

Advancements in non-starch polysaccharides research for frozen foods and microencapsulation of probiotics

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Abstract Conventionally used in the food industry as stabilizing, thickening, gelling, and suspending or dispersing agents, non-starch polysaccharides such as xanthan gum are known to improve the texture of certain frozen products. Another polysaccharide that has received significant attention in recent years is chitosan, a natural biopolymer derived from chitin. In the wake of growing interest in finding ideal encapsulating agents for probiotics, non-starch polysaccharides have been investigated. Scattered research can be found on the effect of each individual polysaccharide, but there remains a void in the literature in terms of closely comparing the characteristics of non-starch polysaccharides for these applications, especially when more than one biopolymer is employed. A good understanding of the tools capable of elucidating the underlying mechanisms involved is essential in ushering further development of their applications. Therefore, it is this review's intention to focus on the selection criteria of non-starch polysaccharides based on their rheological properties, resistance to harsh conditions, and ability to improve sensory quality. A variety of critical tools is also carefully examined with respect to the attainable information crucial to frozen food and microencapsulation applications.

Keywords microencapsulation, xanthan, galactomannan, chitosan, curdlan

1 Introduction

Non-starch polysaccharides stem from several sources, including botanical, algal, microbial, and animal origins [1–3]. They are commonly used as thickeners [4,5], emulsifiers, and stabilizers [6–8], as well as microencapsulating agents for flavor compounds and enzymes [9,10].

In frozen foods, these non-starch polysaccharides help maintain or control textural stability through such means as ice recrystallization, minimization of water distribution, and viscosity enhancement [11,12]. A number of non-starch polysaccharides, namely, carrageenan, xanthan gum, locust bean gum, guar gum, microcrystalline cellulose (MCC), and carboxymethyl cellulose (CMC), can serve as texture modifiers that protect products from heat shock and deteriorations, function as bodying agents [13,14], help stabilize frozen foods [15], and reduce recrystallization [12]. For instance, locust bean and guar gum are used to give and improve the texture of ice cream [13,14]; MCC can be used to reduce ice crystals in frozen desserts [14] or as a low-fat texture modifier [16]; and, when used as an emulsifier, stabilizer or thickener, xanthan gum has the ability to keep its viscosity after being defrosted [15,17]. Nevertheless, there are limited studies in the literature characterizing the textural effects of different gum combinations under freeze-thaw conditions.

On the other hand, an increasing number of studies have been directed towards non-starch polysaccharides and their use for microencapsulation. Microencapsulation is the process by which a pure material or a mixture is coated or entrapped into another, which is called wall material, membrane, carrier or shell, to produce capsules in the micrometer to millimeter range known as microcapsules. The purpose of microencapsulation is to protect the functional core ingredient to be separated from the surrounding destructive environment until its release is desired [18]. Natural examples of encapsulation include egg shells, plant seeds, and bacterial spores [19]. Microencapsulation has been used for a variety of functional materials like cells, enzymes, and pharmaceutical drugs. Food ingredients like oleoresins, oxidation-sensitive vitamins, sweeteners, minerals, antioxidants, and proteins are often found encapsulated as well. The food industry mainly relies on microencapsulation technology for controlled release applications, enhanced stability, flavor masking, protection against harsh conditions, and

improved nutrition [20]. Release of the core material can be designed to be triggered by temperature, pH changes, osmotic shock, or a combination of factors. Wall material for capsule formation usually utilizes a combination of one or more of sugars, proteins, natural and modified polysaccharides, lipids, and synthetic polymers. Different techniques used for encapsulation include spray drying, spray chilling or cooling, extrusion coating, fluidized bed coating, liposome entrapment, coacervation, inclusion complexation, and centrifugal extrusion [19]. Additionally, chitosan, a natural biopolymer derived from chitin, has received significant research attention as an encapsulating agent due to its unique positive charges on the side chains [21,22]. The ability of chitosan to form polyionic complexes with negatively charged polysaccharides has been used for microencapsulation of probiotics.

Probiotics are defined as “Live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host” [23]. Currently, the standard for any food sold with health claims from the addition of probiotics follows the FAO/WHO recommendation that it must contain, per gram, at least 10^6 to 10^7 cfu of viable probiotic bacteria. It is well recognized that the ability of probiotic microorganisms to survive and multiply in the host strongly influences their probiotic benefits. The bacteria need to reach the intestine in large number in order to provide the health benefits. For instance, probiotic cells have to pass through the strong acidity in the stomach (pH 1–2) and the high concentration of bile for a long period of time [24]. Besides the adverse conditions in the gastrointestinal (GI) tract, food processing parameters such as dissolved oxygen content, temperature, and moisture content could also greatly impact the viability of probiotics. Therefore, in the last decade attention has been given to the development of microencapsulation techniques to improve the viability of probiotics during processing steps and storage, and against harsh conditions after ingestion. There is a pressing need for biodegradable natural polymers that are resistant to degradation in the upper GI tract and capable of releasing probiotic cells in the intestine.

The ideal polysaccharide for frozen food and microencapsulation applications needs to 1) have a gel or gel-like properties either through junction zone formation or polyionic complexes; 2) have the ability to withstand harsh conditions of processing and/or the gastrointestinal tract; and 3) retain the sensory qualities of the food. The junction zones or ionic bonds formed between two materials that produce the gel or gel-like properties give the polysaccharides the ability to control ice recrystallization for frozen foods and provide the strength needed to encapsulate a material. Proper selection of polysaccharides is necessary because they differ in their ability to resist pH, temperature, electrolyte concentration, enzymatic degradation, and storage conditions [2]. However, while these abilities are

known in some polysaccharides, it is not uncommon to encounter certain limitations in order not to negatively impact the product’s sensory quality. For example, polysaccharides must be added in an effective amount that provides cryoprotection, inhibition of water recrystallization during freezing and thawing conditions, but should not affect mouth-feel or result in significant syneresis in frozen foods. It is also desirable that, when used to protect and deliver probiotics, the polysaccharide capsules should be as small as possible in order to be applicable in a variety of foods without being noticed. This review will provide an intimate overview of several non-starch polysaccharides that are utilized in both frozen foods and the microencapsulation of probiotics, while providing some insight on the reasons they are used in these particular sectors of the food industry. The key techniques capable of characterizing the conformation, structure, and textural function of these polysaccharides are also highlighted.

