ISOLATION AND CHARACTERIZATION OF SOLUBLE POLYSACCHARIDES OF *DENDROCALAMUS BRANDISII*: A HIGH-YIELDING BAMBOO SPECIES

Zheng-Jun Shi,[a,b] Ling-Ping Xiao,[a] Jia Deng,[a] Feng Xu,[a] and Run-Cang Sun[a,c,]*

Nine soluble polysaccharide fractions were sequentially extracted with hot water at 80, 100, and 120 °C for 3 h, and 60% aqueous ethanol containing 0.25, 0.50, 1.00, 2.00, 3.00, and 5.00% NaOH at 80 °C for 3 h from dewaxed bamboo (*Dendrocalamus brandisii*) sample, and their chemical compositions and physicochemical properties were examined. The sequential treatments yielded 20.6% soluble polysaccharides of the dry dewaxed bamboo material. Molecular weight and neutral sugars analysis revealed that the soluble polysaccharides were mainly composed of arabinoglucuronoxylans and amylose starch. Spectroscopy (FT-IR, ^{1}H, ^{13}C, and 2D-HSQC NMR) analyses suggested that the isolated arabinoglucuronoxylans from bamboo (*D. brandisii*) could be defined as a linear (1→4)-β-linked-xylopyranosyl backbone to which α-L-arabinofuranose units and/or short chains of 4-*O*-methyl-glucuronic acid were attached as side residues via α-(1→3) and/or α-(1→2) linkages. In addition, it was found that the thermal stability of polysaccharides increased with an increment of their molar mass.

Keywords: Bamboo; NMR; Biopolymers; Polysaccharides; Structure; Characterization

*Contact information: a: Institute of Biomass Chemistry and Technology, Beijing Forestry University, Beijing, 100083 China; b: College of Material Science and Technology, Southwest Forestry University, Kunming, 650224 China; c: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, 510640 China; * Corresponding author: rcsun3@bjfu.edu.cn (R.C. Sun)*

INTRODUCTION

In order to meet the energy demand and maintain global climate stability, efforts to exploit a variety of plants have been made particularly for agricultural and forestry biomass resources. The bamboo species *Dendrocalamus brandisii*, belonging to *Bambusoideae* of *Gramineae*, having strong and abundant woody stems, is mainly distributed in Southeast Asia, including the southwest region of China. Traditionally, as a raw material, this kind of bamboo species is widely used in construction, paper making, and man-made board industries. Due to its easy propagation, fast growth, and high productivity, *D. brandisii* is considered as one of the most potential renewable non-woody forestry feedstock for the production of value-added products from its lignocellulosic components, such as hemicelluloses and starch.

Hemicelluloses are the second most abundant plant renewable materials after celluloses (Sun et al. 2001). Unlike cellulose, hemicelluloses are not chemically homogeneous but rather a family of polysaccharides, which consist of various different sugar units, arranged in different proportions and with different substituents (Aspinall et al. 1954). The principal sugars of hemicelluloses are D-xylose, L-arabinose, D-glucose, D-

galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fucose, and various *O*-methylated neutral sugars (Sun et al. 2005). The most abundant hemicelluloses in annual plants are arabinoglucuronoxylans, which contain a backbone of D-xylopyranosyl residues, linked together by β-(1→4)-glycosidic bonds (Bendahou et al. 2007). In the plant cell walls, hemicelluloses are associated with cellulose and lignin by hydrogen bonds and covalent bonds (mainly ether and ester linkages). Therefore, isolation of hemicelluloses in a pure form from plant cell walls involves hydrolysis of ester and ether linkages followed by extracting them into aqueous media (Xu et al. 2007). Starch, the main source of carbohydrate in food and non-food industry, is composed of two types of polysaccharide: the almost linear α-(1→4)-glucan amylose and α-(1→4)-glucan with α-(1→6)-branched amylopectin (Cheetham et al. 1998). Currently, with the development of biomass ethanol industry, much attention has been devoted to exploring various new non-food biomass resources with high contents of starch.

Traditionally, for quantitative isolation of hemicellulosic polysaccharides from both hardwood and softwood, materials have been first delignified, generally with chlorine (Timell et al. 1951), chlorine dioxide (Yang et al. 1978), or sodium chlorite (Fengel et al. 1989), after which the left holocellulose has then been treated with various procedures. However, the treatments of delignification often oxidize some reducing-end residues to aldonic acid residues and cause partial depolymerization, and some loss of components is also inevitable (Aspinall et al. 1961). For these reasons, numerous methods have been developed in an attempt to extract as much of the hemicellulosic polysaccharides as possible while minimizing the extent of chemical modification. These methods include extraction with concentrated solutions of sodium or potassium hydroxide (Dupont et al. 1987), alkaline hydrogen peroxide solution (Doner et al. 1997), solutions of barium or calcium hydroxides at elevated temperatures (Bergmans et al. 1996), or with hot water (Sun et al. 2004). Among these methods, hot water and alkali extractions are preferred to be chosen due to the various advantages, such as environmentally friendly characteristics, simplicity of performance, and cost-effectiveness. In addition, previous studies have shown that the plant hemicellulosic preparations consist of several hemicellulosic polymers that vary in structural characteristics (Cyran et al. 2007), and one step of treatment could only extract part of the hemicelluloses from the raw materials (Bergmans et al. 1996). In view of these facts, fractionation techniques, such as sequentially extracted with hot water at different temperatures and alkaline aqueous ethanol with different alkali concentrations, were worth attempting to obtain more homogeneous polysaccharide fractions and thus to further explore their structural properties.

Although *D. brandisii* is considered to be one of the most potential renewable biomass resources, the detailed physicochemical properties of soluble polysaccharides present in this kind of bamboo species have not been reported in the previous literature. For that, the present work was aimed at fractionally isolating soluble polysaccharides from the cell walls of *D. brandisii* and comparatively elucidating their physicochemical properties. In this work, the isolated polysaccharide fractions were investigated by acid hydrolysis, gel permeation chromatography (GPC), Fourier transform infrared (FT-IR), thermal analysis, proton magnetic resonance (^{1}H NMR), carbon-13 magnetic resonance

(^{13}C NMR) spectroscopies, and heteronuclear single quantum correlation (HSQC) spectroscopy, as well as thermogravimetric analysis (TGA) and differential thermal analysis (DTA).

EXPERIMENTAL

Materials

Bamboo (*D. brandisii*), 3 years old, was obtained from Yunnan Province, China. It was first dried in sunlight and then chipped into small pieces. The air-dried pieces of bamboo were ground and screened to obtain a 40-60 mesh fraction. This fraction was subjected to extraction with toluene/ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h, and then the dewaxed powder was further dried in an oven under circulated air at 60 °C for 16 h before use. The compositions of *D. brandisii* were cellulose, 53.19%, Klason lignin, 23.06%, xylan, 20.47%, arabinan, 0.75%, galactan, 0.28%, rhamnan, 0.15%, glucuronic acid, 0.17%, and galacturonic acid, 0.05%, on a dry weight basis, determined by use of National Renewable Energy Laboratory's standard analytical method (Sluiter et al. 2008). All standard chemicals, such as monosaccharide and chromatographic reagents, were purchased from Sigma Chemical Company (Beijing, China).

