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Characterization of fractional precipitation behavior of galactomannan gums with ethanol and isopropanol



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Hong-Lei Jian^{a,b}, Xue-Jiao Lin^a, Wei-An Zhang^a, Wei-Ming Zhang^c, Da-Feng Sun^c, Jian-Xin Jiang^{a,*}

^a Department of Chemistry and Chemical Engineering, Beijing Forestry University, Beijing 100083, China

^b Department of Food Engineering, Anhui Agricultural University, Hefei 230036, China

^c Nanjing Institute for the Comprehensive Utilization of Wild Plant, Nanjing 210042, China

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ABSTRACT

Three galactomannans of guar gum (GG), locust bean gum (LBG) and fenugreek gum (FG) were fractionally precipitated from pure solutions by the addition of either ethanol or isopropanol. The galactomannan precipitates were formed when the bulk solutions containing >18.6% (v/v) ethanol or 13.5% (v/v) isopropanol. The galactomannan recovery of GG, LBG and FG in 33.3% ethanol solvent was 93.1, 86.1 and 94.5%, respectively, while that in 28.8% isopropanol solvent was 94.1, 92.5 and 97.7%, respectively. To achieve the same precipitation efficiency, the usage amount of isopropanol was much lower than that of ethanol, owing to the low dielectric constant for isopropanol (20 vs. 25). It was demonstrated that the precipitation behavior of galactomannans in polar organic solvents was dependent on the molecular structure, such as molecular weight and galactose substitution.

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1. Introduction

Plant gums are high molecular weight carbohydrates which serve a variety of functions in value-added products. Galactomannans are commercially important hydrocolloids found in numerous plants, particularly in the endosperm cell walls of Leguminosae seeds (Buckeridge, Pessoa dos Santos, & Tiné, 2000). Galactomannans have a typical structure of β -(1 \rightarrow 4)-p-mannan backbone to which single p-galactopyranosyl residues are attached at O-6 of the mannose units via α -(1 \rightarrow 6) linkages (Singh, Sethi, & Tiwari, 2009). The galactomannan gums from different sources differ in the degree and pattern of galactose substitution and molecular weight distribution (Robinson, Ross-Murphy, & Morris, 1982).

Galactomannans have widespread applications in various industries, including mining, paper, textile, cosmetic and oil drilling, mainly based on their rheological properties. Guar gum (GG, *Cyamopsis tetragonolobus*) and locust bean gum (LBG, *Ceratonia siliqua*) are extensively used in the food industry as thickening and stabilizing agents, usually in amounts of <1% of the food weight (Izydorczyk, Cui, & Wang, 2005). For instance, they have been used to improve shelf-life and prevent creaming or settling in salad dressings, soft drinks, and fruit juices. Fenugreek (*Trigonella foe-num-graecum*) is a leguminous plant grown in central and south-eastern Europe, northern Africa, the Mediterranean, western Asia, and northern India. Fenugreek seeds have been used as medicinal and food ingredients for many years. The storage polysaccharide in the fenugreek seed endosperm is found to be a galactomannan, similar to GG and LBG, but more heavily substituted by side chains of single galactose. In recent years, fenugreek gum (FG) is finding numerous applications in various industries (Brummer, Cui, & Wang, 2003).

When dispersed in an aqueous solution, galactomannans show a folded or a random-coil conformation, since inter-chain hydrogen bonding is considerably reduced due to the steric hindrance by galactose side chains (Mathur, 2011, chap. 1). The addition of dehydrating agents such as ethanol and isopropanol can promote intra-molecular associations of water-soluble polymers through water competition (Huggins, 1942). The dielectric constant of a solvent is closely related to its ability to increase the attractive forces between solute molecules which eventually bring about precipitation (Gonzales, Johns, Greenfield, & Pace, 1990). Ethanol and isopropanol have much lower dielectric constants than water (80), 25 and 20 at 25 °C, respectively (Bilati, Allémann, & Doelker, 2005). Adding alcohol solvents can lower the dielectric constant of the polysaccharides solution and induce conformational changes



^{*} Corresponding author. Tel.: +86 10 62338152; fax: +86 10 62336061. *E-mail address:* jiangjx2004@hotmail.com (J.-X. Jiang).

of the polysaccharides, thus allowing polymer molecules to aggregate and precipitate.

