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Application of spray-drying and freeze-drying for microencapsulation of lactic acid bacteria: A review

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Article Info	Abstract
Article history Received 13 April 2023 Revised 1 June 2023 Accepted 2 June 2023 Published Online 30 June-2023	Lactic acid bacteria (LAB) are well known for the production of fermented foods and their beneficial effects on consumers. However, they are very sensitive to environmental conditions, and their viability and functionality can be affected during different processing methods and storage. Therefore, encapsulation is essential to avoid injuries to bacterial cells and improve their survivability. Freeze-drying and spray-drying are two commonly used drying techniques for microencapsulation. Freeze-drying has been the
Keywords Microencapsulation Spray-drying Freeze-drying Lactic acid bacteria Probiotic food powders	conventional drying process for encapsulation and production of bacterial cultures in dried form, but it has some limits, such as low production yield and longer drying time. On the other hand, spray-drying technique has benefits such as fast, higher, and continuous productivity. Nowadays, due to increasing urbanization and consumer awareness, the beneficial LAB is dried in various food matrices apart from dairy food to produce functional food powders in ready-to-reconstitute form. These products have beneficial effects on live microbes as well as nutritional and functional properties of carrier media. This article provides a comprehensive overview of the application of freeze-drying and spray-drying methods for the encapsulation of LAB. It highlights the importance of the encapsulation of LAB and the production of functional probiotic food powders using different wall materials.

1. Introduction

Lactic acid bacteria (LAB) are common microflora of the human intestine and fermented foods. They play an important role in improving the nutritional and flavoring attributes of food and providing health benefits to humans (Meena et al., 2023). Many LAB has been categorized as probiotics, which mainly belongs to Lactobacillus (e.g., Lactobacillus plantarum, L. acidophilus, L. rhamnosus) and Bifidobacterium (e.g., Bifidobacterium bifidum, Bifidobacterium longum) genera (Meena et al., 2022). Other microbes such as Enterococcus faecium, Lactococcus lactis ssp. lactis, and yeasts such as Saccharomyces boulardii also reported to exhibit probiotic attributes (Barbosa and Teixeira, 2017). The LAB group has a "generally recognized as safe (GRAS)" status (Frakolaki et al., 2021). These species have been extensively studied for their healthpromoting attributes, and a number of lactic acid bacterial based probiotic foods are sold in the market (Diez-Ozaeta and Astiazaran, 2022; Sharma et al., 2022). Probiotic LAB have been shown to enhance digestive health, improve immune function (Meena et al., 2008, 2017), and reduce the risk of certain diseases such as inflammatory bowel

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Copyright © 2023 Ukaaz Publications. All rights reserved. Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com disease and colorectal cancer (Sumathi *et al.*, 2021; Chaudhari and Dwivedi, 2022). They achieve these health benefits through various mechanisms, such as the production of antimicrobial compounds, strengthening of the gut barrier function, and modulation of the immune system (Diez-Ozaeta and Astiazaran, 2022; Meena *et al.*, 2023). However, the efficacy of probiotic LAB can be influenced by various factors such as the strain, dose, delivery method, and host factors. Therefore, it is essential to select the appropriate strain and delivery method based on the targeted health benefits and individual needs of the consumer (Gao *et al.*, 2021). To make these bacteria more accessible for industrial applications, most LAB are commercialized in dried form, as it is easier to transport, control its quality, and has lower overall costs when compared to liquid form (Jiang *et al.*, 2022).

At present, the major carrier of probiotics is refrigerated perishable foods like fermented milk and milk products. However, customers demand healthy products with long storage stability due to changing lifestyles (Sharma and Sarwat, 2023). Dried foods with viable probiotics having long shelf-life at room temperature is one of the promising alternatives; however, due consideration should be taken care to maintain sufficient probiotic count in foods till the end of declared shelf-life (Minimum 10⁶ cfu/g or 10⁸ cfu/serving). According to the World Health Organization (WHO), probiotic products should possess a minimum quantity of live bacteria, specifically at least 10⁶ cfu/g, in order to have a positive impact on health. The characteristics of the dried cells, such as the number of live cells and their biological activity, typically vary based on the drying method employed. Despite proven beneficial health effects, probiotic bacteria have some undesirable deficiencies, especially strain survivability during preservation and processing (Agudelo-Chaparro *et al.*, 2021).

