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Fractional isolation and structural characterization of hemicelluloses from *Caragana korshinskii*

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ABSTRACT

Sequential treatments of dewaxed *Caragana korshinskii* with dimethyl sulfoxide and dioxane-triethylamine (9:1, v/v) at 80 °C for 5 h, saturated barium hydroxide, 1 M potassium hydroxide and 1 M sodium hydroxide at 50 °C for 5 h, and 3 M potassium hydroxide at 50 °C for 4 h released 9.9%, 12.1%, 19.2%, 27.7%, 13.2% and 12.9% of the original hemicelluloses, respectively. The DMSO-soluble and four alkali-soluble hemicellulosic fractions contained higher amounts of xylose (73.6–91.5%), but were lower in rhamnose (0.5–2.3%) and arabinose (2.8–17.6%) than dioxane-triethylamine (9:1, v/v) soluble hemicellulosic fraction, in which xylose (37.9%), rhamnose (25.9%) and arabinose (25.0%) were the major sugar components. In comparison, the molecular-weight analysis showed that hemicelluloses were substantially degraded with a value of 13,930 g mol^{-1} under the organic alkaline extraction condition (dioxane-triethylamine, 9:1) used, whereas saturated $Ba(OH)_2$ treatment favored the solubilization of macromolecular hemicelluloses (69,910 g mol^{-1}). It is confirmed that the hemicelluloses from *C. korshinskii* are (1 → 4)-linked β-D-xylans with L-arabinofuranosyl group attached based on both ^1H and ^{13}C NMR spectra.

1. Introduction

Caragana korshinskii, a long-lived grassland and desert shrub species belonging to Leguminosae (Fabaceae), is indigenous to half-fixed and fixed sandy regions in the northwest of China and Mongolia. The species plays a critical role in reducing wind erosion, controlling desertification, fixing atmospheric nitrogen and enhancing water conservation, and has considerable economical and ecological importance (Wang, Gao, Han, & Wu, 2006). The stems of the shrub are cut once every 3 years to make it flourish, which generates a large amount of residues. At present, only a small amount of the stubble is used for the production of fiberboard, and most of the remainder is burnt as firewood, which cause serious environmental pollution (Xu, Sun, & Zhan, 2004). In order to meet the energy demand and global climate stability, the efforts to exploit a variety of plants have been made particularly for agricultural crops and wood wastes (Luque et al., 2008). Hence, these residues from *C. korshinskii* should have a great potential for utilization because of the high content of cellulose and hemicelluloses (Xu, Sun, & Lu, 2006).

Hemicelluloses are a large group of low-molecular-weight polysaccharides found in the primary and secondary cell walls, which comprise roughly one-fourth to one-third of most plant materials of all land and fresh water plants, and in some seaweed. Unlike cellulose, hemicelluloses are not chemically homogeneous; they consist of various different sugar units, arranged in different proportions and with different substituents (Aspinall & Mahomed, 1954). The principal sugars are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fucose, and various *O*-methylated neutral sugars. Structures of the hemicelluloses vary significantly in different plants and have been hot topics of great academic interest. Previous studies have shown that plant xylans form a family of polysaccharides which consist of the backbone of β-(1 → 4)-D-xylopyranose residues which can be substituted in C-2 and/or C-3 by short and flexible side chains. Depending on their origin, hardwood xylans are mainly constituted of units of α-D-glucuronic acid, or 4-*O*-methyl-α-D-glucuronic acid. Among the side groups, the commons are acetyl groups, phenolic acids, ferulic and coumaric acids (Bendahou, Dufresne, Kaddami, & Habibi, 2007). Besides, hemicelluloses associate with cellulose and lignin in plant cell walls. They form hydrogen bonds with cellulose, covalent bonds (mainly α-benzyl ether linkages) with lignins and ester linkages with acetyl units and hydroxycinnamic acids. Therefore, isolation of hemicelluloses involves alkaline hydrolysis of ester linkages to liberate them from the lignocellulosic matrix followed by extraction into

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aqueous media. However, the liberation of the xylan component from the cell wall is restricted by the lignin network as well as by ester and ether lignin–hemicelluloses linkages. Furthermore, extensive hydrogen bonding between the individual polysaccharide cell wall components may impede isolation of the hemicellulosic component (Ebringerová & Heinze, 2000). Previously, hemicelluloses were isolated by direct extraction of fully lignified wood without delignification. Nowadays as for quantitative isolation of hemicelluloses from both hardwood and softwood, the material should firstly be delignified, generally, with chlorine (Timell & Jahn, 1951), chlorine dioxide (Yang & Goring, 1978), or sodium chlorite, after which the left holocellulose could be treated with various procedures. However, no completely satisfactory method for preparation of holocellulose is available up to now. Delignification by acid-chlorite may oxidize some reducing-end residues to aldonic acid residues and cause partial depolymerization, and some loss of components is apparently inevitable (Aspinall, Greenwood, & Sturgenon, 1961). In addition, before delignification, the material is pre-extracted, preferably with chloroform, acetone, methanol and toluene–ethanol (2:1, v/v). All these pretreatments are used to remove lipophilic and hydrophilic non-structural components.

