Chapter X

Polysaccharide Nanostructures

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X.1 Introduction

Polysaccharides are carbohydrate polymers where sugar units are linked together through glycosidic linkages. In living organisms polysaccharides are the structural polymers that provide support (*e.g.*, cellulose in plants or chitin in arthropods) or the sources of energy for plant development (*e.g.*, starch). Polysaccharides are routinely used in the food industry, most frequently as thickeners, stabilizers of dispersions (emulsions, foams) or structuring agents of water and air. Thickening solutions and stabilizing dispersions against creaming are two of the most common industrial applications of polysaccharides. These functional properties are used to create formulations with reproducible flow properties not only during processing but also during the specified shelf life of the product. The viscosity of a polysaccharide solution exhibits a remarkable increase above the critical polymer concentration (c^*). Polysaccharides normally show Newtonian or pseudoplastic flow behavior at concentrations below or above c^*, respectively. As is evident, concentration along with other factors is critical and can be used to control the functionality of polysaccharides. Common polysaccharides that are used to enhance viscosity include xanthan, galactomannans, starches or cellulose derivatives. Apart from thickening solutions and conferring desirable textural properties, polysaccharides can be also used in more technologically demanding applications that require structuring of water, air or emulsifying a hydrophobic compound. They can be used for partial or total replacement of fat in reduced fat formulations by structuring water in the form of a gel. The textural and functional characteristics of the gelled structure should be comparable to those of fat. This is a particularly difficult task considering the extensive dissimilarities in the chemical structure and physical

properties of fats and hydrocolloids. Of particular importance in fat replacement is the melting behavior of the gel that should resemble that of fat *i.e.*, a melting point that is close to the body temperature (~37 ^{o}C) with a sharp melting transition so as to impart mouth-melting characteristics in the structure. Another important feature would be the structural stability of the gel in order to provide the desirable shelf life to the product. Quality losses are usually manifested by the presence of a thin layer of water that is expelled out of the structure. This is known as syneresis and is due to rearrangements of the microstructure with time. Syneresis not only results in losses of visual qualities but in most cases in accompanied by losses in texture of the product. Some polysaccharides that are used as fat replacers are polydextrose, microcrystalline cellulose, maltodextrins, and modified starches. As discussed later, mixed polysaccharide systems or mixtures of a polysaccharide with a protein solution could provide in some cases superior structuring of water. Air structuring using polysaccharides is another functionality that is exploited in the baking industry and specifically in gluten-free formulations. Hydrophobically functionalized cellulose derivatives (*e.g.*, methylcellulose (MC) or hydroxypropylmethylcellulose (HPMC)) are used in applications where thermoreversibility of the gel is required. These polysaccharides self assemble on heating by means of weak reversible hydrophobic interactions, which lead to gel formation. In gluten-free formulations, the leavening agent (*e.g.*, bicarbonate) creates CO_2 bubbles in the dough, which makes it rise. The polysaccharide network that forms on heating during baking (see Figure X.3 for mechanism) not only entraps CO_2 but also provides structural rigidity to the newly formed microstructure. On cooling, the gel reverses to the sol state and the polysaccharide now acts as water management agent. Formulations of deep fried products (*e.g.*, chicken nuggets, fish fingers etc) may also require similar functionality

to prevent oil migration and structure disintegration during frying at high temperatures. [1] Finally, stabilization of flavor oils (*e.g.*, limonene) is also possible with the use of appropriate polysaccharides. In this case, the polysaccharide should be able to create fine emulsions without enhancing viscosity of the solution. This can be achieved by polysaccharides that have been properly functionalized so as to arrange at the oil-water interface. A typical polysaccharide with this functionality is gum arabic that is able to create fine emulsions with minimum increase in viscosity even at concentrations as high as 20%. [2]

Understanding structure formation mechanisms demands departure from the traditional approach of analytical and chemical descriptions of polysaccharides and utilization of concepts from materials science. Such an approach is imperative as research in the last two decades shows that many aspects of food ingredient functionality can be controlled by the interaction of distinct structural elements at various length scales rather than simply by their chemical characteristics. [3] The mesoscopic scale plays central role in engineering food structure that for all practical purposes ranges from a 1 nm to 1 μm, although the exact boundaries are not well defined. [4] At this scale the properties of the material cannot be described adequately by continuum mechanics because interactions among discrete particles come into play. [4, 5] The interplay between attractive *vs.* repulsive forces and molecular mobility dictates the stability of the material. Usually, the system is considered stable when the energy barrier between the particles is larger than the thermal fluctuations. [6] Such stability may refer, for instance, to stability against flocculation in emulsions, phase separation in mixed biopolymer systems, gelation in single biopolymer solutions or stabilization of biopolymer matrices below their glass transition temperature. As foods are metastable materials (out of equilibrium) they are susceptible to structural

re-organization through various relaxation mechanisms. [7] Consequently, stability refers to "kinetic stability" emphasizing that the system is arrested at a temporarily stable molecular arrangement that usually matches the technological requirements of the material. Typical examples of such behavior are the α-relaxation in biopolymer glasses in the vicinity of glass transition temperature or the enhancement of inter-chain interactions in gels. The loss of stability in the former example is manifested with the loss of the structural integrity of the material as it enters the rubbery state. In the latter case, syneresis occurs with expulsion of water from the structure accompanied by significant changes in the mechanical properties of the gel.

The physicochemical responses that influence the functionality and industrial performance of polysaccharides are controlled by the fine structure of the chains at molecular level. The objective of this chapter is to outline how structure is created and controlled in a wide range of polysaccharide-based systems that are utilized in food applications.

X.2. Polysaccharide sources and composition

Polysaccharides can be obtained from plants with minimal processing (*e.g.*, rice or potato starch) or as a result of processing of agricultural wastes (*e.g.*, pectin). Other sources include extraction from algae (*e.g.*, alginates, carrageenan), processing of by-products of the shellfish industry (*e.g.*, chitin), or from microbial fermentation (*e.g.*, xanthan, gellan). It should be noted that extraction from natural sources or culture media results inevitably in the presence of proteins that depending on their content may affect to a various degree the properties of the polysaccharide extract. Irrespectively of the protein content in the extract the fine structure of the isolate heavily depends on the isolation protocol that was followed. For example, choice of

pH, salt concentration, temperature, choice of solvent for precipitation or drying technique (*e.g.*, freeze drying *vs.* spray drying) can modify the molecular characteristics of polysaccharides. Modifications may include changes in molecular weight and its distribution, presence and extent of branching and extend of functionalization (*e.g.*, methyl, acetyl etc). In many cases the isolated polysaccharide has totally different chemical and physical properties than at its source. A typical example is pectin where although extraction procedures are optimized to tailor the isolates having various highly specific functional properties, the structure within the plant cell wall is still largely unknown. [8]

