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Isolation and structural characterization of hemicelluloses from the bamboo species *Phyllostachys incarnata* Wen

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ABSTRACT

Water and alkali-soluble polysaccharides, isolated by sequential extractions with distilled water, 0.5% and 1% NaOH, 60% ethanol containing 1% NaOH, and 3%, 5% and 8% NaOH, were prepared at 60 °C for 3 h from dewaxed bamboo *Phyllostachys incarnata* Wen. The yields of the seven fractions together accounted for 80.1% of total available hemicellulosic polysaccharides. Sugar composition studies showed that the water-soluble polysaccharides consisted mainly of glucose units, while xylose, arabinose and glucuronic acid were the major sugars in alkali-soluble hemicelluloses. Moreover, the molecular weights of these polymers varied between 3760 and 36,000 g/mol as revealed by GPC. Furthermore, the structure of the hemicellulosic fraction extracted with 3% NaOH was determined by ^{1}H and ^{13}C NMR spectroscopy along with 2D HSQC. It was found that the bamboo hemicelluloses were *O*-acetyl-(4-*O*-methylglucurono)-arabinoxylans, and the structural element could be identified as below: 1,4-*β*-D-[2-*O*-Ac][2,3-di-*O*-Ac][4-*O*-Me-α-D-Glc*p*A-(1 → 2)][α-L-Ara*f*-(1 → 2)(1 → 3)]-xyl*p*.

1. Introduction

Hemicelluloses, next to cellulose, refer to a large group of complex polysaccharides in cell wall of plants. Unlike cellulose, they are branched polymers of low molecular weight with a degree of polymerization of 80–200. The two major classes of hemicelluloses are glucomannans and xylans that are further classified as linear homoxylan, arabinoxylan and glucuronoarabinoxylan. Generally, hemicelluloses are attached to cellulose microfibrils by hydrogen bonds and cross-link between cellulose microfibrils. On the other hand, hemicelluloses are linked to lignin by ferulic acid that simultaneously esterifies its carboxyl group with *O*-5 of arabinose substituents of xylan and etherifies its hydroxyl group with phenyl hydroxyls of lignin (Jeffries, 1990). Because of this complex and structure, studies on the utilization and properties of hemicelluloses are not straightforward.

Bamboo belongs to Gramineae Bambusoideae and is widely distributed in China, Japan, and South-East Asian countries. As one of the most important and fast growing forestry plants, bamboo is a rich resource. It is traditionally used for in house construction and for furniture. However, in recent years, much attention has been denoted to utilization of bamboo as a potential natural resource for pulp and paper, food industry, medicine and so on. For that reason, great interest has focused on the chemical composition and structural characterization of bamboo hemicelluloses. The hemicelluloses of bamboo (*Phyllostachys reticulata* C. Koch) were characterized as a 1,4-linked linear structure of D-xylopyranose residues with 4-*O*-methyl-D-glucuronic acid and L-arabinose as side units in a molar ratio of 25:1.3:1.0 (Maekawa, 1976). Meanwhile, Maekawa (1976) reported a xylan containing 6.5% of native acetyl groups in a yield of 3.1% (on the defatted bamboo meal) by extraction with DMSO from bamboo holocellulose. Furthermore, Ishii (1991) considered that acetylation occurred at *O*-2 of the feruloylated arabinofuranosyl residue in the arabinoxylan in cell walls of bamboo shoot. In addition, the methods used for structural studies of the xylan from bamboo were also various, including partial hydrolysis, methylation, periodate oxidation and acetolysis. On the other hand, enzymatic hydrolysis together with a LiChrospher 100 NH_2 column and paper chromatography was also applied to analyze structure of xylan from bamboo species *Sasa senanensis* Rehd. (Yoshida, Kuno, Saito, Aoyama, & Kusakabe, 1998). However, little is known about the hemicelluloses of bamboo species *Phyllostachys incarnata* Wen, which is the most widely planted in Zhejiang and Fujian provinces in China and usually grows up 4–8 m in height and 3–5 cm in diameter.

In this study, the sequential treatments of dewaxed bamboo powder (*P. incarnata* Wen) with various concentrations of alkaline solution were performed to isolate the hemicelluloses. The

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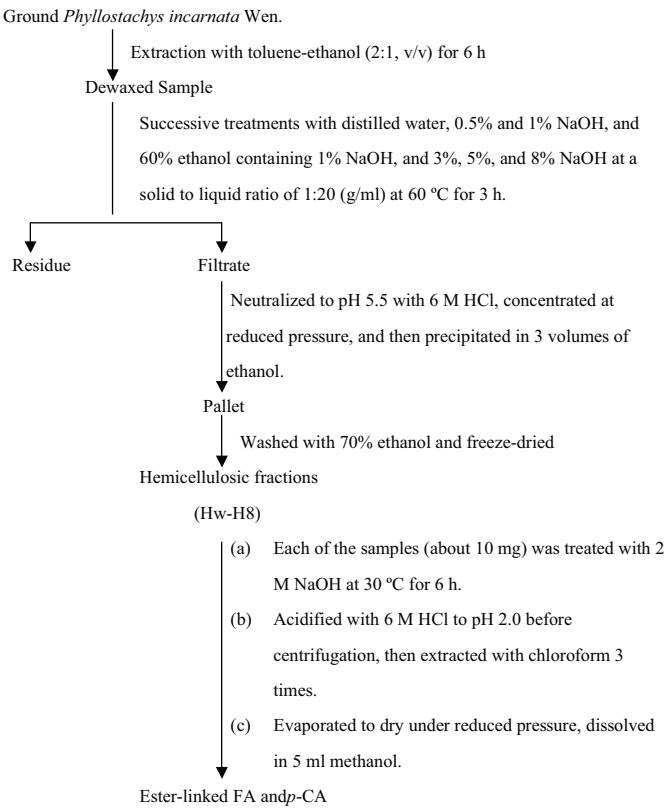

Fig. 1. Scheme for fractional isolation of hemicelluloses from bamboo species *Phyllostachys incarnata* Wen.

chemical composition and the structural features of the hemicellulosic fractions were determined and characterized by high performance anion exchange chromatography (HPAEC), gel permeation chromatography (GPC), Fourier transform infrared (FT-IR), and hydrogen-1 and carbon-13 nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C NMR) along with 2D ^{1}H–^{13}C hetero-nuclear single quantum coherence (HSQC) spectra to pave the way for the use of bamboo hemicelluloses in further industrial application.

