Food Science of Animal Products

Review Article

Diversified modification strategies for sodium alginate-based probiotic-embedded gels and their potential for food applications

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Abstract: Sodium alginate (SA), due to its unique biodegradability and gel-forming properties, is used as an excellent wall material. It can achieve targeted delivery of probiotics into the body for the purpose of improving human microecology. However, SA hydrogels alone tend to have weak mechanical properties and are susceptible to external environmental attack, making it difficult to adequately protect the probiotic core. In order to overcome this bottleneck, in recent years, there have been literature reports on the modification of SA-based hydrogels based on physical, chemical, and synergistic modification of both. And other novel wall materials were introduced to be blended with SA to form a composite wall material with a denser structure and superior performance, which enhances the resistance and storage period stability of probiotics. Based on this, this paper outlines the structural properties and advantages and disadvantages of SA, and systematically summarises the effects and differences of different modification methods on the performances of SA-based microcapsules. It also meticulously combs out the wide application of SA-based probiotic microcapsules in the field of food, showing its great potential in improving food quality and promoting health. Finally, this paper objectively points out the problems and challenges facing the current research on SA-based microencapsulation delivery system. It provides new perspectives and strategies for the upgrading of the wall material of SA-based microencapsulation and the improvement of the efficiency of the probiotic delivery system.

Keywords: sodium alginate; probiotics; microencapsulation; modification; application

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1 Introduction

In the post epidemic era, the demand for probiotics as emerging functional factors is growing rapidly. Foods and beverages containing probiotics account for 82% of market sales and are expected to reach a total of $69.3 billion by 2030, according to 2023 statistics[1]. Probiotics need to be taken orally and reach a certain unit biomass (10^6 CFU/g) in the colon to provide significant benefits to the host. However, commercial probiotic products are often susceptible to inactivation and thus affect their health benefits due to adverse factors such as processing condition, storage environment and host digestion[2]. Microencapsulation is a widely used technology for the delivery of bioactive substances such as probiotics, macromolecular proteins and sensitive drugs. It can encapsulate the target substance in a protective wall material to improve the bioavailability of the core material and achieve the purpose of targeted controlled release[3].

Currently, polysaccharides (chitosan, xanthan gum, dextrin, and carrageenan) and animal and plant proteins have been used to prepare microencapsulated-probiotic delivery systems. Among them, sodium alginate (SA) has attracted much attention due to its excellent properties such as high biocompatibility, biodegradability, good mechanical properties and promotion of intestinal adhesion of probiotics[4]. Our relevant search on web of science found that the number of publications of SA-related studies ranked in the top three in the field of probiotic encapsulation. However, pure SA gels face certain limitations in practical applications due to their inherent porosity and easy permeability[5]. In recent years, as the research on microencapsulation of probiotics has increased dramatically, the design of walls based on SA has become more subtle[6]. By combining SA with other materials using physical, chemical and both synergistic approaches, the aim is to maximise the advantages of SA-based gels while effectively compensating for their shortcomings[7]. These novel strategies not only enable fine tuning of the gel structure, but also endow the microcapsules with excellent antioxidant properties and long-term storage stability. They ensure the effective retention of probiotic activity in complex food matrices such as dairy products, beverages or breads, while also demonstrating good controlled release in oral gastrointestinal delivery[8]. These strategies open up broader application prospects for probiotic products in the food industry.

Given that previous reviews have focused more on the potential of a single microencapsulated wall material for encapsulation and delivery, or the interaction between alginate gels and probiotics. However, with the continued emergence of novel materials and the diversification of probiotic embedding targets, the key to microencapsulation improvement has shifted to the combinatorial effects of alginate. Based on these, our paper outlines the structural properties and application limitations of SA, and focuses on different modification methods of SA-based probiotic-embedded

gels, including physical, chemical, and synergistic modification of the two, in order to clarify the advantages of modified SA-based composite microcapsules and their protective properties in probiotics. The paper also summarizes the applications of microcapsules in food field and finally looks forward to the challenges of microencapsulation research of probiotics, which is instructive for the future research of SA-based microencapsulation of probiotics.