Although in nature there are numerous monosaccharides, the number of those comprising the polysaccharides is relatively small (Table X.1). Common sugar units include glucose and mannose that form the backbone of some of the most important commercial polysaccharides. Other sugars or sugar acids such as galactose, xylose, arabinose or galacturonic, guluronic and mannuronic acids are commonly found in industrially relevant polysaccharides (Table X.1). However, the type of linkages, isomeric forms, functionalization of sugars as well as branching and periodicity of the monomers in the backbone result in great structural diversity. Slight structural modifications usually change the functionality of the polysaccharide. These modifications are, for example, methylation or acetylation at various positions, presence of sulfate or other functional groups or differences in the anomeric type of monosaccharides that make up the polysaccharide (Table X.1). A notable example is that of amylose and cellulose that both consist of glucose. Glycosidic linkages between glucose units in amylose are α-D-(1→4) whereas in cellulose are β-D-(1→4) resulting in totally different functional properties not only within the plant (structural *vs.* source of energy) but also when they are used as food ingredients. This difference

at the molecular level has also implications to the higher level of structure. For example cellulose chains are able to assemble and form fibrous semi-crystalline structures with unique mechanical properties whereas amylose is a flexible chain that has the ability to form crystals under certain conditions (*e.g.*, in bread staling). By further varying the linkage type and anomeric form between glucose units a range of different polysaccharides can be obtained with various functional properties (Table X.1).

Table X.1 around here

X.3 Polysaccharide conformations

Polysaccharide structuring starts at the molecular level where they are generally encountered with either ordered or disordered conformations.[9] A polysaccharide forms when several monosaccharide units, usually more than 20, are connected together *via* glycosidic linkages. Polysaccharides are commonly divided into homopolysaccharides or heteropolysaccharides based on the number of different sugars in the structure (Figure X.1a). Homopolysaccharides contain a single sugar unit on the backbone (*e.g.*, amylose) whereas heteropolysaccharides more than one (*e.g.*, pectin). The sequence of sugar residues in the chain forms the *primary structure* of the polysaccharide. For example, in homopolysaccharides that contain only one sugar residue the primary structure would consist of a sequence of the same sugar unit (Figure X.1a). In heteropolysaccharides the repeating motif may be a disaccharide or longer segment (*e.g.*, in carrageenan or gellan) resulting in more complex primary structures (Figure X.1a and Table X.2). Further classifications are possible, for example, according to the source, type of sequence, charge etc. Table X.2 shows

examples of repeating patterns of common polysaccharides that are used in industrial applications.

Table X.2 around here

The sugar units have the ability to rotate around the glycosidic linkage with two torsion angles (φ, ψ) (Figure X.1b). Although the pyranose ring also shows flexibility, its effect on the conformation of polysaccharides is negligible when compared to the effect of the rotations around the glycosidic bonds. [10] Therefore, the conformations that affect the interactions of polysaccharides at the molecular level can be understood by studying the conformations of disaccharides. Figure X.1b shows the different possible torsion angles in a polysaccharide. Angle φ is located between the anomeric carbon and the oxygen of glycosidic linkage of the first monomer and ψ between the oxygen of glycosidic linkage and the non-anomeric carbon of the second monomer. Introduction of branching at C6 gives one more possible angle of rotation (ω) about the C-5 and C-6 bond (Figure X.1b). The conformation of a polysaccharide chain can be specified by the relationship between the φ, ψ torsion angles. Because of the ability of sugar monomers to rotate about the linkages, polysaccharides may adopt *secondary structures*. When $\varphi_1 = \varphi_2$ and $\psi_1 = \psi_2$ (and all the subsequent φ, ψ sets in the chain) the chains adopts a helical conformation in the solid state. However, in solutions $\varphi_1 \neq \varphi_2$ and $\psi_1 \neq \psi_2$ and the chains generally tend to adopt random coil conformations. [10] The most stable conformation is usually the one that results in the lowest energy, as some are not allowed due to steric hindrances. These steric hindrances are short range between neighboring residues or long range by sugar units that are remote in chain but near in space (Figure X.1c). The long-range interactions result in excluded volume effects that depend on the quality of the solvent (ionic strength, pH). As a result of these interactions polysaccharides may adopt one or more of the three

idealized conformations (*secondary structures*): random coil (*e.g.*, pullulan), ribbons (*e.g.*, cellulose) or helices (*e.g.*, κ-carrageenan).[9, 11, 12] Interactions of polysaccharide chains at the molecular level depend most commonly on the quality of the solvent. In good solvent, interactions between solvent and chain-segments are favorable resulting in extended conformations and high solubility. In poor solvents interactions of chain segments with themselves are favored resulting in aggregation. At a specific temperature called θ-temperature, the long-range interactions no longer influence the conformations of the chains and the short-range interactions become predominant (Figure X.1c). The interplay between the interactions of polysaccharide and solvent molecules determines if the biopolymer will be able to form stable structures at greater length scale, most commonly gels. In the case where polysaccharides are charged the situation becomes more complex as charges also affect chain conformation. To control these interactions it is possible to manipulate a range of factors such as concentration, temperature, polydispersity, ionic strength and pH, or addition of crosslinkers such as calcium cations as in the case of low methoxylated pectins or alginates. It is very important to understand how the various factors depend on each other as deviations from optimum conditions usually influence the ability of the polysaccharide chains to associate into a three-dimensional network.

Figure X.1 around here

It is evident from the above discussion that polysaccharide structures do not fit into a simple description due to the multitude of factors that need to be controlled simultaneously. Various experimental techniques are available to study conformations at various length scales such as X-ray diffraction, light scattering, small angle X-ray scattering, NMR or atomic force microscopy (AFM). AFM is one of the few techniques that allows for visual observation of a single polysaccharide chain. AFM

generates images by sensing the surface of the molecule with the aid of a sharp probe. Because this technique minimizes sample preparation it is possible to image polysaccharides in a "near native" state of the macromolecule. [13] Images that were obtained using AFM under various experimental conditions (Figure X.2) illustrate the great diversity in chain conformations of polysaccharides. Xanthan [14], κ-carrageenan [15] or pectins[16] (Figure X.2a, b and c) form elongated structures whereas gellan (Figure X.2d) forms short rods [17] each one of them corresponding to polysaccharide-specific conformations. On the other hand, intrachain aggregation in *β*-glucan [18] dispersions is evident by the presence of large aggregates with linear chains protruding away of the structure (Figure X.2e). This is a typical behavior when intrachain interactions are strong. Finally, arabic gum [19] shows globular structures as a result of the presence of protein moieties on the polysaccharide backbone (Figure X.2f). It should be stressed that these images represent the conformations of polysaccharides under the specific conditions that were used to capture them and they tend to change depending on the composition of aqueous medium. However, they demonstrate the complexity that is involved in polysaccharide structuring at nanometer length scales.

