ISOLATION AND FRACTIONATION OF HEMICELLULOSES FROM *SALIX PSAMMOPHILA*

F. PENG [*,**], N. JIA,[*] J. BIAN,[*] P. PENG,[**] R. C. SUN[*,**] and S. J. LIU[**,***]

[*]*Institute of Biomass Chemistry and Technology, Beijing Forestry University, Beijing 100083, China*
[**]*State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China*
[***]*Department of Paper Bioprocess Engineering, SUNY College of Environmental Science and Forestry, Forestry Drive, Syracuse, NY 13210, USA*

Received November 21, 2011

Water- and alkali-soluble hemicelluloses were isolated with hot water and 10% KOH at 25 °C from dewaxed and delignified *Salix psammophila*, respectively. The alkali-soluble hemicelluloses were then successively subfractionated by neutralization and subjected to gradual precipitation in the end, using ethanol concentrations of 15, 30, 45, 60, 75 and 90%, respectively. Chemical composition, physico-chemical properties and structures of the 8 precipitated hemicellulosic fractions obtained were established by sugar analysis, molecular weight determination, FT-IR, ^{1}H, ^{13}C and 2D HSQC NMR. It was found out that the water-soluble hemicelluloses were more branched, having a low molecular weight (6060 g mol^{-1}), whereas the alkali-soluble hemicelluloses were less branched, having higher molecular weights (17110-85540 g mol^{-1}), with xylose and uronic acid as the main sugar components. In addition, the less branched hemicelluloses with large molecules were precipitated in lower ethanol percentages while, with increasing ethanol concentration, more branched hemicelluloses with low molecular weights were obtained. That is why, linear hemicelluloses could be recovered at lower ethanol concentrations, and more branched hemicelluloses could be obtained at higher ethanol concentrations. According to FT-IR, ^{1}H, ^{13}C and 2D HSQC NMR studies, the alkali-soluble hemicelluloses had a main structure composed of a (1→4)-linked β-D-xylopyranosyl backbone with 4-*O*-methyl-α-D-glucuronic acid attached to the *O*-2 of the xylose residues.

Keywords: *Salix psammophila*, hemicelluloses, alkali, fractionation, xylans

INTRODUCTION

A growing interest is now manifested for exploiting renewable resources for the production of numerous industrial and non-food consumer products, such as fuels, chemicals and biodegradable polymers. The lignocellulosic waste materials from agriculture and forestry appear as promising for replacing the environmentally unfriendly hydrocarbons.[1] *Salix psammophila*, a relatively new potential wood source of fiber – planted in the Northwestern and Northeastern desert region of China to prevent wind erosion and control desertification – has a great economical and ecological importance. This forest activity generates a large amount of residues, because the stems of the plant are cut once every 3 or 4 years to enable its flourishing. Usually, these residues can provide wood or fodder. At present, only small amounts of stubble are used for the production of fiber board, the rest remaining underutilized.[2] Therefore, *S. psammophila* has a great potential, due to its high content of cellulose, hemicelluloses and lignin.

After cellulose, hemicelluloses are the second most abundant polysaccharides in plants. They are non-cellulosic and short-branched chain hetero-polysaccharides consisting of various

sugar units, arranged in different ratios and with different substituents.[3] Hemicelluloses are partially linked to cellulose microfibrils by hydrogen bonds, and also to the lignin, pectins and proteins from the cell wall matrix.[4-6] Hemicelluloses may have wide direct food and non-food applications. First, hemicelluloses can be easily hydrolyzed into pentoses (xylose and arabinose) and hexoses (glucose, galactose and mannose) and transformed into fuel ethanol and other high value-added chemicals, such as 5-hydroxymethylfurfural (HMF), furfural, levulinic acid and xylitol.[7] In addition, hemicelluloses can be also converted into various biopolymers, with wide applications, such as viscosity modifiers, food packaging films, wet strength additives in papermaking and tablet binders.[8] In plant cell walls, there are large amounts of hemicelluloses with different contents and chemical structures. Hemicelluloses generally consist of several populations of polysaccharide molecules with various structural characteristics. Some fractionation techniques have been employed in the attempt of obtaining more homogeneous fractions and exploring the structure–property relationships for hemicellulosic polymers. The present study aimed at fractionating hemicelluloses from the cell walls of *S. psammophila*, at determining comparatively their structural features, for a complete understanding of their chemistry and of their structure–property relationships as hemicellulosic polymers.

