TECNOCIENCIA CHIHUAHUA
Revista de ciencia y tecnología
ISSN-e: 2683-3360

UACH
UNIVERSIDAD AUTÓNOMA DE CHIHUAHUA

Artículo de Revisión

Recent developments on wall materials for the microencapsulation of probiotics: A review

Desarrollos recientes en materiales de pared para la microencapsulación de probióticos: Una revisión

José Gabriel Montoya-Soto[1], Rubén Francisco González-Laredo[1], Luis Medina-Torres[2]*, Olga Miriam Rutiaga-Quiñones[1], José Alberto Gallegos-Infante[1], Luz Araceli Ochoa-Martínez[1],

[1] Tecnológico Nacional de México/IT de Durango. Felipe Pescador 1830 Ote., 34080 Durango, Dgo., México.
[2] Universidad Nacional Autónoma de México. Facultad de Química. Circuito Exterior S/N, Coyoacán, Cd. Universitaria, 04510 Ciudad de México, CDMX.

*Correspondencia: luismt@unam.mx (Luis Medina-Torres)
DOI: https://doi.org/10.54167/tch.v17i1.1140
Recibido:: 09 de enero de 2023; **Aceptado**: 18 de abril de 2023
Publicado por la Universidad Autónoma de Chihuahua, a través de la Dirección de Investigación y Posgrado

Abstract

In recent decades a surge in demand for better and healthier foods has sprung up. One category of products under such increases in demand is probiotic products, both in the form of foodstuffs and dietary supplements. These are living microorganisms that when consumed provide a variety of health benefits to the host, regarding the health of the gastrointestinal tract. The main technological hurdle this presents is to provide them alive in enough quantity. Therefore, microencapsulation methods are often employed to enhance their survivability. A critical point in the design of the encapsulation processes is the adequate selection of an encapsulating agent, which must comply with a series of requirements such as being food grade, being able to envelop the probiotic, and being of low cost to name a few. Thus, this presents an area of opportunity regarding the formulation and exploration of different wall materials. In this paper, some of the developments regarding new wall materials for microencapsulated probiotics are presented and discussed.

***Keywords*: microencapsulation, probiotic, wall material, *Lactobacillus*, mucilage, gum**

Resumen

En las últimas décadas ha surgido un aumento en la demanda de alimentos mejores y más saludables. Entre ellos, los productos probióticos, ya sea en forma de productos alimenticios o como suplementos dietéticos. Los probióticos son microorganismos vivos que cuando se consumen en cantidades adecuadas brindan una variedad de beneficios para la salud del huésped, en particular,

a la salud del tracto gastrointestinal. El principal obstáculo tecnológico que esto plantea es proporcionarlos vivos en cantidad suficiente. Por ello, a menudo se emplean métodos de microencapsulación para aumentar su capacidad de supervivencia. Un punto crítico en el diseño de los procesos de encapsulación es la selección adecuada de un agente encapsulante, el cual debe cumplir con una serie de requisitos como ser grado alimenticio, poder envolver al probiótico y ser de bajo costo, por mencionar algunos. Por lo tanto, esto presenta un área de oportunidad en cuanto a la formulación y exploración de diferentes materiales de pared. En este artículo, se presentan y discuten algunos de los desarrollos relacionados con nuevos materiales de pared para probióticos microencapsulados.

***Palabras clave*: microencapsulación, probiótico, material de pared, *Lactobacillus*, mucílago, goma.**

1. Introduction

The importance of probiotics in several sectors such as the pharmaceutical and food industries has resulted in an ever-increasing interest in its application to both food systems and food supplements and drugs. A microorganism is considered a probiotic by the United Nations Food and Agriculture Organization (FAO) and World Health Organization (WHO) if it provides measurable and verifiable health benefits to the host when it is administered in a sufficient dose. A sufficient dose is considered to be around 10^6 CFU per milliliter or gram (although this may vary from individual to individual) (FAO/WHO, 2002, 2006). Ensuring that the right amount of living cells is administered is one of the challenges that technologists and engineers face when working with probiotics due to the harsh conditions during both processing and delivering into the gastrointestinal tract.

Microencapsulation is the group of technologies in which a sensitive but biologically active component is enveloped using a much more resistant material known as wall material. Some of the features of the encapsulated products are consumer safety, such as the "generally recognized as safe (GRAS)" label given by the Food and Drug Administration (FDA); ability to envelop adequately the active material, preserving its bioactive and/or organoleptic features; protection against adverse environmental conditions such as humidity, temperature changes, or UV radiation; and controlled release of the active material after its consumption (Kandasamy and Naveen, 2022). Thus, one of the main concerns when designing a probiotic microencapsulation process is the adequate selection of the encapsulating material (wall material) because it must comply the aforementioned characteristics and be compatible with the probiotic physiological characteristics, site of action, preferred release mechanism and the encapsulation technique used.

Thus, one of the food engineers' main concerns is seeking new wall materials sources that fulfill technological features. Among them, meeting GRAS requirements, good thermal, rheological, and physicochemical in addition to other effects such as prebiotic potential or anti-inflammatory properties (Macías-Cortes *et al*., 2020). The objective of this review is to collect works about the different materials used for the microencapsulation of probiotics; with an emphasis in novel or previously unexplored materials such as mucilage, gums, vegetable proteins.

2. Probiotics

As stated in Probiotics in Food: Health and nutritional properties page 2, the FAO and WHO (2002), define a probiotic as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." The use of probiotics in food systems is quite old, dating back centuries, mainly for the production of fermented milk products such as yogurt and kefir. Some of the microorganisms responsible for the production of these food products include several genera of lactic acid bacteria such as *Lactobacillus*, *Streptococcus*, *Lactococcus*, and yeasts such as some species of the genus *Kluyveromyces*. In broad terms, the idea behind the consumption of probiotics is to change the composition of the normal and potentially harmful microbiome into one that provides benefits to its host. This is due to multiple mechanisms by which probiotics benefit their host, as shown in Figure 1. These include enhancing the epithelial barrier, increasing adhesion to the gastrointestinal mucosa, inhibition of pathogen adhesion, competitive exclusion of pathogens, secretion of antimicrobial substances, and modulation of the immune system (Bermudez-Brito *et al*., 2012)

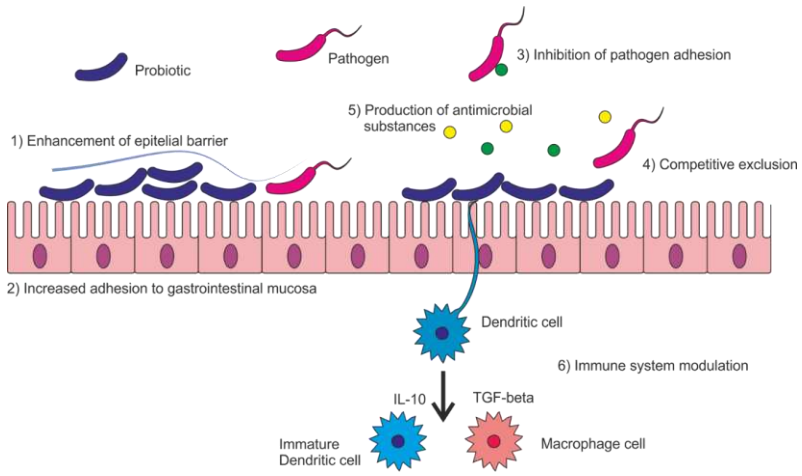

Figure 1. Probiotic's mechanisms of action.
Figura 1. Mecanismos de acción de los probióticos.

2.1 Common probiotics

2.1.1 Lactic acid bacteria

Lactic acid bacteria are a group of facultative anaerobic gram-positive bacteria, commonly found in human mucosal surfaces and fermented foods, such as some dairy products and fermented vegetables (Vinderola *et al*., 2019). These include *Lactobacillus*, *Lactococcus*, *Streptococcus*, etc. Lactic acid bacteria have been used for at least a century in the production of products such as fermented dairy foods like yogurt, cheese, and kefir, and fermented vegetables such as pickles, sauerkraut, or

kimchi, and some fermented meats like salami. Some species of *Lactobacillus* used as probiotics are *Lactobacillus acidophilus, Lb. rhamnosus, Lb. casei, Lb. helveticus* (Azad *et al*., 2018). Regarding their morphology, they can be classified into bacilli and cocci. According to their metabolism they can be separated into homofermentative, bacteria that produce almost exclusively lactic acid, and heterofermentative which can produce other metabolic products such as ethanol, acetic acid, and CO_2. Figure 2 shows a diagram of the main metabolic pathways from which homofermentative and heterofermentative bacteria obtain energy.

