Chemical Composition and Structural Feature of *Populus gansuensis* Hemicellulosic Polymers

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Received 22 March 2011; accepted 5 May 2011
DOI 10.1002/app.34835
Published online 4 November 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Hemicellulosic polymers A and B were isolated from *Populus gansuensis* by extracting the chlorite-delignified residue with 10% KOH. Fractional precipitation of hemicellulose B by graded ethanol resulted in six subfractions varying in yield, molecular size distribution, and sugar composition. Macromolecular and more linear hemicellulosic polymers with higher yields were preferentially precipitated in relatively lower ethanol concentrations, while more branched and complex hemicellulosic polymers with lower molecular weights were obtained in relatively higher ethanol concentrations. Chemical and spectral evidence suggested that H30 subfraction obtained by 30% ethanol precipitation was assumed to be 4-*O*-methyl-D-glucurono-D-xylans, with 4-*O*-methyl-D-glucuronic acid linked to C-2 of (1→4)-β-D-xylan. On average for every six D-xylopyranosyl residues, there is one 4-*O*-methyl-D-glucuronic acid group. Hemicellulosic subfraction H75 precipitated at the ethanol concentration of 75% was more branched, and presumed to be heterogeneous mixture of arabinoglucurnoxylan and galactoglucomannan. In addition, the thermal stability of the linear large molecular hemicellulosic subfraction H30 appeared higher than the branched subfraction H75. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 3154–3164, 2012

Key words: *Populus gansuensis*; hemicelluloses; GPC; FTIR; NMR

INTRODUCTION

Hemicelluloses, the most complex component of lignocellulose, are heteropolysaccharide polymers consisting of pentoses, hexoses, and glucuronic acids.[1] At the present time, considerable interest has been directed to hemicelluloses since they have already been separated by various extraction and isolation methods, and then utilized in a number of ways.[2] Generally, in monomeric form, hexoses are converted to ethanol in high yields, xylose can be fermented to xylitol which has beneficial health properties and represents an alternative to current conventional sweeteners.[3–5] In oligomeric form they can be used in food, pharmaceutical, agriculture, and feed products and in polymeric form they can be used as hydrogels and bio-degradable barrier films for food packaging.[6–9]

Xylans are the most abundant hemicelluloses found in the cell wall of the land plants. They are known to occur in several structural varieties and even in different plant tissues within one plant.[10] Depending on the botanical source, xylans possess a backbone of β-(1→4)-D-xylopyranosyl residues which carry short glycosyl side chains in various proportions on O-2 and/or O-3. The branches are mostly composed of α-D-glucuronic acid (GlcA), 4-*O*-methyl-α-D-glucuronic acid (MeGlcA), *O*-acetyl groups, and some neutral sugar units such as D-galactose, L-arabinose, and D-glucose.[11] Ebringerová et al. suggested that the structural variability of xylans affect their biological properties.[12] The structural variability may originate from the degree of polymerization (DP) of the polysaccharide, the monosaccharidic composition, and the random or regular distribution of substituents along the xylosyl backone. More precisely, 4-*O*-methylglucuronoxylan (MGX) is the major hemicellulose component of the cell wall of hardwood.[13] The most representative structure of this polysaccharide is a linear chain of β-(1→4)-xylopyranosyl units of which a few

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Contract grant sponsor: Specific Programs in Graduate Science and Technology Innovation of Beijing Forestry University; contract grant number: BLYJ201109.
Contract grant sponsor: State Forestry Administration; contract grant numbers: 200804015, 948-2010-16.
Contract grant sponsor: Ministry of Science and Technology; contract grant numbers: 973, 2010CB732204.
Contract grant sponsor: Natural Science Foundation of China; contract grant number: 30930073.
Contract grant sponsor: China Ministry of Education; contract grant number: 111.

unevenly distributed residues are substituted at C-2 by 4-*O*-methylglucuronic acid.[14] The xylose (Xyl) to MeGlcA ratios of glucuronoxylan isolated from different hardwoods range from 4 : 1 to 16 : 1.[13] In addition, acetyl groups located at O-2 and/or O-3 are often found on the backbone of xylopyranosyl residues. Based on the hitherto published results it can be deduced that the structural diversity of MGX is contributed to the botanical origin of the material and the isolation and extraction procedure.[15–17] In general, for the extraction of hemicelluloses, the use of dilute aqueous alkaline hydroxide, hot water, and steaming have been studied. The fractionation techniques, such as ammonium sulphate precipitation, and anion-exchange chromatography, have been employed in an attempt to obtain more homogeneous fractions.[18]

The Gansu Poplar (*Populus gansuensis*; C. Wang and H.L. Yang), which is characterized by rapid growth and development, has been planted as farmland shelter-belts, and covers an area of about 18,000 ha.[19] The Gansu Poplar is not only extremely useful in controlling desertification, but also rich in hemicelluloses. As is known, the utilization efficiency of hemicelluloses is highly dependent on their structure and physicochemical properties. In this work, we have isolated, fractionated, and partially characterized hemicelluloses from *P. gansuensis*. The obtained hemicellulosic subfractions were studied by sugar analysis, Fourier transform infrared (FTIR), 1D and 2D NMR spectroscopy, and gel permeation chromatography (GPC) to provide significant evidence on elucidation of the original *P. gansuensis* hemicellulose structures. Besides, the present study of isolation and characterization of hemicelluloses is believed to be important in providing a method for subfraction and purification of xylans.

EXPERIMENTAL

Materials

P. gansuensis was harvested in 2006 in Gansu Province, China. After removing the leaves and bark, the trunks were dried in sunlight and first chipped into small pieces and then ground to pass a 0.8-mm-size screen. The components (%, w/w) of the *P. gansuensis* were cellulose 41.8%, hemicelluloses 31.2%, and lignin 20.9% on a dry weight basis.