2 Non-starch polysaccharides

2.1 Seed-derived gums

2.1.1 Guar gum

Native to India and Pakistan, guar gum is obtained from the guar plant, *Cyamopsis tetragonolobus* of the *Leguminosae* family [1,25]. Currently, the plant is commercially grown annually in Texas, Oklahoma, Arizona, and the southern hemisphere in response to the fluctuations in availability of obtaining guar bean gum from foreign sources [1,25]. Made up of nearly all galactomannan, the nonionic structure of guar gum consists of a backbone with (1→4)-linked β -D6 mannopyranosyl unit with a side chain unit consisting of (1→6)-linked α -D-galactopyranosyl at a ratio of 1.8:1, respectively (Fig. 1). The large amount of galactose substitutions prevents strong cohesion of the main backbone, and therefore extensive crystalline regions cannot be formed, allowing hydration at and above room temperature [25]. Hydration of guar gum can be correlated to the intermolecular and intramolecular hydrogen bonding through the unsubstituted regions of the backbone [26,27] and thus makes guar gum soluble in cold, highly agitated water while exhibiting pseudoplastic behavior. It is used as a formulation aid, stabilizer, firming agent, and thickener [13] with a classification of Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration (FDA) [28].

Wielinga et al. [25] first reported that guar gum can help enhance the freeze-thaw stability of solutions. It was found that guar gum can be stable over two freeze-thaw cycles in aqueous, NaCl, CaCl₂, citric acid, acetic acid, and milk serum solutions at a concentration of 1.0%. Another study found that when added to curdlan gum in the same amount

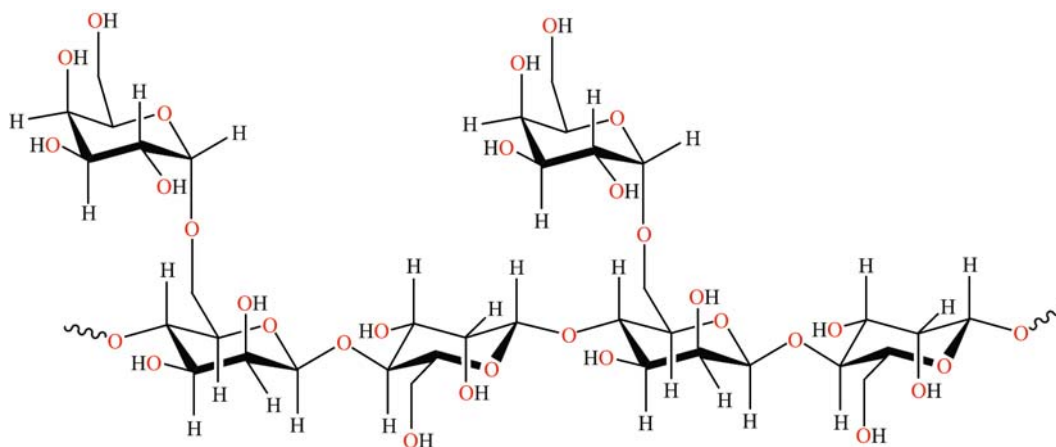


Fig. 1 Structure of guar gum

totaling 2.0% (w/v), syneresis was undetectable over five freeze-thaw cycles [29]. Galactomannans such as guar gum and locust bean gum are amongst the most frequently used ingredients for the stabilization of ice cream [14]. In fresh and frozen vegetable puree, guar gum was found effective in reducing drip loss in potatoes, carrots, and turnips [30]. Moreover, when added to frozen dough, guar gum has shown improvements in bread texture, volume, and crumb structure [31].

The ability of guar gum to form a polymeric matrix that is resistant to degradation in the upper GI tract and susceptible to enzymatic action of colonic bacteria has led to extensive studies for its colon-specific drug release properties by various researchers [32–35]. These studies utilized varying compositions of guar gum (20%–98%) for preparation of compression-based tablets. Tablets with more than 40% guar gum resulted in limited release of drug even after 24-hour dissolution study in the presence of rat caecal contents; thus, 20% to 30% was recommended for colon-specific release [32]. Despite the ability of guar gum to retain the bioactive material in harsh conditions of stomach gastric and intestinal fluids, very few studies have been reported on guar gum for intestinal delivery of probiotic bacteria. In a recent study, however, Ding et al. [36] reported microencapsulation of various probiotic strains using guar gum. Encapsulation was achieved by emulsion technique using CaCl_2 followed by 1 hour of exposure to gastric conditions at pH 2 where viability decreased from 10^{10} to $<10^7$. The viability was decreased to $<5 \log \text{ cfu/mL}$ after 8 h incubation in 3% tauchloric acid. Permeability of encapsulated fluorescent dye was also least retained by guar gum compared with other gums, which could be due to high pore size of guar gum, resulting in poor protection of probiotic bacteria.

