Water-insoluble and soluble glucuronoxylans from Eucalyptus pulp and their behaviors in alkaline pulping

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Abstract

Hemicellulose plays a versatile role in plant cell wall by linking cellulose and lignin or impeding lignin removal from sustainable cellulosic fibers. Herein, hemicellulose fractions (glucuronoxylans) from Eucalyptus pulps under various cooking degree were extracted by potassium hydroxide solution and purified with ethanol. The glucuronoxylans are divided into water-insoluble (GXI) and -soluble (GXS) fractions. The release or degradation behavior of glycosylated side-chain of hemicellulose in alkaline pulping was explored according to the separate discussion of GXI and GXS, and take consideration of the effect of extraction temperature on polysaccharide degradation. The major difference is that GXI is constructed of basically straight xylan chain with high linearity, and GXS contains multi-branches xylan chain. The harsher cooking conditions with higher H factor and alkaline-extraction with higher temperature significantly influenced the composition and structure, leading more glycosyl side chains removal. Higher content glucuronic acid (6.04–11.26%) in GXS, which may relate to high branch on xylans and great solubility, while less of glucuronic acid (MeGlcA, 2.28–5.48%) in GXI was accounting for the poor solubility. The conversion of MeGlcA to hexenuronic acid (HexA) is significant during pulping, which may affect the subsequently bleaching. The glycosyl contents and structure of the two fractions extracted from pulps with different cooking degrees (corresponding to H factors 317, 586 and 904) showed that the HexA converted by MeGlcA in GXI was retained during cooking, while that in GXS was easier to be degraded in pulping process.

1. Introduction

Hemicellulose is the second abundant components of lignocellulose biomass. There are heterogeneous polysaccharides in the plant cell wall, which interconnected with cellulose by hydrogen bonds and linked to lignin by covalent bonds. (Sun, Wen, & Ma, 2013). Hemicellulose affects the strength properties and wettability of final paper due to the interactions between xylan and cellulose (Silva, Colodette, & Lucia, 2011). Hemicellulose is mainly composed of O-acetyl-(4-O-methylglucurono) xylan in Eucalyptus (Gullón, González-Munoz, & Gool, 2011). The backbone consists of xylan chain of approximately 200 linear (1,4)-linked-β-D-xylopyranosyl (Xyl) residues, and a (1,2)-linked (4-O-methyl)-α-d-glucopyranosyl uronic acid (MeGlcA) sidegroup linked with per ten xylopyranosyl residues, substituted at position C-2 position (Vignon & Gey, 1998), where is also a substitution by acetyl groups.

In the process of pulp and paper making, it is often necessary to remove lignin and part of hemicellulose to retain more cellulose. There are some studies considered that partial reservation of hemicellulose could increase the paper swelling and reduce the power consumption during pulp beating (Silva, Colodette, & Lucia, 2011). However, it is necessary to take into consideration that the formation of hexenuronic acid (HexA) from the MeGlcA side group of xylan chains in the cooking may consume more bleaching agent in the subsequent stages and do harm to pulp properties (Zhang, Nie, & Qin, 2019). Therefore, it is worth further studied about the hemicellulose changes pattern and how to effectively control the transformation of MeGlcA to HexA in the process of cooking, which will be helpful to reduce the degradation of polysaccharides and retain better paper properties. Marques, G. et al. (2010) isolated

the heteroxylan by extraction of peracetic holocellulose and studied its behavior during soda/AQ pulping of sisal fibers, and the results showed that MeGlcA and glucuronosyl are mostly removed or converted to HexA. However, there is still 15% of the initially present MeGlcA was maintained intact upon cooking.

Nowadays, there are many studies on the extraction of hemicellulose from wood meal, but the extraction and separation of hemicellulose from pulp has not drawn wide attention (Hakala, Liitiä, & Suurnäkki, 2013). Compared with wood meal, the connections between hemicellulose and lignin closely on pulp have preliminarily been damaged (Borrega, Tolonen, & Bardot, 2013). The removal of lignin exposed more hemicellulose and increased the hemicellulose dissolution in alkaline extraction (Peng, Peng, & Bian, 2011). At present, there are many extraction methods for hemicellulose, such as acid-base extraction (Goldmann, Ahola, & Mikola, 2017), microwave assisted extraction (Yuan, Zou, & Zhou, 2019), organic solvent extraction (Valoppi, Lahtinen, & Bhattarai, 2019) and so on. Among these approaches, alkali extraction is widely used because the alkaline treatment could disrupt the cell wall and cleave the hydrogen bonds and covalent bonds like ester and ether linkages in the lignocellulose (Miller, & Fry, 2001), though hemicellulose extracted from aqueous alkali contains a small number of bound lignin (Wen, Sun, & Xu, 2010).

