Food Hydrocolloids 41 (2014) 319–324

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Isolation and characterization of a novel polysaccharide from seeds of *Peltophorum pterocarpum*

Louis M. Nwokocha [a,*], Peter A. Williams [b]

[a] Department of Chemistry, University of Ibadan, Ibadan, Nigeria
[b] Center for Water Soluble Polymers, Glyndwr University, Wrexham, North Wales LL 11 2AW, UK

ARTICLE INFO

Article history:
Received 19 August 2013
Accepted 6 April 2014
Available online 19 April 2014

Keywords:
Peltophorum pterocarpum seed
polysaccharide
Molecular weight
Monosaccharide composition
Solution rheology

ABSTRACT

Peltophorum pterocarpum seeds yielded ~20% water soluble polysaccharide. The polysaccharide is a galactomannan with mannose:galactose ratio of 4.4:1. The polysaccharide had an intrinsic viscosity of 3.14 dl/g and weight average molecular weight of 2.49 × 10^5 g/mol. The polysaccharide solutions were non-Newtonian at concentrations above 1%. A double logarithmic plot of the zero shear specific viscosity versus volume concentration gave a coil overlap concentration, c^* of 2.6/[η], with slope $c^{1.4}$ in the dilute regime and $c^{4.3}$ in the concentrated regime. The experimental data were fitted to different viscosity models with the Martin model giving the best fit. The Cox–Merz empirical rule gave close superimposition of the data from steady shear viscosity $\eta(\dot{\gamma})$ and complex viscosity $\eta^*(\omega)$.

1. Introduction

Plants of the *leguminosae* family continue to dominate the food industry as a source of water soluble polysaccharides. The polysaccharide is located mainly in the endosperm and regulates the physiological functions of the seeds by preventing the seed from completely drying out thus avoiding the denaturation of essential enzymes (Srivastava & Kapoor, 2005). Polysaccharides are safe, replenishable and biodegradable. Hence they are ideal for application in food uses. They are employed as viscosity modifiers, gelling agents, suspending agents, encapsulating agents, water crystal and sugar crystal growth inhibitors, in flavour release control and to reduce syneresis when added to starch products.

Water soluble plant polysaccharides from guar and locust bean are among the most commonly applied industrially. They are also among the most studied and their properties are well documented in the literature (Fernandes, Gonçalves, & Doublier, 1993; Gong et al., 2012; Kök, 2007; Labeau, 2012; Mao & Chen, 2006; Patel, Ranjan, & Patel, 1987; Prabaharan, 2011). However, there are other plants of the leguminosae family which show potential for application but little is known about their polysaccharide properties, hence they have remained largely unexplored. *Peltophorum pterocarpum* is one of these plants. *P. pterocarpum* is a legume of the subfamily: Fabaceae. *P. pterocarpum* is used as an ornamental plant; it is widely referred to as Golden flamboyant tree. It produces yellow flowers and short flat elongated pods that contain the seeds. Mayworm, Salatino, and Buckeridge (2004), who worked on *Peltophorum dubium* have reported that the seed endosperm contains a galactomannan with mannose:galactose ratio of 4.5:1. We have not found any report on the molecular characteristics and rheology of the seed polysaccharide. In this study we reported the molecular characteristics and the solution properties of *P. pterocarpum* polysaccharide investigated in the dilute and concentrated regimes. The potential of this polysaccharide has been highlighted elsewhere (Nwokocha & Williams, 2012a).

2. Materials and methods

2.1. Seed composition and polysaccharide isolation

Whole seeds were selected and the weights determined. These were soaked in water to hydrate and the swollen seeds cut through with a razor, the hull, germ and endosperm were carefully separated and left to air dry to a constant weight. These were weighed and the weights reported as percentages of the whole seed. Since the seeds were small in size, polysaccharide isolation was based on defatted whole seed flour prepared as follows: Whole seed was coarsely crushed in a Warring blender and defatted in a soxhlet by extracting with hexane for 8 h. The residue was left in a fume chamber to get rid of residual hexane. A known weight of sample

* Corresponding author.
E-mail address: lmnwokocha@yahoo.com (L.M. Nwokocha).

http://dx.doi.org/10.1016/j.foodhyd.2014.04.016
0268-005X/

(~20 g) was dispersed in 400 ml of water and left in a water bath maintained at 60 °C to fully hydrate overnight. This was blended in a Warring blender, poured into a centrifuge bottle and centrifuged at 2500 rpm for 2 h. The supernatant was collected. The residue was mixed with 200 ml water and blended and the blender rinsed with some water and both transferred into a centrifuge bottle and centrifuged. The supernatants were pooled, concentrated using a Rotavapor and the polysaccharide precipitated with isopropanol. The polysaccharide was purified by dispersing in water and re-precipitating with isopropanol. The sample was reconstituted in a small amount of water and freeze dried.