Figure X.2 around here

X.4 Structuring using polysaccharides - High moisture regime

A bottom-up approach to structuring requires the biopolymer chains to assemble and form well-defined "building blocks" at nanoscale level that may interact and further develop to a macroscopic structure at higher length scales. The macroscopic structure is usually "soft" due to the characteristic mechanical properties of the resulting material (*e.g.,* low yield point, viscoelasticity). Such structuring occurs *via* weak, reversible, non-covalent interactions *i.e.,* hydrogen bonding, hydrophobic, ionic,

and van der Waals interactions, steric and excluded volume effects. The aggregated system represents a minimum energy structure or equilibrium phase and exhibits short range, localized ordering in contrast to the long-range atomic order of crystals. [20] The previously mentioned forces that are responsible for the ordering between molecules are both attractive and repulsive and the balance between them determines the stability of the structure. Repulsive interactions in polysaccharides in aqueous solvent are mostly due to steric and excluded volume effects. [21] Excluded volume is the volume that one part of a long chain cannot occupy when it is already occupied by another part of the same chain. Furthermore, when atoms in a chain are too close to each other their electron clouds overlap a situation resulting in steric repulsion. Both events influence the polysaccharide conformation and its ability to form macrostuctures. Attractive forces are the result of van der Waals interactions and hydrogen bonding that stem from dipole-dipole interactions. These forces are important in gel formation of polysaccharides particularly if we consider the multitude of hydroxyl groups in polysaccharide chains that are available to interact with water or with each other. Ionic forces predominate when polysaccharides are charged. This occurs very frequently when monomers have reactive groups available such as carboxyl or sulfate (*e.g.*, carrageenan, pectin or alginate). Bridging of adjacent chains and subsequent gel formation is frequently mediated by the presence of cations (*e.g.*, Ca^{++}, K^{+}). Finally, the hydrophobic effect is important when polysaccharides are functionalized with hydrophobic groups such as methyl, acetyl, propyl etc. (*e.g.*, cellulose derivatives or pectin). This confers to polysaccharides new properties such as gel formation on heating or ability to arrange at interfaces and act as emulsifiers. At this juncture, we should stress an important difference between polysaccharides and other biological molecules that have the propensity to self-assemble at nanoscale.

Self-assembling of polysaccharides is not as easy as in small amphiphilic molecules (*e.g.*, mono- or di- glycerides, surfactants) or proteins, because dispersions of polysaccharides in aqueous solutions exhibit very low interfacial tension conferring water solubility to the molecule. To contrast them with casein micelles, the most characteristic self-assembled food nanostructure, the specific balance of the hydrophobic to hydrophilic amino acids not only allows formation of the nanostructure but also helps retaining the individual character of micelles. In polysaccharides, self-assembling requires modifying the chemistry of the monomers by introducing appropriate functional groups. For biomedical applications and drug-delivery, self-assembled polysaccharide nanostructures are currently being used in a wide range of applications. These are mostly based on chitosan or dextran derivatives and various glycosaminoglycans. [22, 23] In such applications, the polysaccharide nanoparticle is usually required to deliver a specific functionality to cells or tissues but is not required to build macroscopic superstructures. In these cases the individuality of the nanoparticles should be retained and aggregation phenomena must be avoided. On the contrary, food structuring with polysaccharides requires creation of structures up to the macroscopic length scale with specific mechanical properties and technological functionality. Therefore, the individual character of the nanoparticle is rarely required in food structuring applications and association at atomic or mesoscale require further aggregation to create a three dimensional macrostructure, namely, a gel. Gelation involves attractive interactions among polysaccharide chains, which convert the solution into a three-dimensional metastable viscoelastic "soft" solid occupying the same volume as the solution. As discussed above, polysaccharide chains in water will interact with each other (inter-chain interactions), with themselves (intra-chain interactions) and with water molecules (chain-solvent

interactions). Interchain interactions usually lead to gel formation whereas intrachain interactions result in aggregation of the polysaccharide and precipitation. In gels formed by neutral polysaccharides, the length scale is controlled to some extend by the mesh size of the network. Similarly to semi-dilute polymer solutions the mesh size can be adjusted by the polysaccharide concentration affecting directly their mechanical properties. In gels formed by charged polysaccharides, mesh size can be also adjusted by carefully tuning pH and ionic strength or by addition of crosslinking ions. [3, 24] pH influences in most cases the degree of dissociation of the carboxyl group of uronic acid residues whereas in chitin and chitosan pH influences the dissociation of the amino group. When the pH is above the pK of the charged group, repulsive interactions maintain the chains in extended conformations. Ionic strength can also be used to tailor the interactions and conformations in polyelectrolytes. Charged polysaccharides at low salt concentrations (low ionic strength) tend to adopt extended conformations as electrostatic repulsion keeps charged groups apart. Electrostatic screening provided by counterions at higher concentrations (usually 0.1 M NaCl) contracts chains to more compact conformations affecting solubility and the ability to gel. [25, 26]

Gels are classified depending on the nature of their interactions into covalently crosslinked, entanglement or physical networks. [27] In food systems, the most predominant gels are those that are formed *via* physical interactions. Interactions at the molecular level involves the creation of structures with short-range order such as helices, “egg boxes”, ion assisted bridging or junction zones. Depending on the strength of these interactions gelation may be reversible or irreversible. Figure X.3 illustrates three different mechanisms of gel formation using representative examples for κ-carrageenan, methylcellulose and mixed linkage (1→3)(1→4)-*β*-D-glucan. κ-

Carrageenan gelation mechanism initiates with helix formation and ion-assisted crosslinking of the helices. [28] In κ-carrageenan solution, above ~60 ^{o}C, chains are in random coil conformation. Cooling below ~60 ^{o}C induces a coil-to-helix transition and κ-carrageenan coils are able to form double helices. Aggregation proceeds with formation of hydrogen bonding between helices, which in turn enable formation of a weak three-dimensional network. The introduction of potassium cations in the solution allows crosslinking of helices owing to the presence of sulfate groups. Mechanical properties of the final gel depend not only on the molecular properties of κ-carrageenan that is used to create the gels (*e.g.*, sulfate content, molecular weight, polydisperisty etc.) but also on the concentration of K^{+}, ionic strength and pH of solution. As is evident, in this case there are several parameters available that can be used to fine-tune the structure and the properties of the gel. Other polysaccharides that gel by means of coil-helix transition include gellan, agar and curdlan. Hydrophobic interactions among polysaccharides can be also exploited to create gels for food applications as in the case of hydrophobically modified celluloses (*e.g.*, methylcellulose (MC), hydroxypropylmethylcellulose (HPMC)). Polymer chains of MC solutions are in disordered conformation at room temperature. On heating, MC chains are capable of interacting with each other to form thermally reversible gels. [29] The mechanism of gel formation is based on the extent of hydrophobic interaction among MC chains that associate to form a fibrilar gel. [30] As temperature increases, hydrophobic interactions strengthen and chains are able to assemble and form the gel. This gel is thermally reversible and the sol form is recovered as temperature drops below the critical temperature for association. In consequence, the degrees of freedom to control characteristics of the network are the molecular weight and its distribution and the degree of substitution with hydrophobic groups. A third mechanism of