Compared with sodium hydroxide and lithium hydroxide, potassium acetate formed by potassium hydroxide during neutralizing alkali extract is more soluble in ethanol, so potassium hydroxide is more suitably selected for alkali treatment of lignocellulose materials (Sun, Wen, & Ma, 2013). The hemicellulose from sweet sorghum stem was obtained by extracted with 0.3%-2.0% potassium hydroxide aqueous solution followed with 60% ethanol containing 2.5% potassium hydroxide with the yield of 76.3% (Sun, Wen, & Ma, 2013). The solubility of hemicellulose extracted is different due to its various structural composition. Studies showed that the water-soluble polysaccharides consisted mainly of glucose units, while xylose, arabinose and glucuronic acid (UA) were the major glycosyl in alkali-soluble hemicelluloses (Peng, Peng, & Bian, 2011).

The purpose of this study is to extract and character water-soluble and -insoluble hemicellulose fractions from the pulps after different cooking degree with potassium hydroxide and precipitated with ethanol solution. The aim is to explore the changing patterns of glycosyl contents and distribution, molecular weight and structural characteristics of the above two components along with the deepening of cooking procedure, and hemicellulose degradation during the extraction process were considered by exploring the extraction temperature. The results would provide ideas for further utilization of hemicellulose and a hint to process optimization of pulping and papermaking for fiber recycling and paper aging.

2. Materials And Methods

2.1. Materials

Eucalyptus chips (3cm*5cm*2cm) were donated by Juntai Paper Ltd Co, Huaihua, Hunan, China. They were dried in sunlight and then seal saved. The chemicals NaOH, Na_2S, KOH, ethyl alcohol, DMSO,

arabinose, xylose, xylobiose, glucose, furan 2-carboxylic acid, trifluoroacetic acid (TFA), glucuronic acid, were bought from Sigma-Aldrich Company.

2.2. Preparation of pulps

The pulps from Eucalyptus wood chips were cooked in 1 L rotary multi-pot digester 2201-6 (USA) with temperature control at liquid ratio of 4:1 L/kg. The white liquor was prepared with 18% alkali concentration and 26% sulfidity. The temperature in the digester rose to 165°C within 2h and kept it for 2h. A series of pulps closing to the predetermined H-factor (nearly 300, 600, or 900) was obtained. The H-factor is an intensity factor that combines the effects of pulping temperature and time into a single variable (Mašura, V., 1998). The final pulps with various H factors 317, 586 and 904 which represented different cooking degrees were obtained.

2.3. Extraction and purification of hemicelluloses

The sequential extraction of hemicelluloses procedures used was shown in Fig. 1. The hemicelluloses from the pulp with H factor 586 were extracted with 2 M KOH at 30°C, 50°C and 80°C for 12 h with a liquor ratio of 1:30, in order to study the effect of temperature on the structure of hemicellulose so that control the extraction temperature with more diverse structures and less sugar degradation for subsequent analysis. The same extraction procedure was used in pulps with H factors 317 and 904 under 50 and 80°C to study the influence of H factor on hemicellulose structure corresponding to the different cooking condition under the same extraction condition. The consideration of extraction under 80°C is to explore the pattern of polysaccharide degradation under high temperature. The alkaline extracts were then neutralized with 6 M acetic acid and adjust pH to 5.5, standing for 12h to form sediment that was collected as insoluble fraction **(GXI)** by centrifugation. The soluble fraction **(GXS)** in supernatant was adjusted with ethanol to the solution containing ethanol 75% to precipitate. The precipitates obtained by the above methods were centrifuged (6500 x, 10 min at 25°C) and washed with 95% ethanol (x 3) until the supernatant is clarified, followed by freeze dried. The precipitates **GXI** and **GXS** from different stages were purified by dialysis for further determination.

2.4. Chemical characterization of hemicelluloses

2.4.1. Carbohydrates and lignin compositions

The compositions of pulp were determined after a two-stage acid hydrolysis, according to the analytical method NREL/TP-510-42618 issued by the National Renewable Laboratory (NREL) (Li, Knierim, & Manisseri, 2010). Neutral monosaccharides were determined by high-performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) in a Dionex ICS-3000 (Sunnyvale CA, USA). The content of Klason lignin was quantified gravimetrically, and the amount of acid soluble lignin (ASL) was determined in a Shimadzu (Kyoto, Japan) UV-1900 spectrophotometer at a wavelength of 205 nm, using an adsorption coefficient of 110 L/ (g cm). The identified chemical compositions of the pulps were showed in Table 1.

