BACTERIAL CELLULOSE AS A SUSTAINABLE MATRIX FOR PROBIOTIC IMMOBILIZATION IN FUNCTIONAL FOODS

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The probiotics market was valued at USD 99.98 billion in 2024 and is projected to reach USD 374.57 billion by 2034, growing at a CAGR of 14.12%. Live, non-pathogenic microorganisms known as probiotics are beneficial for health, particularly in digestive, neurological, and immune functions. The main drivers of market growth include increased consumer awareness, technological advancements, and the demand for non-dairy probiotic products. However, the viability of probiotics depends on their manufacturing and storage conditions. Various encapsulation technologies are being explored to enhance stability and functionality, focusing on bacterial cellulose, a GRAS-approved, indigestible dietary fiber that provides excellent protection. Bacterial cellulose helps maintain probiotic viability as they pass through gastrointestinal conditions in a controlled manner. This study highlights encapsulation methods such as electrospinning, cross-linking, *in-situ* composite development, solvent casting, and spray drying. These technologies offer effective solutions for improving the functions of foods and probiotic delivery systems in the food industry.

Keywords: probiotics, bacterial cellulose, functional foods, immobilization

INTRODUCTION

The global food industry is poised for a significant shift toward functional, sustainable, and health-boosting food products that offer additional physiological benefits beyond basic nutrition.[1] Probiotics are among the most popular bioactive food components, as they have established roles in immune function, metabolic balance, gut health, and disease prevention.[2,3] Probiotics are live microorganisms that, when consumed in sufficient amounts, are beneficial or even essential for health.[4] Originally discovered in fermented foods, such as kefir, kimchi, and yogurt, probiotics are now found in a variety of functional foods, beverages, and nutraceuticals.[5] An increasing demand for clean-label products that are naturally sourced, the focus on preventive healthcare, and greater consumer awareness of the gut-microbiome axis are the main factors driving this rise in popularity.[6] However, the commercialization of probiotics faces certain challenges in the food industry. Primarily, the stability and viability of probiotic organisms during processing, storage, and passage through the gastrointestinal tract are major concerns.[6,7,11]

The activity of probiotics can be negatively affected by exposure to oxygen, extreme temperatures, low pH, and digestive enzymes, which can significantly reduce the number of viable cells. Therefore, it remains technologically challenging to ensure that enough live probiotics reach the colon.[8,9] To address this, encapsulation processes have been extensively researched as a method to protect probiotic cells, enhance their survival in hostile environments, and enable controlled release at the site of action.[10] By encapsulating probiotic cells within a barrier, this technique preserves their biological activity without exposing them to damaging environmental factors.[8,11]

The search for suitable encapsulation materials has mainly focused on natural and biodegradable polymers. Among these, bacterial cellulose (BC) stands out as the most promising.[12] It is a biopolymer with unique structural and physicochemical features, which is also renewable, sustainable, and multifunctional.[13] Certain strains of *Komagataeibacter* and other acetic acid bacteria produce BC extracellularly.[14] BC differs from plant cellulose as it has high crystallinity, an ultra-fine nanofibrillar network, a high capacity for water retention, and strong mechanical properties. Additionally, it is free of lignin and hemicelluloses.[14,15] Its ideal applications are in the food, biomedical, and pharmaceutical industries due to its ability to be chemically modified, its non-toxicity, and biocompatibility.[16] Most importantly, BC has a three-dimensional porous structure that serves as an effective encapsulation matrix for protecting probiotic cells. Using BC-based encapsulation technology offers several sustainability benefits.[14-17] First, BC production aligns with bioeconomy principles, utilizing inexpensive substrates like agro-industrial waste for microbial fermentation.[18] Second, BC encapsulation improves consumer acceptance, reduces food loss, and extends the shelf life of probiotic-enriched foods.[19] Third, as a biodegradable and natural material, BC meets consumer and regulatory expectations for clean-label and eco-friendly foods.[20] Consequently, BC improves probiotic delivery and supports the broader sustainability goals of the food industry.[12]

For probiotic encapsulation, several strategies have been developed and engineered, beginning with conventional methods, such as electrospinning, spray drying, extrusion, and emulsification.[6] In terms of cost-effectiveness, scalability, cell viability, and encapsulation efficiency, all methods have advantages.[21] To tailor delivery systems, BC can either be applied *in situ*, where cells of probiotics are immobilized during BC biosynthesis, or *ex situ*, where cells are entrapped following synthesis.[22] The targeted release profile, food application, and industrial viability all determine the method and material choice. The applications of probiotics entrapped in BC are witnessing growing trends in the food industry.[23] Probiotics can be incorporated in almost all types of food items, such as dairy, non-dairy, bakery, beverages, and functional foods, owing to encapsulation, which also facilitates survival under adverse food matrices and intestinal conditions.[6,21] Encapsulation allows controlled release in the intestine to ensure that the highest number of viable cells reach the colon and are capable of conferring health benefits. This encourages innovation in the functional food industry and broadens the applications of probiotics beyond conventional fermented foods.[22,24] In addition, BC-based encapsulation systems can be applied to edible films and food coating packaging to improve food safety and quality and provide probiotics in a novel way.[25]

Overall, probiotic encapsulation with bacterial cellulose is a convergence of food technology, microbiology, and green biomaterials. In addition to addressing the serious issue of probiotic viability, it provides sustainable processes and applications with higher value in the food industry. This review discusses the basic aspects of probiotics and their application in sustainable food systems, together with the definition of bacterial cellulose as a possible encapsulation matrix and a summary of the various methods of probiotic encapsulation, with an outline of possible lines of future research and commercial opportunities.

