Encapsulated Probiotics for Non-Dairy Food Applications

Subjects: Food Science & Technology

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The incorporation of probiotics in non-dairy matrices is challenging, and probiotics tend to have a low survival rate in these matrices and subsequently perform poorly in the gastrointestinal system. Encapsulation of probiotics with a physical barrier could preserve the survivability of probiotics and subsequently improve delivery efficiency to the host.

encapsulation non-dairy probiotics stability storage

1. Introduction

The growing awareness among consumers regarding healthy lifestyles has increased the demand for food that could provide additional specific health benefits beyond nutrition. Functional food is one of the leading trends in today's food industry. The term "functional food" refers to foods containing (either present naturally or added by manufacturers) ingredients or bioactive compounds that provide extra health benefits over its adequate nutritional effects, which can beneficially affect one or more physiological mechanisms in the body, resulting in an enhancement in health and reduction in risk for disease, in the amount consumed in a diet [1]. For example, probiotics are one of the dominant groups of functional foods [2].

Probiotics, from the Greek word, "for life", are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host" by a joint United Nations Food and Agricultural Organization/World Health Organization working group in 2001 and The International Scientific Association for Probiotics and Prebiotics (ISAPP). Probiotics have also been considered functional foods due to their health-promoting abilities [3]. Among probiotic strains in use today, strains from genera of *Lactobacillus* and *Bifidobacterium* are the most frequently used. In addition, other non-pathogenic microorganisms that occur within the host gut or tissues have also been developed as probiotics. These include strains from genera *Propionibacterium*, *Pediococcus*, *Bacteroides*, *Bacillus*, *Streptococcus*, *Escherichia*, *Enterococcus*, and *Saccharomyces*. Lately, *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, and *Eubacterium hallii* have also been identified as potential next-generation probiotics with promising health-promoting functionalities [1][4].

By regulating the natural balance of gut bacteria in the human gastrointestinal tract, probiotics have been shown to promote a wide range of health benefits such as improving intestinal health, improving lactose digestion, enhancing the host's immune response, reducing serum cholesterol, diarrhea diseases, and inflammatory bowel disease, counteracting allergies, and lowering the risk of certain cancers [5]. For a potential probiotic strain to exert

therapeutic effects on the host, the viability of probiotics in food should be at least 6 to 7 log CFU/mL (or CFU/g) when reaching the small intestine and colon. In this regard, the viability of at least 8 to 9 log CFU/mL (or CFU/g) of probiotics in food before ingestion is necessary [3][6].

Probiotics must be stable throughout the digestive tract and able to adhere to human epithelial cells when they reach the intestine. However, the survival of probiotics is greatly affected by the harsh conditions of the gastrointestinal tract, including the acidic pH of the gastric environment and bile acids (a loss of around 2 log CFU/mL or CFU/g during digestion) [7]. Several intrinsic (e.g., pH, water activity, molecular oxygen, the composition of the food, food additives added, and oxidation-reduction potential) and extrinsic factors (e.g., temperature, relative humidity, and gas composition) have also been observed to negatively affect the viability and stability of probiotics during food preparation and food processing, as well as over a prolonged storage period [5][7][8].

Traditionally, dairy products have been recognized as the best carriers of probiotics. Current probiotics have been formulated into numerous dairy products, such as fermented milk, yogurt, cheese, and ice cream. However, consumers' preferences today lie more with non-dairy-based probiotic products because of the ongoing trend of vegetarianism and awareness of drawbacks associated with the intake of dairy products, such as lactose intolerance, high cholesterol content, and milk protein allergy [2][9]. In recent years, non-dairy matrices, such as fruits [10][11][12], fruit and vegetable juices [7][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25][26], fermented rice beverages [27], tea [28][29], jelly-like desserts [30], bakery products [31][32][33], cereal bars [34], sauces [35], gum products [36], and powdered functional drink [37] have been explored as vehicles to deliver probiotics. Although non-dairy food matrices are more versatile (absent of lactose, dairy allergens, and cholesterol) than dairy food matrices, the delivery of probiotics using non-dairy food matrices is more challenging. As an example of a dairy food matrix, milk, which is rich in proteins and fats, could effectively act as a protective matrix to protect the probiotics throughout the digestive tract [38]. In contrast, non-dairy food matrices, such as fruit and vegetable juices, have considerable amounts of organic acids, dissolved oxygen, and inherently low pH values that could negatively affect the viability of inoculated probiotics [9]. Dairy food matrices are usually stored at refrigerated temperature (4 °C), and therefore, the viability of probiotics can be well-maintained throughout the product's shelf life. In contrast to dairy food matrices, non-dairy food matrices are often stored at ambient temperature, which could adversely affect the viability of probiotics [2]. The sensory qualities of non-dairy food matrices could also be enhanced or deteriorated by the metabolic compounds produced through the interaction between the probiotics and food matrices [2][9].