Table 1
The chemical compositions of the pulp with different H-factors.

Pulp	Chemical components (%)						
	Xylose	Glucose	Glucuronic acid	Arabinose	Galactose	Klason lignin	ASL
H1 [a]	18.40	72.83	0.19	2.00	1.23	4.48	0.86
H2	14.09	82.66	0.10	1.57	0.36	1.03	0.18
H3	9.52	87.74	0.04	1.40	0.24	0.94	0.12

[a] H1, H2, H3, the pulps with various H factors corresponding to H1 = 317, H2 = 586, H3 = 904

2.4.2. Analysis of polysaccharides

Glucuronoxylan components (GXI, GXS) were hydrolyzed to analyze the carbohydrate composition. Hemicelluloses components were firstly subjected to hydrolysis in 2 M TFA for 6 h at 120°C. Then TFA was removed by vacuum evaporation at 35°C under a nitrogen gas stream and solute was dissolved in deionized water. For each hydrolysate, three replicates were realized.

2.5. Structural characterization of hemicelluloses

2.5.1. Molecular weight analysis

The information on the molecular weights of the hemicelluloses from pulps was provided in detail by gel permeation chromatography (GPC). The samples were analyzed in a Dionex Ultimate 3000 (Sunnyvale CA, USA) system, equipped with a guard and four analytical Agilent (Santa Clara, USA) PL-gel Mixed-A columns (7.5 × 300 mm), and coupled with a Shodex (Tokyo, Japan) RI-101 refractive index detector.

2.5.2. FT-IR spectroscopy

FT-IR measurements were performed on a Thermo Scientific Nicolet iN10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI, USA) equipped with a KBr beam splitter and a deuterated triglycine sulfate room temperature detector. Dried samples were ground and tableted with KBr and the spectra were collected in the range of 4000 to 500 cm^{-1} at 4 cm^{-1} resolutions with 128 scans per hemicellulose sample. The measurement time for each spectrum was ~60 s.

2.5.3. Nuclear magnetic resonance (NMR) spectroscopy

Mono-dimensional (^{13}C- and ^{1}H-) and bi-dimensional (coupled HSQC) NMR spectra were acquired at 25°C on Bruker AVANCE III 600 NMR spectrometer, operating at 9.5 T, and observing ^{1}H at 600 MHz and ^{13}C at 151 MHz, equipped with a 5-mm multinuclear inverse detection probe with z-gradient. The resonances of $-CH_3$ groups of acetone (^{1}H at δ 2.22; ^{13}C at δ 30.20) are used as internal standard. All pulse programs were supplied by Bruker.

3. Results And Discussion

3.1. Extraction and fractionation of the polysaccharides and their composition

3.1.1 Contents of polysaccharides and side-chain saccharides

The yield and monosaccharide components of hemicellulose extracted were shown in Table 2. The results showed that the hemicellulose in Eucalyptus pulp was mainly 4-O-methyl-glucuronoxylans. And a small amount of arabinose might be due to galactoarabinoxylans and arabinogalactans (Blackwood, Salter, & Dettmar, 2000). From the data shown in the Table 2, the water-insoluble fraction GXI extracted from pulp with H2 (H2-GXI) was mainly composed of xylose (82.09–84.81%), glucuronic acid (2.28–5.48%), xylobiose (1.09–3.94%), hexenuronic acid (3.86–6.52%), arabinose (1.99–3.81%) and glucose (1.48–2.44%). Compared with GXI, the contents of water-soluble components H2-GXS presented lower xylose (70.14–83.98%) higher glucuronic acid (7.28–11.26%) contents. These diversities may be caused by the distribution of branches along the xylan backbone and the molecular weight (Sun, Wen, & Ma, 2013).

Each fraction's monosaccharide is various as extraction temperature changes. It has been reported that xylose has a lower activation energy (35.1 kcal mol^{-1}) than those of galactose (38.0 kcal mol^{-1}), glucose (39.0 kcal mol^{-1}), and arabinose (41.6 kJ mol^{-1}) (Wang, Ru, & Lin, 2013). According to the data of activation energy, the xylose and glucose are easier to dissolute than arabinose, which means at a lower temperature more xylan with glucose and glucuronic acid as branched chains is obtained. However, with the rising of temperature, the linkage bonds of side chains are breaking, performing for a lower monosaccharide contents. Besides, the glucose group extracted at high temperature (e.g. H3-80) may be derived from the partly degradation of cellulose.

