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Review Article

Synbiotic Encapsulation: A Trend towards Increasing Viability and Probiotic Effect

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Probiotics are effective coadjuvancy against human affections. To confer their beneficial effects to humans, probiotics adhere and colonize the intestine. Then, they must survive the gastrointestinal conditions (~10^8–10^{10} cfu/day). However, their concentration and the dose to produce the beneficial effect are reduced. Synbiotics are the combination of probiotics and prebiotics, and they can increase the beneficial effect of probiotics. Microencapsulation is an efficient approach to protect synbiotics during their passage through the intestinal tract. In this article, we thoroughly reviewed the different encapsulation techniques of synbiotics. The most common were ionic gelation, emulsification, extrusion, spray drying, coacervation, freeze drying, and their combination in some cases. These techniques focus on survival under gastrointestinal conditions. The aim of this work was to review the different techniques of synbiotic encapsulation and discuss the effect of microencapsulation on viability and probiotic properties in in vitro and in vivo models of microencapsulated synbiotics.

1. Introduction

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. They are commonly administered to humans through foods [2]. However, probiotic viability can be affected by the stress during food production and storage [3, 4]. Factors such as presence of oxygen, high temperatures, antimicrobials, and present microbiota, among others intrinsic and extrinsic, can affect probiotic viability [3]. There is a hostile environment in the gastrointestinal tract (GIT) due to the low pH and the presence of salts and enzymes [5]. Probiotics exert a beneficial effect in the intestine when the concentration of viable cells is ~10^8–10^{10} cfu/day (considering 100 g or 100 ml of ingested food), corresponding to ~10^6–10^8 cfu/g or ml in the product when ingested [4, 6–10]. It must be noted that several clinical studies have proven that a mix of probiotics is better than only one strain when improving the characteristics of the endogenous microbiota [5, 11, 12].

Prebiotics are defined as nondigestible food ingredients that benefit the host by selectively stimulating growth and/or the activity of one or more bacteria in the GIT [13]. Supplementation with prebiotics can stimulate the growth of probiotic bacteria hosted in the host's intestine, including administered probiotic strains [14, 15]. Carbohydrates, as dietary fiber, are the most commonly used prebiotics to stimulate growth and normal gut microbiota activity; they

also provide a health benefit to the host [7]. Prebiotics include compounds resistant to digestive enzyme hydrolysis that are not absorbed in the upper GIT, including the small intestine [16, 17]. These compounds have to reach the large intestine, where the microbiota is found, and stimulate the growth of some beneficial microorganisms [18, 19].

Oligosaccharides, often found in agroindustrial waste, are currently the most important prebiotics [20]. Fructooligosaccharides (FOS), xylooligosaccharides (XOS), polydextrose, and galactooligosaccharides (GOS) are some oligosaccharides used as prebiotics [21, 22]. As probiotics, prebiotics must be ingested daily to guarantee a continuous effect [23]. Favorable changes have been observed in gut microbiota with inulin and/or FOS (4–20 g/day) [23–25].

The role of probiotics and prebiotics as modulators of gut microbiota has been widely investigated regarding disease treatment and prevention [26–28]. Synbiotics were first defined by [29] as the mix of probiotics and prebiotics. Some alternatives to increase the viability of probiotic bacteria passing through the GIT have been proposed. The aim is to obtain adequate concentrations to achieve a beneficial effect in the host's health. Probiotic bacteria blends mixed with prebiotics and then microencapsulated are good candidates. Microencapsulation arose as a support to improve probiotic survival during the processing, storage, and consumption [4, 30, 31].

Microencapsulation is a process through which droplets or microscopic particles of liquid or solid materials are surrounded, covered, or embedded in a continuous film of polymeric material, homogeneous or heterogeneous, to produce small capsules with useful properties [32–35]. Microcapsules are particles consisting of an internal core, mostly central, containing the active substance and covered with a polymer coat that constitutes the barrier or wall material of the capsule [36]. The wall material of microcapsules protects the active compound (as bacteria) from dangerous environmental conditions, such as acids, alkalinity, heat, humidity, and even the interaction with other compounds [3, 37, 38]. The capsule size depends on the technique used; still, it ranges between 0.2 μm and 5 mm [32, 39]. The wall material is absolutely important since it impacts encapsulation efficiency and microcapsule stability [36]. There are several microencapsulation techniques, such as spray drying, spray freezing, fluidized bed coating, electrostatic coating, emulsification, extrusion, and coacervation [4, 40, 41]. After microencapsulation, it is the key to check the probiotic viability under simulated GIT conditions in vitro and in vivo. The goal is to identify whether microencapsulation protects the microorganisms against unfavorable conditions and during their passage through the stomach. It also helps to determine if they can exit the microcapsule and colonize the intestine. There are thorough reviews on probiotics microencapsulation [4, 10, 40–47]. Still, only few articles deal with the established relationships between the encapsulation mechanism, the use of prebiotics, the probiotic bacteria blends, the encapsulating material, the viability of probiotic bacteria, the molecular interactions in microcapsules, and the probably increased potential of their beneficial effect in the host's health. Therefore, the aim of this review is to discuss the influence of synbiotic microencapsulation and its effects in *in vivo* and *in vitro* models.

2. Microencapsulation of Synbiotic Blends and Its Effect *In Vitro*

Currently, there is little information on the simulation of synbiotic microcapsules passing through the GIT and their use in foods [38]. Raddatz, et al. [48] indicate that the addition of prebiotics to probiotic microcapsules is beneficial since it produces a functional capsule. Additionally, it contributes to the protection of probiotics against adverse events as the passage through the GIT and different storage conditions.

Probiotics have been encapsulated with the prebiotic inulin, a nondigestible carbohydrate that selectively stimulates probiotic strains and promotes their survival and implantation in the colon [49]. Furthermore, GOS and FOS protect probiotics during microencapsulation and increase their resistance against simulated gastric conditions [8, 50–52].

Nowadays, there are several techniques to microencapsulate synbiotics. However, to select one, it is necessary to consider physical aspects that affect their survival, such as temperature, humidity, and agitation [23]. The microencapsulation techniques for probiotic bacteria most commonly cited in the literature are ionic gelation, spray drying, coacervation, and freeze drying (Figure 1) [3, 38, 53–60] (Figure 1).

2.1. Ionic Gelation. The production of microcapsules by ionic gelation does not demand the use of high temperatures nor organic solvents [43, 61, 62]. There are two gelation techniques: external and internal. The first starts with the diffusion of a calcium ion from a source surrounding the hydrocolloid towards the neutral pH alginate solution [63, 64]. The particle size obtained using this technique is 400 μm–1 mm [65]. Internal gelation is based on the controlled release of the calcium ion from an internal source of insoluble or partially soluble calcium salt dispersed in the sodium alginate solution. The release of the calcium ion can occur in the presence of a calcium salt insoluble at neutral pH but soluble at acidic pH [64, 66]. This technique produces particles measuring 50 μm approximately [65].

The combination of calcium alginate and prebiotics offers an improved protection for probiotics in food systems and eventually in the GIT due to a synbiotic relationship [52, 67]. This is explained by the three-dimensional microcrystal networks, created by prebiotics, interacting with each other. They do not harm the cell and form small aggregates that contribute to a better probiotic protection [52, 68].

