1   **Characterization of Gum Isolated from Camelina Seed**

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3   Ningbo Li^a, Guangyan Qi^b, Xiuzhi Susan Sun^b, Donghai Wang^{a,*}

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5   ^a*Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS 66506.*

6   ^b*Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.*

7   **Corresponding author. Tel: 785-532-2919. Fax: 785-532-5825. Email: dwang@ksu.edu (Donghai*

8   *Wang).*

9   **ABSTRACT**

10   Camelina gum (CG) was characterized on the physicochemical properties including rheological
11   properties, chemical compositions, morphological property, and thermal properties. The results showed
12   that dried CG has porous structure and polysaccharides and protein content of 75.1% and 12.3%,
13   separately. CG solution showed high apparent viscosity, strong temperature and pH tolerance. CG also
14   showed higher viscosities, storage (G'), and viscous modulus (G'') than commercial gums such as κ -
15   carrageenan and hydroxyethylcellulose (HEC) gums. At concentration of 1.0 %, the viscosity of CG was
16   more than 2 times and 4 times higher than that of κ -carrageenan and HEC gums (0.01 1/s), respectively,
17   and G' was more than 3 times and 30 times higher than that of κ -carrageenan and HEC gums (0.01 Hz),
18   respectively. CG showed modulus reversibility, 100% of G' could be recovered in 180 s. Those properties
19   indicate camelina gum is an excellent candidate for food and industrial uses as thickener or stabilizer.

20   **Keywords:**   Camelina   gum,   Rheological   property,   DSC,   TEM,   FTIR,   SEM

1. Introduction

Natural gums, mucilage, or hydrocolloids are polysaccharides from natural products, capable of causing a large viscosity increase in solution, even at low concentrations. They are widely used in both food and non-food industries as thickening agents, crystal inhibitors, swelling agents, foam stabilizers, gelling agents, emulsifying agents, water bonding agents, etc. (Walter, 1998). Most of the gums are derived either from the terrestrial or the marine plants. For instance, agar, alginic acid, sodium alginate and carrageenan gums were isolated from seaweeds, and guar, locust bean, beta-glucan, gum arabic gums were harvested from land plants (Walter, 1998). Some other natural gums were also harvested from the agricultural product, such as yellow mustard gums and flaxseeds gums were isolated from the coat layers of their seeds (Cui, 2000).

As a member of the mustard family genetically, camelina is a new but rapidly spreading high-oil flowering annual or winter annual crop in the United States even though its cultivation history could be traced back to as early as 600 BC in the Rhine River Valley. Camelina was primarily known in North America as a weed species and was not evaluated to some extent until 1980s (Putnam et al., 1993). Compared to the extensive researches on the other oil seeds such as soy bean or canola, little has been conducted on camelina, and its agronomic potential remains largely unknown. Currently, researches on camelina are mainly focusing on improving camelina yield, developing camelina oil as source of biodiesel or omega-3 supplements and utilizing camelina meal as a supplement of animal feed (Budin et al., 2005).

Only fairly few detailed researches were focusing on the camelina gums. Taasevigen (2010) stated that camelina meal showed a distinct quality of binding in water. Grady and Nleya (2010) indicated that the camelina gum can be used as a soil amendment to help preventing erosion of exposed soils during construction projects. Our preliminary studies indicated that camelina gums showed fairly high viscosity. We speculated that the camelina gums might have the functions as other commercial gums that would benefit both the food and non-food industries. Therefore, the objective of this research was to characterize

the physicochemical properties of camelina gums including rheology properties, chemical compositions, morphological property, and thermal properties.

2. Materials and methods

2.1. Materials

Camelina gum (CG) was provided by SBT LLC (Manhattan, KS, USA). NATROSOL®HEC was generously provided as free sample from Ashland (Wilmington, DE, USA). κ -carrageenan was purchased from Sigma (St. Louis, MO, USA). All chemicals used in this research were of analytical grade and purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

2.2. Chemical analysis

Moisture content was measured using the V30 Compact Volumetric KF Titrator (Columbus, OH, USA). Nitrogen (N) was measured via a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (Shelton, CT, USA). Nitrogen was converted to protein using a factor of 6.25. All tests were performed with duplications.