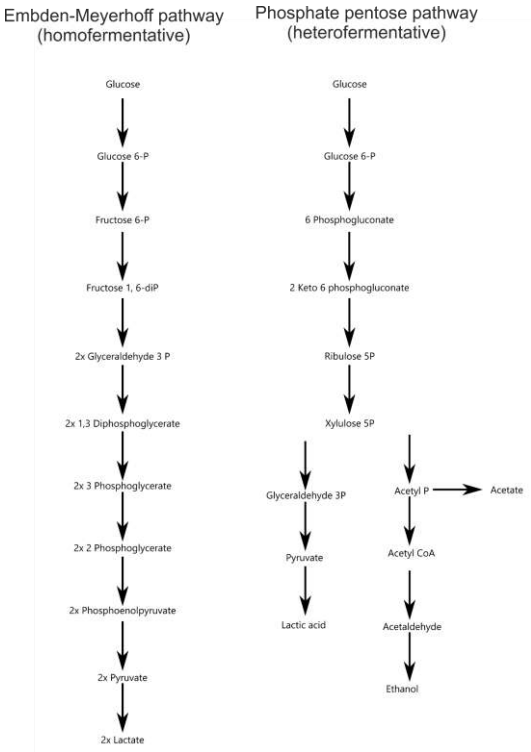

Figure 2. Embden-Meyerhoff (left) and Phosphate pentose (right) metabolic pathways.
Figura 2. Rutas metabólicas Embden-Meyerhoff (izquierda) y pentosas fosfato (derecha).

Lactobacilli are rod-shaped bacteria often found in food such as dairy and fermented vegetables. In the same way lactobacilli can be classified by their metabolism as homofermentative and heterofermentative.

Homofermentative bacteria's main characteristic is that the primary product of their fermentation is lactic acid, with little to no presence of other metabolites such as CO_2, acetic acid, or ethanol. This is due to a preference of species within this classification for the Embden-Meyerhoff pathway that uses glucose as its main substrate, ending with pyruvate as final product which then acts as an acceptor of protons by reduced nicotinamide adenine dinucleotide (NADH) and leads to the production of lactate and NAD+ (Poltronieri *et al*., 2017). While strict homofermentative bacteria exist within the *Lactobacillus* genus, they are fewer in number than heterofermentative species. Some

homofermentative species within the genus are *Lb. acidophilus, Lb. delbrueckii, Lb. helveticus, Lb. salivarius* (Vinderola *et al*., 2019).

Heterofermentative includes all lactic acid bacteria that produce other metabolites aside from lactic acid while fermenting sugars, this includes CO_2, ethanol, and acetic acid. Bacteria within this group, have a predilection for the pentose phosphate pathway that uses sugars (e.g., glucose) as a substrate to produce NADPH, pentoses, and ribose (used for the synthesis of nucleotides), and the final product is xylulose, which is metabolized into ethanol, acetate, and lactate by its catabolism. Within this classification exist both obligate heterofermentative and facultative heterofermentative. *Lactobacillus* species such as *plantarum, sakei, curvatus,* and *casei,* are considered facultative heterofermentative; while species such as, *fermentum, pontis, reuteri, brevis,* and *buchneri*, are considered obligate heterofermentative. These bacteria have shown several probiotic activities such as immune system modulation, production of antimicrobial substances and direct competition with pathogens (Vinderola *et al., 2019*).

2.2.1 Other bacteria

Even when the most studied probiotics are lactic acid bacteria, other genera such as *Propionibacterium*, or *Bifidobacterium* can be found inhabiting the same places. In addition, other genera typically associated with the human gut as *Escherichia* and *Clostridium*, may have probiotic strains (George Kerry *et al*., 2018; Crook *et al*., 2019; Guo *et al*., 2020).

2.3.1 Yeast

Yeast is a heterogeneous denomination given to single-celled organisms belonging to the fungi kingdom. They are widely used in the food industry and are involved in the production of many food products including wine, beer, bread, kefir, cider, sake, cocoa, etc. Some species of yeast that have shown potential as probiotics include *Saccharomyces cerevisiae, S. cerevisiae* var. *boulardii, Kluyveromyces marxianus, K. lactis, Pichia kluyveri, P. pastoris*, *Debaromyces spp., Torulaspora spp., Hanseniaspora spp.,* etc. These have shown various activities such as denaturing *Clostridium difficile*'s toxins and modulation of cytokines production (Gut *et al*., 2018; Staniszewski and Kordowska-Wiater, 2021).

3. Microencapsulation

This is the name given to technologies whose final goal is to envelop on a microscopic scale any given active material (often called core material) in a layer that protects it from reactions with the environment, increasing its shelf life, stabilizing, and ensuring a gradual release of the active material when consumed. All microencapsulation technologies have three steps: the wall must envelop the core material, the shell must maintain its integrity, and finally, the crust must subside at the right moment (and right place) ensuring the release of its contents at an adequate rate (Macías-Cortes *et al*., 2020). The release of the active material inside the microcapsule is as important as its microencapsulation. There are different mechanisms, as shown in Figure 3. The microcapsules release their content mediated by factors such as temperature, pressure, and concentration gradient,

and subjected to the adequate stimulus, the enveloping material will dissolve, expand or rupture (Hu *et al*., 2017). There are different methods of microencapsulation, the most common are mentioned ahead.

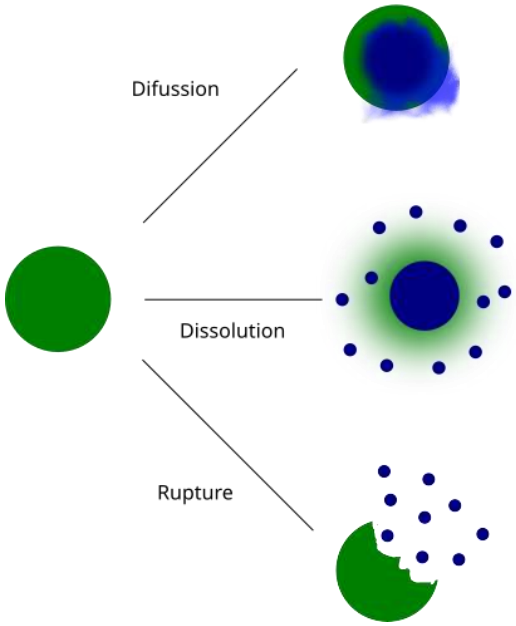

Figure 3. Different release mechanisms of the active component in a microcapsule.
Figura 3. Diferentes mecanismos de liberación del componente activo de una microcápsula.

3.1 Extrusion

This technique typically involves mixing the probiotic along with a hydrocolloid such as a carbohydrate dispersion. Thereafter, it is extruded through a nozzle producing a small droplet, afterwards, these droplets fall into a bath containing a solution that hardens the wall material enveloping its core. The main advantage of this procedure is that it does not involve the use of either high temperature or solvents, thus ensuring a high survival rate for the probiotic. The most common material used to microencapsulate with this method is sodium alginate. However, the main disadvantage for this process to be used at an industrial scale lies within its inability to produce large quantities of microcapsules, the relatively high particle size and its higher costs compared with other methods (Lee *et al*., 2019; Yang *et al*., 2020).

3.2 Coacervation

The definition of coacervation is the separation into two liquid phases of a colloidal solution. There are two main forms of coacervation, simple and complex. Simple coacervation involves the use of a single polymer and a salt or dissolving agent, such dissolving agents can be either alcohol

or acetone. In complex coacervation, two polymers with opposing charges interact producing two immiscible phases of liquid, the dense phase, which is polymer-rich, and the continuous phase which is not. This method often uses animal proteins such as gelatin, whey protein or sodium caseinate. Even though this technique provides advantages over other methods such as very stable shells, with very high encapsulation efficiency and great controlled release of the core material, it poses several disadvantages mainly due to the time required to encapsulate and its cost. This is a relatively new technology, and it is considered to still be in experimental phase (Dhakal and He, 2020; Yang *et al*., 2020).

