CHAPTER 1

Tailor-made novel polymers for hydrogel encapsulation processes

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1.1 Introduction

Natural polymers are materials of large molecular weight and natural origin such as plants, animals, or microorganisms. They have been known for centuries and have found widespread use in various industries, such as food, cosmetics, pharmaceuticals, textiles, plastics, and paper. They are of considerable importance because they are generally biodegradable and are generally recognized as safe (GRAS), which is a significant advantage, especially in recent times, when "pro-nature" policies and goals to reduce "chemicals" and synthetic materials in our lives, including food, have become popular. This makes the interest in natural polymers unabated and still increasing. One successful way of using these materials is in encapsulation processes, including spray-drying, emulsion techniques, coacervation, and ionotropic gelation. The utility of polymers as encapsulants is determined by their specific properties. These can include film-forming properties, emulsifying properties, high resistance to the environment of the gastrointestinal tract, biodegradability, low viscosity at high solids contents, low hygroscopicity, and availability and low cost (Özkan and Bilek, 2014).

Generally, among the natural polymers two main groups can be distinguished: polysaccharides and proteins. The next section presents the most popular polymers, commonly used as materials to form capsule matrix (Table 1.1 and Table 1.2). Despite the many well-known encapsulants, there is still a need to look for novel polymers and new means to use the old ones in other ways to create ideal capsules with excellent resistance and mechanical properties and wide applicability; this topic is also presented in this chapter.

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Table 1.1 Selected carbohydrate polymers commonly used for hydrogel encapsulation processes.

	Properties								
Origin/ Isolation	**Structures**	**Solubility in Water**	**Viscosity**	**Gel Formation/ Gelation**	**Synergistic Effects with**	**Other**	**Micro-encapsulated Active Substances**	**Micro-encapsulation Techniques**	**References**
Alginate									
Mainly from marine brown alga Also from exocellular material of some bacteria	Linear anionic polysaccharide Copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate and α-L-glucuronate residues	Depends on the rate of dissociation and the type of the counter-ion Alginic acid: insoluble Salts of alginic acid (sodium alginate): soluble	High viscosity at relatively low concentration Exponential increase with the molar mass Highly dependent on ionic strength	Ionotropic gels in the presence of polyvalent cations (Ca^{2+} most commonly used) The α-L-glucuronate blocks are responsible for gelation	—	—	Folic Acid (Alginate-Starch)	Coacervation	Madziva *et al.* (2006)
							Lactobacillus Acidophilus, Bifidobacterium lactis (Alginate/ Hi-Maize Starch)	Emulsion	Kailasapathy (2006)
							Lactobacillus, Acidophilus (Alginate, $CaCl_2$, chitosan)		Krasaekoopt *et al.* (2006)
Carrageenan									
Red seaweed (Rhodophycae) *Chondrus crispus, Gigrtina, Furcellaria* (κ, ι) *Eucheuma cottoni* (K) *Euchema spinosum* (ι)	Anionic polyelectrolytes Structure can vary with the source and extraction and purification conditions Three types of carrageenan are commercially available: ι- (iota), κ- (kappa), λ- (lambda) chains contain alternating (1-3)- linked β-D-galactopyranosyl and (1-4)-linked α-D-galactopyranosyl units	Depends on the carrageenan type Solubility in cold/hot water: ι-Carrageenan: Na-salt soluble; K-, Ca-, ammonium salts from limited to high swelling/ soluble >70 °C κ-Carrageenan: Na-salt soluble; Ca-salts give thixotropic dispersions, soluble >70 °C		ι-Carrageenan: thermo-reversible gels during cooling in the presence of specific counter-ions; strongest gels are obtained with Ca^{2+} Gels are elastic, have high freeze–thaw stability, do not undergo syneresis	Other gums, e.g., κ-carrageenan brittle gels became softened with locust bean gum	—	*Bifidobacterium longum*	Two-phase (water/oil) system	Adhikari *et al.* (2003)

	Some (1-3)-linked units are like the 2- and 4-sulfates Some (1-4)-linked units are like the 2- and 6-sulfates, the 2,6 disulfates, the 3,6-anhydride, the 3,6-ahydride-2-sulfate	λ-Carrageenan: All salts soluble, create viscous, pseudoplastic solutions/ soluble		κ-Carrageenan: thermo-reversible gels during cooling in the presence of specific counter-ions; strongest gels are obtained with K^+ Gels are brittle, have low freeze–thaw stability, undergo syneresis λ-Carrageenan: non-gelling					
Xanthan Produced by bacteria (*Xanthomonas campestris*) in aerobic fermentation	Anionic polyelectrolyte mixed salt of sodium, potassium and calcium Main chain consists of β-(1-4)-D-glucuronosyl units Every second unit at 3C position has the trisaccharide side chain (one D-glucuronosyl unit between two D-mannosyl units	Soluble in cold water	Progressive reduction with increasing shear stress; reversible after eliminating shear stress Stable in a broad range of pHs (2–12) and temperatures Increases after salt addition to a salt-free xanthan solution in concentration >0.15% Increases during heating salt-free xanthan solution	—	Guar gum: enhancement of viscosity Locust bean gum and konjac mannan: obtain soft, elastic thermo-reversible gels at higher concentration	Undergoes cryogelation Not degraded enzymatically	*Bifidobacterium lactis*	Extrusion	McMaster *et al.* (2005)

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Table 1.1 (Continued)

	Properties								
Origin/ Isolation	**Structures**	**Solubility in Water**	**Viscosity**	**Gel Formation/ Gelation**	**Synergistic Effects with**	**Other**	**Micro-encapsulated Active Substances**	**Micro-encapsulation Techniques**	**References**
Gellan									
Produced by some bacteria strains (e.g., *Sphingomonas elodea*)	Linear, anionic polyelectrolyte Tetrasaccharides repeating unit consisting of one rhamnose and glucoronic acid unit, and two glucoses The 3-linked glucose unit is substituted with glyceryl and acetyl at O(2) and O(6), respectively Designated →4)-L-rhamnopyranosyl-A-(1→3)-D-glucopyranosyl-B-(1→4)-D-glucuronopyranosyl-B-(1→4)-D-glucopyranosyl-B-(1→	Depends on the degree of acetylation and the type and amount of ions Low acyl gellan depends more on the ion concentration (needed sequestrant addition) High acyl gellan depends less on the ion concentration Generally in hot water (85–95 °C)	Increases with the degree of acetylation	Thermo-reversible gels during cooling Low acyl gellan: needed gelling cations High acyl gellan without cations Low acyl gellan gels: hard, non-elastic, brittle High acyl gellan gels: soft, elastic, flexible, transparent	—	Resistant to heating to 120 °C	*Bifidobacterium lactis* (hydrated gellan) *Lactobacillus* sp.	Extrusion Interfacial polymerization	Mcmaster *et al.* (2005) Yáñez-Fernández *et al.* (2008)
Chitosan									
Main source of chitosan is chitin Mainly obtained by alkaline deacetylation of shrimp and other crustacean shells Isolation from fungi has been carried out	Linear, polyelectrolyte composed of randomly distributed β-(1-4)-linked D-glucosamine and n-acetyl-D-glucosamine (deacetylated and acetylated unit, respectively)	Depends on degree of acetylation Chitosans with acetylation <40% are soluble in acidic pH		With tripolyphosphate and alginate	—	Film-forming properties Antimicrobial activity	Astaxanthin/ multiple emulsion Tuna o/w emulsion (lecithin-chitosan)	Solvent evaporation layer-by-layer deposition + spray-drying	Higuera-Ciapara *et al.* (2004) Klinkesorn *et al.* (2006)

Gum Arabic									
Obtained from stems and branches of *Acacia senegal* or *Acacia seyal*	Branched neutral or slightly acidic compound of arabinogalactan oligosaccharides, polysaccharides, glicoproteins Composition is highly variable due to climate, source, season, rainfall, etc. Generally the main chain contains β-(1-3)-linked D-galactopyranosyl units Side chains contains two to five β-(1-3)-linked D-galactopyranosyl units combined with the backbone by 1,6-linkages Main and side chains consist of α-l-arabinofuranosyl, α-l-rhamnopyranosyl, 4-*O*-methyl-β-glucuronopyranosyl	Highly soluble in cold and hot water to 50 wt%	Depends on gum arabic type, pH, ionic strength Maximum viscosity is noted between 6 and 7 pH 40 wt% solutions behave like Newtonian liquids <40% solutions are pseudoplastic	—	Majority of plant hydrocolloids, proteins, modified starches Gum tragacanth: lowering the viscosity	Creation of a strong protective film around oil droplets because of presence in the branched structure both protein (hydrophobic) and polysaccharide (hydrophilic) moieties Obtained film/layer protect against, e.g., aggregation, oxidation, evaporation, and moisture absorption	Betacyanin	Spray-drying	Pitalua *et al.* (2010)
							Betalain	Spray-drying	Janiszewska & Włodarczyk (2013)
							Lycopene	Spray-drying	Shu *et al.* (2006)
							Turmeric	Spray-drying	Martins *et al.* (2010)
							Limonene (gum arabic-sucrose-gelatin)	Freeze-drying	Kaushik & Roos (2007)
							Lactobacillus sp./	Interfacial polymerization	Yáñez-Fernández *et al.* (2008)
							Linoleic acid	Spray-drying	Fang et al., (2005)

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Table 1.1 (Continued)