EXPERIMENTAL

Materials

The samples of *S. psammophila* were collected in October 2002 from Shalin arboretum, in Yikezhao League of Inner Mongolia, China. The leaves and the bark of *S. psammophila* were removed at harvest time. The trunks were dried in sunlight, and then chipped into about 1-2 cm small pieces. After subsequent drying at 60 °C for 16 h, the powder was dewaxed with 2:1 (v/v) toluene-ethanol in a Soxhlet apparatus for 6 h. The dewaxed sample was further dried in a cabinet oven with air circulation at 60 °C for 16 h and stored. All standard chemicals, such as sugars, purchased from Sigma Chemical Company (Beijing), were of analytical grade.

Isolation and fractionation of hemicelluloses

Hemicellulosic fractions of *S. psammophila* were obtained by sequential extractions and fractionations (Fig. 1). The dewaxed powder (15 g) was treated with distilled water at 80 °C for 2 h under stirring. After filtration, the filtrates were concentrated to about 50 mL, then mixed with three volumes of 95% ethanol, for isolating the water-soluble polysaccharides (Hw). The water-insoluble residue was then delignified with 6% sodium chlorite at pH 3.6-3.8, adjusted with 10% acetic acid, at 75 °C for 2 h. The residue, holocellulose, was subsequently extracted with 10% KOH at 25 °C for 16 h. The filtrate was neutralized with 6.0 M acetic acid to pH 5.5, and concentrated to 150 mL. After standing for 12 h, the solution was centrifuged at 3500 rpm for 15 min. The precipitate (H_A) obtained was washed with 70% ethanol and freeze-dried. The supernatant was further subfractionated into six subfractions by gradual ethanol precipitation in the end, at ethanol concentrations of 15, 30, 45, 60, 75 and 90%, labelled as H_1, H_2, H_3, H_4, H_5, and H_6, respectively.

Chemical characterization

The constituent neutral sugar in the isolated hemicellulosic fractions was determined by high performance anion exchange chromatography (HPAEC). The hemicelluloses (4-6 mg) were hydrolyzed by 10% H_2SO_4 at 105 °C, in a sealed tube, for 2.5 h. After hydrolysis, the samples were diluted 30 times, filtered and injected into the HPAEC system (Dionex ISC 3000, U.S.) with an amperometric detector, an AS50 autosampler and a CarbopacTM PA1 column (4×250 mm, Dionex).[9] The molecular weights of the hemicellulosic fractions were determined by gel permeation chromatography (GPC), on a PL aquagel-OH 50 column (300×7.7 mm, Polymer Laboratories Ltd), calibrated with PL pullulan polysaccharide standards (peak average molecular weights of 783, 12200, 100000, 1600000, Polymer Laboratories Ltd).[9]

Spectroscopic characterization

FT-IR spectra of the hemicellulosic samples were obtained on an FT-IR spectrophotometer (Tensor 27), using a KBr disc containing 1% finely ground samples. Solution-state ^{1}H-NMR spectra were recorded on a Bruker NMR spectrometer at 400 MHz, using 15 mg of hemicelluloses in 1.0 mL of D_2O. The chemical shifts reported were calibrated relatively to the signals from D_2O, used as an internal standard, at 4.7 ppm for the ^{1}H NMR spectra. The ^{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), and left overnight at room temperature. The ^{13}C-NMR spectra were recorded at 25 °C after 30000 scans. Chemical shifts (δ) are expressed

relatively to the resonance of Me_4Si ($\delta = 0$). A 30° pulse flipping angle, a 3.9 μs pulse width and a 0.85 s delay time between scans were used. The proton-detected heteronuclear single quantum (HSQC) spectra were obtained in the HSQCGE experiment mode, over a t_1 spectral width of 10000 Hz and a t_2 width of 1800 Hz, at an acquired time (AQ) of 0.1163 s. The scanning time (NS) is 32. The delay between transients was of 2.6 s and the delay for the polarization transfer was set to correspond to an estimated average $^1H–^{13}C$ coupling constant of 150 Hz. Data processing was performed using standard Bruker Topspin-NMR software.