2. Experimental

2.1. Materials

Bamboo culms (3 years old) were obtained from Bamboo Garden of the North-Western University of Agricultural and Forest Sciences and Technology (Yangling, China). They were dried in an oven for 16 h and then cut into small pieces. The cut culms were ground to pass a 1.0 mm size screen. Chemical analysis revealed the composition to be 71.9% holocellulose, 25.0% pentosans, 22.5% Klason lignin, 3.2% extractives, and 2.4% ash on a dry weight basis according to the method of Leopold (1961) and Tappi standards T 223 cm-01, T 222 om-02, T 204 cm-97, T 211 om-02 (Tappi 2002), respectively.

2.2. Sequential extractions

To remove the extractives, the ground bamboo was first extracted with toluene-ethanol (2:1, v/v) in a Soxhlet for 6 h. The dewaxed bamboo was dried in a cabinet oven with air circulation at 55 °C for 16 h and then kept at 5 °C before treatments. The procedure used to isolate hemicelluloses from bamboo species *P. incarnata* Wen is illustrated in Fig. 1. The extractive free powder (15.0 g) was sequentially extracted with distilled water, 0.5% and 1% NaOH, and 60% ethanol containing 1% NaOH, and 3%, 5% and 8% NaOH with a solid to liquid ratio of 1:20 (g/ml) at 60 °C for 3 h. Each of the residues was filtered off and washed thoroughly with 70% ethanol until the filtrate was neutral, and then dried in an oven at 55 °C for 16 h, respectively. The combined supernatant was neutralized to pH 5.5 with 6 M HCl, concentrated on a rotary evaporator under reduced pressure to about 80 ml, and then mixed with three volumes of 95% ethanol for isolating the solubilized hemicelluloses, respectively. The fractions obtained with distilled water, 0.5% and 1% NaOH, 60% ethanol containing 1% NaOH, and 3%, 5% and 8% NaOH were freeze-dried and labeled as H_w, $H_{0.5}$, H_1, $H_{60\text{-}1}$, H_3, H_5, and H_8, respectively.

2.3. Chemical analyses

High performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detector was used to analyze the monomeric sugars that were liberated by hydrolysis with 1 M H_2SO_4 for 2.5 h at 105 °C. After hydrolysis, the sample was diluted 50-fold, filtered and then injected into the HPAEC system (Dionex, ICS-3000, USA) with an AS50 autosampler and a Carbopac^{TM} PA-20 column (4 mm × 250 mm, Dionex). Neutral sugars and uronic acid were separated in a 5 mM NaOH (carbonate free and purged with nitrogen) for 20 min, followed by a 0–75 mM NaAc gradient in 5 mM NaOH for 15 min. Then the column was washed with 200 mM NaOH to remove carbonate for 10 min, and followed a 5 min elution with 5 mM NaOH to re-equilibrate the column before the next injection. The total analysis process was performed at 30 °C.

The lignin content in hemicellulosic samples was measured as acid-insoluble lignin (Klason lignin, Tappi standard T 222 om-02). The phenolics were analyzed by the method of Xu et al. (2005) with a slight modification. In brief, the polysaccharides (about 10 mg) were treated with 2 M NaOH at 30 °C for 6 h. Upon completion, after acidifying with 6 M HCl to pH 2.0, and centrifugation, the supernatant was extracted with 3 × 30 ml of chloroform, and then evaporated to dry under reduced pressure. The released ester-linked ferulic and *p*-coumaric acids were dissolved in 5 ml methanol and analyzed by high-performance liquid chromatography (Agilent 1200, USA) with a ZORBAX Eclipse XDB-C_{18} column (250 mm × 4.6 mm), which was kept at 30 °C and eluted with methanol–water gradient.

2.4. Gel permeation chromatography

The molecular weights of all fractions were determined by gel permeation chromatography on a PL aquagel-OH 50 column (300 mm × 7.7 mm, Polymer Laboratories Ltd.) with a differential refractive index detector (RID), which was eluted with 5 mM sodium phosphate buffer (pH 7.5) containing 0.02 N NaCl at a flow rate of 0.5 ml/min and kept at 30 °C. The molecular weight calibration cure was obtained using PL pullulan polysaccharide standards (M_w of 783, 12,200, 100,000, and 1,600,000, Polymer Laboratories Ltd.).

2.5. FT-IR and NMR spectroscopy

The FT-IR spectra of all samples were obtained on a spectrophotometer (Tensor 27) in the range 4000–400 cm^{-1} using a KBr disc containing 1% of finely ground samples. The solution-state ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AVIII 400 MHz spectrometer operating in the FT mode at 100.6 MHz. The hemicellulosic sample (20 mg for ^{1}H, 80 mg for ^{13}C) was dissolved in 1 ml D_2O. The chemical shifts were calibrated relative to the signal 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 30,000 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 spectra

Table 1
Yield and the chemical composition of water and alkali-soluble polysaccharides from bamboo.