Isolation of Polysaccharide Fractions

A scheme for separation of bamboo (*D. brandisii*) polysaccharide fractions is shown in Fig. 1. The dewaxed bamboo sample (15.00 g) was sequentially extracted with hot water (at 80, 100, and 120 °C) for 3 h, 60% alkaline aqueous ethanol (containing 0.25, 0.50, 1.00, 2.00, 3.00, and 5.00% NaOH) at 80 °C for 3 h with a solid to liquid ratio of 1:20 (g/mL). The extracted solutions were filtrated with a Buchner funnel. After filtration, the filtrate was neutralized with 6 M hydrochloric acid solution to pH 5.5 (note that the three water-extractable solutions do not need to be neutralized to pH 5.5 since the water-extractable solution showed weak acidity), and then concentrated to about 20 mL with a rotary evaporator under reduced pressure. After that, three volumes of ethanol were added to each concentrated solution with continuous stirring, and then the flocculent precipitate appeared. The precipitated polysaccharide fractions were centrifuged and washed with 70% ethanol at room temperature and freeze-dried. Subsequently, the three polysaccharide preparations extracted by hot water (at 80, 100, and 120 °C) were labeled as H_1, H_2, and H_3, and the six polysaccharide preparations extracted by alkaline aqueous ethanol (containing 0.25, 0.50, 1.00, 2.00, 3.00, and 5.00% NaOH) were labeled as H_4, H_5, H_6, H_7, H_8, and H_9, respectively. It should be noted that the bamboo sample and water were transferred into an autoclave when extracted with hot water at 120 °C to obtain the polysaccharide fraction H_3. All the experiments were performed at least in duplicate. Yields of the polysaccharide fractions were calculated on a dry weight basis related to the dewaxed bamboo samples. The relative standard deviation was observed to be lower than 4.50%.

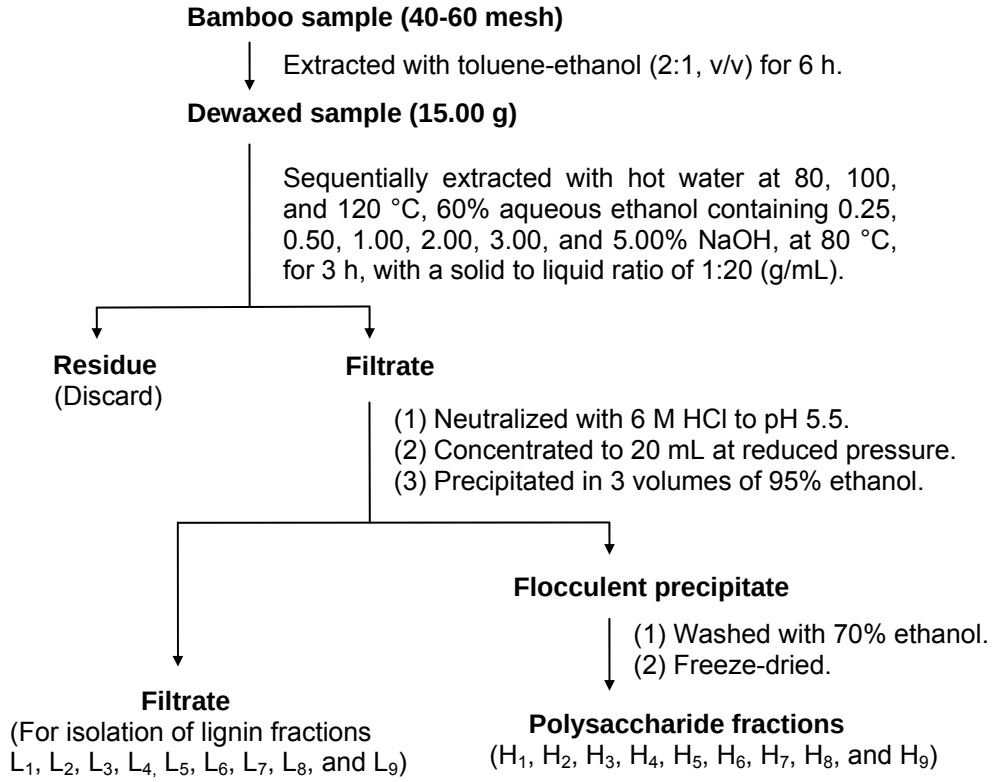

Fig. 1. Scheme for extraction of polysaccharide fractions from bamboo *D. brandisii*

Characterization of the Polysaccharide Fractions

The molecular weights of the polysaccharide preparations were determined by gel permeation chromatography (GPC) on a PL aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories Ltd.), calibrated with PL pullulan polysaccharide standards (peak average molecular weights of 783, 12,200, 100,000, and 1,600,000 g/mol, Polymer Laboratories Ltd.). A flow rate of 0.5 mL/min was maintained. The eluent was 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5. Detection was achieved with a Knauer differential refractive index detector (RID). The column oven was kept at 30 °C. Polysaccharides were dissolved with 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5, at a concentration of 0.10%.

The neutral sugar compositions of the polysaccharide fractions were determined by hydrolysis with dilute sulfuric acid. A 4-6 mg sample of polysaccharides was hydrolyzed with 1.475 mL of 6.10% H_2SO_4 for 2.5 h at 105 °C. After hydrolysis, the mixture was filtered, and the filtrate containing the liberated neutral sugars was analyzed by high-performance anion exchange chromatography (HPAEC) system (Dionex ICS 3000, U.S.) with pulsed amperometric detector and an ion exchange Carbopac PA-1 column (4 × 250 mm). Neutral sugars were separated in 18 mM NaOH (carbonate-free and purged with nitrogen) with post-column addition of 0.30 M NaOH at a rate of 0.5 mL/min. Run time was 45 min, followed by 10 min elution with 0.2 M NaOH to wash the column and then a 15 min elution with 18 mM NaOH to reequilibrate the column. Calibration was performed with standard solutions of L-rhamnose, L-arabinose, D-

glucose, D-galactose, D-mannose, D-xylose, glucuronic acid, and galacturonic acid. The analyses were run twice, and the average value calculated for all of the polysaccharide fractions.

The FT-IR spectra of the polysaccharide fractions were obtained using a KBr disk containing 1% finely ground samples on a FT-IR Spectrometer (Bruker Tensor 27) in the range of 4000 to 400 cm^{-1}. The solution-state ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AVIII 400 MHz spectrometer. The sample (15.00 mg for ^{1}H, 80.00 mg for ^{13}C) was dissolved in 1.00 mL D_2O. The chemical shifts were calibrated relative to the signals from D_2O, used as an internal standard, at 4.7 ppm for the ^{1}H NMR spectra. ^{13}C NMR spectra were obtained at 25 °C after 30000 scans. A 30° pulse flipping angle, a 9.2 μs pulse width, and a 2 s delay time between scans were used. The heteronuclear single quantum coherence (HSQC) spectra were acquired over a t_1 spectral width of 20000 Hz and a t_2 width of 3600 Hz, and the acquired time per scan (AQ) is 0.1409 s. The numbers of scan (NS) was 64. The delay between transients was 1.5 s, and the delay for polarization transfer was set to correspond to an estimated average ^{1}H-^{13}C coupling constant of 145 Hz.

