Rheological properties of a polysaccharide isolated from *Adansonia digitata* leaves

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ABSTRACT

The rheological properties of *Adansonia digitata* leaf polysaccharide were studied in dilute and semi-dilute solutions. The intrinsic viscosity of the polysaccharide obtained by Fedors equation and the combined Huggins and Kraemer extrapolations was ~3.27 dL/g. The polysaccharide contained random coil macromolecules with mass average molecular mass of 4.01×10^6 g/mol. The polysaccharide in semi-dilute concentrations exhibited strong shear thinning property, and viscoelastic behaviour was observed with solutions within (3–5% (w/w)) consistent with the formation of entangled random coil macromolecules in solution. The polysaccharide solutions were sensitive to temperature and the minimum energy to initiate flow in 4.0% polysaccharide solution calculated from Arrhenius plot of zero shear viscosity as a function of temperature was 48.6 kJ/mol. The FTIR spectral studies of the polysaccharide confirmed the presence of uronic acid groups.

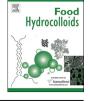© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Many of the hydrocolloids currently available as industrial gums were first used in an empirical way in domestic cookery (Ndjouenkeu, Goycoolea, Morris, & Akingbala, 1996). There are many others, however, that are not yet exploited commercially but are extensively used in local recipes, particularly in Nigeria and other tropical regions of the world. These materials are obtained from plants that grow wild or are cultivated only on a limited scale, and their functional properties as hydrocolloids remain largely unexplored. The performance of these plants in local cookery indicates they have potential for exploitation; however, there is lack of information on the properties of the hydrocolloids. Recently, some of them are receiving attention: *Afzelia Africana* (Ren, Ellis, Sutherland, & Ross-Murphy, 2003; Ren, Picout, Ellis, Ross-Murphy, & Grant Reid, 2005), *Irvingia gabonensis* (Ndjouenkeu et al., 1996; Nwokocha & Williams, 2014a; Uzomah & Ahiligwo, 1999), *Brachystegia eurycoma* (Nwokocha & Williams, 2014b; Uzomah & Ahiligwo, 1999), *Corchorus olitorius* (Yamazaki, Kurita, & Matsumura, 2009), *Detarium microcarpum* (Picout, Ross-Murphy, Errington, & Harding, 2003), *Mucuna flagellipes*

(Nwokocha & Williams, 2009) and *Hibiscus esculentum* (Ndjouenkeu et al., 1996).

Adansonia digitata L.(Bombacaceae) is an African plant known as baobab tree. The baobab tree grows in most parts of West Africa as well as in parts of North and East Africa where the leaves are used as vegetable and as a soup thickener (Builders, Okeke, & Egieye, 2007; Woolfe, Chaplin, & Otchere, 1977). Woolfe et al., (1977) who analysed the sugar composition reported it contained mainly galacturonic and glucuronic acids with minor quantities of galactose, rhamnose, glucose and arabinose; they also reported that the mucilage viscosity decreased with increase in temperature. If the polysaccharide is to find expanded application in the food industry, a detailed study is required to provide information on its application properties. Presently, we have not seen any studies on the molecular characteristics, intrinsic viscosity and mechanical spectra of this important food thickener. In this work, we studied the molecular characteristics of the polysaccharide isolated from the leaves of *A. digitata* using GPC-MALLS coupled to RI and UV detectors and the rheological properties using capillary viscometry in the dilute regime, and steady shear and small angle deformation oscillatory measurements in the semi dilute regime. We also studied the effect of temperature on the rheological properties of *A. digitata* polysaccharide and confirmed the presence of uronic acid groups using FTIR. Some preliminary characteristics of this polysaccharide have been highlighted elsewhere (Nwokocha &

Williams, 2012).

2. Materials and methods

2.1. Sample preparation and isolation of polysaccharide

Fresh leaves were collected from *A. digitata* tree and air dried. The leaves were pulverized and defatted in a soxhlet extractor using hexane as solvent. The defatted flour was rid of the solvent by leaving to dry in a vacuum chamber. The flour, 10 g/L (w/v) was dispersed in deionised water by means of a mechanical stirrer for 3 h. The resulting dispersion was poured into centrifuge bottles and centrifuged at 2500 rpm for 2 h at 25 °C. The supernatant was pooled together. The resulting supernatant was treated with excess isopropanol to precipitate the polysaccharide. The powdered polysaccharide was recovered by freeze drying.

2.2. Fourier transform infrared spectroscopy

FTIR spectra of *A. digitata* polysaccharide and its H-form were obtained on a KBr disc using FTIR spectrophotometer (Perkin Elmer Spectrum Two, USA). The disc was prepared by grinding 2 mg of the native polysaccharide with 200 mg of FTIR grade KBr and pressing into a disc. The polysaccharide in H-form was prepared by acidifying the polysaccharide with hydrochloric acid solution during which all carboxylate groups (-COO⁻) were converted to free carboxylic acid groups (-COOH); this was precipitated with ethanol and washed with the same and dried. The polysaccharide (H-form) was prepared for FTIR as previously described for the native polysaccharide.

