Characterization of Hemicelluloses Extracted from *Populus tomentosa* Carr. by the Hydrothermal Method with Ethanol

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Abstract: The aim of this study was to investigate the hemicelluloses extracted from *Populus tomentosa* Carr. by the hydrothermal method with ethanol. The influence of ethanol concentration on the hemicellulosic fractions was systematically studied. The chemical compositions and structural features of the hemicellulosic fractions were investigated by a combination of sugar analysis, gel-permeation chromatography, Fourier transform infrared spectroscopy, and one-dimensional proton and carbon-13 nuclear magnetic resonance, and two-dimensional heteronuclear single quantum coherence spectroscopy. Neutral sugar analysis of the hemicellulosic fractions revealed that a higher ethanol concentration (45%~80%) favored the isolation of hemicelluloses with more side chains and lower glucose contents. The molecular weights of these polysaccharides ranged between 2842 g/mol and 5101 g/mol. The results of this study indicate that the hydrothermal ethanol process provides a new pretreatment strategy for the isolation and extraction of biomass.

Keywords: hemicelluloses; isolation; hydrothermal; ethanol; characterization



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1 Introduction

Hemicelluloses are the second most abundant polysaccharides on the earth. They are consisted of various sugar units including *D*-xylose, *L*-arabinose, *D*-glucose, *D*-galactose, *D*-mannose, *D*-glucuronic acid, 4-O-methyl-*D*-glucuronic acid, *D*-galacturonic acid, *L*-rhamnose, *L*-fucose, and various O-methylated neutral sugars[1].

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In recent years, hemicelluloses have received increasing research attention in academia and industry, and have been widely used in the conversion of fuel ethanol and chemicals[2-6]. It is well known that cellulose, hemicelluloses, and lignin are the major components of plant cell walls, in which hemicelluloses associate with cellulose and lignin to form tight compact structures[7]. Hemicelluloses form hydrogen bonds with cellulose and covalent bonds with lignin, making them difficult to separate from the lignocellulosic matrix, thus restricting their application[8]. Therefore, the development of pretreatment methods for the extraction and isolation of hemicelluloses from lignocellulosic matrices is of great importance for broadening their industrial application.

Rapid progress has been made in the extraction and isolation of hemicelluloses using various pretreatment strategies, such as hot water extraction[9], dilute-acid pretreatment[10-11], alkali extraction[12], steam explosion extraction[13-14], ultrasound-assisted extraction[15], hydrothermal treatment[16-17], microwave irradiation[18], and ultrafiltration[19]. Among these methods, the hydrothermal method is a particularly promising pretreatment strategy owing to its short reaction time, high percent conversion, and relatively low reaction temperature. Consequently, it has been employed by several researchers[16-17,20-22]. For example, hemicelluloses were extracted from triploid of *Populus tomentosa* Carr. through hydrothermal pretreatment (160℃, 12~24 h) using distilled water, 1 mol/L NaOH and 1 mol/L KOH aqueous solutions, respectively. The alkaline extractions were found to favor the extraction of xylose, and yielded more hemicelluloses than the extraction with distilled water[16]. Hydrothermal pretreatment can be performed without the addition of chemicals, making it a promising strategy for the pretreatment of large quantities of lignocellulosic feedstock, such as straw[17]. Furthermore, Thomsen et al[17] established a pilot plant consisting of three reactors for the hydrothermal pretreatment of wheat straw (120~150 kg/h) aiming at co-production of bioethanol (from sugars) and electricity (from lignin). Moreover, a semi-pilot scale continuous-flow-type hydrothermal reactor has been used to investigate the separation of hemicelluloses from corncob[20]. In environmental terms, moderate hydrothermal pretreatment in the absence of chemicals (such as acid or alkali) is highly desirable. It is generally agreed that polysaccharides with high glucose concentrations and low molecular weights can be obtained via hydrothermal pretreatment[22]. However, obtaining polysaccharides with low glucose concentrations and high molecular weights via hydrothermal pretreatment is yet to be achieved.

