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REVIEW ARTICLE

From Preparation to Product: Factors Influencing Probiotic Viability in Spray Drying

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ABSTRACT - With growing health awareness, particularly amid the SARS-CoV-2 pandemic, consumers increasingly value nutrition, diet, and food safety. Probiotic-based foods and beverages are widely recognized for their health benefits, including improved gut health and immune function. Spray drying is a scalable and efficient method for encapsulating, enhancing the stability and shelf life of probiotics. This review explores strategies to optimize the spray drying process, with a particular focus on factors influencing probiotic viability during and after drying. Key considerations included strain-specific thermal tolerance, feed composition, and critical process parameters such as drying temperature and feed rate. Notably, encapsulating agents play a vital role in maintaining the physicochemical quality of the final product while protecting probiotics from environmental stress. Recent advancements in encapsulation technologies, including biopolymers, hybrid materials, and emerging nanotechnology-based solutions have shown significant potential for enhancing probiotic survival under harsh processing conditions. Future research should integrate molecular-level insights, such as omics-based approaches, to better understand stress responses and optimize encapsulation strategies. Genome-editing tools and high-throughput screening techniques could accelerate the creation of thermotolerant probiotic strains, enabling more robust formulations. In parallel, the development of environmentally sustainable encapsulating agents with superior protective properties is essential to advance both efficiency and scalability. By addressing these challenges, spray drying can be further refined to produce durable, high-quality probiotic formulations that meet the growing demand for functional foods and beverages, while aligning with evolving consumer health priorities.

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1. INTRODUCTION

In an era where modernization and heightened health consciousness are paramount, consumers are increasingly focused on nutrition, diet, and food safety to enhance their quality of life. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, which has persisted for over three years, has also underscored the importance of nutritional status as a major determinant of severe illness outcomes. Probiotic-based foods and beverages represent a prominent category of functional foods that have garnered widespread acceptance among consumers due to their health benefits to consumers. Probiotics are live microorganisms that administered in adequate amounts confer a health benefit on the host [1].

Some well-known probiotic strains include *Lactobacillus*, and *Bifidobacterium* [2]. These beneficial bacteria can be sourced naturally from fermented food or enriched in commercial products. Some examples of naturally probiotic-rich food that naturally contain beneficial bacteria include yoghurt, kefir, kombucha, and lassi [3]. A few studies have highlighted the beneficial properties of probiotics, including immune system enhancement, gut microflora regulation, promotion of gut-barrier function, and antibacterial activity through metabolite production during fermentation [4,5]. Additionally, probiotics have demonstrated efficacy in treating gastrointestinal issues, allergies, ischemic heart disease, hypertension, and reducing the risk of certain cancers such as colon and skin cancer [6,7].

However, natural fermented products often have limited storage lifespans, therefore the processing of these products into powder is a necessary step in obtaining products with enhanced quality and stability. The powdered probiotic powder not only facilitates storage and transportation but also offers ease of handling [8,9]. Spray drying is currently a popular and important drying system, particularly in the food industries and pharmaceutical industries. The spray drying process transforms the liquid state feed into a solid product, usually in powdered form in only one step. Spray drying commonly works in combination with the microencapsulation technique to protect active ingredients and thermally sensitive compounds, especially in probiotics. Different carriers have been found used in the microencapsulation process such as maltodextrin, arabic gum, skim milk, whey protein and etcetera [8,10,11]. The major advantages of this drying process are rapidity, ease of operation, continuous, cost-effective, and scalable production of dry powder [12,13]. Despite the advantages claimed for this preservation method, the short residence time in the drying chamber can significantly cause

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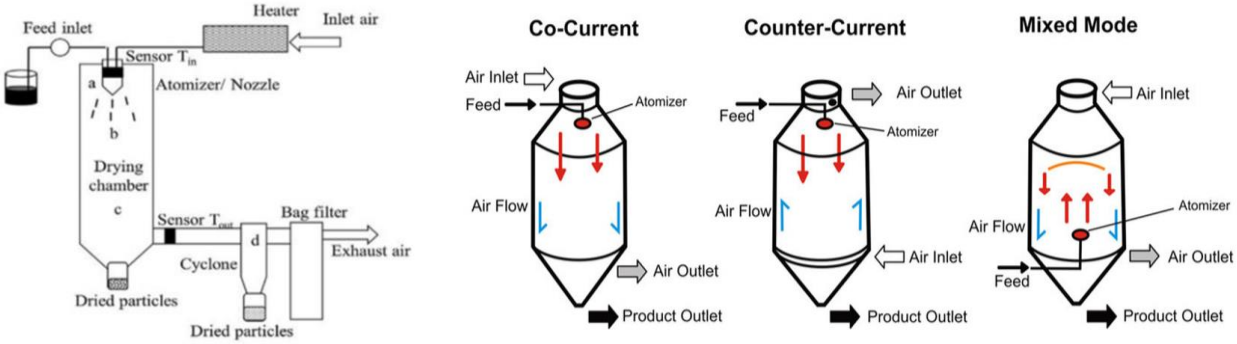
inactivation of the cells during the process and subsequent storage. This review highlights the application of spray drying in the production of probiotics from preparation to product. Furthermore, we also illustrate the factors affecting the spray drying process and strategies that can improve the viability and efficacy of probiotics during spray drying as well as at the pre-and post-drying stages.