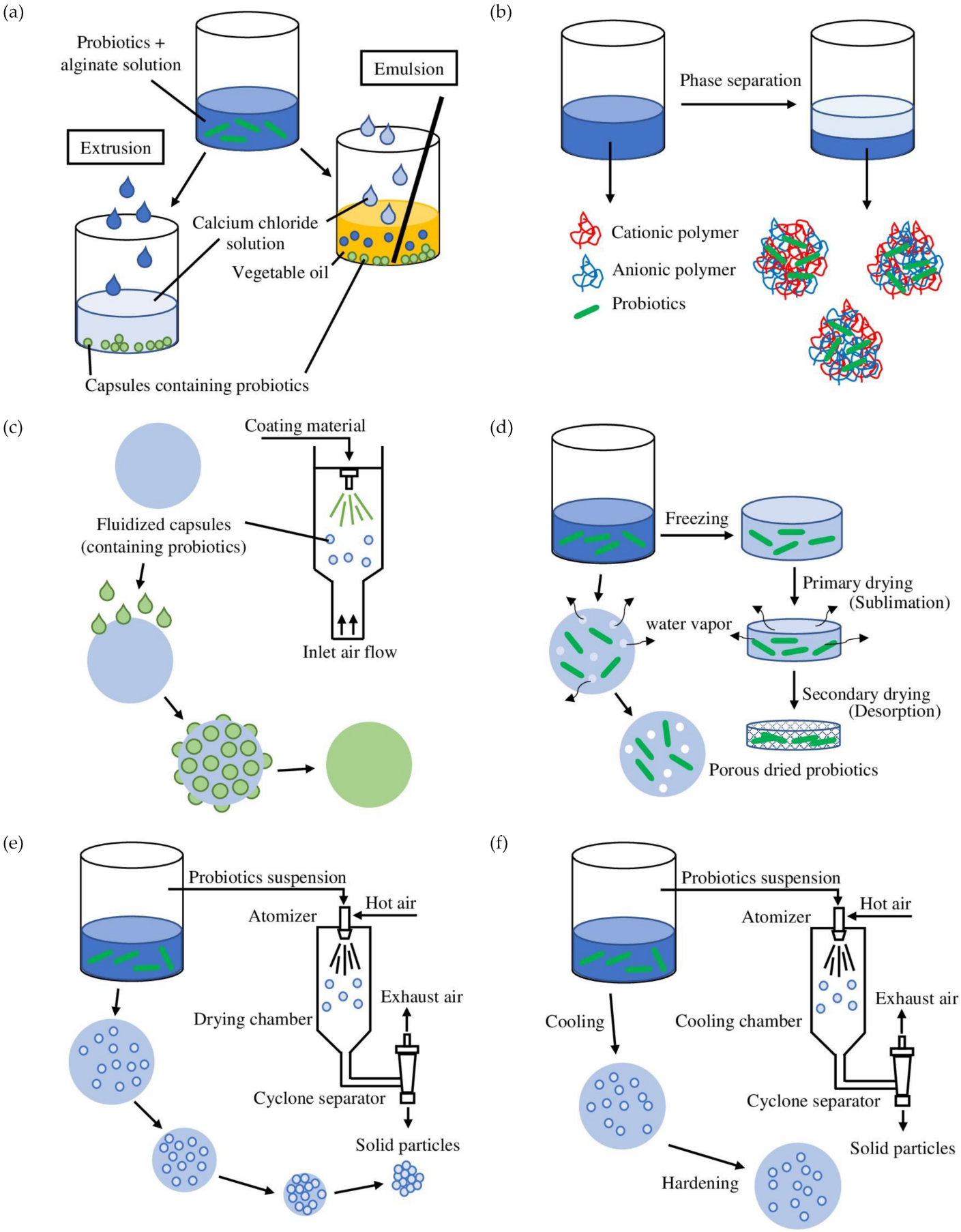
2. Encapsulation

To date, encapsulation is one of the most promising techniques in protecting active compounds against adverse environments. Encapsulation technology has been widely used in the pharmaceutical, medicine, nutritional, food science, biological, agriculture, toiletries, and cosmetics industries for over 50 years. The goal of encapsulation is to protect the encapsulated active compound (core material) against unfavorable or adverse environments (such as light, moisture, temperature, and oxygen). In food industries, a broad range of products (including probiotics, antioxidants, antimicrobials, flavors, enzymes, and nucleic acids) are encapsulated to (a) prevent the core material from degradation, (b) slow down the evaporation rate of volatile core material, (c) separate the components that

would otherwise react with each other, (d) modify the nature of the core material for easier handling, (e) increase the stability, (f) to mask undesired tastes, colors, and odors, (g) enable sustained and controlled release (release slowly over time at a constant rate), (h) control oxidative reactions, (i) use with bacteriophages to control foodborne pathogens, and (j) extend the shelf life. Indeed, encapsulation is one of the new and effective methods to protect probiotics from the harsh conditions they encounter throughout food processing, shelf storage, and gastrointestinal transit [1][39][40][41].

