Structure and Thermal Stability of Polysaccharide Fractions Extracted from the Ultrasonic Irradiated and Cold Alkali Pretreated Bamboo

Ming-Fei Li,[1] Yong-Ming Fan,[1] Feng Xu,[1] Run-Cang Sun[1,2]

[1]*Institute of Biomass Chemistry and Technology, Beijing Forestry University, Beijing 100083, China*
[2]*State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China*

Received 1 August 2010; accepted 29 September 2010
DOI 10.1002/app.33491
Published online 17 February 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Polysaccharide fractions were extracted from partially delignified bamboo (*Neosinocalamus affinis*) culms pretreated with ultrasonic irradiation for varied times and cold sodium hydroxide/urea solution, and their structure and thermal stability were comparatively characterized. In this case, ball-milled bamboo culms were treated with ultrasonic irradiation for varied times (0, 5, 15, and 25 min), dissolved with 7% sodium hydroxide/12% urea solution at −12°C, and then extracted with ethanol and dioxane to obtain partially delignified solid fractions. Subsequently, the solid fractions were subjected to be extracted with dimethyl sulfoxide followed by precipitation in ethanol and yielded the polysaccharide fractions. Sugar analysis indicated that the total sugar content increased from 60.63% in the polysaccharide fraction prepared without ultrasonic irradiation to 81.26% in the polysaccharide fraction prepared with an ultrasonic irradiation time of 25 min. Glucose (∼ 50–55%) was the major sugar component, and xylose (∼ 41–44%) was the second major sugar in polysaccharide fractions in all cases. Spectroscopy (FTIR, ^{1}H-NMR, ^{13}C-NMR, and HSQC) analysis suggested that the polysaccharide fractions were mainly composed of (1→4)-linked α-D-glucan from amylose and (1→4)-linked β-D-xylan attached with minor amounts of branched sugars from hemicelluloses. In addition, thermal analysis showed that the main degradation stage of the polysaccharide fractions occurred between 210 and 320°C. Compared to the polysaccharide fraction prepared without ultrasonic irradiation, the polysaccharide fraction prepared with ultrasonic irradiation had a slightly lower thermal stability.
 J Appl Polym Sci 121: 176–185, 2011

Key words: NMR; HSQC; polysaccharides; structure; thermal properties

INTRODUCTION

Lignocelluloses are natural renewable feedstocks that can be converted into materials, chemicals, and fuels. Bamboo, a perennial woody grass belonging to the Gramineae family and Bambuseae subfamily, is widely distributed in tropical and subtropical areas over many countries with a total annual production of 6–7 million tons.[1] Being a natural polymeric composite, it consists mainly of carbohydrate based polymers combined with lignin and lesser amounts of extractives, protein, and inorganics. The main components of bamboo are as follows: cellulose 40–48%, hemicelluloses 24–28%, and lignin 25–30%.[2] Conventionally, bamboo is widely used as feedstocks of paper, textile, food, and construction fibers. In addition, cellulose isolated from this lignocellulose is a feedstock for the production of cellulose derivatives such as cellulose acetate[3,4] and hydroxypropyl cellulose.[5] For instance, cellulose acetate with a high degree of substitution of 2.90–2.93 was successfully synthesized from high-grade bamboo dissolving pulp.[4] Recently, much attention has been paid to its application as the raw material for chemicals and fuels, such as bioethanol,[6,7] methane,[8] and lactic acid.[9] Principally, bamboo is considered to be a substitute for wood to produce bioethanol because of its advantages of high crop productivity and richness in carbohydrates.[2,6]

In the process of bioethanol production, pretreatment is necessary to make the cellulose more accessible to cellulolytic enzymes that convert the

Correspondence to: F. Xu (xfx315@bjfu.edu.cn) and R.-C. Sun (rcsun3@bjfu.edu.cn).
Contract grant sponsor: Science and Technology Innovation Program of Beijing Forestry University; contract grant number: BLJC200901/TD2010-1.
Contract grant sponsor: Major State Basic Research Projects of China; contract grant number: 973-2010CB732204.
Contract grant sponsor: National Natural Science Foundation of China; contract grant numbers: 30930073, 31070526.
Contract grant sponsor: Ministries of Education; contract grant number: 111.
Contract grant sponsor: State Forestry Administration; contract grant number: 200804015/948-2010-16.

carbohydrate polymers into fermentable sugars, owning to the recalcitrant characteristic of the feedstock. Pretreatment is mainly achieved by disrupting the crystalline structure of cellulose and breaking the lignin seal and noncellulosic polysaccharide (hemicelluloses, starch, etc.) sheathing over cellulose.[10] In addition to obtaining a less recalcitrant cellulose fraction, liberating lignin with less degradation and recondensation and limiting the degradation of hemicelluloses are the key issues to achieve a full recovery of all lignocellulosic components for utilization.[11] Recently, it was found that cold 7% NaOH/12% urea solution can dissolve cellulose directly for its capacity of breaking the intra- and intermolecular hydrogen bonding of cellulose.[12] However, fewer works have been conducted on applying such a novel system to treat highly lignified feedstock to facilitate the separation of the components for bioethanol production. Zhao et al.[13] have reported that the enzymatic efficiency was remarkably improved after mechanically pretreated spruce was soaked with either NaOH alone or NaOH/urea solution at −15°C for 24 h. In our previous study, the bamboo has been subjected to cold NaOH/urea solution pretreatment followed by lignin extraction and the dissolved lignin fractions has been structurally characterized for further use.[14] However, some recalcitrant polysaccharide fractions were still retained in the solid fraction, which hindered the interaction of enzyme and cellulose in the subsequent enzymatic hydrolysis. Therefore, a further release of these components is needed to achieve a more hydrolysable substrate.

Polysaccharides have been found numerous applications in a variety of fields, including paper and textile, food, cosmetics, and chemical industries, etc. Thus, the aim of this study was to explore potential applications of the released polysaccharide fraction dissolved during the pretreatment process. In this study, the partially delignified cellulose rich fractions prepared with varied ultrasonic irradiation times were extracted with a neutral solvent. The polysaccharides liberated were comparatively characterized with a set of wet chemistry, spectroscopy, and thermal analysis methods.

