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REVIEW

Emerging Technologies and Coating Materials for Improved Probiotication in Food Products: a Review

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Abstract

From the past few decades, consumers' demand for probiotic-based functional and healthy food products is rising exponentially. Encapsulation is an emerging field to protect probiotics from unfavorable conditions and to deliver probiotics at the target place while maintaining the controlled release in the colon. Probiotics have been encapsulated for decades using different encapsulation methods to maintain their viability during processing, storage, and digestion and to give health benefits. This review focuses on novel microencapsulation techniques of probiotic bacteria including vacuum drying, microwave drying, spray freeze drying, fluidized bed drying, impinging aerosol technology, hybridization system, ultrasonication with their recent advancement, and characteristics of the commonly used polymers have been briefly discussed. Other than novel techniques, characterization of microcapsules along with their mechanism of release and stability have shown great interest recently in developing novel functional food products with synergetic effects, especially in COVID-19 outbreak. A thorough discussion of novel processing technologies and applications in food products with the incorporation of recent research works is the novelty and highlight of this review paper.

Keywords Probiotics · Microencapsulation · Drying · Functional foods · Storage · Packaging condition

Introduction

Probiotics are live microorganisms when administrated in an adequate amount confer a health benefit on the host (FAO/WHO, 2002). In the last century, different species of microorganisms have been used as probiotics due to their potentiality to prevent and cure diseases such as diabetes, gastric cancer, and inflammatory bowel disease (IBD) by enhancing the gut barrier function, producing antimicrobial compounds and immunoprotective responses that cause elimination of pathogens such as rotaviruses, *Helicobacter pylori*, *Salmonella*, and modulation of host immunity (Razavi et al., 2021). Consumption of probiotics (as food products or as dietary supplements) is a major concern among consumers nowadays. The increased awareness of consumers for improving the immune system widened the application of probiotics in food products rather than the consumption of chemically synthesized drugs as the consumption of probiotics in higher quantities is an alternative way to prevent several health problems despite taking antibiotics treatment (Reid et al., 2003).

The most used probiotic microorganisms belong to *Lactobacillus* and *Bifidobacteria* group; however, other species such as *Bacillus cereus* and *Escherichia coli* Nissle 1917 (a non-pathogenic strain) also have been employed as probiotics to accomplish the objectives such as preventing relapse of ulcerative colitis, improving the immune system, and inducing positive effects on allergy or inflammatory diseases (Solanki et al., 2013). To fulfill these beneficial health effects, probiotics should be metabolically active during the transit of the stomach to the intestine in an adequate amount (Sanz, 2007). The probiotic bacteria should be metabolically active at the time of consumption with a population of more than 10^{6-7} CFU/mL or g end products to fulfill numerous physiological functions in the animal/human body (Adhikari et al., 2000). Under the functional food category, probiotic foods have the widest global market accounting for 60–70% of the global foods and rise to $176.7 billion in 2013 from $33 billion in 2000 (Granato et al., 2010; Hennessy, 2014; Holzapfel et al., 2006). The probiotics market is predicted

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to have a compound annual growth rate (CAGR) of 7.0% from 2018 to reach USD 69.29 billion by 2023 (Liu et al., 2020). The consumption of probiotic food products is a natural alternative to the administration of drugs to prevent many diseases, especially in the COVID-19 situation (Misra et al., 2021).

To achieve the probiotics in a metabolically active state and targeted release in the colon of the host with the desired quantity ($> 10^6$ CFU/mL or g of end product) for fulfilling the physiological functions in the human body, probiotication of food products, i.e., incorporation of microencapsulated probiotics with suitable coating materials in food matrices, can be an advantageous and cost-effective concept as probiotics loss their viability due to the exposure to several harsh conditions such as acidic conditions of the stomach, thermal stress of the heating process, high shearing action, freezing/thawing process, complex food systems, and environmental stress during storage of food products. So, the purpose of this review is to emphasize on health benefits of probiotics, unexplored/less commonly used novel microencapsulation technologies with the suitable coating materials for encapsulation of probiotics, their release mechanism, application of the microencapsulated powder in different food products as effective carriers of probiotics with the recent trends, and the packaging/storage conditions to enhance the shelf-life of products along with the embedded probiotics.

Probiotics

The "probiotics" is a broad term that includes some other terminologies of different functionalities such as postbiotics, prebiotics, and synbiotics. Postbiotics can be defined as metabolic by-products of the probiotic origin or non-viable bacterial products which include organic acids, bacteriocins, acetaldehydes, ethanol, diacetyl, and hydrogen peroxide (Vallianou et al., 2020). These metabolic products show a wide inhibitory property toward pathogens so that they can be used as an alternative to antibiotics. Different probiotic species such as *Bifidobacterium lactis*, *Bacteroides fragilis*, *Lactobacillus*, *Escherichia coli*, and *Bifidobacterium breve* are characterized by the properties of postbiotics such as nontoxicity, nonpathogenic, and resistance to hydrolysis by mammalian enzymes (Cicenia et al., 2014). Prebiotics are defined as such nutrients which can able to modify the gut microflora and help in the growth of beneficial microorganisms in the gut (Rastall & Gibson, 2015). Prebiotics can be extracted from natural resources like grains, fruits, and vegetables. Some of the common prebiotics are fructooligosaccharides, lactulose, inulin, galactooligosaccharides, arabinoxylan, and resistant starch-1, 2, 3, 4 (Hutkins et al., 2016). These carbohydrates supply energy to epithelial cells in the colon by the fermentation process due to the action of gut microbiota. The fusion of prebiotics and probiotics results in the formation of synbiotics. The synergistic effect comes to an action when both prebiotics and probiotics work together in the living system and provide several benefits such as enhancing the survival of probiotics, disease prevention, and improvement in nutritional status. Commercial functional foods containing synbiotic relationship is gaining popularity from the point of improving gut health. Past studies have illustrated the potentiality of consumption of probiotic-containing fermented foods to prevent upper respiratory tract infection among children and adults (Makino et al., 2010). The probiotics *Lactobacillus delbrueckii* ssp. *bulgaricus OLL1073R-1* showed positive effects against viral infection such as the common cold by increasing the natural killer cell activity in elderly people (Sindhu et al., 2014). Another study reported that the probiotic strain *Lactobacillus rhamnosus GG* reduced the severity and occurrence of rhinovirus infection in subjects after consumption for 6 months (Kumpu et al., 2015). Probiotics inhibit angiotensin-converting enzyme (ACE), a potent vasoconstrictor by converting food proteins to bioactive peptides during the fermentation process; produce short-chain fatty acids (SCFAs) that helps in the activation of the intestinal mucosal immune cells in the human gastrointestinal (GI) tract; improve the host defense system by strengthening the communication between the gut microbiota with the epithelial and mucosal lymphoid elements (Ayyash et al., 2020). Different probiotic strains such as *Lactobacillus gasseri*, *Lactobacillus rhamnosus*, *Pediococcus pentosaceus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, and *Bifidobacterium longum* have been recognized to combat COVID-19 (Baud et al., 2020). Thus, the administration of probiotics can be an alternative therapy during the COVID-19 pandemic. Probiotics when administrated orally have to survive in the harsh conditions during their passage through the GI tract with the potentiality to modulate the gut microbiota. The boosting of immunity by probiotics can be explained by their mechanism of action in the gut of the host.

Mechanism of action

The mechanism of action of probiotics is strain-dependent which can be categorized into three modes of action. Firstly, probiotics may affect the host's immune system (generally the gut-associated immune cells and the gut epithelial cells). The adhesion between host immune cells and probiotics leads to immune modulation (Burgain et al., 2011). Secondly, probiotics possess the ability to compete with the pathogens thus arresting their adherence to the intestine; thirdly, probiotics are capable to alter some host products such as bile salt, food ingredients, and microbial products such as toxins (Frakolaki et al., 2021). Probiotics also produce metabolites such as amino acids, peptides, and vitamins which regulate the host immune system thus the immune

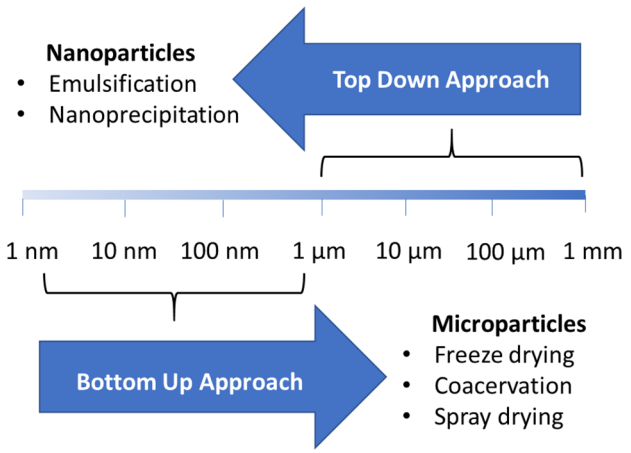

Fig. 1 Top-down and bottom-up approaches in encapsulation techniques (adapted from Ezhilarasi e 2013)

response can challenge the risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections (Koirala & Anal, 2021).

Microencapsulation

Several parameters such as oxygen, pH, storage temperature, hydrogen peroxide, and many others affect the viability of probiotics (Reque & Brandelli, 2021). To improve the resistance against the harsh conditions, different methodologies have been suggested including the use of oxygen-impermeable containers, appropriate selection of acid, and bile resistant strain with their adaptation to different stresses, microencapsulation, and addition of micronutrients such as peptides and amino acids (Sarkar, 2010). Among the abovementioned methods, microencapsulation is the most preferred method which is the primary consideration of many researchers.

Microencapsulation is a novel preservation method in which probiotic strains are entrapped within a selective supporting membrane to avoid the deterioration of cell mass or cell injury and to achieve a targeted release in the gut in an adequate amount (Ermis, 2021). The end product of this technology is encapsulated powder which is easier to use and the homogeneity is maintained throughout the process (Mortazavian et al., 2007). Based on the size of the polymeric beads, cell immobilization by encapsulation techniques can be broadly categorized as macroencapsulation and microencapsulation (John et al., 2011). The polymeric beads formed by macroencapsulation process range from a few millimeters to centimeters whereas; beads size of 1–1000 µm is produced by the microencapsulation process. Figure 1 shows the size ranges of microcapsules produced by different encapsulation technologies. The microencapsulation process is more advantageous as macroencapsulation results in lower cell viability (Uludag et al., 2000).

The formed microcapsules are classified as matrix and reservoir types. The dispersion of active agents over the carrier material forms matrix type microcapsule wherein the case of reservoir type, a shell is coated around the core material. Different types of microcapsules viz. core–shell, mononuclear, polynuclear, multilayer, matrix, irregular prepared using various encapsulation methods are shown in Fig. 2. The encapsulation efficiency is dependent on the survivability of microorganisms and their ability to multiply. The viability of encapsulated probiotic strain depends on the concentration and type of coating material, physicochemical properties of capsules, initial cell number, as well as the type of bacterial strain. The carrier material must be able to protect the encapsulated substances as well as it must be food grade for incorporation in food products. The objective of encapsulation is to protect against adverse conditions and allow the release of viable and metabolically active probiotics in the intestinal part (Picot & Lacroix, 2004). The microparticle should be water-soluble to maintain the integrity in the upper part of the GI tract as well as in the food matrix with a progressive liberation of the entrapped cells during the passage of the intestinal phase.

Coating material

Different polymeric materials such as polysaccharides (alginate, starch, chitosan, cellulose acetate phthalate, k-carrageenan, plant gum), protein (gelatin, milk protein), and fat have been incorporated for the development of microparticles. Sodium alginate is a widely used polymer for encapsulation as it forms a nontoxic, biocompatible, and highly versatile matrix for the protection of microorganisms against adverse conditions during processing and storage. The sensitivity of the formed gel by sodium alginate to extreme pH values can affect the protection of encapsulated materials as well as the release of core materials (Mortazavian & Sohrabvandi, 2006). The application of prebiotics such as

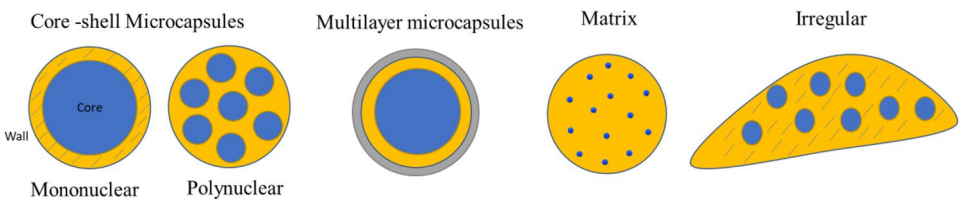

Fig. 2 Different types of microencapsulation: core–shell microcapsules mononuclear, polynuclear, multilayer microcapsules, matrix, irregular

resistant starch in the formulation can overcome this drawback and enhance the stability of probiotic bacterial cells (Chen et al., 2005). Alginate has been used as a wall material for probiotics due to its sensitivity towards pH (Allan-Wojtas et al., 2008). Nami et al. (2020) observed that *L. lactis* encapsulated with alginate, Persian gum, and inulin showed only a 1.46 log CFU/g decrease in viability, while the unencapsulated cells indicated a reduction of 6.52 log CFU/g after 120 min of simulated digestive condition (pH 2.5). Guimarães et al. (2013) encapsulated *Bifidobacterium animalis* DN-173 010 and *L. rhamnosus* GG ATCC 53,103 with calcium alginate which showed higher viability (above 5 log CFU/g) while the free cells were found to be nonviable at pH 2.0 and 2.5 after 180 min in a simulated gastric fluid condition. Similarly, gelatin-alginate microspheres provided higher stability of *Bifidobacterium adolescentis* 15703 T with a 1.21 log CFU/mL decrease in the viability as compared to free cells with a loss of 3.45 log CFU/mL in the harsh environment of gastric conditions (Annan et al., 2008). *Enterococcus faecium* MC13 encapsulated with alginate-chitosan capsules showed no release of cells after 144 h in simulated gastric fluid (SGF) condition (Kanmani et al., 2011). The addition of prebiotics such as galactooligosaccharides enhanced the viability of *Bifidobacterium breve* cells to 8.0 log CFU/mL in the chitosan-coated multiparticulate system added with prebiotic poly(D,L-lactic-co-glycolic acid) and showed an improved result over alginate-chitosan microencapsulation system (1.4 log CFU/mL) (Cook et al., 2014). Thomas et al. (2014) revealed that the strong electrostatic interaction between chitosan and dextran sulfate protected *S. boulardii* cells at low pH of the gastric condition with higher survivability of 7.19 log CFU/100 mg, where only 4.24 log CFU/100 mg of uncoated cells were survived after 2 h of the exposure period. Pandey and Mishra (2021) optimized the shell composition for microencapsulation of probiotic *Lactobacillus plantarum* as 0.4%, 4.6%, and 8.4% of inulin, dextran, and maltodextrin, respectively, and obtained an encapsulation efficiency of 99.21% as well as the produced microcapsules were highly thermostable. Barajas-Álvarez et al. (2021) observed that gum arabic and trehalose microparticles exhibited greater protection to the encapsulated *Lactobacillus rhamnosus* with a viability loss of 3.02 log CFU/g, whereas the loss of 6.21 log CFU/g was found for free bacterial cells after 2 h of simulated intestinal conditions.

