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Fractionation of bamboo hemicelluloses by graded saturated ammonium sulphate

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ABSTRACT

The hemicelluloses were isolated with 10% KOH at 25 °C from dewaxed and delignified bamboo powder. The alkali-soluble hemicelluloses from *Sinocalamus affinis* were fractionated by ammonium sulphate precipitation method. The bamboo alkali-soluble hemicelluloses yielded seven hemicellulosic fractions obtained at 0, 5, 15, 25, 40, 55, and 70% saturation with ammonium sulphate. It was found that the more branched hemicelluloses were precipitated at higher ammonium sulphate concentrations (55 and 70%), the more linear hemicelluloses were precipitated at lower ammonium sulphate concentrations (0, 5, 15, 25, and 40%). The molecular weights of hemicellulosic fractions become lower from 35,270 (H_0) to 18,680 (H_{70}) g mol^{-1} with the increasing concentrations of saturated ammonium sulphate from 0 to 70%. Based on the FT-IR, ^{1}H, ^{13}C and 2D HSQC NMR studies, the alkali-soluble hemicelluloses were 4-*O*-methyl-glucuronoarabinoxylans composed of the (1 → 4)-linked β-D-xylopyranosyl backbone with branches at *O*-3 of α-L-arabinofuranosyl or at *O*-2 of 4-*O*-methyl-α-D-glucuronic acid.

1. Introduction

Lignocellulosic materials, the most abundant biomass resource on the earth, represent a major source of carbon for biofuels, materials, and biochemicals. The biorefinery concept is based on the selective separation of the major lignocellulosic materials components (cellulose, hemicelluloses, and lignin), thus achieve the goal of high value-added conversion of the separated components (Haraguchi & Li, 2006; Sun, 2009). Bamboo is a perennial woody grass belonging to the *Gramineae* family and *Bambuseae* subfamily encompassing about 1250 species within 75 genera, distributed in the Asian countries, such as China, and other Southeast Asian countries, with a total annual production of 6–7 million tons (Scurlock, Dayton, & Hames, 2000). The culms of bamboo are reproduced asexually every year with a rapid growth rate of sprouts. Because of their high productivity, much attention has been paid to energy feedstock and bio-based materials (Li, Fan, Sun, & Xu, 2010; Peng et al., 2013; Peng & She, 2014; Shao, Jin, Wen, & Iiyama, 2009; Zhang, Yu, Huang, & Liu, 2007).

In lignocellulosic materials, hemicelluloses rank second to cellulose in abundance, comprising roughly one-fourth to one-third of most plant materials (Sun, 2009). Hemicelluloses are usually associated with various other cell-wall components such as cellulose, cell-wall proteins, lignin, and other phenolic compounds by covalent and hydrogen bonds, and by ionic and hydrophobic interactions. They are branched polymers of low molecular weight with a degree of polymerization of 80–200. Studies on utilization of hemicelluloses have shown them to be a potential fermentation feedstock in production of sugars, fuel ethanol, and other value-added chemicals, such as 5-hydroxymethylfurfural (HMF), furfural, levulinic acid, and xylitol (Canilha, Silva, Felipe, & Carvalho, 2003). In addition, the properties of hemicelluloses being worth exploiting are their ability to serve as adhesives, thickeners, stabilizers, as well as film formers and emulsifiers. Moreover, hemicelluloses have also been investigated for their possible medical uses due to their ulcer protective (Cipriani et al., 2006), antitussive (Kardošová, Malovíková, Pätoprstý, Nosál'ová, & Matáková, 2002), immunostimulatory (Kulicke, Lettau, & Thielking, 1997), and antitumor (Kitamura et al., 1994) properties. Although the hemicelluloses have a very wide variety of applications, the hemicelluloses removal is desired to improve the accessibility of cellulose material to hydrolytic enzymes in the process of ethanol production. Therefore, efficient utilization of the biomass components by pre-extraction and isolation of hemicelluloses will increase revenue streams for the lignocellulosic biorefinery and help to maximize the utilization of biomass in the production of fuels, chemicals, and materials.

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The isolated hemicellulosic preparations consist of different hemicellulosic molecules which vary in structural characteristics. Owing to the heterogeneity and complex structure of hemicelluloses, the properties of hemicelluloses and their derivative are unstable. In general, the hemicelluloses were purified by ion-exchange chromatograph and gel chromatography. But these methods are very complicated, expensive, time-consuming, and difficult for industry. In view of the facts, several fractionation techniques, such as graded ethanol precipitation (Peng et al., 2009) and iodine-complex precipitation (Peng et al., 2010) have been proposed in order to obtain more homogeneous fractions. Salting-out procedures, such as ammonium sulfate precipitation, commonly utilized in the isolation and fractionation of proteins (Stec, Bicka, & Kuzmak, 2004), have received less attention in the area of fractionation and purification of polysaccharides. In the previous work, acetyl and non-acetyl hemicelluloses were fractionated by precipitation at 50% and 80% saturated ammonium sulphate precipitation (Peng et al., 2012). The present work was primarily carried out to gain a new knowledge on the structural variation and physicochemical properties of hemicellulosic fractions from alkali-soluble hemicelluloses by graded saturated ammonium sulphate precipitation, and thus explore relationships between structure and method for hemicelluloses.

2. Materials and methods

2.1. Materials

Sinocalamus affinis was harvested in January 2011 in Sichuan Province, China. After removal of leaves, the trunks were chipped into small pieces. The chips were dried in sunlight and then grounded to pass a 0.8-mm-size screen. After being further dried at 60 °C for 16 h, the powder was dewaxed with 2:1 (v/v) toluene–ethanol in a Soxhlet apparatus for 6 h. The dewaxed sample was further dried in a cabinet oven with air circulation at 60 °C for 16 h. The components (%, w/w) of the *S. affinis* were holocellulose 72.1%, pentosans 25.0%, Klason lignin 23.5%, extractives 2.2%, and ash 2.4%, which were determined according to Tappi standards (Tappi 2002) for measuring the chemical composition. All standard chemicals were analytical grade, purchased from Sigma Chemical Company (Beijing).

