

Efficient extraction and characterization of polymeric hemicelluloses from hybrid poplar



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ABSTRACT

Hemicelluloses were isolated from hybrid poplar with the single-step alkaline extraction. Neutralization and subsequent ethanol precipitation were used to recover them. Their structures were investigated using sugar analysis, Fourier transform infrared and ¹³C nuclear magnetic resonance spectroscopy. The hemicelluloses were characterized by particle size analyzer, gel permeation chromatography and thermogravimetric analyzer. 36.4% and 50.4% of the original hemicelluloses were isolated in the form of polymer by neutralization and precipitation, respectively. The hemicellulosic fraction (HA) by neutralization was more homogeneous in sugar components than the one (HB) by precipitation. However, hemicellulose isolated by both of the process has chemical structure, as were (4-O-methylglucurono)-D-xylans. The weight average molecular mass of HA was 17,480 g mol⁻¹, higher than one of HB (14,670 g mol⁻¹) and they were both with a low polydispersity. HA had lower zeta potential and higher thermal stability than HB. Therefore, the procedures in this study were efficient for the isolation and the recovery of polymeric hemicelluloses with different properties.

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1. Introduction

Because of the continuous exhaustion of the ready sources of feedstocks, such as coal and petroleum-based compounds, there is a need for developing alternative renewable feedstocks. Hemicelluloses are the second most abundant renewable biopolymers after celluloses. It is considered as branched heteropolysaccharides present in lignocelluloses and of low molecular weight with a degree of polymerization of 80–200. At present, hemicelluloses are underutilized. In kraft pulping, hemicelluloses, degraded into the waste pulp liquor, are concentrated and burned in the recovery furnace although the heating value of carbohydrates is approximately half of that of lignin (McKendry, 2002). Hemicelluloses can be hydrolyzed into sugars and then fermented into ethanol, but the industrial fermentation of pentoses has not yet been achieved in a cost-efficient way (Ragauskas, Williams et al., 2006). The general trend toward sustainability in the forest industry, and a growing interest for the biorefinery concept, has recently brought forth the issue of utilizing wood hemicelluloses as a natural resource of higher value-added products (Ragauskas, Nagy, et al., 2006). Hemicelluloses have been used for the synthesis of ionic polymers (Schwika, Heinze, Ebringerova', & Petzold, 2006; Šimkovic,

Gedeon, Uhliariková, Mendichi, & Kirschnerová, 2011), hydrogels for controlled drug release (Lindblad, Ranucci, & Albertsson, 2001; Sun, Wang, Jing, & Mohanathas, 2013), and oriented derivatives (Hettrich et al., 2006; Jain, Sjöstedt, & Glasser, 2001). There are two main factors determining the practical use of hemicellulose isolates. First, the chemical (sugar) composition, which is primarily a plant specific feature, needs to be considered. Second, the methods of isolation of hemicelluloses influence their structures, and hence the possible fields of their applications (Benko et al., 2007). Therefore, interest in the isolation and characterization of hemicelluloses from biomass has greatly increased day by day.

To date, a variety of effective pretreatment methods to hydrolyze and fractionate hemicellulose components have been investigated. However, alkaline extractions have been proved to be a promising process to extract hemicelluloses with higher molecular weight although they deacetylate hemicelluloses (Benko et al., 2007; Huang, Ramaswamy, Tschirner, & Ramarao, 2008). Two aqueous alkali processing methods have been proposed. One is to extract hemicelluloses from the holocellulose by aqueous alkali, and another is to extract the hemicelluloses from the dewaxed materials directly. The latter is an eco-friendly extraction process (Peng, Peng, Xu, & Sun, 2012). In order to increase the hemicellulose yield, ultrasound was applied during the alkali extraction (Hromadkova & Ebringerov, 2003). However, the ultrasound would also result in degradation of the hemicelluloses to oligomeric and monomeric forms, which significantly limits

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the possible applications of the hemicelluloses (Vodeničarová, Dřimalová, Hromádková, Malovíková, & Ebringerová, 2006). Therefore, the ultrasound was also applied in the delignification before the alkali extraction to reduce the degradation of dissolved hemicelluloses (Yuan, Xu, He, & Sun, 2010).

Hybrid poplar is planted extensively for paper and plywood in China, and it provided good resources for producing hemicelluloses. Hemicelluloses can be preextracted from hybrid poplar wood before pulping or isolated from the residues such as twigs, wood shaving and sawdust in the processing hybrid poplar wood. The hemicellulose fractions of hybrid poplar have recently been investigated and characterized using multi-step extraction by Yuan et al. (2010), but the procedures for extraction of hemicellulose were time-consuming and complex, and much process liquid was produced. The processes would face some challenges when they are applied in industrial scale. In our study, ultrasound treatment was applied in the partial delignification rather than in the alkali extraction in order to increase the yield of high-molecular mass hemicelluloses. Then, polymeric hemicelluloses were extracted from hybrid poplar using a single-step process to simplify the isolation process. The isolated hemicelluloses were recovered by neutralization of hemicellulose extract and subsequent ethanol-precipitation, respectively. Until now, we still have little information about the efficiency of the extraction procedure used in our study and the difference in the properties of the isolated hemicellulose fractions. The objective of this work was to investigate the efficiency of the modified procedures for the extraction of polymeric hemicelluloses with high molecular weight, to characterize the isolated hemicellulose fractions. The primary results based on the modified procedures provide the basis for further study of economically producing polymeric hemicelluloses from hybrid poplar.