Whey protein is a mixture of globular protein isolated from whey and exhibits a wide opportunity from the point of protection and reversion of the binding of active substances before their targeted release in the host. The high temperature leads to deterioration of probiotic bacteria thus heat-treated, denatured whey proteins have been used to encapsulate probiotics at 35–40 °C (Razavi et al., 2021). Many current studies illustrated the microencapsulation of probiotics by employing whey protein in the form of whey protein isolate (WPI), whey protein concentrate (WPC), and sweet whey (the product containing both casein and whey protein). Li et al. (2019) encapsulated *Lactobacillus casei* by using WPI, gellan gum, and cellulose acetate phthalate matrixes and observed the highest viability of probiotics (8.25 log CFU/g) in the WPI matrix as compared to free cells (8.15 log CFU/g) after freeze drying as well as found long term stability of bacteria (7.59 log CFU/g) up to 19 weeks at 4 °C. Loyeau et al. (2018) reported that incorporation of high molecular weight dextran (450 kDa) with WPI as coating materials resulted in only a 0.01 log reduction of viability of probiotic strain *Bifidobacterium animalis* after spray drying. Similarly, Agudelo-Chaparro et al. (2021) observed improved viability of 8.74 log CFU/g after spray drying of *Lactobacillus rhamnosus* with WPC, maltodextrin, and trehalose.

Sweet whey behaves as efficient carrier material for probiotics in liquid form and enables the protection of the microbes in the simulated GI condition (De Castro-Cislaghi et al., 2012). In this study, the viability of free cells of *Bifidobacterium animalis* decreased by 1.51 log CFU/g whereas, microencapsulated cells with sweet whey showed only 0.73 log CFU/g loss after exposure to pH 2.0 for 3 h. Similarly, at low pH, sweet whey maintained the survival of *Lactobacillus acidophilus* La-5 but the free cells presented a significant reduction of 3.11 log cycles after 7 h of GI simulation study (Maciel et al., 2014).

Gharibzahedi and Smith (2021) emphasized legume proteins such as soy protein concentrate, soy protein isolate, pea protein concentrate, pea protein isolate, chickpea protein isolate, lentil protein isolate, and faba bean protein isolate as potential and alternative probiotic encapsulants to animal proteins because of their better gel-forming and emulsifying activity, superior solubility properties, and thermal stability.

Raddatz et al. (2020) observed that pectin microparticles containing probiotic strain *Lactobacillus acidophilus* presented higher encapsulation efficiency of 68.1% when prebiotic such as inulin was incorporated in the encapsulation matrix, while the control (pectin alone) exhibited an efficiency of 64.9% after the freeze drying process. Beads made up of sodium alginate and amidated low-methoxyl pectin in the ratios of 1:4 and 1:6 provided improved resistance to the entrapped *Lactobacillus casei* in simulated gastric and bile salt condition significantly (Sandoval-Castilla et al., 2010). Moghanjougi et al. (2021) reported that pectin nanoparticles were resistant to both enzymatic and acidic conditions thus the microcapsules could be released in the colon environment, whereas alginate was suitable for encapsulation of probiotic bacteria *Lactobacillus acidophilus* under acidic conditions due to the conversion into insoluble alginic acid. The coating of whey protein concentrate and pectin did not provide additional protection to the probiotics in simulated GI conditions despite the higher survivability of probiotics (8 log CFU/g) after encapsulation (Gebara et al., 2013). Application of Hi-maize starch as a protective

agent in freeze dried pectin-rice bran capsule entrapped with *Lactobacillus plantarum* showed the highest survivability (i.e., 8.63 ± 0.01 log CFU/g) as compared to the uncoated ones (5.63 ± 0.02 log CFU/g) after the encapsulation process (Chotiko & Sathivel, 2016).

Application of resistant starch in microencapsulation technology can solve many technical challenges such as increasing the shelf-life stability of sensitive compounds, improving the thermal stability, as well as controlling the release of bioactive molecules (Mirzaei et al., 2012). The encapsulation efficiency of probiotics *Lactobacillus acidophilus* was improved to above 94% by the addition of potato, rice, and maize resistant starch with the control medium comprising of WPC, maltodextrin, and D-mannose which showed a lower efficiency of 79.90% after spray drying (Muhammad et al., 2021). Co-encapsulation of resistant starch (Hi-maize) of the bacterial cell has been carried out to accomplish the synbiotic approach (Nugent, 2005). Resistant starch (Hi-maize) at a concentration of 1% provided better protection to *L. acidophilus* microencapsulated with sodium alginate beads in simulated GI juice. It also preserved the viability of probiotics (6 log CFU/g) up to 135 days of storage period whereas the microcapsules coated only with alginate lost the viability after 60 days of storage (de Araújo Etchepare et al., 2016). Co-encapsulation of *Lactobacillus* F19 and *Bifidobacterium* Bb12 with corn starch in casein-based microcapsules affected the physical barrier of the matrix negatively and resulted in the reduction of protective effect (Heidebach et al., 2010).

There are some other polymers available for microencapsulation of probiotics which are enlisted in Table 1 with their formations, mechanisms, and barrier properties that will be helpful to researchers for screening and evaluation of protective wall materials.

Novel technologies for microencapsulation of probiotics

Sophisticated shell materials and technologies have been developed for the encapsulation of sensitive bioactive compounds with an extremely wide variety of functionalities. The factors affecting the release of encapsulated ingredients are mechanical stress, change in temperature, pH, time, enzymatic activity, osmotic pressure, etc. The innovativeness of microencapsulation technologies is a great benefit to society, which cannot be achieved by the non-encapsulating method so that the consumer can compromise the cost-in-use and also get improved value-added products.

There are many research works and reviews already available on microencapsulation of probiotic bacteria by spray and freeze drying. So, this review paper emphasizes other novel emerging technologies with their application in the controlled release of probiotics as well as improving the storability of microencapsulated particles to develop a comparative study by considering their merits and demerits.

Vacuum drying

Vacuum drying is similar to freeze drying with a difference in the removal of water vapor through the evaporation process rather than sublimation. Generally, vacuum dryers operate at a higher temperature and higher pressure (above 10 mbar) as compared to freeze dryers but the process parameters are lower than spray drying. Loss of viability of heat-sensitive probiotics is less in vacuum drying as the process can handle the thermal stress gently. Oxygen-sensitive probiotics such as *Bifidobacteria* can be effectively handled by this drying process but severe viability loss can also be happened due to dehydration stress. Cell wall and cell membrane of vacuum dried cells are two main sites of damage that have been observed by atomic force microscopy and Fourier transform infrared spectroscopy (Santivarangkna et al., 2007). From the point of targeting to the cellular membrane, the process parameters should be optimized to improve cell viability and preserve the probiotics from dehydration stress. There are many possible ways to improve the viability such as alteration of process parameters, the addition of protecting agents, or pre-treatment of cells with sub-lethal stress before vacuum drying.

The application of protective agents such as sugars or polyalcohol is beneficial for bacterial viability. Different sugars such as trehalose, lactose, and polyalcohol such as sorbitol have been used in the vacuum drying of probiotics (Crowe, 2007; Foerst et al., 2012). With the addition of 25% (w/w) of trehalose or sorbitol, the survivability of vacuum dried *L. paracasei* enhanced from 29 to 70% and 54%, respectively (Foerst et al., 2012). Conrad et al. (2000) studied vacuum dried *L. acidophilus* strain with the addition of 20% (w/w) trehalose. The survivability increased from 18.9% to 37.9% within 4 days at room temperature. Santivarangkna et al. (2007) revealed that the viability of *L. helveticus* WS1032 was doubled by the addition of 1% (w/w) sorbitol to the cell suspension after vacuum drying and there was a negative correlation between the additions of protectant from 10 to 100% (w/w) and probiotic viability. It has been studied that by increasing the concentrations of trehalose from 50 to 250 mM, the viability increased whereas, the survivability showed the lower value at a higher concentration of trehalose due to an increase in an osmotic gradient (Gómez Zavaglia et al., 2003).

Bacterial viability and water activity were greatly influenced by the process parameters of vacuum dryers such as drying time and temperature. Chances of bacterial damage

Table 1 Characteristics of commonly used polymers for microencapsulation of probiotics

Wall material	Source	Chemical structure	Mechanism	Remark	References
Alginate	Brown seaweed	• Linear heteropolysaccharide composed of α-L-guluronic and β-D-mannuronic acids	• Ionotropic gelation due to cross-linking and exchange of sodium ions from the guluronic acids with divalent cations (Ca^{2+}, Ba^{2+}, or Sr^{2+})	• The concentration of alginate commonly used to form the gel ranges from 0.6 to 3%, whereas that of calcium chloride from 0.05 to 1.5 M • Provides a gentle environment for the entrapped materials, exhibits excellent biocompatibility properties with low toxicity and low cost • Its bioadhesive property facilitates coating with other polymer and produces capsules with an average diameter of lesser than 40 µm • The produced microparticles lose and crack their mechanical stability in acidic conditions due to porous structure	Janksowki et al. (1997); Krasaekoopt et al. (2003); Sankalia et al. (2007); Takka and Gürel (2010); Rathore et al. (2013); Sohail et al. (2011)
Milk protein (casein and whey protein)	Milk	• Casein contains lactose and soluble proteins • Whey protein consists of compact globular proteins, with major constituents of β-lactoglobulin and α-lactalbumin	• Casein proteins are used as soluble sodium- or calcium-caseinate to encapsulate suspensions rich in polyphenols by acid coagulation • Heating above 90 °C results in the unfolding of protein which allows protein–protein interaction, hydrogen-bonding, disulfide cross-linking that results in aggregation, coagulation, and precipitation	• A combination of probiotics with whey protein isolate (WPI) will be a valuable addition to processed food products due to biocompatibility and an ideal nutritional source for microorganisms to grow and multiply • Widely used in food products due to its broad functionality such as gelation, emulsification, foaming, and water-binding capacity • Potential device for the controlled release of pharmaceuticals and completely biodegradable • The gel preparation does not require any chemical cross-linking agents	Burgain et al. (2013); Doherty et al. (2011); De Castro-Cislaghi et al. (2012); Raei et al. (2018); Khem et al. (2016); Picot and Lacroix (2004); Gbassi et al. (2011); Rajam et al. (2012); Ying et al. (2013); Gunasekaran et al. (2006)

Table 1 (continued)

Wall material	Source	Chemical structure	Mechanism	Remark	References
Chitosan	Crustacean shell	• The linear polysaccharide of glucosamine with a positive charge which is obtained by deacetylation of chitin extracted from crustacean shells	• Ionotropic gelation • At pH less than 6, it is water-soluble and forms a gel by ionotropic gelation	• Used as immobilizing agent for probiotic bacteria because of its ease of handling, low cost, non-toxicity and it is an accepted food additive • Provides low encapsulation efficiency for probiotics and is preferably used as a coating agent but not as a capsule • Protects probiotics in simulated GI conditions due to the controlled release and therefore, provides targeted delivery of viable bacterial cells to the colon when associated with alginate • Low-concentration chitosan solution (2–4 g/m^3) has been applied for shell-making on alginate and gelatin capsules	Chen et al. (2017); Gandomi et al. (2016); Mirtič et al. (2018); Chávarri et al. (2010)
Starch	Maize, potato, barley, oat, etc	• Polysaccharides consisting of α-D-glucose units (mainly amylose and amylopectin) connected by glycosidic linkages, produced by all green plants	• Thermal gelation • Calcium ions are interacted with the hydroxyl and phosphate groups of starch to produce dense structures	• Exhibit film-forming ability, which may protect encapsulated substances • Resistant starch (RS) is that portion of the starch, which is not affected by digestion in the small intestine and thus may be subjected to fermentation in the colonic condition which shows its efficacy of target delivery of probiotics • Provides thermal stability, and controlled release of bioactive molecules thus enhancing the shelf life of sensitive compounds • Protects probiotic cells when used in conjunction with alginate and exhibits the synergistic effect	de Araújo Etchepare et al. (2016); Benavent-Gil et al. (2018); Ziar et al. (2012)
k-carrageenan	Marine macroalgae, red algae	• Neutral polysaccharide comprising of alternating 1–4 linked α-D galactopyranose units and 1–3 linked β-D-galactopyranose	• Thermal gelation	• Temperatures between 60 and 90 °C and concentrations of 2–5% are required for dissolution • Excess potassium employed for cross-linking may cause toxicity to microbial cells and the produced gels are brittle	Martín et al. (2015)

Table 1 (continued)

Wall material	Source	Chemical structure	Mechanism	Remark	References
Gelatin	Collagen	• Protein gum comprising of 4-hydroxyproline residues and glycine proline	• Physical cross-linking using irradiation, high pressure/thermal/cross-linking using glutaraldehyde or formaldehyde	• Used as an encapsulating agent due to its thermo-reversible gelling behavior • Forms strong interaction with anionic gellan gum due to its amphoteric nature, when adjusting the pH below its isoelectric point	Yao et al. (2018); Picone et al. (2017); Vaziri et al. (2018); Martín et al. (2015); Rathore et al. (2013)
Pectin	Apple pomace, citrus peels, and sugar beet pulp	• Anionic water-soluble polysaccharide contains large amounts of methylated carboxylic groups of D-galacturonic acid formed by the esterification process	• Ionotropic gelation of ionic polysaccharides due to certain divalent cations • High-ester pectin (degree of esterification, DE, > 50%) forms gels due to hydrophobic interaction and hydrogen bonding at pH below 3.5 • Low-ester pectin (DE < 50%) forms gels with divalent cations such as calcium ions	• Produces a stable emulsion at a concentration between 1 and 2% • Resistant to active enzymes such as amylase and protease in the upper gastrointestinal tract but digested easily by pectinase produced by colonic microflora • Sugar beet pectin has good emulsifying properties but exhibits a poor gelling ability • The high solubility property limits the release and diffusion rates but the application of insoluble film-forming polymers such as ethylcellulose can overcome this problem • Gel strength depends on the concentration of calcium, which is an important parameter to control the drug release	Zhang et al. (2016); Dafe et al. (2017a); Sun et al. (2019); Prezotti et al. (2014); Jung et al. (2013); Martín et al. (2015); Li et al. (2016); Sandoval-Castilla et al. (2010); Souza et al. (2012); Rodrigues et al. (2015); Chotiko and Sathivel (2016)
Carboxymethylcellulose	Agricultural waste (Sugarcane bagasse, sugar beet pulp, sago waste, papaya peel, Mimosa pigra peel, etc.)	• Water soluble-cellulose ether derivative consisting of β-linked glucopyranose residues with varying levels of carboxymethyl substitution	• Cross-linking with aluminum ions	• Exhibits gastric acid resistance and intestinal solubility properties • Natural and biocompatible polymer, used as food gum	Singh et al. (2018); Dafe et al. (2017b); Chitprasert et al. (2012); Singh et al. (2017); Demitri et al. (2017); Yonekura et al. (2014); Solanki and Shah (2016); Asl et al. (2017)
Cellulose acetate phtalate	Chemical synthesis (reaction of a partially substituted cellulose acetate (CA) with phthalic anhydride in the presence of an organic solvent and a basic catalyst)	• A cellulose ester derivative contains anhydrous glucose units, of which about one-half of hydroxyl groups are acetylated and about one-fourth are esterified with one of the two acidic groups of phthalic acid	• Coacervation phase-separation technique	• Insoluble at pH ≤ 6 but soluble at pH ≥ 6 due to the presence of phthalate groups • Provides good protection for microorganisms in simulated GI conditions	Martín et al. (2015); Rao et al. (1989); Fávaro-Trindade and Grosso (2002); Ganguly et al. (2011); Chaturvedi et al. (2011)

Table 1 (continued)

