Separation and Characterization of Lignin and Sugars in the Hydrolysate of Hot Water Extraction of Poplar Wood by Membrane Filtration and Activated Carbon Adsorption

Guoyu Tian,[a] Yaqi Chu,[c] Xiaoqian Chen,[b] Xiuhong Zhong,[b] Zhaojiang Wang,[b] and Tongtong Zhang [a,b,*]

Hot water extraction of poplar wood was conducted at temperatures from 190 to 200 °C for 5 to 8 min. A hemicellulose yield of 81% and a lignin yield of 38% were obtained at 200 °C for 8 min. A combined process of microfiltration, ultrafiltration, and activated carbon adsorption was developed to separate lignin and sugars in the hydrolysate of hot water extraction. Lignin recovery efficiencies of 56.7%, 26.0%, and 13.2% were attained for microfiltration, ultrafiltration, and activated carbon adsorption, respectively. The characterization of lignin revealed diversity in molecular weight and functional groups, which is beneficial for high-value valorization. The obtained hemicellulose sugars from the combined process showed a good recovery rate of 43.8% and remarkable purity of 97.5%. The purified sugars were a mixture of monomers and oligomers that consisted of arabinose, galactose, xylose, glucose, and mannose. Sugar oligomers with degrees of polymerization from 2 to 6 accounted for 21.6% of all sugars.

Keywords: Woody biomass; Hot water extraction; Filtration; Lignin; Hemicelluloses

*Contact information: a: Key Laboratory of Green Printing and Packaging Materials and Technology in Universities of Shandong, School of Light Industry Science and Engineering, Qilu University of Technology, Shandong Academy of Sciences, Jinan, 250353, China; b: Key Laboratory of Pulp and Paper Science and Technology of Ministry of Education, Qilu University of Technology, Shandong Academy of Sciences, Jinan, 250353; c: School of Bioengineering, Qilu University of Technology, Jinan 250353, China; *Corresponding author*: zhangtongtongabc@163.com*

INTRODUCTION

Hot water extraction (HWE) is an autohydrolysis process, and it can be used to extract hemicelluloses from lignocellulosic biomass. No chemicals are used for HWE except for water, which makes it technically and economically advantageous (Pu *et al.* 2011; Qiang *et al.* 2015). The acetic acid produced by the deacetylation of hemicellulose generates hydronium ions, which provides a weakly acidic environment for the HWE process. In addition to the hydrolysis of hemicelluloses, lignin can be extracted from biomass as a result of the depolymerization reactions that occur in acidic conditions (Zhang *et al.* 2018). The hydrolysate of HWE contains hemicellulose-derived sugars, lignin fragments, lignin-carbohydrates complex (LCC), and other water-soluble extracts (Gong *et al.* 2013; Dieste *et al.* 2016).

Hemicelluloses-derived sugars are potential raw materials for food and pharmaceutical production and the production of biodegradable plastics, coatings, tablets, and chitosan-xylan hydrogels (Gabrielii *et al.* 2000; Kurian *et al.* 2010; Zhang *et al.* 2013; Snhma *et al.* 2020). Sugars in the hydrolysate of HWE can be considered as the

raw material for a variety of products, such as xylitol (Vallejos *et al.* 2015), ethanol (Oliveira *et al.* 2014), rheology control agents (Shi *et al.* 2011), and packaging films (Al Manasrah *et al.* 2012). Many studies have been conducted on the recovery of sugars in the hydrolysate of HWE. However, macromolecular lignin and lignin fragments from the depolymerization of lignin impede the separation and utilization of sugars. To obtain high purity sugars, lignin must be effectively removed. Many methods for removing lignin have been reported, such as polymer precipitation (Yasarla and Ramarao 2012), potassium aluminum sulfate dodecahydrate precipitation, ethanol extraction (Liu *et al.* 2011), membrane filtration (Zhuang *et al.* 2017), ion exchange, enzyme treatment (Jiang *et al.* 2018), and gel chromatography (Moniz *et al.* 2014). Membrane technology is an effective purification method due to its low technical energy requirements and the fact that its key operating variables can easily be changed (Cano and Palet 2007; Arkell *et al.* 2014). Membrane filtration can be roughly divided into reverse osmosis, ultrafiltration, nanofiltration, and microfiltration according to the difference of molecular retention. Membrane technology can effectively concentrate and separate hemicellulose from different solvents. Beyond filtration, adsorption using activated carbon is among the most widely used methods due to its effectiveness and simple operational procedure (Melo *et al.* 2013; Wang *et al.* 2016). Shen *et al.* (2013) reported a combined process that used activated carbon, anion exchange resin, and nanofiltration membranes to recover dissolved organics from pre-hydrolysis liquor (Shen *et al.* 2013). The results showed that the content of lignin, acetic acid, and furfural were reduced to 0.32%, 0.71%, and 0.02%, respectively, and the removal percentage of hemicellulose sugar was 22.13%. Although lignin was removed in high amounts, the loss of carbohydrates should not be underestimated.