2 SA

2.1 Structures and properties

The molecular formula of SA is usually expressed as $(C_6H_7O_6Na)_n$ or $(C_6H_9NaO_7)_m$. The molecular weights are in a wide range, usually between 32 and 250 kDa, depending on the source of the SA and the extraction process. It is a natural anionic polysaccharide consisting of *α-L*-guluronic acid (G-unit) and *β-D*-mannuronic acid (M-unit) relying on *β*-1,4 glycosidic bonds. The film-forming mechanism of SA is primarily contingent upon the prevalence of carboxyl and hydroxyl groups within its molecular chain. The aforementioned functional groups are capable of forming hydrogen bonds with water molecules, which results in the aggregation of SA molecules in water, thereby forming colloidal particles. As the concentration of the solution increases, the particles form a mesh structure through intermolecular interactions, which ultimately results in the formation of a stable film[9]. When divalent cations such as Ca^{2+} and Cu^{2+} are present, the Na^+ on the G-units undergoes a substitution reaction with the divalent cations, and the G-units pile up to form a cross-linking network structure, resulting in the formation of a hydrogel, which is often described by the 'egg carton model'[10]. Typically, alginates with a high G-unit content form stiffer, more porous gels that maintain their integrity over a longer period of time. Conversely, alginates with high M-unit content form softer, less porous gels that tend to disintegrate over time[11]. It can be seen that the intrinsic properties of SA, i.e., M/G ratio, order of arrangement, length of G-G chain segments, and molecular mass are the key factors affecting the properties of hydrogels prepared from it, which significantly influences the stability of the gels, the rate of release, and so on.

SA microencapsulation formula is divided into two types: external gelation and internal gelation[12]. External gelation occurs when free divalent cations and the molecular chains of carboxyl groups in sodium alginate rapidly cross-link upon contact, forming an insoluble shell. This cross-linking then continues to diffuse from the outside inwards, increasing the thickness of the shell[13]. This gelation method is easy to operate and has a high encapsulation rate, and almost does not cause mechanical damage to the core material, however, there may be problems such as uneven ion diffusion, difficult to regulate the capsule structure, and poor stability. Internal gelation, on the other hand, involves mixing insoluble calcium salts with SA, and then free calcium ions released *in situ* under acidic environment combine with SA to form calcium alginate, which forms a more homogeneous and stable gel structure, and is mostly used for the development of new pH-responsive hydrogels[14].

2.2 Advantages and limitations

SA, since its approval by the U.S. Food and Drug Administration (FDA) in 1983 for direct application in the food and pharmaceutical industries, has increased its production significantly[15]. Among them, China is the world leader in the production of SA, accounting for 40% of the world's total production. In particular, the ion-derived gelling properties of SA have great potential for application in the field of gastrointestinal delivery of bioactive substances. Studies have shown that both small molecule bioactive components such as anthocyanins[16], *β*-carotene[17], and riboflavin[18], large molecule proteins[19], and cellular metabolites[20] can be encapsulated in alginate gels. This not only improves the high stability of the active molecules in the complex gastrointestinal environment and effectively protects against digestive enzymes and bile salts, but also enables the targeted release of the core. In addition, the structure of SA contains a large number of carboxyl groups, which are capable of hydrogen bonding with mucus glycoproteins with high bioadhesion properties, adhering to the surface of the intestinal mucosal targeting site. This can prolong the residence time of the core in the colon and improve its bioavailability[21]. As the efficacy of probiotics is becoming more widely recognized by the general public, SA-based gels are being developed for the delivery of probiotics.

However, SA microcapsules alone have certain limitations, including relatively weak stability, limited barrier properties, loose and porous structure, variable affinity for heavy metal ions, and instability during heat treatment. To overcome these shortcomings, it is necessary to establish effective combination strategies, where SA is combined with novel materials by physical, chemical or co-mingling methods, such as cross-linking, lamination or nanocomposites.

3 SA-based composite microcapsules prepared by different modification methods

3.1 SA-based composite microcapsules prepared by physical method

3.1.1 SA-non-cationic polysaccharide

Non-cationic polysaccharides refer to polysaccharide molecules without positive charge and often do not form cationic forms in water. They can add protective utility to core probiotics by filling gel pores, forming gel outer films, acting as prebiotics, and improving the adhesion of probiotics.

