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Spray drying encapsulation of probiotics and enzymes

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Abstract

Spray drying is one of the most frequently used encapsulation techniques. The incorporation of different active compounds in small capsules contributes to their protection and stability. Applications of spray drying of food ingredients are constantly being developed for the food industry due to the simplicity, low cost, effectiveness and versatility of this technique. Probiotics and other active compounds, such as enzymes, can be encapsulated by spray drying by combining various carrier materials, such as maltodextrins, gums, modified starch or alginate. However, exposure to high temperatures can be injurious to the integrity of probiotic cells or enzyme activity and can cause irreversible changes. Approaches such as enhancing pre- and post- spray drying steps are crucial to maintaining the integrity of these active compounds in the dried powders. This review focuses mainly on two major factors affecting the survival of probiotics and the activity of enzymes during spray drying, namely, the choice of carrier/wall material and drying temperature, bringing new light on how these influence post-drying characteristics of the final products.

Key Words: enzyme activity, enzyme encapsulation, probiotics, drying carriers, outlet air temperature, survival

15.1. Introduction

Spray cooling, coacervation, coextrusion, emulsification, and spray drying (SD), are some of the techniques currently used for the microencapsulation of compounds of

interest in the food industry (Arepally et al., 2020a; Singh et al., 2010). Microencapsulation enables the addition of various compounds, ranging from vitamins and minerals to phenolic compounds and proteins to food matrices (Choudhury et al., 2021). Furthermore, creating a protective wall around compounds of interest can preserve their characteristics for extended periods. Moreover, it not only confers protection from environmental reactions, such as those caused by oxygen, moisture and pH values in the matrix but also facilitates the application of such products in foods without the sensory impact they might otherwise cause (Choudhury et al., 2021). In addition, microencapsulation is particularly useful to protect susceptible products, like probiotics and proteins, from the highly acidic environment of the stomach and to guarantee that they reach the intestine in significant concentrations. For example, as reported in the study of de Araujo Etchepare et al. (2020), the successfully encapsulated bacteria *Lactobacillus acidophilus* were protected during exposure to gastric acids since bacterial death was considerably reduced from over 7 log Colony Forming Units (CFU)/g in free bacterial samples to 3-4 log CFU/g when encapsulated.

Spray drying was first patented in the last half of the XIX century, and it has since become a staple technique of the food and pharmaceutical industries for drying liquids (Santos et al., 2017; Samborska et al., 2022). Briefly, this technique consists of atomising a liquid solution and contact with the controlled flow of hot air at temperatures up to 200 ºC (Bednarska et al., 2020). As bacterial cell suspension is atomized into droplets, water is evaporated. During the passage through the spray dryer atomizer, shear forces can cause mechanical damage to cells (Ghandi et al., 2012) or can even, at a molecular level, accelerate protein denaturation, blocking bacterial metabolism (Minic, 2015). Due to the small dimension of the droplets (10-150 μm), and its significant surface-area-to-volume ratio, the energy transfer between the gas inside the equipment results in the elimination of most of the moisture in the solution's particles, resulting in the production of dry spherical powder particles (Both et al., 2022; Santos et al., 2017). This process is associated with the cooling caused by the evaporation of the water, which allows to keep low temperature inside the droplet - crucial for thermosensitive compounds (Phisut, 2012). The powder is then separated from the gas particles by centrifugal force, filtration, or electrostatic precipitation. The low moisture property of spray-dried products significantly extends their shelf-life while permitting the transportation of increased quantities of dried products due to their low volume compared to the non-dried solution (Santos et al., 2017).

Apart from the application of this technique for drying, it is also efficient in the microencapsulation of compounds. The compound of interest, being suspended in a protective solvent called a carrier or wall material, is encapsulated inside a shell of solidified solvent molecules (Arepally et al., 2020a). SD can be continuously operated as long as a constant flux of feed solution is injected into the equipment and conditions are maintained. This, in addition to the simplicity of the process and the affordability of the equipment, results in the fact that this technique is regarded as a reliable, efficient, and financially advantageous method for drying solutions and the microencapsulation of probiotics and other active compounds (Arepally et al., 2020a; Samborska et al., 2021). Nonetheless, the adaptation of the drying process must be performed depending on the characteristics of the solution. Several process parameters must be adapted, such as the inlet (IAT) and outlet air temperature (OAT) and the atomization pressure. In addition, characteristics such as the viscosity of the solution and its glass transition temperature, protein denaturation conditions, and the stability of bioactive compounds must be taken into consideration since high temperatures might result in improper drying or the destruction and/or inactivation of encapsulated compounds (Truong et al., 2005).

The growing interest in probiotic-rich diets, owing to the medical value these appear to provide, is creating a demand for spray-dried and encapsulated probiotics in foods. Therefore, several studies regarding the SD of probiotic bacteria and its application in food products have been published in the last decades (Desmond et al., 2001; Lian et al., 2002; Wang et al., 2004; Simpson et al., 2005; Mestry et al., 2011; Barbosa et al. 2015a; Bustamante et al., 2020). Adequate IAT and OAT appear to be key parameters for the efficient encapsulation of microorganisms. While certain conditions can produce microcapsules with probiotic bacteria internalized, the use of high temperatures may result in the loss of cell viability and/or cellular injury resulting in the inability of the microorganism to thrive after ingestion. Since the temperature used during the SD process can be significantly higher than those tolerated by most microorganisms, there is a need for the application of heat protectants. The selection of wall materials with solubility properties at an acceptable level for the SD process is rather limited, as an aqueous solution is required for this drying process (de Araújo Etchepare, 2020). Typically, skim milk (SM), maltodextrin (MD), hydrophobically modified starch and their mixtures, gum acacia, alginate, carboxymethyl cellulose, guar gum, soy protein, whey protein, gelatin and sodium caseinate are employed as coating materials to encapsulate probiotics (Chavez and Ledeboer, 2007; Jackson and Lee, 1991; Silva et al., 2011).