3.3 Liposomes

This encapsulation technique requires the formation of a bilayer, typically of a phospholipid, in an aqueous solution to which agitation, and heat are applied to form vesicles. Since phospholipids are amphiphilic molecules, the liposomes can trap polar active substances in an aqueous solution with their polar ends. It is also possible as well to trap nonpolar substances with the lipophilic end of the phospholipid, making them more suitable for an aqueous delivery system. A special application of this technique is the entrapment of core materials that require high water activity such as enzymes or probiotics. Although one of the main disadvantages of this method of encapsulation is the low resistance of the encapsulating material, being a phospholipid, it is prone to oxidation and hydrolysis as well as sensitive to changes in pH and temperature (Dhakal and He, 2020; Mehta *et al*., 2022).

3.4 Spray drying

Spray drying is one of the most widely spread microencapsulation techniques in use due to its short processing time and the overall quality of the encapsulated product, mainly uniform particle size, and defined morphology (see Figure 4). Spray drying is a technology that consists in the pulverization or atomization of a suspension or solution into a chamber filled with hot gas and finally recover it in the form of powder. The way in which spray drying microencapsulation works is to prepare a dispersion of both the active and enveloping materials in an adequate solvent, pumping the dispersion into the drying chamber, wherein it is atomized, and the solvent is quickly evaporated. The dehydrated product is collected either in powder or agglomerated form from a cyclone (Ceja-Medina *et al*., 2020). This method consists of the next main steps: preparation of the dispersion, homogenization, atomization, dehydration of the atomized particles and collection. Some of the advantages the final product provides when working with spray drying is an increased shelf life due to its low water activity, thus reducing the need of refrigeration, cutting costs in both storage and transport. The main elements present in spray drying equipment (see Figure 5) are a feeding pump, an atomizer, a heating device, a drying chamber, and a cyclone. Spray drying uses a variety of compounds from which polysaccharides such as starches with varying grades of modification, gums, and plant-based mucilage are of particular interest. This technique's main disadvantages are due to the high temperature involved. Because of this a wall material that is highly resistant to heat, has high water solubility and a relatively low viscosity such as Gum Arabic, modified starches, maltodextrin, and plant mucilage (Macías-Cortes *et al*., 2020; Yang *et al*., 2020).

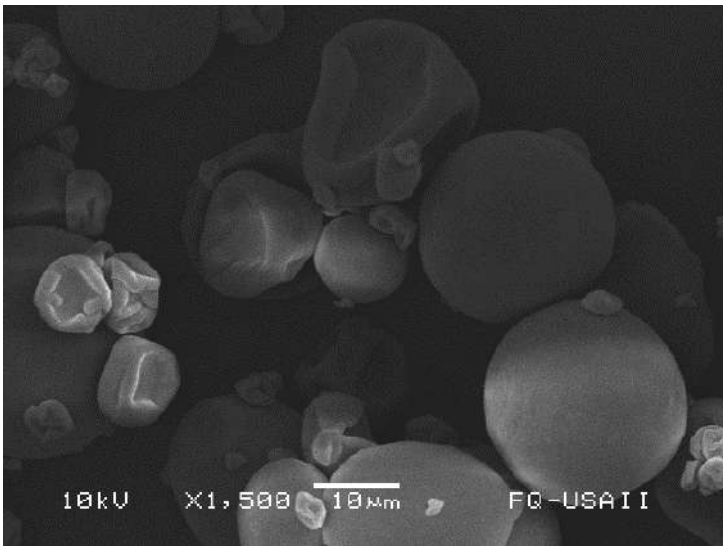

Figure 4. SEM micrography of *Lb. plantarum* microencapsulated in an *Aloe vera* mucilage/agave fructans/gum Arabic microcapsule

Figura 4. Micrografía por SEM de *Lb. plantarum* microencapsulado en una microcápsula de mucílago de *Aloe vera*/fructanos de agave/goma Arábiga (Ceja-Medina *et al*., 2020).

3.5 Spray chilling

Spray chilling, also known as spray cooling, is typically employed to encapsulate hydrophilic materials into a hydrophobic shell. The wall material is most often a lipidic material such as waxes, with a relatively high melting point, and similarly to spray drying is atomized into a chamber filled with cool gas, which causes a fast solidification of the lipidic material around the core. This technique is typically used in the encapsulation of water-soluble vitamins, enzymes, and flavor agents. It is also possible to encapsulate hydrophobic core materials with hydrophobic wall materials such as the encapsulation of liposoluble vitamins with fats, waxes, and oils as wall materials. The main disadvantage of this method lies in the melting point of the material used as it should be low enough as to not thermally kill the probiotic and high enough to not melt at room temperature. For this reason, mixtures of waxes and other materials such as polysaccharides are under study (Choudhury *et al*., 2021).

3.6 Spray freeze-drying

This is a combination of spray chilling and lyophilization in which a mixture of both the encapsulating agent and the core material is sprayed into a cooling chamber in which it is instantly frozen, producing a current of tiny ice spheres in which both materials are contained. Thereafter, the ice particles are freeze-dried through regular lyophilization. Lyophilization is the removal of moisture from a frozen matrix without going through the liquid phase, through a process known as sublimation. Sublimation involves the use of very low pressure to turn ice into water vapor without raising its temperature to the melting point. Its main use is to dry materials that are particularly

sensitive to high temperature. Lyophilization consists of the next phases, the initial freezing phase, and two drying phases. The initial phase in lyophilization is freezing, wherein most of the moisture is frozen. Afterwards during the first drying step, frozen free moisture is removed by sublimation. The two parameters that play a crucial role during this step are the shelf temperature and the chamber pressure. The second drying phase cycle involves the elimination of unfrozen bound water through desorption. The wall materials needed for this technique are similar to those needed in spray drying, with the exception of resistance to high temperature (Kandasamy and Naveen, 2022).

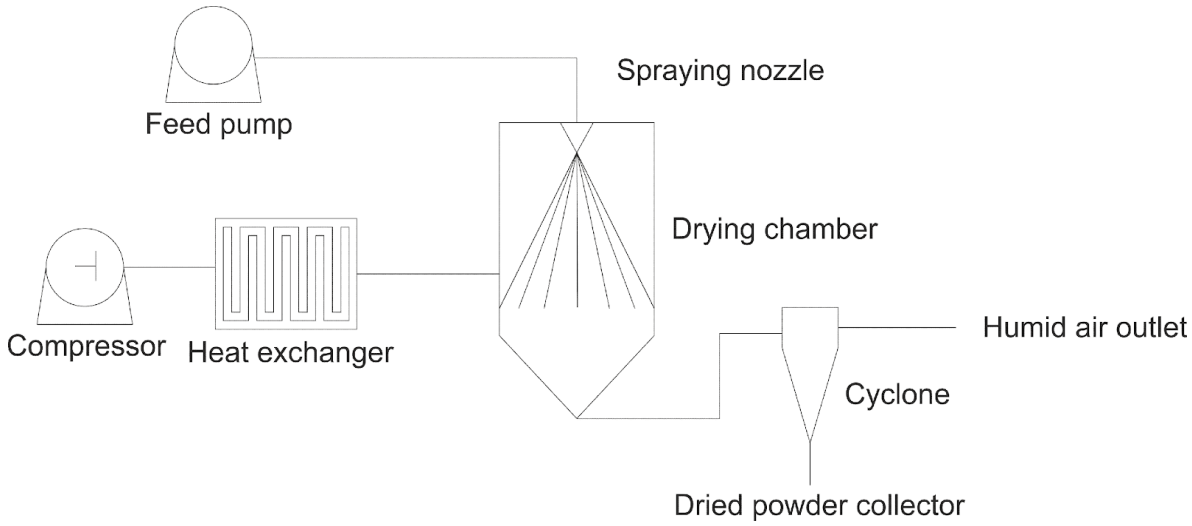

Figure 5. A typical arrangement of spray drying equipment.
Figura 5. Un arreglo típico de un equipo de secado por aspersión.