2. Materials and methods

2.1. Materials

A hybrid poplar tree, 6 years old, was provided by Shihong Wood Processing Factory in Jiangsu Province. The air-dried and bark-free wood sample was cut into small pieces and then ground to pass through a 2 mm size screen and the fraction between 1.8 and 0.7 mm was collected for experimental work. The main components of samples were as follows: cellulose (43.4%), hemicelluloses (28.5%), and lignin (22.5%) on a dry weight basis.

2.2. Extraction and isolation of hemicellulose

A scheme for alkaline extraction of hemicelluloses is shown in Fig. 1. The dried sample was first extracted with toluene–ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h. After the dry, the dewaxed sample was treated with 70% ethanol solution containing 1% (w/v) NaOH aided by ultrasound at 75 °C for 3 h in a 250 mL round-bottomed flask using the Sonic system Scientz-IID (NingBo, Zhejiang) with the sonic power of 400 W and irradiating frequency of 30 kHz. The ratio of liquid to solid was 20 and the ultrasound irradiated for 4 s every 2-s pause. After filtration, the filtrate was used for the determination of hemicellulose content after dilute acid hydrolysis as mentioned below, and the hemicellulosic fraction was named as HDL. The solid residue was dried in an oven at 40 °C for 20 h. The partially delignified sample was treated with 8% NaOH solution at 90 °C for 10 h using the ratio of liquid to solid of 20, and then was filtrated. The residue was analyzed for hemicelluloses using two-step hydrolysis. The filtrate was collected for recovery of solubilized hemicellulose. At 25 °C, the filtrate was neutralized using 2 M acetic acid solution to pH 6.5 under violent stirring, and

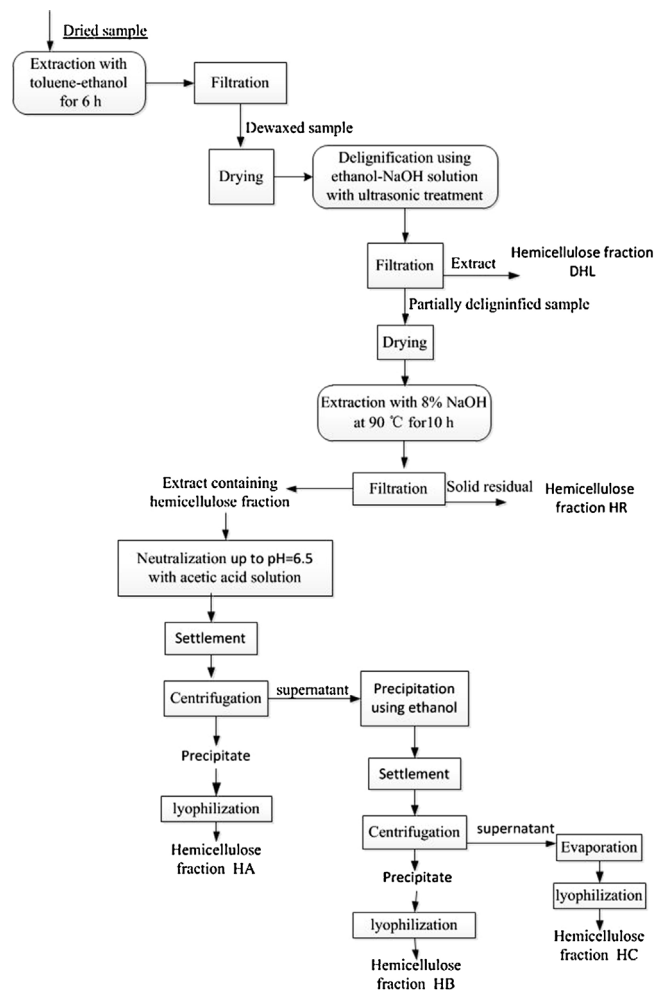


Fig. 1. Scheme for extraction and recovery of hemicelluloses from hybrid poplar.

then stood overnight at 4 °C. The mixture was centrifuged at Allegra 64R centrifugator (Beckman Coulter, USA.) with 20,000 rpm, and the precipitate was washed using some ultrapure water and centrifuged three times. Finally the precipitate was lyophilized at Heto Powerdry LL3000 freeze dryer (Thermo, Denmark) and was named as HA. All the supernatant after centrifugation was pooled and precipitated by 3-fold volumes of 95% ethanol. The mixture stood overnight, and then was centrifuged. The precipitate was lyophilized and named as HB. The supernatant was evaporated under reduced pressure for hemicelluloses to be determined; the hemicelluloses were named as HC.

2.3. Analysis and characterization

The sugar compositions in dewaxed samples and residue after alkaline extraction were analyzed using two-step hydrolysis (Sluiter et al., 2005) followed by high-performance liquid chromatography (HPLC) analysis of the sugars mentioned below. The sugar compositions of the four hemicellulosic fractions (HA, HB, HC and HDL) were determined using HPLC after hydrolysis with dilute sulfuric acid. A ~4–6 mg sample of hemicelluloses was hydrolyzed with 84 mL of 4% H₂SO₄ for 1 h at 121 °C (Tunc & van Heiningen, 2008).

After hydrolysis, the mixture was filtered, neutralized, and then analyzed by HPLC system (Agilent 1100, USA) with refractive index detector, AS50 autosampler, the Aminex HPX-87P column (Bio-Rad, USA), and it was operated at 80 °C using ultrapure as the mobile

phase at 0.4 ml min^{-1} . Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-mannose, and D-galactose (Fluka Chemie AG, Buchs, Switzerland).

