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Rheological approach to test plant-based polymer alternatives to gelatin in food products

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Abstract

In recent years, the global food industry has shifted towards plant-based products, led by increasing consumer awareness and concern for ethical, environmental, and dietary as well as for those concerned with sustainability and the ecological footprint of animal-based products. This transition in the food industry has created a pressing need for alternatives to animal derived ingredients in many food products. More specifically, there is an increasing demand to find a possible plant-based alternative to gelatin, a key component in many food products. For a long time, gelatin has been utilized as a food component, such as a gelling and foaming agent. It is a substance made from animal skin, white connective tissue, and bones that has been obtained through partial hydrolysis of collagen. Currently, the primary sources of commercial gelatin are restricted to cow or pig bones and skins, likely because the finished gelatin product is very inexpensive[1].

Finding an ideal substitute for mammalian gelatin is evidently a formidable, if not impossible, task. Many attempts have been made to replace gelatin in a generic way, but the versatility of gelatin has prevented this approach. In this work we selected certain plant-based polymers that according to the literature are potential candidates for replacing gelatin in the food industry and we demonstrate that small amplitude oscillatory shear as well as Large amplitude oscillation shear can be used to find a “plant-based rheological twin” of gelatin. We decided to solely use the polymer concentration as a tuning parameter, but one could also use the molecular weight (Mw) and temperature. In this work we utilize the rheological phase diagram, obtained through small amplitude oscillatory shear, to mimic the Linear Viscoelastic response of gelatin with the plant-based polymers in terms of Viscosity. In order for our work to be even more detailed and comprehensive we decided to use Large amplitude oscillation shear experiments to explore the Non-Linear Viscoelastic region (NLVE). With this set of experiment, we were able to extrapolate information’s regarding the Yield Strain and Stress of the samples as well as the hysteresis of the material under constant shear.

This work can be used as a guide for future works on the topic of plant-based polymers alternatives to gelatin in the food industry as we demonstrate the properties plant-based polymers must posses in order to replace gelatin. This work could be a milestone when the final goal is reached.

Acknowledgement

I want to start by expressing my gratitude to my supervisor, Dr. Daniele Parisi, for his unwavering support and belief in my abilities. His mentorship combined with the essential flexibility to do my research provided me the confidence to reach my full potential. Without Daniele's encouragement and belief in me, I could not have been able to do this challenging task. I am deeply appreciative of the stimulating discussions, constructive criticism, and valuable advice provided by Daniele, which have greatly enriched my learning experience.

I would also want to express my gratitude to Prof Patrizio Raffa for his support during this challenging journey.

I am particular thankful to the rest of my lab mates, for the continuous encouragement and care. These past months I felt that they were more than just colleagues and lab mates. I want to specifically thank Doctoral Researcher, Roshan Akdar. Roshan made himself available whenever I needed help and assistance during my project and for that I am ever grateful. His patience and attention to detail is always inspiring.

Finally, i would like to thank all my friends and family for their undying support and kindness that made it possible for me to strive towards my goals. Their support and belief in me were outstanding and for that I am grateful.

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1. Introduction

In recent years, the global food industry has shifted towards plant-based products, led by increasing consumer awareness and concern for ethical, environmental, and dietary as well as for those concerned with sustainability and the ecological footprint of animal-based products. This transition in the food industry has created a pressing need for alternatives to animal derived ingredients in many food products. More specifically, there is an increasing demand to find a possible plant-based alternative to gelatin, a key component in many food products. For a long time, gelatin has been utilized as a food component, such as a gelling and foaming agent. It is a substance made from animal skin, white connective tissue, and bones that has been obtained through partial hydrolysis of collagen. Currently, the primary sources of commercial gelatin are restricted to cow or pig bones and skins, likely because the finished gelatin product is very inexpensive[1].

Importance of gelatin in food industry

Before diving in the possible rheological approach suggested in this work for the development of gelatin plant-based alternatives one must fully understand what are the properties of gelatin that makes it such an ideal material to be used in the food industry. Out of all the biopolymers available on the market, gelatin is the one that is used the most. Gelatin is by far a multifaceted, useful component in modern life. Based on current consumption trends, Grand View Research's analysis projects that the gelatine market will reach a valuation of USD 5.0 billion by 2025. The gelatin market is expected to reach a valuation of USD 6.7 billion by the end of 2027. In seven years, this market size is expected to drive demand for 868.9 kilotons of gelatin due to functional food and beverage goods as well as medicinal uses[2]. Gelatin exhibits numerous unique properties that are not readily replicated by other biopolymers. On of the most unique and important properties of gelatin is the “melt in the mouth”. The "melt-in-the-mouth" property of gelatin arises from its physical characteristic of melting slightly below the human physiological temperature. This quality imparts a distinctive "melt-in-the-mouth" sensation, leading to an intensified release of flavor and aroma. This behavior is a crucial attribute of gelatin-based systems, such as gelatin-based gel desserts, and is challenging to replicate in other biopolymer systems. Additionally, another unique property of gelatin is the ability to form thermoreversible gels. Gelatin gels are unique among protein and polysaccharide gels in their thermoreversibility, dissolving upon warming. On top of that gelatin is recognized as one of the most adaptable biopolymers in the food industry. It performs multiple functions, including gelling, thickening, water-binding, emulsifying, foaming, and film-forming. Finally, Gelatin is available in various gel strengths and particle sizes, allowing it to be tailored for specific applications. It is easy to use, as it gels within the typical pH range of most foods and does not necessitate the addition of salts or sugars for setting[1].It is commonly recognized that pig skin gelatin is less expensive and requires less processing time than other sources of extracted gelatin. However, eating edible gelatin containing cow or animal bones increases the chance of developing diseases like mad cow disease, spongiform encephalopathy (BSE), or foot-and-mouth disease (FMD) with anaphylaxis being the main risk to avoid the gelatin used in daily life . Therefore, plant-based polymers are a cleaner alternative to gelatin in the food industry[2].

Plant-based alternatives to gelatin in the food industry

Finding an ideal substitute for mammalian gelatin is evidently a formidable, if not impossible, task. Many attempts have been made to replace gelatin in a generic way, but the versatility of gelatin has prevented this approach. As a result, researchers and industry have been working for a long time to create gelatin substitutes that have most or all of the special functional qualities of mammalian gelatin. Industries are now working to create gelatin-free products, meaning that mammalian gelatin is not utilized as an ingredient or as a processing aid[3]. In this work we will discuss the properties of gelatin that makes it unique in the food industry as well as provide an in-depth rheological analysis on some of the possible plant-based polymers that according to the literature can replace gelatin in the food industry. The plant-based polymers are normally developed from plant biopolymers. These include Carrageenan, a polysaccharide extracted from red seaweed, Agar, also called Agar-Agar, a polysaccharide biopolymer primarily sourced from red marine seaweeds of the Rhodophyta species. Xanthan gum which is also a polysaccharide, also known as glycans, which is a naturally occurring polymer produced by microorganisms, plants, and animals. Xanthan gum specifically is extracted from cell wall surface of the microorganism Xanthomonas campestris and is commercially produced through fermentation. Pectin polysaccharide substance present in cell walls of all plants and fruits, Guar gum, also known as cluster bean, is a natural non-ionic, water-soluble polysaccharide extracted from the seeds of the guar plant. . Locust bean gum, a type of galactomannan, is a white to creamy white powder produced by milling the seed endosperm of the carob tree and last but not least Satiagum which is a type λ-carrageenan. In this work we assess the linear and nonlinear shear rheology of gelatin and the potential alternative plant-based polymers. An in-depth analysis and comparison between gelatin and the plant-based alternatives are highlighted in this work. We decided to solely use the polymer concentration as a tuning parameter, but one could also use the molecular weight (Mw) and Temperature (T). In this work we decided to compare everything in room temperature since it's a typical condition for preparing a representative food product. Nonetheless, varying Mw would only result in different polymer concentrations needed for mimicking the gel response of gelatin. For the selected plant-based polymers alternatives to gelatin we show the rheological dynamic phase diagram for a fixed temperature (room temperature) and Mw. Additionally, the selected plant-based polymer alternatives were incorporated in an actual food product and the rheological response was investigated and compared to the same food product with gelatin.

1. Theoretical Background

Gelatin

Gelatin, a clear, colorless, and flavorless substance derived from the collagen in the body parts of living organisms, is brittle when dry and turns gel-like and rubbery when moist[3]. Gelatin is created by partially breaking down collagen, which is obtained from animal skin, connective tissue, and bones, and is mainly produced using by-products from the meat and leather industries on a large scale[4]. Two varieties of gelatin, Type A and Type B, are produced depending on the pre-treatment method of collagen. Type A gelatin, which undergoes acid treatment and has an isoelectric point between pH 6 to 9, is typically made from the less covalently crosslinked collagen found in pig skin. Conversely, Type B gelatin is produced through alkaline treatment, has an isoelectric point at pH 5, and is suitable for the more complex collagen found in bovine hides[2]. The outcome of using an alkaline pre-treatment on gelatin is that it becomes electrically distinct from gelatin processed with acid. This difference arises because alkaline-processed gelatin contains a higher proportion of carboxyl groups, giving it a negative charge and reducing its isoelectric point (IEP) compared to acid-processed gelatin, which has an IEP similar to that of collagen. Utilizing these techniques, manufacturers are now able to produce gelatin with a variety of IEP values[5].

Chemical structure of gelatin

Based on the chemical composition, gelatin and collagen are completely different. Collagen's basic structure features a triple helix extending from the center, typically made up of three parallel α-chains, each with a left-handed conformation[6]. The conversion of collagen to gelatin acid or alkaline treatment, discussed above, breaks the hydrogen bonds leading to the disruption of the triple-helix structure found in collagen[7]. Therefore, gelatin cannot be regarded as a single chemical entity with a fixed molecular weight, instead, it comprises mixtures of polypeptide chains with varying molecular weights within a specific range. Depending on the production process, gelatin may consist of different types of chains characterized by their molecular weights. These include (i) individual α-chains, (ii) two α-chains covalently crosslinked to form β-chains, and (iii) three covalently crosslinked α-chains known as γ-chains, shown in Figure 1 below[8].

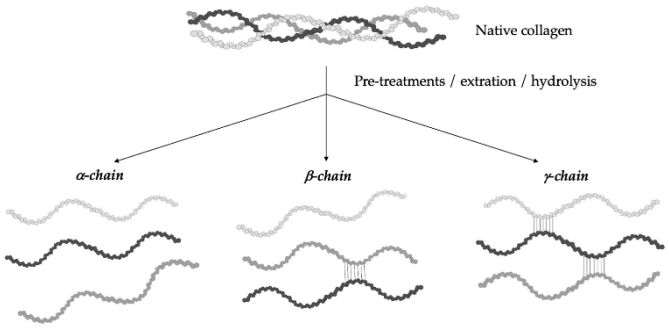


Figure 1. Different types of gelatin chains, α-chains , two α-chains covalently crosslinked to form β-chains and three covalently crosslinked α-chains known as γ-chains[8].

Gelatin is formed by assembling several polypeptide chains into a triple helical structure, where each of the 3 chains in this configuration needs approximately 21 rotations to complete one full turn[9].

Gelatin gels and properties

Gelation refers to the process where a liquid solution (sol) transforms into a disordered solid (gel), which is composed of a three-dimensional network formed through chemical or physical bonds between molecules[10]. The development of gelling systems is a key focus in gelatin rheology. During the sol-gel transition, a crosslinking polymer transitions from a liquid to a solid state at a critical juncture known as the "gel point." The gel strength and melting point of gelatin gels are significant attributes, affected by the gelatin's concentration, molecular weight, and the ratio of α/β-chains present. Additionally, the content of α-chains in gelatin determines the gel strength, with a higher number of α-chains leading to stronger gels[11]. Rheology is extensively utilized to study the gelation process of gelatin gels under both isothermal and non-isothermal conditions. The gelation mechanism of aqueous gelatin solutions can be described through four distinct rheological phases. Initially, the system is in a liquid state. Progressing to the second stage, junction zones emerge within the sample, thus strengthening the gel's structure. In the third phase, elasticity mildly increases due to the expansion of pre-existing crosslinks without generating new ones. The final stage is marked by a significant enhancement in the gel's elasticity, attributed to the ongoing growth and reorganization of helices over an extended timeframe[12]. The Bloom index, assessed through gel strength, serves as a crucial physicochemical parameter indicating the content of triple-helix structures in gelatin gels, which in turn influences their mechanical, rheological, and thermal properties. A higher Bloom index typically implies an increased presence of triple-helix structures, resulting in decreased swelling and enhanced elastic Young's modulus. Moreover, the Bloom index is frequently correlated with gel strength and is deemed a vital parameter for the research and development of gelatin-based gels, significantly impacting the structural integrity, stability, and functionality of gelatin-based gels[13]. Gelatin presents multiple advantages including affordability, widespread availability, biodegradability, and low immunogenicity, in addition to its high biocompatibility and inherent bioactivity. It is characterized by various functional groups amenable to chemical modifications, such as attachment of crosslinkers or target ligands, making it a popular choice in the production of substrates across numerous sectors including biomedical, pharmaceutical, cosmetic, and food industries. Specifically, in the food industry, gelatin is extensively utilized as a thickener in products like sweets and jams, a clarifying agent in beverages such as wine and juices, an emulsifier in confectioneries, a stabilizer in ice creams and cheeses, and a texturizer and film former in meat and confectionery coatings[8].

2. Plant-based alternatives to gelatin

Within the array of commercial biopolymers employed in the food industry, gelatin is considered particularly special and unique. It serves multiple functions and has a broad spectrum of applications, highlighting its versatility and integral role in food processing and product formulation. The challenge of finding gelatin substitutes has been prominent for years in vegetarian, halal, and kosher markets, and has seen a surge of interest over the past decade, especially within Europe. Since most commercial gelatins are derived from pigskin or cow hide, there has been a marked effort to identify and utilize alternative sources. Dietary restrictions based on religious beliefs or vegetarian lifestyles prevent certain consumer groups from consuming products containing gelatin. Consequently, the development of gelatin alternatives is increasingly important for food processors, particularly as the global market for halal-certified foods expands rapidly. In response, both academic and industrial sectors are actively working to develop substitutes that replicate the unique functional properties of mammalian gelatin[1].

Carrageenan

Algal polysaccharides are attracting attention for their sustainability and abundant availability, along with their unique chemical composition, which is not found in any other organisms. These biopolymers, derived from various types of seaweed, green seaweeds like ulvans, brown seaweeds such as alginates, and red seaweeds like carrageenan's, are notable for their specific functional properties, such as thickening and gelling. Additionally, they exhibit biological activities including antiviral, anti-inflammatory, and anticoagulant effects. As a result, carrageenan and alginates are primarily utilized in the pharmaceutical, cosmetic, and food industries[14]. Carrageenan refers to a group of gel-forming and viscosifying polysaccharides extracted from various seaweeds belonging to the Rhodophyceae class. Common genera from which carrageenan is derived include Eucheuma, Solieria, Cripus, Agardhiella, Chondrus, Hypnea, Sarconema, Iridaea, Gigartinastellate, and Agardhiella. These particular types of seaweeds are predominantly found in the Atlantic Ocean near Britain, Europe, and North America[15]. Carrageenan is categorized into six primary types based on its sulfate content, source of extraction, and solubility: kappa (κ), iota (ι), lambda (λ), mu (μ), nu (ν), beta (β), and theta (θ) carrageenan. The most significant variants are kappa-carrageenan (κ-carrageenan), iota-carrageenan (ι- carrageenan), and lambda-carrageenan (λ-carrageenan). These types are distinguished by their unique chemical structures and resultant physical properties[16]. For this report, we will only consider κ-Carrageenan, ι-Carrageenan, and λ-Carrageenan, since it contains superior gelling abilities compared to the other structures.

Chemical structure of carrageenan

Carrageenan consists of alternating 3-linked and 4-linked D-galactose residues, which are modified by 3,6-anhydro bridges and substitutions with ester sulfate, methyl, or pyruvate groups. This structure may also incorporate a variety of additional carbohydrate residues including galactose, sulfate, xylose, glucose, and uronic acids. The presence of 3,6-anhydrogalactose units along with the sulfation pattern are key factors that significantly influence the variations in the structural composition of different types of carrageenan[17]. The molecular weight of carrageenan generally remains high, although it can vary depending on a range of factors such as the species of seaweed, the seaweed's age, the time of harvest, and the extraction techniques and conditions employed. These elements significantly affect the final characteristics of the carrageenan[18]. κ-carrageenan consists of alternating units of 3-linked β-D-galactose 4-sulfate and 4-linked 6-anhydro-α-galactopyranose. Each disaccharide

repeating unit in this structure carries one negative charge. While ι-carrageenan contains two sulfate groups per disaccharide repeating unit. On the other hand, λ-carrageenan is characterized by having three sulfate groups per disaccharide unit. Unlike κ- carrageenan and ι-carrageenan, λ- carrageenan does not exhibit any 3,6-anhydride bridges[14]. Figure 2 bellow depicts the chemical structure of κ-carrageenan, ι-carrageenan and λ-carrageenan.