EXPERIMENTAL

Materials

Bamboo (*Neosinocalamus affinis*) was collected from the experimental farm of North-Western University of Agriculture and Forestry, Yangling, China. The air-dried bamboo culms were cut into small pieces, then ground and sieved to obtain a 20–40 mesh fraction. This fraction was subjected to extraction with methylbenzene/ethanol (2 : 1, v/v) in a Soxhlet apparatus for 6 h, and then air-dried. The composition of the dewaxed bamboo was glucose 50.82%, xylose 22.94%, arabinose 1.13%, galactose 0.51%, mannose 0.37%, rhamnose 0.02%, glucuronic acid 0.94%, lignin 19.46% (Klason lignin 16.97% and acid-soluble lignin 2.49%), and ash 2.52%.[14]

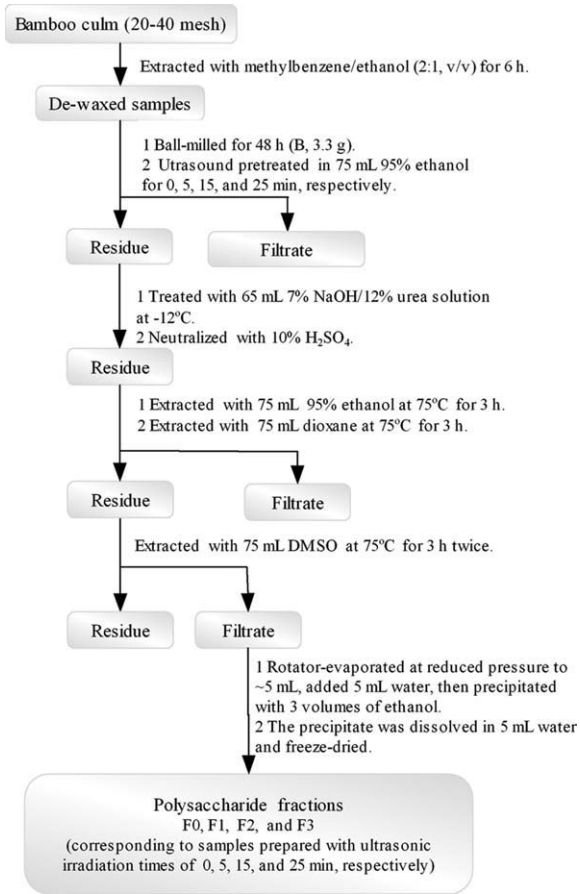

Figure 1 Scheme for extraction of polysaccharide fractions from bamboo culms.

Isolation of the polysaccharide fractions

A scheme for pretreatment and extraction of polysaccharides from the bamboo is illustrated in Figure 1. The dewaxed sample was ball-milled with a rotary ball mill at room temperature for 48 h. After that, the milled sample of 3.3 g (oven-dried weight) was suspended in 75 mL of ethanol (95%) solution for 5 min, and then the ultrasonic irradiation pretreatment was performed in a ultrasonic cell crusher machine with a sonic power of 100 W at 20°C for 0, 5, 15, and 25 min, respectively. Next, the samples were filtered, and the residues were air-dried, respectively. The four residues were submitted to treatments as follows. For each treatment, 65 mL aqueous solution of 7% NaOH/12% urea was precooled to −12°C, and the sample was dispersed into it and then stirred vigorously at a

rotational speed of 1200 rpm for 10 min at −12°C. Subsequently, the mixture was neutralized with 10% H_2SO_4 to pH 7. After the solid was precipitated from the aqueous mixture, it was washed with water and filtered. Next, the recovered solid preparation was extracted with 75 mL of 95% ethanol at 75°C for 3 h, and then with 75 mL of dioxane at 75°C for 3 h to obtain partially delignified residue. Then the residue was extracted with 75 mL dimethyl sulfoxide (DMSO) at 75°C for 3 h twice. The two DMSO fractions rich in polysaccharides were filtered, combined, and rotator-evaporated to ~ 5 mL under reduced pressure at 110°C, and then 5 mL water was added. After that, the solution was dripped into 30 mL ethanol to precipitate the polysaccharides. Subsequently, the precipitated polysaccharide fractions were dissolved in 5 mL water and then freeze-dried for weight determination and characterization (Note F0, F1, F2, and F3 corresponding to the samples prepared with ultrasonic irradiation times of 0, 5, 15, and 25 min).

Structural characterization of the polysaccharide fractions

The composition of neutral sugars and uronic acids in the polysaccharide fractions was determined by high-performance anion exchange chromatography (HPAEC).[15] The neutral sugars and uronic acids in the polysaccharide fractions were liberated by hydrolysis with 72% H_2SO_4 for 45 min at 25°C followed by a high temperature hydrolysis at 105°C for 2.5 h by dilution to 3% H_2SO_4. After hydrolysis, the samples were diluted and injected into the HPAEC system (Dionex ISC 3000, USA) with an amperometric detector, a CarbopacTMPA-20 column (4 mm × 250 mm, Dionex), and a guard PA-20 column (3 mm × 30 mm, Dionex). Neutral sugars and uronic acids were separated in isocratic 5 m*M* NaOH (carbonate free and purged with nitrogen) for 20 min, followed by a 0.75 m*M* NaAc gradient in 5 m*M* NaOH for 15 min with a flow rate of 0.4 mL/min. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-glucose, D-mannose, D-galactose, glucuronic acid, and galacturonic acid.

The molecular weights of the polysaccharide 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, and 1,600,000; Polymer Laboratories Ltd.).[15] A flow rate of 0.5 mL/min was maintained. The eluents were 0.02*M* NaCl in 0.005*M* sodium phosphate buffer, at pH 7.5. Detection was achieved with a Knauer differential refractometer. The column oven was kept at 30°C. Samples were dissolved in 0.005*M* sodium phosphate buffer with 0.02*M* NaCl, pH 7.5, at a concentration of 0.1%.

