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Advances in food-grade hydrogel encapsulation of probiotics with next-generation prebiotics for targeted synbiotic delivery

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ABSTRACT

Background. Hydrogel-based encapsulation represents a successful method for preserving probiotics and their functionality during processing, storage, and gastrointestinal transit. Although conventional systems offer protection, advances in next-generation prebiotics such as polyphenols and non-digestible carbohydrates have shifted the focus toward multifunctional synbiotic delivery systems. These materials provide sustainable, food-grade design and improve both structural integrity and bioactivity. Additionally, fabrication and crosslinking methods such as ionic interactions, Maillard conjugation, and enzymatic or pH-responsive techniques facilitate precise hydrogel customization for targeted probiotic release. Scope and approach. This review discusses recent progress in natural and composite hydrogels for probiotic encapsulation, with particular attention to the influence of next generation prebiotics on hydrogel functionality. A decision-oriented design framework is presented, aligning target sites and release triggers with materials and crosslinking strategies, exemplified with quantitative results. This framework offers a systematic approach for selecting food-grade matrices and encapsulation methods. Key findings and conclusions. Natural and composite hydrogels provide effective protection against oxygen, heat, acidity, bile salts, and digestive enzymes, maintaining a hydrated and biocompatible microenvironment. Protein–polysaccharide combinations increase mechanical and rheological stability, while next-generation prebiotics further reinforce structural integrity and bioactivity. Encapsulation efficiencies above 90 percent and enhanced cell viability in simulated digestion demonstrate promising performance of these systems. The integration of quantitative mapping and design principles establishes a practical framework for developing scalable, food-grade, multifunctional synbiotic hydrogels, supporting advancements in probiotic delivery technology. Adopting standardized digestion models and prioritizing in vivo validation will aid the development of synbiotic hydrogels acceptable in real food systems.

1. Introduction

Encapsulation refers to a process involving mainly physical or chemical methods to enclose a specific active compound (usually termed ‘core material’) within another substance (known as ‘wall material’), thus resulting in particles at nanoscale (nanoencapsulation), microscale (microencapsulation), or millimeter scale. This technique involves surrounding or coating the core material with a shell of wall material, which serves to provide protection, controlled delivery, and release; above that, it enhances stability, handling, and utilization of the active substance (Bu et al., 2025; Chen & Chen, 2007; Nezamdoost-Sani et al., 2024). Various encapsulation methods are now commonly used in different fields, including chemistry, biotechnology, medicine, pharmacy, agriculture, and the food industry (Gong et al., 2024; Liu et al., 2022; Xu et al., 2024; Xu et al., 2024).

In this review, focusing on the food sector, encapsulation technology finds an effective application in food processing to address relevant challenges. These include the stabilization and protection of certain food ingredients (e.g., proteins, lipids, vitamins, enzymes) from oxidation, heat, light, acids, or bases (Alfatama et al., 2024; Hu et al., 2022; Sarabandi & Jafari, 2020; Weng et al., 2024); masking undesirable flavors, such as the bitterness of peptides or the astringency of tea polyphenols (Gao et al., 2022; Sarabandi & Jafari, 2020; Xie et al., 2025); regulating the delivery and release of target food ingredients (e.g., probiotics, bioactive compounds) (Chávez-Falcón et al., 2022; Yu et al., 2024; Yue et al., 2020); or uniformly combining incompatible components (Bajaj

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et al., 2021; Banasaz et al., 2020; Khosh Manzar et al., 2020).

In addition, encapsulation technology can significantly contribute to the development of functional foods, understood as 'foods containing significant levels of biologically active components which provide desirable health benefits beyond basic nutrition' (Food and Agriculture Organization). Lately, functional foods, from energy-boosting snacks to dairy products and immune-boosting drinks, are gaining interest and demand among consumers that adopt healthier eating habits and lifestyle (Gupta et al., 2023; Sgroi et al., 2024). Probiotics, which earned great attention due to their link to better intestinal health, immunity, and overall well-being, are estimated to dominate the market of functional food ingredients (Kuo et al., 2022; Vicentini et al., 2016). Functional drinks rich in probiotics, prebiotics, or fibers occupy one of the largest parts of the functional beverage market after energy and sports drinks (Gupta et al., 2023). However, the integration of free probiotic cells into food matrices such as dairy, fermented, and non-fermented products may induce adverse changes in the physicochemical properties, sensory attributes, shelf life, probiotic viability, and overall functionality of the product. Moreover, probiotic cultures can encounter difficulties surviving the gastrointestinal tract (GIT) before successfully colonizing large intestine (Barajas-Álvarez et al., 2023).

Consequently, diverse probiotic encapsulation methodologies are being investigated to solve these issues. Probiotic encapsulation methods include hydrogels, freeze-drying, spray-drying, emulsification, layer-by-layer self-assembly, and others (Xie et al., 2023; Yang et al., 2024). Among them, hydrogels are recognized as highly suitable and effective delivery systems, requiring greater efforts to optimize conditions, delivery and release of probiotics using food- and consumer-friendly materials, as well as evaluate their effectiveness and functionality *in vivo*.

Hence, this review focuses on probiotic encapsulation within hydrogel systems, exploring potential food-grade materials suitable for the structural formulations to achieve specific physicochemical and functional properties. It additionally examines the potential and impact of prebiotic substances, highlighting next-generation prebiotics, for their integration into hydrogels to develop targeted synbiotic delivery systems.

2. Overview of traditional and next-generation probiotics

Probiotics, as suggested by the World Health Organization and the Food and Drug Administration in 2001 and further updated by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2014, are abbreviated as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' (Hill et al., 2014). Such live microorganisms, namely bacteria and yeasts, are present in dietary supplements and different fermented products such as sauerkraut, kimchi, kombucha, kefir, yogurt, and others. Nonetheless, fermented foods typically have a variety of uncharacterized microorganisms with no verified health benefits and thus lack the necessary evidence to be classified as probiotic products. Consequently, only the strains that are well-characterized, safe to use, and have scientifically proven health benefits can be termed probiotics (Binda et al., 2020; ISAPP).

Lactobacillus (*Lacticaseibacillus*) (*L. rhamnosus, L. acidophilus, L. casei, L. reuteri, L. plantarum, L. delbrueckii*) and Bifidobacterium (*B. longum, B. lactis, B. bifidum, B. breve, B. infantis*) are the primary genera of probiotic bacteria that have been thoroughly investigated for their safety and health benefits (Qualified Presumption of Safety | EFSA). Other strains of *Bacillus* (*B. coagulans, B. subtilis, B. cereus*), *Streptococcus* (*S. thermophilus*), and some *Saccharomyces* yeast species, namely *S. boulardii*, have also recently been studied as probiotics (Binda et al., 2020). When administered properly, probiotics support healthy gut microbiota, strengthen the immune system, contribute to the nutrition and metabolism processes, and relieve various GIT related conditions or diseases (e.g., diarrhea, colorectal cancer, lactose maldigestion, intestinal infections, irritable bowel syndrome, and inflammatory bowel disease) (de Souza et al., 2024). Besides, growing research also emphasizes probiotics' potential for improvements in oral (Beattie, 2024), liver (Liu et al., 2023), skin (Mei et al., 2022; Xu et al., 2024), and vaginal (Scillato et al., 2021) health.

Furthermore, the concept of Next-Generation Probiotics (NGPs) has been recently introduced. NGPs, unlike traditional probiotics, aim to treat specific ailments, such as bowel diseases, neuropsychiatric disorders, chronic inflammation, metabolic syndromes, inflammatory asthma, dysbiosis, or cardiovascular diseases. Therefore, are considered to be more applicable in pharmacy for the development of live biotherapeutic products (Table 1). The latter is defined as a product containing live microorganisms that prevent, treat, or alleviate human diseases or conditions but is not a form of vaccination (Jan et al., 2024). Genera of *Akkermansia, Faecalibacterium, Bacteroides, Clostridium, Eubacterium, Propionibacterium,* and *Roseburia* were proposed as NGPs candidates, thus expanding the scope of probiotic research (Han & Zhuang, 2021; Jan et al., 2024). On the other hand, NGPs, in contrast to classical *Lactobacillus* or *Bifidobacterium*, do not have a history of use, nor are they qualified as generally recognized as safe or have a qualified presumption of safety. Hence it requires testing for safety, functional profile, and efficacy, as well as setting strict regulatory guidelines before reaching the market. What is more, a number of proposed NGPs are highly oxygen sensitive and require unusual growth conditions, which poses challenges in their cultivation, maintenance, large-scale production, or integration into food products (Lalowski & Zielińska, 2024), and here encapsulation could also be a solution. Although the field of NGPs is rather new, it is very promising and therefore requires extensive research to be practically adopted (Singh & Natraj, 2021).

Returning to the definition of probiotic, another important key aspect is the requirement of adequate amounts, indicating that a certain amount of probiotics is necessary to provide the intended health benefits (Hill et al., 2014). Even though the functional dose needs to be based on the induction of the claimed physiological effect with human studies and may vary between probiotic strains and products (Probiotics - International Scientific Association for Probiotics and Prebiotics (ISAPP); World Gastroenterology Organization Global Guidelines Probiotics and Prebiotics, 2023), the generally accepted minimum count of probiotics is equal to or higher than 10^6-10^7 CFU per milliliter or gram of the food product at consumption time (Bu et al., 2025; Nezamdoost-Sani et al., 2024; Somera et al., 2024; Yang et al., 2024). Although some countries, such as the Italian Ministry of Health and Health Canada, require a level of 10^9 CFU per serving (Hill et al., 2014).