gelation that is commonly encountered in proteins is displayed by mixed linkage (1→3)(1→4)-*β*-D-glucan. This polysaccharide exhibits random coil conformation in hot aqueous solutions. In this case, gel formation progresses by interactions of at least three consecutive cellotriosyl residues that result in conformational ordering with inter- and intra- chain associations, at chain segmental level. [31] Such interactions lead to formation of a fractal network of particular aggregates that has been described using scaling concepts. [32] Particulate aggregates interact mainly with hydrogen bonding creating fractal clusters resulting in the gelled structure. The particulate nature of *β*-glucan gels has been recently reinforced by AFM imaging [18] (Figure X.2f) and particle tracking microrheology [33] revealing microheterogeneities during microstructural evolution of the network. Controlling gelation for this type of gel usually requires tailoring the molecular properties of *β*-glucan chains to specific molecular weight and cellotriosyl-to-cellotetraosyl ratio. Particulate gels are most commonly encountered in proteins where denaturation under specific conditions allows aggregation of the particles producing colloidal-type, usually irreversible, networks. It is evident that in all cases manipulations are directed towards influencing the interactions at the molecular level and affect the conformational properties of the chains.

Figure X.3 around here

Microstructure engineering in polysaccharide systems can be also achieved by varying the processing conditions during gel formation. Application of shear is a pathway to create new microstructures and should be applied during the conformational ordering process resulting in *fluid gels*. [34] In that case, the polysaccharide solution is sheared while it undergoes conformational transition resulting in the production of gel particles *via* a nucleation and growth mechanism.

The gel particles grow to a specific droplet size and stability is obtained if the particles are kept below the gel melting temperature so that re-aggregation is prevented. [35] In order to control the formed microstructures a range of tools is available that can be used such as cooling rate, strength of shear field or concentration and type of polysaccharide. These factors control the droplet size distribution, their shape and the interactions among the droplets that in turn affect the stability and mechanical properties of the fluid gels. [34] Microstructures can be also fabricated starting from mixtures of phase-separated biopolymers when at least one component is able to gel. [36, 37] In that case shear field with simultaneous cooling can be used to fabricate the droplet. Shear forces deform the droplet and cooling induces gelation that kinetically arrests the formed droplets. An example of the effect of the shear field on the morphology of fluid gels can be seen with gellan–κ-carrageenan mixtures (Figure X.4). From a-f the strength of shear field increases with concomitant changes in the particle morphology. For instance, at low shear droplet coalescence takes place before gelation and the particles are bigger (Figure X.4b) than the fluid gel created at quiescent conditions (Figure X.4a). At higher shear rates the particles become elongated (Figure X.4c-e) and beyond a specific value the particles obtain non-specific morphology (f). [36] Fluid gels can be used to improve rheological properties of various products in food and personal care industries and control the release of nutrients in the gut to improve satiety.

Figure X.4 around here

Mixing two different biopolymer species can also achieve microstructure manipulation and tuning of gel properties. In most cases mixtures include two different polysaccharides or a polysaccharide and a protein. Mixing two biopolymers brings about new physicochemical responses to the systems. The mixtures are broadly

classified into two groups depending on the nature of interactions between the biopolymer species. Interactions are either *segregative* or *associative* and lead to phase separation or creation of complexes, respectively. Phase separation creates phases that are enriched in one of the two biopolymers whereas complexation creates complexes that are either soluble or insoluble. The demixing of the biopolymer species depends on the interplay of the interactions between the biopolymer species consisting the mixture, as described previously. Phase separation primarily depends on the concentration of the biopolymers in the mixture and on the structural characteristics of the chains (*e.g.*, molecular weight, charge etc). Below a concentration threshold the two biopolymers co-exist whereas beyond the threshold value they phase-separate. The phase behavior is better understood with the use of isothermal phase diagrams of biopolymer mixtures. [38] Figure X.5a illustrates the phase diagram of mixtures of sodium caseinate with β-glucan varying in molecular weight. Solid line represents the binodal, which sets the boundaries of the compatible (below the curve) and the incompatible (above the curve) regions. Compatibility generally increases as molecular weight of the polysaccharide and nominal concentration of biopolymers in the mixtures decrease. This is a general behavior that is observed in protein polysaccharide mixtures and influences the stability and rheology of the systems. [37-39] These system properties can be adjusted by modifying the concentration and molecular characteristics of consisting biopolymers, solvent quality or temperature. The phase behavior also plays a dramatic role on microstructure of phase-separated mixtures (Figure X.5b). It is evident that as β-glucan molecular weight decreases a remarkable change in the morphology of the mixtures occurs. The coarse β-glucan-enriched microphases, in the high molecular weight samples, are gradually transformed into fine droplets as size of chains

decreases, a situation that influences rheology and textural properties of the mixtures. [40] Furthermore, a remarkable change in the continuity of the mixtures occurs as polysaccharide concentration increases (from left to right). [40] Mixtures where sodium caseinate is the continuous phase progressively change to β-glucan continuous systems passing from its bi-continuous counterparts. When such a mixture is gelled under the appropriate conditions as a result of microstructure manipulation the thermal and mechanical properties vary greatly. [41] At this stage the gels will have distinct mechanical properties depending on the continuity of the system. We can distinguish three classes where the gel is a) biopolymer-A continuous, b) biopolymer-B continuous or c) bi-continous. The three gels will have completely different rheological, thermal and microstructural properties. [42] Gels that involve synergistic interactions between polysaccharides can be also created in a similar manner. Interaction creates gels with properties distinct from those that were created in the absence of the second polysaccharide. For example, mixtures of galactomannans with carrageenans create firmer gels compared to those without galactomannans. Furthermore, interactions between xanthan and galactomannans lead to gelation although neither of the single solutions is able to gel alone. [26] Mixed polysaccharide systems have been explored extensively in the literature for various applications such as reduction of fat and calories, control of texture and mouthfeel of various food formulations or simply reduction of cost of existing formulations. [42]

Figure X.5 around here

X.5 Structuring using polysaccharides - Low moisture regime

The previous discussion focused on the behavior of polysaccharides in solution under conditions that promote gelation. We saw that microstructural elements of

polysaccharides may form disordered or short-range ordered structures. The typical level of solids in such a gelling system depends on the chemical properties of the polysaccharide but in most cases is in the range of 0.5-2%. However, in low moisture systems that contain biopolymers, water fails to hydrate them adequately. This restricts molecular mobility and conformational rearrangements and the structure of the material is distinct from its high-moisture counterparts. We can normally distinguish two solid states in polysaccharide systems, that is the *crystalline* and the *amorphous*. In most cases, branching and chemical heterogeneity restricts crystallization. However, some polysaccharides either in their native state or under appropriate sample preparation conditions may give distinct X-ray diffraction patterns revealing formation of structures with long-range order. On the other side of the spectrum, amorphous solid state lacks long-range order and polysaccharide chains are in a completely disordered state. In that case, glass transitions dominate the physicochemical and mechanical responses of the systems. At this point we should mention that this solid state is not encountered in lipid systems.