		Properties							
Origin/ Isolation	**Structures**	**Solubility in Water**	**Viscosity**	**Gel Formation/ Gelation**	**Synergistic Effects with**	**Other**	**Micro-encapsulated Active Substances**	**Micro-encapsulation Techniques**	**References**
Gum Karaya (Sterculia Gum)									
Dried exudate from the stems and braches of *Sterculia* or *Cochlospermum* spaces (e.g., *Sterculia urens*)	Compound partly acetylated polysaccharide received as Ca- and Mg-salts Branched structure Main chain contains α-D-galacturonic acid and α-L-rhamnose units Side chains are linked to the galacturonic acid of the backbone by (1-2)-linkage of β-D-galactose or (1-3)-linkage of β-D-guluronic acid Half of the backbone rhamnose units are (1-4)-linked to β-D-galactose units	Native gum soluble up to 10%/up to 30% Deacetylated gum soluble up to 90%	—	—	Other plant hydrocolloids, carbohydrates, proteins	—	—	—	—
Mesquite Gum									
Obtained from mesquite tree (*Prosopis* spp.) or shrub	Neutral salt of a composite acidic polysaccharide Branched structure Main chain contains (1-3)-linked β-D-galactose units	Comparable to gum arabic Possible concentration of solution up to 50%	Increases with concentration	—	—	Good film-forming properties	Cardamom oil *Lactobacillus* sp.	Spray-drying Interfacial polymerization	Beristain *et al.* (2001) Yáñez-Fernández *et al.* (2008)

	Side chains are linked with backbone by (1-6) bond Side chains consist of single sugar or oligosaccharide such as L-arabinose (pyranose and furanose ring forms), l-rhamnose, β-D-glucuronate, and 4-*O*-methyl β-D-glucuronate Small amount of proteins								
Pectin									
Main sources: citrus fruits and apples	Hetero-polysaccharide Presents a highly complex, nonrandom structure with linear homo-poly(galacturonic acid) blocks) (smooth regions) and strongly branched blocs (hairy regions) Contains at least 65 wt% α-(1-4)-linked D-galacturonic acid–based units units can occur as free acid or salts (Na-, K-, Ca-, ammonium), naturally methanol esterified or as acid amide in amidated pectins	Soluble Possible concentration at range of 6%–12% Most stable at pH 3–4	Low viscosity in comparison with plant gum Close to Newtonian flow at low concentration Pseudoplastic behavior at higher concentration	Depends on the degree of esterification *HM pectins:* Gel in the presence of sugars and low pH range Gel strength is inversely related to pH level *LM pectins:* Gel in the presence of calcium ions	—	—	Fish oil and fish oil–extra virgin olive oil Lycopene (complex with gelatin)	Spray-drying Coacervation	Polavarapu *et al.* (2011) Silva *et al.* (2012)

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Table 1.1 (Continued)

		Properties							
Origin/ Isolation	**Structures**	**Solubility in Water**	**Viscosity**	**Gel Formation/ Gelation**	**Synergistic Effects with**	**Other**	**Micro-encapsulated Active Substances**	**Micro-encapsulation Techniques**	**References**
	Polymer chain contains some neutral sugars (e.g., L-rhamnose, D-galactose, L-arabinose, D-xylose) L-Rhamnose unit exists only as (1-2)-linked in the backbone Other sugar units preferably at the rhamnose and galactose residues to the backbone Two types of pectin depending on the esterification degree: high methocylated (HM) >50% esterification and low methoxylated (LM) <50%			Gelation depends also on the proportion and arrangement of the carboxyl groups in the pectin chain Ca^{2+} with pectin interaction increases with decreasing esterification degree Amide groups increase the range of the calcium ion concentration where the LM pectins form gel					
Starch									
Produced by most green plants as an energy store	Polymer of α-D-glucose Two architecturally different molecules in the structure: linear (amylose) and branched (amylopectin)	Insoluble in cold water Swelling Swelling power decreases with decrease in granule size and increased amylose content	Amylose is responsible for the solution's high viscosity	*MC:* Occurs on heating above 50 °C Reversible on cooling substitution	—	—	*Bifidobacterium* Pl1 Fish oil Chlorophyll	Spray-coating, spray-drying Spray-drying Spray-drying	O'Riordan *et al.* (2001) Tan *et al.* (2005) Porrarud and Pranee (2010)

Commercial sources: cereal grain seeds (corn, wheat, rice, sorghum), roots and tubers (potato, tapioca, arrowroot), stems and pith (sago)	Generally the content of amylose is about 20%–30% and amylopectin 70%–80% *Amylose* contains from 500 to 6000 D-glucose units, which are linked by α-(1-4)-glycosidic bond *Amylose* occurs in the form of a double helix *Amylopectin* contains up to 2 million D-glucoses Side chains include about 30 D-glucoses and occur approximately every 20 to 30 glucose units along the chain The point of chain branching has α-(1-6) glycosidic bond Present in small grains of different shapes (spherical or lentil-shaped) and size (e.g., 1–100 µm, 5–900 µm)	Crystalline becomes amorphous in water at 60–70°C *Amylose:* Specific dissolution behavior because of helical structure Soluble in hot water In dilute solution it can bind with itself in a double helix Undergoes retrogradation and, as a consequence, after drying it becomes insoluble Retrogradation is faster with lower concentration, temperature, molar mass; the fastest is between pH5 and 7 *Amylopectin:* Insoluble in cold and hot water No tendency to retrogradation and crystallization	Gelation temperature decreases with high degree of substitution *HPMC:* Creating thermo-reversible gels Gel transition temperature is in range of 50 to 90 °C and depends on the ratio of methyl to hydroxypropyl derivatization Gel texture is changeable with increasing hydroxypropyl substitution

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Table 1.1 (Continued)

		Properties							
Origin/ Isolation	**Structures**	**Solubility in Water**	**Viscosity**	**Gel Formation/ Gelation**	**Synergistic Effects with**	**Other**	**Micro-encapsulated Active Substances**	**Micro-encapsulation Techniques**	**References**
		Derivatives of native starch: Modification of structure (in chemical, biological, biochemical way) and controlled effect on hydrogen bonding to improve starch properties, e.g., improvement of heat and shear stability, inhibition of swelling, reduction of retrogradation							
Cellulose and Derivatives (Methylcellulose (MC), Hydroxypropyl Methyl Cellulose (HPMC)									
Major structural plants material Sources: wood, cotton, straw, flax, jute, hemp Other: acetic bacteria and many algae synthesize cellulose	Linear polymer of β-D-glucose Chain units are bonded by β-(1-4)-glycosidic linkage	Native cellulose: Insoluble Crystalline become amorphous in water at 320°C And 25 MPa Derivatives: Swelling and soluble Decreases with increase in polymerization and substitution degrees Solutions are surface active	MC solutions are stable from 3 to 11 PH Mixture of MC and HPMC exhibit pseudoplastic non-thixotropic flow properties Deviation from Newtonian behavior increases with molar mass			MC and HPMC have good film-forming properties	Fish oil/spray-drying		Kolanowski *et al.* (2007)

From Wandrey et al., 2010; Milani & Maleki, 2012.

Table 1.2 Selected proteins commonly used for hydrogel encapsulation processes.

	Properties							
Origin/Isolation	**Structures**	**Solubility in Water**	**Viscosity**	**Gel Formation/ Gelation**	**Other**	**Microencapsulation of Active Substances**	**Microencapsulation Techniques**	**References**
Gelatin								
Produced from collagens by destruction of their secondary and higher structures Main sources of collagen to gelatin production: skin, hides, bones of cattle, pigs, horses; skin of fish Depending on the using processes are obtained two types of gelatins: type A by acid treatment (pH 1.5–3.0) and type B by alkaline treatment (pH 12) of collagens	Heterogeneous mixture of single- or multistranded polypeptides consisting of 300 to 4000 amino acids Each polypeptide has extended left-handed proline helix conformations Generally, every third unit in all chains is glycine then proline and 4-hydroxyproline as next often occurring residues *Type A gelatin (obtained from pigskin):* Typical composition of amino acids (residues per 100 units): glycine 33, proline 13, alanine 11, hydroxyproline 9, arginine 5, serine 3.5, aspartic acid 3, lysine 3, glutamic acid 2.5, and further leucine, valine, phenylalanine, threonine, isoleucin, hydroxylysine, methionine, histidine, and tyrosine *Type B gelatin:* Aspargine and glutamine are conversed into aspartic acid and glutamic acid	Soluble in hot water In cold water, swelling to an elastic mass after 5–10 min	Solutions characterized by high viscosity, viscoelastic flow, streaming birefringence	Thermo-reversible, elastic, transparent gels Mammalian gelatins gel below 35–40 °C Fish skin gelatins gel at 5 °C Or 12 °C, depending on the fish type from which skin originated: cold-, warm-water fish respectively	Amphiphilic nature Good emulsifying properties	Lycopene	Spray-drying	Rocha *et al.* (2012)
						Fish Oil (Fish Gelatin/Corn Starch)	Spray-drying	Kolanowski *et al.* (2007)

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Table 1.2 (Continued)

		Properties						
Origin/Isolation	**Structures**	**Solubility in Water**	**Viscosity**	**Gel Formation/ Gelation**	**Other**	**Microencapsulation of Active Substances**	**Microencapsulation Techniques**	**References**
Whey Proteins								
Obtained from whey As powders of different quality (demineralized, delactosed, and demineralized-delactosed), whey protein concentrate (WPC: low level of fat and typically 35%–80% protein), whey protein isolate (WPI: fat and lactose removed, >90% protein), lactalbumin, individual whey protein fractions -β-lactoglobulin isthe most important whey protein	Globular proteins Main components: α-lactoalbumin (0.07%–0,15%), β-lactoglobulin (0.2%–0.4%), immunoglobulins (0.06%–0.1%), serum albumin (0.01%–0.04%) α-Lactalbumin: calcium metalloprotein with four intramolecular disulfide cross-links β-lactoglobulin has two intramolecular disulfide cross-links and one free SH group	Depends On pH Insoluble at isoelectric point at about pH 5 and also after denaturing at >70 °C	Less viscous than caseinate solutions Newtonian flow at concentration from 4%–12% exhibit pseudoplastic behavior in the range of 18%–29%	Thermally irreversible gels after denaturing (>70 °C) Cold-induced gelation of preheated mixture in presence of calcium	Form films after thermally induced disulfide cross-linking Films 'tensile strength is like synthetic films Good surface-active properties Amphiphilic Ability to be adsorbed at the emulsion interface and coat oil droplets, preventing coalescence and floculation	Astaxantin *Bifidobacterium. breve* (milk fat, denaturated whey proteins) Conjugated linoleic acid (CLA)/	Multiple emulsion/solvent evaporation Emulsion, spray-drying Spray-drying	Higuera-Ciapara *et al.* (2004) Picot and Lacroix (2004) Jimenez *et al.* (2006)

Caseins								
Obtained from skim milk by destabilizing of micelles Main products: mineral or lactic acid casein, rennet casein Possible fractionation Most commonly used is Na-caseinate	Heterogeneous group of phosphoproteins Distinguishes the following four main groups: A_{s1}-casein (0.9%–1.5%) A_{s2}-casein (0.3%–0.4%) B-casein (0.9%–1.1%) K-casein (0.3%–0.4%) In milk they occur in the form of micelles	Depends on pH Varies according to the fractions Generally insoluble in isoelectric point, at about pH 4.6	Solutions of 10%–15% prepared at pH 6–7: high viscosity At pH <3.5: solution highly viscous, even gel-like	Only Ca-salt of caseins exhibit reversible thermal gelation	Film-forming properties Na-caseinate And β-caseinate surface of films have the lowest viscoelasticity and best flexibility: highly heat-stable, no coagulation by heat Good fat emulsifiers	Curcumin	Spray-drying	Pan *et al.* (2013)

From Wandrey *et al.* (2010).