Silva et al. [38] obtained similar results when encapsulating *Lactobacillus acidophilus* in alginate-gelatin (AG) and alginate-gelatin-FOS (AGF) microbeads by external ionic gelation. The addition of FOS to the AG matrix improved the network since FOS filled the interstitial spaces in the matrix, leading to smaller pores and a more interconnected network. The results showed that AG and AGF microbeads protected the probiotics, improving their survival under storage (4°C for 20 days) and digestibility (pH 3 to simulate gastric fluid *in vitro* and pH 7 to simulate intestinal fluid at 0, 60, and 120 min conditions evaluated vs. free probiotics

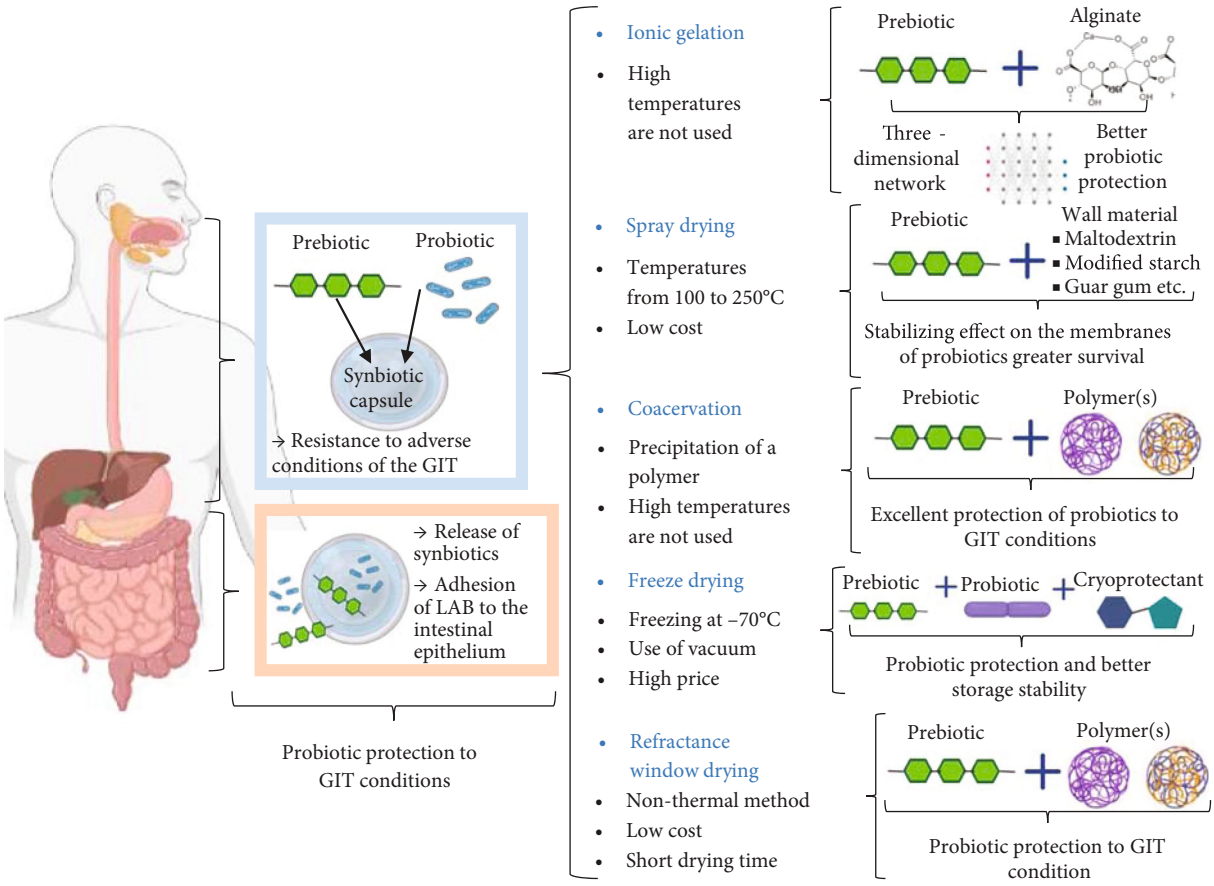


Figure 1: Microencapsulation techniques commonly used for synbiotic.

(Figure 2). FOS added to microbeads also improved *L. acidophilus* viability in yoghurt since they acted as a substrate. They promoted the formation of an encapsulation matrix more resistant to disintegration when subjected to gastrointestinal conditions (Figure 2)

Fratianni et al. [7] microencapsulated *Saccharomyces cerevisiae boulardii*, a probiotic yeast, in a xanthan gum-alginate-inulin blend. The growth capacity of the microencapsulated probiotic in berry juice was assessed along with its survival after four weeks of storage at 4°C. They also evaluated the exposure of microencapsulated yeast to simulated gastrointestinal conditions (gastrointestinal fluid pH 2 and intestinal fluid simulated with pancreatin and bile salts).

Regarding the microencapsulation of the synbiotic, it was observed that the viability of the yeasts was significantly improved after the fermentation and storage process compared to the free yeast (7.59 log cfu/ml versus 6.98 log cfu/ml, respectively), and a protective effect was observed during exposure to simulated gastrointestinal transit after a storage period of four weeks. On the other hand, the free yeast exhibited a considerable loss of viability after storage, presenting a cell viability close to zero (0.23 log cfu/ml) after exposure to simulated gastrointestinal tract conditions. The synbiotic microcapsules exposed to the berry juice were able to absorb a certain amount of polyphenols and anthocyanins. It is concluded that anthocyanins and polyphenols could reach the intestine in their native form and be transformed by the microflora into less complex molecules, providing beneficial effects on the microflora and human health.

2.2. Emulsification. Emulsification is one of the most common microencapsulation techniques [69]. It consists in the dispersion of two immiscible liquid phases in the presence of a stabilizing or emulsifying compound [70, 71]. When microencapsulating probiotics using this technique, it is recommended to use a discontinuous aqueous phase constituted by the polymer-bacterial suspension blend added to a large volume of vegetable oil (continuous phase) with agitation [71]. The mix is homogenized to create a water-in-oil (w/o) emulsion, and the aqueous phase must be insolubilized by adding a reticular agent. Microparticles are formed in the middle of the oil phase and collected by filtration [58]. The size of the capsules obtained with this method ranges between 25 μm and 2 mm, depending on the agitation speed and the water/oil proportion [43]. This encapsulation technique is usually used in laboratories but poses some disadvantages for applications in the food industry and probiotic cells [72]. For instance, the presence of residual oils on the capsule surface is detrimental to the texture and sensory properties of food products. In addition, the surfactants or emulsifiers used can be toxic to probiotic cells [72].

Raddatz et al. [48] determined that microcapsules with prebiotics prepared by emulsion/internal gelation efficiently promote *Lactobacillus acidophilus* LA-5 viability under

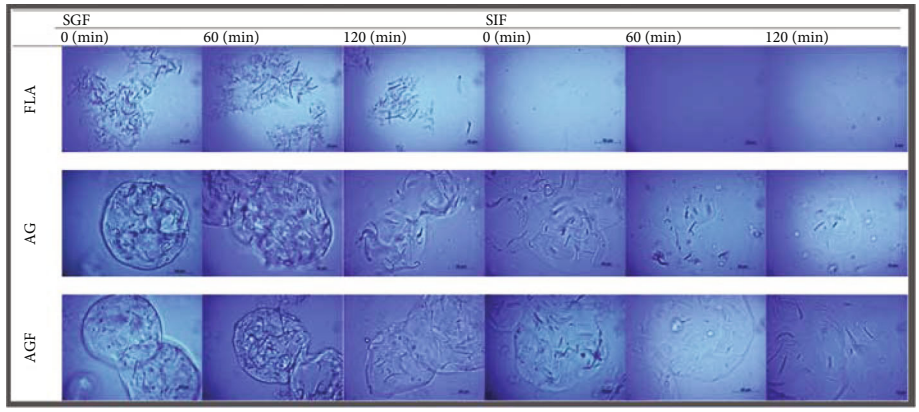


Figure 2: Optical micrographies of free *L. acidophilus* (LL) and microbeads of *L. acidophilus* in alginate-gelatin (LAG) and alginate-gelatin-fructooligosaccharide (LAGF) during simulated digestion in vitro of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) [38].

simulated GIT conditions. Resistant maize starch, inulin, and rice bran compounds are attributed not only prebiotic effects in microcapsules but also the increased viability of *L. acidophilus* LA-5 by internal gelation, which confers a protective effect.

It must be noted that some compounds used as coating or wall material also show prebiotic properties, as pectins that promote *Bifidobacterium* and *Lactobacillus* spp. growth [9, 73]. This agrees with the findings by Raddatz et al. [48] indicating the use of pectin in microencapsulation (emulsion/internal gelation) increased probiotic (*L. acidophilus*) viability to obtain functional capsules. Pectin also improved *L. acidophilus* protection in a model simulating adverse conditions in different sections of the GIT, such as esophagus/stomach (added pepsin, pH adjusted to 2 for 90 min), duodenum (added pancreatin and bile salts, pH adjusted to 5 for 20 min), and ileum (pH adjusted to 6.5 for 90 min). Different storage conditions were also simulated (temperatures of $-18 \circ C \pm 2.7 \circ C \pm 2$ and $25 \circ C \pm 2$) to match freezing, refrigeration, and room temperature conditions. The addition of the prebiotic to the capsules at 25°C and −18°C promoted the viability of probiotic microorganisms.

2.3. Extrusion. Extrusion is one of the most common encapsulation techniques for probiotics since it demands soft conditions. It allows for high cell viability rates and is a low-cost option [74, 75]. This technique consists of several stages, among which are hydrocolloid preparation, addition of cells to the hydrocolloid, and extrusion of the mixture (hydrocolloid-probiotic) through a nozzle to promote the formation of droplets on the gelling solution and hardening of the mixture [76–79].