The inadequate selection of the temperature and the carrier can be detrimental to the survivability of the probiotic. This was reported in the study of Farahmandi et al. (2021), where the increase in IAT from 150 ºC to 170 ºC reduced the survivability of *Lacticaseibacillus rhamnosus* from around 78% to 48%. Also, in Arepally et al. (2019), the increased gum Arabic (GA) concentration in a maltodextrin-rich carrier solution guaranteed higher survivability rates for *L. acidophilus*. Since higher process temperatures result in lower moisture content in the final product, which leads to increased stability and shelf-life of the powder, selecting a suitable carrier is an important parameter to consider.

The demand for enzymes and similar compounds in both the pharmaceutical and food industries is ever-increasing, as is the dependency of these sectors on these proteins. While enzymes are not directly applied to food to improve its nutritional value, like other proteins, they have become fundamental to industrial processes such as fermentation, deesterefication, and hydrolysis, among others (Zhu et al. 2011). As such, the need for stable, long-lasting enzymes demands the development of techniques capable of protecting these sensitive compounds. Easily inactivated by adverse pH conditions and extremely susceptible to denaturation by high temperatures, enzyme stability benefits considerably from microencapsulation by SD. However, proper carriers and temperature are required to ensure that enzymatic activity is not lost during the process (Furuta and Neoh, 2020). Unsuitable carriers might result in the inactivation of the protein, as reported by Costa-Silva et al. (2011): the enzymatic activity of lipase after encapsulation appeared to be directly dependent on the carrier used. Therefore, the encapsulation of proteins, and in specific, enzymes, through spray drying also demands significant consideration.

In this chapter, an extensive study on adequate conditions, focusing on the selection of carriers and IAT and OAT for the microencapsulation of probiotic cultures and enzymes through SD are presented. Through the review and compilation of several studies regarding this topic, published since the beginning of the XXI century, conditions for the correct and successful encapsulation of these highly demanded compounds shall be exposed and commented upon.

15.2. Probiotics and probiotic foods

The term “probiotic” was first used in 1953 to describe active substances able to restore the health of malnourished patients (Kollath., 1953; Hamilton-Miller et al., 2003). Nowadays, probiotics are defined by the Food and Agriculture Organization of the United

Nations and the World Health Organization as "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Those health benefits can vary among probiotic strains and are related to the control of irritable bowel syndrome and inflammatory bowel diseases (Coqueiro et al., 2019; Whelan et al., 2013), to reduce the risk associated with cardiovascular disease (Liu et al., 2017), to the improvement of lactose tolerance (Gingold-Belfer et al., 2020), to the lowering serum cholesterol (Nguyen et al., 2007), to antimutagenic and anticarcinogenic properties (Ahmadi et al., 2014), to prevent and treat obesity (Kobyliak et al., 2016), to improve mental health (Barbosa and Vieira-Coelho, 2020), among others.

The most commonly used probiotics belong to the Lactic Acid Bacteria group (LAB), in particular to the genera *Bifidobacterium* and *Lactobacillus, Lactiplantibacillus* or *Lacticaseibacillus* (both previously *Lactobacillus*; Zheng et al., 2020), such as *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus* and *Lacticaseibacillus rhamnosus* and also to the *Lactococcus* and *Enterococcus* genera (Bermúdez-Humarán et al., 2008; Barbosa et al., 2014; Brenner and Chey, 2009; Corcoran et al., 2004; Kim et al., 2010; Lian et al., 2002). Yeasts such as *Saccharomyces boulardii* are also used (Kelesidis and Pothoulakis, 2012).

The adequate number of probiotics ingested is not specified, but high levels of viable microorganisms are recommended to ensure their effectiveness (~10^6-10^9 CFU/g or mL) (FAO/WHO, 2002; Ishibashi and Shimura, 1993; Knorr, 1998; Williams, 2010). Probiotics can be ingested in foods, such as yoghurts (Lourens-Hattingh and Viljoen, 2001), cheeses (da Cruz et al., 2009) or frozen fermented dairy desserts (Shah and Ravula, 2000); or in different formats of dietary supplements or pharmaceuticals, such as capsules, tablets or powders (Kim et al., 2003; Klayraung et al., 2009; Sutton, 2008). The production of probiotic foods is challenging since these microorganisms must survive during processing and along shelf-life. Additionally, the ingested microorganisms need to be able to survive and populate the gastrointestinal tract of the host to exert their beneficial effects (Ghelardi et al., 2022). Since probiotics' survival is essential, encapsulation has been proposed to increase their resistance to adverse conditions.

15.3. Spray drying encapsulation of probiotics

Cell encapsulation is a common strategy to protect and improve the survival of probiotics during long periods (Lasta et al., 2021). It is either a physicochemical or a mechanical

process involving cell entrapment in encapsulating/wall materials that protect and reduce cellular losses of the encapsulated microorganisms (Rodrigues et al., 2020).

A compilation of studies on the probiotic encapsulation by SD in the last 20 years is presented in Table 1, where the growth and drying parameters are detailed, as well as the survival rate obtained for each strain investigated.