Wall material	Source	Chemical structure	Mechanism	Remark	References
Locust bean gum	Seeds of the carob tree	• Polysaccharide composed of mannose and galactose units	• Ionotropic gelation	• The strength of carrageenan gel can be enhanced using locust bean gum • The best ratio of carrageenan to locust bean gum is 2:1, which can make strong gel beads with synergistic effects	Cheow et al. (2014); Shi and et al. (2013a, 2013b); Damodharan et al. (2017)
Xanthan gum	*Xanthomonas campestris*	• Anionic polysaccharide consists of a backbone of β-(1–4)-D-glucopyranose glucan with side chains of (1–3)-α-D-mannopyranose–(2–1)-β-D-glucuronic acid–(4–1)-β-D-mannopyranose on alternating 4,6-pyruvated and 6-acetylated mannose residues	• Ionotropic gelation in the presence of calcium ions	• Highly resistant to enzymatic degradation, and variations in pH • Forms stable microspheres with gellan gum at low pH	Albertini et al. (2010); Ding and Shah (2009); Rathore et al. (2013)
Gellan gum	bacterium *Sphingomonas elodea*	• Extracellular anionic heteropolysaccharide consists of tetrasaccharide (1,3-β-D-glucose, 1,4-β-D-glucuronic acid, 1,4-β-D-glucose, 1,4-α-L-rhamnose) repeating units	• Ionotropic gelation (thermo-reversible gel is formed in the presence of divalent cations (Ca^{2+} and Mg^{2+}) at low concentrations (0.01–0.20%)	• The gel characteristics depend mainly on pH, the degree of substitution, temperature, and concentration • Resistant to an acidic condition	Moslemy et al. (2002); Muthukumarasamy et al. (2006); Rosas-Flores et al. (2013)
Human-like collagen	Recombinant *E. coli* BL21 containing human-like collagen cDNA	• Consists of amino acids bound together to form a triple helix of elongated fibril	• Cross-linking	• Used as scaffolding and hemostatic biomaterial for the regeneration of organs or tissue and functional food product development • Exhibits low immunogenicity, excellent water solubility, good product stability, and is virus-free	Martín et al. (2015); Su et al. (2011); Song et al. (2017)
Flaxseed mucilage	Flaxseed	• Contains a mixture of rhamnogalacturonan I and arabinoxylan	• Cross-linking	• Contains prebiotic properties • Reduces glucose level and cholesterol in diabetics	Bustamante et al. (2015)
Gum Arabic	*Acacia senegal*	• A primary group with polysaccharide of galactose unit as backbone along with rhamnose and arabinose branching units, whereas a second group consists of arabinogalactan-protein complex	• Cross-linking	• Exhibits a higher survival and the least population reduction of probiotics • Enhances the survival of *Bifidobacteria* in the adverse stomach condition	Nie et al. (2013); Lian et al. (2003); Arslan et al. (2015)

Table 1 (continued)

Wall material	Source	Chemical structure	Mechanism	Remark	References
Polymers (dextran, maltodextrin)	Starch hydrolysis	• High molecular weight polysaccharides	• Cross-linking	• Increases the stability of spray-dried capsules in terms of moisture content, water activity, hygroscopicity, pH, glass transition temperature, solubility, color, nutritional composition, and fluidity • Caking and stickiness can be reduced by the addition of maltodextrin to the coating material thus resulting in a free-flowing spray dried powder • The powder recovery can be increased by reducing the maltodextrin ratio but it may result in low encapsulation efficiency	Reyes et al. (2018); Semyonov et al. (2010)
Disaccharides (lactose, trehalose)		• Combination of monosaccharides by glycosidic linkage	• Cross-linking	• Maintains the membrane integrity by replacing water molecules with hydroxyl groups of saccharides and interacting with phospholipid bilayers of the bacterial cell membrane • Trehalose is a well-known dehydration protectant • Accumulation of sucrose as an osmolyte is advantageous for stressed bacteria • Prevents Maillard reactions due to its non-reducing nature	Nunes et al. (2018); Zhang et al. (2016); Broeckx et al. (2017)
Polyol (mannitol, sorbitol)	Hydrogenation of sugars	• Sugar alcohol containing multiple hydroxyl groups		• Exhibits stress protectant ability • Protect against osmosis, temperature, salt, and oxidative stress in microbial cells by maintaining a crystalline structure	Perdana et al. (2014); Semyonov et al. (2010)
Prebiotics (inulin, oligofructose, and oligofructose-enriched inulin)	Chicory roots	• Inulin consists of chains of fructose units • Oligofructose is obtained through partial hydrolysis of inulin	• Cross-linking	• Provide a synbiotic effect and improve the viability of probiotics during drying and processing conditions • Due to the low glass transition temperature of inulin, it results in sticky powder	Fritzen-Freire et al. (2012); Ananta et al. (2005)

Table 1 (continued)

Wall material	Source	Chemical structure	Mechanism	Remark	References
Polyacrylamide	Chemical synthesis	• Consists of acrylamide subunits	• Cross-linking using N,N-methylenebis (acrylamide), ammonium persulfate, N,N,N,N-tetramethylenediamine	• The hardness of formed gel is affected by the ratio of cells to acrylamide content and produces porous and stable gel • Causes loss in cell viability due to the radical-dependent polymerization reaction and the reagent used for cross-linking may provide toxicity • Used as a synthetic polymer	Calvet et al. (2004); Wyss et al. (2004)
Polyvinyl alcohol (PVA)		• Composed of vinyl alcohol subunits	• Cross-linking using boric acid	• Boric acid causes damage to the microbes enclosed in the PVA matrix during the bead preparation process • The addition of sodium sulfate in boric acid can reduce toxicity during the cross-linking process	Gao et al. (2004)

can be prevented by shortening the processing time as well as the temperature. Tymczyszyn et al. (2008) observed an increase in the damage of the cell membrane and decreasing in water activity, which was due to an increase in temperature from 30 to 70 °C in the vacuum drying of *L. delbrueckii* subsp. *bulgaricus* CIDCA333. A similar trend was observed with an increase in treatment time at a particular temperature. The viability of *L. helveticus* WS1032 during vacuum drying and after 12 h of the storage period sharply declined due to rupture on the cell surface as well as cell lysis (Santivarangkna et al., 2006). The survival rate of *L. plantarum* CIF17AN2 was higher at 4 mbar pressure and 57 °C temperature up to 12 h drying time. Thermal damage of probiotic cells can be avoided by reducing the vacuum pressure and the viability can be enhanced with the addition of glycerol (Bauer et al., 2012). Freeze drying has been proved better than vacuum drying from the point of attaining high survivability of probiotic strains, so fewer research papers are available on vacuum drying.

Fluidized bed drying

Fluidized bed drying is a process involving the application of conditioned air usually heated gas passed through a suspended bed of solid particles at a controlled velocity. The problems arise in the drying of the bacterial cell due to its relatively small size (several micrometers). It is difficult to keep the dried particles of low density in the suspended state which may result in a lowering of yield. Moreover, it is better to combine the fluidized-bed drying with other techniques such as spray drying or freeze drying. However, this technique is a preferred encapsulating method due to the formulated probiotics of reduced moisture content.

In the fluidized bed drying method, different carrier or matrix molecules such as casein, cellulose, maltodextrin, NaCl particles, or lactose have been used for encapsulation of probiotics (Bensch et al., 2014; Mille et al., 2004). This drying method involves the spraying of bacterial cell suspension on the fluidized bed carrier followed by spray or freeze drying to obtain bacterial pallets and subsequently, encapsulation of the particles with the protective carrier or shells by fluidized bed drying to enhance the cell viability (Azim et al., 2012). To improve the storability in the long term, the probiotic cells are encapsulated with different materials such as fats, proteins, or polysaccharides as coating agents. The coating layer protects the encapsulated probiotic cell by minimizing moisture diffusion over the storage period. This method has rarely been used for encapsulation of probiotics but the produced powder exhibits lower cohesiveness thereby improving higher flowability, which is the main advantage of this technique.

The viability of probiotics is influenced by the selection of protectants and controlling the process parameters as well

as the stress response of bacterial cells before drying. Different protectants such as saccharides, skim milk, or alginate matrix have been used with probiotics in fluidized bed drying. Wu et al. (2021) optimized top fluidized bed drying conditions to encapsulate probiotic culture *Lactobacillus brevis* RK03 and found that the highest survival of 95% was found at the processing time of 40 min at 50 °C by employing casein and whey protein isolate (5% w/v) as carrier materials. Strasser et al. (2009) studied the survivability of *L. plantarum* IFA No. 278 by adding glucose, sucrose, trehalose, or maltodextrin (32% w/v) and the protectants enhanced viability more than 5 times whereas; the survivability is reduced to 0.2% without any protectants. The addition of trehalose and sucrose (32% w/v) enhanced the survivability to 36.9 and 36.4%, respectively. The probiotic strain *L. helveticus* CNRZ 303 encapsulated in alginate beads showed the highest viability of 70.7% with the addition of reconstituted nonfat milk solids as compared to 0.5 M odonitol and glycerol (Selmer-Olsen et al., 1999). *Enterococcus faecium* IFA No. 278 had lower survivability (11%) without any application of protective carriers during the drying process (Strasser et al., 2009). The addition of sucrose or skim milk (10% w/w) resulted in no increase in survivability of *E. faecium* M74 as compared to the unencapsulated cells (Stummer et al., 2012).

Controlling process parameters is a considerable aspect of improving the survivability of probiotic cells. Fluidized bed drying influences the moisture level of the dried product. During the drying process, when the moisture level is above 15%, the drying temperatures have no significant impact on the survivability of probiotics (Bayrock & Ingledew, 1997). Other parameters such as water activity, atomizing air pressure as well as spraying time greatly influence the survivability of the encapsulated bacteria. There were 4 log units of viability loss of *E. Faecium* M74 when the spray time and pressure were increased above 30 min and 1.5 bar, respectively (Stummer et al., 2012). Some other parameters such as loading rate, hot air humidity, and temperature have been taken into considerations by analyzing the heat and mass transfer and applying different mathematical and empirical models (Akbari et al., 2012; Debaste et al., 2008). The prestressing of probiotics has been proved beneficial from the point of enhancing survivability. The probiotic *L. casei* CRL 431 cells were stressed osmotically and showed better survivability after fluidized bed drying as compared to unstressed cells (Nag & Das, 2013).

Higher temperature causes a higher declination in the survival rate of entrapped microbial cells during the storage period. When dried *E. faecium* IFA No. 278 and *L. plantarum* IFA No. 045 powder were stored at 4 °C the survival rate was higher as compared to the storage temperature of 22 and 35 °C (Strasser et al., 2009). The increased storage temperature of 20 °C resulted in a greater reduction of viability of *L. plantarum* than 4 °C during the storage period of 3 months (Bensch et al., 2014). It has been observed that vitamin E has a crucial role in enhancing the stability of probiotics from oxidative damage. *L. casei* CRC 431 maintained its stability during the storage period of 20 weeks at a temperature of 25 °C with the addition of 0.5% (w/w) vitamin E (Nag & Das, 2013). Rehydration temperature is a critical index from the point of recovery of probiotics. It has been observed that a higher rehydration temperature causes better recovery. For example, dried *L. helveticus* CNRZ 303 showed higher recovery at temperatures of 20 and 30 °C as compared to the temperature of 5 °C, and higher rehydration temperature (30–37 °C) showed better results in terms of improving the survivability of probiotic strains *L. bulgaricus* RD 546 and *L. plantarum* RD 263 (Mille et al., 2004; Selmer-Olsen et al., 1999).

Microwave drying

An efficient and effective dehydration method is required for successfully balancing between physiological activity and higher cell viability of dried cultures. The oxidative potential at minimal pressure levels and low dehydration temperature in freeze dryer leads to instant rehydration capacity and high survivability of probiotic cells as well as provides the favorable application of microorganisms in food industries (Bozoğlu et al., 1987). At the same time, the cost of freezing equipment causes an increase in the cost of the drying process. In contrast, to freeze drying, vacuum drying results in dried starter cultures with improved storability (Foerst et al., 2012). The freeze and vacuum drying methods are time-consuming processes, which limits the throughput capacity and ultimately results in high production costs.

Microwave (MW) energy could help in lowering production costs by significantly shortening the drying time. The availability of microwave radiation to penetrate the product of its whole volume (i.e., volumetric heating) and to heat evenly depends on the geometry and composition of the exposed product. Due to internal heat generation, drying rates become higher which leads to a shortening of drying times (Ambros et al., 2018a, b, c). As opposed to conventional drying methods, drying of microcapsules by microwave radiation results in the generation of inverse thermal gradient that favors the drying of particles from inside out and fast elimination of water which involves much lower temperature and processing time (Mardaras et al., 2021). Nevertheless, microwave radiation might be harmful to probiotics during encapsulation if the processing conditions are not optimized properly (Yoha et al., 2021). The higher processing time and product temperature cause product degradation and loss of bioactivity. Therefore, the short drying time and low product temperature should be targeted to prevent the deterioration of bacterial cells. The effect of microwave energy on the survival of bacteria culture is scanty in

literature. However, some authors mentioned that instead of continuous, intermittent application of microwave energy in pulsed mode improved the product quality and enhanced energy efficiency (Ambros et al., 2018a, b, c). Different researchers applied microwave coupled with other drying techniques such as vacuum and freeze drying to lower the processing time thus preserving the viability of encapsulated probiotics (Ambros et al., 2018a, b, c). In vacuum drying, the heat transfer rate from hot shelves to the product is low due to the lower conduction of heat in vacuum conditions which increases the drying time, whereas microwave energy targets the water molecules directly due to dipolar rotation and ionic interaction. This accelerates the drying process. Combining the microwave energy with a vacuum drying process can shorten the drying time considerably (Karimi, 2010). Microwave combined with vacuum drying caused energy reduction (32–71%) as well as decreased drying time (25–90%) as compared to conventional drying (Duan et al., 2010; Durance & Wang, 2002; Sharma & Prasad, 2006; Varith et al., 2007). Microwave energy can be assisted with freeze drying process to give an energy-efficient output (Ambros et al., 2018a, b, c). The microwave-freeze drying process is similar to the conventional lyophilization process in which frozen water is removed by the sublimation process from the product and microwave vacuum drying is working on the same principle as a conventional vacuum drying method. The major difference is the source of energy supply as in the microwave heating process, electromagnetic energy is converted to heat energy (Haghi & Amanifard, 2008), and removal of water, as well as the transportation of energy, is not affected by heat conductivity barriers (Bouraoui et al., 1994). The microwave-freeze drying maintains the product structure during the entire process by drying in the frozen state. Initially, as the water is present in the frozen state, the resulting structure contains pores after the drying process. After rehydration, the water occupies the pore space instantly and resolves the bacterial cultures leading to the instant character. The microwave-assisted with freeze drying process reduces the time to 50–75% as that of the conventional freeze drying process due to the improved heat and mass transfer (Ambros et al., 2018a, b, c).

The overheating or nonhomogeneous heating of the material and degradation or loss of the bioactivity of probiotics depends on the processing temperature and time of microwave heating (Wang et al., 2014). To avoid the deterioration of bacterial cells, low product temperature, as well as short drying times should be targeted (Bauer et al., 2013). Ahmad et al. (2012) observed that the viability of probiotic strain *Lactobacillus salivarius* 417 was improved when exposed to lower microwave power input and absolute pressure levels; however, the application of skim milk powder as a protectant affected the survivability. Microwaves used in pulse mode enhanced the efficiency of input energy levels as well as the quality of the exposed product (Gunasekaran & Yang, 2007). Kim et al. (1997) studied the D-value for a mixture (1:1) of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* by taking different temperatures and water activities. This study concluded that microwave-vacuum dried yogurt showed higher survivability as compared to spray and freeze dried samples and survival rates declined when dehydration temperature increased. Ambros et al. (2018a, b, c) tested the microwave-vacuum drying process for high retention of bioactivity of the bacterial strain, *Lactobacillus paracasei* subsp. *paracasei* by considering three parameters such as chamber pressure, microwave power level, and minimum product temperature. The study concluded that drying time and microwave efficiency were not affected significantly by the maximum product temperature and vacuum pressure level, but both parameters greatly affected the survival rate of the probiotic strain. The authors observed that microwave power levels between 2 and 3 W/g, a low chamber pressure of 7 mbar with the product surface temperature of 35 °C were found to be most suitable for retention of the viability of probiotics as the low chamber pressure reduced the absorption of microwave energy but power level above 4 W/g resulted in overheating of the product.