2. PRINCIPLE OF SPRAY DRYING

Spray drying is a method used to transform liquid solutions into dry powders efficiently and quickly. It is widely applied in industries such as food production and pharmaceuticals because it produces consistent and high-quality results. The drying process works by breaking the liquid into tiny droplets, exposing them to hot air to remove moisture, and collecting the resulting dry particles.

Figure 1 (A) and (B) show the operation of a spray dryer and the flow patterns of spray dryer. Generally, the operation of a laboratory-scale spray dryer can be described by three major mechanisms: 1) atomization, 2) droplet-to-particle conversion and 3) particle collection (Figure 1(A)). The atomization process commences when the liquid feed is atomized in small droplets due to the decrease of surface tension, and later forming a large number of droplets. The drying process proceeds when the atomized droplets are exposed to hot air within the drying chamber, causing rapid evaporation. Once the droplet-to-particle conversion is completed, the solid particles are dissociated from the drying air and are ready to be collected in the cyclone [12]. The spraying air is an important part of the spray dryer operation regarding the liquid spray direction. Three types of flow patterns inside the spray dryer include co-current, counter-current, and mixed flow (Figure 1(B)).



(A) (B)

Figure 1. (A) Schematic representation of a spray dryer operation, illustrating key components and stages. Liquid feed enters through a nozzle/atomizer (a), where it is converted into fine droplets within the drying chamber (b). The droplets undergo rapid drying, with dried particles collected at the cyclone (c) or further separated in a collection chamber (d). Adopted from Broeckx et al. [12]; (B) Flow patterns in spray drying. i) Co-current (droplets and hot air move together), ii) Counter-current (air flows opposite to droplets), iii) Mixed flow (a combination of both). Adopted from Peighambardoust et al. [14]

In the co-current flow, both the atomized droplets and hot air pass through the dryer in the same direction. The dried particles will be dropped at the bottom of the chamber and released together with the hot air [15]. Such a flow pattern is suitable for heat-sensitive microorganisms. In the counter-current design, the sprayed droplets and hot air are introduced in the opposite direction, with the atomizer positioned at the top and supplied air from the bottom. Therefore, the dried particle will be released at the bottom of the chamber while the hot air leaves through the upper part of the chamber [15]. Mixed flow is the combination of co-current and counter-current flow patterns. Atomized droplets are fed from the bottom of the chamber in the counter-current pattern. The dried particles and hot gas are then released at the bottom of the chamber. Most dryers operate in co-current airflow pattern and mixed flow pattern [16]. The principle of efficient spray drying is highly dependent on the contact area between the desired substances to be dried and the hot air. Therefore, the importance of applying sufficient energy to produce hot air and a high enough temperature to allow vaporization of the liquid and remove moisture is crucial [17]. Low temperature reduces the productivity and quality of the final product.

3. BIOLOGICAL PARAMETERS AFFECTING VIABILITY OF PROBIOTICS AT PRE- SPRAY DRYING STAGE

3.1 Characteristic of Probiotic Strains

The harsh conditions such as high temperature, oxidation, and osmotic stress are often attributed to the loss of viability of cells [18]. Previous studies declared that the heat-sensitive strain is subjected to cell death after spray drying. However, there was also another case study by Soukoulis et al. [19] reported that some strains of *lactobacilli* have greater heat resistance which belonging to *Lactobacillus plantarum, L. salivarius, and L. paracasei* species with survival rates up to 80% after spray drying. Furthermore, *L. acidophilus* NRRL B-4495 *and L. rhamnosus NRRL B-442* also obtained a

satisfactory survival rate which was 81.17% when spray drying at 100 ℃ [20]. Besides, the cell resistance of *L. casei* and *L. paracasei* was higher when compared to *L. plantarum* and *L. acidophilus* [21].

3.2 Growth Medium

Growth media can affect the viability of probiotics. As an example, it has been reported that compatible solutes in the growth medium could increase cell survival during spray drying. During the drying process, the probiotics are subjected to low water activity conditions. Under such conditions, some microorganisms take up or synthesize compatible solutes to regulate the osmotic balance with the extracellular environment. Those solutes may help the probiotics to stabilize the cell components during stressful conditions [14,22,23].