Fractionation of hemicelluloses by graded ethanol precipitation

The dried powder was first extracted with toluene–ethanol (2 : 1, v/v) in a Soxhlet apparatus for 6 h, and then dried in an oven at 60°C for 16 h.[20] The delignification of this extractive-free material was performed using sodium chlorite in acidic solution

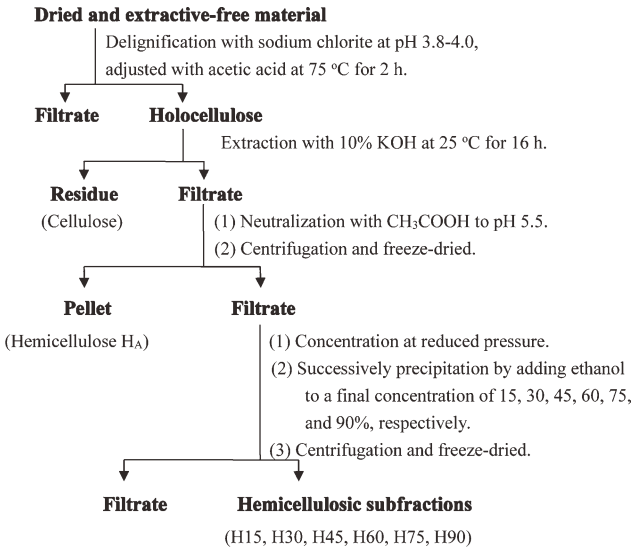

Figure 1 Scheme for fractional isolation of hemicelluloses from *P. gansuensis.*

(pH 3.8–4.0, adjusted by acetic acid) at 75°C for 2 h. After treatment, the residue (holocellulose) was filtered off and washed thoroughly with distilled water, and further dried in a cabinet oven with air circulation for 16 h at 50°C. The holocellulose was first extracted with 10% KOH with a solid to liquor ratio of 1 : 25 (g mL^{-1}) for 16 h at room temperature. The filtrate was acidified with 6*M* acetic acid until the pH reached 5.5 and the mixture was centrifuged. The precipitate obtained was identified as hemicellulose fraction H_A. Then appropriate volumes of 95% ethanol was slowly added with continuous stirring to the filtrate (H_B), until a final concentration of 15% (v/v) was achieved and the solution was allowed to stand overnight at room temperature. The precipitate was recovered by centrifugation and freeze-drying and referred to as hemicellulosic subfraction H15. Then, the supernatant was brought to an ethanol concentration of 30%, leading to hemicellulosic subfraction H30. This procedure was repeated with stepwise increase in ethanol concentration (increments of 15%) until a final ethanol of 90% was reached. The precipitates were collected and designated hemicellulose subfractions H45, H60, H75, and H90, respectively. The scheme for fractionation of hemicelluloses by graded ethanol precipitation from *P. gansuensis* was illustrated in Figure 1. To reduce errors and confirm the results, each experiment in this study was conducted in duplicate under the same conditions, with 5–10% standard error. All weights and yields were given on a moisture-free basis.

Characterization techniques

The neutral sugars and uronic acids in the seven hemicellulosic subfractions were determined by

high-performance anion-exchange chromatography (HPAEC) using a Dionex ICS3000 gradient pump, amperometric detector, AS50 autosampler and a Carbopac^{TM} PA-20 column (4 × 250 mm, Dionex). The neutral sugars were liberated by hydrolysis with 10% H_2SO_4 for 2.5 h at 105°C. After hydrolysis, the sample was diluted 50-fold, filtered, and separated in a 5 m*M* NaOH isocratic eluent (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. Then the columns were washed with 200 m*M* NaOH to remove carbonate for 10 min, and followed a 5 min elution with 5 m*M* NaOH to re-equilibrate the column before the next injection. The total analysis time was 50 min, and the flow rate was 0.4 mL min^{-1}. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-glucose, D-mannose, D-galactose, glucuronic acid, and galacturonic acids.[21] FTIR spectra were recorded on a Tensor 27 FTIR spectrophotometer in the range 4000–400 cm^{-1}. Pellets were prepared from mixture of finely samples and KBr in 1 : 100 (w/w) ratio. The spectra were obtained by subtracting the spectrum of KBr. The solution-state ^1H NMR spectra were recorded on a Bruker AVIII NMR spectrometer at 400 MHz using 20 mg hemicelluloses in 1.0-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 ^1H NMR spectra. The acquired time (AQ) is 4 s and the relaxation time is 1 s. ^{13}C NMR spectra were obtained on a Bruker spectrometer at 100 MHz. The sample (80 mg) was dissolved in 1.0-mL of D_2O (99.8%D) overnight at room temperature. The ^{13}C NMR spectra were recorded at 25°C after 30,000 scans. A 30° pulse flipping angle, a 9.2 μs pulse width, and a 4-s delay time between scans were used. The proton-detected heteronuclear single quantum (HSQC) spectra were acquired by HSQCETGP experiment mode, over a *t*1 spectral width of 10,000 Hz and a *t*2 width of 1800 Hz and the AQ is 0.13 s. The scanning time (NS) is 64. The delay between transients was 1.5 s and the delay for polarization transfer corresponds to an estimated average ^1H–^{13}C coupling constant of 145 Hz. Data processing was performed using standard Bruker Topspin-NMR software. GPC and thermal stability of the seven hemicellulosic subfractions were determined as described previously.[21]

RESULTS AND DISCUSSION

Yield and neutral sugar composition of hemicellulosic subfractions

The ideal experimental procedures for isolation of hemicelluloses are to sufficiently extract a major class of molecules and also limit the degradation during the extraction process. The *P. gansuensis* hemicelluloses investigated here were isolated by potassium hydroxide extraction of chlorite-delignified materials since the literature reported that delignification was performed not only to remove the lignin but also to thereby render the hemicelluloses more accessible to extraction.[22] The yields and neutral sugar composition of each subfraction are listed in Table I. From a quantitative point of view, the hemicellulose H_A and the precipitates collected at 15, 30, 45, 60, 75, and 90% ethanol yielded 4.0, 2.1, 10.4, 10.3, 0.4, 0.5, and 1.3% of dry matter of the initial amount of dewaxed material. This represented 11.5, 6.0, 29.8, 29.6, 1.1, 1.4, and 3.7% of the overall hemicelluloses, respectively. Clearly, considerable amounts of hemicelluloses were obtained at the ethanol concentration of 30 and 45%. In contrast, the lower yields were detected with the higher ethanol concentrations. This phenomenon is probably due to the multiple steps involved in the precipitation. However, these minor subfractions were still required for providing information to establish the whole structure of *P. gansuensis* hemicelluloses. The major subfractions H30 and H45 contained as much as 83.6 and 74.3% of xylose, and were also rich in uronic acids. Meanwhile, small amounts of other monosaccharides were also identified at these two hemicellulosic preparations. Hemicellulosic subfractions H_A and H15 were also rich in xylose and uronic acids, along with minor contributions from arabinose, rhamnose, galatose, and glucose. From these data the hemicellulosic subfractions H_A, H15, H30, and H45 were implied a structural model of glucuronoxylan. Apparently, the content of the xylose was comparatively much higher in the lower ethanol concentrations than in the higher ones. In the hemicellulosic subfractions H60, H75, and H90, the content of xylose was detected ranging from 21.4 to 43.7%, while a relatively high content of other monosaccharides were noted. More precisely, fractions H60 and H75 contained a large proportion of galactose, while a substantial amount (23.2%) of glucose was present in H90. Besides, mannose was not detected in the lower ethanol concentrations, whereas enriched in the hemicellulosic subfractions H75 and H90 (15.9 and 26.1%, respectively). On the basis of the composition of the subfractions, it can be concluded that the hemicellulosic subfractions H60, H75, and H90 were highly branched and may contain complex structures. This also indicated that more branched hemicelluloses appeared to be more soluble in aqueous solution, which required a much higher ethanol concentration to precipitate from the solution.