Thermogravimetric analysis of polysaccharide fractions was performed using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (DTG-60, Shimadzu, Japan). Samples of approximately 10 mg weight were heated in an aluminium crucible from room temperature to 600 °C at a heating rate of 10 °C/min while the apparatus was continually flushed with a nitrogen flow of 20 mL/min.

RESULTS AND DISCUSSION

Fractional Yield of Polysaccharides

It is well known that the soluble polysaccharide is mixture of a number of different macromolecular substances, and the yield and composition of the polymer can vary depending on the methods of isolation (Morrison et al. 1974). Table 1 shows that the successive treatments of the dewaxed bamboo sample with hot water at 80, 100, and 120 °C for 3 h, and with 60% aqueous ethanol containing 0.25, 0.50, 1.00, 2.00, 3.00, and 5.00% NaOH at 80 °C for 3 h, resulted in a dissolution of 3.20, 0.80, 0.60, 1.50, 2.90, 6.30, 2.30, 1.40, and 1.60% of the bamboo polysaccharides (percent dry matter), respectively. The total yield of the nine soluble polysaccharide preparations was 20.60% of the initial dry weight.

As can be seen, the hot water-soluble polysaccharides accounted for 22.33% of the totally extracted polysaccharides, implying that the bamboo (*D. brandisii*) probably contained a relatively high content of glucose-rich substances. It should be noted that when the bamboo residue was extracted with hot compressed water at 120 °C, the yield of polysaccharides was low. This fact indicated that almost all of the water-soluble polysaccharides had already been released from the bamboo material after three steps of hot water treatments. However, it was found that when the bamboo residue was treated with alkaline aqueous ethanol, some amounts of polysaccharides in bamboo cell wall were dissolved, suggesting that alkaline aqueous ethanol at high temperature could

significantly dissolve polysaccharides from plant cell wall. The high solubility of bamboo polysaccharides in alkaline aqueous ethanol resulted from the alkali function, because hydroxyl ions liberated from alkaline solutions could cause swelling of cellulose, disruption of intermolecular hydrogen bonds between cellulose and hemicelluloses, and hydrolysis of ester bonds that most likely play an important role in connecting the cell wall polysaccharides and lignin (Bergmans et al. 1996). It could be speculated that these differences in extractability of polysaccharides were the results of different structural properties of these polymers in the bamboo cell walls.

Table 1. Yield of Polysaccharides Solubilized during the Successive Treatments of *D. brandisii* with Hot Water and Alkaline Aqueous Ethanol

Polysaccharide fractions	Yield (% dry matter)
80 °C water-soluble polysaccharides (H_1)	3.20
100 °C water-soluble polysaccharides (H_2)	0.80
120 °C water-soluble polysaccharides (H_3)	0.60
60% ethanol and 0.25% NaOH extractable polysaccharides (H_4)	1.50
60% ethanol and 0.50% NaOH extractable polysaccharides (H_5)	2.90
60% ethanol and 1.00% NaOH extractable polysaccharides (H_6)	6.30
60% ethanol and 2.00% NaOH extractable polysaccharides (H_7)	2.30
60% ethanol and 3.00% NaOH extractable polysaccharides (H_8)	1.40
60% ethanol and 5.00% NaOH extractable polysaccharides (H_9)	1.60
Total solubilized polysaccharides	20.60

Molecular Weight Distribution

Generally, the molecular weight values of soluble polysaccharides are dependent on the method of isolation performed. To evaluate the difference of the nine isolated soluble polysaccharide fractions, in this study, the weight-average (M_w) and number-average (M_n) molecular weights, and polydispersity (M_w/M_n) of polysaccharide fractions were analyzed by gel permeation chromatography (GPC), and the results are listed in Table 2. It was found that the three steps treatments of dewaxed bamboo sample with hot water resulted in dissolution of polysaccharides with lower average molecular weights (ranging from 18330 to 19730 g/mol), and the subsequent treatments of bamboo residue with alkaline aqueous ethanol led to release of polysaccharides with relatively higher molecular weights (ranging from 22870 to 41180 g/mol). Additionally, it should be noted that as the NaOH concentration increased from 0.25% to 5.00%, the M_w value of the isolated polysaccharides increased from 35700 g/mol (H_4) to 41180 g/mol (H_6), and then decreased to 22870 g/mol (H_9), implying that noticeable degradation occurred under the conditions of higher alkali concentrations.

The polydispersity is an important parameter of macromolecules in the chemical industry. In general, narrow polydispersity means better physicochemical stability. Therefore, it is important to get polymers with a relatively narrow polydispersity from plants. As shown by the data in Table 2, the three polysaccharide fractions isolated from the dewaxed bamboo with hot water had wider distribution of molecular weights (from 3.40 to 5.60), and the six polysaccharide fractions isolated from the bamboo residue with alkaline aqueous ethanol showed narrow distribution of molecular weights (from 1.12 to 1.96).

Table 2. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Polysaccharide Fractions Isolated from Bamboo (*D. brandisii*)

	Polysaccharide fractions [a]								
	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9
M_w	19170	19730	18330	35700	35910	41180	31320	23220	22870
M_n	3420	2490	5430	18240	25310	27200	22470	19120	20470
M_w/M_n	5.60	4.60	3.40	1.96	1.41	1.51	1.42	1.21	1.12

[a] Represents the bamboo polysaccharide fractions isolated by hot water at 80, 100, and 120 °C for 3 h, aqueous ethanol containing 0.25, 0.50, 1.00, 2.00, 3.00, and 5.00% NaOH, at 80 °C for 3 h

Content of Neutral Sugars and Uronic Acids

To analyze the difference among these polysaccharide fractions sequentially isolated from bamboo (*D. brandisii*), the contents of neutral sugars and uronic acids of the nine polysaccharide fractions were detected, and the data are illustrated in Table 3. Interestingly, the analysis results showed that the three polysaccharide preparations isolated from *D. brandisii* with hot water, had a similar high content of sugars. Glucose was the absolutely dominant component (95.70 to 96.19%), only minor amount of xylose (1.48 to 2.74%), arabinose (0.70 to 1.29%), and trace of uronic acid were observed. The predominance of glucose in the isolated polysaccharide fractions (H_1, H_2, and H_3) probably resulted from the released starch under the condition of increasing temperature. The existence of starch in bamboo was supported by Toledo et al. (1987), in which the authors stated that the yield of starch isolated from bamboo culm *(Guadua flabellata)* with alkaline solution was 8.50%. Coincidentally, our recently published study has also reported that water-soluble starch was present in bamboo species *Phyllostachys bambusoides f. shouzhu Yi* (Peng et al. 2011).