Fraction[a]	Weight percentage (wt%)				Sugar composition (mol%)[d]				Molar ratio		
	Yield[b]	Klason lignin[c]	*p*-Coumaric acid[c]	Ferulic acid[c]	Ara	Xyl	Gal	Glc	GlcA	Ara/Xyl	GlcA/Xyl
H_w	1.19	6.31	0.16	0.17	6.56	2.64	6.19	84.16	0	2.49	0
$H_{0.5}$	1.85	4.05	0.18	0.19	9.48	69.28	2.14	14.53	4.57	0.14	0.07
H_1	2.82	3.52	0.12	0.15	7.17	71.44	0.94	16.00	4.44	0.10	0.06
$H_{60\text{-}1}$	1.75	4.19	0.04	0	9.16	68.40	1.50	11.64	9.29	0.13	0.14
H_3	7.93	3.35	0.07	0.10	6.25	80.82	0.38	9.17	3.37	0.08	0.04
H_5	2.95	2.58	0.02	0.03	5.97	85.73	0.33	5.10	2.86	0.07	0.03
H_8	1.52	2.22	0.04	0.04	6.02	86.03	0.28	4.43	3.24	0.07	0.04

[a] H_w, $H_{0.5}$, H_1, $H_{60\text{-}1}$, H_3, H_5, and H_8 represent the fractions obtained by sequential treatments with distilled water, 0.5% and 1% NaOH, 60% ethanol containing 1% NaOH, and 3%, 5% and 8% NaOH at a solid to liquid ratio of 1:20 (g/ml) at 60 °C for 3 h, respectively.
[b] Based on the amount of initial dewaxed bamboo sample.
[c] Based on the each fraction.
[d] Expressed in relative molar percentages.

were acquired by HSQC experiment mode, over a t_1 spectral width of 20,000 Hz and a t_2 width of 3600 Hz, and the acquired time per scan (AQ) was 0.1409s. 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 ^1H–^{13}C coupling constant of 145 Hz.

2.6. Thermogravimetric analysis

Thermal analysis of the hemicellulosic samples was performed using thermogravimetric analysis (TGA) on a simultaneous thermal analyzer (SDT-60, Shimadzu). The apparatus was continually flushed with a nitrogen flow of 25 ml/min. The sample was weighed between 8 and 12 mg and heated from room temperature to 600 °C at a rate of 10 °C/min.

3. Results and discussion

3.1. Yield and chemical composition

The yield and sugar composition of the seven fractions are given in Table 1. As can be seen, the sequential extractions of defatted bamboo with distilled water, 0.5% and 1% NaOH, 60% ethanol containing 1% NaOH, and 3%, 5% and 8% NaOH at 60 °C for 3 h yielded 1.19%, 1.85%, 2.82%, 1.75%, 7.93%, 2.95% and 1.52% (% dry matter, w/w), respectively, of the initial amount of bamboo, which together accounted for 80.1% of total available hemicellulosic polysaccharides. This indicated that most of hemicelluloses were solubilized from the bamboo meal employing this procedure. Obviously, the yield of water-soluble fraction was much lower than those of the alkali-soluble fractions. The reason was believed to be that the hydroxyl ions resulted in swelling of cellulose, disruption of hydrogen bonds between cellulose and hemicelluloses, and hydrolysis of ester bonds most likely connecting other polymers in the cell wall. Moreover, it should be noted that H_3 followed to $H_{60\text{-}1}$ gave the highest yield (7.93%), suggesting that the alkaline organic solvent extraction can be followed by an alkali step to obtain more hemicelluloses. The reason was probably due to removing more lignin and exposing more hemicelluloses by organic solvent treatment.

After acid hydrolysis of the hemicelluloses, the released monomeric sugars were monitored with HPAEC. As shown in Table 1 the monosaccharides were identified as mainly of the xylose, arabinose, glucuronic acid and glucose along with a small amount of galactose, which suggested that the hemicelluloses from bamboo were composed mainly of glucuronoarabinoxylans-type polysaccharides. This observation is also similar to those obtained from other bamboo species (Maekawa, 1976; Yoshida et al., 1998), indicating that bamboo hemicelluloses share the same basic chemical structure. Generally, the arabinose to xylose ratio is considered to be a measure for the degree of linearity or branching of hemicelluloses, a high arabinose to xylose ratio indicates a short-chain polymer with a large amount of branching with other monosaccharides while a low arabinose to xylose ratio suggests a linear hemicellulosic polymer with little bonding with other monosaccharide constituents. Obviously, the decreasing arabinose to xylose molar ratios from 0.14 to 0.07 with increasing NaOH concentrations from 0.5% to 8% reflected the different manner of substitution of the xylan backbone among all hemicellulosic fractions. These results also confirmed that the hemicelluloses extracted with a higher alkaline concentration appeared to be more linear. On the contrary, a lower concentration led to more highly branched hemicelluloses. This can be explained by the fact that the presence of larger unsubstituted regions in the xylan chains can lead to the strong hydrogen bonds, causing interchain aggregation and more difficult isolation (Höije, Gröndahl, Tømmeraas, & Gatenholm, 2005). Consequently, a higher concentration of alkali is required. On the other hand, the content of glucuronic acid in $H_{60\text{-}1}$ was the highest (9.29%) while in H_w was the lowest (0%), indicating that the acidic hemicelluloses were preferred to be dissolved during treatment with aqueous alkaline ethanol solution. It was also found that the relative lower molar ratios of glucuronic acid to xylose (0.03–0.17) were observed in the alkali-soluble hemicellulosic fractions ($H_{0.5}$–H_8). These glucuronic acids are originated from 4-*O*-methylglucuronic acids as side-chains, which can be confirmed by NMR later. 4-*O*-methl-D-glucuronic acid was also found in both bamboo species *Sasa senanensis* Rehd. and *Phyllostachys reticulata* C. Koch by Yoshida et al. (1998) and Maekawa (1976) who gave a similar molar ratio of 4-*O*-methylglucuronic acid to xylose (0.04), respectively.