In recent years, graded (or stepwise) ethanol precipitation, which has been proved to be a promising process for the isolation of hemicelluloses from the cell walls of agricultural wastes, has been widely used in fractionating hemicelluloses and obtaining deeper insight into the diversity of wheat bran arabinoxylans[23-25]. For example, Peng et al[26] have reported the treatment of dewaxed sugarcane bagasse with H_2O and 1% or 3% NaOH aqueous solution at a solid-to-liquid ratio of 1∶25 at 50℃ for 3 h, after which all of the hemicellulosic fractions were extracted by graded precipitation in ethanol at concentrations of 15%, 30%, and 60% (V/V). They found that the arabinose-to-xylose (Ara/Xyl) ratio increased with increasing ethanol concentration from 15% to 60%, and that arabinoxylans with higher Ara/Xyl ratios had higher molecular weights. Additionally, Bian et al[27] isolated *Caragana korshinskii* hemicelluloses with 10% KOH at 25℃ for 10 h from delignified materials. The alkali-extractable hemicelluloses were then subfractionated by graded ethanol precipitation with ethanol concentrations of 10%, 20%, 30%, 45%, 60%, and 80%. The experimental results revealed that more branched hemicelluloses with low molecular weights could be obtained with increasing ethanol concentration. Moreover, Nie et al[28] obtained hemicellulosic subfractions by graded ethanol precipitation from sweet sorghum stem by using 10% KOH during the extraction process. Thus, the graded ethanol precipitation technique allows hemicellulosic subfractions to be selectively precipitated[29]. However, the use of alkali with high concentration can corrode the extraction

equipments. Therefore, the development of simple, low-cost, and environmentally friendly pretreatment methods for the isolation of hemicelluloses is of great importance for exploring and promoting their potential industrial application.

The triploid of *Populus tomentosa* Carr. is a fast-growing poplar widely cultivated in China to prevent wind erosion and control desertification. In recent years, rapid progress has been made in the extraction and isolation of hemicelluloses from *Populus tomentosa* Carr.[22,30]. In the authors' previous studies, hemicelluloses were successfully extracted from *Populus tomentosa* Carr. via hydrothermal pretreatment in the dimethylformamide or aqueous NaOH or KOH solutions[16,22]. Herein, hydrothermal pretreatment was used to extract the degraded hemicellulosic fractions from *Populus tomentosa* Carr. in the presence of ethanol. The aim of the present study was to explore the effects of hydrothermal treatment and solvent on the hemicellulosic fractions. The chemical compositions and structural features of the isolated hemicellulosic fractions were investigated by a combination of sugar analysis, gel-permeation chromatography (GPC), Fourier transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (^{1}H NMR), carbon-13 nuclear magnetic resonance (^{13}C NMR), and two-dimensional heteronuclear singular quantum correlation (2D-HSQC) spectroscopy.

2 Experimental

2.1 Materials

Timbers of four-year-old triploid *Populus tomentosa* Carr. were obtained from Shandong Province, China. The leaves and bark were removed, and the stems were cut into small pieces. The main components of the raw material were determined to be cellulose (51.3%), hemicelluloses (20.7%), lignin (27.2%), waxes (0.5%), and ash (0.3%) on a dry weight basis, according to the Laboratory Analytical Procedure (LAP) provided by National Renewable Energy Laboratory[31].

The chips were ground with a plant crusher and passed through a mesh sieve with a pore size of 40~60 wires-per-inch. The obtained wood powder was oven dried at 50℃ for 16 h and dewaxed with ethanol/toluene (1∶2, V/V) for 8 h. Prior to mild alkali treatment, the dewaxed material was first treated with distilled water at a solid-to-liquid ratio of 1∶25 at 80℃ for 2 h. The residue was filtered, washed with distilled water, and oven dried at 50℃ for 16 h. Delignification of the dewaxed material was performed in a round-bottom flask, in which a 15 g sample was added to 300 mL distilled water containing 15 g $NaClO_2$. The mixture was then acidified to pH value of 3.8~4.0 with acetic acid, and the flask was heated in a water bath at 75℃ with continuous stirring for 1 h. Then, 7.5 g $NaClO_2$ and half the volume of the acetic acid used previously were added to the flask, which was kept for another 1 h under the same conditions. After delignification, the residue (holocellulose) was filtered off with a nylon cloth and washed thoroughly with distilled water until the pH value of the filtrate was neutral. The resulting solid was oven dried at 50℃ for 16 h.

All chemicals used were of analytical grade and purchased from Sigma Chemical Company (Beijing, China).

2.2 Fractionation of degraded hemicelluloses via hydrothermal pretreatment with ethanol

The scheme for the sequential treatment of *Populus tomentosa* Carr. and the isolation of hemicelluloses is illustrated in Fig.1. The obtained holocellulose was mixed with 90 mL of 10%, 20%, 30%, 45%, 60%, or 80% ethanol at a solid-to-liquid ratio of 1∶20. The mixture was transferred into a Teflon-lined stainless steel autoclave, which was sealed and maintained at 160℃ for 16 h. After being cooled naturally to room temperature, the product was separated by centrifugation (4000 r/min, 10 min), and the supernatant was adjusted to the pH value of 5.5 with 6 mol/L acetic acid, followed by concentration under reduced pressure (–0.1 MPa, 40℃) to about 30 mL. Subsequently, the concentrated solution was mixed with a 3-fold volume of anhydrous ethanol, and the mixture was kept still