The results of the present fractionation scheme also showed that *P. gansuensis* hemicelluloses comprise populations of polysaccharides differing in the weight ratio of uronic acids to xylose (UA/Xyl). The

TABLE I
Yield (of Dry Matter of the Initial Amount of Dewaxed Material) and Sugar Composition of Hemeicellulosic Subfractions

Fraction[a]	Yield[b]	Molar composition (%)							
		Rha	Ara	Gal	Glc	Man	Xyl	UA	UA/Xyl
H_A	4.0	1.7	3.8	1.5	0.4	ND	81.4	11.2	0.14
H15	2.1	1.2	1.3	1.4	1.5	ND	73.7	20.9	0.28
H30	10.4	0.9	0.5	0.4	0.4	ND	83.6	14.3	0.17
H45	10.3	1.8	1.9	1.7	0.4	ND	74.3	19.8	0.27
H60	0.4	3.1	8.7	18.8	8.1	ND	43.7	14.2	0.32
H75	0.5	2.4	12.3	12.9	12.2	15.9	30.4	13.9	0.46
H90	1.3	2.1	7.9	6.4	23.2	26.1	21.4	12.8	0.60

Rha, rhamnose; Ara, arabinose; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose; UA, uronic acids; UA/Xyl, weight ratio of uronic acids to xylose; ND, not detected.

[a] H_A was precipitated from the KOH supernatant by acidifying to pH 5.5 with 6*M* acetic acid; H15, H30, H45, H60, H75, and H90 represent the hemicellulosic subfractions obtained by precipitation in 15%, 30%, 45%, 60%, 75%, and 90% ethanol, respectively.

[b] Dry matter of initial amount of dewaxed material.

UA/Xyl ratio increased from 1 : 7.3 to 1 : 1.3 with the increasing ethanol concentration from 0 to 90%. It could be concluded that with the increasing ethanol concentration, more branching and complex fractions would be obtained. The most significant discovery in the sugar analysis of these hemicellulosic subfractions was that the virtually pure glucuronoxylan could be achieved at the lower ethanol concentration, while with the increasing ethanol concentration, some side chains and hemicelluloses with more complex structure would be obtained.

Molecular size distribution

The weight and number average molecular weights (M_w and M_n) together with the polydispersity indices (M_w/M_n) values for the seven hemicellulosic polymers precipitated are documented in Table II. The M_w of the seven subfractions exhibited varying between 15,640 and 78,320 g mol^{-1}. To be more specific, an increase in ethanol concentration from 15% to 30% resulted in growth of M_w from 61,860 to 78,320 g mol^{-1}, while a further increasing ethanol concentration from 45 to 90% led to a decrease in M_w from 60,890 to 15,640 g mol^{-1}. This indicated that the hemicelluloses of higher molecular weights were precipitated at lower ethanol concentrations, while with increasing ethanol concentration, the average molecular weights of the precipitated hemicellulosic subfractions decreased. From this observation and the noticed UA/Xyl ratio, it can be concluded that the hemicellulosic subfractions with higher molecular weights and lower UA/Xyl ratios may have a linear but longer chain and preferentially precipitated in lower ethanol concentrations. Figure 2 depicts the molar mass distribution curves obtained from these hemicellulosic subfractions. As can be seen, the molar mass distribution curves for the hemicelluloses obtained from various ethanol concentrations were quite dissimilar. H_A and hemicellulosic subfractions obtained in the lower ethanol percentage clearly exhibited a higher molar mass than the hemicelluloses precipitated in the higher ethanol percentage. Apparently, the distribution curves of H75 and H90 shift towards lower mass relative to the curves of the hemicellulosic subfractions H15, H30, and H45. This is in accordance with the M_w values in Table II. The narrow peaks of all curves in this figure also revealed that the polydispersity values of all of the hemicelluloses analyzed were low (i.e., M_w/M_n values of approximately 1.11–1.56), especially of H75 and H90.

FTIR spectroscopy

FTIR spectroscopy has been proven to be a useful tool for the study of the physicochemical and

TABLE II
Weigt-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Hemicellulosic Subfractions

	Hemicellulosic subfraction[a]						
	H_A	H15	H30	H45	H60	H75	H90
M_w	58,100	61,860	78,320	60,890	47,760	23,040	15,640
M_n	40,620	62,850	57,840	40,560	30,644	18,570	14,120
M_w/M_n	1.43	2.12	1.35	1.50	1.56	1.24	1.11

[a] Corresponding to the hemicellulosic subfractions in Table I.