Table 3. Contents of Neutral Sugars and Uronic Acids (% Polysaccharides Sample, w/w) in the Isolated Polysaccharide Fractions

	Polysaccharide fractions [a]								
	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9
Rhamnose	0.07	0.09	0.15	0.41	0.22	0.02	0.67	0.19	0.07
Arabinose	1.07	0.70	1.29	10.04	6.85	3.77	4.83	4.93	4.46
Galactose	0.45	0.24	0.58	3.80	2.02	0.87	2.35	2.42	ND [b]
Glucose	95.70	96.04	96.19	49.94	48.28	40.75	35.48	21.90	21.20
Xylose	2.45	2.74	1.48	26.21	37.54	50.85	52.42	64.88	70.28
galacturonic acid	0.14	0.09	0.19	2.65	1.36	0.39	0.81	0.93	0.46
glucuronic acid	0.12	0.10	0.13	6.95	3.73	3.36	3.45	4.74	3.53
Xylose/Arabinose	2.29	3.91	1.15	2.61	5.48	13.49	10.85	13.16	15.76

[a] Represents the bamboo polysaccharide fractions isolated by hot water at 80, 100, and 120 °C for 3 h, aqueous ethanol containing 0.25, 0.50, 1.00, 2.00, 3.00, and 5.00% NaOH, at 80 °C for 3 h ; [b] ND = not detectable

The data in Table 3 also showed that the major sugar components of the six polysaccharide preparations isolated from bamboo residue after hot water treatments, were xylose (26.21-70.28%) and glucose (21.20-49.94%), followed by small amounts of

arabinose (3.77-10.83%) and uronic acid (3.75-9.60%). These analysis results implied that the isolated polysaccharide preparations (H_4, H_5, H_6, H_7, H_8, and H_9) probably contained significantly amount of arabinoglucuronoxylans, which was in agreement with those found in other bamboo species (Meakawa et al. 1976; Fengel et al. 1984; Yoshida et al. 1998). In addition, starch was also presumed to be present in the six alkaline aqueous ethanol-extractable polysaccharide preparations due to their high glucose unit contents. However, it should be noted that with the increase of alkali concentration from 0.25% to 5.00%, the content of glucose decreased from 49.94% in H_4 to 21.20% in H_9, and in contrast to this decreasing trend, the content of xylose increased from 26.21% in H_4 to 70.28% in H_9, indicating that the contents of arabinoglucuronoxylans increased from polysaccharide fraction H_4 to H_9.

To confirm the assumption of starch presenting in the isolated polysaccharide fractions, all the hot water-soluble and alkaline aqueous ethanol-soluble polysaccharide fractions were tested with starch-iodine paper. The results demonstrated that amylose starch appeared both in the water-soluble and alkaline aqueous ethanol-soluble polysaccharide fractions. This result implied that *D. brandisii* may be considered as a new starch source for food and non-food industries.

Xylose to arabinose ratios are indicative of the degree of linearity or branching of polysaccharides (Wedig et al. 1987). A higher xylose to arabinose ratio would indicate a high degree of polymerisation with little bonding with other monosaccharide constituents, whereas a lower xylose to arabinose ratio would suggest a short-chain polymer with a large amount of branching with other monosaccharide constituents. As the data show in Table 3, the xylose to arabinose ratios increased from 2.61 in H_4 to 15.76 in H_9 with the increment of alkali concentration from 0.25% to 5.0%, and the similar trend of xylose to acidic sugars ratios were also observed. These results indicated that the polysaccharide polymers isolated by aqueous ethanol with higher alkali concentrations seemed to be more linear, while those of the polysaccharide fractions extracted by hot water and aqueous ethanol with lower alkali concentrations seemed to be more branched.

FT-IR Spectra

The FT-IR spectra of the isolated polysaccharide fractions obtained from bamboo (*D. brandisii*) are shown in Figs. 2 and 3. It was found that most of absorption bands of the isolated polysaccharide fractions were rather similar. The absorption at 3349 cm^{-1} is attributed to the stretching of -OH groups. The C-H stretching vibration gives signals at 2930 cm^{-1}. The small shoulder peak at 1732 cm^{-1} is due to the C=O stretching of acetyl groups in the polysaccharide polymers. The intense absorption band at 1635 cm^{-1} corresponds to the bending mode of the absorbed water, because the polysaccharides usually have a strong affinity for water, and in the solid state these macromolecules may have disordered structures, which can easily be hydrated (Kacurákováet al. 2000). Absorptions at 1149 and 1076 cm^{-1} are both assigned as the coupling of C-O, C-C and O-H bond stretching, bending and asymmetric stretching of the C-O-C glycosidic bridge (Goodfellow et al. 1990; Van Soest et al. 1994). Absorbance at 1021 cm^{-1} is assigned to the vibration of C-O-H deformation (Van Soest et al. 1995), and absorbance at 930 cm^{-1} is assigned for C-H bending (Irudayaraj et al. 2002).