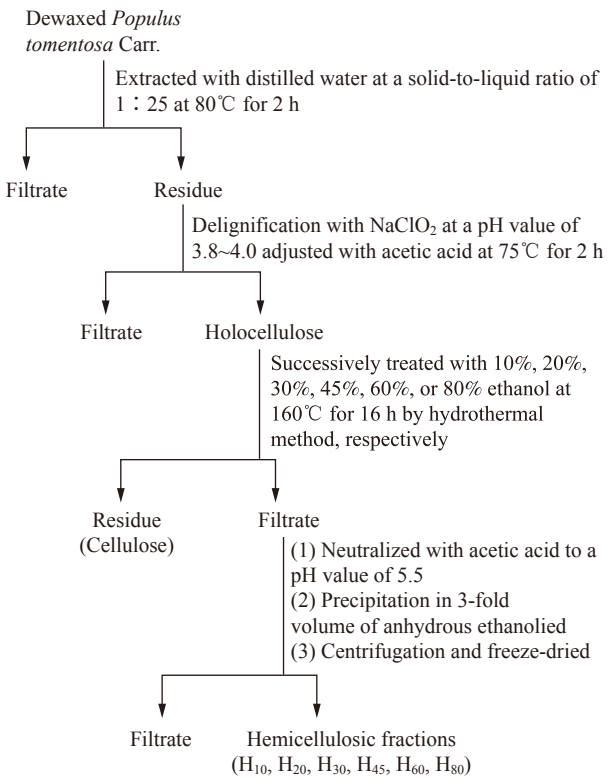


Fig.1 Scheme for the isolation of hemicelluloses from *Populus tomentosa* Carr.

overnight. The precipitates formed were recovered by centrifugation and freeze-dried, and these hemicellulosic fractions were labeled as H_{10}, H_{20}, H_{30}, H_{45}, H_{60}, and H_{80}, respectively.

2.3 Chemical characterization

The neutral sugar and uronic acid contents of the six hemicellulosic fractions were determined using a high-performance anion-exchange chromatography (HPAEC) system (Dionex ICS3000, USA) equipped with a Dionex ICS3000 gradient pump, an amperometric detector, an AS50 autosampler, and a Carbopac™ PA-20 column (Dionex). The monosaccharides in the hemicellulosic fractions were obtained by hydrolyzing 5 mg samples with 10% H_2SO_4 for 2.5 h at 105℃. Then, the samples were diluted 50-fold, filtered, and separated in a 5 mmol/L NaOH isocratic eluent (carbonate-free and purged with nitrogen) for 20 min, followed by a 0~75 mmol/L NaAc gradient in 5 mmol/L NaOH over 15 min. The columns were then washed with 200 mmol/L NaOH for 10 min to remove carbonate, followed by a 5 min elution with 5 mmol/L 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. Calibration was performed with standard solutions of *D*-rhamnose, *L*-arabinose, *D*-galactose, *D*-glucose, *D*-xylose, *D*-mannose, galacturonic acids, and galacturonic acids.

The molecular weights of the samples were estimated using GPC with a PL aquagel-OH 50 column (300×7.7 mm, Polymer Laboratories Ltd.), and calibrated with PL pullulan polysaccharide standards (peak average molecular weights 738, 12200, 100000, and 1600000 g/moL, Polymer Laboratories Ltd.). A flow rate of 0.5 mL/min was maintained, and the eluent was 0.02 mol/L NaCl in 0.005 mol/L sodium phosphate buffer (pH value of 7.5). Detection was achieved with a Knauer differential refractive index detector (RID). The column oven was kept at 30℃. Polysaccharides were dissolved in 0.02 mol/L NaCl in 0.005 mol/L sodium phosphate buffer (pH value of 7.5) at a concentration of 0.1%.

2.4 Spectroscopic characterization

FT-IR spectra were performed on a Thermo Scientific Nicolet iN10 FTIR Microscope (Thermo Nicolet Corporation, USA), which was equipped with a liquid-nitrogen-cooled mercury cadmium telluride (MCT) detector. The spectra were recorded in the wave number range of 675~4000 cm^{-1} at a resolution of 4 cm^{-1} with 64 scans per sample.