It should be noted that, although prebiotics in microcapsules (obtained by any technique) seek to stimulate probiotic activity in the intestine, some prebiotics protect probiotic bacteria in the microcapsule. For example, Ergİnkaya et al. [77] microencapsulated a blend of *L. rhamnosus* with inulin or FOS by extrusion. They observed that the viability of *L. rhamnosus* microencapsulated with inulin was higher than those of *L. rhamnosus* microencapsulated with FOS and *L. rhamnosus* microencapsulated without any prebiotic. On the other hand, Atia et al. [53] evaluated several formulations containing 2% w/v alginate and different inulin concentrations (0, 5, 10, 15, and 20% w/v) to encapsulate *Pediococcus acidilactici* UL5, *Lactobacillus reuteri* ATCC 53608, and *Lactobacillus salivarius*, using extrusion/ionotropic gelation. When 15% w/v inulin was used to form the capsules, a greater amount of inulin was captured in the matrix. Additionally, the capsules made with 5% w/v inulin provided bacteria a more efficient protection against bile salts. Krasaekoopt and Watcharapoka, [8] studied the effect of GOS and inulin in *Lactobacillus acidophilus* 5 and *Lactobacillus casei* 01 microcapsules using 2% w/v alginate and chitosan in the coating. The prebiotic concentrations used in the formulation of the microcapsules were 0%, 0.5%, 1.0%, and 1.5%. The GOS concentration (1.5%) provided a better protection; after the treatment under simulated conditions of gastric fluid (pH 1.55), followed by intestinal simulation of 0.6% bile salts, there was a reduction of only 3.1 and 2.9 log cfu/g in *L. acidophilus* 5 and *L. casei* 01, respectively. The microencapsulates were then added to yoghurt with fruit. The treatment with 1.5% GOS provided a better protection and improved the growth of microencapsulated probiotics in the yoghurt and during storage at 4°C for four weeks. The number of probiotic bacteria with GOS in yoghurt was higher than that without GOS in approximately 1.1 and 0.4 log cfu/g of *L. acidophilus* 5 and *L. casei* 01, respectively. The concentration was above the minimum therapeutic level (10^7 cfu/g or ml of the product).

Iyer and Kailasapathy [80] encapsulated *L. acidophilus* CSCC 2400 or CSCC 2409 in a blend with three different prebiotics separately, Hi-maize® starch, Raftiline®, or Raftilose®, by extrusion/ionic gelation. They determined the *in vitro* viability of probiotic bacteria encapsulated at pH 2. The highest probiotic survival or viability was found in microcapsules containing Hi-maize® after 3 h of contact with a solution (pH 2) vs. microcapsules containing Raftiline® or Raftilose®. Furthermore, the probiotic concentration was significantly higher ($p < 0.05$) when Hi-maize® was used at 1.0% w/v than at higher concentrations. In the same study, the researchers evaluated the effect of three different polymers used as wall material (chitosan, L-

polylysine, and alginate) in the survival of encapsulated probiotics when subjected to contact with a pH 2 solution. They also evaluated the effect of bile salts at concentrations of 0.5–1.0%. The Hi-maize® (1.0% w/v) and chitosan blend significantly ($p < 0.05$) increased the survival of encapsulated probiotic bacteria under acidic conditions with bile salts *in vitro* as well as in yoghurt stored for six weeks. They compared these against microencapsulated probiotics with the other two polymers.

2.4. Emulsion/Extrusion. Peredo et al. [81] evaluated the survival of *Lactobacillus casei Shirota* (Lc) and two strains of *Lactobacillus plantarum* (Lp33 and Lp17) in microcapsules obtained by emulsion/extrusion containing three different prebiotics: papaya starch (PS), *Plantago psyllium* (PSY), and inulin (INU). The authors obtained a greater encapsulation yield when using PSY (94% in Lp17) and INU (78% in Lp33). In addition, the survival of the three probiotic bacteria at 4°C during storage for 30 days was higher (8.37 ± 0.50 log cfu/ml) in microcapsules containing PSY. The same microcapsules showed better probiotic survival when they were in contact with simulated gastric fluid (pH 2) and simulated intestinal fluid (pH 7) containing pancreatin and bile salts. The concentrations exhibited were higher than 6 log cfu/ml, as recommended by FAO (Food and Agriculture Organization of the United Nations). The authors concluded that the addition of prebiotics to probiotic bacteria microcapsules promotes probiotic survival. This would allow for the addition of the microcapsules to different food products without affecting their viability or the physicochemical characteristics of the foods.

Valero-Cases and Frutos, [82] compared the survival of *L. plantarum* in microcapsules obtained by extrusion or internal emulsion and containing inulin (0.1% and 2%). The microcapsules were stored at 4°C for 0, 15, and 30 days and were subjected to simulated gastrointestinal digestion. The authors found that *L. plantarum* survived better in both types of microcapsules containing 2% inulin. There was a reduction of only 0.71 and 0.47 log cfu/g in the probiotic in microcapsules obtained by extrusion and emulsion, respectively. From day 15 of storage, the internal emulsion microcapsules did not maintain their structure. At the end of the simulated gastrointestinal conditions (30 days), the number of cells was 7.40 and 6.53 log cfu/g in microencapsulation by extrusion and emulsion, respectively. In both microencapsulation methods, *L. plantarum* showed a survival greater than 10^6 cfu/g. However, during extended periods of storage (30 days), the method that best conserves the viability of *L. plantarum* during gastrointestinal digestion was microencapsulation by extrusion (Figure 3).

2.5. Spray Drying. The microencapsulation by spray drying consists in elaborating a suspension, containing microorganisms and coating agents, atomized with hot air or nitrogen [83, 84]. This technique is convenient in terms of energy demand and operational costs; it leads to a high yield of the process and is often used to microencapsulate probiotics [57, 84, 85]. The microcapsules obtained by this technique can protect probiotics against the effect of hydrochloric acid in the stomach, which considerably reduces the damage of probiotic cells [86, 87]. As in any other microencapsulation technique, the material used as coating must not show cytotoxicity or antimicrobial activity [88]. The microencapsulated probiotic must also maintain the viability of the product and its release in the intestine must be controlled [37]. It is worth mentioning that the high temperatures used in the process of spray drying can stress probiotic cells, reducing their viability [67, 89].

In microencapsulation by spray drying, some prebiotics (as inulin) have been widely used as coating or wall material to protect probiotic cells during spray drying [90]. Inulin is thermally stable and little soluble due to its high degree of polymerization [91, 92].

Nunes et al. [56] microencapsulated *L. acidophilus* La-5 with inulin, trehalose, or Hi-maize® by spray drying. The highest encapsulation percentages of the probiotic were obtained with inulin and Hi-maize® (93.12% and 94.26%, respectively). The microparticles were subjected to different thermal treatments (63°C/30 min and 72°C/15 s) to identify the protective effect of the different encapsulating matrices on the viability of *Lactobacillus acidophilus* LA-5. The matrix with trehalose (Figure 4) provided the greatest protection for this microorganism at concentrations of 9.43 and 10.33 log cfu/g after treatments of 63°C/30 min and 72°C/15 s, respectively. *L. acidophilus* LA-5 survived best in microcapsules produced with Hi-maize® when these microcapsules were the subject of simulated gastrointestinal conditions corresponding to the different GIT sections: esophagus/stomach (contact with pH 2 pepsin for 90 min), duodenum (contact with pancreatin and bile salts at pH 5 for 20 min), and ileum (pH 7.5 fluid for 90 min) (Figure 4).

In a different study, Pinto et al. [93] microencapsulated *Bifidobacterium* BB-12 with sweet whey and two different prebiotics (inulin and polydextrose). The study evaluated the probiotic survival during spray drying and after microcapsule exposure to simulated gastrointestinal conditions. To do so, they used several enzymes and different pH values, contact times, and agitation intensity. The authors also evaluated the effect of different thermal treatments (60, 65, and 70°C for 5, 10, and 15 min) on the viability of the microencapsulated probiotic. They found that the highest survival of *Bifidobacterium* BB-12 after spray drying was obtained in microcapsules produced only with sweet whey (9.54 log cfu/g). Sweet whey also produced the best yield in microencapsulation (95.43%) of the probiotic. On the other hand, the researchers observed that microcapsules prepared only with sweet whey and those produced with a sweet whey-inulin blend provided a better viability of bifidobacteria vs. free cells; the reduction was 0.49 and 0.97 log cfu/g, respectively. The authors concluded it is better to use only sweet whey to microencapsulate *Bifidobacterium* BB-12 since the viability of a wide number of probiotic bacteria is maintained after spray drying. It is even maintained after exposure to simulated gastrointestinal conditions and thermal treatments. Therefore, the performance is better than when it is used in a blend with prebiotics like inulin in encapsulation.

