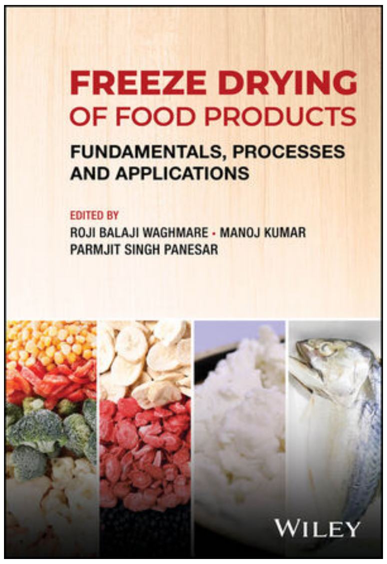
WILEY

To purchase this product, please visit https://www.wiley.com/en-br/9781119982081



Freeze Drying of Food Products: Fundamentals, Processes and Applications

Roji Balaji Waghmare (Editor), Manoj Kumar (Editor), Parmjit Singh Panesar (Editor)

E-Book	978-1-119-98208-1	January 2023		**$144.00**
Hardcover	978-1-119-98206-7	January 2024	*Print-on-demand*	**$180.00**
O-Book	978-1-119-98209-8	January 2024		**Available on Wiley Online Library**

DESCRIPTION

An accessible guide to safely dehydrating food

Freeze drying, or lyophilization, is a method for dehydrating food or other substances through the use of pressure instead of heat. This allows for the preservation and storage of high-value food products without altering their essential properties or causing a reduction in quality or value. For these reasons, freeze drying is the most reliable method for preserving and distributing high-quality products.

Freeze Drying of Food Products provides a concise, accessible overview of freeze-drying techniques and their modern applications. Beginning with the basic principles and processes of freeze drying, it incorporates specific discussion of freeze-drying different categories of food products, before moving to an analysis of recent developments in freeze-drying technology. The result is a key publication in the fight to extend the shelf-life of food products and expand the distribution of high-quality freeze-dried foods.

Freeze Drying of Food Products readers will also find:

- An editorial team with a wide range of pertinent research experience
- Detailed discussion of different freeze-drying processes such as vacuum drying, atmospheric drying, and spray drying
- Commercial Applications of freeze-dried food products

Freeze Drying of Food Products is ideal for researchers and industry professionals involved in food production, food distribution, or food biotechnology, as well as students studying these and other related fields.

✍ ABOUT THE AUTHOR

Roji Balaji Waghmare is an Assistant Professor in the Department of Food Engineering and Technology, Institute of Chemical Technology, Mumbai, India. She has worked extensively on different drying techniques of food products, including freeze-drying.

Manoj Kumar is a Scientist in the Chemical and Biochemical Processing Division at the Central Institute for Research on Cotton Technology, Mumbai, India. His research focuses on the extraction and functional characterization of bioactive compounds from plant-based matrices.

Parmjit Singh Panesar is a Professor in the Food Biotechnology Research Laboratory, Department of Food Engineering and Technology, as well as Dean of Planning and Development, Sant Longowal Institute of Engineering and Technology, Longowal, India. His research focuses on agro-industry bioproducts as incorporated into the production of high-value food products.

To purchase this product, please visit https://www.wiley.com/en-br/9781119982081

6

Freeze-Drying of Probiotics for the Incorporation in Functional Foods: Drying Process, Viability, and Powder Properties

*Yasemin Ş. Küçükata, Hatice E. Kırtıl, and Ertan Ermiş**

Department of Food Engineering, Faculty of Engineering and Natural Sciences, Istanbul Sabahattin Zaim University, Istanbul, Turkey

6.1 Introduction

Probiotics are defined as live microorganisms administered in adequate amounts as part of foods or supplements to benefit host health (Arellano et al. 2021). Since probiotics improve the intestinal microbial balance, foods containing probiotics are classified as functional foods. The beneficial impact of probiotics on human health includes alleviating inflammatory bowel disease, immune disorders, type 2 diabetes, and atherosclerosis (Arellano et al. 2021). Even though the daily dosage intake or the minimal viable cell number of probiotics is not well defined, it is suggested around $10^8\,g^{-1}$, or ml^{-1}, to exert health benefits in the gastrointestinal system (Her et al. 2015).

Fermented foods (i.e. fermented dairy products) are natural sources of probiotics. On the other hand, freeze-dried and spray-dried probiotic powders in capsules or sachets are available in the market, which is expanding rapidly. Probiotics in the package should maintain viability during transportation and storage. Different drying techniques have been used to obtain probiotic powders. The freeze-drying (FD) method has been preferred over others, such as spray-drying (SD), for higher viability and extended shelf life (Meireles Mafaldo et al. 2022). However, FD takes a long time and is more costly than spray drying. The drying conditions need to be optimized, and appropriate protective/cryoprotectant materials should be used to maintain the cell viability as high as possible during harsh conditions in drying processing steps such as pumping (shear and oxygen stresses),

---

* Corresponding Author: ertan.ermis@gmail.com

Freeze Drying of Food Products: Fundamentals, Processes and Applications, First Edition.
Edited by Roji Balaji Waghmare, Manoj Kumar, and Parmjit Singh Panesar.

drying (shrinkage and ice crystals), growth to high cell densities, and grinding (shear and heat stress) after drying (Béal and Fonseca 2015; Her et al. 2015).

Microencapsulation might be employed to protect the sensitive probiotic strains using FD and wall materials such as proteins (i.e. whey protein), alginates, polysaccharides, resistant starch, and milk, during processing and gastrointestinal stress conditions (Arellano et al. 2021). Maillard reaction products can improve the solubility, emulsifying properties, and thermal stability of proteins; hence protein and polysaccharide conjugates are functional wall materials for encapsulating probiotics (Li et al. 2023). The rehydration of dried probiotics is a vital step in producing functional foods. The rehydration conditions significantly influence the viability of the probiotics in the food matrix.

This chapter aims to provide a general overview of the FD (lyophilizing) probiotics process, focusing on the significance of protective material, process optimization, characterization, and factors affecting the powder properties and viability of freeze-dried probiotics. It also reviews recent works incorporating freeze-dried probiotic powders into functional food products.

6.2 Functional Foods

Since ancient times, food has been not only for energy and alimentation but also sort of medicine and health-promoting source of life. Greek physician Hippocrates II, the great phenomenon in the history of medicine, once said, “Let food be thy medicine and let medicine be thy food” (King 2020). Food’s role in life and its contribution to health through its numerous bioactive properties are gaining the utmost importance (Chugh and Kamal-Eldin 2020). The more the health consciousness develops in society, the more expectation of food efficacy increment. Therefore, there is an increased demand for functional foods (Dinkçi et al. 2019). The term functional food can be simply explained as food for supplemental health use (Lin 2003). Natural foods, foods with additional components, and foods with omitted components can all be functional foods through biotechnological procedures (Figure 6.1) (Ashaolu 2020).

Consuming bioactive and nutritious components, such as metabolites, fatty acids, probiotics, prebiotics, vitamins, polyphenols, and flavonoids, through functional foods is more effective than taking medicine or supplements (Peng et al. 2020). Increasing demand for healthy and functional foods has opened new paths for developing bioactive components like probiotics and nutraceuticals (Reque and Brandelli 2021). Among all the bioactive compounds in functional foods, food products supplemented with probiotic bacteria are of particular interest (Siciliano et al. 2021). Probiotics are best known for restoring gut microbes and controlling gastrointestinal system activities (Lin 2003). A list of probiotic microorganisms can be seen in Table 6.1.

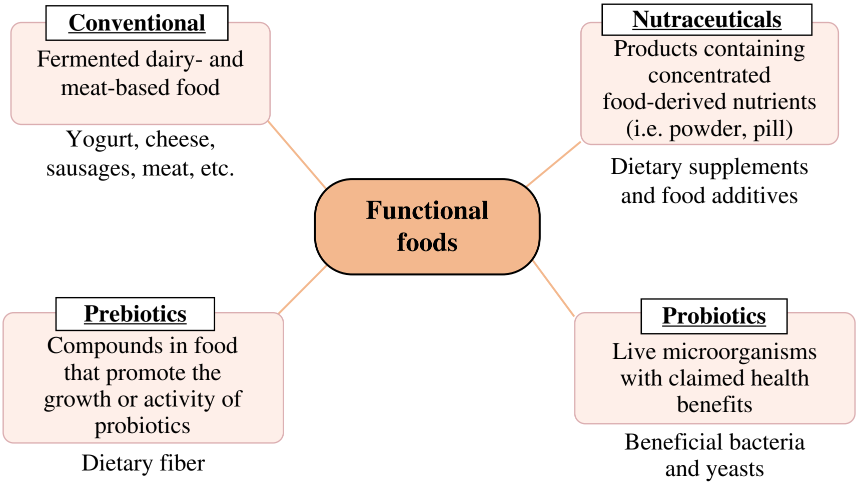


Figure 6.1 Categories of functional foods. *Source:* Adapted from Damián et al. (2022).

Table 6.1 Probiotic microorganisms (Song et al. 2012).