Aqueous solutions of potassium and sodium hydroxide have been found by far to be the most extensive application for extraction of hemicelluloses (Cyran & Saulner, 2007; Fares, Renard, Rzina, & Thibault, 2001; Höije, Gröndahl, Tømmeraas, & Gatenholm, 2005; Moine et al., 2007; Rao & Muralikrishna, 2006). In general, one step of dilute alkali treatment only extracts part of the hemicelluloses from both the holocellulose and lignified material. Successive treatments with alkali of initially low and then higher concentration avoid unnecessary exposure of hemicellulosic material to alkali that are more concentrated than those required to extract it (Buchala, Fraser, & Wilkie, 1971). In this case, the hemicellulosic materials from plant cell walls are frequently fractionated to give polysaccharides with different structural features. More importantly, studies of such fractionated materials have led to much structural information on molecules in those of the populations of hemicellulosic molecules recovered by the most commonly used procedures.

Since the reports on the structures and the physiochemical properties of the hemicelluloses from *C. korshinskii* are limited, the present work focus on this issue. For that, the extractive-free material was delignified by sodium chlorite, and then successively extracted with dimethyl sulfoxide (DMSO), dioxane-triethylamine (9:1, v/v) and alkali of increasing concentration. The work aimed at fractionally isolating hemicellulosic polysaccharides from the cell walls of *C. korshinskii*, comparatively determining their structural features, and having a more complete understanding of their chemistry. The isolated hemicellulosic preparations were comparatively investigated by acid hydrolysis, gel permeation chromatography (GPC), Fourier transform infrared (FT-IR), thermal analysis, proton magnetic resonance (^{1}H NMR) and carbon-13 magnetic resonance (^{13}C NMR) spectroscopy.

2. Experimental

2.1. Materials

The *C. korshinskii* was obtained from Shalin arboretumin Yikezhao League of Inner Mongolia, China. It was harvested in October 2005, with an average stem height of 3.5 m. The leaves and the capitula were removed and only the stalks were collected. Fats, waxes and oils were removed from *C. korshinskii* in a Soxhlet apparatus for 6 h with 2:1 (v/v) toluene–ethanol. The extractive-free material was weighed into a flask. Then distilled water (300 ml) and 15 g sodium chlorite were successively added. The mixture in the flask was acidified to pH 4.0 with glacial acetic acid whose volume used was recorded. The flask was then covered with continuous stirring in a water-bath at 75 °C. After 60 min, additional 7.5 g sodium chlorite and the half quantity of the foregoing recorded glacial acetic acid were added and the mixture was kept for a further 60 min under the same conditions. After treatment, the residue (holocellulose) was filtered with a nylon cloth and washed thoroughly with distilled water, and then oven-dried at 50 °C overnight.

2.2. Fractionation of hemicelluloses

The hemicelluloses were obtained from the holocellulose firstly extracted with dimethyl sulfoxide at 80 °C for 5 h. The filtrate was concentrated under reduced pressure to about 50 ml, subsequently mixed with 3 volumes of 95% ethanol (24 h, 20 °C), the precipitates were obtained by freeze-drying after centrifugation and the hemicellulosic fraction was labeled as H_1. After that, the residues were successively extracted with dioxane-triethylamine (9:1, v/v) at 80 °C for 5 h, saturated barium hydroxide, 1 M KOH, and 1 M NaOH at 50 °C for 5 h, and 3 M KOH at 50 °C for 4 h with a solid to extractant ratio of 1:25 (g ml^{-1}). The filtrates in each of the extraction were neutralized with 6 M hydrochloric acid or 6 M acetic acid solution to pH 5.5, and then were concentrated under reduced pressure to about 50 ml. Subsequently, the concentrated solution was mixed with 3 volumes of 95% ethanol (24 h, 20 °C). The precipitates formed were recovered by centrifugation and freeze-dried and these hemicellulosic fractions were considered to be H_2, H_3, H_4, H_5 and H_6, respectively. The scheme for sequential treatments of *C. korshinskii* and isolation of hemicelluloses were illustrated in Fig. 1. To reduce errors and confirm the results, each experiment in this study was conducted in duplicate under the same conditions, and all weights and yields were given on a moisture-free basis.

2.3. Structural and physiochemical characterization of hemicelluloses

The neutral sugars in the six hemicellulosic fractions were liberated by hydrolysis of the polymers with 6% H_2SO_4 for 2.5 h at 105 °C. The analysis of the neutral sugars in the hydrolyzate was

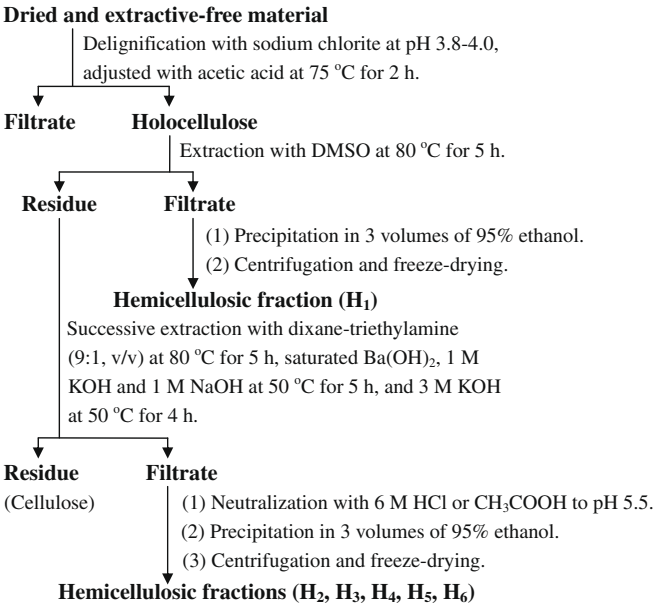

Fig. 1. Scheme for isolation of hemicelluloses from *Caragana korshinskii*.