The acid-soluble lignin content of the hemicellulose fraction was measured by UV-vis absorbance at 280 nm using an extinction coefficient of $20.3 \text{ (L g}^{-1} \text{ cm}^{-1})$ for hardwoods (Tunc & van Heiningen, 2008), and the acid-insoluble lignin content was determined gravimetrically. The uronic anhydride content in the hemicellulose fraction was determined using the method proposed by Li, Kisara, Danielsson, Lindstrom, and Gellerstedt (2007). The analysis of sugar composition was run in triplicate, and average values were reported here.

The mass of hemicelluloses was defined as the sum of all detectable hemicellulosic sugars including xylose, arabinose, mannose, galactose and uronic acid in the sample. The hemicellulose percentage was defined as the ratio of its mass in the obtained stream to the one in the dewaxed sample.

The molecular mass distribution of the obtained hemicelluloses was determined using a size-exclusion chromatography system (AKTA explorer 100, GE, Sweden). The chromatograph was equipped with a reflective index (RI) detector (XY-4000K, Beijing, China), an ultraviolet (UV) detector (GE, Sweden) operating at 280 nm and two column (16 mm I.D.) in series, packed with 60 cm of Superdex 75 and 60 cm of Superdex 200 (both from GE, Sweden), respectively. 200 mg of hemicellulose fraction was dissolved in 5 ml of 2% NaOH solution, and then filtrated through $0.22 \mu\text{m}$ syringe filter. The columns were operated at 25°C , and eluted with 0.1 M NaCl in 0.1 M NaOH solution at a flow rate of 0.4 ml min^{-1} . The system was calibrated with polyethylene glycol standards with peak molecular masses of 400, 1000, 2000, 6000, 10,000, 20,000 and $30,000 \text{ g mol}^{-1}$ (Sigma, USA) (Persson, Ren, Joelsson, & Jönsson, 2009).

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet 6700, USA) using a KBr translucent disk containing 1% finely ground samples.

The solution-state ^{13}C NMR spectra were obtained on a Bruker DRX600 (Bruker BioSpin GmbH) spectrometer at 600 MHz. The spectrum was recorded at 35°C from 80 mg of samples dissolved in 1.0 ml DMSO containing 1% LiCl after 30,000 scans. A 12-ms pulse width and a 2-ms relaxation delay were used.

The size distribution and zeta potential were determined on Zetasizer ZEN 3600 (Malvern, UK), 100 mg of samples was completely dispersed in 10 ml 10% DMSO solution at room temperature, and stirred for 8 h. All the data were recorded at 25°C .

The thermal stability analysis was performed using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (DTG-60, Shimadzu). The apparatus was continually flushed with a nitrogen flow of 30 mL min^{-1} . The sample weighed between 9 and 11 mg and was heated from room temperature to 600°C at a rate of $10^\circ\text{C min}^{-1}$.

3. Results and discussion

3.1. Percentage of hemicelluloses after the alkaline extraction

The extraction and recovery procedure of hemicelluloses from hybrid poplar is presented schematically in Fig. 1. HA was isolated from the extracts by neutralization to pH 6.5 with acetic acid. HB was precipitated from the neutralized extracts, which the HA had already been recovered from, by the addition of ethanol. HC was ethanol-soluble hemicellulose fraction after the vacuum evaporation of ethanol. HR was the hemicellulose fraction remained in the residues after the alkaline extraction. HDL was the hemicelluloses dissolved during the partial delignification. The hemicellulose

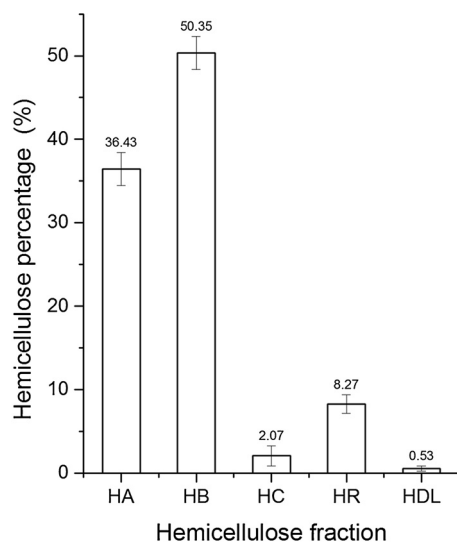


Fig. 2. Hemicellulose profile after the alkaline extraction, HA obtained from the alkaline extract by neutralization, HB from the extract by ethanol-precipitation after the neutralization, HC from the extract after the neutralization, and ethanol-precipitation, HR left in the residual after the alkaline extraction, HDL, obtained from partial delignification.

percentage in each stream, on the basis of the amount of hemicellulose in dewaxed sample, is presented in Fig. 2.