3.7 Fluidized bed coating

It is like spray drying technology, although with some modifications. It consists of three stages, the absorption of the core materials onto a support material, a solid porous material, which is being fluidized; the coating which is done by spraying the liquid wall material into the fluidized bed; and finally, the hardening of the shell by either vaporization of the wall material solvent or by chilling, being air the most common gas used to both fluidize and cooling. This method provides flexibility in wall materials using both water soluble materials such as protein and polysaccharides and lipophilic materials such as waxes (Mehta *et al*., 2022).

4. Wall materials

The selection of adequate wall material is essential given that it will determine both the efficiency of encapsulation and the stability of the capsules. Some of the qualities that wall material must possess are, non-reactivity towards the core material, it must be able to contain the core integrally inside of the capsule, being economically viable, it must meet standards such as FDA's GRAS, and depending on whether its application is in food or not, it must not have any unpleasant smells or flavors (Dhakal and He, 2020). While most of the commonplace materials nowadays meet the criteria of being safe for consumption, some of the more common problems are that resistance of capsules and the price of the material are often at odds. Another avenue for new sources of materials is that some might have prebiotic effects or come from vegan or vegetarian sources. This opens new avenues for development of new developments. A brief list of recent developments regarding common wall materials can be seen in Table 1. There are different wall materials of microencapsulation, the most common are:

4.1 Protein

Protein wall materials coming from both animal and vegetable sources have been used with success to encapsulate probiotics, some of these sources range from dairy, gelatin, and various legumes. Protein solubility varies according to a variety of extrinsic factors, like pH and temperature, and intrinsic factors mainly regarding the structure of the protein and its amino acid profile. Their molecular weight varies drastically ranging from around 10 kDa upwards to 50 kDa or more. The capacity of proteins to form emulsions is variable depending on factors such as molecular weight, source, structure, and adsorption capacity (Kim *et al*, 2020). Proteins are typically sourced from animal byproducts such as gelatin, obtained from the thermal hydrolysis of collagen from waste products of the meat and leather industry such as bones, horns, hooves, skins, cartilage. One of the most used sources for microencapsulating proteins is milk, usually milk whey. Milk whey is the liquid remaining from the curdling of milk during cheese elaboration. It contains a mix of minerals, and proteins such as alpha-lactalbumin, beta-lactoglobulin, and lactose. It is mainly used along with wall materials from other sources such as carbohydrates like maltodextrin, alginates, etc. (Bhagwat, *et al*., 2020; Obradović *et al*., 2022). Due to the rise in demand of vegan and vegetarian ingredients, advances in vegetable protein have also been made. Regarding vegetable protein works like González-Ferrero *et al*. (2020) worked with soy protein isolate, and maltodextrin to produce microcapsules loaded *Lactobacillus plantarum* CECT 220, using a Büchi spray dryer, with a survival rate of 93 %. Or Qi *et al.* (2021) in which a microcapsule made by coacervation made using pea protein isolate and sugar beet pectin yielded the best results in a gastrointestinal simulated digestion. Nevertheless, the bulk of research on vegetable protein is centered around microencapsulating materials like antioxidants, essential oils, and vitamins. Due to their origin and complex purification, protein wall materials are typically of higher costs than some other materials such as carbohydrates.

Table 1. Common wall materials, and recent developments on probiotic microencapsulation
Tabla 1. Materiales de pared comunes y desarrollos recientes en microencapsulación de probióticos

Polymer	Monomeric units	Encapsulation techniques	Temperature °C (i=inlet, o=outlet)	Encapsulation efficiency %	Probiotic	Reference
Whey protein	Lactalbumin and lactoglobulin (amino acids)	Spray drying (SD)	140(i) 60(o)	70.65+-1.84	*Enterococcus canintestini*	Bhagwat, *et al*., 2020
Gelatin	Hydrolyzed collagen (amino acids)	Emulsification/coacervation	40	97.78	*Lb. plantarum*	Paula *et al*., 2019
Maltodextrin	Glucose	SD	130-150(i) 55+-2(o)	89.15	*Lb. acidophilus*	Arepally *et al*., 2020
		Mixed flow SD	140(i) 80(o)	20.88±0.03	*Lb. rhamnosus*	Jiang *et al*., 2020
Resistant and modified starch	alfa 1-4 and alfa 1-6 linked Glucose	Extrusion	-	48.46 ± 0.98	*Lb. casei*	Ashwar *et al*., 2018
CMC	Carboxy-methyl Glucose	Extrusion	-	94.7±0.78	*Lb. plantarum*	Dafe *et al*., 2017
Chitosan	Glucosamine	SD	120(i) 68(o)	91±0.33	*K. marxianus*	Vanden Braber *et al*., 2020
Fructans	Fructose	SD/Spray Freeze Drying	SD: 110(i), 62(o) SFD: -80	SD: 89.21 SFD: 96.16	*Lb. plantarum*	Yoha, *et al*., 2020

4.2 Lipids

Lipids, which include fatty acids, fats, waxes, sterols, and phospholipids, are an eclectic group of molecules used to designate substances with relatively low polarity, and thus low solubility in polar compounds such as water. As such there are not many studies that work with the encapsulation of probiotics with only lipids as an encapsulating agent. Nevertheless, there are some studies in which an outer crust of materials such as beeswax and stearic acid is used to cover microcapsules made with other materials such as resistant starch or alginate to increase their resistance to moisture. Lipids also ensure a controlled release in the intestine by the way of lipase action while being digested (Rodrigues *et al*., 2020).

4.3 Carbohydrates

Carbohydrates are the group of biopolymers most widely used in the encapsulation of probiotics. They can be identified in any number of ways by size, composition, refining grade, and origin. Being classified regarding their composition in homopolysaccharides when their forming monomers are the same, such as starches, maltodextrin, and inulin, and heteropolysaccharides, when their forming units are non-homogeneous, such is the case of gums such as Arabic and guar gums. Carbohydrate solubility in water varies depending on the degree of polymerization, and crystallinity; being those with a smaller polymerization degree and less crystalline more easily solubilized. While carbohydrates range in molecular weight from around 180 Da for average monosaccharides to 480 kDa for an average polysaccharide, although some polysaccharides can upwards to 788 kDa. The molecular weight of carbohydrates frequently used in microencapsulation usually starts at around 20 kDa (Ushiyama and Shimizu, 2018).

4.3.1 Starch

Starch is the name given to the reserve polysaccharides made by green plants. It is found in foodstuffs such as cereals and tubers and it is one of the main sources of calories in modern human diets. It is made up of glucose units linked together by alpha α (1 ➡ 4) glycosidic bonds, forming long chains, known as amylose, with ramifications of alpha α (1➡ 6) glycosidic bonds known as amylopectin. Starch is typically made up of around 1:3 up to 1:4 parts of amylose to parts of amylopectin by weight. It is used both as an ingredient and raw material for different food products, including modified starches and high fructose corn syrup. It has recently become of interest to technologists the use of resistant starches as novel materials for the encapsulation of probiotics due to their potential as a prebiotic. Resistant starches are starches resistant to enzymatic digestion by intestinal amylase, this is due to a higher proportion of amylose which is less susceptible to these enzymes due to its structure. The categories of resistant starch are as follows RS1) physically inaccessible, RS2) native granules of non-gelatinized starch, RS3) retrograded amylose, RS4) chemically modified starch (Bojarczuk *et al*., 2022). RS4 was studied by Ashwar *et al*., (2018; 2021) using a rice-modified starch in an extrusion system.

4.3.2 Maltodextrin

Maltodextrin is a group of polysaccharides obtained from partial hydrolysis of starches. Like starch, it is made up of glucose monomers bound to each other by **α** (1 → 4) bonds with occasional **α** (1 → 6) bond ramifications. Maltodextrin is one of the most widely used and studied encapsulating agents with works as recent as those made on survivability and stability by Arepally *et al*. (2020), with novel forms of spray drying technology such as mixed flow spray drying by Jiang *et al*. (2020), and its uses with other materials to yield better capsules such as whey protein by Bhagwat *et al*. (2020).