PROBIOTICS - AS A FUNCTIONAL ORGANISM

Probiotics are the microbes that reside in the intestine and are beneficial to the host's health when consumed in appropriate amounts. The broad term for these protective microbes is gut microbiota, which was discovered in the late nineteenth century.[26] All probiotics are believed to be helpful to humans and have been shown to promote the health of both humans and animals.[27,28] Typically, probiotics act in the gastrointestinal tract, possibly influencing the intestinal microbiota. Many microbes colonize the human gastrointestinal tract.[27] Some key probiotic microorganisms, including lactic acid bacteria (LAB) species, are used in food bio-preservation because they can produce bacteriocins. These are substances capable of inhibiting the growth of spoilage and pathogenic bacteria.[29] Bacteriocins as small peptides produced by LAB ribosomes that are toxic to closely related bacterial strains. Probiotic bacteria are also referred to as friendly microorganisms because they exert beneficial effects on the 100 trillion microbiota already colonizing the mammalian intestine.[30] Without impairing the host's immune system, probiotic microbes are also known to enhance cytokine pathways, boost anti-inflammatory responses, and induce cell-mediated immune reactions.[31] Probiotic microbes have been used to treat lactose intolerance, food allergies, Crohn's disease, stimulate the immune system, reduce blood ammonia levels and blood cholesterol levels, exhibit antimutagenic activity, and alleviate symptoms of colon and diarrheal disorders.[29,32] Since LAB bacteria are gram-positive, non-sporeforming rods, non-aerobic, catalase-negative, cytochrome-deficient, aero-tolerant, acid-tolerant, strictly fermentative, and lactic acid producers, they are the most significant probiotic microorganism species.[33]

Typically, probiotics function in the gastrointestinal system, which can influence the gut microbiota. Various microorganisms have colonized the human gastrointestinal tract, but only a few have a significant impact on human health and disease through their influence on the intestinal microbiota.[27,34] The stability and activity of probiotics are considered essential to maximize their benefits.[28] Therefore, to effectively transfer probiotic effects to the consumer, microorganisms must be metabolically stable and active within the food product, survive in sufficiently high numbers through the digestive tract, and produce positive effects within the host's intestine.[31,35] This is important for public health, both in promoting intestinal health and in expanding the range of food products. Since the possible effects regarding liver disease, allergy, or AIDS are still under investigation, health claims of probiotics remain strain-specific or sometimes contradictory.[36] However, most reports highlight the potential of probiotics to address various clinical symptoms, such as diarrhoea, liver diseases, irritable bowel syndrome, immune response deficiencies, urinogenital disorders, gastroenteritis, infant allergies, respiratory and inflammatory bowel diseases, lactase deficiency, hyperlipidaemia, and even serum cholesterol levels.[26,28,36]

One of the most important parameters to fulfil the desired health effects of probiotics is the dosage level. In order to obtain probiotic activity within the human gut, the US FDA and the food industry suggest a level of 10^6 CFU mL^{-1} for probiotic foods at the level of consumption.[37] The demand for probiotic foods is growing at a very rapid rate due to awareness and interest among consumers. However, probiotic-producing bacteria must be able to colonize and sustain metabolic activities in the human intestine and withstand environmental exposure to be useful.[21,26,37]

PROBIOTIC ENCAPSULATION: A SUSTAINABLE SOLUTION FOR THE FOOD INDUSTRY

The food and agriculture industries continue to evolve due to innovation phenomena, which result in ongoing research and new technology. The quality of food is certainly guaranteed as a consequence of technological innovation because consumers' acceptances, needs, and tastes continually change. The evolution of technology used in the food industry would also be driven by consumers' cultural heritage, traditions, and even environmental issues.[38,39] Functional foods are increasingly marketed by manufacturers since consumers are more concerned and concerned about the nutritional quality of food these days. Therefore, to lead the market successfully, relying on added-value food functionalities and the food quality concept along the chain is required. One of the most important research areas for the future food market is the creation of probiotic food formulations.[39]