The disaccharides were observed with their potential to protect the microbial cell membrane and protein during spray drying by several researchers [24]–[26]. Sucrose was proposed as an effective protectant for selective species and strain during spray drying, by improving the thermotolerance of cells [27]. This is evidenced by Zheng et al. [25], where the survival rate of *L. casei* L61 was slightly increased with an addition of 1% of sucrose into the growth medium after drying. Moreover, Jantzen et al. [28] also demonstrated the application of lactose to protect probiotic cells. The growth media containing lactose was observed to increase the survival of lactic acid bacteria with thermo-protectivity.

3.3 Growth Phase

The growth phase of probiotic bacteria plays a critical role in determining their survival during spray drying, with bacteria in the stationary phase generally exhibiting enhanced resistance compared to those in the exponential phase [29]. This resilience is primarily attributed to the activation of stress-response mechanisms, which are triggered by nutrient depletion and environmental stress factors such as acidification, heat, and dehydration [30]. This resilience is largely attributed to the activation of stress-response mechanisms, including the upregulation of heat shock proteins (HSPs) and other protective compounds. For instance, Gao et al. [31] highlighted that the upregulation of HSPs, such as the chaperonins DnaK and GroEL, and their cofactors GroES, plays a central role in enhancing the heat resistance of lactic acid bacteria (LAB), where the expression of genes coding for the chaperonin GroEL and the co-chaperone GroES, which account for normal formation of proteins under stress conditions, increase in the stationary phase [32,33].

This stress response can be further amplified through specific stress-inducing conditions, such as exposure to bile salts. A recent study by Bustos et al. [34] highlighted that bile-induced stress can trigger the upregulation of a broad array of stress-related proteins, including HSPs like GroEL, GroES, DnaK, and DnaJ, across various LAB strains. This method not only reinforces cellular defenses but also prepares the bacteria to withstand the challenging conditions encountered during spray drying. The scalability of this bile-induced stress response offers a promising approach to improving the viability and stability of probiotics on an industrial scale, particularly for formulations undergoing drying or other preservation methods.

3.4 Pre-Treatment

Due to extreme temperatures, pressure, and other process hurdles, even robust probiotics can sometimes be eliminated during industrial applications. However, sub-lethal stress can induce adaptive responses in probiotics, enhancing their resilience to harsh environments. These adaptive responses include mechanisms to withstand heat, cold, bile salts, high pressure, nutrient starvation, and osmotic stress [35]. These strategies increase the survivability of probiotic cells compared to those that are directly propagated into the same lethal stress condition.

Heat shock pre-treatment is one of the most efficient adaptation strategies for improving probiotic survival. For instance, Bommasamudram et al. [36] reported that pre-treating *L. casei* N at 45 °C or 50 °C for 60 mins before spray drying increased survivability by up to 0.26 log cycles compared to untreated cells. Similarly, Paéz et al. [21] observed that applying a mild heat treatment at 52 °C for 15 mins to *L. casei* Nad and *L. plantarum* 8329 significantly enhanced their survival during spray drying. Desmond et al. [37] demonstrated that heat-adapted *L. paracasei* NFBC338, pre-treated at 52 °C for 15 mins, exhibited 300- and 700-fold greater thermotolerance compared to control strains This effectiveness is largely due to the induction of heat shock proteins (HSPs), including DnaK, GroEL, and GroES, which stabilize cellular components, assist in proper protein folding, and protect against protein denaturation under stress [12, 37].

Acid, bile, and salt pre-treatments are also widely used to enhance probiotic resilience. Acid and bile stress stabilize cellular membranes and regulate pH, while salt-induced osmotic stress provides cross-protection against heat [39]. For instance, Desmond et al. [37] reported that salt-treated *L. paracasei* cells exhibited a 16-fold higher survival rate during spray drying compared to untreated controls. Meanwhile, heat-treated cells consistently outperform these methods. Chen et al. [39] found that *L. kefiranofaciens* M1 pre-treated with heat (37 °C for 1 h) showed a 53.51-fold increase in survival after exposure to 52 °C for 2 h, surpassing the 18.04-fold and 46.62-fold increases observed for acid (pH 5.0 for 1 h) and bile salt (0.05% for 1 h) treatments, respectively. Anekella [40] further confirmed that heat pre-treatment produced the highest survival rates compared to salt, peroxide, and bile stress treatments, emphasizing its robustness.

Non-thermal pre-treatments, such as pulsed electric fields (PEFs), offer additional options for improving probiotic resilience. These methods stimulate the production of HSPs and other protective proteins, providing an alternative for heat-sensitive strains. However, their industrial scalability and cost-effectiveness remain underexplored. Ultimately, the

choice of pre-treatment strategy should be guided by the specific strain's stress tolerance profile and the intended application.

4. PROCESS PARAMETERS AFFECTING VIABILITY OF PROBIOTICS DURING SPRAY DRYING

4.2 Drying Temperature

The inlet temperature is the main parameter that determines the drying efficiency as it influences the amount of water to be removed, the content of residual water as well as the physical properties of the final product when working with feed and air flow to evaporate water. In contrast, the outlet temperature is the highest temperature at which the final product is to be heated and it is measured between the drying chamber and the cyclone [15].