2.1.2 Locust bean gum

Locust bean gum (LBG), a.k.a. carob bean or St. John's gum, is obtained from the seeds of the carob tree,

Ceratonia siliqua, grown in Mediterranean countries [1,25]. The galactomannan structure of LBG, similar to guar gum, consists of a backbone with (1→4)-linked β-D-mannopyranosyl units and a side chain consisting of a single (1→6)-linked α-D-galactopyranosyl unit with a ratio of 3.9:1 (Fig. 2). Unlike the structure of guar gum, LBG side chains are very unevenly substituted, with sections of the backbone concentrated with substitutions and sections with no substitutions. Slightly soluble in water at room temperature, LBG must be heated to approximately 60°C to 85°C to achieve complete hydration. LBG solution exhibits pseudoplasticity (shear-thinning behavior), which shows reduced viscosity at increased shear rate, and can form a weak gel network with concentrations as low as 0.5% [37]. Classified as GRAS by the FDA, LBG is used for its stabilizing, thickening, and fat-replacing properties.

Unlike in guar gum, there is little stability in a system containing LBG. For instance, LBG forms a gel after the first freeze-thaw cycle in the presence of NaCl, acetic acid, and milk serum, and syneresis occurs in both aqueous and CaCl_2 solutions [25]. This can be attributed to the unevenly substituted sections of the backbone that create junction zones and thus a gel or gel-like network. It has been shown that freeze-thaw cycles and rate of freezing and/or thawing affect its gel strength [38–41]. Therefore, LBG alone cannot provide the freeze-thaw stability over multiple cycles.

Several studies with LBG and xanthan gum have been performed because of their unique gelling behavior that is not formed with the other galactomannan, guar gum [26,42,43]. Conformational studies via parameters such as intrinsic viscosity that depict molecular occupancy in highly dilute solutions have been used to better understand this interaction. It was reported that the intrinsic viscosity exhibited lower values than weighted averages, suggesting that there is some flexibility in the structure of xanthan that may allow LBG to bond to the backbone [43,44]. Even so, LBG is best known for its use in ice cream to prevent

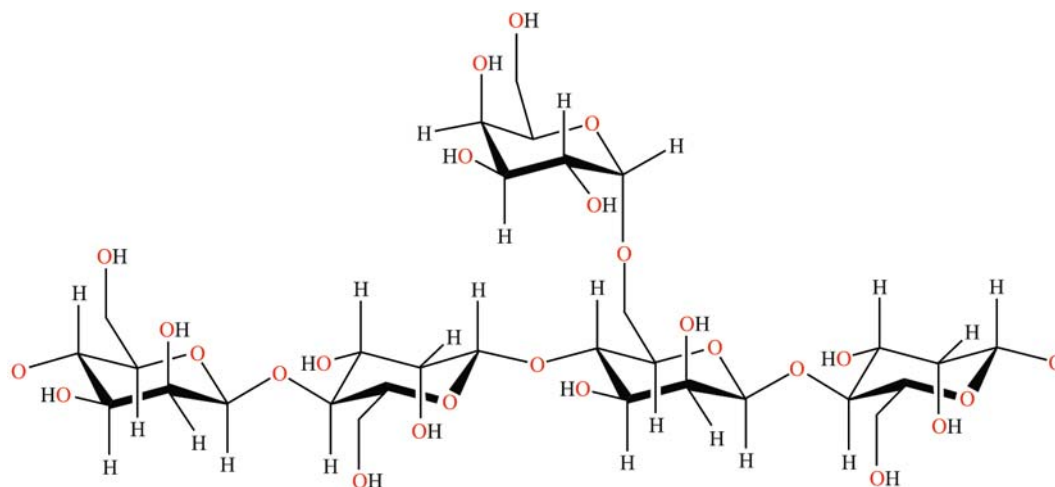


Fig. 2 Structure of locust bean gum

iciness during heat shock [1,13,14] and many times is combined with guar and carrageenan. However, little to no studies have been conducted to examine any potential synergistic effects provided by LBG and xanthan under freeze-thaw abuse. On the other hand, frozen dough containing LBG was found to enhance gluten quality and reduce proof times, while increasing specific loaf volume with improved external and internal bread characteristics [45–47].

Since LBG forms very weak gels, it is not used for encapsulating probiotic bacteria by itself. However, strong gels due to synergistic effect result when LBG is mixed with other gums such as κ -carrageenan and xanthan. A recent study investigating the ability of LBG matrix to protect probiotics reported that the viability of probiotic strains rapidly decreased from 10 to 4.84 log cfu/mL within 2 h in simulated gastric juice (SGJ) at pH 2 and to < 5 log cfu/mL after 8 h incubation in 3% tauchloric acid, both less than the requirement of probiotic bacteria to exhibit their health benefits [36].

2.2 Gums derived from seaweed extracts

2.2.1 Alginate

The extraction and processing of brown algae from a host of species, including *Laminaria hyperborean*, *Macrocystis pyrifera*, *Laminaria gidditata*, *Ascophyllum nodosum*, *Laminaria japonica*, *Eclonia maxima*, and others, can produce the intermediate product, alginic acid. After the process of neutralization with sodium carbonate or sodium hydroxide, alginic acid forms the more stable water-soluble product called sodium alginate [48,49]. The linear structure of alginate consists of either the homopolymeric blocks β -D-mannuric acid (M) and α -L-guluronic acid (G) or the heteropolymeric blocks of alternating M and G linked by (1→4)-glycosidic linkages, making it a

polyanionic hydrocolloid (Fig. 3). The amount of homopolymeric and heteropolymeric blocks present in alginate depends upon the sources previously mentioned. Thermo-irreversible gels can be formed by controlling the amount of calcium and acidity. On the other hand, low-acid solutions below pH 4.0 form thermo-reversible gels when combined with high-methoxyl pectin. There is a variety of different viscosity grades of alginates due to the amount of G-blocks available for Ca^{2+} to form an “egg-box” structure at junction zones creating a strong gel. Due to its ability to form strong gels, they are used to control the shape of foods such as onion rings, pimiento and anchovy olive fillings, apple pieces for pie fillings, cocktail berries, meat chunks for pet food, shrimp-like fish products, and fish patties [48].