Total sugar in CG was determined using the phenol-sulfuric acid method as described by Dubois et al. (1956). To study the monosaccharaides in camelina gums, 0.3 g CG was mixed with 3 mL of 12M H₂SO₄ at 37 °C for 1 h to solubilize the polysaccharides. 15 mL of distilled water was added to dilute the H₂SO₄ to 2 M. The mixture was hydrolyzed at 120 °C for 3 h to give constituent monosaccharides. The hydrolysate was cooled, diluted to 1:2 with distilled water, neutralized with calcium carbonate, filtered through a 0.45 mm filter and analyzed by using an HPLC with a Rezex RPM-monosaccharide column (300 mm × 7.8 mm; Phenomenex, CA, USA) and a Refractive Index Detector (RID -10A, Shimadzu, MD, USA). The column was eluted with double-distilled water at a flow rate of 0.6 mL/min. The temperature of the chromatograph column was maintained at 80 °C.

2.3. Rheological properties

The apparent viscosity, storage (G') and loss (G'') moduli of CG were performed using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA, USA) equipped with a 8 mm parallel

plate head at 0.5 mm gap. A thin layer of low viscosity mineral oil was used to cover the system in order to prevent solvent evaporation during measurements.

2.3.1. Apparent viscosity measurement

The continuous shear test was conducted at 25 °C in the shear rate ranged from 0.01-2 s⁻¹ to measure the apparent viscosity. All experiments were done in duplicate, and average values were reported. Effects of temperature on apparent viscosity of CG were studied between 4 °C to 90 °C at a constant shear rate of 0.1 s⁻¹.

2.3.2. Dynamic viscosity measurement

The frequency sweep tests were performed at 25 °C in a strain-controlled mode wherein the amplitude of shear strain was 1-500%, which was within the linear viscoelastic region. The frequency range was from 0.01 Hz to 10 Hz. The G' and G'' were continuously registered.

Fourier transform infrared spectroscopy

The Fourier transform infrared (FTIR) data was collected in the region of 400 to 4000 cm⁻¹ with a PerkinElmer Spectrum™ 400 FT-IR/FT-NIR spectrophotometer (Shelton, CT, USA). The transmission spectra of 32 scans of each sample were collected at a resolution of 1 cm⁻¹ in the reflectance mode. The information on carbohydrates and protein in sample was given by absorptions. All samples were tested with duplications.

2.4. Transmission electron microscopy

Transmission electron microscopy (TEM) was obtained using a model CM 100 TEM (FEI Company, Hillsboro, OR, USA) operated at 100 kV. CG was first dissolved in distilled water with a solid concentration of 0.05% (w/w). Prepared sample was absorbed for approximately 30 s at room temperature onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Fort Washington, PA, USA) and were stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, VT, USA) for 60 s at room temperature before being viewed by TEM.

2.5. Scanning electron microscopy (SEM)

A Hitachi S-3500 N (Hitachi Science System, Ibaraki, Japan) SEM was used to observe the microstructure of dried CG powder. The ground powder was affixed to an aluminum stub with two-sided adhesive tape and coated with an alloy of 60%gold and 40% palladium with a sputter coater (Desk II Sputter/Etch Unit, Moorestown, NJ, USA). The SEM image of the CG was performed with operation conditions at an accelerating voltage of 5 kV.

2.6. Differential scanning calorimetry (DSC)

Thermal transition properties of CG was measured with a DSC Q200 V24.4 instrument (TA Instruments, New Castle, DE, USA) that was calibrated with indium and zinc before making official measurements. Sample of both gum solution (1.5%) and dried powder weighing approximately 7–15 mg were measured in a hermetic aluminum pan under a nitrogen atmosphere with a gas flow rate of 50 mL/min. Gum solution was heated from 10 °C to 90 °C at a heating rate of 0.5 °C/min in an inert environment. Gum powder was heated from 25 °C to 280 °C at a heating rate of 10 °C/min in an inert environment. All experiments were performed in duplicate.

3. Results and discussion

3.1. Proximate analysis

CG in this work was a mixture of polysaccharides (75.1%) and protein (12.3%). For polysaccharides, it's comprised of six mono sugars, and galactose (58.1%) was the major fraction in CG, followed by glucose (25.0%), rhamnose (11.6%), and xylose (5.2%) (Table 1). Monosaccharaides compositions of other polysaccharides were also listed for comparison. Different gums exhibited varied monosaccharaides patterns. For instance, flaxseed gum was characterized with high arabinose content (42.3%), and galactose (45%) and mannose (67%) were dominated in Arabic gum and guar gum, respectively (Cui, 2000).