The chemical composition of phenolic acids and aldehydes liberated from alkaline nitrobenzene oxidation of the lignin associated with polysaccharide fractions was determined by high-performance liquid chromatography (HPLC, Agilent).[16] Briefly, 2*N* sodium hydroxide (7 mL) and nitrobenzene (0.4 mL) were added to 0.3 g dried polysaccharide samples which were oxidized at 170°C for 2.0 h, respectively. After oxidation, the solutions were filtered and the filtrates were extracted with chloroform (3 × 30 mL), acidified to pH 1 with 20% chlorhydric acid, and then extracted again with chloroform (3 × 30 mL). These last chloroform extracts were evaporated at 40°C to dryness. After dilution with methanol, the lignin oxidation products were analyzed using HPLC on a Hichrom H5ODS column of dimensions 250 × 4.6 mm. Separations were obtained using a linear gradient of two solvent systems: solvent A (water:methanol:acetic acid, 89:10:1) and solvent B (methanol:water:acetic acid, 90:9:1). A linear gradient was run over 30 min from 0 to 40% B at a flow rate of 1 mL/min. Identification of the individual compounds was made at 280 nm by computer comparison of the retention times and peak areas with reference to well-characterized phenolics.

FTIR spectra were obtained on an FTIR spectrophotometer (Bruker Tensor 27) using KBr disks containing 1% finely ground samples. The spectra were obtained in the frequency range of 4000–800 cm^{-1} at a resolution of 2 cm^{-1} in the transmittance mode.

The solution-state ^{1}H-NMR, ^{13}C-NMR, and HSQC spectra of the sample were acquired on a Bruker AVIII 400 MHz spectrometer at 25°C. The ^{1}H-NMR spectrum was recorded at 400 MHz using 15 mg of polysaccharide fraction in 1.0 mL of D_2O. Chemical shifts were referred to the residual signal of HOD at 4.69 ppm. The ^{13}C-NMR spectra were obtained at 100 MHz after 30,000 scans using 80 mg sample in 1 mL of D_2O. A 30° pulse flipping angle, a 9.2 μs pulse width, a 1.36 acquisition time, and a 2 s delay time between scans were used. The HSQC spectrum was acquired by HSQC GE experiment mode using 20 mg sample in 1 mL D_2O. The spectral widths for the HSQC spectra were 2200 Hz and 15,400 Hz for the ^{1}H and ^{13}C dimensions, respectively. The number of collected complex points was 1024 for the ^{1}H-dimension with a recycle delay of 1.5 s. The number of san was 128, and 256 time increments were recorded in the ^{13}C-dimension. The $^1J_{CH}$ was set to 146 Hz. Prior to Fourier transform, the data matrices were zero filled up to 1024 points in the ^{13}C-dimension.

TABLE I
Contents of Sugars in the Polysaccharide Fractions (%)

	Neutral sugars and uronic acid[b]						
Sample[a]	Ara	Gal	Glu	Xyl	GluA	GalA	Total sugar[c]
F0	2.21	0.50	50.82	43.99	2.39	0.10	60.63
F1	1.99	0.43	53.69	42.30	1.49	0.10	75.39
F2	2.32	0.38	54.67	41.07	1.48	0.09	79.39
F3	2.17	0.33	52.93	43.08	1.40	0.10	81.26

[a] F0, F1, F2, and F3 corresponding to the polysaccharide fractions prepared with ultrasound times of 0, 5, 15, and 25 min.
[b] Neutral sugars and uronic acid contents calculated based on the total neutral sugars and uronic acid. Ara: arabinose; Gal: galactose; Glu: glucose; Xyl: xylose; GluA: glucurinic acid; GalA: galacturonic acid.
[c] Total sugar represents all the neutral sugars and uronic acid in the preparation.

Thermal analysis

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) of the polysaccharide fractions were investigated by a simultaneous thermal analyzer (DTG-60, Shimadzu, Japan). The freeze-dried samples were further dried at 60°C for 12 h before thermal analysis. Samples weighted 8–10 mg were heated in an aluminum crucible from room temperature to 600°C at a heating rate of 10°C/min. The apparatus was continually flushed with nitrogen at a flow rate of 30 mL/min.

RESULTS AND DISCUSSION

Yield

In this study, the dewaxed bamboo culms were ball-milled at room temperature to decrease the size of the particles and the crystallinity of the feedstock to facilitate the penetration of solvent. After that, the samples were subjected to ultrasonic irradiation treatment in ethanol solution with varied times (0, 5, 15, and 25 min) to determine the effect of ultrasonic irradiation on the subsequent component separation. Then the solid fractions prepared with varied ultrasonic irradiation times were dissolved in 7% sodium hydroxide/12% urea solutions at −12°C. Subsequently, the samples were extracted with ethanol and dioxane to partially release lignin. After the partially delignified sample were extracted with dimethyl sulfoxide (DMSO) under the conditions given in the experimental section, the DMSO-solubles rich in polysaccharides was evaporated under a reduced pressure, and the condensed DMSO-solubles were precipitated in ethanol to obtain the polysaccharide fractions. The yields of the polysaccharide fractions were 4.0, 3.4, 3.3, and 3.4% (based on the oven-dried weight of the original ball-milled bamboo) for samples prepared with an ultrasonic irradiation time of 0, 5, 15, and 25 min, respectively. Obviously, the yields of the polysaccharide fractions prepared with a prolonged ultrasonic irradiation time were relatively lower than that of the fraction prepared without ultrasound treatment. This was probably due to the preferential degradation (further confirmed by the relatively low molecular weight afterwards) and dissolution of the polysaccharides in the alkaline solution resulting from the more intense breaking force of ultrasonic irradiation. It has been reported that spruce was soaked in cold sodium hydroxide/urea solution at −15°C for 24 h resulted in a partial dissolution of polysaccharides and lignin in the alkaline solution.[13] In this study, the vigorous stirring promoted the dissolution of these components, and the ultrasonic irradiation pretreatment facilitated the mass transfer of the dissolved components from the ball-milled bamboo to the solution, resulting in the enhancement of the release of polysaccharides. Therefore, a slightly lower amount of DMSO-soluble polysaccharides were obtained from the ultrasonic irradiated samples.

