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**SYNTHESIS, CHARACTERIZATION, AND RHEOLOGICAL PROPERTIES OF
MODIFIED XANTHAN GUM**

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ABSTRACT

Xanthan gum (XG) is a microbial exo-polysaccharide (biocompatible, biodegradable, and hydrophilic) produced industrially from the fermentation of simple carbon sources under aerobic conditions by Gram-negative bacteria *Xanthomonas campestris*. As a great commercial significance, it has demonstrated interesting properties like biodegradability, high viscosity at a low shear rate and stability under a wide range of temperature and pH which leads to the wide range of applications such as pharmaceutical, cosmetic, biomedical, petroleum and agriculture, especially in food applications. However, certain limitation such as high solution viscosity even at a lower concentration due to its high molecular weight can hinder its uses in specific functional food applications considering its prebiotic features. Therefore, this review focuses on the rheological properties alterations of xanthan gum modified by various non-thermal emerging technologies, targeting the product with the lower molecular weight to obtain xanthan-derived oligosaccharides with the antibacterial, and antioxidant activities. Xanthan polymer with the low molecular weight also provides a variety of applications. The steady and dynamic flow behavior as rheological properties of XG in aqueous solutions which is closely associated with the polymer concentration has been studied extensively. In this review, high pressure treatment in varied ways, ultrasound and radiation technologies were considered to explain the change of xanthan gum flow behaviors, based on previously published researches.

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1. Introduction

The bacterial exopolysaccharide exhibits a wide range of chemical structures, have gained increasing attention to use instead of traditionally current plant gums. The natural biopolymers which can be modified continue to attract the attention of researchers. The modified gums presents varied applications in food systems with different functional properties such as oligosaccharides with antioxidants against superoxide anion radical, hydroxyl radical, DPPH radical, hydrogen peroxide and ferrous ions because modifications of gums resulted in the high number of generated activated hydroxyl groups (Xiong, Li, Xie, Jin, Xue, & Sun, 2013). Therefore, the food applications of degraded gums are regarded to be products with high added-value (R. Li & Feke, 2015b). They act as dietary fibre, reduce the risk of cardiovascular disease, improve the immune function and help in control of weight and colonic health (Hamdani, Wani, & Bhat, 2018). Xanthan gum as a food hydrocolloid is widely used as stabilizer and thickener in salad dressings, soups and gravies, convenience foods, frozen foods, desserts, toppings, dairy products and beverages (Katzbauer, 1998a). The degradation of the gums to lower their molecular weight or particle size has been aimed to modify rheological properties in order to meet the requirements of its industrial application. In recent years, several xanthan gum degradation methods have been studied including thermal, non-thermal emerging technologies, chemical and enzymatic-bio degradation, which were detailed reviewed in recent published study by Riaz, Iqbal, Jiang, and Chen (2021). This review focuses on the rheological properties of degraded xanthan gums.

2. History, production and characteristics of xanthan gum

Xanthan gum is a water-soluble natural polysaccharide and a commercially important industrial biopolymer. It was the second discovered microbial polysaccharide [after dextran (early 1940s)] by Allene Rosalind Jeanes at National Center for Agricultural Utilization (now called as Northern Regional Research Center) of the United States Department of Agriculture (USDA) in 1950. After industrial production in 1960 by Kelco Company, (today CP Kelco), commercially production was done in 1964, followed by the approval by the FDA (Fed. Reg. 345376) in 1969 as a non-toxic, non-sensitizing, and safe polymer for application as food additives (stabilizer and emulsifier) without any restrictions (Habibi & Khosravi-Darani, 2017; A. Kumar, Rao, & Han, 2018; Petri, 2015). Xanthan gum production is performed in the United States (producers: Merck, Kelco, and Pfizer), France (producers: Rhone Poulenc, Mero-Rousselot-Santia and Sanofi-Elf) and Austria (producer: Jungbunzlauer). As one of the largest xanthan production, China (producer: Saidu Chemical) come into prominence since 2005 (García-Ochoa, Santos, Casas, & Gómez, 2000; Tao, Wang, Ma, Yang, Tang, Gai, et al., 2012). The production amount is estimated approximately 30,000 tons per year and the requirements of this gum exhibits gradually increasing trend with annual rate of 5–10%, and it now exceeds 86,000 tons per annum (Faria, de Oliveira Petkowicz, de Moraes, Terrones, de Resende, de França, et al., 2011; Habibi & Khosravi-Darani, 2017; P. Li, Li, Zeng, Li, Jiang, Wang, et al., 2016).