Additionally, traces of ester-linked ferulic (0–0.19%) and *p*-coumaric (0.02–0.18%) acids with the arabinoxylan were released under the alkaline conditions. The attachments of the ferulic and *p*-coumaric acids at the *O*-5-position of α-arabinosyl residues of arabinoxylans via the ester bonds were proved in bamboo shoots by Ishii (1997). Furthermore, the seven fractions obtained with a stepwise gradient of increasing NaOH concentrations from 0% (water) to 8% contained decreasing Klason lignin content from 6.31% to 2.22%, indicating that the alkaline treatment can significantly break the bonds between lignin and hemicelluloses.

In contrast to alkali-extractable hemicelluloses, water-extractable polysaccharides had a remarkably higher molar ratio of arabinose to xylose (2.49%), suggesting that the water-soluble polysaccharides were more highly branched than alkali-soluble hemicelluloses. This result supports the conclusion that a higher degree of branching of the xylan chains would lead to a higher solubility of the polysaccharides. Moreover, the water-soluble fraction contained relatively high amounts of glucose (84.16%) arising from starch, which contributed to filtration problems, and galactose (6.19%) arising from arabinogalactans (Dervilly, Saulnier,

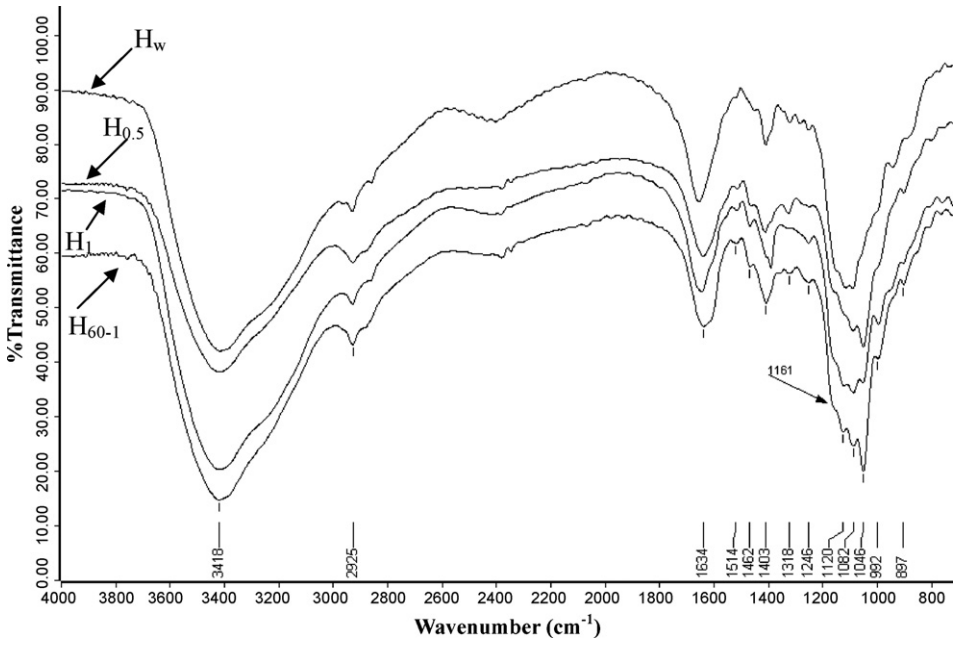

Fig. 2. FT-IR spectra of the fractions H_w, $H_{0.5}$, H_1 and $H_{60\text{-}1}$.

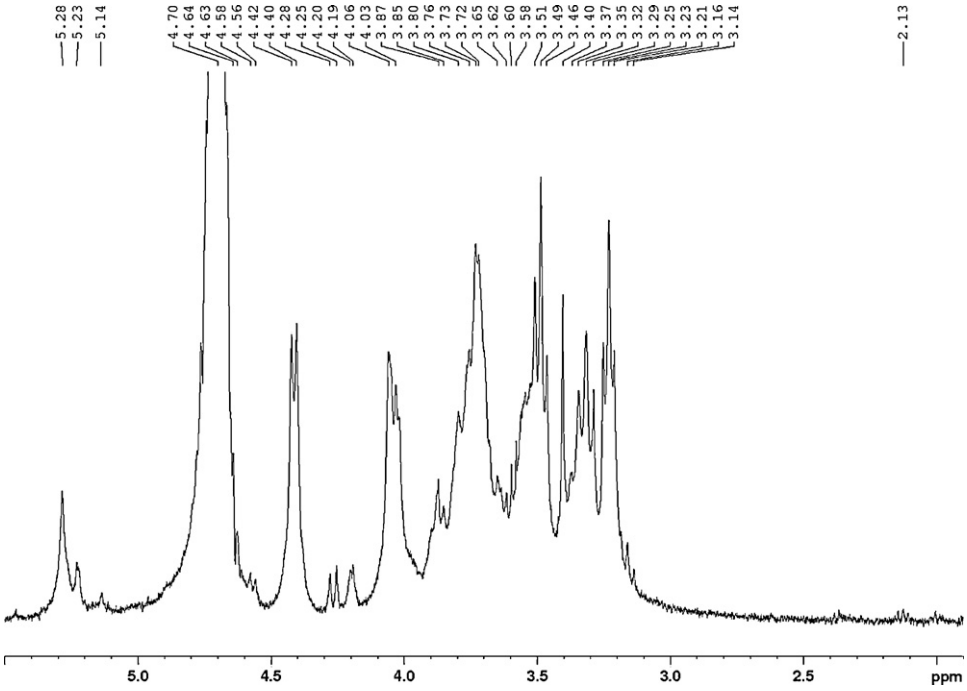

Fig. 3. ^{1}H NMR spectra of hemicellulosic fraction H_3.