Microencapsulation involves packaging or entrapping a cell or bioactive/functional ingredient within a secondary protective coating or matrix, forming a microcapsule. This process minimizes the undesired interactions, degradation, and adverse effects on taste, odor, and health. The microbial cells exhibit enhanced survival after microencapsulation during processing and storage. The design of a microencapsulated ingredient requires the knowledge of the core, encapsulating wall materials, core-matrix-environment interactions, stability during storage and in food, and release mechanisms of the core (Liu et al., 2019). The microencapsulation of probiotic LAB in different food matrices produced by different drying methods produces a stable and convenient product that resembles the original after rehydration to satisfy consumer expectations. There are mainly four drying methods, i.e., spray drying, freeze-drying, vacuum drying, and fluidized bed drying, that can be applied to produce microencapsulated dried probiotic formulations on an industrial scale (Barbosa et al., 2015; Rishabh et al., 2021).

People's eating habits have changed as a result of rapid urbanization and industrialization, which has led to the creation of microencapsulated probiotic food formulations and ready-to-eat convenience foods having added functionality of probiotics. These food formulations are type of food consisting of processed grains, legumes, sauces, spices, or any other food in various combinations. These provide a great deal of comfort to stay-at-home moms who are working and juggling other tasks. Instant mixes have gained popularity due to their consumer-friendliness (Chaturvedi *et al.*, 2021). Instant foods, which are increasingly popular, will contribute significantly to the growth of the packaged food business. Traditional methods of making many dairy cuisine items are cumbersome and time-consuming (Bunkar *et al.*, 2020a, b). Therefore, instant mixes for such products are needed to meet demand in metropolitan areas where traditional preparation methods are inconvenient.

2. Drying methods

Traditionally, freeze-drying has been the most commonly used method for encapsulation and drying probiotic lactic acid bacteria, but it has a high production cost, low productivity, and is limited by a small sample size and a long drying time (more than 24 h), which makes it unsuitable for continuous production (Jiang et al., 2022). The productivity rate of freeze-drying is only 20% of spray drying (Jiang et al., 2022). Spray drying is a highly significant industrial drying system in use today, which costs over ten times less than freeze drying. This method offers the benefits of rapid, efficient, and continuous production by atomizing the emulsion or suspension that contains bacteria and carrier materials into hot dry air, leading to quick water evaporation and the formation of microcapsules (Masum et al., 2020). However, the high temperatures involved in the process, typically above 89°C, can decrease the viability and activity of the probiotic LAB in the final product. As a result, protective agents are added to safeguard the LAB during the spray drying process (Jiang et al., 2022). The basic principle for microencapsulation of live LAB by different drying techniques are illustrated in Figure 1.



Figure 1: Schematic illustrations of basic principles for different drying methods used in microencapsulation.

The vacuum drying contains a vacuum dryer comprised of a chamber containing heated shelves where trays filled with wet biomass are positioned. By employing a vacuum pump and a condenser, the water vapor is eliminated. In this method, bacterial cells remain liquid in contrast to lyophilization or freeze-drying (Mella *et al.*, 2022). Furthermore, vacuum dryers function at higher temperatures and pressures, resulting in 40% lower energy consumption compared to freeze drying. Typically, the pressures used in vacuum drying range above 30-60 mbar, corresponding to a water boiling point of 25-30°C (Richter Reis, 2014). In contrast, freeze drying operates at

pressures below 6 mbar. In contrast to spray or fluid bed drying, the primary drawback of vacuum drying is the extensive processing time, spanning from 20 to 100 h (Kiepœ and Dembczyński, 2022).