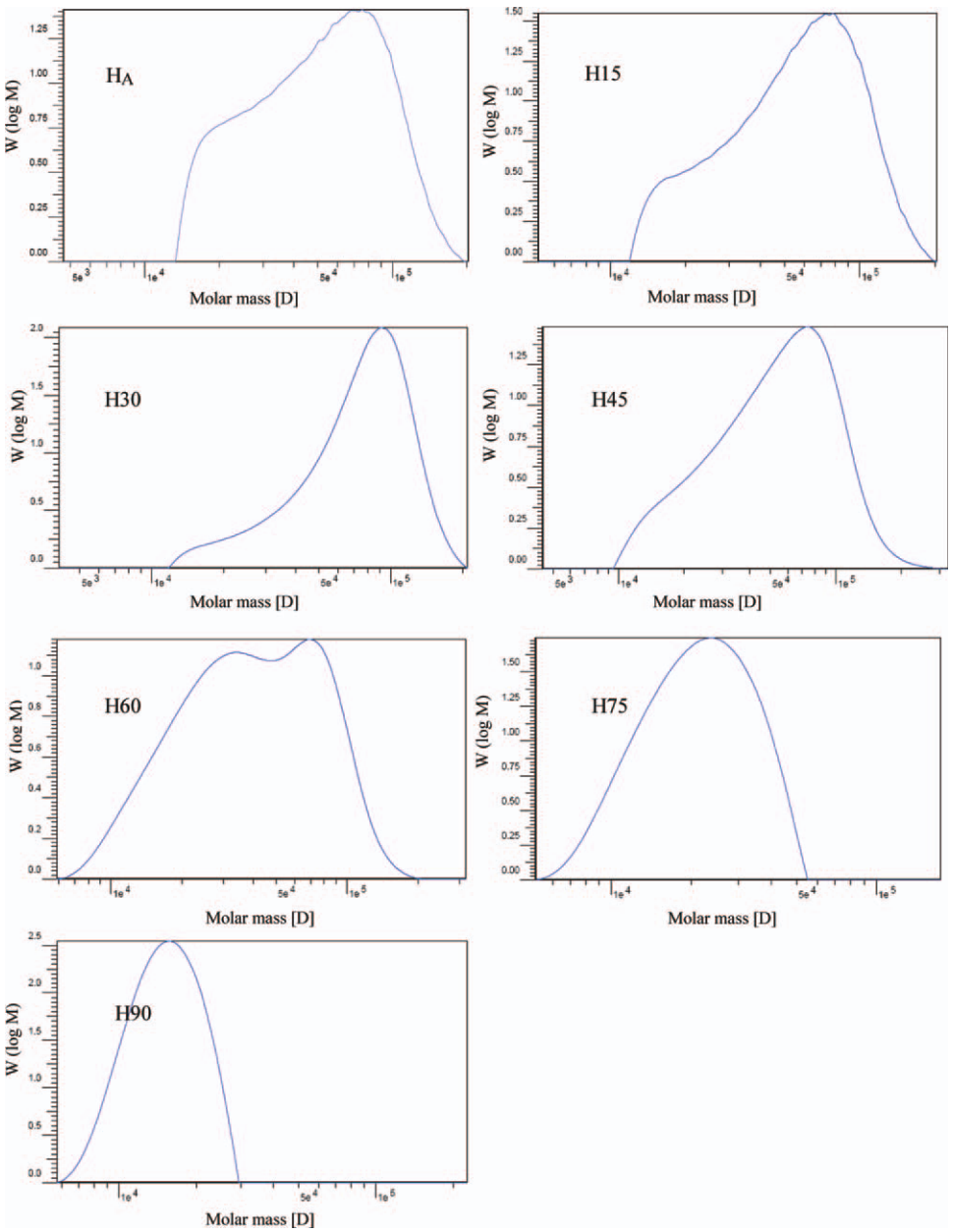

Figure 2 Molecular weight distributions of seven hemicellulosic subfractions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

conformational properties of polysaccharides since clear differences in the infrared spectra related to the intensity, shape, and location of some bands could be detected.[23] Figures 3 and 4 show the FTIR spectra of all the hemicellulosic subfractions obtained by precipitation in aqueous acetic acid at pH 5.5 and various ethanol concentrations. The tentative assignment of the bands is based on the reported data.[24–26] It may be noted that there is an apparent intensity decrease at the band 1047 cm^{-1} from Figure 3 to Figure 4, which is assigned to the β-glycosidic linkage C—O—C contributions in xylans. This was consistent with the results obtained by sugar analysis, in which the relative content of xylose decreased from 83.6 to 21.4%. The presence of the salt form of the uronic acid is manifested mainly by the appearance of the asymmetric stretching mode at 1575 cm^{-1} and symmetric stretching vibration at 1410 cm^{-1}.[24] The strong intensities of these two bands allow some conclusion concerning the orientation of the 4-*O*-methyl glucuronic acid group. Considering the fact that the characteristic "anomeric region" absorption bands for α-linkage (834 cm^{-1}) and β-linkage (898 cm^{-1}) distinguish well between the two glycosidic linkage types of the aldopyranoses, the overall small band at 900 (890) cm^{-1} is due to the β-glycosidic linkages between xylose units in hemicelluloses.[25] The frequency at 1176 cm^{-1} in spectrum 7 is attributed to C—O—C stretching, which

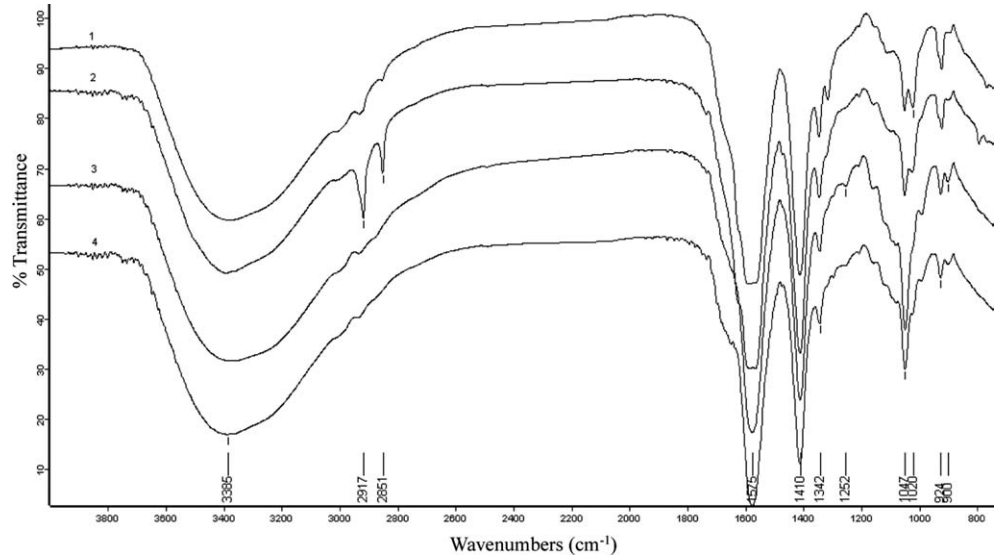

Figure 3 FTIR spectra of the hemicellulosic subfractions H_A (spectrum 1), H15 (spectrum 2), H30 (spectrum 3), and H45 (spectrum 4).

has been reported to mannose by Kačuráková et al.[26] However, this band may result from other glycosyl or the contribution of two or even more kinds of motions by the fact that hemicelluloses present a complex structure. In the higher frequency region, the observed band at 2917 and 2853 cm^{-1} in H15 (spectrum 2) are assigned to the vibrations associated with the CH group. Evidently, the strong broad peak at 3385 (3367) cm^{-1} in the spectra is the characteristic of OH stretching vibration.