2.2. Molecular weight determination

The molecular weight 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 3.9985×10^{-3} g/ml 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 and injected through a rheodyne into a 200 μl loop connected to a combination of Suprema columns (100 Å, 3000Å and 30,000Å) packed with 10 μm beads of poly-hydroxymethacrylate copolymer network. The solvent (0.1 M $NaNO_3$ + 10^{-6} M NaN_3 solution) was pumped (Waters: 515 HPLC Pump, Milford, MA 01757, USA) through a degasser (CSI 6150, Cambridge Scientific Instruments, England) at a flow rate of 0.5 ml/min. The total injected mass was 7.997×10^{-4} g. The chromatogram was analyzed with Astra software using a Zimm first order polynomial and a predetermined dn/dc value of 0.140 ml/g.

2.3. Intrinsic viscosity

A sample for intrinsic viscosity measurement was prepared by dispersing 0.4% (w/w) polysaccharide powder in distilled water overnight on a roller mixer (SRT2, Stuart Scientific, UK) maintained at 25 °C. The polysaccharide solution was passed through a 0.8 micron Nylon filter and 7 ml of the solution transferred into a Canon-Ubbelohde capillary viscometer (75, J379). The viscometer was immersed in a precision water bath maintained at 25.0 ± 0.1 °C and the flow time between the two etched marks determined after equilibrating for 15 min. Serial dilution was performed in situ and three readings were taken for each dilution after equilibration and averaged. The relative viscosity, η_r, was calculated as the ratio of the flow time of the polymer solution to that of water. The specific viscosity, η_{sp}, was obtained as $\eta_r - 1$. The intrinsic viscosity, $[\eta]$, was evaluated by Fedors equations (Eqn. (1)) and combined extrapolation of the Huggins and Kraemer

$$\frac{1}{2\left(\eta_r^{\frac{1}{2}} - 1\right)} = \frac{1}{[\eta]c} - \frac{1}{c_m[\eta]} \quad (1)$$

where c is the polymer concentration, c_m is the upper limit of Fedors concentration.

2.4. Rheological measurements

Polysaccharide solutions (0.49%–5%, w/w) were characterized rheologically using a steady shear stepped flow procedure at shear rates from 0.001/s to 1000/s (AR2000, TA Instruments, Newcastle, UK). Small angle deformation oscillatory measurements were used to study the visco-elastic properties at angular frequency from 0.01 rad/s to 625.8 rad/s at a percentage strain within the linear viscoelastic range. The percentage strain was determined by carrying out an oscillation stress sweep at an angular frequency of 6.283 rad/s. Standard-size recessed end concentric cylinders (gap 4 mm, rotor outer radius 14 mm) was used for 0.49%; 60 mm acrylic plate (ser no 70408, gap 1000 μm) was used for 1%–1.5%, and 40 mm steel plate hatched (ser no 994387, gap 1000 μm) for 2% and higher concentrations. A solvent trap was employed to reduce loss of moisture. The measurements were conducted at a temperature of 25 °C.

3. Results and discussion

3.1. Physical composition of seed and polysaccharide yield

P. pterocarpum seeds (Fig. 1) consist of 42.1% coat, 29.3% endosperm and 28.6% germ (Table 1a). The endosperm is translucent and rubbery in appearance indicating a high content of polysaccharide. The polysaccharide in most seeds is located in the endosperm; hence a high content of endosperm may indicate a high content of the polysaccharide. The polysaccharide yield from *P. pterocarpum* seed was 20.4%. This yield is in the range of 14–30% reported for *Leucaena leucocephala* (Pamplona & Zerrudo, 2008; Soni & Varshney, 2003; Unrau, 1961), but less than 32.6% reported for *Mucuna flagellipes* with 67.15% endosperm (Nwokocha & Williams, 2009) and 59.8% for *Detarium senegalense* (Wang, Ellis, Ross-Murphy, & Reid, 1996). The *P. pterocarpum* seed polysaccharide was found to contain mannose (80.07%), galactose (18.08%), and very small amounts of glucose (0.51%), arabinose (0.29%), rhamnose (0.22%), glucuronic acid (0.46%) and galacturonic acid (0.37%). Therefore *P. pterocarpum* polysaccharide is a galactomannan with mannose:galactose (*M*/*G*) ratio of 4.4:1. The *M*/*G* ratio is similar to the 4.5:1 reported for *P. dubium* galactomannan (Mayworm et al., 2004) but higher than *M*/*G* of: locust bean gum, 4:1; Tara gum, 3:1; guar gum, 2:1 (Mathur & Mathur, 2005; Picout, Ross-Murphy, Jumel, & Harding, 2002). The *M*/*G* ratio has been reported to affect the functionality of galactomannans. Galactomannans with high percentage galactose have high cold water dispersibility and high viscosity but poor gelling properties (Mathur & Mathur, 2005). The high *M*/*G* ratio of *P. pterocarpum* galactomannan indicates that it has potential application in combination with kappa carrageenan to modify to enhance its gel characteristics.

Fig. 1. *Peltophorum pterocarpum* seeds.