Solution-state ^1H NMR spectrum was recorded on a Bruker AVIII spectrometer operating in FT mode at 400 MHz using 20 mg of sample dissolved in 1.0 mL of D_2O. The chemical shifts were calibrated relative to the HOD signal (δ=4.70) from D_2O. The acquisition time was 10 s, and the relaxation time was 1 s. The ^{13}C NMR spectrum was also recorded on the Bruker AVIII spectrometer at 100 MHz using 80 mg of sample dissolved in 1.0 mL of D_2O containing a few drops of NaOD (7.5 mol/L). The ^{13}C NMR spectrum was recorded at 25℃ over 30000 scans. A 30° pulse-flipping angle, a 9.2 μs pulse width, a 1.36 s acquisition time,

and a 1.89 s delay time between scans were employed. HSQC spectrum was obtained over a t_1 spectral width of 20000 Hz and a t_2 width of 3600 Hz, and the time per scan was 0.1409 s. The number of collected complex points was 1024 for the ^{1}H-dimension with a relaxation delay of 1.5 s. The number of scans was 128, and 256 time increments were recorded in the ^{13}C-dimension. The $^1J_{C\text{-}H}$ value used was 146 Hz. Prior to Fourier transformation, the data matrices were zero filled up to 1024 points in the ^{13}C-dimension. Data processing was performed using standard Bruker Topspin NMR software.

3 Results and discussion

3.1 Neutral sugar and glucuronic acid contents

Hemicelluloses are a kind of branched polymer composed of several types of monosaccharides[1], the compositions of which can vary depending on the isolation method, the type of plant, and the part of the plant used. The neutral sugar and glucuronic acid contents of the six hemicellulosic fractions are listed in Table 1. Clearly, xylose is the dominant sugar component (59.72%~80.30%), while glucose and arabinose are the second and third most abundant sugar components, comprising 5.33%~21.96% and 2.50%~8.37% of the total sugars, respectively. Mannose (ND~6.51%), galactose (2.96%~6.15%), glucuronic acid (1.13%~14.37%), and rhamnose (ND~0.50%) are also observed as minor constituents. The high percentage of xylose residues probably originates from xylan backbones, which is consistent with previous reports[26-28]. A significant difference is observed for the H_{80}, in which higher contents of arabinose (8.37%), galactose (6.15%), and glucuronic acid (14.37%), but lower contents of xylose (59.72%) and mannose (3.13%) are observed compared with those of the other hemicellulosic fractions (H_{10}~H_{60}). Compared with H_{10}, the glucuronic acid content of H_{80} increases from 1.13% to 14.37% and the arabinose content increases from 2.50% to 8.37%. These results indicate that a higher ethanol concentration favors the isolation of hemicelluloses with more side chains. Ethanol is miscible with water at any ratio, and the addition of ethanol to water significantly changes its physicochemical properties and dissolution ability. The different ethanol concentrations endow the water with different hemicellulose dissolution abilities, leading to the isolation of hemicelluloses with different structures.

In this study, the content of xylose increases from 65.09% to 80.30% with increasing ethanol concentration from 10% to 45%, implying that increasing the ethanol concentration favors the extraction of hemicelluloses with more linear chains. However, the content of xylose then decreases from 80.30% to 59.72% with increasing ethanol concentration from 45% to 80%, revealing that higher ethanol concentration favors the isolation of hemicelluloses with more branched chains. The contents of arabinose and glucuronic acid increase from 2.50% and 1.13% to 8.37% and 14.37% upon with increasing the ethanol concentration from 10% to 80%, respectively, indicating that hemicelluloses with more branched chains are obtained using higher ethanol concentration. When the ethanol concentration is increased from 10% to 30%, the contents of xylose are

Table 1 Neutral sugar and glucuronic acid contents and glucuronic acid/xylose ratios of the hemicellulosic fractions

Hemicellulosic fraction	Sugers/%							Glucuronic acid/xylose
	Rhamnose	Arabinose	Galactose	Glucose	Xylose	Mannose	Glucuronic acid	
H_{10}	0.50	2.50	2.96	21.96	65.09	5.86	1.13	0.02
H_{20}	0.34	2.63	3.06	18.97	67.38	6.26	1.36	0.02
H_{30}	0.21	2.75	3.14	15.17	70.79	6.51	1.43	0.02
H_{45}	ND	3.38	3.26	5.33	80.30	3.56	4.17	0.05
H_{60}	ND	3.92	3.63	5.84	77.51	ND	9.10	0.12
H_{80}	0.16	8.37	6.15	8.10	59.72	3.13	14.37	0.24

*Neutral sugar and glucuronic acid contents are relative percent of the hemicellulosic samples; ND, not detectable.

lower than those reported in the literature[27]. However, when the ethanol concentration is increased from 45% to 80%, the content of xylose are higher than those reported in the literature[27]. These differences may be due to the different extraction pretreatments.