Rosolen et al. [94] microencapsulated *Lactococcus lactis* subsp. lactis R7 (*L. lactis* R7) with whey and inulin by spray

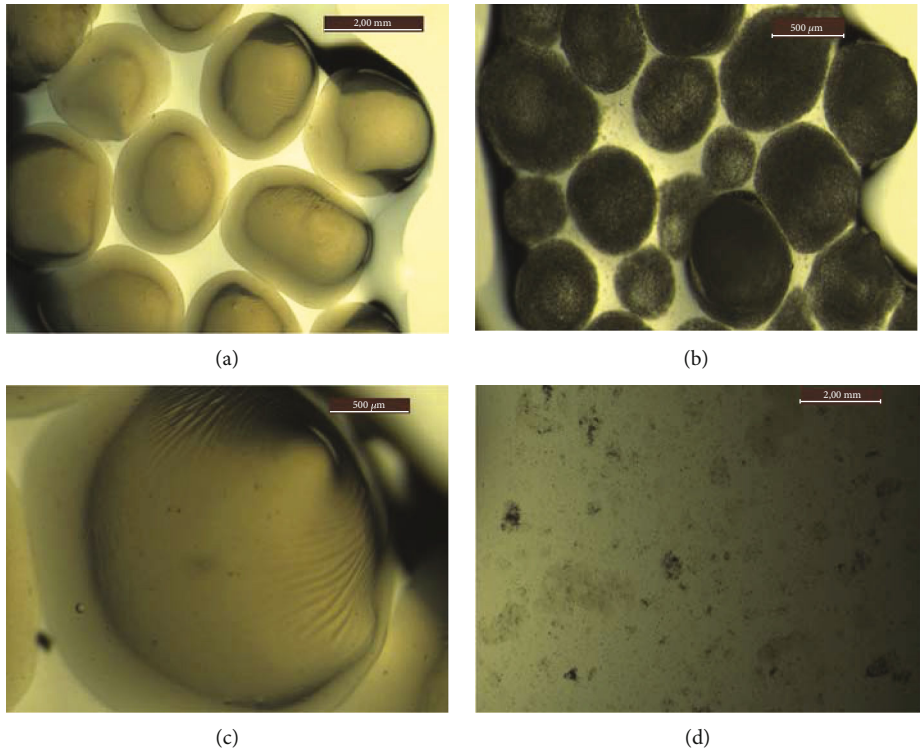


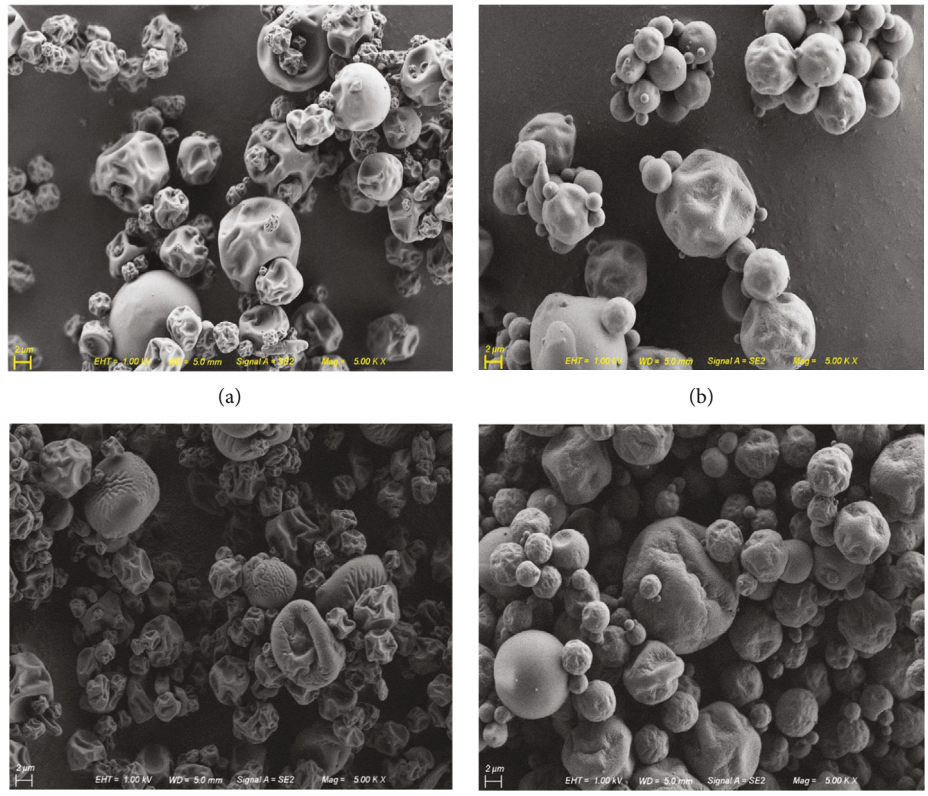
Figure 3: Stereoscopic microscope images: (a) microcapsules obtained by extrusion before gastric digestion in vitro (GDInV), (b) microcapsules obtained by internal emulsion before GDInV, (c) microcapsules obtained by extrusion after GDInV, and (d) microcapsules obtained by internal emulsion after GDInV [82].

drying. They evaluated the survival of the microencapsulated probiotic as it passes through simulated gastric degradation, thermal treatments, and storage (6 months) at $-20 \pm 1 \circ$ C, $4 \pm 1 \circ$ C, and $25 \pm 1 \circ$ C. *L. lactis* R7 microencapsulated with inulin and whey was recovered in high concentrations (13.0 log cfu/g) after microencapsulation, and the yield of the microencapsulation was also high (94.61%). In time-dependent survival studies, the concentration of the microencapsulated probiotic was relatively high (>8.0 log cfu/g) at the three storage temperatures and at least at month 6 of study. In addition, the microcapsule protected *L. lactis* R7 during its passage through simulated GIT (gastric fluid with pepsin at pH 2, 2.5, and 3 and intestinal fluid with pancreatin at pH 8). It conferred resistance against thermal treatments (60, 65, and 70°C for 0, 5, 10, 15, and 30 min), while there were reductions of 1.36, 0.77, and 2.34 logarithmic cycles at pH 2, 2.5, and 3, respectively.

2.6. Coacervation. Coacervation consists in the precipitation of a polymer (simple coacervation) or several (complex coacervation) induced by phase separation. In simple coacervation, proteins are used as encapsulating material and their precipitation can be induced by a change in pH or ionic strength [95–97]. Complex coacervation is carried out by mixing two polymers with opposite charges and the same pH [98]. The polymer crosslink and precipitation is carried out by adjusting the pH and cooling of the blend. After using this encapsulation method, drying is usually necessary to provide stability to the capsules [98].

Interesting coacervation systems are those between proteins and ionic polysaccharides of opposite charge. For instance, pectin is a popular anionic polysaccharide for complex coacervation with β-lactoglobulin [99–101]. Once formed, coacervates look like dynamic, adaptable structures capable of responding to environmental changes (pH, ionic strength, and temperature). They can reorganize to create an adequate charge distribution when the environment is not ideal [102]. Structural reorganizations can also occur during coacervate storage, and such modifications can be relatively slow [103]. Coacervation is a promising encapsulation technology due to its high charge capacity and effective release of encapsulants by mechanical stress, temperature, and pH alterations [95].

Kaewnopparat et al. [96] used complex coacervation to encapsulate *L. rhamnosus* GG in a blend of Bambara groundnut protein isolate (BGPI), alginate, and inulin. The optimal model included BGPI/alginate solution at a 1 : 1 weight ratio to obtain a 2.14% w/v solution with 3.23% w/v inulin. The microcapsules prepared under optimal conditions showed excellent protection since they improved cell survival rate of cell in simulated gastric liquid (pH 2 pepsin for 2 h and simulated intestinal juice with pH 7 pancreatin) in ~4.88 log cfu/ml after 3 h vs. free cells. They demonstrated cell release of 8.53 log cfu/ml in 4 h and under storage conditions at 4 and 30°C for 6 months; the survival rate was 7.82 log cfu/ml and 8.0 log cfu/ml, respectively. It was higher than that of free cells under the same conditions (4.10 log cfu/ml and <1.0 log cfu/ml, respectively). Microcapsules also



(a) (b) (c) (d)

Figure 4: Scanning electron microscopy micrographs of *Lactobacillus acidophilus* microcapsules obtained by spray drying with an initial solution containing 8 g of trehalose [56].

efficiently released lactic acid bacteria (LAB) in the simulated intestinal fluid.