3. Probiotic Encapsulation Techniques

Numerous encapsulation technologies have been developed and adopted to protect probiotics. All the techniques aim to protect the viability and stability of probiotics. However, their concepts, operation methods, and properties of produced capsules are different. Each technique also has its own strengths and drawbacks. **Figure 1** illustrates different types of probiotics encapsulation techniques and the morphologies of corresponding microcapsules obtained. Various aspects must be taken into consideration before the selection of encapsulation techniques. Selecting a suitable encapsulation technique depends on several parameters, such as the nature of the probiotics, the operational conditions of the encapsulation technique, the properties of the biomaterials used, the particle size needed to deliver the adequate probiotics load without affecting the sensory properties, the release mechanism and release rate, the composition of the target food application, the storage conditions of the food products before consumption, and lastly, the cost limitation of production [42][43].


(a)
Probiotics + alginate solution
Emulsion
Extrusion
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Vegetable oil
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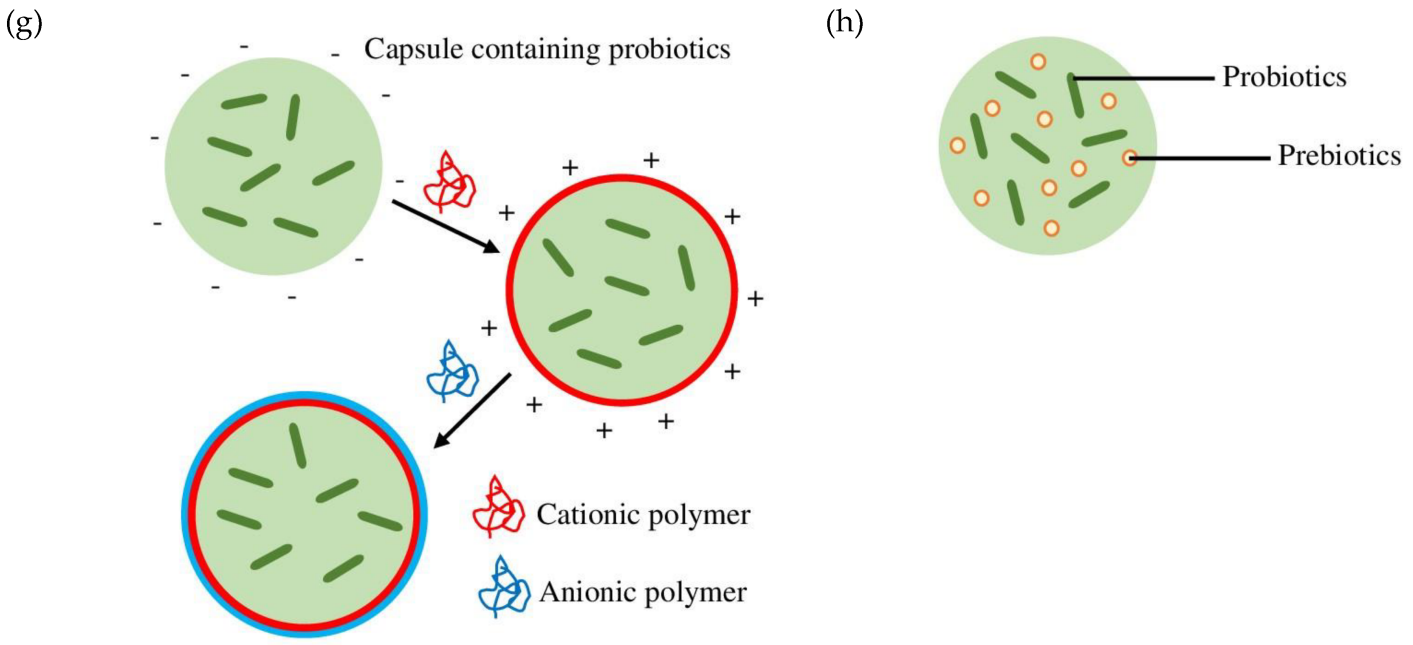

Figure 1. Different types of probiotics encapsulation techniques and the morphologies of corresponding microcapsules obtained: (**a**) ionic gelation (emulsion, extrusion); (**b**) coacervation; (**c**) fluidized bed coating; (**d**) freeze-drying; (**e**) spray-drying; (**f**) spray chilling; (**g**) layer-by-layer method; (**h**) co-encapsulation.

Table 1 shows the main properties, advantages, and disadvantages of encapsulation techniques that can be applied in multilayer and co-encapsulation techniques of probiotics.

Table 1. Overview of common probiotic encapsulation techniques.

Methods	Properties of Encapsulation	Advantages	Disadvantages	References
Extrusion (external ionic gelation)	Produces capsules with sizes of 100 µm to 3 mm. Can encapsulate hydrophilic and hydrophobic/lipophilic compounds.	Monodispersity. Simple and mild process.Can be conducted under both aerobic and anaerobic conditions. Low operation cost. High survival rate of probiotics.	Produces relatively large beads.Slow solidification process. Not suitable for mass production. Additional drying process is required.	[39][40][42][44]