Probiotics have also shown promising results in the prevention as well as therapy of gastrointestinal conditions in numerous scientific research studies. There are almost 50 diverse genera of bacteria, some being hazardous or useful ones, which make up the complex bacterial fauna of the human gut, *i.e.*, the large intestine. The administration of probiotics promotes the development of a favourable gut microbiota, eliminates potentially harmful bacteria, and activates the body's resistance.[12,13] Probiotics regulate the overall health of human beings by normalizing the gut microbiota composition, boosting immunity, and balancing metabolic processes.[40] Therefore, the usage of functional food products as a source for probiotics is an ideal choice. One of the most notable features of probiotics is their capacity to synthesize chemicals with pharmaceutical or health-bestowing qualities, such as antibiotics or anticarcinogens.[41] However, when the manufacturing, processing, transportation, and storage affect the activity of bacteria. Probiotics are also affected by temperature, pH, and light.[39,41]

Cellulose, being an indigestible fiber and gastric juice-resistant, has established a niche in the food sector due to its beneficial mechanical features, biodegradability, biocompatibility, and renewability.[43] Cellulose-encapsulated probiotics show increased survival and resistance to unfavourable environmental conditions. Increased consumer knowledge leads to an immediate boost in the demand for probiotic functional foods, and most of the dairy sector makes use of probiotic cultures when producing functional foods.[38] Yogurt, a fermented milk product, ice cream, powdered milk, chocolates, and flavoured milk are some examples of the numerous foods that have recently been examined for probiotics.[38,42]

Encapsulation techniques

Probiotic particles are presently produced by employing a range of encapsulation processes. There is a requirement, however, that the method should ensure the encapsulated cell's viability and mechanical stability for the target application.[44] Various techniques are currently available for probiotic encapsulation, like freeze-drying, spray drying, extrusion, emulsion, electrospinning, and electro-spraying.[6] Figure 1 illustrates these encapsulation techniques.

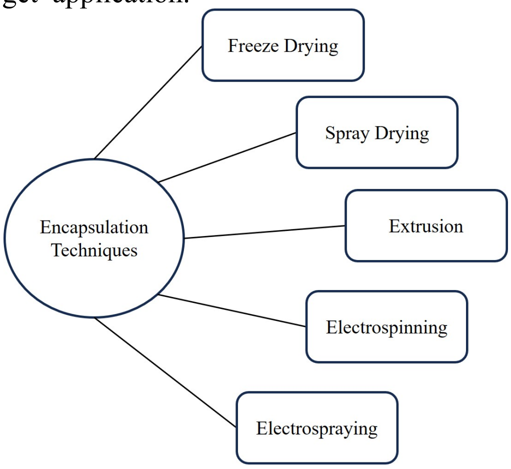

Figure 1: Techniques to encapsulate probiotics

Freeze drying

Freeze drying is a standard way of keeping probiotics in a dehydrated form for extended periods, but it is a very recent innovation to use freeze drying for encapsulation.[44] Probiotic cells are suspended in a liquid medium that is used for encapsulating, at very low temperatures, and then the water is extracted under vacuum as part of the freeze-drying process of microencapsulation. Cell viability is lost even though the processing conditions are milder, especially during freezing. The cooling rate controls cell inactivation during freezing, and the slowest cooling stage results in maximum loss of viability.[45] Because ice crystals are formed during the freezing stage, the cell wall can be subjected to excessive stress and breakage. The primary benefit of freeze drying is the prevention of oxidation and phase transition of the water phase.[46] Freeze-dried products, like vaccines and antibodies, can be readily and quickly reconstituted, making them especially useful in emergencies where prompt administration is required. Additionally, because there are fewer steps involved than with other microencapsulation techniques (such as coacervation, extraction of solvent, supercritical liquid precipitation, and others), this technique is easier to use. This is one of the most widespread storage approaches for storing probiotics.[44,47]

Figure 2 illustrates the process of freeze drying. Cryoprotectants can prevent alteration of the cellular organization during the process, which could be due to a reduction of water activity. To replace the loss of water content during the dehydration process is the role of the cryoprotectants (lactose, sucrose, glycerol, and trehalose). Apart from this, they limit molecular interactions and create a glassy matrix. Thus, for the storage of microcapsules that are freeze-dried, the glass transition temperature (Tg) for varying water content should be considered.[48]

The most important consideration in cell viability is storage temperature; probiotic microcapsules stored above the freezing point can accelerate metabolism and therefore decrease viability.[49] In a recent research, *Lactobacillus acidophilus* and *Lactobacillus casei* freeze-dried with encapsulating material remained viable for 30 days at 4 °C and 25 °C. The viable count of cells of sealed *L. casei* and *L. acidophilus* samples after 30 days at 4 °C was surprisingly greater than 8 CFU/mg.[44,48,49]

Spray drying

Spray drying is one of the most well-known pharmaceutical and food industry processes for the manufacturing of a large number of microcapsules, which are dried in a straightforward, continuous process. The chief benefits of this technique are particle size control and flowable powders that dry quickly.[49] It has been applied extensively by the food industry in the past 70 years as a food ingredient encapsulation process, such as probiotics, vitamins, and flavouring.[50] Figure 3 represents the process of spray drying in a spray dryer. Three steps that involve spray drying are:[49] (i) atomization, which produces droplets; (ii) mixing, which combines droplets with hot air, and water evaporates; and (iii) separation – removes the dried powders with the help of the cyclone separator.