carried out by high performance anion exchange chromatography (HPAEC) using a Dionex ICS3000 gradient pump, ED50 electrochemical detector, AS50 autosampler and a Carbopac™ PA1 column (4 × 250 mm, Dionex). Samples injected into the system were eluted with 0.018 M NaOH (carbonate free and purged with nitrogen) with post-column addition of 0.3 M NaOH at a rate of 0.5 ml/min. Run time was 45 min, followed by 10 min elution with 0.2 M NaOH to wash the column and then 15 min elution with 0.018 M NaOH to re-equilibrate the column. The analysis was quantified against two separated standard solutions using Chromeleon™ computer software. The uronic acids were eluted with 0.4 M NaOH for 20 min at a rate of 1 ml/min with post-column addition of 0.3 M NaOH at a rate of 0.5 ml/min. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-mannose, D-galactose, and galacturonic acids. The average molecular weights of hemicelluloses were estimated 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 738, 12,200, 100,000, 16,00,000, Polymer Laboratories Ltd.). Flow rates of 0.5 ml/min for hemicelluloses were maintained. The eluents were 0.02 N NaCl in 0.005 M sodium phosphate buffer (pH 7.5). Detection was achieved with a Knauer differential refractometer. The column oven was maintained at 30 °C. Hemicelluloses were dissolved with 0.02 N NaCl in 0.005 M sodium phosphate buffer, pH 7.5 at a concentration of 0.1%.

Fourier transform infrared (FT-IR) spectra were recorded on a Tensor 27 FT-IR spectrophotometer in the range 4000–400 cm^{-1} using a KBr disc containing 1% finely ground samples. Background spectra were taken in the empty chamber. The solution-state ^{1}H and ^{13}C NMR spectra were obtained on a Bruker MSL300 spectrometer operating in the FT mode at 74.5 MHz. ^{1}H NMR spectrum was recorded at 25 °C from 20 mg of sample dissolved in 1.0 ml D_2O and the ^{13}C NMR spectrum was recorded at 25 °C from 80 mg of sample dissolved in 1.0 ml D_2O after 30,000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width and a 0.85 s delay time between scans were used. Thermal stability of hemicelluloses was performed using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (DTG-60, Shimadizu, Japan). The samples weighed between 8 and 13 mg and were run from room temperature to 600 °C at a rate of 10 °C/min under continual nitrogen flow. All samples were dried in an oven for 10 h at 50 °C before the spectra were recorded.

3. Results and discussion

3.1. Fractional yield of hemicelluloses

The yields of hemicelluloses (on a dry, extractive-free basis of the *C. korshinskii*) and the extraction conditions are presented in Table 1. In order to isolate the pure hemicelluloses, the delignification with sodium chlorite was examined at 75 °C for 2 h. In this case, the loss of the dry matter was 29.2% including lignin, residual protein, ash, and starch as well as minor quantities of polysaccharides. Since acetyl groups are one of the common side groups in hemicelluloses as mentioned, extraction with alkaline solvents should be ineligible when acetyl groups are to be retained in the hemicelluloses. Therefore, DMSO solution was used before the application of alkaline solutions with different concentrations in this study. In this case, adequate naturally acetylated hemicelluloses kept from the saponification of ester linkages could be obtained (Lawther, Sun, & Banks, 1995). As can be seen from Table 1, with the successive treatments, the yields of the fractions were 3.6%, 4.4%, 7.0%, 10.1%, 4.8% and 4.7% of the initial amount of dewaxed *C. korshinskii*, corresponding to the dissolution of 9.9%, 12.1%, 19.2%, 27.7%, 13.2% and 12.9% of the original hemicelluloses, respectively. Obviously, total yield of the six hemicellulosic fractions accounted for 95.0% of the original hemicelluloses in the cell walls of *C. korshinskii*. The results revealed that the sequential extractions of the *C. korshinskii* were very effective, and the highest extraction yield was obtained with 1 M KOH (10.1%), implying that 27.7% of the total available hemicelluloses were liberated with 1 M KOH at 50 °C. It is believed that hydroxyl ions liberated from alkaline solutions could cause swelling of cellulose, disruption of intermolecular hydrogen bonds between cellulose and hemicelluloses, hydrolysis of ester bonds which most likely play a role in connecting the cell wall polysaccharides and solubilization of substantial amounts of hemicelluloses from the cell wall (Bergmans, Beldman, Gruppen, & Voragen, 1996; Izdorczyk, Macri, & MacGregor, 1998). On the other hand, the lowest yield (3.6%) initially obtained from the extraction with DMSO at 80 °C, accounted for only 9.9% of the hemicelluloses, and it was also apparent that nearly equal amounts of hemicelluloses were obtained by 1 M NaOH and 3 M KOH. It could be speculated that these differences in extractability of the hemicelluloses were the results of different functions of these polysaccharides in the cell walls.

Table 1
Yields of hemicelluloses (% dry matter) solubilized from the extractive-free *Caragana korshinskii*.