[Insert Table 1 here]

15.3.1. Major challenges in probiotic encapsulation

Despite several advantages of the SD method, exposure to high temperatures, and nonthermal drying effects, such as pH, atomization and oxidative stress, reduce the viability of the probiotics during drying and their activity in the subsequent storage (Barbosa et al., 2015b, Marino et al., 2021; Silva et al., 2011). As the production of dried probiotics starts with their growth in a culture medium and ends with their storage in powder form, several parameters can be optimized before, during, and after SD to increase their survival. Intrinsic resistance of the strains, growth media, growth phase and growth temperature are some conditions before drying that can influence cell viability during SD (Wong et al., 2010). Also, the composition of the feed solution and parameters during SD, such as the temperature during and after SD, are extremely important to guarantee the maximum survival of probiotics (Barbosa and Teixeira, 2016; Barajas-Álvarez et al., 2022). Additionally, dehydration inactivation is also an important factor during SD of probiotics and may occur during drying at their physiological temperature or due to very high or very low drying temperature. Since water activity is the main factor in cell integrity and stability maintenance, the drying of cells poses a dichotomy (Santivarangkna et al., 2007; Peighambardoust et al., 2011). While on the one hand, low water activity poses an important aspect on the stability and storage of the final powdered product, on the other, when water removal occurs and low water activity is achieved, cell integrity will be compromised, mainly, loss of lipid bilayer integrity and impairment of cell membrane functions (Peighambardoust et al., 2011).

In this chapter, the composition of feed solution and thermal conditions during SD will be focused due to their direct influence on the survival of probiotics and the consequent quality and stability of the final products.

15.3.1.1. Food solution composition: effect of protective carriers

In SD, protective carriers are of paramount relevance, as they allow the stabilization of the bioactive compounds of interest in their interior (Akbarbaglu et al., 2021). Carriers help with the challenges and difficulties occurring during SD, like shear tension during

atomization, aggregation, thermal stress and degradation during dehydration which can result in a loss of biological activities and functional properties. Also, glass-transition-related changes, including adhesion of the materials to the dryer chamber, collapse, caking, and stickiness, can cause a reduction in powder yields, while the application of carriers can reduce such mechanisms (Bazaria & Kumar, 2016; Sarabandi et al., 2019; Sarabandi & Jafari, 2020). The efficiency of carriers is essential for encapsulating and maintaining physicochemical, structural, functional, and biological activities of the bioactive properties of the probiotics (Akbarbaglu et al., 2021). These properties are affected by the type, composition, and nature of carriers (Shishir et al., 2018). Carriers are intended to exhibit good rheological behaviour, dispersibility of the bioactive within the matrix, no destructive chemical reaction between carrier and core, creation of a stable coating during the process, controlled release of the bioactive, maximum stabilization against destructive environmental factors during storage (moisture, heat, oxygen, light, among others) and to be easily handled. Generally, the core substance is homogenized with the carrier in a ratio of 1:4, and this mixture is fed into the spray dryer (Ray et al., 2016). After atomization into droplets, dry spherical particles (capsules) are formed with the core material retained within the wall material (carrier), and then, the capsules are collected at the bottom of the dryer (Gibbs et al., 1999). Different encapsulating agents used in SD are available based on different materials, such as polysaccharides, proteins (Akbarbaglu et al., 2021), and lipids (Ray et al., 2016). Mainly polysaccharides, proteins and their combinations have aroused interest for use as carriers for microencapsulation of probiotics (Pinto et al., 2015).

Polysaccharide-based carriers have high solubility, low cost, neutral taste, and low viscosity at high concentrations. However, they have no surface activity and have low film-forming capacity (Akbarbaglu et al., 2021). Starches, maltodextrins, corn syrups and GA are polysaccharide-based encapsulating agents used for spray drying (Saénz et al., 2009).

MD has been widely used to microencapsulate various bioactive compounds using SD, conferring protection from environmental degradation factors such as oxygen, light, and heat (Sarabandi et al., 2019). It also increases the stability of the compounds during storage, preventing oxidative stress (Arepally et al., 2020a), and covers their bitterness (Sarabandi et al., 2019). Due to its low emulsifying capacity, the use of MD in combination with other materials is favoured (Arepally et al., 2020a). Gervasi et al. (2022) studied the production of a functional orange powder supplemented with probiotics and

prebiotics using spray drying, combining MD and pectin in a 10:1 weight ratio. Survival of *Lactobacillus casei* Shirota, *Lactobacillus casei* Immunitas and *Lactobacillus johnsonii* during SD of orange juice has been proven, and the viability of the bacteria during storage was demonstrated; encapsulated cells were shown to have higher viability when stored at 4 °C than those stored at 25 °C (Gervasi et al., 2022).

Protein-based carriers are one of the main carriers used in SD for the encapsulation of bioactive compounds, with some being known as food grade and generally recognized as safe (GRAS), digestible, and biodegradable as a source of energy and amino acids with high nutritional value (Pihlanto et al., 2017). The main advantages of using proteins as carriers and drying aids are their surface-active properties and migration to the water/air interface, film formation properties and activity in stabilising bioactive compounds (Fang & Bhandari, 2012). In addition, they also have other functional properties such as excellent emulsification, foaming ability, water/oil holding capacity and high glass transition temperature (Ge et al., 2021). Protein-based carriers are used to minimize shear stresses, structural changes, heat stress, and loss of biological and antioxidant activities of bioactive compounds (Ajmera & Scherließ, 2014; Akbarbaglu et al., 2021). Gelatin, casein, milk serum, wheat, and soy are protein-based encapsulating agents commonly used for SD (Saénz et al., 2009).

Casein is an animal-based protein, and it is present in milk. This protein is already well known and used as a source of functional, nutritive, and bioactive peptides. Casein is the main group of soluble milk proteins (Akbarbaglu et al., 2021) and has a mild taste (Shishir et al., 2018). Its ability to function as a carrier will be affected by its structures, which are related to the mechanism of cluster formation. Casein micelles, which are colloidal particles, can retain hydrophobic substances in their structures (Akbarbaglu et al., 2021). In addition, sodium and calcium caseinate have high water solubility, improving the stability of hydrophobic bioactive compounds (Jarunglumlert et al., 2015). Dairy products are among the most used carriers for the encapsulation of probiotics since they have a high buffering capacity and provide natural protection to the probiotics in the passage through the gastrointestinal tract, increasing their viability. Liu et al. (2016) demonstrated that encapsulating the probiotic *Lactobacillus* zeae LB1 in sodium caseinate and GA improved cell viability during spray drying, storage and *in vitro* digestion. Reconstituted skim milk (RSM), which contains a mixture of proteins, carbohydrates, among others (Lian et al., 2002), is one of the most used carriers with a protective effect on the viability of probiotic cultures during dehydration in SD (Meng et al., 2008; Barbosa and Teixeira,