Fig. 2 showed that about 36.5% (HA) of hemicelluloses in dewaxed sample was isolated after the neutralization of the alkaline extract, and about 50.4% (HB) of them was isolated using the ethanol-precipitation after the recovery of HA. Only about 8.3% of hemicellulose was still left in the solid residues after alkaline extraction, primarily due to the hydrogen and covalent linkages between hemicelluloses and cellulose and lignin, respectively. In addition, only about 2.1% (HC) of hemicelluloses remained in liquid phase after ethanol-precipitation. HC was considered as heavily branched and/or with low molecular weight, probably due to the degradation of hemicelluloses (Verwimp, Craeyveld, Courtin, & Delcour, 2007). These data suggested that polymeric hemicellulose could efficiently be extracted and recovered using the procedures and the degradation of hemicellulose into oligomers or monomers could be negligible. Additionally, only 0.5% of hemicelluloses was dissolved during the partial delignification. This result illustrated that the released hemicelluloses in the delignification was really trivial. It was shown the overall hemicellulose yield (about 87%) using the single alkaline extraction after alkaline-ethanol pretreatment aided with ultrasonic irradiation was higher than the one (75.5%) obtained by multi-step alkaline extraction reported (Yuan et al., 2010). Meanwhile, the overall hemicellulose yields were only 73.6% and 45.8% in control extractions when the raw material was delignified using alkaline-ethanol treatment without ultrasonic irradiation, and when the raw material did not undergo any delignification, respectively. Therefore, it was shown that delignification aided with ultrasonic irradiation could enhance the overall yield of hemicelluloses significantly. It was reported that ultrasonic irradiation could cause the glycosidic linkage among the sugars to cleave and result in the degradation of polysaccharides (Vodeničarová et al., 2006). In our study, the ultrasonic irradiation was applied in the partial delignification of dewaxed samples rather than in the extraction. This layout might be favorable to avoiding the degradation of dissolved hemicelluloses due to radical reaction from ultrasonic irradiation, and preserving their structure, but promoting the rupture of the bond between lignin and hemicelluloses (Garcia, Alriols, & Labidi, 2012). On the other hand, when the pressure waves from ultrasonic irradiation travel through the surface of

Table 1
Composition of obtained polymeric hemicelluloses (%).

Composition	Fractions	
	HA	HB
Glucose	0.18	3.10
Xylose	90.03	79.00
Galactose	0.29	0.65
Arabinose	0.55	0.99
Mannose	0.46	5.47
Glucuronic acid	0.63	6.22
Acid-soluble lignin	3.25	3.18
Acid-insoluble lignin	6.40	0.40

samples, the cavitation bubble will collapse, and cause microfractures in the samples (Vinatoru, 2001; Vinatoru et al., 1997), thus facilitating the subsequent alkaline extraction. Therefore, these results suggested that alkaline extraction preceded by delignification aided with ultrasonic irradiation was effective and feasible for the isolation and recovery of polymeric hemicelluloses from hybrid poplar.

3.2. Composition of hemicellulose fractions

The hemicellulosic polymer is a mixture of a number of different polysaccharides, and composition of the polymer can vary depending on the methods of isolation. The compositions of only HA and HB fraction are presented in Table 1 due to the negligible amount of HC and HDL.

Table 1 showed that xylose was dominant component sugar in HA and HB. Nevertheless, some differences in the chemical contents of these two hemicellulosic fractions were observed. The content of xylose was up to 90% of the total fractions in the HA. Meanwhile, glucose, galactose, arabinose, and glucuronic acid were observed as a minor, all comprised less than 1% of the total fractions. Besides, HA also contained comparable lignin, and the content of acid-insoluble was 6.40%, doubling the one of acid-soluble one (3.25%). In contrast to HA, the content of xylose in the HB was only 79% of the total fractions. Nevertheless, other hemicellulosic sugars were also significant in HB. Compared with the ones in HA, the contents of glucose, mannose and glucuronic acid increased more than ten-fold and galactose and arabinose did two-fold. The contents of acid-soluble lignin were almost equal in both of the hemicellulosic fractions, being about 3%, although the content of acid-insoluble was only 0.4% of the total mass in HB. These data suggested the HA was more homogeneous than HB in terms of sugar components, but it contained more lignin than HB. HB, more branched, could consist of glucuronoxylan mixed with some glucomannan (Gaillard, 1965).

It is well known that glucuronoxylans represents more than 90% of the hemicellulos component in hardwoods, and the mole ratio of methyl-glucuronic group to xylose varies between 1:4 and 1:16. In addition, xylan containing only glucuronic acid side chains in various proportions (the mole ratio of glucuronic group to xylose is 1:6–43) was reported (Ebringerová & Heinze, 2000). The ratio of glucuronic acid to xylose for HB was found to be 1:12. In addition to xylan, hardwoods contain 2–5% of a glucomannan (GM), which is composed of β -glucopyranose and β -mannopyranose units linked by (1 \rightarrow 4)-bonds. However, the mannose/glucose monomer ratio may vary depending on the original source of GM. The ratio of glucose to mannose varies between 1:2 and 1:1 (Sjöström, 1981). The ratio for HB was found to be 1:1.8. It was shown that the ratios of sugar residue in HB were almost consistent with the reported ones. This finding showed that the HB was mainly glucuronoxylans with a low degree of substitution, and contaminated with a small amount of glucomannan and trace amount of other saccharides. Therefore, these results suggested that neutralization and ethanol