4.3.3 Cellulose

Cellulose is a structural polysaccharide found most abundantly in the cell walls of plants, although it is also produced by bacteria such as that found in acetic fermentations. It is made up, like starch, of glucose monomers, with the difference being that the bonds between subunits are **β** (1→6), forming a much more resistant fibrillar structure. In its natural form, it is of little use as an encapsulating agent due to its very low solubility in water and other common solvents, thus some products derived from it such as methylcellulose, hydroxypropyl cellulose, and carboxymethylcellulose (CMC), and other forms of such as micro and nanocrystalline cellulose are used. Multiple works with both modified cellulose and nano/micro crystalline cellulose are at the forefront of the study of new methods of probiotic encapsulation. Such as the study done by Wang *et al*. (2022) where kelp nanocellulose was incorporated in an alginate microcapsule, leading to increased survivability during gastrointestinal digestion simulation in comparison with a system made exclusively with alginate. Regarding modified cellulose Tao *et al*. (2019), used skim milk along with some polysaccharides including several modified celluloses such as CMC, hydroxypropyl methylcellulose, methylcellulose as well as gum arabic and sodium alginate observing increased survivability of *Lb. paracasei*, after spray drying compared to skim milk alone with encapsulation efficiencies between 91 to 97 %.

4.3.4 Chitosan

Chitosan is a food-grade polysaccharide obtained from processing shrimp and other crustaceans' exoskeletons with an alkali such as sodium hydroxide, it is the only carbohydrate used in microencapsulation that is predominantly positively charged. It is composed of **β** (1→ 4) linked units of both acetylated and nonacetylated D-glucosamine. It is widely used in the chemical, pharmaceutical, cosmetic, and food industries (Pech-Canul *et al*., 2020). Chitosan has also been used as an encapsulating agent to encapsulate different probiotics such as *Lb. gasseri, Lb. rhamnosus, Lb casei, Lb. acidophilus, Bifidobacterium animalis* and, *B. bifidum* (Călinoiu *et al*., 2019).

4.3.5 Fructans

Although the bulk of forming units of fructans are as the name suggests fructose the reducing end of the chain is a sucrose unit. There are five different types of fructans according to the kind of bonds they present. The major kinds of fructans are inulin, levan, graminin and neo series inulin and levan. In nature they are found in plants such as onions, garlic, artichokes, asparagus, grasses, and agave. Fructans are widely used in the food industry mainly as a dietary fiber supplement, because of their prebiotic properties (Wang and Cheong, 2023).

Fructans have recently been of interest as encapsulating agents in processes similarly to spray drying such as the study done by Ceja-Medina *et al*. (2020) in which several food gums such as guar, xanthan, and gum Arabic, as well as whey protein concentrate in several encapsulating systems, made up from a mixture of *Aloe vera* mucilage and high molecular weight agave fructans. Being the systems made with *Aloe vera*/fructans/whey and *Aloe vera*/fructans/gum Arabic, the ones with a higher survival rate at about 70 %. Also, the work of Alvarado-Reveles *et al*. (2019) involved the use

of both agave fructans and buttermilk proteins as wall materials yielding $1.08x10^{10}$CFU/mL against $3x10^{9}$CFU/mL using buttermilk proteins alone.

4.3.6 Algae polysaccharides

Some of the most used heteropolysaccharides in the industry are derived from algae. Some of the polymers which are currently under use in the food and pharmaceutical industries as encapsulating material are those of the carrageenan family of polymers from which kappa, iota, and lambda carrageenan are the most used. Another relevant polysaccharide obtained from algae is alginic acid often found in its salt form as sodium alginate is a linear polymer with blocks of (1→4) beta-D-mannuronate and alpha-L-guluronate. Another relevant polysaccharide derived from algae is agar, it is most often associated with microbiological culture media, although not properly microencapsulation since the particle size is bigger than 1mm developments such as the one done by Albadran *et al*. (2020) which used chitosan to coat gelatin-agar particles to encapsulate *Lb. plantarum*, demonstrating survival to a simulated gastric and intestinal digestion, demonstrate possible new applications for agar as a potential microencapsulating agent

4.3.7 Gums and mucilages

Gum is a generic name given to a broad number of heteropolysaccharides that are soluble and have the property of forming gels; gums are often found on the seed epidermis, the leaves, and the bark of plants, although there are some, such as xanthan gum, that are of microbial origin. Gums are a response to damage by some plants or unfavorable environmental conditions. Some of the most common gums include guar, acacia (also known as Arabic), and tragacanth gums. Mucilages are natural components of plant metabolism, they are very thick, viscoelastic, and usually do not have as high solubility as gums, inside the plant they are often involved in water retention, calorie reserves, as well as helping in seed germination (Amiri *et al.*, 2021). A widely used gum is gum Arabic which is a heteropolysaccharide obtained from the bark of *Acacia senegal* (L.), its constituents are arabinose, galactose, rhamnose, and glucuronic acid. It has been thoroughly studied as an enveloping material for probiotics such as the work of Arepally *et al*. (2020); in which a mixture of maltodextrin and gum Arabic are used to encapsulate probiotics in a spray dryer, providing better physical and physicochemical properties, as well as higher encapsulation yields when higher concentrations of gum Arabic was used with a viability percentage higher than 80 % (around 7.3-9.9 log CFU/g). An alternative to gum Arabic that is currently under study is mesquite gum, which is obtained from several species of trees under the *Prosopis* genus such as *P. laevigata, P. juliflora, P. velutina*, and *P. pubescens*. Mesquite gum is made up mainly of monomeric units of L-arabinose and D-galactose with trace amounts of D-xylose, D-mannose, and D-glucuronate, differing from acacia gum because of its lack of L-rhamnose (Mudgil and Barak, 2020). There are few works regarding the use of mesquite gum as a protecting agent for probiotics one of such is the work done by Rodríguez-Huezo *et al*. (2014) a double emulsion process, in which *Lb. plantarum* cells were dispersed in canola oil (continuous phase) and either sweet whey or "aguamiel" (sweet agave juice" were dispersed in water, after that, it was further dispersed in a dispersion of gum Arabic, mesquite gum, and maltodextrin. This emulsion was then integrated into the processing of Oaxaca cheese, exhibiting survival rates of 8.2 and 8.15 log CFU/g for sweet whey and aguamiel respectively versus 6.8 log CFU/g in cheeses prepared with free cells.

Regarding mucilages, while there have been uses in the food industry for them before, it is only recently that such polymers have garnered attention to their potential as new encapsulating materials, although in some cases there has been more development towards the encapsulating of antioxidants or other kinds of ingredients such as the work done by Medina-Torres *et al*. (2019), which involved the encapsulation of gallic acid with nopal (*Opuntia ficus-indica*) and *Aloe vera* mucilage via spray drying, obtaining a higher releasing percentage when encapsulating using *Aloe vera* mucilage while nopal mucilage gave particles with a bimodal particle size distribution and a prolonged release of the active ingredient. The work done by Jannasari *et al*. (2019) in which a mixture of gelatin and cress seed mucilage was used to encapsulate Vitamin D using coacervation as the encapsulation method obtaining up to 67 % of encapsulation efficiency. Cortés-Camargo *et al*. (2017 and 2019), which used a mixture of mesquite gum and nopal mucilage to encapsulate lemon essential oil obtaining the best overall results using a mix of both since nopal mucilage gave greater encapsulating efficiency while mesquite gum allowed for a greater volatile oil retention. Alpizar-Reyes *et al*. (2020) used tamarind seed mucilage to encapsulate sesame seed oil both showed comparable results in peroxide value results for the oil, which was much lower than that of free oil confirming the capabilities of these materials as good encapsulating vectors for essential oils. Although few, work regarding the encapsulation of probiotics using these gums and mucilages, there have been some recent developments such as the works of Lai *et al*. (2020 and 2021). They used flaxseed mucilage to encapsulate *Lb. rhamnosus* GG that yielded a reduction in cellular death during storage between 120-135 % at 4°C and between 52-243 % at 25°C. The work done by Ceja-Medina *et al*. (2020 and 2021), in which *Aloe vera* mucilage and agave fructans (along with some other biopolymers) were used to successfully encapsulate *Lb. plantarum* with a survival rate of above 70 % for the best mixes of polymers (gum Arabic and whey protein concentrate). Homayouni-Rad *et al*. (2021) using *Alyssum homolocarpum* seed mucilage and inulin in spray drying encapsulation to encapsulate *Lb. casei* obtaining a survival rate of around 67 % after gastric simulation as well as showing a good capsule morphology with absent cracks in their walls. Bustamante *et al*. (2020) encapsulating *Lb. plantarum*, *Bifidobacterium longum*, and *B. infantis* using chia and flaxseed mucilages as well as inulin, which exhibited high viability after spray drying with around 98 % of viability and high survivability after storage incorporated with an instant powder juice 10.5-11 log CFU/g for *B. infantis* and between 7.3-8.9 log CFU/g for *Lb. plantarum*.