Sugar component and residual lignin analysis

The sugar components of the polysaccharide fractions were identified and qualified by analysis of the sugars obtained by sulfuric acid hydrolysis method, and the results are presented in Table I. The total sugar content increased from 60.63% in the fraction prepared without ultrasonic irradiation to 81.26% in the fraction prepared with an ultrasonic irradiation time of 25 min. Glucose (∼ 50–55%) was the major sugar component, and xylose (∼ 41–44%) was the second major sugar in the polysaccharide fractions. In addition, noticeable amounts of arabinose (∼ 2%) and glucuronic acid (∼ 2%) were observed and minor amounts of galactose and galacturonic acid were detected. The relatively high quantity of glucose and xylose indicated that the polysaccharide fractions were probably mixed polysaccharides of glucan and xylan. In addition, arabinose, galactose, and uronic acids are regularly attached to the

TABLE II
Yield of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of Bound Lignin in the Polysaccharide Fractions (%)

Phenolic acids and aldehydes	Sample[a]		
	F0	F1	F3
p-Hydroxy benzoic acid	0.96	1.06	1.02
p-Hydroxybenz aldehyde	0.13	0.14	0.12
Vanillic acid	0.14	0.16	0.14
Vanillin	0.49	0.52	0.48
Syringic acid	0.41	0.43	0.39
Syringaldehyde	0.27	0.23	0.23
Acetovanillone	0.15	0.16	0.14
Acetosyringone	0.02	0.01	0.02
p-Coumaric acid	0.02	0.01	0.04
Ferulic acid	0.16	0.28	0.31
Total	2.75	3.00	2.89
Molar ratio (g:s:h)[b]	0.6 : 0.5 : 1	0.6 : 0.4 : 1	0.6 : 0.4 : 1

[a] Corresponding to the fractions in Table I.
[b] g represents the sum of total moles of vanillin, vanillic acid, and acetovanillone; s represents the sum of total moles of syringaldehyde, syringic acid, and acetosyringone; and h represents the sum of total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

backbone of xylan in bamboo.[17] As it can be seen, there was a slight decrease of the contents of arabinose and galactose with the prolonged ultrasonic irradiation time. This suggested that the branched sugars were prone to be released from the polysaccharide backbone to the solution during the cold alkali pretreatment, resulting in the lower content of branched sugars in the polysaccharide fractions.

It has been widely reported that polysaccharides and lignin were bounded in the plant cell walls. To confirm the existence of associated lignin in the fractions, the samples were subjected to alkaline nitrobenzene oxidation, and the contents of the oxidation products are illustrated in Table II. Overall, the yields of the total phenlic monomers released from the bound lignin during the alkaline nitrobenzene oxidation ranged from 2.75 to 3.00%. For all samples, the major oxidation productions can be divided into three types: (a) *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid (derived from *p*-hydroxyphenyl), (b) vanillin, vanillic acid, and acetovanillin (derived from guaiacyl), and (c) syringaldehyde, syringic acid, and acetosyringone (derived from syringyl).[18] It was obvious that the lignin existed in the polysaccharide fractions were mainly composed of *p*-hydroxyphenyl (H) unit, followed by guaiacyl (G) and syringyl (S) unit.[18,19] From the calculated molar ratios, H unit was the predominated unit in the preparation followed by G unit and S unit in all cases. With the prolonged ultrasonic irradiation time, there was a slight decrease of the S/H value from 0.5 to 0.4, whereas the G/H value was 0.6 for all preparations. The results implied that ultrasound treatment could only slightly enhance the disrupting force to the linkages between S unit and polysaccharides. In addition, small amounts of ferulic acid and noticeable amounts of *p*-coumaric acid were detected. This was in well agreement with the widely reported results on the existence of chemical linkages among xylan, lignin, and cinnamic acid derivatives.[17,20] The data above suggested that there were strong linkages between polysaccharides and lignin in the bamboo after pretreatments. The cold alkali treatment could break the linkages to some extent, resulting in a relatively low amount of bound lignin in the preparations.

Molecular weight

The polysaccharide fractions were further studied to determine their molecular weights with size exclusion chromatography, and the data are presented in Table III. The weight-average molecular weight (M_w), number-average molecular weight (M_n), and polydispersity were 5950–6650, 3670–3930, and 1.6–1.8 for the polysaccharide fractions prepared with ultrasonic irradiation for 5–25 min, as compared to 6360, 3560, and 1.8 for the sample prepared without ultrasonic irradiation. Generally, the M_w of polysaccharides (noncellulosic components) existed in bamboo culm were over 25000 (osmotic pressure measurement).[21] The relatively low molecular weight of the obtained polysaccharide fractions in this study indicated that the pretreatment with cold NaOH/urea solution could result in the degradation of the polysaccharides of the raw material to some extent. However, there was no significant decrease of the molecular weights with the increment of the ultrasonic irradiation times. This indicated that the ultrasonic irradiation only disrupted the linkage between lignin and polysaccharides, but it did not significantly degrade the polysaccharides under the conditions given.

FTIR spectra

The FTIR spectra of the polysaccharide fractions are illustrated in Figure 2. The similar spectra profiles

TABLE III
Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersities (M_w/M_n) of the Polysaccharide Fractions

Sample[a]	F0	F1	F2	F3
M_w	6360	5950	6650	6480
M_n	3560	3670	3700	3930
M_w/M_n	1.8	1.6	1.8	1.6

[a] Corresponding to the fractions in Table I.

