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# Advances in bio-polymer coatings for probiotic microencapsulation: chitosan and beyond for enhanced stability and controlled release

Great Iruoghene Edo[a,b,c], Alice Njolke Mafe[d], Nawar. F. Razooqi[b], Ebuka Chukwuma Umelo[e], Tayser Sumer Gaaz[f], Endurance Fegor Isoje[g], Ufuoma Augustina Igbuku[a], Patrick Othuke Akpoghelie[h], Rapheal Ajiri Opiti[c], Arthur Efeoghene Athan Essaghah[i], Dina S. Ahmed[j], Huzaifa Umar[k] and Dilber Uzun Ozsahin[k,l,m]

[a]Department of Chemistry, Faculty of Science, Delta State University of Science and Technology, Ozoro, Nigeria; [b]Department of Chemistry, College of Sciences, Al-Nahrain University, Baghdad, Iraq; [c]Department of Petroleum Chemistry, Faculty of Science, Delta State University of Science and Technology, Ozoro, Nigeria; [d]Department of Biological Sciences, Faculty of Science, Taraba State University Jalingo, Taraba State, Nigeria; [e]Department of Healthcare Organisation Management, Cyprus International University, Nicosia, Turkey; [f]Department of Prosthetics and Orthotics Engineering, College of Engineering and Technologies, Al-Mustaqbal University, Babylon, Iraq; [g]Department of Science Laboratory Technology (Biochemistry Option), Faculty of Science, Delta State University of Science and Technology, Ozoro, Nigeria; [h]Department of Food Science and Technology, Faculty of Science, Delta State University of Science and Technology, Ozoro, Nigeria; [i]Department of Urban and Regional Planning, Faculty of Environmental Sciences, Delta State University of Science and Technology, Ozoro, Nigeria; [j]Department of Chemical Industries, Institute of Technology-Baghdad, Middle Technical University, Baghdad, Iraq; [k]Operational Research Centre in Healthcare, Near East University, Nicosia, Cyprus; [l]Department of Medical Diagnostic Imaging, College of Health Sciences, University of Sharjah, United Arab Emirates; [m]Research Institute for Medical and Health Sciences, University of Sharjah, United Arab Emirates

**ABSTRACT**

This review paper analyzes recent advancements in bio-polymer coatings for probiotic microencapsulation, with a particular emphasis on chitosan and its synergistic combinations with other materials. Probiotic microencapsulation is essential for protecting probiotics from environmental stresses, enhancing their stability, and ensuring effective delivery to the gut. The review begins with an overview of probiotic microencapsulation, highlighting its significance in safeguarding probiotics through processing, storage, and gastrointestinal transit. Advances in chitosan-based encapsulation are explored, including the integration of chitosan with other bio-polymers such as alginate, gelatin, and pectin, as well as the application of nanotechnology and innovative encapsulation techniques like spray drying and layer-by-layer assembly. Detailed mechanistic insights are integrated, illustrating how chitosan influences gut microbiota by promoting beneficial bacteria and suppressing pathogens, thus enhancing its role as a prebiotic or synbiotic. Furthermore, the review delves into chitosan's immunomodulatory effects, particularly in the context of inflammatory bowel disease (IBD) and autoimmune diseases, describing the immune signaling pathways influenced by chitosan and linking gut microbiota changes to improvements in systemic immunity. Recent clinical trials and human studies assessing the efficacy of chitosan-coated probiotics are presented, alongside a discussion of practical applications and a comparison of in vitro and in vivo findings to highlight real-world relevance. The sustainability of chitosan sources and their environmental impact are addressed, along with the novel concept of chitosan's role in the gut-brain axis. Finally, the review emphasizes future research needs, including the development of personalized probiotic therapies and the exploration of novel bio-polymers and encapsulation techniques.

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## 1. Introduction

Probiotic microencapsulation is a technique designed to protect live probiotic microorganisms by enclosing them within a protective coating, typically made from biocompatible materials like polymers [1]. The primary purpose of this method is to shield probiotics from environmental stresses such as oxygen, temperature changes, moisture, and the acidic conditions of the stomach [2]. When used in probiotic formulations, chitosan's antimicrobial activity selectively inhibits harmful pathogens while potentially affecting beneficial microbiota, necessitating careful formulation to maintain microbial balance [3]. By encapsulating probiotics in micro-sized carriers, their viability is preserved throughout the production process, storage, and eventual delivery into the gastrointestinal tract, where they can contribute to gut health [4].

The use of microencapsulation is essential in maintaining the viability and functionality of probiotics for several

**CONTACT** Great Iruoghene Edo greatiruo@gmail.com Department of Chemistry, Faculty of Science, Delta State University of Science and Technology, Ozoro, Nigeria

reasons [5]. During production, probiotics are often exposed to heat, pressure, and other mechanical stresses that can damage them [6]. Microencapsulation acts as a protective barrier, helping probiotics survive these harsh conditions. Also, probiotics are prone to degradation during storage when exposed to air, light, or moisture [7]. Strategies such as chemical modifications, blending with other biopolymers, and the development of chitosan derivatives with enhanced acid resistance are being actively researched to overcome its limitations in highly acidic conditions [8]. Encapsulation extends their shelf life by forming a physical shield that prevents such exposure. The most critical challenge, however, is ensuring probiotics survive the acidic and enzymatic environment of the stomach and small intestine [9]. Microencapsulation protects probiotics from stomach acid (pH 2.0) and bile salts, enhancing their ability to survive and reach the intestines, where they can colonize and exert their beneficial effects [5]. Thus, microencapsulation plays a vital role in ensuring probiotics remains stable and effective from production to consumption [10].

Bio-polymer coatings, which can be natural or synthetic polymers, are biocompatible, biodegradable, and suitable for encapsulating probiotics in a protective matrix [9]. These coatings are chosen for their ability to form stable structures around probiotic cells, protecting them and allowing for controlled release in specific areas of the gastrointestinal tract [11]. Bio-polymers used in encapsulation are generally recognized as safe (GRAS) and are widely applied in the food, pharmaceutical, and nutraceutical industries [12]. The key properties that make bio-polymers ideal for encapsulation include biocompatibility, ensuring they are safe for consumption, and biodegradability, allowing them to break down into non-toxic substances after their protective role is fulfilled [13]. Additionally, bio-polymers are engineered to enable controlled release, ensuring probiotics are delivered to the target site in the digestive system [14].

Chitosan, a natural polymer derived from the shells of crustaceans such as shrimp and crabs, is one of the most commonly used materials in probiotic microencapsulation [15]. As a polysaccharide composed of glucosamine and N-acetylglucosamine units, it offers several properties that make it ideal for encapsulation [16]. Chitosan is biocompatible and biodegradable, meaning it can be safely broken down by the body into simple sugars [17]. Its mucoadhesive properties allow it to adhere to the mucosal surfaces in the gut, improving the retention and colonization of probiotics [18]. Moreover, chitosan has antimicrobial activity, helping maintain probiotic viability by reducing the risk of contamination during processing and storage [19]. Its pH sensitivity is another advantage; chitosan is soluble in acidic environments, making it suitable for protecting probiotics in the stomach, and it forms a gel in more neutral pH levels, allowing for targeted release in the intestines [20]. Despite its advantages, research continues to address chitosan's limitations, such as its instability in highly acidic conditions and limited solubility in neutral or alkaline environments [21].

In recent years, there has been a growing recognition of the multifaceted roles of chitosan in probiotic microencapsulation, extending beyond mere protective barriers to encompass significant mechanistic insights into its influence on gut microbiota and immune system modulation [22]. This review aims to examine recent advancements in bio-polymer coatings for probiotic microencapsulation, focusing particularly on chitosan and its applications, as well as other biopolymers such as alginate, pectin, and gelatin. We will explore how chitosan promotes beneficial bacterial populations while suppressing pathogenic strains, thereby enhancing gut health through its prebiotic and synbiotic properties. Recent studies highlighting the synergistic effects of chitosan with specific probiotics will be examined, particularly in the context of inflammatory bowel disease (IBD) and autoimmune disorders, where chitosan's immunomodulatory effects and associated immune signaling pathways are crucial. Long-term use of chitosan-based products potentially causes side effects such as gastrointestinal discomfort, nutrient malabsorption (particularly fat-soluble vitamins and minerals), or allergic reactions in sensitive individuals, emphasizing the need for careful dosage and monitoring [23]. In addition, we will present innovations in chitosan-based encapsulation techniques, mechanisms of controlled release, and their importance in improving probiotic efficacy. Updated findings from clinical trials and human studies will validate the efficacy of chitosan-coated probiotics, emphasizing their practical applications in the food and pharmaceutical industries. This review will also address the sustainability of chitosan sources and their environmental impacts while introducing the novel concept of chitosan's role in the gut-brain axis, linking microbial interactions to mental health outcomes. Chitosan encapsulation improves the long-term viability of probiotics by shielding them from environmental stressors, with its effectiveness influenced by variables such as the concentration of chitosan and the conditions under which they are stored [24]. Finally, we will identify future research directions, emphasizing the need for personalized probiotic therapies and the exploration of innovative bio-polymers and encapsulation techniques to enhance the functionality of probiotic formulations, ultimately meeting the growing demand for probiotic-based functional foods and therapeutics.

## 2. Chitosan as a bio-polymer coating

### 2.1. Properties of chitosan

Chitosan, a biopolymer derived from the deacetylation of chitin (a structural polysaccharide found in the exoskeletons of crustaceans), possesses several key physicochemical properties that make it highly suitable for probiotic encapsulation [25] (Table 1).

### 2.2. Applications of chitosan in probiotic encapsulation

Specific conditions for chitosan's effectiveness in promoting beneficial gut microbiota include low molecular weight and concentrations typically ranging from 0.1% to 1%, which balance antimicrobial activity against pathogens while preserving probiotic viability [3].

### 2.3. Mechanistic insights into chitosan's role in gut microbiota modulation

Chitosan, beyond its utility as an encapsulating agent, plays a pivotal role in modulating the gut microbiota. Its influence extends to promoting the growth of beneficial bacteria while simultaneously suppressing pathogenic microorganisms. This dual functionality is achieved through a combination of physicochemical interactions, metabolic modulation, and immunomodulatory effects [38]. Understanding these mechanisms is crucial for optimizing chitosan-based probiotic formulations aimed at enhancing gut health and overall immune function.

#### 2.3.1. Promotion of beneficial bacteria

Chitosan acts as a prebiotic, selectively fostering the growth of beneficial gut microbiota such as *Bifidobacteria* and *Lactobacillus*. One of the primary mechanisms through which chitosan promotes these beneficial bacteria is selective fermentation and utilization. Certain beneficial bacteria possess the enzymatic machinery required to degrade chitosan [39]. Enzymes such as chitosanases, produced by *Bifidobacteria* and *Lactobacillus*, hydrolyze chitosan into smaller oligomers and monomers, which these bacteria can utilize as carbon sources and amino acids or ammonium as nitrogen sources [40]. This selective utilization provides a competitive advantage to these beneficial strains over less advantageous or pathogenic bacteria that lack such enzymatic capabilities. Potential side effects or contraindications associated with the use of chitosan as a prebiotic encapsulating agent include gastrointestinal issues such as bloating, constipation, or diarrhea, and it may interfere with the absorption of fat-soluble vitamins and certain medications, particularly in individuals with shellfish allergies [41]. More so, the degradation products of chitosan serve as an energy source, enhancing the growth and metabolic activity of beneficial bacteria. The increased availability of nutrients supports their proliferation and metabolic functions, contributing to a balanced and diverse gut microbiota [42].

Another significant mechanism is the enhancement of short-chain fatty acid (SCFA) production. The fermentation of chitosan by beneficial bacteria leads to the increased production of SCFAs, including acetate, propionate, and butyrate. These SCFAs play multiple roles in gut health. Butyrate, in particular, is a primary energy source for colonocytes, promoting intestinal

**Table 1.** Physicochemical properties of chitosan.

| S/N | Properties | Description |
|---|---|---|
| 1. | Biocompatibility | Chitosan is recognized for its biocompatibility, meaning it is generally well-tolerated by the human body and does not elicit significant adverse immune responses. This property is crucial for ensuring that chitosan-based encapsulation materials are safe for consumption and suitable for use in food and pharmaceutical applications [26]. |
| 2. | Biodegradability | Chitosan is biodegradable, breaking down into non-toxic components that can be metabolized or eliminated by the body. This characteristic ensures that, after serving its purpose as a protective coating, chitosan decomposes into substances that do not accumulate in the body, minimizing potential long-term effects [27]. |
| 3. | Antimicrobial Properties | Chitosan exhibits intrinsic antimicrobial activity, which is effective against a range of microorganisms, including bacteria and fungi. This antimicrobial property helps preserve the viability of probiotics during storage and processing by reducing the risk of contamination and spoilage [3]. |
| 4. | Film-Forming Ability | One of chitosan's notable attributes is its ability to form strong and flexible films. When dissolved in an acidic medium, chitosan forms a gel-like network that can be used to create a thin, continuous film. This film can encapsulate probiotic microorganisms, providing a protective barrier that shields them from environmental stresses [28]. |
| 5. | Mucoadhesive Properties | Chitosan has mucoadhesive properties, meaning it can adhere to mucosal surfaces in the gastrointestinal tract. This characteristic is beneficial for enhancing the retention of encapsulated probiotics in the intestines, improving their chances of colonization and effectiveness [29]. Specific strains like *Lactobacillus plantarum, Bifidobacterium bifidum*, and *Saccharomyces cerevisiae var. boulardii* have shown significant benefits from chitosan coating due to enhanced protection against gastrointestinal conditions and improved survival rates during storage and delivery [30]. |
| 6. | pH Sensitivity | Chitosan is soluble in acidic environments but forms a gel or solidifies at higher pH levels. This pH sensitivity is advantageous for probiotic encapsulation because it allows chitosan to dissolve and protect probiotics in the acidic environment of the stomach while transitioning to a gel state in the more neutral pH of the intestines, where the probiotics are intended to be released [31]. |