4.3.8 Prebiotics as encapsulating agents

Prebiotics are substances, typically carbohydrates, which when ingested promote the growth of desirable bacteria groups within the gut they are often classified as dietary fiber. To be considered a prebiotic a polysaccharide must meet certain criteria which include: 1) high resistance to orogastric digestion which includes resistance to both the enzymes found in the mouth and stomach as well as a low pH, 2) it is fermentable by gut bacteria, 3) provides a health benefit to the consumer, 4) selectively stimulates the growth of certain bacteria, 5) stability in food systems. Some of the ways a prebiotic might be incorporated into new products are, emulsifying agents, foam stabilizers, fat or sugar substitutes, fiber supplements, and encapsulating agents (Behare *et al.*, 2021; López-Castejón *et al*., 2021). The use of prebiotics as an ingredient in products with probiotics has been of interest in recent times, naming such products as *synbiotics*. The importance of research in the realm of symbiotics lies in the synergic effect that the prebiotic has on the probiotic, while not enhancing the growth of other (potentially pathogenic) bacteria. *Synbiotic* show promise as effective tools in managing health outcomes (Maftei, 2019). Typical prebiotics includes fructooligosaccharides (FOS),

galacto-oligosaccharides (GOS), and trans-galacto-oligosaccharides (TOS) (Davani-Davari *et al*., 2019) although some other substances such as xylooligosaccharides, pectic oligosaccharides, resistant starch, and polyphenols are considered in some instances as such (do Nascimento and Marostica Junior, 2021). Previously in this review, some of such materials have been mentioned as being under study by different research groups as new sources of materials for the encapsulation of probiotics, such as fructooligosaccharides and other fructans by Ceja-Medina *et al*. (2020, 2021), Alvarado-Reveles *et al*. (2019); and resistant starches by Ashwar *et al*. (2018, 2021). Some kinds of resistant starches have shown potential as prebiotics, by enabling the growth of lactobacilli and bifidobacteria (Bojarczuk *et al*., 2022). Also, pectins have shown promising activity as probiotics such as reported by Singh *et al.,* (2020) and potential as new encapsulating materials such as reported by Motalebi Moghanjougi *et al*. (2021). *Aloe vera* has shown to be a new possible source of prebiotics as shown by the works of Tornero-Martinez *et al*. (2019) and Quezada *et al*. (2017) which show the potential of *Aloe vera*'s polysaccharides as a possible substrate for probiotics to ferment, fermentation that produces short chain fatty acids. Nopal (*Opuntia ficus-indica*) which has been used as an encapsulating agent for other kind of bioactive materials has also shown potential as a prebiotic, functioning as a substrate for lactobacilli and bifidobacteria (Cruz-Rubio *et al*., 2020).

Regarding new materials that are being studied as encapsulating agents with the potential of being prebiotic components, materials such as gums, pectin, mucilages, and other biomaterials are a promising frontier and an interesting alternative for new developments and satisfy the current demand in the food industry.

Conclusions

Although the encapsulation of probiotics is not a new concept there is still much in the way of developing better encapsulating materials. While some materials that are now commonplace in the food and pharmaceutical industries, such as: starches, maltodextrins, and other polysaccharides, which have already been extensively studied; there are still gaps in our knowledge of possible new wall materials. One of such gaps is in the use of new refined proteins obtained from vegetable sources such as soy, peas, and other plants. Another blind spot is the utilization of some gums and mucilages such as those from tree sap (acacia and mesquite gums), seeds (flaxseed, and chia seeds mucilages), and particularly some cactus and other succulents (dragon fruit, prickly pear cladodes, and aloe).

Acknowledgments

Partial financial support from CONACYT is recognized (Fortalecimiento de Infraestructura-CONACyT 2021, grant No. 317235)

Conflict of interests

All authors declare that they have no conflicts of interest.

References

Albadran, H. A., A. Monteagudo-Mera, V. V. Khutoryanskiy & D. Charalampopoulos. 2020. Development of chitosan-coated agar-gelatin particles for probiotic delivery and targeted release in the gastrointestinal tract. Applied Microbiology and Biotechnology 104: 5749–5757. https://doi.org/10.1007/s00253-020-10632-w

Alpizar-Reyes, E., V. Varela-Guerrero, J. Cruz-Olivares, H. Carrillo-Navas, J. Alvarez-Ramirez, & C. Pérez-Alonso. 2020. Microencapsulation of sesame seed oil by tamarind seed mucilage. International Journal of Biological Macromolecules 145: 207–215. https://doi.org/10.1016/j.ijbiomac.2019.12.162

Alvarado-Reveles, O., S. Fernández-Michel, R. Jiménez-Flores, C. Cueto-Wong, L. Vázquez-Moreno & G. Ramos-Clamont Montfort. 2019. Survival and Goat Milk Acidifying Activity of *Lactobacillus rhamnosus* GG Encapsulated with Agave Fructans in a Buttermilk Protein Matrix. Probiotics and Antimicrobial Proteins 11: 1340–1347. https://doi.org/10.1007/s12602-018-9475-y

Amiri, M. S., V. Mohammadzadeh, M.E.T. Yazdi, M. Barani, A. Rahdar & G.Z. Kyzas . 2021. Plant-Based Gums and Mucilages Applications in Pharmacology and Nanomedicine: A Review. Molecules 26(6):1770. https://doi.org/10.3390/molecules26061770

Arepally, D., R. S. Reddy & T. K. Goswami. 2020. Studies on survivability, storage stability of encapsulated spray dried probiotic powder. Current Research in Food Science 3: 235-242. https://doi.org/10.1016/j.crfs.2020.09.001

Ashwar, B. A., A. Gani, A. Gani, M. Ahmad & A. Shah. 2021. Encapsulating probiotics in novel resistant starch wall material for production of rice flour extrudates. LWT 140: 110839. https://doi.org/10.1016/j.lwt.2020.110839

Ashwar, B. A., A. Gani, A. Gani, A. Shah & F. A. Masoodi. 2018. Production of RS4 from rice starch and its utilization as an encapsulating agent for targeted delivery of probiotics. Food Chemistry 239: 287–294. https://doi.org/10.1016/j.foodchem.2017.06.110

Avila-Reyes, S. V., F. J. Garcia-Suarez, M.T. Jiménez, M. F. San Martín-Gonzalez & L. A. Bello-Perez. 2014. Protection of *L. rhamnosus* by spray-drying using two prebiotics colloids to enhance the viability. Carbohydrate Polymers 102: 423–430. https://doi.org/10.1016/j.carbpol.2013.11.033

Behare, P., S. A. Hussain, D. R. Naranjo, P. Sharma & O. McAuliffe. 2021. Chapter 12 Prebiotic and Probiotic Food Formulations. In Shivani Pathania, Brijesh K. Tiwari (Editors), *Food Formulation: Novel Ingredients and Processing Techniques* (pp. 237–263). Wiley-Blackwell https://doi.org/10.1002/9781119614760.ch12

Bermudez-Brito, M., J. Plaza-Díaz, S. Muñoz-Quezada, C. Gómez-Llorente & A. Gil. 2012. Probiotic mechanisms of action. Annals of Nutrition and Metabolism 61: 160–174. https://doi.org/10.1159/000342079