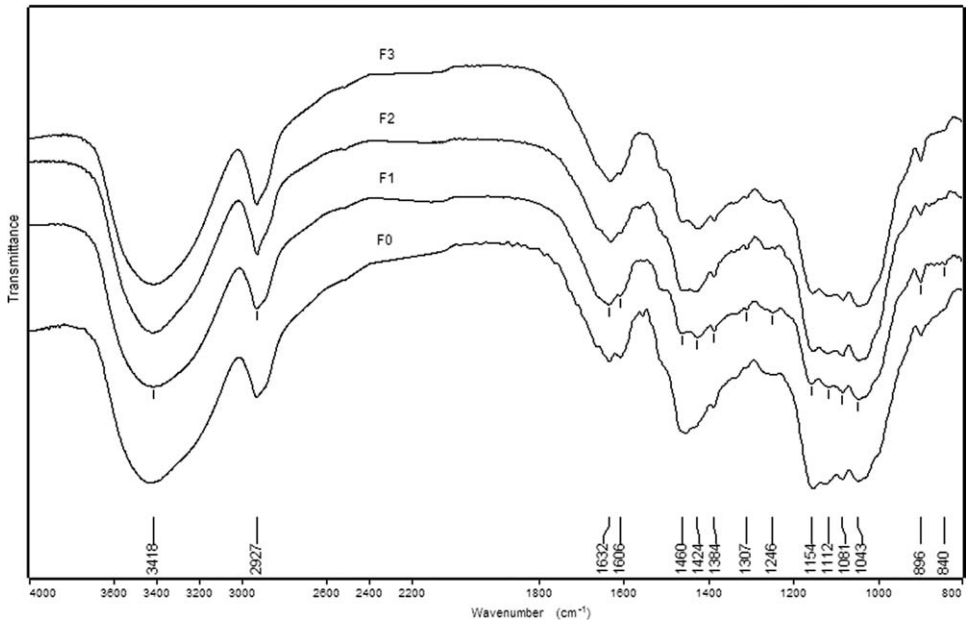

Figure 2 FTIR spectra of polysaccharide fractions prepared without ultrasonic irradiation (F0) and with ultrasonic irradiation times of 5 min (F1), 15 min (F2), and 25 min (F3).

indicated that the polysaccharide fractions exhibit an analogous structure. The predominate band around 3400 cm^{-1} corresponds to the O—H stretching vibrations of OH groups and hydrogen bonding, and the peak at 2900 cm^{-1} is assigned to the C—H stretching vibrations of CH_2 and CH_3 groups. The intense signal at 1632 cm^{-1} is assigned to the adsorbed water, since the polysaccharides have a strong affinity for water, and those macromolecules are easy to be hydrated when they are in solid state.[22] The absorptions at 1460, 1424, 1384, 1307, and 1246 cm^{-1} are attributed to the C—H and C—O bending or stretching.

The absorption between 1200 and 800 cm^{-1} indicates the typical polysaccharide structures of the fractions. The peak at 1043 cm^{-1} is assigned to the C—O, C—C, and glycosidic (C—O—C) stretching of xylans. In addition, glucan gives signal at 1154 cm^{-1} corresponding to the C—O stretching and signals at 1081 and 1008 cm^{-1} corresponding to the glycosidic (C—O—C) stretching.[23] The absorption at 896 cm^{-1}, corresponding to C1 group frequency or ring frequency, is attributed to the β-glycosidic linkages between sugar units.[24] In addition, the shoulder peaks at 941 cm^{-1} and 843 cm^{-1}, probably due to the signal overlapping, revealed the existence of α-glycosidic linkage.[25] These linkages were confirmed by NMR spectra afterwards.

The near absence of signal at 1730 cm^{-1}, corresponding to C=O vibrations of acid or esters, implies that the uronic, acetic, and/or phenolic acids were largely released from the polysaccharide fractions during the pretreatment process, which was in agreement with the low content of uronic acids in the polysaccharide fractions. Moreover, minor amounts of lignin are evidenced by small signals at 1610 and 1510 cm^{-1} corresponding to the aromatic skeletal vibrations, which was consistent with the results of the alkaline nitrobenzene oxidization. Compared to the fraction prepared without ultrasonic irradiation, the fractions prepared with ultrasonic irradiation (F1, F2, and F3) exhibited weaker signals at 1610 and 1510 cm^{-1}, which was in well agreement with their relatively high contents of sugars as determined by sugar analysis.

NMR spectra characterization

The structure of the polysaccharide fractions can be better interpreted on the basis of NMR results. The ^{1}H-NMR (Fig. 3), ^{13}C-NMR (Fig. 4), and 2D HSQC (Fig. 5) spectra of F3 (polysaccharide fraction prepared with ultrasonic irradiation time of 25 min) were recorded and the peak assignments are presented in Table IV by comparing with the literature data.[25–33]

The α-anomeric protons give signals at 5.0–5.3 ppm and β-anomeric protons at 4.4–4.6 ppm in ^{1}H-NMR spectra.[26] In the HSQC NMR spectrum, the δ_C/δ_H signals at 101.2/5.24, 72.5/3.51, 74.1/3.85, 78.7/3.48, 71.7/3.77, and 61.0/3.78 correspond to C1-H, C2-H, C3-H, C4-H, C5-H, and C6-H of (1→4)-linked α-D-glucan.[25,27] The signals of anomeric protons at 5.24 and 5.12 ppm (absence of signal at ~ 5.00 ppm) further confirmed that the polysaccharide fractions contained (1→4)-linked α-D-glucan, i.e.,

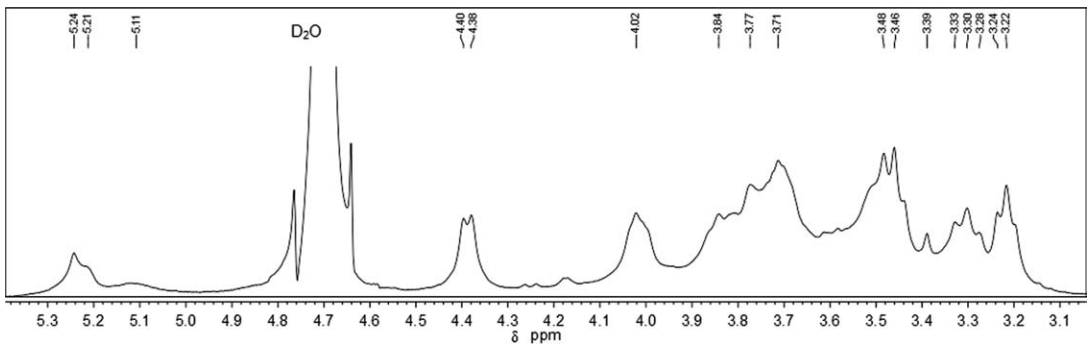

Figure 3 [1]H-NMR spectrum of F3 (polysaccharide fraction prepared with an ultrasonic irradiation time of 25 min).

amylose,[28] since the addition of iodine, the polysaccharide fractions dissolved in water showed the typical violet color of starch/iodine complex. The presence of strong signals of amylose explains the high glucose content in the sugar analysis of the polysaccharide fractions. Previously, Lisboa et al.[27] reported the dissolution of starch in the black liquor from kraft pulping. It was found that glucose accounted for ~ 10% of the polysaccharides in the *Eucalyptus globulus* black liquor, which was confirmed as ramified amylopectin.