Generally, a high inlet temperature can lead to excessive evaporation and may result in cracking problems, degradation of encapsulated materials as well as loss of volatile compounds. In contrast, too low inlet temperature could not sufficiently dry the powder. According to Samantha et al. [17], if temperature differences are high enough, they may result in a product with a high residual moisture content which would influence the stability of the end product upon storage. To maximize the productivity of the final product, some models of spray dryers have a designed control system for outlet temperature whose setting depends on the inlet temperature, to minimize the deviation with inlet temperature.

Table 1. Summary of different inlet and outlet temperatures applied to produce various probiotics products

Product	Inlet temperature [℃]	Outlet temperature [℃]	Encapsulating agents	Outcomes	References
Yoghurt powder with *Lactobacillus helveticus*	120 150	60	Butter milk powder	The inlet/outlet temperatures (120/60°C), respectively were found to keep the survival of probiotics better	[43]
Saccharomyces cerevisiae	110–140	45 – 75	Starch & Maltose	The least cell loss was suspension in soluble starch plus maltose couplet to inlet and outlet temperatures of 110 and 55°C	[44]
Lassi powder with *Streptococcus thermophilus*, *Lactobacillus rhamnosus*, and *Lactobacillus acidophilus*	270 –180	130 – 60	Not stated	Optimised formulation for lactic acid bacteria was 190/ 70℃ for inlet/outlet temperature	[45]
Kefir powder	130 ± 2	55 ± 2 65 ± 2 75 ± 2	Maltodextrin (MD)/ Arabic gum (AG)	The optimum product formula, which simultaneously maximized the microbial viability and product yield, was predicted by the parameters as 0/100 for MD/AG, no inulin addition, and 70°C of outlet temperature	[46]
Probiotic Sohiong powder with *Lactobacillus plantarum*	120- 140	< 60	maltodextrin	Optimum conditions for the production of Sohiong juice powder with acceptable quality were 120°C inlet temperature, 12% w/w maltodextrin concentration	[47]
Lactococcus lactis ssp. cremoris	130 150 170 200	38 45 55 65	Lactose, sodium caseinate, Whey protein isolate	An inlet air temperature of 130℃ and 65℃ as the outlet air temperature maintained high survival of the bacteria	[41]
Russian Olive water kefir powder	120–170	Not stated	Maltodextrin 13-17 Dextrose equivalent /gum Arabic	Optimal spray drying conditions were observed at an inlet air temperature of 120ºC, 35% feed flow rate, and 7% concentration of drying aid.	[48]

Ghandi et al. [41] emphasized that the inlet and outlet temperatures should not be lower than 100 ℃ and 45 ℃ respectively. Chavarri et al. [42] also proposed that the inlet temperature as low as 60 ℃ resulted in poor drying and led to the production of sticky products that accumulated in the cyclone. In the case of probiotics, the outlet temperature

ranging from 40 ℃ to 60 ℃ offers satisfactory cell viability after spray drying and tends to decrease linearly at outlet temperatures of 50 ℃ to 90 ℃ [18]. Table 1 summarizes different inlet and outlet temperatures the researchers applied to spray dry probiotics.

4.2 Feed Flow Rate

A positive correlation was elucidated between the feed flow rate and the microbial survival, where the microbial survival increased as the feed flow rate increased [49]. An increase in feeding flow rate produced larger droplet sizes, lower residence time in the system, and shorter exposure time to high temperatures, thus microbial survival is preserved. Since the residence time of feed in the drying chamber is shortened, the heat transfer is less efficient, eventually producing end-products with higher residual content [50]. As mentioned earlier, the high feed flow rate would influence the residue content because the high feed rate lowers the outlet temperature, leading to the build-up of product in the drying chamber [17,51]. For instance, Behboudi-Jobbehdar et al. [51] reported the effect of three distinct air inlet temperature and feed flow rates on the spray drying of *L. acidophilus* NCIMB 701748, the cyclone recovery decreased as the feed flow rates increased from 6 mL/min to 9 mL/min.

5. PRODUCT PARAMETERS THAT AFFECT THE CELL SURVIVAL DURING SPRAY DRYING

5.1 Encapsulating Agent

The selection of biopolymers used for microencapsulation of probiotics is crucial. They must be biocompatible, biodegradable, processable, and inert to probiotics [52,53]. In addition, selected wall materials must be water soluble, have low viscosity, high glass transition temperatures, and fast dry performance [54]. Furthermore, they must have the capability to release the loaded probiotics under certain conditions, especially in gastrointestinal conditions [55]. Importantly, the selection of additives is crucial because it reflects on the physicochemical properties and the process parameters during spray drying such as drying temperature, feed flow rate, drying air flow rate etcetera [56].

