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Structure characteristics and rheological properties of acidic polysaccharide from boat-fruited sterculia seeds

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ABSTRACT

An acidic polysaccharide (APS) was extracted from the boat-fruited sterculia seeds (*Semen Sterculiae Lychnophorae*). It consisted mainly of galacturonic acid (40.1%, w/w) along with rhamnose, arabinose, galactose, xylose and glucose as minor components, indicating a pectic polysaccharide which was confirmed by FT-IR spectra. The degree of esterification of APS determined by FT-IR method was 68%. The shear-thinning and viscoelastic behaviors of APS have been investigated by steady shear and small amplitude oscillatory experiments, respectively. Steady-shear rheological measurement in a range of shear rate (1–1000 s^{-1}) showed increase in pseudoplasticity (or non-Newtonian shear-thinning flow behavior) with the increase in APS concentration (1.0–10%, w/v). APS itself could not form a gel; however, thermal irreversible gels were obtained in the presence of sucrose at low pH (pH < 3.5). The changes in storage modulus G' and loss modulus G'' during heating and cooling cycles indicated that G' was nearly reversible at temperature >30 °C while G'' exhibited thermal hysteresis.

1. Introduction

As a major structural component of the primary cell walls of plants, pectic polysaccharides are widely studied natural anionic biopolymers with useful and versatile properties and applications. Their gelling capacity has provided the basis for most applications as fine chemicals in the food and pharmaceutical industries. They have been used as extrusion material in the formation of pellets, employed in formulations intended for colon drug delivery and as film coatings and tablet matrix in the pharmaceutical industry (Kjøniksen, Hiorth, & Nyström, 2005). In food systems pectic polysaccharides are used as thickeners, stabilizers, gelling agents, emulsifiers, flavor fixation agents and texture modifiers (Fu & Rao, 2001; Wang, Pagan, & Shi, 2002). All the functional properties of these types of biopolymers are closely related to their rheological properties.

Boat-fruited sterculia seed, *Semen Sterculiae Lychnophorae*, is a tropical herb of the *Sterculiaceae* family, mainly distributed in Vietnam, Thailand, Malaysia, Indonesia, as well as South China (Wang et al., 2003). The aqueous extracts from this plant exhibited some biological functions and are commonly used for the treatment of many diseases such as clearing phlegm (by "clearing heat from the lungs" as explained in Chinese medicine) and relieving sore throat to restore the voice on the upper respiratory tract, relaxing the bowels to relieve constipation (Xiao, 2002). In previous studies, we optimized the extraction process of crude polysaccharides (Wu, Cui, Tang, & Gu, 2007) and identified that the acidic polysaccharide component was the active component responsible for the anti-inflammatory properties for the boat-fruited sterculia seeds (Wu, Cui, Tang, Wang, & Gu, 2007). Although some studies on the preparation, sugar composition and biological activity of water-soluble polysaccharides from boat-fruited sterculia seeds have been reported, there is limited information currently available in the literature on the structural and rheological properties of this material (Chen, Li, Shen, Peng, & Xu, 1994; Chen, Cao, & Song, 1996; Somboonpanyakul, Wang, Cui, Barbut, & Jantawat, 2006). In order to explore further the applications and understand the functional properties of this material, the objectives of current work were to study the flow behavior and viscoelastic properties of this acidic polysaccharide.

2. Materials and methods

2.1. Plant material and chemicals

The boat-fruited sterculia seeds harvested in Vietnam were provided by Shanhe Pharmaceutical Co. Ltd. (Wuxi, China). Pectin