1D and 2D NMR spectra

1D (^1H and ^{13}C) NMR spectra of the hemicellulosic subfractions H30 and H75 (Figures 5 and 6) were collected in order to elucidate the structural features. The main spectral patterns of these two hemicellulosic subfractions are the same. Examination of their proton spectra showed three anomeric protons at δ 4.42 (4.44), 4.57 (4.50), and 5.23 (5.25), which were assigned as non-substituted backbone of D-xylose units, D-xylose units substituted with 4-*O*-methyl-D-GlcA and 4-*O*-methyl-D-GlcA residues, respectively. The relevant signals occurred in two regions, namely, the anomeric region (δ 5.6–4.9 for α-anomers and δ 4.9–4.3 for β-anomers) and the ring proton region (4.5–3.0).[27] This supported that the xylose residues of both substituted and nonsubstitued were shown to be linked via β-glycosidic bonds, which agreed with the presence of IR band at 900 cm^{-1}.

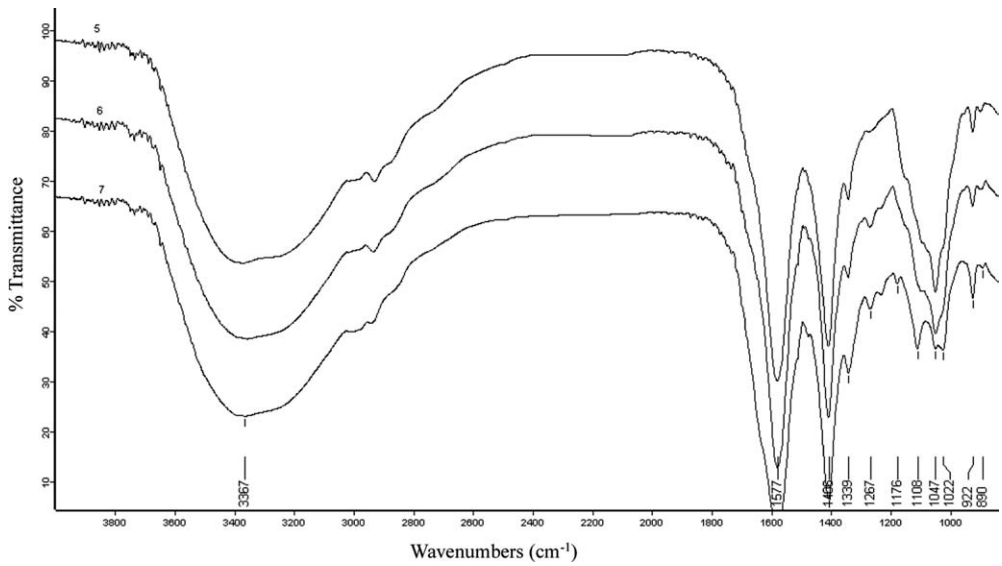

Figure 4 FTIR spectra of the hemicellulosic subfractions H60 (spectrum 5), H75 (spectrum 6), and H90 (spectrum 7).