Although temperature does not hinder gelation of sodium alginate, it affects the final gel properties; however, once a gel it is heat and freeze-thaw stable [48,49]. Sodium alginate has been seen to improve frozen dough stability along with whey by increasing the specific loaf volume [50]. Following each of seven freeze-thaw cycles, alginate increased dough development, water adsorption, and reduced syneresis; however, it yielded different texture profiles with a firmer dough when compared with control samples [51].

Alginate is the most common encapsulating agent used by researchers for probiotic bacteria, which is mainly attributed to its ease of formation of capsules and release of probiotic bacteria using chelation in phosphate buffer. Alginate capsules are made using different techniques like extrusion and emulsification [52]. Alginate capsules made with 1.8% alginate solution provided protection to probiotic bacteria and improved viability up to 3 log cfu/mL during 2 h incubation at pH 1.2 gastric solution [53]. Ding et al. [54] used an extra layer of poly-L-lysine with alginate microcapsules. This technique improved viability by 1 log cfu/mL compared with alginate capsules

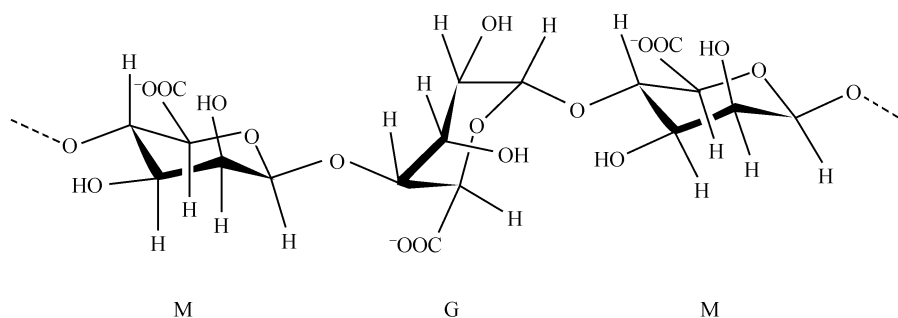


Fig. 3 Structure of alginate gum

in gastric conditions, but no improvement was observed in intestinal solution, suggesting the emulsion-breaking capacity of bile salts. Furthermore, capsules coated in poly-L-lysine retained double the amount of water-soluble fluorescent dye as compared with alginate capsules over a 6-week storage period. In another study, encapsulation in alginate capsules with diameters below 100 μm did not improve survival of the acid-sensitive *Bifidobacteria* exposed to SGJ at pH 2. However, survival during refrigerated storage in milk was significantly improved for *Bifidobacterium longum* Bb-46 [55].

2.2.2 Carrageenan

Three varieties of carrageenan, kappa (κ), lambda (λ), and iota (ι), can be extracted from the red seaweeds. They are primarily extracted from the *Gigartina* species and *Chondrus crispus* that produce kappa and lambda types, and *Eucheuma cottonii* and *spinosum* species that produce kappa and iota types, respectively [3,56]. The general structure of carrageenan contains repeating galactose units and 3,6-anhydrogalactose both with 15% to 40% (w/w) ester sulfate content and side chain consisting of alternating α -(1 \rightarrow 3)- and β -(1 \rightarrow 4)-glycosidic linkages. The three types of carrageenan do not exist singly, but as a combination of two types and available with one predominating type or molecules containing structural components of more than one type. Each type of carrageenan has a unique set of characteristics, including gel strength, viscosity, temperature stability, synergism, and solubility. The solubility of each type of carrageenan depends on the number of sulfate groups, which increases water solubility, compared with anhydro bridges, which is hydrophobic. Having the least water solubility, κ -carrageenan has one sulfate group for every two galactose units and one anhydro bridge; ι -carrageenan has two sulfate groups for every two galactose units along with one anhydro bridge; and with the highest solubility, λ -carrageenan has three sulfate groups for every two galactose units and no anhydro bridges [3,56].

There are distinct differences among each type of carrageenan, but all types are soluble at high temperatures

and are stable above pH 4.5. Though the least soluble, κ -carrageenan provides the strongest, yet brittle, gel that allows for some syneresis. The ι -carrageenan type is more elastic and is freeze-thaw stable, allowing for no syneresis, and λ -carrageenan thickens without gelling. Moreover, due to syneresis, κ -carrageenan has poor freeze-thaw stability; however, by the right combination of κ - and ι -carrageenan, intermediate freeze-thaw stability can be acquired along with a range of gel textures without syneresis [56]. In ice cream and other frozen milk-based products, a small amount of κ -carrageenan (0.01%–0.3%) is used to prevent phase separation of casein [14,57]. κ -Carrageenan alone or in combination with whey exhibited increased specific volume in bread after the dough was frozen during storage [46,50].

Although carrageenan forms gels by itself, gel properties are influenced greatly by addition of ions and other gums due to stabilization effects and synergistic effects, respectively. Potassium ions have been found most effective in increasing the gel strength and have been used for microencapsulation of probiotics. In a recent study, the viability of *Lactobacillus acidophilus* cells was significantly improved when encapsulated with κ -carrageenan during fermentation of tomato juice and storage at 4°C for 10 weeks as compared with free cells [58]. In another study, encapsulation with κ -carrageenan showed increased viability of *Bifidobacteria* in yogurt during storage up to 30 days compared with non-encapsulated bacteria [59]. The viability of 10 different probiotic strains encapsulated in κ -carrageenan was significantly improved in simulated gastric and intestinal solutions [36]. κ -Carrageenan in combination with LBG was able to maintain the viability of entrapped lactic acid bacteria in NaCl, glycerol solutions at 4°C for at least 11 days before reaching 10^5 cfu/mL [60].