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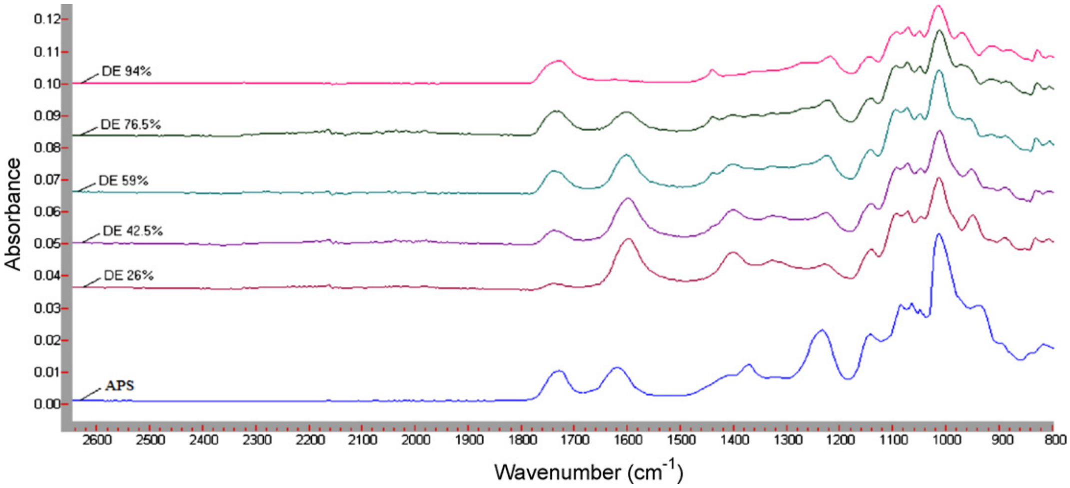

Fig. 1. Fourier transform infrared spectra of APS and pectin standards.

standards with known degree of esterification (DE) (26, 59 and 94%) were obtained from Sigma–Aldrich. Pectin standards (DE: 42.5% and 76.5%) were prepared by mixing accurate amounts of the three commercial pectins.

2.2. *Extraction and fractionation of water-soluble polysaccharides*

Crude polysaccharides were extracted from boat-fruited sterculia seeds powder and fractionated by anion-exchange chromatography on DEAE-Cellulose column (*D* 2.6 cm × 30 cm) into a neutral polysaccharide (NPS) and an acidic one (APS), as described previously (Wu, Cui, Tang, Wang, et al., 2007).

2.3. *Determination of DE value of APS sample*

The pectin standards and samples were dried in a vacuum oven at 80 °C for 3 h and desiccated overnight in a vacuum jar prior to FT-IR analysis. FT-IR spectra of the samples were obtained using a Golden-gate Diamond single reflectance ATR in a FTS 7000 FT-IR spectrometer, equipped with a DTGS detector (Digilab, Randolph, MA). The spectra were recorded at the absorbance mode from 4000 to 400 cm^{-1} (mid infrared region) at a resolution of 4 cm^{-1} with 128 co-added scans (Singthong, Cui, Ningsanond, & Goff, 2004). At least triplicate spectra were recorded for each sample.

2.4. *Rheological properties*

2.4.1. *Preparation of the samples*

Aqueous polysaccharide solutions at different concentrations ranging from 1.0 to 10% (w/v) were prepared by dissolving the dry polysaccharide powder in distilled water with agitation (60 °C, 2 h) for the steady shear tests. In the viscoelastic measurements, the polysaccharide was first dispersed in 0.1 mol L^{-1} citrate buffer (pH 3.0) at room temperature, then heating to 85 °C, and finally 60% (w/w) of sucrose was added under stirring.

2.4.2. *Rheological experiments*

All the rheological measurements were conducted on a strain-controlled ARES Rheometer (TA Instruments, New Castle, USA) using a parallel plate (40 mm in diameter, gap 1.0 mm) for both steady shear and oscillatory tests. Steady shear viscosity was determined at various concentrations at 25 °C. The viscoelastic properties, storage modulus (G') and loss modulus (G''), were determined through small amplitude oscillatory test at frequencies from 0.1 to 10 Hz. Prior to any dynamic experiments, a strain sweep test at a constant frequency of 0.1 Hz was conducted to set the upper limit of the linear viscoelastic zone. All oscillatory tests were performed at a strain value of 2%, which was within the linear viscoelastic region. After the upper plate was lowered onto the gel, a thin layer of low viscosity mineral oil was applied to the exposed edge of samples to prevent evaporation of water during the experiments. For all rheological measurements, values reported were the mean of at least two replicates.