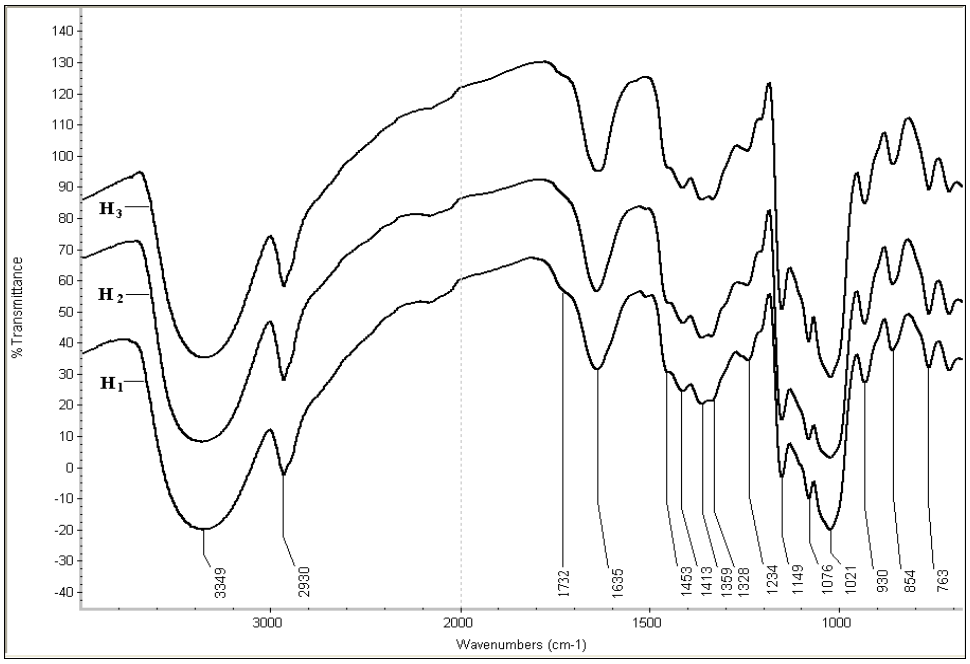

Fig. 2. FT-IR spectra of the isolated polysaccharide fractions H_1, H_2, and H_3

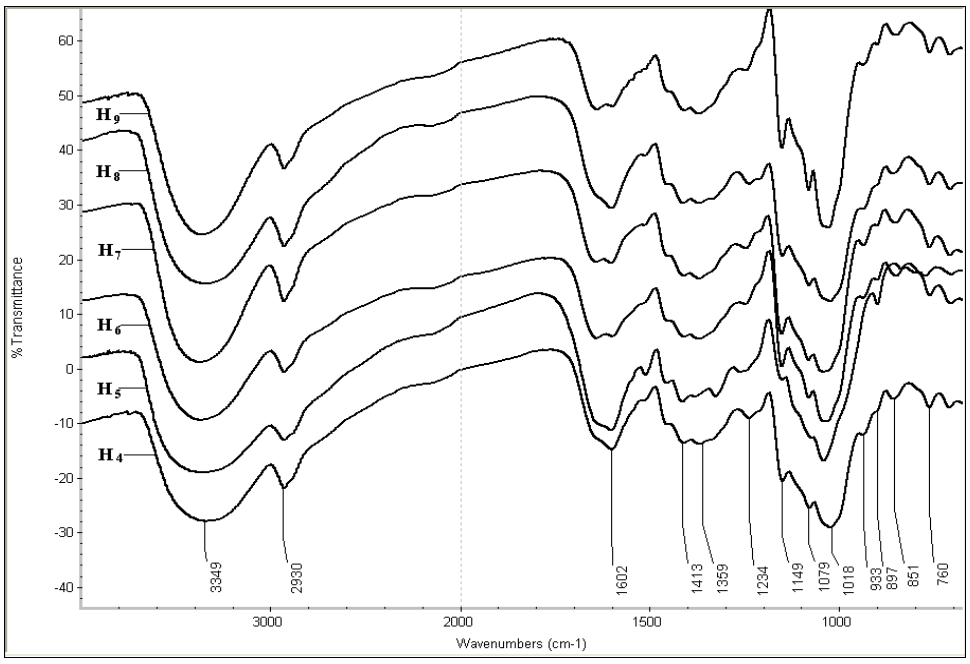

Fig. 3. FT-IR spectra of the isolated polysaccharide fractions H_4, H_5, H_6, H_7, H_8, and H_9

Additionally, an intense absorption band at 854 cm^{-1} in Fig. 2 is indicative of α-glycosidic linkages in the polysaccharide fractions, giving evidence for the high contents of starch in the hot water-soluble polysaccharide fractions. The new absorbance at 897 cm^{-1} presented in Fig. 3 indicates the presence of β-glycosidic linkage between units in the isolated polysaccharides. The disappearance of absorption at 1732 cm^{-1} in Fig. 3

indicates that the ester groups were substantially saponified during the treatments with alkaline aqueous ethanol under the conditions given.

1D and 2D NMR Spectra

NMR spectra are supposed to assay and identify the polymer backbone and the type of side chain branching along the backbone. To further elucidate the structural characteristics of the polysaccharide polymers extracted from bamboo (*D. brandisii*), the polysaccharide preparation H_6 was investigated using 1D and 2D NMR spectroscopy and its ^{1}H, ^{13}C, and 2D-HSQC NMR spectra are shown in Figs. 4, 5, and 6, respectively. The signals for ^{1}H and ^{13}C NMR were assigned on the basis of HSQC spectra and previous literatures (Xu et al. 2007; Chaikumpollert et al. 2004; Vignon et al. 1998). As can be seen from Fig. 4, the relevant signals of H_6 appeared in two regions, the anomeric region (δ 5.60-4.90 for α-anomers and δ 4.90-4.30 for β-anomers) and the ring proton region (δ 4.50-3.00). Therefore, the signals at 5.20 and 4.34 ppm are assigned as α-glycosidic and β-glycosidic bridges, respectively. This confirmed that xylan is linked by β-glycosidic linkages, and starch is linked by α-glycosidic bonds, which is consistent with the presence of absorption peaks at 897 and 851 cm^{-1} in the FT-IR spectrum of polysaccharide fraction H_6 in Fig. 3.

The ^{13}C NMR spectrum (Fig. 5) of H_6 exhibits five major signals corresponding to those of (1→4)-linked-β-D-xylan. The signal at 102.43 ppm corresponds to the anomeric region in a β-configuration, as confirmed by the ^{1}H NMR spectra, while the signals at 75.94, 74.85, 73.29, and 63.29 ppm correspond to C-4, C-3, C-2, and C-5 of (1→4)-linked-β-D-xylopyranosyl units, respectively.

The amylose starch was obviously characterized by six strong signals at 102.83, 80.13, 74.39, 72.72, 71.52, 60.66 ppm, which were assigned to C-1, C-4, C-3, C-5, C-2, and C-6 of α-(1→4)-glycosidic-linked glucose residues, respectively. The signals at 97.54, 82.65, 80.13, 72.19, and 70.39 ppm, arise from C-1, C-4, C-3, C-5, and C-2 of 4-*O*-methyl-α-D-glucuronic acid, respectively. Other less intense signals at 86.40, 80.13, 78.40, and 59.54 ppm correspond to C-4, C-2, C-3, and C-5 of α-L-arabinfuranosyl residues, respectively.

In order to gain a more complete understanding the structure of the isolated polysaccharides, the 2D HSQC technique was used in this study. From the HSQC spectrum of H_6 (Fig. 6), the dominant five cross-peaks could be expressly identified at 102.43/4.34, 75.94/3.76, 74.85/3.35, 73.29/3.17, 63.29/3.97, and 3.23 ppm, which were assigned to C_1-H_1, C_4-H_4, C_3-H_3, C_2-H_2, and C_5-H_5 of the (1→4)-linked-β-D-xylopyranosyl units, respectively.

The marked $^{1}H/^{13}C$ cross-peaks in the HSQC spectrum at 102.83/5.20, 80.13/3.45, 74.39/3.76, 72.72/3.35, 71.52/3.73, and 60.66/3.76 ppm, confirmed the structural element of amylose starch units. Furthermore, the HSQC spectrum also provided the additional evidences for the presence of 4-*O*-methyl-D-glucuronic acid and α-L-arabinose. In summary, all of the detailed chemical shifts of the 2D-HSQC of polysaccharide fraction H_6 are summarized in Table 4.