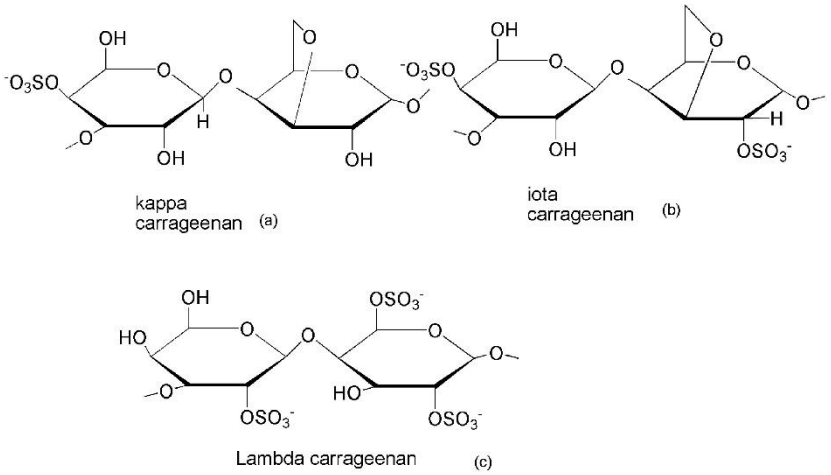


Figure 2. Chemical structure of κ- carrageenan, ι- carrageenan and λ- carrageenan[14]

Carrageenan gels and properties

The gelation process of carrageenans is a multifaceted phenomenon influenced by the type and concentration of carrageenans, the species and concentration of cations, and the temperature. Numerous models have been proposed to elucidate the gelation mechanism of carrageenans. It is widely acknowledged that the gelation process involves an initial transition from coil to helix, followed by the aggregation of helices to form a three-dimensional network[19]. The thermoreversible gelation of κ-carrageenan is understood to involve a coil-helix conformational transition. The gel formation in aqueous solution is a complex process influenced by the polysaccharide's chemical structure, the nature of co- and counterions, polymer concentration, and temperature. Given the wide range of structural factors and the variety of experimental methodologies employed by different researchers, it is unsurprising that several controversies persist regarding the kinetics of gel formation and the nature of the ordered conformations of κ-carrageenans. Specifically, debates continue regarding the type of conformational transition (coil-helix or coil-double helix), the relationship between the conformational transition and gelation, and the sequence of steps leading to gelation[20]. The prevailing consensus in the literature is that the gelation of κ-carrageenan occurs through a two-step process. Initially, at elevated temperatures, κ-carrageenan molecules are dispersed in water. Upon cooling, helices form, which subsequently aggregate, resulting in gelation, as shown in figure 3 bellow. This gelation model referred to as the domain model was originally proposed by Morris et al. In aqueous solutions at elevated temperatures, carrageenans adopt random coil configurations due to electrostatic repulsions between proximate negatively charged polymer chains[21]. Upon cooling, gel formation in carrageenan is initiated by the development of helical structures, which only occurs when a 3,6-anhydro bridge is present on the B unit of the carrageenan molecule. These anhydro bridges are absent in the native carrageenan precursors and are formed during the extraction process. This formation involves the removal of sulfate from the sulfate esters present in the precursors, facilitating the necessary structural change for gelation[22]. Further cooling, along with the presence of cations,

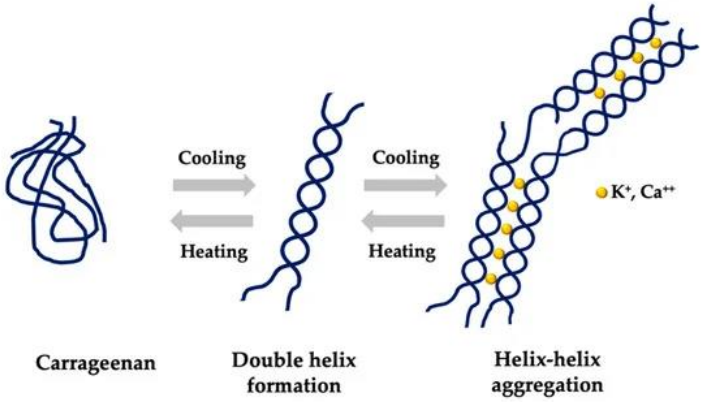


Figure 3. Possible gelling mechanism of κ and ι carrageenan[16].

induces the aggregation of helical carrageenan chains, leading to the formation of a stable three-dimensional network through intermolecular interactions between adjacent carrageenan chains. The mechanisms underlying these conformational transitions remain highly debated and controversial. It is generally accepted that κ-carrageenans transition to a double-helical structure prior to gelation. Helix formation and the consequent gelation likely result from intra- and intermolecular interactions, such as hydrogen bonds and van der Waals forces, between hydroxyl groups and potentially the hemiacetal oxygen, similar to other gelling polysaccharides[21]. The linear helicoidal structures in carrageenan interact and bind with appropriate ions to form a firm and stable three-dimensional gel network[16]. The impact of various cations on carrageenan gelation has been extensively researched. Monovalent cations that promote κ-carrageenan gelation include K^+, Rb^+, Cs^+, and high concentrations of Na^+ and Li^+. Similarly, divalent cations such as Ca^{+2}, Ba^{+2}, and Mg^{+2} are capable of inducing gel formation. Divalent cations generally enhance the gel strength of κ-carrageenan more effectively than monovalent cations, except for potassium, which likely forms specific bonds with κ-carrageenans due to its ability to shield electrostatic repulsion[21]. Different salts can influence the phase transitions and gelling behavior of kappa and iota carrageenan gels differently. For instance, stronger kappa-carrageenan gels have been observed with the addition of KCl. In contrast, λ-carrageenan does not form gels and maintains a random coil conformation at all temperatures[14].

Xanthan gum

Polysaccharides, also known as glycans, are naturally occurring polymers produced by microorganisms, plants, and animals. These compounds find extensive use across various applications, including food, food packaging, and pharmaceutical formulations. Prominent examples of glycans include xanthan gum, guar gum, locust bean gum, and cellulose. Xanthan gum, specifically, was first discovered in the 1960s and commercialized in the 1970s. Xanthan gum is extracted from cell wall surface of the microorganism Xanthomonas campestris and is commercially produced through fermentation. It is a water-soluble polymer with a high molecular weight, typically ranging from 1 to 50 10^6 g/mol[23,24]. Xanthan gum has some unique properties that allow it to have various applications. Xanthan gum is highly soluble in both cold and hot water but has very limited solubility in most organic solvents. This property can vary depending on the intended use of the product. For example, when used in food applications, the levels of bacteriological contaminants and heavy metals must be kept extremely low, whereas such stringent requirements may not apply to other industrial uses of xanthan gum. Xanthan gum exhibits high viscosity even at low concentrations, a 1wt% solution can have a gel-like consistency, yet it remains pourable and easy to mix and pump. The viscosity of xanthan gum solutions is influenced by factors such as temperature, biopolymer concentration, and pH. Xanthan gum is highly regarded for its exceptional emulsifying, stabilizing, and suspending properties. It also demonstrates excellent compatibility with other market-available thickeners like pectin, gelatin, dextrin, alginate, and carrageenan. In solution, xanthan gum exhibits pseudoplastic behavior, meaning its viscosity decreases with increasing shear rate. This property is particularly beneficial in enhancing the sensory qualities of food products, such as flavor release and mouthfeel. Finally, Xanthan gum displays high tolerance to pH variations, effectively remaining stable across a pH range of 2 to 12, making it well-suited for a variety of food products. Additionally, it exhibits strong resistance to temperature fluctuations, maintaining stability even in the presence of acids and salts[25].

Chemical structure of Xanthan gum

Xanthan gum is a heteropolysaccharide with a primary structure resembling cellulose, consisting of a backbone of β-1,4-linked glucose units. This backbone is alternately substituted with a trisaccharide side chain composed of two mannose units flanking a glucuronic acid. In this structure, the internal mannose is predominantly O-acetylated, and the terminal mannose may be modified with a pyruvic acid residue. The inclusion of glucuronic and pyruvic acids in the side chain endows xanthan gum with a highly charged nature, contributing to a very rigid polymer backbone. The secondary structure of XG consists of a five-fold right-handed helix with a pitch of 4.7 nm and a diameter of 1.9 nm. This structure undergoes a thermally-induced transition between ordered and disordered states, which can be facilitated by high temperatures and low salt concentrations. Salt plays a crucial role in stabilizing the ordered conformation, thereby enhancing the exceptional stability of XG[26]. Figure 4 indicates the chemical structure of xanthan gum. The trisaccharide side chain composing the backbone form a right-handed helix, contributing to the structural stability supported by non-covalent and hydrogen bonds. When in solution, these side chains envelop the backbone, thereby protecting the bonds from degradation. This protective shield is crucial for the gum's exceptional stability under adverse conditions.

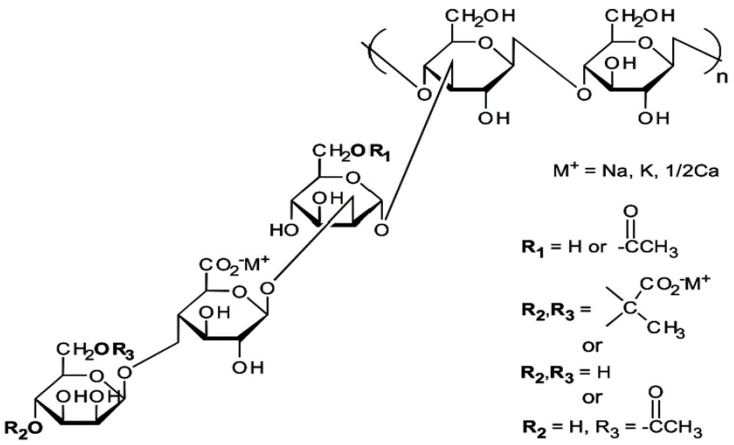


Figure 4. Chemical structure of Xanthan gum[23].

Upon heating, xanthan gum undergoes a conformational change, transitioning from a rigid, orderly state at lower temperatures to a more flexible and disordered state at higher temperatures. This thermally induced transformation is key to its functional properties in various applications[25].

Xanthan gum gels and properties

XG is soluble in both cold and hot water, but requires vigorous agitation when mixed with water to prevent lump formation. XG solutions exhibit non-Newtonian fluid characteristics and display highly pseudoplastic behavior, with apparent viscosity that changes significantly with time and/or shear rate. The thermal stability of XG against hydrolysis is generally superior to many other water-soluble polysaccharides or polymers. This enhanced stability is likely due to its ordered helical structure, which protects the molecules from depolymerization. XG is widely utilized in the food industry due to its effective thickening properties, high solution viscosity at low concentrations, pseudoplastic behavior in aqueous solutions, and high stability across a broad range of temperatures, pH levels, ionic strengths, and shear conditions. XG chains can form physical networks with divalent cations by involving two disaccharide units in the main chain and O-acetyl and pyruvyl residues in side chains, leading to intramolecular crosslinking and chain contraction. The acetyl and pyruvyl residues vary based on the bacterial species and fermentation conditions used to produce XG gum. The secondary structure of XG undergoes an "order-disorder" transformation from a helix to a coil structure depending on the pH, type of electrolyte, ionic strength of the solution, and the content of acetyl and pyruvyl residues. The content of these residues also influences the behavior of XG in aqueous solutions. Generally, lower pyruvyl content results in lower viscosity, while higher pyruvyl content enhances gel behavior through improved macromolecular association. Conversely, higher acetyl content inhibits the gelation behavior of XG aqueous solutions. The conformational transition to a double-helix is a prerequisite for gel formation, leading to stronger materials[27]. In aqueous solution, XG can be considered a highly extended worm-like chain due to the electrostatic repulsion from the deprotonated carboxylic acid groups on its side chains. Non-covalent interactions, primarily hydrogen bonding, between these extended structures render XG a weakly structured material, resulting in weak gel-like behavior. Additionally, the glycosidic oxygen atoms that link monosaccharide units also participate in hydrogen bonding in aqueous solutions. While XG is typically viewed as a non-gelling polysaccharide that exhibits shear-thinning behavior in solution, it can form a gel in the presence of trivalent ions or when

combined with other polysaccharides or proteins[28]. The conformation of xanthan gum in solution is influenced by solvent quality, electrolyte content, and temperature. In aqueous solutions, the trisaccharide side chains interact with the polymer backbone, stabilizing the overall conformation through non-covalent interactions, such as hydrogen bonding. In a good solvent, electrostatic repulsion between charges on the side chains causes the xanthan gum chains to extend. Conversely, in a poor solvent, the chains contract, reducing the hydrodynamic volume and consequently lowering solution viscosity. Xanthan gum also undergoes a conformational transition from a helical structure (considered as a rigid rod) to a random coil (flexible chains) under external stimuli, such as changes in temperature or pH as shown in figure 5. Despite these transitions, xanthan gum solutions exhibit weak gel-like properties due to the 3-D association of its chains. However, xanthan gum does not form true gels at any concentration, largely due to its reliance on weak non-covalent intermolecular interactions, rather than hydrogels. In contrast, hydrogels are crosslinked 3-D networks of hydrophilic polymers that swell in water or biological fluids but do not dissolve[23,29].

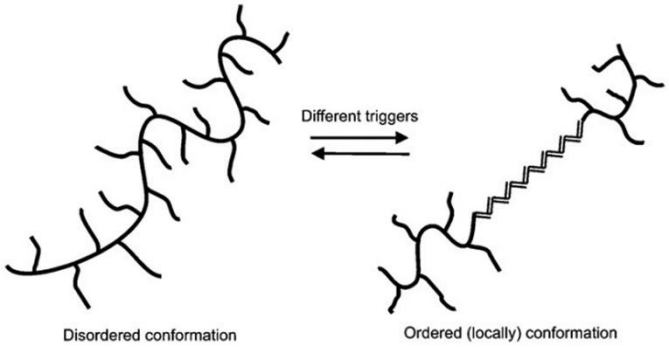


Figure 5. Schematic of conformational transition of xanthan gum chains in aqueous solution: from (left) disordered state to (right) ordered state[23].

Agar

Agar, a polysaccharide hydrocolloid, ranks among the oldest of such materials still employed contemporarily. First identified in 17th century Japan, it was predominantly utilized to produce gelled food substances, presumably serving as a preservation technique. Historically, agar, derived from seaweed, has been a key ingredient in the preparation of jellies from fruits and vegetables[30]. Agar is primarily sourced from red marine seaweeds of the Rhodophyta species. Functionally analogous to cellulose in green plants, agar serves a structural role. It is a fibrous polysaccharide extracted from marine algae species such as Gelidium, Gracilaria, and Pterocladia. Seaweeds from the Gracilariales order generally yield agars of lower quality due to their high sulfate content. However, the quality of these agars can be enhanced through alkaline treatment. Species belonging to the genus Gracilariopsis, which are found across tropical and warm-water regions globally, are predominantly considered suitable for commercial agar production[31]. Agar consists of a mixture of agarose and agaropectin, with Agarose making up about 70% of the mixture, while AgaroPectin makes up about 30% of it, with the latter being slightly branched and sulfated[32]. Agar exhibits noteworthy properties as a hydrocolloid. It remains insoluble in water at room temperature (20°C) but dissolves upon boiling. Agar can form reversible gels by simply cooling a heated aqueous solution. A translucent gel forms when the solution cools to 35°C, which can then be re-liquefied by reheating to 85°C. These properties, including its mechanical strength and the ability to form a reversible translucent gel, make agar highly valuable for use as a substrate in the culturing, enumeration, and identification of microorganisms. Furthermore, the processing characteristics of agar gel, which allow it to melt upon heating and reset upon cooling, can be repeated indefinitely without a loss in the gel's mechanical properties[30,32].

Chemical structure of Agar

The chemical structure of agar comprises a blend of agaropectin, the non-gelling fraction, and agarose, the gelling fraction. Agarose is a linear polysaccharide made up of repeating units of D-galactose and 3-6-anhydro-L-galactose, connected by alternating α-(1→3) and β-(1→4) glycosidic bonds[33] as shown in figure 6 bellow.

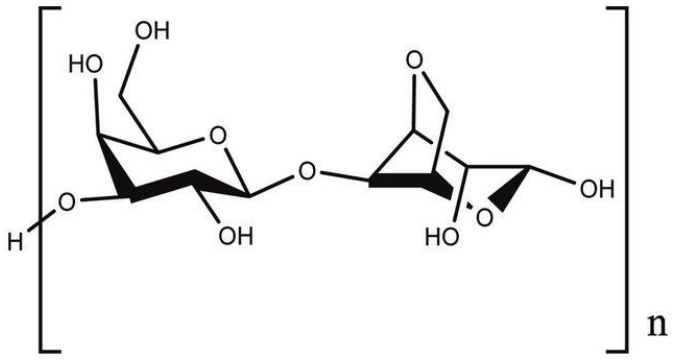


Figure 6. chemical structure of agar[34].

The primary distinction between agaropectin and agarose lies in the presence of negatively charged sulfate groups in agaropectin. Approximately 3% to 10% of agaropectin molecules are sulfated, which imparts a strong negative charge and inhibits its ability to form gels, rendering it functionally limited. Conversely, agarose contains minimal to no sulfate and is highly

effective at forming reversible gels. In aqueous solutions, agarose facilitates the formation of hydrogen-bonded crosslinks that stabilize the three-dimensional gel network. For commercial applications, such as in food products and microbiological media, agar is typically purified to remove any agaropectin, thereby enhancing the gel strength and utility of the final agarose product[30]. Small quantities of sulfate half-ester groups and pyruvate 3,6-cyclic acetal groups may be present in agar, along with considerable amounts of methyl ether groups. These specific structural features render agar the least hydrophilic and the least water-soluble among the polysaccharides derived from red seaweeds. This lower hydrophilicity and solubility differentiate agar in its interactions and functionality compared to other red seaweed polysaccharides[35].

Agar gels and properties

Among seaweed hydrocolloids, the physio-chemical properties of agar stand out. Agar consists of a mixture of agarose and agaropectin fractions in varying proportions, depending on the raw material and the manufacturing process used. Specifically, agarose, the purified form of agar devoid of agaropectin, enables the polysaccharide to form helical structures comparable to those seen in carrageenans. Gelation in agar is solely due to its agarose content, which is formed exclusively through hydrogen bonds. It's tempting to suggest that agar gelation also results from the aggregation of these helices, although unlike carrageenan, it does not require ions for stabilization. Since it does not require any additional substances to form a gel, agar has significant potential for applications as a food ingredient. Agarose forms 'physical gels,' meaning that their structure is entirely formed by the polymer molecules aggregating through hydrogen bonds. This unique gelling property allows these gels to retain a significant amount of water within their network, with the water able to move relatively freely through the macroreticulum. Another fundamental property of agarose gels is their gelling mesh size, which is characterized by very high exclusion limits. The exclusion limit is defined as the largest globular protein size that can traverse the gel in an aqueous solution[36]. The gelation of agarose is believed to occur through the association of molecular chains into double helices, which then aggregate to form a network that can immobilize water. Evidence for the formation of double helices comes from X-ray diffraction patterns of agarose fibers. The gelation mechanism for agar is very similar to the one imposed in the carrageenan[37].Notably, agar forms left-handed helices, whereas kappa and iota carrageenans, which contain 3,6-anhydro-D-galactose, form right-handed (dextrogyre) helices. Additionally, the helical pitch in agar is shorter than in carrageenan, which may be attributed to agar's lower sulfate content, leading to a tighter and more compact network. The aggregation of agar helices (agarose chains) is more readily achieved than in carrageenans, depending solely on intermolecular and intramolecular hydrogen bonding within a structural network. This network has been likened to a tetrahedral ice-like structure, resulting in agar possessing the highest gel strength among the red seaweed hydrocolloids when in pure water[21,38]. The gelation mechanism for agar is shown in figure 7 bellow. Although it is generally accepted that gelling involves aggregation, the details of the mechanism remain unclear. Minute calcium sulfate crystals, onto which agarose has been adsorbed, are thought to be present in agarose solutions and may act as nucleation centers to promote gelation. Spinodal demixing, which is the separation of a homogeneous sol into regions of differing concentration, has been proposed as the mechanism leading to the transition from individual coils to double helices. Studies using differential scanning calorimetry (DSC), rheology, X-ray diffraction, and NMR have provided further evidence that aggregation contributes to the stabilization of the gel[37]. Native agar displays gelling and melting temperatures of 22°C and 60°C, respectively, while modified agar

exhibits these transitions at 37°C and 98°C. As with all seaweed hydrocolloids, a higher molecular weight enhances the likelihood of forming a stable gel network through molecular interactions. Comparative studies of agar samples with varying levels of methyl substitutions have demonstrated that both the quantity and the positioning of these methyl groups significantly influence the gelation mechanism, enabling gelation to occur at different temperatures. This variability in physical properties underscores the impact of chemical modifications on the functional characteristics of agar[21].