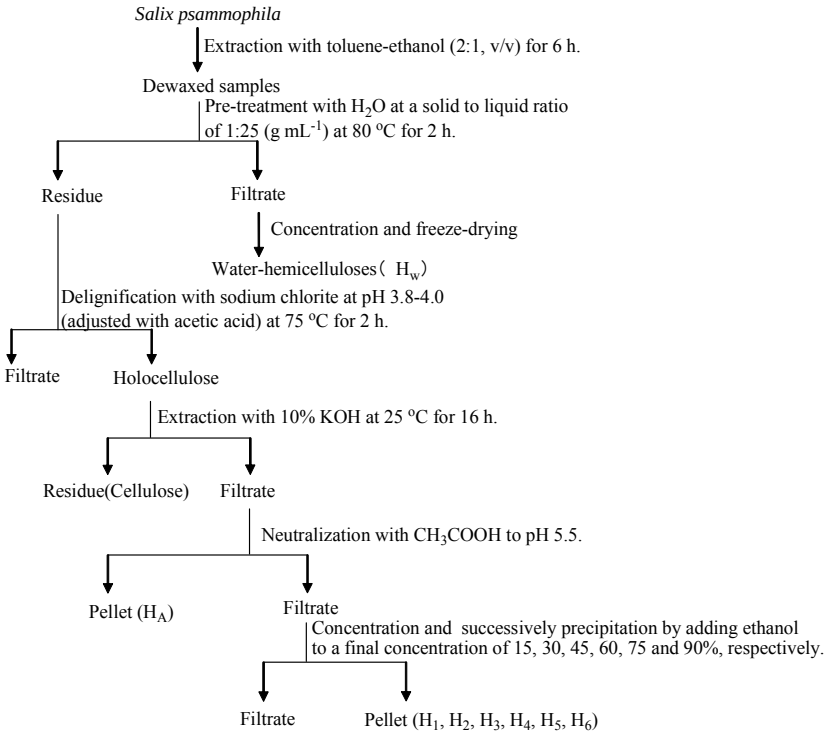

Figure 1: Scheme for fractionation of hemicelluloses from *S. psammophila*

RESULTS AND DISCUSSION

Yield and sugar analysis

Hemicelluloses are usually associated with various other cell-wall components, such as cellulose, cell-wall proteins, lignin and other phenolic compounds, through covalent and hydrogen bonds, as well as through ionic and hydrophobic interactions.[10] Hydroxyl ions cause swelling of cellulose, disruption of hydrogen bonds between cellulose and hemicelluloses, resulting in the solubilization of substantial amounts of hemicelluloses from holocellulose. The isolation procedure yielded 3.6 and 34.3% hemicelluloses (% dry materials) by water and alkali treatment, respectively. The alkali-soluble hemicelluloses were subfractionated into 7 samples (H_A, H_1, H_2, H_3, H_4, H_5 and H_6) by neutralization and gradual ethanol precipitation, the yields of the 7 subfractions being of 15.4, 1.1, 7.6, 1.9, 1.0, 2.1 and 0.4% dry matter, respectively. Evidently, the major hemicellulosic subfractions were obtained by neutralization and 30% ethanol precipitation. Taken together, the total yield of the 7 hemicellulosic subfractions was of 29.42%, which accounts for 85.7% of the total alkali-soluble hemicelluloses. This indicates that the 14.3% hemicelluloses may be mainly degraded into small substances, such as oligosaccharides and monosaccharides, which were not recovered.

