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Minireview

MINIREVIEW – Food Microbiology

Low-moisture food matrices as probiotic carriers

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One sentence summary: This mini-review may contribute to the design of manufacturing strategies aimed to maintain the microbial viability, at relevant levels, in probiotic food products with low or intermediate contents of moisture.

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ABSTRACT

To exert a beneficial effect on the host, adequate doses of probiotics must be administered and maintaining their viability until consumption is thus essential. Dehydrated probiotics exhibit enhanced long-term viability and can be incorporated into low-moisture food matrices, which also possess high stability at refrigeration and ambient temperature. However, several factors associated with the desiccation process, the physicochemical properties of the matrix and the storage conditions can affect probiotic survival. In the near future, an increased demand for probiotics based on functionally dominant members of the gut microbiome ('next-generation probiotics', NGP) is expected. NGPs are very sensitive to oxygen and efficient encapsulation protocols are needed. Strategies to improve the viability of traditional probiotics and particularly of NGPs involve the selection of a suitable carrier as well as proper desiccation and protection techniques. Dehydrated probiotic microcapsules may constitute an alternative to improve the microbial viability during not only storage but also upper gastrointestinal tract passage. Here we review the main dehydration techniques that are applied in the industry as well as the potential stresses associated with the desiccation process and storage. Finally, low- or intermediate-moisture food matrices suitable as carriers of traditional as well as NGPs will be discussed.

Keywords: low-moisture probiotic food; desiccation techniques; protectant agents; microencapsulation

INTRODUCTION

Functional foods can influence the health and well-being of the consumer either naturally or through the addition, removal or modification of specific components (Ozen, Pons and Tur 2012; Brown *et al.* 2018). Probiotics defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' (Hill *et al.* 2014) deserve special attention among those components.

Probiotics have been associated with strain-specific health benefits such as reducing pathogenic bacteria colonization, alleviating diarrhea, reducing intestinal inflammation, lowering blood cholesterol, and for potentially having anti-colon-cancer activity (Chotiko and Sathivel 2016). However, maintaining the viability of probiotics in sufficient numbers during formulation and storage until the time of consumption is critical and represents a technological challenge (Dodoo *et al.* 2017). Furthermore, probiotic microorganisms must withstand harsh conditions during their transit through the upper gastrointestinal tract (GIT) in order to reach their site of action and consequently exert functional benefits on the host (Kim *et al.* 2016).

The survival of probiotic cells during storage as well as during passage of the mammalian upper GIT can be

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positively or negatively affected by the food matrix serving as a carrier (Sanders and Marco 2010). Traditionally, probiotics have been added to fermented dairy products (Rivera-Espinoza and Gallardo-Navarro 2010) and some non-dairy beverages like fruit juice and ice cream (Panghal *et al.* 2018). However, stress factors like low pH, presence of antimicrobial substances of vegetable origin, dissolved oxygen and interaction with starter cultures in the product limit the survival during storage, and therefore probiotics require constant refrigeration (Ranadheera, Baines and Adams 2010; Rivera-Espinoza and Gallardo-Navarro 2010; Shori 2015).

The water activity (a_w) of food, which is the parameter that determines the accessibility of water for chemical reactions and the growth of microorganisms, ranges between 0.15 for very dry food matrices and 0.99 for moist fresh foods (Dianawati, Mishra and Shah 2016). Low- and intermediate-moisture foods like chocolate ($a_w \approx 0.2$–0.3), peanut butter ($a_w \approx 0.35$), cereals (a_w 0.25 – 0.4), dried-fruit paste ($a_w \approx 0.6$), etc. have a long shelf life and are usually stable for years at room temperature (Finn *et al.* 2013). Food products in these categories confer a stable environment for probiotics due to a reduced water activity (a_w), which is a key factor in maintaining their viability as dried metabolically inactive cells (Vesterlund, Salminen and Salminen 2012) during long-term storage. However, stress suffered during the drying processes may negatively affect the survival of probiotic cells and needs to be mitigated through technological optimization of those processes (Broeckx *et al.* 2016).

Probiotics encased in freeze- or spray-dried microcapsules formed by gelation of biopolymers such as alginate, gelatin, chitosan, gelatin, xanthan gum, gellan gum, cellulose acetate phthalate, etc. have been found to possess enhanced survival during storage and upper GIT transit (Dianawati, Mishra and Shah 2016).

Probiotic food products have mainly been supplemented with strains belonging to *Lactobacillus* spp. and *Bifidobacterium* spp. Nonetheless, there is a broader spectrum of species where members have been described as probiotics or to exhibit probiotic properties. This include, among others, strains belonging to *Streptococcus* spp. (Iyer *et al.* 2010; Uriot *et al.* 2017), *Bacillus* spp. (Elshaghabee *et al.* 2017), *Propionibacterium freudenreichii* (Campaniello *et al.* 2015; Le Maréchal *et al.* 2015) and *Escherichia coli* (Secher *et al.* 2017).