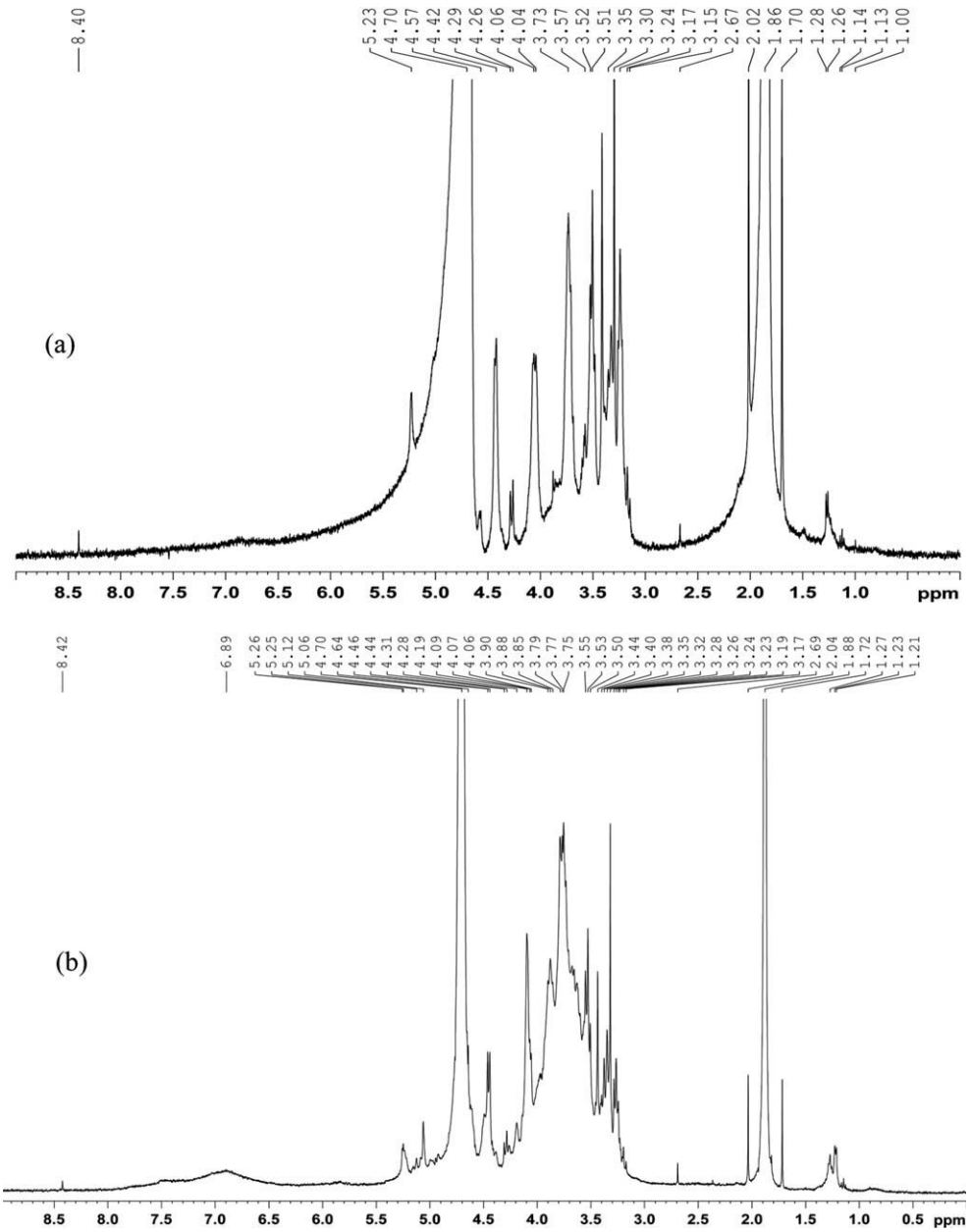

Figure 5 ^{1}H NMR spectra (400 MHz, D_2O) of hemicellulosic subfractions H30 (spectrum a) and H75 (spectrum b).

By using $^{13}C/^{1}H$ shift correlated 2D experiment, the proton spectra were completely assigned from the ^{13}C NMR spectra (chemical shifts reported in Table III). The contour plot of the HSQC spectrum of H30, displayed in Figure 7(a), shows the relative simplicity of the structure. The ^{13}C NMR spectrum gives five major signals at 101.74 (C-1), 72.78 (C-2), 73.72 (C-3), 76.40 (C-4), 63.20 (C-5ax), and 63.03 (C-5eq) ppm, corresponding to (1→4)-linked-β-xylan.[28] The signal at 101.74 ppm corresponding to the anomeric region is a β-configuration, as confirmed by the ^{1}H NMR spectrum.[29] Signals at δ 97.60 (C-1), 71.34 (C-2), 72.23 (C-3), 82.47 (C-4), 72.20 (C-5ax), 176.80 (C-6), and 59.83 (-OCH_3) indicate the presence of α linked 4-*O*-MeGlcA residues.[30] Other less intense signals at δ 101.54 (C-1), 76.82 (C-2), 73.37 (C-3), 76.20 (C-4), 63.20 (C-5ax), and 63.04 (C-5eq) ppm are characteristic of β-D-xylose units substituted in position O-2 with 4-*O*-methyl-α-D-GlcA.[31] Evidently, for substituted xylose units, the C-2 signal downfield shifted to 76.82 ppm which demonstrated O-2 substitution with 4-*O*-Me-D-GlcA. Moreover, the molar proportion of D-Xyl and MeGlcA residue was determined by integration of the corresponding anomeric plot of the HSQC spectra, and was found to be 5.4 : 1 for hemicellulosic subfraction H30. This data obtained by NMR, is in accordance with the classical sugar analysis. For every six D-xylopyranosyl residues in the main chain, there is one uronic acid

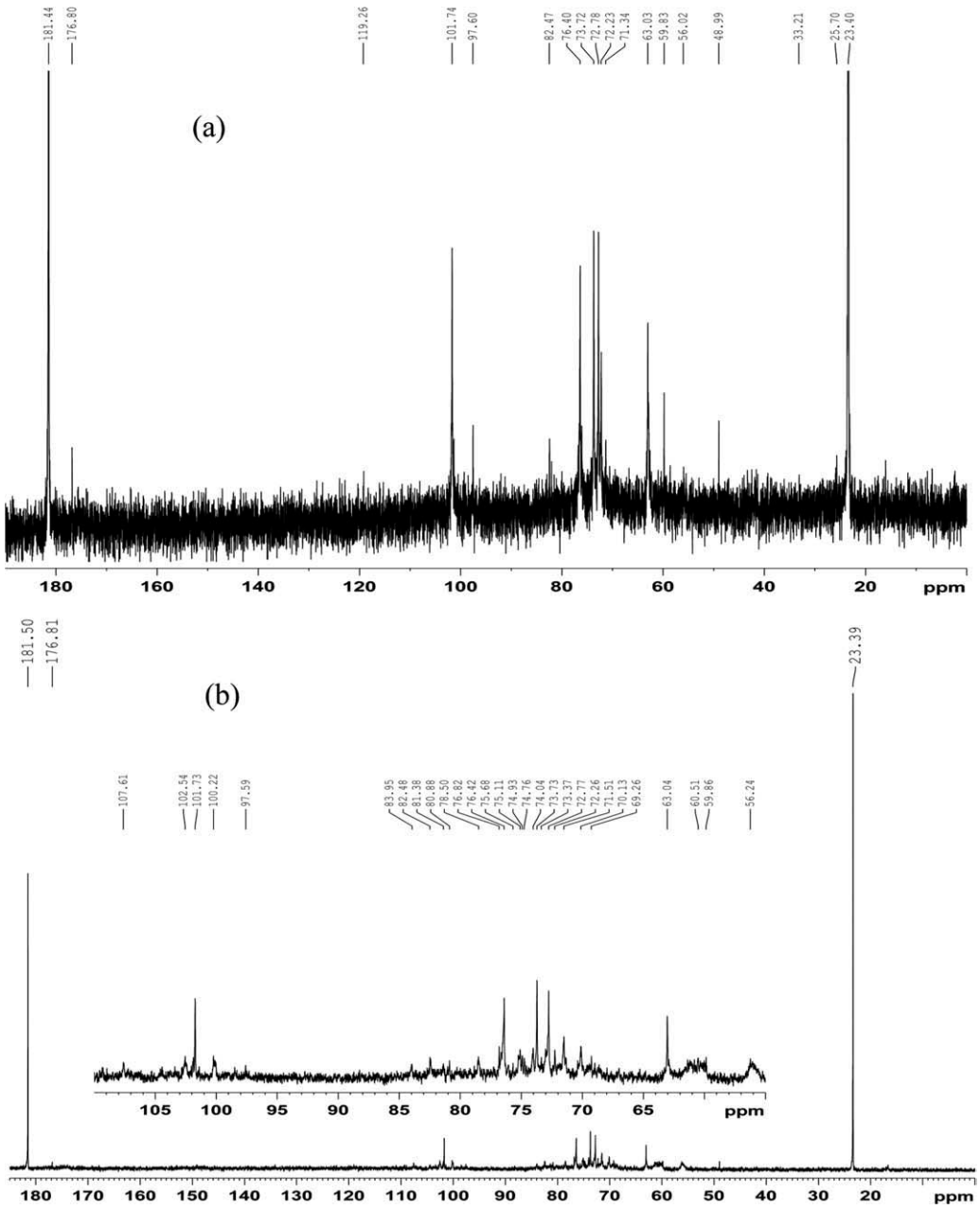

Figure 6 ^{13}C NMR spectra (100 MHz, D_2O) of hemicellulosic subfractions H30 (spectrum a) and H75 (spectrum b).