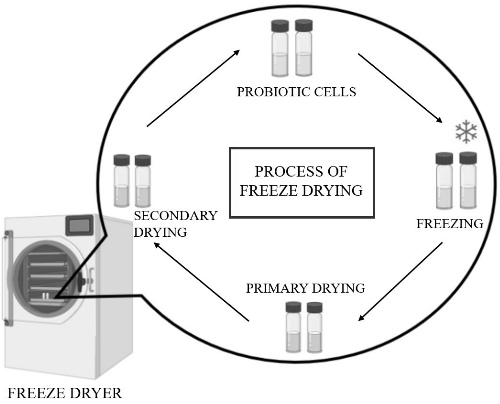

Figure 2: Schematic representation of the freeze-drying process

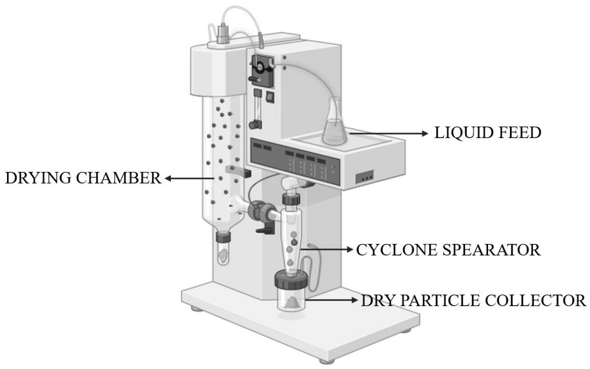

Figure 3: Spray drying process for microencapsulation of probiotics

The microencapsulation process begins with the making of a feed solution, in which probiotic cells are suspended with the encapsulating material. The resulting feed solution is atomized and spray dried to evaporate water content and create dried microcapsules of a size range 10–100 µm. Spray-dried products must have 4-7% moisture content for optimal storage stability.[50] Probiotic viability can be affected by the probiotic bacteria strain's resistance, encapsulating material, and working conditions used for spray drying encapsulation. The lethal killing of the final microbial cells in the hot, dry temperature is the biggest disadvantage of the process.[53]

Based on reports, air temperature, feed flow rate, atomization type, shear range at the time of atomization, mass, and heat transfer between the hot air and the droplets can all influence the survival rate in spray-drying. In addition, shell material formulations and spray-drying air temperature directly influence the bacterial cell viability during drying and preservation.[52] Spray-dried probiotics are very susceptible to damage to the bacterial cell membrane due to the concurrent dehydration and heat stress. Outlet temperature is responsible for cell viability during spray drying, as stated by several studies.[53] Factors like the flow rate of air, the temperature of the inlet, the flow rate of feed, and the concentration of feed solution regulate the outlet temperature. Droplet temperature and retention time of the droplets within the fatal temperature range can be directly accountable for the viability of microbes.[50,51] A higher outlet temperature in the spray-drying process instantly elevates the maximum droplet temperature and ensures that the droplets will spend the greatest time in their lethal range of temperatures. It can be fatal to treat mesophilic microorganisms at temperatures over 55 °C. The temperature of the air outlet could be quite indicative of the temperature of the particles within the drier, but it is not indicative of the overall temperature.[39,40]

In a recent study, researchers have utilized a cellulose-based polymer, hypromellose phthalate (HPMCP), for the microencapsulation of *Lactobacillus plantarum* using spray drying, to address challenges related to processing stress and gastrointestinal transit, resulting in the viability loss.[54] The pH-sensitive solubility of HPMCP causes it to aggregate in acidic conditions and dissolve at intestinal pH. To tackle this, the authors formulated different types of microcapsules: one using HPMCP only and another in combination with hypromellose. The addition of hypromellose is hypothesised to protect during spray drying due to its film-forming properties. Results demonstrated that cellulose-based formulations significantly improved probiotic survival during spray drying compared to free cells, with an increase in cell viability. The study also establishes that cellulose-based coatings provide exceptional protection under simulated gastrointestinal conditions and during storage.[53,54]

Extrusion

The most common and extensively employed process of creating hydrocolloid capsules (such as alginate and carrageenan) is by preparing a hydrocolloid solution, seeding the solution with microorganisms, and extruding the suspension produced from it using a syringe (in a lab) or an extruder (in a pilot plant), so that the droplets can freely fall into a solution, which contains encapsulating material (such as calcium chloride). Figure 4 represents the process of extrusion. The nozzle's diameter and the distance between the nozzle and the $CaCl_2$ control the measurement and shape of the pearl formed.[46] It is easy to work with and cost-effective. It yields high cell viability and is nontoxic to cells. Both aerobic and anaerobic environments can be used with this technology, which is devoid of any dangerous solvents. The only limitation of this process is that, because microspheres develop slowly, it is not possible to use it in mass production.[53]