Fluidized bed drying is a process where a heated gas, typically air, flows through a layer of solid particles at a specific velocity, causing them to enter a state of fluidized flow. This process offers excellent conditions for heat and mass exchange, enabling rapid evaporation of water from the material being dried. The duration of fluidized bed drying (ranging from 1 min to 120 min) is shorter than that of freeze drying and comparable to spray drying (Chen *et al.*, 2018). Moreover, the relatively low drying temperature avoids inducing thermal stress. Instead of drying the cell biomass alone, it is mixed with additional material that serves as a carrier or matrix for the cells to which microbial cells attach. Various loose and powdered materials have been utilized for this purpose, including wheat flour, skim milk powder, starch, casein, maltodextrin, microcrystalline cellulose, inulin, and salt (Kiepœ and Dembczyñski, 2022).

3. Freeze drying

Freeze drying technique is a well proven method to keep viability of bacterial cultures during drying. However, it has limitation due to high drying cost which makes hindrance for large scale application. Freeze drying is to date the best process known to dry bacteria while keeping their viability. This technique also used in food processing industry due to various benefits over other drying process;

- It is best suited for heat labile food products to minimize the compositional change and loss of nutrition (Meera *et al.*, 2016; Bunkar *et al.*, 2020b).
- It maintains the natural attributes of fresh foods such as color, flavour, and overall acceptability, to a larger extent. The freeze dried foods have very low shrinkage rate as compare to origin fresh food product (Rishabh *et al.*, 2021).
- The rehydration speed of freeze dried products are excellent due to none surface hardening, spongy and porous interior (Liu *et al.*, 2022).
- Due to low level of moisture, freeze dried foods can be kept and stored at ambient temperature for longer duration in packed form and considered ideal meal during travelling and gatherings (Barbosa *et al.*, 2015; Liu *et al.*, 2022).

However, cost of freeze-drying and time consumption are some of disadvantage. It works on very complicated heat and mass transfer phenomenon, and its multifarious impact on quality characteristics needs to be investigated thoroughly (Liu *et al.*, 2022).

The dried probiotic powders are manufactured by using this technique for decades. The applications of freeze-drying in food industry are depicted in Figure 2.



Figure 2: Application of freeze-drying in food processing industry.

3.1 Principle

In this technique, drying occurs in three stages; pre-freezing (water is frozen), primary drying (removal of water from food by sublimation), and secondary drying (desorption of water) (Meera et al., 2016). The working principle is based on thermodynamic phase equilibrium theory of water phases, in which at a particular pressure (4.579 mm Hg), the triple point temperature occurs at 0.0098°C. The food must be pre-treated before directly exposing to freezedrying such as cleaning, grading, blanching and concentration depending on type of ingredients. Pre-freezing or quick freezing before primary drying (sublimation drying) converts water of pretreated foods into solid state which is helpful to minimize heat denaturation and avoid frothing during subsequent vacuum drying stage (Zhang 2005a). In the sublimation drying stage, the frozen product is heated which leads to conversion of solid (ice crystals) directly into vapour (through sublimation) and the dehydration and drying removes 90-95% water of the product. The temperature of food should be maintained lower than vitrification temperature to avoid any structural deformation. The sublimation dried foods still have adsorbed water on its dry surface. Therefore, the residual moisture is eliminated by desorption at higher temperature but should be maintained not too high to avoid denaturation of ingredients of products. The final moisture of products commonly found in the range of 0.5-3%.

The sealing and packing of the freeze-dried products in vacuum or inert gas is essential to avoid any foreign moisture absorption and minimize oxidation denaturation during storage after desorption drying. The products are commonly stored in ambient conditions, however, some special products required specific storage conditions $(-4 \text{ to} - 20^{\circ}\text{C})$.