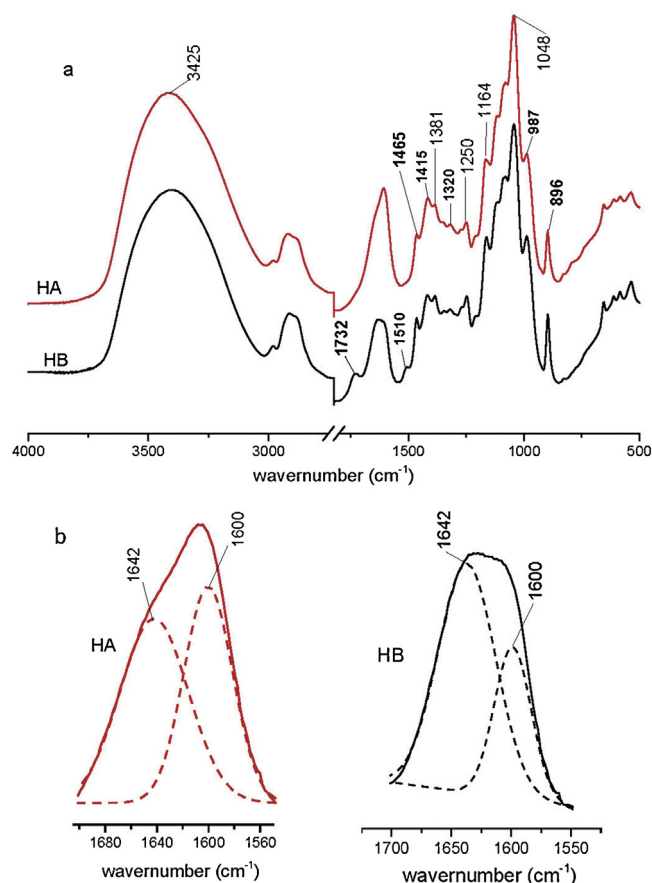


Fig. 3. (a) The FTIR spectra of HA and HB and (b) the peaks (dash) obtained by peak analysis of their absorbance bands (solid) between 1550 and 1700 cm^{-1} .

precipitation could efficiently recover the hemicellulosic fractions with different components.

In general, hemicelluloses in hardwoods hardly contain arabinosic and galactosic residues. However, Table 1 showed that the HA and HB contained a trace amount of arabinose and galactose. Some literatures reported that the hemicellulose from hybrid poplars contained arabinosic and galactosic residues (Yuan et al., 2010). The results might be attributable to the fact that hybrid poplars are crosses between North America's cottonwoods, aspens and Europe's poplars.

3.3. FTIR spectra of hemicellulosic fractions

FTIR spectroscopy, which potentially offers the assignment of absorbance bands to specific molecular structures, when combined with other methods of analysis, can be used for the analysis of lignocellulose. The FTIR spectra of hemicellulosic fractions, HA and HB, are shown in Fig. 3.

A typical signal pattern for hemicelluloses was observed from Fig. 3a. The absorption at 3425 cm^{-1} is attributed to the stretching of the OH groups. The absorptions at 1465, 1415, 1381, 1320, 1250, 1164, 1048, 987, and 896 cm^{-1} are associated with hemicelluloses (Peng et al., 2009). All these absorbance bands occurred simultaneously in the spectra of both HA and HB. These results suggested that main structures of hemicelluloses in HA and HB were similar to each other. The peak, at 896 cm^{-1} in Fig. 3a, is attributable to the beta-(1–4)-glycosidic linkages between the xylose units in the hemicelluloses (Ebringerová, Hromádková, Alföldi, & Berth, 1992; Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). As shown in Fig. 3a, the peak at 1048 cm^{-1} , which is assigned to the