***Lactobacillus* species**	***Bifidobacterium* species**	**Others**
L. acidophilus	*B. adolescentis*	*Bacillus cereus*
L. amylovorus	*B. animalis*	*Clostridium butyricum*
L. brevis	*B. breve*	*Enterococcus faecalis*
L. casei	*B. bifidum*	*Enterococcus faecium*
L. rhamnosus	*B. infantis*	*Escherichia coli*
L. crispatus	*B. lactis*	*Lactococcus lactis* subsp. *cremoris*
L. delbrueckii subsp. *bulgaricus*	*B. longum*	*Lactococcus lactis* subsp. *lactis*
L. fermentum		*Leuconostoc mesenteroides* subsp. *dextranicum*
L. gasseri		*Pediococcus acidilactici*
L. helveticus		*Propionibacterium freudenreichii*
L. johnsonii		*Saccharomyces boulardii*
Lactobacillus lactis		*Streptococcus salivarius* subsp. *thermophilus*
L. paracasei		*Sporolactobacillus inulinus*
L. plantarum		
L. reuteri		
Lactobacillus salivarius		
L. gallinarum		

FAO/WHO's definition of probiotics is "live microorganisms which, when administrated in adequate amounts, confer a health benefit on the host" (FAO/WHO 2001). Foods containing a certain number of live probiotics are considered functional foods that potentially positively affect host health. The commercial interest in probiotic food products and supplements has been growing in recent years. There are numerous probiotics on the market in the form of milk, yogurt, or special freeze-dried pharmaceutical dietary preparations that are also available in tablet form (Zubillaga et al. 2001). The functional foods supplemented with probiotic bacteria directly and indirectly, impact the ecology and health of the gut through the bacteria that live there (Peng et al. 2020). Besides their increasingly growing reputation, probiotic cultures are successfully used in various food matrices to make a variety of functional foods (Table 6.2).

One of the biggest reasons for the expansion of probiotics in the functional food market is people's need for healthy alternatives to their gastrointestinal problems other than medicine and supplements (Reque and Brandelli 2021). In addition to all its favorable properties, it should be kept in mind that there are various parameters for probiotics. The stability and viability of probiotics are two crucial factors; food matrices to deliver probiotics, bacterial ability to adhere intestine, and bacterial strength to gastrointestinal conditions are also essential factors for appropriate and effective probiotic use in functional foods (Siciliano et al. 2021). Besides all, it should be emphasized that

Table 6.2 Some examples of probiotic bacteria used in food products.

Probiotic bacteria	Food products	Reference
Lactobacillus plantarum 299v *Pediococcus acidilactici* HA-6111-2	Orange powder	Barbosa et al. (2015)
Lacticaseibacillus rhamnosus GG	Apple juice, yogurt	Romero-Chapol et al. (2022)
Lactobacillus casei 01	Carrot juice	Petreska-Ivanovska et al. (2014)
Bifidobacterium longum 15708	Cheddar cheese	Amine et al. (2014)
Lactobacillus delbrueckii N102 *Latilactobacillus sakei* H1-5	Dry fermented sausages	Liu et al. (2021)
L. casei ATCC393	Fermented milk	Terpou et al. (2018)
Lactobacillus acidophilus DOWARU	Ice cream	Ferraz et al. (2012)
Bifidobacterium animalis ssp. *lactis* BB12	Kefir	González-Sánchez et al. (2010)
Lactobacillus reuteri E81	Sourdough	Ispirli et al. (2020)

functional foods fortified with probiotics should be available and affordable in the food market (Ashaolu 2020).

Overall, functional food products aim to regulate cellular health and enhance the implementation of cells' endogenous defense mechanisms (Zubillaga et al. 2001). Today, functional foods containing probiotics constitute a developing industry with significant economic interest. The ongoing interest in well-being and healthy lifestyles has led to an expansion of probiotic products in the functional food sector (Siciliano et al. 2021). To meet consumer demands, developing natural, health-supporting, and accessible probiotics and other functional foods as alternative supplements to medicines and drugs should be regarded seriously.

6.3 Cultivation and FD of Probiotics

The trend toward functional foods using probiotic bacteria is increasing as consumers realize the positive effects on health (Fonseca et al. 2020; Haindl et al. 2020; Yao et al. 2020). Lactic acid bacteria (LAB) and related genera (i.e. *Bifidobacterium* species) and some yeasts (i.e. *Saccharomyces cerevisiae*) are generally added to foods as probiotics (Chen et al. 2019; Romero-Chapol et al. 2022; Siaterlis et al. 2009; Vorländer et al. 2020). Besides the advantages of probiotic cultures in these products, some disadvantages limit their use. For this reason, adequate doses of probiotics may not be taken into the body while consuming foods containing these bacteria. During the production and storage period, the cultures may be affected by environmental conditions in the food environment and could not maintain their viability for a long time (Aschenbrenner et al. 2015; Kiepś and Dembczyński 2022). Various applications have been made in different foods to maintain the survival of probiotics from products to reach consumers. These applications include cultivation, drying, and storage period (Haindl et al. 2020; Yao et al. 2020). Cultivation of the probiotics is the first step of the whole process and may ensure to survival of the cells for drying. Specific broths, media, and optimum growth conditions (pH, temperature, etc.) for each probiotic species are used in fermentation (Haindl et al. 2020). Siaterlis et al. (2009) incubated *Lactobacillus rhamnosus* GG (ATCC 53103) and *L. plantarum* (NCIMB 8826) in modified MRS broth (using different ratios of glucose, yeast extract, and peptone) at 37 °C for 15 hours. Then the broth was centrifuged at 3200g and resuspended in PBS. Chen et al. (2019) prepared *Bifidobacterium bifidum* BB01 in MRS medium at 37 °C for 18 hours and harvested by a high-speed centrifuge at 8000 rpm for 15 minutes. In another study, kefir grains were added to UHT (ultra high temperature) milk samples, and this mixture was incubated at 26 °C for 24 hours. Then, kefir grains were washed to separate from the milk residues (Conde-Islas et al. 2019). Haindl et al. (2020) have inoculated *B. longum* ssp. *longum* Reuter

1963 to MRSc medium (MRS medium with 1 g/L cysteine) at 37 °C combined with 0.1 L/min N_2 with a mixing speed of 80 rpm (pH 6.0). Vorländer et al. (2020) activated baker's yeast *S. cerevisiae* onto yeast extract peptone dextrose (YPD) agar plates. Then the cells were suspended in PBS (isotonic phosphate-buffered saline solution, pH 7.4) for the process. *Lactobacillus plantarum* was grown in MRS broth overnight at 37 °C and 140 rpm using a shaker (Oluwatosin et al. 2021). Romero-Chapol et al. (2022) incubated *Lacticaseibacillus rhamnosus* GG cells in MRS broth at 37 °C for 24 hours at 200 rpm.

One of the most used methods recently is FD (lyophilization) as a new concept combined with the microencapsulation technique. FD dramatically increases the survival rates of probiotics in food products and in the gastrointestinal tract (GIT; Fonseca et al. 2020). Although FD is an old method used for the preparation of probiotic preparations, this method has been frequently used in microencapsulation applications in recent years (Aschenbrenner et al. 2015; Kiepś and Dembczyński 2022). FD is a very effective method for preserving sensitive bioactive materials such as probiotic microorganisms by minimizing the damage caused by temperature in living cells due water removal by sublimation (Broeckx et al. 2016). This method is based on freezing microorganisms at a shallow temperature and then drying them under a high vacuum by sublimation far below 0 °C (usually between −50 and −20 °C) (Aschenbrenner et al. 2015; Fonseca et al. 2020).

The FD process consists of three stages. These stages are sublimation-based freezing (crystallization water) and primary (sublimation ice) and secondary drying (desorption water) processes (Aschenbrenner et al. 2015; Broeckx et al. 2016; Rajam and Subramanian 2022). FD's first stage is freezing (to $T \sim -50\,°C$), which starts the formation of ice crystals in the liquid solution and causes ice crystals to separate water molecules from the solution. By decreasing the chamber pressure and gradually raising the shelf temperature, which starts the sublimation of ice, the ice crystals are removed from the frozen product, and unfrozen water (15–20%) remains in the product during primary drying (to $T \sim -35/-20\,°C$). This unfrozen water is desorbed in secondary drying (H bonds breakage) by adjusting the chamber's pressure and temperature. The product's targeted moisture content (2–10%) is finally achieved, thanks to the higher temperature under vacuum. The working principle of the method is based on the code that the material to be dried is first frozen and then dried by sublimation under a high vacuum. Due to not applying high temperatures during the process, the viability of microorganisms can be preserved at a higher rate than spray drying (Aschenbrenner et al. 2015; Broeckx et al. 2016; Rajam and Subramanian 2022). As the crystals formed due to low temperature and high osmotic pressure during the FD process affect cell viability, cryoprotectants/protective solutions are added to the material before this process (Misra et al. 2022). Different researchers have demonstrated that the results of viability are improved when this temperature is lower. Siaterlis et al. (2009) froze *L. rhamnosus* GG (ATCC 53103) and *L. plantarum* (NCIMB 8826) at −80 °C for 24 hours. After 45 hours of lyophilization, the survival rates of *L. rhamnosus* GG and *L. plantarum* ranged between 40–85% and 60–90%, respectively. *Bifidobacterium bifidum* BB01 cells were lyophilized at −50 °C, 7.23 Pa for 24 hours with a vacuum freeze-dryer, and the viability was detected to 88–90% (Chen et al. 2019). FD of the kefir grains was conducted at −20, −40, −60, or −80 °C; and pressure in 0.2, 0.4, 0.6, or 0.8 mbar (Conde-Islas et al. 2019). In another study, the conditions were at 3700 Pa, 24 hours for *B. longum* ssp. *longum* Reuter 1963 after prefreezing at −80 °C overnight (Haindl et al. 2020). Lyophilization for *S. cerevisiae* was carried out at three different freezing temperatures (−20 and −35 °C in the freezer and −196 °C in liquid nitrogen), stored at −20, −35, or −80 °C for 24 hours and primary drying took 24 hours at 0.220 mbar. For secondary drying, the pressure was 0.002 mbar (Vorländer et al. 2020). *Lactobacillus plantarum* suspensions were frozen at −80 °C for 24 hours, and then they were moved to a freeze-dryer that was ran for the same amount of time at −35 °C and 300 mTorr (Oluwatosin et al. 2022).