2.7. Freeze Drying. Freeze drying is an adequate technique to encapsulate thermosensitive microorganisms [104, 105]. In general, it consists of freezing microorganisms at −70°C under a vacuum. Water is eliminated by sublimation during the process, and the result is a dry paste where the bacteria remains stable along time [106, 107]. The technique produces microcapsules of stable biological materials as the effect of temperature and chemical reactions is reduced during storage [59, 108].

Several ecological factors determine microbial survival during and after freeze drying, among which are the bacterial species, the physiological state of microorganisms, cell density, the effect of encapsulating or wall materials [109, 110], freezing rates, and rehydration, among others [109–111]. The effect of these parameters or factors can cause cell damage and reduction in microbial viability and activity at different degrees [112]. Some authors report that the use of prebiotics as resistant starch, inulin, and FOS in wall material blends confers protection to microencapsulated bacteria [105, 110, 113], additionally, to increase probiotic viability during encapsulation by freeze drying, storage, and the subsequent passage through the GIT, cryoprotective agents (saccharides and polyols), and other compatible solutes [105, 106, 110].

Barona et al. [50] evaluated the effect of the prebiotics inulin and FOS (25% w/v) in the freeze-drying microencapsulation and storage of *L. casei* ATCC-393 and *Lactobacillus rhamnosus* ATCC-9469. They also evaluated changes in the physicochemical properties of the powders and the viability of the microencapsulated bacteria. They observed a high hygroscopicity and wettability in the presence of prebiotic agents. They also found an increased viability (>93.94%) of probiotic bacteria encapsulated with inulin and FOS as compared vs. the treatment without prebiotic (89.79% viability) in *L. casei*. Still, the characteristics of the encapsulates hamper the application of powders to a food matrix with low water activity and possible hydration at the moment of consumption. This is because hygroscopicity and wettability are increased in the presence of prebiotic material. The behavior is closely related to the chemical and structural characteristics and the number of free sugars and degree of polymerization. In hydrated matrices, microcapsule solubility could risk microorganism survival and food stability given that probiotics might deteriorate and decompose the food.

Estilarte et al. [113] microencapsulated *Enterococcus durans* (LM01C01, LM05C01 and EP1) by freeze drying using sucrose, lactulose, or maltodextrin as prebiotics. Lactulose provided the best protection for all three strains of *E. durans* after freeze drying and storage at 4 and 25°C for 92 days. However, strain LM01C01 showed the highest survival. They observed that the freeze dried LM01C01 with lactulose

was more resistant at low pH (2.5) and in the presence of bile (3 and 50 g/L), as the probiotic was able to grow.

2.8. Refractance Window Drying. The refractance window drying (RW) encapsulation technique is a nonthermal method that does not require high operating pressures [114]. RW drying is a technique used to concentrate viscous solutions and suspensions, obtaining a product in the form of flakes or film at reasonable costs [115]. The RW drying method consists of uniformly placing the viscous solution or suspension on a thin transparent material (such as Pyrex glass or a polyethylene film known as Mylar®) in the infrared, which is in contact with hot water (95–98°C) [116, 117]. The thin transparent film or material creates a "window" that allows the transport of heat and infrared energy from the hot water to the wet feedstock [118]. The radiation allows the product to settle quickly because the film has low resistance to thermal conductivity [116]. The product temperature is below the hot water temperature, eliminating the effects of excessive drying [119]. The previously mentioned characteristics before RW drying are alternatives for thermosensitive products such as probiotics.

There is little research using the RW drying technique for synbiotics encapsulation. Aragon-Rojas et al. [116] encapsulated *L. fermentum* K73 by RW drying, using maltodextrin and sweet whey (0.6 : 0.4) as an encapsulation matrix using three water temperatures (59.85, 69.85, and 79.85°C). The survival of the microorganisms and the drying kinetics was studied using mathematical models (modified Gompertz and Midilli). They found that the most favorable conditions, according to modeling, were a drying time of 41 min, a temperature of 79.85°C with concentrations of 9.1 log cfu/g and a final humidity of 10%. On the other hand, Yoha et al. [120] encapsulated a synbiotic consisting of *L. plantarum* (NCIM 2083) in combination with FOS, whey protein (WP), and/or maltodextrin (MD). The best viability of the probiotic was found at 40°C with the mixture of prebiotics FOS:WP:MD (2 : 0.5 : 0.5). The moisture content for the different mixtures ranged between 5.25% and 6.51% and the encapsulation efficiency between 88.05 and 93.29%. Regarding the survival of *L. plantarum* (NCIM 2083) under simulated GIT conditions, the results showed that, under oral conditions, there was no significant decrease in cell viability. RW-encapsulated synbiotics showed a decrease in cell viability under gastric conditions. However, these changes were not significant. Under intestinal conditions, there was a reduction in cell viability of the synbiotics (~6 log cfu/g). Prebiotic-encapsulated probiotics were less susceptible to loss of viability compared to cell free. This is because prebiotics protects probiotics. Later, this same group of researchers [121] incorporated the different synbiotic encapsulations into 3D-printed foods.

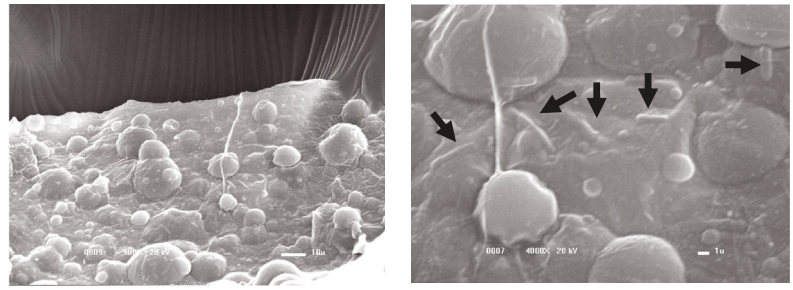
According to the findings in the different investigations, the refractance window drying encapsulation technique could be a technically viable technology for encapsulating probiotics derived at low cost, short drying time, and acceptable characteristics in the encapsulates. It is important to note that this method considers a nonthermal drying approach, ideal for heat-sensitive foods and ingredients.

2.9. Combination of Encapsulation Techniques Applied to Synbiotics. Ribeiro et al. [101] microencapsulated *Lactobacillus acidophilus* LA-5 using whey protein and pectin as wall material and prebiotic, respectively. The microencapsulation included two processes: ionic gelation and complex coacervation. Figure 5 shows the size and shape of the microcapsules obtained, which were added to yoghurt to evaluate the survival of microencapsulated bacteria and changes in microcapsule characteristics. The study also evaluated the survival of probiotics subjected to simulated GIT conditions and sensory acceptance of yoghurt after 35 days of refrigerated storage (5°C). The yoghurt containing encapsulated *L. acidophilus* LA-5 showed a lower acidification and better probiotic survival (62%) as compared against the yoghurt containing free *L. acidophilus* LA-5 cells (10%) after 35 days of refrigerated storage. Additionally, the survival of encapsulated *L. acidophilus* LA-5 was higher than that of the free microorganism in studies on the effect of simulated gastrointestinal conditions (gastric juice simulated at pH 3, intestinal juice simulated at pH 7, and 1% w/v bile solution). After 35 days of storage, there were no significant differences ($p < 0.05$) in appearance, smell, taste, and overall impression of both samples (Figure 5).

Cook et al. [122] microencapsulated *Bifidobacterium breve* with Bimuno™ (GOS), an alginate-chitosan blend, and a double emulsion before freeze drying. They observed the cell survival of *B. breve* encapsulated in multiparticles was 8.0 ± 0.3 log cfu/ml under acidic conditions (pH 2 for 60 min), an improvement vs. microencapsulation with alginate-chitosan (1.4 log cfu/ml).