2016; Dianawati et al., 2017; Teijeiro et al., 2018). It produces a porous structure in the dehydrated product, its proteins develop a protective cover for the bacterial cells (Teijeiro et al. 2018), preventing cell injury by stabilizing cell membrane constituents (Castro et al. 1995), and the calcium in milk increases survival after dehydration (King and Su, 1993). According to Teijeiro et al. (2018), thermal protectants (carriers) significantly increased the survival of microorganisms when kefir was dehydrated by SD. Using SM as a carrier to produce kefir powder resulted in LAB survival greater than 9 log CFU/g and better survival in simulated gastrointestinal conditions (Teijeiro et al., 2018). Gardiner et al. (2002) produced a spray-dried probiotic milk powder with 20% (w/v) RSM with a rifampicin-resistant variant of *Lactobacillus paracasei* NFCB 338, and a survival of 84.5% was obtained at IAT and OAT of 175 ºC and 68 ºC, respectively. This powder was used as an adjunct inoculum during the production of Cheddar cheese and the number of probiotics remained stable after 3 months of maturation, without negatively affecting the quality of the cheese (Gardiner et al., 2002). Ananta et al. (2005) used RSM at a concentration of 20% (w/w) as a carrier in SD and obtained a survival rate of probiotic bacteria *Lactobacillus rhamnosus* GG (ATCC 53103) of 60% at an OAT of 80 °C. In addition, Barbosa et al. (2022) studied the microencapsulation of the live biotherapeutic candidate *Akkermansia muciniphila* by SD. The authors concluded that by using 10% SM as carrier and IAT/OAT of 150/65 ºC, stability of viability is obtained, both during prolonged aerobic storage as well as exposure to simulated gastrointestinal passage (Barbosa et al., 2022).

Whey proteins are important by-products of the dairy industry, being produced in large quantities (Pinto et al., 2015), and applied in a wide range of dairy and non-dairy beverages, meat products and desserts (Duongthingoc et al., 2013). These proteins have biological, amphiphilic, and emulsifying activities, film formability, and high coverage properties, being excellent candidates for the encapsulation of probiotics (Akbarbaglu et al., 2021). Duongthingoc et al. (2013) investigated the effect of whey protein agglomeration on the survivability of *S. boulardii* within spray-dried microcapsules, and it was demonstrated that whey protein agglomeration promotes the survivability of the yeast during SD. Pinto et al. (2015) microencapsulated *Bifidobacterium* BB-12 by SD using liquid whey, or whey retentate obtained from nanofiltration. The viability of the bifidobacteria was determined during 90 days of storage at 4 °C and −20 °C. It was concluded that all microcapsules had high counts of bifidobacteria (8.79-9.56 log CFU/g), low water activity (0.191-0.134) and low moisture content (3.55-2.67%). Colín-Cruz et

al. (2019) co-encapsulated blackberry juice and *L. acidophilus* by SD using three materials: GA, MD and whey protein concentrate, alone and in a 50:50 mixture. In this study, the authors concluded that whey protein concentrate conferred the most protection for probiotic bacteria compared with the others tested.

Gelatin is a denatured protein obtained from animal collagen through thermal treatments or by enzymatic, acidic, and alkaline hydrolysis methods (Shishir et al., 2018). It is used by the cosmetic, food and pharmaceutical industries as a foaming, emulsifying, and wetting agent because it has surface-active properties (Jiang et al., 2020). Gelatin is suitable for encapsulation and to be used as a carrier for SD as it has good functional characteristics: biodegradability, biocompatibility, water retention capacity, film formation and anti-carcinogenicity. While being affordable (Estevinho et al., 2013), the use of gelatin also brings a reduction in biodegradability or toxicity issues related to the use of synthetic compounds. The main disadvantage is the rapid solubility in the aqueous system (Dang et al., 2017). Guergoletto et al. (2017) investigated the effect of GA, MD and gelatin on the survival of *Lactobacillus reuteri* LR92 in fermented juçara pulp after dehydration by SD, using 10% w/v of carrier agents. It was demonstrated that gelatin provided the highest number of viable bacterial cells after drying (8.63 ± 0.11 log CFU/g).

Plant-based proteins and their commercial applications are of increasing interest to the food industry because of their lesser allergenicity, low toxicity, lower cost compared with animal-based proteins and better hydrophobic properties (Akbarbaglu et al., 2021).

Soy protein, one of the main sources of vegetable protein, has significant physicochemical functionalities in gel formation, emulsification, fat absorption, water binding capacity and nutrient protection against oxidation (Shishir et al., 2018). Furthermore, soy protein, although it may be allergenic (Cordle, 2004), is gaining increasing interest because of its high nutritional value (Lohcharoenkal et al., 2014), health benefits (Xiao, 2008), availability (Hadzieva et al., 2017), biodegradability, being relatively inexpensive, exhibiting low immunogenicity (Tansaz & Boccaccini, 2016), and being relatively stable and presenting long storage times (Peles et al. 2013). Studies that refer to soy protein as a carrier in the SD of probiotics generally use it together with other compounds. It can be combined with other synthetic and natural polymers and inorganic materials to improve the mechanical properties and the bioactive behaviour of the probiotic (Tansaz & Boccaccini, 2016). Hadzieva et al. (2017) investigated a formulation for the preparation of *L. casei* 01 encapsulated in soy protein isolate and alginate microparticles using SD. It was demonstrated that probiotic microparticles presented increased stability and viability

of the probiotic above the minimum level of ingestion of 10^7 CFU/g after microencapsulation and in simulated gastrointestinal juice, and a high potential for release of probiotic and prolonged residence when ingested (Hadzieva et al., 2017).