Fraction No.	Extractant	Temperature (°C)/times (h)	Yield (%)
H_1[a]	DMSO	80/5	3.6
H_2[b]	Dioxane-triethylamine (9:1, v/v)	80/5	4.4
H_3[c]	$Ba(OH)_2$	50/5	7.0
H_4[d]	1 M KOH	50/5	10.1
H_5[e]	1 M NaOH	50/5	4.8
H_6[f]	3 M KOH	50/4	4.7

[a] H_1 represents for the hemicellulosic preparation isolated with DMSO at 80 °C for 5 h from the holocellulose.
[b] H_2 represents for the hemicellulosic preparation isolated with dioxane-triethylamine (9:1, v/v) at 80 °C for 5 h.
[c] H_3 represents for hemicellulosic preparation isolated with saturated $Ba(OH)_2$ at 50 °C for 5 h.
[d] H_4 represents for hemicellulosic preparation isolated with 1 M KOH at 50 °C for 5 h.
[e] H_5 represents for hemicellulosic preparation isolated with 1 M NaOH at 50 °C for 5 h.
[f] H_6 represents for hemicellulosic preparation isolated with 3 M KOH at 50 °C for 4 h.

3.2. Content of neutral sugars and uronic acids

To characterize the solubilized hemicelluloses, the six fractions were prepared for the determination of their constituent sugars, and the results are given in Table 2. Obviously, the sugar components of organic alkali dioxane-soluble fraction H_2 can be clearly distinguished from that with DMSO treatment and other alkali-soluble hemicellulosic preparations. A much higher content of rhamnose (25.9%), arabinose (25.0%) and galactose (8.3%), but lower quantities of xylose (37.9%) and uronic acids (0.06%) than those of the hemicellulosic fractions isolated with DMSO and alkali solutions were observed. As to the DMSO-soluble fraction H_1 and the inorganic alkali-soluble fractions H_3–H_6, xylose contents ranging from 73.6% to 91.5% were 2-fold higher compared with organic alkali dioxane-soluble hemicellulosic preparation. The next most abundant sugars in these preparations were arabinose (2.8–17.6%), galactose (1.8–6.5%) and glucose (0.3–4.8%). Uronic acids (0.2–0.8%) appeared as a trace component and mannose was not detected as free monosaccharide. This monosaccharide analysis definitely revealed that *C. korshinskii* hemicelluloses mainly contained arabinoxylans and galactose was probably side chain in or-

Table 2
Content of neutral sugars (relative % hemicellulosic sample, w/w) and uronic acids (% hemicellulosic sample, w/w) in isolated hemicellulosic fractions.

Sugars (%)	Hemicellulosic preparation[a]					
	H_1	H_2	H_3	H_4	H_5	H_6
Rhamnose	2.3	25.9	2.0	1.1	1.0	0.5
Arabinose	11.4	25.0	17.6	9.5	8.8	2.8
Galactose	4.7	8.3	6.5	2.7	4.4	1.8
Glucose	4.8	3.0	0.3	0.4	2.6	3.4
Mannose	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Xylose	76.5	37.9	73.6	86.3	83.2	91.5
Uronic acids	0.6	0.06	0.4	0.6	0.8	0.2

[a] Corresponding to the hemicellulosic preparations in Table 1.
[b] ND, not detectable.

ganic alkali dioxane-soluble hemicellulosic preparation. Interestingly, with the sequential separation stages from saturated $Ba(OH)_2$, to 1 M KOH, to 1 M NaOH, and to 3 M KOH, the content of arabinose decreased from 17.6% in H_3, to 9.5% in H_4, to 8.8% in H_5, and to 2.8% in H_6. This phenomenon provided the evidence that in *C. korshinskii* cell walls, arabinose, probably as a side chain in hemicelluloses, was easily solubilized during the initial and dilute alkaline extraction process, whereas this side chain was partially cleaved or degraded in the strong alkaline solutions. Similar decreasing trend was also observed for galactose. In contrast, the content of xylose appeared the significantly increasing trend, from 73.6% in H_3 to 91.5% in H_6. It was found that the ratio of arabinose to xylose decreased with the increment of alkaline concentrations performed. This lower arabinose content indicated the lower degree of branching of xylan chains (Sun, Lawther, & Banks, 1996). Therefore, it was easy to note that the degree of branching of the arabinoxylans decreased with the increasing extraction strength. This was particularly true in the hemicellulosic fraction H_6, extracted with 3 M KOH at 50 °C for 4 h, which had the lowest arabinose content (2.8%) and the highest xylose content (91.5%), suggesting that the less branched structures were extracted as the alkaline concentration increased.

3.3. Average molecular weight

As reported, the molecular-weight values are considerably affected by the method of estimation (Ebringerová, Hromádková, & Heinze, 2006). In this study, the molecular weights of the six hemicellulosic fractions were analyzed by GPC. GPC sometimes referred to as gel filtration chromatography (GFC) or size exclusion chromatography (SEC), entails the chromatographic fractionation of macromolecules according to molecular size (Gellerstedt, 1992). Among these, GPC is effective in estimating the molecular weight of unknown polymers of similar or identical chemical structures to those used to calibrate columns (Himmel et al., 1989). The weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) are given in Table 3. As expected, the average molecular weight of DMSO-soluble hemicellulosic fraction with an M_w value of 31,590 g mol^{-1} was lower than those of the four alkali-soluble hemicellulosic fractions, ranging from 45,940 to 69,910 g mol^{-1}. This phenomenon suggested that the DMSO treatment only released low-molecular-weight polysaccharides, while alkaline extraction dissolved the hemicelluloses having high molecular weights. The following treatment with dioxane-triethylamine (9:1, v/v) released much lower-molar-mass (13,930 g mol^{-1}) hemicellulosic material than that with the DMSO treatment. This was probably a consequence of the cleavage of glycosidic ether linkages between sugar units in the macromolecular structure of hemicelluloses since triethylamine is a strong organic alkali. Apparently, the lower-molecular-weight polysaccharides

Table 3
Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the hemicellulosic fractions.