Carrageenan, pectin and gum arabic are common polyanionic polysaccharides. Carrageenan has an ionisable sulphate group which can improve the structure of SA gels, resulting in better interconnectivity within the matrix[22]. Pectin is a heteropolysaccharide derived from plant cell walls, and its polymer chains form a reticulated gel with good elasticity and solubility. In the presence of Ca^{2+}, polygalacturonic acid from pectin is able to complex with SA to localise Ca^{2+} at specific sites, resulting in a tighter internal structure of the gel[23]. Gum arabic is not only able to act as a prebiotic to stimulate the growth of lactic acid bacteria. It is also able to be mixed with alginate to increase the hardness of the gel beads and enhance the internal three-dimensional network structure, and gastrointestinal simulations has shown that it can promote a significant increase in the survival rate of probiotic[24].

Starch, a polysaccharide consisting of glucose units linked by *α*-1,4 and *α*-1,6 glycosidic bonds, is a neutral polysaccharide that can interact with SA through hydrogen bonding to improve gel properties. Porous starch is a kind of modified starch with relatively homogeneous pores distributed on the surface, which can achieve controlled release in different environments by adjusting the ratio

between it and SA, and has good pH responsiveness. In particular, the enzyme-modified porous starch has a porous surface and internal cavities, which can improve the adhesion ability of probiotics on the surface of the particles, and at the same time, it can fill the internal space of the gel to retain the probiotics, which can effectively improve the encapsulation efficiency and thermal stability of the syste[25].

In addition, sporopollenin is a natural polymer material derived from the pollen wall of plants. It can form a core-shell structure with SA, which can not only cover the holes on the outer wall, but also control the release of probiotics encapsulated inside[26]. Hydroxymethyl *Poria* polysaccharide, produced by hydroxypropylation reaction, can improve the swelling behaviour and microstructure of SA gel beads, thus regulating the release of the contents[27].

In conclusion, non-cationic polysaccharides, due to their gelling, thickening and stabilizing properties, can significantly enhance the structural strength and mechanical properties of gels when synergistically prepared with SA, which is conducive to the stable delivery of probiotics *in vivo*. However, the distinctive molecular characteristics of various polysaccharides, along with the influences of their sources and extraction techniques, have resulted in disparate gel modification outcomes. Further research is required to elucidate the molecular characteristics of non-cationic polysaccharides and their interactions with SA, with the objective of optimizing gel properties and expanding the range of potential applications.

3.1.2 SA-double emulsion

Double emulsions are a class of multiple emulsion systems that, when mixed with SA-based gels, the oil droplets trapped in the gel network are attracted to each other and thus tightly stacked, increasing the denseness of the gel structure to prevent probiotic leakage. Studies have shown that SA-dual emulsion probiotic capsules demonstrated good stability in simulated gastric digestion. The reason for this is that under acidic conditions of gastric juice, alginate is converted to insoluble alginate, which synergistically encapsulates the probiotics with the emulsion and prevents the penetration of hydrogen ions and enzymes. In addition, compared to *Lactobacillus acidophilus*, *Bifidobacterium animalis* had higher viability in simulated gastric digestion and storage experiments, suggesting that the same encapsulation system has a difference in protective effect for different probiotic core[28].

The composite of double emulsion-SA gel system with other wall materials can also significantly improve the oxidative stability and mechanical strength of the gel system. For example, Šipailienė et al.[29] added lipophilic sea buckthorn pomace extract as an antioxidant to the emulsion gel to reduce the amount of chemical oil degradation during storage, and successfully improved the resistance of the delivery system. The addition of calcium carbonate to the emulsion gel system can achieve endogenous cross-linking, further increase the network density, enhance the gel strength, and also help to reduce the interfacial tension to achieve the purpose of stabilizing the system. It was also pointed out that the introduction of calcium carbonate particles promoted the clustering of probiotic bacteria, played a colony effect, and significantly improved the resistance of probiotic bacteria[30].

In conclusion, the physical modification makes the wall structure of SA-based probiotic microcapsules become dense and stable, which can effectively block the invasion of probiotics by external adverse factors, such as gastric acid and bile salts, and significantly improve the survival rate of probiotics in the gastrointestinal tract (Table 1). Meanwhile, physical modification can also achieve precise control of the release rate of probiotics by adjusting the composition and structure of the wall, which promotes the colonisation and growth of probiotics in the body. In addition, due to the simple, low-cost and environmentally friendly preparation method of the physically modified SA-based probiotic microcapsules, it is easier to achieve industrial production, which significantly enhances the added value and market competitiveness of the products.

Table 1 Encapsulation effect of physically modified SA-based probiotic microcapsules.