3.2. Morphological properties

Morphological structures of CG which were related to its physicochemical and functional properties were studied with SEM and TEM (Fig. 1 A, B). In dry state, CG showed irregular linear shape with a loose and porous structure (Fig. 1 A), which might contribute to its functional properties such as water absorption, water holding, suspending and swelling ability, as well as the rheological properties. As to TEM (Fig. 1 B), batches of intersecting fibrils were observed in CG, which were believed to be polysaccharides. The linear polysaccharides have an average diameter of around 5 nm and several thousand nanometer lengths. The spherical clusters adhered to the linear polysaccharides were believed to be the camelina albumin protein (Li et al., 2014). The adherence of albumin to the linear polysaccharides in CG might restrict the movement or flexibility of camelina gum in aqueous environment, resulting in higher viscosity, which will be discussed in detail in next section.

3.3. FTIR

FTIR absorbance pattern of CG was presented in Fig. 2A. Polysaccharides have specific bands maximum in the 1200-800 cm^{-1} region, which was dominated by ring vibrations overlapped with stretching vibrations of (C-OH) side groups and the (C-O-C) glycosidic bond vibration. Peaks at 1070 cm^{-1} was assigned to galactose unit. Intensive peak at 1070 cm^{-1} indicates higher galactose unit content. The peaks at around 1151 cm^{-1} were ascribed to the glycosidic linkages (C-O-C) contribution. Similar observations were also described in earlier report in which galactose units with any link type and position were found at about 1155 cm^{-1} , xyloglucan at 1153 cm^{-1} , and at lower frequencies (1051-1039 cm^{-1}) were polysaccharides with mannose, arabinose and rhamnose constituents (Kacurakova et al., 2000). The anomeric region between 800 and 1000 cm^{-1} was much complicated due to the peak overlapping. However, this region gave supplemental information to the above descriptions. According to Kacurakova et al. (2000), peaks at 834 cm^{-1} and 898 cm^{-1} were designed for α and β glycosidic linkage types of aldopyranoses and the furanoid compounds, respectively. Also peak at 898 cm^{-1} was sharper than that of 834 cm^{-1} , indicating β glycosidic linkage was dominated in camelina gums.

Camelina protein was also characterized on the curve. Peaks at around 1630 cm^{-1} and 1540 cm^{-1} are dominated by camelina protein's secondary structures, amide I for C=O stretch and amide II for N-H bend, respectively (Yu et al., 2005). A sharp peak at around 1600 cm^{-1} was detected for CG, which is mainly attributed for carboxylate asymmetrical Stretch (Pasqui et al., 2012). The peptide bond of protein is unique containing C=O , C-N , and N-H . The amide I absorption contains contributions from primarily C=O stretching vibration (80%) with a minor C-N stretching vibration, while the amide II absorption appears to be arising from N-H bending vibrations (60%) coupled with C-N stretching vibrations (40%) (Jackson and Mantsch, 1995). A broad and intense band between 2500 and 3600 cm^{-1} was ascribed to the OH stretching of glycoside ring (Pasqui et al., 2012).

3.4. DSC thermal analysis

Fig.2B presented the DSC curves of CG, as a function of heating temperature from $10\text{ }^{\circ}\text{C}$ to $250\text{ }^{\circ}\text{C}$. No thermal behaviors, such as glass transition, gelation, or melting, were observed before $100\text{ }^{\circ}\text{C}$ in CG. One sharp exothermal peak at $235\text{ }^{\circ}\text{C}$ was observed in CG, which was attributed to the thermal decomposition. The initial decomposition of CG occurred at $200\text{ }^{\circ}\text{C}$ and reached the maximum at $235\text{ }^{\circ}\text{C}$. This finding was similar to the results of some other studies on natural gums such as guar gum, xanthan gum and tragacanth gum, which were decomposed at around $250\text{--}300\text{ }^{\circ}\text{C}$ (Cunha et al., 2005; Zohuriaan and Shokrolahi, 2004).

3.5. Rheological properties

3.5.1. Flow behavior

Effects of concentration: The apparent viscosity curves of CG at concentrations of 0.1 , 0.5 , 1.0 , 1.5 , and 2.0% were presented in Fig.3A. The viscosity greatly increased as concentration increased. CG displayed high and stable viscosities when shear rate is less than 0.01 s^{-1} , indicating CG has a Newtonian flow behavior at low shear rate. However, as the shear rate exceeded 0.01 s^{-1} , CG showed shear thinning behaviors where the viscosities decreased as the shear rate increased. The high viscosity of CG at low

shear rates provide them for long-term stability in the application of the colloidal systems, and the reduction in viscosity at relatively high shear rate is important to the pouring and transferring properties of gum suspensions. Similar flow behavior were also shown to xanthan gum and indicated that gum with high viscosity at low shear rate might be a result of intermolecular association among polymer chains which resulted in the formation of a complex network of entangled rod-like molecules. At relatively high shear rates, the weak entanglements were progressively disrupted by the force of shear resulting in the shear thinning behavior. And above a critical shear rate, the disentanglement in gums predominates the rheological behavior and the viscosity drops to a minimum plateau values at infinite shear rate (Williams and Phillips, 2000). Similar flow behavior was also observed to guar and locust bean gums, which were widely used as the thickening agent in food industry (Yamazaki et al., 2009).