In addition, the (1→4)-linked β-D-xylan is indicated by δ_C/δ_H signals at 102.2/4.40 (C1-H), 73.2/3.22 (C2-H), 74.4/3.47(C3-H), 76.6/3.71 (C4-H), and 63.4/4.03 and 3.31 (C5-H), in which two chemical shifts of 4.03 and 3.31 ppm correspond to the equatorial and axial protons linked at C5, respectively.[29,30] Furthermore, arabinose gives very weak signals at 109.4, 78.4, 80.3, 86.3, and 61.7 for C1, C2, C3, C4, and C5 in the [13]C-NMR spectrum.[31,32] Signal corresponding to other sugars was not observed in the spectra because of their low contents in the polysaccharide fractions. In addition, very weak signals about associated lignin and ferulic acid were observed: G unit gives signals at 142.3 (C-4, nonetherified), 131.5(C-1, nonetherified), 119.4 and 118.6 ppm (C-6); S unit gives signals at 135.5 (C1, etherified), 131.5 (C-1, nonetherified, overlapped with G units); H unit gives a signal at 130.0 (C-2/6); etherified ferulic acid gives signals at 121.1 (C-6) and 117.4 ppm (C_β); the two distinct signals at 55.9 and 55.7 ppm are attributed to —OCH_3 of syringyl and guaiacyl.[33] Thus, from the results of NMR and the previous sugar analysis, it can be speculated that the polysaccharide fractions were mainly composed of (1→4)-linked α-D-glucan (amylose) and (1→4)-linked β-D-xylan attached with minor amounts substituted sugars and uronic acids, mainly arabinose, galactose, glucurinic, and galacturonic acids.

Thermal properties

The thermal behavior of polymeric molecules is essential for reasonable industrial application. To illustrate the thermal stability of the polysaccharide fractions, the TG, DTG, and DTA curves of the fraction prepared without ultrasonic irradiation (F0) and the fraction prepared with an ultrasonic irradiation time of 15 min (F2) were recorded (Fig. 6). The TG curves [Fig. 6(a)] shows that the two fractions initiated with a slight decrease of weight below 100°C, which was attributed to the desorption of moisture as hydrogen bonded water to the polysaccharide structure. Then the degradation proceeded with a

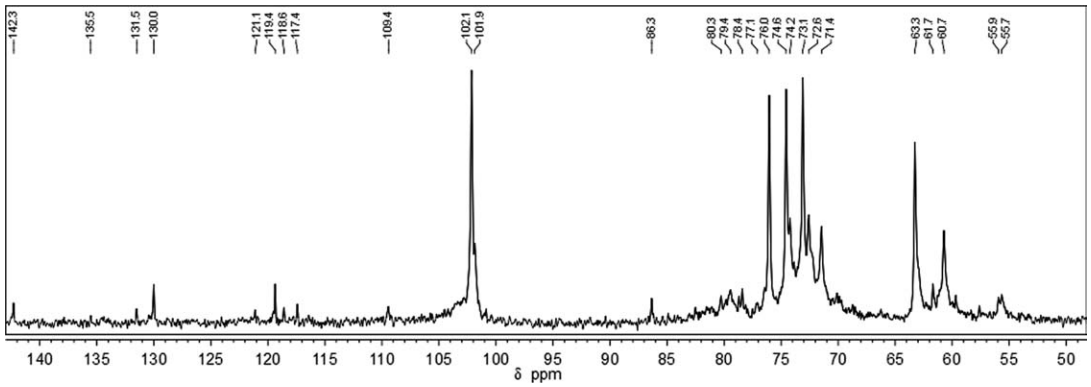

Figure 4 [13]C-NMR spectrum of F3 (polysaccharide fraction prepared with an ultrasonic irradiation time of 25 min).

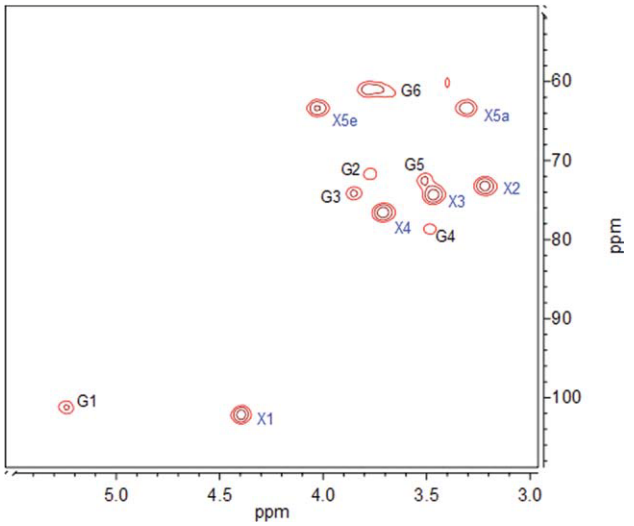

Figure 5 HSQC spectrum of F3 (polysaccharide fraction prepared with an ultrasonic irradiation time of 25 min; G represents (1→4)-linked α-D-glucan and X represents (1→4)-linked β-D-xylan). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