Pea protein has heat stability, good solubility, ability to form a stable film and stabilize fat emulsions, which are characteristics that allow the encapsulation and protection of probiotics from heat and osmotic stress caused by SD (Barać et al., 2015; Dianawati et al., 2017). Moreover, this protein has a low cost (Kowalska et al., 2022). Dianawati et al. (2017) demonstrated that after SD, pea protein-based formulations protected *L. acidophilus* from heat treatment. Furthermore, the pea protein formulation improved the survival of the bacteria when exposed to simulated gastric juice and simulated intestinal fluid. Arslan et al. (2015) concluded that pea protein used as wall material by SD provided high resistance of probiotic *Saccharomyces boulardii* to the gastric solution.

Alongside the main wall materials mentioned above, various compounds can be added to the encapsulating solution as auxiliary protective agents. While these are applied in mostly small concentrations and do not form the bulk of the wall, they are, nonetheless, expected to improve bacteria resistance to thermal, osmotic, oxidative and acidic/alkaline stresses. As seen in Table 1, the presence of amino acids (e.g. inulin) (Wang and Mutukumira, 2022; Barajas-Álvarez et al., 2022), organic acids (e.g. ascorbic acid) (Rodklongtan et al., 2022), and sugars (e.g. trehalose and sucrose) (Arslan-Tontul and Erbas et al., 2017; Barajas-Álvarez et al., 2022; Lapsiri et al., 2012; Zhang et al., 2016) can function as secondary protective agents, improving carrier efficiency in protecting the core material from the extreme external environment.

Antioxidants, such as ascorbic acid, and osmo- and cryoprotectants, such as trehalose and sucrose, act by providing the bacteria with resistance to oxidative stresses (Rodklongtan et al., 2022) by serving as compatible solutes, stabilizing the cell wall to extreme changes in osmolarity (Duong et al., 2006), or by interacting with the phospholipid membrane of the cell contributing to membrane integrity during temperature variations (Daryoush et al. 2010). The use of such compounds improved rates of probiotic survival to SD in the studies of Zhu et al. (2016), Chávez and Ledeboer (2007) and Rodklongtan et al. (2022), while failing to show any beneficial return in Lapsiri et al. (2012) and Zhang et al., (2016).

Amino acids such as glycine and inulin are primarily used in the pharmaceutical industry as buffering, solubilizing, bulking agents and to reduce aggregation, increase stability and maintain enzyme activity during SD (Akbarbaglu et al., 2021; Sarabandi et al., 2019, Oskouei et al., 2010). As seen in the study of Kingwatee et al. (2015), the inclusion of

inulin in a pasteurized lychee juice with MD appears to improve the survivability of *Lacticaseibacillus casei* to SD stresses. However, Perdana et al. (2014) also studied the protective effect of different carriers on the survival of *Lactiplantibacillus plantarum* WCFS1 under SD conditions and subsequent storage and concluded that the residual viability of cells dried with glycine was relatively low. According to the authors, the high molar concentrations of glycine during drying increased the osmotic pressure across the cell membrane, which cannot be tolerated by *L. plantarum* WCFS1, leading to cell death. This variability in encapsulation efficiency shows that while selecting the right carrier is of imperative importance, the addition of thermal, osmotic, and antioxidative protectants and cell wall stabilizers can either improve yield or hinder this technique's performance.

15.3.1.2. Drying parameters: effect of outlet air temperature

SD parameters such as IAT and OAT directly influence probiotic survival as well as the quality of the final product. A high percentage of bacterial survival is one of the most sought-after outcomes when applying this method for probiotic commercialization (Barbosa and Teixeira, 2016). OAT has been identified as the most significant factor influencing the survival of probiotics during the SD process. This parameter is dependent on IAT, drying characteristics of the matrix used, air flow rate and atomized droplet size (Maroof et al., 2020; Souza et al., 2022; Barbosa and Teixeira, 2016). OAT is difficult to predict and unable to be directly controlled, lying ideally between 50-80 ºC. It directly influences powder characteristics such as moisture content, water activity, droplet size, shape and structural integrity (Singh et al., 2022). High OAT has been associated with reduced probiotic survival due to thermal injury and protein denaturation, but also with low water activity and low moisture content, which are fundamental factors in extending shelf-life and ensuring the quality of the final product. Conversely, lower temperature has been linked with increased viable cell counts as well as with pristine atomized droplets, meaning droplets with uniform sizing, without caking and free of damage (Singh et al., 2022; Souza et al., 2022; Maroof et al., 2020).

With this conundrum between the use of higher or lower OAT, a compromise between temperature low enough to avoid bacterial death and high enough to achieve optimal moisture content and water activity is necessary in order to achieve optimized drying settings for probiotic encapsulation.

Various authors have studied the effect of OAT temperature on SD of several probiotics. Barbosa et al. (2022) tested different IAT and OAT for *Akkermansia muciniphila* DSM 22959, finding that the most suitable OAT regarding encapsulation efficiency and

viability was the lowest temperature tested of 65 ºC despite the IAT, stating that the outlet highly influences the outcome (Barbosa et al., 2022). Zhang et al. (2016) tested different OAT for *Lactobacillus salivarius* NRRL B-30514 ranging from 100 to 70 ºC, and observed lower logarithmic reductions for 72-70 ºC as well as higher water activity, as expected. Significant differences were observed from 100-98 ºC to 84-86 ºC, with steep log reductions being observed, albeit lower water activity was also observed for these OAT. From 86-84 ºC to 72-70 ºC, higher viability was observed, proving to be more efficient when it comes to the post-drying survivability and quality of the powder probiotic (Zhang et al., 2016). Kingwatee et al. (2015) studied different OAT for different carrier materials for *L. casei* 01 encapsulation. It was shown that temperatures between 60 and 80 ºC enabled bacterial survival for all carriers tested, while higher temperatures, 90 ºC, severely affected the viability of this probiotic. While lower OAT may allow for greater bacterial survival, powder characteristics were far inferior, with high moisture content being impeditive of long storage solutions. Thus, even though at 90 ºC, higher quality products are attained, low probiotic survival does not allow for it to be feasible. The optimal temperature for *L. casei* 01 encapsulation has been set at 80 ºC due to providing the lowest moisture content with the highest bacterial survival (Kingwatee et al., 2015).