Studying long-range order of polysaccharides is a difficult task, as they cannot provide large crystals for X-ray diffraction studies. Furthermore, powder diffraction X-ray patterns are difficult to interpret due to the molecular complexity and polycrystalline nature of the structures. Polycrystalline materials are those that are composed of aggregated small crystals of different size and orientation. In polysaccharides and some synthetic polymer systems these materials also include amorphous regions in their structure. Typical polysaccharides that acquire a polycrystalline character during their biosynthesis are starch [43], cellulose [44, 45] and chitin. [46] In cellulose and chitin for instance, acid hydrolysis of the amorphous regions results in fabrication of a new materials that consist of aggregates of cellulose or

chitin crystals at various length scales. These materials find applications in food and pharmaceutical industries as fat substitutes, texture modifiers, tablet binders or additives that reinforce polymer composites. Starch granules present another example of the ability of sugar polymers to form complex crystalline structures controlled by the molecular composition of the material. Maize starch powder X-ray diffraction patterns, for example, show the crystalline and non-crystalline regions of the structure (Figure X.6a). [47] Furthermore, increasing in amylose concentration in the granule decreases the crystallinity of starch, which is attributed mostly to the formation of double helices of amylopectin. [48] To overcome some of the difficulties that are posed by the absence of well-defined single polysaccharide crystals, fiber X-ray diffraction may be used to study the molecular orientation of polysaccharides. In that case, a fiber is prepared that consists of oriented microcrystalline and amorphous regions [10, 49] the extent of which depends on the particular architecture of the polysaccharide (Figure X.6b). [50]

When crystalline solids melt form liquids and with subsequent temperature reduction, the liquid may crystallize again. Crystallization can be frequently delayed or inhibited, depending on the cooling rate of the liquid solution. When such a liquid solution is cooled below its melting point, it enters a *supercooled* state. With further reduction of temperature in the absence of crystallization, the viscosity of the liquid increases significantly and eventually undergoes a *glass transition*. The formed amorphous solid-state structure is called "glass". Biopolymer solutions on cooling rarely crystallize (*e.g.*, amylose recrystallization) but glass formation often plays an important role in the physical stability and textural properties of the food matrix. Glasses in food systems may be obtained by either removal of water (*e.g.*, dehydration or extrusion processes) or by cooling of high-solids biopolymer solutions

below a specific temperature. What happens microscopically at the glass transition is that on the time scale of observation the translational and rotational motions of the atoms or the molecules that give rise to the viscous flow have ceased. Below glass transition temperature (T_g), during the measurement period, the atoms are vibrating only about their equilibrium positions. The resulting glassy system is expected to be stable below T_g whereas above T_g, the difference between T_g and the storage temperature T ($T-T_g$) controls the rate of physical and chemical changes. [51] It was stated earlier that below the glass transition molecular motions, albeit restricted, persist. This mobility is mainly local and restricted to atom or bond vibrations, or reorientation of small groups. [51-53] Sub-T_g relaxations are named according to their position relative to the main α-relaxation (glass transition), which is due to cooperative motions of the molecules or polymer chains. At lower temperatures, β- and γ- relaxations take place and are linked to rotation of lateral groups (such as -OH or -NH) or to changes in conformation of the main chain in the case of biopolymers.

Melting and glass transition events can be followed by differential scanning calorimetry that distinguishes between first (melting) and second (α-relaxations) order transitions. Typically, melting of crystals appears as a well-defined endothermic peak whereas glass transition manifests by shifting the heat capacity baseline. Identifying and distinguishing between the two transitions pinpoints processing and storage requirements of polysaccharide based structures. Thermal properties are ultimately controlled by the fine structure of the polysaccharide but also by the water content of the system. For instance, starch gelatinization and glass transition temperature varies with water content which affects the functional characteristics of the material (Figure X.7). At high water contents the major endothermic peak (~70 ^{o}C) is assigned to melting of crystalline regions of amylopectin (gelatinization) whereas the peak at

about 110 ^{o}C is assigned to the melting of amylose-lipid complexes (Figure X.7a). [54] With reduction of moisture content below 30% the gelatinization peak disappears, as starch granule cannot absorb water and hydrate. As water content is further lowered (<18%) glass transition events of the amorphous regions of starch granule appear on heating (Figure X.7b).[55, 56] These move to higher temperatures as the plasticization effect of water (see below) is diminished with water content decrease. Other relevant events that can be followed using calorimetry include gel "melting" and protein denaturation temperature. In the case of gels, melting is not a typical first order transition since there is no actual crystalline structure present. Rather, it refers to the "detachment" of the contact points (*e.g.*, junction zones) with increase in temperature.

Figure X.7 around here

The microstructure of glasses depend on the kinetics of glass formation or in other words on the rate that the system arrives at its pseudoequilibrium (rate of cooling or water removal). High cooling rates (or fast water removal) arrest the system at a more disordered (more "open") state than slower cooling rates (or slow water removal). Such a process results in structures that are not in thermal equilibrium with their surroundings. Due to the low temperature motions or with storage near to the α-relaxation temperature (usually between Tg and the temperature where β-relaxations occur), the thermodynamic properties such as enthalpy, entropy and volume will tend to evolve towards their equilibrium values, a process that is called *physical ageing*. Ageing affects significantly the properties of the glassy materials and preparation of the glassy phase and storage should be carefully controlled, as microstructure and glass transition of biomaterials are interdependent. [57] Because of physical aging, the material is subject also to microstructural rearrangements that may have implications to the stability of the system. [52] To account for the variations in the

dynamics of the material undergoing a glass transition the fragility parameter *m* is introduced [58] to distinguish systems in which relaxation mechanisms (*e.g.*, viscosity) are highly dependent on temperature above T_g (*m* between 100-200, "fragile") from those that are less dependent (*m* between 16-100, "strong"). [59, 60] Such a classification has important implications in various technological processes that may allow tailoring the technological performance of polysaccharide matrices. Variations in parameter *m* between two glassy polysaccharide structures may result in significant changes in stability as the rate of relaxation mechanisms *i.e.*, the speed by which the system approaches equilibrium, is influenced significantly in the vicinity of T_g. Furthermore, various processes that involve fast removal of water (*e.g.*, extrusion, flaking) or rapid cooling (*e.g.*, confectionary industry) may benefit from the understanding of relaxation mechanisms of the materials that are utilized in the formulations. Several polysaccharide systems are reported as "strong" indicating moderate dependence of relaxation mechanisms on temperature. For example, in pullulan,[61] chitosan and chitosan blends [62] or pullulan-starch blends [63] fragility parameter *m* varies between 30-96 depending on the molecular weight and the moisture content of the materials.