When the ethanol concentration is increased from 10% to 20% and 30%, the contents of glucose decrease from 21.96% to 18.97% and 15.17%, respectively. However, when the ethanol concentration is increased to 45%, 60%, and 80%, the contents of glucose dramatically decrease to 5.33%, 5.84%, and 8.10%, respectively. In general, a high content of glucose can be obtained at high temperature owing to the decomposition of cellulose and degradation of hemicelluloses via hydrothermal pretreatment. In a previous study[22], glucose (40.74%~63.40%) was observed during the degradation of lignocelluloses using hydrothermal pretreatment with dimethylformamide. In the current study, the lower ethanol concentrations (10%~30%) provide similar conditions to those used in the dimethylformamide method and favor the degradation of biomass to glucose, which is consistent with previously reported results[22]. Interestingly, a low content of glucose is obtained using higher ethanol concentrations (45%~80%) owing to the inhibition of cellulose decomposition and degradation of hemicelluloses, which is different from previously reported results[22]. These results indicate that hydrothermal pretreatment does not have a positive connection with the degradation of biomass to glucose, and that the pretreatment conditions play an important role in the sugar compositions and structures of the hemicellulosic fractions. Furthermore, these results indicate that some interesting phenomenon occur under complex hydrothermal pretreatment conditions, and that an intrinsic mechanism still needs to be established. It should be noted that the glucuronic acid/xylose ratios in H_{10}~H_{30} are only 0.02, while the values increase to 0.05, 0.12, and 0.24 for ethanol concentrations of 45%, 60%, and 80%, respectively. This result further demonstrates that the hemicellulosic fractions obtained under high ethanol concentrations have more side chains[28].

3.2 Distribution of molecular weights

The weight-average (*M*w) and number-average (*M*n) molecular weights and the polydispersity (*M*w/*M*n) of the six hemicellulosic fractions were investigated using GPC, and the results were shown in Table 2. The values of *M*w were 5101, 4755, 3880, 4167, 2842, and 4728 g/mol with increasing ethanol concentration from 10% to 80%, respectively. The results were completely different from those of the previous study[27], in which the value of *M*w first increased and then decreased with increasing ethanol concentration, demonstrating that hydrothermal pretreatment had a significant effect on the *M*w values of hemicelluloses. Moreover, all six hemicellulosic fractions (H_{10}~H_{80}) had low *M*w values (≤5101 g/mol), indicating that hydrothermal treatment combined with ethanol precipitation favored the solubilization of hemicelluloses with low *M*w values. In a previous study, the hemicellulosic fractions with high *M*w values of 14890~58900 g/mol were obtained through sequential treatments with 10% KOH aqueous solution and graded ethanol precipitation[27]. The current results are consistent with the results of neutral sugars analysis (Table 1), and are probably due to the slight degradation of hemicelluloses during hydrothermal pretreatment at relatively high temperature (160℃). Highly branched xylan fractions with higher *M*w than the less branched fractions were reported by Hoffmann et al[32]. However, the results for the neutral sugars indicate that H_{80} contains species with more branched chains and lower *M*w than those in H_{10}~H_{60}. These differences might be caused by differences in the hydrothermal pretreatment techniques employed. It is generally agreed that hemicellulosic fractions

Table 2 *M*w, *M*n and *M*w/*M*n of the six hemicellulosic fractions

Hemicellulose	*M*w	*M*n	*M*w/*M*n
H_{10}	5101	4339	1.18
H_{20}	4755	3396	1.40
H_{30}	3880	3780	1.03
H_{45}	4167	4026	1.04
H_{60}	2842	1192	2.38
H_{80}	4728	2023	2.34

with low *M*w can be obtained via hydrothermal pretreatment due to the degradation of hemicelluloses. However, in a previous study, hemicellulosic fractions with high *M*w values of 24840 g/mol were obtained via hydrothermal pretreatment[22]. Therefore, hemicellulosic fractions with both high and low *M*w can be extracted by hydrothermal pretreatment. This phenomenon further demonstrates that hydrothermal pretreatment has no direct effect on the degradation of hemicelluloses, and that it is not the hydrothermal pretreatment but the ethanol concentration which has a significant influence on the *M*w value of the hemicelluloses extracted.