2.3 Microbial exopolysaccharides

2.3.1 Curdlan

Alcaligenes faecalis var. *myxogenes*, now classified as *Agrobacterium* biovar. 1, produces the neutral, linear

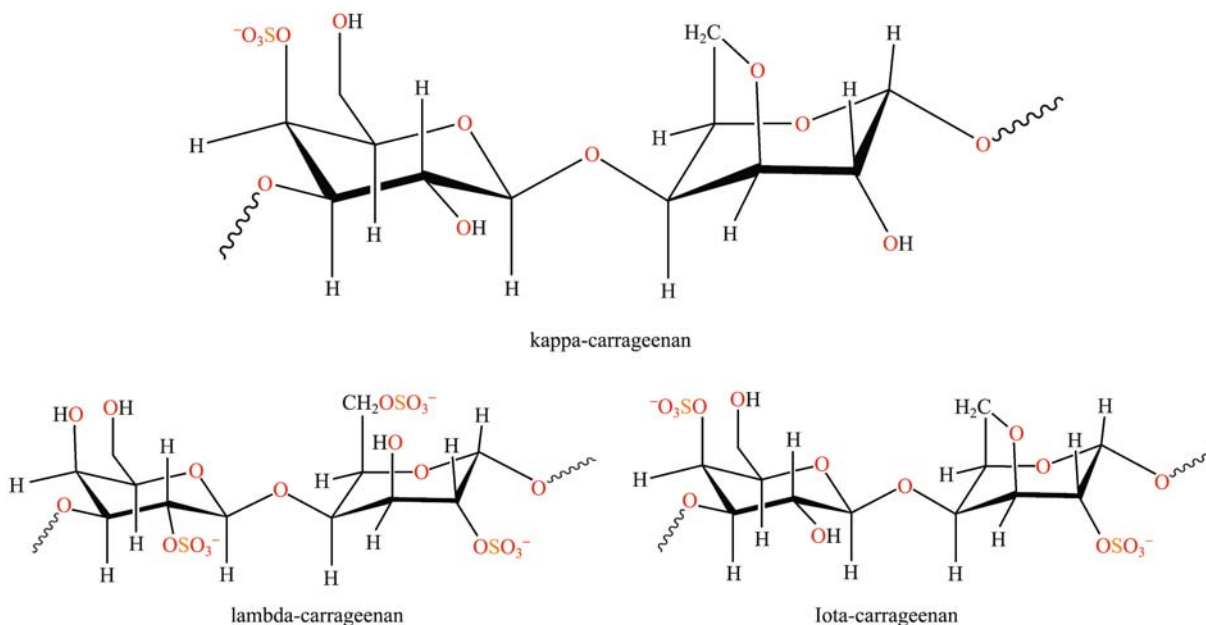


Fig. 4 Structures of kappa-, iota-, and lambda-carrageenan

polysaccharide curdlan. Curdlan is the third microbial extracellular polysaccharide following xanthan gum and gellan gum to be approved for food uses in the United States [28]. Repeating units of (1→3)- β -glucan (Fig. 5) can form a low-set thermo-reversible gel when heated between $\sim 55^{\circ}\text{C}$ and 80°C or a high-set, triple helix, and thermo-irreversible gel when heated above $\sim 80^{\circ}\text{C}$ and then cooled [61–65]. The gelation mechanism of the low- and high-set gels continues to be studied [65–67]. Curdlan lacks solubility in water, alcohols, and most organic solvents, but shows solubility in alkaline solutions [64,68]. It is known to be able to hold moisture in processed meat and flour products. It is stable over a pH range of 2 to 12 and freeze-thaw conditions, except as an aqueous solution there is syneresis [63].

Composed of a three-dimensional structure stabilized by crosslinks that connect junction zones between molecules [69–73], curdlan gum was once the focus of extensive research efforts to expand its applications to improve the texture of various food products [72,74,75] as well as the delivery of medicinal ingredients [76,77]. To date, unfortunately, despite curdlan's ability to form viscous aqueous suspensions with shear-thinning flow behavior

[62,78,79], the applicability of curdlan in the U.S. market remains scarce and limited, due mainly to its less profound viscosity than xanthan gum in solution and inferior gel formation capacity when compared with gellan [80].

Nevertheless, curdlan continues to be used as a texture modifier in Chinese and Japanese noodles and surimi-based products [72,81], as well as in processed meats such as pork, fried battered chicken, hamburger patties, and meatballs to yield juicier and softer products [63,82]. Furthermore, curdlan is also capable of forming tasteless, odorless, and colorless hydrogel complexes with other polysaccharides [83,84]. This unique gelling mechanism can be used to increase retention or absorption of moisture and other ingredients [84] while withstanding the temperature extremes of freezing and retorting processes [25,64].

Although the gel strength of curdlan is consistent after one freeze-thaw cycle compared with carrageenan, agar, and konjac, syneresis occurs [63]. Xanthan in combination with curdlan has been demonstrated by our research group to reduce syneresis to a point where it is not detected and yields stable rheological and physical properties over freeze-thaw cycles when compared with curdlan combined with guar, locust bean, or κ -carrageenan

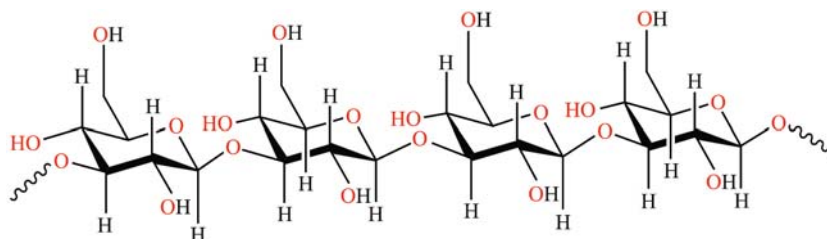


Fig. 5 Structure of curdlan

gums [29]. The study measured viscosity, heat stability, modulus, gel strength, adhesiveness, and syneresis over five freeze-thaw cycles and found a stable system throughout each experiment, in addition to showing good water holding capacity.