In this study, HWE of poplar wood was conducted to increase the sugar yield by elevating the temperature to 200 °C. A combined process of filtration and adsorption was proposed to separate the sugars and lignins in the hydrolysate of HWE. Particularly, lignin in HWE was fractionated into constituents with distinctive molecular weight. The obtained sugars and lignin were then characterized in terms of their molecular weight and structural features.

EXPERIMENTAL

Materials

The 5-year-old poplar wood was harvested in the Shandong province of China. The regenerated cellulose microporous membrane (pore sizes of 0.22 μm, 0.45 μm, 0.8 μm, 1.2 μm, and 5 μm) was purchased from Haining Taoyuan Medical Chemical Instrument Factory (Haining, China). The centrifugal filter (molecular weight cut-off 3k Daltons) was purchased from Millipore. The commercial activated carbon (DARCO G60 in powder form) with a particle size of 100-mesh was supplied by Sigma-Aldrich, Inc. (St. Louis, MO, USA). The DARCO G60 had a total surface area of 600 $m^{2/}g$ with a mean pore radius of 25 Å. The 50% NaOH solution, anhydrous sodium acetate, and xylan standards with 1 to 6 degrees of polymerization were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

Hot Water Extraction

The poplar chips were immersed in water at room temperature for 24 h and then ground into wood fibers using a mechanical refiner (Kumagaya Riki Industry Co., Ltd., Hyogo Prefecture, Japan) equipped with disks of D2B505 (Kumagaya Riki Industry Co., Ltd., Hyogo Prefecture, Japan). The obtained wood fibers were de-watered *via* mechanical extrusion to a solid content of 35% and then stored in a laboratory refrigerator at -18 °C for later use. The wood fibers were composed of 41.1% cellulose, 21.9% hemicellulose (0.4% arabinan, 0.7% galactose, 17.1% xylan, and 3.7% mannan), 24.5% acid-insoluble lignin (AIL), and 2.5% acid-soluble lignin (ASL). An accelerated solvent extraction instrument (ASE350, Thermo Fisher, Sunnyvale, CA, USA) was used for the HWE treatment of wood fibers under high temperature and high pressure. A 7.0 g quantity of wood fibers was put into the sample cell in the reactor. The deionized water was used as the solvent and pumped to the sample cell with a solid:liquid ratio of 1:12. The equilibrium time was 5 min prior to the static extraction. The static extraction was kept at 190 °C for 8 min or at 200 °C for 5 min. At the end of static extraction, hydrolysate was purged out of sample cell using fresh solvent. The extraction was carried out once, twice, or thrice. After extraction, the reactor was cooled and depressurized. The hydrolysate of HWE was collected and stored at 4 °C for further testing.

Microfiltration, Ultrafiltration, and Adsorption

The collected hydrolysate was subjected to microfiltration, ultrafiltration, and adsorption, as depicted in Fig. 1. A syringe filter was employed for microfiltration using microporous membranes with pore sizes of 0.22, 0.45, 0.8, 1.2, and 5 μm. The lignin present in retentate of the microfiltration was collected and defined as high molecular weight lignin (HL). The filtrate from the microfiltration was further treated *via* centrifugal ultrafiltration. The molecular weight cut-off of the ultrafiltration was 3000 Daltons. Centrifugal ultrafiltration was conducted at a centrifuge speed of 8000 r/min for 50 min. The lignin in the retentate of the ultrafiltration was defined as medium molecular weight lignin (ML). The filtrate from the ultrafiltration was further treated by activated carbon at activated carbon dosages from 0.1% to 2%. The adsorption was carried out in a batch reactor at 25 °C for 60 min. After adsorption, the desorption of lignin from the activated carbon was conducted in a column using aqueous ethanol 60% (v/v) at 80 ºC as the eluent. This fraction of lignin was defined as low molecular weight lignin (LL).

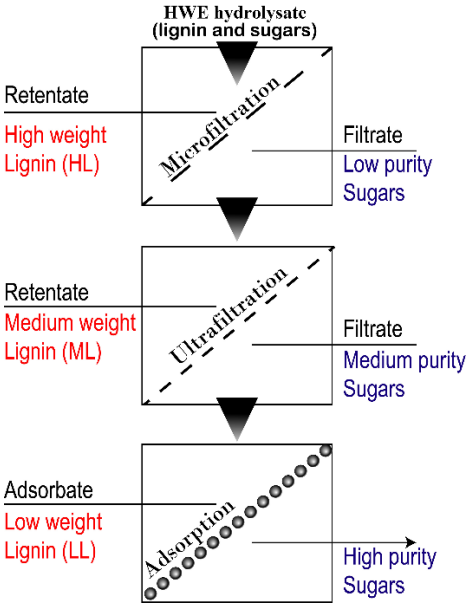

Fig. 1. The schematic flow diagram that describes the separation of sugar and lignin from HWE hydrolysate by the combined process of microfiltration, ultrafiltration, and activated carbon adsorption

Analytical Methods

Lignin samples were diluted with 5% NaOH. The quantities of lignin and lignin-related substances were measured *via* ultraviolet-visible (UV-VIS) spectroscopy at 195 nm until the absorbance value reached 0.2 to 0.7 cm^{-1}. The lignin extinction coefficient used in the measurement was 60 L/(g × cm) according to NREL technical report (NREL/TP-510-42618).