Table 2
Weight-average (M_w) and number-average (M_n) molecular weights (g/mol) and polydispersity (M_w/M_n) of all polysaccharide fractions.

	Fraction[a]						
	H_w	$H_{0.5}$	H_1	$H_{60\text{-}1}$	H_3	H_5	H_8
M_w	3760	26,140	30,140	21,780	27,550	32,830	36,000
M_n	3580	14,990	17,830	13,910	17,080	20,890	25,340
M_w/M_n	1.1	1.7	1.7	1.6	1.6	1.6	1.4

[a] Corresponding to the fractions in Table 1.

Roger, & Thibault, 2000). Furthermore, a relatively lower content of arabinose in H_w appeared to be a plausible reason for a lower content of ferulic and *p*-coumaric acids, because it was found that ferulic and *p*-coumaric acids were esterified their carboxyl group to *O*-5 of arabinose substituents of arabinoxylan (Smith & Hartley, 1983), while NaOH would deesterify the linkages between ferulic and *p*-coumaric acids and arabinose, leading to a lower content of ferulic and *p*-coumaric acids in the alkali-extracted hemicelluloses.

Another interesting aspect was that, compared to the fraction H_1, $H_{60\text{-}1}$ extracted with alkaline organic solvent gave a relatively free of ferulic acid but a higher Klason lignin content with the molar

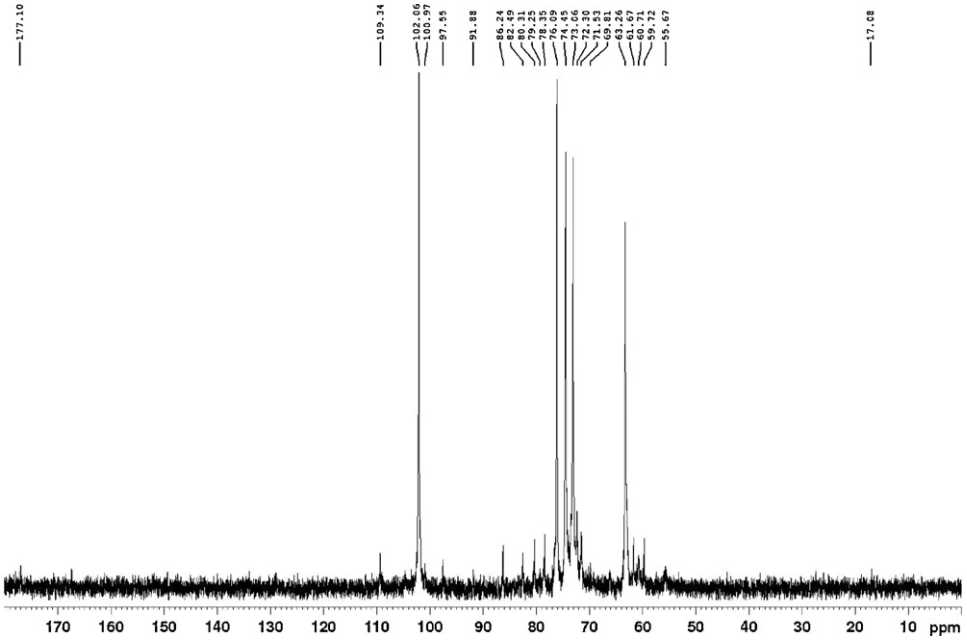

Fig. 4. ^{13}C NMR spectra of hemicellulosic fraction H_3.

ratios of arabinose to xylose (0.13) and glucuronic acid to xylose (0.14). These indicated that the extraction with alkaline organic solvent is a potential way to isolate the ferulic acid free hemicelluloses with more branches and higher amount of lignin residue than those of the alkali-extractable counterpart.

3.2. Molecular weight

The weight-average (M_w) and number-average (M_n) molecular weights determined by gel permeation chromatography (GPC) and polydispersity (M_w/M_n) are presented in Table 2. Evidently, the water-soluble polysaccharides with an M_w value of 3760 g/mol was lower than those of the alkali-soluble hemicellulosic fractions in the range of 21,780–36,000 g/mol, which suggested that the alkali played an important role in releasing the high-molecular-weight hemicelluloses. However, in comparison, the M_w of hemicellulosic fraction $H_{60\text{-}1}$ (21,780 g/mol) extracted with alkaline organic solvent (60% ethanol containing 1% NaOH) was lower than those of the fractions extracted with alkaline solvent. This was probably due to a consequence of the cleavage of glycosidic ether linkages between

Table 3
^1H NMR chemical shifts in ppm (measured at 400 MHz in D_2O; relative to an internal D_2O, δ_H 4.700 ppm) of constituent monosaccharide residues in hemicellulosic fractions H_3 and H_8.

Fraction	Structural	Residue[a]	Chemical shifts (ppm)						
			H-1	H-2	H-3	H-4	H-5a	H-5e	OCH_3-4
H_3	●○	β-X_{red}	4.58	3.23	3.60	3.76	3.34	n.d.[b]	
	●	X_{int}	4.42	3.29	3.51	3.73	3.37	4.06	
	●	X_{term}	4.40	3.25	3.49	3.72	3.35	4.03	
	●△	$X_{MeGlcA2}$	4.64	3.56	3.65	3.80	3.38	4.19	
	●◇◇	X_{A23}	4.56	3.53	3.62	3.65	3.32	n.d.	
	●△	X_{Ac2}[c]	4.63	4.63	3.76	3.87	3.40	4.19	
	●▽△	X_{Ac23}	4.76	n.d.	5.14	n.d.	3.54	4.20	
	◇	A_2	5.23	4.20	n.d.	n.d.	3.73	3.85	
	◇	A_3	5.28	4.25	n.d.	n.d.	3.73	3.87	
	△	MeGlcA	5.27	3.58	3.87	3.23	n.d.		3.46
H_8	●○	X_{red}	4.59	3.19	3.95	3.76	3.35	n.d.	
	●	X_{int}	4.43	3.32	3.51	3.74	3.37	4.06	
	●	X_{term}	4.41	3.24	3.49	3.73	3.35	4.05	
	●△	$X_{MeGlcA2}$	4.64	n.d.	3.67	3.80	3.38	4.20	
	●◇◇	X_{A23}	4.57	3.53	3.62	3.65	3.41	n.d.	
	●▽△	X_{Ac23}	n.d.	n.d.	5.12	n.d.	n.d.	4.20	
	◇	A_2	5.24	4.26	n.d.	n.d.	3.74	3.85	
	◇	A_3	5.28	4.29	n.d.	n.d.	3.74	3.87	
	△	MeGlcA	5.28	3.57	3.87	3.24	4.32		3.49