Coating materials	Probiotics	Preparation method	Experimental condition	Bacterial count change	Reference
SA, citric pectin	*Lactiplantibacillus plantarum* BL011	Electrospray	Gastrointestinal tract simulation for 120 min	Decrease of less than 1 (lg (CFU/mL))	[23]
			Storage at 4 °C for 21 d	Viable bacteria count was 9 (lg (CFU/mL))	
SA, arabic gum	*L. plantarum*, *Lactococcus lactis*	Extrusion	Simulated gastric juice for 4 h	Survival rate of 95.53%	[24]
SA, hydroxypropyl *Ganoderma lucidum* polysaccharide, sporopollenin exine capsules	*L. plantarum*	Vacuum loading technique	Lyophilization	Decrease of less than 1 (lg (CFU/mL))	[27]
			Storage at 4 °C for 90 d	Decrease of less than 1 (lg (CFU/mL))	
SA, canola oil-tween-80	*L. acidophilus* LA-5, *B. animalis* BB-12	Syringe emulsification	Storage at 4 °C for 30 d	Viable bacteria count greater than 6 (lg (CFU/g))	[31]
			Gastrointestinal tract simulation for 270 min	Capsules are resistant to the environment	
SA, sunflower seed oil-tween-80, probiotic mannitol, sea buckthorn pomace extract	*L. plantarum* F1	Syringe emulsification	Storage at 37 °C for 42 d	Survival rate of 80.99%	[29]
			Gastrointestinal tract simulation for 120 min	Decrease of less than 1 (lg (CFU/mL))	
SA, span-80-paraffin oil, calcium carbonate	*Saccharomyces boulardii*, *Enterococcus faecium*	Syringe emulsification	Gastrointestinal tract simulation for 180 min	Survival rate increased by 55.92%	[32]

3.2 SA-based composite microcapsules prepared by chemical methods

3.2.1 SA-chitosan and its derivatives

As shown in Table 2, chitosan-SA complexes are usually prepared by extrusion, and the polyelectrolyte complexes formed by the two are good carriers of bioactives to enhance their stability in unfavourable environments in the gastrointestinal tract. Specifically, chitosan is a linear polysaccharide composed of glucosamine units, and its combination with alginate microcapsules provides a 'double protective barrier' to the core material. That is, the internal barrier of the SA hydrogel layer formed by the dense polymer matrix, and the external barrier formed by the primary amino groups on the molecular chains of chitosan cross-linking with the carboxyl groups on the molecular chain segments of SA through intermolecular forces[38]. This can reduce the permeation of external substances by reducing the porosity of the gel network. The tightness and thickness of the external barrier formed on the surface of microcapsules are proportional to the chitosan concentration[39]. SA and chitosan are also adhesion agents that can adhere to intestinal mucus to prolong the retention time of probiotics and enhance their colonisation in the gut[40]. In addition, chitosanate, a derivative of chitosan, retains the cationic properties of chitosan. Its excellent water solubility under neutral pH conditions breaks through the limitation that chitosan can only be dissolved in organic or inorganic acid solutions.

3.2.2 SA-protein

Protein has good emulsification properties and low viscosity, which is conducive to the formation of a dense network inside the capsule, and can be used to prepare small particle size microcapsules[41]. However, single protein gels have poor mechanical and fluid properties, and are prone to flocculation and aggregation, resulting in a decrease in texture and water retention, which is not conducive to the encapsulation of probiotics[42]. The presence of polysaccharides, on the other hand, can enhance the intrinsic structural features of proteins and form cross-linked interpenetrating networks. Among them, protein and SA composite gels are widely used for probiotic delivery.

Gelatin is a natural protein derived from the partial degradation of collagen in animal tissues, which can form a 'honeycomb' three-dimensional network structure under the combined effect of heating, cooling, and ions. This facilitates the formation of a double-layer encapsulated hydrogel with SA. Gelatin can also improve the toughness and rigidity of the network structure through electrostatic interactions with the carboxyl groups in SA and intermolecular hydrogen bonding[43]. Different kinds of animal proteins have different cross-linking structures produced by endogenous emulsification with polysaccharide composite gels due to their different physicochemical properties. Overall whey protein microcapsules were optimal in terms of encapsulation rate and microcapsule morphology; gelatin microcapsules had the smallest particle size; and casein microcapsules disintegrated most easily in simulated intestinal fluid[44].