Nevertheless, because probiotics are live microorganisms, their survivability is influenced by various factors, including the ingredients, chemical composition, and environment of the food product; its production conditions, temperature, packaging, storage, and transportation (de Souza et al., 2024; Yang et al., 2024). Above that, as was revealed in *in vitro* simulation of GIT conditions, the viability of probiotics is negatively affected by highly acidic gastric juice, digestive enzymes, and bile salts in the small intestine, thus decreasing the final cell count reaching the colon (Suvarna et al., 2018; Zimmermann et al., 2024). Fortunately, the survivability and delivery of probiotics to the target location can now be effectively improved by designing and adapting microencapsulation and delivery systems, particularly hydrogel matrices.

3. Hydrogel as a delivery system for probiotics

Hydrogel is a three-dimensional network produced cross-linking natural or synthetic polymers and using water as a continuous phase. Its polymeric chains with hydrophilic or amphipathic groups allow hydrogel to swell and absorb large amounts of water or hydrophilic biological fluids without dissolving. Consequently, this property makes it valuable in fields like biomedicine, biotechnology, food, and agriculture (El Sayed, 2023; Gyles et al., 2017; Nezamdoost-Sani et al.,

Table 1
Examples of Next-Generation Probiotics, their characteristics and therapeutic potentials based on the present studies.

NGPs	Characteristics	Optimal growth conditions	Limitations	Preventive and therapeutic potential			Refs
				Disease	Effects	Dosage and duration	
Akkermansia muciniphila	Strictly anaerobic, gram-negative, oval-shaped, non-motile, mucus-degrading	37°C; pH of 6.5; atmosphere: 100 % N_2 or 5 % H_2, 10 % CO_2, 85 % N_2. Medium: brain heart soak, porcine gastric mucin medium, Columbia broth, trypsin soy, or synthetic medium (16 g L^{-1} soy protease, 4 g L^{-1} threonine, 25 mM glucose, and 25 mM N-acetylglucosamine).	Challenging cultivation; few strains available; mode of action is not fully understood; effective dose for human remains unknown; safety and regulation issues.	Overweight/ obese insulin-resistant volunteers	↑ insulin sensitivity; ↓ insulinemia; ↓ total cholesterol; ↓ body weight and fat mass; ↓ plasma lipopolysaccharides (LPS), ↑ gut barrier; -no significant change in overall gut microbiota.	10^{10} CFU daily for 3 months (safe and well tolerated)	(Depommier et al., 2019; Zhai et al., 2019)
				Metabolic-associated fatty liver disease	↓ weight gain; ↓ hepatic steatosis, ↓ liver injury markers (ALT, AST); ↓ intrahepatic TGs; ↓ harmful secondary DCA and LCA bile acids ↑ gut barrier (by ↑ occludin, TJP1, ↓ LPS); ↑ adipocyte metabolism and related adipokines; ↓ gut dysbiosis (↓ *Alistipes, Lactobacilli, Tyzzerella, Butyricimonas, and Blautia;* ↑ *Ruminiclostridium, Osclibacter, Allobaculum, Anaeroplasma, and Rikenella*).	Mice – 0.2 mL 1.5×10^9 CFU daily for 21 weeks	(Wu et al., 2023)
				Atherosclerosis	↓ atherosclerotic lesion formation without affecting hypercholesterolemia; ↓ inflammation (by ↓ macrophage infiltration and expression of proinflammatory cytokines and chemokines); ↓ metabolic endotoxemia; ↑ gut barrier (by ↑ zona occludens protein-1, occludin).	Mice – 200 μl 5×10^9 CFU daily for 8 weeks	(Li et al., 2016)
Faecalibacterium prausnitzii	Strictly anaerobic, extremely oxygen sensitive, gram-positive, rod-shaped, nonmotile, non-spore-forming.	37°C; pH 6.7; 80 % N_2, 12 % CO_2, and 8 % H_2; Medium: YCFAG (+resazurin) or M2GSC (+cysteine); Riboflavin, cysteine, or glutathione can enhance survival rate in a microaerobic environment.	Anoxic conditions are necessary; highly sensitive to slight increases in physiological concentrations of bile salts.	Dextran sodium sulfate (DSS)-induced colitis	↓ symptoms: weight loss, diarrhea, bloody stools, and colon shortening; ↑recovery from DSS-induced colitis; ↓the expression of pro-inflammatory cytokines (TNF-α, IFN-γ, and IL-10); ↓ expression of mucosal cytokines; ↑ histological score (↓inflammation, tissue damage).	Mice – 0.2 ml 1.5×10^8 CFU daily for 7 days	(Kawade et al., 2019)
				House dust mite-induced allergic asthma	↑ alleviation of allergic asthma: ↓BALF differential cell counts; ↓inflammatory cell infiltration and haemorrhage; ↓ Th2-driven cytokines (IL-4, IL-5, and IL-13), IgG1 levels; ↑ regulatory T cells; ↓ pro-inflammatory *Turicibacter*;	Mice – 0.25 mL 1×10^9 CFU mL^{-1} once a day for 22 days	(Hu et al., 2021)

(*continued on next page*)

Table 1 (*continued*)

NGPs	Characteristics	Optimal growth conditions	Limitations	Preventive and therapeutic potential			Refs
				Disease	Effects	Dosage and duration	
					↑*Faecalibaculum, Dubosiella, Streptococcus, Lachnoclostridium*; ↑ levels of propionate and butyrate.		
				Impaired intestinal barrier function and intestinal flora disorder by sleep deprivation	↑ goblet cells count and MUC2 levels; ↑ tight-junction protein expression; ↓ macrophage infiltration; ↓ pro-inflammatory cytokine expression; ↓ apoptosis; ↓harmful *Klebsiella, Staphylococcus* ↑ beneficial *Akkermansia;* ↑ fecal butyrate level.	Mice – 0.2 mL 10^8 CFU daily for 14 days	(Wang et al., 2024)
Faecalibacterium duncaniae	Can grow under low-oxygen conditions	YCFA-medium		Influenza	↓weight loss; ↓pulmonary viral load; ↓ lung and gut inflammation (↓ the expression of pro-inflammatory genes); ↑recovery in levels of SCFAs; ↑ lung barrier integrity; ↓systemic secondary bacterial infection.	Mice – 0.2 ml 1×10^9 CFU daily for 12 days	(Chollet et al., 2024)
Bacteroides thetaiotaomicron	Anaerobic, gram-negative, non-spore-forming, rod-shaped.	37°C; pH 6.5-7.5; Medium: Columbia blood, chopped meat, fastidious anaerobe broth.	As these bacteria are both commensal and opportunistic pathogens, they can cause health issues. The application in supplements or food technology should be carefully monitored.	Crohn's Disease	↑ microbial diversity and evenness in group of multiple doses treatment; -no significant changes in median fecal calprotectin levels (a marker of intestinal inflammation); -no worsening of disease activity.	*Single dose:* 3 capsules, each $10^{7.73\pm1.43}$ CFU (total daily dose of $10^{8.21\pm1.43}$ CFU) *Multiple doses:* every 12 hours for 7.5 days (total dose 8.98×10^7 – 6.5×10^{10} CFU) is well tolerated	(Hansen et al., 2021)
				Alcoholic fatty liver disease	↓ hepatic steatosis; ↓ triglycerides content in the liver; ↑ mucus thickness by ↑mucin2 expression; ↓ LPS translocation; ↓ expression of cldn1, MMP9, ERK phosphorylation, and Hes1; ↑ GLP1 and restored FGF15 hormones; ↓ fatty acid synthesis; ↑ FA oxidation and lipid exportation; -preserved the mitochondrial fitness and redox state in alcohol-fed mice.	Mice – 0.2 ml 3×10^9 bacteria daily for 14 days	(Sangineto et al., 2022)
				Non-alcoholic fatty liver disease	↓body weight, fat accumulation; ↓ hyperlipidemia; ↓ insulin resistance, ↓ hepatic steatohepatitis, liver injury; ↓ metabolic dysfunction (↑ gut-liver folate levels and hepatic metabolites); ↑ microbiota diversity; ↓ *Firmicutes/Bacteroidetes* ratio; ↑ hepatic PUFA, ↓ hepatic MUFA.	Mice – 0.1 mL 1×10^8 CFU three times per week for 12 weeks	(Li et al., 2024)

2024).

Hydrogel delivery systems are well suited method for embedding probiotic cells due to their biocompatibility, biodegradability, hydrophilic and swelling properties. In addition, hydrogels can be designed sensitive to external stimuli like pH or digestive enzymes, allowing for controlled and targeted probiotic release (Argin et al., 2014; Gyles et al., 2017; Yan et al., 2020; Yang et al., 2024; Zheng et al., 2023). Moreover, hydrogel preparation is simple and often includes milder conditions and more gentle processes (e.g., ionic gelation, cross-linking), avoiding osmolarity stress or high temperatures met in methods like freeze and spray drying (Nezamdoost-Sani et al., 2023) (Table 2). Encapsulation of probiotics in hydrogels has been shown to be very efficient in different studies, reaching encapsulation efficiency (EE) of 88.9 % (Yan et al., 2021), 95.95 % (Virk et al., 2024), 97 % (Ni et al., 2023), 87.69-99.98 % (Zhang et al., 2023), or 90.60-92.70 %, depending on biopolymer types, concentrations, ratios, and crosslinking method applied (Praepanitchai et al., 2019).

However, hydrogels still require additional post-treatment for long-term storage (to several months or even >1 year), most usually freeze-drying (Kuo et al., 2022; Sharma et al., 2024; Sun et al., 2021), and often can have low mechanical strength if prepared with a single polymer. The latter issue led to the development of composite hydrogel networks to improve their structural properties (Gyles et al., 2017).