Methods	Properties of Encapsulation	Advantages	Disadvantages	References
Emulsion (internal ionic gelation)	Produces capsules with sizes of 200 nm to 1 mm. Can encapsulate hydrophilic and hydrophobic compounds.	Simple process. Produces relatively small beads. Suitable for mass production. High survival rate of bacteria.	Polydispersity. High operation cost. Conventional emulsions are thermodynamically unstable. Not suitable for low-fat food matrices. Additional drying process is required.	[39][42][44][45]
Coacervation (complex coacervation)	Produces capsules with sizes of 1 µm to 1 mm. Encapsulates hydrophobic compounds.	Simple and mild process. Suitable for the food industry. High encapsulation efficiency. Controlled release potential.	High operational cost. Not suitable for mass production.Animal-based protein is commonly used. Only stable at a narrow pH, ionic strength, and temperature range.	[42][46]
Spray-drying	Produces capsules with sizes of 5–150 µm. Encapsulateshydrophilic and hydrophobic compounds.	Monodispersity. Fast, continuous process.L ow operation cost. Suitable for mass production. Produces dry beads with low bulk density,	Low cell viability. Produces beads with low uniformity.Biomaterials used have to be water-soluble.	[1][39][42][43]

Methods	Properties of Encapsulation	Advantages	Disadvantages	References
		water activity, and high stability.		
Freeze-drying	Produces capsules with sizes of 1–1.5 mm. Encapsulates hydrophilic and hydrophobic/lipophilic compounds.	Suitable for temperature-sensitive probiotics. Dried end product is suitable for most food applications.	High operation cost. Not suitable for mass production. Cryoprotectants are needed.	[39][47]
Spray chilling	Produces capsules with sizes of 20–200 µm. Encapsulates hydrophobic compounds.	Monodispersity. Fast, continuous, mild process. Low operation cost. Suitable for mass production.Promising in controlled release of probiotics.	Low encapsulation efficiency. Rapid release of the encapsulated probiotics. Special storage conditions can be required.	[42][43][48][49]
Fluidized bed coating	Produces capsules with sizes of 5–5000 µm. Encapsulateshydrophilic and hydrophobic compounds.	Mild process.Low operation cost. Suitable for mass production. Can provide multi-coating layers. Suitable for temperature-sensitive probiotics.	Slow process. Probiotics have to be pre-encapsulated and dried.	[42][43][50][51]

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3. FAO/WHO. FAO/WHO Joint Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food (30 April 2002 and 1 May 2002); Scientific Research Publishing: London, ON, Canada, 2002.

4. Biomaterials Utilized for Probiotics Encapsulation

To be an effective encapsulation material (core or wall material), the biomaterial used must be able to protect the encapsulated probiotics along the gastrointestinal tract until reaching the target site (small intestine/large intestine), where they can exert their health-promoting effects. The encapsulation material should only release the encapsulated probiotics when it is exposed and triggered by certain environmental conditions (such as temperature, pH, and enzyme activity). In other words, the capsules containing probiotics should remain protected inside the encapsulation material during the passage through the stomach and only decompose after reaching the target site to release the probiotics. The commonly used biomaterials in probiotic encapsulation include carbohydrates, proteins, and lipids, which will be discussed in detail in the coming subsections. Their specific advantages and limitations in probiotic encapsulation are also summarized in **Table 2**.

Table 2. Common biomaterials for encapsulating probiotics.

Category	Biomaterial	Characteristics and Advantages	Limitations	Remarks	References
Carbohydrate	Alginates	Anionic character, non-toxic, biocompatibility, biocompostability, cell affinity, strong bioadhesion, absorption characteristics, antioxidative, anti-inflammatory, and low in cost. Stable (shrink) in the low acidic stomach gastric solution and gradually dissolve (swell and release encapsulated probiotics) under alkaline conditions in the small intestine.	Sensitive to heat treatment, highly porous, poor stability and barrier properties.	Technique: extrusion, emulsion.Could form a strong gel network by interacting with cationic material (e.g., chitosan). Combination: pectin, starch, chitosan.	[52][53]

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8. Thamacharoensuk, T.; Boonsom, T.; Tanasupawat, S.; Dumkliang, E. Optimization of microencapsulated Lactobacillus rhamnosus GG from whey protein and glutinous rice starch by

16. Naga Sivudu, S.; Ramesh, B.; Umamahesh, K.; Vijaya Sarathi Reddy, O. Probiotication of tomato and carrot juices for shelf-life enhancement using micro-encapsulation. J. Food Sci. Technol.

Category	Biomaterial	Characteristics and Advantages	Limitations	Remarks	References
	Chitosan	Cationic character, non-toxic, biodegradability, bioadhesiveness, antimicrobial, antifungal, low in cost, high film-forming properties, great probiotics biocompatibility, resistance to the damaging effects of calcium chelating and anti-gelling agent, generate strong beads.	Degrade easily in low pH conditions, water-insoluble at pH > 5.4. Pose inhibitory effect against lactic acid bacteria.	Technique: extrusion, layer-by-layer (LbL), emulsion.Normally used as a coating rather than as a capsule. Combination: alginate, pectin.	[53][54]
	Starch and starch derivatives	GRAS is abundant, low in cost, non-allergenic, and biodegradable. Could produce gels with strong but flexible structure, transparent, colorless, flavorless, and odorless gel that is semi-permeable to water, carbon dioxide, and oxygen. Resistant to pancreatic	Exhibit high viscosity in solution.	Technique: extrusion, emulsion. Combination: alginate.	[54][55]

27. Srisuk, N.; Nopharatana, M.; Jirasatid, S. Co-encapsulation of Dictyophora indusiata to improve Lactobacillus acidophilus survival and its effect on quality of sweet fermented rice (Khoa-Mak) sap beverage. J. Food Sci. Technol. 2021, 58, 3598–3610.