Actually, ethanol and isopropanol have been used in many studies to precipitate galactomannans from the water-extracted gum solution (Amid & Mirhosseini, 2012; Cunha, Paula, & Feitosa, 2007: Cunha, Vieira, Arriaga, De Paula, & Feitosa, 2009: Jian, Cristhian, Zhang, & Jiang, 2011). However, galactomannan is not completely precipitated from the solution, due to the presence of low molecular weight fractions which are soluble at high alcohol concentrations (Navarro, Cerezo, & Stortz, 2000). In order to obtain acceptable recovery yields from dilute biopolymer solutions, usually a large amount of alcohol solvent must be added, leading to considerable supernatant recycling or disposal costs (Torrestiana-Sanchez, Balderas-Luna, Brito-De la Fuente, & Lencki, 2007). Precipitation is an important process in the isolation and purification of galactomannan gum. However, there has been little published research on the relationship between the molecular structure of galactomannan and its precipitation behavior by organic solvents.

Therefore, the aim of this work was to investigate the influence of molecular structure of galactomannan gum on the precipitation behavior such as the morphology, the recovery yield, and the physico-chemical properties of the precipitated fraction. Three types of galactomannans (GG, LBG and FG) with different mannose to galactose ratios (M/G ratios) and molecular weight distributions were fractionally precipitated in ethanol- and isopropanol—water solutions. The characterizations of the galactomannan precipitates were carried out, such as monosaccharide composition, galactomannan recovery yield and molecular weight distribution. The results of this study will provide useful information for the isolation and purification of galactomannan gums, with respect to both academic interest and practical applications.

2. Materials and methods

2.1. Materials

GG, LBG and standard monosaccharides (L-rhamnose, L-arabinose, D-glucose, D-galactose, D-mannose and D-xylose) were purchased from Sigma—Aldrich (Saint Quentin Fallavier, France). Fenugreek seeds were obtained from Anhui province, China. The endosperms of Fenugreek seeds were manually separated and ground into powder form (i.e., FG). The three crude gums of GG, LBG and FG were used for the following experiments without any further purification. Dextran standards, with an average molecular weight range of $3.7 \times 10^3 - 1.2 \times 10^6$ Da, were purchased from TosoHaas (Japan). All the other chemicals and reagents used in this study were of analytical or higher grade.

2.2. Rheological properties

Samples for rheological measurement were prepared at a gum concentration of 0.5% (w/w) on the dry weight basis. Then, the gum dispersions were placed in a water bath at 80 °C under mechanical stirring for 30 min to produce uniform dispersion and complete hydration. The apparent viscosity of the crude gums of GG, LBG and FG were determined at 30 °C in an LVDV-III Ultra Rheometer (Brookfield Engineering Laboratories, Stoughton, MA, USA) fitted with a small sample adapter (SC13R) and spindle SC4-31, using exactly 9.0 mL of solution. All the measurements were conducted at least in triplicates. Curves of shear stress (σ) as a function of the shear rate $(\dot{\gamma})$ for the three gum solutions were obtained by the software (Rheocalc V3.2) with the shear rate increasing from 1.7 to 85.0 s⁻¹ within 4 min. Ostwald-de Waele model or Power-law model in Eq. (1) was applied, where the parameters k and n are related to consistency index (Pa s^n) and flow behavior index (dimensionless), respectively.

$$\sigma = k \times \dot{\gamma}^n \tag{1}$$

2.3. Fractionation of the crude gums

Three fractions for each gum of GG, LBG and FG were precipitated by the gradual addition of ethanol/isopropanol, respectively. Initially, the gum flour was dispersed into distilled water to achieve a gum concentration of 0.1% (w/w), and then hydrated at 80 °C for 30 min with mechanical stirring. The gum dispersion after full hydration was centrifuged at 12,000 \times g for 15 min (Mikro 120, Hettich Zentrifugen, Germany) to remove the insoluble residues, and the supernatant was collected for further fractional precipitation by ethanol and isopropanol. Then, a certain volume of alcohol solvent was added slowly to the supernatant under constant stirring at room temperature (25 \pm 3 °C), to yield a precipitate of galactomannan. Afterward, the solution was kept at 4 °C for 1 h to facilitate aggregation, and the precipitate was separated by centrifugation at 12,000 \times g for 15 min. The wet precipitate was weighed and then dried in a vacuum oven at 60 °C for constant

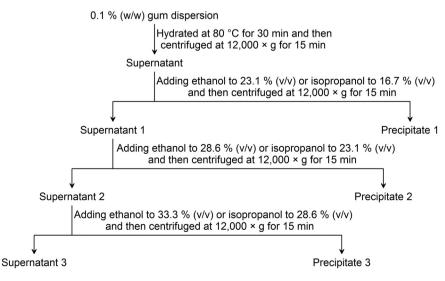


Fig. 1. Scheme of fractional precipitation of three galactomannan gums of guar gum (GG), locust bean gum (LBG) and fenugreek gum (FG) by ethanol/isopropanol.

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