Glass transition temperature depends on the molecular weight of the polysaccharide and the presence of low molecular weight compounds, called *plasticizers*. [59] The most common plasticizer for polysaccharide matrices is water but other small molecules can also show plasticization effects (*e.g.*, glucose, sorbitol or glycerol). Plasticizers increase the free volume of the system thus increasing the molecular mobility of the chains. The result of increased molecular mobility is that the glass transition occurs at lower temperatures than it would in the absence of a plasticizer (Figure X.7b). Consequently, by intelligent manipulation of the water content, the microstructural and textural characteristics can be precisely controlled.

Furthermore, engineering of novel materials such as edible film coatings [64, 65] or encapsulation matrices [66] can be also achieved. Edible films are mostly prepared from polysaccharides although proteins can be also used as starting materials. Such a film is a low-moisture polysaccharide system that comes into direct contact with the surface of the food. Films provide a barrier to moisture loss or uptake and control gas exchange of food with the environment (*e.g.*, O_2 or CO_2). They can be also used to control microbial growth when antimicrobial compounds are introduced. It is easy to realize that the properties of films have a profound dependence on the plasticization effect of water that may migrate from food or the atmosphere to the film. This plasticization may reduce the glass transition temperature to the storage temperature of the product thus altering the effectiveness of the film. Encapsulation of active ingredients such as flavour, colour or nutrients is also accomplished by the use of polysaccharides. This technology allows protection of the encapsulated material form oxidation, losses due to evaporation, light or interactions with food ingredients. Encapsulation usually proceeds with immobilization of the desirable component into a glassy polysaccharide matrix. In the operating environment (*e.g.*, mouth, stomach or intestines) the active component will be released in a controlled manner from the matrix to provide its functionality (*e.g.*, flavor or nutrient release). Similarly to the edible films the capacity of the encapsulating matrix to stabilize the ingredients depends on the properties of the glassy polysaccharide matrix and the plasticization effect of water.

X.6 Conclusions

The evolution of structure formation has been reviewed for a range of polysaccharide systems. Although polysaccharides consist of a relatively small

number of monosaccharides they have the capacity to form a wide range of structures. The interactions among the chains are those that primarily control how the structure will evolve and stabilize. Depending on the water content of the systems it is possible to distinguish two regimes where polysaccharides can form completely different structures with distinct physical and mechanical properties. In the high moisture systems polysaccharides are able to form gels making it possible to structure water or air. On the opposite extreme where moisture content is low, glassy state and the related relaxation phenomena control the structural stability of the material whereas some native materials also show structures with long-range order. The greatest drawback for materials based on polysaccharides is their metastable nature *i.e.*, their sensitivity to structural evolution in time. In food applications it is usually manifested by a limited shelf life stability and changes in functional properties during storage. Further work should focus on exploring how to limit the kinetic processes that influence these changes so as to provide novel polysaccharide materials with improved functional properties.

X.7 References

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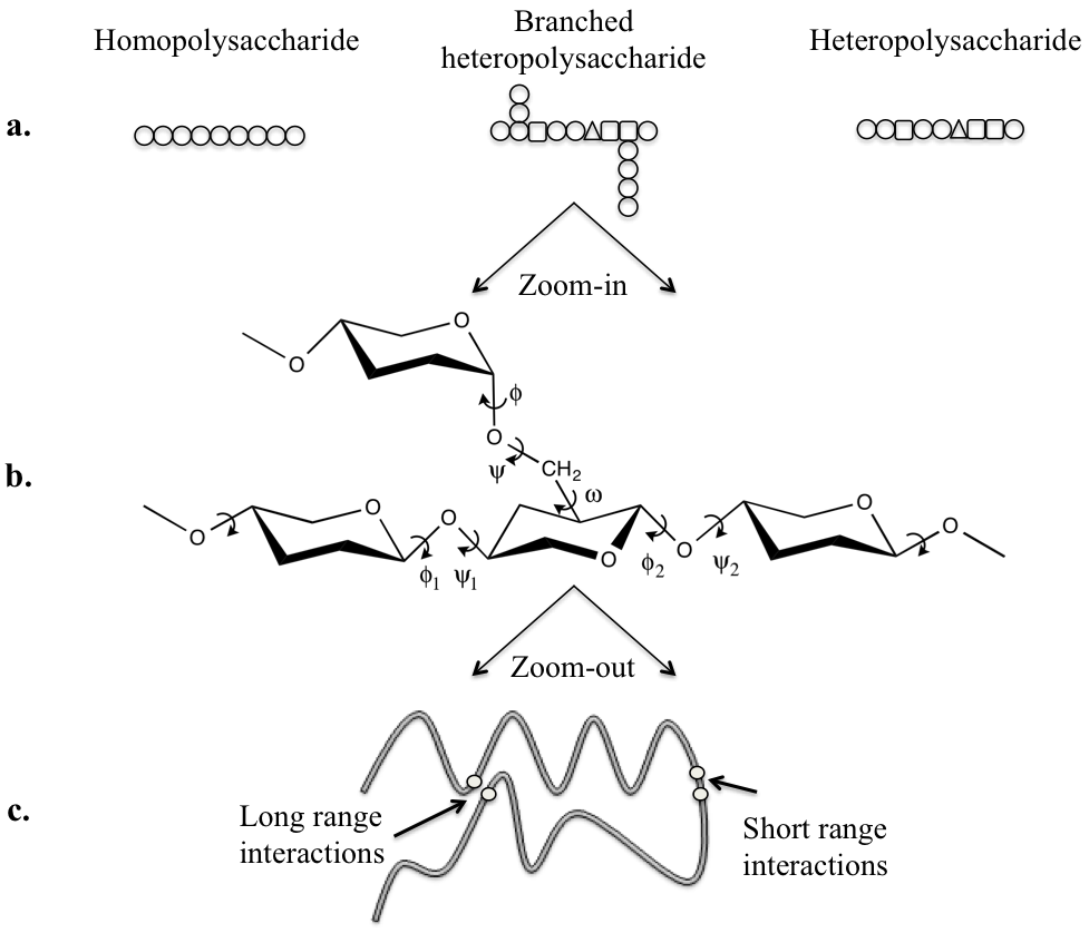

Homopolysaccharide
Branched heteropolysaccharide
Heteropolysaccharide
a.
Zoom-in
b.
Zoom-out
c.
Long range interactions
Short range interactions