Nowadays, due to the recognition of the role that the GM plays in the health of the human host, there is an increasing interest in using indigenous commensal bacteria, which are dominant members of the GM and perform special functions in the complex intestinal environment, as potential next-generation probiotics (El Hage, Hernandez-Sanabria and Van de Wiele 2017). The potential candidates to be considered as next-generation probiotics include extremely oxygen-sensitive bacteria like *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, among others. However, the sensitivity to oxygen, gastric pH and bile salts, together with the difficulties of large-scale propagation, are factors that challenge, from a technological approach, the development of dosage protocols of these novel probiotic candidates (Brodmann *et al.* 2017). Additionally, it has been shown that several commensal *Clostridium* spp. strains belonging to Clostridia clusters IV, XIVa and XVIII are strongly involved in the maintenance of overall gut function and possess potential probiotic properties (Lopetuso *et al.* 2013). The spore-forming capacity of *Clostridium* spp. might represent an advantage, in terms of survival, during industrial processes, storage and GIT passage.

During storage, the survival of probiotics can vary depending on the strain and the food matrix. An overview of physically stable matrices that can provide appropriate conditions in order to maintain their viability at relevant levels for an extended period of time is thus needed. This, particularly due to the emergence of next-generation probiotic candidates once they are approved for human consumption and their beneficial effects are proven, will require an optimal dosage protocol (El Hage, Hernandez-Sanabria and Van de Wiele 2017) that can involve novel microencapsulation techniques or even addition into a proper food matrix.

DRYING TECHNIQUES OF PROBIOTICS

Anhydrobiosis is the state in which an organism stops its vital functions temporarily due to partial or total desiccation (García 2011). The extreme reduction of measurable metabolism in dehydrated bacterial cells allows them to remain viable for a long period of time if stored under appropriate conditions (Perdana *et al.* 2013).

Dehydration of microbial cells can be achieved by the application of methodologies such as freeze-drying, spray-drying, vacuum-drying and fluidized bed-drying. The decision of whether to use one technique or another at industrial scale relies mainly on the cost effectiveness. However, the removal of intracellular water causes a mechanical stress on the cell membrane altering its plasticity (Perdana *et al.* 2013) and desiccation increases the contact of cell surfaces with oxygen molecules, inducing the intracellular accumulation of reactive oxygen species, which cause damages in cell macromolecules (Iaconelli *et al.* 2015). Therefore, careful optimization of the process is essential.

In concert with the general desiccation stress, cells must face several specific stresses, which may cause severe losses in viability, unless the cells are protected by the utilization of protectant compounds and development of efficient specific protocols (Table 1). Furthermore, the intrinsic resistance of strains to the generated stress is also critical when a desiccation method is selected.

The utilization of a specific desiccation technique involves several advantages and drawbacks. For instance, freeze-drying is the preferred long-term preservation method due to the satisfactory survival rates associated with its application; however, it is an expensive and time-consuming batch process (Prakash, Nimonkar and Shouche 2013). In contrast, spray-drying represents a lower energy cost and higher productivity technique, but the continuous exposure to oxygen and heat stress generated during the desiccation process challenge the microbial survival (Huang *et al.* 2017).

The combined application of the above-mentioned techniques can also improve the yield of the process in terms of cost effectiveness, e.g. spray freeze-drying involves spraying a probiotic suspension and immediately freeze-drying the resultant particles and consequently reducing the long drying time (Rajam and Anandharamakrishnan 2015).

MICROENCAPSULATION OF PROBIOTICS IN BIOPOLYMERIC MATRICES

Microencapsulation of probiotics involves the immobilization and coating of cells in covalently or ionically cross-linked polymer networks, or in some cases polymer granules, which are not cross-linked, such as those produced during spray-drying (Cook *et al.* 2012). This coating constitutes a physical barrier that may protect probiotics from oxidative reactions, low pH and bile salts,

Table 1. Main techniques applied for the dehydration of probiotics.

Drying technique	Description	Specific stresses	Mitigation strategies of stress
Freeze-drying	A frozen suspension of microbial cells is first reduced by sublimation and then by desorption to a value that will no longer support biological activity or chemical reaction (Reddy *et al.* 2009). Unless the cells have been previously microencapsulated, the final product is a dry cake that requires further steps to obtain individual particles (Broeckx *et al.* 2016).	The formations of ice crystals can induce mechanical damage leading to cellular death (Santivarangkna, Kulozik and Foerst 2008). Furthermore, osmotic stress is generated due to crystallization of water and consequent concentration of solutes (Coulibaly *et al.* 2010).	Freezing at a proper cooling rate (approximately 5°C min^{-1}) reaching a final temperature <−60°C in order to avoid intracellular crystal formation (Heylen *et al.* 2012; Dimitrellou, Kandylis and Kourkoutas 2016). Compounds like disaccharides, polyalcohols, amino acids and proteins can stabilize cell membranes and proteins during freezing and desiccation (Reddy *et al.* 2009; Siaterlis, Deepika and Charalampopoulos 2009) acting as cryo- and lyoprotectant agents.
Spray-drying	A bacterial suspension is sprayed in 10–150 μm droplets that are directed into a flow of hot and dry (150°C–250°C) air-drying of the sprayed droplets in a few seconds (Huang *et al.* 2017).	Besides cell wall and cell membrane damage, heat stress can also affect DNA and ribosomes, causing high mortality of cells (Desmond *et al.* 2001).	Adding protective agents, mainly disaccharides that stabilize cells when enclosed in a glassy matrix, preserves the structure of proteins and membranes by binding to the sites formerly interacting with water molecules (Perdana et al. 2014). Other protective agents as gelatin, gum Arabic, cellulose acetate phthalate, included in the spray-drying medium, can constitute a physical barrier against hot air exposure (Fávaro-Trindade and Grosso 2002; Hsiao, Lian and Chou 2004; Huang *et al.* 2017)
Fluidized bed-drying	A stream of air (35°C–50°C) blowing upward through the feed material constituted by harvested cells plus the support material (skim milk, starch, alginate, casein, maltodextrin, etc.). As the heated air travels through the particle bed, it provides rapid mixing and dehydration. Particle size of the final product ranges from 200 to 1600 μm (Stummer *et al.* 2012).	An osmotic shock that leads to viability loss may be generated since cells right after harvest are mixed with a low a_w support material (Mille *et al.* 2004).	Conditioning cells with compounds such as sucrose, trehalose, skim milk, etc. can prevent damage due to desiccation stress (Stummer *et al.* 2012; Bensch *et al.* 2014).
Vacuum-drying	As the boiling point of water is lowered under low pressure, the vacuum-drying technique allows the removal of the moisture from materials at a low temperature. The final product is a dry cake, which requires further processing to obtain individual particles (Santivarangkna, Kulozik and Foerst 2007).	An increase in the temperature of the material due to water removal can lead to a viability loss (Bauer *et al.* 2012).	Add protecting agents (sorbitol, sucrose, trehalose, etc.) in order to stabilize the cell membrane during desiccation (Santivarangkna *et al.* 2009, 2010).