Plant proteins are biodegradable, have good emulsification and film-forming properties, and are also suitable for microencapsulation[45]. Compared with animal proteins, plant proteins have fewer allergens. Therefore, microcapsules prepared from plant proteins combined with SA have great potential for probiotic delivery. For example, microencapsulation of *B. bifidum* with SA (1.4 g/100 mL) and corn alkyd soluble protein as the wall material could reach 94.56% encapsulation rate. After *in vitro* digestion, the viability of bacteria in the naked group of bacteria and SA alone group decreased to 10^3 and 10^5 CFU/g, respectively. whereas the viable cell count of *B. bifidum* co-encapsulated with zeinolysin-SA was greater than 10^6 CFU/g[46].

In conclusion, chemical modifications can enhance the stability, controlled release properties, biocompatibility, and survival and activity of probiotics in SA-based microcapsules. This in turn prolongs the survival time of probiotics in complex environments (e.g., gastrointestinal tract) and enhances their health benefits once they reach the target site (e.g., intestinal tract) (Table 2). In addition to the modification methods mentioned above, future modification strategies should be optimized through continuous innovation. This includes the introduction of green cross-linking agents, surface modification, and copolymerization modification to prepare probiotic microencapsulated products with better performance and functionality, which will provide strong technical support and solutions for the food industry.

3.3 SA-based composite microcapsules prepared by synergistic modification

As diversified novel wall materials continue to be discovered, their co-encapsulation of probiotics with SA is expected to break through barriers and achieve complementary advantages and synergistic effects of various materials. This provides a better protective barrier for probiotic delivery (Table 3).

Table 2 Encapsulation effect of chemically modified SA-based probiotic microcapsules.

Coating materials	Probiotic	Preparation method	Experimental condition	Bacterial count change	Reference
Chitosan hydrochloride salt, SA	*Bacillus licheniformis*	Pore polymerization	Gastrointestinal tract simulation for 8 h	Survival greater than 6 (lg (CFU/mL))	[33]
SA, chitosan	*L. plantarum*	Syringe extrusion	Simulated gastric juice for 3 h followed by simulated intestinal juice for 8 h	Release rate of about 80%	[34]
SA, chitosan	Yeast	Syringe extrusion	Simulated gastric juice for 3 h followed by simulated intestinal juice for 4 h	Survival rate of 71%	[35]
			Storage at 4 °C for 30 d	Survival rate of 95%	
Fish gelatin, SA	*B. longum*	Syringe extrusion	Simulated gastric juice for 2 h	Survival rate increased by 15%	[36]
			Water bath at 60 °C for 30 min	Increase of about 2 (lg (CFU/mL))	
SA, gelatin, oligofructose	*L. acidophilus*	Syringe extrusion	Yogurt at 4 °C for 28 d	Viable bacteria count of 4.25 (lg (CFU/g))	[37]
			Simulated gastric juice for 2 h	Decrease of less than 2 (lg (CFU/mL))	

Table 3 Encapsulation effect of SA-based probiotic microcapsules modified by synergistic modification.

Coating materials	Probiotics	Preparation method	Experimental condition	Bacterial count change	Reference
Whey protein isolate, SA, shellac, soybean oil-span-80, sucrose	*Limosilactobacillus reuteri* TMW 1.656	Syringe emulsification	Simulated gastric juice for 2 h	Decrease of about 2 (lg (CFU/mL))	[47]
Whey or whey permeate, SA, pectin	*L. paracasei* ML33, *L. pentosus* ML82, *L. plantarum* ATCC 8014	Vibration extrusion	Storage at 4 °C for 90 d	Survival greater than 6 (lg (CFU/mL))	[48]
			Simulated gastric juice for 3 h followed by simulated intestinal juice for 4 h	Survival greater than 6 (lg (CFU/mL))	
Konjac glucomannan, SA, soybean oil, calcium carbonate	*Bifidobacterium*, *L. plantarum*, *L. casei*	Syringe emulsification	Simulated gastric juice for 120 min followed by simulated intestinal juice for 30 min	Survival greater than 7 (lg (CFU/mL))	[49]
SA, emulsion, chitosan	Lactic acid bacteria	Microfluidic technique	Storage at 18 °C for 150 d	Survival greater than 6 (lg (CFU/mL))	[50]
Carboxymethyl konjac glucomannan, chitosan, SA, soybean oil, whey protein isolate, trehalose, glycerin	*L. reuteri*	Syringe emulsification	Storage at 4 °C for 90 d	Survival greater than 8 (lg (CFU/mL))	[51]
			Simulated gastric juice for 6 h	Survival greater than 7 (lg (CFU/mL))	
SA, span-80-tween-80-liquid paraffin	*B. bifidum* ATCC 35914	Emulsion	Simulated gastric juice for 12 h	Survival greater than 7 (lg (CFU/mL))	[52]