2.3.2 Xanthan gum

Produced by fermentation of *Xanthomonas campestris*, xanthan gum is an anionic microbial polysaccharide with a disaccharide backbone and a trisaccharide side chain. The backbone consists of two repeating (1→4)- β -D-glucose units, the same as cellulose, and located on C3 of every other glucose, α -D-mannose, β -D-glucuronic acid, and β -D-mannose trisaccharide are attached (Fig. 6). Various terminal mannose residues are pyruvated depending on the *X. campestris* strain. Xanthan is soluble in water with the ability to hydrate in cold water, exhibiting pseudoplastic behavior. The viscosity of xanthan is stable over a range of pH values, salt concentrations, temperatures, and enzymatic breakdown [15,86]. Thickening, stabilizing, and emulsifying are some of the well recognized characteristics of xanthan gum. In xanthan gum's function as a thickening agent, low shear rates show stiff, aggregated, highly ordered molecules and, as shear is increased, the stiff molecules separate and align in the direction of shear force. As a consequence, pseudoplasticity is exhibited. Because of its resilience to pH and temperature fluctuations, xanthan gum has been extensively studied for its own properties and synergistic effects with other gums, starches, and food ingredients. The interactions between xanthan and galactomannans have been examined to identify their synergy that alters viscous and gelation properties [15,42–44,87–89]. The synergy of xanthan has also been explored with xyloglucan [90], glucomannan [91], and its positive effects with gum arabic and LBG in emulsions [92], as well as its effect on starch when combined with other gums [93].

While 1.0% xanthan solution produces gel-like consistency, as little as 0.1% xanthan gum can increase viscosity and shows similar rheological properties. Giannouli and Morris [94] suggested that xanthan can form stronger more cohesive networks when frozen and thawed, similar to how Ca^{2+} enhances xanthan's weak gel network. Furthermore, the stability of frozen entrees and sauces was improved by xanthan gum with respect to syneresis and viscosity control over freeze-thaw cycles [15]. The presence of xanthan gum in starch gels has significantly increased its freeze-thaw stability, according to Lo and Ramsden [81], indicating an excellent compatibility with major food components. Xanthan gum has been shown to increase the specific volume of the bread after one week of frozen dough storage and heating via a microwave [95], reducing crust deterioration when compared with LBG and guar [47]. Improved dough quality by the addition of xanthan could be attributed to the reduction of free water as indicated by a reduction in the fusion enthalpy [96].

Xanthan gum was excellent in protecting probiotic bacteria from acidic environment of gastric juice at pH 2 and 3% taurocholic acid [36]. Xanthan has been used in combination with other polymers like gellan for immobilizing cells [97], alginate for encapsulation of urease enzyme [98], and chitosan for immobilizing xylanase [99]. Also, when combining xanthan with chitosan, our research group has shown the combination to be effective in protecting probiotic bacteria against gastric conditions at pH 2 followed by release in the presence of simulated intestinal conditions enabling it as an effective carrier for probiotic bacteria for colon-specific release [100].

2.4 Cellulose derivatives

2.4.1 Sodium carboxymethyl cellulose

Sodium carboxymethyl cellulose (CMC), or cellulose gum (Fig. 7), is produced by treating cellulose with sodium