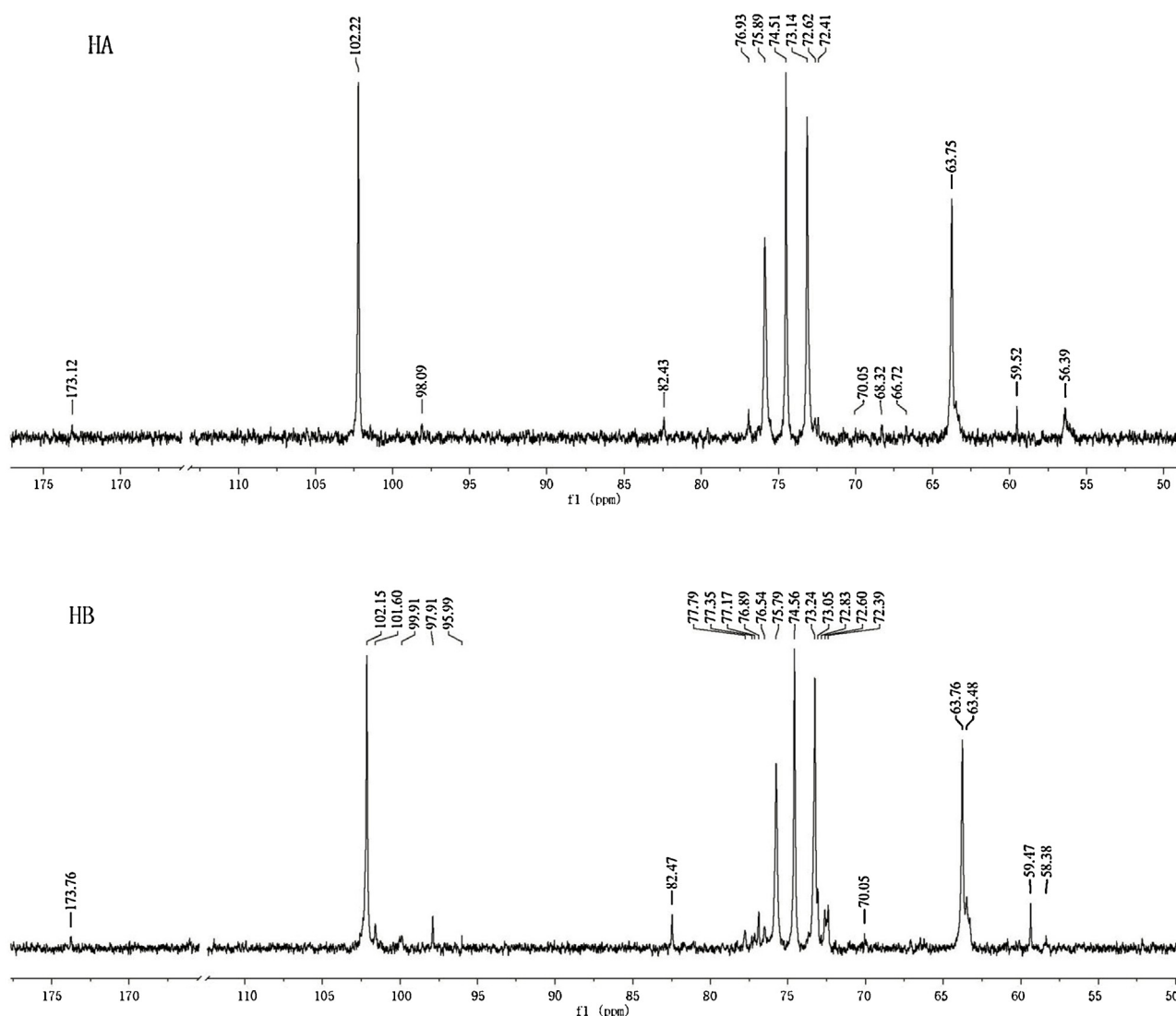


Fig. 4. ^{13}C NMR spectra of hemicellulose fraction HA and HB.

C–O, C–C stretching vibration or C–OH bending vibration, indicates a typical absorbance for xylan in the isolated hemicelluloses. In addition, the peak was stronger in HA spectrum than the one in HB spectrum, indicating that HA had more xylan than HB, (as seen in Table 1). The two peaks at 1164 and 987 cm^{-1} indicate the present of the arabinosyl side chains, which have been reported to be attached only at positions of the xylopyranosyl constituents (Kačuráková, Ebringerová, Hirsch, & Hromádková, 1994). The two peaks in HB spectrum were much stronger than the one in HA spectrum. The result showed the HB contained more arabinose than HA did. On the other hand, Fig. 3a showed a small band at 1732 cm^{-1} in spectra of HB, instead of HA, was observed. The band is characteristic of C=O group (An, Dong, Dung, & Thien, 2011). The result suggested the HB contained more carbonyl acid group than HA did.

Additionally, Fig. 3a showed the occurrence of a very small band at 1510 cm^{-1} largely due to the presence of small amounts of associated guaiacyl nuclei of lignin in the hemicelluloses. It was also shown that the peaks between 1550 and 1700 cm^{-1} were overlapped in the spectra of both HA and HB due to poor resolution. Peak analysis (Gaussian peak fit) was performed to find the overlapped peaks. The results from peak analysis are presented in Fig. 3b, and it was shown the original absorption bands (solid lines) between 1550 and 1700 cm^{-1} in the spectra of both HA and HB were decomposed into two peaks (dash line) at 1642 and 1600 cm^{-1}

(Adj. $R^2 = 0.999$), respectively. The absorption bands at 1642 and 1600 cm^{-1} have been attributed to hydration water present in xylan-type polysaccharides (Oliveira et al., 2010; Sarwar, Nasima, Khalidul, & Iqbal, 2007) and to syringyl nuclei of lignin in the hemicelluloses, respectively. The area under peaks at 1600 cm^{-1} in the spectra of HA and HB was 14.7 and 4.5 by using Gaussian peak fit. These results suggested that the HA contained more lignin than HB and that the lignin properties in HA were different from the ones in HB. Therefore, all these results were in agreement with the results obtained from sugar analysis of HA and HB (Table 1).

3.4. ^{13}C NMR spectra of hemicellulosic fractions

To further confirm the structural features of HA and HB, they were analyzed using NMR spectroscopy. ^{13}C NMR spectra of HA and HB are presented in Fig. 4. The spectra were interpreted on the basis of reported data for structurally defined arabinoxylan-type, and (4-O-methyl-D-glucurono)-D-xylan (Ebringerová et al., 1992; Imamura, Watanabe, Kuwahara, & Koshijima, 1994). As seen from Fig. 4, five strong signals at 102.2, 75.9, 74.5, 73.2, and 63.8 ppm were shown in the spectra of HA and HB. These signals correspond to C-1, C-4, C-3, C-2, and C-5 position of the 1, 4-linked beta-D-xylopyranose units. On the other hand, seven weak signals at 173.2, 98.1, 82.4, 76.9, 72.4, 70.0 and 59.5 ppm were found, which

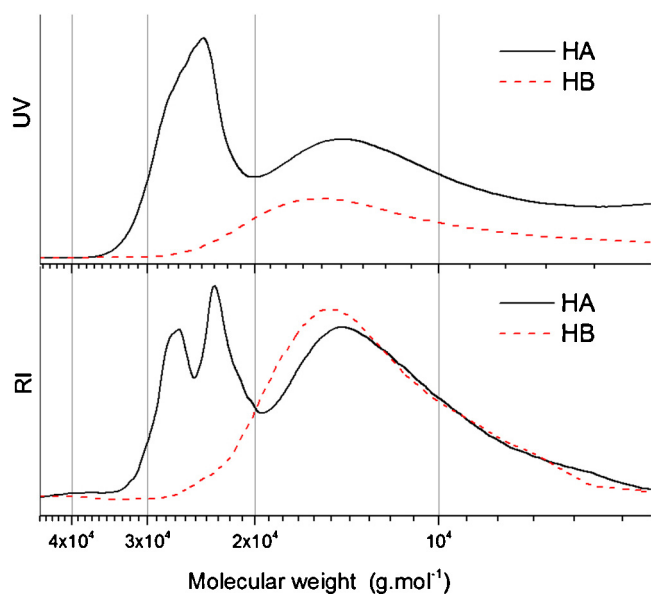


Fig. 5. Molecular weight distribution of HA and HB.

