarinaDosReis ES, Fritzen-Freire CB, Lorenz JG, Sant'Anna ES. Bifidobacterium Bb-12 microencapsulated by spray drying with whey: Survival under simulated gastrointestinal conditions, tolerance to NaCl, and viability during storage. J Food Eng. 2012;113(2):186–93.
112. O'riordan K, Andrews D, Buckle K, Conway P (2001) Evaluation of microencapsulation of a Bifidobacterium strain with starch as an approach to prolonging viability during storage. J Appl Microbiol 91(6):1059–1066
113. Ashwar BA, Gani A, Gani A, Shah A, Masoodi FA (2018) Production of RS4 from rice starch and its utilization as an encapsulating agent for targeted delivery of probiotics. Food Chem 239:287–294
114. Ji R, Wu J, Zhang J, Wang T, Zhang X, Shao L et al (2019) Extending viability of Bifidobacterium longum in chitosan-coated alginate microcapsules using emulsification and internal gelation encapsulation technology. Front Microbiol 10:1389
115. Annan N, Borza A, Hansen LT (2008) Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic Bifidobacterium adolescentis 15703T during exposure to simulated gastro-intestinal conditions. Food Res Int 41(2):184–193
116. Yoha KS, Anukiruthika T, Anila W, Moses JA, Anandharamakrishnan C (2021) 3D printing of encapsulated probiotics: Effect of different post-processing methods on the stability of Lactiplantibacillus plantarum (NCIM 2083) under static in vitro digestion conditions and during storage. LWT. 146:111461
117. Ying D, Sun J, Sanguansri L, Weerakkody R, Augustin MA (2012) Enhanced survival of spray-dried microencapsulated Lactobacillus rhamnosus GG in the presence of glucose. J Food Eng 109(3):597–602

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