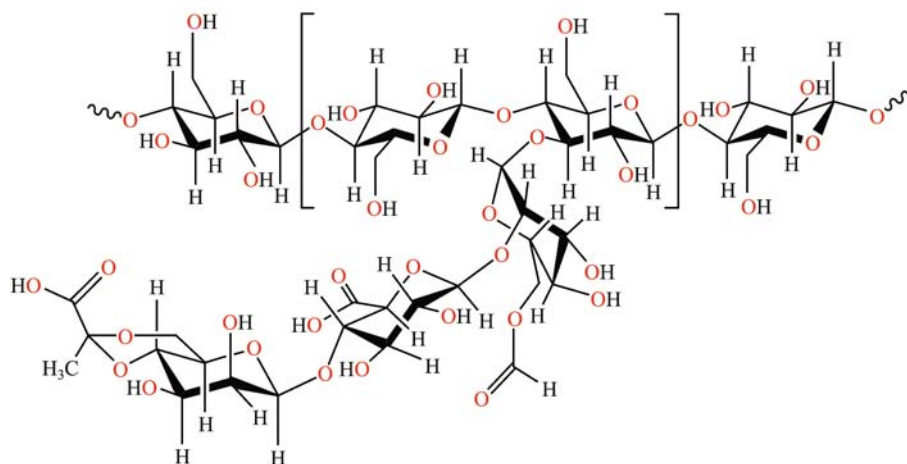


Fig. 6 Xanthan gum

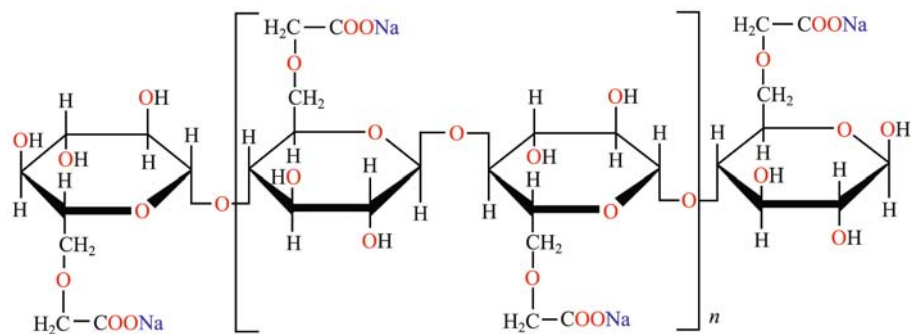


Fig. 7 Structure of carboxymethyl cellulose

hydroxide, having it reacted with sodium monochloroacetate, and finally washed [101]. CMC is water soluble with the ability to increase viscosity to 5 Pas in a 1% aqueous solution, which is considerably lower than that of xanthan gum. CMC solutions exhibit shear-thinning [102] and sometimes Newtonian behavior or shear-thickening [103]. It is currently used as a thickener or bodying agent for instant products, sauces, dressings, and soft drinks; however, high viscosity forms of CMC can cause a “gummy” mouth feel [102]. Similar to carrageenan and locust bean gum, CMC is used to stabilize frozen products like ice cream by inhibiting ice crystal formation [14,102]. Similar to LBG, CMC was found to reduce proof times and to be more resistant to extension in previously frozen dough while improving external and internal characteristics of bread, but also to increase a desired yellow color in the crust compared with control samples [45,46].

2.4.2 Microcrystalline cellulose

Microcrystalline cellulose (MCC) is a linear structure consisting of anhydroglucose units linked by a (1→4) β -glycosidic bond. MCC is water insoluble and thus needs sufficient shear or copolymer network to properly disperse in water [104]. Temperature has little to no effect on the functionality or apparent viscosity of the networks and MCC remains stable during high-temperature processing such as baking or retort. Not only can MCC be applied to high-temperature processes, it can also be used as a texture modifier for low-fat desserts [14] and to prevent the growth of ice crystals in frozen foods during freeze-thaw cycles [16]. Synergy is important to create and improve texture properties, but it is more difficult to achieve with other material or gums due to its highly linear structure. In many cases, co-processing under specific conditions is required to form hydrogen bonds between MCC and the material [1].

2.5 Chitosan

Chitosan is the deacetylated form of chitin, the most

abundant natural biopolymer after cellulose. Chitin is a copolymer of glucosamine and N-acetyl-d-glucosamine linked together by β -(1,4) glycosidic bonds (Fig. 2). Chitin is the major structural component of exoskeleton of invertebrates and the cell walls of fungi [22]. Chitosan is a primary aliphatic amine with a pKa of 6.3 and can be protonated by selected acids. It is a biocompatible polymer and does not result in adverse reactions when in contact with human cells. It has seen many applications in the food industry, including the edible film industry, owing to the anti-fungal properties of chitosan [105], water purification as a chelation ion exchange polymer [106], and clarification and deacidification of fruit juices [107]. Chitosan as a cationic polymer has pharmaceutical applications such as a nasal drug delivery agent due to its bioadhesive nature. It also interferes with the metabolic process of cholesterol and other neutral lipids by binding them with hydrophobic bonds [108].

In 1983 chitosan received a GRAS status by the FDA in the USA for use as an animal feed component. In 1992, the use of chitosan for purification of water was approved by the US Environment Protection Agency (EPA) up to a maximum concentration of $10 \text{ mg} \cdot \text{L}^{-1}$. Japan's health department approved the use of chitin and its derivatives as functional food ingredients [109].

As an edible film, chitosan has been shown to be effective in coating frozen foods to reduce several negative effects of frozen storage. After eight months of frozen storage, 1.0% (w/v) chitosan significantly inhibited lipid oxidation compared with a control sample of salmon fillet [110]. It has also been shown that as an edible coating chitosan reduces drip loss and maintains the textural quality of frozen strawberries after thawing [111].

Chitosan-coated alginate beads provided a viability of at least 1 log cfu/mL greater than free probiotic bacteria in yogurts made with ultra-high temperature (UHT)-treated and conventionally treated milk. In another study, three different chitosans varying in molecular weight were used to coat the alginate capsules. It was found that the viability of probiotic bacteria *L. bulgaricus* KFRI 673 increased with increasing molecular weight of chitosan after

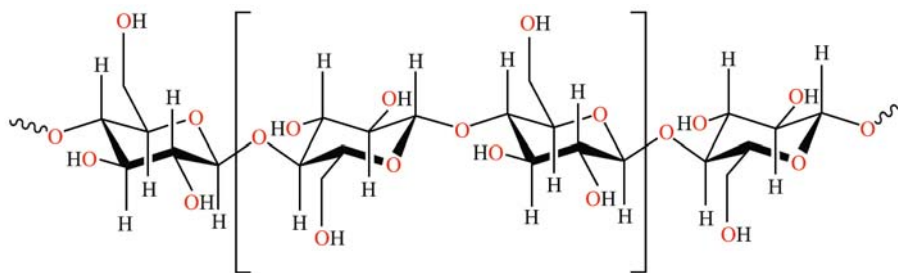


Fig. 8 Structure of cellulose

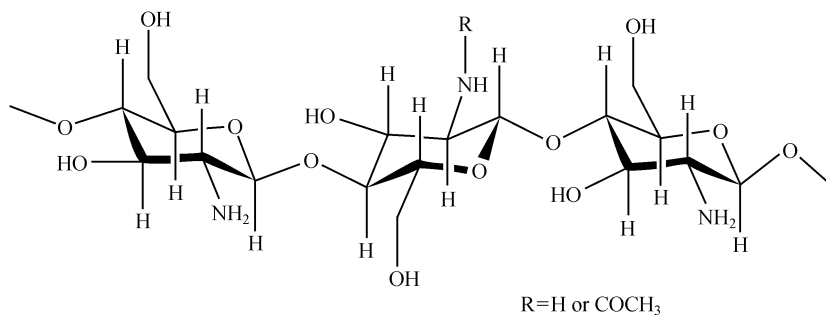


Fig. 9 Structure of chitosan

sequential incubation in simulated gastric and intestinal fluids. However, capsules formed by high-molecular weight chitosan and alginate resulted in non-uniform shaped capsules with partial collapse in the center compared with spherical shaped capsules with low-molecular weight chitosan [51].