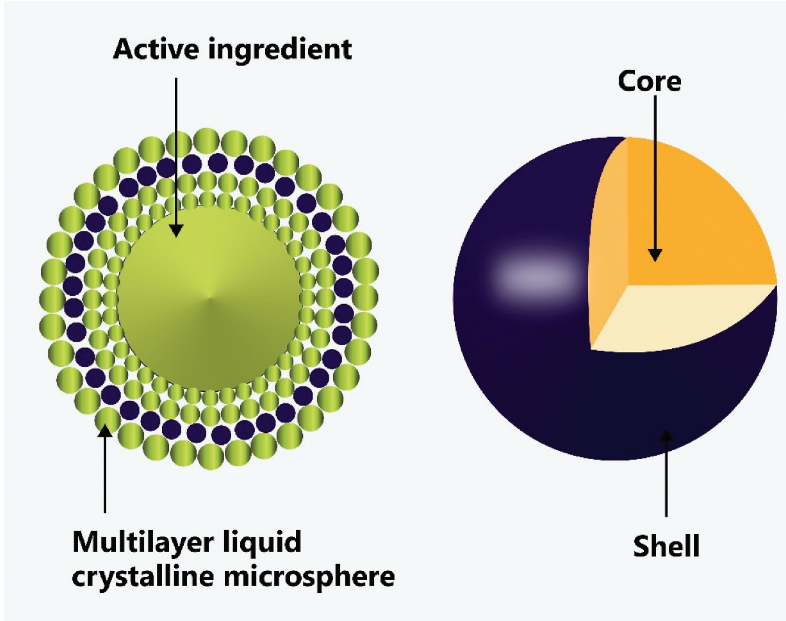


**Figure 1.** Chitosan.

**Table 2.** Current studies and applications.

| S/N | Applications | Description |
|---|---|---|
| 1. | Protection Against Stomach Acid and Bile Salts | Numerous studies have demonstrated chitosan's effectiveness in protecting probiotics from the harsh conditions of the gastrointestinal tract. Chitosan-coated probiotics have shown enhanced survival rates in acidic environments, such as the stomach, compared to non-coated probiotics. The chitosan coating acts as a physical barrier that shields probiotics from stomach acid and bile salts, increasing their viability as they pass through the digestive system [32]. The time for chitosan to exert noticeable effects on gut health typically ranges from a few days to several weeks, depending on factors such as dosage, formulation, individual gut microbiota composition, and overall health status [33]. |
| 2. | Controlled Release Mechanisms | Research has shown that chitosan can be used to develop controlled-release systems for probiotics. By forming a gel-like matrix in response to pH changes, such as transitioning from the acidic environment of the stomach (pH 2) to the more neutral conditions of the intestine (pH 6–7), chitosan enables the gradual release of probiotics in specific regions of the gastrointestinal tract. This controlled release helps ensure that a higher number of probiotics reach the intestines, where they can exert their beneficial effects [34]. |
| 3. | Encapsulation Techniques | Chitosan has been applied in various encapsulation techniques, including spray drying, extrusion, and electrostatic assembly. These methods utilize chitosan's film-forming and gelation properties to create microencapsulated probiotic formulations that can withstand processing and storage conditions [35]. |
| 4. | Synergistic Combinations | Studies have explored the combination of chitosan with other biopolymers, such as alginate and pectin, to enhance encapsulation performance. These combinations leverage the unique properties of each polymer, such as chitosan, alginate, and pectin, to improve the stability, release profile, and overall efficacy of probiotics in capsules, gels, or coated tablets [36]. Potential side effects or contraindications associated with chitosan as a prebiotic encapsulating agent include gastrointestinal discomfort, such as bloating or constipation, and possible allergic reactions, especially in individuals sensitive to shellfish [37]. |

barrier integrity and reducing inflammation [43]. Moreover, SCFAs lower the intestinal pH, creating an unfavorable environment for pathogenic bacteria while favoring the growth of acid-tolerant beneficial strains. SCFAs also influence immune cell function and cytokine production, contributing to an anti-inflammatory environment in the gastrointestinal tract or digestive system [44].

Chitosan further enhances gut barrier function by improving tight junction integrity and mucus production. SCFAs, especially butyrate, strengthen the tight junctions between epithelial cells, reducing intestinal permeability ('leaky gut'). Potential side effects or contraindications associated with chitosan supplementation in different populations include gastrointestinal discomfort (e.g., bloating, constipation), interference with fat-soluble vitamin absorption, and potential allergic reactions in individuals with shellfish allergies; caution is also advised for individuals with diabetes, as chitosan may affect blood sugar levels, and for pregnant or breastfeeding women due to limited safety data [45]. This barrier function is crucial for preventing the translocation of harmful bacteria and toxins into the systemic circulation [46]. Additionally, beneficial bacteria stimulated by chitosan can enhance mucus production by goblet cells, providing an additional protective layer that impedes pathogen adhesion and invasion [47].

Moreover, chitosan facilitates the competitive exclusion of pathogens by promoting the growth of beneficial bacteria that compete with pathogens for essential nutrients and adhesion sites on the intestinal mucosa. This competitive advantage indirectly inhibits the colonization and proliferation of pathogenic microorganisms [48]. The efficacy of chitosan in modulating immune responses varies with dosage, where low doses enhance immune cell production and gut health, moderate doses promote cytokine production and NK cell activity, and high doses may suppress excessive inflammation or cause immune dysfunction [49]. Beneficial bacteria also produce antimicrobial peptides, bacteriocins, and other inhibitory compounds that suppress pathogen growth. The enhanced population of these bacteria, facilitated by chitosan, increases the overall antimicrobial activity within the gut environment [50].

### 2.3.2. Suppression of pathogenic microorganisms

Chitosan exerts both direct and indirect antimicrobial effects that suppress the growth and colonization of pathogenic bacteria in the gut. Direct antimicrobial activity is primarily due to chitosan's polycationic nature, which interacts electrostatically with the negatively charged components of microbial cell membranes. This interaction leads to membrane disruption, increased permeability, and eventual leakage of cellular contents, resulting in cell death [51]. Different bacterial strains respond variably to chitosan's chemical modifications, such as thiolation or quaternization, with modifications enhancing antimicrobial activity against specific pathogens while also potentially influencing the prebiotic effects based on the bacterial strain's ability to tolerate or utilize these modified forms of chitosan [52]. Additionally, chitosan can induce the production of reactive oxygen species (ROS) within pathogenic bacteria, causing oxidative stress and damage to cellular components such as DNA, proteins, and lipids. This oxidative damage further contributes to the antimicrobial efficacy of chitosan [53].

Chitosan also inhibits the formation and stability of biofilms, which are protective layers that enhance the resistance of pathogenic bacteria to antimicrobial agents and the host immune system. By interfering with the initial adhesion of bacterial cells to surfaces and inhibiting the synthesis of extracellular polymeric substances (EPS) essential for biofilm stability, chitosan disrupts biofilm formation [47]. In established biofilms, chitosan can penetrate the matrix and disrupt the structural integrity, leading to the dispersal of bacterial cells and increased susceptibility to antimicrobial substances such as antibiotics or disinfectants [40].

Another critical mechanism is the inhibition of quorum sensing, a bacterial communication system that regulates gene expression related to virulence, biofilm formation, and antibiotic resistance. Chitosan interferes with quorum sensing signals, thereby reducing the expression of virulence factors and inhibiting the coordinated behavior of pathogenic bacteria [46]. Furthermore, chitosan can interfere with the metabolic pathways of pathogenic bacteria, inhibiting essential processes such as DNA replication, protein synthesis, and energy production. This metabolic inhibition reduces the growth rate and virulence of pathogens [54]. By binding to essential nutrients and limiting their availability, chitosan restricts the nutrient uptake of pathogenic bacteria, thereby impeding their growth and survival [43].

### 2.3.3. Modulation of host-microbe interactions

In addition to its direct effects on microbial populations, chitosan modulates host-microbe interactions, contributing to an environment that favors beneficial bacteria and suppresses pathogens. Chitosan activates innate immune cells such as macrophages and dendritic cells, enhancing their phagocytic activity and cytokine production. This immune activation helps in clearing pathogenic bacteria and maintaining a balanced microbial ecosystem [54]. Furthermore, chitosan influences the adaptive immune system by modulating T-cell responses and promoting the production of regulatory T cells (Tregs). This modulation fosters an anti-inflammatory environment that supports the growth of beneficial bacteria while inhibiting pro-inflammatory pathways that can be exploited by pathogens [55].

Chitosan also enhances mucosal immunity by stimulating the production of secretory immunoglobulin A (sIgA) in the gut mucosa. sIgA plays a crucial role in neutralizing pathogens and preventing their adhesion to the intestinal epithelium, thereby reducing infection rates [56]. Optimal ratios of degree of deacetylation (DD) and molecular weight for balancing antimicrobial activity and prebiotic potential typically involve a moderate DD (around 70–85%) and a molecular weight range of 100–500 kDa, which enhances both antimicrobial effects and prebiotic activity by promoting selective fermentation and pathogen inhibition [57]. Additionally, by promoting anti-inflammatory cytokines such as IL-10 and reducing pro-inflammatory cytokines like TNF-α and IL-6, chitosan helps maintain immune homeostasis. This balanced cytokine environment discourages the overgrowth of inflammatory-associated pathogens [58].

### 2.3.4. Structural and functional changes in the gut environment

Chitosan induces structural and functional changes in the gut environment that favor beneficial bacteria and hinder pathogens. The fermentation of chitosan by beneficial bacteria results in the production of SCFAs, which lower the gut pH. A more acidic environment inhibits the growth of acid-sensitive pathogenic bacteria while promoting acid-tolerant beneficial strains [54]. Additionally, chitosan stimulates goblet cells to produce more mucus, increasing the thickness and viscosity of the mucus layer. This enhanced mucus barrier provides a physical impediment to pathogen colonization and offers a habitat for beneficial bacteria [56].

Moreover, SCFAs produced from chitosan fermentation strengthen tight junctions between epithelial cells, reducing intestinal permeability. This reduction prevents the translocation of pathogens and toxins from the gut lumen into the bloodstream, thereby maintaining intestinal integrity [59].

### 2.3.5. Synergistic effects with probiotics

When used in conjunction with probiotic strains, chitosan enhances their efficacy through synergistic interactions. Chitosan-coated probiotics are better protected against gastric acidity and bile salts, ensuring higher survival rates as they transit through the gastrointestinal (GI) tract. This increased survival enhances the colonization potential of probiotics in the gut [44]. Additionally, chitosan provides additional substrates for probiotic metabolism, supporting their growth and metabolic activities. This synergy results in higher SCFA production and more effective suppression of pathogenic bacteria [50]. Different dietary fibers and prebiotics interact with chitosan by synergistically enhancing its effects on gut microbiota, where fibers like inulin and oligosaccharides promote the growth of beneficial bacteria, while chitosan's ability to modulate microbial composition and reduce pathogen growth is complemented, resulting in improved gut health, better nutrient absorption, and more effective immune modulation [60].

Furthermore, both chitosan and probiotics independently stimulate the immune system. When used together, they produce a more robust immune response, enhancing the overall immunomodulatory effects and contributing to a healthier gut microbiota balance [48]. Chitosan promotes beneficial bacteria over pathogenic ones selectively through mechanisms such as its ability to bind to negatively charged bacterial cell membranes, disrupting the integrity of pathogenic bacteria while allowing beneficial, often positively charged, bacteria to thrive; its modulation of gut pH to favor the growth of beneficial microbes; and its prebiotic effects, which enhance the growth of specific beneficial strains like *Lactobacilli* and *Bifidobacteria* by providing substrates for their fermentation [61].

### 2.3.6. Molecular structure-function relationships

The specific structural characteristics of chitosan, such as its proportion of free amino groups (degree of deacetylation, DD) and molecular weight, significantly influence its interactions with gut microbiota. A higher DD results in more free amino groups, increasing the polycationic nature of chitosan. This enhances its ability to interact with negatively charged bacterial cell membranes, leading to more effective antimicrobial activity against pathogens [51]. Additionally, the DD affects chitosan's solubility and its susceptibility to enzymatic degradation by beneficial bacteria. Optimizing the DD can balance antimicrobial activity with prebiotic potential, ensuring selective promotion of beneficial strains [40].

Molecular size also plays a crucial role in chitosan's functionality. Higher molecular weight chitosan forms more viscous solutions and stronger films, providing better protection for encapsulated probiotics. This enhanced film formation ensures sustained release and prolonged protection against GI stresses [40]. Conversely, lower molecular weight chitosan is more easily degraded by microbial enzymes, facilitating quicker utilization by beneficial bacteria. This rapid fermentation supports timely SCFA production and more immediate suppression of pathogens [47]. Additionally, chemical modifications of chitosan, such as thiolation or quaternization, can tailor its interactions with specific bacterial strains. These modifications enhance selective binding and uptake by beneficial bacteria, further promoting their growth while maintaining antimicrobial efficacy [46].

### 2.3.7. Interaction with dietary components

Chitosan interacts with various dietary components, influencing its modulatory effects on the gut microbiota. When combined with dietary fibers, chitosan enhances the fermentation process by providing additional substrates for beneficial bacteria. This synergy results in increased SCFA production and more effective suppression of pathogenic bacteria [42]. Moreover, chitosan can bind to polyphenols and other antioxidants, protecting them from degradation and enhancing their bioavailability. These antioxidants further support gut health by reducing oxidative stress and inflammation, creating a more favorable environment for beneficial bacteria [43].

Additionally, chitosan can bind to dietary proteins, altering their digestion and absorption. This interaction influences the availability of amino acids for beneficial bacteria, supporting their growth and metabolic functions [44].

### 2.3.8. Environmental and host factors

The efficacy of chitosan in modulating gut microbiota is influenced by various environmental and host-related factors. The overall composition of the host's diet affects how chitosan interacts with gut microbiota. Diets rich in fibers and prebiotics can enhance the prebiotic effects of chitosan, while high-fat or high-sugar diets may counteract its benefits by promoting pathogenic bacteria [40]. Genetic differences in the host can also influence the composition of the gut microbiota and its response to chitosan. Individuals with different genetic backgrounds may exhibit varying levels of enzyme production, such as chitosanases, necessary for chitosan degradation, affecting its prebiotic efficacy [50].

Furthermore, the baseline health of the host's gut microbiota determines the extent of chitosan's modulatory effects. Individuals with dysbiosis or compromised gut barriers may experience more pronounced benefits from chitosan supplementation compared to those with a balanced microbiota [48]. Concurrent use of antibiotics can disrupt gut microbiota, reducing the population of both beneficial and pathogenic bacteria. Chitosan's selective promotion of beneficial bacteria may aid in faster microbiota recovery post-antibiotic treatment by outcompeting any remaining pathogenic strains [40].

Chitosan exerts a multifaceted influence on gut microbiota through selective fermentation, enhancement of SCFA production, direct antimicrobial activity, biofilm inhibition, quorum sensing disruption, and modulation of host immune responses. Its structural properties, such as degree of deacetylation and molecular weight, play crucial roles in determining its efficacy in promoting beneficial bacteria like *Bifidobacteria* and *Lactobacillus*, while suppressing pathogens such as *Escherichia* coli, *Salmonella* spp., and *Clostridium difficile* [62]. Additionally, chitosan's interactions with dietary components, environmental factors, and host genetics further refine its modulatory effects, making it a versatile agent in maintaining and enhancing gut health. Future research should focus on optimizing chitosan's structural characteristics and exploring synergistic combinations with other prebiotics and probiotics to maximize its beneficial impacts on the gut microbiota and overall immune function.