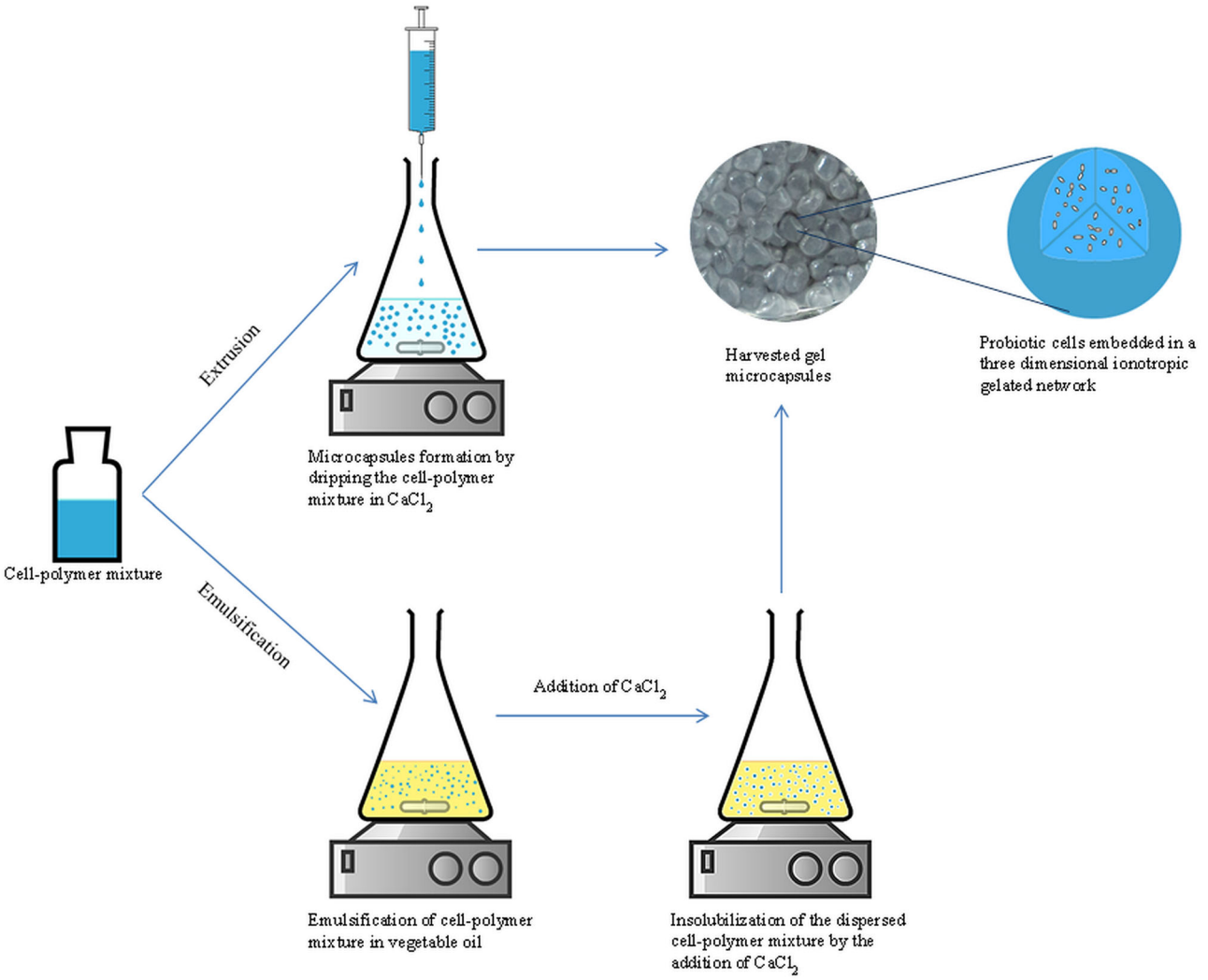

Figure 1. Formation of ionotropic cross-linked biopolymer microcapsules by extrusion or emulsification.

extending the shelf life, enabling controlled release and enhancing the survival throughout GIT transit (Martín *et al.* 2015).