On this notion, both natural (polysaccharide and protein-based) and synthetic (hydrolyzable) biopolymers were employed to microencapsulate probiotics, as displayed in Figure 2. The choice of biopolymer significantly affects encapsulation efficiency, thermal stability, and storage performance. This section also provides a detailed comparison of the efficiency of polysaccharide-based, protein-based, lipid-based, and synthetic biopolymers, focusing on their advantages and limitations. The selection choice of additive for spray drying necessitates careful consideration of the physicochemical properties of the additives themselves. Such criteria include molecular weight, glass transition, and the concentration of additives should be taken into account. Moreover, the type of feed material used together with added additives has a significant impact on the performance of properties [56]. Therefore, selecting additives tailored to specific applications demands a comprehensive understanding of spray drying principles.

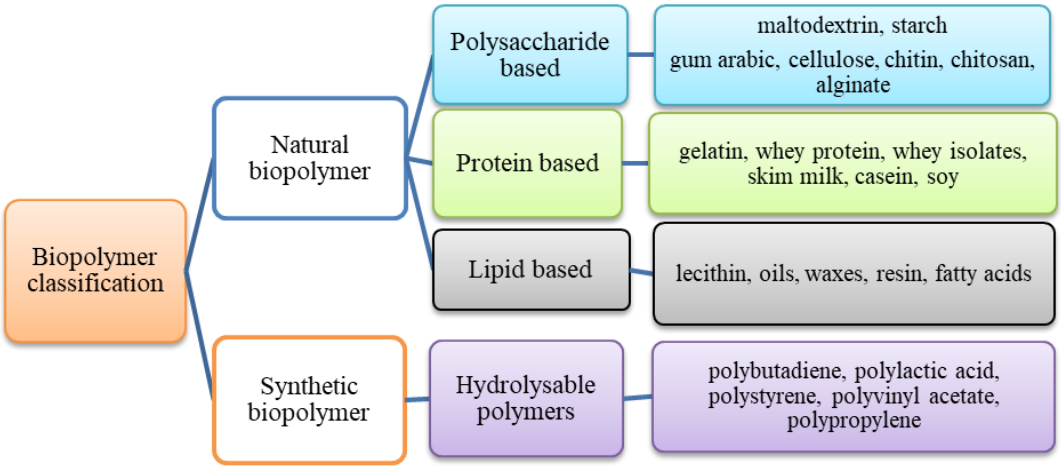


Figure 2. Classification of biopolymers for microencapsulation

5.1.1 Microencapsulation with Polysaccharide-Based Biopolymers

Polysaccharides are among the most commonly used biopolymers in microencapsulation due to their inherent advantages, including low cost, abundant availability, and excellent film-forming properties [57]. Examples included maltodextrin, starch, gum arabic, cellulose, chitin, and alginate, each offering unique characteristics that make them suitable for different encapsulation purposes. Maltodextrin, for instance, is frequently employed because of its low viscosity, which facilitates the formation of stable powders during drying processes. However, its ability to protect sensitive compounds under extreme drying conditions, such as high temperatures or rapid dehydration, is somewhat limited, making it less ideal for certain applications [58].

Starch and its modified forms, such as octenyl succinic anhydride (OSA)-starch, provide better thermal resistance than maltodextrin, which is particularly advantageous in processes for spray drying [59]. Modified starches have demonstrated superior encapsulation efficiency compared to native starch, with some studies reporting over 80% survival rates of probiotics during the drying process [60]. This makes them a preferred choice for encapsulating heat-sensitive materials. Similarly, alginate is another polysaccharide that has garnered significant attention due to its gel-forming capabilities and high encapsulation efficiency, particularly under acidic conditions. Its ability to create a protective matrix around probiotics enhances probiotic's survival during storage and gastrointestinal transit, making it a valuable material in food and pharmaceutical applications [61].

Gum arabic, despite being more expensive than other polysaccharides, offers excellent emulsifying properties that enhance the stability of encapsulated materials. It is often used in combination with maltodextrin to optimize encapsulation efficiency and improve the protective barrier around sensitive bioactive compounds. Previous studies have shown that blends of gum arabic and maltodextrin result in significantly higher probiotic survival rates compared to using maltodextrin alone, highlighting the synergistic effects of combining these biopolymers [46,62].

5.1.2 Microencapsulation with Protein-Based Biopolymers

Protein-based biopolymers such as gelatin, whey protein, casein, and soy protein have unique advantages in microencapsulation due to their excellent emulsifying properties and ability to form crosslinked networks [63]. These characteristics enable proteins to create robust encapsulation matrices, which protect sensitive compounds during processing and storage. Whey protein, for instance, has been widely studied for its high encapsulation efficiency and its ability to provide thermal protection during processes like spray drying. Furthermore, when whey protein is combined with polysaccharides such as maltodextrin, the resulting encapsulation matrix demonstrates enhanced stability, effectively protecting probiotics from environmental stresses [2,64].