[a] The following designations are used: β-X_{red}, β-Xyl reducing end; X_{int}, Xyl internal; X_{term}, Xyl terminal end; $X_{MeGlcA2}$, MeGlcA 2-*O*-linked Xyl; X_{A23}, Ara 2-*O*- and 3-*O*-di-substituted Xyl; X_{Ac2}, 2-*O*-acetylated Xyl; X_{Ac23}, 2,3-di-*O*-acetylated Xyl; A_2, 2-*O*-linked Ara; A_3, 3-*O*-linked Ara; MeGlcA, 4-*O*-methylglucuronic acid.
[b] n.d., not detectable.
[c] The chemical shift was observed for the 2-*O*-acetyl groups at δ_H 2.11 ppm.

Table 4
^{13}C NMR chemical shifts in ppm of constituent monosaccharide residues in hemicellulosic fractions H_3 and H_8.

Fraction	Structural	Residue[a]	Chemical shifts (ppm)						
			C-1	C-2	C-3	C-4	C-5	OCH_3-4	COOH
H_3		X_{red}	n.d.[b]	n.d.	n.d.	77.80	66.50		
		X_{int}	102.06	73.06	74.45	76.09	63.26		
		X_{term}	n.d.	74.02	76.90	70.54	66.61		
		$X_{MeGlcA2}$	103.55	78.39	73.47	n.d.	n.d.		
		X_{A23}	n.d.	n.d.	74.60	76.98	n.d.		
		X_{Ac2}	100.97	n.d.	72.53	n.d.	63.95		
		X_{Ac23}	100.42	72.51	73.97	n.d.	n.d.		
	/	A_2/A_3	109.34	82.49	79.25	86.24	61.67		
		MeGlcA	97.55	72.31	73.76	n.d.	n.d.	59.72	177.10
H_8		X_{red}	n.d.	n.d.	n.d.	77.72	64.95		
		X_{int}	102.20	73.19	74.67	76.08	63.35		
		X_{term}	n.d.	74.05	77.05	69.93	66.65		
		$X_{MeGlcA2}$	103.74	78.44	n.d.	77.55	n.d.		
		X_{A23}	n.d.	73.89	n.d.	77.28	n.d.		
	/	A_2/A_3	109.47	82.57	80.36	86.38	61.75		
		MeGlcA	97.58	72.23	73.89	n.d.	n.d.	59.71	176.99

[a] The following designations are used: β-X_{red}, β-Xyl reducing end; X_{int}, Xyl internal; X_{term}, Xyl terminal end; $X_{MeGlcA2}$, MeGlcA 2-*O*-linked Xyl; X_{A23}, Ara 2-*O*- and 3-*O*-di-substituted Xyl; X_{Ac2}, 2-*O*-acetylated Xyl; X_{Ac23}, 2,3-di-*O*-acetylated Xyl; A_2, 2-*O*-linked Ara; A_3, 3-*O*-linked Ara; MeGlcA, 4-*O*-methylglucuronic acid.
[b] n.d., not detected.

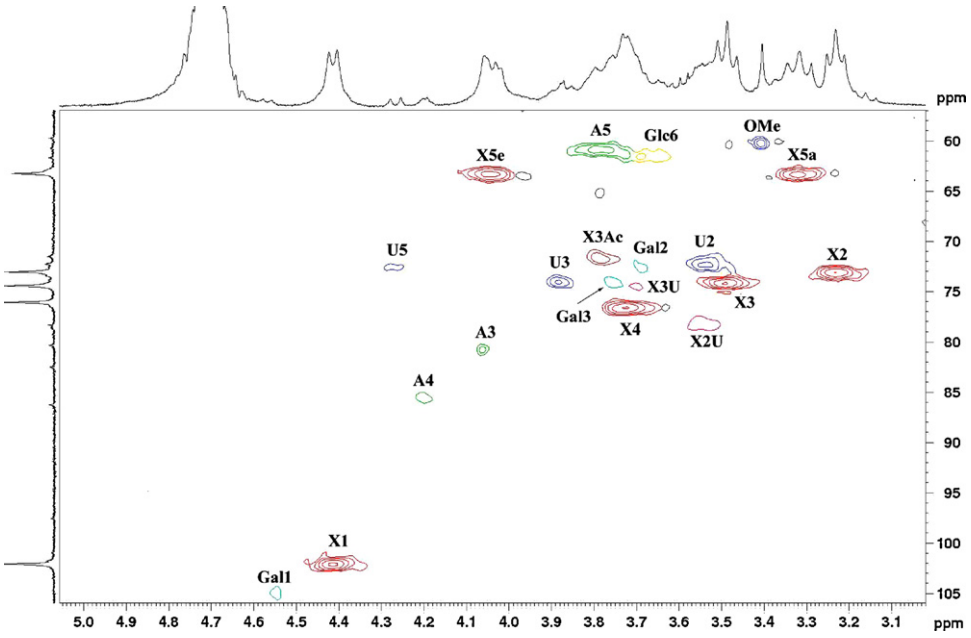

Fig. 5. HSQC (D_2O, 25 °C) spectra of hemicellulosic fraction H_3. Designations are as follows: X, Xyl*p* unit; A, Ara*f* unit; U, 4-*O*-Me-α-D-Glc*p*A unit; Gal, →4)-β-D-Gal*p*-(1→ unit; Glc, (1 → 4)-linked α-D-Glc*p* unit.