	Hemicellulosic preparation[a]					
	H_1	H_2	H_3	H_4	H_5	H_6
M_w	31,590	13,930	69,910	45,940	48,490	49,700
M_n	25,970	10,970	31,960	29,900	21,610	30,670
M_w/M_n	1.22	1.27	2.19	1.54	2.24	1.62

[a] Corresponding to the hemicellulosic preparations in Table 1.

were relatively easy to be extracted under mild extraction conditions such as DMSO treatment. However, it should be noted that the hemicellulosic polymers could be substantially degraded and resulted in the fragmentation under the strong organic alkaline extraction condition used, e.g. treatment with dioxane-triethylamine (9:1, v/v). In contrast, a much higher M_w (69,910 g mol^{-1}) in H_3 preparation implied that the saturated $Ba(OH)_2$ treatment favored the solubilization of macromolecular hemicelluloses from *C. korshinskii*. On the other hand, a slight depolymerization of the native hemicelluloses may occur during the treatment with strong inorganic alkalis such as 1.0 M KOH, 1.0 M NaOH, and 3 M KOH solution as shown by the relative lower values of M_w from 45,940 to 49,700 in Table 3. Additionally, the analysis showed that the first two polymeric hemicellulosic fractions solubilized during DMSO and dioxane-triethylamine (9:1, v/v) treatments have relative lower polydispersity indexes (1.22 for H_1 and 1.27 for H_2) as compared to those of the other four alkaline-soluble hemicellulosic fractions, which have M_w/M_n between 1.54 and 2.19.

3.4. FT-IR spectra

FT-IR spectroscopy, which is quite extensively applied in plant cell wall analysis, offers a potential for the assignment of absorbance bands to specific molecular structures (Kačuráková & Wilson, 2001). The spectra of DMSO-soluble hemicellulosic fraction H_1 (spectrum 1), dioxane-triethylamine (9:1, v/v) soluble hemicellulosic fraction H_2 (spectrum 2), and saturated barium hydroxide soluble hemicellulosic fraction H_3 (spectrum 3) obtained from *C. korshinskii* are illustrated in Fig. 2. As can be seen, the three spectral profiles of the bands are significant different. The occurrence of a small shoulder peak in spectra 1 and 2 at 1510 cm^{-1} (data not shown) originated from aromatic skeletal vibrations in associated lignin, indicating that H_1 and H_2 were slightly contaminated with minimal amounts of bound lignin (Vazquez, Antorrena, Gonzalez, & Freire, 1997). The absorption at 3419 cm^{-1} is attributed to the stretching vibration of —OH groups. The C—H stretching vibration gives signals at 2923 and 2854 cm^{-1}. In the carbonyl stretching region, a small band at 1732 cm^{-1} in spectra 1 and 2 is assigned to the acetyl group of the hemicelluloses. The disappearance of this signal in the spectrum 3 verified that the treatment with saturated barium hydroxide under the condition used completely cleaved this ester bond of hemicelluloses. The intensive band at 1635 cm^{-1} is due to the absorbed water, since the hemicelluloses usually have a strong affinity for water, and in the solid state these macromolecules may have disordered structures which can be easily hydrated (Kačuráková, Belton, Wilson, Hirsch, & Ebringerová, 1998). Band due to CH_2 symmetric bending was observed at 1463 cm^{-1}, and the bands at 1397, 1327 and 1254 cm^{-1} represent C—H stretching and OH or C—O bending vibration in hemicelluloses. Besides, bands between 999 and 1170 cm^{-1} are typical of arabinoxylans (Sun, Sun, Sun, & Su, 2004). The linear and branched (1 → 4)-β-xylans show the main band maximum at about 1048 cm^{-1} (Kačuráková, Ebringerová, Hirsch, & Hromádková, 1994). The absorption band at 1171 cm^{-1} is due to C—O antisym-