3.1. Hydrogel classification

Hydrogels can appear as natural, synthetic, or hybrid depending on their polymer's origin (Fig. 1). The most common examples of natural polymers are starch, gelatine, alginate, chitosan, pectin, cellulose, hyaluronic acid, etc. Natural hydrogels are of special interest, especially in terms of the food sector, due to their non-toxicity, nutritional value, biocompatibility, biodegradability, low immunogenicity, possibility of food-waste valorization, and abundant resources (Kamaci & Kaya, 2023; Nezamdoost-Sani et al., 2023; Ohlmaier-Delgadillo et al., 2021; Zheng et al., 2023). On the other hand, natural hydrogels tend to be less resistant to physiological degradation and have weaker mechanical properties, restricting their application in some areas (Bu et al., 2025). Meanwhile, synthetic hydrogels include chemically synthesized polyacrylamide, polyethylene glycol, polyvinyl alcohol, or polylactic polymers. Although synthetic hydrogels commonly have higher water absorption, better mechanical strength, and are more stable, their applicability in food is limited due to their potential toxicity, environmental and sustainability concerns (El Sayed, 2023; Zheng et al., 2023). Lastly, hybrid hydrogels made from natural and synthetic polymers in combination (for instance, chitosan and polyurethanes (Kamaci & Kaya, 2023)) are a possible choice for improving their mechanical strength, functionality, and stability (Kamaci & Kaya, 2023; Zheng et al., 2023). Nevertheless, consumers are already more inclined to choose products that are food-grade and minimally processed. Therefore, the application of natural materials for proper hydrogel formation with desired properties should be the major interest.

On the basis of polymeric composition, hydrogels are categorized into three types: homopolymeric, copolymeric, and multipolymeric. Homopolymeric hydrogels consist entirely of a single type of monomer that forms the polymer network. Copolymeric hydrogels, on the other hand, are made from two or more different monomer species, with at least one being hydrophilic, organized in a random block, or alternating pattern along the polymer network chain. Finally, multipolymeric network hydrogels, including both interpenetrating (IPNs) and semi-interpenetrating networks (semi-IPNs), were designed to overcome limitations in homopolymeric hydrogels such as low mechanical strength. These networks involve the polymerization of two or more polymers, where a pre-polymerized hydrogel is introduced into a polymeric network solution of monomers and a polymerization initiator (Gyles et al., 2017). Such IPN hydrogel was enzymatically designed by W. Yan et al. (2021), employing biopolymers of soy protein isolate (SPI) and sugar beet pectin (SBP) as a delivery system for probiotics. When no cross-linking agent is used, semi-IPNs are formed (Ahmed, 2015; Gyles et al., 2017).

The mode of network formation is one of the principal determinants affecting the characteristics of hydrogels, including mechanical stability, encapsulation, and deliver efficiency. Hydrogel networks can be cross-linked either chemically or physically. Chemical junctions are based on covalent bonds usually initiated by chemical crosslinkers, thereby conferring stability and durability to hydrogel structure, but at the same time resulting in lower elasticity (Nezamdoost-Sani et al., 2024). Conversely, physical networks rely on non-covalent bonds like ionic interactions (e.g., alginate crosslinked by calcium ions), van der

Table 2
Comparison of probiotic encapsulation methods in terms of efficiency, cell viability and stability.

	Hydrogel	Freeze-drying	Spray-drying
Structural materials	Wide range, e.g., alginate, chitosan, pectin, whey, carrageenan, gelatin, etc.; various combinations	Carriers like whey, maltodextrin, proteins, coatings; gum Arabic, calcium-alginate, chitosan, inulin	
Encapsulation efficiency (%)	High: 80-98 %; Depends on biopolymer types concentration, crosslinking method	Moderate to high: 70-95 %	Moderate: 68-88 %
Survival in simulated gastrointestinal tract	SGF: ↓0.5-0.7 log; survival by 74-98 % SIF: ↓0.4-1 log; survival by 77- 94 %; possible to modify by manipulating materials and construction.	SGF: ↓1-2.8 log; 53-86.8 % survival; SIF: ↓0.1-1.2 log; 50-72-87 % survival (depending on strain and matrix)	SGF: ↓1-4.5 log; 65-75 % survival; SIF: ↓0.1-1.2 log; If additional coating or matrix is used– 83-89 %
Storage stability	Varies among formulations: at 25°C for 90 days and at -18°C for 120 days: $>10^6$ CFU/g; after 30 days: ↓0.87-1.04 log; after 40 days at 4°C: ↓0.5-0.7 log	Higher survival rate than spray-drying: e.g., after 45 days at 25°C: ↓0.5 log; 6 months at 4°C: ↓1.2-3.1 log;	Lower survival rate: e.g., after 45 days at 25°C: ↓3 log; after 2 weeks at 4°C: ↓1.5-2 log;
Advantages	Best for biological protection and functional delivery; enhanced protection to GIT, oxygen; controlled and targeted delivery; biocompatible microenvironment;	Low temperature preserves probiotics and their functionality better than spray-drying (but survival is higher 70-82 %; can be achieved to 98.6 % with a proper carrier design;), best for long-term storage.	Large-scale, low-cost, fast, continuous process
Disadvantages	Require post-treatment for long storage (usually freeze-drying); more complex formulations;	Osmotic and oxidative stress; viability changes (↓0.5-2 log), long, expensive;	High temperatures, lowest viability ↓2–5 log, unless well optimized carrier system (can reach survival 69.25-86.24; 94.4-98.2 %)
References	(L. Ma et al., 2024; Praepanitchai et al., 2019; Premjit et al., 2024; W. Yan et al., 2021, 2021; Yi et al., 2025; Zhang et al., 2023; Z. Zhu et al., 2024)	(Barajas-Álvarez et al., 2022; Buahom et al., 2023; Halim et al., 2017; Ho et al., 2024; Obradović et al., 2022; Wong et al., 2010)	

*SGF, simulated gastric fluid; SIF, simulated intestinal fluid.

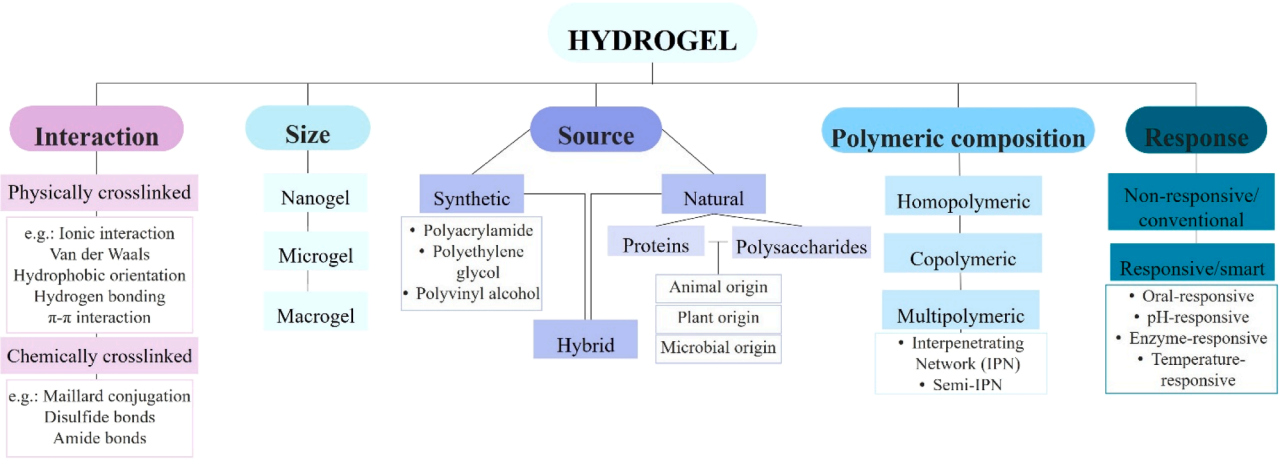

Fig. 1. Hydrogel classification.
Applications: synthetic hydrogels – drug delivery, tissue engineering, wound dressings, superabsorbent; natural – food, pharmaceutical, and nutraceutical industries (encapsulations, edible films & coatings:); hybrid – biomedical, food, and packaging applications.

Waals force, hydrophobic orientation, or hydrogen bonding. These types of hydrogels are easier to operate but have relatively lower mechanical strength (Zheng et al., 2023) (more details in 2.2.3).

Depending on the morphology and internal structure, there are two distinct hydrogel types—microspheres and microcapsules. The former is uniformly mixed and homogeneous, meaning that probiotics are dispersed and entrapped throughout the matrix. The latter have different regions or phases and a characteristic core-shell structure, where probiotics are placed in a core and are surrounded by a protective shell (Nezamdoost-Sani et al., 2024).

Moreover, hydrogels can be designed to respond to specific external stimuli. In this context, external stimuli include various environmental factors, both physical and chemical, with which hydrogel particles may interact at some level. Examples of such factors are temperature, light, pressure, magnetic and electric fields, sound, pH, ionic strength, and specific chemical molecules (e.g., enzymes, bile salts, acids, or oxygen). Based on the response to these factors, conventional and smart hydrogels are distinguished. Conventional, or non-responsive, hydrogels do not undergo significant structural changes in response to external stimuli. Contrary smart, or responsive, hydrogels are designed so that external factors influence their internal network structure and functionality in a beneficial way, exhibiting desirable swelling or shrinkage behavior (Tan et al., 2022). Such hydrogels have received significant attention among researchers, particularly in the area of controllable nutraceutical release and delivery. Depending on the environmental factors to which the hydrogel responds, several main categories can be named, including oral-responsive, pH-responsive, enzyme-responsive, and temperature-responsive hydrogels, among others (Chai et al., 2018).