group. The value examined here is typical of hardwood hemicelluloses.[13]

The HSQC spectrum of H75 presents the predominant structure of 4-*O*-methyl glucuronxylan, the same general feature as already described in H30. The ^{13}C NMR spectrum indeed dominated by signals of D-xylose units, D-xylose units substituted with 4-*O*-methyl-D-GlcA and 4-*O*-methyl-D-GlcA residues. However, ^{13}C NMR spectrum of the H75 [Fig. 7(b)] appeared much more complex than those of the H30, not only differing in the intensity of the signals but also due to the presence of some extra signals. The presence of minor peaks at δ 100.22 (C-1), 70.10 (C-2), and 74.06 (C-3), corresponded to mannaose residues. The anomeric signal at δ 104.10 correlated with proton resonance at 4.60 ppm, may indicate the presence of glucose. The $^{13}C/^{1}H$ cross peaks at δ 73.20/3.34, 74.94/3.53, and 78.34/3.65 can be, respectively, assigned to C-2, C-3, and C-4 of glucose. The C-2 of galactose could be distinguished based on the signals at δ 68.75/3.88. All these data were assigned in accordance with recent literature data for galactoglucomannan models.[32,33] The signal at δ 107.61 is from the anomeric carbon of arabinose, and the signals at 82.20 and 76.90 ppm arise from C-2 and C-3, respectively.[34] The low-field chemical shift at 107.61 ppm indicated that branched arabinose adopted α configuration and furanose form.[35] In

TABLE III
^{1}H and ^{13}C Chemical Shift (ppm) Assignments for Hemicellulosic Subfractions H30 and H75

Fraction	Residue	Chemical shift (ppm)							
		1	2	3	4	5ax	5eq	6	O—CH_3
H30					X[a]				
	^{1}H	4.42	3.24	3.51	3.73	3.30	4.04		
	^{13}C	101.74	72.78	73.72	76.40	63.20	63.03		
					XU[b]				
	^{1}H	4.57	3.42	3.57	3.79	3.35	4.06		
	^{13}C	101.54	76.82	73.37	76.20	63.20	63.04		
					U[c]				
	^{1}H	5.23	3.52	3.70	3.17	4.26			
	^{13}C	97.60	71.34	72.23	82.47	72.20		176.80	59.83
H75					X[a]				
	^{1}H	4.44	3.28	3.53	3.75	3.30	4.06		
	^{13}C	101.73	72.77	73.73	76.82	63.04	63.03		
					XU[b]				
	^{1}H	4.50	3.41	3.60	3.82	3.40	4.08		
	^{13}C	101.70	76.30	73.47	76.18	63.04	63.04		
					U[c]				
	^{1}H	5.25	3.52	3.71	3.23	4.28			
	^{13}C	97.59	71.51	72.26	82.48	72.26		176.81	59.86
					Ara				
	^{1}H	5.06	4.18	3.93		3.80			
	^{13}C	107.61	82.20	76.90		61.80			
					Gal				
	^{1}H		3.88					3.80	
	^{13}C		68.75					61.02	
					Man				
	^{1}H	4.72	4.09	3.64					
	^{13}C	100.22	70.10	74.06				61.60	
					Glc				
	^{1}H	4.60	3.34	3.53	3.65				
	^{13}C	104.10	73.20	74.94	78.34			61.30	

[a] (1→4)-β-D-Xyl*p*.
[b] (1→4)-β-D-Xyl*p*-2-*O*-(4-*O*-Me-α-D-Glc*p*A).
[c] 4-*O*-Me-α-D-Glc*p*A.
ax = axial, eq = equatorial

addition, a broad cross peak encompassing the 3.61–4.10 ppm in the proton dimension and ~ 61.5 ppm in the carbon domain may attribute to the coupling signals of glucose, mannose, galactose, and arabinose residues.[35] It is to be noted that the chemical shift assignment in the present study were analogous to the partial data reported for specific residues.

On the basis of the NMR analysis, H30 was in agreement with those obtained on acidic polysaccharides related to 4-*O*-methyl-α-D-glucuronoxylans. In the case of the hemicellulose H75, due to the presence of characteristic signals of arabinose, it demonstrated that H75 is assumed to be arabinoglucurnoxylan. Besides, small amounts of galactoglucomannan could also be presented in this fraction.

Thermal analysis

The structure–property of the three components of biomass was found to influence the pyrolysis characteristic greatly.[36] To better understand the physicochemical properties of the precipitated hemicelluloses, the hemicellulosic subfractions H30 and H75 are investigated using the techniques of thermogravimetric analysis (TGA) and differential thermal analysis (DTA) between room temperature and 600°C. As illustrated in the figure, the TGA curves of the pyrolysis process exhibit three weight loss phases generally agreed upon: an early dehydration stage (<150°C), followed by fragmentation through random scission of the macromolecular structure, accompanied by the release of volatile components (150–250°C), and finally degradation of the carbohydrate residue, the evolution of gaseous products, their combustion and/or the glowing ignition of a char residue.[37] The pyrolysis of hemicellulose H30 started at 205°C, while H75 started its decomposition at ~ 170°C. At 30% weight loss, the decomposition temperature of the hemicelluloses samples occurred at 437°C for H30 and 295°C for H75. The higher activity of H75 in thermal decomposition might be attributed to its chemical structure and molecular weights. Hemicellulosic subfraction H75 with a low molecular weight was rich in branches (arabinose,