Extrusion involves forcing a material through an orifice whose diameter keeps changing at an even rate in different conditions of high and low temperature, humidity, and velocity to obtain a variety of products as per manufacturer specifications or on market demand.[55] Extrusion is capable of making a variety of products, most of which are difficult to make through other processes. Because most of the bioactive compounds are thermolabile, the only disadvantage of the technology is that the process of extrusion is carried out employing elevated temperatures (80-150 °C) for a short duration of time (one minute).[56] As it can be operated in low-cost production and high capacity in a continuous process, the extrusion process of food ingredient processing is now one of the most significant manufacturing methods.[55] The low-moisture process does not generate large-scale effluents, and the extrusion method is eco-friendly. Thus, there is a low extent of environmental pollution and water consumption.[55,56]

An extruder consists in a single or double rotating screw fitting snugly into a barrel with the die at one end.[57] All extruders are founded on the same principle, which is to feed raw materials into the barrel using a feeder and then push them with the help of screws in the direction of the die and cutter. More specialized extruders are constituted of a system for preconditioning, feeding and cutting, screws, barrel, and die. There are five extrusion technologies:[55,56] melt injection, hot-melt extrusion, electrostatic/electrospinning, centrifugal/co-extrusion, and particles from gas saturated solution (PGSS) process. Melt injection extrusion is a relatively low-temperature extrusion based on entrapment of biologically active compounds in melted encapsulating materials as an encapsulation approach.[58] In centrifugal extrusion, biologically active material is pushed out of orifices into the encapsulating material surrounding the rotating cylinder using centrifugal force. A system employing an arrangement of needles and electrostatic charges is referred to as electrostatic extrusion.[56]

For bacterial cells to be encapsulated by extrusion, various polysaccharides can be used, such as alginate, chitosan, and bacterial cellulose. The concentration of a polysaccharide influences both the shape and size of the produced beads and the protection of encapsulated cells.[55,58] The technique is widely used to develop particles containing natural polysaccharides, like alginate and bacterial cellulose, which extend the viability of encapsulated cells under different storage conditions.[56] In a recent study, natural polysaccharides found in linseed and okra mucilage, Botryosphaeria (an exopolysaccharide produced by the endophytic fungus *Botryosphaeria rhodina* MAMB-05), were combined with alginate to encapsulate strains of *Lactobacillus casei 01* and BGP 93 using an extrusion technique. During the fifteen days of refrigerated storage, the produced beads proved a protective effect on the viability of encapsulated probiotics. Moreover, resistance towards microorganisms was diminished by extruding particles with prebiotics. Despite the potential of the technique, it still needs modifications to address the challenges for larger-scale applications.[6]

Emulsion

An emulsion is a colloidal system, which is made up of more than two immiscible liquids, such as water and oil, with one liquid dispersing into another.[58] Probiotic cells can be encapsulated in emulsions. Two types of emulsions are mostly used: oil-in-water (O/W) and water-in-oil (W/O).[59] Figure 5 depicts the schematic representation of an emulsion. It can be produced by mechanical or chemical techniques. The concentration of the emulsifier and the hydrophilic-lipophilic balance (HLB) value are applied in the chemical methods.

High-shear high-pressure emulsifiers are used in the physical methods.[60] Nano and micro-emulsions can be attained using a microfluidizer, while small particles are created through a homogenizer. Often, physical-chemical techniques are used to produce these types of emulsions.[61]

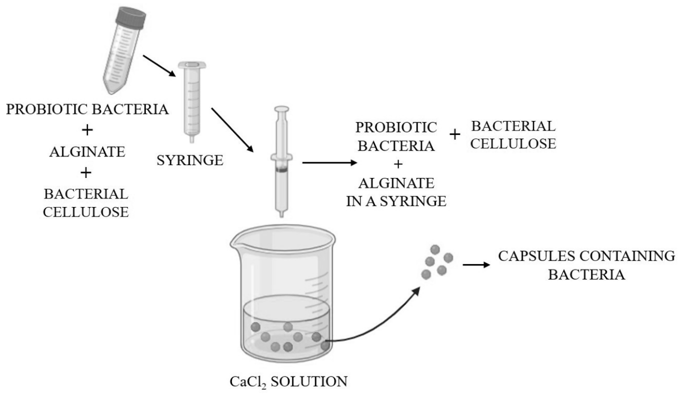

Figure 4: Extrusion process for encapsulation of probiotics

A water-in-oil-in-water emulsion can be created by emulsifying a water-in-oil emulsion in water. This type of emulsion is often called "multiple emulsion" or "double emulsion". There are usually two steps that involve making a W/O/W emulsion. A hydrophilic emulsifier is added to an aqueous solution under lower shear and re-dispersed after making up a stable W/O emulsion under high shear. In such a scenario, after high shear, the water droplets coalesce or burst into the oil phase.[60,61] Shortly, membrane and microfluidic emulsification need to be considered. In membrane emulsification, the targeted emulsion's dispersed phase is introduced into the continuous phase by passing it through a porous membrane. Unique micro- or nanodroplets are formed from each pore when the membrane and process conditions are controlled precisely, resulting in very monodispersed emulsions with, in essence, an extremely high-throughput continuous process.[59,61] Microfluidic emulsification utilizes the microchannels' junction in different arrangements to generate dispersed-phase droplets in a carrier phase. Whith T-junctions, co-flow geometries are the major shapes for that purpose.[59-61]