3.2 Freeze-drying for microencapsulation and production of food powders

The freeze-drying technique is used for manufacturing of powder of viability and stability of probiotic cells during long storage. This technique involves dehydration step which causes structural injury to microbial cells, reduced metabolic activity and cell survivability, especially if carried out without matrices or cryo-protective agents (Rishabh et al., 2021). The main reason of viability loss in this technique is changes in the physical form of membranous lipids accompanied with the disruption of integrity and liquidity. The enhanced cell viability and minimum structural damage can be achieved by blending with simple or complex materials like sugars, skim milk, whey, amino acids, dietary fibers, and glycerol, including those have prebiotic attributes (Araújo et al., 2020). These matrices primarily functions to protect microbial cells by minimizing the disruptive effect resulted due to dehydration. (Rishabh et al., 2021) isolated a potential probiotic strain Enterococcus faecalis (K13) from Gundruk (an indigenous fermented food) and Kanji (a fermented carrot juice). The isolate used in carrot juice for fermentation of carrot juice and microencapsulated using maltodextrin and gum arabic as wall material by freeze-drying method. The obtained carrot powder was studied for moisture, water activity, color, and viability of probiotic cells during storage. The freeze-dried probiotic carrot powder has more storage viability (up to one month) than spray dried powder prepared using same ingredients.

Murali et al. (2019) investigated the storage stability of probiotic black carrot powder encapsulated by spray drying and freeze drying using maltodextrin, gum Arabic and tapioca starch as microenc apsulating ingredients. The antioxidative activity, anthocyanin content, color difference and cell viability was evaluated at 15 days interval during storage up to 90 days. Meera et al. (2016) reported to develop a papaya juice powder by incorporating probiotic strain (Lactobacillus acidophilus) for fermentation at 37°C for 48 h by freeze-drying technique. The processing conditions were set in terms of total sugar in juice, acidity content, freezer condenser temperature, vacuum, and drying temperature to achieve final product with best sensory attributes and probiotic count not less than 10⁷ cfu/ml. The physicochemical characteristics and microbial counts revealed that it had storage stability for 60 days when stored at different temperatures (i.e., 5, 30, and 37°C). The optimized product was found rich in â-carotene and ascorbic acid. The study established and optimized the processing parameters of freeze drying for production of papaya juice powder using probiotic L. acidophilus strain with highest probiotic and functional component stability during storage. Some of important studies have been summarized in Table 1.

able 1: A summary of studies done for the production of freeze-dried problotic food powders using LAB strains							
Probioticmicroorganism	Growth temperature /incubation time	Carrier media /food product	Encapsulation wall material	References			
Enterococcus faecalis (K13)	37ºC/24 h	Carrot juice	Maltodextrin and gum Arabic	Rishabh et al., 2021			
<i>Lactobacillus acidophilus</i> and <i>Lb. casei</i>	-	Banana paste	Whey protein isolate, fructo- oligosaccharides or combination of both	Massounga Bora et al., 2019			
Lactobacillus acidophilus 10307	37ºC/48 h	Papaya juice	-	Meera et al., 2016			
Lactococcus lactis WK11	37°C/48 h	Skim milk	Ca-alginate, soy powder solution 10%	Gwak <i>et al.</i> , 2015			
Lactobacillus plantarum 299v	37°C/48 h	Orange juice	Maltodextrin	Barbosa et al., 2015			
Lactobacillus brevis WK12	37°C/48 h	Skim milk	Trehalose, soy powder solution 10%	Gwak <i>et al.</i> , 2015			
Pediococcus acidilactici HA-6111-2	37°C/48 h	Orange juice	Maltodextrin	Barbosa et al., 2015			

Table 1: A summary of studies done for the production of freeze-dried probiotic food powders using LAB strains

4. Spray drying

Spray drying is one of the most studied alternatives to lyophilization due to its cheaper cost. It has 6 to 10 times more energy efficient and 30 to 50 cheaper than freeze drying, easy availability, ease of maintenance and operation, and can be applied for large-scale production (Sharma et al., 2022). However, the production of probiotic products using spray drying poses the challenge of preserving the viability of cells during and after drying. Probiotic cells are generally not able to tolerate high temperatures of drying, so proper care should be taken to minimize the cell damage while keeping in view reducing the moisture level below 4% for long-term storage stability. The traditional spray drying temperatures (e.g., inlet 200°C and outlet $> 80^{\circ}$ C generally used for milk drying) cannot be used for probiotic microbes considering their heat tolerance limit. During spray drying, various adverse conditions like big thermal shock, osmotic imbalance, dehydration, and change in oxygen availability cause damage to cellular substances (i.e., fatty acids, nucleic acids, and ribosomes), which results in the increased death of probiotic microbiota (Liu et al., 2019). Therefore, its application for probiotic powder preparation has minimal operational flexibility.