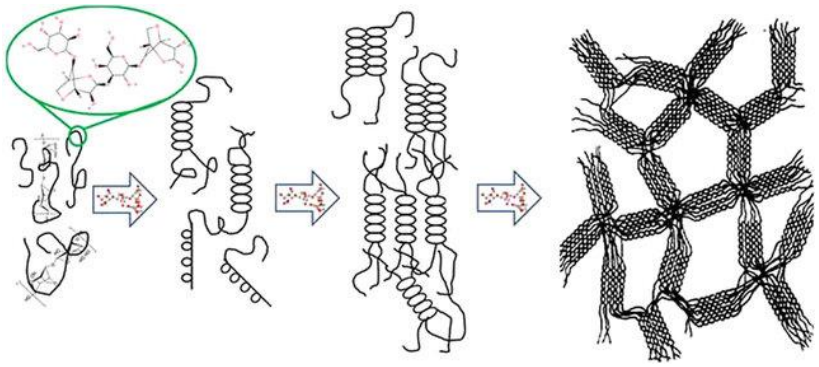


Figure 7. Gelation mechanism of agar[39]

Pectin

Pectin, derived from Greek words implying "to congeal" or "curdle," is a structural heteropolysaccharide embedded in the primary cell walls of terrestrial plants. Its initial isolation and characterization were performed by Henri Braconnot in 1825. Functioning as a versatile component of cell walls, pectin is recognized for its high value as a functional food ingredient, frequently employed both as a gelling agent and as a stabilizer in various culinary applications. Commercially, pectin is produced as a white to light brown powder. It is extracted on a large scale from fruit waste, a byproduct of the fruit processing industry. If not repurposed, this waste would contribute to environmental degradation through microbial breakdown and the release of greenhouse gases in landfills. In the food industry, pectin is primarily utilized as a gelling agent in jams and jellies. Additionally, it is employed in fillings, sweets, as a stabilizer in fruit juices and milk drinks, and as a dietary fiber source. In plant cells, pectin is a complex assembly of polysaccharides found predominantly in the primary cell walls, and is especially abundant in the non-woody parts of plants. It exists not only in the primary cell walls but also in the middle lamella, the layer between plant cells, where it acts as a binding agent that holds cells together. Within the plant cell wall structure, the concentration of pectin gradually diminishes from the primary cell wall to the plasma membrane, with its highest concentration observed in the middle lamella. The quantity, structure, and chemical composition of pectin vary among different plants, change over time within a single plant, and differ across various parts of the same plant[40,41]. Pectic substances are categorized into four distinct types by the American Chemical Society: (a) protopectin, (b) pectic acid, (c) pectinic acid, and (d) pectin. Protopectin is the precursor form, characterized by its relative insolubility and lack of gel-forming capabilities. Pectic acid results from the demethylation of pectin by enzymes, removing a methyl ester group, and does not possess gelling properties. Pectinic acids are composed of long chains of polygalacturonans with less than 75% of their galacturonate units methylated. Both pectin and pectinic acids are soluble forms of pectin polymers essential for gel formation[41].

Chemical structure of pectin

Pectin has a complex structure, its molecules predominantly consist of three types of chains, each primarily formed from rings of methylated D-galacturonic acid units. The structural attributes of pectin can be influenced by the conditions under which it is isolated, as well as by the storage and processing of the plant materials from which it is derived. During the transformation of D-galactose into D-galacturonic acid via oxidation, the sixth carbon in the R group, external to the saccharide ring, is converted from an alcohol group into a carboxylic acid group. This chemical change is crucial in defining the properties and functionality of pectin. Pectin is structured around a homogalacturonan (HG) skeleton to which several sub-domains are attached, including rhamnogalacturonan I (RGI), rhamnogalacturonan II (RGII), and xylogalacturonan (XG). HG is a linear polymer of polygalacturonic acid, typically unsubstituted and often described as "smooth" chains and it comprises about 60% of all pectins found in cell walls. RG-I features a long backbone of alternating galacturonic acid units and D-rhamnose, representing a structurally complex and spatially regulated polymer. XG involves a backbone of galacturonic acid with branches of xylose. Conversely, RG-II is distinguished by a homogalacturonan backbone that is extensively substituted with diverse and complex glycan side chains, which include a wide range of neutral sugars, adding to the

structural diversity and functionality of pectin. Figure 8 provides a schematic representation of pectin chemical structure[41,42].

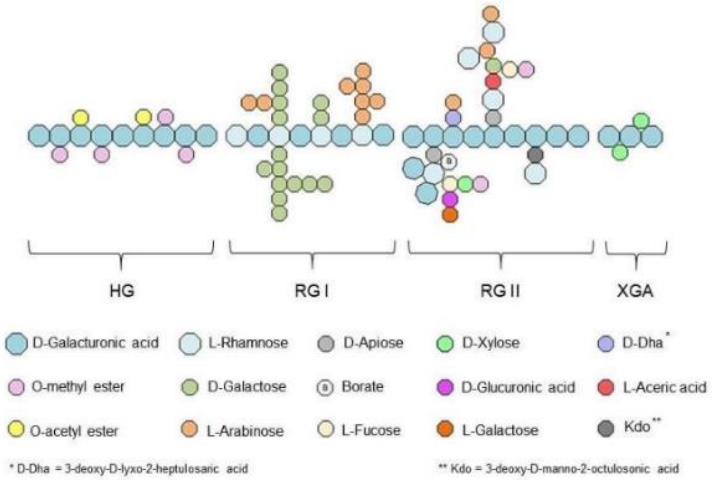


Figure 8. Schematic representation of the chemical structure of pectin[42].

Pectins are classified based on their degree of esterification into high methoxyl (HM) or low methoxyl (LM) pectins, each possessing distinct properties and suited for different industrial applications. HM pectins require a high concentration of soluble solids and an acidic medium (pH < 3.5) to form gels. The gelation process in HM pectins is stabilized by intermolecular hydrogen bonds and hydrophobic interactions between methyl esters. They are commonly used in the production of jellies, sweets, and desserts. Conversely, LM pectins can form gels across a broader pH range (2.0–6.0) and depend on the presence of calcium ions or other multivalent cations for gelation. The specific amount of calcium required for gelation varies with the pH and the concentration of soluble solids. HM pectins can be converted to LM pectins through chemical de-esterification using alkalis or enzymatically using pectin methylesterase, altering their functional properties and expanding their application possibilities. Despite sharing common characteristics, pectins display a wide range of structures influenced by their source and extraction methods. These structures vary in several aspects, including molecular mass and its distribution, degree of esterification, the presence of neutral sugar side chains, ferulic acid, proteins, and the degrees of methoxylation and acetylation, among others. These structural differences significantly impact the gel-forming ability and overall functionality of the pectin, affecting its suitability for various industrial applications[41,42].

Pectin gels and properties

The gelation mechanism of pectins is primarily determined by their degree of esterification. In LM pectins, gelation occurs through specific non-covalent ionic interactions between sequences of galacturonic acid residues in the pectin backbone and divalent cations, such as calcium. The affinity of pectin molecules for calcium is known to increase as the degree of esterification decreases. This affinity also rises with a reduction in ionic strength and an increase in polymer concentration, enhancing the gelation process[43]. Ca-dependent gelation is a critical functional property of pectin, making it a valuable component in the food industry. This gelation process is described by the classic "egg-box" model, which was originally proposed for alginate. This model outlines how two antiparallel polyuronate chains interact with Ca^{+2} ions to form "egg-box" dimers. These dimers then aggregate laterally to create multimers, facilitating gel formation. The model was adapted for pectin after recognizing that the Ca-binding sites in both alginate and pectin exhibit mirror-symmetric spatial

conformations, allowing a similar mechanism to govern the Ca-dependent gelation process in pectin. However, the egg box model was further refined with the introduction of the "shifted egg-box" model. This modification aims to provide a more detailed explanation of the gelation mechanism in pectin. According to the shifted egg-box model, two antiparallel pectin chains engage in gelation but with a notable axial misalignment. This misalignment represents an adjustment to the traditional model, accounting for variations in how pectin chains interact and bind with calcium ions during gel formation. The gelation mechanism of Ca^{+2} with pectin differs from that in other polysaccharides as it does not involve the lateral cross-linking of egg-box dimers. Instead, the process is characterized by two distinct phases: the initial formation of dimers followed by negligible subsequent aggregation of these dimers. This unique behavior in pectin gelation is described using a "dotting" mode of growth, rather than a "zipping" mode commonly observed in other gel-forming processes. This dotting mode of growth is primarily attributed to the block-wise and random distribution of non-methoxylated galacturonic acid units within the pectin molecules. This distribution pattern allows for the immediate formation of Ca-pectin dimers upon the addition of Ca^{+2}, bypassing the need for a critical concentration of Ca^{+2} typically necessary for initiating gelation in other systems. This distinctive feature highlights the unique properties of pectin in calcium-dependent gelation mechanisms. The primary driving force behind the formation of pectin egg-box dimers is electrostatic interaction. Ca^{+2} ions interact with the dissociated carboxyl groups present in pectin, leading to cross-linking between separate polymer molecular chains. This cross-linking effectively reduces the electrostatic repulsion between polymer molecules, thereby facilitating the structuring of the gel. Additionally, other forces such as hydrogen bonding, van der Waals forces, and hydrophobic interactions also play significant roles in the gel formation process. These interactions collectively enhance the stability and integrity of the gel network, contributing to its functional properties in various applications. The egg-box model mentioned above primarily pertains to the Ca-dependent gelation of LM pectin and does not apply to HM pectin[44]. Gelation in HM pectin particularly in the presence of homogalacturonan chains, is enhanced by the addition of cosolutes such as sucrose. This soluble sugar, when added to a pectin dispersion intended for gelation, reduces water activity, thereby favoring chain-to-chain interactions over pectin-solvent interactions. Sucrose may also act as a "coating agent," enhancing the strength of branch points possibly through cooperative interactions. It could influence the self-association of pectin chains by condensing around them or binding to them, facilitating closer proximity of the chains. The junction zones that facilitate gelation are stabilized through interchain hydrogen bonds between the protonated carboxyl groups and secondary alcohol groups of the pectin homogalacturonan chains. Additionally, hydrophobic interactions between the methyl ester groups of these chains further stabilize the network. The resulting gel is a vast three-dimensional network that immobilizes both solvent and cosolutes, forming a thermoreversible cohesive system. This gel resists deformation and exhibits a specific stress/strain relationship during small deformations, indicative of its structural integrity and functionality[45].

Guar Gum

Natural gums are hydrophilic polysaccharides sourced from plants or microorganisms. Based on their origin, they are classified into plant exudate gums, seed gums, microbial gums, or marine gums. Seed gums, such as guar gum, tamarind gum, and locust bean gum, are derived from the embryos of certain seeds, where they serve as a food reserve. Among these polysaccharides, galactomannan is the most commonly used. The primary sources of galactomannan include locust bean, tara, cassia, and guar. Of these, guar gum is the most

readily available and cost-effective source, making it a primary focus for researchers investigating galactomannan. Guar, also known as cluster bean, is an annual crop belonging to the family Leguminosae. It is cultivated in the arid regions of west and northwest India, Pakistan, Sudan, and parts of the USA. Guar gum, also referred to as Cyamopsis gum, Guaran, Guyan, Guarina, or Glucotard, is a natural non-ionic, water-soluble polysaccharide extracted from the seeds of the guar plant. The gum is derived from the endosperm of the seeds, which serves as a reserve food supply for the embryo during germination. Guar gum is a high molecular weight polysaccharide, characterized by its white to yellowish-white appearance and lack of odor. It is insoluble in hydrocarbons, fats, alcohols, esters, and ketones, with few exceptions such as formamide. Water is the primary solvent in which guar gum dissolves effectively. Guar gum is the naturally occurring water-soluble polysaccharide with the highest molecular weight. Its properties are primarily influenced by chemical features such as chain length, the abundance of cis-OH groups, steric hindrances, the degree of polymerization, and the presence of substituents. Advanced analytical techniques have determined that the number average molecular weight of guar gum ranges from 10^6 to $2*10^6$ g/mol[46,47].

Chemical structure of Guar gum

Guar gum is a heterogeneous polysaccharide composed of galactomannans. Polysaccharides are intricate polymers linked by glycosidic bonds, forming large, highly branched or non-branched structures. Guar gum is commonly described as a high-viscosity, water-soluble polysaccharide consisting of linear chains of (1→4)-β-D-mannopyranosyl units with α-D-galactopyranosyl units attached via (1→6) linkages as side chains. The mannose to galactose units are present in a mass ratio of approximately 2:1. Figure 9 bellow depicts the chemical structure of guar gum indicating the mannopyranose backbone and the galactose sidechains[48].

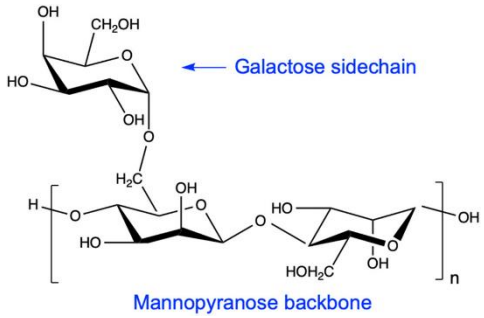


Figure 9. Chemical structure of Guar gum[48].

Guar gum gels and properties

As described in the above section of this work, guar gum is a heterogeneous polysaccharide composed of galactomannans. Its chemical structure consists of a galactose sidechain attached to the mannopyranose backbone. Galactomannans are insoluble in organic solvents such as hydrocarbons, alcohols and esters, with the exception of formamide. Water is the most important solvent for galactomannans, as it not only hydrates them but also forms colloidal solutions with unusually high viscosity. Various factors influence the solubility of guar gum in water. Increasing the temperature, decreasing the pH, and reducing the particle size all enhance its solubility. Conversely, the presence of salt and sugar decreases the solubility of guar gum in water[49]. When guar gum is introduced to water, the hydroxyl groups,

predominantly from galactose side chains attached to the mannose backbone, interact with water molecules, leading to intermolecular chain entanglement. This entanglement between guar gum and water results in increased viscosity, causing gelling and thickening of the solution. The straight-chain guar gum molecules are randomly interconnected by cross-linkers. Commonly used cross-linking agents include derivatives of methylene-bis-acrylamide, ethylene-glycol-di(meth)acrylate, divinylbenzene, and glutaraldehyde. These cross-linking agents have two active sites that form intermolecular bonds with the hydroxyl groups of polymer chains, creating a closed-loop structure. When water is added to this cross-linked material, it becomes entrapped within the network, significantly increasing the water absorption and retention capacity of the hydrogel system[46]. The gel formed by guar gum is an intermediary between solid and liquid, exhibiting both solid (elastic) and liquid (flow) properties. Its formation depends on factors such as temperature, pH, and the concentration of guar gum. The optimal pH range for gel formation is between 7.5 and 10.5. Various compounds, including borate and transition metal ions, enhance cross-linking with guar gum, thereby increasing its gelling power, viscosity, and resistance to high temperatures[49,50].

Locust bean gum

Carbohydrate molecules are the primary source of natural polymers. These polysaccharides are extracted or isolated from plant seed sources such as locust bean gum, guar gum, tara gum, and tamarind. Polysaccharides, also referred to as gums, are derived from the endosperm of various plant seeds, mainly from the Leguminosae family, where they act as reserve materials during germination. The majority of these polysaccharides possess structural similarities known as galactomannans. Locust bean gum, a type of galactomannan, is a white to creamy white powder produced by milling the seed endosperm of the carob tree (Ceratonia siliqua L.), a member of the legume family found in Mediterranean regions. This species is classified under the subfamily Caesalpinioideae of the Leguminosae family[51]. Locust bean gum (LBG) solutions do not form gels on their own but augment the gelation of other hydrocolloids, such as xanthan and carrageenan. Predominantly used as an additive in the food and beverage industry, LBG functions mainly as a thickening, stabilizing, and gelling agent, as well as an emulsifier. The texture, an essential yet intangible property of food, is greatly affected by these roles[52].

Chemical structure of Locust bean gum

Locust bean galactomannan is composed of the monosaccharide's galactose and mannose. The structure features a linear chain of (1→4)-linked β-D-mannopyranosyl units with (1→6)-linked α-D-galactopyranosyl residues as side chains. Figure 10 reported bellow illustrates the chemical structure of LBG. In its solid state, the galactomannan molecule assumes an extended ribbon-like configuration, whereas in solution, it adopts a semi-flexible coil-like structure. The galactose to mannose ratio in locust bean gum ranges from approximately 1:3.1 to 1:3.9. The α-D-galactosyl residues are distributed along the mannose backbone in random, blockwise, or ordered patterns[51].

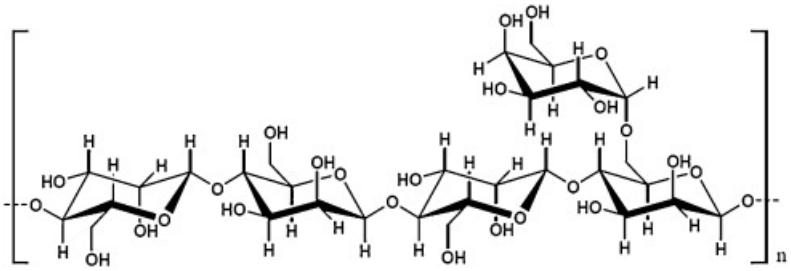


Figure 10. Chemical structure of Locust bean gum[51].

Locust bean gum gels and properties

As mentioned previously in this section on the report, LBG solutions do not form gels on their own but augment the gelation of other hydrocolloids, such as xanthan gum and carrageenan. In this section of the report, we will discuss the synergistic behavior of LBG with Xanthan gum (XG). The synergistic interactions between XG and LBG have been well-documented since their initial discovery, though the exact molecular mechanisms remain debated. Early explanations attributed the synergistic effects to poor or absent interactions due to gum incompatibility, volume exclusion, or weak connections unrelated to specific intermolecular interactions. Another theory suggests the presence of cooperative interactions between the two polysaccharides. The branches of galactomannan in LBG are irregularly spaced along the backbone, with some regions being more branched and others being smoother. Researchers have proposed various sites for these interactions: between the side chains of xanthan helices and the smooth regions of the galactomannan backbone, between the xanthan helix and these smooth regions, or between the disordered xanthan and galactomannan structures. These models are known as the “Tako model,” the “Unilever model,” and the “Norwich model,” respectively[52], shown in figure 11 bellow. Additionally, LBG synergistically interacts with XG to form a thermoreversible gel that exhibits enhanced elasticity and strength, providing a significant advantage for industrial applications of both hydrocolloids. The mass ratio of XG to LBG is crucial in this synergistic interaction. A mixture consisting of 60% XG and 40% LBG demonstrates the strongest synergistic effect, attributed to the more flexible conformation of the XG-LBG complex. It has been found that ordered XG interacts with locust LBG through their side chains, while disordered XG and LBG form gels via backbone-backbone interactions. However, the rheological properties of these gels within food systems and the kinetics of gelation between the XG-LBG binary polymers remain poorly understood[53].

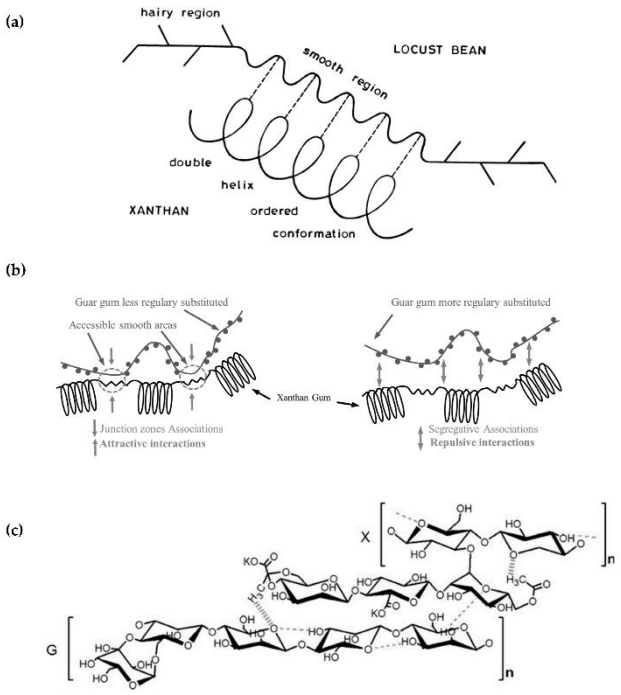


Figure 11. Different models of XG—LBG synergies : (a) Unilever model, (b) Norwich model, (c) Tako model[52].

Table 1 below illustrates some of the chemical characteristics of the plant-based polymers mentioned in the above section of the report , as well as the pricing of these polymers in the European market[54].