Bhagwat, A., P. Bhushette & U. S. Annapure. 2020. Spray drying studies of probiotic Enterococcus strains encapsulated with whey protein and maltodextrin. Beni-Suef University Journal of Basic and Applied Sciences 9:33. https://doi.org/10.1186/s43088-020-00061-z

Bojarczuk, A., S. Skąpska, A. M. Khaneghah & K. Marszałek. 2022. Health benefits of resistant starch: A review of the literature. Journal of Functional Foods 93: 105094. https://doi.org/10.1016/j.jff.2022.105094

Bustamante, M., L. Laurie-Martínez, D. Vergara, R. Campos-Vega, M. Rubilar & C. Shene. 2020. Effect of three polysaccharides (inulin, and mucilage from chia and flax seeds) on the survival of probiotic bacteria encapsulated by spray drying. Applied Sciences 10(13): 4623. https://doi.org/10.3390/app10134623

Călinoiu, L. F., B. E. Ştefănescu, I. D. Pop, L. Muntean & D. C. Vodnar. 2019. Chitosan coating applications in probiotic microencapsulation. Coatings 9(3): 194. https://doi.org/10.3390/coatings9030194

Ceja-Medina, L. I., L. Medina-Torres, M. González-Ávila, J.C. Martínez-Rodríguez, I. Andrade-González, M. Calderón Santoyo, J.A. Ragazzo-Sánchez & R.I. Ortiz-Basurto. 2021. In vitro synbiotic activity of *Lactobacillus plantarum* encapsulated with mixtures of *Aloe vera* mucilage, agave fructans and food additives as wall materials. Revista Mexicana de Ingeniería Química 20(2): 711–723. http://rmiq.org/iqfvp/Numbers/V20/No2/Bio2234.html

Ceja-Medina, L. I., R. I. Ortiz-Basurto, L. Medina-Torres, F. Calderas, M. J. Bernad-Bernad, R. F. González-Laredo, J. A. Ragazzo-Sánchez, M. Calderón-Santoyo, M. González-Ávila, I. Andrade-González & O. Manero. 2020. Microencapsulation of *Lactobacillus plantarum* by spray drying with mixtures of Aloe vera mucilage and agave fructans as wall materials. Journal of Food Process Engineering, 43(8): e13436. https://doi.org/10.1111/jfpe.13436

Choudhury, N., M. Meghwal & K. Das. 2021. Microencapsulation: An overview on concepts, methods, properties and applications in foods. Food Frontiers 2(4): 426–442. https://doi.org/10.1002/fft2.94

Cortés-Camargo, S., J. Cruz-Olivares, B. E. Barragán-Huerta, O. Dublán-García, A. Román-Guerrero & C. Pérez-Alonso. 2017. Microencapsulation by spray drying of lemon essential oil: Evaluation of mixtures of mesquite gum–nopal mucilage as new wall materials. Journal of Microencapsulation 34(4), 395–407. https://doi.org/10.1080/02652048.2017.1338772

Crook, N., A. Ferreiro, A. J. Gasparrini, M. W. Pesesky, M. K. Gibson, B. Wang, X. Sun, Z. Condiotte, S. Dobrowolski, D. Peterson & G. Dantas. 2019. Adaptive Strategies of the Candidate Probiotic E. coli Nissle in the Mammalian Gut. Cell Host and Microbe 25(4): 499–512.e8. https://doi.org/10.1016/j.chom.2019.02.005

Cruz-Rubio, J. M., M. Mueller, R. Loeppert, H. Viernstein & W. Praznik. 2020. The Effect of Cladode drying techniques on the prebiotic potential and molecular characteristics of the mucilage extracted from *Opuntia ficus-indica* and *Opuntia joconostle*. Scientia Pharmaceutica 88(4): 43. https://doi.org/10.3390/scipharm88040043

Dafe, A., H. Etemadi, H. Zarredar & G. Reza-Mahdavinia. 2017. Development of novel carboxymethyl cellulose/k-carrageenan blends as an enteric delivery vehicle for probiotic bacteria. International Journal of Biological Macromolecules 97: 299–307. https://doi.org/10.1016/j.ijbiomac.2017.01.016

Davani-Davari, D., M. Negahdaripour, I. Karimzadeh, M. Seifan, M. Mohkam, S. J. Masoumi, A. Berenjian & Y. Ghasemi. 2019. Prebiotics: Definition, types, sources, mechanisms, and clinical applications. MDPI Foods 8(3): 92. https://doi.org/10.3390/foods8030092

Dhakal, S. P. & J. He. 2020. Microencapsulation of Vitamins in Food Applications to Prevent Losses in Processing and Storage: A Review. Food Research International 137:109326. https://doi.org/10.1016/j.foodres.2020.109326

do Nascimento, R. de P. & M. R. Marostica Junior. 2021. Emerging Prebiotics: Nutritional and Technological Considerations. In A. Gomes da Cruz, C. Senaka Ranadheera, F. Nazzaro, & N. Amir Mortazavian (Editors), Probiotics *and Prebiotics in Foods: Challenges, Innovations, and Advances* (pp. 13–46). Academic Press. https://doi.org/10.1016/B978-0-12-819662-5.00016-1

FAO/WHO. (2002). Guidelines for the Evaluation of Probiotics in Food. http://fanus.com.ar/posgrado/10-09-25/fao%20probiotics.pdf

FAO/WHO. (2006). Probiotics in Food Health and nutritional Properties and guidelines for the Evaluation.

George Kerry, R., J. K Patra, S. Gouda, Y. Park, H. S. Shin & G. Das. 2018. Benefaction of probiotics for human health: A review. Journal of Food and Drug Analysis 26(3): 927–939. https://doi.org/10.1016/j.jfda.2018.01.002

González-Ferrero, C., J. M. I rache, B. Marín-Calvo, L. Ortiz-Romero, R. Virto-Resano & C. J. González-Navarro. 2020. Encapsulation of probiotics in soybean protein-based microparticles preserves viable cell concentration in foods all along the production and storage processes. Journal of Microencapsulation 37(3): 242–253. https://doi.org/10.1080/02652048.2020.1724203

Guo, P., K. Zhang, X. Ma & P. He. 2020. Clostridium species as probiotics: Potentials and challenges. Journal of Animal Science and Biotechnology 11:24. https://doi.org/10.1186/s40104-019-0402-1

Gut, A. M., T. Vasiljevic, T., Yeager & O. N. Donkor. 2018. Salmonella infection – Prevention and treatment by antibiotics and probiotic yeasts: A review. Microbiology 164(11): 1327–1344. https://doi.org/10.1099/mic.0.000709

Homayouni-Rad, A., A. M. Mortazavian, M. G. Mashkani, N. Hajipour, & H. Pourjafar. 2021. Effect of *Alyssum homolocarpum* mucilage and inulin microencapsulation on the survivability of *Lactobacillus casei* in simulated gastrointestinal and high-temperature conditions. Biocatalysis and Agricultural Biotechnology 35: 102075. https://doi.org/10.1016/j.bcab.2021.102075

Hu, M., J. Guo, Y. Yu, L. Cao & Y. Xu. 2017. Research advances of microencapsulation and its prospects in the petroleum industry. Materials, 10(4): 369. https://doi.org/10.3390/ma10040369

Jannasari, N., M. Fathi, S. J. Moshtaghian & A. Abbaspourrad. 2019. Microencapsulation of vitamin D using gelatin and cress seed mucilage: Production, characterization and in vivo study. International Journal of Biological Macromolecules 129: 972–979. https://doi.org/10.1016/j.ijbiomac.2019.02.096

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

Kandasamy, S. & R. Naveen. 2022. A review on the encapsulation of bioactive components using spray-drying and freeze-drying techniques. Journal of Food Process Engineering 45(8): e14059. https://doi.org/10.1111/jfpe.14059

Kim, W., Y Wang & C. Selomulya. 2020. Dairy and plant proteins as natural food emulsifiers. Trends in Food Science and Technology 105: 261-272. https://doi.org/10.1016/j.tifs.2020.09.012