1.2 Well-known and commonly used polymers

1.2.1 Carbohydrate polymers

Carbohydrate polymers are described as natural homo- and copolymers that consist of sugar residues and/or their derivatives. Characteristic linkages that bond specific monosaccharides together in this polymeric structure are the O-glycosidic linkages. The interaction can occur between any of the hydroxyl groups of sugar monomers, resulting in polysaccharides presenting linear or branched-chain construction. The character of a polymer's structure determines its functional properties, such as solubility, gel-forming, and surface properties. Table 1.1 gives an overview of the origin and physicochemical properties of selected carbohydrate polymers. Figure 1.1 shows the principal chemical structure of selected carbohydrate polymers.

1.2.2 Proteins

Proteins are large molecules composed of linear long chains of amino acids that are bonded by amide (or peptide) linkage. The compositions of protein polymers are various combinations of 20 amino acids that give an enormous variety of sequences. These polymers have a natural origin, but some of their properties, such as fibers and hydrogels, can be compared to those of synthetic materials. Table 1.2 gives an overview of the origin and physicochemical properties of selected protein-based polymers used especially in the food industry.

1.3 Novel polymers

1.3.1 Zein

1.3.1.1 Origin and structure

Zein is a major storage protein obtained from natural, sustainable and renewable source of corn or maize seeds (*Zea mays* L.), accounting for 35% to 60% of total proteins present in corn (Luo and Wang, 2014; Patel *et al.,* 2014). Commercial zein is currently separated from corn gluten meal, a coproduct of corn wet milling, and is a mixture of at least four types of proteins: α-, β-, γ-, and δ-zein, each with a different amino acid sequence, molecular weight, and solubility (Shukla *et al.,* 2001; Zhu *et al.,* 2007; Zhong *et al.,* 2009).

1.3.1.2 Properties

Zein is one of the few hydrophobic water-insoluble biopolymers that have been approved for oral use by the U.S. Food and Drug Administration (FDA). Zein is considered a prolamine due to its characteristic solubility. It is insoluble in water unless specifically defined conditions are applied, such as a certain concentration of alcohol, high concentrations of urea, extreme alkaline condition (pH >11), and/or anionic detergents. This unique solubility behavior of zein is attributed to

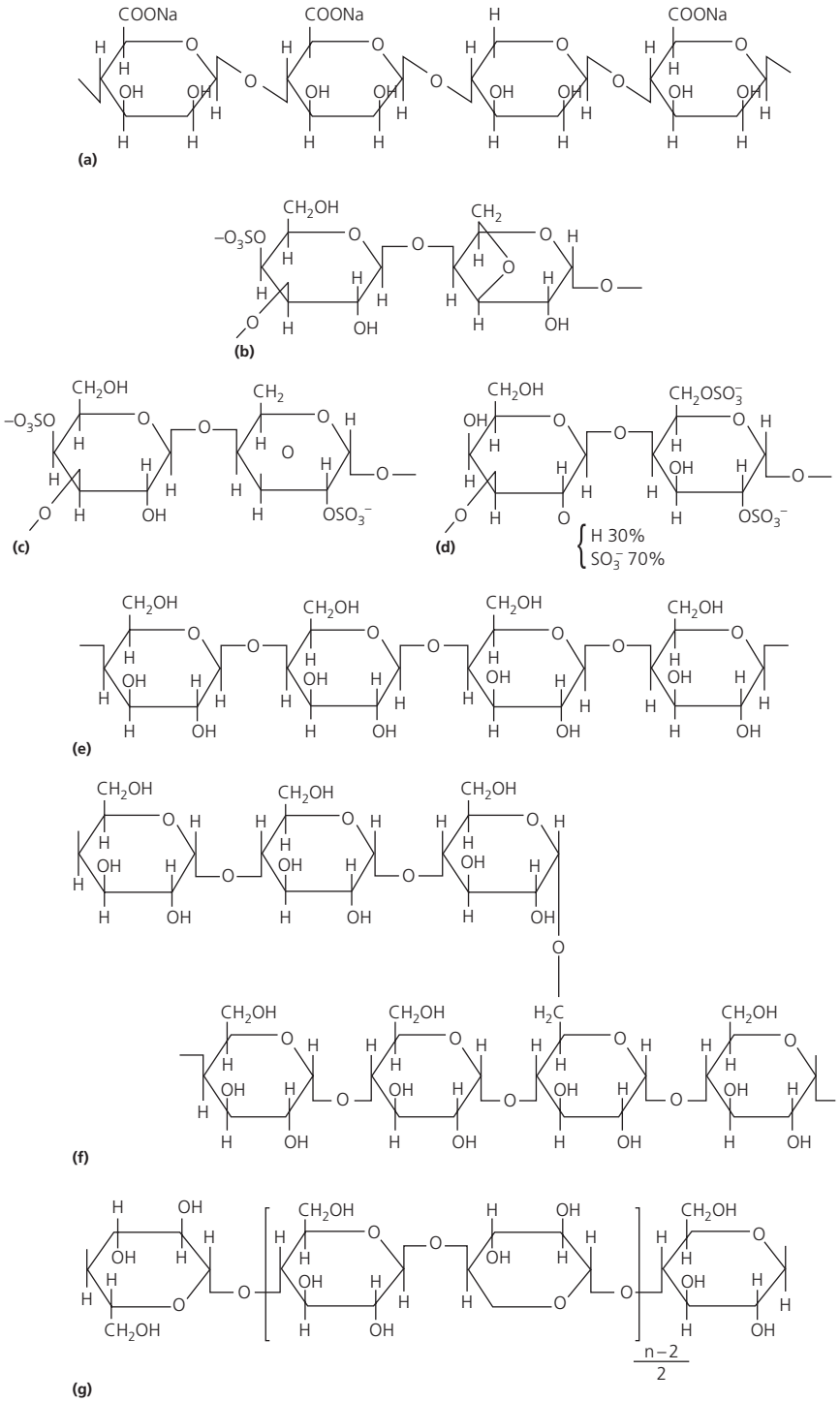

Figure 1.1 Principal chemical structure of selected carbohydrate polymers. **a,** sodium alginate; **b,** κ-carrageenan; **c,** ι-carrageenan; **d,** **λ-**carrageenan; **e,** amylose starch; **f,** amylopectin starch; **g,** cellulose.

the high percentage of nonpolar amino acids, with more than 50% being nonpolar, including leucine, proline, alanine, phenylalanine, isoleucine, and valine (Luo and Wang, 2014; Lawton, 2002; Shukla and Cheriyan, 2001; Patel *et al.*, 2014). The protein structure allows zein to function as a polymeric amphiphile (as it contains nearly an equal amount of hydrophilic and lipophilic amino acid residues), which has been observed to facilitate the encapsulation and dispersion of oil-based microspheres (Torres-Giner *et al.*, 2010; Wang *et al.*, 2008).

Because zein is hydrophobic, this protein can be easily transformed into colloidal particles by simply changing the solubilizing capacity of the primary solvent through dilution with a nonsolvent, the process commonly known as the antisolvent precipitation method. This is achieved due to excellent miscibility of ethanol and water, where the water is not a good solvent for the dissolved material such as zein (Torres-Giner *et al.*, 2010). Zein micro- and nanoparticles have been studied as promising delivery systems, especially for hydrophobic nutrients or drugs. Generally, the hydrophobic bioactives are dissolved together with zein in aqueous ethanol binary solvent and then mixed with an antisolvent, such as water, to coprecipitate bioactives with zein (Luo and Wang, 2014).

Zein has long been a subject of research for scientific interest, as well as industrial applications (as material used in production of coatings, fibers, and printing ink) (Hamakar, 1995; Lawton, 2002; Patel *et al.*, 2014), and it has been employed as an edible coating for foods and pharmaceuticals because it shows low water-uptake values, high thermal resistance, and good mechanical, oxygen, and aroma barrier properties (Shukla and Cheriyan, 2001; Patel *et al.*, 2014). Zein can create a protective layer because of its extremely high surface area and trapping efficiency (Torres-Giner *et al.*, 2010). Zein is also known for its resistance to digestive enzymes, resulting in a slower digestibility in the gastrointestinal tract, which can be exploited for a controlled release of functional components loaded in zein colloidal particles (Patel *et al.*, 2014).

Taken together, these properties make zein an attractive novel material that is used in a wide range of protecting applications of bioactive components such as polyphenols, vitamins, and omega-3 fatty acids. Because zein used in the preparation of colloidal particles is edible (GRAS), encapsulation in zein colloidal particles exhibits potential in the design of novel functional foods or bioactive packaging strategies to enhance the long-term stability of bioactive functional ingredients.