2.3. Molecular mass determination

The molecular mass was determined using gel permeation chromatography coupled to multiangle laser light scattering and refractive index and UV detectors (Optilab DSP, Wyatt Technology Corporation, Santa Barbara Ca93103). The polysaccharide solution (20 mL) containing 4.001×10^{-4} g/mL (w/w) was subjected to microwave bomb treatment for 40 s to ensure complete disaggregation (Ratcliffe, Williams, Viebke, & Meadows, 2005), filtered through a 0.45 μm syringe filter and injected through a rheodyne into a 200 μL loop connected to a combination of Suprema columns (100 Å, 3000 Å and 30000 Å) packed with 10 μm beads of polyhydroxymethacrylate copolymer network through which the degassed (CSI 6150, Cambridge Scientific Instruments, England) eluent (0.1 M NaNO₃ + 10⁻⁶ M NaN₃ solution) was pumped (Waters: 515 HPLC Pump, Milford, MA 01757, USA) at a flow rate of 0.5 mL/min. The total injected mass was 8.002×10^{-5} g. The chromatogram was analysed with Astra software with a predetermined dn/dc value of 0.140 mL/g (Li & Xie, 2006) and the molecular mass, polydispersity and radius of gyration quantified using Berry first order polynomial.

2.4. Determination of intrinsic viscosity

0.2% (dry basis, w/w) *Adansonia digitata* polysaccharide powder was dispersed in 0.1 M NaCl solution at ambient temperature by placing on a roller mixer (SRT2, Staurt Scientific, UK) overnight. 7 mL of solution was transferred into a Cannon-ubbelohde capillary viscometer (no 75), which was immersed in a precision water bath to maintain the temperature at 25.0 ± 0.1 °C. After equilibration for 10 min, the flow time was determined between the two etched marks. Serial isoionic dilution was performed in situ and three readings were taken for each dilution and averaged. The relative viscosity, η_r, was calculated as flow time of the solution divided by

the flow time of the solvent. The intrinsic viscosity, $[\eta]$, was determined by applying Fedors equation (Eq. (1)) (Fedors, 1979)

$$\frac{1}{2\left(\eta_r^{1/2} - 1\right)} = \frac{1}{[\eta]C} - \frac{1}{[\eta]C_{max}} \tag{1}$$

Where C is the concentration of polymer (g/dL), C_{max} is a factor showing Fedors concentration limit.

The specific viscosity, η_{sp}, is related to η_r by $\eta_{sp} = \eta_r - 1$. The $[\eta]$ was also evaluated by the combined Huggins (η_{sp}/C vs C) and Kraemer (ln (η_r)/C vs C) plots.

2.5. Steady shear and small angle deformation oscillation studies

Different concentrations of the *A. digitata* polysaccharide solutions (0.5–6.0%, w/w) were prepared by dispersing the desired amount of the polysaccharide powder in distilled water while continuously solubilizing it overnight or more at ambient temperature by means of a roller mixer (SRT2, Stuart Scientific, UK). The rheological data were generated on a Controlled Stress Rheometer (AR 2000, TA Instruments, Newcastle, UK) at 25 °C using cone and plate geometry (40 mm 2° steel cone, ser no 982525, truncation gap 53 μm) for sample concentrations 3–6% and standard-size recessed end geometry (coaxial cylinders with rotor outer radius 14 mm, gap 4000 μm) for concentrations 0.5 and 2.0%. Solvent trap was used to prevent moisture loss. The sample was allowed to equilibrate for 2 min before each measurement. The flow properties were obtained by subjecting the polysaccharide solutions to a stepped-flow procedure at a shear rate of 0.01–1000 s⁻¹. In the oscillation procedure, strain sweep was performed on each gum solution from 0.003 to 100% at an angular frequency of 1 rad/s to locate the linear viscoelastic region. A frequency sweep was performed on the gum solutions in the region of 0.1–120 rad/s at an amplitude strain within the linear viscoelastic region. The resulting data was analysed using TA Data Analysis Software.

2.6. Effect of temperature

The effect of temperature on the viscosity of *A. digitata* polysaccharide was investigated in water by subjecting a 4% polysaccharide solution to a stepped flow procedure in the temperature range 5° to 55 °C at 10° interval. The Cross model was fitted to the flow curves to determine the zero shear viscosities. The activation energy for viscous flow of 4% polysaccharide in water was determined from the Arrhenius plot of zero shear viscosities (η_o) versus the inverse of absolute temperature (T) (Eq. (2)).

$$\eta_o = \eta_{o,T_\infty} \exp\left(\frac{E_a}{RT}\right) \tag{2}$$

η_o = zero shear viscosity (Pa s), η_{o,T_∞} zero shear viscosity (Pa s) at infinite temperature, E_a = activation energy for viscous flow (J/mol), R = gas constant (8.314 J/mol K), T = temperature (K). Since η_{o,T_∞} represents the zero shear viscosity at infinite temperature, the equation can be written in the natural logarithmic form by choosing a reference temperature, in this case 308 K (Eq. (3)).

$$\ln \eta_o = \ln \eta_{o,308} + \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{308}\right) \tag{3}$$