The survivability of *Lactobacillus paracasei* F19 was maintained (67 to nearly 100%) at all microwave-freeze drying conditions whereas, the highest survivability of *B. lactis* (almost 100%) was observed at lower microwave power input and chamber pressure combination (i.e., 1.5 W/g and 0.6 mbar) with the reduced drying time (up to 80%) as compared to freeze drying process (Ambros et al., 2018a, b, c). In this study, the authors applied microwave energy in pulsed mode when the drying temperature exceeded 30 °C to avoid further increase in the product temperature and thermal shock to microorganisms and also highlighted that the low chamber pressure maintained the membrane integrity and viability of cells as high pressure resulted in higher drying rates, thus internal thermal stress due to the higher dielectric loss factor and ultimately overheating of products. Mardaras et al. (2021) reported that near fluidizing microwave drying technique with adequate control of process temperatures resulted in the viability of more than 90% for yeast cells *Saccharomyces cerivisae* encapsulated in alginate microcapsules and recommended an inside microcapsule temperature of 30 °C and air temperature of 5–20 °C to protect the probiotics as well as to achieve a good-quality product.

Spray cooling/chilling

The spray cooling/chilling process is mainly used for the entrapment of textural ingredients, flavors, enzymes, and

other functional ingredients by targeting a lot of benefits such as improving the heat stability, conversion of a liquid hydrophilic ingredient into free-flowing powder, and delaying the release in a wet environment. This encapsulation technology is the least inexpensive method typically referred to as a "matrix" type method. In the matrix encapsulation process, the coated material generally releases the whole of the content within a short period after incorporation in the foodstuffs. There are few works of literature available regarding the thermal stability of enzymes by spray cooling method. Due to the presence of active ingredients at the surface of microcapsules, there is a direct assessment of the exposed environment. Therefore, when the microencapsulated material comes in contact with the foodstuffs, the release of active ingredients begins. The factors such as osmotic forces, diffusion of water through the shell material, and mechanical dispersion play a significant role in the release mechanism. The spray chilling process is comparable with the freeze drying process from the point of achieving similar encapsulation efficiency due to the effect of lower temperatures with a lower operating cost (Dianawati et al., 2013).

Though there is a process similarity between spray chilling and spray drying, the major difference lies in the atomization of the coating material. The matrix suspension is injected into a chamber in which the chilled air comes in contact with the droplets and the solidified droplets form the microcapsules with probiotics as core material (Chambi et al., 2008). The solution of wall material is a major influencing factor for encapsulating active ingredients by the spray chilling process. Protein- or carbohydrate-based hydrophilic wall materials are generally employed for microencapsulation in spray and freeze drying technology (Dianawati et al., 2016) while fat-based hydrophobic materials such as fatty acid, phospholipids, fatty alcohols, hydrogenated fat, polyethylene glycol, waxes, triacylglycerol, and their mixture are commonly employed in spray chilling process (Chambi et al., 2008; Sillick & Gregson, 2012).

Spray chilling technology has been applied to microencapsulate different probiotic strains for improving viability. This technique was applied to encapsulate different bacterial cells such as *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, and *Saccharomyces boulardii* in a single and double layer with the addition of hydrogenated palm oil. Single-layered microcapsules showed better survivability of strain; however, microcapsules showed lower resistance in simulated intestinal conditions as compared to spray dried microcapsules (Arslan-Tontul & Erbas, 2017). Application of vegetable fat as carrier material can be considered in spray chilling technology for better protection, application, and delivery of probiotic strains such as *Bifidobacterium lactis* and *Lactobacillus acidophilus* (de Lara Pedroso et al., 2012). This study resulted in improved resistance and stability of probiotic strain not only in simulated gastric conditions but also in 90 days of prolonged storage period. Similarly, the efficacy of solid-lipid microparticles entrapped with *Lactobacillus acidophilus* produced by spray cooling method with the addition of prebiotics was evaluated (Okuro et al., 2013). Polydextrose was found to be an effective prebiotic for the protection and delivery of probiotics with the benefits of the synbiotic effect. Spray chilling technique produced microencapsulated powders of lower moisture content and water activity along with smooth and continuous spherical particle-containing probiotics *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus acidophilus* and this method increased the potentiality for the incorporation of strains into savory cereal bars (Bampi et al., 2016). Similarly, cocoa butter as a carrier material improved the resistance of probiotic strain *Lactobacillus acidophilus* by the spray chilling method in GI fluid conditions. The microcapsules produced by this technique showed higher stability up to 90 days of storage period (Pedroso et al., 2013).

Spray freeze drying

This is an advanced method of encapsulation of probiotics that involves the processing steps of both spray and freeze drying. The probiotic solution is atomized into a container containing cryogenic liquid (generally liquid nitrogen) and frozen droplets are formed in the cold vapor phase which is further dried by freeze drying process to produce dried encapsulated powder (Amin et al., 2020). This process is advantageous due to the formation of microcapsules of a larger specific area and controlled size than spray dried microcapsules and the coating of additional shell materials protects the core ingredients against the adverse conditions (Semyonov et al., 2010). This process consumes high energy due to the long processing time with a requirement of high-cost (30 to 50 times expensive than spray drying), so it is lagging behind conventional spray and freeze drying (Zuidam & Shimoni, 2010).

Maltodextrin as a wall matrix helps in minimizing the mobility of probiotic strains in the glassy state (Semyonov et al., 2010). Different cryoprotectants such as trehalose act as a protective agent for improving the cell viability during freezing and enhancing the storability of dried bacteria. Trehalose prevents structural damage during dehydration by forming hydrogen bonds with the polar head groups of the lipid cellular membrane. In general studies, entrapment of probiotic bacteria in a gel matrix of gellan, alginate, xanthan, and k-carrageenan is a common process for encapsulation, which is then followed by extrusion and emulsion process to form droplets of the desired size. The stabilization of probiotics within gel beads may satisfy the desired output but it is difficult to scale up. From this point of view, the extension of the storage life of microcapsules should also be taken into

account. The spray freeze drying method has been popularized in the pharmaceutical industry (Costantino et al., 2000). The particles formed by this method retain spherical shape as well it shows the porous morphology. The spray freeze drying method has been employed to minimize the irreversible damage to proteins such as aggregation and denaturation (Heller et al., 1997). In spray freeze drying, cooling rates are an important point that should be taken into consideration. It has been calculated that the upper limit of the cooling rate is in the order of 300 K/s (Heller et al., 1999). There are limited research works available to dry the probiotic cells by using spray freeze drying technology.

Her et al. (2015) prepared probiotic powder of *Lactobacillus casei* IFO 15,883 by spray freeze drying technique. The viability of probiotics after spray freeze drying was found to be 97.7% under optimized conditions. Trehalose as carrier material was applied to encapsulate *Lactobacillus paracasei* by spray freeze drying and proved as highly effective from the point of increasing the viability (> 60%) of probiotics (Semyonov et al., 2010).

Hybridization system

This is a dry technique to encapsulate probiotics, which comprises a stator, rotor with six blades rotating at high speed, and a power recirculation unit. Inside the vessel, a stream of air is generated due to the high-speed rotating blades that give high impaction to the powder mixture consisting of the host and guest particles. The guest particles are coated on host particles which form an ordered mixture that minimizes the chances of thermal damage of probiotics due to the cooling effect of air (Ishizaka et al., 1993). Different prebiotics have been tested for double microencapsulation of probiotics such as lactulose, sorbitol, mannitol, inulin, xylitol, raffinose, and fructooligosaccharide by utilizing hybridization technique to provide beneficiary effects to the host (Ann et al., 2007). Microencapsulated *Lactobacillus acidophilu*s ATCC 43,121 prepared by hybridization technique showed prolonged stability as compared to uncoated and single-coated ones when exposed to the acidic or heating condition.

Impinging aerosol technology

This technique has been developed to overcome the damage associated with heat or solvent liable probiotics and has a large throughput capacity. This method requires two separate aerosols, i.e., alginate solution with microbial suspension and another is calcium chloride solution to produce microbeads of a diameter of fewer than 40 µm by the cross-linking process (Sohail et al., 2011). The water-insoluble microbeads could be subsequently sprayed or freeze dried. Sohail et al. (2012) produced alginate microbeads with sizes ranging between 10 and 40 µm containing *Lactobacillus acidophilus* NCFM and *Lactobacillus rhamnosus* GG by the dual aerosol method. The encapsulated probiotics did not show a significant increase in viability at the temperature of 4 and 25 °C but this technique reduced the acidification at both the temperatures and prevented the declination in sensory properties as well as improved the storability of orange and other fruit-based juice products. This is an indication of the absence of the buffering capacity of encapsulating material. However, some studies showed acidification in encapsulated probiotic fruit-based products, which might be due to diffusion of sugar components through polymeric coating material that further metabolizes into organic acid in small size microbeads. The macrobeads (approximately 2 mm diameter) produced by extrusion technique provided better protection to encapsulated probiotics than microbeads (10–40 µm) in high acid and bile salt condition but both the techniques offered comparable results which might be due to chitosan coating of the porous alginate gel matrix as a result of controlling diffusion in the acidic condition (Sohail et al., 2011).

Ultrasonication

In recent years, ultrasonication has been used to encapsulate bioactive compounds in food processing (Leong et al., 2017a, b). Encapsulation using ultrasound has the potential to be operated at large scales with a low cost per unit operation and is capable of making nano and microcapsules/droplets with a narrow size distribution (Leong et al., 2018, 2017a, b). Ultrasound frequency of 16 to 3000 kHz is generally suitable for the processing of fluids. Ultrasound passes through liquid medium and cavitation bubbles are generated and collapsed which produces strong shear and mechanical forces resulting in droplet or capsules formation (Fig. 3). Double emulsions were prepared in skim milk using a 20 kHz horn-type ultrasound probe. Flaxseed oil (7–21%) was incorporated in skim milk using low frequency (20 kHz) ultrasound microencapsulated insulin using w/o/w double emulsion via ultrasonication (Mutaliyeva et al., 2017; Shanmugam & Ashokkumar, 2014). The capsules were prepared with shell material chitosan and xanthan gum complexes to preserve insulin stability and biological activity. Also, water-in-oil-in-water emulsions by low-frequency ultrasound (20 kHz) were prepared using skim milk and sunflower oil with a low amount of surfactant (Leong et al., 2017a, b). Della Porta et al. (2012) successfully encapsulated *Lactobacillus acidophilus* in double emulsion using sonication followed by the preparation of microspheres using supercritical emulsion extraction (SEE) technology with 80% encapsulation efficiency. Pandey et al. (2021) prepared double emulsion (W1/O/W2) microcapsules containing probiotic *Lactobacillus plantarum* by a two-step ultrasonication process by employing dextran and whey protein

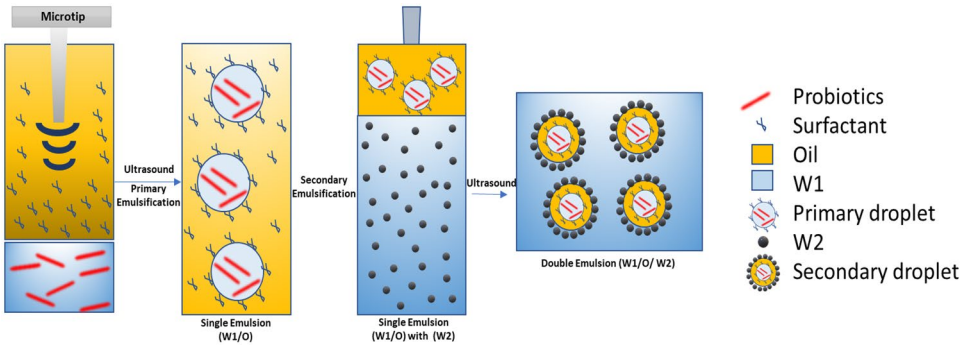

Fig. 3 Double emulsion (W1/O/W2) preparation using ultrasonication (adapted from Pandey et al., 2021)

as encapsulating material and observed that the viability of bacteria remained stable (> 10^9 CFU/mL) up to the treatment time of 200 s.

The optimization of process conditions of different technologies has been briefly represented in Table 2 from the point of characterization of microcapsules and viability of probiotics which is a compilation of recent research studies of individual technologies.

Advantages and disadvantages of microencapsulation technologies.

- *Convective hot air drying*: In this method, the direct exposure of droplet containing probiotic cells to hot air results in cell rupture, lipid oxidation, and shrinkage that ultimately causes a decrease in the cell viability but the operational cost is five times lesser than freeze drying process (Ermis, 2021).
- *Spray drying*: This single unit process is the most economic (approximately 10 times cheaper than freeze drying) with high-throughput capacity and easy to scale up as well as the produced dried microcapsules of higher stability and lower bulk density highlight the wider applicability of the process but the higher inlet temperatures and the exposure of cells to extreme osmotic stress during drying lead to lower survival of probiotics, poor stability during storage (Her et al., 2015). This process produces water-soluble microparticles which may result in an early release of probiotics (Frakolaki et al., 2021).
- *Freeze drying*: This method is suitable for encapsulation of heat-sensitive bacterial strains, and the freeze dried capsules are well stable but need lower storage temperature or an inert atmosphere however the longer processing time, higher operating cost, and the negative effect on cellular lipid membrane as well as cell proteins limit the application (Nag & Das, 2013).
- *Vacuum drying*: This method provides a higher drying rate, lower drying temperature and reduced oxygen concentration under vacuum conditions, and higher viability of probiotic cells as compared to conventional hot air drying (Ermis, 2021). However, the dried product might have shrinkage, denser structure, poor rehydration, and the loss of instant character of probiotics in the food matrix limit its industrial application (Ambros et al., 2018a, b, c).
- *Fluidized bed drying*: This process provides uniform drying with provisions for controlling the temperature and is suitable for encapsulation of probiotics along with multilayer coating of core substances, easy to scale up at comparatively lower processing cost but this technology is difficult to master and requires longer processing time (Martín et al., 2015).
- *Microwave drying*: Opposite to convection air drying, drying of microcapsules by microwave radiation results in the generation of inverse thermal gradient that favors the drying of particles from inside out and fast elimination of water which involves much lower temperature and processing time (Mardaras et al., 2021). Nevertheless, microwave radiation might be harmful to probiotics during encapsulation if the processing conditions are not optimized properly (Yoha et al., 2021). The higher processing time and product temperature cause product degradation and loss of bioactivity. Different researchers applied microwave coupled with other drying techniques such as vacuum and freeze drying to lower the processing time thus preserving the viability of encapsulated probiotics Ambros et al. (2018a, b, c).
- *Spray cooling/chilling*: This technique is the least expensive method and has broad applicability at the industrial level due to higher yield, a wider range of materials used as coating ingredients, production of smaller beads as well as lower processing temperature that eliminates lethality of cells (Pedroso et al., 2013). The size and morphology of produced beads facilitate their incorporation into food products without any negative effect on textural properties (Martín et al., 2015). This method results in lower encapsulation efficiency which is due to the direct exposure of bacterial cells to the outer environment and requires special attention during handling and storage of encapsulated microparticles (Kailasapathy et al., 2008).
- *Spray freeze drying*: This is an advantageous process for providing controlled capsule size and larger specific