Several food-grade biopolymer materials are available to encapsulate microbes in hydrogel matrices, depending on the desired physicochemical properties of the delivery vehicle (Yeung *et al.* 2016). Widely used choices include proteins, such as casein, and carbohydrates, such as starch, pectin, alginate and gums that are largely applied using different microencapsulation techniques (Shori 2017).

The production of cross-linked polymer microcapsules containing probiotics falls into two main mechanisms: extrusion and emulsion (Cook *et al.* 2012; Rathore *et al.* 2013). Figure 1 schematizes the principle behind their formation (laboratory scale illustrated in Fig. 1).

Microencapsulation by extrusion typically involves dripping, by extrusion through a syringe needle, a hydrocolloid solution with suspended probiotic cells into a hardening solution containing cations like Ca^{++} (in the form of $CaCl_2$), forming a three-dimensional network by cross-linking via calcium ions (de Vos *et al.* 2010; Burgain *et al.* 2011). Among the major advantages of this method are the gentle operational conditions, which ensure a high viability of cells (Mortazavian *et al.* 2007) and the uniform size of the microcapsules in a batch (Solanki *et al.* 2013).

Based on the same principle, microdrops can also be formed through spraying systems and hardened in an ionic solution (Cook *et al.* 2012). If the droplet formation occurs in a controlled manner (e.g. vibrating nozzles, spinning-disk atomization and using a coaxial flow or an electrostatic field), the technique is known as prilling (Martín *et al.* 2015). In contrast with needle extrusion, either spraying or prilling can be easily utilized by industry to scale-up microencapsulation operations (Kailasapathy 2009). The particles size obtained by needle extrusion can range from 750 to 5000 μm (depending on the diameter of the orifice of the needle), whereas microcapsules formed through spraying or prilling generally exhibit a diameter of $<$1000 μm (Silva *et al.* 2018). Additionally, the distance between the outlet and the hardening solution and the viscosity of the probiotic suspension also influence the size of particles (Heidebach, Först and Kulozik 2012).

Emulsification consists of dispersing the cell-hydrocolloid suspension in a bigger volume of an immiscible liquid, like vegetable oil for food applications, forming a water-in-oil emulsion where the water soluble polymer is insolubilized after the addition of calcium chloride, by means of cross-linking and thus makes gel particles in the oil phase (Mortazavian *et al.* 2007; Holkem *et al.* 2016). The size of microcapsules produced by

emulsification ranges from 25 to 2000 μm depending on the variation of agitation speed, mixer type, addition and type of emulsifying agents, and the water/oil ratio (Sarao and Arora 2017). The difficulty to obtain uniformly shaped microcapsules in the same batch is a drawback of the emulsification technique (Nazzaro *et al.* 2012).

Hardened microcapsules may be either directly added to a probiotic product or further processed in order to obtain stable dehydrated particles by applying the desiccation technologies described above.

Microcapsule particle size has a paramount role in protecting probiotic survival both during the storage of foods and GIT passage, since the diameter of microcapsules is positively associated with the protective effect toward cells (De Prisco and Mauriello 2016). On the other hand, increasing bead size is also correlated to grainy texture of foods and this could be a limiting factor regarding the sensory acceptance of the food product (De Prisco and Mauriello 2016), since during mastication and swallowing, the tongue and mouth senses only those aggregates greater than 20 μm (Fischer and Windhab 2011).

The efficiency of an encapsulation protocol depends on the strain and its compatibility with the selected polymer matrix as well as the desiccation process and the application of protectant agents to mitigate the stress (Solanki *et al.* 2013). Although microencapsulation has shown promising results, only a narrow spectrum of bacterial species has been tested and it is still far from enough to ensure the obtainment of the claimed protective and targeted release effects in humans or animals (Liu *et al.* 2017).

Below we summarize studies reporting a survival enhancement of probiotics, entrapped in desiccated biopolymer microcapsules, during storage and upper GIT passage.

Alginate

Alginate, widely used as an encapsulation material, is an anionic linear polysaccharide composed of (1-4)-linked β-D-mannuronic acid and α-L-guluronic acid residues arranged as blocks of either type of unit or as a random distribution of each type (Albadran *et al.* 2018).

Calcium and sodium alginate, due to their biocompatibility and low cost, are the most popular biopolymers used for microencapsulation purposes (Chan *et al.* 2011). For instance, Holkem *et al.* (2016) evaluated the viability of *Bifidobacterium animalis* subsp. *lactis* BB-12 embedded in freeze-dried sodium alginate microcapsules. They observed ≈1.7 and a 6.3 log CFU g^{-1} reduction after 120 days of storage at 7°C and 25°C, respectively. During *in vitro* simulated upper gastrointestinal transit, a 0.8 log CFU g^{-1} reduction was observed for the microencapsulated cells, compared with 5.5 log CFU g^{-1} reduction for naked cells (Holkem *et al.* 2016). The enhanced survival during upper GIT passage are in agreement with Ding and Shah (2009), who reported improved survival of several probiotic *Lactobacillus* and *Bifidobacterium* strains encapsulated in alginate during *in vitro* upper GIT transit.