From the 128 studies (2000 - 2022) reviewed and compiled in Table 1, the vast majority of OAT used were comprised within the established optimal temperature (50-80 ºC). This threshold allows for good bacterial recovery after SD as well as keeping water activity values within a certain range, namely, 0.1-0.2, and moisture content under 4%, being these characteristics of utmost importance from a commercial point of view (Broeckx et al., 2016; Barbosa et al., 2022). Regarding the influence of temperature on dehydrative inactivation or damage, it mainly occurs at low drying temperatures, while at high IAT, inactivation is attributed to both dehydration and thermal inactivation, resulting in loss of viability of the probiotics (Hernández-López et al., 2018).

Various factors impact the outcome of the drying process, with an optimal OAT for probiotic SD needing to be established to achieve the best possible product. Since probiotics extended shelf-life must be assured, optimized processing steps during SD are crucial for the best yield. From the data collected and shown in Table 1, the probiotic survival rate was shown to vary considerably with strain, ranging from as high as 100% and as low as 0.01%. Thus, acceptable levels do not fall under a specific threshold but will rather vary with probiotics, matrix and temperatures used during the SD process.

Thermotolerance parameters to study the behaviour of more sensitive strains during this process have been proven to be an important step regarding the need to achieve the highest survival rate possible that will be suitable for industrial needs (Gardiner et al., 2000).

15.4. Enzymes and the industrial enzyme market

Enzymes are proteins that act as biological catalysts in different processes due to the acceleration of various reactions. They are essential in the metabolic pathways of the cells (Sarabandi et al., 2021). As such, enzymes are widely used in various industrial products such as detergents, food processing, animal-feed additives, fine chemistry, pharmacology and drug manufacture, textile, and beverages (Zhu, Wu and Wang, 2011). For example, in medicine, thrombin is used to promote wound healing, while other enzymes are used to diagnose certain diseases. In the food industry, enzymes have a wide range of applications. Some are used for flavour development in dairy products, like lipases (Abdel-Mageed et al., 2021), while others are used to improve the nutritional value, solubility and digestibility of food protein, such as proteases (Nurhayati et al., 2022).

The development of enzymes has been employed since primordial times using a very well-known process - fermentation. In fermentation, the catalyst is a live microorganism, such as bacteria and fungi, that produces a high concentration of extracellular enzymes (Raveendran et al., 2018). Short fermentation cycles and inexpensive culture media are economically pleasing on a large scale.

In the past few decades, the industrial enzyme market has grown at a rapid rate. Improving production and efficiency are some of the desirable market goals. Tailor-made enzymes are developing fast, resulting in new application fields, in protein engineering and site-derived evolution. In Table 2, some leading brands in the industrial enzyme market are listed.

[Insert Table 2 here]

15.5. Spray drying encapsulation of enzymes

The production of commercial enzymes must guarantee their properties (stability and activity) during storage. Degradation of enzymes, physical or chemical, is facilitated by the presence of water. In this way, dry formulations are developed to provide an acceptable protein shelf-life (Alloue et al., 2007).

Enzyme encapsulation aims to improve stability, accessibility, provision and control of the release (Alexakis et al., 1995). SD is one of the most common techniques that allow microencapsulated enzymes to be able to endure several adverse conditions during

transport and storage (Gouin, 2004). Exposure to fluctuations in temperature or pH may lead to denaturation and/or inactivation of enzymes due to loss of protein structure integrity and enzyme ability (Furuta and Neoh, 2020). To achieve stabilization, the use of wall materials that serve as protective compounds and suitable drying conditions is the most common approach in the SD encapsulation method (Alexakis et al., 1995; Gouin, 2004; Lauruengtana et al., 2009).

New approaches have been developed to obtain fine powder formulations/particles (0.3–5 μm) with higher yields. Although SD is easy to scale up and can be used to encapsulate practically any drug, nano-SD is a new process that produces powder at the nanoscale and improves drug formulation and delivery. Choosing the drying techniques will depend mainly on the economics of the drying process and the intended route of drug administration.

15.5.1. Major challenges in enzyme encapsulation

In enzyme encapsulation by SD, the functional properties of the wall materials are a key factor in ensuring the optimal drying condition of enzymes (Bakry et al., 2016). Possible stress factors that the protein experiences during SD are surface adsorption, shear stresses, temperature, and dehydration. Additionally, applied wall materials may influence the activity of enzymes during storage.

The main drawback and difficulty of using enzymes and proteins in pharmaceutical applications are that they are extremely susceptible to physical and chemical stressors. The evaporation of water has been shown to significantly increase the thermal stability of proteins and enzymes when they are in a dry state (Abdel-Mageed et al., 2019; Costa-Silva et al., 2022; Pinto et al., 2021). Protein denaturation temperature is a function of water content, rising rapidly as water content decreases. Water replacement is a mechanism that would prevent protein degradation during SD (Abdel-Mageed et al., 2019). Water molecules form hydrogen bonds that create a layer of hydration, commonly called structured water (Mozhaev, 1998). The presence of bound water helps maintain the native active structured of enzyme proteins (Mozhaev, 1998). As opposed to that, at high water content, conformational changes occur leading to denaturation, i.e., enzyme inactivation (Polakovič et al., 1998). Addition of substances that have the ability to replace bound water is added to reduce the negative effects of water loss. Sugars are stabilizing substances that can compensate for the dehydrative inactivation due to hydrophobic interactions.