4.1 Working principle of spray drying

Spray-dried fermented foods are a new area of research for producing

stable powders at large-scale. In the spray drying process, some characteristics of food, such as nutritional composition, flavor, and color, can be retained largely due to the evaporative cooling effect and very little food exposure time (5-100 s) to heat. The spray dryers have a drying chamber in which liquid streams are injected where the solute is converted into a solid and solvent into a vapor. The injection of liquid stream is done through a spray nozzle, and its size depends on the size of the spray dryer. The laboratory-scale drver has smaller nozzles, while large-size nozzles are assembled in commercial spray dryers. The nozzle decides the droplet size of the powder, and it varies from 20 µm to 185 µm. The spray nozzles are designed in such a way that the heat transfer surface between liquid feed and dry air should be maximum. The nature and viscosity of the liquid and desirable physicochemical properties are some of the criteria which play an important role in deciding the atomizer configuration. The powder formation starts as moisture content rapidly evaporates from liquid droplets of feed. The nozzle size is commonly designed to form droplets of very small size to maximize the heat transfer rate and rate of vapor formation. This technique has the ability to dry liquid products very faster than other drying methods. It completes drying in a single step, which provides added benefits such as maximum profit in minimum processing (Habtegebriel et al., 2018).



Figure 3: An overview of spray drying for preparation of probiotic spray-dried powder.

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4.2 Spray drying for encapsulation and production of food powders

Spray drying is widely studied as a microencapsulation technique for the stability of probiotic microbiota in a variety of food matrices, which usually contain proteins, carbohydrates, polysaccharides, and a mixture of them (Liu *et al.*, 2019). Many studies have been reported for microencapsulated probiotics in dairy products (*i.e.*, cheeses, chocolates) as well as in plant-based foods (fruit juices, sohiong, moringa, bakery, and meat products) as a carrier of probiotic incorporation (Chávez and Ledeboer, 2007; Barbosa *et al.*, 2015; Bustamante *et al.*, 2017; Agudelo-Chaparro *et al.*, 2021; Frakolaki *et al.*, 2021; Vivek *et al.*, 2021). During spray drying, various protective agents are incorporated for the survival of probiotic cells in the formulation (Shori, 2017; Jiang *et al.*, 2020). The application of protective wall material depends on the type of strain, and the use of various colloids or combinations of these colloids has been studied in various food matrices. Some of important studies have been summarized in Table 2.

Probioticmicroorganism	Growth temperature /incubation time	Carrier media /food product	Encapsulation wall material	References
Lactobacillus plantarum	30°C/72 h	Guava juice	Maltodextrin and inulin	Upadhyay and Dass J, 2021
Lactobacillus plantarum subsp. plantarum	37°C/72 h	Sohiong juice	Maltodextrin	Vivek et al., 2021
Lactobacillus acidophilus (LA5) and Bifidobacterium lactis (BB12) and	30°C for 72 hr	Moringa juice	Maltodextrin	Looi et al., 2019
Lactobacillus plantarum MTCC2621	37°C/48 h	Lychee juice	Maltodextrin, oligofructose, and pectin	Kalita et al., 2018
Lactobacillus plantarum CIDCA83114	37°C/48 h	Okra oil	Sodium caseinate	Quintana et al., 2018
Lactobacilluscasei 01 and Lactobacillus acidophilus LA5	37°C/72 h	Maoluang juice	Tiliacora triandra gum, maltodextrin and inulin	Chaikham <i>et al.</i> , 2017
Lactobacillus casei LK-1	37°C/16 h	Skim milk powder	Trehalose, and maltodextrin	Liao <i>et al.</i> , 2017
Lb. bulgaricus + Strepto- coccus thermophilus	42 °C/16 h	Skim Milk	maltodextrin	Seth et al., 2017
Lactobacillus plantarum 299v	40°C/48 h	Orange juice	Maltodextrin	Barbosa et al., 2015
Lactobacillus casei 01	37°C/24 h	Lychee juice	Gum arabic, and inulin	Kingwatee et al., 2015