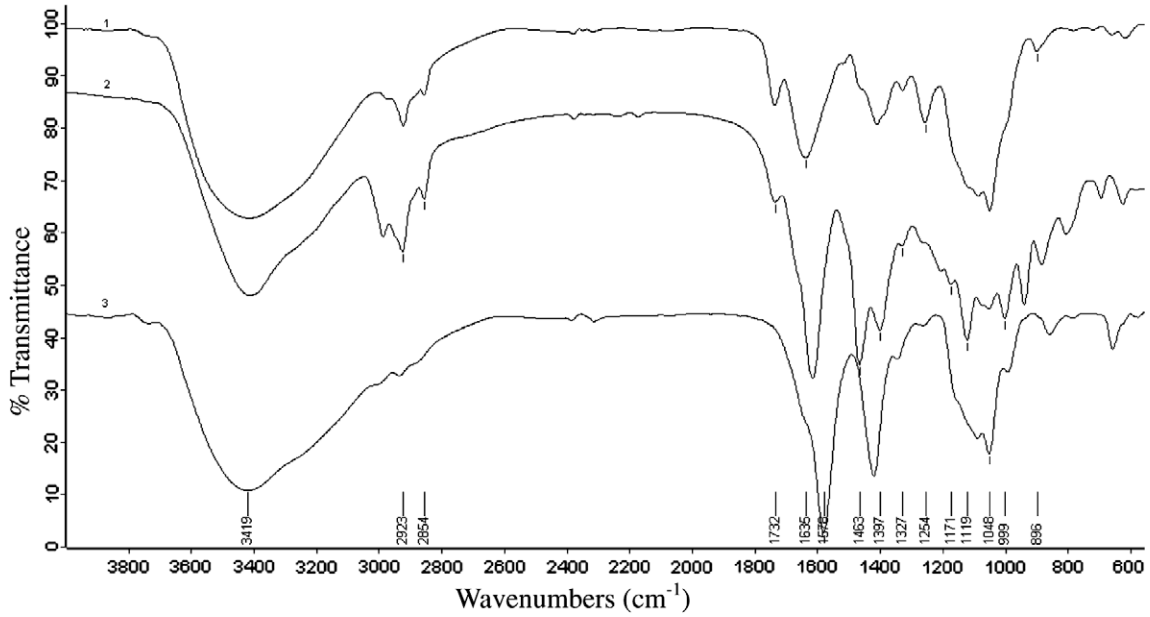

Fig. 2. FT-IR spectra of the hemicellulosic preparations H_1 (spectrum 1), H_2 (spectrum 2), and H_3 (spectrum 3).

metric bridge stretching, 1119 cm^{-1} is attributed to C—OH skeletal vibration, and 1048 cm^{-1} is dominated by C—O, C—C stretching or C—OH bending in hemicelluloses (Chaikumpollert, Methacanon, & Suchiva, 2004). A small band at 896 cm^{-1}, which is due to the C-1 group frequency or ring frequency, indicates β-glycosidic linkages between the xylose units in the hemicelluloses (Gupta, Madan, & Bansal, 1987).

Fig. 3 illustrates the FT-IR spectra of hemicellulosic fractions released during the treatment with 1 M KOH (spectrum 4), 1 M NaOH (spectrum 5) and 3 M KOH (spectrum 6). The most obvious feature is the similarity of the spectra 4 and 6 in Fig. 3 to the spectrum 3 in Fig. 2, indicating that the hemicellulosic fractions extracted with saturated barium hydroxide, 1 M KOH, and 3 M KOH under the conditions used may have a similar structural feature. As expected, the absence of a signal at 1730 cm^{-1} for carbonyl stretching in all the three spectra implied that the alkali-soluble hemicelluloses obtained in this study fully saponified acetyl groups and methyl esters. The small shoulder peak at 1510 cm^{-1} (data not shown) appeared in spectrum 5 caused by aromatic skeleton vibration in bound lignin indicated that the NaOH-soluble hemicellulosic fraction H_5 contained trace of lignin, and the delignification with sodium chlorite at 75 °C for 2 h did not completely remove the lignin from the dewaxed *C. korshinskii*. The absorbances at 1631, 1412, 1343, 1094, 1048, 989 and 899 cm^{-1} in the spectra are associated with hemicelluloses, in which the band at 1048 cm^{-1} is typical of arabinoxylans.

3.4.1. ^1H and ^{13}C NMR spectra of hemicellulosic preparation (H_1)

Nuclear magnetic resonance has significant advances in understanding complicated structure. To obtain further information about the anomeric linkage configuration and relative content of monosaccharide residues constituent as well as the type and number of specific linkage, the hemicellulosic fraction H_1, extracted with DMSO, was analyzed by ^1H and ^{13}C NMR spectrometry in

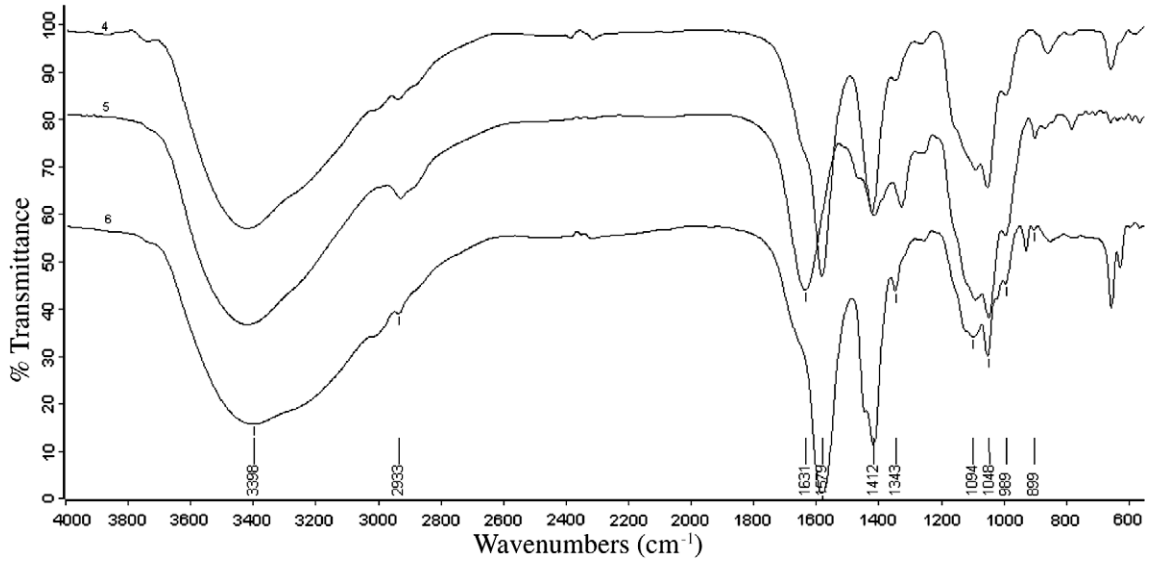

Fig. 3. FT-IR spectra of alkali-hemicellulosic preparations H_4 (spectrum 4), H_5 (spectrum 5), and H_6 (spectrum 6).