Table 2 Optimized conditions of microencapsulation technologies for probiotics and characterization of produced microcapsules

Microencapsulation technology	Operating conditions	Probiotic strains (core material)	Wall material	Characteristics of microcapsules				Survivability/viability of probiotics	Reference
				Moisture content (%)	Water activity (a_w)	Particle size	Morphological characteristics		
Vacuum drying	Shelf temperature: 15 °C for 24 h and chamber pressure: 1500 Pa	*Lactobacillus paracasei* ssp. *paracasei* F19 (100 g)	No supporting material	13.40 ± 1.94; 0.25–0.4%/day water uptake (75% RH)		Exceeding 1 mm	Found to be tightly compacted; The cells were closely aligned, leaving almost no space in between them	Around 20% after drying	Ambros et al. (2018a, b, c)
Vacuum drying	Shelf temperature: 37 °C for 12 h and chamber pressure: 30 mm of Hg	*Lactobacillus plantarum* CIF17AN2 (CC: 1×10^{10} CFU/mL)	Cell suspension of probiotic bacteria and saba banana resistant starch at the ratio of 1:1 (v/w)					85.81% after drying	Hongpattarakere and Uraipan (2015)
Vacuum drying	Shelf temperature: 15 °C for 22 h and chamber pressure: 15 mbar	*Lactobacillus paracasei* F19 (223 g/l; CC: 1×10^{11} CFU/mL)	Trehalose: 25% (w/w)					$7.0 \pm 1.7 \times 10^{10}$ CFU/g after drying	Foerst et al. (2012)
Vacuum drying	Shelf temperature: 15 °C for 24 h, chamber pressure: 15 mbar and maximum product temperature: 6 °C	*Lactobacillus paracasei* ssp. *paracasei* F19 (CC: 1×10^{11} CFU/mL)	No supporting material	6–7				50.6% after drying	Bauer et al. (2012)
Fluidized bed drying	Air inlet temperature: 60 °C Air flow rate: 3.10 m/s	*Lactobacillus lactis* 1464 (25% v/w)	Monosodium glutamate: 5% (w/v), acacia gum: 5% (w/v)	< 11				10^7 CFU/g over 3 months of storage at 30 °C	Wirunpan et al. (2016)
Top fluidized bed drying	Air inlet temperature: 50 °C, spray pressure: 1.5 air/min, spray fluid flow rate: 5 mL/min, drying time: 40 min	*Lactobacillus brevis* RK03 (100 mL)	Casein: 5% (w/v), whey protein: 5% (w/v)	3.33–3.38	Below 0.4		Particles with tight smooth surfaces free of cracks and holes	95% after drying	Wu et al. (2021)

Table 2 (continued)

Microencapsulation technology	Operating conditions	Probiotic strains (core material)	Wall material	Characteristics of microcapsules				Survivability/viability of probiotics	Reference
				Moisture content (%)	Water activity (a_w)	Particle size	Morphological characteristics		
Fluidized bed drying	Atomization pressure: 60 kPa, fluidization air pressure: 40 kPa, drying temperature: 50 °C, spraying rate: 2.2 ml/min, drying time: 15–30 min	*Lactobacillus reuteri* DSM 20,016 (CC: 5×10^8–10^9 CFU/mL)	Sweet whey powder: 100 g, aqueous shellac solution: 25% (w/w)	1.35–2.0		206.01–234.19 µm		3.40E + 07–6.29E + 07 CFU/g after drying	Schell and Beermann (2014)
Fluidized bed drying	Air flow rate: 183 m^3/h, drying temperature: 35 °C, drying time: 60 min	*Lactobacillus plantarum* (CC: 11.5 log CFU/mL)	Alginate: 2% (w/v), chitosan: 0.4% (w/v)	9.72 ± 1.14	0.22 ± 0.01	0.98 ± 0.08 mm	Irregularly shaped capsules with a rough surface, less fragile	9.7 ± 0.05 log CFU/mL after drying	Albadran et al. (2015)
Fluidized bed drying	Air flow rate: 3 ± 0.5 m/s, spraying rate: 7 g/min, spraying time: 15 min, atomizing air pressure: 1:5 bar, product bed temperature: 37 ± 2 °C	*Enterococcus faecium* M74 (NM)	Skim milk: 100% (w/w) or sucrose: 100% (w/w)		0.20–0.22			A maximal loss of 2.5 log units after 2 months storage irrespective of conditions	Stummer et al. (2012)
Microwave vacuum drying	Specific microwave power input: 3 W/g, chamber pressure: 700 Pa, product temperature: 35 °C, drying time: 1.5 h	*Lactobacillus paracasei* ssp. *paracasei* F19 (100 g)	No supporting material	17.72 ± 5.13		Exceeding 1 mm	Appeared as much larger aggregates	Around 20% after drying	Ambros et al. (2018a, b, c)
Microwave freeze drying	Specific microwave power input: 1.5 W/g, chamber pressure: 60 Pa, product temperature: 30 °C, drying time: 4 h, condenser temperature: −50 °C	*Lactobacillus paracasei ssp. paracasei* F19 (100 g)	No supporting material	9.87 ± 0.53; 0.6%/day water uptake (75% RH)		5 to ~ 200 µm	Appeared as "fragile" flakes forming porous aggregates, slightly compact structure	Around 35% after drying	Ambros et al. (2018a, b, c)

Table 2 (continued)

Microencapsulation technology	Operating conditions	Probiotic strains (core material)	Wall material	Characteristics of microcapsules				Survivability/viability of probiotics	Reference
				Moisture content (%)	Water activity (a_w)	Particle size	Morphological characteristics		
Spray chilling	Feed temperature: 45 °C, nozzle temperature: 38 °C, Aspiration rate: 20 m^3/h	*Lactobacillus acidophilus* (A) and *Bifidobacterium bifidum*(B) (5% w/w)	Hydrogenated palm oil (20 g)	4.30 ± 0.17	0.79 ± 0.03	612.54–244.55 µm	Larger and crystallinity form; had an inseparable and uniform appearance	A: 90.15%, B 98.25% after drying	Arslan-Tontul and Erbas (2017)
Spray chilling	Feed temperature: 55 °C, chamber temperature: 15 °C, air pressure: 5 bar	*Bifidobacterium animalis subsp. lactis* (BL) and *Lactobacillus acidophilus* (LA) (4% w/w)	Hydrogenated palm oil	< 5	< 0.60	BL: 85.9 ± 0.08 µm; AL: 60.9 ± 0.09 µm	Spherical and continuous surface	BL: 5.8 ± 0.1 log CFU/g AL: 8.1 ± 0.2 log CFU/g after 120 days of storage at − 18 °C	Bampi et al. (2016)
Spray chilling	Chamber temperature: 15 °C, Air pressure: 5 bar	*Lactobacillus acidophilus* (4% w/w) with prebiotic polydextrose (3% w/w)	Hydrogenated palm oil			1–4 µm	Spherical shape, relatively smooth continuous surface, without pronounced cracks or pores	6.997 ± 0.085 log CFU/g after 120 days of storage at − 18 °C (RH 11%)	Okuro et al. (2013)
Spray freeze drying	Feed rate: 5 mL/min, air pressure: 20 kPa followed by annealing	*Lactobacillus casei* (IFO 15,883) (NM)	Buffered peptone water: 0.1% and glucose solution: 1%			24.8 µm	Spherical and highly porous	99.1% after drying	Her et al. (2015)
Spray freeze drying	Feed rate: 0.3 mL/min, air flow rate: 4.53 L/min, Air pressure: 1.01 bar, spraying height: 10 cm	*Lactobacillus paracasei* (0.25% w/v)	Maltodextrin-trehalose solutions (1:1)	9–15	0.33–0.8	1000–1400 µm	Spherical particles of controllable size	89 ± 7% after drying	Semyonov et al. (2010)
Vacuum-spray-freeze drying	Atomizing spraying nozzle: 0.7 mm, Temperature of the cryogenic chamber: -30 °C, chamber pressure: less than 150 Pa	*Saccharomyces cerevisiae* (CC: 10^{10}–10^{11} CFU/mL)	Sucrose: 7% (w/v), Maltodextrin: 15% (w/v), glycerin: 4% (w/v) and skim milk powder: 11% (w/v)				Spherical shape, irregularly shaped capsules with rough surface and particles with porous aggregates	76.36% after drying	Cao et al. (2020)

Table 2 (continued)

Microencapsulation technology	Operating conditions	Probiotic strains (core material)	Wall material	Characteristics of microcapsules				Survivability/viability of probiotics	Reference
				Moisture content (%)	Water activity (a_w)	Particle size	Morphological characteristics		
Electrospraying	Voltage: 10.24 kV	*Leuconostoc lactis* (NM)	Soy protein isolate (13.13%, w/v) and sunflower oil (4.77%, w/v)				Spherical with a smooth outer surface with the absence of fissures, cracks, and any other disruptions on the exterior surface	92.93% after drying	Premjit and Mitra (2021)
Electrospraying	Voltage: 0–60 kV, D_n: 500 µm	*Bifidobacterium longum* subsp. *infantis* CECT 4552 (CC: 10^{10} CFU/mL)	Whey protein concentrate: 20 wt.%, resistant maltodextrin: 20 wt.%, polyvinylpyrrolidone: 10 wt.%			1.95–2.47 µm	Spherical particles	5.50 log CFU/g after 600 days at room temperature and 23% RH	Librán et al. (2017)
Electrospraying	Voltage: 9.5 kV, flow rate: 5 mL/h, D_n: 0.4 mm, d_{nc}: 10 cm	*Lactobacillus plantarum* (5% w/w)	Chitosan: 0.2% (w/v), Na-alginate: 5 wt.%, $CaCl_2$: 0.3 M			450–550 µm	Very small and highly uniform spherical microcapsules	8.88 ± 0.13 log CFU/g after electrospraying	Zaeim et al. (2017)
Electrospraying	Voltage: 14 kV, flow rate: 0.15 mL/h, D_n: 2.41 mm, d_{nc}: 10 cm	*Lactobacillus plantarum* (CC: 9–10 log CFU/mL)	Whey protein concentrate: 0.3 g/mL of skim milk Fibersol®: 10 wt.% Tween20®: 9 wt.%				Homogeneous structure, where individual and spherical particles could be distinguished	less than 1 $\log_{10}$ CFU/g reduction after 3 weeks of storage at 53% RH	Gomez-Mascaraque et al. (2016)

Dn internal diameter of needle, *dnc* distance of the needle tip-to-collector, *CC* initial cell concentration, *NM* not mentioned

surface area with shorter processing time than spray and freeze drying but this process consumes 30–50 times higher energy than spray drying (Burgain et al., 2011).

- *Extrusion*: The advantages of extrusion technology are low cost, simplicity in operation, and mild processing conditions result in higher viability of probiotics however it involves a few demerits such as difficulty in scaling up of the process, inefficient to produce microsphere less than 500 µm due to clogging of the orifice, and the produced microspheres showed lower long term stability (Rathore et al., 2013).
- *Emulsification*: This technique produces microspheres with a wide shape and size range even below 300 µm as well as offers higher cell viability and can easily be applied at the industrial level but the residues of organic solvent or oil left on the surface of particle that may be toxic to probiotic cells limit the application of the process (Frakolaki et al., 2021).
- *Coacervation*: This process provides higher encapsulation efficiency, high-throughput capacity, and controlled release properties of microcapsules, but the process is complex, expensive, and difficult to scale up (Chávarri et al., 2010).
- *Electrospraying*: This method is cost-effective, adaptable along with the strong electrical field strength has no negative impact on bacterial cells thus providing high encapsulation efficiency and controlled release of core ingredients as well as can produce capsules in nanosize range (1–1000 nm) (López-Rubio et al., 2012). However, the dripping of the solution may occur if the conditions are not optimized well (Gomez-Mascaraque et al., 2016).
- *Hybridization system*: This process is advantageous as compared to spray drying for achieving higher microencapsulation efficiency by minimizing the heat stress by reducing the temperature below 30 °C (Ann et al., 2007). In this method, the single coating of encapsulated capsules might not provide adequate protection to probiotics in gastric conditions but the double-coated microencapsulated matrix could overcome this limitation (Ann et al., 2007).
- *Impinging aerosol technology*: This process can be operated continuously, has a higher production yield that results in small particle size products, and is suitable for heat-sensitive materials such as microbial cells (Sohail et al., 2012). The produced microparticles may result in acidification when incorporated in fruit juices thus there is a need for the selection of suitable polymeric coating materials for the encapsulation of probiotics (Sohail et al., 2011).
- *Ultrasonication*: Ultrasonication is an emerging technology in encapsulating both hydrophilic and lipophilic substances in food and therapeutic products. It can produce stable microbubbles having small droplets with a narrow size distribution with low cost and energy consumption (Cavalieri et al., 2011). Microcapsules can be prepared using less surfactant or no surfactant. However, the high shear force produced during ultrasonication can have a negative effect on the bacteria (Ashokkumar, 2015). Thus, ultrasonication parameters (ultrasonication power, time) need to be optimized before use (Pandey et al., 2021).

Characterization of microcapsules

Residual moisture content

The residual moisture content of microencapsulated powder is an important parameter to affect the survivability of probiotics. The variability of moisture content depends on the type of carrier medium used and microencapsulation technology applied, which ultimately affects the hygroscopicity of the product and molecular mobility of probiotics (Hoobin et al., 2013). The microcapsules having a desirable moisture content in the range of 2.8–5.6% inhibit the deteriorative biochemical reactions thus improving the stability of probiotics in dried powder (Dianawati et al., 2013; Khem et al., 2016). The residual moisture content and hygroscopicity of the microencapsulated powder containing probiotics were influenced by the hygroscopic property of polysaccharide-based carrier medium after drying and storage period (Muhammad et al., 2017). Generally, higher moisture content has been observed in spray dried protein and polysaccharide-based products (Shrestha et al., 2008). The improved shelf life of powder was obtained by minimizing the residual moisture content below 5% (Ananta et al., 2005). The viability of probiotics is affected by both very low and too high moisture contents as the elimination of whole residual water resulted in damage to cellular protein due to over-drying (Zayed & Roos, 2004). In contrast, higher cellular water content caused declination of acidification activity, severe membrane damage, and cell death (Passot et al., 2012). Persian gum (PG) has lower moisture retention capacity and prevents the deterioration of probiotics during excess drying. Microencapsulation of probiotics *Lactobacillus reuteri* by fluidized bed drying with shellac and sweet whey produced microcapsules of 1.35 to 2.00% moisture content with no degradation of the bacterial viability (Schell & Beermann, 2014). The layer of trehalose and maltodextrin protected encapsulated bacteria *Lactobacillus paracasei* by raising the solid concentration and lowering the freezing rate (Semyonov et al., 2010).

Water activity

Water activity is a critical parameter for affecting the long-term stability of the microencapsulated probiotics. It has

been reported that water activity lower than 0.3 is desirable for maintaining the viability and storability of encapsulated probiotics (Tonon et al., 2009). Such lower water activity is required to achieve desired handling properties such as high flowability with lower stickiness and agglomeration in spray dried powder (Behboudi-Jobbehdar et al., 2013). Freeze dried samples resulted in lower water activity of 0.052–0.072 due to the effect of a higher vacuum gradient (Moayyedi et al., 2018). It has been observed that lower a_w was achieved for spray dried powder with a higher inlet temperature (Behboudi-Jobbehdar et al., 2013). Spray dried powder was reported with lower a_w (0.53) as compared to spray chilled powder (0.83) (Arslan-Tontul & Erbas, 2017). The lowest a_w was observed with pectin as wall material when *L. plantarum* was encapsulated by spray drying, as compared to potato resistant starch and potassium alginate (Muhammad et al., 2017). Hydrophilic and hydrophobic properties of wall material also affect the residual a_w of microcapsules as free water is easily released due to the low water-binding capacity of hydrophobic wall materials (Avila-Reyes, Garcia-Suarez, Jiménez, San Martín-Gonzalez, & Bello-Perez, 2014).