Furthermore, the tendency of arabinose in GXI and GXS was slightly different. The difference of the release or degradation rate of arabinose in GXI and GXS components may be related to the peeling reaction of hemicellulose under alkaline condition. As the temperature rises, the peeling reaction intensified and the arabinosaccharides is easy to shed, resulting from the (1→3)-attachment of arabinosyl substituent to the xylan backbone (Jacobs & Dahlman, 2001). The stable structure resistant to peeling reaction is formed during the removal of arabinose group along the xylan chain with higher branching degree, indicating that multi-branched xylan chain structure was more sensitive to degradation in alkaline solution.

The ratios of each glycosyl group to xylose is shown in Table 3. The data represented the linearity and branching of hemicellusic fractions extracted. The ratio of arabinose to xylose (Ara/Xyl) is an indicative of the linearity or branching of arabinoxylans (Cyran, Courtin, & Delcour, 2004). The decrease of Ara/Xyl

ratio implied that the hemicellulose fractions had more linear structure. The hemicellulose fractions with a higher Uro/Xyl ratio (0.01–0.23), extracted by using organic alkaline solvent had more branched structure (Ma, Jia, & Zhu, 2012).

The glycosyl compositions analysis showed that GXS had higher UA/Xyl (0.07–0.16) and Glc/Xyl (0.002–0.08) ratios than GXI (UA/Xyl (0.03–0.06), Glc/Xyl (0.001–0.07)), indicating a higher branching degree for GXS and a higher linearity for GXI. The result supports the conclusion that a higher branching degree of the xylan chains would increase the solubility of hemicellulose polysaccharides (Gullón et al., 2011). The presence of larger unsubstituted regions in the xylan chains can lead to the strong hydrogen bonds, causing interchain aggregation and more difficult isolation (Höije, Gröndahl, & Tømmeraas, 2005). At harsher conditions, the breakage of hydrogen bonds resulting in the decreasing of monosaccharides ratios and more unsubstituted linear xylan was obtained.

Table 2
Sugar components of hemicellulose extracted under various treatment conditions.

Hemicellulose fractions		monosaccharide components(%)[a]						Yield (%)
		Ara	Glc	Xyl	bi-Xyl	UA	HexA	
H1-50 [b]	GXI	2.40	3.25	80.43	9.48	3.95	0.49	30.19
	GXS	1.45	3.66	73.65	8.15	10.10	3.00	16.43
H1-80	GXI	2.94	3.99	80.60	4.27	4.93	3.27	22.90
	GXS	2.98	2.11	77.23	5.71	7.33	4.63	13.89
H2-30	GXI	1.99	2.44	82.31	3.94	4.97	4.36	32.33
	GXS	1.38	5.43	70.14	5.84	11.26	5.94	15.16
H2-50	GXI	2.41	2.26	82.09	3.91	5.48	3.86	30.50
	GXS	0.27	2.60	77.29	4.09	8.61	7.14	16.75
H2-80	GXI	3.81	1.48	84.81	1.09	2.28	6.52	23.92
	GXS	0.07	0.16	83.98	4.53	7.28	3.97	14.63
H3-50	GXI	ND.[c]	0.13	89.38	3.33	2.46	4.70	35.06
	GXS	0.62	4.43	77.29	9.57	7.08	1.00	17.43
H3-80	GXI	ND.	4.47	81.39	2.57	2.86	8.71	28.73
	GXS	0.33	3.17	82.78	6.92	6.04	0.76	11.47

[a] % of mass fraction of monosaccharide components relative to the total polysaccharides, determined by HPAEC.

[b] H1, H2, H3-30,50,80, hemicellulose of pulp with H-factors 317,586 and 904 extracted at 30,50 and 80°C respectively.

[c] Not detected.

3.1.2 Contents of glucuronic acid and hexenuronic acid

The glucuronic acid branch chain of xylan is partially converted into hexenuronic acid (HexA) in cooking (Gellerstedt, 1996), which has adverse effects on the subsequent process of pulping and bleaching. Therefore, it is important to study the change pattern so as to control cooking conditions and improve pulp quality. The degradation products furan 2-carboxylic acid (90%) and 5-formylfuran-2-carboxylic acid (10%) generated by acid reactions of HexA have UV absorption at 245 and 290nm (Evtuguin et al., 2002), and the content of HexA can be indicated by indirect determination of the degradation product (Zhang, Nie, & Qin, 2019). Here, the determination of HexA was obtained indirectly by measuring the value of its acid degradation product furan 2-carboxylic acid as reference sample for HPAEC.