In addition to the advantages of the FD method, such as reducing aroma losses, excellent reconstitution properties of the products obtained, and minimizing the losses caused by the movement of solutes in the food, there are also disadvantages, such as high cost and long processing time. While this method is more moderate than spray drying, its use in microencapsulation applications is limited due to economic reasons (Aschenbrenner et al. 2015; Fonseca et al. 2020; Kiepś and Dembczyński 2022).

6.4 Protection of Probiotics During FD

Lyophilization is used to dry microorganisms and preserves cell viability at a high rate. However, this process may cause a decrease in viability because of the denaturation of some sensitive proteins due to the high osmotic pressure during the freezing stage (Broeckx et al. 2016; Fonseca et al. 2020; Yao et al. 2020). The negative effects of FD can be reduced by adding some cryoprotectants. Appropriate cryoprotectants are added to the medium, facilitating the adaptation of probiotics and accumulating in the cell, reducing the osmotic pressure difference with the external environment. The cryoprotectant used should be of food origin, nontoxic, easy to find, cheap, and edible (Aschenbrenner et al. 2015). Polysaccharide, protein, and lipid-based drying matrices are important to utilize for the probiotics during FD, dehydration, storage, and gastrointestinal digestion. For this purpose, polymers such as bacterial cellulose, alginate, skimmed milk powder, whey proteins, betaine, trehalose, adonitol, lactose, dextran, glycerol, and polyethylene glycerol are some of the cryoprotectants used (Bagad et al. 2017; Gwak et al. 2015; Rajam and Subramanian 2022).

Oluwatosin et al. (2021) used 10% of skimmed milk, inulin, maltodextrin, and sucrose as cryoprotectants for the FD of *L. plantarum*. They detected that skimmed milk demonstrated the highest survival (91%), while inulin showed the least (<1% cell survival). Chen et al. (2019) added 5.5% glycine, 0.8%, sodium bicarbonate, 7% xylooligosaccharides, 4.5% arginine, and 25% skim milk to the process as cryoprotectants. The survival rate of *B. bifidum* BB01 was determined as 88–90%. This research showed that optimizing composite cryoprotectant for freeze-dried powder successfully protected *B. bifidum* cells. However, this does not provide all organisms with the same degree of viability. Due to differences in the colony morphology and the cell membrane, *Lactobacillus* is adversely affected during lyophilization compared to *Bifidobacterium* (Chen et al. 2019). This difference in vitality preservation is observed even in the presence of cryoprotectants. Haindl et al. (2020) declared that *B. longum* cells were inactivated during drying; however, storage at +4 °C with the addition of 75% maltodextrin relative to bacterial dry mass totally prevented cell loss. Besides the addition of protective molecules to the medium, adaptation of bacterial cells during the fermentation process (prestressing factors before FD such as acid, temperature, and media composition), process parameter management (the choice of operating conditions such as freezing kinetics), final water content, and storage conditions are the defense mechanisms created to increase bacterial viability during FD (Aschenbrenner et al. 2015; Broeckx et al. 2016). It is also known that prestressing cells before FD boosts cell membrane integrity (Oluwatosin et al. 2022). Cryoprotectants can restrain the negative effects of membrane integrity loss caused by vitrification or glass formation (Broeckx et al. 2016). Haindl et al. (2020) determined that the effects causing viability losses were better understood with measurements of the glass transition temperature and membrane integrity. For *B. longum* Reuter 1963, cultivation at pH 6.0 caused more durable cell membranes, resulting in the preservation of membranes of 43% after drying as opposed to cultivation at free acidification, which preserved only 1.0% of membranes after drying. They found a direct correlation between survival rate and remaining cell membrane integrity under various cultivation conditions. In this study, the best process parameters were cultivation at pH 6.0, a drying process of 3700 Pa, 24 hours, and storage temperatures at −10, +10, and +35 °C. In addition, the survival rate of probiotics during drying depends on the composition of the growth medium and the presence of carbohydrates.

Siaterlis et al. (2009) investigated the effects of some saccharides on the growth of human-derived *L. plantarum* and *L. rhamnosus* GG. Sucrose provided more protection from the studied cryoprotectants than trehalose and sorbitol. The growth of *L. plantarum* tested using 2% of the saccharides in glucose-free MRS and its prebiotic potential was identified. Sucrose and inulin both encouraged cell proliferation, with sucrose doing so better than either (Oluwatosin et al. 2022). The protective effect varies depending on the type, composition, combination, and sugar ratio. The encapsulation technique is also used for freeze-dried probiotics for this purpose. The wall material used in encapsulation provides a physical barrier around the cells to protect the viability of the microorganisms against adverse environmental conditions (thermal, osmotic, mechanical, and oxidative). In this method, a protective film or coating layer is formed around the active microorganism with various substances. With a successful application, the spoilage that microorganisms can cause in the product is prevented, and microorganisms can preserve their vitality at the highest level during storage (Haindl et al. 2020; Yao et al. 2020). The temperature at which the probiotics are frozen is a crucial process parameter that can significantly affect how viable probiotics are during FD.

Previous research demonstrated that freezing probiotic bacteria at lower temperatures could result in higher freezing rates and smaller ice crystals, preventing cellular harm. Additionally, ice crystals during freezing can lead to mechanical and osmotic stresses that compromise the integrity of cell membranes, and the subsequent removal of bound water during desorption can destabilize cellular components like phospholipids and proteins to result in additional viability losses (Aschenbrenner et al. 2015; Broeckx et al. 2016; Rajam and Subramanian 2022). Wang et al. (2020) used C18:1 to increase the survivability of *L. plantarum* during FD. C18:1 effectively maintains the integrity and fluidity of the cell membrane. It may act as a cryoprotectant to preserve the fluidity and integrity of the cell membrane, hence improving *L. plantarum*'s survival rate after FD. This investigation served as a defense mechanism for bacterial survival.

6.5 Stability and Viability Assessment After FD

Probiotics are live microbial supplements that have been shown to have health benefits (Rajam and Subramanian 2022) and can be found in various foods such as non-fermented milk products, fruit juices, and cereals (Saarela et al. 2006). Probiotic bacteria need to meet the satisfaction of safety and functional criteria. On the other hand, from a technical perspective, they should have high cell intensity and maintain viability during the drying processes, storage conditions, and GIT (Ampatzoglou et al. 2010). There are numerous challenges in developing probiotic-containing food products, such as selecting effective strains and ensuring their survival during processing and storage (Celik and O'Sullivan 2013). Encapsulated probiotic powders and probiotic-filled capsules are commercially used for probiotic supplements (Torp et al. 2022). Encapsulation of probiotics increases the survival rate and keeps the viability at recommended level of cfu/g during the storage (Her et al. 2015; Jouki et al. 2021a,b; Obradović et al. 2022).

In the pharmaceutical and food industries, FD (also known as lyophilization) is a preferred preservation technique for the long-term storage of sensitive products like probiotic bacteria (Nguyen et al. 2022). If conditions are optimized, drying cultures can be stored for a long time and transported without refrigeration (Celik and O'Sullivan 2013; Jalali et al. 2012). In the FD process, probiotics are exposed to low temperatures and desiccation (Jalali et al. 2012). While unbound and bound water are removed from the cell, the decrease in water activity during the drying process causes damage to the cellular structures and reduces bacterial viability (Tymczyszyn et al. 2012). During the freezing process, ice crystals can be formed and cause harm to the cell membrane (Meireles Mafaldo et al. 2022). Even though FD is known to be stressful for live bacteria, this technique is nonetheless regarded as a suitable strategy for providing prolonged shelf life for the most of probiotic microorganisms (Arellano-Ayala et al. 2021).

6.5.1 Factors Affecting Survival and Stability

The viability of probiotic bacteria is a crucial parameter during the FD process and storage. Oxygen concentration, water activity, temperature, and storage time affect probiotics' stability and vitality (Celik and O'Sullivan 2013). The variables affecting probiotic survival during drying and storage are discussed by Ermis (2021).

Moisture content is an important parameter affecting the survival rate after drying and the degree of inactivation during storage (Tymczyszyn et al. 2012). The required moisture content of probiotic powders was reported as 4% or below to improve the viability during storage (Heidebach et al. 2010; Ying et al. 2010). The water activity of freeze-dried probiotic powders ranged from 0.24 to 0.27 (Savedboworn et al. 2019). The moisture content, glass transition temperature (T_g), and storage temperature parameters should be taken into consideration together to evaluate the viability of probiotic cells in freeze-dried powder form. The storage temperature should not be close to the T_g to maintain the viable cell number of probiotic bacteria (Savedboworn et al. 2019).