The monosaccharide composition of hemicelluloses is listed in Table 1. As one can see, the water-soluble hemicelluloses contained significant amounts of glucose (40.8%), arabinose (16.5%) and glucuronic acid (10.9%), together with a small amount of galactose (7.4%), xylose

(8.9%), rhamnose (8.7%) and mannose (7.1%), indicating that the hot water treatment probably released both high branched galactoarabinoxylans, β-glucans and pectic polysaccharides. Evidently, xylose and glucuronic acid are the dominating monomeric sugar comoponents in the seven hemicellulosic subfractions of alkali-soluble hemicelluloses, representing 29.9-85.2% and 7.1-20.8% of the total sugars, respectively. This suggests that the hemicelluloses of *S. psammophila* mainly consisted of glucuronoxylans. In addition, as compared to the 6 hemicellulosic subfractions obtained by gradual ethanol precipitation, the H_A obtained by precipitation with acetic acid at a pH of 5.5 had a higher content of xylose (85.2%) and a relatively lower glucuronic acid content (9.5%) and glucuronic acid/xylose (GlcA/Xyl) ratio (0.14), which indicates that H_A consisted of a very low substituted population, since the GlcA/Xyl ratio is indicative of the degree of linearity or branching of hardwood hemicelluloses. From H_1 to H_4, with increasing ethanol concentration from 15 to 60%, the ratio of GlcA/Xyl increased from 0.14 to 0.38. Compared with H_1, H_2, H_3 and H_4, a significant difference was found in H_5 and H_6, obtained by 75 and 90% ethanol precipitation, in which a relatively higher amount of galactose, glucose and arabinose, and a relatively lower amount of xylose were observed. Moreover, mannose (12.6%) was detected in H_6. Generally, most of the xylose units originated from the backbone of xylan, while arabinose, galactose, glucose and uronic acid mainly resulted from the side chains. Hence, sugar analysis implied that H_5 and H_6 were highly substituted xylans, an aspect still to be investigated since, based on the sugar composition alone, it is difficult to draw conclusions on the structural patterns of hemicelluloses. Similar results previoulsy obtained for *Caragana korshinskii*[10] suggested that, with the increment of ethanol concentration, more branched hemicelluloses were obtained. The present study also indicates that the hemicelluloses containing a high degree of side-chain substitution are more water-soluble, while the less branched hemicelluloses, for example, H_A, had low solubility in water.

Molecular weight

The weight-average (M_w) and number-average (M_n) molecular weights were determined by gel permeation chromatography (GPC). As shown in Table 2, water-soluble hemicelluloses showed a lower degree of polymerization (6060 g mol^{-1}) than those of the other 7 alkali-soluble hemicellulosic subfractions (M_w = 17110-80550 g mol^{-1}). For the alkali-soluble hemicelluloses obtained by ethanol precipitation, an increase in precipitated ethanol concentration from 15 to 30% resulted in a M_w growth from 55970 to 85540 g mol^{-1}. However, when ethanol concentrations were increased from 45 to 90%, M_w decreased from 80550 to 17110 g mol^{-1}. In this case, the results indicated that hemicellulosic subfractions with higher molecular weights could be obtained at ethanol concentrations between 15 and 75%. In addition, the hemicellulosic subfraction (H_3) obtained by 45% ethanol precipitation had a relatively higher polydispersity index (3.3), while the other fractions gave relatively lower polydispersity indices (1.2-2.1), which indicated that the hemicellulosic subfraction with chemical and structural homogeneity could be obtained by gradual ethanol precipitation.

Table 1
Neutral sugars and glucuronic acid (relative percent of hemicellulosic sample, w/w) and GlcA/Xyl ratios of hemicellulosic subfractions

Sugar (%)	Hemicellulosic subfractions							
	H_w	H_A	H_1	H_2	H_3	H_4	H_5	H_6
Rha[b]	8.7	0.7	1.2	0.9	1.3	2.6	4.6	5.3
Ara[c]	16.5	1.3	2.3	0.4	1.7	7.3	4.6	14.2
Gal[d]	7.4	1.7	4.7	0.5	1.3	12.7	23.0	14.7
Glu[e]	40.8	1.3	1.3	0.8	0.8	1.7	8.3	16.3
Man[f]	7.1	Nd[a]	Nd[a]	Nd[a]	Nd[a]	Nd[a]	Nd[a]	12.6

Xyl[g]	8.9	85.2	79.4	80.3	74.2	54.9	39.4	29.9
GlcA[h]	10.9	9.8	11.1	17.1	20.8	20.8	12.2	7.1
GlcA /Xyl[i]	1.22	0.11	0.14	0.21	0.28	0.38	0.31	0.24