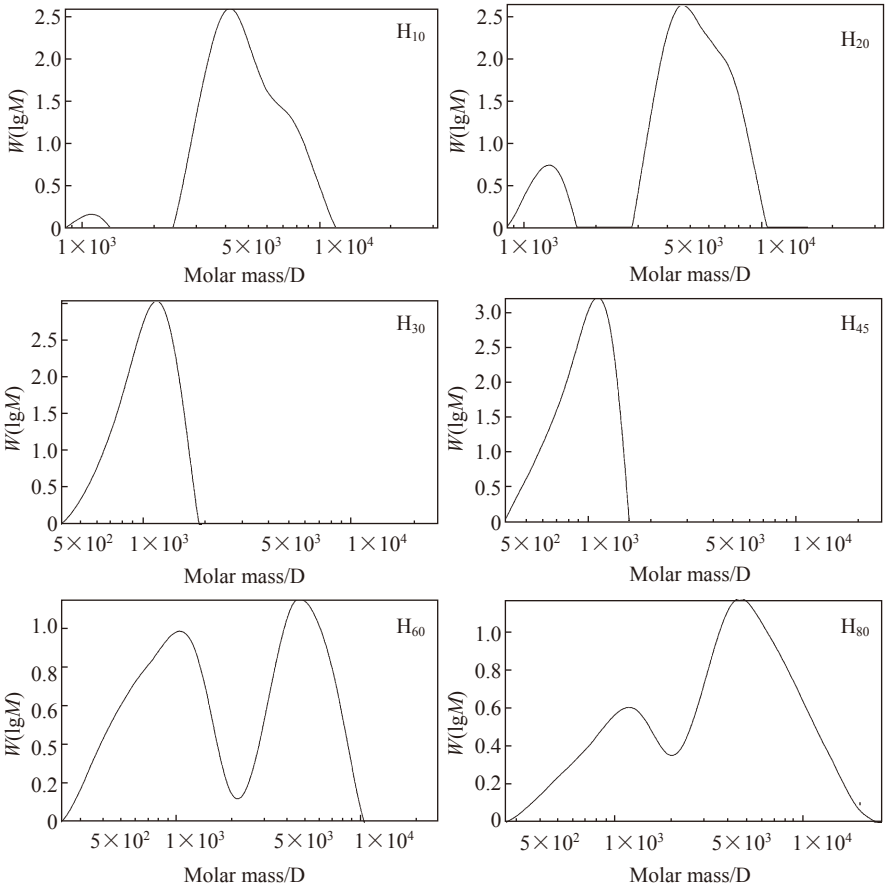


Fig.2 Molecular weight distribution curves of the six hemicellulosic fractions

The molecular weight distribution curves for the six hemicellulosic fractions are shown in Fig.2, and the polydispersity values were calculated as *M*w/*M*n. As seen from Table 2, the data show that the hemicellulosic fractions H_{10}~H_{45} have narrower molar mass distributions corresponding to polydispersity values of 1.18, 1.40, 1.03, and 1.04, respectively. In comparison, the hemicellulosic fractions H_{60} and H_{80} present broader molecular weight distributions with polydispersity values of 2.38 and 2.34, respectively. A low molecular weight (3880 g/mol) and narrow molecular weight distribution (1.03) is observed for H_{30}, and a high molecular weight (5101 g/mol) and narrow molecular weight distribution (1.18) is observed for H_{10}, while a high molecular weight (4728 g/mol) and broad molecular weight distribution (2.34) is observed for H_{80}. These results indicate that hydrothermal pretreatment with ethanol induces some interesting phenomena. This demonstrates that there are many unknown mechanisms needing to be further explored associated with hydrothermal pretreatment.

3.3 FT-IR spectra

The effect of ethanol concentration on the structures of the hemicellulosic fractions was explored using FT-IR spectroscopy, as shown in Fig.3. All of the hemicellulosic fractions have similar absorption peaks, indicating similar structures. The absorption bands at 3358 and 2902 cm^{-1} are attributed to the stretching vibrations of —OH and C—H, respectively. The absorption peaks in the 1200~1800 cm^{-1} region provide information about the polysaccharides. The absorption band at 1727 cm^{-1} is characteristic of the C═O in acetyl and uronic esters in the hemicelluloses, and this signal becomes stronger with increasing ethanol concentration from 10% to 80%, suggesting that the acetyl and uronic ester contents increase when the materials are treated with higher ethanol concentration, which is in good agreement with the increasing glucuronic acid contents revealed in the sugar analysis (Table 1). The absorption

band at 1644 cm^{-1} corresponds to the bending mode of absorbed water. The absorption peaks at 1428, 1370, and 1312 cm^{-1} are the bending vibrations of C—H, —OH, and —CH_2 in the hemicelluloses, respectively. The absorption band at 1245 cm^{-1} represents the —OH or C—O bending vibration in hemicelluloses[33]. The occurrence of the three adsorption peaks around 1727, 1370, and 1245 cm^{-1} in these spectra indicate the acetylation of hemicelluloses resulting from treatment with different ethanol concentrations. The absorption peak at 1160 cm^{-1} is typical peak of arabinoxylans, that at 1107 cm^{-1} is attributed to C—OH skeletal vibration, and that at 1024 cm^{-1} is dominated by C—O, C—C stretching, or C—OH bending in hemicelluloses[34-35]. The small absorption band at 898 cm^{-1} is due to the C-1 group frequency or ring frequency, which is the characteristic of β-glycosidic linkages between xylose units in hemicelluloses[36]. The aromatic skeleton vibrations attributed to lignin fractions located around at 1510 cm^{-1} are not observed in all six samples, indicating that there is no associated lignin in these hemicellulosic fractions[37].