3. Results and discussion

3.1. *Monosaccharide composition of water-soluble polysaccharides*

Protein, total carbohydrate, galacturonic acid and sugar composition of NPS and APS were reported previously (Wu, Cui, Tang, Wang, et al., 2007). NPS was a neutral polysaccharide as it was only eluted out with HAc–NaAc buffer in the anion-exchange chromatography (DEAE–cellulose) and contained no uronic acid; while APS was rich in galacturonic acid (40.1%), rhamnose (11.4%), arabinose (17.5%) and galactose (15.7%), as well as a small amount of xylose (0.6%) and glucose (0.4%). The monosaccharide composition analysis indicated APS from boat-fruited sterculia seeds was a pectic polysaccharide, which was later confirmed by FT-IR spectroscopy (Fig. 1). Since APS demonstrated significant anti-inflammatory activity (Wu, Cui, Tang, Wang, et al., 2007), the focuses of the current study were on the flow behavior and viscoelastic properties of APS and the gelation properties in the presence of co-solutes.

3.2. *FT-IR spectrum and DE value of APS*

Carbohydrates in the IR spectrum show high absorbencies in the region 1200–950 cm^{-1} (i.e., the so-called fingerprint region), where the position and intensity of the bands are specific for each polysaccharide, allowing its possible identification (Filippov, 1992). For pectic polysaccharides, the unique spectral shape in the fingerprint region is due to the high galacturonan content and the main absorbance regions of galacturonic acid (GalA) are at 1140, 1070, and 1030 cm^{-1} (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). The FT-IR spectrum of APS was compared with commercial pectin standards (Fig. 1). The shape of spectrum of APS was similar to that of commercial pectin standards confirming that APS was a pectic polysaccharide.

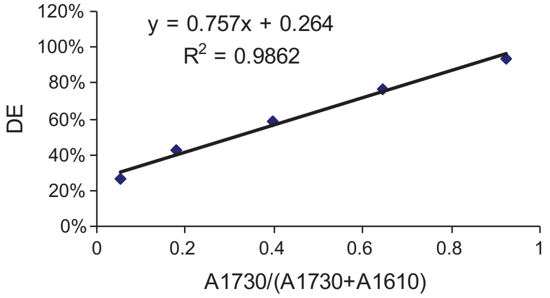

Fig. 2. Calibration curve of the degree of esterification (DE) determined by FT-IR spectra of pectin standards.

Pectic polysaccharides are composed mainly of GalA residues that can be partially esterified. The degree of esterification (DE) is defined as the percentage of carboxyl groups esterified with methanol (Barros et al., 2002). Stronger bands occurring between 1720–1740 cm^{-1} and 1600–1620 cm^{-1} are derived from the ester carbonyl (—COOR) groups and carboxylate ion stretching band (—COO$^-$), respectively. It was observed that the ester carbonyl groups increased in their intensity and band area as the DE increased, while the intensity of the carboxylate stretching band decreased (Gnanasambandam & Proctor, 2000). These observations established the basis for quantitative analysis of DE in pectic polysaccharides by FT-IR. Therefore, quantitative analysis of the bands representing ester carbonyl (1720–1740 cm^{-1}) and free carboxylate groups (1600–1620 cm^{-1}) would be important for quantitation of DE and identification of pectin samples (Singthong et al., 2004). The calibration curve was established from the ratio of $A_{1730}/(A_{1730} + A_{1610})$ based on pectin standards of known DE (Fig. 2). Using this calibration curve, the DE value of APS from boat-fruited sterculia seeds was found to be 68%.

3.3. Rheological properties

3.3.1. Flow behavior

The effect of shear rate on apparent viscosity for 1.0%, 2.0%, 4.0%, 7.0% and 10.0% (w/v) APS solutions at 25 °C is shown in Fig. 3. The apparent viscosity of high concentration solution (7.0% and 10.0%) obviously decreased with the increase of shear rate. This phenomenon indicated high concentration polymers of APS exhibited pseudoplastic flow behavior at high shear rate region (shear rate > 10 s^{-1}); whereas low concentration solutions (1.0%, 2.0% and 4.0%) exhibited almost Newtonian flow behavior coupled with relative low apparent viscosity. In concentrated solutions, interpenetration of polymer chains forms a dynamic 'entangled' network structure. At low rates of shear, those entanglements which are disrupted by the imposed deformation are replaced by new interactions between different chains, with no net change in the extent of entanglement, and hence no reduction in viscosity. This corresponded to the horizontal 'Newtonian plateau' in viscosity–shear rate plots such as those shown in Fig. 3. The onset of shear thinning occurs when the rate of externally imposed disruption rate becomes greater than the rate of formation of new entanglements. Under such conditions, the density of the temporally formed 'crosslink' of the network is depleted, hence the viscosity reduced (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). As shear rate increased, the crosslink density further decreased, as evidenced by the decrease of apparent viscosity.