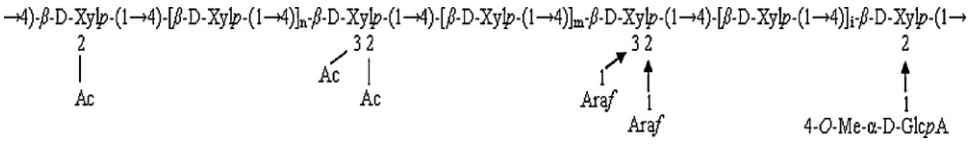

Fig. 6. Abbreviated structural formulae for the acetylated (4-*O*-methylglucurono)arabinoxylans of alkali-soluble hemicelluloses isolated from bamboo species *Phyllostachys incarnata* Wen.

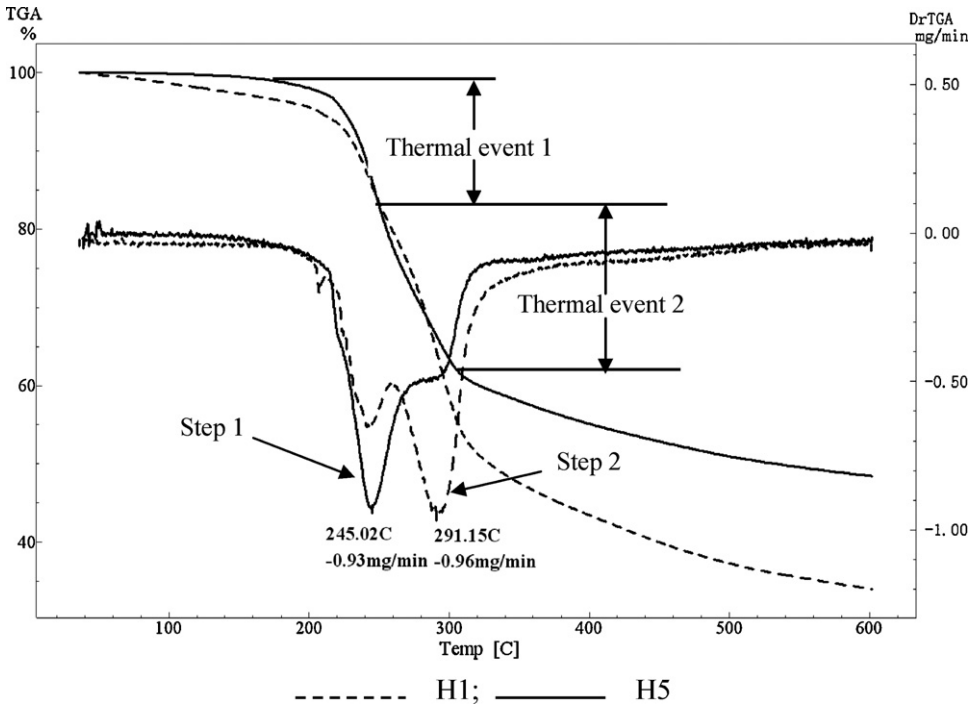

Fig. 7. TGA and DTG curves of hemicellulosic fractions H_1 and H_5.

sugar units, which resulted in the substantial degradation of the hemicelluloses under the organic alkaline condition (Bian, Peng, Xu, Sun, & Kennedy, 2010). In addition, the relatively low polydispersity indices (1.1–1.7) indicated a narrow distribution of molecular sizes for the polysaccharides obtained from the successively alkaline treatments.

3.3. FT-IR spectra

Fig. 2 shows the FT-IR spectra of fractions H_w, $H_{0.5}$, H_1, and $H_{60\text{-}1}$ that appeared to be rather similar absorption of typical polysaccharide structures. Generally, FT-IR spectra in the 1200–800 cm^{-1} region give the information about the main polysaccharides (Kačuráková, Ebringerová, Hirsch, & Hromádková, 1994). As can be seen from Fig. 2, the absorptions at 1462 and 1403 cm^{-1} arise from the CH_2 and CH or OH bending, respectively. A very small band at 1161 cm^{-1} is assigned to C–O and C–O–C stretching with some contribution of OH bending in arabinoxylans (AX). The C–C stretching gives the absorption at 1120 cm^{-1}. Furthermore, the band at 1082 cm^{-1} is originated from the ring vibration. The main band maximum at 1046 cm^{-1} is attributed to C–O–H bending modes (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). A weak band at 992 cm^{-1} is characterized by the presence of lower substituted AX. Moreover, the most important band is at about 897 cm^{-1}, corresponding to the glycosidic C_1–H deformation mode with ring vibration contribution for β-anomer form of the pyranoid ring, which was characteristic of β-glycosidic linkages between the sugar units. In addition, the broad band at 1634 cm^{-1} has been assigned to the H–O–H angle vibration, since the hemicelluloses usually have a strong affinity for water. The occurrence of a band at 1514 cm^{-1} is due to the associated lignin with the hemicelluloses (Roy, Sen, Bag, & Pandey, 1991). The band at 1246 cm^{-1} is attributed to the C–O linkage in the acetyl group in xylan (Robert, Marquis, Barron, Guillon, & Saulnier, 2005). The bonds at 3418 and 2925 cm^{-1} are originated from O–H and the C–H_2 stretching vibrations, respectively.