Table 1: Properties and pricing of polymers explored in this work

Polymer	**Melting temperature (T_m) [°C]**	**Price[USD/MT]**
Gelatin	31.7–34.2[55]	8850
κ-Carrageenan	68.8[56]	11360
Xanthan gum	64.43	4738
Agar	35-40[57]	19040
Pectin	152[58]	10280
Guar gum	0[59]	1620
Locust bean gum	–10 and 5[60]	-
Satiagum	31.7–34.2[55]	-

A possible rheological approach for the development of gelatin plant-based alternatives in the food industry

Before delving into the potential rheological method outlined in this study for the identification of plant-based gelatin substitutes, it is important to comprehend the characteristics of gelatin that make it such a perfect material for usage in the food industry. Gelatin exhibits numerous unique properties that are not readily replicated by other hydrocolloids. Consequently, an ideal gelatin substitute should possess all or at least some of these properties. The "melt-in-the-mouth" property of gelatin arises from its physical characteristic of melting slightly below the human physiological temperature. This quality imparts a distinctive "melt-in-the-mouth" sensation, leading to an intensified release of flavor and aroma. This behavior is a crucial attribute of gelatin-based systems, such as gelatin-based gel desserts, and is challenging to replicate in other biopolymer systems. Additionally, another unique property of gelatin is the ability to form thermoreversible gels. Gelatin gels are unique among protein and polysaccharide gels in their thermoreversibility, dissolving upon warming. Although some plant biopolymers, discussed in previous sections of this report, such as carrageenan and agar, also exhibit thermally reversible gelation, their melting points are considerably higher than those of gelatin gels. On top of that gelatin is recognized as one of the most adaptable hydrocolloids in the food industry. It performs multiple functions, including gelling, thickening, water-binding, emulsifying, foaming, and film-forming. No other biopolymers match this range of functionalities. Finally, Gelatin is available in various gel strengths and particle sizes, allowing it to be tailored for specific applications. It is easy to use, as it gels within the typical pH range of most foods and does not necessitate the addition of salts or sugars for setting. Finding an ideal substitute for mammalian gelatin is evidently a formidable, if not impossible, task. The development of gelatin alternatives in the food industry should be tailored to specific applications and processes. It is unlikely that a single polymeric compound will universally replace gelatin across all food applications[1]. While there are different aspects that should be considered to replace gelatin in food products, obtaining a nearly identical viscoelastic response from the potential plant-based substitutes certainly plays a pivotal role. In this work we selected certain plant-based polymers that according to the literature are potential candidates for replacing gelatin in the food industry and we demonstrate that small amplitude oscillatory shear as well as Large amplitude oscillation shear can be used to find a “plant-based rheological twin” of gelatin. We decided to solely use the polymer concentration as a tuning parameter, but one could also use the molecular weight (Mw) and temperature. Nonetheless, varying Mw would only result in different polymer concentrations needed for mimicking the gel response of gelatin. For the selected plant-based polymers alternatives to gelatin we use small amplitude oscillatory shear to obtain the rheological dynamic phase diagram for a fixed temperature (room temperature) and Mw. The rheological dynamic phase diagram indicates at which concentration range the sol-gel transition occurs for each plant-based polymer at a given temperature. In the phase diagram polymers can exhibit either Newtonian, viscoelastic liquid or viscoelastic solid (gel) behavior. Ideally viscous flow behavior (Newtonian flow behavior) means that the measured viscosity is independent of the shear rate. Viscoelastic liquids with G'' > G' have a higher loss modulus than storage modulus. The reason for this is that, in most of these materials, there are no such strong bonds between the individual molecules. On the other hand, viscoelastic solids with G' > G'' have a higher storage modulus than loss modulus. This is due to links inside the material, for example chemical bonds or physical-chemical interactions. Utilizing the rheological phase diagram for all polymers in this study we were able to mimic the Linear

Viscoelastic response of gelatin with the plant-based polymers in terms of Viscosity. In order for our work to be even more detailed and comprehensive we decided to use Large amplitude oscillation shear experiments to explore the Non-Linear Viscoelastic region (NLVE). With this set of experiment, we were able to extrapolate information's regarding the Yield Strain and Stress of the samples as well as the hysteresis of the material under constant shear.

3. Materials and Methodology

The goal of this section is to elaborate in detail on the experimental protocol utilized to characterize our plant-based polymeric samples alongside gelatin. Gelatin, Xanthan gum(XG), locust bean gum(LBG), pectin and Satiagum were kindly provided by Fortuin, a Dutch company specializing in the food industry since 1842. Carrageenan was obtained by Sigma-Aldrich, Guar Gum was also obtained by Sigma-Aldrich, and finally Agar (courtesy of Kamperman Lab, University of Groningen) was procured from Boom Lab .

Preparation Protocol

Samples were prepared using Mili Q Pure Water. Different concentrations were prepared for all plant-based polymers as shown in Table 2 bellow.

Table 2: Gelatin and plant-based polymers concentrations tested in this work

Polymers	**Gelatin**	**Carrageenan**	**Xanthan Gum (XG)**	**Agar**	**Blend of XG and LBG**	**Pectin**	**Satiagum**	**Guar Gum**
Concentration of Polymer in Water [% wt/wt]	1	0.05	0.1	1	0.1	3	0.8	0.3
	1.5	0.1	0.3	2	0.3	5	1	0.5
	2	0.3	0.5	3	0.5	7	1.5	0.8
	3	0.4	0.8	4	0.8	9	2	1
	4	0.5	1	5	1	10	3	2
	6	0.6	2	6	2		5	3
	8	0.8	4	8	5		7	4
	10	1	6	10	6		9	5
		1.3	8		8			6
			10					
			13					
			15					
			20					

Polymer solutions were quantified via wt% which is represented as:

$$\text{Wt/wt\%} = \frac{Weight\ of\ Soluble\ (g)}{Weight\ of\ Soluble\ (g) + Weight\ of\ Solvent\ (g)}$$

Solutions were prepared in 20ml glass vials. Based on the polymer concentration wanted to obtain, the desired amount of the polymer was added to the vial. Afterwards, the required amount of mili q pure water was added in order to obtain the concentration in need. The magnetic bead was added to the polymer/water solution and the vial was placed in the magnetic and heating pad. The stirring was set to 150-200 rpm and the heating temperature ranged from 60-80 °C depending on the polymer. The polymer solutions were left at the heating and stirring plates for 20-30 minutes depending once again on the polymer.

Note: A higher temperature than 80°C was avoided in order not to degrade the polymeric solution

Rheological measurements

Rheological measurements were performed using a TA's Discovery Hybrid Rheometer -2 (DHR-2). DHR-2 is a prevalently stress-controlled rheometer with a combined motor and transducer function (CMT). The rheometer employs a single plate (the top plate in this case) to induce stress into the system under scrutiny while simultaneously recording the back stress that the system applies on the plate. While the system is highly sensitive and versatile in its applications, the rheometer does have certain limitations. One of the main constraints is the oscillation torque it measures as the plate reaches the target strain. According to the specifications, the rheometer can detect a minimum oscillation torque of 0.1 μN.m, data obtained below this specific value become unreliable. As a result, measurements below this torque are avoided. When preparing the rheometer for an experiment, it is crucial to consider the geometry used to apply shear to the system. We have several geometries available, all made from stainless steel, which are connected to the motor through a spindle. In this study we only used parallel plates geometry. Parallel plates come in different sizes. Adopting conventional standards, smaller diameter plates are used for highly viscous liquids and soft solids, while, larger diameter plates are used for low-viscosity liquids. Using large plates for less viscous samples improves the oscillation torque noted by the rheometer and therefore provides more accurate data.

In this study we employed two different parallel plates geometries : 40 mm parallel plate(PP) and 25mm parallel plate. As mentioned above the 40mm PP were utilized when the polymer solution was liquid-like in order to obtain the best possible oscillation torque therefore acquiring accurate data out of our experiment. 25mm PP were utilized for polymer solutions that had a solid-like appearance and for highly viscous gels.

Rheological protocol

Before conducting our rheological experiments, we start by calibrating the geometry for inherent inertial, frictional constants of the geometry and map the rotation of the geometry. Once this step is done, we zero the gap, between the two plates. The gap is zeroed to calibrate the height so that the rheometer can precisely measure the gap between the two plates. Since our polymeric solutions had the tendency to evaporate, especially the low concentrations solutions, wet paper towels were placed on either side of the rheometer where the heating would usually take place. This was done to mitigate the evaporation issue and slow down the evaporation of the sample enough so that there was sufficient time to perform the experiments needed. When using the 25mm PP a trap was installed in the bottom plate of the geometry which allowed us to fill this with oil to surround the sample, even further slowing down the evaporation of the polymeric solution. The sample is then loaded on the stage of the base plate and the top plate attached to the spindle is lowered till the sample bulges out from the side into a parabolic ring (donut shape).

After the preparation and loading of the sample in the rheometer was completed a set of desired rheological experiments were performed to characterize the sample.

1) Flow sweep tests.

Flow sweep tests were performed at ambient room temperature when the polymeric solution samples were visually liquid like. Each sample was loaded at room temperature with a loading gap around 1µm. The shear rate regime ranged from 10 to 500 reciprocal seconds and the viscosity of the samples was observed.

2) Dynamic Strain Sweep (DSS)

DSS tests were performed at a constant angular frequency and room temperature and varying strain amplitude to determine the (strain amplitude) range of linear viscoelastic (LVE) response, where the values of the storage and loss moduli, G'(ω) and G"(ω), respectively, do not depend on the strain. This is the linear viscoelastic (LVE). Beyond a critical value of the strain the moduli of G' and G'' are not constant anymore. This marks the non-linear viscoelastic region, where the structure (e.g., chain conformation) of the sample is affected. From the DSS tests we were able to determine the value of strain in which we are in the LVE.

3)Dynamic Frequency Sweep (DFS)

This experiment is conducted after probing the linear regime of the material via DSS. The DFS is performed with a fixed strain mentioned above and a fixed temperature, in the case of this work room temperature. The angular frequency varies from 100 rads/s to 0.1 rads/s. The DFS test provides the viscoelastic properties of a sample at different frequencies, which reflect relevant material timescales. Several parameters can be obtained, such as the Storage (Elastic) Modulus [G'], the Loss (Storage) Modulus [G"], and the Complex Viscosity η*[Pa s].

4)Large amplitude oscillatory shear experiment (LAOS)

This test was performed for polymeric solutions whose rheological response was similar to that of the reference concentration of gelatin. A DSS was performed at three different frequencies(100 rad/s, 10 rad/s and 1 rad/s). A DSS test was performed starting from low percentage of strain to high percentage of strain and immediately after another DSS was performed starting from high percentage of strain to low. This test will allow to check the self-healing properties and hysteresis of the materials as well as draw conclusions on the yielding of the system at different time scales (inverse of frequency) and further shed light on the processability of the samples.

4. Experimental results and Discussion

A comprehensive rheological analysis was performed on the prepared polymeric solutions, with this section dedicated to presenting and interpreting the obtained findings.

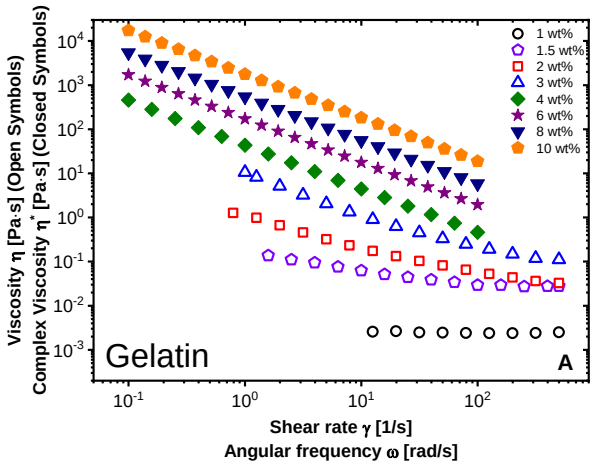
Dynamic Frequency Sweep and Flow Sweep

The dynamic frequency sweep(DFS) is a fascinating concept that utilizes the time-to-length scale equivalence, which suggests that the time required for the plate to complete its oscillation corresponds to the length of the polymer chain segment being examined. We know that the time scale is equivalent to the length scale thus, when analyzing at higher frequencies, we are examining a smaller segment of the polymer chain, whereas at lower frequencies, a larger segment of the chain is being investigated.

The DFS was performed at the angular frequency range of 0.1 rad/s to 100 rad/s. For each concentration of a specific plant-based polymeric solution as well as gelatin, an oscillation strain value was chosen from the linear viscoelastic regime(LVE) obtained through the DSS experiment. The oscillation strain chosen for all the DFS experiments in this work is fixed in the LVE in order to characterize the innate properties of the polymers without introducing any shear stress effect.

In this work it is rather important to understand the dynamic rheological phase diagram of gelatin since it is the polymer in need of replacement. The rheological dynamic phase diagram indicates at which concentration range the sol-gel transition occurs for gelatin aqueous solutions in room temperature (25 °C). DFS and flow sweep data obtained for the various concentrations of gelatin in aqueous solutions are reported in Figure 35 in the appendix.

Low concentrations of gelatin (1 wt%, 1.5 wt%, 2 wt% and 3 wt%) were too liquid like to be measured using the DFS test. The nature of the samples would result in poor oscillation torque and poor signal form the rheometer, hence the flow sweep test was selected for these specific concentrations. As illustrated from Figure 35A in the appendix, 1 wt% of gelatin behaved completely Newtonian with its viscosity being independent of any deformation from the shear rate. For the concentration range of 1.5wt%-3wt% the system behaved like a weak gel. On the other hand, Figure 35B in the appendix shows that from 4 wt% and above gelatin showed gel like behavior with Storage modulus (G') being higher than Loss modulus (G''). This could suggest that the gelation concentration of gelatin in aqueous solution lies between 3 wt% and 4 wt%.



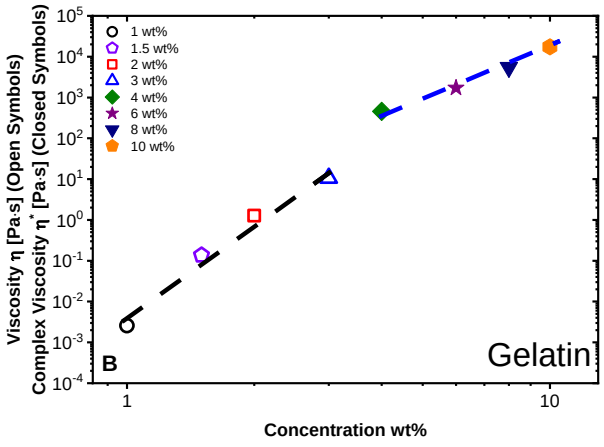


Figure 12.(A) Viscosity and Complex Viscosity as a function of shear rate and angular frequency for gelatin-aqueous solutions. (B) Viscosity and Complex Viscosity as a function of sample concentration, black and blue dotted lines represent the slope for liquid like and gel like samples respectively ,for gelatin-aqueous solutions

Complex Viscosity as well as Viscosity are shown in Figure 12 are obtained through the data of the DFS test. Complex viscosity is a measure of a materials resistance to deformation when subjected to oscillatory (dynamic) shear. Complex viscosity provides an overall idea of the systems viscoelastic properties. As the material gets closer to a Newtonian liquid the complex viscosity slope reduces to 0, represented by a straight horizontal line shown for 1wt%. As the shear rate/ angular frequency for a particular strain is increased the viscosity/complex viscosity of the sample drops, still the sample regains its viscoelastic properties since the strain applied is in LVE. This effect alongside the fact that with an increase in polymer concentration, the viscosity of the sample also increases, can be clearly observed in Figure 12A. Additionally, Figure 12B was prepared by obtaining the viscosity and complex viscosity data for each concentration at the smallest shear rate and smallest frequency accordingly. Figure 12B illustrates the change in the viscosity of the samples when the concentration increases. Black dotted line represents the slope of the liquid like samples and blue dotted lines represents the slope of the gel like samples. It is clear that there is a change in the slope of the liquid like samples compared to the gel like samples. This further confirms our belief that the gelation concentration of gelatin lies between 3wt% and 4wt%.

Similarly to the gelatin-water samples for this work it is key to understand the dynamic rheological phase diagram of all the plant-based polymers that can be used as an alternative to gelatin. Therefore, a rheological study similar to gelatin samples was performed in room temperature (25 °C) for all plant-based polymers studied in this work.

Carrageenan

DFS and flow sweep data obtained for the various concentrations of Carrageenan in aqueous solutions are reported in Figure 36 in the appendix. Similar to gelatin samples low concentrations of carrageenan in aqueous solution were too liquid like to be measured using the DFS test, therefore a flow sweep test was performed. Figure 36A in the appendix illustrates the results of the flow sweep test, with all concentrations having a Newtonian behavior. Their viscosity is completely independent of the any deformation applied through the shear rate. Furthermore, Figure 36B shows the results obtained thorough the DFS test for the remaining concentrations. From 0.4 wt% and above all carrageenan samples showed a gel like behavior with Storage modulus (G') being higher than Loss modulus (G''). This could suggest that the gelation concentration of carrageenan in aqueous solution lies between 0.3 wt% and 0.4 wt%.

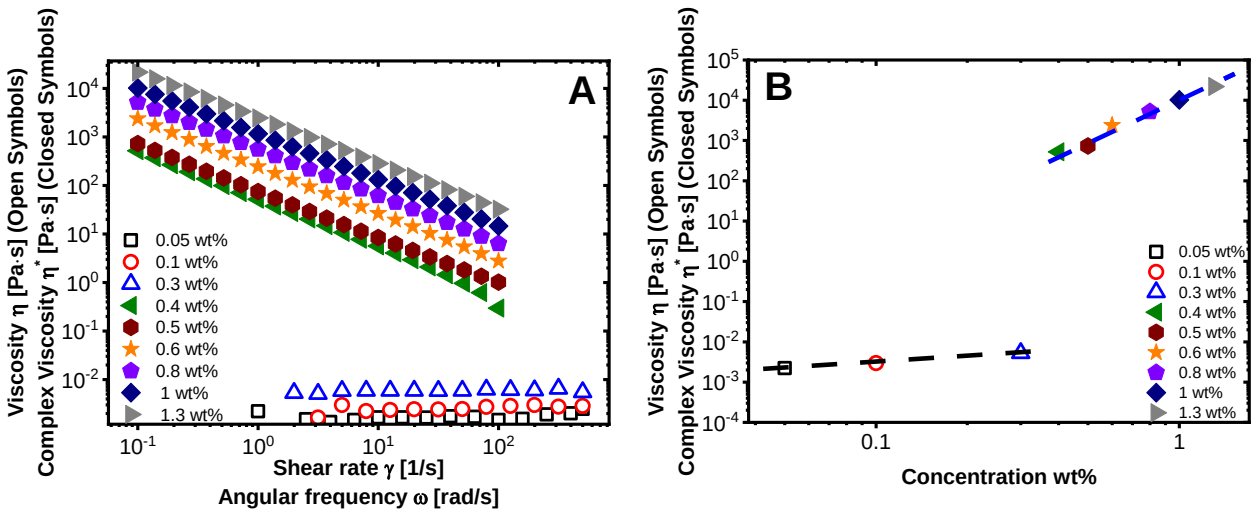


Figure 13. (A) Viscosity and Complex Viscosity as a function of shear rate and angular frequency for carrageenan-aqueous solutions. (B) Viscosity and Complex Viscosity as a function of sample concentration, black and blue dotted lines represent the slope for liquid like and gel like samples respectively ,for carrageenan-aqueous solutions

Figure 13A above reports the complex viscosity and viscosity, which are obtained through the DFS test. As the material gets closer to a Newtonian liquid the complex viscosity's slope reduces to 0, represented by a straight horizontal line indicated by samples 0.05wt%, 0.1wt% and 0.3wt%. The remainder concentrations showed gel like behavior and similar to gelatin samples, the viscosity increased with an increased concentration. Additionally, Figure 13B was prepared by obtaining the viscosity and complex viscosity data for each concentration at the smallest shear rate and smallest frequency accordingly. Figure 13B illustrates the change in the viscosity of the samples when the concentration increases. Black dotted line represents the slope of the liquid like samples and blue dotted lines represents the slope of the gel like samples. It is clear that there is a change in the slope of the liquid like samples compared to the gel like samples. This further confirms our belief that the gelation concentration of carrageenan lies between 0.3wt% and 0.4wt%. Carrageenan samples show a big change in the slope of the liquid like samples compared to the gel like samples. Since in this work we keep a constant room temperature and the Mw was not manipulated, this severe change in the slope can only be attributed in the gelation mechanism of Carrageenan. As discussed in previous section of this work, Carrageenan gelation involves an initial transition from coil to helix, followed by the aggregation of helices to form a three-dimensional network. κ-carrageenans, which is the biopolymer studied in this work, transitions to a double-helical structure prior to gelation. Helix formation and the consequent gelation likely result from intra and intermolecular interactions, such as hydrogen bonds and van der Waals forces, between hydroxyl groups and potentially the hemiacetal oxygen. Hence, this sudden change in viscosity observed in Figure 13B is mainly attributed to this gelation mechanism.