Lastly, these are by no means the only categories of classification. In addition to the previous categories, hydrogels can also be classified based on size (nanogels, microgels, and macrogels), water content (low water <30 % or high water >30 %), functionalization (whether it is modified to have functional groups or not), application areas (biomedical, food, agricultural), network electric charge (nonionic, ionic, or amphoteric), and structural configurations (amorphous, semicrystalline, or crystalline) (Ahmed, 2015; Gyles et al., 2017; Tan et al., 2022). In this context, Table 3 provides a decision-oriented design framework aligning target sites and triggers with materials and crosslinking strategies.

3.2. Selection of hydrogel structural components

Selecting the right structural components is one of the most crucial steps in creating hydrogels. Essentially, the materials are selected, and the system is engineered so that it provides the desired properties and functionality for the intended application. Characteristics such as hydrogel particle size, shape, porosity, rheology, encapsulation efficiency, stability, strength, biocompatibility, and probiotic viability can be controlled by using appropriate polymers, cross-linking methods, and processing techniques (Naaz et al., 2024; Nezamdoost-Sani et al., 2024; Šipailienė & Petraitytė, 2018). Such aspects as cost, availability, biocompatibility, and safety need to be considered as well (Yang et al., 2024). In terms of the food sector, hydrogels for probiotic encapsulation are usually prepared from natural, non-toxic, food-grade biopolymers, such as proteins and polysaccharides or their combinations (Nezamdoost-Sani et al., 2024).

3.2.1. Proteins as biopolymers for hydrogel network

As a popular macromolecular polymer in the food industry, proteins are biocompatible, biodegradable, renewable, and possess excellent emulsifying, gelling, film-forming, and usually cryoprotective properties along with high nutritional value (Nguyen et al., 2015; Pinpimai et al., 2015). These qualities make them ideal wall materials for encapsulation (Ma et al., 2024). Proteins can come from different sources, including animals, plants, and microorganisms. Widely used animal-origin proteins are whey proteins (Duongthingoc et al., 2013; Gedik & Karahan, 2024; Hébrard et al., 2010; Hernández-Rodríguez et al., 2014; Naaz et al., 2024; Sompach et al., 2022; Ying et al., 2013), casein (Burgain et al., 2013; Nag et al., 2011), and gelatin (Annan et al., 2007; Arslan et al., 2015; Kuo et al., 2022; Ni et al., 2023; Patarroyo et al., 2020). Given the increasing concerns about self-health, animal welfare, ethics, and the environment protection, alternative proteins currently receive significant attention (Nezamdoost-Sani et al., 2024). Moreover, the use of animal-origin proteins in food products immediately excludes a large consumer group—vegans. Therefore, plant-derived proteins from soybean (Praepanitchai et al., 2019; Yan et al., 2020, 2021; Yao et al., 2023), pea (Arslan et al., 2015; Yi et al., 2025), and cereals (Ma et al., 2024) are great alternatives employed for hydrogel formation (Liu et al., 2021; Ma et al., 2024).

However, hydrogels prepared with a single type of protein tend to result in crumpled, cracked, and microporous gel surfaces, which can reduce mechanical strength, storage and loss modulus, water-holding capacity, and thermal stability (Zheng et al., 2023). In addition, protein-based hydrogels instantly suffer rapid degradation upon digestive enzymes like pepsin. To overcome these limitations, additional polymers (other proteins (Burgain et al., 2013) or polysaccharides (Hébrard et al., 2010; Nag et al., 2011; Ying et al., 2013)) are typically used in combination with proteins, leading to more complex hydrogel systems (Ma et al., 2024).

Table 3
Design framework for developing a hydrogel with desired properties and targeted delivery.

Steps		Options/examples			Design implication
1.	Target sites	Stomach Small intestine Large intestine		released in the stomach retained in the stomach, released in the small intestine retained in the stomach and small intestine, released in the large intestine	Analyze all the conditions in each location, find the key differences; determine required resistance (acid/bile/enzymes) and release profile (fast, delayed, sustained); match polymer degradability to trigger for targeted release.
2.	Triggers	pH responsive: • stomach – 1.5-3.5 • intestine – 6-7		• Gum Arabic (pKa ~ 2.2) + whey protein isolate (WPI) – at pH 2 electrostatic attraction disappears; • Gelatin+ carboxymethyl cellulose; only alginate gel • Chitosan+Konjac glucomannan; Peach gum polysaccharide+β-glucans;Corn fiber gum+SPIAlginate + chitosan	Polymers should have carboxyl, sulfonic, or amino groups, that could vary with pH ion strength in aqueous media. The response is based on electrostatic repulsion and swelling caused by group ionization. Unstable under high physiological salt concentration around 150 mM; Used for intestinal release mainly.
		Digestive enzymes: • pepsin • pancreatin		• Gelatin, casein, WPI, SPI, zein • Protein-polysaccharide, lipid-based, chitosan; WPI+alginate	Use protein-based natural polymers; remains stable in acidic gastric conditions but breaks down under pancreatin due to enzymatic digestion.
		Colonic microbial enzymes (pectinase, glycosidase, mannanase)		Pectin/POS, inulin, resistant starch, β-glucan, dextran based systems; Konjac glucomannans + xanthan; lactulose+alginate; lactosucrose+alginate	Use for colon targeting release; wall materials are restricted to intestinal amylase specific and colonic microbial enzymes; protein alone not suitable.
3.	Material families	Origin (natural/ synthetic/hybrid) Prefer natural / hybrid for food applications	Polysaccharides	Alginate, chitosan, carrageenan, xanthan, pectin, gellan gums, dextran, cellulose, etc.	Provide mucoadhesion, pH/enzymatic responsiveness, controlled release, and prebiotic function; but insufficient mechanical strength and structural integrity
			Proteins	WPI, SPI, casein, gelatin, pea protein.	Give gelling matrix and cryoprotection but are digestive enzyme-sensitive, have lower mechanical strength and stability
			Protein–polysaccharide composites	Alginate-SPI; alginate-WPI; alginate-gelatin; carrageenan-WPI; gellan gum-alginate, dextran-WPI; etc.	Enhance hydrogel structure, viscoelastic properties, transportation and delivery; choose for combined functionality;
			Polyphenol-enriched or other next-generation prebiotic addition	Inulin, FOS, IMO, XOS, GOS, POS, β-glucan; resveratrol, tannic acid, gallic acid, curcumin, quercetin, rutin.	Synergistic hydrogel-probiotic-prebiotic system; carefully consider polyphenols type and concentration (suggested range is 0.04-0.9 %)
4.	Crosslinking (consider probiotic viability)	Physical → non-covalent bonds		Ionic interactions (e.g., Ca^{2+}-alginate), van der Waals force, hydrophobic interaction, H-bonding, chain entanglement	Easier to operate but have relatively lower mechanical strength; mild processing (good for probiotics), reversible hydrogel, sensitive to environmental, good for food-grade colon-targeting systems.
		Enzymatic		Laccase, transglutaminase	Mild conditions (good for probiotics); less toxic, more biocompatible, improved stability and durability without harsh reagents.
		Chemical → covalent bonds		Chemical crosslinkers: polyethylene glycol, glutaraldehyde, N-hydroxysuccinimide esters; Natural alternatives: genipin, citric, tannic acid, aldehydes Maillard conjugation (protein–polysaccharide covalent conjugates)	Consider carefully for food-grade applications, better choose natural options; irreversible, stable, durable hydrogel, fast-swelling, but lower elasticity; Cost-effective, natural and spontaneous formation of covalent bonds with modified protein properties; potential of prebiotic effect (but control to avoid harmful reaction by-products).
5.	Post-treatment	Freeze-drying		Sublimation under vacuum, −40 to −50°C; takes hours to days; very low moisture content <2–5 %; excellent storage stability.	Low temperatures, slow, batch process, requires cryoprotectants, preserves probiotics better, but more expensive.
		Spray-drying		Atomization into fine droplets dried by hot air at 150–200°C; moisture content 3–8 %; good storage stability.	Cost-effective, fast, continuous, economical and scalable, but stress heat-labile cells, wall-material degradation or core loss at high temp., need for thermal protectants.

*FOS, fructooligosaccharides; IMO, isomaltooligosaccharides; XOS, xylooligosaccharides; GOS, galactooligosaccharides; POS, pectic oligosaccharides.

3.2.2. *Polysaccharides as biopolymers for hydrogel network*

Similarly to proteins, polysaccharides also have good biocompatibility and biodegradability, are easily metabolized, and are abundant in nature, originating from animals, plants, microorganisms, and algae. Because of diverse functional groups (-OH, -COOH, $-NH_2$, and $-SO_4$) present in polysaccharide chains, polysaccharide-based hydrogels can feature unique properties, including bio-adhesion, affinity to the mucus layer, stability, self-healing, and shape memory (Xie et al., 2023). On the other hand, having insufficient mechanical strength, structural integrity, and EE when a single polysaccharide is used (Huang et al., 2024; Zheng et al., 2023).

Due to its great biocompatibility, availability, non-toxicity, and low cost, the salt form of alginic acid, namely sodium alginate, is the most widely used natural polysaccharide for encapsulation (Bevilacqua et al., 2020; Kuo et al., 2022; Ni et al., 2023; Pinpimai et al., 2015). Besides alginate, other so-called marine polysaccharides like chitosan and κ-carrageenan are of interest (Ghiorghita et al., 2024), in addition to abundant natural plant and microbial polysaccharides such as cellulose (El Sayed, 2023; Gong et al., 2024), starch, pectin (Ohlmaier-Delgadillo et al., 2021), gellan and xanthan gums (Gyles et al., 2017; Hughes et al.,

2024; Kuo et al., 2022; Liu et al., 2021; Nezamdoost-Sani et al., 2023; Srivastava et al., 2023; Xie et al., 2023).