Figure 1

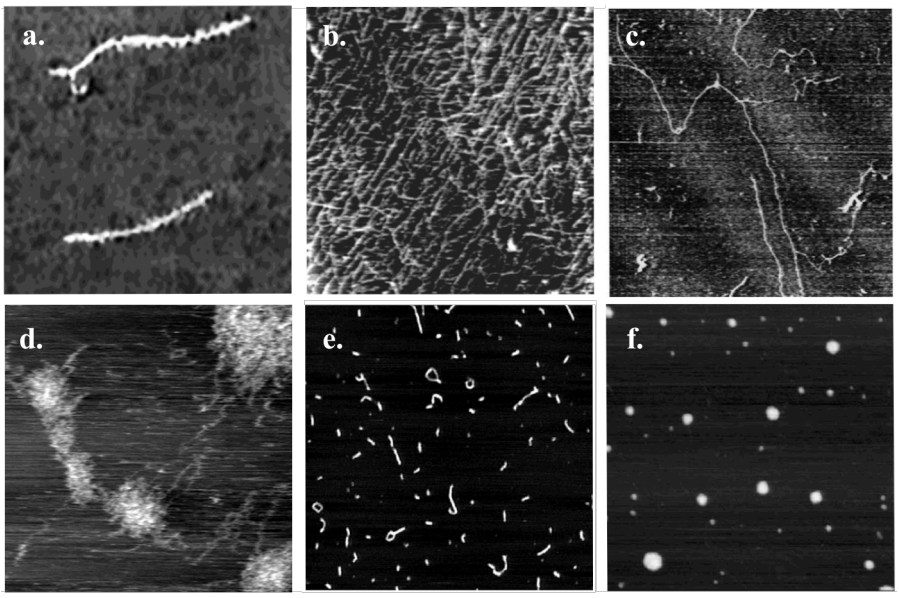


Figure 2

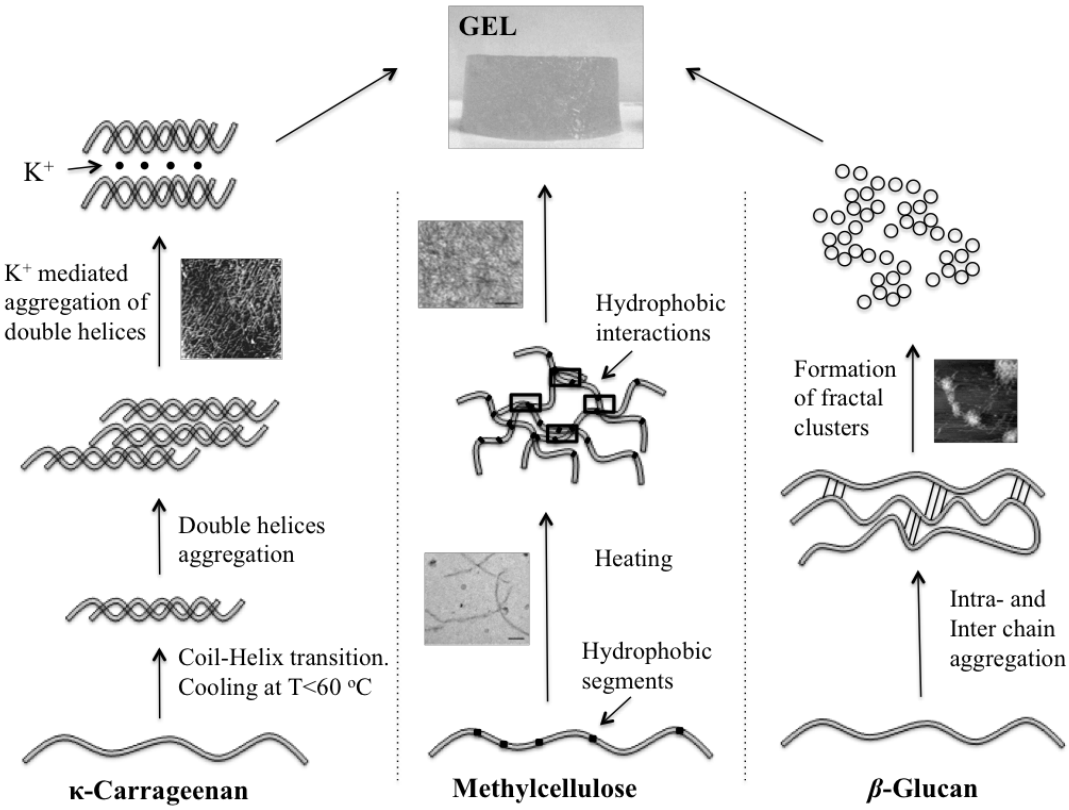


Figure 3

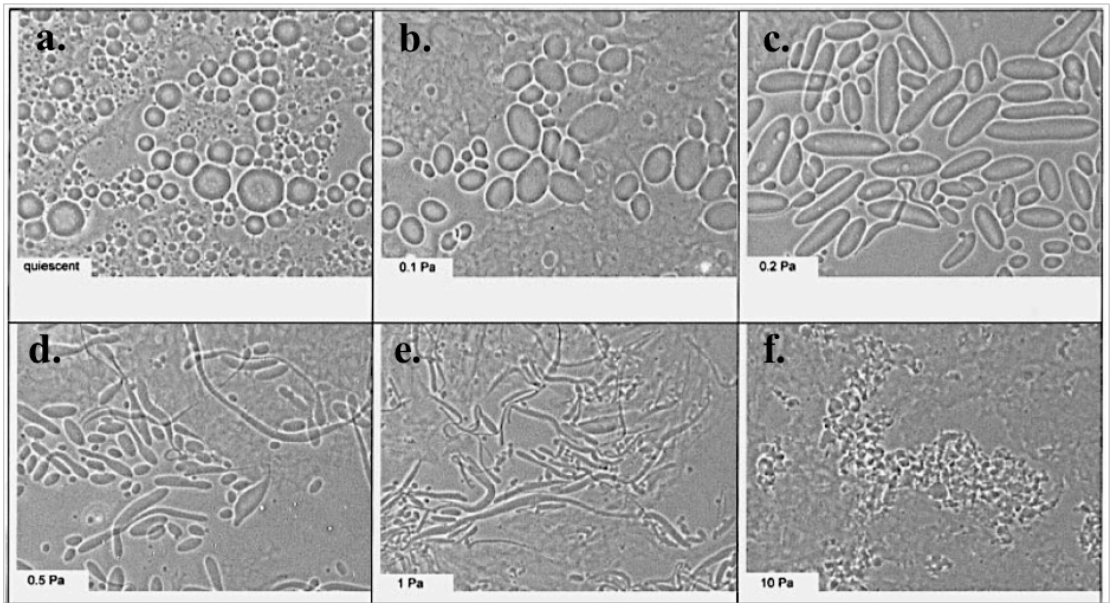
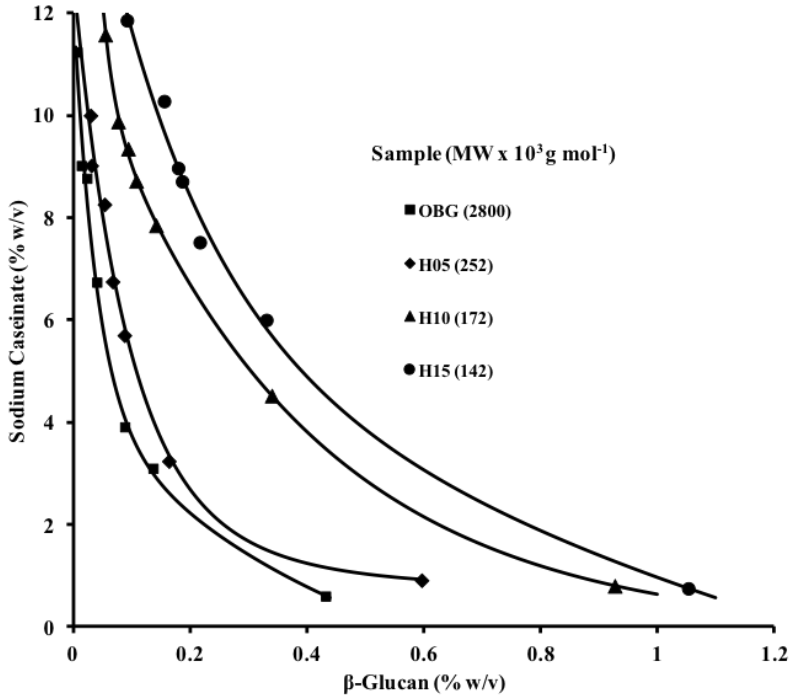


Figure 4

a.



b.