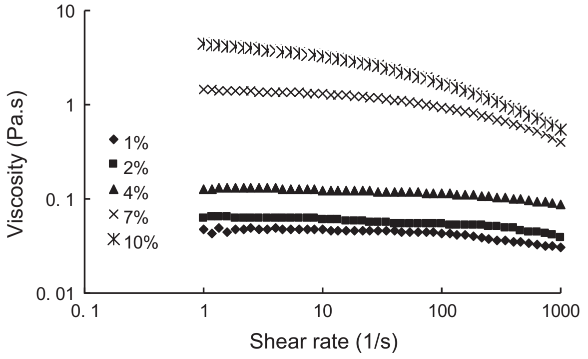

Fig. 3. Steady shear flow curves of different concentration of APS at 25 °C.

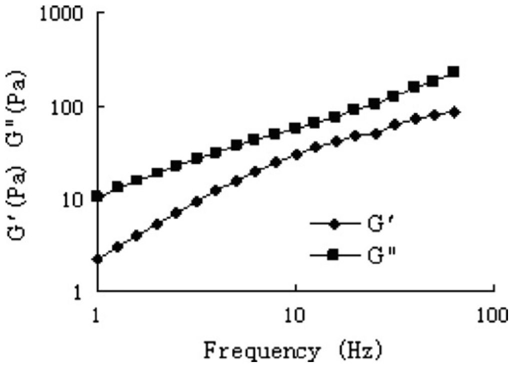

Fig. 4. Frequency dependence of storage (G') and loss (G'') modulus of APS (10%, w/v) at 25 °C.

Compared with the pectic polysaccharide from Krueo Ma Noy (Singthong, Ningsanond, Cui, & Goff, 2005), the viscosity of APS was much lower. This may indicate a low molecular weight (Mw) and branched polysaccharide structure for APS, which needs to be confirmed by structural and conformational analysis.

3.3.2. Viscoelastic behavior

The viscoelastic properties of APS were evaluated by oscillatory experimental measurements, and the results are shown in Fig. 4. At a concentration as high as 10%, the mechanical spectra of APS at 25 °C still exhibited a liquid-like property with loss modulus (G'') higher than storage modulus (G') over the entire accessible frequency range. This result indicated APS itself could not form a gel network. Based on the results of monosaccharide analysis and the FT-IR spectroscopy, APS was confirmed as a pectic polysaccharide with high degree of esterification. It is reasonable to assume that ASP may resemble high-methoxy (HM) pectin in forming gels. For HM pectin to gel, it requires an acidic environment (pH < 3.5) and in the presence of co-solutes, usually sugars, typically more than 60%, to form a gel (Löfgren, Walkenström, & Hermansson, 2002; Löfgren, Guillotin, Evenbratt, Schols, & Hermansson, 2005). The gelation mechanism is based on hydrophobic interactions and hydrogen bonds (Oakenfull & Scott, 1984). The hydrophobic interactions mainly occur between methyl esters at reduced water activity promoted by the co-solute; hydrogen bonds are favored at low pH levels due to suppression of electrostatic repulsion between pectin chains (Morris, Gidley, Murray, Powell, & Rees, 1980). For HM pectin, the gel formation depends strongly on both polymer concentration and temperature.