Gelatin, a protein derived from collagen, forms stable capsules with excellent film-forming properties, making it an attractive material for encapsulation [65]. However, it is sensitive to high temperatures, which may limit its use in thermally intensive like spray drying process. Nonetheless, its ability to create a protective layer around encapsulated materials has made it a popular choice in the pharmaceutical and food industries. On the other hand, soy protein, a plant-based alternative, has emerged as a competitive encapsulating material due to its emulsifying properties and compatibility with other biopolymers. When combined with polysaccharides, soy protein isolates exhibit promising encapsulation efficiencies, providing an effective and sustainable solution for encapsulating probiotics and other sensitive compounds [66].

5.1.3 Microencapsulation with Lipid-Based Biopolymers

Lipid-based biopolymers are less commonly employed for probiotic microencapsulation in spray drying compared to polysaccharides or proteins. This is largely due to their limited compatibility with aqueous systems, which are critical to spray drying. Besides, this limitation also arises from the inherent challenges associated with lipid materials, such as their poor solubility in aqueous systems and susceptibility to oxidation under high-temperature conditions. However, some studies have explored co-encapsulation strategies, where lipids, particularly oils, are incorporated alongside probiotics to enhance their protection during spray drying [67].

In co-encapsulation systems, oils such as sunflower, fish, or coconut oil act as protective agents, mitigating thermal and oxidative damage during spray drying. Liquid oils with high unsaturated fatty acid content reduce oxidative stress, while solid oils with higher saturated fats, such as palm or coconut oil, help absorb heat, minimizing thermal degradation of probiotics [68]. For instance, the study by Ngamekaue et al. [69] demonstrated that encapsulating *L. reuteri* KUB-AC5 in coconut oil emulsified with whey protein isolate significantly enhanced their survival during spray drying and gastrointestinal digestion. Similarly, Eratte et al. [70] investigated the co-microencapsulation of omega-3 fatty acids and *L. casei*, finding that the lipid-based matrix not only improved the oxidative stability of omega-3 oils but also enhanced probiotic viability and surface characteristics of the spray-dried powders. Despite these advantages, oxidation of oils during spray drying and challenges in emulsion stabilization require the use of wall materials, such as gum arabic, maltodextrin, or modified starch, to form a stable encapsulation matrix [68].

5.1.4 Microencapsulation with Synthetic Biopolymers

Synthetic biopolymers such as polylactic acid (PLA) and polyvinyl acetate (PVA) are rarely utilized in spray drying-based probiotic encapsulation due to their reliance on organic solvents for dissolution. These solvents are incompatible with the high temperatures involved in spray drying, leading to potential loss of probiotic viability, and their residual toxicity poses significant challenges for food-grade applications [71,72]. Despite these limitations, the use of synthetic biopolymers in encapsulation has shown promise under specific conditions. For example, Akanny et al. [73] successfully encapsulated *L. rhamnosus GG* using Eudragit® S100, an enteric polymer, in an aqueous-based spray drying process. This polymer's pH-dependent dissolution properties enable the targeted release of probiotics in the colon. They have also suggested that incorporating protective agents like trehalose and maltodextrin into the formulation would significantly enhanced bacterial viability during the spray drying process.

5.2 Molecular Weight of Additives

The molecular weight of additives is a critical factor influencing their effectiveness in enhancing spray drying processes. It directly impacts the transition temperature, with shorter chain molecules exhibiting lower transition temperatures compared to longer chain molecules [58,74]. According to Perdana et al. [75], lower molecular weight carbohydrates carriers, including maltose, lactose, and trehalose often render better stabilization in respect to thermal inactivation. Higher dextrose equivalent (DE) values also indicate lower molecular weight, due to the variation in the degree of polymerization, leading to the loss of viability during drying [76].

Souza et al. [77] illustrated this relationship by comparing maltodextrin with DE 5, 10, and 15 to the survival of probiotic *lactobacilli* in the mixed juice of acerola and ciriguela, found that an increase in the DE value of maltodextrin reduced microbial viability. Meanwhile, increased DE can impact the product yield. An increase in DE often has higher hygroscopic and with faster water adsorption and lower moisture in the powders. The differences in water adsorption can also be explained by the chemical structure of each agent [78]. The phenomenon of water adsorption by a carbohydrate was attributed to the links between the hydrogen present in water molecules and the hydroxyl groups available in the amorphous regions of the substrate as well as the surface crystalline regions [79]. Therefore, higher hygroscopicity attributed to maltodextrin with high DE is because of a great number of ramifications with the hydrophilic groups that make moisture from the ambient air easily adsorbed by them[79].