Table 2: A summary of studies done for the production of spray-dried probiotic food powders using LAB strains

*NR- Not reported; **NM- Not mentioned

4.3 Encapsulation with proteins during spray-drying

In the past few years, proteins as protective wall material have attracted the interest of researchers and industry because of their ability to enhance probiotic viability as a natural carrier and delivery vehicle in the gut due to their structure and physicochemical attributes (Maleki et al., 2020; Agudelo-Chaparro et al., 2021). Proteins improve the survivability of probiotic strain during spray-drying due to their film-forming ability that minimizes thermal stress and improves moisture availability (Khem et al., 2015, 2016). The microencapsu lation of Lactobacillus rhamnosus during spray-drying was done using whey protein isolate and maltodextrin, which improved survivability during storage. The addition of sugar or trehalose further improved the probiotic shelf-life during the storage study and proved to be a better combination for probiotic carriers (Agudelo et al., 2017). Bazaria and Kumar (2016) reported that adding whey protein concentrate to beetroot juice helps maintain the functional content (betalain) and physicochemical properties of the finished powdered product during and after spray-drying.

4.4 Encapsulation with sugars during spray-drying

The incorporation of sugars also affects the viability of probiotics during and after drying. Various researchers reported the influence of sugars (*i.e.*, inositol, glucose, trehalose, lactose, and fructose) on dried microbial cells, but the survivability varies according to the sugar variety used (Zhu *et al.*, 2013; Li *et al.*, 2017; Agudelo-Chaparro *et al.*, 2021). The sugars as protective wall material exhibit various useful properties such as good solubility, lower viscosity in higher concentrations, quick drying, and high glass transition temperature property which make them appropriate microencapsulation ingredients during the drying process.

Recently, Upadhyay and Dass (2021) developed a probiotic (*Lactobacillus plantarum*) incorporated spray-dried guava juice powder using maltodextrin and inulin as an encapsulating material. This study determined probiotic survival, water activity, color, and rehydration period during a storage study for 45 days under refrigerated conditions (4°C) and ambient temperature (25°C). 80% probiotic survival was found under refrigerated conditions (4°C) up to 45 days, whereas 85% survival was reported up to 15 days at 25°C in the powder prepared using 10% maltodextrin and 10% inulin. There was no color and water activity difference reported in any of the tested combinations; however, the maximum yield and lowest rehydration time were found in the product having 20% maltodextrin.

Arepally et al. (2020) used the spray drying technique to encapsulate Lactobacillus acidophilus cells to develop a probiotic powder by admixing constant concentration of maltodextrin (20%) and varied gum arabic (0-10%). This study studied the effect of processing temperature (inlet air temperature-130 to 150°C) and variable gum arabic concentration on the physical attributes and encapsulation yield of probiotics in the powdered products. The different tested formulations had encapsulation efficiency in the range of 65-89.15%, whereas moisture content (4.59-9.05%), water activity (0.33-0.52), hygroscopicity (12 to 21.15 g H₂O/100 g dry weight), and wettability 116 s to 353 s was also assessed. The structural stability and probiotic cell viability were studied using fourier transform infrared (FTIR) and simulated gastric intestinal conditions, respectively. During storage studies of the tested formulations, the probiotic cell viability was found to be higher under refrigerated conditions (4°C) than stored at room temperature (25°C). Vivek et al. (2021), developed a probiotic sohiong powder using probiotic culture (Lactobacillus plantarum subsp. plantarum) in the sohiong juice and blended varied concentrations of Maltodextrin by spray-drying. The processing conditions of spray-drying, including inlet air temperature (120-140°C), inlet feeding rate (120-240 ml/h), and different levels of maltodextrin (12-25%), were optimized using Box-Behnken design. The drying yield, color, moisture, and encapsulation efficiency were taken as response parameters in the study. The optimum conditions for product preparation with acceptable sensory quality attributes were observed at 120°C inlet temperature, 12% (w/w) maltodextrin level, and 201 ml/h feeding rate. The storage stability of probiotic count was 36 and 104 days at ambient temperature and under refrigerated conditions, respectively.