**Table 3.** Challenges and limitations.

| S/N | Challenge | Description |
|---|---|---|
| .1 | Sensitivity to pH Changes | While chitosan's pH sensitivity is advantageous for controlled release, it can also be a limitation. Chitosan's solubility in acidic environments means that its protective effects may be compromised if the pH conditions are not adequately managed. In highly acidic conditions, such as those found in the stomach, chitosan may dissolve too rapidly, potentially reducing its protective efficacy [63]. |
| 2. | Instability in Neutral or Alkaline Environments | Chitosan's tendency to form a gel in neutral or alkaline environments is beneficial for controlled release, but it can also pose challenges. In some cases, the gelation may be insufficient or inconsistent, leading to variations in release rates and affecting the overall performance of the encapsulated probiotics [64]. |
| 3. | Limited Solubility and Processing Issues | The solubility of chitosan is limited to acidic conditions, which can complicate its use in formulations where neutrality or alkalinity is required. Additionally, the processing of chitosan can be challenging, as it often requires the use of acids or specific conditions to dissolve and form coatings. This can affect the scalability and cost-effectiveness of chitosan-based encapsulation methods [65]. |
| 4. | Variability in Chitosan Sources | Chitosan is derived from natural sources, and its properties can vary depending on the source of chitin, the degree of deacetylation, and the processing methods used. This variability can lead to inconsistencies in the performance of chitosan-based encapsulation systems [66]. |

**Table 4.** Research on combining chitosan with other biopolymers.

| S/N | Combinations | Description |
|---|---|---|
| 1. | Chitosan and Alginate | Chitosan and alginate are frequently combined to enhance encapsulation efficiency. Alginate, derived from brown seaweed, is a natural polysaccharide that forms gel beads in the presence of calcium ions. When combined with chitosan, alginate provides an additional layer of protection and enhances the stability of the encapsulated probiotics. The combination of these biopolymers (Figure 2) can improve the overall encapsulation efficiency by creating a dual-layered structure that offers better resistance to gastrointestinal conditions. Studies have shown that chitosan-alginate systems can achieve a more controlled and sustained release of probiotics, making them suitable for targeted delivery applications [67]. |
| 2. | Chitosan and Gelatin | Gelatin, a protein derived from collagen, is often used in conjunction with chitosan to leverage its film-forming and gelation properties. Chitosan-gelatin composites can be used to create encapsulation matrices that are both flexible and strong. The combination improves the mechanical properties of the coating and provides a more controlled release rate or pattern. Research has demonstrated that chitosan-gelatin systems can enhance the stability of probiotics during storage and increase their survival rate through the gastrointestinal tract. Additionally, the mucoadhesive properties of chitosan complement the gelatin's ability to form stable gels, leading to better retention and colonization of probiotics in the gut [68]. |
| 3. | Chitosan and Pectin | Pectin, a polysaccharide found in fruits, is used in combination with chitosan to create encapsulation systems that offer enhanced stability and targeted release. Pectin is known for its gel-forming ability in the presence of calcium ions, and when paired with chitosan, it can provide additional structural integrity and resistance to acidic conditions. Chitosan-pectin systems have been shown to improve the encapsulation efficiency of probiotics by forming a robust, multi-layered coating that protects the probiotics and facilitates their controlled release in the intestines [69]. |

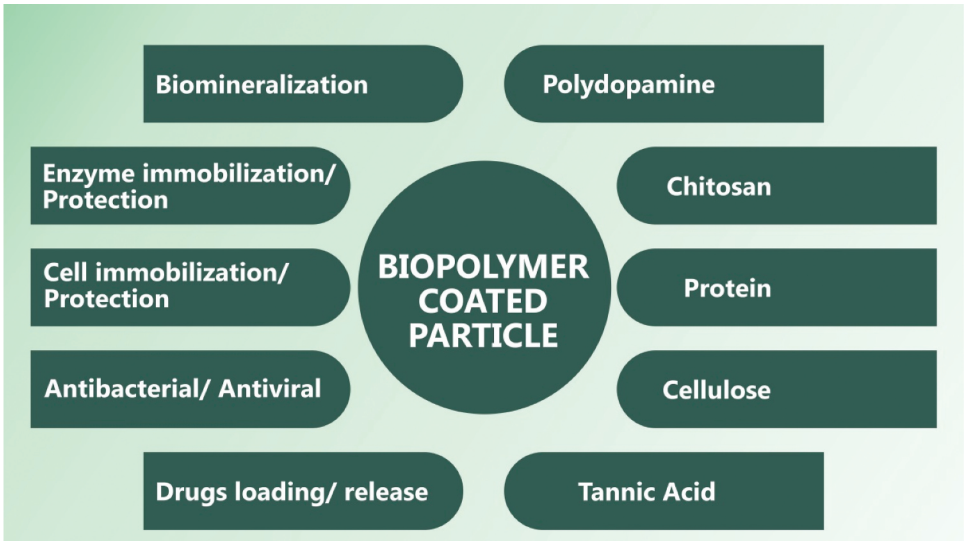


**Figure 2.** Bio polymer coatings.

While chitosan (Figure 1) offers numerous advantages for probiotic encapsulation, including biocompatibility, biodegradability, antimicrobial properties, and pH-sensitive controlled release, it also presents challenges related to its sensitivity to pH changes, stability in different environments (Tables 2–4), and processing requirements. Addressing these limitations through further research and technological advancements will be crucial for optimizing the use of chitosan in probiotic microencapsulation and improving its overall efficacy and practicality.

### *2.4. Limitations of chitosan*

## 3. Advances in chitosan-based encapsulation

### *3.1. Combination with other materials*

### *3.2. Nanotechnology in chitosan encapsulation*

### *3.3. Innovative methods in chitosan coating*

Advances in chitosan-based encapsulation have been driven by the combination of chitosan (Tables 5–9) with other biopolymers, the application of nanotechnology, and the development of innovative encapsulation techniques [82]. These advancements have improved the stability, bioavailability, and controlled release of probiotics, leading to more effective and practical probiotic formulations [83]. Continued research and development in these areas are essential for further enhancing the performance and applications of chitosan-based encapsulation systems.

## 4. Beyond chitosan: other bio-polymer coatings

Each biopolymer – alginate, pectin, gelatin, and carrageenan – offers unique properties that can be utilized for probiotic encapsulation (Figure 3) [92]. By understanding their individual characteristics and combining them with other materials (Tables 10–11), researchers can develop advanced encapsulation systems that enhance the stability, controlled release, and overall efficacy of probiotics [93]. A comparative analysis of these bio-polymers highlights their strengths and limitations, providing valuable insights for selecting the most suitable materials for specific applications in probiotic microencapsulation [94].

## 5. Controlled release mechanisms

Controlled release mechanisms play a crucial role in the effectiveness of probiotic formulations (Tables 12 and 13). By utilizing pH-sensitive, enzyme-triggered,

**Table 5.** Benefits of combining chitosan with other biopolymers.

| S/N | Benefits | Description |
|---|---|---|
| 1. | Enhanced Encapsulation Efficiency | Combining chitosan with other biopolymers can result in coatings that offer improved encapsulation efficiency, protecting probiotics more effectively during processing and storage [70]. |
| 2. | Better Targeting and Controlled Release | Multi-material coatings can provide more precise control over the release of probiotics, allowing for targeted delivery to specific regions of the gastrointestinal tract [71]. |
| 3. | Increased Stability | The synergistic effects of combining chitosan with other materials can enhance the overall stability of the encapsulated probiotics, ensuring their viability and efficacy [72]. |

**Table 6.** Advancements in nanotechnology.

| S/N | Advancements | Description |
|---|---|---|
| 1. | Nanoscale Encapsulation Systems | Nanotechnology has led to the development of nanoscale chitosan-based encapsulation systems that offer improved stability and bioavailability of probiotics. These nanoscale systems utilize chitosan nanoparticles or nanocarriers to encapsulate probiotics, providing a high surface area-to-volume ratio that enhances the protective effects of the coating. Nanoparticles can create a more uniform and controlled release profile, improving the delivery and efficacy of probiotics [73]. |
| 2. | Chitosan Nanoparticles | Chitosan nanoparticles are created by dissolving chitosan in an acidic solution and then using techniques such as ionic gelation or solvent evaporation to form nanosized particles. These nanoparticles can encapsulate probiotics with high efficiency and provide a protective barrier that improves their stability. Studies have shown that chitosan nanoparticles can effectively protect probiotics from environmental stresses and enhance their bioavailability by facilitating their absorption in the gastrointestinal tract [74]. |
| 3. | Nanocomposite Systems | The integration of chitosan with other nanomaterials, such as nanoparticles of metals or metal oxides, can create nanocomposite systems with enhanced properties. These systems can offer additional functionalities, such as antimicrobial activity or improved mechanical strength, which further improve the encapsulation and delivery of probiotics. For example, chitosan-nanoparticle composites can provide both protective and therapeutic benefits, enhancing the overall effectiveness of probiotic formulations [74]. |

**Table 7.** Benefits of nanotechnology in Chitosan Encapsulation.

| S/N | Benefits | Description |
|---|---|---|
| 1. | Enhanced Stability | Nanoscale systems offer superior stability for encapsulated probiotics, protecting them from degradation during storage and processing [75]. |
| 2. | Improved Bioavailability | Nanotechnology can enhance the bioavailability of probiotics by facilitating their absorption and uptake in the gastrointestinal tract [76]. |
| 3, | Controlled Release | Nanoscale encapsulation systems allow for precise control over the release of probiotics, improving their targeted delivery and efficacy [71]. |

**Table 8.** New Encapsulation Techniques.

| S/N | Technique | Description |
|---|---|---|
| 1. | Spray Drying | Spray drying is a widely used method for creating chitosan-based encapsulation systems. In this process, a solution containing chitosan and probiotics is atomized into small droplets, which are then dried to form powder particles. Spray drying offers several advantages, including the ability to produce large quantities of encapsulated probiotics with controlled particle size. Research has shown that spray-dried chitosan formulations can effectively protect probiotics from environmental stresses and provide a controlled release profile [77]. |
| 2. | Layer-by-Layer Assembly | Layer-by-layer (LbL) assembly involves the sequential deposition of alternating layers of chitosan and other materials onto a substrate to create multilayered coatings. This technique allows for precise control over the thickness and composition of the coating, enabling the development of advanced encapsulation systems with enhanced stability and release properties. LbL assembly can be used to create complex chitosan-based coatings that offer improved protection and controlled release of probiotics [78]. |
| 3. | Electrostatic Deposition | Electrostatic deposition is a technique that uses electrostatic forces to deposit chitosan onto a substrate or onto probiotics. This method allows for the creation of uniform and adherent coatings with controlled thickness. Electrostatic deposition can enhance the encapsulation efficiency and stability of probiotics by forming a strong and cohesive chitosan layer. Studies have demonstrated that electrostatic deposition techniques can produce high-quality chitosan coatings that offer effective protection and controlled release [22]. |

**Table 9.** Benefits of Innovative Encapsulation Methods.

| S/N | Benefits | Description |
|---|---|---|
| 1. | Improved Encapsulation Efficiency | Advanced techniques such as spray drying, LbL assembly, and electrostatic deposition can enhance the encapsulation efficiency of chitosan-based systems, ensuring better protection and stability of probiotics [79]. |
| 2. | Controlled Release | Innovative methods allow for precise control over the release profile of probiotics, improving their targeted delivery and effectiveness [80]. |
| 3. | Scalability and Versatility | These techniques offer scalable and versatile solutions for producing chitosan-based encapsulation systems, making them suitable for various applications in the food and pharmaceutical industries [81]. |

and time-controlled release strategies, as well as ensuring targeted delivery and enhanced stability, bio-polymer coatings can significantly improve the viability and efficacy of probiotics [103]. Continued research and development in these areas are essential for optimizing probiotic delivery systems and meeting the growing demand for effective probiotic products.

**Table 10.** Beyond chitosan: other bio-polymer coatings.

| S/N | Bio-polymers | Role in Probiotic Encapsulation | Applications and Benefits |
|---|---|---|---|
| 1. | Alginate [84] | Alginate is a natural polysaccharide extracted from brown seaweed, and it is widely used in probiotic encapsulation due to its unique gel-forming abilities and protective properties. Gel-Forming Abilities: Alginate forms gels in the presence of divalent cations, such as calcium ions. This property is harnessed to create a gel matrix that can encapsulate probiotic microorganisms effectively. The gel formation occurs through ionic gelation, where calcium ions cross-link alginate molecules, resulting in a stable network that can protect probiotics from environmental stresses. Protective Properties: Alginate coatings provide a protective barrier against mechanical stresses, oxidation, and moisture, which helps maintain the viability of encapsulated probiotics during processing and storage. Alginate's ability to form a gel at physiological pH makes it particularly suitable for encapsulating probiotics intended for gastrointestinal delivery. | Improved Encapsulation Efficiency: Alginate's gel-forming property allows for the creation of microbeads or capsules that can effectively encapsulate and protect probiotics. Controlled Release: Alginate-based systems can be designed to release probiotics in response to specific environmental triggers, such as pH changes, making them suitable for targeted delivery to different parts of the gastrointestinal tract. Compatibility with Other Polymers: Alginate is often combined with other biopolymers, such as chitosan, to enhance its performance and provide additional functionality. |
| 2. | Pectin [85] | Pectin is a complex polysaccharide found in the cell walls of fruits and vegetables, and it plays a significant role in enhancing the encapsulation and release of probiotics. Gel Formation: Pectin forms gels in the presence of calcium ions or under acidic conditions. This gel-forming ability is utilized to create encapsulation matrices that protect probiotics from environmental stresses. The gel structure provides a stable environment for probiotics, enhancing their viability during storage and processing. Targeting the Colon: Pectin is particularly effective in targeting the colon due to its gel-forming properties in the presence of specific ions. Pectin-based capsules can be designed to remain intact in the upper gastrointestinal tract and disintegrate in the colon, where they release probiotics. This targeted release is beneficial for probiotic strains intended to exert their effects in the colon. | Enhanced Encapsulation and Stability: Pectin's gel-forming ability helps improve the encapsulation efficiency and stability of probiotics. Controlled and Targeted Release: Pectin-based systems can be engineered for controlled and targeted release, ensuring that probiotics are delivered to specific regions of the gastrointestinal tract. Natural and Biodegradable: Pectin is a natural and biodegradable biopolymer, making it an attractive option for use in food and pharmaceutical applications. |
| 3. | Gelatin [86] | Biodegradability and Film-Forming Capabilities: Gelatin is a protein derived from collagen, and it is known for its biodegradability and ability to form flexible films. In probiotic encapsulation, gelatin is used to create protective coatings that can encapsulate probiotics and provide a controlled release. Synergies with Chitosan: Gelatin is often combined with chitosan to create composite coatings that leverage the unique properties of both biopolymers. The combination of gelatin's film-forming capabilities and chitosan's antimicrobial and mucoadhesive properties results in enhanced encapsulation efficiency and stability. | Biodegradability: Both gelatin and carrageenan are biodegradable and biocompatible, making them suitable for use in food and pharmaceutical applications. Enhanced Encapsulation: Gelatin and carrageenan coatings provide effective protection and controlled release for probiotics. Synergistic Effects: Combining gelatin and carrageenan with chitosan can lead to improved encapsulation efficiency and functionality. |
| 4. | Carrageenan [87] | Film-Forming and Gelation: Carrageenan is a polysaccharide extracted from red seaweed, and it is known for its ability to form gels and films. Carrageenan can be used in probiotic encapsulation to create protective coatings that offer stability and controlled release. Combination with Chitosan: Carrageenan is sometimes combined with chitosan to improve the encapsulation performance. The synergistic effects of chitosan and carrageenan can enhance the stability and controlled release of probiotics. | Biodegradability: Both gelatin and carrageenan are biodegradable and biocompatible, making them suitable for use in food and pharmaceutical applications. Enhanced Encapsulation: Gelatin and carrageenan coatings provide effective protection and controlled release for probiotics. Synergistic Effects: Combining gelatin and carrageenan with chitosan can lead to improved encapsulation efficiency and functionality |

## 6. Chitosan as a prebiotic or synbiotic

### *6.1. Introduction to chitosan and its role in gut health*

Chitosan is a biopolymer derived from chitin, commonly found in the exoskeletons of crustaceans like shrimp and crabs. It has garnered significant attention for its wide range of applications in various fields, including biomedical and pharmaceutical industries, water purification, and food technology [104]. Recently, its potential as a prebiotic or synbiotic agent has been explored due to its positive effects on gut health. As a prebiotic, chitosan promotes the growth and activity of beneficial gut bacteria. When combined with probiotics, it can function as a synbiotic, enhancing the probiotic's ability to colonize and exert positive health effects on the host [105].