However, other studies have reported poor survival of probiotics microencapsulated in alginate when exposed to low pH (Sultana *et al.* 2000; Gbassi *et al.* 2009). In this context, coating alginate-based microcapsules with a shell of polymers like chitosan and poly-L-Lysine (Cui *et al.* 2000; Chávarri *et al.* 2010; Yeung *et al.* 2016; Bernucci *et al.* 2017) or the addition of other materials like gelatin, pea protein or starch (Li *et al.* 2009; Varankovich *et al.* 2017; Yao *et al.* 2017) into the polymer mixture have been found to enhance protection to probiotic cells during storage and throughout *in vitro* upper GIT transit. Likewise, it has been reported that the dissociation of Ca^{++} from $CaCO_3$ powder (through the addition of organic acids), homogeneously dispersed in an alginate solution of water-in-oil emulsion, internally gelate the alginate micelles producing symmetrical and homogeneous spheres (Song *et al.* 2013). This approach may also lead to an enhanced survival of probiotics encapsulated in alginate beads during storage and GIT passage (Holkem *et al.* 2016).

Pectin

Pectin is a heteropolysaccharide, mainly extracted from fruits and resistant to low pH, which is composed of α-(1-4)-linked D-galacturonic acid and 1,2-linked L-rhamnose residues (Yasmin *et al.* 2018). Li *et al.* (2016) reported high stability during storage at room temperature of *Lactobacillus rhamnosus* GG, encapsulated in freeze-dried pectin beads, and a moderate 2 log CFU mL^{-1} reduction throughout *in vitro* gastric passage (pH 1.6), which was significantly lower than the reduction suffered by non-encapsulated cells.

Resistant starch

Resistant starch is the portion of starch that can resist digestion by human pancreatic amylase in the small intestine and thus reach the colon where it can be fermented (Fuentes-Zaragoza *et al.* 2011). Encapsulation in resistant starch has the often-desired property that it leads to release of the bacterial cells in the large intestine (Sarao and Arora 2017). When resistant starch is used in conjunction with alginate, it can promote a synergistic effect on gelation, providing further protection to probiotic cells as reported by Etchepare *et al.* (2016), who observed a survival enhancement of *Lactobacillus acidophilus* La-14 entrapped in alginate plus resistant starch compared with bacteria encapsulated in alginate only, during 30 days of storage at 7°C (Etchepare *et al.* 2016).

Xanthan and gellan gum

Also, bacterial exopolysaccharides have shown promising potential as encapsulation matrices for protecting encapsulated probiotics from the harsh acid and bile conditions of the upper GIT (Cook *et al.* 2012; Corona-Hernandez *et al.* 2013).

Xanthan gum, produced by *Xanthomonas campestris*, is an extracellular heteropolysaccharide composed of a linear (1-4) linked β-D-glucose backbone with a trisaccharide side chain on every other glucose at C-3 containing two units of mannose and a terminal glucuronic acid residue (Habibi and Khosravi-Darani 2017). Xanthan gum is known to possess high stability at a wide range of pH and temperatures (Leela and Sharma 2000).

Likewise, *Sphingomonas elodea* produces gellan gum, a linear exopolysaccharide composed of repetitive units of two D-glucose molecules, one L-rhamnose and one D-glucuronic acid (Zia *et al.* 2018). Gellan gum has the ability to bear heat and gels composed of this polymer are highly stable at low pH (Zia *et al.* 2018).

A ratio of 1:0.75 in a mixture of xanthan and gellan gum has been described as giving optimal gelling properties when using an extrusion encapsulation technique at room temperature and consequently protecting probiotics efficiently against low pH (Sun and Griffiths 2000). The use of this polymer mix in the form of freeze-dried microcapsules preserved the viability of *Lactobacillus plantarum* and *L. rhamnosus* during long exposure (6 h) to pH 2 while free lactobacilli suffered a total loss of

viability under the same conditions (Jiménez-Pranteda *et al.* 2012). Moreover, the survival of encapsulated *L. rhamnosus* throughout *in vitro* simulated upper GIT was significantly higher than that of naked cells (Jiménez-Pranteda *et al.* 2012).

We have recently applied xanthan/gellan gum (1:0.75) to microencapsulate and subsequently freeze-dry *A. muciniphila*, a next-generation probiotic candidate, observing a viability loss corresponding to approximately 0.6 and 4.06 log CFU g^{-1} during 30 days of storage at 4°C or 25°C, respectively (Marcial-Coba *et al.* 2018). In the same study, microencapsulated *A. muciniphila* was exposed to *in vitro* upper GIT conditions at fasted (gastric phase pH 2) and fed (gastric phase pH 4) state, suffering a total reduction of 2.9 and 1.3 log CFU mL^{-1}, respectively, reflecting a 1.03 and 1.6 log CFU mL^{-1} better survival than that of free cells under the same conditions (Marcial-Coba *et al.* 2018).

Milk proteins

Milk proteins including caseins, whey proteins and milk fat globule membrane proteins, offer excellent properties such as high solubility and low viscosity in solution, allowing a homogenous dispersion of probiotic cells in the matrix (Heidebach, Först and Kulozik 2009). Furthermore, milk proteins can form capsules, under mild conditions, through different mechanisms including extrusion, emulsification, spray-drying and enzyme-induced gelation (Abd El-Salam and El-Shibiny 2015).