The molecular weight of lignin was measured via gel chromatography (GPC) on a Waters Alliance Separations Module e2695 system equipped with three tandem 300 mm × 7.8 mm (L. × I.D.) Phenogel 5U columns (10000, 500, and 50 Å, respectively) and a 50 mm × 7.8 mm (L. × I.D.) Phenogel 5U guard column (Phenomenex, Torrance, CA) (Zhou *et al.* 2019). The lignin was acetylated with tetrahydrofuran eluent to increase its solubility prior to the molecular weight analysis. (Liu *et al.* 2018). The lignin sample (2 mg) was mixed with pyridine (0.5 mL) and acetic anhydride (0.5 mL) in a glass tube. The test tube was filled with nitrogen and tightened quickly at room temperature for 72 h. The acetylated lignin was dried in a fume hood and dissolved in tetrahydrofuran (1.0 mL) for molecular weight analysis.

The lignin and sugars in hydrolysate were freeze-dried for 2D-HSQC NMR analysis using a Bruker Avance III 500 MHz NMR spectrometer (Karlsruhe, Germany) equipped with a Prodigy (liquid N2-cooled) 5 mm gradient TCl (inverse configuration) 1H/13C/15N cryogenic probe (Karlsruhe, Germany). A 60 mg quantity of the sample was fully dissolved in 0.6 mL of dimethyl sulfoxide-d6 (DMSO-d6), and it was then added into a 5 mm NMR tube. The 1H NMR spectrum was recorded on the spectrometer with a minimum of eight scans, a sweep width of 600 MHz, an acquisition time of 2.0 s, and a relaxation delay time of 3 s, whereas the 13C NMR spectrum was acquired with a minimum of 20,000 scans, a sweep width of 150 MHz, an acquisition time of 0.4 s, and a relaxation delay of 1.5 s.

The concentration of sugar monomers was determined using a Dionex ICS-5000+ ion chromatography system (Thermo Fisher Scientific, New York, NY, USA), equipped with a CarboPacTM PA20 (Thermo Fisher Scientific, New York, NY, USA) column (150 mm × 3 mm), an ED40 electrochemical detector (Thermo Fisher Scientific, New York, NY, USA), and an AS50 automatic sampler (Thermo Fisher Scientific, New York, NY, USA). The column was maintained at 30 °C. Water that contained 4% of 50 mmol/L NaOH was used as the eluent at a flow rate of 0.4 mL/min.

The concentration of sugar oligomers was determined by ion chromatography using a Dionex ICS-5000+ chromatography system (Thermo Fisher Scientific, New York, NY, USA), equipped with a CarboPac PA1 (Thermo Fisher Scientific, New York, NY, USA) column (250 mm × 4 mm), an ED40 electrochemical detector (Thermo Fisher Scientific, New York, NY, USA), and an AS50 automatic sampler (Thermo Fisher Scientific, New York, NY, USA). The column was maintained at 30 °C, with a gradient eluent A of 100 mmol × L^{-1} NaOH solution and eluent B of 100 mmol × L^{-1} NaOH/300 mmol × L^{-1} NaAc solution, and the flow rate was 1.0 mL/min.

RESULTS AND DISCUSSION

Sugars and Lignins in Hydrolysate of Poplar Wood

Native lignin is a hydrophobic phenolic polymer in the plant cell walls. After the hydrothermal reaction, the lignin was depolymerized into soluble polyphenols, which were soluble in aqueous solutions. The concentrations of sugars and lignin fragments in the HWE hydrolysate of wood under different treatment conditions are shown in Table 1. As the reaction temperature increased, the yield of hemicellulose-derived sugars increased from 64% for T190t8-thrice to 81% for T200t5-thrice. At the same temperature, as extraction time increased, the sugar yield increased from 57% for T190t8-once to 64% for T190t8-thrice. As the number of HWE repetitions increased, the lignin yield increased noticeably from 22% at T190t8-once to 39% at T190t8-thrice. The highest sugar yield of 81% was achieved with T200t5-thrice, which was the production of 177.4 g of sugars from 1.0 kg of poplar wood. The highest lignin yield of 38% was attained with T200t5-thrice, which was the production of 102.2 g of lignin from 1.0 kg of poplar wood. Lignin concentration increased more than the concentration of sugars as reaction severity increased. Table 1 lists the concentrations of formic acid, acetic acid, furfural, and 5-HMF in short time extracts. Acetic acid had the highest concentration, whereas 5-HMF had the lowest concentration among the degradation products. Notably, short time extraction showed a much lower concentration of degradation products than conventional hot water extraction, which lasted over 1 h. Thus, short-time extracts have lower biotoxicity, as these degradation products inhibit the biological fermentation of sugars into biofuels (Huang *et al.* 2020). The acetic acid concentration in conventional hot water extraction was reported to reach 1.6 to 4.1 g/L, which was much higher than the acetic acid concentrations in the extracts of the short-time treatments listed in Table 1.