Agar

DFS and Flow sweep data obtained for various concentrations of agar in aqueous solutions are reported in Figure 37 in the appendix. 0.1wt% concentration behaved completely Newtonian as samples viscosity remained completely independent from the deformation applied through the shear rate. From 0.3wt% and above all agar samples showed a gel like behavior with Storage modulus (G') being higher than Loss modulus (G"). One can draw the conclusion that the gelation concentration of agar in aqueous solutions lies between 0.1wt% and 0.3wt%.

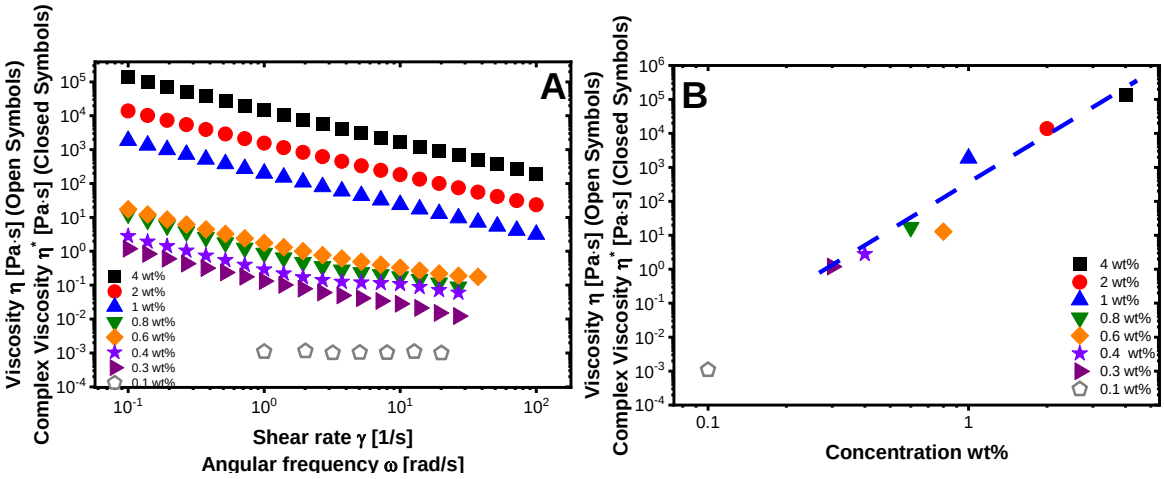


Figure 14.(A) Viscosity and Complex Viscosity as a function of shear rate and angular frequency for Agar-aqueous solutions. (B) Viscosity and Complex Viscosity as a function of sample concentration, blue dotted lines represent the slope for gel like samples, for Agar-aqueous solutions.

Figure 14A above reports the complex viscosity and viscosity, which are obtained through the DFS test. As the material gets closer to a Newtonian liquid the complex viscosity's slope reduces to 0, represented by a straight horizontal line indicated by sample 0.1wt%. The remainder concentrations showed gel like behavior, also supported from the data shown in figure 37 in the appendix. Additionally, Figure 14B was prepared by obtaining the viscosity and complex viscosity data for each concentration at the smallest shear rate and smallest frequency accordingly. Figure 14B illustrates the change in viscosity of the samples when the concentration is increased. Blue dotted lines represent the slope of the gel like samples. Agar samples in aqueous solution have the ability to form gels from a very small concentration. Similar to Carrageenan this can be attributed to the gelation mechanism of Agar which is reported in previous section of this work. Gelation in agar is solely due to its agarose content, which enables the polysaccharide to form helical structures comparable to those seen in carrageenans. Agar gelation also results from the aggregation of these helices, although unlike carrageenan, it does not require ions for stabilization. Agarose forms 'physical gels,' meaning that their structure is entirely formed by the polymer molecules aggregating through hydrogen bonds. This results in a cross-linked network with the agarose chains adopting helical conformation. These helices play a role in creating the junction zones required for the formation of the gel network. Upon cooling, these higher-order structures coalesce to generate bundles, leading to the establishment of robust gel structures. The gelation mechanism for agar is very similar to the one imposed in the carrageenan. Notably, agar forms left-handed helices, whereas kappa and iota carrageenans form right-handed helices. Additionally, the helical pitch in agar is shorter than in carrageenan, which may be attributed to agar's lower sulfate content, leading to a tighter and more compact network.

Guar Gum

DFS data obtained for guar gum for various concentrations in aqueous solution are reported in Figure 38 in the appendix. Guar gum at low concentrations such as 0.3 wt% and 0.5 wt% behaved like a liquid with Loss modulus (G'') being higher than Storage modulus(G'). On the other hand, samples showed a gel like behavior for concentrations above 2 wt%. The concentration range of 0.8wt% to 1 wt% is believed to be the transition concentration for the gelation of the Guar gum samples.

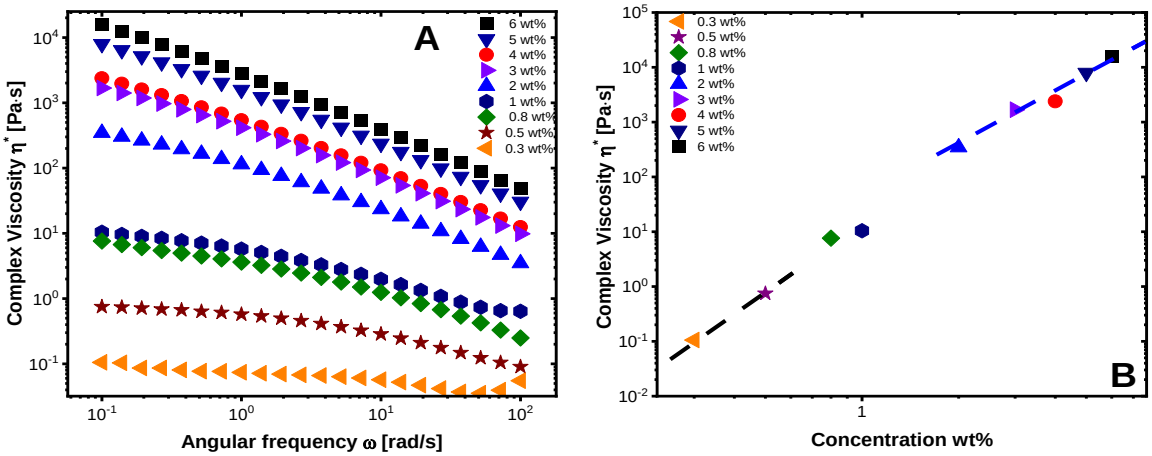


Figure 15. (A) Complex Viscosity as a function of angular frequency for Guar gum-aqueous solutions. (B) Complex Viscosity as a function of sample concentration, black and blue dotted lines represent the slope for liquid like and gel like samples respectively ,for Guar gum-aqueous solutions

Figure 15A above reports the complex viscosity, which is obtained through the DFS test. In the case of Guar gum none of the concentrations showed any Newtonian behavior. It is evident that high concentrations of Guar gum showed a gel like behavior with lower concentrations behaving like a weak gel. Similar to other biopolymer samples studied in this work the viscosity of the polymer increases with polymer concentration. Figure 15B was prepared by obtaining the viscosity and complex viscosity data for each concentration at the smallest shear rate and smallest frequency accordingly. Figure 15B illustrates the change in the viscosity of the samples when the concentration increases. Black dotted line represents the slope of the liquid like samples and blue dotted lines represents the slope of the gel like samples. It is clear that there is a change in the slope of the liquid like samples compared to the gel like samples. This further confirms our belief that the gelation concentration of Guar gum lies between 0.8wt% and 1wt%. This change in slope is mainly attributed to the gelation mechanism of Guar gum which is reported in previous section of the report. Guar gum gelates when the hydroxyl groups, predominantly from galactose side chains attached to the mannose backbone, interact with water molecules, leading to intermolecular chain entanglement. This entanglement between guar gum and water results in increased viscosity, causing gelling and thickening of the solution. The straight-chain guar gum molecules are randomly interconnected by cross-linkers. These cross-linking agents have two active sites that form intermolecular bonds with the hydroxyl groups of polymer chains, creating a closed-loop structure. When water is added to this cross-linked material, it becomes entrapped within the network, significantly increasing the water absorption and retention capacity of the hydrogel system. The gel formed by guar gum is an intermediary between solid and liquid, exhibiting both solid (elastic) and liquid (flow) properties as shown in figure 15B.

Pectin

DFS data obtained for pectin in aqueous solutions for various concentrations are reported in Figure 39 in the appendix. Pectin samples behaved like a liquid for the smallest concentration of 3wt% with Loss modulus (G") being above Storage modulus (G'). All the above concentrations, 5wt% -10wt%, showed a gel like behavior. The 3wt% sample was measured from the initial frequency of 100 rad/s but not all data were selected to be presented as a result of poor signal from the instrument and the low torque values outside of the limits of the rheometer, making these data unreliable to be used in our study. This could suggest that the gelation concentration for pectin lies between 3wt% and 5 wt%.

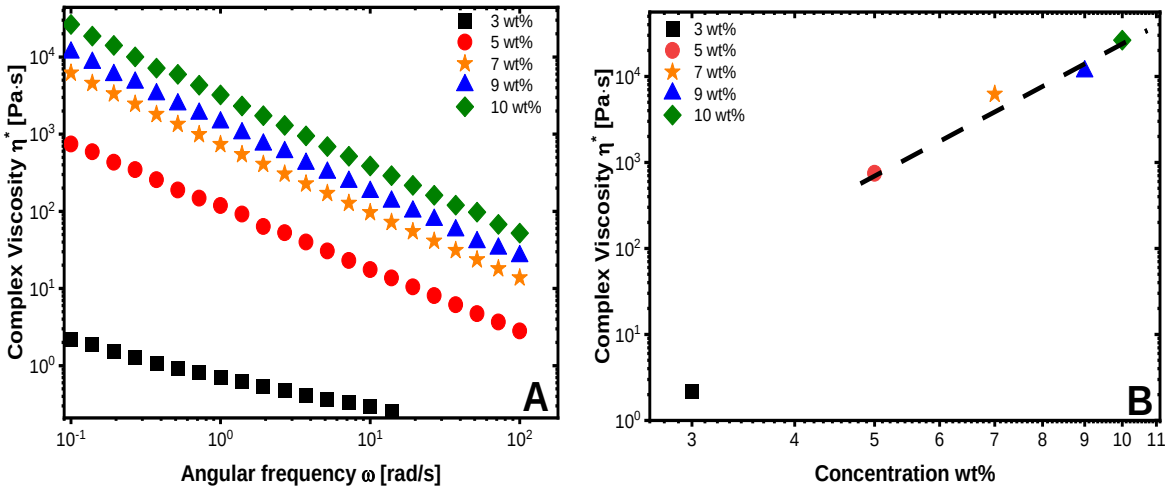


Figure 16.(A) Complex Viscosity as a function of angular frequency for Pectin-aqueous solutions. (B) Complex Viscosity as a function of sample concentration, black dotted lines represent the slope for gel like samples, for Pectin-aqueous solutions.

Figure 16A above reports the complex viscosity, which is obtained through the DFS test. In the case of pectin none of the concentrations showed any Newtonian behavior. It is evident that high concentrations of pectin showed a gel like behavior with lower concentrations, 3wt%, behaving like a liquid. Similar to other samples studied in this work the viscosity of the polymer increases with polymer concentration. Figure 16B was prepared by obtaining the viscosity and complex viscosity data for each concentration at the smallest shear rate and smallest frequency accordingly. Figure 16B illustrates the change in the viscosity of the samples when the concentration increases. Black dotted line represents the slope of the gel like samples. It is evident that the gelation concentration of Pectin samples lies between 3 and 5wt%. The gelation mechanism of Pectin differs from other biopolymers. As reported previously in this work, the gelation mechanism of pectins is primarily determined by their degree of esterification. In LM pectins, gelation occurs through specific non-covalent ionic interactions between sequences of galacturonic acid residues in the pectin backbone and divalent cations, such as calcium. On the other hand, gelation for HM pectins is described by the "shifted egg-box" model. In the "shifted egg-box" model two antiparallel pectin chains engage in gelation but with a notable axial misalignment. The process is characterized by two distinct phases: the initial formation of dimers followed by negligible subsequent aggregation of these dimers. The primary driving force behind the formation of pectin egg-box dimers is electrostatic interaction, leading to cross-linking between separate polymer molecular chains. This cross-linking effectively reduces the electrostatic repulsion between polymer molecules, thereby facilitating the structuring of the gel. Additionally, other forces such as hydrogen bonding, van

der Waals forces, and hydrophobic interactions also play significant roles in the gel formation process. These interactions collectively enhance the stability and integrity of the gel network

Satiagum

DFS data obtained for Satiagum in various concentrations are reported in Figure 40 in the appendix. Satiagum in aqueous solutions showed a liquid like response for the concentration range of 0.8wt% - 1.5wt%. One can observe that the transition concentration for the gelation of Satiagum is the 2wt%. All the remaining concentrations between 3wt% and 9wt% showed a gel like response.

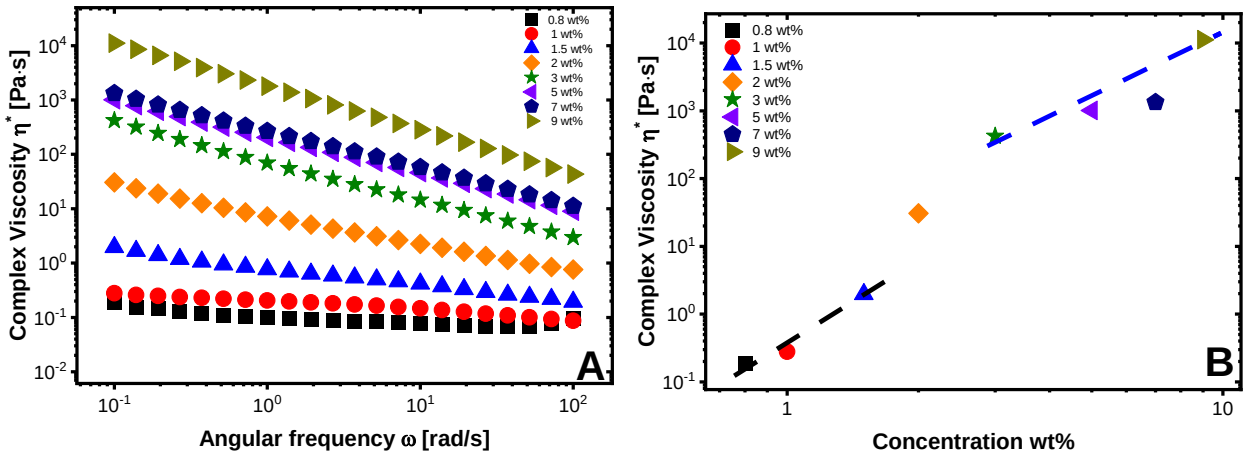


Figure 17. (A) Complex Viscosity as a function of angular frequency for Satiagum-aqueous solutions. (B) Complex Viscosity as a function of sample concentration, black and blue dotted lines represent the slope for liquid like and gel like samples respectively ,for Satiagum-aqueous solutions

Figure 17A above reports the complex viscosity, which is obtained through the DFS test. In the case of Satiagum none of the concentrations showed any Newtonian behavior. It is evident that high concentrations of Satiagum showed a gel like behavior with lower concentrations behaving like a liquid. Similar to other samples studied in this work the viscosity of the polymer increases with polymer concentration. Figure 17B was prepared by obtaining the viscosity and complex viscosity data for each concentration at the smallest shear rate and smallest frequency accordingly. Figure 17B illustrates the change in the viscosity of the samples when the concentration increases. Black dotted line represents the slope of the liquid like samples and blue dotted lines represents the slope of the gel like samples. It is clear that there is a change in the slope of the liquid like samples compared to the gel like samples. This further confirms our belief that the gelation concentration of Satiagum is the 2wt%. This change in slope is mainly attributed to the gelation mechanism of Satiagum which is the same as the Carrageenan, since Satiagum is a type of Carrageenan.

Xanthan Gum

DFS data obtained for Xanthan gum in aqueous solutions in various concentrations are reported in Figure 41 in the appendix. The DFS data are split in two separate graphs to make them easier to understand. For the whole concentration range Xanthan gum in aqueous solutions behaves like a gel.

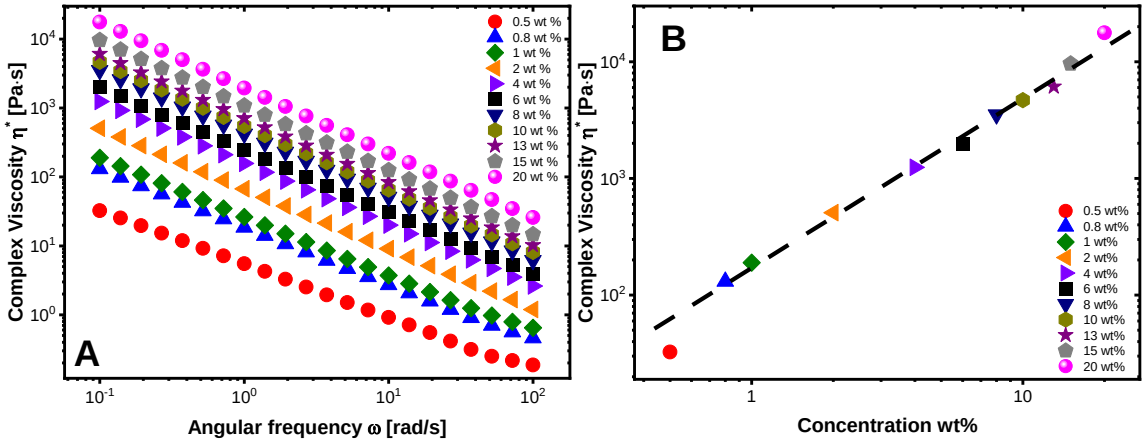


Figure 18. (A) Complex Viscosity as a function of angular frequency for Xanthan Gum-aqueous solutions. (B) Complex Viscosity as a function of sample concentration, black dotted lines represent the slope for gel like samples, for Xanthan Gum-aqueous solutions.