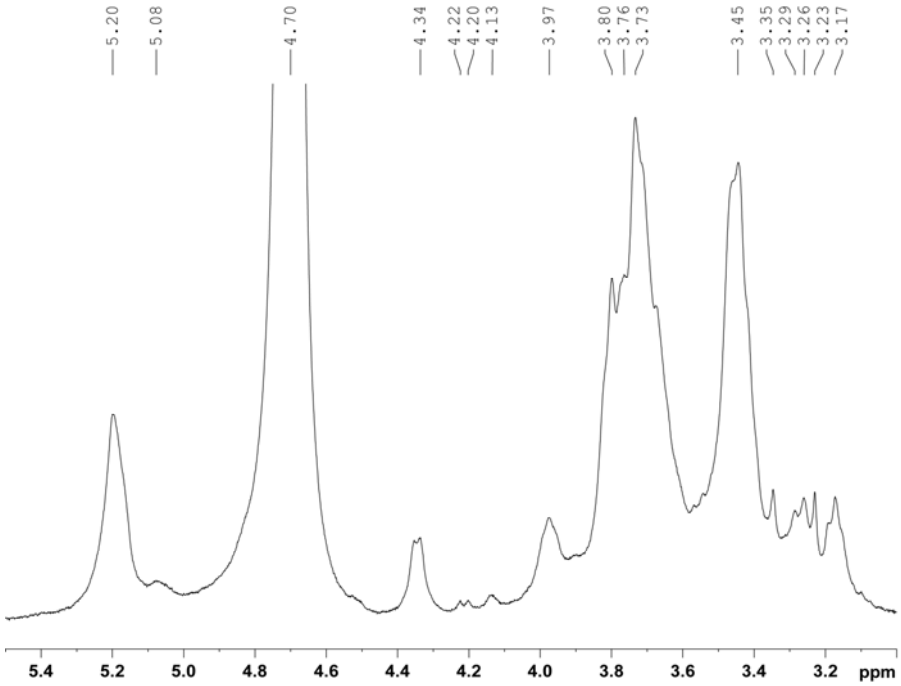

Fig. 4. ^{1}H-NMR spectrum (in D_2O) of the polysaccharide fraction H_6 isolated with 60% aqueous ethanol containing 1.00% NaOH

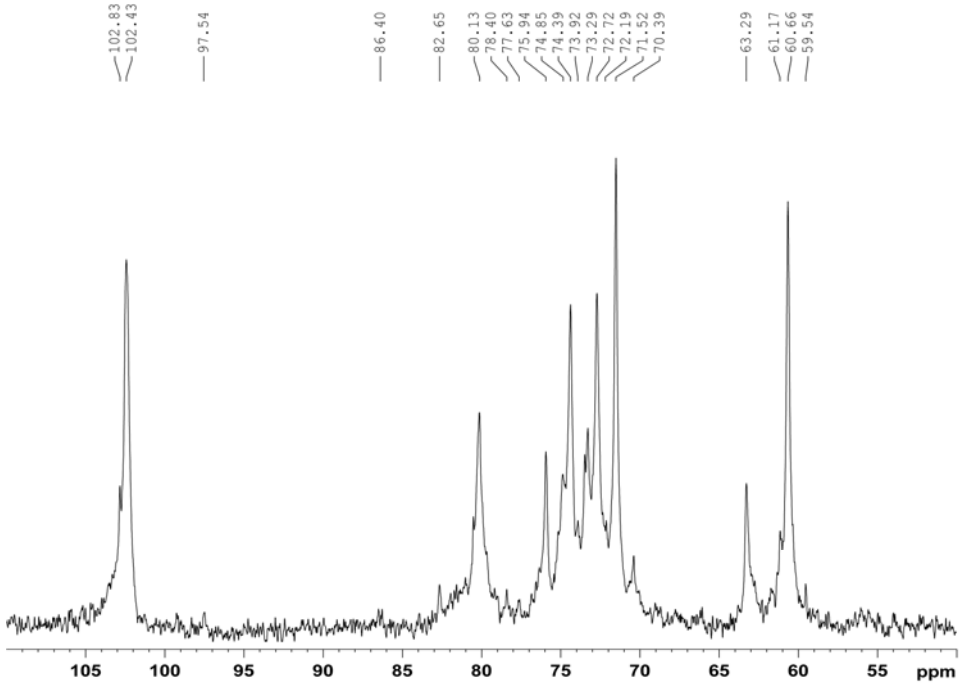

Fig. 5. ^{13}C-NMR spectrum (in D_2O) of the polysaccharide fraction H_6 isolated with 60% aqueous ethanol containing 1.00% NaOH

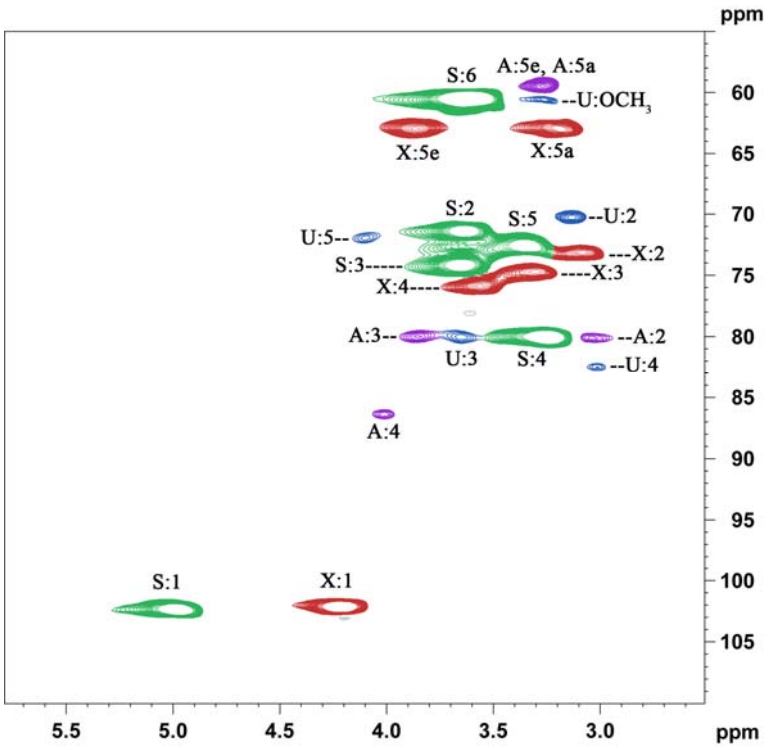

Fig. 6. $^{1}H/^{13}C$ NMR (HSQC) of the polysaccharide fraction H_6 isolated from with 60% aqueous ethanol containing 1.00% NaOH

Table 4. Assignment of $^{1}H/^{13}C$ Cross-signals in the HSQC Spectrum of the Polysaccharide Fraction H_6 Isolated with 60% Aqueous Ethanol Containing 1.0% NaOH

Saccharide	NMR	Assignment							
		1	2	3	4	5eq [a]	5ax [b]	6	OCH_3
X [c]	^{13}C	102.43	73.29	74.85	75.94	63.29	63.29	-	-
	^{1}H	4.34	3.17	3.35	3.76	3.97	3.23	-	-
S [d]	^{13}C	102.83	71.52	74.39	80.13	72.72	-	60.66	-
	^{1}H	5.20	3.73	3.76	3.45	3.35	-	3.76	-
A [e]	^{13}C	-	80.13	78.40	86.40	59.54	59.54	-	-
	^{1}H	5.08	3.80	3.73	4.13	-	-	-	-
U [f]	^{13}C	97.54	70.39	80.13	82.65	72.19	-	-	60.66
	^{1}H	-	3.17	3.73	3.17	4.22	-	3.26	1.06