3.4. ^1H and ^{13}C NMR

NMR spectroscopy as an important non-destructive tool was used to investigate the fine structure of polysaccharides. Fig. 3 shows the ^1H NMR spectrum of hemicellulosic fraction H_3. The signals of α-anomeric protons were seen in the spectral region of 5.20–5.40 ppm and β-anomeric protons at 4.40–4.65 ppm. The relative complexity of the structures were exhibited by (i) major signals at δ 4.42 (H-1), 4.06 (H-5eq), 3.73 (H-4), 3.51 (H-3), 3.37 (H-5ax) and 3.29 ppm (H-2), corresponding to non-substituted (1 → 4)-β-D-Xyl residues; (ii) minor signals at δ 3.58 (H-2), 3.87 (H-3), 3.23 (H-4), and 3.46 (OCH_3-4) ppm, originating from 4-*O*-methyl-α-D-Glc*p*A acid (1 → 2) residues (Ebringerová, Alföldi, Hromádková, Pavlov, & Harding, 2000); (iii) weak signals at δ 5.28 and 5.23 ppm, arising from Ara*f* units attached to 3-*O*- and 2-*O*- of the same Xyl*p* residue, respectively (Hoffmann, Geijtenbeek, Kamerling, & Vliegenthart, 1992); (iv) a very small signal at δ 2.11 ppm, indicating that the hemicellulosic polymers are mono-acetylated at C-2 position of Xyl*p* (Teleman, Lundqvist, Tjerneld, Stålbrand, & Dahlman, 2000). Maekawa (1976) isolated a xylan containing 6.5% of native acetyl groups and considered that the bamboo xylan had native acetyl groups, which was characteristic in common with the hardwood xylans originally having native *O*-acetyl groups. However, the signal at δ 2.11 ppm in H_3 is very weak, which can be ascribed to removing seriously of acetyl groups under alkaline condition used; (v) signal at 5.14 ppm is assigned to H-3 of 2,3-di-*O*-acetylated β-D-Xyl units (Teleman et al., 2000), indicating the presence of 2,3-di-*O*-Ac units.

The ^{13}C NMR spectrum of H_3 (Fig. 4) shows five main signals at δ 102.06 (C-1), 76.09 (C-4), 74.45 (C-3), 73.06 (C-2) and 63.26 ppm (C-5), corresponding to (1 → 4) linked β-D-Xyl residues (Habibi & Vignon, 2005). Other less intense signals at δ 177.10, 97.55, 72.31 and 59.72 ppm, are characteristic, respectively, of COOH, C-1, C-2 and OCH_3-4 of 4-*O*-methy-α-D-glucuronic acid residues (Vignon & Gey, 1998). Small signals at 109.34 (C-1), 82.49 (C-2), 79.25 (C-3), 86.24 (C-4) and 61.67 ppm (C-5) are attributed to Ara*f* units (Roubroeks, Andersson, & Åman, 2000). The detailed chemical shifts

of the ^{1}H and ^{13}C NMR of fractions H_3 and H_8 are summarized in Tables 3 and 4, respectively.

In order to gain a more complete understanding of the hemicellulosic structure, the 2D HSQC technique was used in this study. The HSQC spectra of H_3, displayed in Fig. 5, gives the cross-peaks characteristic of the structural element: (1 → 4)-β-D-Xyl residues (i.e., $^{1}H/^{13}C$ chemical shifts of 4.42/102.06), Ara*f* units (4.20/82.49), 4-*O*-Me-α-D-Glc*p*A -(1 → 2) units (3.58/72.31 and 3.46/59.72), Xyl*p* units attached with acetyl groups (3.76/72.53), Xyl*p* units attached with GlcA (3.56/78.39), (1 → 4)-α-D-Glc*p* units (3.69/61.52) (Lisboa, Evtuguin, Neto, & Goodfellow, 2005), and (1→4)-β-D-Gal*p* units (4.54/105.10) (van Hazendonk, Reinerink, Waard, & van Dam, 1996), which are also confirmed by the sugar composition. Therefore, abbreviated structural formulae for alkali-soluble hemicelluloses isolated from bamboo species *P. incarnata* Wen is potentially shown in Fig. 6.

3.5. Thermogravimetric analysis

Thermal degradation of hemicelluloses has been investigated using the techniques of thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG) between room temperature and 600 °C. TGA–DTG analysis as the most common measurement of weight loss allows us to describe the distinct thermal events recorded during the process, in which hemicelluloses were decomposed to monomer sugars, furfural, furan, aldehyde and acetic acid. As shown in Fig. 7, the TGA curves show an initial decrease in the weight of samples between 100 and 160 °C due to the release of moisture remaining in the samples. Furthermore, two thermal events in TGA curves corresponding to two decomposition steps (step 1 and step 2) in DTA curves were observed around between 180 and 360 °C. Evidently, the major decomposition temperature of H_5 occurred at 245 °C whereas that of H_1 was at 291 °C. This was due to the different thermal stability of fractions H_1 and H_5, which depended on many factors such as crystalline and amorphous regions, molecular weights, linear and branched structure of hemicelluloses.

4. Conclusions

The above results revealed that the treatments of the bamboo with sequential alkalis were suitable ways to isolate bamboo hemicelluloses with a high yield and traces of ester-linked ferulic and *p*-coumaric acids. Moreover, the water-soluble polysaccharides contained predominantly glucose, and the water-soluble xylans were more branched than the alkali-soluble ones, indicating that a higher degree of branching xylan chains would lead to a higher solubility of the polysaccharides, while the alkali-soluble hemicelluloses were composed mainly of glucuronoarabinoxylans-type polysaccharides. Furthermore, the hemicelluloses extracted with a higher alkaline concentration had the features of more linear structure and a higher value of M_w.

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