1.3.1.3 Application of zein in the encapsulation process

1.3.1.3.1 Zein–chitosan complex nanoparticles

A water-soluble chitosan derivative, carboxymethyl chitosan (CM-chitosan), is used to form coatings on the zein surface. CM-chitosan forms a gel at an acidic pH and thus provides greater protection of zein protein against enzymatic degradation. A CM-chitosan coating also confers thermal stability to zein

nanoparticles, so that the complex nanoparticles can provide excellent protection of labile nutraceuticals against thermal degradation and oligomerization (Luo and Wang, 2014).

The zein–chitosan complex nanoparticle is a specific design for encapsulation and delivery of nutrients or drugs and can be tailored in two ways for different applications. The first example of the zein–chitosan complex delivery system is in the design of chitosan nanoparticles as hydrophilic core and zein coating as hydrophobic shell. In this method, chitosan–tripolyphosphate (TPP) nanoparticles are first fabricated through ionic gelation, and then zein (predissolved in 70% ethanol aqueous solution) is added into nanoparticles, dispersing with gentle stirring. Because of the acidic condition (pH <5.5) of chitosan–TPP nanoparticles, zein forms films spontaneously upon removal of ethanol by nitrogen stream or rotary evaporation under reduced pressure. When compared with chitosan nanoparticles without a zein coating, the zein–chitosan complex nanoparticles provide significant improvement in functionalities, namely, higher encapsulation efficiency and slower sustained release of hydrophilic nutrients or drugs in the gastrointestinal tract, owing to the hydrophobic zein shell, which prevents dissolution of chitosan in the acidic condition of the stomach and helps the complex maintain its structure. The second example of a zein–chitosan complex delivery system is encapsulating and delivering hydrophobic drugs and nutrients, where zein nanoparticles, along with hydrophobic bioactives such as fat-soluble vitamins, are poured into chitosan solution to induce phase separation and form zein–chitosan complex nanoparticles (Luo and Wang, 2014).

Vitamin E (α-tocopherol) is the main dietary fat-soluble antioxidant and is widely considered to help reduce the risk of many chronic diseases, such as cardiovascular diseases (Herrera and Barbas, 2001; Tucker and Townsend, 2005; Luo *et al.,* 2011). This vitamin, like other lipophilic nutraceuticals, is poorly soluble in water and is biologically unstable when exposed to environmental factors, such as light, high temperature, and oxygen (Miquel *et al.,* 2004; Sabliov *et al.,* 2009; Luo *et al.,* 2011).

Physicochemical analyses suggest that electrostatic interactions, hydrogen bonds, and hydrophobic interactions are the main forces in an α-tocopherol–zein–chitosan complex. Chitosan coating does not affect the encapsulation efficiency but greatly improves the controlled-release properties of α-tocopherol in release profile in the presence of enzymes. This result indicates that α-tocopherol–zein–chitosan complex can be developed as a novel nano-scale delivery system of α-tocopherol supplementation or treatment (Luo *et al.,* 2011).

In the case of encapsulation of vitamin D_3 into zein–chitosan complex nanoparticles prepared by phase separation, it was possible to achieve a controlled-release property and improve the stability of labile nutrients (Luo *et al.,* 2012). Vitamin D is one of the fat-soluble vitamins and has two major physiologically active forms, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Dietary sources of vitamin D_3 are very limited, and only fish are an abundant

source. Vitamin D is an essential nutrient for human health, not only for calcium absorption and homeostasis regulation but also for the prevention of many chronic diseases, such as type 2 diabetes, hypertension, and cardiovascular disease (Picciano, 2010; Pittas *et al.*, 2010; Luo *et al.*, 2012). Vitamin D_3 was first encapsulated into zein nanoparticles, and then chitosan was applied to coat zein nanoparticles and hardened by calcium ions. Photostability of vitamin D_3 against ultraviolet (UV) light was significantly improved after encapsulation of hydrophobic nutrients in zein nanoparticles with chitosan coatings (Luo *et al.*, 2012).

1.3.1.3.2 Zein–polyphenol composite colloidal particles

Polyphenols are known to strongly interact with proline-rich proteins via noncovalent interactions such as H-bonding and hydrophobic interactions. Moreover, polyphenols have excellent solubility in lower alcohols, and thus they can be encapsulated in zein colloidal particles using the process of antisolvent precipitation (Zhang *et al.*, 2008; Zheng *et al.*, 2005; Patel *et al.*, 2012; Patel *et al.*, 2014).

Quercetin is a natural flavonol known to possess a wide range of physiological benefits in humans, including antioxidant, anticancer, and antiviral activities (Zheng *et al.*, 2005). Use of this polyphenol for food and clinical applications is limited due to its low oral bioavailability, owing to its limited aqueous solubility and degradation in the physiological alkaline pH of the intestinal tract (Zhang *et al.*, 2008).

Novel zein–quercetin composite colloidal particles were prepared by simultaneous precipitation of zein and quercetin by adding their hydroalcoholic solution to aqueous solution in the presence of sodium caseinate as an electrosteric stabilizer. Electrosteric stabilization of zein colloidal particles using an oppositely charged protein (sodium caseinate) results in the surface interaction between positively charged zein particles and negatively charged caseinate and provides protection against aggregation in physiologically relevant conditions and due to the hydrophilic nature of sodium caseinate (Patel *et al.*, 2014).

The precipitation of quercetin from an organic solvent generally results in the formation of needle-like crystals. Incorporation of quercetin in zein matrix results in the formation of spherical particles, with complete disappearance of needle-like particles at a zein-to-quercetin ratio of 25:1 wt/wt, suggesting effective encapsulation of quercetin. The entrapment of quercetin in zein colloidal particles led to its enhanced molecular stability to alkaline pH and UV irradiation. The positive effect of encapsulation was successfully demonstrated by comparing the antioxidant activity of quercetin in alkaline medium (Patel *et al.*, 2012; Patel *et al.*, 2014).

Curcumin is a natural polyphenol that exhibits a range of pharmacological activities including antioxidant, antiinflammatory, antiproliferative, and antiangiogenic activity (Patel *et al.*, 2010; Aggarwal and Sung, 2009). It is a very powerful antioxidant, but the formulation and delivery of curcumin in oral products is a very challenging task due to a combination of factors including low solubility

in aqueous medium, photodegradation, susceptibility to enzymatic degradation, and instability in alkaline intestinal conditions (Patel *et al.,* 2014; Patel *et al.,* 2010). Zein–curcumin composite colloidal particles were successfully prepared using an antisolvent precipitation method. Encapsulation of curcumin in zein colloidal particles was carried out by coprecipitating different ratios of zein to curcumin (50:1 to 5:1 wt/wt) in the presence of sodium caseinate as a stabilizer. Curcumin in colloidal particles showed enhanced water dispersibility. Zein colloidal particles led to enhanced stability of curcumin at all physiologically relevant pH levels and to UV irradiation (Patel *et al.,* 2010; Patel *et al.,* 2014).

Procyanidins are known to have antioxidant capacities and might reduce the risk of chronic diseases, such as cardiovascular diseases and cancers (Lou *et al.,* 2012), and cranberry procyanidins exhibit preventive effects against urinary tract infections. Cranberry procyanidins have been encapsulated in zein colloidal particles using a modified liquid–liquid dispersion method to enhance their stability as well as improve their bioavailability through controlled *in vivo* delivery (Lou *et al.,* 2012; Patel *et al.,* 2014).

1.3.1.3.3 Zein–protein nanoparticles and microparticles

Zein–β-lactoglobulin nanoparticles

To design a colloidal delivery system to encapsulate the poorly water-soluble bioactive flavonoid tangeritin, a hydrophobic protein (zein) was used as a core for forming protein nanoparticles based on antisolvent precipitation (Chen *et al.,* 2014).

Tangeritin is a flavonoid found in citrus fruits and has beneficial effects that include anticarcinogenic activity and antiinflammatory effects (Li *et al.,* 2009). However, the extensive application of this flavonoid is currently limited because of its low water solubility, which means it may be present in foods as crystals, making it difficult to incorporate into many aqueous-based foods and beverages (Li *et al.,* 2009; Patel *et al.,* 2012; Chen *et al.,* 2014).

Tangeritin-loaded protein nanoparticles were produced by mixing an organic phase containing zein and tangeritin with an aqueous phase containing β-lactoglobulin, then converting it into powder by freeze-drying. When dispersed in water, this powder formed a colloidal suspension that was relatively stable to particle aggregation and sedimentation. To the authors' knowledge (Chen *et al.,* 2014), this was the first time that zein nanoparticles had been used as a delivery system for tangeritin, which is an important nutraceutical. Thus, bioactive flavonoid tangeritin incorporated into small protein nanoparticles that consisted of a hydrophobic zein core and an amphiphilic β-lactoglobulin shell could be used in various food products as a functional ingredient. These zein–protein nanoparticles behaved similarly to β-lactoglobulin–coated fat droplets under different environmental conditions: They were stable at low salt concentrations at pH values far from the isoelectric point, but they aggregated at higher salt levels and pH values near the isoelectric point. In addition, they were stable to aggregation at temperatures below the thermal denaturation temperature of β-lactoglobulin,

but they aggregated at higher temperatures, particularly in the presence of salt (Chen *et al.*, 2014).

Zein–soy protein microparticles

A novel technique, the cold gelation method, has been reported to produce zein–soy protein isolate (SPI) complex microparticles for delivery of hydrophilic nutraceuticals, such as riboflavin (vitamin B_2) (Chen and Subirade, 2009). In this method, zein was dissolved at pH 11.0 in the absence of alcohol and then mixed with preheated SPI and calcium carbonate. The mixture was then emulsified in soybean oil to form a water-in-oil emulsion, followed by addition of acetic acid to lower the pH and induce the gelation of the zein–SPI matrix. Blending of SPI and zein provides a convenient method of adjusting the hydrophobicity and crystallinity of the protein matrix. Interestingly, in this process without any alcohol involvement, phase separation did not occur between zein and SPI, which suggested excellent compatibility and miscibility.