To better understand and acknowledge the main risk factors that influence enzyme activity, this section will review some studies where SD was used as an encapsulation enzyme strategy (Table 3).

[Insert Table 3 here]

15.5.1.1. Feed solution composition: effect of protective carriers

Enzyme preparation requires controlled conditions. In practice, enzymes should be immobilized on a solid carrier, on carrier-free cross-linked enzyme aggregates or by inclusion (microencapsulation) (Rother and Nidetzky, 2014). The carrier is an encapsulating agent that facilitates the SD process. The selection of carriers for encapsulation is vital since it may affect the development of protein formulations.

As stated, sugar-based encapsulating agents may be beneficial by their water-replacement properties. Regarding sugar-based carriers, trehalose is the most preferred in SD technique among all disaccharides excipients used (Pinto et al., 2021). When compared with, for example, sucrose, trehalose demonstrates a higher ability to form H-bond links with proteins (Malferrari et al., 2016). Trehalose at 10 % (w/v) as an encapsulating agent provided 100% and 63.28% of relative enzyme activity (ReA) at high IAT for two enzymes, β-galactosidase and lipase, respectively. Trehalose provided full protein activity preservation for β-galactosidase at lower OAT (89 ºC). However, at higher OAT (101 ºC), 39 % of activity loss was observed (Costa-Silva et al., 2011; Lipiäinen et al., 2018). In contrast, at low OAT (70.5 ºC), 63.28% of residual lipase activity was obtained. For trypsin, trehalose (1:1) showed to be capable of providing protein stability (more than 97% of ReA at 180 ºC) (Costa-Silva et al., 2011). The selection of the encapsulation agent, and its concentration, should be accounted for not only by the enzyme to be encapsulated but also for its ability to support storage stability. The usage of trehalose is preferable to mannitol (the most used monosaccharide excipient) regarding storage stability. Trehalose showed no enzymatic activity loss, but almost 53% activity loss was observed for mannitol after eight months of storage at 5 ºC (Costa-Silva et al., 2011).

Abdel-Mageed et al. (2019) observed that when lower IAT used (80 ºC), high ReA of α-amylase activity was observed (99%). However, ReA values were carrier-dependent since different efficiencies were obtained at the same temperature. The effect of carriers may be reflected in the yield value and ReA even after an extended period of storage (Lipiäinen et al., 2018; Abdel-Mageed et al., 2021).

Enzymes undergo different inactivation mechanisms, from denaturation to shear and dehydration stresses. Enzyme encapsulation by SD is widely used in the food industry due to conferring enzymes with protection from moisture and heat, allowing for higher viability and stability (Lauruengtana et al., 2009). MD is widely used as an encapsulating agent for several types of heat-sensitive compounds in SD due to its protection against environmental factors, low cost, high digestibility and for conferring powders with reduced stickiness and diminishing wall deposition, as well as glassy amorphous powders (Sarabandi et al., 2019; Kurozawa et al., 2009). High glass transition temperature is important to achieve minimum activity loss during drying and storage, with this type of matrix making it difficult for proteins to change their shape, and thus maintaining activity (Costa-Silva et al., 2011; Schutyser et al., 2012). Nurhayati et al. (2022) have reported that MD has a significant effect on enzyme activity and stability during SD for the encapsulation of trypsin, allowing for a higher powder yield due to conferring the enzyme with thermal protection from high IAT (Nurhayti et al., 2022). Another study by Costa-Silva et al. (2011) showed that lipases produced by endophytic fungus spray dried with MD were able to maintain 100% of their activity when in the presence of MD. GA is also one of the most used carriers for enzyme encapsulation. Ramakrishnan et al. (2007) reported that GA used as the encapsulating agent for endoglucanase was crucial for maintaining enzymatic activity and stability regarding temperature and pH changes (Ramakrishnan et al., 2007). Costa-Silva et al. (2011) have also reported that lipase spray dried with GA as a carrier was able to maintain 87.2% of enzymatic activity, proving to be a good encapsulating agent for drying and stabilizing enzymes (Costa-Silva et al., 2011).

15.5.1.2. Drying parameters: effect of air temperature and feed flow rate

Temperature poses a risk when handling thermolabile proteins. Consequently, high temperatures may lead to enzyme denaturation during the falling rate period, i.e., protein would lose its structure as the OAT increases (Schutyser et al., 2012). The objective of drying is to produce stable and solid forms of the enzyme. Critical water content and dehydrative inactivation in the dry formula is an important downfall, given that the degradation of the hydration shell and 3D structure has a direct impact on the loss of enzyme activity and poor storage stability (Schäfer, 2015; Pinto *et al.*, 2021). Rapid changes in temperature when droplets enter the chamber, low contact time, i.e., feed flow speed (Banga, 2015), in addition to the use of non-aqueous volatile solvents, may

counteract this problem (Pinto *et al.*, 2021). A study by Samborska *et al.* (2005) reported that after SD, the dried formula had 51.9 to 91.8 % ReA of α-amylase (Table 2). However, ReA was affected not by the elevated temperatures (160 to 220 ºC), but by the differences in feed ratio speed and resulting water content. It was observed that the lower feed ratio speed resulted in a reduction of residual activity and final water content, and the removal of bound water caused the degradation of the 3D structure. This corroborates that the effect of drying air temperature on the residual enzyme activity depends on the feed flow speed. A combination of high temperatures and specific feed ratio speed is preferable, and they may, however, be detrimental to the effect of ReA. Undesirable operational conditions, e.g., low IAT and low feed ratio speed may influence the moisture content leading to poor storage stability (Schäfer, 2015). Protein degradation during SD may be prevented by mechanisms of vitrification, or water replacement (Estevinho et al., 2014; Schüle et al., 2008; Mensink et al., 2017). The vitrification theory says that carbohydrates in their glassy solid state immobilise and stabilise the conformation of enzymes (Mensink et al., 2017). According to water replacement theory, some sugars (trehalose and sucrose) prevent aggregation and improve protein stability due to the replacement of bound water evaporated from protein structure during drying (Schüle et al., 2008).