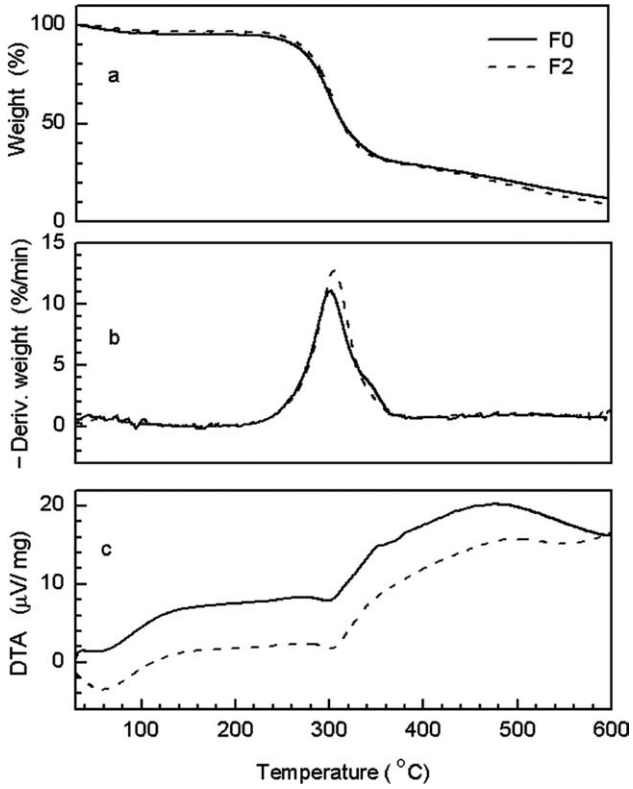

Figure 6 TG, DTG, and DTA curves of F0 (polysaccharide fractions prepared without ultrasonic irradiation) and F2 (polysaccharide fraction prepared with an ultrasonic irradiation time of 15 min).

slow rate. After that, the main degradation stage took place in the temperature range of 210 and 380°C. Correspondingly, the DTG curves [Fig. 6(b)] show that the big peak occurred at 300°C with the degradation rate of 11.0%/min for F0, whereas at 306°C with the degradation rate of 12.7%/min for F2. A shoulder peak appeared at 349°C with the degradation rate of 2.9%/min for F0. Subsequently, the degradation preceded with lower weight losses. When temperature reached to 600°C, the residual weight of the original weight decreased to 11.9% and 8.9% for F0 and F2. It was obvious that the polysaccharide fraction prepared with ultrasonic irradiation decomposed more rapidly than that prepared without ultrasonic irradiation.

DTA curves of the two polysaccharide fractions (F0 and F2) initiated with a small endothermic region, which is attributed to the release of adsorbed water [Fig. 6(c)]. Then the curves were in favor of the exothermic direction. The negative peaks at 303°C for F0 and at 306°C for F2 were in well agreement with the maximum degradation rate in Figure 6(b). An intense exothermic peak around this temperature has been observed from several natural polysaccharides such as starch,[34] xylans,[35] and xanthan gum.[36] It was reported that polysaccharides with amorphous structure were easy to degrade to volatize gases (CO, CO_2, and some hydrocarbon, etc) at a lower temperature.[37] The peak at 348°C for F0 and the peak at 345°C for F2 were probably from

TABLE IV
^{1}H and ^{13}C Chemical Shift (ppm) Assignments for Polysaccharide Fractions (F2)

	Chemical shift (C/H)							
Sugar residues[a]	C1	C2	C3	C4	C5	C5ax[b]	C5eq[c]	C6
G	101.2	72.5	74.1	*78.7*	71.7			61.0
	5.24	3.51	3.85	3.48	3.77			3.78
X	102.2	73.2	74.4	76.6		63.4	63.4	
	4.4	3.22	3.47	3.71		3.31	4.03	
Ara	109.4	78.4	80.2	86.3	61.7			

[a] G: (1→4)-linked α-D-glucan; X: (1→4)-linked β-D-xylan; Ara: arabinose.
[b] Axial.
[c] Equatorial.

the degradation of xylan and starch. In addition, the peak at 476°C for F0 and the peak at 490°C for F2 were probably from the degradation of bound lignin.[38] The reason for the high resistance of degradation of lignin was mainly due to the aromatic structures of lignin.[39]

From the above-mentioned observation, it can be speculated that the fractions prepared with ultrasonic irradiation has a relatively lower thermal stability compared to the fraction prepared without ultrasonic irradiation. This was probably due to the lower proportion of nonsugar components (mainly lignin) in the fractions prepared with ultrasonic irradiation, as indicated by the previous sugar analysis. It has been reported that the higher amount of aromatic substances (like lignin) in the sample resulted in a higher thermal stability of the sample.[38]

The obtained polysaccharides with a mixture of hemicelluloses and starch could be separated further to single fractions for use. Especially, the purified hemicellulosic fractions with relative low molecular weights are useful for chemicals and polymer materials. There are preferential feedstocks for the production of xylitol and its derivates.[40] The current uses of hemicelluloses have been achieved though conversion to xylose, xylitol, and furfural in an industrial scale. In addition, the degraded hemicellulosic fractions rich in OH groups can be chemically modified though esterification, etherification, and graft polymerization, etc., for potential applications as adhesives, thickeners, stabilizers, film formers, and emulsifies.[41]

CONCLUSIONS

In this study, polysaccharide fractions prepared from bamboo culms with varied ultrasonic irradiation times were comparatively characterized. The results showed that the cold NaOH/urea solution pretreatment resulted in degradation of the polysaccharides, while the ultrasonic irradiation treatment afterwards did not degrade significantly the molecules with the increase of the ultrasonic irradiation time. For the obtained polysaccharide fractions, glucose (~ 50–55%) was the major sugar component, and xylose (~ 41–44%) was the second major sugar in all cases. The polysaccharide fractions were mainly composed of (1→4)-linked α-D-glucan from amylose and (1→4)-linked β-D-xylan attached with minor amounts of branched sugars from hemicelluloses. In addition, the polysaccharide fractions were bound with lignin of H unit, followed by G and S unit. In accordance with the sugar content, the polysaccharide fraction prepared with ultrasonic irradiation had a slightly lower thermal stability as compared to that prepared without ultrasonic irradiation. The above structural and thermal analysis of the polysaccharide fractions indicated that they were preferential feedstocks for chemicals and polymer materials.