Xanthan gum is an exo-heteropolysaccharide that is produced with the fermentation of glucose saccharose and/or alternative low-cost fermentation substrates (such as starch, hydrolyzed starch, corn syrup, hydrolyzed rice, barley and corn flour, acid whey) by *Xanthomonas campestris* (Gram-negative bacteria, plant pathogen) in controlled aerobic conditions, with a molecular mass ranging from 1 to 7×10^6 Da. Regarding the product yield, supply, and product quality, glucose is still considered the best carbon sources for gum production (Faria, et al., 2011; Milas, Reed, & Printz, 1996). This bacterium is widely found on the leaves of plants such as those belonging to the cabbage family (Park, Mun, & Kim, 2018). In fact, this bacteria generates gum for exploiting different functions comprising adhesion, cell-to-cell interactions, and protection against extreme environmental conditions (A. S. Kumar, Mody, & Jha, 2007). The purposive production of gum with high quality and yield is directly linked to the several factors such as the settings of the bioreactor, continuous or batch operation, type and concentration of nutrients in the fermentation medium and controlling the production conditions of temperature, pH, agitation speed, aeration and fermentation time (Lopes, Lessa, Silva, Filho, Schnitzler, & Lacerda, 2015). At the end of fermentation process, microorganisms in broth medium are eliminated by filtration or centrifugation, xanthan is precipitated by decreasing the solubility of polymer molecules with using isopropanol (by force of FDA regulation) for further purification. The dried product is milled and stored into airtight containers (García-Ochoa, Santos, Casas, & Gómez, 2000; Kurt & Kahyaoglu, 2015).

The main structure of xanthan gum is based on a linear backbone of β -(1 \rightarrow 4) linked D-glucose similar to cellulose backbone (cellobiose) as repeating unit. As a branched polysaccharide, every alternate glucose residue has a charged trisaccharide side chain at the C(3) position consisting of a glucuronic acid residue of every alternate glucose residue as schematically represented in Figure 1. The anionic trisaccharide side-chains wrapped around the main chain is responsible for the formation of relatively stiff rod xanthan molecule, providing the stability against heat, acid and alkali conditions (García-Ochoa, Santos, Casas, & Gómez, 2000). The rod-like conformation is more sensitive to shear than a random-coil conformation that is the explanation of the pronounced shear-thinning behavior of xanthan solutions (Urlacher & Noble, 1997). The glucuronic acid linked via β -1,4 to the terminal β -D-mannose unit and α -(1,2) to the second α -D-mannose. The structure contains these sugar units in a 2:2:1 molar ratio with varying proportions of *O*-acetyl and pyruvyl residues (Abbaszadeh, Lad, Janin, Morris, MacNaughtan, Sworn, et al., 2015; Faria, et al., 2011; Habibi & Khosravi-Darani, 2017; Petri, 2015). Approximately one-half of the terminal D-mannose residues include a pyruvic acid moiety linked via ketal linkage to the O(4) and O(6) positions, with an unknown distribution. Acetate groups present at position O(6) of D-mannose unit as substituents (Habibi & Khosravi-Darani, 2017; Lopes, Lessa, Silva, Filho, Schnitzler, & Lacerda, 2015). The composition of xanthan is affected by the *X. campestris* strain and fermentation conditions. In general, the degree of pyruvate substitution at the terminal mannose residues varies between 30 and 50%, whereas 60-70% of the internal mannose residues may contain

acetate groups (Abbaszadeh, et al., 2015). Ordered (helix)-disordered (coil) transition of xanthan configuration in aqueous solution occurs based on the increase of temperature. This conformational transition type and temperature depends on acetate-pyruvate contents, pH, ionic strength and polymer concentration. The changes in the extent of acetate and pyruvate residues affect flow behavior properties of xanthan solutions, consequently its applications. Acetate groups have an effect on the stabilization of the xanthan helical conformation while pyruvate groups destabilize the helical conformation which can be explained with the developing internal electrostatic repulsions between the charged groups on the side chains (Abbaszadeh, et al., 2015; Khouryieh, Herald, Aramouni, & Alavi, 2007; Kool, Gruppen, Sworn, & Schols, 2014). Low pyruvate content yields low viscosity, whereas its high content promotes gel behavior (Lopes, Lessa, Silva, Filho, Schnitzler, & Lacerda, 2015).