Table 1. Concentrations of Sugars, Lignin, Formic Acid, Acetic Acid, Furfural, and 5-HMF in the HWE Hydrolysate of Poplar Wood under Different Treatment Conditions (g/L)

Labels [a]	Temp. (°C)	Time (min)	Repetitions	Sugars	Lignin	Formic Acid	Acetic Acid	Furfural	5-HMF [d]
T190t8-once	190	8	once	10.3(57%) [b]	4.9(22%) [c]	0.067	0.207	0.048	0.004
T190t8-twice	190	8	twice	11.3(62%)	5.6(25%)	0.061	0.225	0.073	0.005
T190t8-thrice	190	8	thrice	11.6(64%)	6.5(29%)	0.066	0.251	0.095	0.008
T200t5-once	200	5	once	11.3(62%)	5.8(26%)	0.072	0.241	0.072	0.005
T200t5-twice	200	5	twice	14.5(79%)	7.8(35%)	0.067	0.229	0.063	0.006
T200t5-thrice	200	5	thrice	14.8(81%)	8.5(38%)	0.071	0.267	0.128	0.007

a: The numbers after T and t are temperature and duration of static extraction; Once, twice, and thrice mean the number of repetitions of static extraction
b: The numbers in brackets are sugar yields on the basis of hemicelluloses in untreated wood
c: The numbers in brackets are the lignin yields on the basis of lignin in untreated wood
d: 5-Hydroxymethylfurfural

Microfiltration of Hydrolysate under Different Pore Sizes

Microfiltration was used to preliminarily separate the lignin and sugars in the hydrolysate of HWE. The HL in the hydrolysate of HWE was retained on the membrane as filter cake, whereas the rest of the lignin and sugars passed through the membrane as illustrated in Fig. 1. In this study, membranes with pore sizes of 0.22, 0.45, 0.8, 1.2, and 5 μm were used. Figure 2a shows the retention percentages of sugar and lignin for membranes with different pore sizes. As the pore diameter decreased, the retention rate of lignin increased slowly, and it reached a maximum of 61.2% at 0.22 μm. The interception of sugars on membranes was quite weak, which was indicated by the retention rates of sugars that ranged from 0.1% to 5.1% for membranes with pore sizes from 0.22 to 5.0 μm. For membrane filtration, smaller pore size results in a longer filtration time. For practicality, a membrane with a pore size of 5 μm was chosen for microfiltration to recover HL from the hydrolysate of HWE. The retention rate of lignin was 56.7% for the membrane with pore size of 5 μm. This meant that over half of the lignin was recovered as retentate from membrane interception. The molecular weight distribution of the recovered lignin was determined by GPC, as shown in Fig. 2b. Molecular weight analysis determined a weight-average molecular weight (M_w) of 1545 g/mol and a number-average molecular weight (M_n) of 840 g/mol. This fraction of lignin was named HL. The molecular weight profile of HL had five peaks at 259, 396, 517, 670, and 932 g/mol. These five peaks may correspond to lignin monomers, dimers, trimers, tetramers, and pentamers. Song *et al.* (2013) reported that lignin fragments with a molecular weight of 1511.5 g/mol had 7 phenylpropane units. Figure 2b shows that the

tetramer and pentamer fractions accounted for a large proportion of HL, whereas the peaks of monomer and dimer were relatively smaller.