References

1. Vantomme, P.; Markkula, A.; Leslie, R. N. Non-wood Forest Products in 15 Tropical Countries-An Overview; FAO Regional Office for Asia and the Pacific. Bangkok, 2002.
2. Scurlock, J M. O.; Dayton, D. C.; Hames, B. Biomass Bioenerg 2000, 19, 229.
3. He, J. X.; Cui, S. Z.; Wang, S. Y. J Appl Polym Sci 2008, 107, 1029.
4. He, J. X.; Zhang, M.; Cui, S. Z.; Wang, S. Y. J Appl Polym Sci 2009, 113, 456.
5. Sharma, R.; Varshney, V. K.; Chauhan, G. S.; Naithani, S.; Soni, P. L. J Appl Polym Sci 2009, 113, 2450.
6. Yamashita, Y.; Shono, M.; Sasaki, C.; Nakamura, Y. Carbohydr Polym 2010, 79, 914.
7. Sathitsuksanoh, N.; Zhu, Z. G.; Ho, T. J.; Bai, M. D.; Zhang, Y. H. P. Bioresour Technol 2010, 101, 4926.
8. Kobayashi, F.; Take, H.; Asada, C.; Nakamura, Y. J Biosci Bioeng 2004, 97, 426.
9. Asada, C.; Nakamura, Y.; Kobayashi, F. Biochem Eng J 2005, 23, 131.
10. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y. Y.; Holtzapple, M.; Ladisch, M. Bioresour Technol 2005, 96, 673.
11. Brosse, N.; Sannigrahi, P.; Ragauskas, A. Ind Eng Chem Res 2009, 48, 8328.
12. Cai, J.; Zhang, L. Macromol Biosci 2005, 5, 539.
13. Zhao, Y. L.; Wang, Y.; Zhu, J. Y.; Ragauskas, A.; Deng, Y. L. Biotechnol Bioeng 2008, 99, 1320.
14. Li, M. F.; Fan, Y. M.; Sun, R. C.; Xu, F. Bioresources 2010, 5, 1762.
15. Peng, F.; Ren, J. L.; Xu, F.; Bian, J.; Peng, P.; Sun, R. C. J Agric Food Chem 2010, 58, 5743.
16. Peng, F.; Ren, J. L.; Xu, F.; Bian, J.; Peng, P.; Sun, R. C. J Agric Food Chem 2009, 57, 6305.
17. Azuma, J.; Nomura, T.; Koshijima, T. Agric Biol Chem 1985, 49, 2661.
18. Billa, E.; Tollier, M.-T.; Monties, B. J Sci Food Agric 1996, 72, 250.
19. Bach Tuyet Lam, T.; Iiyama, K.; Stone, B. A. Phytochemistry 1990, 29, 429.
20. Fujii, Y.; Azuma, J.; Marchessault, R. H.; Morin, F. G.; Aibara, S.; Okamura, K. Holzforschung 1993, 47, 109.
21. Maekawa, E. Wood Res 1976, 59/60, 153.
22. Kaîcuráková, M.; Belton, P. S.; Wilson, R. H.; Hirsch, J.; Ebringerová, A. J Sci Food Agric 1998, 77, 38.
23. Pal, S.; Mal, D.; Singh, R. P. Carbohydr Polym 2005, 59, 417.
24. Sun, X. F.; Sun, R. C.; Tomkinson, J.; Baird, M. S. Cell Chem Technol 2003, 37, 283.
25. Huang, Z. P.; Zhang, L. N. Carbohydr Res 2009, 344, 1136.
26. Hoffmann, R. A.; Geijtenbeek, T.; Kamerling, J. P.; Vliegenthart, J. F. G. Carbohydr Res 1992, 223, 19.
27. Lisboa, S. A.; Evtuguin, D. V.; Neto, C. P.; Goodfellow, B. J Carbohydr Polym 2005, 60, 77.
28. Sugiyama, H.; Nitta, T.; Horii, M.; Motohashi, K.; Sakai, J.; Usui, T.; Hisamichi, K.; Ishiyama, J. Carbohydr Res 2000, 325, 177.
29. Evtuguin, D. V.; Tomas, J. L.; Silva, A. M. S.; Neto, C. P. Carbohydr Res 2003, 338, 597.
30. Sun, X. F.; Fowler, P.; Rajaratnam, M.; Zhang, G. C. Phytochem Analysis 2010, 21, 406.
31. Bendahou, A.; Dufresne, A.; Kaddami, H.; Habibi, Y. Carbohydr Polym 2007, 68, 601.

32. Bradbury, J. H.; Jenkins, G. A. Carbohydr Res 1984, 126, 125.
33. Xu, F.; Sun, R. C.; Zhai, M. Z.; Sun, J. X.; She, D.; Geng, Z. C.; Lu, Q. Separ Sci Technol 2008, 43, 3351.
34. Lawal, O. S.; Lechner, M. D.; Kulicke, W. M. Polym Degrad Stabil 2008, 93, 1520.
35. Ren, J. L.; Sun, R. C.; Peng, F. Polym Degrad Stabil 2008, 93, 786.
36. Zohuriaan, M. J.; Shokrolahi, F. Polym Test 2004, 23, 575.
37. Yang, H. P.; Yan, R.; Chen, H. P.; Lee, D. H.; Zheng, C. G. Fuel 2007, 86, 1781.
38. Chen, W. H.; Tu, Y. J.; Sheen, H. K. Int J Energ Res 2010, 34, 265.
39. Xie, X. F.; Goodell, B.; Zhang, D. J.; Nagle, D. C.; Qian, Y. H.; Peterson, M. L.; Jellison, J. Bioresour Technol 2009, 100, 1797.
40. Ebringerová, A.; Heinze, T. Macromol Rapid Comm 2000, 21, 542.
41. Doner, L. W.; Hicks, K. B. Cereal Chem 1997, 74, 176.