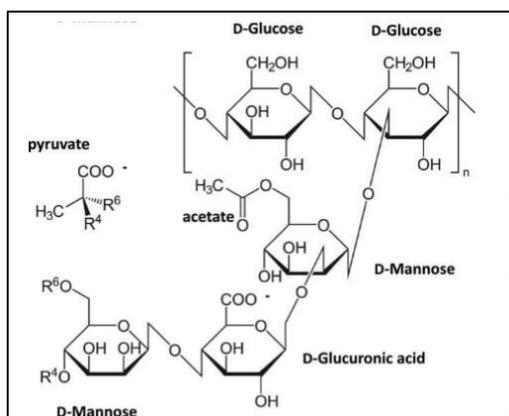


Figure 1. Representation of the chemical structure of xanthan repeating unit and nomenclature in xanthan

Xanthan gum dispersion in water is quick and the stable, transparent aqueous solution (around pH 7) has a high-intrinsic viscosity with

yield stress even at low concentrations as a result of the formation of aggregated clusters due to its high molecular weight, interaction of helicoidal structure by hydrogen bonds. The presence of many strong polar groups like hydroxyl and carboxyl are responsible for the formation of intramolecular and intermolecular hydrogen bonding interactions in xanthan aqueous solution (Morris, 1977; Shi, Wei, Luo, Tan, & Cao, 2018). The solution exhibits excellent rheological and thickening properties that enhance sensory qualities (flavor release, mouth feel) in food products. The stability in acidic and alkaline solutions, resistance to degradation at elevated temperatures and added ions make it excellent as compared with many other water-soluble polysaccharides (Faria, et al., 2011; Katzbauer, 1998b). Therefore, these behaviors of xanthan gum has led to a wide range of industrial applications such as food, cosmetic, oil recovery and pharmaceutical industries (H. Li, Hou, & Li, 2012; P. Li, et al., 2016; Lopes, Lessa, Silva, Filho, Schnitzler, & Lacerda, 2015). Although xanthan gum in solution can form intra-intermolecular associations, Xanthan gum will not form a rigid or strong gel by itself. Blending xanthan with other polymers (such as mannans, gellan gum and chitosan) results in the formation of hydrogen bonds and electrostatic interactions to give a thermoreversible physical gel, allowing to stabilize the compound in formed true gel with the properties of fascinating texture, being very elastic and having high rupture strength (Huang, Deng, Ren, Chen, Wang, Wang, et al., 2018; Martínez-Ruvalcaba, Chornet, & Rodrigue, 2007).

Moreover, xanthan gum has been shown to be non-digestible in human that swelling and viscosity increment ability during gastric

digestion provides a product with low caloric value and with low transition time through the upper gastrointestinal tract (Espert, Constantinescu, Sanz, & Salvador, 2018; Fan, Wang, Liu, & He, 2008). The other well-known properties of xanthan gum are drug release retarding characteristic and synergistically interaction with galacto- and gluco-mannans to form gel with enhanced behaviors (Annable, Williams, & Nishinari, 1994; Fan, Wang, Liu, & He, 2008).

Determining rheological properties of food products is critical for the product development, quality control, sensory evaluation, and to ensure processing requirements and machinability. It also plays an important role in heat transfer to the fluid food which presents variations according to its structural organizations. The factors of temperature, pressure, concentration, and physical state of dispersion are responsible for the wide range of rheological properties of gum solutions (Ahmed & Ramaswamy, 2004). Apparent viscosity, consistency coefficient, flow behavior index are derived from the steady flow behavior of solutions, while storage (G' , elastic behavior), loss modulus (G'' viscous behavior) and $\tan \delta$ (G''/G') were the parameters of the frequency sweep tests. These are considered as an important quality control parameters of the process industries and their end product (Kurt, 2021). The changes in these parameters of the solutions including modified xanthan molecules produced from the emerging non-thermal technologies were evaluated by reviewing the published researches in the rest of this article.

3. Modifications of xanthan gum

3.1. High-hydrostatic pressure

High-pressure (HP) treatment is an emerging technology which is a promising alternate to thermal processing without significantly affecting color, flavor, and sensory attributes of food as approved by the United States Food & Drug Administration (2004) and Canadian Food Inspection Agency (2018). As a non-thermal treatment, HP was applied to the xanthan gum solutions previously. HP-treated xanthan gum (0.25–1.25% w/v; 80–400 MPa/15 min) exhibited that both the pressure and concentration affected the consistency coefficient and apparent viscosity, whereas, the flow behavior index and yield stress were not changed (Ahmed & Ramaswamy, 2004).