Category	Biomaterial	Characteristics and Advantages	Limitations	Remarks	References
		enzymes. Pose prebiotic properties.			
	Cellulose and cellulose derivatives	Abundant, low in cost, biodegradability, biocompatibility, tunable surface properties. Insoluble at pH ≤ 5 but soluble at pH ≥ 6, effective in delivering probiotics to the colon.	Cannot form gel beads by extrusion technique.	Technique: emulsion, spray-drying. Combination: alginate, protein.	[56]
	Maltodextrin	Non-toxic, bland in taste, abundant, low in cost, good solubility, low viscosity even at high solid content. Excellent thermal stability. Pose (moderate) prebiotic properties.	Low emulsifying capacity.	Technique: spray-drying. Combination: gum Arabic, sodium caseinate.	[40][57]
	Carrageenan (κ-carrageenan)	Pose thermosensitive and thermoreversible characteristics, the probiotic release can be controlled with temperature.	The gel beads produced are irregular in shape, brittle and weak, and their probiotic release rate is much slower	Technique: extrusion, emulsion. Dissolves at 80–90 °C. Addition of probiotics at 40–50 °C. Gelation at room temperature.	[40][58]

39. Oberoi, K.; Tolun, A.; Sharma, K.; Sharma, S. Microencapsulation: An overview for the survival of probiotic bacteria. J. Microbiol. Biotechnol. Food Sci. 2021, 2021, 280–287.

Category	Biomaterial	Characteristics and Advantages	Limitations	Remarks	References
			than alginate beads.	Combination: milk protein, alginate, locust bean gum (LBG), carboxymethyl cellulose.	
	Pectin	Anionic character, abundant, non-toxic, water-soluble, biocompatibility, biodegradability, bioadhesiveness, antimicrobial, antiviral, good gelling, emulsifying, thickening and water binding properties, prebiotic effect.	Low in thermal stability, poor mechanical properties. High water solubility. High concentration of sucrose contents.	Technique: spray-drying. Combination: a variety of carbohydrate-based biomaterials.	[53][59][60]
Gums	Xanthan gum	Anionic character, non-toxic, biodegradable, biocompatible, excellent gelling properties, highly soluble in both cold and hot water. Excellent heat and acid stability. Resistant to gastrointestinal digestion and enzymatic	High susceptibility to microbial contamination, unstable viscosity, and uncontrollable hydration rate. Gels produced solely using xanthan gum are relatively weak.	Technique: spray and freeze-drying. Combination: alginate, chitosan, gellan, and β-cyclodextrin.	[40][61][62]

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Category	Biomaterial	Characteristics and Advantages	Limitations	Remarks	References
		decomposition. Could also act as a source prebiotic.			
	Gellan gum	Anionic character, non-toxic, biocompatible, biodegradable, water-soluble, and low in cost. High resistance against heat, acidic environments, and enzymatic degradation. Swell at high pH.	High gel-setting temperatures (80–90 °C) cause heat injuries to probiotics.	Technique: spray-drying. Combination: gelatin, sodium caseinate, and alginate.	[40][43][63]
	Gum Arabic	Anionic character, acid stability, highly water soluble, low in viscosity. Exhibit surface activity, foaming, and emulsifying abilities. Could prevent complete dehydration of probiotics during the drying process and storage.	Restricted availability and high cost. Show only partial protection against oxygen.	Technique: spray-drying. Combination: maltodextrin, gelatin, whey protein isolates.	[40][57][63]
Animal-based proteins	Gelatin	Amphoteric character, could form complexes with anionic	High solubility.	Technique: extrusion, complex coacervation, spray chilling,	[1][40][64]

proteins in nutritional supplement powders: A review. Int. Food Res. J. 2019, 26, 1651–1664.

Category	Biomaterial	Characteristics and Advantages	Limitations	Remarks	References
		polymers. Could produce beads with strong structure and impermeable to oxygen.		spray-drying, lyophilization. Combination: alginate, pectin.	
	Whey protein	Amphoteric character, highly nutritious, high resistance and stability against pepsin digestion, great gelation properties, thermal stability, hydration, and emulsification properties.	The gel beads or matrices produced are weak.	Technique: extrusion. Combination: gum Arabic, pectin, maltodextrin.	[40][65][66]
	Milk protein (casein)	Amphiphilic character, abundant, low in cost, possess excellent gelling and emulsifying properties, self-assembling properties, biocompatibility, biodegradability, produce gel beads with varying sizes (range from 1 to 1000 µm), higher density and better protection, high	Immunogenicity and allergenicity.	Technique: extrusion, emulsification, spray-drying, enzyme-induced gelation. Combination: a variety of carbohydrate-based biomaterials.	[40][67][68]