Encapsulated and dried probiotics should be stored in an environment without light and moisture exposure (Rajam and Subramanian 2022). Water activity is one of the significant parameters affecting freeze-dried probiotics. It is essential not only for durability but also for color and solubility. Alteration in color and solubility at higher a_w can be explained by nonenzymatic browning as the Maillard reaction (Celik and O'Sullivan 2013). The survival kinetics revealed that probiotics' viability decreases more quickly over the water activity of 0.33, which is essential for keeping them alive (Rascón et al. 2018). The moisture level of freeze-dried probiotic bacteria is critical for stability and vitality during storage. The moisture content values are proportional to the cryoprotectant molecular weights, with the lowest molecular weight resulting in the highest moisture content. Generally, it is suggested that for probiotic bacteria, moisture content should be under 5% (Oluwatosin et al. 2022). Relative humidity (RH) is another parameter affecting cell viability. The cells were observed to lose their vitality as the RH and temperature increased (Tymczyszyn et al. 2012). *Lactobacillus salivarius* subsp. *salivarius* showed higher viability when stored at 2.8% and 5.6% RH than stored at 8.8% RH (Zayed and Roos 2004). Maintaining probiotics in a dry state with low water content is vital to improve long-term stability (Broeckx et al. 2016). It should be noted that 0% moisture is unnecessary because of the harmful effect of over-drying on the cells. Another reason might be attributed to the free radicals formation from lipid oxidation and membrane damage caused by phospholipid degradation leading to a decrease in the viability of freeze-dried probiotics during storage (Savedboworn et al. 2019).

6.5.2 Protective Agents to Improve Viability

The probiotic strain stability during storage is an essential concern for the functional food industry. Protecting and maintaining the viability of cells by protective agents is a crucial challenge for promoting cellular vitality during storage (Savini et al. 2010). Health-promoting impact of dried probiotic bacteria depends on high viability in foods (Santivarangkna et al. 2011). The most thoroughly researched techniques for protecting probiotic microorganisms are the inclusion of protectants and the alteration of processing parameters (Broeckx et al. 2016).

Maintaining the viability and activity of freeze-dried probiotic bacteria after storage, finding proper protective agents, and controlling the drying process are necessary (Bergenholtz et al. 2012). Cryoprotectants protect the cell in the freezing phase, while cryoprotectants protect it in the drying phase (Broeckx et al. 2016). The use of cryoprotective substances such as skim milk, whey milk, sucrose, lactose, trehalose, sorbitol, proteins, amino acids, dietary fibers, or prebiotics is the subject of extensive research to increase probiotic cell viability in food products (Ampatzoglou et al. 2010). Some studies showed that algae and microalgae could also be potential preservatives for probiotics (Kuo et al. 2022; Meireles Mafaldo et al. 2022). Cryoprotectants are added to the carrier medium to promote cell viability during FD and to stabilize them during storage (Rajam and Subramanian 2022). The choice of wall material for probiotic microencapsulation is critical since the chemicals used to encapsulate probiotics must be food-grade, biodegradable, and capable of forming a protective wall between the cell and its surroundings (Rajam and Subramanian 2022). These protective materials during FD lead to improved viability and high yields (Broeckx et al. 2016). It should be noted that all these cryoprotectants can be strain dependent.

Skim milk is a convenient cryoprotectant that enhances the viability of probiotic bacteria during the FD process. It was found that adding 6% skim milk to medium as a cryoprotective agent increased the vitality of bacteria by up to 20% compared to the absence of any other cryoprotective substance (Jalali et al. 2012). Oluwatosin et al. (2021) showed that probiotics treated with 10% skimmed milk showed 91% viability, while those treated with distilled water had only 22%. Under refrigerated settings, a high degree of product stability was observed, with no decrease in cell viability. It is reported that 8% of skim milk can protect the cell wall and the probiotic *Enterococcus faecalis* during and after FD (Romyasamit et al. 2021). It has been observed that using trehalose and lactose together with skim milk preserves cell viability at room temperature for 39 weeks (Broeckx et al. 2016). Due to the commonness of milk-based probiotic formulations, new probiotic protective agent demands are increased. For customers with lactose intolerance or allergies, non-milk-based probiotic formulations are crucial (Savini et al. 2010). Using dairy products as protective agents is not always adequate owing to their short shelf-life and refrigeration requirements (Kuo et al. 2022). Therefore, it is critical to find and optimize suitable protective agents and convenient parameters for the vitality of probiotic bacteria.

Trehalose, a non-reducing disaccharide, is another suitable cryoprotectant for FD probiotics and is quite effective when combined with other excipients (Celik and O'Sullivan 2013). Several studies show a higher cell number of bacteria when trehalose is added as a cryoprotective agent to the medium. When 24% skim milk, 4% sucrose, 0.3% ascorbic acid, and 5% trehalose were added as cryoprotectants, the initial viable count of *B. longum* was 10^{10} cfu/g, and it decreased only to 10^9 cfu/g after 56 days of storage at 4 °C (Izquierdo-López et al. 2017). This indicates that cell viability and counts can be maintained by combining different cryoprotectants. In the presence of 10% cellobiose and trehalose, the activity of β-glucosidase, produced by *Bifidobacterium*, was observed to be 98.1% and 97.6%, respectively. In this study, it should be noted that cellobiose is a good cryoprotectant that can protect cells during the drying stages (Basholli-Salihu et al. 2014). Besides being economically reasonable, it was found that during room temperature, maltodextrin, glycerol, and mannitol provide significant stabilization for the viability of the cells (Arellano-Ayala et al. 2021; Savini et al. 2010). Sucrose, on the other hand, is suggested to have a noteworthy lyo- and cryoprotective effect on cell survival (Bergenholtz et al. 2012). Compared to cells without cryoprotectants, adding cryoprotectants dramatically improved the survival of *L. reuteri* at both 4 and 30 °C. The survival rates of cells treated with combined cryoprotectants (8–24% trehalose, 0.13–0.53% Na_2HPO_4, 3–12% lactose, 15–25% skim milk) after six months in storage were 96.4% at 4 °C and 73.8% at 30 °C, respectively (Shu et al. 2018). According to Rascon et al. (2018), hypertonic sucrose solutions had the potential as a cryoprotective agent in infusing fruits loaded with the *L. rhamnosus*. Freeze-dried banana slices infused with probiotic bacteria were successful due to cell count remaining constant at 10^9 cfu/g for 20–28 days. In another study, apple slices were inoculated with *L. plantarum* and dried using different drying methods. When the bacterial loads of apple slices using sucrose solution as cryoprotectant were examined after drying, it was observed that the best efficiency was obtained from FD (Cui et al. 2018). It is reported that ascorbic acid, skim milk, and sorbitol helped freeze-dried probiotics to remain viable and maintain their probiotic qualities while being stored. In the control group (without protectants), the survivability percentage of strains was 70%, while strains with excipients had a long-term survival rate of 73–93% (Bagad et al. 2017). Hu et al. (2022) showed that isomaltose oligosaccharide (IMO) increases the survival rate of *Pediococcus pentosaceus* by 11.02% during FD and provides protection in different temperatures storage. It was discovered that IMO might boost the survival rate of *P. pentosaceus* during storage and ideal temperatures were between −20 and 4 °C. da Silva Guedes et al. (2019) evaluated the potential use of brewer yeast β-glucans (YβG) as cryoprotective on different *Lactobacillus*, and they found YβG were effective protectives for *L. plantarum*. YβG added probiotics maintained the initial probiotic counts for all strains evaluated for up to 60 days of storage. It should be mentioned that the activity and interaction between the microorganism and cryoprotectants seen *in vitro* may differ when tested *in vivo*. The significance of incorporating functional foods fortified with freeze-dried probiotics in emergency relief strategies is to ensure the provision of essential nutrients and support digestive health among affected populations. It may explore the practical implications of this technology in facilitating the distribution, storage, and consumption of nutritionally fortified products during critical times. The recent focus has also brought attention to the significance of managing various emergency situations and events (Wang et al., 2021). Primarily used cryoprotective agents and viability rates of probiotics are given in Table 6.3.

Although the FD technique is known to be challenging for live bacteria, it is nonetheless regarded as a suitable method for ensuring a longer shelf life for most probiotic products (Arellano-Ayala et al. 2021). In the vast majority of studies, it has been shown that the inclusion of certain cryoprotectants with prebiotic qualities in the medium (such as skim milk) can increase the viability of cells throughout the FD process in addition to their potential function with probiotics (Fatemeh 2011). Despite the availability of a few marketed probiotic medicines, sustaining cell viability for an extended length of time remains challenging. Extending cell viability during storage, processing, and digestion should be investigated for effective probiotic production (Rajam and Subramanian 2022). Therefore, besides *in vitro* studies, more *in vivo* studies are needed on the subject.

6.6 Properties of Freeze-Dried Probiotic Powders

The liquid probiotic preparations require low storage and transport temperature to reduce the effect of environmental stresses such as pH, water activity, and dissolved oxygen acting on cells. Drying probiotics into powder form is a helpful way to minimize the costs and exhibits convenience in handling, storage, and transportation (Ermis 2021). However, it is challenging to produce probiotic powders containing a sizeable bacterial population having high viability during the dehydration process and storage time in complex environments. For this reason, encapsulation techniques are employed to maintain microbial bioactivity by using protective materials

Table 6.3 Different cryoprotectants and their effect on the survival rate of probiotic bacteria in various storage conditions.