The relationship between the oscillatory rheological behavior of xanthan and conformational change as the order-disorder transition of xanthan molecule under the high-pressure conditions were studied by Ahmed (2022), performing the time-pressure/temperature superposition principle. Three independent variables, concentration (0.75, 1 and 1.25%), pressure (0.1, 300 and 600 MPa), and temperature (40, 55 and 70 °C), were considered in RSM design. In that study, the elastic behavior (G') of xanthan was significantly affected by concentration and temperature but the pressure had the least effect on mechanical rigidity.

Alternative treatments to enzymatic and chemical modification, high-pressure homogenization (HPH) applied for mechanically modification of xanthan solutions at low (69 MPa) and high (276 MPa) pressures (Eren, Santos, & Campanella, 2015). The irreversible changes through inter and intra molecular arrangements altered the rheological properties of solutions. The viscosity and storage-loss modulus of the

treated solutions were lowered by HPH depending on its severity, which was attributed to the loss of structured network due to partial loss of junction zones and increased disordered structure, by authors. The rise in polydispersity and hydrodynamic radius induced by HPH were also correlated with the reduction of viscoelastic behavior.

Laneville, Turgeon, and Paquin (2013) have used the microfluidization as a dynamic high pressure treatment to modify 1 wt% xanthan gum solutions prepared at ionic strengths of 10^{-4} M or 0.1 M NaCl, circulating 1, 4, or 12 passes through a microfluidizer. Microfluidization decreased the intrinsic viscosity and pseudoplastic behavior which was explained by the disruption of aggregates and increasing treatment severity caused to dissociate the double helical structure into single chains of xanthan molecules. The degradation determined in samples in 0.1 M NaCl was higher than the degradation induced in 10^{-4} M NaCl solution which exposed to the high shear and cavitation forces in the microfluidization chamber.

As another type of high pressure application, microfluidization technology was performed at different pressure and number of passes for xanthan gum solutions to investigate its effects on the flow behavior, hydration rate, water uptake and molecular weight of xanthan (Lagoueyte & Paquin, 1998). They found that high pressure treatment reduced the properties of thickening and stabilizing for xanthan, owing to the polymer modifications and the ordered–disordered conformational transition via the high shear, turbulence forces and cavitation of process. Microfluidized xanthan exhibited lower viscosity, pseudoplastic behavior, hydration rate and

water uptake compared to untreated xanthan. The increase of the number of passes reduced the pseudoplastic character (n values showed increment) and consistency coefficient (K) values. As compared to untreated xanthan, the dramatic decrease of K values of xanthan solution passed 2 times became slightly decrease trend up to the pass number of 8.

3.2. Ultrasound

Ultrasonication degrades the polymers primarily through a mechanical mechanism associated with the violent implosion of cavitation-generated microbubbles during compression and rarefaction of the acoustic waves create localized high temperature/pressure along with generation of liquid circulation currents correlated with the high hydrodynamic forces. This method is considered to be a promising technique for modifying the molecular weight of gums to produce a permanent reduction in molecular weight without changing its chemical properties. Moreover, the ultrasound application is regarded as greener, rapid, energy-saving, effective and by product-free compared to other modification methods to degrade xanthan gum (R. Li & Feke, 2015a; Price, West, & Smith, 1994; Saleh, Annuar, & Simarani, 2017).

Degradation of xanthan via ultrasound at varied environmental conditions (salt types, NaCl or Na_2SO_4) was applied at the frequency of the amplifier of 25 kHz and the amplitude of 35% (92W) for different durations to observe degradation kinetic of xanthan (R. Li & Feke, 2015b). The ultrasonic degradation of xanthan gum in aqueous solutions varied related to the salting-in and salting-out salts. The intrinsic viscosity of xanthan gum derived by Huggins equation decreased from 16.91 dL/g to 3.77

dL/g after 30 min sonication indicated the decrease of the molecular weight of xanthan gum. The results were attributed to the shorter and stiffer molecular chains that their effects on the molecular interaction for intrinsic viscosity become smaller and less important.