In one study, the viability loss of probiotics contained in freeze-dried sodium caseinate microcapsules was limited to approximately 1 and 2 log CFU g^{-1} for *Lactobacillus paracasei* subsp. *paracasei* F19 and *Bifidobacterium lactis* Bb12, respectively, during 3 months of storage at 4°C (Heidebach, Först and Kulozik 2010). Similarly, Zou *et al.* (2012) observed that *Bifidobacterium bifidum* F35 embedded in freeze-dried whey protein microcapsules maintained the initial concentration of live cells when stored at 4°C during one month of storage, but showed a loss close to 1 log CFU g^{-1} at 25°C. Additionally, the survival of encapsulated cells was reduced by only 1.2 log CFU mL^{-1} when exposed, for 2 hours, to simulated gastric juice (pH 2) without pepsin, which contrasted a 4.6 log CFU mL^{-1} reduction when pepsin was added to the simulated gastric fluid (Zou *et al.* 2012). The digestion of milk proteins by pepsin may constitute a drawback of using it as encapsulation material (Hébrard *et al.* 2010). This can be improved by coating the microspheres with polymers, e.g. carrageenan and locust bean gum (Shi *et al.* 2013) or by combining milk proteins with polysaccharides, e.g. gellan gum (Nag, Han and Singh 2011).

READY-TO-EAT LOW-MOISTURE FOOD MATRICES

Once the dehydrated particles are prepared, either as powders or potentially microcapsules, it is necessary to package and store them until its addition into a food product, serving as a probiotic carrier, which once again requires to be packaged and stored up to the time of consumption.

The survival of probiotics is not only challenged during drying processes but also during storage. Along with the protectant used during desiccation, the residual moisture content, atmospheric oxygen level, exposure to light, relative humidity and storage temperature, among others, have significant influence on the viability of probiotics, as briefly explained in Fig. 2 and previously reviewed (Zayed and Roos 2004; Chávez and Ledeboer 2007; Fu and Chen 2011; Santivarangkna *et al.* 2011; Vesterlund, Salminen and Salminen 2012; Tripathi and Giri 2014). Moreover, the diameter of particles, apart from having a crucial effect on probiotic viability and sensory properties of the final product, affects the distribution of microcapsules and their stability over time in the food product (Huq *et al.* 2013).

The physicochemical parameters related to the stability of probiotics during storage can vary as a function of the food matrix serving as probiotic vehicle (da Cruz, Faria and Van Dender 2007). Below we describe the specific properties of some low-moisture food products that have been used as probiotic carriers.

Peanut butter

Peanuts are consumed all over the world in different forms, e.g. raw and roasted peanuts and as peanut butter. The major components of peanuts are lipids 40%–50% (mainly monounsaturated fatty acids), proteins 27%–29%, carbohydrates 16% and dietary fiber 8.5% (Arya, Salve and Chauhan 2016). Peanuts have been considered as a functional food (Francisco and Resurreccion 2008), due to its high content of bioactive compounds such as vitamin E, folate, coenzyme Q10, minerals, resveratrol, phenolic compounds and flavonoids (Isanga and Zhang 2007).

Peanut butter is formed by grinding roasted peanuts into a paste and stabilized by the addition of vegetable oil, which prevents the separation of the peanut oil and solid fractions (Ma *et al.* 2013). The a_w of peanut butter is close to 0.35 and the pH is approximately 6.3 (He *et al.* 2013). Therefore, peanut butter is a shelf stable low-moisture food matrix that offers promising properties as probiotic carrier.

In this regard, Klu *et al.* (2012) reported that *L. rhamnosus* GG embedded in peanut butter, with an initial concentration of approximately 7 log CFU g^{-1}, showed a viability loss <1 log CFU g^{-1} in samples stored at 4°C for at least 48 weeks and at 25°C for 27 weeks. Likewise, a mixture of 16 *Lactobacillus, Bifidobacterium and Streptococcus* probiotic strains incorporated into peanut butter, in a concentration of 7 log CFU g^{-1}, suffered an approximately 1 and 3 log CFU g^{-1} reduction during 12 months at 4°C and 25°C, respectively (Klu, Phillips and Chen 2014). Finally, an approximately 1.5 log CFU mL^{-1} reduction was observed when cells encased in peanut butter were exposed to *in vitro* upper GIT conditions, showing a higher survival than that of free cells, which suffered a 3.5 log CFU g^{-1} reduction when exposed to the same conditions (Klu and Chen 2015). These findings suggest a high stability of probiotic strains during long-term storage and *in vitro* GIT passage when embedded in peanut butter. However, the concentration of embedded bacterial cells should be increased in order to obtain efficient daily doses (10^9–10^{10} CFU) by consuming this probiotic formulation.

Cereal bars

Cereal bars are consumed worldwide and can be considered as nutritious fast snacks, since cereals are conceived as sources of non-digestible fiber and minerals and a remarkable amount of proteins and carbohydrates can be provided by other ingredients (Siró *et al.* 2008). This snack food is mainly composed of oat, wheat and/or barley plus other ingredients like dried fruits and nuts, which are agglutinated by different syrups (Bchir *et al.* 2018). Depending on the ingredients and the drying process, the a_w of the product can vary between 0.25 and 0.56, and the moisture content can range from 7.5% to 9.5% (Estévez *et al.* 1995; Ouwehand, Kurvinen and Rissanen 2004).