Figure 18A above reports the complex viscosity, which is obtained through the DFS test. In the case of Xanthan gum none of the concentrations showed any Newtonian behavior. It is evident that the whole concentration range of Xanthan gum showed a gel like behavior. Similar to other samples studied in this work the viscosity of the polymer increases with polymer concentration. Figure 18B was prepared by obtaining the viscosity and complex viscosity data for each concentration at the smallest shear rate and smallest frequency accordingly. Figure 18B illustrates the change in the viscosity of the samples when the concentration increases. Black dotted line represents the slope of the gel like samples. It is evident that the gelation concentration of Xanthan Gum samples lies below 0.5wt%. It is rather remarkable that Xanthan Gum behaves like a gel thorough the whole concentration range explored in this work, but this is once again attributed to the gelation mechanism of Xanthan Gum. While Xanthan Gum is typically viewed as a non-gelling polysaccharide that exhibits shear-thinning behavior in solution, it can form a gel in the presence of trivalent ions or when combined with other polysaccharides or proteins. In aqueous solutions, the trisaccharide side chains interact with the polymer backbone, stabilizing the overall conformation through non-covalent interactions, such as hydrogen bonding. In a good solvent, electrostatic repulsion between charges on the side chains causes the xanthan gum chains to extend. Conversely, in a poor solvent, the chains contract, reducing the hydrodynamic volume and consequently lowering solution viscosity. Xanthan gum undergoes a conformational transition from a helical structure (considered as a rigid rod) to a random coil (flexible chains) under external stimuli, such as changes in temperature. However, xanthan gum does not form true gels at any concentration, largely due to its reliance on weak non-covalent intermolecular interactions, such as hydrogen bonding, rather than hydrogels. In contrast, hydrogels are crosslinked 3-D networks of hydrophilic polymers that swell in water or biological fluids but do not dissolve.

Locust Bean Gum

DFS data acquired for the blend of XG and LBG (ratio 50:50) at various concentrations are reported in Figure 42 in the appendix . One can observe than even for very small concentrations, 0.1wt%, the blend behaves completely gel like. The 0.1wt% sample was measured from the initial frequency of 100 rad/s but not all data were selected to be presented as a result of poor signal from the instrument and the low torque values outside of the limits of the rheometer, making these data unreliable to be used in our study. The blend behaves completely like a gel throughout the whole concentration range explored in this work. This is something expected from the literature since Locust bean gum typically enhances the properties of the polymer it is blended with.

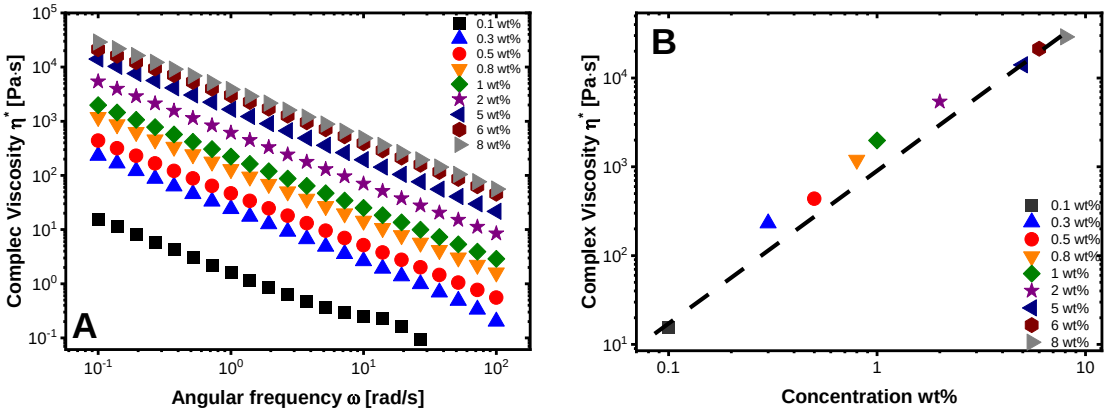


Figure 19. (A) Complex Viscosity as a function of angular frequency for the Blend-aqueous solutions. (B) Complex Viscosity as a function of sample concentration, black dotted lines represent the slope for gel like samples, for the Blend-aqueous solutions

Figure 19A below reports the complex viscosity, which is obtained through the DFS test. In the case of the blend of xanthan gum and locust bean gum none of the concentrations showed any Newtonian behavior. It is evident that the whole concentration range of the blend showed a gel like behavior. Similar to other samples studied in this work the viscosity of the polymer increases with polymer concentration. Figure 19B was prepared by obtaining the viscosity and complex viscosity data for each concentration at the smallest shear rate and smallest frequency accordingly. Figure 19B illustrates the change in the viscosity of the samples when the concentration increases. Black dotted line represents the slope of the gel like samples. It is evident that the gelation concentration of the Blend samples lies below 0.1wt%. It is rather remarkable that the Blend of XG and LBG behaves like a gel thorough the whole concentration range explored in this work, but this is once again attributed to the combination of the gelation mechanism of XG and LBG. As mentioned previously, XG is typically viewed as a non-gelling polysaccharide that exhibits shear-thinning behavior in solution, it can form a gel when combined with other polysaccharides. LBG solutions do not form gels on their own but augment the gelation of other hydrocolloids, such as xanthan gum. LBG synergistically interacts with XG to form a thermoreversible gel that exhibits enhanced elasticity and strength, providing a significant advantage for industrial applications of both hydrocolloids. The mass ratio of XG to LBG is crucial in this synergistic interaction. It has been found that ordered XG interacts with locust LBG through their side chains, while disordered XG and LBG form gels via backbone-backbone interactions. However, the rheological properties of these gels within food systems and the kinetics of gelation between the XG-LBG binary polymers remain poorly understood.

Since gelatin used in the food industry is primarily used in the form of a gel, we selected a concentration of gelatin that has the ability to form strong gels (10 wt%) and tried to mimic the viscosity properties of that specific concentration with the plant-based polymers able to replace it in the food industry. Hence, Figure 21 below reports data obtained from the dynamic frequency sweep test, as well as from flow sweep test for each concentration that the samples were measured at the smallest angular frequency and the smallest shear rate. The green dotted line represents the viscosity value of 10 wt% gelatin sample. In this plot we can observe what concentration of the plant-based polymers matches the best the viscosity of our reference sample (10wt% gelatin). From Figure 21 one can extract the optimal concentration of the plant-based polymers that will match the refence concentration of Gelatin. The best carrageenan concentration that will match the one of gelatin is 1.3wt% , xanthan gum is the 20wt%, Agar is the 2wt%, the blend of Xanthan Gum and Locust bean gum is 5wt%, Satiagum as well as Pectin are 9wt% and finally Guar gum is 5wt%.

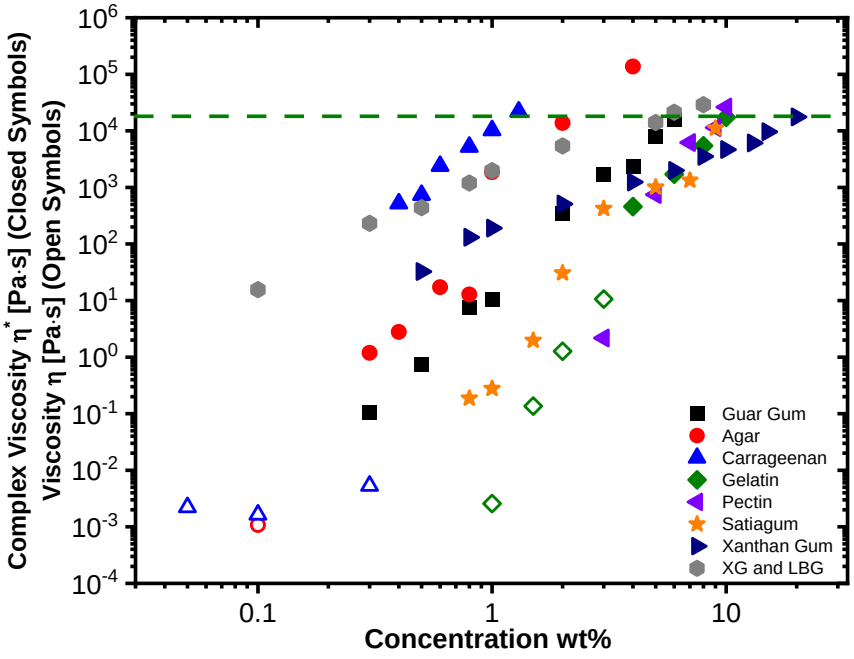


Figure 20. Complex Viscosity and Viscosity as a function of sample concentration for all samples, green dotted line represents the Viscosity Value of the 10wt% Gelatin.

On continuation of figure 21 above, we have matched the viscosity of the reference sample, gelatin 10wt%, with the plant-based polymers for the whole frequency spectrum of 100rad/s to 0.1 rad/s. Data shown in Figure 22 below depicts exactly that, the comparison between the selected reference concentration of gelatin (10 wt%) and the concentrations of the plant-based polymers that match the viscosity of that specific concentration.

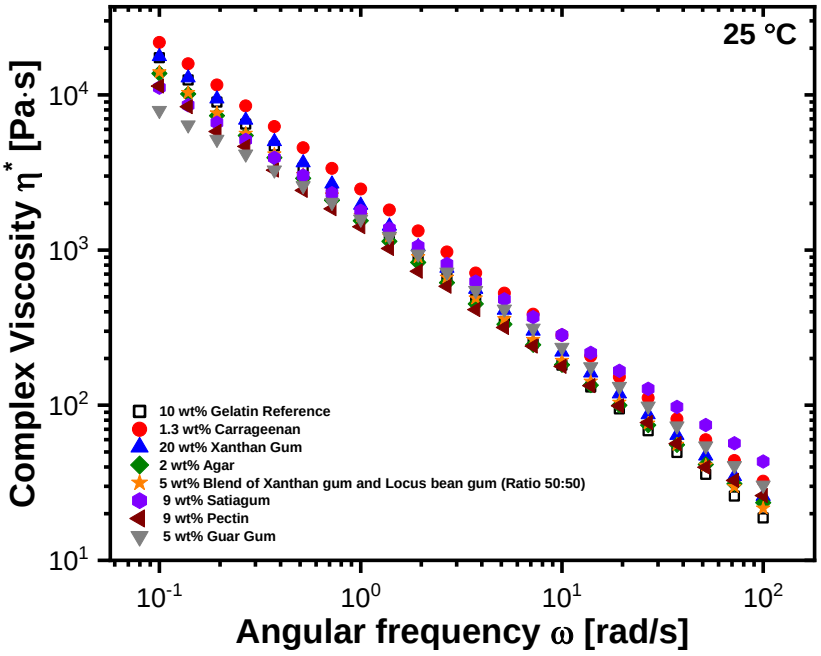


Figure 21. Comparison of the complex viscosities between the reference concentration of gelatin 10wt%(open symbols) and the plant-based polymers (Closed symbols)

Since the goal of this work is to find a plant-based polymer suitable to fully replace gelatin in the food industry, in collaboration with Fortuin we managed to develop one of the companies commercially available product by employing the plant-based polymers studied in this work and incorporating them in the actual recipe for making the product. The plant-based polymers replaced the gelatin found in the recipe of the product but the rest of the ingredients found in the recipe remained the same. A rheological study was performed in the product dough for selected plant-based polymers in this work as well as gelatin. A DFS test was performed on all product doughs, the temperature was set to room temperature(25°C) and to make the comparison even fairer, the gap between the two plates in the rheometer was fixed at 2000μm. At this gap the plates were fully covered by the product dough. The product doughs were prepared and measured the same day to avoid dehydration issues that could appear by leaving the product doughs at room temperature for extended period of time. The DFS test results are illustrated in Figure 23 below.

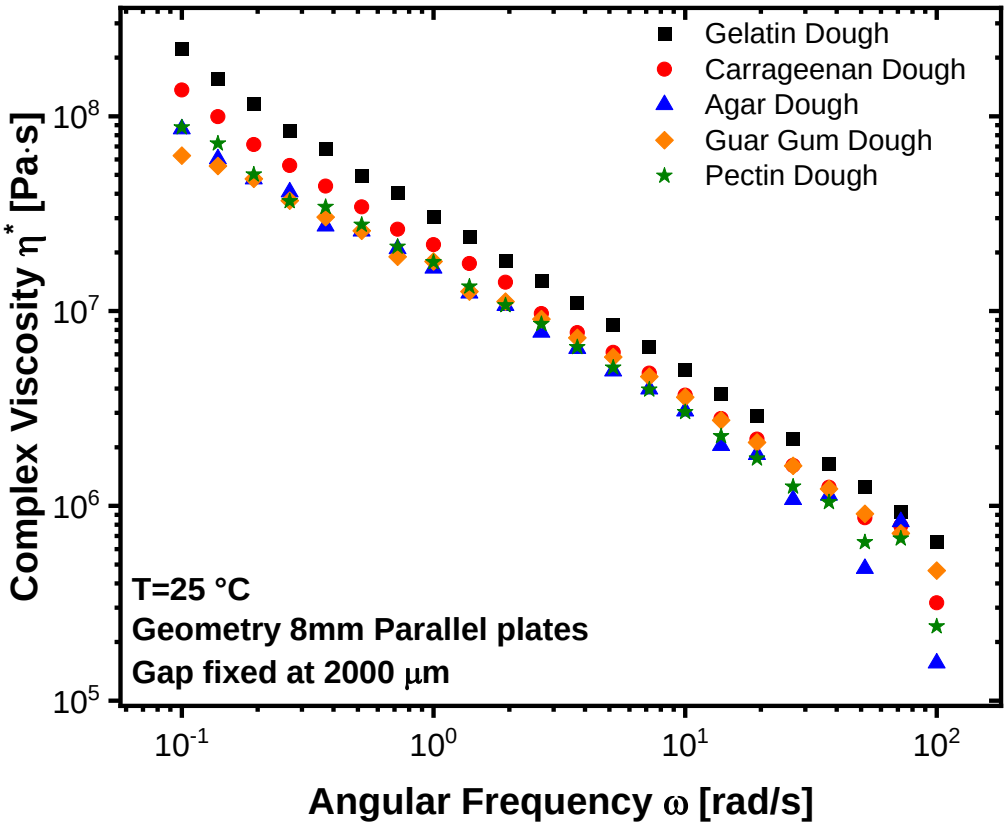


Figure 22. Comparison between the gelatin and plant-based product doughs

One can observe that the viscosity of gelatin dough doesn't fully match the one of plant-based polymers doughs. Even though the only difference in the preparation of the dough was the alteration of gelatin with the plant-based polymers. As a result of this change in the dough recipe, the plant-based polymer doughs required extra amount of water in order to form a dough, compared to the gelatin dough. The extra amount of water needed for the doughs ,reported in table 3 below, made them softer and very brittle in the texture compared to the elastic texture of the gelatin dough. We believe that the extra amount of water used is the reason for difference in the viscosity of the doughs simply because when a large amount of water is added extra the water is acting as the binding agent in the recipe and not the polymer.

Table 3: Extra amount of water needed for each product dough

Polymer	Gelatin	Carrageenan	Agar	Guar gum	Pectin
Extra amount of Water [g]	-	50	40	30	50

Nonlinear Viscoelastic Region (NLVE)

In order to further understand the behavior of gelatin as well as the plant-based polymers we decided to perform a set of Large amplitude oscillatory shear(LAOS) experiments for all the concentrations of the plant-based polymers that matched our reference of 10wt% Gelatin. We tried to explore the NLVE region of all the samples to get a better understanding of the behavior and the mechanical properties of samples in order to provide further information on which plant-based polymer rheologicaly is the best candidate to replace gelatin in the food industry.

The LAOS experiment is in principle a DSS experiment starting from a small percentage of strain all the way to the highest percentage of strain possible for each sample. Immediately after the first run of the DSS test, another DSS experiment was performed but this time the experiment started from the highest percentage of strain achieved in the first run all the way to the initial strain of the first test. The LAOS test was performed at three different frequencies, 100 rad/s, 10 rad/s and 1 rad/s. 100 rad/s represents a processing speed, typical for industries, 1 rad/s is associated with the polymer being in a relaxed state and finally 10 rad/s is something in between processing speed and relaxing of the sample.

Figure 24 below depicts the result of the LAOS experiment for Gelatin at the three different frequencies stated above. One can observe that gelatin sample showed impeccable behavior with no hysterics observed as the second DSS run almost matched the values of the first DSS run throughout the whole frequency range explored.

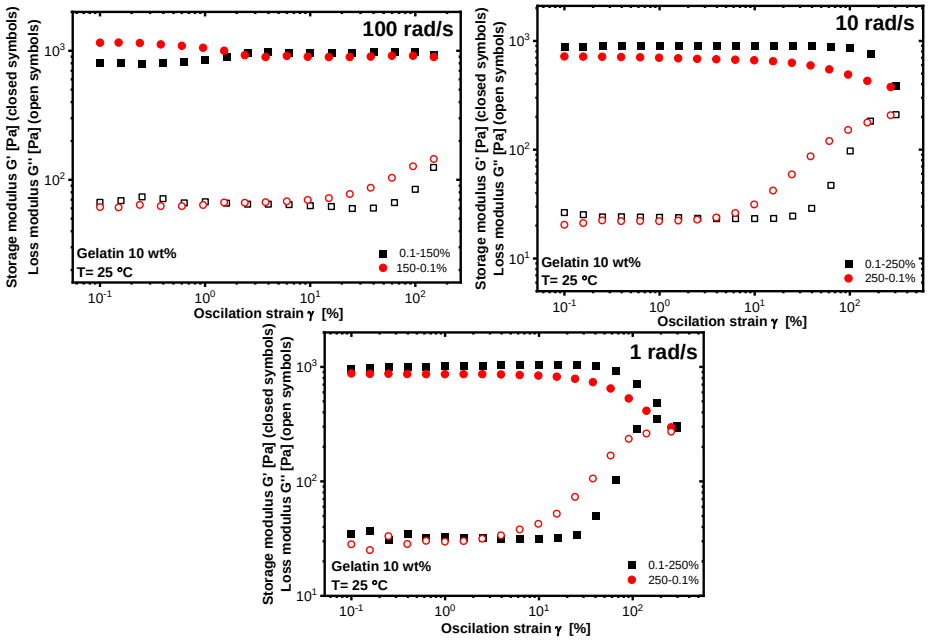


Figure 23. LAOS experiment for 10wt% gelatin at 100,10 and 1 rad/s (First run black symbols, second run Red symbols)

Similarly to gelatin, the same experiment was performed for all the plant-based polymers investigated in this work. Figure 25 below shows the results for the LAOS experiment for 1.3 wt% Carrageenan. For carrageenan at 100rad/s a small hysteric is observed especially at the higher percentage of strain, the NLVE region, but as the frequency becomes lower carrageenan showed incredible overlapping of two DSS runs.