[a] eq, equatorial; [b] ax, axial; [c] X, (1→4)-β-D-Xylp; [d] S, amylose starch; [e] A, α-Araf residues; [f] U, Uronic acid

In short, based on the results of sugars analysis, FT-IR spectra, NMR spectra, and the existing literature concerning the structural properties of bamboo hemicelluloses (Wilkie et al. 1976, 1977), it could be concluded that the polysaccharide fractions isolated from the bamboo (*D. brandisii*) were mainly composed of arabinoglucuronoxylans and amylose starch. The structure of arabinoglucuronoxylans could be defined as a linear (1→4)-linked-β-xylopyranosyl backbone to which α-L-arabinofuranose units and/or short chains of 4-*O*-methyl-D-glucuronic acid were attached as side residues via α-(1→3)

and/or α-(1→2) linkages, with a ratio of arabinose/uronic acid/xylose of 1:1:14. Therefore, the potential structures of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan isolated from bamboo species *D. brandisii* with alkaline aqueous ethanol could be illustrated as in Fig. 7.

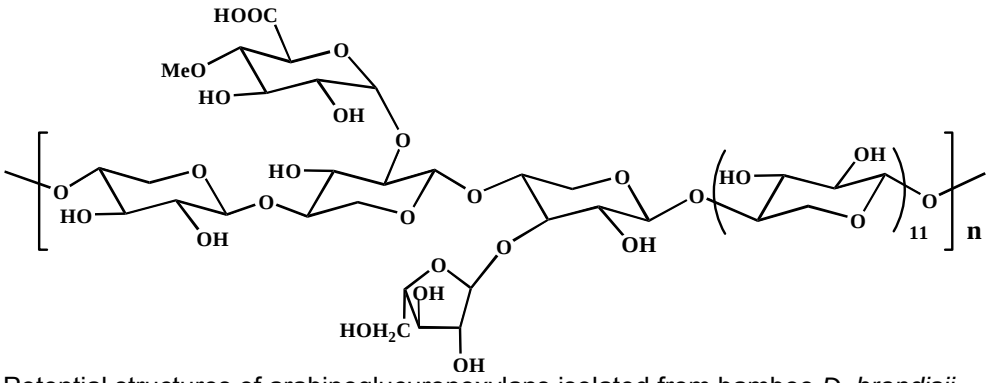

Fig. 7. Potential structures of arabinoglucuronoxylans isolated from bamboo *D. brandisii*

Thermal Stability Analysis

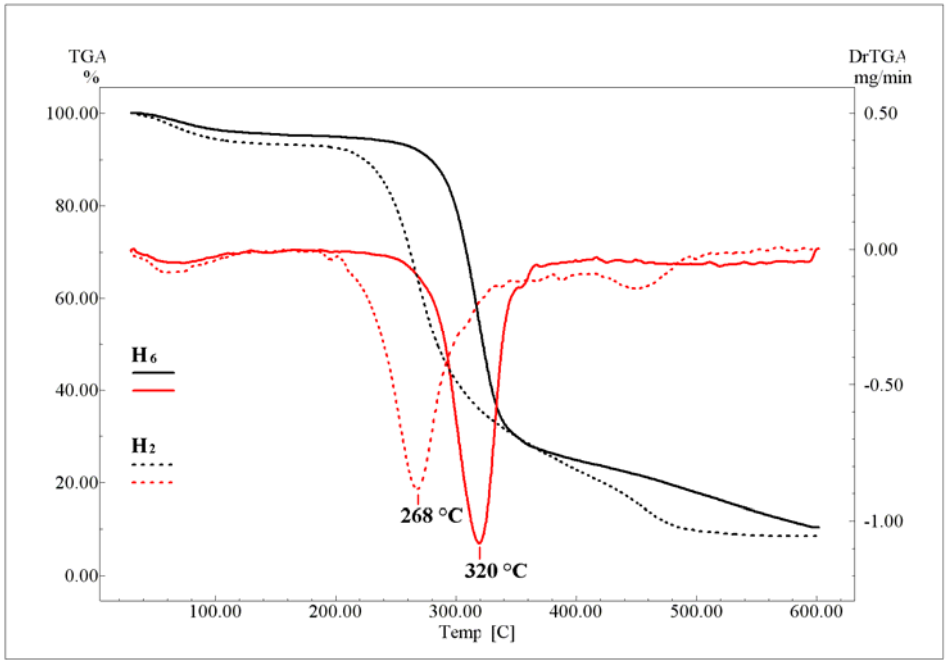

Fig. 8. Thermograms of polysaccharide fractions (H_2, H_6) isolated from bamboo (*D. brandisii*)

Thermogravimetric analysis (TGA) is one of the most common techniques used to rapidly investigate the mass loss, and it is used to compare the thermal behavior during pyrolysis of materials. Figure 8 shows the TGA/DTA curves of the polysaccharide preparations H_2 and H_6 isolated from bamboo species *D. brandisii*. As shown in the Fig. 8, the polysaccharide fractions H_2 and H_6 began to decompose at 215 and 270 °C, respectively, and the maximum rate of weight loss was observed at 268 °C for H_2 and

320 °C for H_6 in DTA thermograms. That is, the thermal stability of the polysaccharide fraction H_6, which had higher molecular weight (41180 g/mol), appeared to be higher than that of the polysaccharide fraction H_2, which had lower molecular weight (19730 g/mol).

CONCLUSIONS

1. The sequential treatments of dewaxed bamboo (*D. brandisii*) samples with hot water at 80, 100, and 120 °C, and 60 % aqueous ethanol containing 0.25, 0.50, 1.00, 2.00, 3.00, and 5.00 % NaOH yielded 20.60% soluble polysaccharides.
2. The dominant components of the soluble polysaccharides from bamboo (*D. brandisii*) were arabinoglucuronoxylans and amylose starch.
3. The isolated arabinoglucuronoxylans from bamboo (*D. brandisii*) could be defined as a linear (1→4)-β-linked-xylopyranosyl backbone to which α-L-arabinofuranose units and/or short chains of 4-*O*-methyl-glucuronic acid were attached as side residues via α-(1→3) and/or α-(1→2) linkages.
4. It was found that the thermal stability of the soluble polysaccharides increased with an increment of their molar mass.

ACKNOWLEDGMENTS

The authors are grateful for the financial support from State Forestry Administration (200804015), Major State Basic Research Projects of China (973-2010CB732204), National Natural Science Foundation of China (30930073, 30710103906), China Ministry of Education (111), Education Department of Yunnan Province, China (2011Z040), and Southwest Forestry University, China.

REFERENCES CITED

Aspinall, G. O., Greenwood, C. T., and Sturgenon, R. J. (1961). "The degradation of xylans by alkali," *J. Chem. Soc.* 3667-3677.