#### *6.1.1. Prebiotic properties of chitosan*

Prebiotics are non-digestible food components that selectively stimulate the growth and activity of beneficial gut microbiota. Several studies have demonstrated chitosan's ability to act as a prebiotic by promoting the

**Table 11.** Comparative analysis of bio-polymers.

| S/N | Bio-polymer | Encapsulation Efficiency | Release Properties | Stability Under Different Conditions |
|---|---|---|---|---|
| 1. | Alginate [88] | High encapsulation efficiency due to its ability to form stable gels in the presence of calcium ions. It is particularly effective in creating microbeads and capsules. | Offers controlled release in response to pH changes and ionic conditions. Suitable for targeted delivery to different parts of the gastrointestinal tract. | Stable under acidic and neutral conditions, but may be less effective in highly alkaline environments. Combining with other biopolymers can improve stability. |
| 2. | Pectin [89] | Good encapsulation efficiency, especially for targeted release applications. Its gel-forming ability is enhanced in the presence of calcium ions or under acidic conditions. | Allows for controlled and targeted release, particularly effective in the colon. Its release properties can be tailored based on the formulation. | Stable in acidic conditions and effective for targeting the colon. Its stability can be affected by the presence of calcium ions and pH changes. |
| 3. | Gelatin [90] | Effective in forming flexible films, but encapsulation efficiency may vary depending on the formulation and processing conditions. | Provides controlled release but may be less effective in highly acidic environments. The release profile can be adjusted through formulation and processing. | Generally stable but may be sensitive to temperature and pH changes. Combining with other biopolymers can enhance stability. |
| 4. | Carrageenan [91] | Provides good encapsulation efficiency and stability, especially when used in combination with other biopolymers. | Offers controlled release with good stability, especially when combined with other biopolymers. Its release properties can be optimized through formulation. | Stable under a range of conditions, including acidic and neutral environments. Its stability can be improved when used in combination with other biopolymers. |

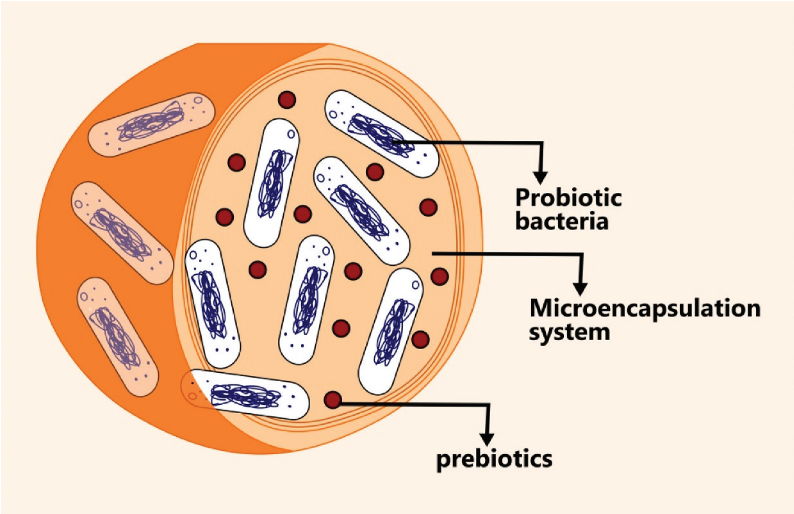


**Figure 3.** Probiotic microencapsulation.

growth of certain probiotic species, such as *Lactobacillus* and *Bifidobacterium* [105]. Chitosan oligosaccharides (COS), the low molecular weight degradation products of chitosan, are particularly recognized for their ability to enhance gut microbiota composition. These oligosaccharides resist digestion in the upper gastrointestinal tract and reach the colon intact, where they become available to gut microbes for fermentation, thereby modulating the gut microbiome [106]. The synergistic effects of chitosan and probiotics can vary among different probiotic strains, as each strain exhibits unique characteristics, such as its capacity to interact with chitosan, endure the conditions of the gastrointestinal tract, and utilize specific nutrients, resulting in varying responses to chitosan's antimicrobial properties and prebiotic effects. Similarly, in the context of layer-by-layer coating with chitosan and liposomes, the stability and antioxidant properties of probiotics are enhanced, which can be further optimized depending on the strain-specific interactions and environmental conditions in vitro [72].

Recent research has shown that COS can increase short-chain fatty acids (SCFAs) production, particularly butyrate, acetate, and propionate, which play critical roles in maintaining gut health and reducing inflammation. SCFAs provide energy to colonocytes, regulate the immune system, and help maintain the integrity of the gut barrier. This fermentation process, coupled with a favorable shift in gut microbiota, reinforces chitosan's role as a prebiotic [107].

### *6.1.2. Synbiotic applications of chitosan*

Synbiotics refer to the combination of prebiotics and probiotics, where the prebiotic component supports

**Table 12.** Mechanisms of probiotic release.

| S/N | Release type | Mechanism | Example | Advantages |
|---|---|---|---|---|
| **1.** | pH-Sensitive Release [95] | pH-sensitive release mechanisms involve the use of bio-polymer coatings that respond to changes in pH along the gastrointestinal tract. Many biopolymers, such as alginate, chitosan, and pectin, exhibit pH-dependent solubility. In the acidic environment of the stomach, these polymers remain intact to protect the probiotics. However, as they move into the more neutral to alkaline environment of the small intestine, the polymers dissolve or change their structure, allowing for the release of probiotics. | Alginate beads, often cross-linked with calcium ions, maintain their structure in the acidic stomach environment. However, they begin to dissolve as they reach the higher pH of the small intestine, releasing the probiotics contained within. | Provides protection to probiotics from the acidic conditions of the stomach.<br>Ensures targeted release in the intestinal environment where probiotics can be more effective. |
| **2.** | Enzyme-Triggered Release [96] | Enzyme-triggered release relies on the activity of specific enzymes present in the gastrointestinal tract to break down the bio-polymer coating. For instance, enzymes like amylases, proteases, or lipases can cleave bonds in the polymer matrix, leading to the release of encapsulated probiotics. | Chitosan-based coatings can be designed to degrade in the presence of certain enzymes. In the gastrointestinal tract, proteolytic enzymes can break down chitosan, leading to the gradual release of probiotics. | Provides a more controlled and gradual release of probiotics.<br>Ensures that probiotics are released in regions where the specific enzyme is active, which may be beneficial for targeting. |
| **3.** | Time-Controlled Release [97] | Time-controlled release mechanisms involve designing bio-polymer coatings that degrade or dissolve over a specific period. This can be achieved through the use of polymers with known degradation rates or by incorporating additives that influence the coating's release profile. | Gelatin-based capsules can be engineered to dissolve at a predetermined rate, releasing probiotics over an extended period. This allows for sustained delivery of probiotics throughout the gastrointestinal tract. | Provides a sustained release of probiotics, ensuring prolonged activity.<br>Useful for formulations requiring a steady release of probiotics over time. |
| **4.** | Colon-Specific Delivery [98] | Targeted delivery systems are designed to ensure that probiotics are released at specific locations within the gastrointestinal tract, particularly in the colon. This is achieved through various strategies, such as using coatings that are resistant to stomach acid and small intestine enzymes but degrade in the colon. | Pectin-based coatings are often used for colon-targeted delivery. Pectin forms a gel in the presence of calcium ions, which can be stable in the upper gastrointestinal tract but break down in the colon, where the pH is different and specific bacteria can degrade the polymer. | Ensures that probiotics are delivered to the site where they are most needed and where they can have the greatest impact.<br>Reduces premature release of probiotics in the upper gastrointestinal tract, where they may not be as effective. |
| **5.** | Targeting by Microbial Degradation [99] | Some bio-polymer coatings are designed to be degraded by specific gut microbiota, which can be more prevalent in certain regions of the gastrointestinal tract, such as the colon. By using polymers that are selectively degraded by these microbes, the release of probiotics can be targeted to specific areas. | Chitosan-pectin composites can be formulated to be selectively degraded by specific bacterial enzymes found in the colon, allowing for targeted probiotic delivery. | Utilizes the natural microbial environment of the gut to achieve targeted delivery.<br>May enhance the effectiveness of probiotics by ensuring they reach their intended site of action. |

the survival, growth, and metabolic activity of the probiotic. Chitosan's incorporation into synbiotic formulations enhances the overall efficacy of probiotics by providing both structural protection and growth-enhancing properties [108].

Chitosan has been shown to protect probiotics during gastric transit, which is often a limiting factor in the effectiveness of probiotic therapies. The acidic conditions in the stomach can degrade probiotic cells, reducing their viability. Studies have demonstrated that chitosan can form a protective gel matrix around probiotic cells, shielding them from stomach acidity and bile salts, and increasing their chances of colonizing the intestines [109]. Strains such as *Lactobacillus* and *Bifidobacterium* are most effectively supported by chitosan, as it enhances their stability, viability, and gut adhesion due to its ability to protect them from harsh gastrointestinal conditions. The concentration of chitosan plays a crucial role in its synergistic effects with probiotics; lower concentrations may support probiotic growth by facilitating adhesion and providing a protective barrier, while higher concentrations can improve the encapsulation efficiency and stability of probiotics, but excessive chitosan may hinder microbial activity by altering the gut pH or interfering with nutrient availability [109].

### *6.1.3. Synergistic effects of chitosan with specific probiotics*

Several recent studies have highlighted the synergistic effects of chitosan, especially in the form of oligosaccharides, when combined with specific probiotic strains. One such study by [110] found that the combination of COS with *Lactobacillus plantarum* significantly improved gut microbiota diversity and increased the abundance of beneficial bacteria in

**Table 13.** Enhanced stability for probiotics.

| S/N | Stability | Mechanism | Example | Advantages |
|---|---|---|---|---|
| 1. | Improved Stability During Storage [100] | Bio-polymer coatings provide a protective barrier that shields probiotics from environmental factors such as moisture, oxygen, and temperature fluctuations. This helps to maintain the viability and potency of probiotics during storage. | Alginate and chitosan coatings have been shown to extend the shelf life of probiotics by protecting them from moisture and oxidation. Encapsulated probiotics in alginate beads or chitosan nanoparticles exhibit improved stability compared to non-encapsulated strains. | Enhances the shelf life of probiotic products.<br>Reduces the need for additional preservatives or refrigeration. |
| 2. | Protection During Processing [101] | During food and pharmaceutical manufacturing processes, probiotics are often exposed to harsh conditions such as heat, pressure, and mechanical stresses. Bio-polymer coatings protect probiotics from these conditions, ensuring their survival and efficacy. | Gelatin-based encapsulation systems can protect probiotics from heat during the spray-drying process. The gelatin forms a protective layer around the probiotics, preventin heat damage and preserving their viability. | Ensures the survival of probiotics through processing stages.<br>Maintains the quality and effectiveness of the final product. |
| 3. | Protection During Gastrointestinal Transit [102] | Bio-polymer coatings protect probiotics from the harsh acidic conditions of the stomach and bile salts in the small intestine. By providing a barrier that withstands these conditions, the probiotics are able to reach the intestines in a viable state. | Pectin and chitosan coatings offer protection against stomach acid and bile salts, allowing probiotics to survive transit through the gastrointestinal tract and reach the colon. | Increases the likelihood that probiotics will reach their target site in the gut.<br>Enhances the effectiveness of probiotics by ensuring their survival through the gastrointestinal tract. |

animal models. The study reported enhanced anti-inflammatory effects in the gut, with reduced levels of pro-inflammatory cytokines like TNF-α and IL-6, pointing to the potential of chitosan to amplify the immunomodulatory effects of probiotics.

Another study by [44] explored the use of chitosan in combination with *Bifidobacterium bifidum* in a synbiotic yogurt formulation. The researchers observed a substantial improvement in the survival rate of the probiotic strain during storage and simulated gastrointestinal digestion. Additionally, the combination led to a significant increase in SCFA production and a reduction in pathogenic bacteria, demonstrating the efficacy of chitosan in enhancing probiotic functionality.

In a similar vein [42], examined the interaction between chitosan and *Lactobacillus rhamnosus*. Their findings revealed that chitosan oligosaccharides enhanced the adhesion of *L. rhamnosus* to the intestinal epithelium, a crucial step for colonization and long-term persistence in the gut. This adhesive property is vital for maintaining a stable probiotic population, which in turn can support gut barrier function and immune health.

#### 6.1.4. Mechanisms underlying synergistic effects

The synergistic effects of chitosan and probiotics are largely attributed to their complementary mechanisms of action. Chitosan serves as a scaffold for probiotics, enabling their survival during digestion and facilitating their colonization in the gut [101]. Furthermore, chitosan's ability to modulate gut microbiota creates a favorable environment for probiotic strains to thrive. Chitosan also has antimicrobial properties, which help in suppressing the growth of pathogenic bacteria, thereby giving probiotics a competitive advantage [111].