Microorganism	Cryoprotective agent (g/ml)	Storage condition	Survival rate (%)	Reference
Lactobacillus paracasei	8% Trehalose	4 °C, 3 months	76	Jalali et al. (2012)
	6% Skim milk			
Lactobacillus delbrueckii	4% Sodium ascorbate		72	
Bifidobacterium infantis	5% Cellobiose	37 °C, 48–72 h	86	Basholli-Salihu et al. (2014)
Enterococcus faecalis	8% Skim milk	4 °C, 30 days	95–99	Romyasamit et al. (2021)
Lactobacillus plantarum	10% Skim milk	4 °C, 12 weeks	91	Oluwatosin et al. (2021)
L. plantarum	32% Sucrose	35 °C, 3 months	34	Strasser et al. (2009)
	32% Trehalose		40	
L. plantarum	8% Brewer yeast β-glucan	4 °C, 120 days	87	da Silva Guedes et al. (2019)
Pediococcus pentosaceus	8% Fructose	4 °C, 120 days	77	Bagad et al. (2017)
	2.5% Ascorbic acid		93	
Lactobacillus acidophilus	13% Trehalose	4 °C, 180 days	93	Shu et al. (2018)
	0.33% Na_2HPO_4			
	7.5% Lactose			
	21% Skim milk			
P. pentosaceus	1% Isomaltose oligosaccharide	25 °C 9 months	63	Hu et al. (2022)
		4 °C	87	
		−20 °C	84	
		−80 °C	86	

(Wang et al. 2022). Various wall materials such as whey protein concentrate, whey, hydrocolloids (i.e. maltodextrin, gums, and agarose-based solutions), and sodium alginate could be used to improve the powder properties (Alehosseini et al. 2019; Jouki et al. 2021a; Obradović et al. 2022).

The amount of total soluble solids in the food mixture is the key factor affecting the powder yield after FD. More soluble solids result in an increased amount of powder. The size of the probiotic cells (1–5 μm) limits the cell loading for beads/capsules at the nanoscale (Frakolaki et al. 2021). The size of the particles containing probiotic cells ranges from 2 to 1000 μm after FD depending on the milling/

grinding method used, such as spiral jet mill (Jiang et al. 2020; Picot and Lacroix 2003). Larger particles confer better protection for the probiotics, while they show poor dispersibility in the food matrix. An optimal microcapsule size should be identified to provide reasonable protection, improved shelf stability, and good food product distribution.

In general, physical, physicochemical, and biological properties of probiotic powders such as flowability, bulk density, size distribution, reconstitution (wettability, dispersibility, stability), chemical properties, particles' microstructural and surface properties, and viability influence the functionality during processing, storage (under varying temperatures and moisture content), and digestion in the host. The formulation of the mixture, the drying method used, and delivery forms (powder beads, tablets, capsules, liquids, etc.) play vital roles in chemical structure, powder quality, and particle properties. The cell viability and performance (i.e. adhesion capacity and growth/colonization) of dried probiotics in the powder state are influenced by biological (e.g. surface membrane adhesion and aggregation), physical (e.g. crystal formation and size and storage temperature), and physicochemical (e.g. oxygen permeability and water activity) factors (Ali et al. 2020). Further characterization of probiotic powders, such as thermal profiling and particle-specific surface area, which is related to the developed surface to react with dispersing liquid media, should be done to evaluate the functional properties better. Increased surface area-to-volume ratio of solid particles of small size accelerates the oxidation and powder permeability affecting powder stability during storage. Particle size, shape, and wettability properties can be correlated to this ratio. The moisture absorption and oxygen transfer rate from the surrounding air are altered by the degree of porosity, permeability, and surface hydrophobicity of the particles (Ali et al. 2020).

Adding encapsulated probiotic cells to yogurt yielded yogurt powder with increased bulk density (around $0.29\,g/cm^3$). Yogurt powder without probiotics provided porous and rougher particles (Jouki et al. 2021b). The yield and the bulk density of skim milk powder inoculated with probiotics were reported as around 15% and 16.67 g/ml, respectively (Hameed et al. 2021). Plant byproducts such as grape pulp, pomegranate, and beetroot peel extracts can produce synbiotic powder using FD technology (Jouki et al. 2021b).

6.6.1 Comparison of Spray-Dried and Freeze-Dried Probiotic Powders

Probiotic cells can be microencapsulated using FD and SD techniques (Vaessen et al. 2020). Spray and FD techniques were used by Luangthongkam et al. (2021) to produce limestone powder containing *Bacillus amyloliquefaciens*. They report 100% survival after spray drying when adjusting the outlet temperature at 80 °C, while it is reported as 60% after FD. In addition, a lower temperature (4 °C) resulted in more extended stability during storage. Her et al. (2015) employed spray freeze-drying (SFD) to produce *Lactobacillus casei* fine probiotic powder. They reported varying particle shapes and sizes (average 24.8 μm) with porous structures depending on the drying conditions. Previous studies report that irregular shapes and surfaces (porous and spongy flakes) with sharp edges and spikes (Figure 6.2) were produced from the FD process (Jiang et al. 2020; Obradović et al. 2022; Rajam and Anandharamakrishnan 2015; Ying et al. 2010). This irregularity might be attributed to the milling process after the FD of probiotic suspensions. Jouki et al. (2021b) reported the particle size of freeze-dried alginate microcapsules of *L. plantarum* ranged from 350 to 1000 μm.

SFD has been introduced to eliminate the adverse effect of heat during SD and develop a fine powder with a controlled capsule size and specific surface area. Figure 6.3 exhibits the processing steps of SD, FD, and SFD. The SFD method includes spraying liquid media containing probiotic cells through an atomization nozzle into the cold vapor phase of a cryogenic liquid, such as liquid nitrogen.

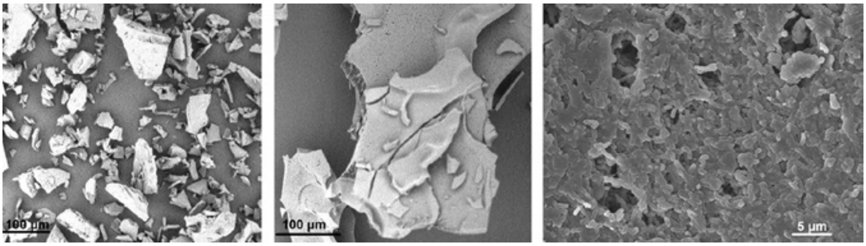


Figure 6.2 SEM images of freeze-dried probiotic powder particles (protective materials: gum Arabic and maltodextrin). *Source:* Shobuz Mahmud et al. 2022/Reproduced with permission from John Wiley & Sons.

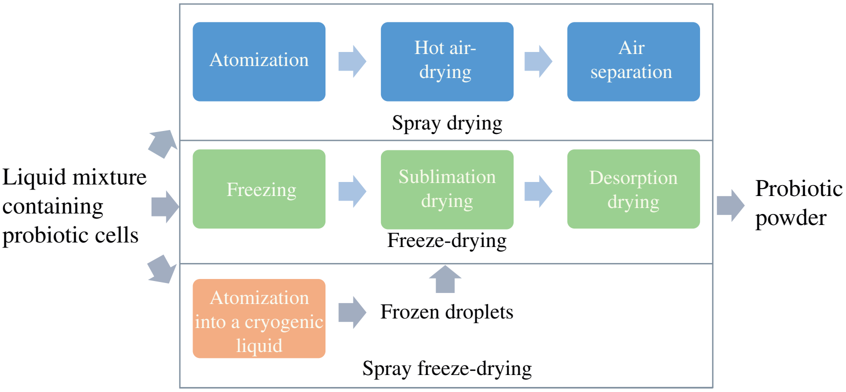


Figure 6.3 The comparison of processing steps of SD, FD, and SFD. *Source:* Adapted from Frakolaki et al. (2021).

Dispersed frozen droplets in cryogenic liquid are dried through the FD technique (Kiepś and Dembczyński 2022). Even though the SFD requires less time than FD, the operating cost is 30–50 times higher than SD (Frakolaki et al. 2021). In addition, the encapsulation efficiency was found to be lower compared to regular FD due to the additional stress factors that occurred during atomization and freezing (Kiepś and Dembczyński 2022).

6.7 Rehydration of Freeze-Dried Probiotics

Rehydration of probiotic powders is required before adding them to food formulations. The wetting time and the solubility analyses are often performed to assess the solubility (Hameed et al. 2021; Jouki et al. 2021b). The hydration of freeze-dried probiotic cells has a significant impact on their viability. Regardless of the drying process, rehydration is a critical stage in the recovery of dehydrated microorganisms. Even when all precautions are taken to ensure survival during freezing, drying, and storing, an inadequate rehydration phase may result in poor cell viability (Chen et al. 2005). Thus, retaining the viability of the cells is essential for effective rehydration (Arellano-Ayala et al. 2021). Careful selection of medium or excipients containing the probiotic culture can be used to identify the least amount of harm caused to microorganisms during rehydration (Nagashima et al. 2013).

The rehydration method and the surrounding media's conditions determine the cell's potential after rehydration (Arellano-Ayala et al. 2021). Slow rehydration of freeze-dried probiotics provides slow water flow through the cell membrane, thus maintaining bacterial viability (Broeckx et al. 2016). However, the viability of probiotic powders by rehydration depends on the strain and the medium in which it is dissolved. When freeze-dried cells were rehydrated directly with water instead of skim milk solution, the death rate increased by 0.65 log cfu/ml (Fatemeh 2011). Skim milk protects cells by stabilizing the components of the cell membrane, forming a porous structure in the freeze-dried product that facilitates rehydration, and having proteins that act as a protective layer for the cells (Jofré et al. 2015). Rehydration of freeze-dried *L. brevis* cells in distilled water resulted in lower recovery than rehydration in a medium containing sugar and minerals (Zhao and Zhang 2005). *Enterococcus faecalis* isolates were freeze-dried with skim milk powder and stored at 4 °C for 30 days. It might imply that freeze-dried *E. faecalis* with skim milk has a high potential for regrowth following rehydration (Romyasamit et al. 2021). On the contrary, no difference in the recovery of the *Lactobacillus helveticus* cells was observed between distilled water and 10% (w/v) skimmed milk medium. This result was attributed to the preservation of probiotics during rehydration by milk components found in kefir (Chen et al. 2005).