The microencapsulation study using spray drying and freeze-drying was also performed for *Lactobacillus plantarum* Dad-13 with skim milk and sucrose as wall material and obtained probiotic powder with significant antibacterial activity against pathogens (Kamil *et al.*, 2020). The developed powdered product was examined for physicochemical attributes (color, water activity, particle diameter, and microstructure), sublethal injury, and probiotic survival during storage. The results showed that freeze-dried probiotic cells (*Lb. plantarum*) had higher storage stability (8 weeks) than spray-dried cells during the storage study. Therefore, freeze-drying is a more suitable technique than spray-drying for better survivability of probiotic cells.

4.5 Encapsulation with starch during spray-drying

The starch has been used as encapsulating material to protect compounds such as lipids, proteins, vitamins, flavoring compounds, drugs, essential oils, polyphenols, pigments, herbicides, fragrance compounds, and also for bacterial cells (Hoyos-Leyva *et al.*, 2018). Starch was also used for the encapsulation of probiotic *Lactobacilli* strains during spray drying for better cell viability (Paéz *et al.*, 2012).

Avila-Reyes *et al.* (2014) studied the effect of two encapsulating materials, *viz.*, native rice starch (NRS) and inulin (IN), for the survival of *Lactobacillus rhamnosus* during spray-drying and reported that product yield was found in the range of 65-74% with NRS, whereas 43-54% was with inulin. The study deducted that NRS provided better survival of probiotic cells than inulin during the drying process and storage study. Similar findings have been reported by other researchers using starch as encapsulating material to protect probiotic cells during dehydration (Crittenden *et al.*, 2001; O'Riordan *et al.*,

2001; Lahtinen *et al.*, 2007). The native starch and modified form (majorly octenyl succinate starch obtained from different plants) and its hydrolyzed derivatives (maltodextrin or dextrans) have been studied as microencapsulating agents. The wider application of starch as a wall encapsulating material depends on its ease of availability, cheaper cost, and diverse functional properties (increase or decrease in viscosity, water retention capacity, *etc.*) (Hoyos-Leyva *et al.*, 2018).

4.6 Encapsulation with skim milk during spray-drying

Skim milk contains milk solids, mainly lactose and protein, in addition to ash, minerals, and other trace elements. It has been considered good microencapsulating material for probiotics due to nutritional components and technological attributes such as emulsification and gel formation. Earlier, various researchers used skim milk as carrier media for probiotics and encapsulating material singly or blending with wall materials (Paéz et al., 2012; Khem et al., 2015; Liao et al., 2017; Duangkhamchan and Itsaranuwat, 2020; Kamil et al., 2020) and the resultant product observed with high survivability of probiotics. The high survivability of probiotic cells might be due to the lactose and different fractions of milk protein (caseins, α lactoglobulin, α -lactoglobulin, bovine serum albumin, lactoferrins) which can avoid and minimize the structural cell damage and to protect cell functioning during dehydration (Maciel et al., 2014; Mis Solval et al., 2020). Furthermore, few researchers reported interaction between lactose and polar groups of phospholipids and proteins present in the cell membrane of bacteria, while milk proteins decrease the leakage of the cell membrane and maintain cellular integrity, thereby minimizing the cellular injury and inactivation during spray drying (Santivarangkna et al., 2008; García, 2011).