Category	Biomaterial	Characteristics and Advantages	Limitations	Remarks	References
		resistance to thermal denaturation (sodium caseinate).			
Plant-based proteins	Zein protein	Amphiphilic character, biocompatible, biodegradable, water-insoluble, high resistance against gastric juice.	Highly unstable, aggregate in aqueous solutions.	Technique: electro-spinning, electro-spraying, spray-drying. Combination: sodium caseinate, alginate, pectin.	[68]
	Soy protein	High nutritional value, less allergenic, surface active, good emulsifying, absorbing, film forming properties, high resistance against gastric juice.	Heat-induced gel formation.	Technique: extrusion, spray-drying, coacervation. Combination: carrageenan, pectin.	[40][68][69]
Lipids	Natural waxes, vegetable oils, diglycerides, monoglycerides, fatty acids, resins	Low in polarity, excellent water barrier properties, thermally stable, and could encapsulate hydrophilic substances.	Weak mechanical properties, chemically unstable, might negatively affect the sensory characteristics of food products	Technique: spray chilling, spray coating.Have melting points ranging from 50–85 °C. Combination: polysaccharides or proteins.	[70]

Category	Biomaterial	Characteristics and Advantages	Limitations	Remarks	References
			due to lipid oxidation.		

5. Application of Probiotics Encapsulation in Non-Dairy-Based Food and Beverage Products

The growing demand for non-dairy probiotic food products has encouraged scientists and researchers to explore more new non-dairy food matrices (**Table 3**). Recent studies have proved that non-dairy food matrices (known to be free of lactose, dairy allergens, and cholesterol and rich in nutrients) are promising vehicles for probiotic delivery. Furthermore, the probiotics were also observed to adapt well to encapsulation using non-dairy food matrices owing to their richness in nutrients. However, researchers still face some challenges, such as the maintenance of probiotic viability and sensory properties of probiotic food products [2][9]. For instance, the composition, pH value, and storage condition of the non-dairy food substrate could negatively affect the viability of inoculated probiotics. Under certain conditions, the metabolic compounds produced through the interaction between the probiotics and food matrices could negatively affect the sensory qualities of non-dairy food products. While probiotics do not usually replicate in non-dairy matrices, it is necessary to keep the viability of probiotics at an adequate level. In addition, components such as carbohydrates, proteins, and flavoring agents in the food matrix could also negatively affect the viability of probiotics. Encapsulated probiotics with bigger particle sizes were also reported to be adverse to the mouthfeel sensation.

Table 3. Examples of recent application of probiotics encapsulation in non-dairy-based products.