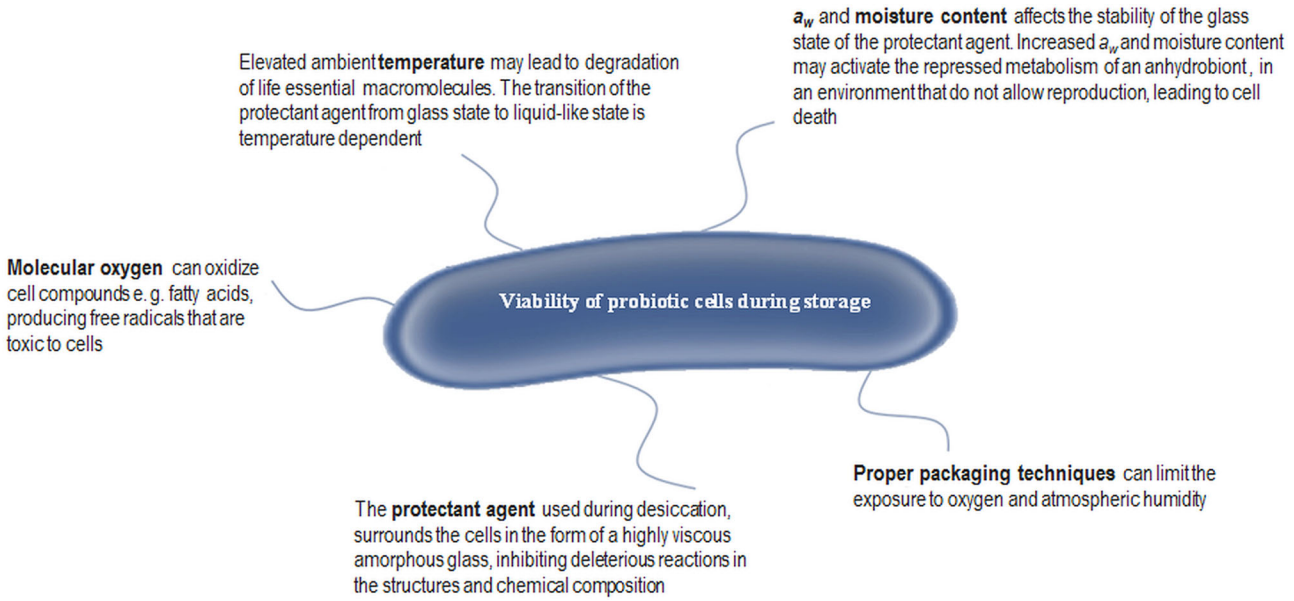

Figure 2. Factors affecting the viability of dried probiotic bacteria during storage.

In one study, oat-based cereal bars ($a_w = 0.25$) were described as efficient carriers of *B. animalis* subsp. *lactis* Bb-12 for administering probiotics to human subjects (Ouwehand, Kurvinen and Rissanen 2004). Moreover, Bampi *et al.* (2016) added *L. acidophilus* or *B. animalis* subsp. *lactis*, contained in solid lipid microcapsules (particle size: 61 and 86 μm for *L. acidophilus* and *B. animalis* subsp. *lactis*, respectively), into savory cereal bars ($a_w \approx 0.6$) and observed that after 90 days of storage at 4°C *L. acidophilus* was reduced by 2.3 log CFU g^{-1} and *B. animalis* subsp. *lactis* suffered a 0.9 log CFU g^{-1} reduction from an initial concentration of 10.5 and 10.3 log CFU g^{-1}, respectively. Although, the a_w of this product is out of the optimal range for maintaining the viability of freeze-dried probiotics (Vesterlund, Salminen and Salminen 2012), it seems that the lipid-based matrix of the microcapsules and the temperature of storage are associated with a relatively high survival of probiotic microorganisms in this study.

Dried-fruit snacks

Fruit derived products are an emerging area within functional foods (Betoret *et al.* 2012). In this connection, dried fruits constitute a healthy snack, since they possess an acceptable taste and provide concentrated compounds such as vitamins, minerals and phytochemicals (Morais *et al.* 2018). Furthermore, fruits in a dried form become more energy dense than fresh fruits and are highly stable during a long shelf life at room temperature (Sun-Waterhouse 2011).

Fruits exhibit a highly porous structure due to the occurrence of intercellular spaces naturally filled with gases and liquid (Puente, Betoret and Cortés 2009). Vacuum impregnation has been reported as an industrial technique that removes the material filling the intercellular spaces by means of vacuum and replaces it by diffusion with bioactive ingredients such as probiotic microorganisms suspended in an impregnation solution (Gras *et al.* 2002). Depending on the desiccation mechanism applied (air-drying or freeze-drying), a final product with a a_w ranging 0.3–0.35 can be obtained (Betoret *et al.* 2003).

Several studies show promising results in terms of survival of probiotics, impregnated in dehydrated apple slices or cylinders, during storage. In one study, the viability loss during 15 days of storage at 20°C was <1 log CFU g^{-1} for *Lactobacillus casei* impregnated in cylindrically air-dried apple portions and using milk or apple juice as impregnation solution (Betoret *et al.* 2003). Likewise, Noorbakhsh, Yaghmaee and Durance (2013) found that *L. rhamnosus*, impregnated in air-dried plus radiant energy vacuum-dried apple slices, suffered a 1 log CFU g^{-1} reduction after 23 days of storage, while the same viability loss occurred after 14 and 12 days for freeze- and air-dried samples, respectively, reflecting that the drying method affected the survival performance of this bacterial strain.