When compared with pure SPI microparticles that showed first-order release kinetics of riboflavin, zein–SPI microparticles demonstrated a zero-order release kinetics in simulated gastric and intestinal conditions. Microspheres with zein–SPI blended at ratios of 5:5 and 7:3 displayed near-zero-order release kinetics, and less than 20% of the riboflavin was released from the microspheres after 30 minutes in gastric fluid, which is the expected time for a food product to pass from the stomach into the intestine and suggests that most of the capacity could reach the intestine without being exposed to gastric conditions. The remaining riboflavin was analyzed after complete enzymatic degradation of the protein matrices and found to be 91% to 96% active, indicating that the nutrient was well preserved in the zein–SPI microspheres (Chen and Subirade, 2009).

Research results showed that zein–SPI microparticles were surprisingly better than pure zein or SPI microparticles in terms of slowing the release rate and increasing the absorption availability of riboflavin in the jejunum, the main site of absorption. Zein–SPI complex microparticles encapsulating riboflavin were further tested in a food product (yogurt). Suspending microparticles in yogurt significantly delayed nutrient release, which would increase the likelihood of gastric-sensitive nutrients passing intact into the intestine for absorption. Thus, zein–SPI complex microparticles exhibited features for delivery of hydrophilic nutrients or drugs with significantly improved bioavailability. Moreover, because no organic solvent was involved in this cold gelation method, the zein–SPI microparticles were proposed as a system for delivering hydrophilic nutrients for food applications (Luo and Wang, 2014).

1.3.1.3.4 Zein–omega-3 polyunsaturated fatty acids

Fish oil, flax oil, and, more recently, algae oil are the most commonly used sources of omega-3 polyunsaturated fatty acids (omega-3 PUFAs). Omega-3 PUFAs have been associated with a variety of health benefits, such as reducing

the risk of coronary heart disease, hypertension, arthritis, and immune response disorders (Quispe-Condori *et al.,* 2011; Rubio-Rodríguez *et al.,* 2010). However, one of the major drawbacks of oils rich in PUFAs is rapid oxidation of multiple unsaturated carbon–carbon double bonds of PUFAs, which involves the formation of toxic products such as peroxides or undesirable off-flavor compounds (Quispe-Condori *et al.,* 2011).

For encapsulation of fish oil in solid zein particles, a liquid–liquid dispersion process was used that could provide a simple method to produce submicrometer-sized solid particles for incorporating lipophilic bioactive compounds as alternative delivery systems to emulsions (Zhong *et al.,* 2009).

The liquid–liquid dispersion process involved the preparation of stock solutions by dissolving different amounts of zein and fish oil (zein-to-oil ratios of 2:1, 4:1, 6:1, and 8:1) in 90% isopropanol; the stock solution was then sheared into deionized water. The decrease of overall isopropanol concentration resulted in the precipitation of oil-loaded zein particles with diameters of 350 to 450 nm. After freeze-drying, samples of the encapsulated fish oil in solid zein particles (with a zein-to-oil ratio of 4:1 or lower) showed good oxidative stability, as assessed by the development of lipid hydroperoxide values during storage. This result showed that solid zein nanoparticles may be incorporated into food products, such as beverages, snacks, and cereals, to supplement bioactive compounds beneficial to human health (Zhong *et al.,* 2009).

Similarly, flax oil (which is rich in PUFAs and hence has low stability and high susceptibility to oxidation) was stabilized by encapsulation in zein microparticles prepared by spray-drying and freeze-drying (Quispe-Condori *et al.,* 2011).

1.3.2 Inulin

1.3.2.1 Origin and structure

Inulin is a natural polysaccharide belonging to the fructans group. It is a plant-derived compound occurring as storage carbohydrate in many members of the Asteraceae family including chicory, Jerusalem artichoke, and dahlia (Barclay *et al.,* 2010; Beirao-da-Costa *et al.,* 2013). This polysaccharide is also produced by bacteria (*Streptococcus mutans;* Wolff *et al.,* 2000) and fungal species, mainly members of the *Aspergillus* species (Kurakake *et al.,* 2007). Chicory (*Cichorium intybus* L., var. *sativum*) is the main natural source of inulin, which is characterized by a substantial fraction of inulin compounds with a high degree of polymerization (Van Loo *et al.,* 1995; Beirao-da Costa *et al.,* 2013).

Chemically, inulin is a polymer built of linear chains of fructosyl groups bonded by β-2,1 glycosidic linkage, with the reducing end terminated by an α-D-1,2 glucopyranoside ring group. It is described as α-D-glucopyranosyl-[α-D-fructofuranosyl](n-1)-D-fructofuranoside (Figure 1.2) (Dan *et al.,* 2009; Kurakake *et al.,* 2007; Barclay *et al.,* 2010). In general, inulins derived from plants have chains containing from 2 to 100 or more units of fructose. Both origin (the species of plant) and the time of harvest affect inulin's length of chains and

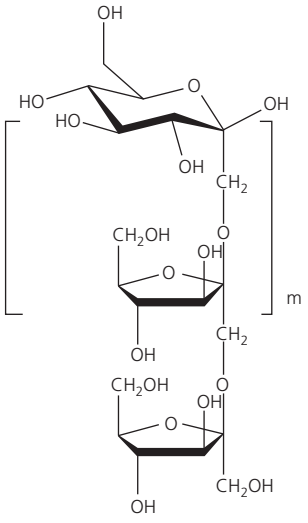

Figure 1.2 Chemical structure of inulin (Dan *et al.,* 2009).

polydispersity (Ronkart *et al.,* 2007; Barclay *et al.,* 2010). The degree of polymerization of inulin produced by microorganisms varies between 10,000 and 100,000 (Franck and De Leenheer, 2002; Barcley *et al.,* 2010).

1.3.2.2 Properties

Multiple application possibilities of inulin are the result of its special physiochemical properties. Inulin is characterized by its biochemical neutrality and nontoxicity (Barclay *et al.,* 2010). In addition, it is described as a substance with a bland neutral taste and without any off flavors or aftertaste. In general, normal inulin has a slightly sweet taste, about one tenth of the sweetness of sugar, but removing from inulin's structure the fraction with polymerization degree below 10 leads to total loss of this flavor (Franck, 2002). Noteworthy is that the presence of β-2,1 glycosidic linkages cause this polymer to be indigestible by humans and higher animals. In the gastrointestinal tract, inulin is digested before it reaches the colon by the activity of inhabiting *Bifidobacterium* species (Lopez-Molina *et al.,* 2005), and hence it acts as dietary fiber and a prebiotic. Its health-promoting effects include improvement of the immune system, increase in calcium and magnesium assimilation, and reduction of cholesterol and serum lipid levels (Coudray *et al.,* 1997; Niness, 1999; Lopez-Molina *et al.,* 2005).

A significant advantage of inulin with respect to its application is its solubility and gelation. Solubility of this polysaccharide is inversely dependent on the chain length, and solubility decreases with increasing chain length. Generally, it is rather poorly water soluble, about 10% at room temperature, creating solutions with quite low viscosity (Franck, 2002). However, inulin with short chains is dissolved in aqueous solution in a concentration up to 80%, whereas longer chain

fractions are much less dissolved and even precipitate in the crystalline forms (Kim *et al.*, 2001; Franck and De Leenheer, 2002). Inulin gelation can be performed by cooling a hot solubilized solution or by shearing suspensions of this polymer; the thermal method gives gels that are stronger and smoother and that have smaller particle size. The concentration of inulin to form gels in aqueous solutions, depending on the chain length, is greater than 13% for longer chains and 25% for shorter chains (Kim *et al.*, 2001; Franck and De Leenheer, 2002; Barclay, 2010). This kind of gel consists of a three-dimensional network of insoluble submicrometer crystalline inulin particles in water. Physical gel stability is guaranteed by a high amount of water immobilized in this network (Franck, 2002). Inulin interacts with other gelling agents, including alginate, gellan gum, κ- and ι-carrageenans, gelatin, and maltodextrins, and despite its lack of emulsifying properties, it can be used in encapsulation processes as a stabilizer of matrix (de Barros Fernandes *et al.*, 2014b).

1.3.2.3 Application in the encapsulation process

Inulin is widely used, especially in the food and pharmaceuticals industry. Application of this polysaccharide in food technology is based on its gelation properties. The texture, mouth feel, and even glossy appearance of inulin gels is similar to that of fat, and hence it is mainly used as its replacement. Because of its sweet taste, inulin can also replace sugar. As a result, inulin can be used to produce a low-calorie food (Stevens *et al.*, 2001; Kim *et al.*, 2001; Franck and De Leenheer, 2002; Robertfroid, 2005; Dan *et al.*, 2009; Barclay *et al.*, 2010). In the pharmacy, inulin functions mainly as a stabilizer and excipient (Fuchs, 1987; Dan *et al.*, 2009; Barclay *et al.*, 2010).

A group of scientists has focused on the inulin as an encapsulant agent. They studied the spray-drying encapsulation process of essential oils from oregano and rosemary using inulin separately (Beirao-da-Costa *et al.*, 2012 and 2013) or in complex with other gelling substances (de Barros Fernandes *et al.*, 2014a and 2014b). Researchers noticed that obtained inulin microcapsules were regular, smooth, uninjured, and spherical, with size in the range of 3 to 4.5 μm (Beirao-da-Costa *et al.*, 2013). Those capsules also were more stable in comparison with gelatin–sucrose microparticles (Beirao-da-Costa *et al.*, 2012). Whey protein–inulin mixture in ratios of 1:1 and 3:1 created a good–quality wall matrix of microcapsules with immobilized rosemary essential oil (de Barros Fernandes *et al.*, 2014a). Moreover, in studying the impact of the partial or total replacement of gum arabic by modified starch, maltodextrin, or inulin on properties of microcapsules with rosemary essential oil, it was noticed that the particles containing inulin were characterized by smoother surface. The addition of inulin also had a positive influence on the particles' wettability and decreased the hygroscopicity under high relative humidity. However, the encapsulation process was less efficient (de Barros Fernandes *et al.*, 2014b).

1.3.3 Angum gum

1.3.3.1 Origin and structure

A native biopolymer, Angum gum, is a natural exudate of *Amygdalus scoparia* Spach, which is grown mainly in the southern and western rangelands of Iran. Local people use it as a functional ingredient for nutritional and medicinal purposes (Jafari *et al.,* 2013).