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
Fruit and vegetable-based	Emulsion	*Bifidobacterium bifidum*	60 mL sodium alginate, κ-carrageenan, 5 g Tween 80	Grape juice	The viability of *B. bifidum* was enhanced from 6.58 log CFU/mL (free) to 8.51 log CFU/mL (sodium alginate-encapsulated) and 7.09 log CFU/mL (κ-carrageenan-	[7]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
					encapsulated) after 35 days of storage.	
	Extrusion	*Enterococcus faecium*	2% (*w*/*w*) sodium alginate	Cherry juice	Encapsulated probiotics had higher viability during storage (4 and 25 °C) and stronger tolerance against heat, acid, and digestion treatments than free probiotics.	[13]
	Emulsion	*Lactobacillus salivarius* spp. *salivarius* CECT 4063	100 mL of sodium alginate (3%), 1 mL Tween 80	Apple matrix	Encapsulated *L. salivarius* spp. *Salivarius* had higher survivability (3%) than those non-encapsulated (19%) after 30 days of storage.	[10]
	Complex coacervation	*Bifidobacterium animalis* subsp. *lactis*	6% whey protein concentrate, 1% gum Arabic, 5% (*w*/*w*) proanthocyanidin-rich cinnamon extract (bioactive compound)	Sugar cane juice	Co-encapsulation of compounds was effective in protecting the viability of *B. animalis* and the stability of proanthocyanidins during storage and allowing simultaneous delivery.	[14]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
	Emulsion	*Lactobacillus acidophilus* PTCC1643, *Bifidobacterium bifidum* PTCC 1644	2% (*v*/*w*) sodium alginate, 5 g/L Span 80 emulsifier	Grape juice	The survivability of *L. acidophilus* and *B. Bifidum* in the encapsulated samples (8.67 and 8.27 log CFU/mL) was higher than free probiotics (7.57 and 7.53 log CFU/mL) after 60 days of storage at 4 °C.	[15]
	Emulsion followed by coating	*Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Lysinibacillus sphaericus*, *Saccharomyces boulardii*	Emulsion: 20 mL of sodium alginate (2%), 0.1% Tween 80 Coating: 0.4% chitosan in acidified distilled water	Tomato and carrot juices	Encapsulated probiotics had higher viability than free probiotics during storage of 5–6 weeks at 4 °C. *Lys. sphaericus* was observed to have higher viability and stability than other probiotics.	[16]
	Co-encapsulation (extrusion)	*Lactococcus lactis* ABRIINW-N19	1.5, 2% alginate-0.5% Persian gum (hydrogels), 1, 1.5, 2% fructooligosaccharides (FOS; prebiotic), and 1, 1.5, 2% inulin (prebiotic)	Orange juice	All formulations used were able to retain the viability of *L. lactis* during 6 weeks of storage at 4 °C. Encapsulated *L. lactis* were only released after 2 h and remained stable for up to 12 h in colonic conditions.	[17]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
	Vibrating nozzle method (evolved extrusion)	*Lactobacillus casei* DSM 20011	2% sodium alginate	Pineapple, raspberry, and orange juices	After 28 days of storage at 4 °C, some microcapsules were observed as broken in pineapple juice, but the viability was 100% (2.3×10^7 CFU/g spheres). 91% viability (5.5×10^6 CFU/g spheres) was observed in orange juice. Raspberry juice was not a suitable medium for *L. casei*.	[18]
	Co-encapsulation (spray-drying)	*Lactobacillus reuteri*	60 g maltodextrin, 0−2% gelatin	Passion fruit juice powder	The use of gelatin in combination with maltodextrin was more efficient in maintaining the cellular viability and retention of phenolic compounds than maltodextrin alone.	[19]
	Spray-drying	*Lactobacillus plantarum*	0.5% (*w*/*w*) magnesium carbonate, 12% (*w*/*w*) maltodextrin	Sohiong (*Prunus nepalensis* L.) juice powder	The quality of probiotic Sohiong juice powder and viability of *L. plantarum* (6.12 log CFU/g) could be maintained for 36 days without refrigeration (25 °C	[20]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
					and 50% relative humidity).	
	Fluidized bed drying	*Bacillus coagulans*	Mixture of 0.0125 g/mL hydroxyethyl cellulose and 1.17 µL/mL polyethylene glycol	Dried apple snack	Encapsulated *Bacillus coag-ulans* in dried apple snacks had high viability (>8 log CFU/portion) after 90 days of storage at 25 °C.	[11]
	Extrusion	*Lactobacillus plantarum*	Mixtures (1:2, 1:4, 1:8, 1:12) of 4% (*w*/*v*) sodium alginate and 20% (*w*/*v*) soy protein isolate	Mango juice	Homogenous aqueous solutions of alginate and soy protein isolate (1:8) increased the thermal resistance of *L. plantarum* against pasteurization process. The viability of *L. plantarum* remained high after the pasteurization process (8.11 log CFU/mL; reduced 0.99 log CFU/mL).	[21]
	Layer-by-layer (Coating)	*Lactobacillus plantarum* 299v	First layer: 1% (*w*/*v*) carboxymethyl cellulose (CMC) and 50% *w*/*w* (based on CMC weight) glycerol; Second layer: 5% (*w*/*v*) zein protein	Apple slices	The viability of CMC-zein protein-coated *L. plantarum* 299v was higher than CMC-coated *L. plantarum* 299v in apple slices under simulated gastrointestinal	[12]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
					conditions (120 min digestion; CMC-zein protein-coated: 1.00 log CFU/g reduction, CMC-coated: 2.18 log CFU/g reduction).	
	Complex coacervation (associated with enzymatic crosslinking)	*Lactobacillus acidophilus* LA-02	Complex co-acervation: 2.5% gelatin, 2.5% gum Arabic; Crosslinking: 2.5, 5.0 U/g transglutaminase	Apple and orange juices	Encapsulated *L. acidophilus* LA-02 incorporated in fruit juices was able to survive throughout the storage period of 63 days (4 °C).	[22]
	Freeze-drying, spray-drying	*Enterococcus faecalis* (K13)	Gum Arabic and maltodextrin	Carrot juice powder	Heat injuries to the probiotics are lower in the freeze-drying technique compared to spray-drying. After being stored for 1 month, the viability of freeze-dried *E. faecalis* remained high (6–7 log CFU/g).	[23]