Chávarri et al. [42] produced alginate microspheres coated with chitosan to encapsulate *Lactobacillus gasseri* (L) and *Bifidobacterium bifidum* (B) as probiotics and prebiotic quercetin (Q), using extrusion and freeze drying technique. The encapsulation yield of viable cells in alginate microspheres coated with chitosan-quercetin was low: 3.90 ± 0.86 log cfu/g in L+Q and 2.99 ± 0.97 log cfu/g in B+Q. These results, along with the study on probiotic survival in microspheres with quercetin during storage at 4°C, proved that probiotic bacteria microencapsulated with quercetin did not survive. Therefore, quercetin and *L. gasseri* or *B. bifidum* were microencapsulated separately. Microencapsulated *L. gasseri* and *B. bifidum* were resistant to simulated gastric conditions (pH 2, 2 h) and bile solution (3%, 2 h); this resulted in 95% and 94% of survival in alginate microspheres coated with chitosan, respectively. The elevated survival of probiotics encapsulated with alginate and chitosan after being exposed to simulated gastric conditions proves that this complex protects the bacterial cell. It reduces the porosity of alginate capsules, which reduces the leakage of the encapsulated probiotic, stable at wide pH ranges.

Raddatz et al. [58] evaluated the effect resistant starch of maize, inulin, and rice brain as prebiotics on *L. acidophilus* LA-5 viability in pectin microparticles obtained by emulsion/internal gelation, followed by freeze drying. The encapsulation matrix pectin+inulin showed the highest encapsulation efficiency (68.1%) as compared against the other treatments. The microparticle size went from



(a) (b)

Figure 5: Scanning electron microscopy (SEM) of microcapsules obtained by ionic gelation and complex coacervation with *L. acidophilus* LA-5. (a) Microcapsules at a magnification of 900x and bar of 10 μm. (b) *L. acidophilus* LA-5 cells (marked with arrows) are randomnly distributed within microcapsules at a magnification of 4000x and bar of 1 μm [101].

$166 \pm 2\,\mu$m (pectin+Resistant maize starch) to $345 \pm 9\,\mu$m (pectin+inulin). Generally, the microparticles with added prebiotics presented higher levels of microorganisms. In addition, bacterial survival in microcapsules with prebiotics was higher in gastrointestinal simulation studies across sections as esophagus/stomach (added pepsin, pH adjusted to 2 for 90 min), duodenum (added pancreatin and bile salts, pH adjusted to 5 for 20 min), and ileum (pH adjusted to 6.5 for 90 min). Similarly, during storage at 25 and −18°C, a greater survival of probiotic microorganisms (above 6.0 log cfu/g) was observed in microparticles containing resistant starch maize, inulin, and rice bran. Probiotics in microcapsules containing pectin+rice bran survived at least 120 days at 7°C.

It has been demonstrated that microencapsulation protects probiotics from adverse conditions during food processing and passage through the digestive system in conditions *in vitro* when combined with prebiotics. Table 1 presents studies on synbiotic encapsulation [7, 8, 38, 42, 45, 48, 53, 56–58, 60, 77, 79–82, 93, 94, 96, 101, 122–124].

2.10. Antimicrobial Effect of Synbiotic Microencapsulates on Pathogens. Gut microbiota is a complex ecosystem [125], and introducing new organisms to this highly competitive environment is difficult [126]. Therefore, microorganisms that can create a product to inhibit the growth or displace the existing microorganisms in the gut are at a distinctive disadvantage [46]. LAB are a group of bacteria that can potentially establish in the intestine and displace part of the gut microbiota by producing bactericide compounds [127]. These compounds can also affect the viability of pathogen bacteria [128]. Several *Lactobacillus* spp strains inhibit different pathogen bacteria, both Gram positive and negative [129–131]. This is because of the production of antimicrobial compounds as bacteriocins, hydrogen peroxide, and organic acids [128, 131]. Different LAB are probiotic bacteria [132–134]. The ability of probiotic LAB to establish in the GIT is reinforced by their capacity to eliminate their competitors [46]. The microcapsules containing antimicrobial compounds provide a controlled release, ensuring the stability of the compound [135]. Table 2 describes some works on the antimicrobial effect of some microencapsulated synbiotics.

2.11. Emerging Technologies: 3D Food Printing. Three-dimensional (3D) food printing technology offers personalized products with complex geometries and designed internal structures, controlled composition, and personalized textures tailored to meet each person's taste preferences and specific dietary needs [136–138]. The main 3D food applications are based on extrusion technology and refer to natively printable materials, such as cereal derivatives, chocolate, doughs, and pasta [136, 137]. According to the nutritional requirements of the current population, a new range of healthy food products has been developed through 3D printing of foods added with nutraceuticals and functional food ingredients [119, 139, 140]. For example, Liu et al. [141] incorporated probiotics (*Bifidobacterium animalis* subsp. *lactis* BB-12) into 3D-printed mashed potatoes. This puree was stored for 12 days, after which the count of viable microorganisms was above (9.77 log cfu/g) the recommended dose. This research suggests that attractive 3D foods can be made with probiotic microorganisms. In 2018, Zhang et al. printed 3D food structures based on cereals containing probiotics. Two types of structures with different surface-to-volume ratios were printed, that is, 9.20 cm^2/cm^3 for the "honeycomb" design and 7.25 cm^2/cm^3 for the "concentric" design, finding that the increase in the surface-to-volume ratio of the structure accelerated its firing process. Thus, the viable counts of probiotics in the "honeycomb" structure exceeded 10^6 cfu/g, concluding that the survival of probiotics could be improved by increasing the surface-to-volume ratio of the structure.

On the other hand, [121] combined the encapsulation (synbiotic) and the 3D printing process on the viability of probiotics by making food based on flour rich in fiber and protein. The stability of the probiotics during storage in two different temperature conditions (4°C and room temperature) was evaluated. No significant loss of probiotic viability was observed during the 3D printing process. In addition, the encapsulation by lyophilization followed by the lyophilization postprocessing method presented the best viability of probiotics (8.18 log cfu/g); the best survival rates of 6.43 log cfu/ml and 7.98 log cfu/g were also obtained under static conditions of in vitro digestion and during 35 days of storage, respectively. 98-99% survival was obtained for all encapsulated probiotics after the 3D printing process,

Table 1: Synbiotic encapsulation and resistance to simulated gastrointestinal conditions.

Probiotic	Prebiotic	Encapsulation technique	Wall material	Resistance to low pH	Resistance to bile salts	References
			Ionic gelation			
Lactobacillus acidophilus	Fructooligosaccharides	External ionic gelation	Alginate (AG) and alginate -gelatin-fructooligosaccharides (AGF)	Viable *L. acidophilus* cell count in microbeads (AG and AGF) meets requisites for classification as probiotic benefits	Reduction of 0.62 log cfu/g and 1.51 log cfu/g in AG and AGF encapsulates, respectively	Silva et al. [38]
Saccharomyces cerevisiae boulardii	Inulin	Ionic gelation	Alginate-inulin-xanthan	Greater resistance against gastric juices vs. free yeast	Microencapsulation of yeast with alginate increases survival in bile solution vs. free yeast	Fratianni et al. [7])
			Emulsification			
Lactobacillus plantarum	0.1 and 2% inulin	Internal emulsion	Alginate	After gastrointestinal digestion *L. plantarum* survival was well above the minimum recommended therapeutic dose (10^6 cfu/g or ml) but microcapsules obtained from internal emulsion did not resist gastric digestion from day 15 of storage and lost integrity, releasing the microorganism into the environment		Valero-Cases and Frutos, [82]
			Extrusion			
Pediococcus acidilactici, lactobacillus Reuteri, Lactobacillus salivarius	0, 5, 10, 15, and 20% w/v inulin	Extrusion/ ionotropic gelation	2% w/v alginate	Bacterial protection vs. acidity (pH 1.2, 3, and 4,5) increased by adding inulin vs. free probiotic bacteria	Beads with 5% w/v inulin provided the most effective bacterial protection vs. bile salts	Atia et al. [53]
Lactobacillus rhamnosus	Inulin and FOS	Extrusion	3% alginate and 1% gelatin	Viability of *L. rhamnosus* microencapsulated with inulin was higher than those of *L. rhamnosus* microencapsulated with FOS and *L. rhamnosus* microencapsulated without prebiotic		Ergİnkaya et al. [77]
Lactobacillus plantarum	0.1 and 2% inulin	Extrusion	Alginate	*L. plantarum* survival was high after gastrointestinal digestion, above minimum recommended therapeutic dose (10^6 cfu/g or ml)		Valero-Cases and Frutos, [82]
Lactobacillus fermentum L7	GOS, isomaltooligosaccharides, FOS, and xylooligosaccharides	Extrusion	Alginate	Viability of microencapsulated probiotic (reduction of 2.76 log cfu/ml) after exposure to simulated gastric and intestinal juices was better than that of free cells and viability of probiotic co-encapsulated with FOS (10 ± 0.05 log cfu/g) was better than that of probiotic cells encapsulated with only alginate (8.17 ± 0.33 log cfu/g)		Liao et al. [123]
Lactobacillus casei LC-01 and *Lactobacillus casei* BGP 93	FOS	Extrusion	Flaxseed mucilages (FM), aokra (O), and fungal exopolysaccharide (FE) botryosphaeran sodium alginate (BSA)	The use of FOS combined with flaxseed mucilage confers a more effective protection to *L. casei* LC-01 and *L. casei* BGP 9 cells	The highest survival after gastrointestinal simulation was obtained with 1.5% FOS + FM and 1.5% FOS+ FE, reaching 6.11 and 6.19 log cfu/g, respectively	Rodrigues et al. [79]

Table 1: Continued.