1.3.3.2 Properties and application in the encapsulation process

Angum gum was used as a food flavor encapsulant in spray-drying encapsulation of D-limonene. After gum extraction, gum dispersions with maltodextrin were prepared in water (in 1%–5% concentrations) and emulsified with 5% and 10% D-limonene using high-pressure homogenization. The emulsification properties of this novel biopolymer in comparison with a model Arabic gum (Arg) showed that the increase in the level of Arabic gum leads to a decrease in emulsion droplet size, whereas increasing Angum gum content results in bigger droplet sizes. Gums such as Angum gum have the advantage of being independent of pH and ionic strength of the emulsion, as compared with proteins, which lose their emulsifying abilities in different emulsion environment conditions (Jafari *et al.,* 2013).

Extensive polymer interactions at the interface lead to the formation of an interfacial membrane, which may therefore provide better protection against droplet recoalescence, and this results in more-stable emulsions of Angum gum than Arabic gum. Native and unrecognized biopolymers such as Angum gum can be a good alternative for various applications, such as emulsification and microencapsulation of food flavors and oils, due to their film- and wall matrix–forming properties for covering the active ingredients and producing encapsulated powders (Jafari *et al.,* 2013).

1.3.4 *Opuntia ficus-indica*

1.3.4.1 Origin and structure of mucilage

The cactus pear, *Opuntia ficus-indica* (a member of the Cactaceae family, and colloquially known as prickly pear or nopal), is characterized by the production of a hydrocolloid commonly known as *mucilage* (nopal mucilage), which forms molecular networks that are able to retain large amounts of water (Saag *et al.,* 1975; McGarvie and Parolis, 1981; Medina-Torres *et al.,* 2000; Sepulveda *et al.,* 2007). *O ficus-indica* mucilage is a high-molecular-weight polysaccharide that behaves as a polyelectrolyte and contains a molecular structure of up to 30,000 different sugars. Chemical composition of *O. ficus-indica* mucilage is a complex mixture of polysaccharides, such as L-arabinose, D-galactose, D-xylose, and L-rhamnose, and D-galacturonic acid, which represent up to 10 g per 100 g of total sugars (McGarvie *et al.,* 1981; Medina-Torres *et al.,* 2000; Saenz *et al.,* 2004). In *O. ficus-indica,* the water-soluble polysaccharide fraction with thickening properties, represents less than 10% of the water-soluble

material (Majdoub *et al.,* 2001). The mucilage structure is proposed as two distinctive water-soluble fractions, where one is a pectin with gelling properties with Ca^{2+} and the other is a mucilage without gelling properties (Sepulveda *et al.,* 2007).

1.3.4.2 Properties and application of mucilage in the encapsulation process

Nopal mucilage, due to its emulsifying properties and rheological behavior, is an interesting option for use as a carrier of active substances (Medina-Torres *et al.,* 2000). Its use as an edible coating has been reported in strawberry preservation, where it achieved good results in increasing shelf life (Del-Valle *et al.,* 2005) and improving optical properties and water-vapor transport (Espino-Díaz *et al.,* 2010).

This mucilage has also been studied for its capacity for encapsulating bioactive compounds by spray-drying (Medina-Torres *et al.,* 2013; Saenz *et al.,* 2009). An antioxidant compound (gallic acid) was encapsulated using aqueous extracts from *O. ficus-indica* mucilage as wall material. The mucilage presented a macromolecular dispersion that became less agglomerated by the addition of gallic acid. The intermolecular mucilage–gallic acid interactions became favorable and considerably reduced the size of the aggregate, which confirmed the encapsulation properties of nopal mucilage. The results showed that using spray-drying to process nopal mucilage extract produced a stable powder with small particle size and, consequently, higher viscosity, while also exhibiting higher resistance to flow, mainly due to encapsulated structures (Medina-Torres *et al.,* 2013).

The controlled release of microcapsules of mucilage with gallic acid was designed with respect to the conditions of the small intestine, which is where gallic acid is absorbed. The controlled release indicated that 65% of gallic acid was released in 2.47 days, and the microcapsules of mucilage gum showed high efficiency (>60%). The nopal mucilage represents a promising and effective encapsulating agent of bioactive additives for incorporation into functional foods (Medina-Torres *et al.,* 2013).

1.3.5 Shellac

1.3.5.1 Origin and structure

Shellac is a natural biodegradable polymer. It is a resin secreted by the female lac insect *(Laccifer Lacca,* also called *Kerria lacca),* which parasitizes some types of trees in India, Thailand, and China. Shellac is a heterogeneous compound of polar and nonpolar components consisting of polyhydroxy polycarboxylic esters, lactones, and anhydrides, with the main acid components being aleuritic and terpenic acids (Krause and Muller, 2001; Patel *et al.,* 2013a). Its chemical structure is presented in Figure 1.3. Shellac is a nontoxic and harmless substance and GRAS (Okamoto and Ibanez, 1986; Chauhan *et al.,* 2013).

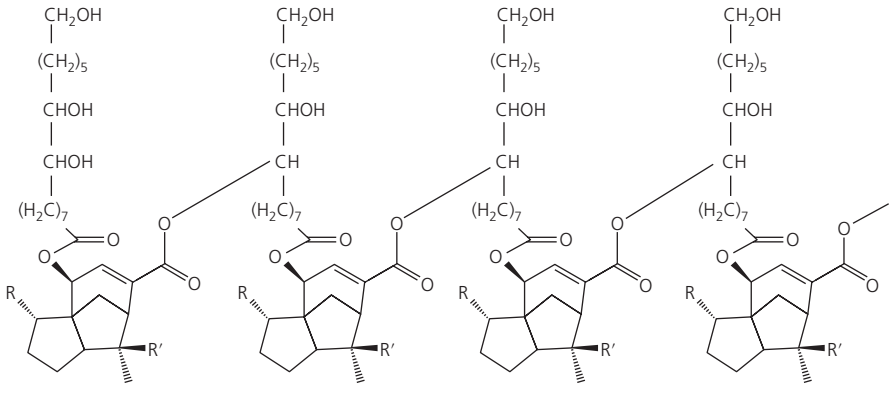

Figure 1.3 Chemical structure of shellac (Limmatvapirat, 2004).

1.3.5.2 Properties

Shellac, as a polymer containing carboxylic groups, is practically insoluble in acidic and pH-neutral aqueous media. Aqueous solutions can be prepared by using alkali salts (Leick *et al.,* 2011). Despite those solubility problems, shellac has some attractive properties, including cohesiveness, thermoplasticity, and insulating and film-forming ability. It is lipophilic and has a tendency to self-assemble into colloidal structures based on its solvent properties (Patel *et al.,* 2013b). Moreover, this resin can interact with some hydrocolloids, including pectin, xanthan gum, and cellulose derivatives (Patel *et al.,* 2011) based on non-covalent interplays, which are attributed to hydroxy aliphatic fatty acid–aleuritic acid, the main component of shellac. Because of the large amounts of carboxylic and hydroxyl groups in the structure of shellac and a strong negative charge, aleuritic acid participates in hydrogen bonding and electrostatic interactions (Coelho *et al.,* 2012; Patel *et al.,* 2013a).

1.3.5.3 Application in the encapsulation process

All of these properties make shellac widely used in industry, especially in pharmaceuticals as an enteric coating material and in the food industry as a glazing agent for confections and nutritional supplements (Boonsongrit *et al.,* 2006; Bouchemal, 2008; Leick *et al.,* 2011). Shellac is applied as an encapsulating agent of active substances (Patent no. 5,164,210; Leick *et al.,* 2011; Patel *et al.,* 2013b).

Patent no. 5,164,210 (1991) discloses the encapsulation of high-intensity sweetener ingredients applied in chewing gum by using as encapsulant mixture of shellac and zein. The encapsulant composition was prepared by dissolving components in ethyl alcohol, mixing them in an appropriate proportion, and then adding sweetener. Finally, the ethyl alcohol was removed from the sweetener–encapsulant mixture by air-drying in a fume hood for 16 hours at room temperature. Obtained capsules had a more positive effect on the shelf life of the chewing gum and sweetener than shellac or zein used separately.

Other researchers have worked on novel all-natural polymeric microcapsules composed of gelatin and shellac. They were obtained using a simple extrusion method without any cross-linkers, which was based on the strong interactions between two oppositely charged polymers and the immediate precipitation of acid-resistant shellac. The mixture of gelatin and shellac was dropped in an acidic medium, causing an instant solidification of liquid drops into solid microcapsules that retained their spherical shape on air-drying. Some possible applications of these novel capsules have been successfully demonstrated for pharmaceuticals (loading and release of bioactives such as silibinin and epigallocatechin gallate), the food industry (encapsulation of colorants and flavors, e.g., curcumin and D-limonene), and biotechnology (immobilization of enzymes) (Patel *et al.,* 2013a).

Leick's group (2010) studied thin-walled, liquid-filled composite capsules where matrix was based on calcium pectinate and shellac. Capsules were also prepared by extrusion. It was shown that the addition of shellac improved mechanical properties of the capsules, which were stronger and showed less deformation than pure pectin capsules. These results are promising for industrial applications in the future.

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References

Adhikari, K., Mustapha, A., and Grün, I. U. (2003). Survival and metabolic activity of microencapsulated *Bifidobacterium longum* in stirred yogurt. *Journal of Food Science: Food Microbiology and Safety* 68(1): 275–280.

Aggarwal, B. B., and Sung, B. (2009). Pharmacological basis for the role of curcumin in chronic diseases: An age-old spice with modern targets. *Trends in Pharmacological Sciences* 30: 85–94.

Barclay, T., Ginic-Markovica, M., Cooper, P., and Petrovsky, N. (2010). Inulin - a versatile polysaccharide with multiple pharmaceutical and food chemical uses, *Journal of Excipients and Food Chemicals* 1(3): 27–50.