Nevertheless, it was shown that only alginate-based hydrogel is pH sensitive and lacks mechanical strength and probiotic protection, especially in the presence of calcium ion chelators or monovalent ions (Bosnea et al., 2017; Luo et al., 2022; Qi et al., 2020; Santos & Machado, 2021). Meanwhile, chitosan alone is not able to form gel and solubilizes under acidic pH (Thomas et al., 2014); gellan gum gel is usually soft and weak in contrast to κ-carrageenan gel that is hard and brittle (Hughes et al., 2024; Srivastava et al., 2023). Therefore, combinations of few polysaccharides are being used to overcome such limitations (Nezamdoost-Sani et al., 2023; Suvarna et al., 2018; Wang et al., 2024). For instance, insightful observations were achieved with chitosan–κ-carrageenan and chitosan–gellan bi-composite hydrogels resulting in an easy and high release rate of probiotics and lower EE due to large pores within their loose network compared to a more compact tri-composite hydrogel containing all three polymers (Srivastava et al., 2023). Chitosan, the only cationic polysaccharide, is usually used as a coating material, for example, for alginate beads (Nezamdoost-Sani et al., 2023; Qi et al., 2020; Qi et al., 2020; Rahimi et al., 2024; Santos & Machado, 2021; Sharma et al., 2024; Suvarna et al., 2018). Given that chitosan has antibacterial activity, its application for direct probiotic encapsulation is limited, unless chitosan molecules are separated from the cells, as was achieved by Huang et al., 2024. Their experiment presented a double-layered polysaccharide hydrogel structured from a carboxymethyl cellulose inner layer and a carboxymethyl chitosan outer layer, successfully maintaining *Lactiplantibacillus plantarum* viability and controlling its delivery and release rate.

The correct combination of polysaccharides at a suitable ratio may result in a hydrogel formation with enhanced functions. For example, the combination of xanthan gum as an anionic polymer and chitosan as a cationic polymer gave a highly enzyme-resistant and pH-responsive hydrogel for controlled and targeted delivery of *Pediococcus acidilactici* (Argin et al., 2014). Meanwhile, carrageenan-pectin gel beads with a carrageenan fraction ≤ 0.25 exhibited good rheological and mechanical properties, being less brittle, more cohesive, and harder. They effectively resisted simulated gastric conditions and maintained high EE (87-96 %), protecting *Lacticaseibacillus rhamnosus* during storage and digestion. In contrast, carrageenan-rich gels with equal polymer fractions had the lowest storage modulus and hardness, thus a weaker and more brittle structure (Hughes et al., 2024).

3.2.3. Polysaccharide-protein composite hydrogels

Although polysaccharides and proteins have been individually utilized to form hydrogels, the combination of both is now becoming a common strategy to develop composite hydrogels with enhanced physicochemical properties (Obradović et al., 2022; Xie et al., 2023; Zheng et al., 2023). Proteins, with strong gelling properties yet sensitive to enzymes and pH, combined with polysaccharides, which form a network of physical barriers adding mechanical strength and mucoadhesion, can enhance hydrogel structure and functionality. Moreover, polysaccharides can protect proteins from enzymatic and acidic degradation in the stomach, thus enhancing transportation and delivery of the probiotics to large intestine (Yan et al., 2020).

Proteins and polysaccharides can interact covalently through enzymatic or chemical cross-linking and non-covalently through physical cross-linking – via van der Waals forces, hydrogen bonds, electrostatic and hydrophobic interactions, thus forming a more compact network with better viscoelastic properties (Hernández-Rodríguez et al., 2014). Cross-linking limits the movement of the polymer chains, typically improving mechanical strength, barrier properties, and water resistance while also decreasing solubility and swelling (Azeredo & Waldron, 2016). However, restrictions of polymer chains segmental mobility and an increase in mechanical strength are accompanied by reduced permeation (Castro-Muñoz & Boczkaj, 2025).

Regarding chemical cross-linking, tannic acid, citric acid, epigallocatechin gallate, genipin, and procyanidin are natural cross-linking agents frequently employed in the synthesis of biopolymer-based hydrogels. In addition to conventional cross-linkers, certain small molecules from natural green sources are now being employed as cross-linking agents in biopolymer systems (explore more in Sapuła et al., 2023, for matching linker with specific polymer). Among these, plant-derived aldehydes stand out as particularly suitable cross-linkers. These compounds, which are present in essential oils extracted from plants such as cinnamon, citrus peels, pepper, and vanilla, offer notable advantages (Maurya et al., 2021). Incorporating aldehyde-containing compounds, such as vanillin, cinnamaldehyde, citronellal, etc. into biopolymer hydrogels not only enables cross-linking via Michael addition/Schiff base reactions between aldehyde and amino groups but also introduces additional functional properties. These features enhance their value in the formulation of advanced hydrogel systems for both food and biomedical applications (Khin et al., 2025). For instance, such hydrogels demonstrate improved resistance to UV radiation and possess antimicrobial properties, making them suitable for use in food packaging (De Carvalho & Grosso, 2004).

Another cross-linking method – enzymatic cross-linking – represents a noteworthy technique among traditional chemical agents and physical cross-linking. It presents a promising approach due to the high specificity of enzymes and the mild reaction conditions required and can be used in food systems (Azeredo & Waldron, 2016). Chen et al., 2019, developed a double network gel composed of SPI and SBP using thermal treatment and laccase catalysis. The resulting gel demonstrated superior mechanical performance and enhanced stability. Alongside laccase, microbial transglutaminase is also a commonly employed enzyme for cross-linking applications. According to the literature, these hydrogels demonstrate decreased solubility in water along with enhanced tensile strength and elevated melting temperatures (De Carvalho & Grosso, 2004).

The main physical cross-linking methods used in food systems include electrostatic interactions between cations and anions, hydrogen bonding, crystallization, and complex coacervation (Zheng et al., 2023). Besides these typical cross-linking methods, Maillard reaction-based conjugation of polysaccharide and protein have emerged as a novel approach to designing food systems for encapsulation and delivery of bioactive components (Sun et al., 2022; Urango et al., 2024), but they haven't been widely applied for probiotics protection yet. The Maillard reaction is a cost-effective non-enzymatic browning reaction based on the natural and spontaneous formation of covalent bonds between carbonyl groups of reducing carbohydrates and amino groups of proteins. Such reactions under the control of time, temperature, pH, moisture, material type, and ratio enable researchers to modify functional and technological properties of proteins. It was noticed that conjugation increases proteins' solubility, thermal stability, emulsification, resistance to GIT digestion and even decreases their allergenicity or unfavorable flavor (Urango et al., 2024). On the other hand, later stages of the Maillard reaction produce compounds—for instance, acrylamide, heterocyclic aromatic amines, or advanced glycation end-products—associated with mutagenicity, carcinogenicity, and cytotoxicity; therefore, the reaction conditions should be carefully adjusted to avoid such compounds (Sun et al., 2022). An additional approach to enhance these conjugates functionality is to include prebiotic carbohydrates, such as inulin, FOS, XOS, GOS, IMO, β-glucan, or pectin, thus achieving a potential prebiotic effect in addition to antioxidative, antimicrobial, and anti-inflammatory properties (Urango et al., 2024; Urango et al., 2024; Zhong et al., 2021). Peled et al. (2024) produced a Maillard conjugate from 2′-fucosyllactose (human-milk-oligosaccharide) and lactoferrin hydrolysate resulting in a promising so-called next-generation prebiotic (more in Chapter 3). The latter contains a protein fraction, that significantly increased short-chain fatty acids (SCFAs) production by promoting beneficial SCFAs-producing bacteria, while reducing gut permeability markers more effectively than conventional carbohydrate-only prebiotics. In this context, future studies could also

investigate the possible application of such prebiotic conjugates for probiotic encapsulation and synbiotic delivery.

3.3. Probiotic encapsulation within composite hydrogels

Numerous studies have investigated the encapsulation of probiotic cultures in composite systems. For instance, in alginate-gelatine gel network (Kuo et al., 2022; Ni et al., 2023), a combination of peach gum with SPI (Yao et al., 2023), alginate-SPI-based hydrogel beads (Praepanitchai et al., 2019), WPI and dextran conjugate (Guo et al., 2022), whey protein concentrate-pullulan-trehalose hydrogel (H. Sun et al., 2021), and WPI with κ-carrageenan (Hernández-Rodríguez et al., 2014) have been tested to encapsulate *L. plantarum.* Such systems improved cell viability, storage and thermal stability, provided effective protection during processing and GIT digestion. Additionally, WPI and gum Arabic complex coacervate have successfully improved the viability of *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum* probiotic cultures in low pH (Bosnea et al., 2017). The lactic acid bacteria culture *L. rhamnosus* was microencapsulated within resistant starch and WPI formulations via spray drying, resulting in better protection under all storage conditions compared to the use of resistant starch alone (Ying et al., 2013). Similarly, the lignin improved the viability of *Limosilactobacillus reuteri* loaded in a WPI-lignin complex basically due to improved protein structure (Diệp Huy Vũ et al., 2021). *Lacticaseibacillus casei* was successfully entrapped in a sodium caseinate and gellan gum gel matrix, achieving a significant EE of 89.5 % and providing protection against SGF and bile salts (Nag et al., 2011). It is evident that diverse polysaccharide–protein combinations have been designed for the encapsulation of various probiotic bacteria.