Glass transition temperature

Glass transition temperature (T_g) indicates the phase transition of polymeric material from a glassy state to a rubbery state. The molecular weight, moisture content, and chemical structure of the material affect its glass transition temperature. It possesses great significance during the spray drying process and it is directly linked by the primal properties of products such as the variation in textural and rehydration ability, caking stability, adhesiveness, controlling biochemical reactions like enzymatic reaction, color, and textural modification during storage (Soukoulis et al., 2014). The lowering of T_g prevents the leakage of the cell membrane during phase transition and maintains the membrane structure in a crystalline state during the drying process. The variation in T_g depends on residual water content and both are negatively correlated. The storage stability of probiotics is greatly influenced by this temperature. The molecular mobility is restricted in the glassy state matrix that ultimately lowers the rate of various detrimental processes and resulted in the long-term preservation of probiotics (Broeckx et al., 2016; Roos, 2002). Application of low molecular weight disaccharides such as trehalose as coating material avoids the problem of increasing the stress on immobilized probiotics by minimizing the size of crystal in the inter-membrane space and maintaining the integrity of the lipid membrane (Koster et al., 2000). Coating materials play an important role in controlling the moisture content, thereby glass transition temperature as trehalose and maltodextrin possess the ability to form a glass matrix (Semyonov et al., 2010). Potato-resistant starch contains higher amylose content that results in high crystallinity (35–38%) (Xu et al., 2013). Trehalose has a lower molecular weight thus lower T_g than maltodextrin, so more effective for preserving probiotics during the freezing stages (Semyonov et al., 2010).

Stickiness

Stickiness is also a primary characteristic of powder which affects the yield and applicability in processing. This property develops during spray drying if the outlet air temperature reaches 10 °C higher than the T_g of disaccharides such as sucrose and lactose (Vega & Roos, 2006). The applicability of mannitol, dextran, and inulin as wall material has been limited due to sticky powder and lower yields (Broeckx et al., 2017). The introduction of high molecular weight carrier polymers can overcome the stickiness problem by altering the glass transition temperature during spray drying. The usage of whey proteins results in lower aggregation of samples as compared to Persian gum (Adhikari et al., 2009). Fluidized bed drying produces dry microcapsular powder with good handling characteristics as compared to freeze drying (Albadran et al., 2015).

Morphological characteristics

The processability, flowability, and physicochemical characteristics of microencapsulated powder are influenced by their morphological behavior. The incorporation of sugars in the drying medium caused an increase in the concentration, thereby viscosity of solution resulting in particles with a smoother surface (Paramita et al., 2010), and drying of individual droplets resulted in the accelerated skin or crust formation (Kim et al., 2009). Application of pectin produced microcapsules with a smoother surface than other polysaccharide-based microcapsules (Chan et al., 2011), whereas wrinkle and irregular ellipsoidal shapes were observed in potato resistant coated microcapsules (Xu et al., 2013). While potassium alginate resulted in a rough and shriveled surface due to the rapid removal of moisture during spray drying (Mokarram et al., 2009). Spray dried sample was characterized by spherical aggregates in the presence of concavities without any mechanical fissures due to the sharp loss of moisture (Fritzen-Freire et al., 2012). Rodríguez-Huezo et al. (2007) reported that the operation at an inlet drying temperature of 140 °C could produce concavities in spray dried powder with a higher shielding effect against solute diffusion and mechanical fracture. D'Alessandro et al. (2021) reported that the increase in the protein ratio in the composition of coating materials could minimize the concavities and shrinkage of spray dried particles, consequently preventing the structural disruption and also observed that the presence of soy protein in wall material prevented the roughness of particles which could be advantageous for consumer preference when incorporating the microcapsules in food matrices. Gum Arabic

powder particles exhibited both shrinkage and smoothness on the spherically shaped surface than maltodextrin powders (Reyes et al., 2018). The larger and smaller size of microcapsules produced with higher solution feed rate and airflow rate, respectively, and airflow rate had a greater impact on the size of microcapsules than the solution flow rate during spray freeze drying (Semyonov et al., 2010). Spray freeze drying produced powders with a highly porous surface with smaller particles than the freeze drying method and also showed its utility in the food processing industry (Her et al., 2015). Vacuum drying resulted in lower survivability of probiotics and rehydrability of powder than freeze drying as it was more prone to mechanical ruptures due to the compactness of cells, as well as samples produced by freeze drying and microwave freeze drying, were porous and fragile structured of size ranging between 5 and 200 µm (Ambros et al., 2018a, b, c). Electrospraying produced more spherical microcapsules as compared to spray and freeze drying, in which the solvent used affected the morphology of microcapsules by altering the viscosity and surface tension of the matrix solution (Gomez-Mascaraque et al., 2016). The freeze dried capsules were more fragile and less robust than fluid bed dried capsules, which indicated the industrial application of fluidized bed drying (Albadran et al., 2015). The microcapsules with a single layer of fructooligosaccharide (FOS) appeared as a rounded shape with some concavities on the surface, whereas a single layer of lactulose displayed irregular surfaces with convex lattices during the hybridization system (Ann et al., 2007).

Thermal stability

The addition of oligofructose enriched inulin enhanced the thermal resistance of the *Bifidobacterium* BB-12 during spray drying (Fritzen-Freire et al., 2013) and flaxseed mucilage at 0.2% w/v acted as thermoprotectant for spray dried *Lactobacillus acidophilus La-05* (Bustamante et al., 2015). Rama et al. (2020) reported that bovine cheese whey as coating material protected the encapsulated probiotic strain *Lactobacillus paracasei* better than ricotta whey during spray drying. The authors stated that the higher protein content in bovine cheese whey resulted in better thermal stability to encapsulated probiotics. The protein structure unfolds due to the high temperature of spray drying which causes the free carboxylic and amino groups of denatured protein available for reaction, increasing hydrophobic forces, hydrogen, and sulfide bonds resulting in aggregation, coagulation, and precipitation. This phenomenon creates microparticles capable of preventing the loss of encapsulated probiotics from harsh conditions (Rama et al., 2020). Trehalose could prevent damage to the cell membrane during the freezing–thawing process because of higher T_g (Han & Bischof, 2004). Cryoprotectants play a vital role to protect the probiotics from freezing stress. Skimmed milk contains proteins, which act as a protective shield for the cell and minimize cellular injury by stabilizing the cell membrane (Jofré et al., 2015). Oligosaccharide acts as a protectant by inducing plasmolysis while the cell membrane becomes more plastic due to glycerol and minimizes the formation of ice crystals within the cell during freezing (Carvalho et al., 2004). Xanthan-gellan gum was efficient to protect the probiotics but unable to provide adequate resistance against the developed stress during freeze drying (Tomás et al., 2015). Fung et al. (2010) studied that soluble dietary fiber provided sufficient thermal resistance to *L. acidophilus* during the electrospinning technique. Dumont et al. (2003) classified four definite ranges of cooling rate based on studies on yeast. Low viability was observed in the very slow cooling rate (< 5 °C/min) range; low cooling rate (5–100 °C/min) did not cause any injury to the cells; rapid cooling rate (100–2000 °C/min) caused lethality due to considerable outflow of water and ultra-high cooling rate (> 5000 °C/min) preserved the viability of probiotics (Semyonov et al., 2010). It was found that concentrated solutions that exerted high osmotic pressure (30–150 MPa) contributed to the high survivability of *Lactobacillus paracasei* even at low and moderate cooling rates during spray freeze drying (Semyonov et al., 2010). Hao et al. (2021) found that culturing LAB strains at elevated temperatures was proved to be useful to improve the survivability of probiotics at higher heat stress conditions.

Storage stability

Dehydration of bacterial cells gives rise to serious oxidative stress and the cell membrane gets damaged due to the reactive oxygen species (ROS), which in turn leads to protein denaturation, lipid peroxidation along with desertification, and damage in cell nucleic acid (García, 2011). Cell membranes are more vulnerable to ROS attack during the prolonged dry storage period (França et al., 2007). Aerobic organisms use oxygen as electron acceptor; however, during respiration, oxygen could be partially reduced by forming ROS, such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and hydroxyl radicals (OH•) and these free radicals would be trapped by antioxidants defense system of probiotic cells under normal metabolic conditions. The dehydration causes water stress and results in the dysfunction of specific enzymes as well as the defense mechanism for which bacterial cells are ultimately affected by the attack of ROS (França et al., 2007). In a desiccated state, the packing density of polar head groups of cellular membrane phospholipids increases that leading to the strengthening of van der Waals interaction between the carbon chains and increasing the phase transition temperature, consequently lipid will be in gel phase at room temperature. Upon rehydration, the lipids suffer a phase transition, resulting in extensive leakage and cell death (García, 2011). Further, the higher degree of unsaturation of desiccated cells encourages lipid

oxidation, as well as the polar heads of the phospholipids, generate ROS by the Fe^{+2} autoxidation process which lowers the shelf life of probiotics (França et al., 2007). The oxygen reduction process produces ROS such as superoxide or H_2O_2 and the protein-Fe^{+2} complex reacts with H_2O_2 to produce ferryl ions which cause proteolysis or inactivation of protein, as well as protein dehydration induces conformational changes of protein, consequently decreasing biological activity and damaging the bacterial cells after rehydration (García, 2011). Decreasing storage temperature and water activity result in increased storability of dried encapsulated cells. Rodrigues et al. (2011) reported that high temperature and relative humidity were detrimental to the survival of probiotics entrapped in microcapsules, which limited their application in food products. Several studies found that optimum survivability of probiotics in spray dried powder was observed at either 4 or 20 °C temperature with water activity between 0.11 and 0.23 during the storage period (Chávez & Ledeboer, 2007). Spray dried powder with Gum Arabic and maltodextrin protected *L. acidophilus* in 97% vacuum condition at 4 °C (Reyes et al., 2018), whereas skim milk provided higher protection to probiotic *L. casei* than maltodextrin and trehalose during spray drying and storage (Liao et al., 2017). The electrosprayed *Bifidobacterium longum* subsp. *infantis* CECT 4552 powder showed higher viability after10 days at 37 °C and after 600 days at 23% RH conditions (Librán et al., 2017). Azizi et al. (2021) found that spray and freeze drying provided an improved storability of probiotics *Lactobacillus rhamnosus* than the electrospraying technique at both ambient and refrigerated conditions. A thumb rule has been proposed that the T_g should be 10–20 °C higher than the storage temperature to maintain the glassy state thus maintaining the structural integrity of powder during storage (Roos, 2002). The ability to encapsulate materials for protecting the spray dried probiotic *L. plantarum KLDS* 1.0344 powder stored at 25 °C with a_w of 0.11 was observed in the following order: potato resistant starch > pectin > potassium alginate > whey protein isolate and D-mannose (Broeckx et al., 2017). Freeze drying has been practiced for the preservation of probiotics during long-term storage but causes cell damage during the drying process. The protective agents increase the glass transition temperature to avoid intracellular ice formation and reduce cell damage during drying (Meng et al., 2008). The survival rate of fluidized bed dried *Lactobacillus lactis* 1464 was maintained (> 10^7 CFU/g) over 3 months of storage period after drying at 50–60 °C, with the application of monosodium glutamate and acacia gum (Wirunpan et al., 2016). Fluidized bed drying provided better stability of *Enterococcus faecium* M74 than freeze drying process (Stummer et al., 2012), whereas bacterial cells exposed to thermal or osmotic stress in growth media before harvesting resulted in higher survivability of *Lactobacillus reuteri* after fluidized bed drying (Schell & Beermann, 2014). The osmotically stressed *L. casei* CRL 431 cells were found to be most stable even when stored at 30 or 37 °C after 24 weeks (Nag & Das, 2013). Similarly, double microencapsulation of *Lactobacillus acidophilus* ATCC 43,121 by hybridization system with FOS and lactulose resulted in the highest survival rate at 25 °C (Ann et al., 2007). The incorporation of vitamin E powder as a coating material provided higher stability to *L. casei* against oxidative damage during dry storage due to its antioxidant property (Nag & Das, 2013).

Mechanism of release in microencapsulation processes

Control release is a method to deliver core material at a targeted site at a specific rate and time. The core material release properties depend upon the core wall morphologies like mononuclear, polynuclear microcapsules, or microspheres. The release of active material may be a combination of the following release mechanisms (Pothakamury & Barbosa-Cánovas, 1995).

- *Diffusion controlled release:* Volatile or non-volatile active material diffuses through carrier or pores present in the wall.
- *Pressure activated release:* Wall rupture due to external pressure like the release of sweetener or flavor in the gum.
- *Shear / compressive force:* (Mechanical release) chewing, blending.
- *Solvent activated release:* Water or solvent penetrates the wall material result in swelling of microcapsules.
- *Osmotically controlled release:* Core material released due to osmotic pressure created inside the microcapsules.
- *Temperature-sensitive release*: Expand or collapse due to temperature change.
- *Dissolution or melting activated release:* Fat or wax as wall material melts during heating.
- *pH-sensitive release:* An enzyme or bacteria release at specific pH in the intestine.
- *Biodegradation:* Oil coating can be degraded by the action of lipase enzymes.

The controlled release of probiotics can be judged by the effect of wall material and microencapsulation technologies on the survival of probiotics during the simulated GI condition, which has been illustrated in Table 3.

Mechanism of diffusion

The release rate depends upon the choice of wall material, morphology, thickness of wall material, physicochemical properties of wall and core material, and permeability of the shell.

Table 3 Effect of wall material and microencapsulation technologies on the survival of probiotics during simulated GI condition

Probiotic	Types of wall material	Encapsulation technology	Probiotic viability/ survivability	Time of incubation	Reference
Bifidobacterium lactis	Casein-pectin complex	Complex coacervation and spray drying	10^8 CFU/mL	3 h	Oliveira et al. (2007)
Lactobacillus rhamnosus	Whey protein isolate	Extrusion technology	10^9 CFU/mL	3 h	Doherty et al. (2011)
Lactobacillus acidophilus	Resistant starch (Hi-maize)-chitosan-sodium alginate	Extrusion technology	6.35 log CFU/g	2 h	de Araújo Etchepare et al. (2016)
Lactobacillus bulgaricus	Alginate–milk	Extrusion technology	8 log CFU/g	2 h	Shi et al. (2013a, b)
Lactobacillus reuteri DSM 20,016	Shellac	Fluidized bed drying	76.74%	100 min	Schell and Beermann (2014)
Saccharomyces boulardii	Hydrogenated palm oil	Spray chilling	96%	180 min	Arslan-Tontul and Erbas (2017)
Lactobacillus acidophilus NCFM	Alginate	Impinging aerosols method	3.83 log CFU/mL	90 min	Sohail et al. (2011)
Lactobacillus casei LK-1	Trehalose	Spray drying	6.5 log CFU/mL	4 h	Liao et al. (2017)
Lactobacillus acidophilus	Alginate-citric acid-modified zein	Electrospraying	Reduced by 1-log CFU/mL	2 h	Laelorspoen et al. (2014)
Lactobacillus plantarum	Sodium alginate-citric pectin matrix	Electrospraying	Decreased by 2.7 log CFU/mL	2 h	Coghetto et al. (2016)
Lactobacillus plantarum CIF17AN2	Resistant starch from unripe saba banana	Vacuum drying	Reduced by 40.83%	6 h	Hongpattarakere and Uraipan (2015)

The release kinetics follows the following models.