In a recent work, authors introduced a novel method for creating biodegradable microcapsules by using the ability of *Gluconacetobacter xylinus* to produce natural biofilm (bacterial cellulose). This approach uses an emulsion templating strategy, where bacteria are inoculated in water-in-oil droplets. The aerobic bacteria need oxygen, which allows them to self-assemble at the water-oil interface, where they synthesize and secrete cellulose nanofibers, which form a dense network that encapsulates the droplets' content. This process allows the formation of the cellulose based matrix, which offers a protective layer that could be an alternative to synthetic polymer capsules. Though this study focuses on the fundamental mechanics of capsule formation, this approach could be investigated for probiotic encapsulation. The method directly addresses the key challenges, such as protecting sensitive bioactive cargoes like probiotic cells, during storage or gastrointestinal transit. The resulting capsule is composed solely of bacterial cellulose, a material known for its biocompatibility, mechanical strength, *etc*., making it an ideal shell for targeted delivery. Authors successfully demonstrated the encapsulation of cellulose-producing bacteria within a self-generated shell.[62]

Electrospraying and electrospinning

Electrospraying and electrospinning are categorized under electrohydrodynamic techniques, which are different from each other. In most cases, the needle, with the help of a syringe pump, extrudes the polymer solution. Figure 6 represents the electrospinning and electrospraying setups. When a drop of the polymer solution appears from the nozzle, the surface tension and electrical repulsion forces become balanced, which results in the formation of a Taylor cone at the needle tip. As the surface tension and the viscoelastic forces are overcome by repulsive forces, jets erupt from this cone, carrying the projected material droplets toward the collector.[64] Most of the studies conducted so far have classified these techniques inaccurately. They can be easily differentiated according to the collector used. The collector can be either a liquid bath (wet electrospraying) or a hard plate (dry electrospraying).

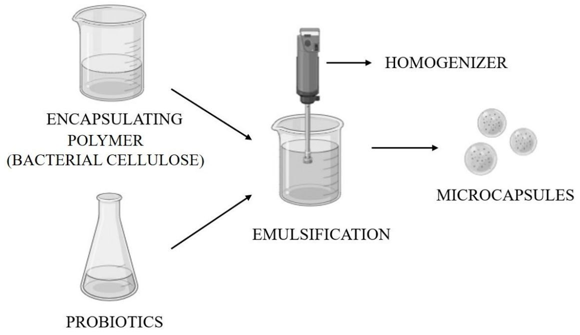

Figure 5: Schematic representation of an emulsion

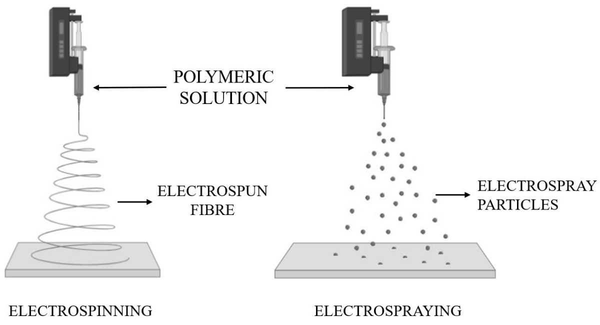

Figure 6: Schematic representation of electrospraying and electrospinning setups for probiotic encapsulation

Depending on the type of nozzle used, electrospinning and electrospraying can be further divided into three types: uniaxial, coaxial, and triaxial.[65]

Even though they are called “sister” technologies, electrospinning and electrospraying have many differences. In electrospinning, a high voltage inverts the free charges in the polymeric solution at the capillary. Coulombic force of the external electric field and the electrostatic repulsion of like charges deform the drop’s hemispherical surface to give it the shape of a cone, which is defined as a Taylor cone. The tip of the Taylor cone emits the charged polymer jet when surface tension equals the electrostatic force.[64] The jet bends or whips due to the net charge acting in the direction toward the collector. With that, the jet extends out while the solvent evaporates almost instantaneously to leave behind a thin, solid fiber that is oriented randomly and non-woven on the grounded collector. In contrast, electrospraying is a process in which the liquid is atomized utilizing an electric force. The concentration of the polymer solution governs how these methods differ. In fact, at high concentrations, the jet elongation that arises from the Taylor cone is stabilized, while whipping instability, being the elongation mechanism, comes into play.[66] At lower concentrations, a situation arises where varicose instability acts to completely destabilize the jet, which results in the formation of fine drops. As the electrically charged drops disperse themselves in space, coagulation and droplet agglomeration are avoided.[65]