Beirão da Costa, S., Duarte, C., Bourbon, A. I., Pinheiro, A. C., Serra, A. T., *et al.* (2012). Effect of the matrix system in the delivery and *in vitro* bioactivity of microencapsulated oregano essential oil. *Journal of Food Engineering* 110: 190–199.

Beirão-da-Costa, S., Duarte, C., Bourbon, A. I., Pinheiro, A. C., Januário, M. I. N., *et al.* (2013). Inulin potential for encapsulation and controlled delivery of oregano essential oil. *Food Hydrocolloids* 33: 199–206.

Beristain, C. I., Garcia, H. S., and Vernon-Carter, E. J. (2001). Spray dried encapsulation of cardamom *(Elettaria cardamomum)* essential oil with mesquite *(Prosopis juliflora)* gum. *LWT – Food Science and Technology* 34: 398–401.

Boonsongrit, Y., Mitrevej, A., and Mueller B. W. (2006). Chitosan drug binding by ionic interaction. *European Journal of Pharmaceutics and Biopharmaceutics* 62: 267–274.

Bouchemal, K. (2008). New challenges for pharmaceutical formulations and drug delivery systems characterization using isothermal titration calorimetry. *Drug Discovery Today* 13(21–22): 960–972.

Chauhan, O. P., Nanjappa, C., Ashok, N., Ravi, N. Roopa N., and Raju P. S. (2013). Shellac and aloe vera gel based surface coating for shelf life extension of tomatoes. *Journal of Food Science and Technology* 52(2): 1200–1205.

Chen, L., and Subirade, M. (2009). Elaboration and characterization of soy/zein protein microspheres for controlled nutraceutical delivery. *Biomacromolecules* 10: 3327–3334.

Chen, J., Zheng, J., McClements, D. J., and Xiao, H. (2014). Tangeretin-loaded protein nanoparticles fabricated from zein/β-lactoglobulin: Preparation, characterization, and functional performance. *Food Chemistry* 158: 466–472.

Coelho, C., Nanabala, R., Ménager, M., Commereuc, S., and Verney, V. (2012). Molecular changes during natural biopolymer ageing: The case of shellac. *Polymer Degradation and Stability* 97: 936–940.

Coudray, C., Bellanger, J., Castiglia-Delavaud, C., Remesy, C., Vermorel, M., and Rayssignuier, Y. (1997). Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *European Journal of Clinical Nutrition* 51: 375–380.

Dan, A., Ghosh, S., and Moulik, S. P. (2009). Physicochemical studies on the biopolymer inulin: A critical evaluation of its self aggregation, aggregate-morphology, interaction with water, and thermal stability. *Biopolymers* 91: 687–699.

de Barros Fernandes, R. V., Borges, S. V., Botrel, D. A., and de Oliveira, C. S. (2014a). Physical and chemical properties of encapsulated rosemary essential oil by spray drying using whey protein–inulin blends as carriers. *International Journal of Food Science and Technology*, 49: 1522–1529.

de Barros Fernandesa, R. V., Borgesa, S. V., and Botrel, D. A. (2014b). Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. *Carbohydrate Polymers* 101: 524–532.

Del-Valle, V., Hernandez-Munoz, P., Guarda, A., and Galotto, M. J. (2005). Development of a cactus-mucilage edible coating *(Opuncia ficus-indica)* and its application to extend strawberry *(Fragaria ananassa)* shelf-life. *Food Chemistry* 91: 751–756.

Espino-Diaz, M., Ornelas-Paz, J. J., Martinez-Tellez, M. A., Santillan, C., Barbosa-Canovas, G. V., *et al.* (2010). Development and characterization of edible films based on mucilage of *Opuntia ficus-indica* (L.). *Journal of Food Science* 75: 347–352.

Fang, X., Shima, M., and Adachi S. (2005). Effects of drying conditions on the oxidation of linoleic acid encapsulated with gum arabic by spray-drying. *Food Science and Technology Research* 11(4): 380–384.

Franck, A., and De Leenheer, L. (2002). Inulin. In: *Polysaccharides II: Polysaccharides from Eukaryotes*, pp. 439–479. A. Steinbüchel and E. J. Vandamme, eds. Berlin: Wiley–VCH Verlag GmbH.

Franck, A. (2002). Technological functionality of inulin and oligofructose. *British Journal of Nutrition* 87(Suppl 2): S287–S291.

Fuchs, A. (1987). Potentials for non-food utilization of fructose and inulin. *Starch-Starke*, 39: 335–343.

Hamakar, B. R, Mohamed, A. A, Habben, J. E, Huang, C. P, and Larkins, B. A. (1995). Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. *Cereal Chemistry* 72: 583–588.

Herrera, E., and Barbas, C. (2001). Vitamin E: Action, metabolism and perspectives. *Journal of Physiology and Biochemistry* 57: 43–56.

Higuera-Ciapara, I., Felix-Valenzuela, L., Goycoolea, F., and Arguelles-Monal W. (2004). Microencapsulation of astaxanthin in a chitosan matrix. *Carbohydrate Polymers* 56: 41–45.

Jafari, S. M., Beheshti, P., and Assadpour, E. (2013). Emulsification properties of a novel hydrocolloid (Angum gum) for D-limonene droplets compared with Arabic gum. *International Journal of Biological Macromolecules* 61: 182–188.

Janiszewska, E., and Włodarczyk, J. (2013). Influence of spray drying conditions on beetroot pigments retention after microencapsulation process. *Acta Agrophysica* 20(2): 343–356.

Jimenez, M., Garcia, H. S., and Beristain, C. I. (2006). Spray-dried encapsulation of conjugated linoleic acid (CLA) with polymeric matrices. *Journal of the Science of Food and Agriculture* 86: 2431–2437.

Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT – Food Science and Technology* 39: 1221–1227.

Kaushik, V., and Roos, Y. H. (2007). Limonene encapsulation in freeze-drying of gum arabic–sucrose–gelatin systems. *LWT – Food Science and Technology.* 40: 1381–1391.

Kim, Y., Faqih, M. N., and Wang, S. S. (2001). Factors affecting gel formation of inulin. *Carbohydrate Polymers* 46: 135–145.

Klinkesorn, U., Sophanodora, P., Chinachoti, P., Decker, E. A., and McClements, D. J. (2006). Characterization of spray-dried tuna oil emulsified in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. *Food Research International* 39(4): 449–457.

Kolanowski, W., Jaworska, D., Weissbrodt, J., and Kunz, B. (2007). Sensory assessment of microencapsulated fish oil powder. *Journal of the American Oil Chemists' Society* 84(1): 37–45.

Krasaekoopt, W., Bhandari, B., and Deeth, H. C. (2006). Survival of probiotics encapsulated in chitosan-coated alginate beads in yoghurt from UHT- and conventionally treated milk during storage. *LWT – Food Science and Technology* 39(2): 177–183.

Krause, K. P., and Muller, R. H. (2001). Production of aqueous shellac dispersions by high pressure homogenization. *International Journal of Pharmaceutics* 223: 89–92.

Kurakake, M., Ogawa, K., Sugie, M., Takemura, A., Sugiura, K., and Komaki, T. (2007). Two types of β-fructofuranosidases from *Aspergillus oryzae* KB. *Journal of Agricultural and Food Chemistry* 56: 591–596.

Lawton, J. W. (2002). Zein: A history of processing and use. *Cereal Chemistry* 79: 1–18.

Leick, S., Kott, M., Degen, P., Henning, S., Pasler, T., *et al.* (2011). Mechanical properties of liquid-filled shellac composite capsules. *Physical Chemistry Chemical Physics*, 13: 2765–2773.

Li, S., Pan, M. H., Lo, C. Y., Tan, D., Wang, Y., *et al.* (2009). Chemistry and health effects of polymethoxyflavones and hydroxylated polymethoxyflavones. *Journal of Functional Foods* 1: 2–12.

López-Molina, D., Navarro-Martinez, M. D., Rojas-Melgarejo, F., Hiner, A. N. P., Chazarra, S., and Rodríguez-López, J. N. (2005). Molecular properties and prebiotic effect of inulin obtained from artichoke (*Cynara scolymus* L.). *Phytochemistry* 66(12): 1476–1484.

Luo, Y., Teng, Z., and Wang, Q. (2012). Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D_3. *Journal of Agricultural and Food Chemistry* 60: 836–843.

Luo, Y., and Wang, Q. (2014). Zein-based micro- and nano-particles for drug and nutrient delivery: A review. *Journal of Applied Polymer Science* 131: 40696.

Luo, Y., Zhang, B., Whent, M., Yo, L., and Wang, Q. (2011). Preparation and characterization of zein/chitosan complex for encapsulation of α-tocopherol, and its *in vitro* controlled release study. *Colloids and Surfaces B: Biointerfaces* 85: 145–152.

Madziva, H., Kailasapathy, K., and Phillips, M. (2006). Evaluation of alginate–pectin capsules in cheddar cheese as a food carrier for the delivery of folic acid. *LWT – Food Science and Technology* 39: 146–151.

Majdoub, H., Roudesli, S., Picton, L., Le Cerf, D., Muller, G., and Grisel, M. (2001). Prickly pear nopal pectin from *Opuntia ficus-indica:* Physico-chemical study in dilute and semi-dilute solutions. *Carbohydrate Polymers* 46: 69–79.

Martins, R., Pereira, S., Siqueira, S., Salomao, W., and Freitas, L. (2013). Curcuminoid content and antioxidant activity in spray dried microparticles containing turmeric extract. *Food Research International* 50: 657–663.

McGarvie, D., and Parolis, H. (1981). Methylation analysis of the mucilage of *Opuntia ficus-indica*. *Carbohydrate Research* 88: 305–314.

McMaster, L. D., Kokott, S. A., and Slatter, P. (2005). Micro-encapsulation of *Bifidobacterium lactis* for incorporation into soft foods. *World J Microbiol Biotechnol* 21:723–728.

Medina-Torres, L., Brito-De La Fuente, E., Torrestiana-Sanchez, B., and Katthain, R. (2000). Rheological properties of the mucilage gum *(Opuntia ficus-indica)*. *Food Hydrocolloids* 14: 417–424.