	Spray-drying	*Lactobacillus casei* Shirota, *Lactobacillus casei* Immunitas, *and Lactobacillus*	Maltodextrin and pectin at weight ratio of 10:1	Orange juice powder	The combination of pectin and maltodextrins effectively protected the probiotics during the spray-drying	[24]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
		acidophilus Johnsonii			process and storage (4 °C)	
	Freeze-drying	*Lactobacillus acidophilus*, *Lactobacillus casei*	Whey protein isolate, fructooligosaccharides, and combination of whey protein isolate, fructooligosaccharides (1:1)	Banana powder	*L. acidophilus* and *L. casei* encapsulated with the combination of whey protein isolate and fructooligosaccharides had higher survivability after being stored for 30 days at 4 °C and more resistant to the simulated gastric fluid intestinal fluid than free probiotics.	[25]
	Fluidized bed drying	*Lactobacillus plantarum* TISTR 2075	3% (*w*/*w*) gelatin and 5% (*w*/*w*) of monosodium glutamate, maltodextrin, inulin, and fructooligosaccharide	Carrot tablet	Encapsulated *L. plantarum* TISTR 2075 in carrot tablet (survivability: 77.68–87.30%) had higher tolerance against heat digestion treatments than free cells (39.52%).	[26]
Other beverages	Spray-drying	*Lactobacillus rhamnosus* GG (LGG)	Mixtures (1:1.6 (*w*/*w*)) of 7.5% (*w*/*v*) whey protein isolate and 20% (*w*/*v*) modified huauzontle's starch (acid hydrolysis-extrusion),	Green tea beverage	The viability of LGG remained above the recommended 7 log CFU/mL after 5 weeks of storage at 4 °C.	[28]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
			supplemented with ascorbic acid			
	Co-encapsulation (extrusion)	*Lactobacillus acidophilus* TISTR 2365	Alginate, egg (0, 0.8, 1, and 3%, *w*/*v*), and fruiting body of bamboo mushroom (prebiotic)	Sweet fermented rice (Khoa-Mak) sap beverage	All formulations used were able to provide high encapsulation yields (95.72−98.86%) and high viability of *L. acidophilus* (>8 log CFU/g) in Khoa-Mak sap beverages for 35 days of storage at 4 °C. Encapsulation with involvement of 3% egg of bamboo mushroom increased the survival of *L. acidophilus* the most.	[27]
	Co-encapsulation (extrusion)	*Lactobacillus acidophilus* NCFM (L-NCFM)	Co-extrusion: 0–2% (*w*/*v*) LBG, 0–5% (*w*/*v*) mannitol (prebiotic) Coating: sodium alginate	Mulberry tea	L-NCFM encapsulated with LBG and mannitol (0.5% (*w*/*v*) and 3% (*w*/*v*), respectively) showed microencapsulation efficiency and viability of 96.81% and 8.92 log CFU/mL, respectively. Among other samples, L-NCFM microencapsulated	[29]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
					with mannitol showed the highest survivability (78.89%) and viable count (6.80 log CFU/mL) after 4 weeks of storage at 4 and 25 °C.	
Bakery products	Double-layered microencapsulation, combination of spray chilling and spray-drying	*Saccharomyces boulardii*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*	Spray chilling: 5% (*v*/*w*) blend of gum Arabic and β-cyclodextrin solution (9:1 (*w*/*w*), 20 g in total), 1% lecithin Spray-drying: 5% (*v*/*w*) blend of gum Arabic and β-cyclodextrin solution, 20 g hydrogenated palm oil, 2% Tween 80 emulsifier	Cake	The survivability of probiotics during the cake baking process was improved by double-layered microencapsulation.	[31]
	Fluidized bed drying	*Lactobacillus sporogenes*	First layer: 10 g microcrystalline cellulose powder and alginate or xanthan gum Second layer: gellan or chitosan	Bread	Encapsulated *L. sporogenes* in alginate (1%) capsule tolerated the simulated gastric acid condition the best. The incorporation of chitosan (0.5%) as an outer layer improved the heat tolerance of *L. sporogenes*.	[32]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
					Encapsulated *L. sporogenes* with an outer layer coated with 1.5% gellan showed the highest survivability 24 h after baking.	
	Emulsion	*Lactobacillus acidophilus* ATCC 4356	1. Alginate 2%; 2. Alginate 2% + maltodextrin 1%; 3. Alginate 2% + xanthan gum 0.1%; 4. Alginate 2% + maltodextrin 1% + 0.1% xanthan gum	Bread	Among the encapsulation agents, probiotics encapsulated using the combination of maltodextrin, xanthan gum, and alginate (4) had the highest survivability under storage (7.7 log CFU/bread) and simulated gastrointestinal conditions.	[33]
Sauce	Co-encapsulation (extrusion)	*Lactobacillus casei* Lc-01, *Lactobacillus acidophilus* La5	4% (*w*/*v*) sodium alginate and 2% alginate mixture in distilled watercontaining 2% high amylose maize starch (prebiotic), 0.2% Tween 80	Mayonnaise	The viability of *L. casei* and *L. acidophilus* encapsulated with high amylose maize starch (7.204 and 8.45 log CFU/mL, respectively) was higher than free probiotics (6.23 and 6.039 log CFU/mL, respectively) and	[35]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
					those without high amylose maize starch (7.1 and 7.94 log CFU/mL, respectively) after 91 days of storage at 4°C.	
Others	Extrusion followed by freeze-drying	*Lactobacillus casei* (*L. casei* 431)	3% (*w*/*v*) quince seed gum, sodium alginate, quince seed gum-sodium alginate	Powdered functional drink	Quince seed gum-alginate microcapsules provided encapsulation efficiency of 95.20% and increased the survival rate of *L. casei* to 87.56%. The powdered functional drink was shelf stable for 2 months.	[37]
	Spray chilling	*Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis*	Vegetable fat (Tri-HS-48)	Savory cereal bars	The viabilities of spray-chilled probiotics were higher than freeze-dried and free probiotics in the savory cereal bars after being stored for 90 days at 4 °C.	[34]
	Co-encapsulation (extrusion)	*Lactobacillus reuteri*	2% (*w*/*v*) sodium alginate, 5 mL of inulin and lecithin solution (0, 0.5, and 1%)	Chewing gum	After storing for 21 days with encapsulation, *L. reuteri* remained viable. The viability of the probiotic	[36]

Category	Technology	Probiotic/LAB Strain	Encapsulating Agent	Food Product	Results	Reference
					increased with the concentration of inulin and lecithin.	