Aspinall, G. O., and Mahomed, R. S. (1954). "The constitution of a wheat-straw xylan," *J. Chem. Soc.* 76, 1731-1734.

Bendahou, A., Dufresne, A., Kaddami, H., and Habibi, Y. (2007). "Isolation and structural characterization of hemicelluloses from palm of *Phoenix dactylifera* L.," *Carbohydr. Polym.* 68(3), 601-608.

Bergmans, M. E. F., Beldman, G., Gruppen, H., and Voragen, A. G. J. (1996). "Optimisation of the selective extraction of (glucurono)arabinoxylans from wheat bran: Use of barium and calcium hydroxide solution at elevated temperatures," *J. Cereal Sci.* 23(3), 235-245.

Chaikumpollert, O., Methacanon, P., and Suchiva, K. (2004). "Structural elucidation of hemicelluloses from Vetiver grass," *Carbohydr. Polym.* 57(2), 191-196.

Cheetham, N. W. H., and Tao, L. (1998). "Variation in crystalline type with amylose content in maize starch granules: an X-ray powder diffraction study," *Carbohydr. Polym.* 36(4), 277-284.

Cyran, M. R., and Saulnier, L. (2007). "Association and structural diversity of hemicelluloses in the cell walls of rye outer layers: Comparison between two ryes with opposite breadmaking quality," *J. Agric. Food. Chem.* 55(6), 2329-2341.

Doner, L. W., and Hicks, K. B. (1997). "Isolation of hemicellulose from corn fiber by alkaline hydrogen peroxide extraction," *Cereal Chem.* 74(2), 176-181.

Dupont, M. S., and Selvendran, R. R. (1987). "Hemicellulosic polymers from the cell walls of beeswing wheat bran: Part I, Polymers solubilized by alkali at 2 °C," *Carbohydr. Res.* 163(1), 99-113.

Fengel, D., and Shao, X. (1984). "A chemical and ultrastructural study of the bamboo species *Phyllostachys makinoi* Hay," *Wood Sci. Technol.* 18(2), 103-112.

Fengel, D., Wegener, G., and Greune A. (1989). "Studies on the delignification of spruce wood by organosolv pulping using SEM-EDXA and TEM," *Wood Sci. Technol.* 23(2), 123-130.

Goodfellow, B. J., and Wilson, R. H. (1990). "A Fourier transform IR study of the gelation of amylose and amylopectin," *Biopolymers* 30(13-14), 1183-1189.

Irudayaraj, J., and Yang, H. (2002). "Depth profiling of a heterogeneous food-packaging model using step-scan Fourier transform infrared photoacoustic spectroscopy," *J. Food Eng.* 55(1), 25-33.

Kacuráková, M., Ccpek, P., Sasinková, V., Wellner, N., and Ebringerova, A. (2000). "FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses," *Carbohydr. Polym.* 43(2), 195-203.

Meakawa, E. (1976). "Studies on hemicellulose of bamboo," *Wood Research* 59/60, 153-179.

Morrison, I. M. (1974). "Changes in the hemicellulosic polysaccharides of rye-grass with increasing maturity," *Carbohydr. Res.* 36(1), 45-51.

Peng, P., Peng, F., Bian, J., Xu, F., and Sun, R. C. (2011). "Studies on the starch and hemicelluloses fractionated by graded ethanol precipitation from bamboo *Phyllostachys bambusoides* f. shouzhu Yi.," *J. Agric. Food. Chem.* 59(6), 2680-2688.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). "Determination of structural carbohydrates and lignin in biomass," In: Laboratory Analytical Procedure (LAP), NREL/TP-510-42618, National Renewable Energy Laboratory Golden, CO.

Sun, J. X., Sun, X. F., Sun, R. C., and Su,Y. Q. (2004). "Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses," *Carbohydr. Polym.* 56(2), 195-204.

Sun, R. C., Fang, J. M., Tomkinson, J., Geng, Z. C., and Liu, J. C. (2001). "Fractional isolation, physicochemical characterization and homogeneous esterification of hemicelluloses from fast-growing poplar wood," *Carbohydr. Polym.* 44(1), 29-39.

Sun, X. F., Sun, R. C., Fowler, P., and Baird, M. S. (2005). "Extraction and characterization of original lignin and hemicelluloses from wheat straw," *J. Agric. Food. Chem.* 53(4), 860-870.

Timell, T. E., and Jahn, E. C. (1951). "A study of the isolation and polymolecularity of paper birch holocellulose," *Svensk Papperstidn* 54, 831-846.

Toledo M. C. F., Azzini A., and Reyes, F. G. R. (1987). "Isolation and characterization of starch from bamboo culm (*Guadua flabellata*)," *Starch* 39(5), 158-160.

van Soest, J. J. G., De Wit, D., Tournois, H., and Vliegenthart, J. F. G. (1994). "Retrogradation of potato starch as studied by Fourier transform infrared spectroscopy," *Starch* 46(2), 453-457.

van Soest, J. J. G., Tournois, H., De Wit, D., and Vliegenthart, J. F. G. (1995). "Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy," *Carbohydr. Res.* 279, 201-214.

Vignon, M. R., and Gey, C. (1998). "Isolation, ^{1}H and ^{13}C NMR studies of (4-*O*-methyl-D-glucurono)-D-xylans from luffa fruit fibres, jute bast fibres and mucilage of quince tree seeds," *Carbohydr. Res.* 307(1-2), 107-111.

Wilkie, K. C. B., and Woo, S. L. (1977). "A heteroxylan and hemicellulosic materials from bamboo leaves, and a reconsideration of the general nature of commonly occurring xylans and other hemicelluloses," *Carbohydr. Res.Carbohydrate Research* 57, 145-162.

Wilkie, K. C. B., and Woo, S. L. (1976). "Non-cellulosic β-D-glucans from bamboo, and interpretative problems in the study of all Hemicelluloses," *Carbohydr. Res.* 49, 399-409.

Wedig, C. L., Jaster, E. H., and Moore, K. J. (1987). "Hemicellulose monosaccharide composition and in vitro disappearance of orchard grass and alfalfa hay," *J. Agric. Food. Chem.* 35(2), 214-218.

Xu, F., Sun, J. X., Geng, Z. C., Liu, C. F., Ren, J. L., Sun, R. C., Fowler, P., and Baird, M. S. (2007). "Comparative study of water-soluble and alkali-soluble hemicelluloses from perennial ryegrass leaves (*Lolium peree*)," *Carbohydr. Polym.* 67(67), 56-65.

Yang, J. M., and Goring, D. A. I. (1978). "The topochemistry of reaction of chlorine dioxide with lignin in black spruce wood," *Holzforschung* 32(5), 185-188.

Yoshida, S., Kuno, A., Saito, N., Aoyama, M., and Kusakabe, I. (1998). "Structure of xylan from culms of bamboo grass (*Sasa senanensis* Rehd.)," *J. Wood Sci.* 44(6), 457-462.

Article submitted: August 17, 2011; Peer review completed: October 5, 2011; Revised version received and accepted: October 26, 2011; Published: October 28, 2011.