Medina-Torres, L., García-Cruz, E. E., Calderas, F., González Laredo, R. F., Sánchez-Olivares, G., *et al.* (2013). Microencapsulation by spray drying of gallic acid with nopal mucilage *(Opuntia ficus-indica)*. *LWT – Food Science and Technology* 50: 642–650.

Milani, J., and Maleki, G. (2012). Hydrocolloids in Food Industry. In: *Food Industrial Processes – Methods and Equipment*. B. Valdez, ed. Croatia: InTech.

Miquel, E., Alegria, A., Barbera, R., Farre, R., and Clemente, G. (2004). Stability of tocopherols in adapted milk-based infant formulas during storage. *International Dairy Journal* 14 (11): 1003–1011.

Niness, K. R. (1999). Inulin and oligofructose: What are they? *Journal of Nutrition* 129: 1402S–1406S.

O'Riordan, K., Andrews, D., Buckle, K., and Conway, P. (2001). Evaluation of microencapsulation of a *Bifidobacterium* strain with starch as an approach to prolonging viability during storage. *Journal of Applied Microbiology* 91:1059–1066.

Okamoto, M. Y., and Ibanez, P. S. (1986). Final report on the safety assessment of shellac. *Journal of the American College of Toxicology* 5: 309–327.

Özkan, G., and Bilek, S. F. (2014). Microencapsulation of natural food colourants. *Internationa. Journal of Nutrition and Food Sciences* 3(3): 145–156.

Pan, K., Zhong, Q., and Baek, S. J. (2013). Enhanced dispersibility and bioactivity of curcumin by encapsulation in casein nanocapsules. *Journal of Agricultural and Food Chemistry* 61(25): 6036–6043.

Patel, A. R., Heussen, P. C. M., Hazekamp, J., Drost, E., and Velikov, K. P. (2012). Quercetin loaded biopolymeric colloidal particles prepared by simultaneous precipitation of quercetin with hydrophobic protein in aqueous medium. *Food Chemistry* 133: 423–429.

Patel, A., Heussen, P., Hazekamp, J., and Velikov, K. P. (2011). Stabilisation and controlled release of silibinin from pH responsive shellac colloidal particles. *Soft Matter* 7: 8549–8555.

Patel, A., Hu, Y., Tiwari, J. K., and Velikov, K. P. (2010). Synthesis and characterisation of zein–curcumin colloidal particles. *Soft Matter* 6: 6192–6199.

Patel, A. R., Remijn, C., Mulet Cabero, A.-I., Heussen, P. C. M., Jack, W. M., *et al.* (2013a). Novel all-natural microcapsules from gelatin and shellac for biorelated applications. *Advanced Functional Materials* 23: 4710–4718.

Patel, A. R., Schatteman, D., De Vos, W. H., Lesaffer, A., and Dewettinck, K. (2013b). Preparation and rheological characterization of shellac oleogels and oleogel-based emulsions. *Journal of Colloid and Interface Science* 411: 114–121.

Patel, A. R., and Velikov, K. P. (2014). Zein as a source of functional colloidal nano- and microstructures. *Current Opinion in Colloid & Interface Science* 19: 450–458.

Patent number 5,164,210. (1991). Zein/shellac encapsulation of high intensity sweeteners in chewing gum.

Picciano, M. F. (2010). Vitamin D status and health. *Critical Reviews in Food Science and Nutrition* 50: 24–25.

Picot, A., and Lacroix, C. (2004). Encapsulation of bifidobacteria in whey protein–based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *International Dairy Journal* 14(6): 505–515.

Pitalua, E., Jimenez, M., Vernon-Carter, E., and Beristain, C. (2010). Antioxidative activity of microcapsules with beetroot juice using gum arabic as wall material. *Food and Bioprocess Processing* 88(2–3): 253–258.

Pittas, A. G., Chung, M., Trikalinos, T., Mitri, J., Brendel, M., *et al.* (2010). Systematic review: Vitamin D and cardiometabolic outcomes. *Annals of Internal Medicine* 152: 307–314.

Polavarapu, S., Oliver, Ch. M., Ajlouni, S., and Augustin, M. A. (2011). Physicochemical characterisation and oxidative stability of fish oil and fish oil–extra virgin olive oil microencapsulated by sugar beet pectin. *Food Chemistry* 127: 1694–1705.

Porrarud, S., and Pranee, A. (2010). Microencapsulation of Zn–chlorophyll pigment from Pandan leaf by spray drying and its characteristic. *International Food Research Journal* 17: 1031–1042.

Quispe-Condori, S., Saldana, M. D. A., and Temelli F. (2011). Microencapsulation of flax oil with zein using spray and freeze drying, *LWT – Food Science and Technology* 44: 1880–1887.

Roberfroid, M. B. (2005). Introducing inulin-type fructans. *British Journal of Nutrition* 93(Suppl. 1): S13–S25.

Rocha, G., Favaro-Trindade, C., and Ferreira Grosso, C. (2012). Microencapsulation of lycopene by spray drying: Characterization, stability and application of microcapsules. *Food and Bioproducts Processing* 90: 37–42.

Ronkart, S. N., Deroanne, C., Paquot, M., Fougnies, C., Lambrechts, J.-C., and Blecker, C. S. (2007). Characterization of the physical state of spray-dried inulin. *Food Biophysics* 2: 83–92.

Rubio-Rodríguez, N., Beltrán, S., Jaime, I., de Diego, S. M., Sanz, M. T., and Rovira-Carballido, J. (2010) Production of ω-3 polyunsaturated fatty acid concentrates: A review. *Innovative Food Science and Emerging Technologies* 11: 1–12.

Saag, L., Sanderson, G., Moyna, P., and Ramos, G. (1975). Cactaceae mucilage composition. *Journal of the Science of Food and Agriculture*, 26: 993–1000.

Sabliov, C. M., Fronczek, C., Astete, C. E., Khachaturyan, M., Khachatryan, L., and Leonardi, C. (2009). Effect of temperature and UV light degradation of α-tocopherol in free and dissolved form. *Journal of the American Oil Chemists' Society* 86(9): 895–902.

Saenz, C., Sepulveda, E., and Matsuhiro, B. (2004). *Opuntia* spp. mucilages: A functional component with industrial perspectives. *Journal of Arid Environments* 57: 275–290.

Saenz, C., Tapia, S., Chávez, J., and Robert, P. (2009). Microencapsulation by spray drying of bioactive compounds from cactus pear *(Opuntia ficus-indica)*. *Food Chemistry* 114: 616–622.

Sepulveda, E., Saenz, C., Aliaga, E., and Aceituno, C. (2007). Extraction and characterization of mucilage in *Opuntia* spp. *Journal of Arid Environments* 68: 534–545.

Shu, B., Yu, W., Zhao, Y., and Liu, X. (2006). Study on microencapsulation of lycopene by spray-drying. *Journal of Food Engineering* 76: 664–669.

Shukla, R., and Cheryan, M. (2001). Zein: The industrial protein from corn. *Industrial Crops and Products* 13: 171–192.

Silva, D. F., Favaro-Trindade, C. S., Rocha, G. A., and Thomazini, M. (2012). Microencapsulation of lycopene by gelatin–pectin complex coacervation. *Journal of Food Processing and Preservation* 36: 185–190.

Stevens. C. V., Meriggi, A., and Booten, K. (2001). Chemical modification of inulin, a valuable renewable resource, and its industrial applications. *Biomacromolecules* 2: 1–16.

Tan, L. H., Chan, L. W., and Heng, P. W. S. (2005). Effect of oil loading on microspheres produced by spray drying. *Journal of Microencapsulation: Micro and Nano Carriers* 22(3): 253–259.

Torres-Giner, S., Martinez-Abad, A., Ocio, M. J., and Lagaron, J. M. (2010). Stabilization of a nutraceutical omega-3 fatty acid by encapsulation in ultrathin electrosprayed zein prolamine. *Food Science* 75: 69–79.

Tucker, J. M., and Townsend, D. M. (2005). Alpha-tocopherol: Roles in prevention and therapy of human disease. *Biomedicine and Pharmacotherapy* 59: 380–387.

Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H., and Smits, G. (1995). On the presence of inulin and oligofrutose as a natural ingredients in the Western diet. *Critical Reviews in Food Science and Nutrition* 35: 525–552.

Wang, Q., Yin, L. L., and Padua, G. W. (2008). Effect of hydrophilic and lipophilic compounds on zein microstructures. *Food Biophysics* 3(2): 174–181.

Wolff, D., Czapla, S., Heyer, A. G., Radosta, S., Mischnick, P., and Springer, J. (2000). Globular shape of high molar mass inulin revealed by static light scattering and viscometry. *Polymer* 41: 8009–8016.

Yáñez-Fernández, J., Ramos-Ramirez, E. G., and Salazar-Montoya, J. A. (2008). Rheological characterization of dispersions and emulsions used in the preparation of microcapsules obtained by interfacial polymerization containing *Lactobacillus* sp. *European Food Research and Technology* 226(5): 957–966.

Zhang, Y., Yang, Y., Tang, K., Hu, X., and Zou, G. (2008). Physicochemical characterization and antioxidant activity of quercetin-loaded chitosan nanoparticles. *Journal of Applied Polymer Science* 107: 891–897.

Zheng, Y., Haworth, I. S., Zuo, Z., Chow, M. S., and Chow, A. H. (2005). Physicochemical and structural characterization of quercetin–beta-cyclodextrin complexes. *Journal of Pharmaceutical Sciences* 94: 1079–1089.

Zhong, Q., and Jin, M. (2009). Zein nanoparticles produced by liquid–liquid dispersion. *Food Hydrocolloids* 23: 2380–2387.

Zhong, Q., Tian, H., and Zivanovic, S. (2009). Encapsulation of fish oil in solid zein particles by liquid–liquid dispersion. *Journal of Food Processing and Preservation* 33: 255–270.

Zhu, F., Kale, A., and Cheryan, M. (2007). Fractionation of zein by size exclusion chromatography. *Journal of Agricultural and Food Chemistry* 55: 3843–3849.