3 Characterization tools

Several tools have been reported in the literature as capable of characterizing different gums to aid in understanding any positive effects that could exist when a system has more than one polymer. Rheological properties, including viscosity and dynamic modulus, are often used to characterize the behavior of gum, starch, and gum/starch combinations [43,44,86,87,90,94,112,113]. Along with rheological properties, certain physical properties such as gel strength and adhesiveness can also provide a broader view of the behavior and stability of the gum system.

In order to better describe conformational and structural characteristics of non-starch polysaccharides, methods such as magnetic resonance, light scattering, and microscopy can be useful (Table 1). Time correlation functions (TCF) via dynamic light scattering (DLS) can provide clues to the gelation behavior, as demonstrated by investigations with xanthan and LBG [89,114] or xanthan in the presence of metal ions [115]. Differential scanning calorimetry (DSC) can also provide gelation information through thermal analysis. DSC measures change in difference in the heat flow rate to the sample and to a

reference sample while they are subjected to a controlled temperature program. It measures a heat flow rate difference due to a temperature change, where a spike in heat flow provides the glass transition, crystallization, and melting temperatures.

Surface morphology can be investigated using scanning electron microscopy (SEM) to elucidate the network properties and microstructure of the combination. It has been successfully employed for investigating skim milk with xanthan and locust bean gum [116], tapioca starch modified by guar and xanthan gum [93], effects of xanthan, LBG, and guar on sucrose [117], chitosan and methylcellulose [118], κ -carrageenan and locust bean gum [119], calcium-induced κ -carrageenan [120], and carrageenan/*O. ficus indica* [121]. In several of these studies, a cryo-chamber was in place with the SEM that freezes the sample at around -160°C followed by sublimation, then the sample may be coated and examined through SEM. Morphology characteristics can also be found with atomic force microscopy (AFM), which uses a technique called single-molecule force spectroscopy. A fractal analysis can be performed based on the image gathered by AFM; however, it requires proper adsorption of sample materials onto a supporting surface, thus some affinity for a specific surface such as mica is needed before the samples can be examined.

To delve further into the nature and adjustments in the molecular structure of polysaccharides, nuclear magnetic resonance (NMR) has been used for molecular analysis in carbohydrates [122], including xanthan and xyloglucan [90], xanthan and locust bean gum [114], or fructose with

Table 1 Investigative methodology of hydrocolloidal systems

methodology	test properties	reference
rheological	flow characteristics, elasticity classification	[43,66,86,87,90,94,112,113,128]
differential scanning calorimetry	thermal analysis, gelation mechanisms explored	[90,112,113,124,128,129]
nuclear magnetic resonance	molecular structure and structural changes	[90,114,130]
electron spin resonance	molecular structure and structural changes	[112,127,129]
dynamic light scattering	particle size	[89,114,115,131]
atomic force microscopy	surface morphology	[113,128]
scanning electron microscopy	surface morphology	[116,132]

locust bean gum [123]. In these studies the spin-spin relaxation time, T_2 , was monitored to identify changes in structure that could be attributed to the combining of two substances using ^1H NMR and could also be related to bound water and water mobility [124–126]. Similar to NMR, electron magnetic resonance (ESR) can be used to measure structural adjustments using the free radicals inherent in the substance being investigated or the free radicals that can be attached to a substance [127].

4 Conclusions

It is apparent that non-starch polysaccharides play an important role in the texture, quality, and functionality of many foods. They are used in both frozen foods and microencapsulation due possibly to their gel or gel-like forming ability and capability of withstanding a wide range of processing conditions and surviving in the gastrointestinal tract. Frozen foods utilize them for viscosity enhancement just as they are in conventional foods, but also for the purpose of cryoprotection in helping to maintain the quality of frozen foods. There is an exhaustive number of studies on these polysaccharides, along with many in combination with each other, but few examine their stability over low-temperature abuse. These studies could provide information about the gums and gum combinations and their behavior as they have with xanthan and curdlan [29]. The strength of ionic complexation or junction zones and enzyme resistance of polysaccharides are ideal for carrying and delivering probiotics through the gastrointestinal tract.

Although not perfectly meeting each criterion for utilization in frozen foods and the microencapsulation of probiotics, alginate, xanthan, guar and chitosan are well suited for both instances. They form gel or gel-like materials, with the ability to withstand harsh conditions of processing and/or the GI tract. However, few studies have examined their sensory qualities in frozen foods and foods containing probiotics. Guar gum could also be an excellent candidate, yet it lacks the ability to provide a firmer gel that could allow better protection of bacteria. Curdlan may be good candidate for further microencapsulation

experiments with its excellent freeze-thaw stability, gelling properties, and resistance to harsh conditions. Although both locust bean gum and carrageenan gums are used in frozen foods, they lack freeze-thaw stability and require further studies of ionic complexations or combining them with other materials to improve their applicability. Carrageenan can provide good protection for probiotics, but not LBG, thus making it the least applicable for both applications. CMC and MCC, like curdlan, have not been extensively studied for microencapsulation, most likely due to their inability to form gels.

Several tools can be used to help identify materials that are suitable for use in both frozen foods and microencapsulation. One such tool is rheology, particularly the modulus data that can show how elastic or gel-like a material is and give an idea of consistency if the polysaccharide is subject to many types of abuse, including temperature and pH. For frozen foods it will then be important to identify any syneresis. Also, scanning electron microscopy can show surface morphology that exhibits the network formed with the polysaccharide alone or in a food system. Furthermore, with NMR, water mobility can be examined when polysaccharides are involved in a system.

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