Whey isolate protein has good buffering capacity and can improve the tolerance of the encapsulation system to gastrointestinal fluids. However, the SA-based capsules added whey isolate protein did not provide significant protection to probiotics during freeze-drying compared to the unadded ones. This may be due to the fact that the polysaccharide gel system has a water holding content and the ice crystals formed during freezing cause more significant damage to the cells than that can be compensated for by whey isolate proteins[47]. Whey permeate is the liquid fraction obtained after whey separation during the production of cheese or similar dairy products. It contains large amounts of water, lactose, proteins (mainly whey proteins), minerals, and vitamins, which are able to bind to alginate and pectin and improve the stability of the composite wall[48]. In addition, the addition of prebiotics in composite gel systems not only improves the structural characteristics of biopolymers, but also stimulates the growth of probiotics. For example, the addition of oligosaccharides to alginate-gelatin mixtures is beneficial to enhance the viscosity of the crystal structure, fill the intermolecular space created by the interaction between alginate and gelatin, and form a more cohesive structure[53]. Similarly, oligofructose has been used to improve the wall structure of SA-based gels[54].

Konjac glucan is a water-soluble dietary fibre, and when the volume of the oil phase in the composite gel is sufficient, the wall can be sufficiently emulsified and dispersed, and it can be combined with SA with weak hydrogen bonding and electrostatic interactions. This contributes to the formation of microspheres with uniform particle size and rich surface folds, which improves the probiotic encapsulation rate[55]. The high concentration of SA can also form viscosity and flow rate differences with the emulsion, and the interaction with calcium ions to form a composite gel with a higher shell/nucleus ratio can provide more effective encapsulation. Similarly, the large size of the multilayer microencapsulated gel also slows down the penetration of environmental agents into the capsule and helps the survival of encapsulated probiotics[56]. Although the double emulsion mentioned earlier is thermodynamically more stable than single emulsion, it is prone to problems such as oil droplet aggregation, internal aqueous phase polymerization, and two-phase migration.

Self-assembled carboxymethyl konjac glucan-chitosan nanogels formed by intermolecular electrostatic interactions were able to synthesise new carbonyl amide groups via ionic cross-linking. This further stabilised the double emulsion structure and effectively slowed down the release of probiotics from the gel beads to the digestive fluid[49].

However, multilayer synergistic encapsulation can significantly enhance the protection effect of probiotics, but the increased particle size is not favourable for product application. Therefore, ensuring the protection effect on probiotic cores at the minimum number of encapsulation layers is the focus of research. A study has used anionic polysaccharides to assemble layer-by-layer to form multilayer emulsions on the basis of double emulsions, and finally determined that three times coated emulsions are the suitable wall materials to maintain the viability of probiotics[57].

Both physical modification and chemical modification, or synergistic modification of both, are performed based on SA. The fundamental purpose is to improve the properties of SA gels, such as improving their mechanical strength, stability, biocompatibility, etc., in order to meet the application in the field of probiotic foods. Physical modifications such as co-mixing and heat treatment improve the properties without changing its chemical structure. Chemical modifications, on the other hand, change the molecular structure by complexing with cations, cross-linking, etc., to give new properties. Synergistic modification combines the advantages of both to enhance performance, but the process is more complex and requires consideration of the interactions.

4 Applications of microcapsules in food field

4.1 Dairy products

The post-acidification of yoghurt and the presence of oxygen make the survival of probiotics low, and the SA-based microencapsulation technology is a good anticorrosive strategy. In one study, probiotic capsules consisting of a composite wall of SA, chitosan, and whey protein concentrate were placed in curdled yoghurt for 28 d of storage, and the viability of the encapsulated probiotics was still higher than 6 (lg (CFU/mL))[52]. In another study, *L. acidophilus* was co-encapsulated with oligofructose in alginate-gelatin microgels and blended into liquid, Greek and frozen yoghurt. The results showed that after 21 d of storage, all three types of yoghurt had viable bacteria counts greater than

6 (lg (CFU/mL)), with the highest viable bacteria counts in the liquid yoghurt. Although the addition of gel beads decreased the apparent viscosity of Greek yoghurt, it had no effect on liquid and frozen yoghurt and significantly increased the bacterial viability of both in *in vitro* digestion[58].