3.3.2.1. Effect of polymer concentration on gelation. The mechanical spectra of various concentrations of APS with 60% (w/w) sucrose at pH 3.0 and 25 °C are shown in Fig. 5. At low polymer concentrations (0.5–1%), the solution showed typical viscous fluid properties where the loss modulus G'' was significantly higher than the storage modulus G' (Morris et al., 1981); both G'' and G' showed a remarkable dependency on frequency. With the increase in polysaccharide concentrations (e.g., 2.0–4.0%), the storage

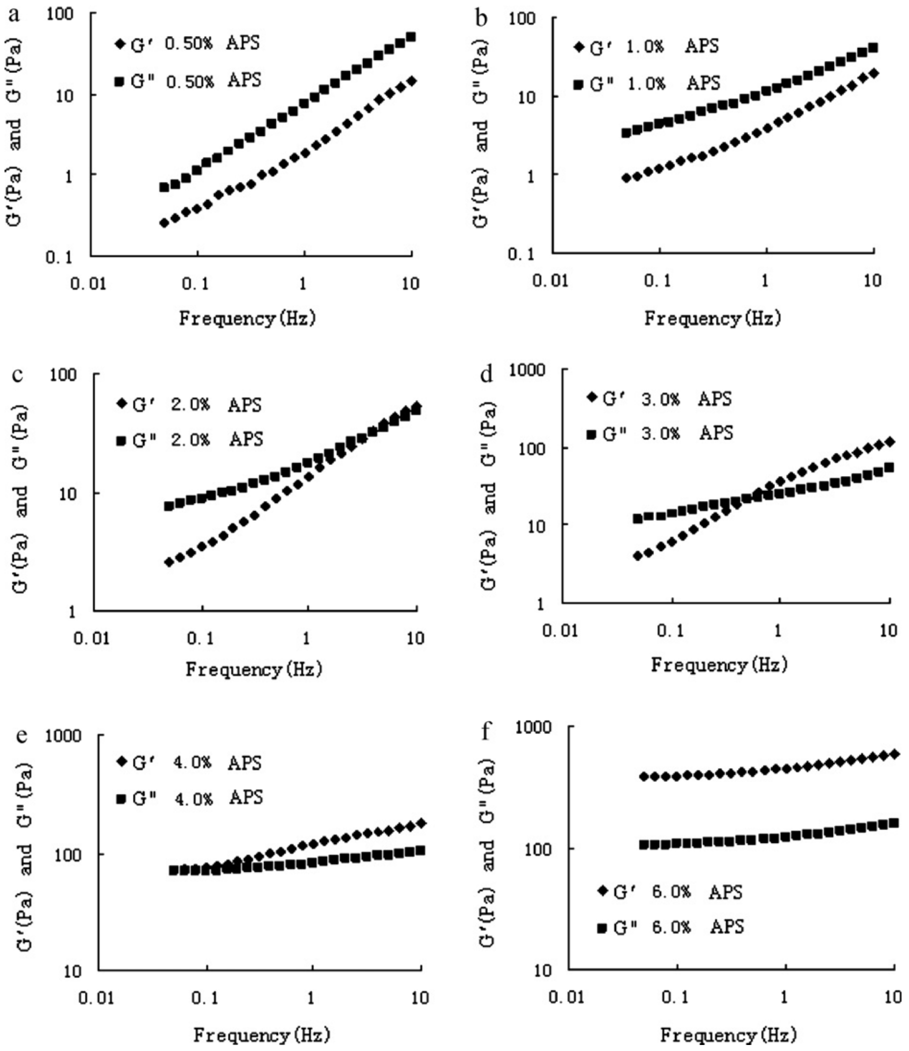

Fig. 5. Frequency dependence of storage (G') and loss (G'') modulus of APS solutions at different concentrations (at pH 3.0, with sucrose 60%, w/w) at 25 °C: (a) 0.50% APS solution, (b) 1.0% APS solution, (c) 2.0% APS solution, (d) 3.0% APS solution, (e) 4.0% APS solution, and (f) 6.0% APS solution.

modulus increased more rapidly than the loss modulus; the polymer solutions showed typical viscoelastic properties, i.e., $G' < G''$ at low frequencies and $G' > G''$ at high frequencies. A gel was formed at high APS concentration (6%), as shown in Fig. 5f, where the storage modulus G' was significantly higher than the loss modulus G'' over the entire accessible frequency range, however, the two moduli exhibited slight dependence of frequency.