Probiotic	Prebiotic	Encapsulation technique	Wall material	Resistance to low pH	Resistance to bile salts	References
			Emulsion/extrusion			
Lactobacillus casei Shirota and two *Lactobacillus plantarum* strains *(Lp33* and *Lp17)*	Potato starch (PS), plantago psyllium (PSY), and inulin (INU)	Emulsion/ extrusion	Alginate	Probiotic bacteria coencapsulated with PSY and INU showed greater viability ($p < 0.05$) after intestinal simulation than capsules with PS without prebiotics	Viability above 6 log cfu/g	Peredo et al. [81]
			Spray drying			
Lactobacillus plantarum (NCIM 2083)	Fructooligosaccharides	Spray drying (SD)	Whey protein (WP) and maltodextrin (MD)	Synbiotics encapsulated by SD shoed significant loss in cell viability (2–3 log reductions) after exposure to gastric conditions (pH 3 for 2 h)	4 log reductions were observed in cell viability of synbiotics encapsulated by SD after exposure to small intestine conditions (pH 7 for 2 h)	Yoha et al. [60]
L. acidophilus	Inulin, trehalose, Hi-maize®	Spray drying	Gum Arabic, maltodextrins	Microcapsules produced with resistant maize starch showed the greatest viability under simulated gastrointestinal conditions in different GIT sections as esophagus/stomach, duodenum, and ileum, protecting *Lactobacillus acidophilus* vs. use of trehalose and inulin		Nunes et al. [56]
Lactobacillus acidophilus La-5	Inulin	Spray drying	Inulin	Survival rate of 78.7% under acidic conditions (reduction of 1.9 log cfu/g). *L. acidophilus La-5* showed a high survival in gastric environment *in vitro* due to a low degradation of inulin microcapsules under acidic conditions, resulting in a late reduction in probiotic survival		Xavier et al. [124]
			Coacervation			
L. rhamnosus GG	Inulin	Complex coacervation	Bambara groundnut protein isolate and alginate	Microcapsules prepared under optimal conditions showed excellent protection to encapsulated cells under gastric conditions vs. unprotected free cells. Microcapsules also efficiently released probiotic cells in simulated intestinal fluid		Kaewnopparat et al. [96]
			Freeze drying			
Lactobacillus plantarum (NCIM 2083)	Fructooligosaccharides, milk protein	Freeze drying (SFD)	Whey protein (WP) and maltodextrin (MD)	Viability of probiotic cells of SFD sample was maintained (~8 log cfu/g) after exposure to simulated acidic conditions	Synbiotics encapsulated by SFD showed a final viability loss of 2 log reductions after simulated oral-gastrointestinal digestion	Yoha et al. [60]
			Combination of encapsulation techniques			
Bifidobacterium breve	Bimuno™ (Galactooligosaccharides)	Double emulsion and freeze drying	Poly (lactic-coglycolic acid) (PLGA)	Cell survival was higher when exposed to simulated gastric solution likely due to increased material hydrophobia after addition of PLGA microcapsules		Cook et al. [122]
Lactobacillus acidophilus LA-5	Pectin	Ionic gelation and coacervation	Whey protein	Encapsulated *L. acidophilus* LA-5 showed higher survival vs. free microorganism during simulated gastrointestinal conditions, simulated pH 3 gastric juice, and simulated pH 7 intestinal juice and bile solution (1%, w/v)		Ribeiro et al. [101]

Table 1: Continued.

Probiotic	Prebiotic	Encapsulation technique	Wall material	Resistance to low pH	Resistance to bile salts	References
Lactobacillus gasseri *Bifidobacterium bifidum*	Quercetin	Ionic gelation (extrusion)/ freeze drying	Alginate Chitosan	Microencapsulated *L. gasseri* and *B. bifidum* were resistant to simulated gastric conditions (pH 2, 2 h)	Encapsulated probiotics also showed resistance to bile solution (3%, 2 h)	Chávarri et al. [42]
Lactobacillus acidophilus LA-5	Inulin	Emulsion/ internal gelation	1% pectin (PECL), 10% Hi-maize®+1% pectin (PHML), 10% inulin 10%+1% pectin (PINL), 10% rice bran+1% pectin (PRBL)	Microcapsules of all treatments conferred probiotics greater protection at the end of gastrointestinal simulation reductions of 0.3 ± 0.2, 0.1 ± 0.0 , 1.6 ± 0.2, and 1.0 ± 0.2 log cfu/g in PECL, PHML, PINL, and PRBL microcapsules, respectively) vs. free microorganism (reduction of 3.4 ± 0.1 log cfu/g)		Raddatz et al. [58]
L. acidophilus LA-5	Hi-maize®, inulin, and rice bran	Emulsion/ internal gelation followed by freeze drying	Pectin	Microparticles with different prebiotics conferred microorganisms a better protection vs. treatment without prebiotics; they also presented a higher viability in gastrointestinal simulation through sections as esophagus/stomach, duodenum, and ileum		Raddatz et al. [48]

Table 2: Antimicrobial effect of microencapsulated synbiotics against pathogens.

Probiotic	Prebiotic	Encapsulation technique	Inhibited microorganisms	Main findings	References
Lactobacillus rhamnosus	Inulin and FOS	Extrusion	*Enterococcus faecalis* *E. faecium*	The culture with added prebiotic showed an inhibitory effect on *Enterococcus faecalis* growth. The survival rate of probiotic culture cells depends on the prebiotic. Inulin was more efficient in *L. rhamnosus* cell viability than FOS	ErgİNkaya et al. [77]
Pediococcus acidilactici *Lactobacillus reuteri* *Lactobacillus salivarius.*	0, 5, 10, 15, and 20% inulin	Extrusion/ionotropic gelation	*Salmonella Montevideo* *Escherichia faecalis* *Staphylococcus aureus* *Listeria monocytogenes* LSD 530 L. *innocua* *E. coli* MC4100	Antimicrobial capacity was significantly lower ($p < 0.05$) in bacteria encapsulated in beads vs. free bacteria	Atia et al. [53]
Lactobacillus reuteri DPC16	Chitosan	Emulsion	*E. coli* O157:H7 S. *typhimurium*	No attenuated antimicrobial effect was observed in immobilized *Lactobacillus reuteri* DPC16 vs. free cells. Microencapsulation provided improved protection in probiotics added	Chen et al. [161]

proving that the 3D printing process does not have a negative impact on the viability of encapsulated probiotics. In this way, it is concluded that the incorporation of encapsulated synbiotic powders in 3D foods can significantly improve the stability of probiotic cells. On the other hand, the incorporation of encapsulated synbiotics into 3D foods could be less complex since, in these foods, the surface-to-volume ratio can be controlled, offering benefits for the survival of probiotic microorganisms.

3. Effect of Microencapsulated Synbiotics in Animal Models

There are currently limited studies on the effect of microencapsulated synbiotics in animal models. Only four studies are found in literature regarding synbiotics encapsulated using different techniques and their effect in animal models. This represents an area of opportunity in scientific research related to the effectiveness of microencapsulated synbiotics and their real effect in a living organism.