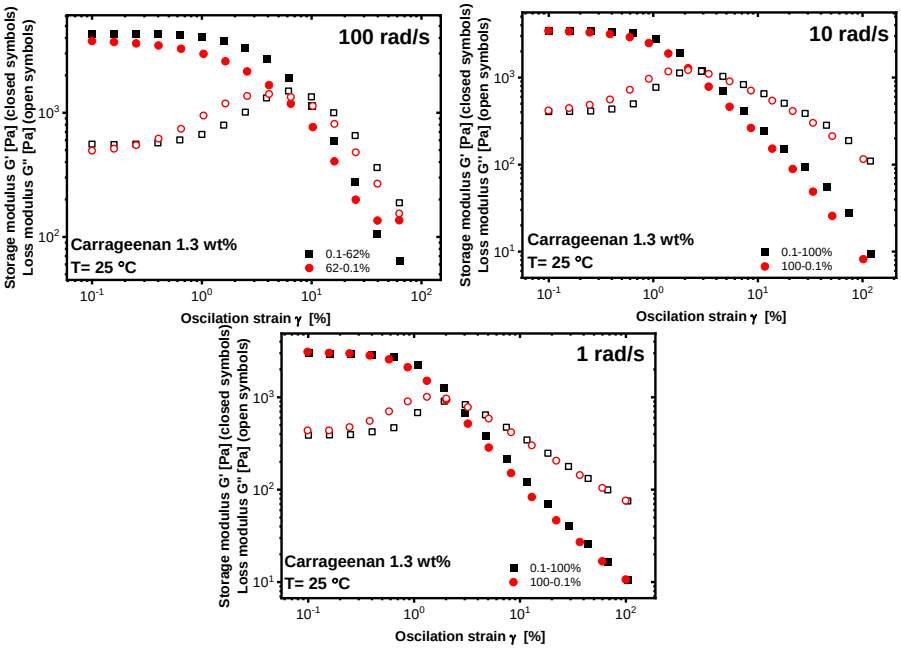


Figure 24.LAOS experiment for 1.3wt% Carrageenan at 100,10 and 1 rad/s (First run black symbols, second run Red symbols)

The next plant-based polymer subjected to the LAOS experiment was Agar. Figure 26 below illustrates the results obtained from the LAOS experiment. Agar showed similar behavior to carrageenan with a hysteric at the NVLE region at 100 rad/s but at 10rad/s and 1 rad/s showed identical behavior between the two DSS runs.

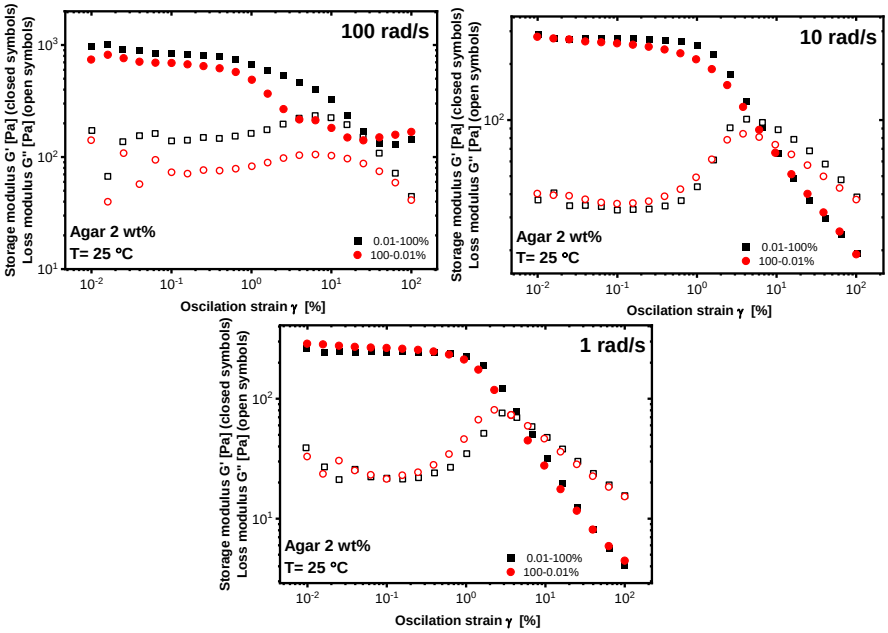


Figure 25.LAOS experiment for 2wt% Agar at 100,10 and 1 rad/s (First run black symbols, second run Red symbols)

LAOS experiments were also performed for Guar gum. Figure 27 below shows the results obtained from these experiments. Guar gum behaved completely identical between the two DSS runs throughout the whole oscillation strain spectrum and the whole frequency range explored in the experiments.

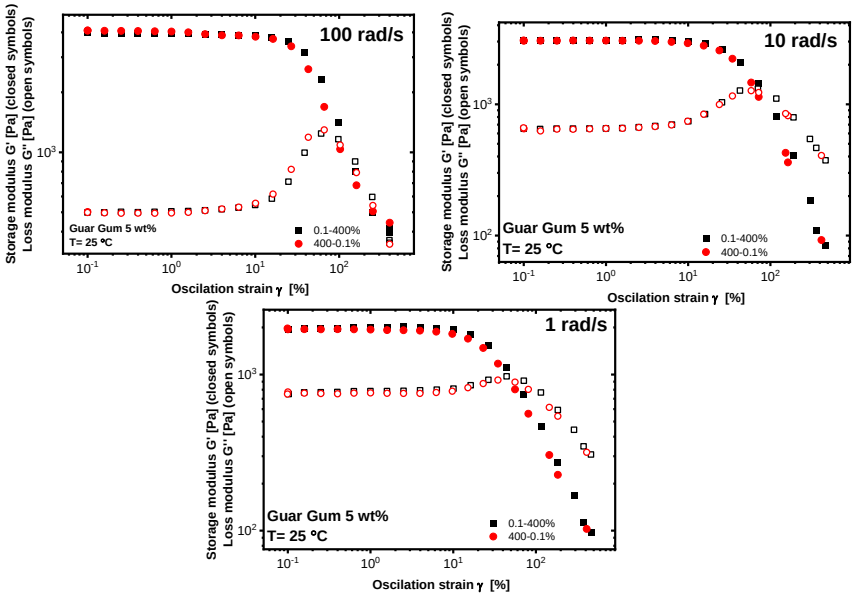


Figure 26.LAOS experiment for 5wt% Guar Gum at 100,10 and 1 rad/s (First run black symbols, second run Red symbols)

The following plant-based polymer to be measured using the LAOS technique was 9wt% pectin. Figure 28 below illustrates the results of the experiment. At 100 rad/s pectin showed a substantial difference between the two DSS runs both in the LVE region of the strain percentage but also in the NLVE region. This behavior was continued in the 10 rad/s but at a less extent. Finally at 1 rad/s Pectin showed similar behavior in the LVE region of the strain percentage but some hysterics was still observed at the NVLE region.

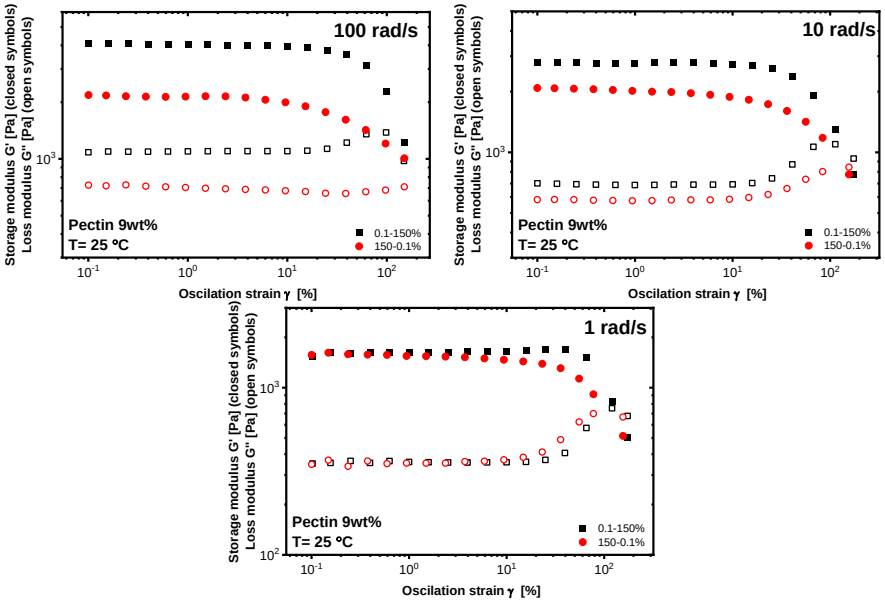


Figure 27. LAOS experiment for 9wt% Pectin at 100,10 and 1 rad/s (First run black symbols, second run Red symbols)

The next plant-based polymer that we performed the LAOS experiment was Satiagum. Figure 29 below illustrates the results of the experiment. Satiagum in all three frequencies showed some correlation between the two DSS runs in the low percentage of strain, LVE region, but at the same time had a substantial deviation between the two DSS runs in the higher percentage of strain, NLVE region.

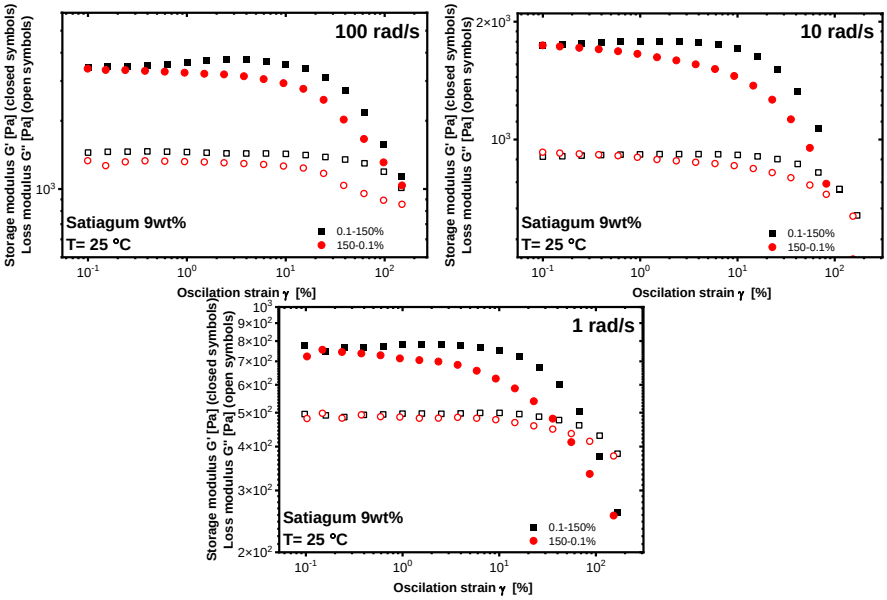


Figure 28.LAOS experiment for 9wt% Satiagum at 100,10 and 1 rad/s (First run black symbols, second run Red symbols)

Xanthan gum was the next polymer to be subjected in the LAOS experiment. Figure 30 below illustrates the results obtained from the LAOS experiment. 20wt% of Xanthan gum was able to be measured at very high percentage of strain but for all three frequencies tested and the whole strain range, LVE and NLVE, there was a significant difference between the two DSS runs.

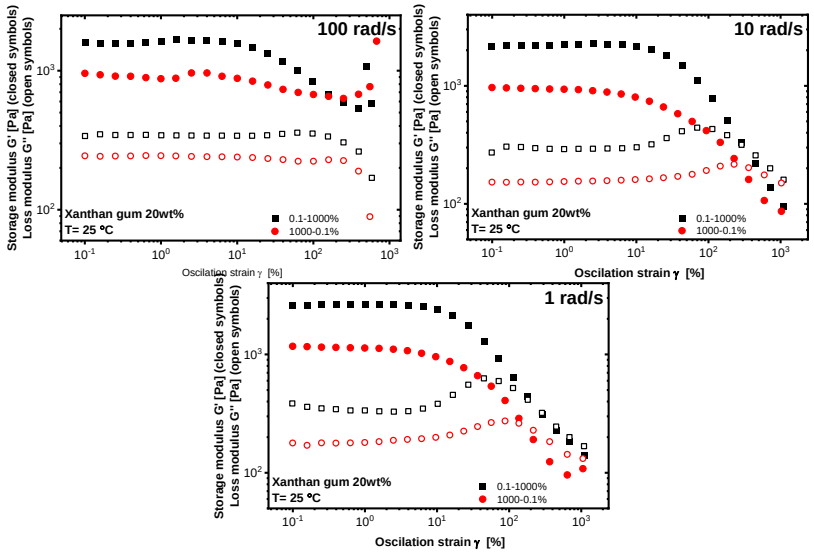


Figure 29.LAOS experiment for 20wt% Xanthan Gum at 100,10 and 1 rad/s (First run black symbols, second run Red symbols)

Last but not least, the blend of Xanthan gum and Locust bean gum (ratio 50:50) was also explored using the LAOS experiment. Figure 31 below illustrates the results of this test. When Xanthan gum is blended with Locust bean gum the behavior of the polymer changes completely. With the incorporation of Locust bean gum the blend showed incredible overlapping between the two DSS runs for the whole strain percentage range both in the LVE and in the NLVE region. Similar behavior was observed in all three frequencies tested.

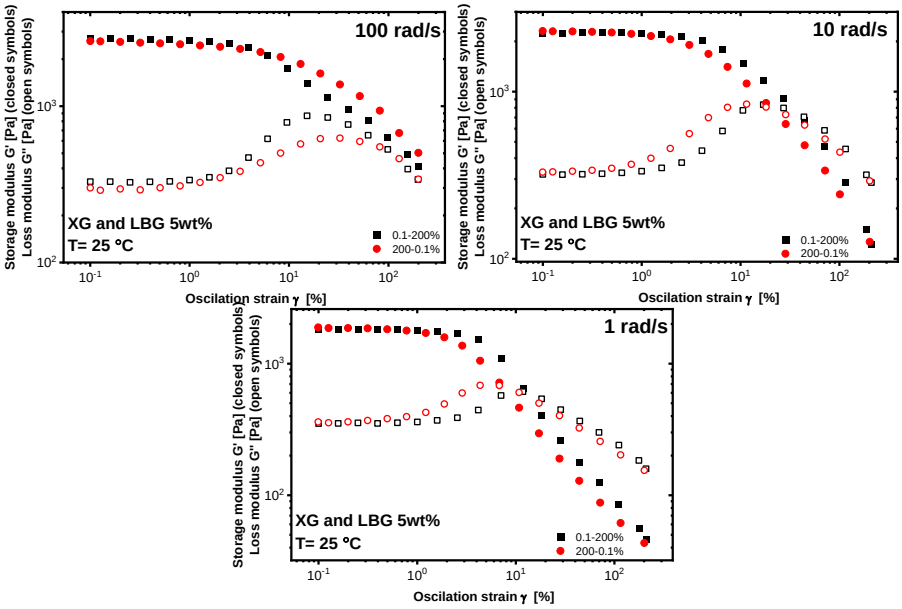


Figure 30.LAOS experiment for 5wt% Blend of Xanthan Gum and Locust bean gum (ratio 50:50) at 100,10 and 1 rad/s (First run black symbols, second run Red symbols)

In order for someone to attempt to replace gelatin with any plant-based polymer for the food industry, there are two main factors to be considered. As a first parameter is the ability of the plant-based polymer to have similar LVE compared to gelatin. In this report this is demonstrated in the begging of this section of this report. We matched the Complex viscosity of a specific concentration of gelatin with the corresponding concentration of plant-based polymers that match that specific reference concentration. The second and also important parameter that a plant-based polymer must possess in order to successfully replace gelatin is a very large yield strain. We were able to obtain the yield strain as well as the yield stress from the LAOS experiments performed.

In order to obtain the yield strain and yield stress we based off our approach to the work of C Christopoulou, G Petekidis et al[61]. Figure 32 below is a DSS test for 2wt% Agar at 100 rad/s illustrating how we obtained the Yield Strain and Stress . The yield point is determined from the change of the slope between the low strain and high strain regions of the Stress-Strain curve, shown in red Symbols-line below. The blue dotted line represents the Yield Strain (γ_y) and the green dotted line represents the Yield Stress (σ_y). The same method was used to acquire the Yield stress and strain for all plant-based polymer and gelatin at the three different frequencies of 100 rad/s, 10 rad/s and 1 rad/s.

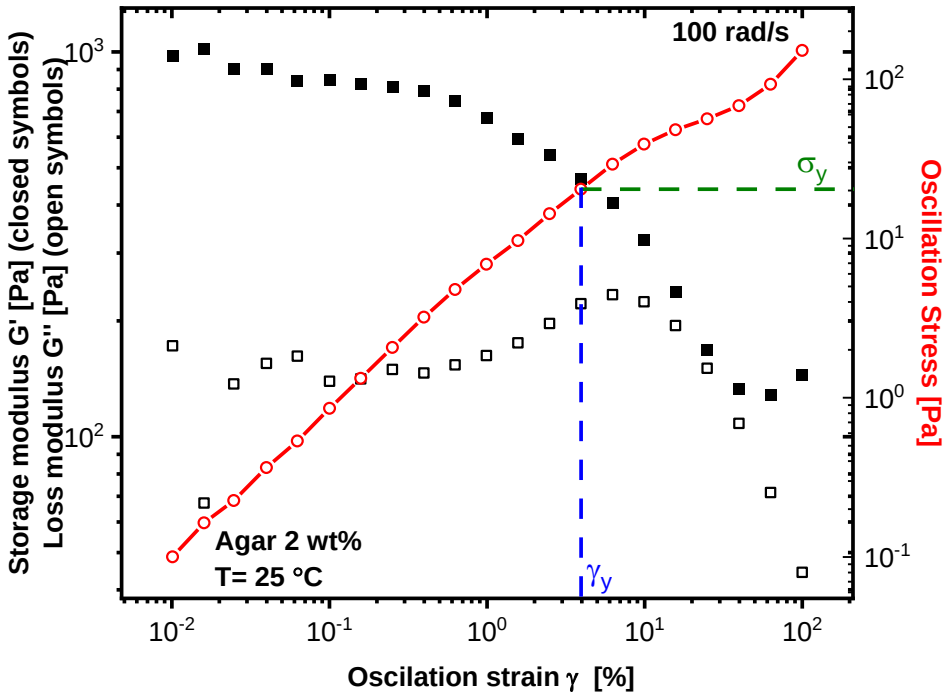


Figure 31. DSS test to characterize Yielding for 2wt% Agar at 100 rad/s, green dotted line represents the Yield Stress, Blue dotted line represents the Yield Strain.

After obtaining the values for the Yield Strain, we plotted them in the form of histogram. Figure 33 below shows these results for all plant-based polymers at the concentration that match our reference concentration of 10wt% gelatin at the three different frequencies of 100 rad/s , 10 rad/s and 10 rad/s. From the figure we can extract that Gelatin has the highest Yield strain with Pectin having the highest Yield strain from all the plant-based polymers throughout the three different frequencies of 100 rad/s , 10 rad/s and 10 rad/s.