5.3 Glass Transition Temperature

Moreover, the glass transition temperature (*Tg*) is a critical factor influencing the stability of spray-dried powders. A higher *Tg* contributes to improved powder stability by maintaining the material in a glassy state, which minimizes issues like caking, stickiness, and moisture absorption [80]. In the glassy state, molecular mobility is significantly restricted, thereby reducing the rate of physical, chemical, and biological changes. This stability is only maintained if the storage temperature remains below the *Tg* [49]. Different carriers used in spray drying exhibit varying *Tg* values. For instance, powders based on whey protein concentrate (WPC) and skim milk powder (SMP) typically have lower Tg compared to those containing maltodextrin. This difference is attributed to the higher lactose and water content in WPC and SMP, which act as plasticizers, reducing the *Tg* of the material [19]. Plasticizers, such as water and sugars, increase molecular mobility and decrease the *Tg* by disrupting the structural rigidity of the matrix [81]. Furthermore, the addition of simple sugars like D-glucose exacerbates the reduction of *Tg* due to their plasticizing effect, which further lowers the energy required for molecular motion [82]. This phenomenon highlights the importance of carefully selecting carrier materials and additives during the formulation of spray-dried powders to ensure sufficient *Tg* for storage stability

5.4 Thermoprotective Capacity of Additives

The compositions of certain carrier agents are known for excellent protection of bacteria cells during spray drying. For example, reconstituted skim milk (RSM) which contains abundant calcium ions and milk protein helps in enhancing the stability of cellular structure. Three different milk proteins (skim milk powder, sodium caseinate, and whey protein concentrate) were tested by Soukoulis et al. [19] on the *L. acidophilus*, and found that these carrier agents provide enhanced stability, up to 70% during drying. Additionally, *L. cremoris*, *L. acidphilus*, and *L. rhamnosus GG* cultured in Ca^{2+}-supplemented growth media also revealed that cells obtained enhanced heat stability after drying [30,83]. Besides RSM, the presence of proteins, namely arabinogalactan proteins (AGP) and glycoprotein (GP) on carrier agents such as arabic gum, is also reported to have protection for the bacterial cells during spray drying. This is done by providing partial replacement of sites for molecules in bacterial cells [84].

5.5 Feed Concentration

Nevertheless, the concentration of feed has a significant effect on the final product yield. The product yield percentage can be supported by the fact that increases the additive concentration, increases the total solid content, and decreases the total water level for evaporation. Typically, 10-20% (w/w) additive concentration is used for the microencapsulation of probiotics [1, 62, 85]. It has been reported that increased total solid content of additives leads to the formation of larger particles which require longer drying times. The probiotics entrapped in the particles would be subjected to more heat damage subsequently leading to cell loss. Fazaeli et al.[86] also concluded that larger maltodextrin particles produced when the concentration increased, made it difficult for water molecules to diffuse, so ended up with increased moisture content after spray drying. This was in agreement with the results of Vivek et al. [87], low encapsulation efficiency was observed when 25% maltodextrin concentration was used to spray dry non-dairy Sohiong fruit powder.

6. KEY FACTORS AFFECTING VIABILITY OF PROBIOTICS AT POST- SPRAY DRYING

6.1 Storage Condition

Probiotic viability in a powder is inversely related to the storage conditions. This has been demonstrated extensively in spray-dried probiotic powder [43, 88, 89]. During storage, the viability of probiotics is strongly affected by the storage temperature. The cell viability can be destroyed at high temperatures such as above 37 ℃. There was an observation by Soukoulis et al. [19] on the survival rate of probiotic *L. acidophilus* at different storage temperatures. At 4 ℃, it was observed that the probiotic cell experienced a slight growth when stored for a week under constant temperature. The

probiotic *L. acidophilus* showed a three-fold and seven-fold reduction in survival rate at temperature of 25 °C and 35 °C respectively. In a recent study, Arepally et al. [20] reported that encapsulated *L. acidophilus* was more viable at 4 ℃ than at temperature of 25 ℃ for a storage period of 12 weeks. The drastic reduction in cell viability that happened under the storage of high temperature is mainly attributed to the denaturation of protein, and oxidation of membrane lipids that lead to the degradation of macromolecules in the probiotic cell [1].

6.2 Water Activity

Water activity (a_w) is defined as the free water in the product, which is available for microbial metabolic purposes. Water activity is the critical parameter for probiotic survival, but the effect of water activity on cell death has not often been covered [90]. a_w is a more useful parameter than moisture content to determine the quality of food products [91]. Regarding water activity, high a_w induces lower stability in the probiotic cells in powdered form, increasing the inactivation rate and the risk of contamination [92, 93]. A study performed on *L. rhamnosus GG* showed a relatively poorer survival of bacteria in dried flaxseed detected during long-term storage attributed to the high water activity of the dried product, with the a_w at 0.43 and 0.22 [94]. Similar behaviour was observed when higher aw was encountered causing the viability of bifidobacterial reduced in the spray-dried *B. animalis ssp. lactis* Bb12 and *L. acidophilus* 2401 powders reported in the study of Dianawati et al. [95]. Results showed a dramatic reduction in bacterial viability from approximately 9 log CFU/g to approximately 7 log CFU/g due to an increase in a_w. According to Huang [96], the lowest water activity limit for bacterial growth is about 0.6 while the optimal range of a_w value for the storage of probiotic powder should be as low as 0.25. The reference line for water activity is aimed at reducing the available water for microorganisms to propagate.