Another crucial aspect of probiotic functioning is its capacity to stick to mucosal surfaces and the intestinal epithelium, which is necessary for its establishment in the human GIT (Schillinger et al. 2005). There are specific *in vitro* tests determined by FAO (The Food and Agriculture Organization and WHO (the World Health Organization)). These tests can be listed as resistance to gastric and bile acidity, adherence to mucus and human epithelial cells, antimicrobial activity against potentially pathogenic bacteria, and bile salt hydrolase activity (FAO/WHO 2022). A key aspect in maintaining freeze-dried probiotics' viability and physiological activity is the careful selection of appropriate cryoprotectants when creating new probiotic supplements. Protecting cells from osmotic stress during rehydration and maintaining probiotic viability is essential for such formulations (Arellano-Ayala et al. 2021). In order to provide a favorable environment for probiotics and restore their effectiveness, it is crucial to confirm the strain's adaptation to rehydration in GIT.

6.8 Conclusion and Future Trends

The viability of probiotic bacteria is essential for ensuring the functional activity of probiotic foods. Protecting the stability and viability of probiotics during and after FD is a severe industry challenge. FD is one of the main techniques for keeping probiotics for prolonged storage. The activity of dried probiotics can be improved by selecting the best bacterial strain and modifying the production, processing, storage, and rehydration parameters with precise approaches (i.e. the use of adequate cryoprotection, optimization of freezing rate and freezing temperature, and adaptation procedures applied before freezing to prepare the cells to freezing stress). In addition, investigating the effect of various cryoprotectants on probiotics is necessary for freeze-dried probiotics. Optimizing and maintaining probiotics' stability with newly improved drying and storage techniques is possible. Advanced fermentation, encapsulation, drying, rehydration, and storage technologies can be used to prepare freeze-dried probiotics with prolonged stability. However, further research needs to be done to test new ingredients, improve the cryotolerance of probiotic bacteria, and improve the stability and functionality of probiotic powders.

References

Alehosseini, A., Gomez del Pulgar, E.-M., Fabra, M.J. et al. (2019). Agarose-based freeze-dried capsules prepared by the oil-induced biphasic hydrogel particle formation approach for the protection of sensitive probiotic bacteria. *Food Hydrocolloids* 87: 487–496. https://doi.org/10.1016/j.foodhyd.2018.08.032.

Ali, M.A., Razafindralambo, H.L., Conti, G., and De Coninck, J. (2020). Bulk and surface wettability characteristics of probiotic powders in their compressed disc and packed-bed column forms. *ACS Omega* 5: 22348–22355. https://doi.org/10.1021/acsomega.0c02728.

Amine, K.M., Champagne, C.P., Raymond, Y. et al. (2014). Survival of micro-encapsulated Bifidobacterium longum in Cheddar cheese during production and storage. *Food Control* 37: 193–199. https://doi.org/10.1016/j.foodcont.2013.09.030.

Ampatzoglou, A., Schurr, B., Deepika, G., Baipong, S., Charalampopoulos, D., 2010. Influence of fermentation on the acid tolerance and freeze drying survival of *Lactobacillus rhamnosus* GG. *Biochemical Engineering Journal* 52, 65–70. doi:https://doi.org/10.1016/j.bej.2010.07.005

Arellano, K., Park, H., Kim, B. et al. (2021). Improving the viability of freeze-dried probiotics using a lysine-based rehydration mixture. *Microbiology and Biotechnology Letters* 49: 1–10. https://doi.org/10.48022/mbl.2012.12007.

Arellano-Ayala, K., Lim, J., Yeo, S. et al. (2021). Rehydration before application improves functional properties of lyophilized *Lactiplantibacillus plantarum* HAC03. *Microorganisms* 9: 1013. https://doi.org/10.3390/microorganisms9051013.

Aschenbrenner, M., Foerst, P., and Ulrich, K. (2015). Freeze-drying of probiotics. In: *Advances in Probiotic Technology* (ed. C. Santivarangkna), 187–220. CRC Press https://doi.org/10.1201/b18807-15.

Ashaolu, T.J. (2020). Immune boosting functional foods and their mechanisms: a critical evaluation of probiotics and prebiotics. *Biomedicine & Pharmacotherapy* 130: 110625. https://doi.org/10.1016/j.biopha.2020.110625.

Bagad, M., Pande, R., Dubey, V., and Ghosh, A.R. (2017). Survivability of freeze-dried probiotic *Pediococcus pentosaceus* strains GS4, GS17 and *Lactobacillus gasseri* (ATCC 19992) during storage with commonly used pharmaceutical excipients within a period of 120 days. *Asian Pacific Journal of Tropical Biomedicine* 7: 921–929. https://doi.org/10.1016/j.apjtb.2017.09.005.

Barbosa, J., Borges, S., Amorim, M. et al. (2015). Comparison of spray drying, freeze drying and convective hot air drying for the production of a probiotic orange powder. *Journal of Functional Foods* 17: 340–351. https://doi.org/10.1016/j.jff.2015.06.001.

Basholli-Salihu, M., Mueller, M., Salar-Behzadi, S. et al. (2014). Effect of lyoprotectants on β-glucosidase activity and viability of *Bifidobacterium infantis* after freeze-drying and storage in milk and low pH juices. *LWT - Food Science and Technology* 57: 276–282. https://doi.org/10.1016/j.lwt.2014.01.011.

Béal, C., Fonseca, F., 2015. Freezing of probiotic bacteria, in: Petra ger Chalat Santivarangkna Advances in Probiotic Technology. CRC Press pp. 179–212. https://doi.org/10.1201/b18807-14

Bergenholtz, Å.S., Wessman, P., Wuttke, A., and Håkansson, S. (2012). A case study on stress preconditioning of a *Lactobacillus* strain prior to freeze-drying. *Cryobiology* 64: 152–159. https://doi.org/10.1016/j.cryobiol.2012.01.002.

Broeckx, G., Vandenheuvel, D., Claes, I.J.J. et al. (2016). Drying techniques of probiotic bacteria as an important step towards the development of novel pharmabiotics. *International Journal of Pharmaceutics* 505: 303–318. https://doi.org/10.1016/j.ijpharm.2016.04.002.

Celik, O.F. and O'Sullivan, D.J. (2013). Factors influencing the stability of freeze-dried stress-resilient and stress-sensitive strains of bifidobacteria. *Journal of Dairy Science* 96: 3506–3516. https://doi.org/10.3168/jds.2012-6327.

Chen, H.-C., Lin, C.-W., and Chen, M.-J. (2005). The effects of freeze drying and rehydration on survival of microorganisms in Kefir. *Asian-Australasian Journal of Animal Sciences* 19: 126–130. https://doi.org/10.5713/ajas.2006.126.

Chen, H., Tian, M., Chen, L. et al. (2019). Optimization of composite cryoprotectant for freeze-drying *Bifidobacterium bifidum* BB01 by response surface methodology. *Artificial Cells, Nanomedicine, and Biotechnology* 47: 1559–1569. https://doi.org/10.1080/21691401.2019.1603157.

Chugh, B. and Kamal-Eldin, A. (2020). Bioactive compounds produced by probiotics in food products. *Current Opinion in Food Science* 32: 76–82. https://doi.org/10.1016/j.cofs.2020.02.003.

Conde-Islas, A., Jiménez-Fernández, M., Cantú-Lozano, D. et al. (2019). Effect of the freeze-drying process on the physicochemical and microbiological properties of Mexican kefir grains. *Processes* 7: 127. https://doi.org/10.3390/pr7030127.

Cui, L., Niu, L., Li, D. et al. (2018). Effects of different drying methods on quality, bacterial viability and storage stability of probiotic enriched apple snacks. *Journal of Integrative Agriculture* 17: 247–255. https://doi.org/10.1016/S2095-3119(17)61742-8.

Damián, M.R., Cortes-Perez, N.G., Quintana, E.T. et al. (2022). Functional foods, nutraceuticals and probiotics: a focus on human health. *Microorganisms* 10: 1065. https://doi.org/10.3390/microorganisms10051065.

Dinkçi, N., Akdeniz, V., and Akalin, A.S. (2019). Survival of probiotics in functional foods during shelf life. In: *Food Quality and Shelf Life* (ed. C.M. Galanakis), 201–233. Elsevier https://doi.org/10.1016/b978-0-12-817190-5.00006-9.

Ermis, E. (2021). A review of drying methods for improving the quality of probiotic powders and characterization. *Drying Technology* 40: 1–18. https://doi.org/10.1080/07373937.2021.1950169.

FAO/WHO 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report, Cordoba, Argentina.

Fatemeh, S. (2011). Optimization of a cryoprotective medium and survival of freeze-dried *Bifidobacterium infantis* 20088 throughout storage, rehydration and gastrointestinal tract transit for infant formula probiotic applications. *African Journal of Microbiology Research* 5: https://doi.org/10.5897/ajmr11.319.

Ferraz, J.L., Cruz, A.G., Cadena, R.S. et al. (2012). Sensory acceptance and survival of probiotic bacteria in ice cream produced with different overrun levels. *Journal of. Food Science* 77: S24–S28. https://doi.org/10.1111/j.1750-3841.2011.02508.x.