Bhatia et al. [142] studied the hypoglycemic potential of microencapsulated prebiotics (lactulose) with a probiotic (*L. casei* subsp. *casei* 17 at a concentration of 10^9 cfu/ml) *in vivo*. The microencapsulation was carried out using sodium alginate (3.5%) and calcium chloride (75 mM). The synbiotic capsules obtained were administered to diabetic albino mice, and the researchers investigated their effect in the reduction of glucose levels as compared against the nonmicroencapsulated synbiotic and glibenclamide. The results showed that synbiotic microcapsules reduced glucose levels in blood by 54% in diabetic mice. This decrease was sharper than that obtained with the nonencapsulated synbiotic (51%) and glibenclamide (46%). Wang et al. [143] microencapsulated *L. plantarum* and FOS by emulsion. The synbiotic microcapsules were administered to weaned piglets, and their effect was identified in the growth of the animals, immune response, and gut microbiota. The piglets administered with the synbiotic showed a greater weight gain and food intake as well as a lower rate of diarrhea ($p < 0.05$) vs. piglets that did not receive the synbiotic. Additionally, piglets given the microencapsulated synbiotic showed higher plasma concentrations of immunoglobulin A (IgA) and G (IgG) ($p < 0.05$) of LAB in colon as compared against the group that did not receive the synbiotic.

Wang et al. [144] used the emulsion technique to microencapsulate *E. faecium* (10^8 cfu/g), *L. plantarum* (10^8 cfu/g), and *B. subtilis* (10^9 cfu/g) with prebiotics β-mannose (250 U/g) and FOS (250 mg/g). The microencapsulated synbiotic was administered to male broiler chickens aged 1 day, and its effect was assessed in growth, immune response, antioxidant capacity, and fecal Lactobacillus concentration. The study included chickens administered with an antibiotic instead of the synbiotic and those that were not administered with synbiotic nor antibiotic. The average daily weight gain, serum levels of immunoglobulin M, and total serum antioxidant capacity (T-AOC) increased significantly ($p < 0.05$) in chickens given the synbiotic and the antibiotic vs. those that did not receive any treatment. Furthermore, the chickens administered with the synbiotic showed the highest levels of serum T-AOC, IgA, serum interleukin-2 (IL-2) and IL-6, and fecal Lactobacillus concentration.

da Silva et al. [145] microencapsulated the probiotic *Lactobacillus casei* 01 (11–12 log cfu/ml) alone, in a blend with inulin enriched with oligofructose (Synergy 1), and as synbiotic microparticle (*L. casei* 01+Synergy 1). They used a blend of chitosan, calcium, and alginate as wall material to obtain the microcapsules by spray drying. The anti-inflammatory effect of the microencapsulated bacteria was evaluated in a trinitro benzenesulfonic (TNBS) acid model of rat colitis. The animals given probiotics/synbiotics (8.5–

8.9 log cfu/ml *L. casei* 01 and 1.5% Synergy 1) showed a reduction in colon damage and increased levels of lactobacilli in feces vs. rats with colitis that received not treatment. Additionally, rats given synbiotic microcapsules presented the highest anti-inflammatory effect and fewer colon lesions, linked to a significant decrease in myeloperoxidase activity.

4. Polymers Used as Wall Material

An important step in the microencapsulation process is the selection of appropriate encapsulation materials [47]. The materials must be chemically compatible and nonreactive with the material to be microencapsulated and provide the desired coating properties such as resistance, flexibility, impermeability, and stability [146, 147].

The materials commonly used to make microcapsules are typically biopolymers, such as alginate, starch, alginate, carrageenan, gelatin, and protein, which generally have good thermal stability, high biocompatibility, low toxicity, and low cost [4]. However, in recent years, new encapsulating biomaterials have emerged, such as gums, mucilages, prebiotic compounds, and microbial exopolysaccharides, which improve the protection and survival of encapsulated microorganisms, allowing their incorporation into dairy and nondairy food products [75]. On the other hand, these biomaterials provide potential health benefits.

Gums and mucilages are polysaccharides that are obtained from plants through natural exudation produced by injury or are extracted from different tissues using extraction processes [148]. The hydrophilic nature of these compounds makes them easy to extract by soaking the seeds or shells in water [149]. The encapsulation of probiotic microorganisms using gums and mucilages as wall material has shown improvement in the viability of the encapsulated cells during storage and their passage through GIT [79].

Psyllium mucilage is extracted from the shells of Plantago ovata Forssk seeds with gelling capacity, swelling capacity, and water absorption properties, properties attributed to the presence of arabinoxylans in its structure [150]. The polysaccharides present natural antioxidant and purifying activity [151]. Peredo et al. [81] encapsulated *L. casei Shirota* and two strains of *L. plantarum* (Lp33 and Lp17) with potato starch, *Plantago psyllium,* and inulin, finding that probiotic bacteria coencapsulated with *Plantago psyllium* and inulin showed the highest viability ($p < 0.05$) after of bowel simulation.

Guazuma ulmifolia Lam (Malvaceae), commonly known as mutamba, has a black fruit with dry skin and seeds inserted in a mucilaginous pulp [152]. The seeds present in the fruit, if soaked in water, release copious amounts of mucilage, forming "gelatinous capsules" [75]. In a mature state, the fruit represents a source of fibers, proteins, vitamins, minerals, and phenolic compounds [153]. Mutamba has structural characteristics that indicate that it could be used as an emerging biopolymer in food and pharmaceutical applications [153]. However, so far, there is no scientific evidence of the use of mutamba mucilage to encapsulate probiotic microorganisms.

Microbial exopolysaccharides such as xanthan gum produced by Xanthomonas campestris are considered nontoxic, presenting hydrosolubility in hot and cold water [75]. Xanthan gum, in combination with another polysaccharide, improves the encapsulating properties. For example, Fratianni et al. [7] microencapsulated a probiotic yeast, *Saccharomyces cerevisiae boulardii*, in a xanthan gum-alginate-inulin mixture, improving yeast survival during storage and under simulated gastric fluids.

On the other hand, prebiotics has been gaining popularity among the biomaterials used to encapsulate probiotics due to the fact that they function as a substrate for microorganisms. Such is the case of inulin, which, being made up of fructose monomers linked by β-glucosidic bonds, makes it resistant to hydrolysis in the digestive system [154]. The use of inulin-trehalose-Hi-maize® to encapsulate L. acidophilus provided protection for bacteria under simulated gastric and intestinal juices [56].

Importantly, the functional performance of microcapsules can be improved when multiple layers or shells are formed on the microcapsule with the same or different biopolymer. Chitosan is one of the most used polysaccharides for this purpose since, due to its positive charge, it can be combined with negatively charged polysaccharides [155]. It has been proposed that the microcapsules formed by layers of alginate-chitosan have good potential since they can resist the conditions of the gastrointestinal tract until they reach the colon, where both the chitosan and the alginate are degraded by the colonic microbiota, thus releasing probiotics [156].

5. Synbiotic Encapsulation Patents

It is important to note that research can sometimes lead to a new product, process, or service that can be applied or used immediately to solve a problem or need in the food industry. Furthermore, these new inventions can generate economic resources for the inventors and/or for the owners. New inventions can be legally protected as industrial property or intellectual property, for example, in the form of patents.

In the case of microencapsulates, even though there are numerous articles published on the different types of microencapsulation of probiotic bacteria and synbiotics, as well as the different wall materials and on the incorporation of encapsulates containing probiotics or synbiotics to a food matrix, compared to this, there are few patents or patent applications on probiotics or microencapsulated synbiotics [157–160]. It is necessary to increase the number of patents or applications for new patents throughout the world, as this increases the probability that a new discovery can be used immediately to solve problems or needs in the food industry.

6. Conclusions

This review provides evidence on the microencapsulation of synbiotics using different techniques, such as ionic gelation, emulsification, extrusion, spray drying, coacervation, freeze drying, and their combination in some cases. They confer probiotics protection as they pass through the gastrointestinal

tract, both in simulated conditions and animal models. This work also demonstrates that microencapsulation helps to reach an adequate concentration of prebiotics and probiotics in the microcapsule to exert a beneficial effect in the host's health. In addition, the available evidence proves that synbiotics are more beneficial when administered in microcapsules than in their free form. On the other hand, microencapsulation allows for the controlled release of antimicrobial compounds that produce most of the probiotic bacteria, ensuring their stability through the gastrointestinal tract. Several works have proven the effectiveness of encapsulated synbiotic models in vivo and revealed the synergy between probiotic, prebiotic, and capsule to counter chronic, degenerative diseases in some cases. Still, further research is necessary both in animals and humans regarding the beneficial effect in health after microencapsulated synbiotics are administered given that the existing studies suggest their use might have a beneficial impact in the consumer's health.

Data Availability

Data will be available upon request.

Ethical Approval

This review adheres to ethical standards since human research ethics approval was not necessary.

Disclosure

There is a permission to reproduce material from other sources. The images of other authors used in this investigation have the pertinent permissions.

Conflicts of Interest

The authors declare no conflict of interest.

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