Moreover, the ability of chitosan to enhance the production of SCFAs creates a feedback loop where the gut environment becomes more conducive for probiotic growth, and the host benefits from enhanced immune modulation, gut barrier integrity, and reduced inflammation. This synergy is further amplified by the bioadhesive properties of chitosan, which can help probiotics establish themselves on the intestinal mucosa, promoting prolonged probiotic activity [112].

#### 6.1.5. Clinical implications and future directions

The potential of chitosan as a prebiotic or synbiotic offers promising avenues for the development of functional foods and nutraceuticals aimed at improving gut health. Given its natural origin, biocompatibility, and non-toxicity, chitosan is well-suited for human consumption. Its prebiotic and synbiotic properties could be leveraged in treating conditions related to gut dysbiosis, such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and obesity [113].

Future research should focus on optimizing the molecular structure of chitosan to enhance its prebiotic effects and identify the most effective probiotic combinations. Moreover, human clinical trials are needed to confirm the findings of animal studies and to explore chitosan's long-term safety and efficacy in synbiotic formulations. Advances in nanotechnology could also play a role in the development of novel chitosan-based delivery systems for targeted probiotic therapy [114].

Chitosan, particularly in the form of chitosan oligosaccharides, shows immense potential as a prebiotic and synbiotic agent. Its ability to promote the growth of beneficial gut bacteria, protect probiotics from harsh gastrointestinal conditions, and enhance the immunomodulatory effects of probiotics makes it a valuable component in gut health management. Recent studies have highlighted its synergistic effects with specific probiotics, underscoring the importance of continued research to unlock its full potential in functional foods and therapeutic applications [115].

## 6.2. Immune system modulation & mechanisms of action

Chitosan not only influences the gut microbiota but also plays a significant role in modulating the host immune system. Its immunomodulatory effects are particularly noteworthy in the context of inflammatory diseases such as Inflammatory Bowel Disease (IBD) and various autoimmune disorders [116]. By interacting with both the gut microbiota and the host's immune cells, chitosan orchestrates a complex network of immune responses that contribute to maintaining gut homeostasis and preventing excessive inflammation. This section delves into the depth of chitosan's immunomodulatory capabilities, especially in disease contexts, and elucidates the immune signaling pathways it influences, thereby linking gut microbiota alterations to systemic immune improvements [117].

## 6.3. Chitosan's immunomodulatory effects in disease contexts

### 6.3.1. Inflammatory bowel disease (IBD)

IBD, encompassing conditions like Crohn's disease and ulcerative colitis, is characterized by chronic inflammation of the gastrointestinal tract, resulting from an inappropriate immune response to intestinal microbiota. Chitosan has been extensively studied for its potential therapeutic effects in IBD due to its anti-inflammatory properties and ability to modulate gut microbiota composition [118].

Studies have demonstrated that chitosan supplementation can significantly reduce inflammation markers in animal models of IBD. For instance [44], reported that chitosan oligosaccharides (COS) administration in mice with induced colitis led to a marked decrease in pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, while simultaneously increasing anti-inflammatory cytokines like IL-10. This cytokine modulation helps in mitigating the inflammatory response, thereby alleviating IBD symptoms.

Furthermore, chitosan's ability to enhance the production of SCFAs, particularly butyrate, plays a crucial role in IBD management. Butyrate serves as an energy source for colonocytes and promotes the repair of the intestinal epithelium, thereby restoring barrier function and reducing intestinal permeability. By fostering a healthier gut barrier, chitosan helps prevent the translocation of pathogenic bacteria and toxins that can exacerbate inflammation [43].

### 6.3.2. Autoimmune diseases

Autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and type 1 diabetes, result from the immune system mistakenly attacking the body's own tissues. The dysregulation of immune responses and the imbalance of pro-inflammatory and anti-inflammatory cytokines are central to the pathogenesis of these conditions. Chitosan has shown promise in modulating immune responses to alleviate autoimmune symptoms [119].

Research indicates that chitosan can influence the differentiation and activity of T-helper cells, particularly promoting the generation of regulatory T cells (Tregs) that suppress autoimmune reactions. Chitosan administration in a mouse model of multiple sclerosis resulted in an increased population of Tregs and a corresponding decrease in Th17 cells, which are known to drive inflammation and autoimmunity. This shift towards a more regulatory immune environment helps in controlling excessive immune responses and reducing tissue damage [120].

Moreover, chitosan's anti-inflammatory effects extend to reducing oxidative stress and inhibiting the activation of nuclear factor kappa B (NF-κB), a key transcription factor involved in the expression of pro-inflammatory genes. By downregulating NF-κB activation, chitosan effectively decreases the production of inflammatory mediators, thereby protecting against autoimmune-mediated tissue damage [53].

### 6.3.3. Immune signaling pathways influenced by chitosan

Chitosan interacts with various immune signaling pathways to exert its immunomodulatory effects. These interactions are often mediated through changes in the gut microbiota, which in turn influence systemic immunity. The following sections outline the key immune signaling pathways influenced by chitosan and how these pathways contribute to immune system improvements [17].

Toll-Like Receptor (TLR) Pathways: Toll-like receptors (TLRs) are crucial components of the innate immune system, recognizing pathogen-associated molecular patterns (PAMPs) and initiating immune responses. Chitosan has been shown to modulate TLR signaling pathways, thereby influencing immune cell activation and cytokine production [121].

Chitosan can act as a ligand for TLRs, particularly TLR4, which is involved in recognizing lipopolysaccharides (LPS) from Gram-negative bacteria. Binding of chitosan to TLR4 can lead to the activation of downstream signaling pathways such as the MyD88-dependent pathway, resulting in the activation of NF-κB and the production of pro-inflammatory cytokines. However, in the context of chitosan's immunomodulatory role, it appears to selectively modulate TLR signaling to promote anti-inflammatory responses. For example, chitosan has been observed to inhibit excessive TLR4 activation in inflammatory conditions, thereby reducing the overproduction of pro-inflammatory cytokines and mitigating inflammation [46].

Nuclear Factor Kappa B (NF-κB) Pathway: The NF-κB pathway is a central regulator of immune and inflammatory responses. It controls the transcription of various genes involved in inflammation, immune cell proliferation, and survival. Chitosan's interaction with the NF-κB pathway is pivotal in its ability to modulate immune responses [122].

Chitosan inhibits the activation of NF-κB by preventing the phosphorylation and subsequent degradation of its inhibitor, IκBα. This inhibition blocks the translocation of NF-κB to the nucleus, thereby reducing the transcription of pro-inflammatory genes such as TNF-α, IL-6, and IL-1β. By downregulating NF-κB activity, chitosan effectively attenuates inflammatory responses, which is beneficial in managing conditions like IBD and autoimmune diseases [123]. Studies have provided clinical evidence that chitosan-based formulations can modulate immune responses, showing potential benefits in autoimmune and inflammatory diseases. Chitosan's ability to enhance immune cell activity, reduce inflammation, and regulate cytokine production has been observed, particularly in conditions like rheumatoid arthritis and inflammatory bowel disease. However, the potential side effects or limitations of using chitosan in immunomodulation include its dose-dependent effects, where high concentrations might cause gastrointestinal discomfort, interfere with nutrient absorption, or trigger allergic reactions in sensitive individuals. Additionally, its efficacy may vary based on the formulation and the individual's immune system response, requiring careful optimization of dosage and delivery methods [124].

Mitogen-Activated Protein Kinase (MAPK) Pathway: The MAPK pathway plays a crucial role in transmitting extracellular signals to the nucleus, influencing gene expression related to cell growth, differentiation, and immune responses. Chitosan has been found to modulate the MAPK pathway, contributing to its immunomodulatory effects [125].

Chitosan can inhibit the activation of key MAPKs such as ERK, JNK, and p38, which are involved in the production of pro-inflammatory cytokines and the activation of immune cells. By suppressing MAPK signaling, chitosan reduces the inflammatory response and prevents the overactivation of immune cells that can lead to tissue damage in autoimmune and inflammatory diseases [53]. Potential side effects or contraindications associated with the use of chitosan for immune modulation include gastrointestinal discomfort such as bloating, constipation, or diarrhea, especially at high doses. Also, chitosan interferes with the absorption of fat-soluble vitamins and other nutrients due to its ability to bind to lipids. In some cases, individuals with shellfish allergies may experience allergic reactions, as chitosan is derived from chitin found in shellfish. Moreover, excessive immune modulation could lead to immune dysregulation or hypersensitivity, particularly in individuals with pre-existing autoimmune conditions, making it important to use chitosan-based formulations with caution and under professional supervision [126].

Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) Pathway: The JAK/STAT pathway is essential for the transmission of cytokine signals from the cell membrane to the nucleus, influencing immune cell differentiation and function. Chitosan's influence on the JAK/STAT pathway contributes to its ability to modulate immune responses [127].

Chitosan has been shown to downregulate the phosphorylation of STAT proteins, which are critical for the expression of genes involved in inflammation and immune cell proliferation. By inhibiting the JAK/STAT pathway, chitosan reduces the production of pro-inflammatory cytokines and supports the development of regulatory immune responses. This modulation is particularly beneficial in autoimmune diseases, where the overactivation of the JAK/STAT pathway leads to excessive immune cell proliferation and inflammation [40].

### 6.4. Gut-brain axis and systemic immunity

Different strains of gut bacteria respond to chitosan treatment in varying ways, which can have significant implications for systemic immunity. Chitosan, being a biopolymer with antimicrobial properties, can inhibit the growth of harmful pathogens like *Escherichia* coli or *Salmonella* while promoting the growth of beneficial strains such as *Lactobacillus* and *Bifidobacterium* [128]. These beneficial bacteria contribute to gut health by producing short-chain fatty acids (SCFAs) like butyrate, which help maintain intestinal barrier integrity and modulate local immune responses. As a result, chitosan treatment can influence systemic immunity by improving gut microbiota composition, enhancing the production of immune-modulating metabolites, and promoting a balanced immune response [129]. This can potentially reduce inflammation and the risk of autoimmune diseases by promoting a more resilient and regulated immune system, particularly by modulating T-cell and cytokine responses. However, the exact impact varies depending on the strain of gut bacteria and the dose and form of chitosan used [130].

Chitosan's effects on the gut microbiota have far-reaching implications for systemic immunity through the gut-brain axis. The gut-brain axis is a bidirectional communication system between the gastrointestinal tract and the central nervous system, involving neural, hormonal, and immune signaling pathways. By modulating the gut microbiota, chitosan influences the production of neurotransmitters, neuropeptides, and immune mediators that affect systemic immune responses [131].

Chitosan-induced changes in the gut microbiota lead to altered SCFA production, which not only benefits colonocytes but also acts as signaling molecules that influence immune cell function throughout the body. SCFAs can enter the bloodstream and interact with immune cells in various organs, promoting anti-inflammatory responses and enhancing systemic immune regulation. This connection highlights how chitosan's modulation of the gut microbiota can lead to improvements in systemic immunity, reducing the risk of autoimmune and inflammatory diseases [42].

Interaction with Gut-Associated Lymphoid Tissue (GALT): GALT is a critical component of the immune system, housing a large number of immune cells that monitor and respond to pathogens in the gut. Chitosan influences GALT by enhancing the activation and function of immune cells within this tissue [132].

Chitosan stimulates dendritic cells and macrophages in GALT, enhancing their antigen-presenting capabilities and promoting the activation of T cells. This stimulation leads to a more robust immune surveillance system that can effectively respond to pathogenic threats while maintaining tolerance to commensal microbiota. By strengthening GALT's immune functions, chitosan helps in maintaining a balanced immune response, preventing excessive inflammation and autoimmune reactions [48].

Linking Gut Microbiota Changes to Systemic Immunity Improvements: Chitosan's ability to modulate the gut microbiota is intrinsically linked to its effects on systemic immunity. The alterations in gut microbial composition and activity influence immune signaling pathways, leading to widespread immune system improvements [133].

Enhancement of Microbial Diversity and Immune Homeostasis: Chitosan promotes the growth of a diverse and balanced gut microbiota, which is essential for maintaining immune homeostasis. A diverse microbiota ensures the presence of various microbial metabolites, such as SCFAs, that play critical roles in regulating immune cell function and cytokine production. This diversity helps in preventing the overgrowth of pathogenic bacteria that can trigger systemic inflammatory responses [134].

### 6.5. SCFA-mediated immune modulation

Short-chain fatty acids (SCFAs) play a crucial role in immune modulation, serving as metabolic products of gut microbiota fermentation of dietary fibers and prebiotics like chitosan. The three primary SCFAs; acetate, propionate, and butyrate act as signaling molecules that influence both local gut immune responses and systemic immunity. Chitosan's ability to increase the production of these SCFAs is a key mechanism by which it exerts its immunomodulatory effects [134].

Increased SCFA Production through Chitosan Supplementation: Chitosan promotes the growth of beneficial gut bacteria, such as *Bifidobacterium* and *Lactobacillus*, which are known to produce SCFAs. As a prebiotic, chitosan not only enhances microbial diversity but also provides fermentable substrates that the gut microbiota can convert into SCFAs. Studies have shown that chitosan oligosaccharides (COS), due to their smaller molecular size, are more easily fermented by gut bacteria, resulting in higher levels of SCFAs compared to native chitosan. This increase in SCFA levels has profound effects on immune regulation, especially in inflammatory and autoimmune diseases [135].

Butyrate's Role in Immune Modulation: Among the SCFAs, butyrate has the most significant impact on the immune system. Butyrate is a primary energy source for colonocytes, promoting the integrity of the gut barrier. A healthy gut barrier prevents the translocation of harmful pathogens and toxins, reducing the likelihood of systemic inflammation. In addition to its role in gut health, butyrate has been shown to exert direct immunomodulatory effects on immune cells [129].

Butyrate influences the differentiation and function of regulatory T cells (Tregs), which are crucial for maintaining immune tolerance and preventing autoimmunity. Studies have demonstrated that butyrate enhances the production of Tregs by increasing the expression of the transcription factor FOXP3, which is essential for Treg development. These regulatory cells help suppress excessive immune responses, reducing inflammation in autoimmune diseases such as IBD, multiple sclerosis, and type 1 diabetes [120].

Additionally, butyrate inhibits the activation of histone deacetylases (HDACs) in immune cells, leading to changes in gene expression that favor anti-inflammatory responses. This mechanism helps shift the immune system from a pro-inflammatory to an anti-inflammatory state, making butyrate a critical player in controlling immune-mediated tissue damage [136].