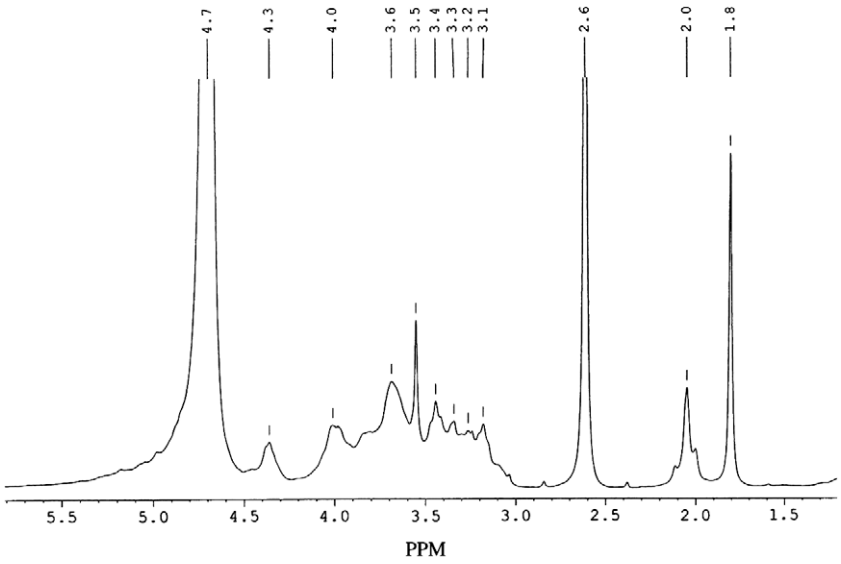

Fig. 4. ^{1}H NMR spectrum of hemicellulosic preparation H_1 extracted with DMSO for 5 h at 80 °C from the holocellulose.

D_2O. Obviously, a strong signal at 4.7 ppm in Fig. 4 was originated from the residual solvent (HDO) and the chemical shifts of 3.2–4.4 ppm were caused by β-D-xylose residues. Anomeric protons of terminal α-arabinofuranosyl residues give a shoulder at 5.2 ppm (data not shown) (Teleman, Lundqvist, Tjerneld, Stalbrand, & Dahlman, 2000). The peaks between 2.1 and 1.8 ppm correspond to the methyl or methylene protons adjacent to double bonds or carbonyl groups (Xu et al., 2008b). A sharp signal at 2.6 ppm is related to the protons in dimethyl sulfoxide (Xu et al., 2008a), which probably originated from the residual DMSO as extractant in the hemicellulosic fraction H_1.

The ^{13}C NMR spectrum of DMSO-soluble hemicelluloses is illustrated in Fig. 5. Most of the major resonances were assigned to data in literature (Gabrielli, Gatenholm, Glasser, Jain, & Kenne, 2000; Imamura, Watanabe, Kuwahara, & Koshijima, 1994). The main 1,4-linked β-D-xylopyranosyl units were characterized by five main signals at 101.6, 76.3, 73.6, 72.6 and 62.5 ppm which were, respectively, assigned to C-1, C-4, C-3, C-2 and C-5 of β-D-xylopyranosyl units. The signals at 75.5 (data not shown) and 62.9 ppm correspond to C-3 and C-5 of α-L-arabinofuranosyl residues linked to β-D-xylans. Similarly, a sharp signal at 38.7 ppm is probably due to the protons in the residual solvent of DMSO (Xu et al., 2008a). The signal at 23.3 ppm is most likely due to CH_3 from acetyl groups in hemicelluloses (Chaikumpollert et al., 2004). All data from NMR stated that the anomeric configuration of the D-xylopyranose residues is β while the L-arabinofuranosyl is α.

3.5. Thermal analysis

Fig. 6 illustrates the TGA/DTA curves of hemicellulosic fractions of H_1 (a) isolated with DMSO at 80 °C for 5 h, and H_4 extracted with 1 M KOH at 50 °C for 5 h from *C. korshinskii*. As can be seen from the figure, the weight loss occurred at the beginning is attributed to the evaporation of water (Ren et al., 2006). This indicated the water probably adsorbed on the surface of the material. The two hemicellulosic fractions began to decompose at 202 and 245 °C,

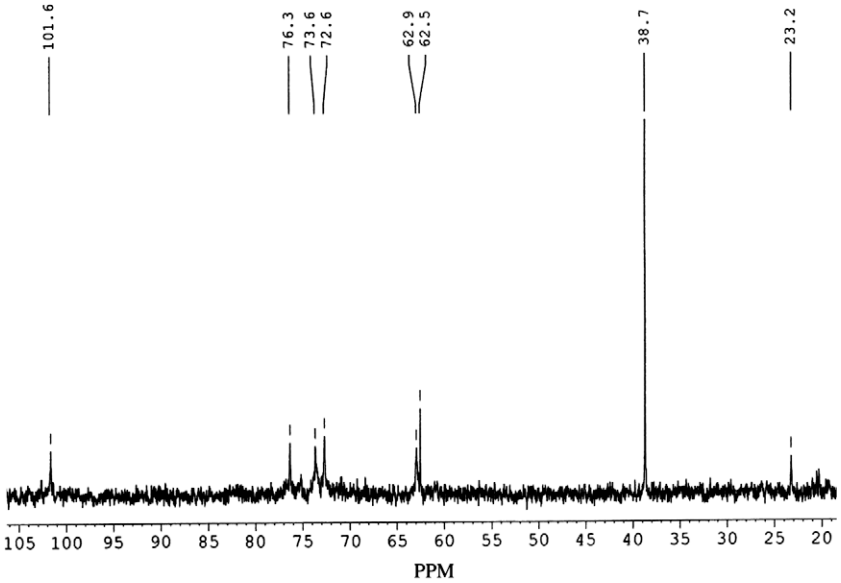

Fig. 5. ^{13}C NMR spectrum of hemicellulosic preparation H_1 extracted with DMSO for 5 h at 80 °C from the holocellulose.