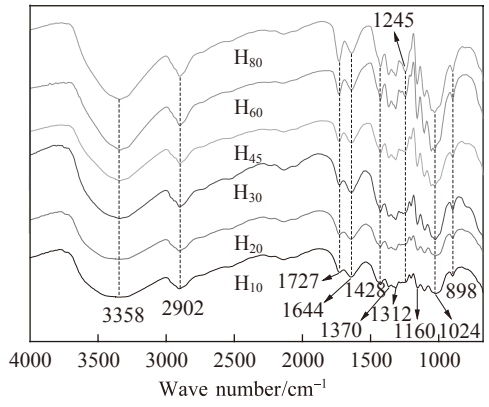


Fig.3 FT-IR spectra of the six hemicellulosic fractions

3.4 ^{1}H NMR, ^{13}C NMR and 2D-HSQC spectroscopy

In order to characterize the structural features of the hemicelluloses, the hemicellulosic fraction H_{60} was investigated by ^{1}H NMR, ^{13}C NMR, and 2D-HSQC spectrometry, as shown in Fig.4~ Fig.6. As seen from Fig.4, the signals at 4.32 (H-1), 3.95 (H-5eq), 3.64 (H-4), 3.40 (H-3), 3.25 (H-5ax), and 3.16 ppm (H-2) in the ^{1}H NMR spectrum are assigned to the (1→4)-β-*D*-