[a]Nd – Not detected; [b]Rha – rhamnose; [c]Ara – Arabinose; [d]Gal – Galactose; [e]Glc – Glucose; [f]Man – Mannose; [g]Xyl – Xylose; [h]GlcA – Glucuronic acid; [i]GlcA/Xyl – Glucuronic acid/xylose; [j]Res – Residue

Table 2
Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of hemicellulosic subfractions

	Hemicellulosic fractions							
	H_w	H_A	H_1	H_2	H_3	H_4	H_5	H_6
M_w	6060	71860	55970	85540	80550	74140	40670	17110
M_n	5110	38450	31720	43930	24760	36170	28120	13020
M_w/M_n	1.18	1.87	1.76	1.95	3.25	2.05	1.45	1.31

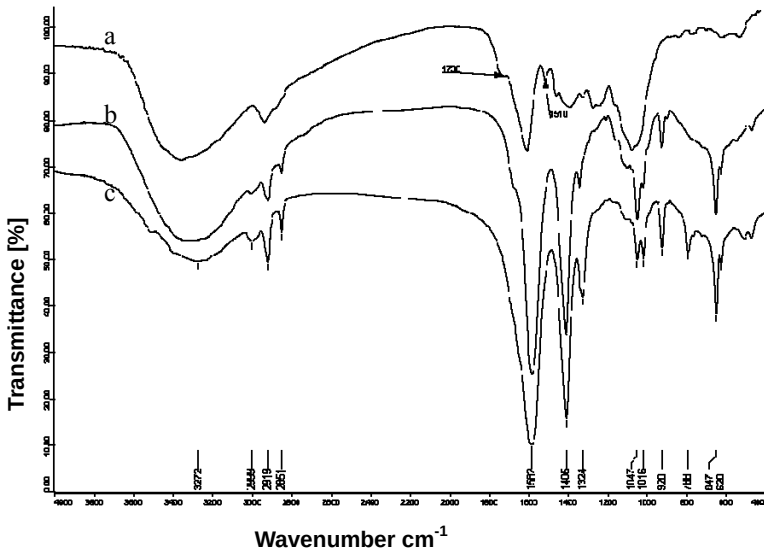

Figure 2: FT-IR spectra of hemicellulosic fractions H_w (spectrum a), H_A(spectrum b), and H_1 (spectrum c)

FT-IR Spectra

The FT-IR spectra of fractions H_w (spectrum a), H_A (spectrum b), and H_1 (spectrum c) are shown in Fig. 2. As seen, the 3 fractions give rather similar characteristic absorptions of typical polysaccharides in the 800-1500 cm^{-1} region. The adsorption at 3272 cm^{-1} is related to stretching of the OH groups, while the three bands at 2999, 2919 and 2851 cm^{-1} – to C-H stretching. The presence of a shoulder signal at 1730 cm^{-1} in water-soluble hemicelluloses H_w (spectrum a) is explained by the acetyl and ester groups of the hemicelluloses. However, the absence of the signal at 1730 cm^{-1} in the other two spectra of alkali-soluble hemicellulosic subfractions (H_A and H_1) implies that the 10% NaOH treatment cleaved the ester bond from the hemicelluloses. Obviously, a small absorption at 1510 cm^{-1} in spectrum a is characterized by aromatic skeleton vibration belonging to lignin, indicating the presence of small amounts of associated lignin in the water-soluble hemicelluloses.[11] A major absorbance at 1047 cm^{-1} is assigned to the C-O-C stretching of glycosidic linkage, which is typical of xylan.[12] Another important signal at about 920 cm^{-1} in spectra b and c is characteristic of the

β-glycosidic linkages among the sugar units present in the hemicellulosic subfractions. Furthermore, two intense signals at 1582 and 1405 cm^{-1} are due to glucuronic acid carboxylate,[13,14] whereas the increasing intensity corresponds to an increased uronic acids content (Table 1).