In terms of the encapsulation of probiotic yeast within such systems, the research is relatively limited and narrow. Species of the *Saccharomyces* genus, predominantly *S. cerevisiae* and *S. boulardii,* are typically encapsulated within polysaccharides. Sodium alginate is the predominant wall material, followed by maltodextrin and chitosan (Bevilacqua et al., 2020; Grambusch et al., 2024; Suryabhan et al., 2019; Suvarna et al., 2018; Thomas et al., 2014). Only a small number of studies have conducted experiments on *S. boulardii* encapsulation utilizing composite hydrogels made from sodium alginate in combination with gelatine (H. Du Le & Trinh, 2018) or whey proteins (Gedik & Karahan, 2024; Hébrard et al., 2010; Poloni et al., 2021). The encapsulation of *S. boulardii* within WPI and alginate microcapsules gave a high EE of 95 %. Moreover, additional coating, either with WPI or alginate, improved yeast survival from 40 % to 60 %; additionally, alginate coat gave delayed release at intestinal pH (Hébrard et al., 2010). *S. cerevisiae* cells loaded in sodium alginate-skim milk capsules demonstrated significantly higher viability during storage and in GIT conditions, in comparison to cells presented in alginate-only capsules (Pinpimai et al., 2015). Moreover, individual encapsulation of *S. cerevisiae* cells using β-lactoglobulin and alginate has been achieved using the layer-by-layer technique, protecting yeast during subsequent freeze-drying (Nguyen et al., 2015).

In addition to the commonly used proteins and polysaccharides mentioned before, marine-derived proteins (e.g., myosin, actin, actinomyosin, and gelatin) from fish, scallops, shellfish, shrimp, or roe and polysaccharides like alginate, carrageenan, and chitosan have recently gained even more interest for the development of marine-derived protein-polysaccharide hydrogels. It is because of their abundance, safety, affordability, edibility, and satiety-enhancement property. Moreover, in this way the resources of natural biopolymers are broadened, and religious issues are avoided along with reducing the risk of zoonotic disease (J. Yan et al., 2024).

4. Concept of prebiotics and their integration in hydrogels encapsulating probiotics

Beyond enhancing structural, physicochemical stability, and functional properties of composite hydrogels as wall materials, the integration of specific polysaccharides may also serve as prebiotics, allowing the creation of synbiotic encapsulation systems (Table 4). Generally, the currently available prebiotics are solely indigestible carbohydrates like inulin, oligosaccharides, and resistant starch, which are resistant to stomach enzymes but are degraded by colon bacteria. Therefore, such compounds in the form of raw materials are of special interest for their application in the development of colon-targeted delivery systems (Yan et al., 2020, 2021). The most important criteria are that a potential prebiotic should firstly withstand the host upper GIT environment (gastric acid, bile salts, degradation by mammalian enzymes, and gastrointestinal absorption), be fermented by gut microbiota, and, moreover, should selectively stimulate the growth and/or activity of beneficial gut bacteria, primarily *Bifidobacteria* and *Lactobacilli,* linked to human health (Kaur et al., 2021).

The hydrogel-probiotic-prebiotic system can be designed to work synergistically, as prebiotics (e.g., inulin, FOS, polyphenols) contribute both to the overall hydrogel network integrity, providing structural protection, and metabolic support by nourishing the encapsulated probiotic cells. Firstly, the hydrogel matrix provides physical and biochemical barriers from harsh GIT conditions, oxygen and processing stress, while providing a favorable hydrated microenvironment for their stability. Secondly, manipulating with the choice of structural materials, pH- or enzyme-responsive behavior can be achieved, enabling targeted colon delivery and release. For example, hydrogels containing GIT-resistant prebiotic polysaccharides, such as pectin or inulin, can be only decomposed in the presence of colonic bacteria enzymes (Tan et al., 2022). Such synbiotic formulation not only improves the functionality and viability of loaded probiotics during storage and digestion but also benefits host intestinal health (Nezamdoost-Sani et al., 2024; Thinkohkaew et al., 2024).

4.1. Fructans

In scientific literature, fructans (FOS and inulin) are generally recognized as safe food ingredients (FDA), which have been widely acknowledged as traditional prebiotics due to their well-documented ability to stimulate growth and activity of beneficial gut bacteria, particularly *Lactobacillus* and/or *Bifidobacterium* species (Gibson et al., 2017; Nagy et al., 2023). As such, EFSA has authorized the health claim of native chicory inulin that 'contributes to normal bowel function by increasing stool frequency' (Commission Regulation (EU) 2015/2314). Inulin is a soluble linear polysaccharide composed of β-D-fructose units connected via β-(2→1) glycosidic bonds and a terminal D-glucose residue (Qin et al., 2023). Several studies have exploited inulin with other wall materials, e.g., whey, sodium alginate, SPI, and sodium caseinate, for probiotic encapsulation, thereby demonstrating its assistance in improving EE, microorganisms' protection, and stability due to its characteristic properties like resistance to pH, low hydrolysis, thermal stability, prebiotic activity, and cryoprotection, among others (Poletto et al., 2019; Qin et al., 2023; Virk et al., 2024) (Table 4).

Besides inulin, other fructan-type carbohydrates were also explored. In agave fructans, or simply agavins, fructose units are linked via β-(2→1) and β-(2→6) bonds, therefore having a more branched structure and being a good alternative to linear inulin (Shi et al., 2023). Agavins, for instance, were used alongside whey protein for the encapsulation of *S. boulardii* (Chávez-Falcón et al., 2022), *L. rhamnosus* (Alvarado-Reveles et al., 2019), *L. acidophilus, B. longum subsp. infantis* (Juárez-Trujillo et al., 2024), and *Enterococcus faecium* (Cancino-Castillo et al., 2020). These encapsulations provided better storage, thermal, and structural stability, improved probiotics survival during particle processing and *in vitro* digestion, additionally resulting in a controlled release compared to the encapsulation with agavins or whey proteins alone (Alvarado-Reveles et al., 2019; Cancino-Castillo et al., 2020; Juárez-Trujillo et al., 2024) (Table 4). Meanwhile, microbial exopolysaccharide levan, another fructan with linear β-(2→6) fructose units'

Table 4
Examples of synbiotic systems and their effects on probiotic encapsulation with inclusion of next-generation prebiotics.

Prebiotics	Partners in hydrogel	Probiotic	Crosslinking method	Effects on:			Refs.
				Structure/rheology	EE (%)	Survival	
Inulin	Sodium caseinate, SPI, carboxymethyl cellulose	*L. plantarum CICC 21804*	Physical ultrasonicated complexation	Strong to hold the water and remain stable; smooth and appealing; Moderately soft, viscous and flexible.	95.95	↑ Thermal resistance at 63 °C for 15 min: 6 vs 2 lg CFU for free cells; after 30 min – ~4.25 lg. ↑ protection against H_2O_2, at 1 mM: 7 lg CFU vs <4 lg for free cells; ↑storage viability: more than 4.58 lg CFU higher viability than free cells; ↑ viability in SGF: > 7.9 vs 5 lg CFU for free cells; in SIF: > 6.9 vs 4 lg CFU	(Virk et al., 2024)
Inulin	Alginate+chitosan coating	*L. casei TISTR 1463*	Physical – ionic crosslinking with $CaCl_2$ + electroactivity	Uniform in size and shape, spherical, more intricate network of creases and wrinkles, denser structures	83-88	↑viability under SGF: ~7 log CFU/g; under SIF: ~6.5 log CFU/g, while free cells did not survive the entire digestion process; ↑initial release and sustained elevated release levels over time ↑ stability at 4 °C after 4 weeks: ~7.6 vs ~5 log CFU/g for free cells	(Thinkohkaew et al., 2024)
Inulin	Pectin	*L. acidophilus*	Physical – ionic crosslinking with $CaCl_2$	Elliptical shape with a wrinkled surface; water activity <0.3	68.1	↑viability after GIT digestion: after 200 min a reduction (from the initial count) of 1.6 (final 6.1) vs 3.4 log CFU/g for free cells; kept viability >10^6 CFU/g at 25°C for 90 days and -18°C for 120 days	(Raddatz et al., 2020)
Rice bran (22.9 % dietary fiber)					66.2	↑viability after GIT digestion: after 200 min a reduction (from the initial count) of 1.0 (final 6.1) vs 3.4 log CFU/g for free cells; kept viability >10^6 CFU/g at 7°C for 120 days	
Hi-maize (amylose-rich resistant starch)					66.7	↑viability after GIT digestion: after 200 min a reduction (from the initial count) of 0.1 (final 7.4) vs 3.4 log CFU/g for free cells	
Agavin (agave fructan)	WPI, sodium alginate	*S. boulardii CNCM-I 745*	Physical – ionic crosslinking with $CaCl_2$	Heterogeneous internal structure + smoother homogeneous external structure	94-97	88.5 % cell survival (7.3 × 10^7 CFU/mL) after the simulated GIT digestion vs 70.37, 75.58 and 72.66 % in control beads of only alginate, fructans, and whey proteins, respectively; controlled-release behavior.	(Chávez-Falcón et al., 2022)
IMO XOS GOS	SPI	*L. casei*	Maillard conjugation between polymers; physical linkage – pH-driven internal gelation (with glucono-delta-lactone) to form microbeads	Cohesive, continuous surface and smooth protuberances	73.22 70.68 62.54	↑survivability under heat treatment at 68°C for 30 min: > 6 vs 2.15 log CFU/mL for free cells (no statistical difference among three prebiotics); ↑ survivability under mechanical forces at 12,000 g for 20 min: almost fully retained at 8 vs 6.16 log CFU/mL for free cells; ↑ survivability during storage: decreased by only 0.87−1.04 to 7 log CFU/mL vs to 3.45 log CFU/mL for free cells after 30 days; ↑ survivability after SGF+SIF: remained at 7.4	(Zhong et al., 2021)