The high fat content of cheese may slow down the damage caused by probiotics in gastrointestinal digestion. However, under microbial fermentation and metabolism in long-term storage, it still affects its quality and safety. In a study, *L. plantarum* was co-encapsulated with SA, soya isolate protein, and oligofructose and applied to high salt white brine cheese. The results demonstrated that microencapsulation inhibited the metabolism of probiotics, and their viable counts still increased by 2.6 (lg (CFU/g)) compared with free bacteria, and the survival of probiotics remained at 6 (lg (CFU/g)) after simulating the gastrointestinal tract[59]. Synergistic encapsulation of *L. casei* and *B. longum* using SA, oligofructose, and inulin has also been studied and applied to goat white cheese. Compared with free probiotics, the decline rate of encapsulated live bacteria was slowed down during storage, and a small amount of free probiotics also effectively prevented protein hydrolysis in cheese[60].

In recent years, the application of probiotics in butter has also received widespread attention. da Silva et al.[61] added *L. acidophilus* and *B. bifidum* encapsulated with SA to butter, and the product showed good organoleptic acceptability. The team also added *L. acidophilus* encapsulated with SA and calcium chloride to low sodium salt butter. The results demonstrated that the butter had stable physicochemical properties and could be considered as a functional butter with sufficient probiotics, low sodium levels and good sensory acceptability[62]. In addition, it was also found that SA microcapsules significantly increased the viability of probiotics in the simulated gastrointestinal tract compared to carrageenan microcapsules. And the quality parameters and sensory properties of ice cream containing encapsulated probiotics were significantly improved[63].

4.2 Beverages

Due to the allergenic nature of dairy products, beverages serve as another major category of food matrices to which probiotic capsules can be easily added. One study added SA capsules loaded with *L. casei* to apple juice. The results showed that the gastrointestinal-digested probiotic retained 100% viability, and the viability of the encapsulated bacteria remained significantly higher than that of the free bacteria after 21 d of storage at 4 °C[64]. Another study encapsulated *L. casei* and a variety of plant by-products (potential prebiotics) with SA, papaya seed gum, and glycerol as wall materials. The results proved that the synbiotic beverage powder had high total phenolic content and oxygen radical absorbance capacity and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity[65]. Whey, a cheese by-product, has been used to develop whey-based probiotic beverages due to its high yield and nutrient richness. SA gel was studied to encapsulate lactic acid bacteria in combination with whey protein concentrate and protein hydrolysate, respectively, and applied to beverages. The results demonstrated that both gel beads effectively enhanced the antioxidant properties and stability of the fermented products, and the protein hydrolysate gel beads exhibited better encapsulation rate and antioxidant capacity[66]. Another study encapsulated a variety of lactic acid bacteria in a composite wall consisting of SA and whey protein concentrate, and the results presented that the microencapsulated probiotics were stably maintained in the milk beverage and also showed good viability in the simulated gastrointestinal tract[67]. However, Wang et al.[68] encapsulated *B. adolescentis* in a composite of pea protein, SA with emulsion and applied it in different fruit juices. It was found that the number of viable bacteria within the probiotic microcapsules decreased to different degrees in pineapple and white grape juices over a period of six weeks, and no cells survived in orange juice in the fourth week. This may be due to the unfavourable effect of substances such as organic acids and sugars in the fruit juices on the microcapsules.

4.3 Other products

Bakery products are an emerging category of probiotic applications in the food sector. In the study of Mirzamani et al.[69], *L. acidophilus* was successively coated with xanthan gum-alginate and chitosan-gelatin layers and mixed with dough to prepare bread. The results showed that encapsulation enhanced the resistance of the probiotic to acidic conditions and improved stability of probiotics in bread at 90 °C. Another study found that encapsulation of *L. acidophilus* in alginate-gelatin microcapsules enhanced its survival during bread baking and storage, and could serve as an effective bread enhancer[70]. In addition, chocolate is an excellent carrier for probiotic microcapsules due to its low moisture content and high oil content, which allows the bacteria to maintain their bioactivity and functional properties during storage. In one study, probiotics encapsulated with SA and oligofructose were added to chocolate and demonstrated good tolerance during both gastrointestinal and thermal processing[71]. Another study also verified the protective ability of the chocolate matrix, and the chocolate encapsulated with *L. rhamnosus* microcapsules maintained a viable count of 7 (lg (CFU/g)) when stored at 4 and 25 °C for 180 and 120 d, respectively[72].