NMR Spectra

Nuclear magnetic resonance (NMR) spectroscopy was used to obtain structural information on high molecular weight materials and their building blocks. ^{1}H and ^{13}C and 2D HSQC NMR spectra of the hemicellulosic subfraction H_3 are given in Figures 3 to 5. The signals for ^{13}C and ^{1}H NMR were assigned from the HSQC spectrum, following the sugar composition and published literature (Table 3).[15-19] The anomeric ^{1}H NMR signals were found in the spectral region of 4.4-5.4 ppm. The signals of α-anomeric protons were observed in the spectral region of 5.0-5.3 ppm, while β-anomeric protons – at 4.4-4.6 ppm. The signal at 4.43 ppm confirmed that xylose is linked by β-glycosidical linkages, the sharp signal at 3.40 ppm confirming the presence of the methyl group of 4-*O*-α-D-Glc*p*A.

Table 3
^{1}H and ^{13}C chemical shift (ppm) assignments for hemicellulosic subfraction H_3

Sugar units	Chemical shift (ppm) H/C							
	1	2	3	4	5ax[d]	5eq[e]	6	OCH_3
X[a]	4.43	3.25	3.51	3.74	3.33	4.05		
	101.8	72.8	73.7	76.8	63.1	63.1		
XU[b]	4.59	3.40	3.59	3.72	3.36	4.07		
	101.4	76.9	72.3	76.3	63.0	63.0		
U[c]	5.24	3.53	3.72	3.18	4.27			3.42
	97.6	71.4	72.2	82.5	72.3		176.8	59.8

[a]X – (1→4)-β-D-Xyl*p*; [b]XU – (1→4)-β-D-Xyl*p*-2-Glc*p*A; [c]U – 4-*O*-Me-α-D-Glc*p*A; [d]ax – axial; [e]eq – equatorial