In another study, degradation of xanthan polymer in aqueous solution by ultrasonic irradiation was investigated at different factors including sonication intensity ($W\text{ cm}^{-2}$), irradiation time (min), concentration of xanthan gum (g L^{-1}) and concentration of NaCl (M) (Saleh, Annuar, & Simarani, 2017). Xanthan gum degraded by ultrasound with the change of an exponential decay function with higher rate constant in the salt free solution. The degree of degradation reduced drastically from 39.8% to 5.8% when the gum concentration was increased fivefold and was further reduced when the concentration $>0.5\text{ g L}^{-1}$. The lowest concentration of 0.1 g L^{-1} exhibited low viscosity at $1.1\text{ mPa}\cdot\text{s}$, and relatively high viscosity at $93\text{ mPa}\cdot\text{s}$ when the xanthan gum concentration was increased fifty-fold, indicated that the lower degradation observed at high concentration resulted in higher viscosity than the relatively dilute solution having higher degradation due to the difficulty of ultrasound based cavitation in viscous xanthan solution.

3.3. Radiation

Gamma irradiation is a non-thermal method of preservation and has been extensively researched for the shelf life improvement of the various foods. Irradiation, a physical treatment, can be utilized to alter the structure and functional properties of the hydrocolloids as well as their end products except to be as a potential technique for sterilization of natural gums. Radiation could possibly responsible for

the conformation change and morphology of polymer chains or degrade the bonds of the long chain polysaccharide molecules affecting their physical properties (Ravat, Yardi, Mallikarjunan, & Jamdar, 2019). Thus, such properties are known to affect the functionality of gums.

Xanthan gum in solid state was degraded by ionizing radiation treatment with gamma rays (Co-60 gamma) at different dose rates (0.1, 3.3 and 7.0 kGy/h) and doses (2.5, 5.0, 10, 20, 30 and 50 kGy) (Şen, Hayrabolulu, Taşkın, Torun, Demeter, Cutrubinis, et al., 2016). Rheological properties of xanthan gum solutions were studied and flow model parameters were determined for all treatments. In reported research, the dose rate was found as an important factor controlling the chain scission yield and degradation rate of xanthan gum. The elastic behavior of untreated xanthan ($G' > G''$) preserved its material characteristic but both modulus became closer up to 20 kGy , meaning that $\tan \delta$ value increased and elastic form of xanthan was diminished when radiation dose increased. The degradation of xanthan was more pronounced at further radiation dose and 50 kGy resulted in modified xanthan with the viscous behavior ($G' < G''$), as result of the significant decrease in molecular weight with dose. In the aforementioned study, for the comparison of steady rheological characteristics of samples, the magnitudes of parameters (K and n) that obtained from Oswald-de Waele model were successfully used. The viscosity of unirradiated and irradiated xanthan solutions decreases exponentially with shear rate which indicates that xanthan solution shows a non-Newtonian behavior ($n < 1$). This pseudoplastic character of xanthan solution decreased with the increment of irradiation dose, which was

consistent with the decrease of K values, suggesting degradation or depolymerizations of polysaccharide macromolecules.

Xanthan gum in aqueous solution was also degraded by using (Co-60 gamma) in at different dose rates (0 and 120 kGy) (Y. J. Li, Ha, Wang, & Li, 2011). Apparent viscosity of xanthan gum solutions reduced with increasing irradiation dose and its stability to shear time was enhanced which was found consistent with the change trend of molecular weight of xanthan gum. Newtonian flow behaviors were observed for irradiated xanthan solutions unlike pseudoplastic character of control sample.

4. Conclusions

Recently, the reduction of xanthan gum molecular weight within a narrow range has attracted much attention to adjust and extend its use in different food systems achieving concentration-independent benefits. In this

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- review, the modification methods such as various pressure treatments and radiation, which don't based upon chemical treatment for altering ingredient functional and rheological properties, were considered to summarize their effects on the flow properties of xanthan gum in order to develop some desirable properties by adjusting the extent of the xanthan polymer degradation. The decrease in the rheological parameters by aforementioned technologies was attributed to the loss of structured network due to partial loss of junction zones and increased disordered structure which can be correlated with the diminished molecular mass and radius of gyration. Therefore, to ensure significant benefits from the functional properties of degraded xanthan gum such as oligosaccharide as a prebiotic, flow behavior characteristics is a key factor which must be considered to demonstrate process-function relationship in the real food systems.

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