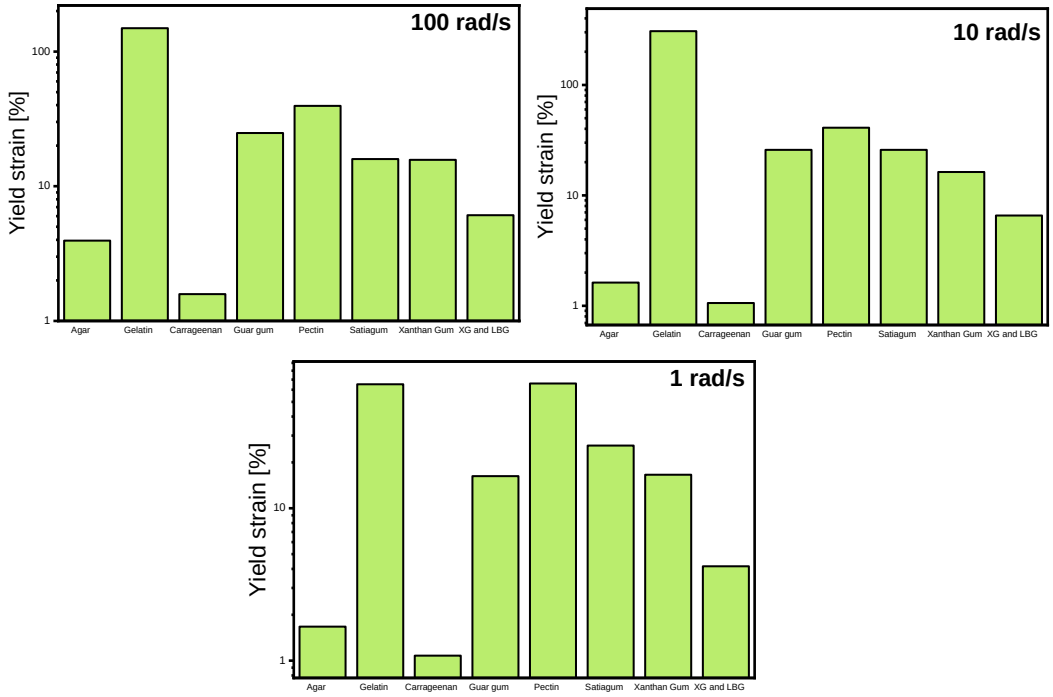


Figure 32. Histogram plot for Yield Strain for all polymers

Similarly to the Yield Strain, the Yield Stress was also plotted in the form of a histogram. Figure 34 below shows the results these results for all plant-based polymers at the concentration that match our reference concentration of 10wt% gelatin at the three different frequencies of 100 rad/s , 10 rad/s and 10 rad/s. From the figure we can extract that once again Pectin has the highest Yield stress from all the plant-based polymers throughout the three different frequencies of 100 rad/s , 10 rad/s and 10 rad/s. Pectin also surpasses the Yield Stress of Gelatin at 100 rad/s which is the processing speed and at 1 rad/s which is usually attributed to relaxing of the polymer.

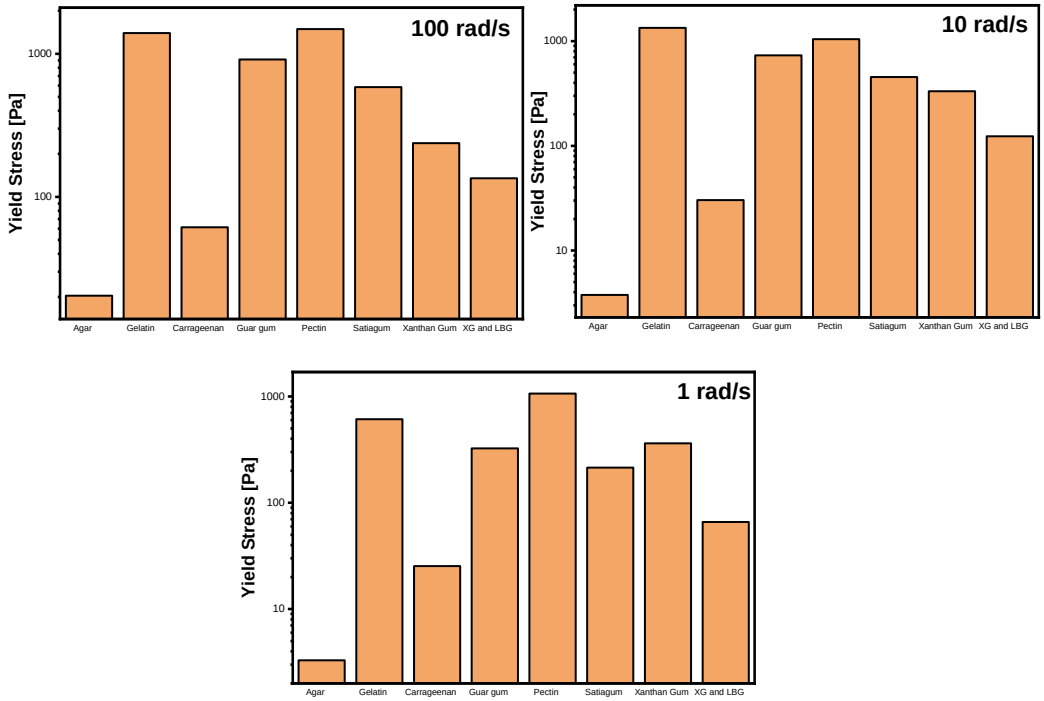


Figure 33.Histogram plot for Yield Stress for all polymers

As mentioned earlier in this section of the work, in order for someone to attempt to replace gelatin with any plant-based polymer for the food industry, there are two main factors to be considered. The material must have similar LVE (Complex Viscosity) and a very large Yield Strain. Out of all the plant-based polymers explored in this work, Pectin is the one that stands out among the rest. Pectin is rheologicaly the best candidate to replace gelatin in the food industry.

5. Conclusion and future Perspectives

The food and non-food sectors employ gelatin, a multifunctional polymer, widely for a range of purposes. However, because gelatin is frequently derived from animal sources, there is an increasing concern among customers over the origin of these products, mostly because of religious beliefs. Therefore, in order to get over this obstacle, research is being done to find a better source of gelatin that isn't derived from animals, such as microorganisms or plants, but instead has comparable or even better physicochemical, functional, and sensory qualities.

This thesis provides a rheological characterization of gelatin and selected plant-based polymers which according to the literature are possible candidates to replace gelatin in the food industry. Characterization of the samples started with a DSS test in which the linear viscoelastic region(LVE) and then non-linear viscoelastic region (NLVE) of the samples were obtained. We were able to explore the extent of gelation concentrations of gelatin as well as plant-based polymers that could be a potential replacement of gelatin in the food industry.

Since gelatin is mainly utilized in the food industry in the form of a strong gel, we selected 10wt% gelatin to be our reference sample for this work. With the information obtain from the DFS test we were able to match the viscosity properties of 10wt% gelatin with various concentrations of the plant-based polymers studied in this work. The exact concentrations of the plant-based polymers, as well as a comparison of them with our reference sample of 10wt% gelatin is reported in the previous section of the report.

We believe that in order for someone to fully replace gelatin the food industry with a plant-based polymer, the plant-based polymer must have similar LVE (viscosity properties) as well as a very large Yield Strain.

Therefore, we performed DSS experiments for the whole viscoelastic spectrum(LVE and NLVE) for all samples which matched our reference concentration of 10wt% gelatin. The experiment was performed at three different frequencies. 100 rad/s which is associated with processing speeds, 1 rad/s which is associated with the materials in a relaxed form and 10 rad/s which is a situation in-between processing and relaxed for the material. Through this test we obtained important information for the NLVE region and extracted the Yield Strain and Stress of the samples. This experiment also shed light in the materials self-healing properties.

Another important aspect of the material properties as mention earlier was the large Yield strain. Throughout the frequency range explored in this work gelatin showed the largest yield strain with Pectin having the highest Yield Strain among the plant-based polymers. As mentioned, before we were able to match the viscosity of pectin with our reference concentration of 10wt% gelatin and the fact that Pectin has the highest Yield Strain and Stress among the plant-based polymers makes it the best rheologicaly plant-based polymer to replace gelatin.

In order to put our experimental finding to the test, in collaboration with Fortuin we implemented selected plant-based polymers in one of the company’s best-selling products. The plant-based polymers replaced gelatin in the product recipe with the rest of the ingredients remained intacted. The product dough with gelatin and the plant-based polymers was explored rheologicaly though the DFS experiment.

None of the plant-based polymers were able to form a uniform dough similar to gelatin without the addition of extra water. This is something that doesn't come out of surprise since the majority of the plant-based polymers are polysaccharides which tend to absorb all the water leaving almost none for the dough to be formed. As a result of the extra water required to form the dough, the doughs were a lot softer compared to the one of gelatin and at the same time lacked the elasticity of gelatin dough. The plant-based polymer doughs had different texture compared to gelatin dough, they were quite brittle when pulling apart the dough. Out of all plant-based polymers Pectin dough was the most promising which is something that corelates with our experimental findings.

Putting everything together, we were able to provide the gelation point of gelatin and various plant-based biopolymers was assessed via linear shear rheology. Additionally, viscosity curve, Yield stress and Yield strain that a plant-based polymer must have to replace gelatin have been identified. These findings can help us understand better what properties a possible plant-based substitute of gelatin must possess in order to fully replace it. This work can be utilized as a possible guide for future attempts to find a possible plant-based polymer to replace gelatin in the food industry.

With our extensive knowledge on the gelation as well as on the mechanical properties of the plant-based polymers it is important for future works to focus on the chemical interactions between the plant-based polymers and the ingredients in the commercial products that can perhaps play an important factor in obtaining a commercial product without gelatin in the food industry. Additionally, a blend of the plant-based polymers could be a possible approach to attain the same rheological characteristics of gelatin. Finally, a possible approach for future works would be to explore further on the possible plant-based polymers that were not explored in this work such as, Konjac and Gellan Gum and modified cornstarch. Plant-based polymers are endless and this work is worth pursuing as it will allow for more products to target largest audience making both the consumers and the companies satisfied.

6. Appendix

Gelatin

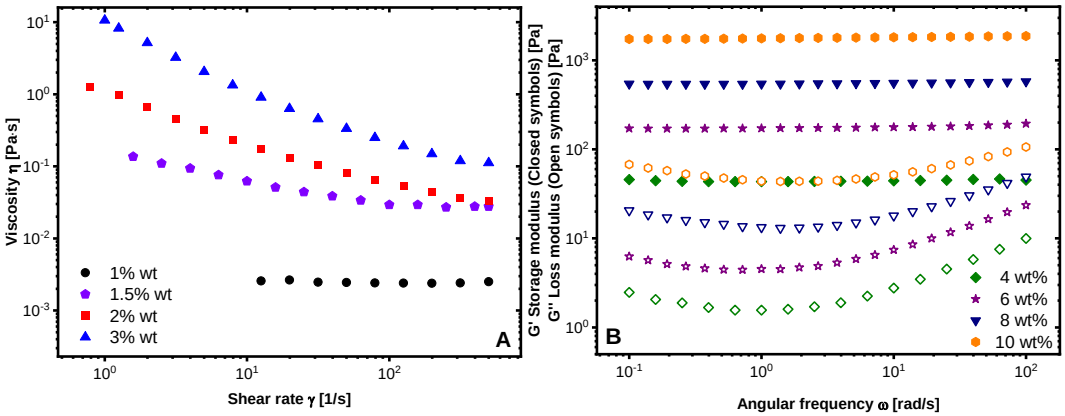


Figure 34. (A) Viscosity as a function of shear rate for gelatin-aqueous solutions. (B) Storage and Loss modulus as a function of Angular frequency for gelatin-aqueous solutions

Carrageenan

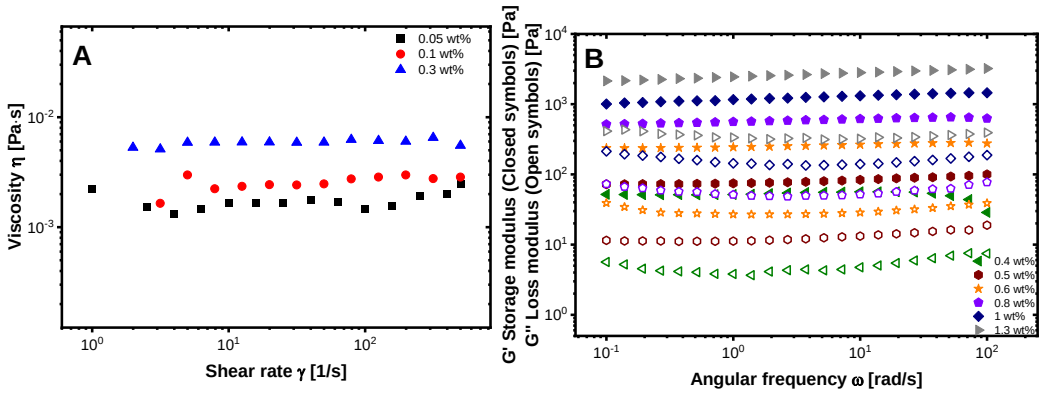


Figure 35. (A) Viscosity as a function of shear rate for Carrageenan-aqueous solutions. (B) Storage and Loss modulus as a function of Angular frequency for Carrageenan-aqueous solutions

Agar

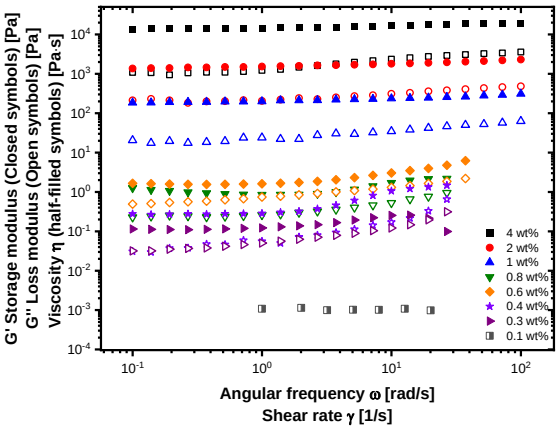


Figure 36.Storage and Loss modulus, viscosity as a function of angular frequency and shear rate for agar aqueous solutions

Guar Gum

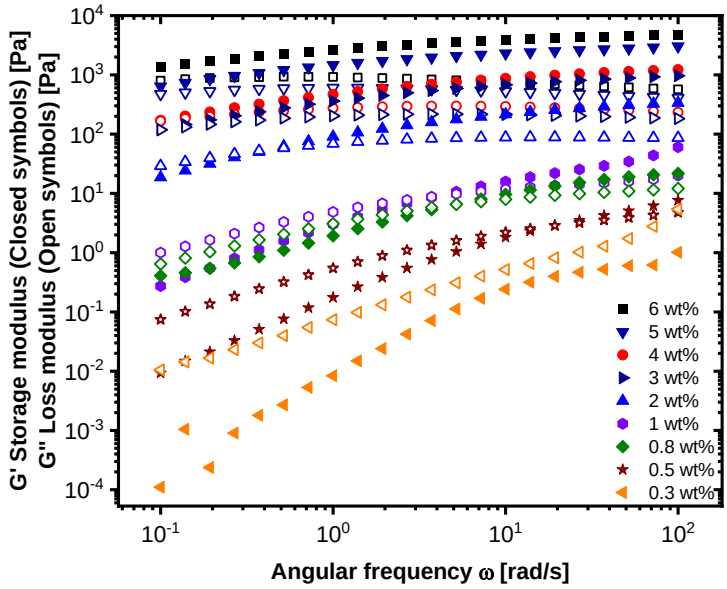


Figure 37.Storage and Loss modulus as a function of Angular frequency for guar gum aqueous solutions

Pectin

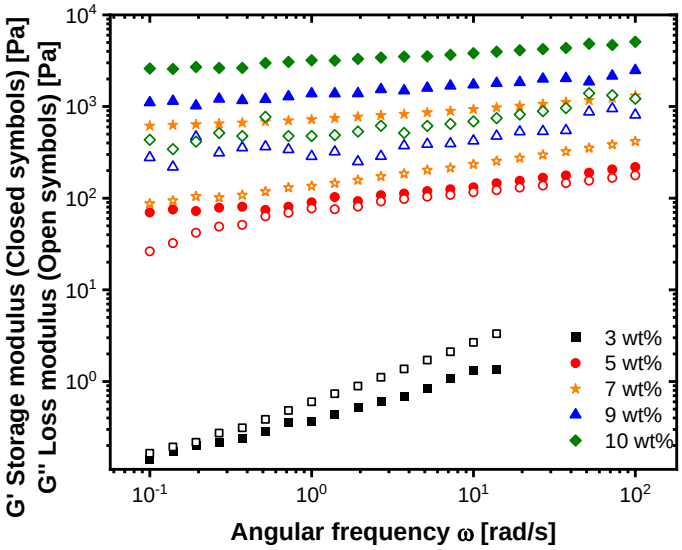


Figure 38.Storage and Loss modulus as a function of angular frequency for pectin aqueous solutions

Satiagum

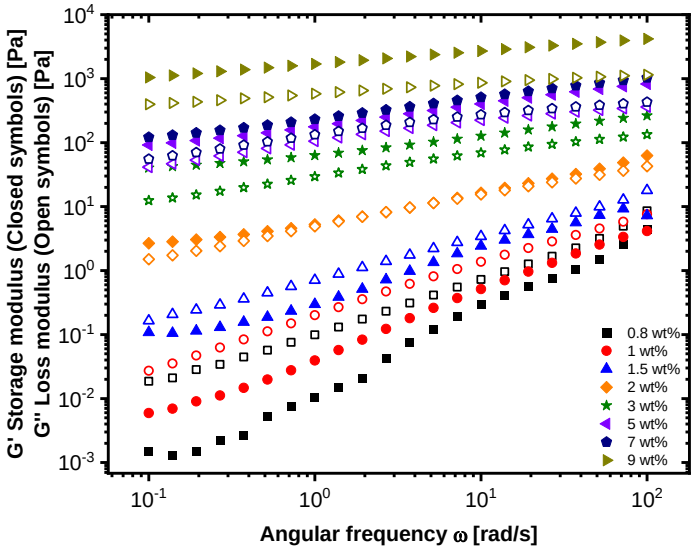


Figure 39. Storage and Loss modulus as a function of angular frequency for Satiagum in aqueous solutions

Xanthan Gum

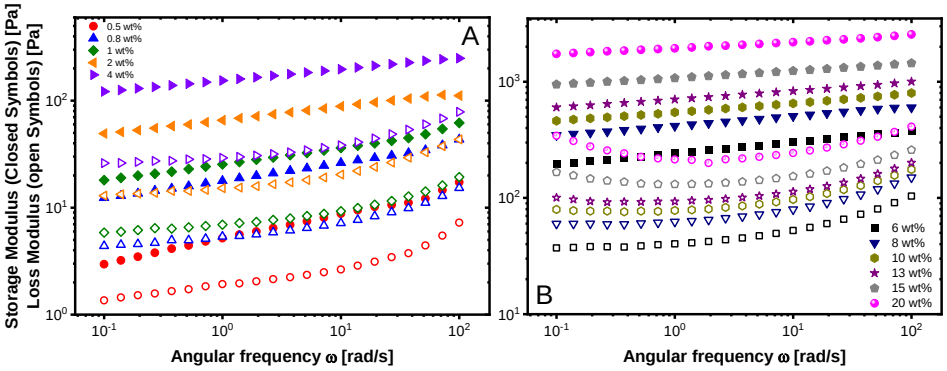


Figure 40. (A and B) Storage and Loss modulus as a function of angular frequency for Xanthan gum in aqueous solutions.

Blend of XG and LBG

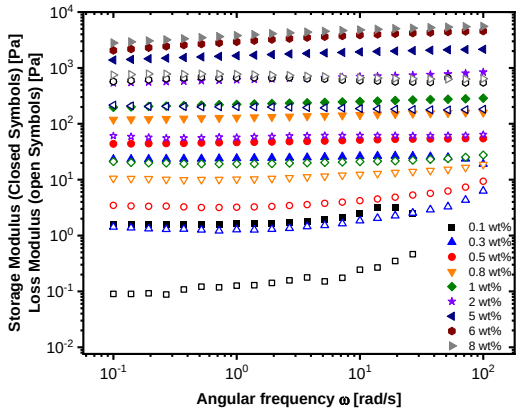


Figure 41.Storage and Loss modulus as a function of angular frequency for Xanthan gum and Locust bean gum blend (ratio 50:50) aqueous solution.

7. References

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