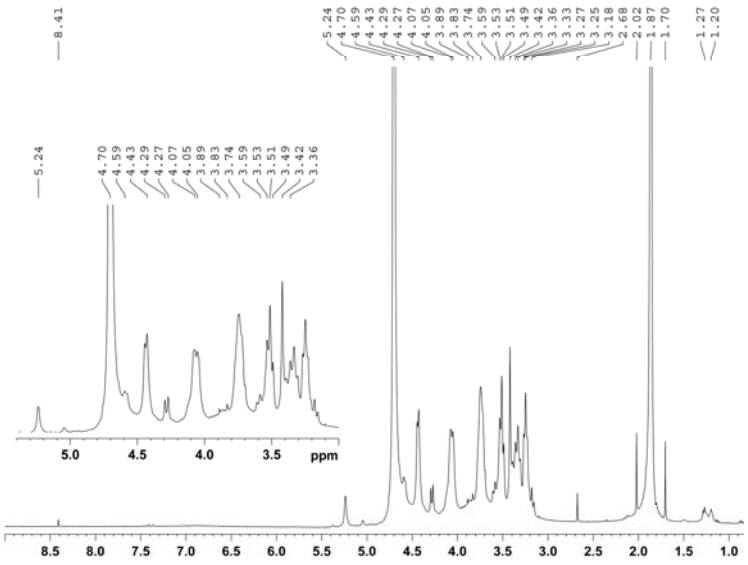

Figure 3: ^{1}H NMR spectra (in D_2O) of hemicellulosic subfraction H_3

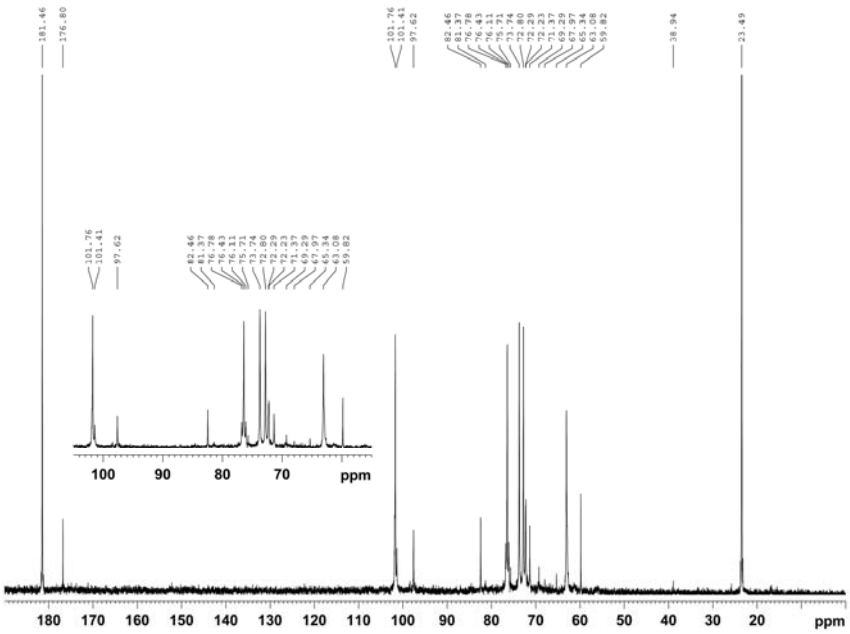

Figure 4: ^{13}C NMR spectra (in D_2O) of hemicellulosic subfraction H_3

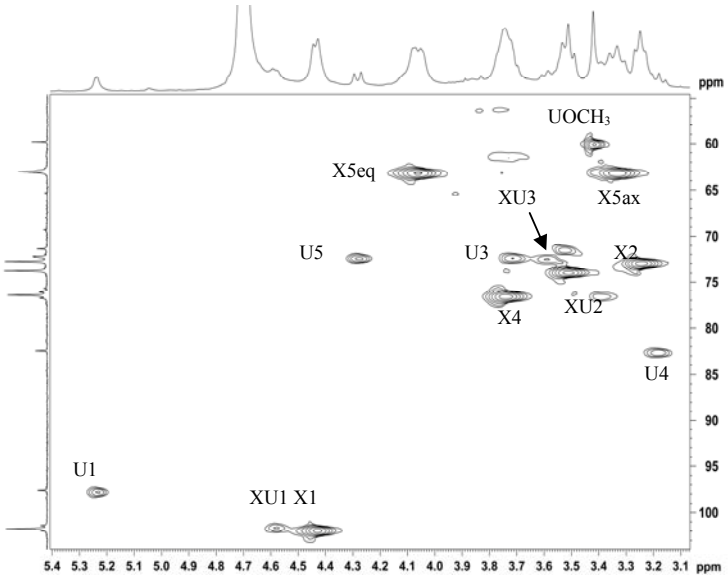

Figure 5: $^{1}H/^{13}C$ NMR spectra (in D_2O) of hemicellulosic subfraction H_3

The examination of ^{1}H NMR data revealed three important groups of protons: unsubstituted (1→4) linked β-D-xylopyranosyl ((1→4)-β-D-Xyl*p*, X), substituted β-D-xylopyranosyl ((1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A, XU) and (1→2) linked 4-*O*-methyl-α-D-glucopyranosyl uronic acid (4-*O*-Me-α-D-Glc*p*A, U) residues. The ^{13}C NMR spectrum of H_3 showed 5 main signals at 101.8 (C-1), 72.8 (C-2), 73.7 (C-3), 76.8 (C-4), 63.1 (C-5) ppm,

corresponding to the (1→4) linked β-D-Xyl*p* residues. In addition, the presence of 4-*O*-Me-α-D-Glc*p*A is confirmed by small signals at 176.8, 97.6, 82.5 and 59.8 ppm, characteristic of C-6, C-1, C-4, and by the methoxyl group. It should be noted that the signal at 181.5 ppm is assigned to the carbonyl groups from the acetyl groups.

The HSQC spectra of H_3 (Fig. 5) show that the dominant 5 cross-signals at 101.8/4.43, 72.8/3.25, 73.7/3.51, 76.8/3.74 and 63.1/4.05 and 3.33 ppm (Table 3) are attributed to the C-1, C-2, C-3, C-4 and C-5 of the β-D-xyl*p* units, respectively. In addition, the presence of the methyl group (OCH_3) of 4-*O*-methyl-D-glucuronic acid was confirmed by cross-peak at 59.8/3.42 ppm. Therefore, it can be concluded that the alkali-soluble hemicelluloses from *S. psammophila* had a main structure composed of a (1→4)-linked β-D-xylopyranosyl backbone with 4-*O*-methyl-α-D-glucuronic acid attached to the *O*-2 of the xylose residues.