Propionate and Acetate in Immune Regulation: While butyrate is particularly known for its potent immunomodulatory effects, propionate and acetate also contribute to immune regulation. Propionate has been shown to inhibit the migration and activation of neutrophils, a type of immune cell involved in inflammation. By reducing neutrophil recruitment to sites of inflammation, propionate helps prevent excessive tissue damage, which is crucial in conditions like IBD and rheumatoid arthritis [137].

Acetate, though less studied, has been found to enhance IgA production in the gut, a crucial component of the mucosal immune system. IgA antibodies play a key role in neutralizing pathogens and preventing their adhesion to the gut epithelium. By boosting IgA production, acetate supports the first line of defense in the gut, enhancing immune protection against infections [51].

Systemic Immune Modulation via SCFAs: SCFAs produced in the gut can enter the bloodstream and exert effects on immune cells in distant tissues, thereby linking gut health to systemic immunity. For example, butyrate has been shown to modulate the function of macrophages and dendritic cells in peripheral tissues, promoting the production of anti-inflammatory cytokines such as IL-10 while inhibiting pro-inflammatory cytokines like TNF-α and IL-6 [138]. This systemic immune modulation is beneficial not only for managing autoimmune diseases but also for enhancing the body's overall immune resilience.

In addition, SCFAs can cross the blood-brain barrier and influence the central nervous system's immune response. This interaction is part of the gut-brain axis, where gut health influences brain function and immune responses in the brain. SCFAs have been implicated in reducing neuroinflammation and improving immune surveillance in neurodegenerative diseases such as multiple sclerosis and Alzheimer's disease [40]. By modulating systemic immunity, chitosan-induced SCFA production has the potential to impact a wide range of disease processes beyond the gut.

Chitosan's ability to enhance SCFA production in the gut is a key mechanism through which it modulates immune responses. The increased levels of butyrate, propionate, and acetate not only strengthen gut barrier integrity but also influence immune cell differentiation, cytokine production, and inflammation regulation [137]. These effects are critical in managing inflammatory diseases such as IBD and autoimmune conditions like rheumatoid arthritis and multiple sclerosis. By linking changes in gut microbiota to systemic immunity, chitosan emerges as a powerful tool in the prevention and management of immune-related disorders.

## 7. Applications in food and pharmaceutical industries

Chitosan has garnered significant attention in both the food and pharmaceutical industries due to its versatile

**Table 14.** Probiotic-enriched foods.

| S/N | Probiotic-Enriched Foods | Mechanism | Example | Advantages |
|---|---|---|---|---|
| 1. | Yogurt and Kefir [143] | In yogurt and kefir production, probiotics are often added to fermented milk products to enhance their health benefits. Bio-polymer coatings help protect these probiotics from the harsh conditions during fermentation and storage, ensuring that they remain alive and active. | Alginate-coated probiotics are commonly used in yogurt and kefir. The alginate coating protects probiotics from the acidic conditions during fermentation and the cold temperatures during storage. This ensures that the probiotics retain their health benefits, such as improving digestion and enhancing the immune system. | **Enhanced Viability**: Bio-polymer coatings help maintain the viability of probiotics during the fermentation process and storage.<br>**Extended Shelf Life**: Protecting probiotics from environmental stresses extends the shelf life of probiotic-enriched products.<br>**Health Benefits**: Probiotic-enriched yogurt and kefir can offer health benefits such as improved gut health, enhanced digestion, and immune support. |
| 2. | Dietary Supplements [144] | In dietary supplements, bio-polymer-coated probiotics are used to create capsules, tablets, or powders that can be consumed for their health benefits. The coating ensures that probiotics survive the manufacturing process and remain effective until ingestion. | Chitosan-coated probiotics are used in dietary supplements to protect them from moisture and oxidation. The chitosan coating helps ensure that the probiotics are delivered to the gut in a viable state, providing benefits such as gut health improvement and immune support. | **Improved Stability**: Bio-polymer coatings protect probiotics from environmental factors that could degrade them.<br>**Controlled Release**: Coatings can be designed for controlled release, ensuring that probiotics are delivered effectively to the target site in the gastrointestinal tract.<br>**Enhanced Efficacy**: Supplements with bio-polymer-coated probiotics can provide more consistent health benefits. |

properties, particularly as a coating material for probiotics [139]. Chitosan's biocompatibility, biodegradability, and antimicrobial activity make it an ideal candidate for protecting probiotics during food processing, storage, and passage through the harsh gastrointestinal (GI) tract [10]. Furthermore, its ability to enhance the stability and viability of probiotics translates into better health outcomes, making it a highly valuable resource in functional food development and pharmaceutical formulations [140].

### 7.1. Probiotic-enriched foods

Bio-polymer-coated probiotics are increasingly used in the food industry to create functional foods that promote gut health [141]. These products leverage the protective capabilities of bio-polymer coatings to ensure that probiotics remain viable and effective throughout the manufacturing process, storage, and consumption [142].

### 7.2. Pharmaceutical applications

Encapsulated probiotics are also used in pharmaceutical formulations, where they offer various health benefits, including immune modulation, gastrointestinal health, and disease prevention [145].

### 7.3. Latest clinical trials and human studies on chitosan-coated probiotics

Chitosan-coated probiotics work to reduce gastrointestinal infections by providing a protective barrier around the probiotics, shielding them from harsh stomach acids and bile, which enhances their survival and activity in the gastrointestinal tract. The chitosan coating also improves the adhesion of probiotics to the intestinal lining, promoting their colonization and activity, while the antimicrobial properties of chitosan can help inhibit the growth of harmful pathogens. This combination enhances the probiotics' ability to balance the gut microbiota, support immune responses, and prevent infection [72].

Recent clinical trials and human studies have focused on assessing the efficacy of chitosan-coated probiotics in promoting gut health, immune function, and even treating gastrointestinal disorders like irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Chitosan coatings help improve the survival of probiotics in low pH environments like the stomach, enabling their safe transit to the intestine, where they can exert their beneficial effects [149].

A 2024 study by [150] evaluated chitosan-coated *Lactobacillus acidophilus* in a randomized clinical trial involving patients with IBS. The results showed that the chitosan coating significantly increased probiotic viability in the gut, leading to improved symptom management, reduced inflammation, and enhanced gut microbiota balance compared to non-coated probiotics. Participants receiving the chitosan-coated probiotics reported higher levels of relief from abdominal pain and bloating, along with a better quality of life after eight weeks of supplementation.

Another trial conducted explored the efficacy of chitosan-coated *Bifidobacterium bifidum* in improving immune function in elderly adults. The results demonstrated that the coated probiotics led to a marked increase in the production of anti-inflammatory cytokines and a decrease in pro-inflammatory markers.

**Table 15.** Pharmaceutical applications.

| S/N | Applications | Mechanism | Example | Advantages |
|---|---|---|---|---|
| 1. | Immune Modulation [146] | Probiotics can influence the immune system by interacting with gut-associated lymphoid tissue (GALT). Bio-polymer coatings help protect these probiotics from the gastrointestinal environment, allowing them to exert their immune-modulating effects. | Gelatin-coated probiotics are used in pharmaceutical formulations to enhance their stability and efficacy. These probiotics can help modulate immune responses, potentially reducing the risk of infections and inflammatory conditions. | **Enhanced Immune Function**: Probiotics can improve immune responses and help prevent infections and diseases.<br>**Consistent Efficacy**: Bio-polymer coatings ensure that probiotics remain effective throughout the gastrointestinal tract. |
| 2. | Gastrointestinal Health [147] | Probiotics help maintain a healthy balance of gut microbiota, which is crucial for digestive health. Bio-polymer coatings protect probiotics from degradation and ensure their delivery to the intestines. | Pectin-coated probiotics are used in pharmaceutical formulations to promote gastrointestinal health. These coatings ensure that probiotics survive transit through the digestive system and reach the intestines where they can enhance gut health. | **Improved Digestive Health**: Probiotics can alleviate symptoms of gastrointestinal disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).<br>**Effective Delivery**: Bio-polymer coatings enhance the delivery and stability of probiotics, maximizing their health benefits. |
| 3. | Disease Prevention [148] | Probiotics have been studied for their potential role in preventing various diseases, including infections, allergies, and even some cancers. Encapsulation with bio-polymers helps maintain the viability of probiotics, allowing them to exert their preventative effects. | Alginate-chitosan composite coatings are used to enhance the stability and effectiveness of probiotics in disease prevention applications. | **Potential Disease Prevention**: Probiotics may offer protective effects against certain diseases.<br>**Stable Formulation**: Bio-polymer coatings ensure that probiotics remain viable and effective, contributing to disease prevention. |

These findings align with earlier in vitro studies showing that chitosan's interaction with probiotics can modulate the immune system, offering therapeutic potential for elderly populations with compromised immunity [151].

In pediatric populations, a clinical trial examined the effect of chitosan-coated probiotics in reducing the incidence of gastrointestinal infections. Children receiving these chitosan-coated probiotic supplements experienced fewer episodes of diarrhea and showed faster recovery from GI infections compared to the control group. These findings highlight the potential of chitosan-coated probiotics as preventive and therapeutic agents, particularly in vulnerable populations such as children and the elderly [152].

#### *7.3.1. Practical applications in the food industry*

In the food industry, the encapsulation of probiotics with chitosan is widely employed to enhance the stability and viability of probiotics in functional foods such as yogurts, cheeses, and fermented beverages. Probiotics are sensitive to environmental stressors like temperature, moisture, and pH fluctuations, which can significantly reduce their efficacy when incorporated into food products. Chitosan coating provides a protective barrier, ensuring that probiotics maintain their viability throughout food processing, packaging, and shelf life [4].

Recent innovations have further improved the application of chitosan-coated probiotics in foods. For instance, advanced microencapsulation techniques using chitosan in combination with other biopolymers, such as alginate or pectin, have shown promising results in extending the shelf life of probiotic-enriched products [22]. A 2021 study demonstrated that combining chitosan with alginate for probiotic encapsulation in dairy products resulted in a 50% increase in probiotic survival rates after 30 days of storage, compared to probiotics encapsulated with chitosan alone. This synergistic approach has practical implications for the functional food market, where probiotic longevity is crucial for product efficacy and consumer satisfaction [153].

Chitosan also offers antimicrobial properties that enhance food safety by inhibiting the growth of pathogenic bacteria such as *Escherichia coli* and *Salmonella* in probiotic-enriched foods. This dual functionality protecting probiotics while safeguarding the food from contamination makes chitosan-coated probiotics especially valuable in ensuring the quality and safety of perishable food items [154].

#### *7.3.2. Practical applications in the pharmaceutical industry*

In the pharmaceutical industry, chitosan-coated probiotics are being explored for their therapeutic potential in treating gut-related diseases and supporting overall health. One of the main challenges in probiotic delivery is ensuring that sufficient numbers of live probiotics reach the intestine to exert their beneficial effects [155]. The harsh acidic environment of the stomach and exposure to bile salts in the small intestine can kill or damage many probiotics before they reach their target site. Chitosan coatings address this issue by forming a protective layer around probiotic cells, enabling their survival during GI transit.

In the context of IBD and other inflammatory conditions, chitosan-coated probiotics have shown promising results. In a 2022 study, Gallo et al. evaluated the use of chitosan-coated *Lactobacillus plantarum* in an animal model of colitis. The study found that the chitosan coating significantly improved the anti-inflammatory properties of the probiotic, leading to a reduction in intestinal inflammation and mucosal damage. These preclinical findings are now being explored in human trials, with preliminary results indicating similar benefits in patients with mild-to-moderate IBD [5].

Chitosan-coated probiotics are also being explored in personalized medicine. Recent advancements in the field suggest that tailoring chitosan formulations to the specific gut microbiota profile of individuals could optimize probiotic efficacy. A 2024 demonstrated that personalized chitosan-coated probiotic formulations based on individual microbiota composition led to superior outcomes in terms of immune modulation and gut health improvement, compared to standardized probiotic formulations [156].

#### *7.3.3. Comparison of in vitro and in vivo findings*

Comparing in vitro and in vivo findings reveals some key differences, but also important synergies. In vitro studies have consistently shown that chitosan coatings protect probiotics from acidic conditions and bile salts, enhancing their viability. However, translating these findings to in vivo conditions often introduces additional variables, such as individual differences in gut microbiota composition and host immune responses [22]. In vivo studies have demonstrated that while chitosan coatings do improve probiotic survival and efficacy in humans and animals, the extent of this benefit can vary based on factors such as diet, health status, and microbial diversity.

For example, while in vitro studies on chitosan-coated *Lactobacillus rhamnosus* showed significant protection from acidic environments, an in vivo study in healthy adults found that the survival rates of the probiotics varied between participants. The variability in probiotic efficacy was linked to individual gut microbiota profiles, suggesting that in vivo outcomes can be influenced by host factors that cannot be fully replicated in vitro [157].

Nonetheless, both in vitro and in vivo research confirms that chitosan-coated probiotics offer substantial improvements in probiotic viability and function, with promising implications for health applications. As research advances, the development of more personalized and targeted chitosan-coated probiotic formulations could further bridge the gap between laboratory findings and real-world efficacy [158].

The application of chitosan in the food and pharmaceutical industries, particularly as a coating material for probiotics, holds significant potential for enhancing probiotic viability, stability, and efficacy. Recent clinical trials and human studies have demonstrated the real-world benefits of chitosan-coated probiotics in promoting gut health, reducing inflammation, and preventing infections [22]. Both the food and pharmaceutical sectors are increasingly adopting chitosan-based technologies to improve probiotic delivery, with promising results that suggest wide-ranging benefits for public health. The comparison of in vitro and in vivo findings underscores the importance of continued research to optimize these formulations for maximum efficacy in practical applications.

### 7.4. Challenges in industrial applications

Bio-polymer-coated probiotics have significant applications in both the food and pharmaceutical industries [161] (Tables 14–16). They are used in creating probiotic-enriched foods and dietary supplements that promote gut health, as well as in pharmaceutical formulations for immune modulation, gastrointestinal health, and disease prevention [41]. However, scaling up bio-polymer encapsulation methods presents challenges such as cost, regulatory approval, and ensuring consistent probiotic viability [162]. Addressing these challenges is essential for advancing the application of bio-polymer-coated probiotics and meeting the growing demand for functional foods and therapeutic products [163].

## 8. Sustainability & gut-brain axis

### 8.1. Sustainability of chitosan sources and environmental impact

Chitosan, a natural biopolymer, is primarily derived from chitin, which is abundantly found in the exoskeletons of crustaceans such as shrimp, crabs, and lobsters. This makes chitosan a sustainable resource, as its production typically utilizes waste materials from seafood processing industries [104]. The large volumes of shellfish waste generated worldwide have spurred interest in extracting valuable by-products like chitosan, transforming what would otherwise be a disposal problem into an environmentally friendly solution [164].