15.6. Nano spray drying of probiotics and enzymes

Nano SD of bioactive compounds has been on the rise as a possible new technology to meet the necessity of an optimized method to conventional SD for the production of nanoencapsulated particles (Jafari et al., 2021). While some advantages of this novel technique lie on the ability to produce nano particles with high powder yields, its major downfall lies on requiring redesigned SD apparatus, inability to handle solutions with higher viscosity and difficulty in scaling up for industrial use (Piñón-Balderrama et al., 2020).

Conventional SD and nano-SD characteristics differ in several aspects. Drying gas stream in nano-SD is laminar for allowing gentle drying of the delicate nanoparticles, while for conventional SD it is turbulent due to the greater dimensions of the sprayed particles. Particle separation also plays an important differential factor, while conventional SD separation technology relies on a cyclone, nano spray driers rely in electrostatic particle collectors. Collection of nanoparticles or even submicron particles is particularly difficult by cyclone separation at sufficient yield, thus the need of the redesigned collection method used in nano-SD (Jafari et al., 2021; Piñón-Balderrama et al., 2020). Droplet size

and dried particle sizes also differ greatly. While droplet sizes for conventional SD threshold is 5-100 µm with a broader nature, nano-SD droplet size threshold is 3-15 µm, with a narrower profile. Dried nanoparticle size in notably smaller, 0.2-5.0 µm, while microparticles depend on the nozzle used. Two-fluid nozzles will originate particles with size ranging from 2-25 µm, and ultrasonic nozzles particle size ranges from 10 to 60 µm (Jafari et al., 2021). Size particle is dependent on other factors such as OAT and IAT, feed concentration and spray rate (Jayaprakash et al., 2022). The main reluctance from the food industry to employ this novel technology is that it is currently limited to laboratory scale, due to having been developed and optimized for products with a high cost to benefit ratio and not for larger, industrial scale production.

Regarding probiotic nanoencapsulation, due to their dimension, between 1 and 5 µm, nanoencapsulation is still limited, being conventional SD the adequate technique. Studies respecting nano-SD of probiotics are still scarce, with future research needed to assure that existing limitations are mitigated (Centurion et al., 2021).

On the contrary, nanoencapsulation of some enzymes by nano SD has been reported (Table 4). For example, Abdel-Mageed et al. (2019) performed the series of experiments to determine the drying and formulation parameters to produce α-amylase nano powder with highest ReA at the highest yield. Optimized production of smooth spherical nanoparticles (600 nm, yield 94%, and ReA 99%) was achieved using 7-mm spray cap and sucrose concentration 0.15% (w/v), drying flow rate 100 L/min, and inlet temperature of 80°C, revealing the promising role of this technology. Abdel-Mageed et al. (2021) studied a technique to enable the production of stable lipase nano powder. A wide range of physical characteristics and yield value was achievied depending on the additive type (210–1313 nm, yield 24–99% and ReA 80.5–100%). Optimized conditions from the data obtained to achieve higher powder yield and residual enzyme activity are attained using 4 µm nozzle diameter, 4% (w/v) dextrin concentration, and IAT of 80 °C. In a study by Bürki et al. (2011), it is reported that β-Galactosidase from *Aspergillus oryzae* can withstand nano SD with relatively no loss of activity if an aptimized protocol is employed, IAT of 80 °C and nozzle diameter of 4.0 µm, achieving particles of respirable size and high yields (2–4 µm, yield 90% and ReA 86%-96%).

[Insert Table 4 here]

Advantages and disadvantages of nano-SD of bioactive compounds have been reported. While several studies reported the efficient nanoencapsulation of enzymes, the fact that probiotic nanoencapsulation is limited and that this novel SD technology is not suited for

industrial scale production are a few reasons why this technique has not launched within the food industry. Its use in the pharmaceutical industry has been further studied, with application being far more forthcoming.

16. Conclusions

Probiotic encapsulation is a well-established method for the production of functional food products. Since the pharmaceutical and food industries have cast interest in this technology, studies and research in this field have been in high demand. Spray drying has been regarded as cost-effective, feasible, high-yield and suitable at an industrial scale. The choice of drying parameters will reflect directly on the quality and stability of the powder. Protective carriers are usually added to achieve higher bacterial survival and viability through the storage period. There is a myriad of options to choose through, depending on the matrix and the microorganism used, being the efficiency of the carrier essential for the encapsulation effectiveness. Skim milk is the most frequently used carrier, followed by maltodextrin and gum Arabic due to their functional properties. Novel encapsulating systems such as lipid carriers are expected to be at the forefront of spray drying carrier innovation in the next decades. Another parameter that is crucial to the efficiency of this method is the outlet air temperature, having been set at a threshold, between 50-80ºC, for optimal bacterial survival as well as optimal powder characteristics for probiotic viability during storage. Future studies, including extensive research of these drying parameters, will allow for better optimization of this process, allowing for its profitable use in the pharmabiotic and food industry.

Spray drying encapsulation of enzymes requires a careful selection of operational parameters due to their thermolabile properties. Opposingly to probiotic encapsulation by the same methodology, choosing an inlet air temperature not so high but just enough to volatilize soluble excipients and not cause enzyme deactivation is extremely important to protein formulation stability. Just as important as the temperature, depending on the enzyme, the selection (regarding type and concentration) and addition of encapsulating agents to avoid thermal stress of protein formulations is one of the main approaches for the encapsulation and preparation of enzymes by spray drying. New approaches are being studied to apply renewable carriers to improve enzyme performance falling into the eco-sustainable development fever.

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