(*continued on next page*)

Table 4 (*continued*)

Prebiotics	Partners in hydrogel	Probiotic	Crosslinking method	Effects on:			Refs.
				Structure/rheology	EE (%)	Survival	
						vs 1.6 log CFU/mLfor free cells	
POS/ low methoxyl pectin (LMP)	Pea protein isolate (PPI)	*L. reuteri ATCC 23272*	Microgel was produced by crosslinking PPI with both transglutaminase and Ca2+ ions; Hydrogel beads were prepared with additional coating of POS and LMP (crosslinking anionic pectin molecules with Ca2+ ions)	Rough surfaces with some pores; with POS increase – smoother and more compact network; amorphous structure.	89.10-96.08	↑viability after SGF: retained >90 % of the encapsulated cells vs reduction to 59.02 % for free cells; after SIF: survival rates of 86.06 % 93.52 %, while none of the free cells survived; Sustained *L. reuteri* release in simulated colonic conditions by 54.62–65.36 % after 4 h, and 82.67–92.36 % after 48 h. ↑viability under thermal stress and during storage at 4 °C for 14 days	(Yi et al., 2025)
SBP	SPI	*L. paracasei LS14*	Enzymatic cross-linking with polymerase laccase	Hydrogels induced by 2 U/g laccase gave relatively low gel strength; disordered and relatively loose network; 10 U/g – the largest storage modulus; highest gel strength, hardness and lowest swelling ratio; more organized and stable microstructure	88.9	↑viability after SGF: over 96.4 % (initial 7.44-7.67 log CFU/ml), while no viability for free cell; ↑viability in SIF in comparison to already dead free cells, but lower than after SGF–decreased by 0.45-1.25 log CFU/ml. Final viability after simulated GIT conditions were above 84.5 %.	(Yan et al., 2021)
Barley β-glucan	WPI-gum Arabic	*Lactobacillus*	Physical – electrostatic and physicochemical interactions	Improved the morphology and structure	21.33-51.69	↑viability after simulated GIT digestion: with 3 % of glucan, only 0.84 log CFU/g was lost during the entire digestion (2-6 h), while free cells lost all the viability within 1.5 h in SGF;	(Yuan et al., 2023)
Resveratrol (Res)	Phycocyanin+ pectin	*L. plantarum*	Physical – ionic crosslinking with $CaCO_3$	Res and GA – more haphazard, inhomogeneous morphology, larger pores; GA – highest hardness, but low water holding capacity; TA- homogeneous, denser pores; All solid form and more elastic than viscous.	n.d.	From initial 8.72 log CFU to ~8 after gastric, to ~7 after intestinal digestion vs from 8.64 to ~ 7.7, to ~6.8 without any polyphenol. after 40 days at 4 °C: decreased to ~8 vs to ~8 log CFU without any polyphenol	(Zhu et al., 2024)
Gallic acid (GA)						From initial 8.33 log CFU to 7.72 after gastric, to 6.44 after intestinal digestion; after 40 days at 4 °C: decreased to 7.66 log CFU	
Tannic acid (TA)						From initial 8.88 log CFU to 8.39 after gastric, to 7.51 after intestinal digestion; after 40 days at 4 °C: decreased to 8.44 log CFU;	
Curcumin	Zein-chitosan	*L. casei (LCG)*	Physical – electrostatic and physicochemical interactions	Bilayer structure, uniform shape, relatively dispersed and evenly distributed cells	58.13	n.d.	(Ma et al., 2024)
Quercetin					68.44	↑survival after SGF: 75 % survival vs 22.63 % for free cells	
Rutin					57.35	↑survival after SGF: 86 % survival 7.30 log CFU/mL vs 22.63 %	
Tea polyphenol					41.5	n.d	

linkage and occasional β-(2→1) branches, has received less attention in studies involving probiotic encapsulation. Except that it was added to skim milk during freeze-drying of *Lacticaseibacillus* strains as an extra cryoprotectant (Yavuz et al., 2024). Levan has excellent emulsifying, thickening, stabilizing, thermal resistance properties and prebiotic activity confirmed *in vivo* (Cheng et al., 2021). In addition it can self-organize in water, which makes it an increasingly desirable material for encapsulating active compounds. Nevertheless, limited availability and high manufacturing costs currently limit its use, especially on an industrial scale (Domżał-Kędzia et al., 2023).

4.2. Next-generation prebiotics

In 2017 the ISAPP updated and defined prebiotic as 'a substrate that is selectively utilized by host microorganisms conferring a health benefit' (Gibson et al., 2017). Suggesting that compounds other than traditional non-digestible carbohydrates (e.g. inulin, FOS, resistant starch) may also possess prebiotic properties, consequently stimulating research on the next-generation prebiotics (Shang, 2022). Next-generation prebiotics meet the same requirements as traditional prebiotics, but they encompass a wider range of molecules, including new carbohydrates like XOS, mannanoligosaccharide, pectin, plant-derived compounds such as polyphenols (Haş et al., 2023; Zhao et al., 2023; Zhu et al., 2023), and polyunsaturated fatty acids (Cardoso et al., 2021; Chaplin et al., 2015; Lee et al., 2023; Rinninella & Costantini, 2022). Furthermore, next-generation prebiotics support the growth of specific beneficial microorganisms beyond merely *Bifidobacteria* and *Lactobacilli*, including species such as *A. muciniphila, F. prausnitzii* or *Clostridioides difficile* (Hirano et al., 2021) (Temis-Cortina et al., 2025). Additionally, beyond serving as fermentable substrates, these prebiotics may also have multi-mechanistic roles in enhancing the gut barrier function, modulating immune or metabolic signaling (Peled et al., 2024). Therefore, they may be used not only for overall gut health, but also for addressing metabolic disorders, immune modulation, neurological health, and personalized nutrition (Tuohy et al., 2024; Kaur et al., 2021). Nevertheless, this research topic is rather new and the beneficial mechanisms of next-generation prebiotics have not been fully elucidated, so detailed investigations are highly necessary (Shang, 2022).

4.2.1. Non-digestible carbohydrates as next-generation prebiotics

In addition to fructans, short-chain carbohydrates, such as IMO, XOS, or GOS, are considered as prebiotic oligosaccharides. As a matter of fact, carbohydrates, including XOS, pectin, β-glucans, mannooligosaccharides and polydextrose (L. Zhu et al., 2022), are now emerging as next-generation prebiotics (Global Prebiotic Association, 2025; Shang, 2022). Regarding their application in hydrogel networks, IMO, XOS and GOS were coupled with SPI to encapsulate *L. casei,* leading to higher survivability under heat treatment, mechanical forces, simulated digestion conditions, and storage in comparison to free cells (Zhong et al., 2021).

Pectin, which is abundantly found in the cell walls of many plants, especially fruits and vegetables, is an anionic soluble polysaccharide with α-(1,4)-linked D-galactosyluronic acid and monosaccharides, most likely arabinose, galactose, and rhamnose, as side chains. Additionally, due to its chemical structure being resistant to protease and amylase but fermented by colon microflora pectinases, pectin, as a dietary fiber was shown to stimulate the growth of *Bifidobacterium* and *Lactobacillus* species (Raddatz et al., 2020). Moreover, due to its great gelling properties at specific conditions, it has great potential in the application of hydrogels for colon-targeted delivery. In line with this, Raddatz et al. (2020) investigated the influence of prebiotics like resistant starch (from high-amylose corn 'hi-maize'), inulin, and rice bran on the viability of *L. acidophilus* loaded in pectin microparticles (Table 4). Eventually it was demonstrated that pectin microparticles in combination with these prebiotics enhanced viability both at 25°C and -18°C storage temperatures, as well as during simulated GIT treatment.

Recently, pectic oligosaccharides (POS), obtained from a partial hydrolysis of pectin and showing comparable or even better prebiotic activity than FOS, have been applied to develop synbiotic encapsulation systems. A study by Yi et al. (2025) showed that the incorporation of POS into pea protein microgel-reinforced LMP hydrogel beads made the network more uniform and less porous, thus preventing *L. reuteri* escape and increasing EE. Consequently, according to the *in vitro* digestion test, such hydrogel beads containing POS in a concentration of 0.4 %, exhibited the highest survival rate across the upper GIT. Its prebiotic activity was associated with the highest rate of probiotic release over 48 hours simulating colonic conditions.

Furthermore, utilization of industrial waste-derived pectin in the development of hydrogels for probiotic encapsulation presented not only promising results but also an opportunity to transform waste materials into valuable products. A few such applications include Bael pulp residue pectin utilized for the extrusion of microparticles embedding *B. clausii* and *S. boulardii*, thus providing a significant effect on their survivability and stability during refrigerated storage (Surolia et al., 2024). Sugar beet solid waste pectin alongside ferulated arabinoxylan derived from maize bioethanol waste was used in the development of hydrogels embedding *S. boulardii* (Ohlmaier-Delgadillo et al., 2021) and in combination with SPI for *L. paracasei*, resulting in high EE and improved bacteria viability in simulated gastric and intestinal fluids (Yan et al., 2021).