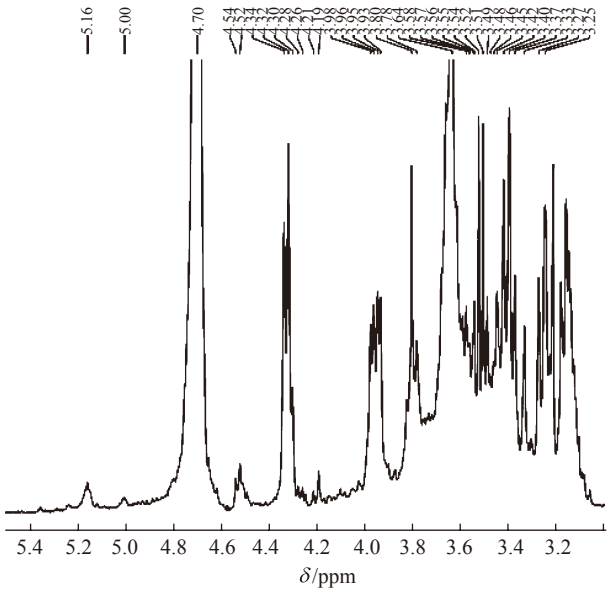


Fig.4 ^{1}H NMR spectrum (in D_2O) of H_{60}

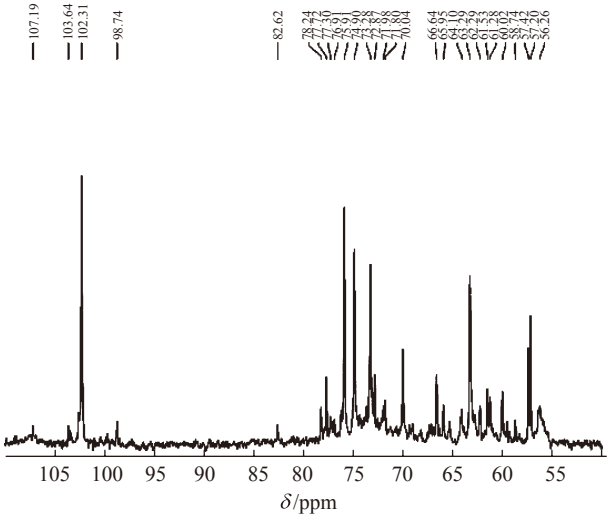


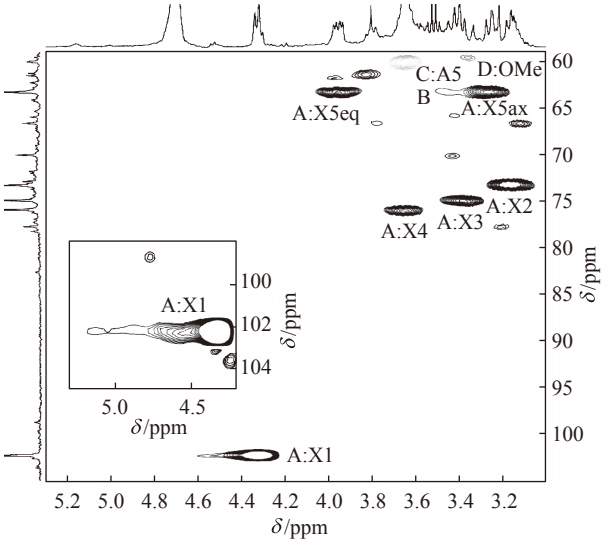
Fig.5 ^{13}C NMR spectrum of H_{60}

xylan backbone. The minor signals at 4.19 (H-5), 3.46 (H-2), and 3.33 ppm (OCH_3) can be attributed to 4-O-methyl-α-*D*-glucuronic acid residues[38], and the signal at 3.64 is derived from the protons at C-5 of the α-*L*-arabinofuranosyl residue. In general, the signals for the anomeric protons of arabinofuranosyl units often appear in the 5.2~5.4 ppm region, while those from the xylopyranosyl residues are presented at 4.4~4.7 ppm[29]. From Fig.4, the weak signal at 5.16 ppm is an overlapped signal that originates from arabinofuranosyl units attached to 2-O- and 3-O- of the same xylopyranosyl residue and 4-O-methyl-α-*D*-glucuronic acid (1→2) residues[32]. In addition, the strong signal at

4.70 ppm originates from the solvent (HDO).

^{13}C NMR spectroscopy was employed to obtain deeper insight into the branched structure of the hemicelluloses, as shown in Fig.5. The five main signals at 102.31, 75.91, 74.90, 73.28, and 63.29 ppm are assigned to the C-1, C-4, C-3, C-2, and C-5 positions of the (1→4) linked *β-D*-xylopyranosyl residues, respectively. The signals at 98.74, 82.62, 73.28, 71.98 and 58.74 ppm are the characteristics of C-1, C-4, C-5, C-2, and OCH_3-4 in the 4-O-methyl-*α-D*-glucuronic acid residue, respectively. Combined with the ^{1}H NMR, FT-IR, and sugar analysis results, one can conclude that H_{60} has a primary structure of (1→4)-*β-D*-xylan attached with minor amounts of substituted sugars and glucuronic acid.

Fig.6 shows the 2D-HSQC spectrum of sample H_{60}. The dominant signals at δ_C/δ_H 102.31/4.32, 75.91/3.64, 74.90/3.40, 73.28/3.16, 63.29/3.25, and 63.29/3.95 ppm are attributed to C-H_1, C-H_4, C-H_2, C-H_3, C-H_{5ax}, and C-H_{5eq} of the (1→4)-*β-D*-xylopyranosyl units, respectively. The signals at 3.25 ppm and 3.95 ppm are assigned to the axial and equatorial protons linked to C-5, and the signal at δ_C/δ_H 60.02/3.25 ppm is assigned to the methyl group of 4-O-methyl-*α-D*-glucuronic acid. Although the major peaks are distinct, there are regions



∗ A, (1→4)-*β-D*-xylopyranosyl; B, (1→4)-*β-D*-xylan-2-O-(4-O-methyl-*α-D*-glucuronic acid); C, *α-L*-arabinofuranosyl; D, 4-O-methyl-*α-D*-glucuronic acid.

Fig.6 2D-HSQC spectrum of H_{60}

in the spectrum where minor peaks overlap. Thus, they may still contain a small number of sugar units such as glucose and galactose, which are not assigned in this spectrum. This result is in good agreement with those for the of neutral sugar and glucuronic acid contents (Table 2).

4 Conclusions

The hemicellulosic fractions were isolated via hydrothermal pretreatment in ethanol. The hemicelluloses with smaller molecular weights were precipitated in 30% and 60% ethanol, and were rich in xylose (70.79% and 77.51%, respectively), principally resulting from 4-O-methyl-*D*-xylans. FT-IR spectroscopy show that these hemicellulosic fractions belong to the xylan family. Higher molecular weights were obtained using 10% and 20% ethanol. The hydrothermal method with ethanol is a simple and environmentally friendly pretreatment strategy for the extraction of biomass. It is hoped that this pretreatment will provide new opportunities for the isolation and application of biomass.

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