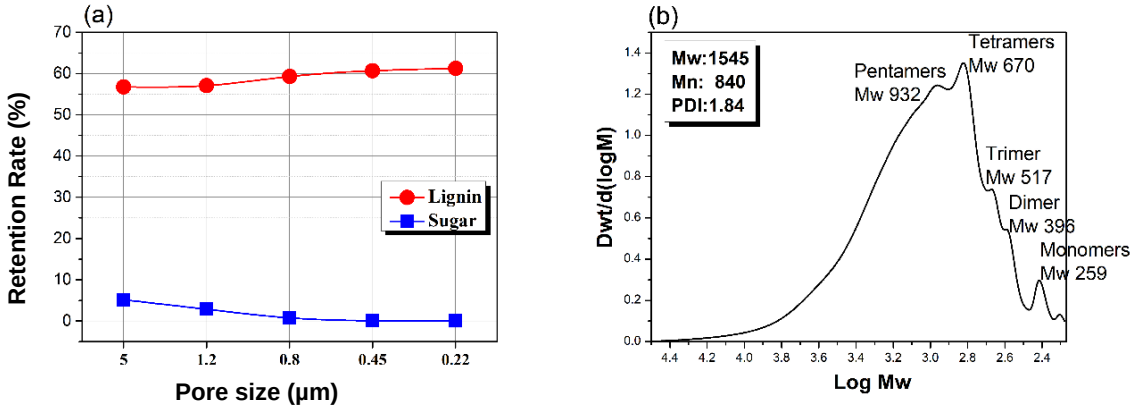

Fig. 2. Microfiltration of the HWE hydrolysate using membranes with different pore sizes: (a) performance of microfiltration in terms of lignin retention and sugars retention; (b) molecular weight distribution of HL obtained from microfiltration

Ultrafiltration Produces Sugars with Good Purity

The filtrate from 5 μm microfiltration of T200t5-twice hydrolysate was used as feed stream for ultrafiltration. The 5 μm microfiltration retained approximately 56.7% of the original lignin and 5.1% of the original sugar in T200t5-twice hydrolysate. The concentrations of sugars and lignin in the ultrafiltration feed stream were 13.8 g/L and 3.4g/L, respectively. The ultrafiltration used a membrane with a 3000 Dalton molecular weight cut-off. During ultrafiltration, a large part of the lignins and sugars were intercepted as retentate, whereas smaller molecules of sugar and lignin penetrated the membrane. The contents of lignin and sugar in the retentate and the filtrate after ultrafiltration are shown in Table 2. In a 10 mL sample of hydrolysate, the filtrate after ultrafiltration was 5 mL, which is twice the concentration of the hydrolysate. The lignin content of the retentate was relatively high, as it accounted for 60.0% of total lignin, which suggested that the retention capacity of ultrafiltration for lignin was greater than that for sugars (Chen *et al.* 2016). The sugar content in the filtrate was greater than that of lignin, as it accounted for 63.1% of total sugars. The ultrafiltration retentate showed a recovery of 25.9% of the orignial lignin in hydrolysate. This fraction of lignin was defined as medium molecular lignin (ML). Molecular weight analysis of ML showed a M_w of 760 g/mol and a M_n of 665 g/mol, as listed in Table 4.

Table 2. Sugar and Lignin Contents in the Retentate and the Filtrate from Ultrafiltration

	Volume (mL)[a]	Sugars (g/L)	Lignin (g/L)
Retentate	5	Arabinose 0.1 Galactose 0.3 Glucose 0.8 Xylose 7.1 Mannose 1.9 SUM 10.2 (36.9%) [b]	4.1 (60.0%)
Filtrate	5	Arabinose 1.5 Galactose1.1 Glucose 0.8 Xylose 11.9 Mannose 2.0 SUM 17.4 (63.1%)	2.7 (40.0%)
a: 10 mL microfiltration filtrate of T200t5-twice hydrolysate was taken as feed stream for ultrafiltration. b: The numbers in the brackets are the values of recovery.			

Purification of Sugars Using Activated Carbon

The effects of activated carbon dosage on the removals of lignin and sugar are shown in Fig. 3. The sugar removal ranged from 26.8% to 31.5%, whereas the lignin removal ranged from 58.7% to 87.9% after the activated carbon treatments. This suggested that activated carbon has a stronger ability to adsorb lignin. However, sugar loss was not low enough to be neglected, since that the lowest sugar removal was 26.8% at activated carbon of 2 g/L. The limited selectivity of activated carbon had been reported previously (Wang *et al.* 2016). Ion exchange resin is alternative adsorbents for lignin adsorption, and showed slightly lower sugars loss compared with activated carbon (Shen et al. 2013). The application of activated carbon in purification of sugars in hydrolysate is competitive due to the lower cost of activated carbon compared to ion exchange resin. The removal of lignin noticeably increased as the dosage of activated carbon increased. The lignin removal rate reached its peak of 87.9% at the activated carbon dosage of 20 g/L. The ultrafiltration filtrate of T200t5-twice hydrolysate was used as feed stream of activated carbon treatment. The concentrations of sugar and lignin were 12.7 g/L and 0.3 g/L in the treated hydrolysate, respectively. This corresponds to a sugar purity of 97.5%.