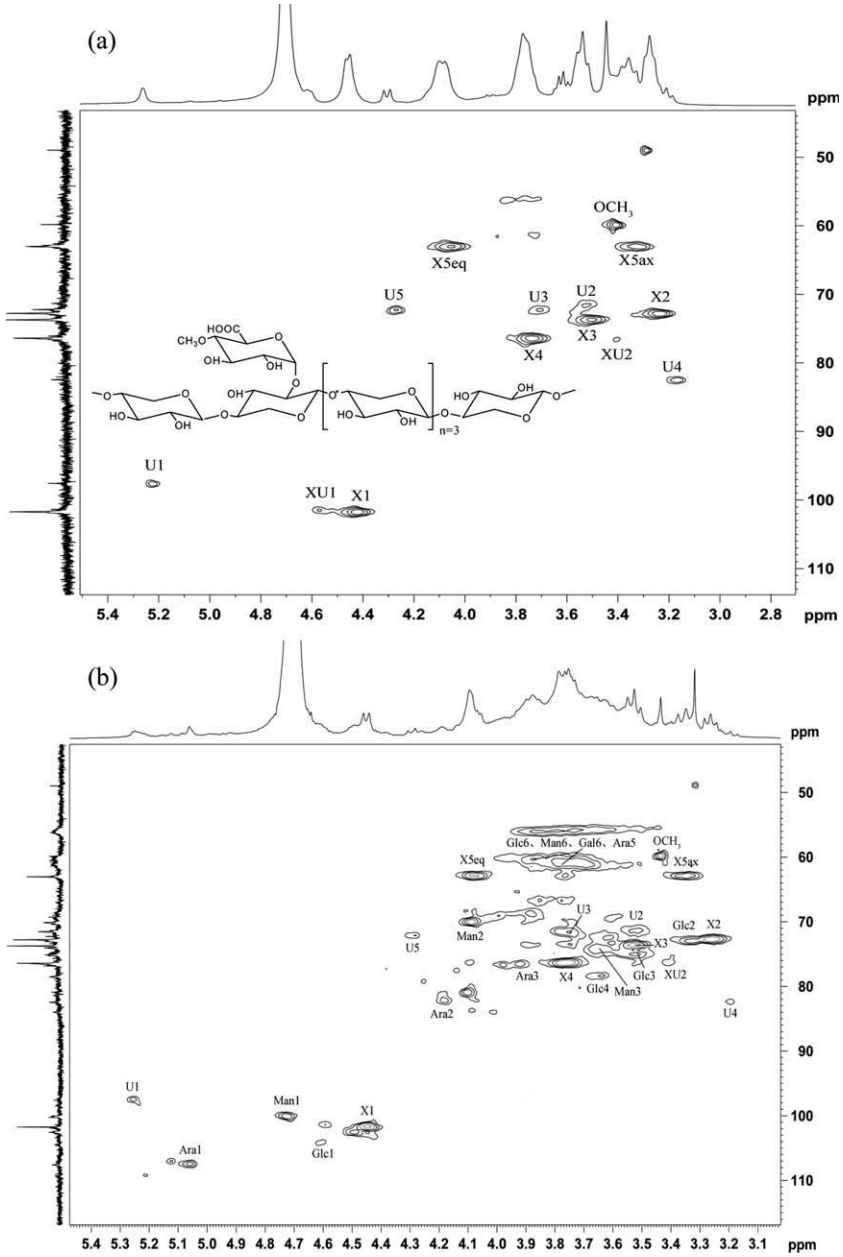

Figure 7 $^{1}H/^{13}C$ NMR (HSQC) spectra of hemicellulosic subfraction H30 (spectrum a) and H75 (spectrum b).

galatose, etc.), which were very easy to be removed from the main stem and to be degraded to volatiles evolving out (CO, CO_2, and some hydrocarbon, etc.) at low temperatures. In addition, the amounts of solid residue left were high even at 600°C for both fractions. This is probably due to the salts formed during the extraction processes.[38] Another reason for this higher content of residues at 600°C is also probably due to the higher thermal stability of these hemicellulosic subfractions, which require much higher temperatures for decomposition. A similar conclusion was reached by Yang et al., who found that hemicellulose component was still ~ 20% solid residue left even at 900°C.[39]

Figure 8 also illustrates DTA tracing for hemicellulose H30 indicating a single broad endotherms at about 100°C and a series of overlapping exotherms, while hemicellulose H75 shows two exothermic effects. The 100°C endothermic nadir is mainly attributed to the evaporation of absorbed water which could not be removed completely on drying. It is reported that in the high temperature range, the overlapping processes of depolymerization, thermooxidation, dehydration, and combustion of the released gaseous products indicated a net small weight loss change. Besides, the high net exothermic effect was probably due to the high combustion heat relative to the low heat needed for degradation of the

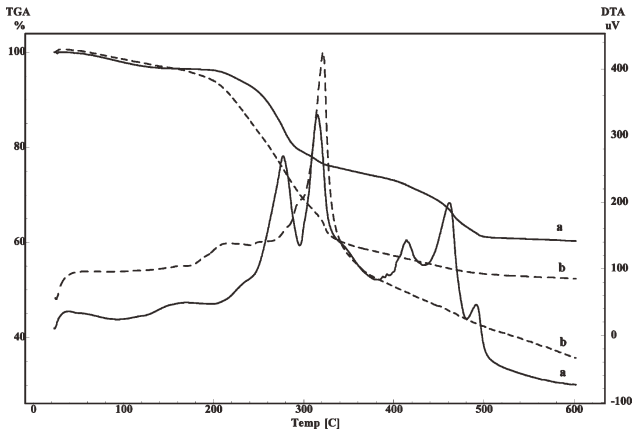

Figure 8 Thermogram of hemicellulosic subfractions H30 (a) and H80 (b).

macromolecular structure and/or fragmentation and dehydration.[37] However, this difference between hemicelluloses must await further investigation. As a result of the above, the hemicellulosic subfraction H30 had a higher thermal stability than that of the H75.

CONCLUSIONS

On the basis of the above results, the following conclusions were made: The larger molecular weights and more linear *P. gansuensis* hemicelluloses with higher yields were precipitated in 30% and 45% ethanol, which were rich in xylose (74.3% and 83.6%), principally resulting from 4-*O*-methyl-D-glucurono-D-xylans. When the ethanol concentration increased, small amounts of more complex hemicelluloses with lower molecular weights were obtained. Hemicellulosic subfraction H75 could be structurally defined as heterogeneous mixture of arabinoglucurnoxylan and galactoglucomannan. Moreover, the thermal analysis suggested that hemicellulose H30 had a higher thermal stability than H75. It is hoped that the graded ethanol precipitation will be a useful guideline in obtaining deeper insight in the structure of hemicelluloses as well as toward isolating desirable purity of xylan, which could be utilized for food or nonfood applications.

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