Zero-order diffusion model

The release rate is constant means increasing or decreasing the concentration will not change the diffusion rate (Eq. 1).

$$C \propto t$$

$$^{dC_t}/_{dt} = k_0 \quad (1)$$

where C_t is the amount of core material released in time t, and k_o is a zero-order rate constant.

First-order diffusion model

The release rate is dependent on the concentration of the active material (Eq. 2).

$$^{dC}/_{dt} = k_1 C \quad (2)$$

where k_1 is the first-order rate constant.

Mechanism of osmosis

Osmosis is the process in which a molecule transfers from a less concentrated region to a higher concentration through a semipermeable membrane. In osmotic control release, the core is released due to osmotic pressure from the microcapsules. From the Van't Hoff equation, we can calculate osmotic pressure as follows (Eq. 3).

$$\Pi = MRT \quad (3)$$

where Π = osmotic pressure (atm), M = molar concentration of solution (mol/L), R = gas constant 0.0821 L atm/mol K, T = temperature in K.

Mechanism of biodegradation/erosion

For microspheres systems, the core is dispersed within the matrix and is released when the matrix degrades or erodes. So, the surface area of the microsphere decreases with time, resulting in decreasing release rates. The release can be controlled by diffusion, erosion, or a combination of both. Erosion can be controlled by homogeneous or heterogeneous processes. The rate of erosion is constant in the homogeneous erosion process throughout the matrix, whereas for heterogeneous erosion, degradation is limited to a thin layer at the surface of the delivery system (Pothakamury & Barbosa-Cánovas, 1995).

Mechanism of swelling

The core is dissolved or dispersed in a thermodynamically compatible medium, the polymer swells absorbing the fluid from the medium. The core in the swollen part of the matrix then diffuses outs. The membrane undergoes a transition

from a glassy to a gel state and core material diffuse out (Madene et al., 2006).

Application of microencapsulated powder for probiotication in food products

The development of food products with probiotic effects has emerged as functional foods (Table 4) intending to provide potential health benefits to mankind. Several factors influencing the survivability of probiotic strains in food products are the type of strain, the method for preparation of culture, state of cell inoculum, oxygen level, and storage temperature (Ying et al., 2013). Microencapsulated probiotic powder formulation has been proved as a more convenient delivery format compared to wet gelled formulations. However, the selection of appropriate microencapsulation technology and coating materials are crucial for the protection of probiotics as well as retention of their functionalities in different food matrices (Fig. 4).

Beverages

Pourjafar et al. (2020) encapsulated *L. acidophilus* by extrusion method with sodium alginate and chitosan and added (1 g) in 10 mL of Iranian Doogh beverage. It was observed that the microencapsulated beverage showed higher viability of probiotics (7.4×10^7 CFU/g) compared to the beverage containing free cells (5.8×10^4 CFU/g) during the storage period (at 5 °C up to 42 days) without any considerable change in pH, acidity, and sensory properties of the beverage. The application of probiotics *Lactobacillus casei* encapsulated by extrusion technology with sodium alginate in orange juice was studied by Olivares et al. (2019). The authors reported higher viability of probiotics (10^6 CFU/mL) during 28 days of cold storage (at 4 °C) and the higher concentration of survivability was achieved due to the presence of ascorbic acid, an antimicrobial compound, antioxidant, and important nutrient for probiotics despite the lower pH (3.45) of juice. Probiotic apple juice was prepared by adding spray dried microencapsulated *Lactobacillus rhamnosus* GG (1% w/v) and resulted in higher survivability at lower pH and higher temperature of 25 °C as compared to a lower temperature of 4 °C (Ying et al., 2013). Dias et al. (2018) observed that the higher proportion of inulin improved the viability of spray dried encapsulated probiotic strain *Bifidobacteria animalis* (above 6.5 log CFU/g) in passion fruit juices during storage at 4 and 25 °C for 30 days whereas the microcapsules containing maltodextrin showed complete loss of viability of bacteria in stored juices at 25 °C after 30 days. Encapsulated bacteria incorporated in orange and apple juice showed higher stability, average malic acid concentration, and Brix than free cells throughout the six weeks of storage as the sugars in the orange juice could be more readily utilized by free probiotic bacteria than the entrapped cells (Ding & Shah, 2008). King et al. (2007) found similar results and observed that the sensory score was higher for fermented tomato juice containing immobilized *L. acidophilus* than free cells due to inhibition of unfavorable reactions during the storage period.

Cheese

Many studies have been performed on the application of microencapsulated bacterial strains in different types of cheese. There are certain advantages of selecting cheese as a good carrier of probiotic organisms due to its relatively high-fat content and good buffering capacity against adverse GI conditions (Frakolaki et al., 2021). The application of microencapsulation of *L. acidophilus* by freeze drying with alginate and chitosan in spreadable goat Ricotta cheese improved the bacterial viability (> 6 log CFU/mL) and quality attributes such as lowering proteolysis, no moisture loss, lowering gumminess, and adhesiveness (Lopes et al., 2021). Sharifi et al. (2021) co-encapsulated probiotic culture *Lactobacillus plantarum* and phytosterol by complex coacervation followed by freeze drying by applying whey.

protein isolate and Gum Arabic as coating materials and incorporated in Iranian white cheese. It was observed that the combination of probiotic bacteria with phytosterol showed higher viability, i.e., 8.14 log CFU/g compared to microencapsulated probiotic strain alone (7.95 log CFU/g), and free cells (6.44 Log CFU/g) in cheese after 61 days of storage. Vasile et al. (2020) comicroencapsulated probiotic strain *Lactobacillus casei* with black beans aqueous extract by freeze drying using biopolymers such as whey protein isolate, chitosan, and inulin which provided improved viability of 2 log increase in bacterial cells as well as a higher stability of phytochemicals and biological parameters such as higher α-glucosidase and α-amylase inhibitory activity in 21 days stored soft cheese sample. Another study revealed that no difference was found between two microencapsulation technologies such as emulsion and extrusion techniques regarding proteolysis, bacterial counts, and organoleptic properties of Kesar cheese (Özer et al., 2008). In this study, the cheese with microencapsulated probiotics *Lactobacillus acidophilus LA-5* and *Bifidobacterium bifidum BB-12* showed the desired viability (above 10^7 CFU/g) whereas nonencapsulated probiotics were found to be continuously decreasing due to scalding throughout the 90 days of storage. In the case of feta cheese, the incorporation of microencapsulated probiotics *Bifidobacterium lactis* and *L. acidophilus* did not significantly influence textural properties such as cohesiveness and springiness ($p < 0.05$) but chewiness, gumminess, and hardness were greatly affected (Kailasapathy & Masondole, 2005).

Table 4 Application of microencapsulation of probiotics in different food products

Microencapsulated probiotic strains (rate of inoculation in food matrices)	Wall material	Method	Food material	References
Lactococcus lactis ABRIINW-N19 (2% (w/v); CC: 5.2–8.5×10^9 CFU/g)	Alginate (1.5% w/v), Persian gum (0.5% w/v) and inulin (1% w/v)	Extrusion technique	Orange juice	Nami et al. (2020)
Enterococcus faecalis (0.1 mL; CC: 7.0 log CFU/g)	Gum Arabic (30 g min 100 mL distilled water) and maltodextrin (40 g min 100 mL distilled water)	Freeze drying	Carrot juice powder	Rishabh et al. (2021)
Lactobacillus paracasei (3% (w/v); CC: 1.10×10^{10} CFU/g)	Sodium caseinate (5% w/w)	Emulsifiation/internal gelation	Yogurt	Li et al. (2021)
Lactobacillus casei and *Bifidobacterium longum* mixture (1% (w/v))	Fructooligosaccharide (2% w/v), sodium alginate (1% w/v)	Extrusion technique	Goat cheese	Kavas et al. (2021)
Lactobacillus paracasei KS-199 (CC: 1.2×10^8 CFU/mL)	Polyvinyl alcohol and sodium alginate in a ratio of 7:1 (v/v)	Electrospinning technique	Kefir	Yilmaz et al. (2020)
Lactobacillus acidophilus La-05 (2.5 g)	Calcium alginate (30 g/L) and chitosan (5 g/L)	External ionic gelation technique	Vegan milk (Rice milk and soybean milk)	Lopes et al. (2020)
Lactobacillus casei ATCC393 (2 g in 500 mL; CC: 10^9 CFU/mL)	Chios mastic gum (1% w/v)	Freeze drying	Fermented milk	Terpou et al. (2018)
Lactobacillus plantarum Lp 115 (33 g/L)	Ternary blend of mesquite gum, maltodextrin DE 10 and gum Arabic in 66:17:17 ratio	Double emulsions	Oaxaca cheese	Rodríguez-Huezo et al. (2014)
Lactobacillus plantarum TISTR 050 (NM)	Sodium alginate and soy protein isolate (1:8% w/w)	Extrusion technique	Mango juice	Praepanitchain et al. (2019)
Lactobacillus rhamnosus GG (1%, w/v)	Whey protein isolate-resistant starch (4:1)	Spray drying	Apple juice	Ying et al. (2013)
Lactobacillus acidophilus (6%, w/v; CC: > 10^6 CFU/mL)	Sodium alginate (3%), inulin (1%), xanthan gum (0.15%)	Gelation	Carrot juice	Nazzaro et al. (2009)
Bifidobacterium longum 15,708 (CC: 10^7 CFU/mL)	Alginate (2% w/v)	Droplet extrusion	Cheddar cheese	Amine et al. (2014)
Lactobacillus reuteri (1%, w/w; CC: 7.0 log CFU/g)	Alginate (3% w/v)	Extrusion	Sausage batter	Muthukumarasamy and Holley (2006)
Lactobacillus paracasei L26 (1 g in 10 mL)	Sodium alginate (2% w/v)	Extrusion	Orange juice and peach juice	Rodrigues et al. (2012)
Lactobacillus plantarum NCIMB 8826 and *Bifidobacterium longum* NCIMB 8809 (CC: 3×10^8 CFU/mL for both strains)	Pectin solution (0.2% w/v)	Extrusion	Pomegranate and cranberry juice	Nualkaekul et al. (2013)
Lactobacillus acidophilus La-5 (1%, w/v)	Sodium alginate (10 g/L), fructooligosaccharide (8% w/w)	External gelation	Yogurt-ice cream	Ahmadi et al. (2014)
Lactobacillus casei (10 g in 100 mL)	Blend of sodium alginate and amidated low-methoxyl pectin (1:4)	Extrusion	Yogurt	Sandoval-Castilla et al. (2010)

Table 4 (continued)

Microencapsulated probiotic strains (rate of inoculation in food matrices)	Wall material	Method	Food material	References
Lactobacillus rhamnosus LBRE-LSAS and *Bifidobacterium animalis* subsp. *lactis* Bb12 (1%, w/v; CC: 7.0 log CFU/mL)	Sodium alginate (18 g/L) and resistant starch (20 g/L)	Emulsification	Yogurt	Ziar et al. (2012)
Lactobacillus acidophilus LA-5 and *Bifidobacterium bifidum* BB-12 (1%, w/v; CC: 5×10^9 CFU/mL)	Sodium alginate (2% w/v), k-carrageenan (2% w/v)	Extrusion and emulsification	Kasar cheese	Özer et al. (2008)
Bifidobacterium animalis ssp. *lactis* BB12 (CC: 1.2×10^8 CFU/mL)	Sodium alginate (3% w/v)	Cell immobilization	Kefir	González-Sánchez et al.(2010)
Lactobacillus acidophilus ATCC 4356 (CC: 8.26 log CFU/g)	Sodium alginate (4% w/v)	Extrusion	Yogurt	Ortakci and Sert (2012)
Lactobacillus casei Lc 01 (1%, w/v; CC: 5.8×10^9 CFU/g) and *Bifidobacterium lactis* BB 12 (1%, w/v; CC: 6.1×10^9 CFU/g)	Hi-maize resistant starch (2%) and Sodium alginate (2%)	Gelation	Ice cream	Homayouni et al. (2008)
Lactobacillus acidophilus LA-5 and *Bifidobacterium lactis* Bb-12 (NM)	Sodium alginate (2% w/v)	Gelation	Yogurt drink	Mortazavian et al. (2008)
Bifidobacterium bifidum F-35 (NM)	Whey protein isolate (10% w/v) and alginate solution (0.5% w/v)	Double emulsion	Yogurt	Mousa et al. (2014)
Lactobacillus acidophilus LA-5 (10%, w/v)	Pectin (2% w/w) and whey protein concentrate (4% w/v)	Ionic gelation and complex coacervation	Yogurt	Ribeiro et al. (2014)
Bifidobacterium bifidum BB-12 and *Lactobacillus acidophilus* LA-5 (CC: 6×10^{10} CFU/mL)	Sodium alginate (2% w/v) and k-carragenan (2.0% w/v)	Extrusion and emulsification	White-brined cheese	Özer et al. (2009)
Lactobacillus acidophilus CCRC 10,695 (4%, v/v)	k-carrageenan (4% w/v)	Cell immobilization	Banana	Tsen et al. (2004)
Lactobacillus acidophilus (1.5% of yogurt)	Pectin and casein (1:1, total solids content of 8%, w/v)	Complex coacervation	Buffalo milk yogurt	Shoji et al. (2013)
Lactobacillus paracasei NFBC 338 (0.1%, w/v)	Reconstituted skim milk (20% w/v)	Spray drying	Cheddar cheese	Gardiner et al. (2002)
Lactobacillus helveticus CNCM I-1722 () and *Bifidobacterium longum* CNCM I-3470 (CC: 10^9 CFU per portion of chocolate, corresponding to 13.5 g)	Fatty acid	Spray coating	Chocolate	Possemiers et al. (2010)
Lactobacillus rhamnosus R011 (CC: 1.3×10^7 bacteria/g of dry matter)	Whey protein isolate (70% w/w)	Extrusion	Biscuit, cranberry and vegetable juice	Reid et al. (2007)
Lactobacillus casei ATCC 393 (50 g/L of milk; CC: 10 log CFU/L)	Whey protein	Cell immobilization	Feta cheese	Dimitrellou et al. (2014)

Table 4 (continued)

Microencapsulated probiotic strains (rate of inoculation in food matrices)	Wall material	Method	Food material	References
Lactobacillus rhamnosus, *Bifidobacterium longum*, *L. salivarius*, *L. plantarum*, *L. acidophilus*, *L. paracasei*, *B. lactis type Bi-04* and *B. lactis type Bi-07* (NM)	Sodium alginate (3% w/v)	Emulsion	Orange and apple juice	Ding and Shah (2008)
L. acidophilus BCRC 10,695 (4%, v/v)	k-carrageenan (4% w/v)	Cell immobilization	Tomato juice	Tsen et al. (2008)
Lactobacillus plantarum (50 g in 450 g of wheat flour)	Fructooligosaccharide and denatured whey protein isolate (ratio of 1:1, 20% w/w)	Freeze drying	Noodle	Rajam et al. (2015)

CC initial cell concentration, *NM* not mentioned

Yogurt

Similarly, probiotic yogurt has been developed by incorporating living strains to increase the therapeutic value (Chen & Chen, 2007). Unlike cheese, it is a poor carrier of probiotics due to the lower pH (4.2 to 4.6). The problem has been resolved by different authors by microencapsulating the bacterial strains. The coating of whey protein and xanthan gum resulted in higher viability of the encapsulated probiotic strain *Lactobacillus acidophilus*, i.e., 6.98 log CFU/g as that of free cells (approximately 2 log CFU/g) in yogurt during 28 days of storage but the addition of microencapsulated probiotics increased adhesiveness, firmness, and viscosity as well as deceased syneresis of yogurt samples (Khorshidi et al., 2021). De Prisco et al. (2017) encapsulated *Lactobacillus delbrueckii* in the alginate-chitosan capsule by extrusion technology and used it as a starter culture for yogurt which showed improved protection to probiotics during 28-day long storage (7.70 log CFU/g) as well as digestion process (2 h of gastric condition) having 100% survivability, whereas the free cells lost their viability completely. Li et al. (2020) observed that the addition of microencapsulated probiotics *Lactobacillus* strains with galactooligosaccharides and lactitol by extrusion showed an adverse effect on textural properties and syneresis of yogurt samples during storage though improved viability of 9.95 log CFU/g was achieved compared to the control sample. Microencapsulated *Bifidobacteria* when incorporated into stirred yogurt, the grainy texture was developed with particle size ranging from 22 to 50 µm which retarded the sensory quality (Adhikari et al., 2003). With the addition of prebiotic ingredients such as resistant starch and glycerol as encapsulating material, the viability was enhanced but failed to protect the probiotic cells in simulated GI conditions (Sultana et al., 2000). This stimulates the growth of probiotics with enhancing the functionality of food products due to a synergistic effect of prebiotic and probiotic. A negative correlation was observed between survivability of probiotics and post storage pH due to the presence of fruit pulp in yogurt despite higher survivability after 35 days of storage period (Kailasapathy et al., 2008). The oxygen-sensitive probiotics were also protected effectively by incorporating microencapsulated powder in yogurt (Talwalkar & Kailasapathy, 2004).