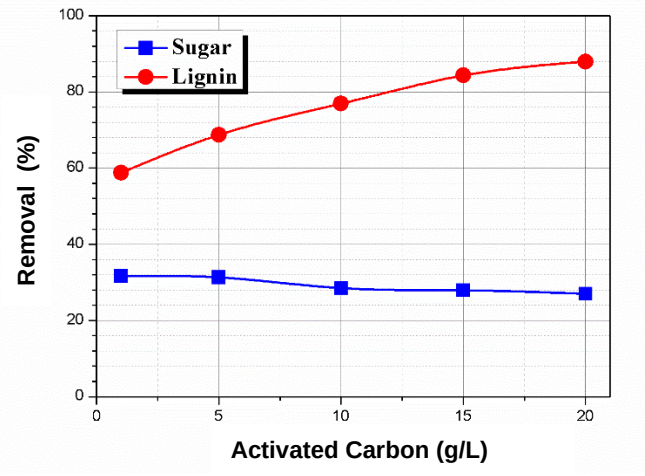

Fig. 3. The removal percentages of lignin and sugars with different levels of activated carbon during adsorption treatment

Characterization of Lignin and Sugars

The untreated hydrolysate and the purified hydrolysate were freeze-dried for NMR analysis. Two-dimensional ^{1}H-^{13}C heteronuclear single quantum coherence NMR (2D-HSQC-NMR) provides important structural information on lignins at the side-chain region (δC/δH 50 to 10/2.5 to 6.0) and the aromatic region (δC/δH 100 to 135/5.5 to 8.5). The distribution of its main structure and the corresponding displacements (shown in Table 3) have been assigned and clarified the HSQC cross signals of lignin and related carbohydrates using published literature (Tian *et al*. 2019). In Fig. 4, the HSQC cross-signals of lignin and carbohydrates were assigned and clarified based on the literature (Wu *et al.* 2020). In the side-chain region (δC/δH 50 to 110/2.5 to 6.0) of lignin, the major inter-unit linkages, such as the lignin substructures of aryl ether (β-O-4, A), resinol (β-β, B), phenylcoumaran (β-5, C), and cinnamyl alcohol (I) inter-unit linkages, were identified in the HSQC spectra of the untreated hydrolysate and purified hydrolysate. Comparison of the signal intensity between the purified hydrolysate and the untreated hydrolysate showed that the lignin in purified hydrolysate had fewer inter-unit linkages. The linkages of aryl ether (β-O-4, A), resinol (β-β, B), and phenylcoumaran (β-5, C) were noticeably reduced. The substructure of cinnamyl alcohol (I) disappeared in the HSQC spectra of the purified hydrolysate. Further, the cross-signals of sugars, such as L-arabinofuranoside ($Ara_{2,3,5}$) and D-xylopyranoside ($X_{2,3,5}$), were observed in the spectrum of both untreated hydrolysate and the purified hydrolysate. The presence of $X2_2$, $X3_3$, U_1, and U_4 also indicated the presence of 2-O-acetyl, 3-O-acetyl, and 4-O-methyl groups in the sugars. In addition, lignin-carbohydrate complex (LCC) in the forms of γ-ester (Est+A′γ), benzyl ester (BE1), and phenyl glycoside (PhGlc1) was identified in the spectra of the untreated hydrolysate (Dong *et al.* 2019). Other researchers have reported that LCC is useful in the treatment of periprosthetic osteolysis *via* scavenging the intracellular and endogenous reactive oxygen species. The amount of LCC in the purified hydrolysate was noticeably reduced. In the aromatic region, cross-signals relating to the syringyl unit (S), guaiacyl unit (G), and *p*-hydroxybenzoate (PB) unit were observed, such as $S_{2,6}$, G_2, G_5, G_6, and $PB_{2,6}$. Finally, all unit bonds of the purified hydrolysate were reduced.