Effects of temperature: The effect of temperature on CG viscosity was measured at a constant shear rate of 0.05 1/s. The viscosities of CG decreased slowly as the temperature increased from 4 °C to 90 °C for all tested concentrations (Fig. 3B). Lower apparent viscosity of camelina gum at higher temperature gives better transfer, easier pumping and more accurate filling properties for both food or non-food applications. Several reasons were given to explain viscosity decrease of gum at increased temperature (Srichamroen 2007; Nep and Conway, 2010; Monsanto, G. S. 2000). First, the decrease in viscosity was considered to be a result of gum not being able to retain the network structure at high temperature and changing its configuration into the random coil. Second, as temperature increased, the water molecular loosed their order around gum, thus affecting the conformation of the system and resulting in viscosity reduction. Third, at higher temperature, the inter-molecular interaction in gum solutions decreased, or the polymer chain disentanglement was promoted by increased temperature, resulting in decreasing of gum apparent viscosity. In addition, gum solutions were capable of retaining their viscosity until a definite “melting temperature” was reached due to a reversible molecular conformation change, and the melting temperature was dependent on factors such as ionic strength of the solution.

Effects of pH: According to Fig.3C, viscosity of CG is pH dependent. Viscosities at alkali conditions were higher than that at neutral and acidic conditions. Viscosities of CG at pH 10 and pH 12 were higher than that of pH 7, followed by pH 3. Alkali conditions might promote the gelling behavior of CG, resulting in higher viscosity. On the other hand, acid conditions reduced the viscosity of CG by hydrolysis effect. However, detailed information on gelling properties of CG toward pH values needs to be further studied. pH affecting the rheological property were reported elsewhere. For example, carrageenan solution loses viscosity and gel strength in systems with pH below 4.3 due to auto hydrolysis as a result of molecule cleaves at the 3,6-anhydrogalactose (Imeson, 2000). However, on the other hand, xanthan gum showed stable viscosities at pH range of 2-12 (Monsanto, 2000).

3.5.2. Viscoelastic properties

Effects of concentration: The viscoelastic properties of camelina gum was characterized by oscillatory experimental measurements and illustrated by the elastic/storage modulus (G') and the viscous/loss modulus (G'') (Fig.4). G' was a measure of energy that is stored elastically in the gel structure suffering from a periodic stress, and G'' was a measure of the energy dissipated or the viscous response (Saha and Bhattacharya, 2010). Analysis of G' and G'' provides information on the degree of solid-like and liquid-like behavior of CG solutions, which is related to molecular structure and intermolecular networks (Singthong, 2004). Solid-like behaviors are those with a G' larger than G'' , whereas liquid-like behaviors are with G'' larger than G' . The larger the G' , the firmer is the gel.

As it's illustrated in Fig.4, CG showed a typical liquid-like behavior at 0.1% and 0.25% concentrations, where G'' was greater than G' at low frequencies, and G' exceeded G'' at high frequencies. Solid-like behaviors were observed at the concentrations higher than 0.5%, where G' was larger than G'' over the entire frequency examined. Both G' and G'' increased as the concentration increased. The initial G' of CG was 80 Pa, 36 Pa, 30 Pa, 4 Pa, 2 Pa, and 0.6 Pa at concentrations of 2.0%, 1.5%, 1.0%, 0.5%, 0.25%, and 0.1%, respectively. Intermolecular entanglement does not occur in dilute gum solution, resulting in a behavior that G'' was higher than that of G' . As the gum concentration increased, more gum

molecules were involved in the intermolecular entanglement, and gum exhibited the gel properties with the G' higher than the G'' (Williams and Phillips, 2000). At low concentrations, CG's storage modulus G' showed a crossover with loss shear modulus G'' at a certain frequency, and this crossover frequency shifted to lower values with increasing gum concentrations, which was interpreted as follows: the macromolecular chains can be disentangled during a long period of oscillation at a lower frequency and thus the system behaves as a solution. While the entangled macromolecular chains do not disentangle during a short period at a higher frequency and then the entanglement point plays a role of a knot of three-dimensional networks and the system shows a solid-like behavior (Nishinari, 2006).