### 8.2. Factors that underscore the sustainability of chitosan

Resource Utilization: Chitosan production repurposes shellfish waste, reducing the environmental burden of seafood processing. On a larger scale, fungal-derived chitosan is generally more cost-effective compared to marine-derived chitosan. Fungal chitosan is produced from easily cultivated fungi like *Mucor* species, which have faster growth rates and lower cultivation costs, making it more affordable to produce. In contrast, marine-derived chitosan, sourced from crustacean shells (such as shrimp and crab), often incurs higher costs due to the extraction process, which involves collection, processing, and purification from shellfish, as well

**Table 16.** Scaling up bio-polymer encapsulation methods.

| S/N | Criteria | Challenge | Solutions |
|---|---|---|---|
| 1. | Cost [159] | Scaling up bio-polymer encapsulation methods for industrial applications can be expensive due to the costs of high-quality biopolymers, specialized equipment, and quality control measures. | **Cost-Effective Materials**: Research into more cost-effective biopolymers or alternative materials could help reduce costs.<br>**Optimized Processes**: Streamlining encapsulation processes and improving efficiency can help lower production costs. |
| 2. | Regulatory Approval [149] | Obtaining regulatory approval for bio-polymer-coated probiotic products involves meeting stringent safety and efficacy standards set by regulatory agencies. | **Rigorous Testing**: Conducting thorough safety and efficacy testing to meet regulatory requirements.<br>**Compliance with Standards**: Ensuring that bio-polymer coatings and probiotic formulations comply with relevant regulations and standards. |
| 3. | Ensuring Consistent Probiotic Viability [160] | Maintaining consistent probiotic viability throughout the manufacturing process, storage, and distribution is crucial for ensuring the effectiveness of probiotic products. | **Quality Control**: Implementing rigorous quality control measures to monitor and ensure the stability and viability of probiotics.<br>**Advanced Encapsulation Techniques**: Developing advanced encapsulation techniques and formulations to enhance the stability and consistency of probiotics. |

as seasonal variability. Additionally, marine chitosan may have environmental and sustainability concerns due to the reliance on marine resources, which further contributes to higher production costs [165].

According to a study, the global seafood industry produces over 6 million tons of shell waste annually, which can be converted into chitosan, alleviating waste management challenges and promoting a circular economy. This extraction process is relatively low-cost, especially in coastal regions with abundant seafood processing operations, making chitosan a renewable and accessible resource [166].

Eco-Friendly Production Processes: Modern chitosan extraction techniques have shifted towards more eco-friendly methods, reducing the use of harsh chemicals traditionally involved in the deacetylation of chitin to chitosan. A study demonstrated a greener extraction process using enzyme-assisted techniques, which not only lower the environmental footprint but also improve the quality of the chitosan produced. This has led to reduced energy consumption, lower carbon emissions, and minimized waste in the production chain [167].

Biodegradability: Chitosan is highly biodegradable and non-toxic, making it a suitable material for applications where environmental sustainability is critical. Unlike synthetic polymers, chitosan decomposes naturally, with minimal environmental impact. This characteristic is especially beneficial when considering its applications in food packaging and pharmaceuticals, as products utilizing chitosan coatings do not contribute to long-term plastic pollution. A 2021 report by [51] highlighted that chitosan-based bioplastics, used for food packaging, fully decompose within months under typical environmental conditions.

Marine-Sourced vs. Fungal-Sourced Chitosan: While marine-derived chitosan remains the most common, there is a growing interest in fungal-derived chitosan as a more sustainable alternative. Fungal chitosan, derived from the cell walls of certain fungi, bypasses the need for marine harvesting, potentially reducing the environmental impact on marine ecosystems [168]. This alternative source is gaining traction as a vegan-friendly and ecologically responsible option for industries aiming to reduce their reliance on animal-derived materials. A study by found that fungal chitosan offers similar physicochemical properties to marine-derived chitosan, with the added benefit of being produced under more controlled and sustainable conditions, minimizing ecological impact [169].

Despite its advantages, chitosan production does face challenges, particularly around scaling up for industrial use and ensuring consistency in quality. As demand for chitosan increases, especially in pharmaceutical and food packaging applications, more sustainable and efficient extraction methods must be developed to mitigate potential environmental impacts, such as overfishing or unsustainable shellfish farming. Research into alternative sources, such as fungal chitosan, and optimizing production processes will be critical in maintaining chitosan's status as a sustainable biopolymer [170]. Studies on the long-term effects of chitosan consumption on mental health in mice are limited, but some research suggests that chitosan may have a positive impact on mental health through its effects on gut microbiota and inflammation. Since chitosan can modulate gut bacteria and reduce gut permeability, it may indirectly influence the gut-brain axis, potentially alleviating symptoms of anxiety or depression [171]. However, there are concerns that prolonged chitosan consumption could affect nutrient absorption, which might lead to deficiencies that could negatively impact brain function over time. More research is needed to definitively determine the long-term mental health effects of chitosan in animal models [172].

### 8.3. *Chitosan's role in the gut-brain axis*

In recent years, the gut-brain axis, a complex communication network between the gastrointestinal (GI) tract and the central nervous system (CNS); has emerged as a critical area of research in understanding the link between gut health and mental well-being. This axis relies heavily on the gut microbiota, which play a central role in modulating mood, cognition, and behavior through the production of neurotransmitters, metabolites, and immune signaling molecules. Chitosan, due to its prebiotic and synbiotic properties, is now being investigated for its potential role in influencing the gut-brain axis and, by extension, mental health outcomes [173].

Modulation of Gut Microbiota: Chitosan has demonstrated the ability to alter the composition of the gut microbiota, promoting the growth of beneficial bacteria like *Lactobacillus* and *Bifidobacterium* while inhibiting pathogenic bacteria. This microbial modulation is significant because gut dysbiosis (an imbalance in gut microbiota) has been linked to mental health disorders such as anxiety, depression, and even neurodegenerative diseases like Parkinson's and Alzheimer's. By fostering a more balanced and healthy microbiome, chitosan may play an indirect but crucial role in regulating mood and cognitive function [174]. The impact of chitosan on gut microbiota diversity and SCFA production is highly dose-dependent. At low dietary concentrations, chitosan supports microbial diversity by promoting the growth of beneficial bacteria such as *Lactobacillus* and

*Bifidobacterium*, which ferment fibers and chitosan to produce SCFAs like acetate, propionate, and butyrate, essential for gut health and reducing inflammation. Moderate concentrations further boost SCFA production while maintaining a healthy microbiota balance, as chitosan's antimicrobial effects effectively suppress pathogens without significantly affecting beneficial microbes [175]. However, at high concentrations, chitosan's strong antimicrobial activity may reduce microbiota diversity, inhibiting both harmful and beneficial bacteria, leading to lower SCFA production and potential dysbiosis. Therefore, optimizing chitosan dosage is crucial to harness its benefits while avoiding adverse effects on gut health [57].

A 2022 study by [114] explored the effect of chitosan-enriched diets on gut microbiota in mice. The study found that chitosan increased the abundance of *Lactobacillus plantarum*, a probiotic known for its role in producing gamma-aminobutyric acid (GABA), a neurotransmitter that reduces neuronal excitability and is associated with feelings of calm and reduced anxiety. Mice fed with chitosan exhibited reduced anxiety-like behaviors, suggesting that chitosan-mediated changes in the gut microbiota can positively influence mental states.

Production of Short-Chain Fatty Acids (SCFAs): Chitosan also influences the production of short-chain fatty acids (SCFAs), which are microbial metabolites with known roles in both gut and brain health. SCFAs, particularly butyrate, propionate, and acetate, can cross the blood-brain barrier and modulate inflammation and neurotransmission. Butyrate, for example, has been shown to possess neuroprotective properties and can regulate the expression of brain-derived neurotrophic factor (BDNF), a key molecule involved in neuroplasticity and cognitive function [107].

A 2024 study by [176] demonstrated that chitosan supplementation in rats led to increased levels of SCFAs in the gut, particularly butyrate, which was associated with improved cognitive function and reduced markers of neuroinflammation. This highlights the potential of chitosan to influence mental health through its effects on SCFA production, linking gut microbial activity to brain health via the gut-brain axis. Chitosan's influence on neurotransmitter systems in individuals with depressive disorders is likely to vary due to differences in gut microbiota composition, metabolic activity, and the underlying causes of depression [177]. Chitosan can modulate the gut microbiota, which in turn affects the gut-brain axis and neurotransmitter production, such as serotonin, dopamine, and gamma-aminobutyric acid (GABA). Variations in individual microbiota profiles may result in different levels of neurotransmitter precursors being available. Additionally, genetic, environmental, and dietary factors that influence both gut health and neurotransmitter pathways could lead to varying responses to chitosan among individuals with depressive disorders [176]. This suggests that personalized approaches are necessary when considering chitosan as a potential adjunct for mood modulation.

Inflammation and the Gut-Brain Connection: Inflammation, both systemic and localized in the gut, plays a pivotal role in the pathogenesis of mood disorders and cognitive decline. The gut microbiota, influenced by dietary components like chitosan, modulate inflammatory pathways, including the production of pro-inflammatory cytokines. Chitosan's known anti-inflammatory properties in the gut may thus have downstream effects on brain inflammation, helping to prevent or alleviate mental health conditions like depression, which are often characterized by chronic low-grade inflammation [177].

A 2024 study by [178] investigated the impact of chitosan on systemic inflammation in a model of depression in mice. The researchers found that chitosan supplementation reduced levels of circulating pro-inflammatory cytokines like IL-6 and TNF-α, which are often elevated in individuals with depressive disorders. These anti-inflammatory effects were attributed to chitosan's ability to improve gut barrier integrity and prevent translocation of inflammatory molecules from the gut to the bloodstream, a process known as 'leaky gut.' By stabilizing gut health and reducing systemic inflammation, chitosan may play a crucial role in mitigating inflammation-related mental health conditions.

Neurotransmitter Regulation: Emerging research suggests that chitosan may directly or indirectly influence neurotransmitter systems, particularly serotonin, which is heavily produced in the gut and plays a critical role in mood regulation. The gut microbiota help regulate serotonin synthesis by influencing tryptophan metabolism, and by promoting a healthy microbial environment, chitosan could contribute to more balanced serotonin levels. This link between gut microbiota, chitosan, and serotonin production is an exciting area of research for potential treatments for mood disorders like depression and anxiety [179].

A 2024 study by [180] on the role of chitosan in serotonin regulation demonstrated that chitosan supplementation in rodents led to increased serotonin levels in the hippocampus, a brain region crucial for mood regulation and cognitive function. These effects were mediated by changes in gut microbial composition and function, particularly through the enhancement of tryptophan availability for serotonin synthesis.

Chitosan's sustainability, largely driven by its origin in seafood waste and potential fungal sources, positions it as an environmentally friendly biopolymer with far-reaching applications. Its eco-friendly production processes, biodegradability, and potential to repurpose waste products align with global efforts towards sustainability in industrial practices [167].

In addition, the novel concept of chitosan's role in the gut-brain axis is gaining traction. Through its prebiotic effects, modulation of SCFA production, anti-inflammatory properties, and potential impact on neurotransmitter systems, chitosan appears to be a promising candidate for influencing mental health outcomes [181]. Continued research is necessary to fully elucidate the mechanisms by which chitosan impacts the gut-brain axis and to explore its potential applications in both preventative and therapeutic contexts for mental health disorders.

## 9. Challenges, future directions and future research needs

### 9.1. Challenges and future directions

While there are significant advancements in bio-polymer coatings for probiotic microencapsulation, challenges such as ensuring consistent release, impacts of manufacturing processes, and variability in probiotic strains need to be addressed [100] (Tables 17–19). Future research should focus on exploring new bio-polymers, optimizing combination coatings, and developing advanced controlled release systems. Additionally, the potential for personalized probiotic therapies offers an exciting direction for future research, with the promise of tailored treatments that cater to individual health needs and gut microbiota profiles [190].

### 9.2. Future research needs: personalized probiotic therapies and novel bio-polymers & encapsulation techniques

The potential of chitosan as a versatile biopolymer for probiotic encapsulation, gut health modulation, and therapeutic applications is well-established. However, several gaps in the research landscape remain, particularly in the areas of personalized probiotic therapies, novel bio-polymers, and advanced encapsulation techniques. Addressing these areas will help unlock the full potential of chitosan and similar materials for tailored health interventions and broader industrial applications [17].

#### 9.2.1. Personalized probiotic therapies

Individual Variability in Microbiota: One of the most significant challenges in the application of probiotics and prebiotics like chitosan is the variability in individual microbiomes. Each person's gut microbiota is unique, shaped by factors such as genetics, diet, lifestyle, and environmental exposures. As a result, the same probiotic

Table 17. Limitations in current encapsulation techniques.

| S/N | Limitation | Challenge | Impact | Solutions |
|---|---|---|---|---|
| **1.** | Ensuring Consistent Release [182] | Achieving consistent release of probiotics from bio-polymer coatings remains a significant challenge. Variability in release profiles can occur due to differences in the thickness or uniformity of the coating, the type of bio-polymer used, or the conditions within the gastrointestinal tract. | Inconsistent release can lead to either premature release in the upper gastrointestinal tract or incomplete release in the targeted areas, reducing the effectiveness of probiotics. | **Enhanced Formulation**: Developing more precise and uniform coating techniques to ensure consistent release.<br>**Advanced Testing**: Implementing rigorous testing protocols to assess and optimize release profiles across different conditions. |
| **2.** | Impact of Manufacturing Processes [183] | The manufacturing processes for producing bio-polymer-coated probiotics can affect the quality and viability of the final product. Factors such as heat, pressure, and mechanical stress during production can impact the stability of probiotics and the integrity of the coatings. | Variations in manufacturing processes can lead to inconsistencies in product quality and efficacy. | **Process Optimization**: Refining manufacturing processes to minimize stress on probiotics and coatings.<br>**Quality Control**: Implementing stringent quality control measures throughout the production process to ensure product consistency. |
| **3.** | Variability in Probiotic Strains [184] | Different probiotic strains may have varying sensitivities to encapsulation techniques and environmental conditions. This variability can affect the overall efficacy of probiotic products. | Strain-specific differences can result in uneven performance and health benefits across different probiotic formulations. | **Strain-Specific Research**: Conducting detailed research on the specific needs and characteristics of different probiotic strains to tailor encapsulation strategies.<br>**Customization**: Developing encapsulation methods that can be adapted for various probiotic strains to ensure consistent performance. |