3.3.2.2. Effect of temperature on APS gelation. The mechanical spectra of 3.0% APS with 60% sucrose at pH 3.0, at 5 °C and 37 °C are shown in Fig. 6. The dynamic moduli were very sensitive to temperature. At 5 °C, the behavior of the system was characteristic of a weak gel structure in which G' was higher than G'' throughout the frequency range (0.01–10 Hz), but the two moduli were dependent on frequency. This gel network formation was due to the aggregation of polymer chains involving hydrogen bonding. At 37 °C, APS–sugar system exhibited remarkable viscoelastic fluid behavior, largely caused by the weakening of the hydrogen bond due to the increase of temperature. With the increase of frequency G' crossed G'' and the moduli were significantly dependent on frequency.

The heating and cooling curves of 4% APS with 60% sucrose at pH 3.0 were obtained at a heating and cooling rate of 1 °C/min at constant frequency (0.1 Hz), as shown in Fig. 7. At the beginning of heating, storage modulus G' slightly decreased with the increase of temperature. When temperature was above 30 °C, storage modulus G' remained almost constant, while loss modulus G'' decreased slowly but consistently with the heating. During cooling period, G' was nearly reversible with the heating curve with no significant thermal hysteresis observed. However, G'' increased with the

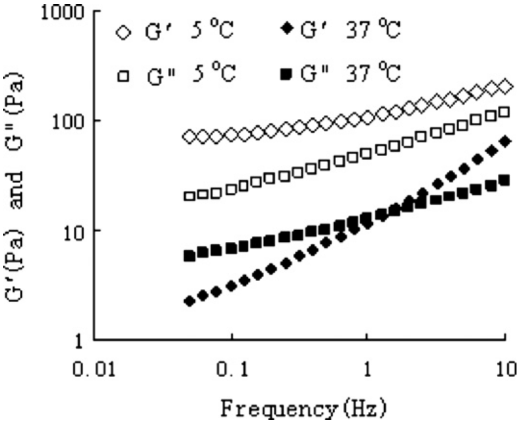

Fig. 6. Frequency dependence of storage (G') and loss (G'') modulus of 3.0% APS with 60% sucrose (pH 3.0) at 5 °C and 37 °C.

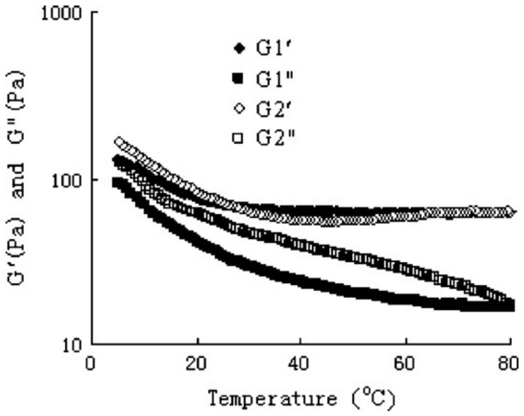

Fig. 7. Changes of storage (G') and loss (G'') modulus during initial heating from 5 to 80 °C (filled symbols) and subsequent cooling to 5 °C (open symbols) at a rate of 1 °C/min for 4.0% of APS with 60% sucrose at pH 3.0.

decrease of temperature and a thermal hysteresis was evident. APS gel did not show a melting point at temperature up to 80 °C.

4. Conclusions

An acidic polysaccharide from boat-fruited sterculia seeds described in the current paper has a potential as a commercial hydrocolloid for the food industry. The monosaccharide analysis and FT-IR spectroscopy confirmed that APS was a pectic polysaccharide with DE value of 68%, hence, belonging to HM pectin. Steady-shear rheological measurements showed a non-Newtonian shear-thinning flow behavior at high concentrations and a Newtonain flow behavior at low concentrations. Similar to other HM pectin, APS could not form a gel on its own, however, gelation occurred when a co-solute was added under acidic conditions (pH < 3.5). The mechanical spectra within the linear viscoelastic region, was a function of APS concentration and temperature. Both fluid-like (G'' dominant) and solid-like (G' dominant) behaviors were observed depending on concentration and frequency. The current paper provided a preliminary exploration of the gelation potential of the material. Extensive research is needed in order to fully explore this new material and its potential application in food and pharmaceutical industries. A detailed structure and conformation study on APS is in progress.

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