Ananta et al. (2005) reported using reconstituted skim milk for microencapsulation of probiotic strain Lactobacillus rhamnosus GG as a spray-drying carrier matrix in which 60% survival was observed at an outlet temperature of 80°C. The storage stability was found to be increased with increased concentration of total solids in skim milk. Gul (2017) evaluated the physical properties, probiotic viability, and storage stability of Lb. casei Shirota when microencapsulated with different wall materials (maltodextrin, skim milk, and gum arabic) by spray-drying. In this study, 94% cell survival was observed with 30% reconstituted skim milk while maintaining outlet temperature in the range of 64-68°C. However, this type of effect was not observed across strains; L. plantarum CIDCA 83114 showed only 10% cell viability when reconstituted skim milk was taken as microencapsulating material in a spray drying process at an outlet temperature of 70°C (Golowczyc et al., 2010). In a similar study, Bifidobacterium BB-12 was spray dried with encapsulating material as reconstituted skim milk (RSM) with prebiotics (viz., inulin and oligofructose or a combination of them), and viable counts and physicochemical properties of probiotic powder was evaluated during storage. The study deducted that higher counts were observed in spray-dried products with prebiotics compared to those prepared using reconstituted skim milk only (Fritzen-Freire et al., 2012).

4.7 Encapsulation with lipids during spray-drying

In the past few years, lipids, especially low melting lipids, is also gaining research attention as a protective agent for probiotic bacteria to reduce heat injuries of viability losses during the preparation of spray-dried probiotic products. During the high-temperature conditions of spray drying, the temperature reaches above the melting point, and fats convert from the solid to the liquid stage, which leads to the absorption of a significant amount of thermal energy during the drying process (Liu *et al.*, 2019). A study reported that isolated *Lactobacilli* strains showed higher survival during spray-drying when sodium caseinate and low melting fats were used as protective agents compared to control samples (Liu *et al.*, 2015). However, the effect of low melting fats may be strain dependent as no response was observed for *Lactobacillus reuteri* strains after spray drying (Liu *et al.*, 2019).

5. Market potential

In the past decade, there has been a notable rise in the development and utilization of microencapsulated ingredients. This growth is attributed to the emergence of cost-effective materials and production processes suitable for food applications. Microencapsulation technology provides enhanced structures that serve as functional ingredients in various industries, such as pharmaceuticals, food and beverages, home and personal care, construction materials, and textiles. The growth of the microencapsulation industry is expected to be driven by its widespread adoption in the food and pharmaceutical sector throughout the world. This technology primarily aims to mask the taste, odor, and activity of the encapsulated structures, which are utilized as functional ingredients in sectors like pharmaceuticals and healthcare. The size of the total global microencapsulation market is estimated to be USD 11,896.2 million in 2022 and is expected to grow at a compound annual growth rate (CAGR) of 10.3% from 2023 to 2030 in all segments, including pharmaceuticals and healthcare. However, the global microencapsulated food ingredient market is expected to grow at a CAGR of 4.8% from 2020 to 2030 (Grandview Research, 2021). The market is driven by the expanding applications of microencapsulated products in different industries, the increasing demand for pharmaceutical and agrochemical products, and the rising popularity of functional food products. However, the market growth is hindered by the high costs associated with the microencapsulation process and the research and development resources, which pose challenges for many market players looking to adopt this technology in various industries.

6. Conclusion

The food industry has incorporated probiotic bacteria into a wide range of food products to offer customers a number of options. However, the primary challenge is to maintain the viability of probiotics during the shelf-life of the products in a cost-effective manner to ensure the beneficial effect after ingestion. To maintain high levels of viability for probiotics, the utilization of microenca psulation techniques and the incorporation of prebiotic substances into the formed beads have shown potential. These approaches explore the possibility of improving the survivability of probiotic cells during harsh conditions such as drying temperature, storage, or the gastrointestinal tract. However, despite numerous studies in this field, the existing microencapsulation technologies and encapsulating materials are unable to consistently ensure optimal viability preservation in all situations, indicating the need for further investigation. Specifically, more effective encapsulating agents and combinations of materials are required to successfully trap probiotic cells and maximize their survival under different stress conditions, such as low pH and high temperature conditions. Moreover, novel

microencapsulation methods suitable for probiotic LAB bacteria need to be explored to facilitate their incorporation into more challenging and less common food products like bakery and meat items. Further, it is essential to explore new technologies that can generate identical food quality throughout commercial production and identify strategies to reduce cost.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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