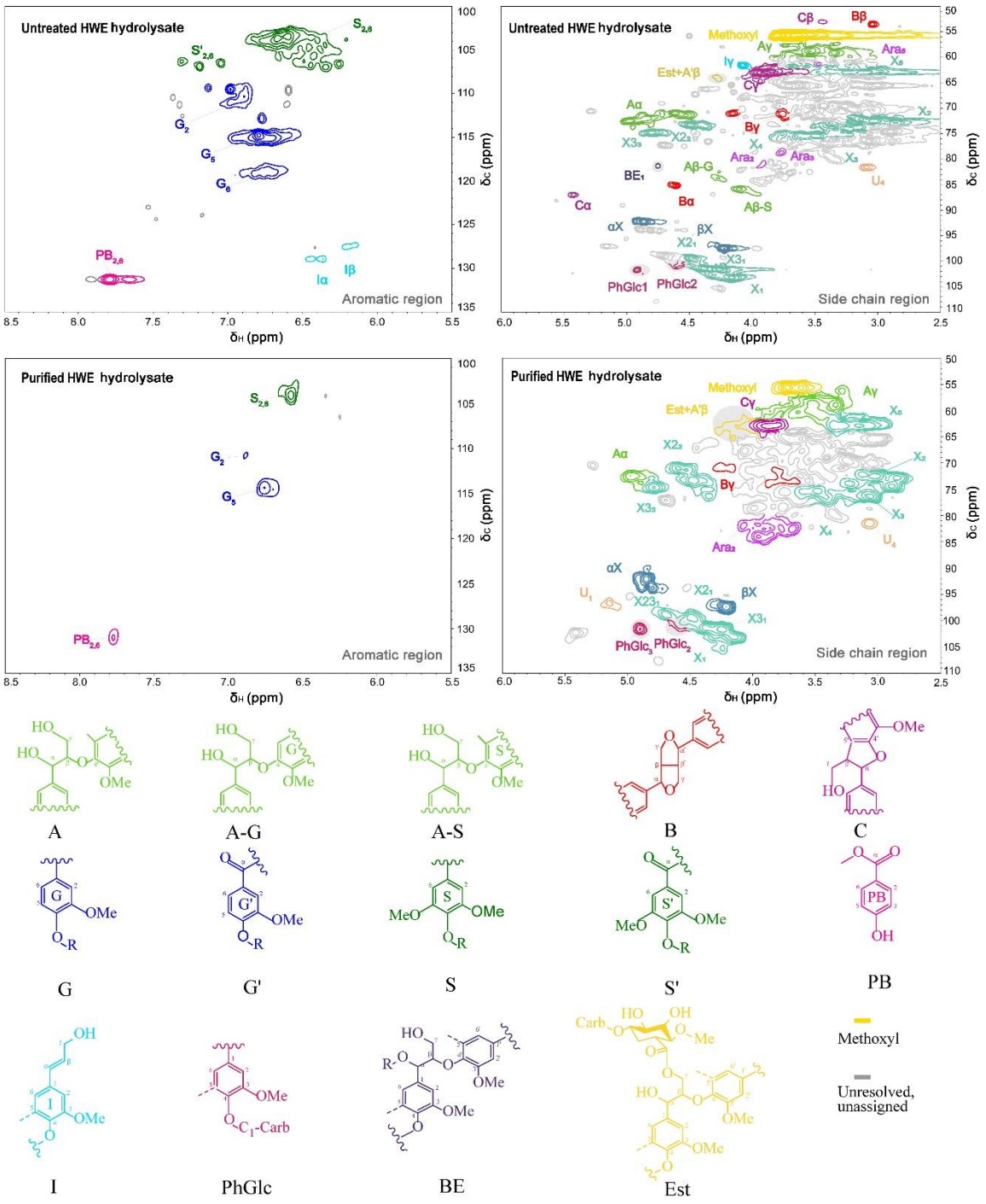

Fig. 4. The aromatic region (δC/δH 100 to 135/5.5 to 8.5) and side-chain region (δC/δH 50 to 110/2.5 to 6.0) of the HSQC spectra of untreated HWE hydrolysate and purified HWE hydrolysate and the main substructures for spectral elucidation

Table 3. ^{13}C-^{1}H Corresponding to the Main Lignin Structural Unit in the HSQC Map

label	δ_C/δ_H (ppm)	assignment
C_β	53.1/3.42	C_β−H_β in phenylcoumaran substructure (C)
B_β	53.5/3.04	C_β−H_β in β−β′ resinol substructure (B)
Methoxyl	55.6/3.72	C−H in methoxyl
A_γ	59.5/3.40 and 3.71	C_γ−H_γ in γ-hydroxylated β-O-4′ substructure (A)
I_γ	61.4/4.08	C_γ−H_γ in cinnamyl alcohol end-group (I)
C_γ	62.6/3.68	C_γ−H_γ in phenylcoumaran substructure (C)
A'_γ	63.6/3.83 and 4.29	C_γ−H_γ in γ-acylated β-O-4′ substructure (A′)
$A_{\alpha(G)}$	70.9/4.72	C_α−H_α in β-O-4′ substructure (A) linked to a G unit
B_γ	71.1/3.82 and 4.17	C_γ−H_γ in β−β′ resinol substructure (B)
$A_{\alpha(S)}$	71.7/4.84	C_α−H_α in β-O-4′ substructure (A) linked to a S-unit
$A_{\beta(G)}$	83.3/4.28	C_β−H_β in β-O-4′ substructure (A) linked to a G unit
B_α	84.9/4.66	C_α−H_α in β−β′ resinol substructure (B)
$A_{\beta(S)}$	85.9/4.11	C_β−H_β in β-O-4′ substructure linked (A) to a S-unit
C_α	86.9/5.43	C_α−H_α in phenylcoumaran substructure (C)
$S_{2,6}$	103.9/6.69	$C_{2,6}$−$H_{2,6}$ in etherified syringyl unit (S)
$S'_{2,6}$	106.2/7.24 and 7.08	$C_{2,6}$−$H_{2,6}$ in oxidized (C_α=O) syringyl units (S′)
G_2	110.9/6.98	C_2−H_2 in guaiacyl unit (G)
G_5	114.9/6.73 and 6.93	C_5−H_5 in guaiacyl unit (G)
G_6	118.8/6.77	C_6−H_6 in guaiacyl unit (G)
$H_{2,6}$	127.8/7.23	$C_{2,6}$−$H_{2,6}$ in p-hydroxyphenyl unit (H)
I_β	128.3/6.25	C_β−H_β in p-hydroxycinnamyl alcohol end group (I)
I_α	128.5/6.44	C_α−H_α in p-hydroxycinnamyl alcohol end group (I)
$PB_{2,6}$	131.3/7.67	$C_{2,6}$−$H_{2,6}$ in p-hydroxybenzoate substructure (PB)