**Table 18.** Future research directions.

| S/N | Directions | Potential | Focus Areas |
|---|---|---|---|
| **1.** | Exploring New Bio-Polymers [185] | Research into new and innovative bio-polymers could lead to the development of more effective and versatile encapsulation materials. New bio-polymers may offer improved biocompatibility, controlled release properties, and enhanced stability. | **Novel Materials**: Investigating natural and synthetic polymers with unique properties for probiotic encapsulation.<br>**Sustainability**: Exploring bio-polymers that are environmentally friendly and sustainable. |
| **2.** | Optimizing Combination Coatings [186] | Combining different bio-polymers in encapsulation coatings can provide synergistic benefits, such as improved stability, enhanced release control, and better protection against environmental stresses. | **Hybrid Materials**: Developing hybrid coatings that combine the strengths of multiple bio-polymers.<br>**Customization**: Tailoring combination coatings for specific probiotic strains and applications. |
| **3.** | Developing Advanced Controlled Release Systems [187] | Advancements in controlled release systems can improve the precision and effectiveness of probiotic delivery. Innovative techniques may include stimuli-responsive systems, such as those triggered by pH, temperature, or enzymatic activity. | **Smart Delivery Systems**: Creating encapsulation systems that respond to specific physiological conditions for targeted release.<br>**Nanotechnology**: Leveraging nanotechnology to enhance the stability and bioavailability of probiotics. |

**Table 19.** Potential for personalized probiotic therapies.

| S/N | Therapy | Potential | Focus Areas |
|---|---|---|---|
| **1.** | Personalized Probiotic Treatments [188] | Personalized medicine, including personalized probiotic therapies, holds promise for addressing individual health needs based on specific gut microbiota profiles. Tailored probiotic treatments can be designed to target individual health conditions more effectively. | **Gut Microbiota Profiling**: Developing techniques to analyze individual gut microbiota profiles for personalized probiotic recommendations.<br>**Customized Encapsulation**: Designing bio-polymer coatings that are tailored to the specific needs of individual patients, based on their gut microbiota and health conditions. |
| **2.** | Tailored Encapsulation Strategies [189] | Personalized encapsulation strategies can enhance the effectiveness of probiotic therapies by addressing individual variations in gastrointestinal conditions and probiotic needs. | **Personalized Coatings**: Creating bio-polymer coatings that are optimized for individual gut conditions and probiotic requirements.<br>**Targeted Health Benefits**: Developing encapsulation methods that provide targeted health benefits based on personalized health assessments. |

strain or prebiotic compound may have different effects across individuals [157]. Future research should prioritize the development of personalized probiotic therapies, where the selection of probiotic strains or prebiotics like chitosan is tailored to an individual's specific microbiome composition. This approach could optimize the efficacy of probiotics, reduce side effects, and enhance therapeutic outcomes in both preventive health and disease management.

Personalized therapies would require advancements in microbiome profiling technologies. Techniques such as 16S rRNA sequencing and metagenomics are already being used to map microbial communities, but more affordable and accessible tools will be necessary to make personalized probiotic therapies widely available. Furthermore, clinical trials should aim to stratify participants based on their microbiome composition to better understand how different populations respond to chitosan-coated probiotics and synbiotics [191].

Targeted Probiotic Strains: There is growing interest in developing probiotic strains that target specific diseases or health conditions, such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and metabolic disorders like obesity and diabetes. Recent studies suggest that probiotics can exert a range of effects on the host, from improving gut barrier function to modulating immune responses. However, many current therapies use a 'one-size-fits-all' approach [41]. The future of probiotic research must focus on designing probiotics that are tailored to specific health needs, taking into account individual microbiome compositions and disease states. This can be achieved through synthetic biology approaches that engineer probiotics to produce specific bioactive molecules or modulate key metabolic pathways.

For example, engineered strains of *Lactobacillus* or *Bifidobacterium* could be combined with chitosan encapsulation techniques to ensure the targeted delivery of therapeutic compounds to specific areas of the gastrointestinal tract. These customized probiotics could be especially beneficial for conditions like IBD or Crohn's disease, where local modulation of inflammation is critical [149].

Role of the Gut-Brain Axis in Personalized Probiotic Therapies: As research on the gut-brain axis evolves, there is a growing need to understand how personalized probiotic therapies can influence mental health outcomes. Different strains of probiotics affect the production of neurotransmitters and short-chain fatty acids

(SCFAs) in the gut, which in turn have systemic effects, including modulation of brain function. Personalized probiotic therapies targeting the gut-brain axis could help manage mental health conditions such as anxiety, depression, and even neurodegenerative diseases [60]. Future research should investigate how chitosan-encapsulated probiotics, tailored to individual microbiome compositions, could optimize mental health interventions, especially for mood and cognitive disorders.

### 9.2.2. Novel bio-polymers and encapsulation techniques

Development of New Bio-Polymers: While chitosan has demonstrated significant potential in the encapsulation of probiotics due to its biocompatibility, biodegradability, and antimicrobial properties, there is still room to explore novel bio-polymers with enhanced functionalities. Future research should focus on developing bio-polymers that improve upon chitosan's limitations, such as its solubility at certain pH levels and sensitivity to environmental conditions like moisture and temperature [24]. These bio-polymers should be designed to offer enhanced protective barriers for probiotics, ensuring that they remain viable throughout the shelf life of the product and can withstand the harsh conditions of the gastrointestinal tract [1].

Some promising candidates for novel bio-polymers include alginate, pectin, xanthan gum, and pullulan, all of which have demonstrated potential in encapsulating probiotics. These materials, either used alone or in combination with chitosan, could provide enhanced protective capabilities, especially under varying pH conditions in the stomach and intestines [31]. Combinatory bio-polymer systems could offer synergistic effects, combining the strengths of multiple bio-polymers to improve stability, controlled release, and functional performance.

Multi-Layer Encapsulation Techniques: Current encapsulation methods often rely on a single protective layer, which may not always provide sufficient protection for probiotics during digestion or industrial processing. Future research should focus on multi-layer encapsulation techniques, where probiotics are coated with multiple layers of bio-polymers with different functional properties [10]. For example, an outer layer could be designed to withstand gastric acidity, while an inner layer could be tailored to degrade in response to intestinal pH or the presence of specific enzymes, ensuring that the probiotics are released at the optimal location in the gut.

Multi-layer encapsulation could also incorporate bioactive molecules like antioxidants, prebiotics, or anti-inflammatory agents into the layers, offering additional health benefits. A study by [10] highlighted the potential of combining chitosan with alginate in a double-layer encapsulation system, which provided enhanced protection to *Bifidobacterium* strains during simulated gastrointestinal transit. Such innovations could pave the way for more robust and effective probiotic delivery systems.

Smart Encapsulation Systems: Another area ripe for exploration is the development of smart encapsulation systems that respond to specific physiological cues. These systems could be designed to release probiotics in response to changes in pH, temperature, or the presence of certain metabolites or enzymes in the gut. For instance, a chitosan-based smart system could be engineered to degrade in response to microbial activity, ensuring that the probiotics are released only when they reach the appropriate part of the gastrointestinal tract [192]. These systems could be particularly useful for treating diseases like IBD, where targeted delivery to inflamed areas is crucial.

Smart encapsulation systems could also incorporate biosensors that detect changes in gut microbiota composition or health status. In this way, the system could adjust the release of probiotics based on the individual's current gut health, providing a more dynamic and personalized approach to probiotic therapy [1].

Scaling Up for Industrial Application: While many encapsulation techniques show promise in laboratory settings, translating these methods to industrial scales remains a challenge. Future research must address the scalability of novel bio-polymer and encapsulation techniques to ensure that they can be efficiently produced at large volumes while maintaining quality and cost-effectiveness [193]. Studies should focus on optimizing production processes, minimizing energy and resource consumption, and ensuring that encapsulated probiotics can be integrated into various food matrices without compromising their viability or functional properties.

Clinical Trials for Efficacy and Safety: While chitosan and other bio-polymers have shown promise in protecting probiotics, more clinical trials are needed to assess the efficacy and safety of these encapsulation systems in real-world settings. Studies should compare in vitro results with in vivo findings to determine how well encapsulated probiotics survive gastrointestinal transit and colonize the gut in human subjects. These trials should also investigate potential side effects, such as allergic reactions or unwanted interactions with other medications, and explore how encapsulated probiotics impact long-term gut health and immune function [20].

Bio-Polymer Degradation and Environmental Impact: While chitosan and other bio-polymers are biodegradable, their degradation rates and environmental impact when used at scale need further study. Future research

**Table 20.** Novelty focus.

| S/N | Novelty Focus | Description |
|---|---|---|
| 1. | Integration of New Bio-Polymers with Chitosan for Synergistic Effects [1] | The integration of new bio-polymers with chitosan represents a cutting-edge approach to enhancing probiotic microencapsulation. Combining chitosan with other bio-polymers can leverage the unique properties of each material to create synergistic effects that improve encapsulation efficiency and functionality. For instance, the incorporation of alginate, with its excellent gel-forming abilities, with chitosan can yield composite coatings that provide enhanced protection against environmental stresses and better controlled release of probiotics. Similarly, combining chitosan with pectin, known for its ability to form stable gels and target the colon, can offer a dual-action encapsulation system that optimizes both stability and targeted delivery. Research into novel bio-polymers such as polysaccharides derived from seaweeds or agricultural by-products may also uncover new combinations with chitosan that enhance its performance. These innovative combinations can address specific challenges such as improving probiotic viability under various conditions, extending shelf life, and ensuring effective delivery to targeted sites within the gastrointestinal tract. |
| 2. | Exploration of Innovative Encapsulation Technologies [10] | Innovative encapsulation technologies, such as nanotechnology and layer-by-layer (LbL) coating techniques, are revolutionizing the field of probiotic microencapsulation. Nanotechnology enables the creation of nanoscale encapsulation systems that offer superior stability and bioavailability of probiotics. Nanoparticles or nanocapsules can protect probiotics from environmental factors and provide precise control over their release. For example, chitosan nanoparticles can be engineered to encapsulate probiotics with enhanced stability and controlled release profiles.<br>Layer-by-layer (LbL) assembly, an advanced coating technique, involves the sequential deposition of alternating layers of bio-polymers onto a probiotic core. This method allows for the creation of multilayered coatings with tailored properties, such as improved protection, controlled release, and targeted delivery. The LbL technique can be used to develop coatings that respond to specific physiological conditions, such as pH changes or enzymatic activity, to release probiotics at the optimal site within the gastrointestinal tract. |
| 3. | Development of Advanced Controlled Release Mechanisms [196] | The development of advanced controlled release mechanisms is critical for enhancing probiotic delivery and efficacy. Traditional encapsulation methods often face limitations in providing precise control over release rates and targeting specific areas of the gastrointestinal tract. Innovative controlled release systems, including stimuli-responsive coatings and smart delivery systems, offer promising solutions.<br>Stimuli-responsive coatings can be engineered to release probiotics in response to specific triggers, such as pH changes, temperature variations, or enzymatic activity. For instance, pH-sensitive coatings can protect probiotics from acidic conditions in the stomach and release them in the more neutral environment of the intestines. Temperature-sensitive coatings can respond to the body's internal temperature, providing targeted delivery based on physiological conditions.<br>Smart delivery systems, utilizing technologies such as microencapsulation combined with responsive materials, can offer tailored release profiles that align with individual digestive needs. These systems can be designed to ensure that probiotics are released at optimal times and locations within the gastrointestinal tract, maximizing their health benefits and improving overall therapeutic outcomes. |

should investigate how bio-polymer coatings behave in different environments, such as in water or soil, to ensure that they do not contribute to pollution or harm ecosystems [194]. As demand for sustainable materials grows, it will be important to develop bio-polymers that are not only effective but also environmentally friendly, minimizing waste and resource consumption throughout their life cycle.

The future of chitosan and bio-polymer research lies in the development of personalized probiotic therapies that take individual microbiomes into account, as well as the creation of novel bio-polymers and encapsulation techniques that enhance the stability, efficacy, and targeted delivery of probiotics. Advances in microbiome profiling, synthetic biology, and smart encapsulation systems will be key to optimizing probiotic therapies for specific health conditions, including those related to the gut-brain axis [31]. Additionally, ensuring the scalability and environmental sustainability of these technologies will be critical for their successful integration into food, pharmaceutical, and health industries. By addressing these research needs, we can unlock the full potential of chitosan and bio-polymers in promoting gut health, supporting disease treatment, and improving overall well-being [195].

## 10. Novelty focus

The integration of new bio-polymers with chitosan, exploration of innovative encapsulation technologies, and development of advanced controlled release mechanisms represent key areas of novelty in probiotic microencapsulation [197] (Table 20). These advancements promise to enhance the stability, release, and efficacy of probiotic formulations, addressing current challenges and meeting the growing demand for effective functional foods and therapeutic products [198].

## 11. Conclusion

Significant advancements have been made in the field of bio-polymer coatings for probiotic microencapsulation, with chitosan emerging as a key material due to its biocompatibility, antimicrobial properties, and ability to form robust, flexible films. Chitosan has been shown to directly influence gut microbiota by promoting beneficial bacteria while suppressing pathogens, enhancing overall gut health and exhibiting prebiotic and synbiotic effects, particularly with probiotics such as Lactobacillus and Bifidobacterium, which may alleviate gastrointestinal disorders like inflammatory bowel disease (IBD). Furthermore, chitosan's immunomodulatory properties are increasingly recognized for their role in improving systemic immunity, particularly in contexts of autoimmune diseases and IBD, where it influences immune signaling pathways and alters gut microbiota composition, subsequently affecting the production of short-chain fatty acids (SCFAs) that regulate inflammation and support mucosal integrity. Recent studies in food and pharmaceutical industries underscore the efficacy of chitosan-coated probiotics in clinical trials, revealing promising in vitro and in vivo results that warrant further large-scale investigations to validate these findings for practical applications in functional foods and medical treatments. Sustainability considerations regarding the sourcing of chitosan from environmentally friendly materials are essential for its long-term industrial application, while emerging research on the gut-brain axis highlights the potential for chitosan to influence mental health outcomes through microbial interactions, paving the way for future exploration in this area. Continued research should focus on personalized probiotic therapies and novel bio-polymers and encapsulation techniques, with innovations in multi-layered encapsulation, smart-release systems, and biosensors crucial for tailoring probiotics to individual microbiomes and enhancing therapeutic efficacy. Overall, while significant progress has been made in optimizing chitosan and bio-polymer coatings, ongoing innovation is vital to meet the growing demand for functional foods and personalized probiotics, ensuring that these products effectively contribute to health, wellness, and disease prevention.

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## ORCID

Great Iruoghene Edo http://orcid.org/0000-0002-2048-532X

## Author's contributions

GE, AM, NR, EU, TG, EI, UI, PA, RO, AE, DA, HU, DO were responsible for the conception and design of the study; GE, AM performed data collection. GE, AM performed data analysis and drafted the article. GE supervised the study, contributed to data analysis, interpretation, and critical revisions. All authors approved the final manuscript.

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