Effects of pH: The viscoelastic properties of CG solutions at different pH values varied from acidic to alkaline conditions at concentration of 1.5 % were examined (Fig.5 A-D). CG showed solid-like behaviors at all examined pH levels. Both G' and G'' of CG under alkaline conditions were higher than that of under neutral and acidic pH conditions, and G' and G'' increased as pH increased from 7 to 12. At pH 10 and 12, the G' were 82 Pa and 121 Pa, respectively, at 0.01 Hz, which were higher than 35 Pa and 30 Pa to gums at pH 7 and pH 3, separately. Alkali conditions might promote the gelling behavior of CG, resulting in higher elastic and viscous modulus. And the reduction of viscoelastic modulus of CG at acidic condition might be attributed to hydrolysis effect.

Modulus recoverability: Storage modulus reversibility of gum indicating its “self-healing” ability and is closely related to the molecular structure of gums. After the gel structure was destroyed by shear, the gel systems that showed high viscoelastic properties might need certain time to recover to its original state. And in general, the longer the interval the recovery stage takes, the more elastic than viscous is a gel (Walter, 1998). According to Fig.5 E-F, both G' and G'' of CG were reversible over shear strength at room temperature in 180 s. The storage modulus recovered when the structure of CG return to its initial state that the polysaccharides molecules were highly entangled and the three dimensional structure of the gum system was reconstructed.

3.6. Comparison of camelina gum with commercial gums

It's worth noting that CG showed higher viscosities than commercial gums such as κ -carrageenan and HEC at the same concentrations (Fig. 6A). At 1.0% concentration and shear rate of 0.01 1/s, CG showed the viscosity of 350 Pa.s which was significantly higher than 100 Pa.s and 80 Pa.s of κ -carrageenan and HEC gums, separately. HEC had the lowest slope of the ratio of viscosity/shear rate, indicating it was more resistant to shear force compared to CG and carrageenan gums. CG showed also the highest G' , followed by κ -carrageenan and HEC, indicating CG had very promising potentials as high viscosity gums (Fig. 6B). Without considering external effects, the viscosity and viscoelastic property of polymer solutions could be positively and significantly influenced by the polymer mass. And the molecular structure of gums could also affect their viscosities. Normally, the linear and stiff molecules have a larger hydrodynamic size than the highly branched and flexible polymers with the same molecular mass and hence give rise to a much higher viscosity. Besides, charged polymers usually have a higher viscosity than non-ionic polymers of similar molecular mass due to the fact that their molecular coils are expanded because of intermolecular charge repulsions (Williams and Phillips, 2000). However, specific reasons that determine rheological properties of camelina gum need to be further studied.

4. Conclusions

The physiochemical and rheological properties of CG was studied. Similar to most of hydrocolloids, CG showed shear thinning behavior. CG, comprising of six mono sugars, exhibited high viscosity, storage modulus, temperature, and pH tolerance, which might be attributed CG's linear molecular structure. Compared to commercial gums such as κ -carrageenan and HEC, CG showed superior rheological behaviors. These properties indicated that CG has the potential as the thickening agent, emulsion, or suspending agents in both non-food and food industry.

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Table 1. Comparison of analytical data of camelina gum and other gums

	Camelina gum	Other gums ¹		
		Flax	Arabic	Guar
Total protein (%)	12.3			
Total sugar (%)	75.1			
Relative monosaccharide composition (%)				
Xylose	5.2	21.1		
Galactose	58.1	28.4	45	33
Glucose	25.0	8.2		
Rhamnose	11.6	25.6	34	
Fucose		5.8		
Arabinose		11.0	24	
Mannose				67

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¹ Cui, 2000.

321   **Figure captions**

322   Figure 1. SEM images (X 300) and TEM images (X 64,000).

323   Figure 2. FTIR analysis (A) and differential scanning calorimetry curves (B).

324   Figure 3. Apparent viscosities at different concentrations (A), temperatures measured at a constant shear
325   rate of 0.05 1/s (B), and pH values at 1.5% concentration (C).

326   Figure 4. Viscoelastic properties at concentrations 0.1%-2.0%.

327   Figure 5. Viscoelastic properties at pH 3-12 (A-D), and the viscoelastic recovery properties at frequency
328   0.5 Hz (E-F).

329   Figure 6. Viscosities (A) and viscoelastic (B) properties of camelina gum and commercial gums.