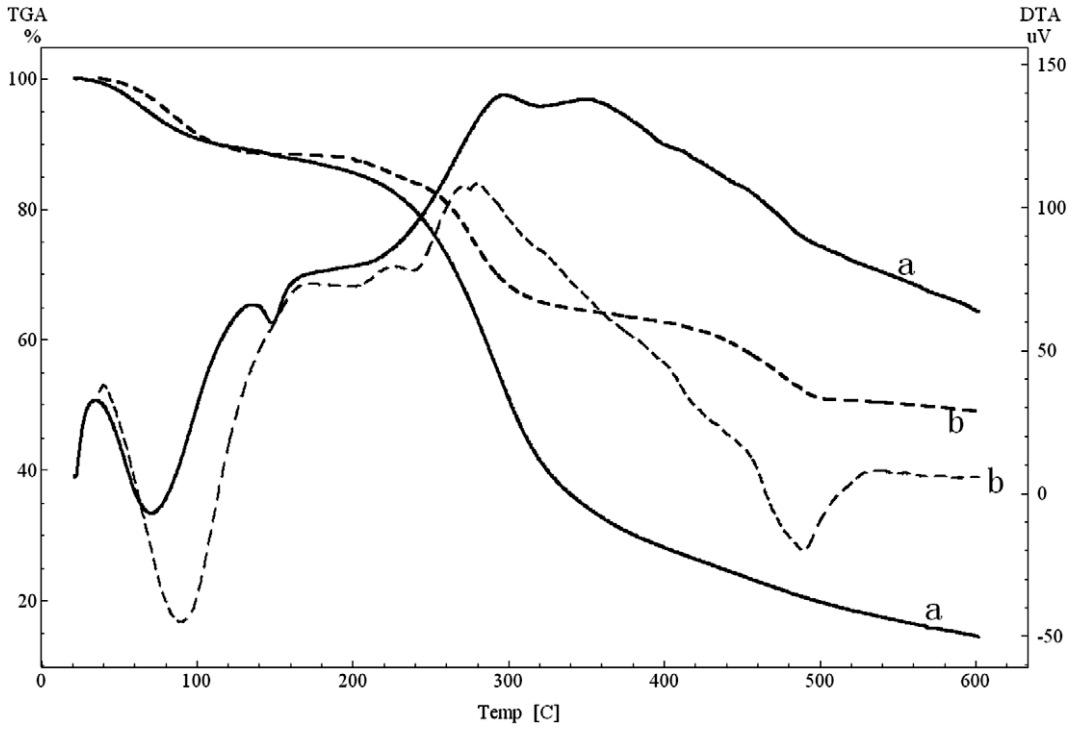

Fig. 6. Thermogram of hemicellulosic preparations H_1 (a) and H_4 (b).

respectively, and the maximum rate of weight loss was observed between 220 and 320 °C for H_1 and between 260 and 320 °C for H_4. That is, the thermal stability of the hemicellulosic fraction, isolated with 1 M KOH, appeared to be higher than that of the fraction extracted with DMSO, which corresponded to the increasing trends of their molecular weights from 31,590 to 45,940 g mol^{-1} in Table 3. This is also presumed due to the different chemical composition between the DMSO-soluble and the alkali-soluble hemicelluloses. Finally, it can be clearly noted that the hemicellulosic fraction H_4 has a higher content of residue (48 wt%) than that of the fraction H_1 at 600 °C. This is probably due to the end-products of the decomposition of hemicelluloses which are carbonaceous residues in an inert atmosphere and the salts formed during the extraction processes contributed to the ash and the inorganic compounds taken by the plant during growing (Devallencourt, Saiter, & Capitaine, 1996). Another reason for this higher content of residue at 600 °C is also presumed due to the higher thermal stability of this hemicellulosic fraction H_4, which needs to be decomposed at much higher temperatures.

4. Conclusion

Successive treatments with DMSO, dioxane-triethylamine (9:1, v/v) and inorganic alkalis under the conditions used was an effective technique for isolation of large proportions of *C. korshinskii* hemicelluloses, which together solubilized 95.0% of the original hemicelluloses in the cell wall. It was found that the DMSO-soluble and the other four alkali-soluble hemicellulosic preparations contained higher amounts of xylose (73.6–91.5%), but lower rhamnose (0.5–2.3%) and arabinose (2.8–17.6%) contents than dioxane-triethylamine (9:1, v/v) soluble hemicellulosic fraction in which xylose (37.9%), rhamnose (25.9%) and arabinose (25.0%) were the major sugar components. In comparison, the treatment with dioxane-triethylamine (9:1, v/v) favored to release much lower molecular weights (13,930 g mol^{-1}) of the hemicellulosic fraction than other five hemicellulosic preparations (31,590–69,910 g mol^{-1}). ^{1}H and ^{13}C NMR spectroscopy confirmed that *C. korshinskii* hemicelluloses consisted of (1 → 4)-linked β-D-xylan backbone and α-L-arabinofuranosyl units were attached as side residues. The results from thermal analysis showed that the thermal stability increased with increasing molecular weight.

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