Lastly, glucans, which are diverse and complex polysaccharides structured from glucose monomers, can be obtained from different sources, including cereals (C. Yuan et al., 2023), yeast cells (Pi et al., 2022), or algae (Dai et al., 2022). Yuan et al. (2023) successfully incorporated barley β-glucan into a WPI-gum Arabic matrix, significantly improving the morphology and structure of probiotic microcapsules. Such inclusion of glucan increased *Lactobacillus* survival during spray drying and *in vitro* digestion, as well as effectively improved bioavailability of cells. Thus, demonstrating the role of glucan not only as a prebiotic but also as a structural enhancer.

4.2.2. Non-carbohydrate plant-derived compounds as next-generation prebiotics

Polyphenols have always attracted attention for their extensively studied antioxidant and anti-inflammatory properties, which give them a preventive effect against many diseases. And more recently, their prebiotic potential has also been recognized, given that most polyphenols remain unabsorbed at the small intestine and reach the colon (Cardoso et al., 2021; Rodríguez-Daza et al., 2021; Yuan et al., 2021). As thoroughly reviewed by Plamada and Vodnar (2022) and Petersen and Mansell (2025), polyphenols in addition to their antimicrobial properties, both through their structure and resulting metabolites, serve as prebiotic substrates for gut microbiota, consequently modulating its composition. First of all, plant polyphenols usually occur naturally in the form of glycosides, which makes them resistant to the GIT and able to reach the large intestine. Considering this, the first possible prebiotic mechanism could be backed by the gut bacteria having β-glucosidases, which are specific to the hydrolysis of glycosylated polyphenols rather than disaccharides (e.g., *L. acidophilus, Levilactobacillus hammesii, Furfurilactobacillus milii, Furfurilactobacillus rossiae*) (Dymarska et al., 2024). These enzymes cleave glycosidic bonds and release simple sugars for further bacterial growth, additionally freeing lipophilic aglycone form of polyphenol, which can then exert its biological activity easier. Microbial polyphenol-active enzymes (e.g., lavone reductase, isoflavone reductase, tannases, catechol dehydroxylases) catabolize polyphenols through reduction, ring fission, and decarboxylation, producing smaller phenolic metabolites like phenolic acids that aid microbial growth and host metabolism. In parallel, polyphenols exhibit selective antimicrobial effects – inhibiting pathogens like *Escherichia coli, Clostridium perfringens* and *Helicobacter pylori*, while promoting beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (Plamada & Vodnar, 2022; Zhu et al.,

2023), which creates ecological niches for these beneficial bacteria. What is more, new studies support polyphenolics contribution to the enhanced SCFAs production by increasing the abundance of SCFA-producing bacteria like *A. muciniphila, F. prausnitzii*, and *L. plantarum.* Consequently, it improves intestinal barrier integrity and reduces inflammation (Petersen & Mansell, 2025). For example, cranberry-rich extracts stimulated *A. muciniphila* and *Lactobacillus* while inhibiting nonbeneficial bacteria (Prasain & Barnes, 2020), and blueberry polyphenols increased the amount of *Akkermansia* and reduced *Proteobacteria* (Della Lucia et al., 2023).

In this context, Plamada and Vodnar (2022) and Petersen and Mansell (2025) some of polyphenols have been studied within the probiotic encapsulation systems to create a synbiotic environment. As it's going to be exemplified further, different polyphenols exhibit varied effects on hydrogel structure, and thus on EE and probiotic viability as well, therefore it is necessary to carefully consider the type of polyphenol utilized. Zhu et al. (2024), investigated the influence of resveratrol, tannic, and gallic acid (0.9 % (w/v)) incorporation within phycocyanin-pectin-based hydrogels embedding *L. plantarum.* Polyphenols rich in hydroxyl groups interact with protein and pectin through hydrogen bonds and hydrophobic association, resulting in enhanced mechanical, protective, and functional properties. Overall, tannic acid, having the most -OH groups, was the only fully encased in a hydrogel matrix and performed best by compacting hydrogel pores and preventing premature release. It also improved bacteria viability during simulated digestion (7.5 vs 6.8 log/CFU without polyphenol) and 40-day refrigerated storage (8.5 vs 8.0 log/CFU without polyphenol) (Table 4). As an antioxidant, it protected bacteria from oxidative stress and, and its potential prebiotic role served as a bacteria's nutrient during the storage period (Zhu et al., 2024). In another study, gallic acid (0.04 % (w/v)) co-microencapsulated with *Lactobacillus kefiranofaciens* provided antioxidant, anti-inflammatory, and prebiotic effects while strengthening microcapsule structure through hydrogen bonding with outer-shell polysaccharides. Similarly, the antioxidant activity of gallic acid protected probiotics from heat and oxidative stress, while serving as a nutrient also improved cells viability for 4 weeks at 4°C (from 9 log CFU/g at the initial day to 4.59 ± 0.06 log CFU/g) in comparison to free cells completely lost by 3rd week. Moreover, gallic acid acted synergistically with both probiotics and outer-shell prebiotic Fu brick tea polysaccharides, thus promoting gut colonization and extending retention to 48 hours *in vivo* mice model (Sun et al., 2024).Another critical consideration is the appropriate polyphenol concentration that provides probiotic benefits. This is important, as elevated concentrations may restrict growth and reproduction due to disruption of intracellular redox balance. Ma et al. (2024) designed a unique bilayer capsule by zein-chitosan complex coacervation to co-deliver hydrophilic *L. casei* and hydrophobic polyphenols (quercetin, curcumin, rutin, and tea polyphenols). Here, low concentrations of polyphenols (0.05 %), particularly quercetin, enhanced probiotic viability (to $\sim 1\times10^{10}$ vs $\sim 4\times10^{9}$ CFU/mL in a blank without polyphenols) and EE (from 50 % to ~70 %), while maintaining good radical scavenging activity at 60 %. Contrary, high concentrations (1.0 %) maximized radical scavenging activity to ~82 % but significantly reduced probiotic survival to $\sim 3.2\times10^{9}$ CFU/mL, likely due to excessive antioxidant activity (Table 4).

Taking everything into account so far, as referenced studies suggest, a recommended polyphenol concentration, being still beneficial to probiotics, could be in the range of 0.04-0.9 % (w/v). Nevertheless, the influence of only a few polyphenols on a narrow range of probiotic bacteria species have yet been investigated, necessitating more extensive research.

5. Conclusions and future scopes

Hydrogel-based encapsulation is a great approach for preserving the viability of probiotic cells during production, storage, and digestion in the gastrointestinal tract. Despite the wide range of materials and methods available for encapsulation, many challenges are still confronted with the encapsulation of live bacteria cells. Employing natural and composite hydrogel networks—combinations of protein and polysaccharides like soy protein isolate–pectin, whey protein isolate–dextran or whey protein isolate–κ-carrageenan —offers structural integrity, durability under challenging conditions, mechanical strength, and compatibility with biological systems, while facilitating gentle, food-safe processing and adjustable delivery mechanisms.

Moreover, incorporating next-generation prebiotics such as polyphenols, pectic oligosaccharides, and agave fructans expands the function of system from protective matrix to bioactive synbiotic platform, capable of influencing gut microbiota and metabolic outputs. However, in the context of polyphenols, it is important to critically consider a dosage that is both prebiotic-efficacious and safe, ensuring it does not perturb the cellular redox balance.

Future research should focus not only on elucidating and scaling up the design solutions for hydrogel-probiotic-prebiotic systems but, more importantly, prioritize the *in vivo* validation of such systems. This includes an examination of their prebiotic-driven effects with the aim of clarifying their role in microbiota modulation, metabolite production, and host physiological responses. Moreover, it is essential to confirm the delivery efficiency and transit time in the gastrointestinal tract to guarantee functional efficacy, aiming for at least 10^{8} CFU per dose reaching the colon. In parallel, standardized dynamic digestion models and unified reporting metrics are necessary to ensure comparability and reproducibility of encapsulation results. Eventually, another important objective is to assess food-grade crosslinking agents such as calcium ions, genipin, aldehydes, transglutaminase and laccase, not only in terms of their effectiveness but also for their effects on texture, sensory attributes, nutritional value, and consumer acceptance. Strengthening these interdisciplinary links among structural composition, function attributes, and perceptual evaluation will enable the development of synbiotic hydrogels that are scientifically sound, nutritionally advantageous, and acceptable in real food systems. For that, future industrialization efforts are expected to depend on materials and fabrication strategies that balance cost-effectiveness, biocompatibility, and large-scale production rather than on a single specific polymer system.

Glossary

ISAPP – International Scientific Association for Probiotics and Prebiotics
EFSA – The European Food Safety Authority
FDA – Food and Drug Administration
GIT – Gastrointestinal Tract
NGPs – Next-Generation Probiotics
SGF – Simulated Gastric Fluid
SIF – Simulated Intestinal Fluid
CFU – Colony Forming Unit
EE – Encapsulation Efficiency
IPNs – Interpenetrating Networks semi-IPNs – semi-Interpenetrating Networks
SCFAs – Short-Chain Fatty Acids
XOS – Xylooligosaccharides
FOS – Fructooligosaccharides
IMO – Isomaltooligosaccharides
GOS – Galactooligosaccharide
POS – Pectic Oligosaccharides
LMP – Low Methoxyl Pectin
SBP – Sugar Beet Pectin
SPI – Soy Protein Isolate
WPI – Whey Protein Isolate
TA – Tannic Acid
Tp – Tea polyphenol
Que – Quercetin

Ethical Statement - studies in humans and animals

This review article does not involve any studies with human participants or animals performed by the authors. All data discussed are from previously published studies.

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CRediT authorship contribution statement

Vidmantė Minelgaitė: Writing – original draft, Visualization. **Sigita Jeznienė:** Writing – review & editing. **Aušra Šipailienė:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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