Ice cream

The growth of probiotics in ice cream is dependent on pH as neutrality tends to support the growth of microorganisms, whereas fermented ice cream affects the metabolic activity of probiotic bacteria but frozen injury, oxygen toxicity, and higher osmotic pressure mainly cause the loss in viability of strains in frozen dairy desserts (Frakolaki et al., 2021). Zaeim et al. (2020) prepared ice cream with the incorporation of

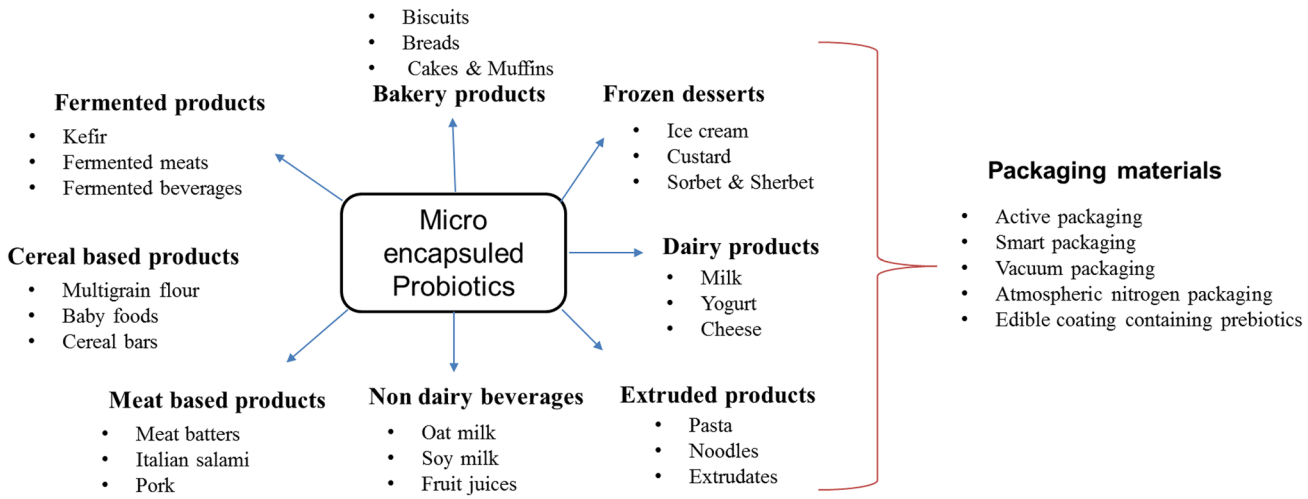

Fig. 4 Application of microencapsulated probiotics in different food products and suitable packaging materials for probioticated foods

electrosprayed microcapsules containing probiotic bacterium *Lactobacillus plantarum* with alginate, chitosan, inulin, and resistant starch as prebiotics. It was observed that starch-containing microcapsules provided better viability (7.82 log CFU/g) as compared to inulin (7.37 log CFU/g) in ice cream after 90 days of storage. The co-encapsulation of probiotics with prebiotics improves their viability in harsh conditions of the food matrix and digestive system by accelerating their growth and proliferation (Zaeim et al., 2020). Similarly, Afzaal et al. (2020) observed that microencapsulated probiotic *Lactobacillus casei* in calcium alginate and whey protein concentrate hydrogel exhibited better survival (> 8 log CFU/mL) compared to non-encapsulated cells (6.41 log CFU/mL) in ice cream samples after 80 days and the microencapsulated cells resulted in grittiness as well as the hydrocolloids affected the texture and appearance of ice cream. The authors also reported that the viscosity of ice cream was greatly affected by the properties of microcapsules such as shape, size, and type of coating materials used for encapsulation.

Other food products

dos Santos et al. (2019) developed synbiotic mousse by incorporating the microencapsulated *Lactobacillus acidophilus* with inulin by spray drying technique and subjected to in vitro gastrointestinal condition. It was observed that the lowest reduction of bacterial cell count occurred in mousse with encapsulated cells (1.3 log cycles), followed by microencapsulated cells (2 log cycles), mousse with free cells (3 log cycles), and the highest reduction occurred in free cells (7.4 log cycles) after 6 h of in vitro study.

Probiotic chocolate was developed by Hossain et al. (2021) by fortifying with freeze dried *Lactobacillus casei* encapsulated with cocoa powder, alginate, and fructooligosaccharides. Higher viability of more than 7 log CFU/g in chocolate after 90 days of storage at 25 °C and gastrointestinal digestion process (8 log CFU/g), as well as cell count of above 10.50 log CFU/g during the colonic fermentation, showed the potential delivery of probiotics through chocolate. Similarly, the addition of microencapsulated bacterium *S. thermophiles* prepared by emulsification process with encapsulants (pectin, carboxymethylcellulose, cellobiose, and gum Arabic) showed good viability in dark (6.90 log CFU/g) and milk (7.12 log CFU/g) chocolates stored at 4 °C up to 180 days without affecting the sensory attributes and moisture content of chocolates (Ozturk et al., 2021). In this study, the higher viability of probiotics during storage might be attributed to the higher protein and carbohydrate content of milk chocolate along with the presence of antioxidant compounds in chocolates preventing the loss of bacteria in gastrointestinal conditions. Introduction of microencapsulated powder (prepared by emulsification process) into chocolate exhibited protection to probiotic strains in environmental stress conditions due to the protective action of lipid components present in cocoa butter (Lahtinen et al., 2007).

Muzzafar and Sharma (2018) microencapsulated probiotics *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Bifiobacterium bifidum* by emulsification followed by freeze drying using xanthan gum, maltodextrin, and sunflower oil as wall materials and incorporated the microencapsulated powder in the cream layer of biscuit. It was observed that the probiotic biscuits retained the viability (above 8 log CFU/g) over 8 weeks (stored at 25 °C) and higher fat, fiber content with higher taste and overall acceptability was found in probiotic biscuits due to the influence of ingredients in wall materials for encapsulation. Similarly, functional biscuits have been developed containing prebiotic inulin and

probiotic *Lactobacillus plantarum* encapsulated by emulsification process using alginate solution (González-Forte et al., 2014). The additional coating layer of starch–glycerol extended the higher survivability of probiotic (8.7 log CFU/mL) as compared to uncoated sample (7.8 log CFU/mL) up to 1 month of storage as well as protected the viability during the passage of the simulated GI tract (5.5 log CFU/mL) as that of the control sample (3.5 log CFU/mL). Another researcher incorporated microencapsulated spray dried *L. acidophilus* to bread surface through the starch-based edible coating and evaluated the change in physicochemical parameters (Altamirano-Fortoul et al., 2012). The probiotic count was not affected by the baking process (at 180 °C for 16 min) exhibiting survival of 63.2% due to an adhesive interaction between probiotics and starch macromolecules. The developed bread exhibited similar characteristics to the common bread with reduced failure force and better acceptability.

de Oliveira Gomes et al. (2021) prepared Italian salami (a dry fermented meat product) with the incorporation of spray dried probiotic *Bifidobacterium animalis* encapsulated using alginate, β-cyclodextrin, and xanthan gum. The addition of 0.020% curing salt with encapsulated probiotic resulted in higher viability (> 8 log CFU/g) during storage (at 25 °C up to 45 days) without affecting the lipid oxidation, nutritional profile, fatty acid profile, textural properties of salami, as well as the probiotication improved the organoleptic properties. Camargo et al. (2021) probioticated coppa (an industrialized meat product obtained from the pork carcass) with probiotic *Bifidobacterium bifidum* encapsulated by emulsification process with curing salt, black pepper, garlic, and nutmeg. There was a declination of viability from 10.60 to 7.3 log CFU/g after 30 days of ripening period along with lower lipid oxidation and greater weight loss were observed in probiotic added coppa as compared to control samples (without the addition of probiotic) over the storage period.

Yoha et al. (2021) developed 3D printed synbiotic-composite flour construct comprising of green gram, fried gram, ajwain seeds, and barnyard millet with the incorporation of probiotic *Lactiplantibacillus plantarum* encapsulated by spray drying, freeze drying, spray freeze drying, and refractance window drying, and subjected the 3D constructs to post-processing methods such as freeze drying, hot air drying, and microwave drying. In this study, the incorporation of spray-freeze dried probiotic powder (flour and probiotic powder in a ratio of 9:1, w/w) followed by freeze dried 3D printed construct showed the highest viability 6.43 log CFU/mL with 79% survivability under in vitro digestion process as well as retained the probiotic viability with 96–98% of survival rate after 35 days of storage period (stored at 4 °C) but the microwave drying (360 W for 5 min) post-processed flour construct showed the complete loss of cell viability due to the rapid rise in product temperature.

Rajam et al. (2015) microencapsulated *Lactobacillus plantarum* with carrier matrices such as denatured whey protein isolate and fructooligosaccharide. The freeze dried powder was incorporated into noodle formation and the functional properties were evaluated. Declination of survivability from 93.63 to 62.42% was observed in the hot dried noodle. Cooking time was reduced by the incorporation of probiotic microcapsules which might be due to a decrease in the gluten level that allows the rapid gelatinization and increased solid loss occurred due to disturbance in the gluten network that resulted in a discontinuous protein matrix. The probiotic noodle was a little brown color due to the addition of microcapsules, but the overall acceptability was higher.

Packaging of probiotic food products

The selection of packaging material is an important factor for the shelf life extension of probiotic fortified functional food products (Fig. 4). Some anaerobes, in particular *Bifidobacteria*, are more susceptible to oxygen; therefore, plastic material with higher oxygen permeability should be avoided while handling the probiotic products. Application of oxygen scavenging agent in packaging material with good oxygen barrier properties provided favorable conditions for preserving probiotics in yogurt (Miller et al., 2003). Glass containers with greater thickness resulted in higher survivability of *Bifidobacteria* and *L. acidophilus* in yogurt sample as compared to plastic containers (Shah, 2000). The higher cost due to the glass containers could be overcome by the insertion of oxygen scavengers, active packaging, and vacuum packaging with higher barrier properties to air and moisture (Tripathi & Giri, 2014). Edible coating or film with the application of prebiotics such as oligosaccharide and inulin have been shown to improve the viability of probiotics in food materials (Asaithambi et al., 2021). Singu et al. (2020) observed higher viability of probiotics *Saccharomyces boulardii* in synbiotic corn flakes kept in nitrogen gas packaging system (7.81 log CFU/g) as compared to vacuum (7.62 log CFU/g), and atmospheric air (7.46 log CFU/g) packaging on the 90th day of storage. In this study, vacuum packaging caused the breaking of corn flakes thus rupture of the embedded cell membrane of probiotics during the process of creating the vacuum whereas, nitrogen as inert gas prevented the oxidation of stored product thus retaining the survival of probiotics in the stored corn flakes. The storage conditions such as crystallinity of the packaging material, temperature, and relative humidity may influence the permeability of packaging material thus may alter the survival rate of the encapsulated probiotic strains (da Cruz et al., 2007; Miller et al., 2002).

Conclusion

Generally, oral administration of probiotics causes severe loss of viability during the transit of the GI tract. From this point of view, incorporation of microencapsulated probiotic

bacteria in the formulation of food products is an alternative and cheapest way to provide functional benefits to human beings as well as animals despite taking antibiotic treatment. Microencapsulation is an effective approach to maintain the viability of probiotics during manufacturing, storage, and gastrointestinal condition. Different technologies such as spray drying, freeze drying, extrusion have been commonly used but other drying methods such as vacuum, fluidized bed, microwave drying can also be employed effectively to encapsulate the probiotics with suitable coating materials at an industrial scale. The incorporation of microencapsulated probiotics into food formulations is a safe pathway to reach them in the targeted delivery system. This process is also associated with several technological, microbiological, and economic challenges.

Many efforts of researchers have succeeded to overcome the hurdles to some extent but future research work is needed to explore heat-resistant strains and coating materials for encapsulation by employing different cost-effective technologies. This solution will lead to a novel approach for the preparation of food products with functional features but the additional manufacturing cost should be within an acceptable limit to remain competitive in the globalized market of functional foods. The microcapsules containing probiotics should not alter the sensorial properties of formulated products as well as the polymers used for encapsulating should be food grade and certified with GRAS status preferably of plant origins. Emulsion and extrusion technologies are easier to scale up, and these methods avoid high temperatures during the encapsulation process thus providing higher survivability of probiotics with a smaller size of beads. The emulsification process limits the application in food industries as residual oil on the capsule surface hampers the survivability of probiotics and is also detrimental to the sensory properties of food products. Spray chilling has been considered as the least expensive microencapsulation technology for probiotics and fluidized bed drying produces microcapsules of the desired flowability with lower manufacturing cost as compared to spray and freeze drying. Optimization of process conditions is required for different probiotic strains to popularize these technologies on an industrial scale.

Different probiotic fortified dairy and nondairy-based products and beverages, meat-based products, and ready-to-eat cereal products are available in the global market. A consistent effort and research should be put in for the commercial production of probiotic enriched bakery and extruded products for all age groups. Proper labeling and health claims should be informed which includes types of the genus, species, and strain; a minimum viable number of probiotics at the end of shelf-life; serving size to provide an adequate amount of probiotic to satisfy the health claim; description of physiological effect certified by the law with scientific evidence and storage conditions for storing the product. From the overview, it is concluded that microencapsulation technology has future benefits despite the extra cost by incorporating the microencapsulated probiotics for the formulation of higher value-added products.

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Code availability Not applicable.

Declarations

Ethics approval We confirm that the research has been conducted in an ethical and responsible manner. This manuscript has not been published elsewhere and is not under consideration by any other journal. All authors have approved the manuscript and agreed with its submission to the journal. The manuscript has been prepared according to the journal's format as provided in the instructions for authors. There is no conflict of interest among authors regarding this article.

Consent to participate Not applicable.

Consent for publication Not applicable.

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