A dried-fruit bar is a snack-like product consisting of a paste obtained from dried-fruit pulp and optionally mixed with other ingredients such as sugar, vegetable oil, pectin, among others (Sharma *et al.* 2013). Most of fruit bars fall into the category of intermediate-moisture fruits, having a_w around 0.6 and a moisture content between 8% and 15% (Orrego, Salgado and Botero 2014), exceeding the optimal a_w range (0.07-0.2) to maintain the viability of dehydrated probiotics during long-term storage at room temperature (Vesterlund, Salminen and Salminen 2012). However, the proposed probiotic *Bacillus coagulans* BC4, in the form of spores, embedded in a dried-date paste ($a_w \approx 0.48$–0.59) showed only neglectable variation in its viability during 45 days of storage either at 4°C or 25°C (Marcial-Coba *et al.* 2019), suggesting that the physical properties of this matrix did not lead to spore germination and consequently the viability remained stable during storage.

Based on these results, dried fruit-based matrices may constitute attractive novel carriers for the administration of probiotics. However, the cited studies assessed the microbial viability during relatively short periods of storage (15–45 days). Therefore, further studies should be performed in order to determine the applicability of this type of matrix for the formulation of probiotic food with longer shelf life.

Chocolate

Chocolate in their main categories, dark, milk and white, is consumed all over the world in all segments of society and by

people of all ages (Konar *et al.* 2016). In essence, chocolate is a dense semisolid suspension of fine particles of cocoa mass, sugar and milk (depending on type) in a fat continuous phase, mostly of cocoa butter (Afoakwa *et al.* 2008). The high fat content in chocolate is associated with a low a_w (≈0.3), oxygen tension and moisture permeability, which consequently confer high stability to the product matrix during its shelf life (Kemsawasd, Chaikham and Rattanasena 2016; Gutiérrez 2017). Besides that, it has been observed that the lipid fraction of cocoa butter provides protection to probiotics during storage and during upper gastro-intestinal tract passage (Lahtinen *et al.* 2007).

Several studies have generated promising outcomes regarding the stability of probiotics embedded in a chocolate matrix during storage. Nebesny *et al.* (2007), supplemented dark chocolate with freeze-dried *L. casei* and *L. paracasei* (approximately 8 log CFU g^{-1}) and observed that ≥80% of cells survived during 12 months of storage either at 4°C or 18°C. Similarly, *L. acidophilus* NCFM and *B. lactis* HN019 embedded in dark or milk chocolate (initial concentration 9 log CFU g^{-1}) showed to be stable during 14 months of storage at 15°C, after a 1.1–1.6 log CFU g^{-1} reduction in the initial period after production (Klindt-Toldam *et al.* 2016). Lalicic-Petronijevic *et al.* (2015) also evaluated the survival of the same strains in dark and milk chocolate and confirmed a high stability of *L. acidophilus* NCFM in both matrices during 6 months of storage at 4°C, while under the same conditions, the viability of *B. lactis* HN019 was reduced with >2 log CFU g^{-1} in both dark or as well as milk chocolate after 5 months of storage.

Klindt-Toldam *et al.* (2016) also observed that the above-mentioned strains encased in milk or dark chocolate exhibited an approximately 9 and 5 log CFU g^{-1} higher survival than that of probiotic cells in yoghurt and juice, respectively, when exposed to simulated gastric fluid (pH 1.4–2.9) for 65 min. Similarly, *L. casei* encased in dark, milk or white chocolate showed a 1 log CFU mL^{-1} better survival than that of free cells when exposed to simulated gastric fluid (pH 1.4) for 90 min (Kemsawasd, Chaikham and Rattanasena 2016).Chocolate in other words not only provides protection during storage, but also during upper GIT passage.

Sensorially dark, milk and white chocolate supplemented with 8–10 log CFU g^{-1} dried probiotic bacteria are generally found acceptable and generally indistinguishable from chocolate without probiotics (Nebesny *et al.* 2007; Lalicic-Petronijevic *et al.* 2015; Kemsawasd, Chaikham and Rattanasena 2016; Klindt-Toldam *et al.* 2016). Chocolate thus offers a good alternative for administering effective doses of probiotics.

CONCLUSION

Low-moisture food products represent an attractive alternative for long shelf life at ambient temperature and demand low costs and simplified logistics for transportation and manipulation. Due to the stability, dehydrated foods as vehicles of probiotics can be capable of enhancing the microbial viability at relevant levels until the time of consumption and even during upper GIT passage. However, the survival of probiotics is challenged by several stresses during manufacturing and storage. Hence, the efficiency of a low-moisture food matrix, as a probiotic vehicle, will not only depend on its physicochemical properties, but also on the intrinsic resistance of strains to environmental stresses, the selection of a proper desiccation method, the application of protectant agents in order to mitigate the stress during dehydration and storage, and the storage conditions. Microencapsulation of probiotics in biopolymers can constitute a promising strategy to provide stability during storage and enhance the viability throughout the upper GIT transit. Some low- or intermediate-moisture food matrices, e.g. chocolate or peanut butter, have shown promising results regarding their applicability as probiotic vehicles. In this connection, the use of this type of probiotic carriers can represent an alternative for administering effective dosses of probiotic microorganisms that can be contained in small portions of the food product. This mini-review may contribute to the design of manufacturing strategies aimed to maintain the microbial viability, at relevant levels, in probiotic food products with low or intermediate content of moisture during processing, storage and upper GIT passage.

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