6.3 Packaging Condition

The effects of different packaging materials on the physical and chemical properties of spray-dried products have been investigated by researchers [46, 88]. Appropriate selection of the packaging material and the storage conditions are varied depending on the spray-dried compositions. Packaging materials with increased barrier properties are aimed to stop the exchange of oxygen, carbon dioxide, water vapor, and light through the packaging material to extend the shelf life of probiotic products. Notably, some probiotics are anaerobic and microaerophilic, it should be mindful that exposure to oxygen would lead to the loss of viability and the functionality of the product. Thus, the level of oxygen could be maintained at low levels using vacuum packaging to effectively control the loss of probiotic viability [97]. Chaikham et al. [98] observed probiotic-incorporated Mamao juice powder in the laminated vacuum-sealed package which showed higher viability of *L. casei* compared to normal sealing conditions during the entire storage period. However, this type of packaging conditions might not be useful for all types of bacteria. Nale et al. [46] demonstrated results using vacuum packaging of spray-dried kefir microcapsules. The findings resulted in a reduction of cell viability for yeast during 3 months of storage. A similar statement was revealed by Cabello-Olmo et al. [88], who stated that the effect of standard packaging seemed to be superior to vacuum packaging on the viability of bacteria and yeast. This phenomenon happened probably because yeasts require oxygen for growth. In terms of the packaging material, the authors also concluded that the glass package was more favorable than the plastic package in maintaining the viability of probiotics [92, 93].

7. LIMITATIONS OF CURRENT METHOD AND FUTURE RESEARCH PERSPECTIVE

Spray drying is a widely adopted technique for preserving probiotics due to its efficiency and scalability. However, a critical limitation is its reliance on high temperatures, which can compromise probiotic viability by causing cellular denaturation and damage to essential structures such as proteins and membranes [96]. Most probiotics are inherently heat-sensitive, and exposure to temperatures beyond their thermal tolerance results in irreversible losses in functionality. Balancing optimal feed flow rates, drying temperatures, and energy efficiency without compromising probiotic survival remains a significant technical challenge, especially for industrial-scale applications.

Additionally, a significant technical bottleneck is the strain-specific variability in resilience to thermal and oxidative stress encountered during spray drying. For example, certain *Lactobacillus* species demonstrate thermotolerance with survival rates exceeding 80% under controlled drying conditions. In contrast, *Bifidobacterium* and yeast species and other sensitive strains often experience drastic reductions in viability, even under similar conditions [46, 99]. This highlights the necessity of customized, strain-specific optimization strategies to enhance the robustness of probiotics during the spray drying process. Furthermore, the heterogeneity in the cellular responses among different strains necessitates deeper investigation into stress adaptation mechanisms, including the roles of heat shock proteins, membrane lipid composition, and intracellular antioxidant systems.

Future advancements could be realized by integrating novel encapsulation strategies, such as microencapsulation and nanotechnology, to create protective barriers that mitigate thermal and oxidative damage. Recent study of Zhang [100] highlighted the potential of nanoencapsulation to enhance cytoprotection of probiotics during processing and storage. Besides, the application of hybrid encapsulation materials combining natural biopolymers (e.g., alginate, carrageenan, or chitosan) with synthetic or functionalized polymers may provide enhanced thermal resistance and sustained protection [101].

To advance strain-specific approaches, the application of omics technologies (e.g., transcriptomics, proteomics, metabolomics) holds great potential. These techniques could elucidate the molecular pathways and regulatory networks involved in stress tolerance, enabling the identification of biomarkers for thermotolerance or stress resistance [102]. Furthermore, leveraging genome editing tools, such as CRISPR-Cas9, may allow the development of engineered probiotic strains with enhanced robustness tailored to spray drying conditions [103]. High-throughput screening methods for assessing probiotic viability under diverse drying conditions would also accelerate the optimization process.

8. CONCLUSION

In conclusion, probiotic survival during spray drying is influenced by a complex interplay of factors, including strain-specific thermal tolerance, process parameters, and encapsulation agent compositions. While advancements in biopolymer-based encapsulation, hybrid materials, and nanotechnology have significantly improved probiotic viability under harsh conditions, challenges persist in customizing processes to accommodate strain variability and achieving industrial scalability. Future research should prioritize molecular-level investigations, such as omics-based approaches, to elucidate probiotic stress responses and develop targeted encapsulation strategies. Additionally, the integration of genome-editing tools and high-throughput screening techniques could accelerate the development of thermotolerant and robust probiotic strains. Efforts to innovate encapsulation materials must also emphasize environmental sustainability and biodegradability, balancing efficiency with ecological responsibility. By addressing these challenges, spray drying can be optimized to produce resilient, high-quality probiotic formulations, meeting the rising consumer demand for functional foods and beverages while aligning with global sustainability goals.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

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