CONCLUSIONS

In short, the alkali-soluble hemicelluloses of *S. psammophila* could be fractionated by gradual ethanol precipitation. The less branched hemicelluloses with large molecules were precipitated in lower ethanol percentages, while with increasing ethanol concentration, more branched hemicelluloses with low molecular weight were obtained. The alkali-soluble hemicellulosic subfraction H_3, obtained by 45% ethanol precipitation, mainly consists of (4-*O*-methyl-α-D-glucurono)-β-D-xylans.
Therefore, gradual ethanol precipitation is very useful in biomass industries in obtaining more homogeneous hemicelluloses with different degrees of branching and molecular weights.

ACKNOWLEDGEMENTS: This work was supported with the grants provided by the Fundamental Research Funds for the Central Universities (No. YX2011-37), Ministry of Science and Technology (973 project, 2010CB732204), the Natural Science Foundation of China (No. 30930073, 31110103902), Beijing Forestry University Young Scientist Fund (2010BLX04) and State Key Laboratory of Pulp and Paper Engineering (201029).

REFERENCES

[1] P. Methacanona, O. Chaikumpollertb and P. K. Thavornitia, *Carbohydr*. *Polym*., **54**, 335 (2003).
[2] F. Xu, R. C. Sun and H. Y. Zhan, *Pap*. *Sci*. *Technol*., **23**, 17 (2004).
[3] W. G. Glasser, W. E. Kaar, R. K. Jain and J. E. Sealey, *Carbohydr*. *Polym*., **43**, 367 (2000).
[4] T. Ishii, *Plant Sci*., **127**, 111 (1997).
[5] J. E. Thompson and S. C. Fry, *Planta*, **221**, 275 (2000).
[6] T. B. T. Lam, K. Kadoya and K. Iiyama, *Phytochemistry*, **57**, 987 (2001).
[7] L. Canilha, J. B. A. E. Silva, M. G. Felipe and W. Carvalho, *Biotechnol*. *Lett*., **25**, 1811 (2003).
[8] S. A. Watson, in "Polysaccharides and Their Derivatives", edited by R. L. Whistler and J. N. BeMiller, New York, Academic Press, 2002, pp. 299-306.
[9] F. Peng, J. L. Ren, F. Xu, J. Bian, P. Peng and R. C. Sun, *J. Agric. Food Chem*., **57**, 6305 (2009).
[10] J. Bian, F. Peng, P. Peng, F. Xu and R. C. Sun, *Carbohydr*. *Res*., **345**, 820 (2010).
[11] R. C. Sun, J. M. Fang and J. Tomkinson, *J. Agric. Food Chem*., **48**, 1247 (2000).
[12] R. C. Sun and J. Tomkinson, *Eur*. *Polym*. *J*., **39**, 751 (2003).
[13] R. H. Marchessault and C. Y. Liang, *J*. *Polym*. *Sci*., **59**, 357 (1962).
[14] A. K. Chatjigakisa, C. Pappasa, N. Proxeniab, O. Kalantzib, P. Rodisb and M. Polissioua, *Carbohydr*. *Polym*., **37**, 395 (1998).
[15] C. Morine, P. Krausz, V. Chaleix, O. Sainte-Catherine, M. Kraemer and V. Gloaguen, *J*. *Nat*. *Prod*., **70**, 60 (2007).
[16] A. Kardosova, M. Matulova and A. Malovikova, *Carbohydr*. *Res*., **308**, 99 (1998).
[17] D. Nabarlatz, A. Ebringerova and D. Montane, *Carbohydr*. *Polym*., **69**, 20 (2007).
[18] A. Teleman, J. Lundqvist, F. Tjerneld, H. Stalbrand and O. Dahlman, *Carbohydr*. *Res*., **329**, 807 (2000).
[19] H. Gruppen, R. A. Hoffmann, F. J. M. Kormelink, A. G. J. Voragen, J. P. Kamerling and J. F. G. Vliegenthart, *Carbohydr*. *Res*., **233**, 45 (1992).