correspond to C-6, C-1, C-4, C-3, C-2, C-5 and 4-O-methoxyl group of glucuronic acid residues in the xylan. And a weak signal at 72.6 ppm was also found, corresponding to C-2 of the beta-D-xylopyranose units linked to glucuronic acid. These suggested that the main structures of HA and HB were (4-O-methyl-D-glucurono)-D-xylan. The areas of peak at 59.5 ppm were 0.52 and 1.12 for HA and HB, respectively. This suggested the HB contained more glucuronic acid than HA, as seen from Table 1. Comparison of NMR spectra of HB with that of HA, signals between 55–57 ppm occurred only in HA spectra rather than in HB spectra, arising from $-\text{OCH}_3$ syringyl and $-\text{OCH}_3$ guaiacyl groups of lignin, respectively (Sun, Xu, Sun, & Wang, 2004). These results suggested the HA contained more lignin than HB. By comparison, some weak signals at 101.6, 77.8, 76.5, 73.0, 71.9 and 63.5 ppm occurred only in the HB spectra rather than HA spectra, indicative of C-1, C-4, C-3 and C-6 of mannose moieties. At least, this result suggested that HB contained more mannan than HA. Therefore, the NMR data illustrated that (4-O-methyl-D-glucurono)-D-xylan was main component in HA and HB, and that the xylan in HA was less substituted by glucuronic acid than one in HB. Meanwhile, HA contained a small amount of lignin and HB comprised a noticeable amount of mannan. They were also in agreement with the results of sugar analysis and IR.

3.5. Molecular weight distribution of hemicellulosic fractions

In order to investigate the difference in molecular weight between hemicellulosic fractions HA and HB, the hemicellulosic preparations were analyzed by gel-permeation chromatography (GPC) using dual detectors for the simultaneous detection of lignin (UV absorbance) and carbohydrates (refractive index), it is possible to get information about the composition and molecular mass distribution of the different types of polymers constituting the hemicellulosic fractions. The GPC results are shown in Fig. 5. As seen from Fig. 5, it was shown that the molecular weight of HA was between 3.3×10^4 and $0.5 \times 10^4 \text{ g mol}^{-1}$, and its chromatogram had three peak signals corresponding to the molecular weights of 2.7×10^4 , 2.3×10^4 and $1.4 \times 10^4 \text{ g mol}^{-1}$, respectively. On the other hand, the one of HB was between 3×10^4 and $0.5 \times 10^4 \text{ g mol}^{-1}$, and its chromatogram had only one peak signal corresponding to the molecular weights of $1.5 \times 10^4 \text{ g mol}^{-1}$. In comparison with HB, there were much more polysaccharides with molecular weight of greater than $2 \times 10^4 \text{ g mol}^{-1}$ in HA.

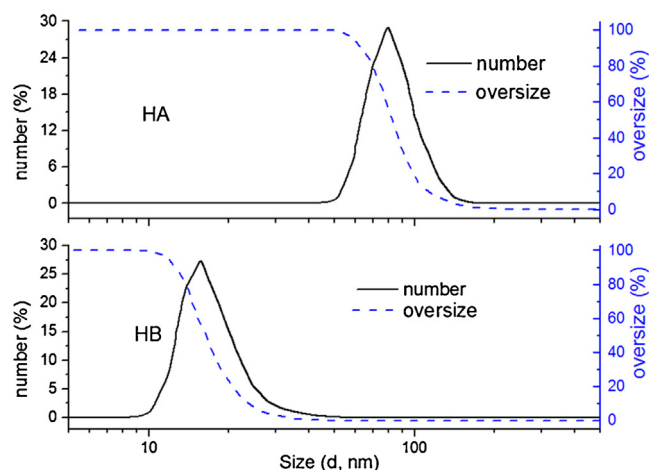


Fig. 6. The size distribution of HA and HB.

However, the UV signal between 2×10^4 and $3 \times 10^4 \text{ g mol}^{-1}$ for HA was also strongest of all signals. These data suggested that HA had a bit higher molecular weight than HB, but the portion of higher molecular weight in HA contained more lignin fragment. Compared with both RI and UV signals, they had the similar pattern to each other. Maximum or/and minimum UV signals occurred at the same retention time as corresponding RI signals did. In addition, UV absorbing components were distributed over all the hemicellulose molecules. The result illustrated that both the hemicelluloses and lignin are eluted together in the two hemicellulose fractions. This further suggested that these polymers were linked together whereas the hemicellulose fractions only contained very small amounts of UV absorbing material (lignin fragment) as seen in sugar analysis.