Fonseca, F., Girardeau, A., and Passot, S. (2020). Freeze-drying of lactic acid bacteria: a stepwise approach for developing a freeze-drying protocol based on physical properties. *Cryopreservation and Freeze-Drying Protocol* https://doi.org/10.1007/978-1-0716-0783-1_38.

Frakolaki, G., Giannou, V., Kekos, D., and Tzia, C. (2021). A review of the microencapsulation techniques for the incorporation of probiotic bacteria in functional foods. *Critical Reviews in Food Science and Nutrition* 61: 1515–1536. https://doi.org/10.1080/10408398.2020.1761773.

González-Sánchez, F., Azaola, A., Gutiérrez-López, G.F., and Hernández-Sánchez, H. (2010). Viability of microencapsulated Bifidobacterium animalis ssp. lactis {BB}12 in kefir during refrigerated storage. *International Journal of Dairy Technology* 63: 431–436. https://doi.org/10.1111/j.1471-0307.2010.00604.x.

Gwak, H.J., Lee, J.-H., Kim, T.-W. et al. (2015). Protective effect of soy powder and microencapsulation on freeze-dried *Lactobacillus brevis* WK12 and *Lactococcus lactis* WK11 during storage. *Food Science and Biotechnology* 24: 2155–2160. https://doi.org/10.1007/s10068-015-0287-5.

Haindl, R., Neumayr, A., Frey, A., and Kulozik, U. (2020). Impact of cultivation strategy, freeze-drying process, and storage conditions on survival, membrane integrity, and inactivation kinetics of *Bifidobacterium longum*. *Folia Microbiologia (Praha)* 65: 1039–1050. https://doi.org/10.1007/s12223-020-00815-3.

Hameed, A.M., Elkhtab, E., Mostafa, M.S. et al. (2021). Amino acids, solubility, bulk density and water holding capacity of novel freeze-dried cow's skimmed milk fermented with potential probiotic *Lactobacillus plantarum* Bu-Eg5 and *Lactobacillus rhamnosus* Bu-Eg6. *Arabian Journal of Chemistry* 14: 103291. https://doi.org/10.1016/j.arabjc.2021.103291.

Heidebach, T., Först, P., and Kulozik, U. (2010). Influence of casein-based microencapsulation on freeze-drying and storage of probiotic cells. *Journal of Food Engineering* 98: 309–316. https://doi.org/10.1016/j.jfoodeng.2010.01.003.

Her, J.Y., Kim, M.S., and Lee, K.G. (2015). Preparation of probiotic powder by the spray freeze-drying method. *Journal of Food Engineering* 150: 70–74. https://doi.org/10.1016/j.jfoodeng.2014.10.029.

Hu, D., Wu, J., Li, J. et al. (2022). Effect of isomaltose oligosaccharide on the freeze-drying process and shelf-life of *Pediococcus pentosaceus*. *LWT* 163: 113583. https://doi.org/10.1016/j.lwt.2022.113583.

Ispirli, H., Özmen, D., Yilmaz, M.T. et al. (2020). Impact of glucan type exopolysaccharide ({EPS}) production on technological characteristics of sourdough bread. *Food Control* 107: 106812. https://doi.org/10.1016/j.foodcont.2019.106812.

Izquierdo-López, D., Goulet, J., and Ratti, C. (2017). Foam-mat freeze-drying of *Bifidobacterium longum* RO175: viability and refrigerated storage stability. *Journal of Food Science* 82: 90–96. https://doi.org/10.1111/1750-3841.13571.

Jalali, M., Abedi, D., Varshosaz, J. et al. (2012). Stability evaluation of freeze-dried *Lactobacillus paracasei* subsp. *tolerance* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in oral capsules. *Research in Pharmaceutical Sciences* 7: 31–36.

Jiang, N., Dev Kumar, G., Chen, J. et al. (2020). Comparison of concurrent and mixed-flow spray drying on viability, growth kinetics and biofilm formation of *Lactobacillus rhamnosus* GG microencapsulated with fish gelatin and maltodextrin. *LWT* 124: 109200. https://doi.org/10.1016/j.lwt.2020.109200.

Jofré, A., Aymerich, T., and Garriga, M. (2015). Impact of different cryoprotectants on the survival of freeze-dried *Lactobacillus rhamnosus* and *Lactobacillus casei/paracasei* during long-term storage. *Beneficial Microbes* 6: 381–386. https://doi.org/10.3920/bm2014.0038.

Jouki, M., Khazaei, N., Rashidi-Alavijeh, S., and Ahmadi, S. (2021a). Encapsulation of *Lactobacillus casei* in quince seed gum-alginate beads to produce a functional synbiotic drink powder by agro-industrial by-products and freeze-drying. *Food Hydrocolloids* 120: 106895. https://doi.org/10.1016/j.foodhyd.2021.106895.

Jouki, M., Khazaei, N., Rezaei, F., and Taghavian-Saeid, R. (2021b). Production of synbiotic freeze-dried yoghurt powder using microencapsulation and cryopreservation of *L. plantarum* in alginate-skim milk microcapsules. *International Dairy Journal* 122: 105133. https://doi.org/10.1016/j.idairyj.2021.105133.

Kiepś, J. and Dembczyński, R. (2022). Current trends in the production of probiotic formulations. *Foods* 11: 2330. https://doi.org/10.3390/foods11152330.

King, H. (2020). Let food be thy medicine. In: *Hippocrates Now: The 'Father of Medicine' in the Internet Age*, 111–132. Bloomsbury Academic https://doi.org/10.5040/9781350005921.ch-006.

Kuo, C.-C., Clark, S., Qin, H., and Shi, X. (2022). Development of a shelf-stable, gel-based delivery system for probiotics by encapsulation, 3D printing, and freeze-drying. *LWT* 157: 113075. https://doi.org/10.1016/j.lwt.2022.113075.

Li, W., Zhao, Y., Zhao, Y. et al. (2023). Food hydrocolloids improving the viability of *Lactobacillus plantarum* LP90 by carboxymethylated dextran-whey protein conjugates: the relationship with glass transition temperature. *Food Hydrocolloids* 134: 108102. https://doi.org/10.1016/j.foodhyd.2022.108102.

Lin, D.C. (2003). Probiotics as functional foods. *Nutrition in Clinical Practice* 18: 497–506. https://doi.org/10.1177/0115426503018006497.

Liu, Y., Wan, Z., Yohannes, K.W. et al. (2021). Functional characteristics of Lactobacillus and yeast single starter cultures in the ripening process of dry fermented sausage. *Frontiers in Microbiology.* 11: https://doi.org/10.3389/fmicb.2020.611260.

Luangthongkam, P., Blinco, J.-A., Dart, P. et al. (2021). Comparison of spray-drying and freeze-drying for inoculum production of the probiotic *Bacillus amyloliquefaciens* strain H57. *Food and Bioproducts Processing* 130: 121–131. https://doi.org/10.1016/j.fbp.2021.09.010.

Mahmud, S., Khan, S., Khan, M.R. et al. (2022). Viability and stability of microencapsulated probiotic bacteria by freeze-drying under *in vitro* gastrointestinal conditions. *Journal of Food Processing & Preservation* https://doi.org/10.1111/jfpp.17123.

Meireles Mafaldo, Í., de Medeiros, V.P.B., da Costa, W.K.A. et al. (2022). Survival during long-term storage, membrane integrity, and ultrastructural aspects of *Lactobacillus acidophilus* 05 and *Lacticaseibacillus casei* 01 freeze-dried with freshwater microalgae biomasses. *Food Research International* 159: 111620. https://doi.org/10.1016/j.foodres.2022.111620.

Misra, S., Pandey, P., Dalbhagat, C.G., and Mishra, H.N. (2022). Emerging technologies and coating materials for improved probiotication in food products: a review. *Food and Bioprocess Technology* 15: 998–1039. https://doi.org/10.1007/s11947-021-02753-5.

Nagashima, A.I., Pansiera, P.E., Baracat, M.M., and Gómez, R.J.H.C. (2013). Development of effervescent products, in powder and tablet form, supplemented with probiotics *Lactobacillus acidophilus* and *Saccharomyces boulardii*. *Food Science and Technology* 33: 605–611. https://doi.org/10.1590/s0101-20612013000400002.

Nguyen, T.-T., Nguyen, P.-T., Nguyen, T.-T.-V. et al. (2022). Correlation between the amount of extracellular polymeric substances and the survival rate to freeze-drying of probiotics. *Current Microbiology* 79: https://doi.org/10.1007/s00284-022-02862-0.

Obradović, N., Volić, M., Nedović, V. et al. (2022). Microencapsulation of probiotic starter culture in protein–carbohydrate carriers using spray and freeze-drying processes: implementation in whey-based beverages. *Journal of Food Engineering* 321: 110948. https://doi.org/10.1016/j.jfoodeng.2022.110948.

Oluwatosin, S.O., Tai, S.L., and Fagan-Endres, M.A. (2021). Sucrose, maltodextrin and inulin efficacy as cryoprotectant, preservative and prebiotic – towards a freeze dried *Lactobacillus plantarum* topical probiotic. *Biotechnology Reports (Amsterdam, Netherlands)* 33: e00696–e00696. https://doi.org/10.1016/j.btre.2021.e00696.