2.2. Extraction and fractionation

The fractional isolation of hemicelluloses from *S. affinis* is illustrated in Fig. 1. The dewaxed powder was delignified with 6% sodium chlorite at pH 3.6–3.8, adjusted with 10% acetic acid, at 75 °C for 2 h. The residue, holocellulose, was subsequently washed with distilled water, and dried at 60 °C for 16 h. Then the holocellulose was extracted with 10% KOH at 25 °C for 16 h. The filtrate was neutralized with 6.0 M acetic acid to pH 5.5, and concentrated to 160 mL. After standing for 12 h, the solution was centrifuged at 3500 rpm for 15 min. The precipitated hemicelluloses A was recovered and labeled as H_A. Ammonium sulphate was slowly added to the supernatant of hemicelluloses up to 5% saturation; the solution was allowed to stand overnight at 25 °C. The precipitated hemicelluloses were collected by centrifugation (3500 rmp, 15 min), redissolved in distilled H_2O, and dialyzed until free of $(NH_4)_2SO_4$, freeze-dried, and designated as H_5. Then the saturation level of ammonium sulphate in the remaining filtrate was subsequently adjusted stepwise to 15, 25, 40, 55, and 70%. The corresponding precipitated hemicellulosic fractions were labeled as H_{15}, H_{25}, H_{40}, H_{55}, and H_{70}, respectively. The unprecipitated hemicelluloses by 70% saturated ammonium sulphate remaining in the supernatant were obtained by precipitating in three volumes of ethanol, and labeled as H_R.

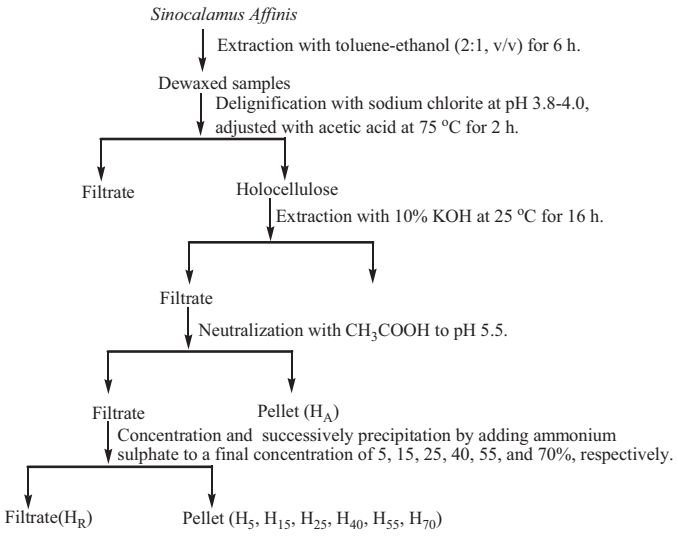

Fig. 1. Scheme for fractionation of hemicelluloses from *Sinocalamus affinis*.

2.3. Chemical characterization

The constituent neutral sugar in the isolated hemicellulosic fractions was determined by high performance anion exchange chromatography (HPAEC). The hemicelluloses (4–6 mg) were hydrolyzed by 10% H_2SO_4 at 105 °C in a sealed tube for 2.5 h. After hydrolysis, the samples were diluted 30-fold, filtered, and injected into the HPAEC system (Dionex ISC 3000, USA) with amperometric detector, AS50 autosampler and a CarbopacTM PA1 column (4 mm × 250 mm, Dionex) (Peng et al., 2010). The molecular weights of the hemicellulosic fractions were determined by gel permeation chromatography (GPC) on a PL aquagel-OH 50 column (300 mm × 7.7 mm, polymer laboratories Ltd), calibrated with PL pullulan polysaccharide standards (peak average molecular weights 783, 12,200, 100,000, 1,600,000, Polymer Laboratories Ltd) (Peng et al., 2010).

2.4. Spectroscopic characterization

FT-IR experiments were conducted using a Thermo Scientific Nicolet iN 10 FT-IR Microscopy (Thermo Nicolet Corporation, Madison, WI) equipped with a liquid nitrogen cooled MCT detector. Dried samples were grounded and pallertized using BaF_2, and their spectra were recorded from 4000 to 650 cm^{-1} at a resolution of 4 cm^{-1} and 128 scans per sample. The solution-state ^1H NMR spectra were recorded on a Bruker NMR spectrometer at 400 MHz using 15 mg of hemicelluloses in 1.0 mL of D_2O. The chemical shifts reported were calibrated relative to the signals from D_2O, used as an internal standard, at 4.7 ppm for the ^1H NMR spectra. The ^{13}C NMR spectra were recorded at 25 °C after 30,000 scans. The sample (80 mg) was dissolved in 1.0 mL of D_2O (99.8% D) with overnight at room temperature. Chemical shifts (δ) were expressed relative to the resonance of Me_4Si ($\delta = 0$). A 30° pulse flipping angle, a 3.9 μs pulse width, and a 0.85 s delay time between scans were used. The proton-detected heteronuclear single quantum correlation (HSQC) spectra were acquired by HSQCGE experiment mode, over a t_1 spectral width of 10,000 Hz and a t_2 width of 1800 Hz, and the acquired time (AQ) was 0.1163 s. The number of scan (NS) was 32. The delay between transients was 2.6 s and the delay for polarization transfer was set to correspond to an estimated average ^1H–^{13}C coupling