By numerical processing of HA and HB chromatograms (relationship of signal with eluent volume, not shown), the number average molecular mass (M_n) and weight average molecular mass (M_w) of HA were 13,370 and $17,480 \text{ g mol}^{-1}$, respectively; and the M_n and M_w of HB were 11,550 and $14,670 \text{ g mol}^{-1}$, respectively. Their polydispersity defined by the ratio M_w/M_n , was 1.31 and 1.27, respectively. Their polydispersity values are always under 1.40. This is a rather small value for a natural polymer. Therefore, it was suggested the isolated hemicellulosic fractions were relatively homogeneous in molecular mass. The molecular mass of the isolated hemicellulosic fractions was lower than the ones reported (Yuan et al., 2010). The plausible reasons might be the distinction in raw materials, method of calibration during GPC, and extraction method.

3.6. The size distribution and zeta potential

Laser diffraction was employed to analyze the size distribution of both HA and HB (Fig. 6). Fig. 6 showed that about 90, 50, 10 and 0% of the HA was larger than 62.7, 82.9, 112.5 and 160 nm, respectively; that about 90, 50, 10 and 0% of the HB was larger than 12.6, 16.4, 23.4 and 32.8 nm, respectively. The span index was used to analyze the polydispersity in the particle size distribution. It is defined as $(D_{10}-D_{90})/D_{50}$, where D_{10} , D_{50} , and D_{90} are the respective particle sizes at 10, 50, and 90% cumulative percentage oversize. The span index of both HA and HB was about 0.6. These results illustrated that the particle size of both samples was uniformly distributed, and that had a low polydispersity. These results were in agreement with the ones of molecular weight distributions obtained by GPC.

The zeta-potentials of HA and HB were about minus 35 and minus 52 mV, respectively, indicating that HB had more negative

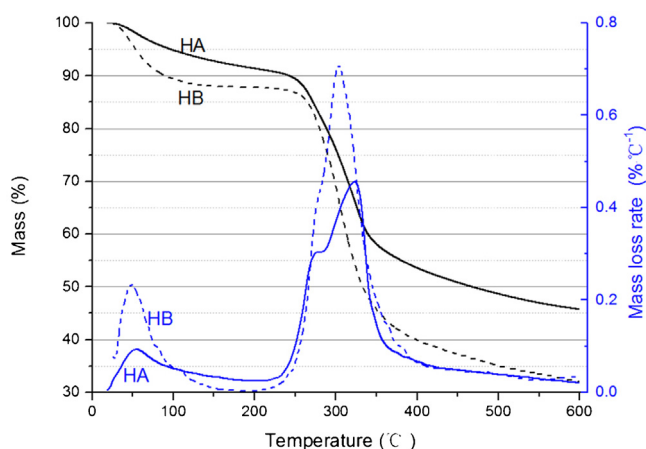


Fig. 7. The TG and DTG curves of HA and HB at $10^{\circ}\text{C min}^{-1}$.

charge than HA did. The result might be explained by the fact that HB contained more uronic acid than HA did (Table 1).

3.7. Thermal analysis of HA and HB

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) are used to study the thermal stability of the hemicelluloses isolated. Fig. 7 shows the TG and DTG curves of HA and HB under nitrogen at the heating rate of $10^{\circ}\text{C min}^{-1}$. Both TGA and DTG curves of the pyrolysis process appeared to be divided into two weight loss stages. For the two hemicellulosic fractions, the first weight loss ($\sim 10\%$) below 125°C was mainly due to the removal of moisture when the samples were heated up. The second weight loss stage mainly happened at $230\text{--}400^{\circ}\text{C}$, and it got the maximum mass loss rate of $0.46\% ^{\circ}\text{C}^{-1}$ at 325°C for HA, and $0.71\% ^{\circ}\text{C}^{-1}$ at 304°C for HB. As seen from Fig. 7, there was a noticeable difference in thermal behaviors of both HA and HB during the second weight loss stage. The weight loss rates of both HA and HB between 230 and 275°C were similar to each other. However, the loss rate of HA was lower than one of HB between 275 and 325°C and they were almost similar to each other again after 325°C . Finally, the char residue of HA and HB were 45 and 33% of the initial amount of hemicellulosic fractions, respectively. The data indicated that the HA with higher molecular weight and higher lignin content had higher thermal stability than HB. The reasons for the different thermal behavior might arise from the differences in the inherent structures and chemical nature of the two hemicellulosic fractions. It was known that HA exhibited high molecular weight and high lignin-content in comparison with HB. The initial weight loss was probably due to generation of noncombustible gases such as CO , CO_2 , formic acid, and acetic acid from hemicellulosic sugars (Sun & Tomkinson, 2003), which might cause the lignin component contained in the HA to condense and form complex polymers with the higher thermo-stability. They inhibited the subsequent pyrolysis. Therefore, higher percentage of xylose and lignin may also contribute to the stability of the HA fraction.

4. Conclusion

The single-step alkaline treatment extracted 91.7% of the total hemicelluloses from the dewaxed hybrid poplar. Neutralization and subsequent ethanol precipitation recovered 36.4 and 50.4% of the total hemicelluloses from the extract, respectively. HA was more homogeneous than HB in term of sugar components, and the physical-chemical properties of HA and HB were noticeably different, such as their weight average molecular mass, their particle sizes, their zeta potentials and their thermal stability. However,

their polydispersity was less than 1.5 in the aspects of molecular weight and particle size. Therefore, polymeric hemicelluloses with high molecular weight and lower polydispersity can efficiently be isolated from hybrid polar with single-step alkaline extraction after alkaline-ethanol treatment aided with ultrasound. Additionally, the negligible amount of oligomers from hemicelluloses in the alkaline extract indicated membrane separation process could be used to recover the used alkali from the extract, and the membrane process is under way.

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