These techniques have wide application domains in areas such as electronics, drug delivery systems, mass spectrometry, nanodevices, tissue engineering, textiles, and filtration. However, there hasn’t been much research done on its application in the food industry. The research published confirms the potential of these techniques in the encapsulation of biologically active food substances and enzymes. Moreover, using these techniques, purposeful materials for active food packaging can be manufactured.[67] The application of electrospun fibers for filtration membranes is another area of low research, but one that has potential use in the processing of food and beverages.[64-67]

In a recent study, authors, while addressing the challenges of maintaining cell viability during storage, explored the potential of bacterial cellulose nanofibers (BCNF) as a carrier material for probiotic immobilization. Pure bacterial cellulose was unable to be electrospun on its own because of the consistent formation of a beaded structure rather than a fibre. To overcome this, authors fabricated a composite by blending 5% of bacterial cellulose solution with an equal proportion of PVA (polyvinyl alcohol), using TFA (trifluoroacetic acid) as a common solvent. The resulting composite exhibited the desired characteristic for a delivery system, including the diameter of 576 nm, high thermal stability and a porous structure. The FTIR analysis confirmed complete evaporation of the TFA solvent, eliminating toxicity concerns and confirming its safety for food and pharmaceutical applications.

The study also demonstrated the efficacy of BCNF for immobilizing *Lactobacillus acidophilus* 016 onto the fibre mat, and the TFA solvent makes it possible for bacteria to be directly encapsulated within fibres during the spinning. The nanofibrous mat served as a supportive scaffold for surface immobilization. The composite maintained a high viable cell count. This makes BC-PVA composite nanofibers a promising and practical platform for developing long shelf life and stable probiotic formulations.[68]

APPLICATIONS OF PROBIOTICS

Encapsulated probiotics in food packaging

To extend food shelf life and ensure safety, packaging must prevent microbial, physical, or chemical contamination.[69] Traditional packaging materials are petroleum-based plastics that have caused serious environmental issues because they are not biodegradable and can release harmful chemicals during recycling. Conversely, cellulose-based products have gained significant interest in the food packaging industry due to their low cost, biodegradability, and eco-friendliness. Since probiotics and their derivatives exhibit strong antimicrobial activity, packaging can be made bioactive by encapsulating them in cellulose-based films.[70,71] In a recent study, to prevent *Aspergillus niger* growth on cheese, a probiotic bacterial cellulose film, with either free or microencapsulated *Bifidobacterium animalis* or *Lactobacillus acidophilus*, was developed.[70] The research showed that microencapsulated probiotic bacterial cellulose films, with high probiotic viability, effectively prevented *Aspergillus niger* growth in cheese. The results indicated that probiotics microencapsulated with sodium alginate within bacterial cellulose films maintained high viability and were most effective at inhibiting fungal growth. Additionally, prebiotics could be incorporated into cellulose-based probiotic films to further improve probiotic survival. Meanwhile, in another study, a carboxymethyl cellulose (CMC) film was used to encapsulate probiotics (*L. plantarum*) for protecting chicken tenders, offering a new method to extend shelf life. In this system, the probiotics showed a 36% increase in survival when inulin was added to the film.[72] Research has shown that, to keep chicken fillets fresh, innovative probiotic cellulose-based packaging films can be used.[73] These films combined probiotics (*B. coagulans and L. casei*) with gelatin, nanocellulose, and nanochitosan. Also, findings revealed that the nanocomposite probiotic film could prolong the shelf life of frozen fish fillets by preventing the growth of *Listeria monocytogenes*. Synbiotics, which combine probiotics and prebiotics, are also increasingly popular for their enhanced health benefits.[74]

Generally, probiotic films based on cellulose could serve as a new platform for bioactive packaged foods, providing protection for probiotics against harmful processing conditions and improving their stability.[75] Although extensive basic research on cellulose-based probiotic encapsulation has been reported, translating these probiotic-containing cellulose films from the lab to food applications currently seems challenging. This difficulty stems from the complex technology required to produce cellulose-based probiotic films, which leads to higher production costs and, consequently, increased costs for packaged foods.[25,75] For example, large-scale production of electrospun probiotic films involves significant investments in expensive advanced equipment that also requires future maintenance. This substantially raises production costs, making industrial applications less desirable.[75,76] In summary, before cellulose-based probiotic films can be practically implemented, further development of suitable and low-cost cellulose preparation techniques, effective encapsulation methods using cellulose or cellulose materials, and new probiotic strains is necessary.[25]