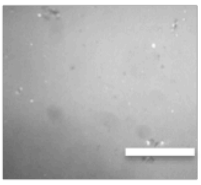
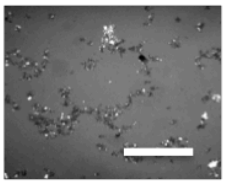
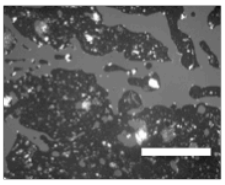
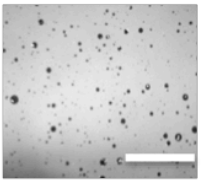
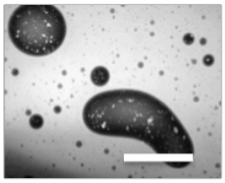
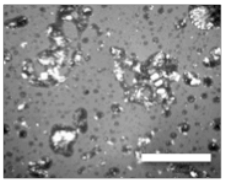
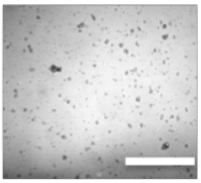
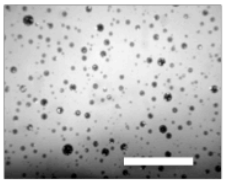
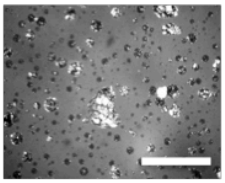
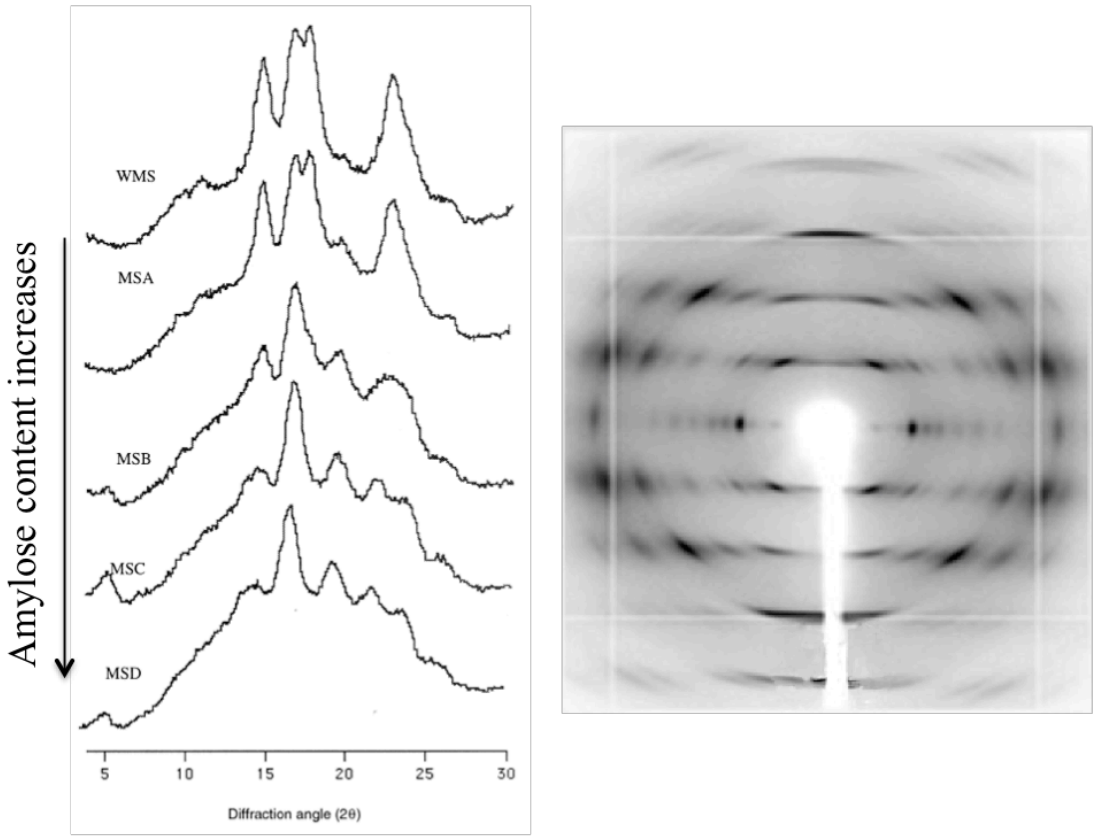
MW (x10^3) OF β-GLUCAN	SODIUM CASEINATE RICH PHASE	BICONTINUOUS PHASE	β-GLUCAN RICH PHASE
2800			
172			
142			

Figure 5



a. b.

Figure 6

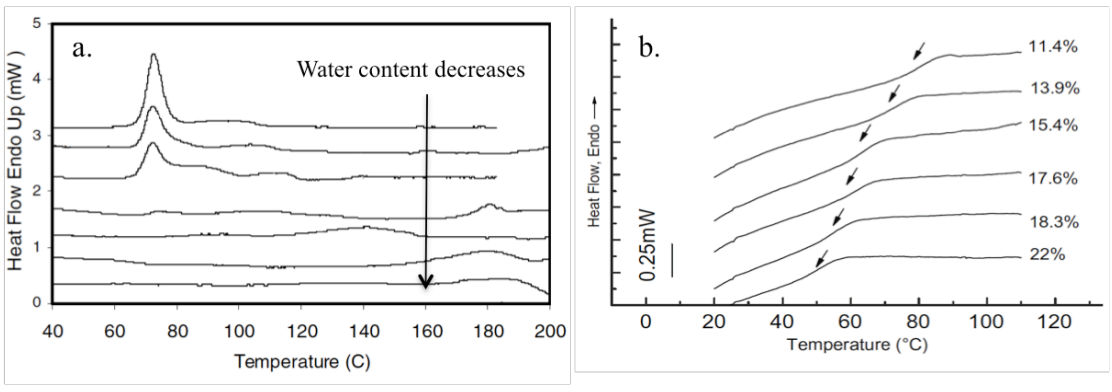


Figure 7