The molecular weight analysis of lignins from the different treatments was performed by GPC. The M_w values of HL from the microfiltration process, ML from the ultrafiltration process, and LL from the activated carbon treatment are shown in Table 4. The M_w of HL, ML, and LL showed a declining trend from 1545 g/mol of HL to 760 g/mol of HL and 705 g/mol of LL. In addition, the polydispersity index (PDI) of HL, ML, and LL showed a decreasing trend toward 1.0, which indicated that the structure of these lignin fractions became more and more uniform.

Table 4. The Weight Average Molecular Weight (M_w), Number Average Molecular Weight (M_n), and Polydispersity (PDI) of the Lignin Fractions from Different Treatments

Lignin Fractions*	M_w (g/mol)	M_n (g/mol)	PDI (M_w/M_n)
HL	1006	510	1.97
ML	760	665	1.14
LL	705	647	1.09
Note: *HL lignin is high molecular weight lignin, ML lignin is medium molecular weight lignin, and LL lignin is low molecular weight lignin			

The sugars in the original hydrolysate of T200t5-twice and the purified hydrolysate were determined *via* ion chromatography (Table 5). The sugars in the hydrolysate consisted of monomers and oligomers that were composed of arabinose, galactose, xylose, glucose, and mannose. Generally, the harsher the treatment condition is, the more sugar monomers will be present in hydrolysate. In the investigated conditions, the percentage of monomers ranged from 8.3% to 30.0%, depending on the treatment severity. The total sugars concentration of original hydrolysate was 14.5 g/L. After the combined processes of purification, the sugars concentration was 12.7 g/L, which means a 43.8% retention of sugars considering the volume reduction of 50% in the ultrafiltration process. Lignin concentrations in original and purified liquid are also listed in Table 5. The lignin removal attained 96.2%. The degree of polymerization (DP) of sugars in the hydrolysate and the purified hydrolysate were analyzed, and the results are shown in Fig. 5. The DP was determined according to the peak time of the standard sample. In original hydrolysate, sugars 2<DP<6 accounted for 28.9% of total sugars. Sugars 2<DP<6 accounted for 21.6% in the purified hydrolysate, which indicated that minimal removal of sugar oligomers during the purification process.

Table 5. Compositions of the Sugars and Lignin in Unpurified Original Hydrolysate and Purified Hydrolysate by the Combined Processes (g/L)

	Arabinose	Galactose	Glucose	Xylose	Mannose	Total sugars	Sugars (2 < DP [b] < 6) (%)	Lignin
Unpurified Original Hydrolysate [a]	0.9	0.7	0.9	10.0	2.0	14.5	28.9	7.8
Purified Hydrolysate by Combined processes	1.2	0.9	0.6	9.1	1.0	12.7	21.6	0.3
a: HWE hydrolysate was obtained at conditions of T200t5-twice b: DP is the degree of polymerization								

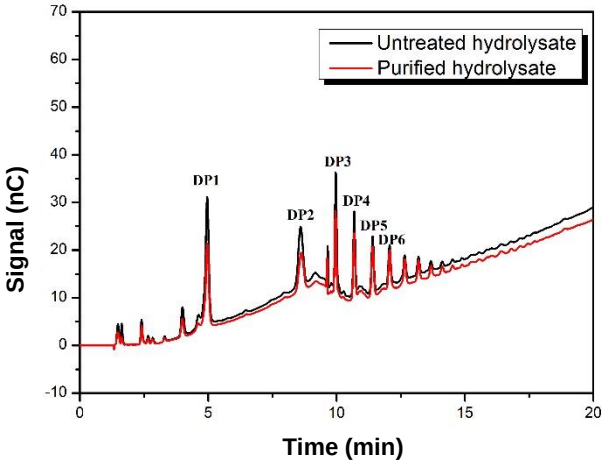

Fig. 5. Distribution of the degree of sugar polymerization in untreated hydrolysate and purified hydrolysate

CONCLUSIONS

Hydrolysate containing sugars and lignin was extracted from poplar wood efficiently at 190 and 200 ºC. A combined process of microfiltration, ultrafiltration, and activated carbon adsorption was developed to separate sugars from lignin in the hydrolysate. Lignins were fractionated into HL, ML, and LL fractions with distinguishable molecular weight. Sugars in the HWE hydrolysate were recovered with remarkable purity and yield.

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