Encapsulated probiotics in dairy products

Fermented dairy products contain live organisms, which are responsible for the fermentation process. The survival of probiotic strains, such as *Lactobacillus casei* or *Lactobacillus acidophilus*, during product shelf life and gastrointestinal transit measures the specific therapeutic efficacy of the strain. Though the dairy matrix offers protection to the strain, the intrinsic acidity of fermented products and digestive stresses reduce the viability of the probiotics. Therefore, the encapsulation of probiotics is necessary, so that probiotics can reach the gut in sufficient quantities to confer health benefits, without compromising the quality of the food.[77] It has been emphasized that to overcome consumption limitations by consumers due to possible intolerances or allergies to compounds present in cow's milk, raw materials with high digestibility, such as goat's milk, can be an alternative to develop dairy foods containing probiotics.[75] In general, dairy foods are acidic and, according to the pH of the medium, they may not be the ideal environment for the stability of probiotic microorganisms. Thus, encapsulation techniques can be used to improve the survival of encapsulated bacteria exposed to stress conditions, ensuring that they fulfil their purpose.[75,78]

A study has reported the production and refrigerated storage of fermented milks containing *Lactobacillus casei* ATCC 393 encapsulated in alginate by the extrusion technique. The encapsulated cells showed greater cell viability than the free cells after 28 days of refrigerated storage.[80] Encapsulation also improved the survival of the encapsulated probiotics in ice cream, as compared to non-encapsulated cells, under cold storage and passage through the simulated gastrointestinal tract. Another study investigated the production of fermented milk with probiotics encapsulated by spray drying.[80] The cell viability of the probiotics was improved during the production, and the addition of encapsulated cells to the product did not influence its sensory aspects.[25] However, the use of particles containing probiotic bacteria produced by spray drying in foods with high moisture and water activity is complex, since the number of materials available to be used as an encapsulating agent is limited and present solubility in water, allowing the entrapped cells present in the particles to easily migrate to the product under humid conditions.[81]

Encapsulated probiotics in the biomedical field

Biopolymer-encapsulated probiotics give a significant advantage to biomedical applications. These encapsulated systems utilize biocompatible polymers, such as bacterial cellulose, alginate, and chitosan, to create a protective matrix that protects probiotic strains from the harsh conditions during the GI transit.[81,82] The layer-by-layer assembling of the biopolymer onto the bacterial surface enhances the probiotic resistance to oxygen, gastric acids and other digestive enzymes. The technology has demonstrated remarkable benefits in preclinical models where the viability of the probiotics has been increased significantly. Furthermore, these systems can be engineered to provide controlled and targeted release in specific regions of the gut, such as the colon, which is often the desired site of action for treating inflammatory bowel conditions and modulating the gut microbiome.[82]

Fabricating a biopolymer system incorporating probiotics offers an improvement in the performance of probiotics during storage and the gastrointestinal transit, which also enhances the mucosa adhesive properties. The novel method for creating biodegradable microcapsules by using the ability of *Gluconacetobacter xylinus* to produce natural biofilm (bacterial cellulose) could provide a protective layer for the encapsulation of probiotics.[62] The use of biopolymer coated probiotic microcapsules, containing different strains of probiotics, has demonstrated health benefits, including cardiovascular health by reducing the cholesterol level, and certain benefits to the digestive health and metabolic health, which could also contribute to faster wound healing.[83] The microencapsulation of *Ligilactobacillus salivarius* Li01 serves to maintain probiotic viability, also being a good option for treating bowel diseases. The encapsulation of biopolymer and probiotic cells also showed a positive result in inhibiting urogenital pathogens. Furthermore, chitosan-based encapsulation systems exhibit prebiotic effects by promoting beneficial bacteria, while suppressing pathogens, and demonstrate immunomodulatory activity relevant to inflammatory bowel disease and autoimmune conditions through specific immune signalling pathways. Controlling the release of probiotics in the urogenital tract offers benefits to human health.[81,84]

CONCLUSION

The growing interest in probiotics in foods has led to the creation of new methods of safeguarding their stability, life, and delivery. The microorganisms known as probiotics must be protected against oxygen, heat, moisture, and the conditions of the digestive system. Thus, encapsulation is important from the time of manufacturing to the time of consumption of the products. Emulsification is simple and inexpensive to use. Spray drying is the most widespread method of industrial production, although the method is damaging because of the high temperatures used. Freeze drying is the best with the use of cryoprotectants in preserving the viability of the microorganism. Extrusion is versatile, especially with alginate, enabling protection in the digestive system. Electrospinning is a new encapsulation technique that allows tailored release, but is limited by the use of solvents and regulatory issues.

The selection of biopolymers, such as alginate, starch, gelatin, pectin, and cellulose derivatives, has a direct impact on the encapsulation efficacy. It affects the product's stability, the degree to which consumers accept it, and also determines how well the product adheres to sustainability principles. The delivery of probiotics can be made more advanced and precise through new formulations, such as multi-layer coatings, symbiotic systems, and stimuli-responsive designs. Issues continue to persist, and they include viability throughout processing, achieving targeted release, and masking undesirable flavours, as well as consumer compliance. From the commercial perspective, food compatibility, cost, and scale are primary concerns. Taken together, microencapsulation represents an essential technology to enable probiotic foods, and there's reason to believe that developments in materials and hybrid systems will define functional foods in the years to come.

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