Oluwatosin, S.O., Tai, S.L., and Fagan-Endres, M.A. (2022). Sucrose, maltodextrin and inulin efficacy as cryoprotectant, preservative and prebiotic – towards a freeze dried *Lactobacillus plantarum* topical probiotic. *Biotechnology Reports* 33: e00696. https://doi.org/10.1016/j.btre.2021.e00696.

Peng, M., Tabashsum, Z., Anderson, M. et al. (2020). Effectiveness of probiotics, prebiotics, and prebiotic-like components in common functional foods. *Comprehensive Reviews in Food Science and Food Safety* 19: 1908–1933. https://doi.org/10.1111/1541-4337.12565.

Petreska-Ivanovska, T., Petrushevska-Tozi, L., Grozdanov, A. et al. (2014). From optimization of synbiotic microparticles prepared by spray-drying to development of new functional carrot juice. *Chemical Industry and Chemical Engineering Quarterly* 20: 549–564. https://doi.org/10.2298/ciceq130218036p.

Picot, A. and Lacroix, C. (2003). Effects of micronization on viability and thermotolerance of probiotic freeze-dried cultures. *International Dairy Journal* 13: 455–462. https://doi.org/10.1016/S0958-6946(03)00050-5.

Rajam, R. and Anandharamakrishnan, C. (2015). Spray freeze drying method for microencapsulation of *Lactobacillus plantarum*. *Journal of Food Engineering* 166: 95–103. https://doi.org/10.1016/j.jfoodeng.2015.05.029.

Rajam, R. and Subramanian, P. (2022). Encapsulation of probiotics: past, present and future. *Beni-Suef University Journal of Basic and Applied Sciences* 11: https://doi.org/10.1186/s43088-022-00228-w.

Rascón, M.P., Huerta-Vera, K., Pascual-Pineda, L.A. et al. (2018). Osmotic dehydration assisted impregnation of *Lactobacillus rhamnosus* in banana and effect of water activity on the storage stability of probiotic in the freeze-dried product. *LWT* 92: 490–496. https://doi.org/10.1016/j.lwt.2018.02.074.

Reque, P.M. and Brandelli, A. (2021). Encapsulation of probiotics and nutraceuticals: applications in functional food industry. *Trends in Food Science and Technology* 114: 1–10. https://doi.org/10.1016/j.tifs.2021.05.022.

Romero-Chapol, O.O., Varela-Pérez, A., Castillo-Olmos, A.G. et al. (2022). Encapsulation of *Lacticaseibacillus rhamnosus* GG: probiotic survival, *in vitro* digestion and viability in apple juice and yogurt. *Applied Sciences* 12: 2141. https://doi.org/10.3390/app12042141.

Romyasamit, C., Saengsuwan, P., Boonserm, P. et al. (2021). Optimization of cryoprotectants for freeze-dried potential probiotic *Enterococcus faecalis* and evaluation of its storage stability. *Drying Technology* 40: 2283–2292. https://doi.org/10.1080/07373937.2021.1931294.

Saarela, M., Virkajärvi, I., Alakomi, H.-L. et al. (2006). Stability and functionality of freeze-dried probiotic *Bifidobacterium* cells during storage in juice and milk. *International Dairy Journal* 16: 1477–1482. https://doi.org/10.1016/j.idairyj.2005.12.007.

Santivarangkna, C., Aschenbrenner, M., Kulozik, U., and Foerst, P. (2011). Role of glassy state on stabilities of freeze-dried probiotics. *Journal of Food Science* 76: R152–R156. https://doi.org/10.1111/j.1750-3841.2011.02347.x.

Savedboworn, W., Teawsomboonkit, K., Surichay, S. et al. (2019). Impact of protectants on the storage stability of freeze-dried probiotic *Lactobacillus plantarum*. *Food Science and Biotechnology* 28: 795–805. https://doi.org/10.1007/s10068-018-0523-x.

Savini, M., Cecchini, C., Verdenelli, M.C. et al. (2010). Pilot-scale production and viability analysis of freeze-dried probiotic bacteria using different protective agents. *Nutrients* 2: 330–339. https://doi.org/10.3390/nu2030330.

Schillinger, U., Guigas, C., and Heinrich Holzapfel, W. (2005). *In vitro* adherence and other properties of lactobacilli used in probiotic yoghurt-like products. *International Dairy Journal* 15: 1289–1297. https://doi.org/10.1016/j.idairyj.2004.12.008.

Shu, G., Wang, Z., Chen, L. et al. (2018). Characterization of freeze-dried *Lactobacillus acidophilus* in goat milk powder and tablet: optimization of the composite cryoprotectants and evaluation of storage stability at different temperature. *LWT* 90: 70–76. https://doi.org/10.1016/j.lwt.2017.12.013.

Siaterlis, A., Deepika, G., and Charalampopoulos, D. (2009). Effect of culture medium and cryoprotectants on the growth and survival of probiotic *Lactobacilli* during freeze drying. *Letters in Applied Microbiology* 48: 295–301. https://doi.org/10.1111/j.1472-765x.2008.02529.x.

Siciliano, R.A., Reale, A., Mazzeo, M.F. et al. (2021). Paraprobiotics: a new perspective for functional foods and nutraceuticals. *Nutrients* 13: 1225. https://doi.org/10.3390/nu13041225.

da Silva Guedes, J., Pimentel, T.C., Diniz-Silva, H.T. et al. (2019). Protective effects of β-glucan extracted from spent brewer yeast during freeze-drying, storage and exposure to simulated gastrointestinal conditions of probiotic lactobacilli. *LWT* 116: 108496. https://doi.org/10.1016/j.lwt.2019.108496.

Song, D., Ibrahim, S., and Hayek, S. (2012). Probiotics. In: *Recent Application of Probiotics in Food and Agricultural Science* (ed. E.C. Rigobelo). INTECH https://doi.org/10.5772/50121.

Strasser, S., Neureiter, M., Geppl, M. et al. (2009). Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria. *Journal of Applied Microbiology* 107: 167–177. https://doi.org/10.1111/j.1365-2672.2009.04192.x.

Terpou, A., Nigam, P.S., Bosnea, L., and Kanellaki, M. (2018). Evaluation of Chios mastic gum as antimicrobial agent and matrix forming material targeting probiotic cell encapsulation for functional fermented milk production. *LWT* 97: 109–116. https://doi.org/10.1016/j.lwt.2018.06.045.

Torp, A.M., Bahl, M.I., Boisen, A., and Licht, T.R. (2022). Optimizing oral delivery of next generation probiotics. *Trends in Food Science and Technology* 119: 101–109. https://doi.org/10.1016/j.tifs.2021.11.034.

Tymczyszyn, E.E., Sosa, N., Gerbino, E. et al. (2012). Effect of physical properties on the stability of *Lactobacillus bulgaricus* in a freeze-dried galacto-oligosaccharides matrix. *International Journal of Food Microbiology* 155: 217–221. https://doi.org/10.1016/j.ijfoodmicro.2012.02.008.

Vaessen, E.M.J., den Besten, H.M.W., Leito, K.M.N., and Schutyser, M.A.I. (2020). Pulsed electric field pre-treatment for enhanced bacterial survival after drying: effect of carrier matrix and strain variability. *Innovative Food Science and Emerging Technologies* 66: 102515. https://doi.org/10.1016/j.ifset.2020.102515.

Vorländer, K., Kampen, I., Finke, J.H., and Kwade, A. (2020). Along the process chain to probiotic tablets: evaluation of mechanical impacts on microbial viability. *Pharmaceutics* 12: https://doi.org/10.3390/pharmaceutics12010066.

Wang, G., Chen, P., Yu, X. et al. (2020). C18:1 improves the freeze-drying resistance of *Lactobacillus plantarum* by maintaining the cell membrane. *ACS Applied Bio Materials* 3: 4933–4940. https://doi.org/10.1021/acsabm.0c00444.

Wang, G., Chen, Y., Xia, Y. et al. (2022). Characteristics of probiotic preparations and their applications. *Foods* 11: 2472. https://doi.org/10.3390/foods11162472.

Wang, Z., Gong, J., Wang, Q., & Qiao, X. (2021). Emergency management science and technology: An international transdisciplinary platform. *Emergency Management Science and Technology*, 1(1), 1–3.

Yao, M., Xie, J., Du, H. et al. (2020). Progress in microencapsulation of probiotics: a review. *Comprehensive Reviews in Food Science and Food Safety* 19: 857–874. https://doi.org/10.1111/1541-4337.12532.

Ying, D.Y., Phoon, M.C., Sanguansri, L. et al. (2010). Microencapsulated *Lactobacillus rhamnosus* GG powders: relationship of powder physical properties to probiotic survival during storage. *Journal of Food Science* 75: E588–E595. https://doi.org/10.1111/j.1750-3841.2010.01838.x.

Zayed, G. and Roos, Y.H. (2004). Influence of trehalose and moisture content on survival of *Lactobacillus salivarius* subjected to freeze-drying and storage. *Process Biochemistry* 39: 1081–1086. https://doi.org/10.1016/s0032-9592(03)00222-x.

Zhao, G. and Zhang, G. (2005). Effect of protective agents, freezing temperature, rehydration media on viability of malolactic bacteria subjected to freeze-drying. *Journal of Applied Microbiology* 99: 333–338. https://doi.org/10.1111/j.1365-2672.2005.02587.x.

Zubillaga, M., Weill, R., Postaire, E. et al. (2001). Effect of probiotics and functional foods and their use in different diseases. *Nutrition Research* 21: 569–579. https://doi.org/10.1016/s0271-5317(01)00281-0.