Lai, K., Y. How & L. Pui. 2021. Microencapsulation of *Lactobacillus rhamnosus* GG with flaxseed mucilage using co-extrusion technique. Journal of Microencapsulation 38(2):134-148. https://doi.org/10.1080/02652048.2020.1863490

Lai, K. W., Y. H. How & L. P. Pui. 2020. Storage stability of microencapsulated *Lactobacillus rhamnosus* GG in hawthorn berry tea with flaxseed mucilage. Journal of Food Processing and Preservation 44(12): e14965. https://doi.org/10.1111/jfpp.14965

Lee, Y., Y. R. Ji, S. Lee, M. J. Choi & Y. Cho. 2019. Microencapsulation of probiotic *Lactobacillus acidophilus* kbl409 by extrusion technology to enhance survival under simulated intestinal and freeze-drying conditions. Journal of Microbiology and Biotechnology 29(5): 721–730. https://doi.org/10.4014/jmb.1903.03018

López-Castejón, M. L., C. Bengoechea, J. M. Alguacil & C. Carrera. 2021. Prebiotic food foams stabilized by inulin and β-lactoglobulin. Food Hydrocolloids 119: 106829. https://doi.org/10.1016/j.foodhyd.2021.106829

Macías-Cortes, E., J.A. Gallegos-Infante, N.E. Rocha-Guzmán, M.R. Moreno-Jiménez, L. Medina-Torres, R. F. González-Laredo. 2020. Microencapsulation of phenolic compounds: Technologies and novel polymers Phenolic compounds. Revista Mexicana de Ingeniería Química 19(2): 491–521. https://bit.ly/3oxjyRd

Maftei, N. M. 2019. Probiotic, prebiotic and synbiotic products in human health. In Frontiers and new trends in the science of fermented food and beverages. In R. L. Solís-Oviedo & A. de la Cruz (Editors). Frontiers and new trends in the science of fermented food and beverages. IntechOpen. http://dx.doi.org/10.5772/intechopen.81553

Mehta, N., P. Kumar, A. K. Verma, P. Umaraw, Y. Kumar, O. P. Malav, A. Q. Sazili, R. Domínguez, J. M. Lorenzo. 2022. Microencapsulation as a Noble Technique for the Application of Bioactive Compounds in the Food Industry: A Comprehensive Review. Applied Sciences 12(3): 1424. https://doi.org/10.3390/app12031424

Motalebi Moghanjougi, Z., M. Rezazadeh Bari, M. Alizadeh Khaledabad, S. Amiri & H. Almasi. 2021. Microencapsulation of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* BB-12 in pectin and sodium alginate: A comparative study on viability, stability, and structure. Food Science & Nutrition 9(9): 5103-5111. https://doi.org/10.1002/fsn3.2470

Mudgil, D. & S. Barak. 2020. Mesquite gum (*Prosopis* gum): Structure, properties & applications - A review. International Journal of Biological Macromolecules 159: 1094–1102. https://doi.org/10.1016/j.ijbiomac.2020.05.153

Obradović, N., M. Volić, V. Nedović, M. Rakin & B. Bugarski. 2022. Microencapsulation of probiotic starter culture in protein–carbohydrate carriers using spray and freeze-drying processes: Implementation in whey-based beverages. Journal of Food Engineering 321: 110948. https://doi.org/10.1016/j.jfoodeng.2022.110948

Paula, D. de A., E. M. Furtado-Martins, N. de Almeida-Costa, P. Martins de Oliveira, E. Basílio de Oliveira & A. Mota-Ramos. 2019. Use of gelatin and gum arabic for microencapsulation of probiotic cells from *Lactobacillus plantarum* by a dual process combining double emulsification followed by complex coacervation. International Journal of Biological Macromolecules 133: 722–731. https://doi.org/10.1016/j.ijbiomac.2019.04.110

Pech-Canul, A. D. L. C., D. Ortega, A. García-Triana, N. González-Silva & R. L. Solis-Oviedo. 2020. A brief review of edible coating materials for the microencapsulation of probiotics. Coatings 10(3): 197. https://www.mdpi.com/2079-6412/10/3/197#

Poltronieri, P., G. Battelli & N. P. Mangia. 2017. Metabolism and biochemistry of LAB and dairy-associated species. In Palmiro Poltronieri (Editor) Microbiological Opportunities and Challenges in the Dairy Industry, 97–122. Wiley online library. https://doi.org/10.1002/9781119115007.ch6

Qi, X., Y. Lan, J. B. Ohm, B. Chen & J. Rao. 2021. The viability of complex coacervate encapsulated probiotics during simulated sequential gastrointestinal digestion affected by wall materials and drying methods. Food & Function 12(19): 8907-8919. https://doi.org/10.1039/D1FO01533H

Quezada, M. P., C. Salinas, M. Gotteland & L. Cardemil. 2017. Acemannan and fructans from *Aloe vera* (*Aloe barbadensis* Miller) plants as novel prebiotics. Journal of agricultural and food chemistry 65(46): 10029-10039. https://doi.org/10.1021/acs.jafc.7b04100

Rodríguez-Huezo, M. E., A. G. Estrada-Fernández, B. E. García-Almendárez, F. Ludeña-Urquizo, R. G. Campos-Montiel & D. J. Pimentel-González. 2014. Viability of *Lactobacillus plantarum* entrapped in double emulsion during Oaxaca cheese manufacture, melting and simulated intestinal conditions. LWT - Food Science and Technology 59(2P1): 768–773. https://doi.org/10.1016/j.lwt.2014.07.004

Singh, R. P., S. Prakash, R. Bhatia, M. Negi, J. Singh, M. Bishnoi & K. K. Kondepudi. 2020. Generation of structurally diverse pectin oligosaccharides having prebiotic attributes. Food Hydrocolloids 108: 105988. https://doi.org/10.1016/j.foodhyd.2020.105988

Staniszewski, A. & M. Kordowska-Wiater. 2021. Probiotic and potentially probiotic yeasts—characteristics and food application. Foods 10(6): 1306. https://doi.org/10.3390/foods10061306

Ushiyama, T. & N. Shimizu. 2018. Microencapsulation using spray-drying: The use of fine starch solution for the wall material. Food Science and Technology Research 24(4): 653–659. https://doi.org/10.3136/fstr.24.653

Vanden Braber, N. L., L. I. Díaz Vergara, Y. E. Rossi, C. A. Aminahuel, A. N. Mauri, L. R. Cavaglieri, & M. A. Montenegro. 2020. Effect of microencapsulation in whey protein and water-soluble chitosan derivative on the viability of the probiotic *Kluyveromyces marxianus* VM004 during storage and in simulated gastrointestinal conditions. LWT 118: 108844. https://doi.org/10.1016/j.lwt.2019.108844

Vinderola, G., A. Ouwehand, S. Salminen & A. von Wright. (Eds.). 2019. Lactic Acid Bacteria: Microbiological and Functional Aspects. 5th ed. CRC Press. https://doi.org/10.1201/9780429057465

Wang, M. & K. L. Cheong. 2023. Preparation, structural characterisation, and bioactivities of fructans: A review. Molecules 28(4): 1613. https://doi.org/10.3390/molecules28041613

Wang, K., J. Ni, H. Li, X. Tian, M. Tan & W. Su. 2022. Survivability of probiotics encapsulated in kelp nanocellulose/alginate microcapsules on microfluidic device. Food Research International 160: 111723. https://doi.org/10.1016/j.foodres.2022.111723

Yoha, K. S., J. A. Moses & C. Anandharamakrishnan. 2020. Effect of encapsulation methods on the physicochemical properties and the stability of *Lactobacillus plantarum* (NCIM 2083) in synbiotic powders and in-vitro digestion conditions. Journal of Food Engineering 283: 110033. https://doi.org/10.1016/j.jfoodeng.2020.110033

Yang, M., Z. Liang, L. Wang, M. Qi, Z. Luo & L. Li. 2020. Microencapsulation delivery system in food industry—Challenge and the way forward. Advances in Polymer Technology. Special Issue (Article ID 7531810) https://doi.org/10.1155/2020/7531810