5 Prospects and challenges

However, in exploring the application of SA-based microcapsules in probiotic foods, researchers are still facing a number of complex and challenging dilemmas, including how to determine the appropriate microcapsule particle size, drying method, and synergistic effects of the system.

First of all, manufacturing microencapsulated probiotics with appropriate particle size is one of the most important challenges. Numerous studies have shown that capsules with a particle size of 0.2–3 mm are required to provide good protection for the core material. However, the smallest microcapsules that can be perceived in the mouth have a particle size of 22 μm in margarine and 55 μm in ice cream[73]. Some researchers have suggested that microcapsules should be added to foods with a particle size as small as possible, less than 100 μm, in order to avoid a 'gritty' sensation during consumption[39,74]. Therefore, gels with good sensory properties should be actively sought as wall materials, and suitable encapsulation techniques should be used to improve the protection of probiotics and to make the particle size controllable. Encapsulated wall materials with similar texture should be matched to the food matrix to ensure the beneficial effects of microcapsules while improving their organoleptic evaluation to meet consumer preferences.

Secondly, spray drying and vacuum freeze drying are commonly used drying and storage methods for probiotic microcapsules. SA-based gel drying has a low survival rate and very limited commercial application. Studies have exhibited that the addition of freeze-drying protective agents such as alginate, hyaluronic acid[75], complex defatted milk[76], and sucrose[77] can reduce the permeability of cell membranes, retain water, prevent membrane damage caused by osmotic pressure and ice crystals as well as protein denaturation, and effectively inhibit the reduction of intracellular metabolic activity after freeze-drying. For example, fish oil was added to

L. plantarum capsules encapsulated with SA-pectin-soybean isolate protein, which successfully improved the survival rate of probiotics in electrospray[51]. However, the current studies are relatively fragmented and there is no unified strategy to prevent desiccation damage.

Finally, despite our attempts to generalise the type of hydrogel, the type of probiotic encapsulated and the conditions of the study. Yet, each study was conducted at its own independent temperature, concentration, pH, time and with different strains of bacteria, making it difficult to summarise the optimal combinatorial effect of the gel system. Altamirano-Ríos et al.[78] reviewed encapsulation strategies for *L. acidophilus* and found that extruded and chitosan-coated alginate capsules provided the best protection. Oberoi et al.[79] coated SA-*L. rhamnosus* gel beads with xanthan gum, gum arabic, sodium caseinate, chitosan, starch, and carrageenan solutions, respectively, it was found that alginate-xanthan gum microcapsules had the highest encapsulation rate of 95.13% and the highest bacterial survival rate of 87.3% after gastrointestinal simulation. In conclusion, future studies should focus on the unification of gastrointestinal simulation procedures, storage experimental conditions, and various resistance test conditions. And matching with probiotic strains and food matrix, suitable microencapsulated probiotic products based on SA should be developed scientifically and rationally.

6 Conclusion

SA hydrogels have great potential for application in encapsulating probiotics and prebiotics due to their structural and chemical similarity to extracellular matrices, desirable biocompatibility, biodegradability, and cost-effectiveness. However, in addition to traditional dairy products and beverages, there is still a need to actively explore and expand products enriched with probiotics and related bioactive ingredients, as well as to expand the applicable population and provide personalised probiotic products. Looking towards the future, in-depth research on the physiological properties and antiretroviral performance of probiotics can be carried out by combining genetic engineering groups, molecules and proteomics. SA can be cleverly combined with other biopolymers to develop a more efficient probiotic hydrogel encapsulation system to improve encapsulation performance and probiotic survival rate. In the meantime, health foods containing microencapsulated probiotics should undergo thorough sensory evaluation, promote commercialisation, ensure scalability of production and implement stringent quality control measures. It is foreseeable that with the increasing awareness of the health benefits of probiotics and the improvement of probiotic delivery systems, the market share of SA-based microencapsulated probiotics will be increasing in the future, and will contribute to the progress of human intestinal health.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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