Table 3
The ratios of glycosyl groups to xylose of the extracted hemicelluloses

Hemicellulose fractions[a]		Ara/Xyl	Glc/Xyl	UA/Xyl	HexA/Xyl	(HexA + UA)/Xyl
H1-50	GXI	0.03	0.04	0.05	0.01	0.06
	GXS	0.02	0.05	0.14	0.04	0.18
H1-80	GXI	0.04	0.05	0.06	0.04	0.10
	GXS	0.04	0.03	0.10	0.06	0.16
H2-30	GXI	0.02	0.03	0.06	0.05	0.11
	GXS	0.02	0.08	0.16	0.09	0.25
H2-50	GXI	0.03	0.07	0.05	0.03	0.11
	GXS	ND.[b]	0.03	0.11	0.09	0.20
H2-80	GXI	0.05	0.02	0.03	0.08	0.10
	GXS	ND.	ND.	0.09	0.05	0.13
H3-50	GXI	ND.	ND.	0.03	0.05	0.08
	GXS	0.01	0.06	0.09	0.01	0.11
H3-80	GXI	ND.	0.06	0.04	0.11	0.14
	GXS	ND.	0.04	0.07	0.01	0.08

[a] Corresponding to the hemicellulose fractions in Table 2.

[b] Not detected

At the extraction temperature 50°C, the content of HexA of H1 to H3 was increasing from 0.49 to 4.70% in GXI, but that of GXS increased firstly and then decreased (3.00, 7.14 to 1.00%), indicating a process that generation firstly and then degradation of HexA. At 80°C, the HexA content in GXI from H1 to H3 was continually increasing (3.27 to 8.71%), while that of GXS gradually decreased (4.63 to 0.76%).

Table 4
The molarity ratios of UA to sum of UA and HexA in hemicelluloses

		UA/(HexA + UA)		
T[a]	Hemicellulose fractions	H1	H2	H3
50	GXI	0.89	0.59	0.34
	GXS	0.77	0.55	0.88
80	GXI	0.60	0.26	0.25
	GXS	0.61	0.65	0.89

[a] Extracting at temperatures 50 and 80°C.

The ratio of the total contents of UA and HexA to xylose (Table 3), compared with their respective proportion, is used to analyze the degradation pattern. Under the same extraction condition, the ratio (HexA + UA)/Xyl of GXI showed an increasing trend on the whole (e.g. 0.06 to 0.11% at 50°C) when UA content was reducing and HexA content was increasing, indicating the content of UA converted to HexA was more than that of degraded in GXI. However, there was an opposite pattern in GXS (e.g. 0.18 to 0.11% at 50°C). The dynamic relationship between the degradation or transformation of glucuronic acid and the generation or degradation of HexA is represented by the molarity ratio of UA to the sum of UA and HexA (Table 4). The decreasing of the ratio may be due to higher conversion rate of UA or less degradation of HexA. At the harsher conditions, higher ratio in GXS than that in GXI showed the HexA of multi-branches xylan chains are more likely to remove during the alkaline extraction, corresponding to a lower HexA content.

3.2 Molecular weight analysis

The gel permeation chromatography (GPC) method was applied to evaluate the molecular weight of hemicellulose components (Himmel et al., 1990). The insoluble hemicellulose was acetylated (Fundador et al. 2012) for determination, so did the GXS to keep the consistency of experiment. The molecular weight of GXS was decreased by 1,150-3,255 g/mol during acetylation. The weight-average (M_w), number-average (M_n) molecular weights and polydispersity index (PDI) of the hemicellulose fractions GXS and GXI were shown in Table 5. At the same extraction temperature, the M_w of GXI (68,740 – 85,430 g/mol) was higher than that of GXS (69,980 – 82,540 g/mol), indicating that straight-chain xylan had a higher M_w than multi-branched xylan chains. Hoffmann et al. (1990) indicated that the highly branched xylan fractions had higher M_w than their less branched fractions. The conclusion is different in this study, and the causes may be related to the raw materials, extraction solvents and species of branched groups. Compared with wood meal, the pulp cooked has removed part of the branched chains and acetyl group, so the M_w of highly branched xylan chains was lower than that with higher linearity.

With the rising of extraction temperature, the M_w of hemicellulose was decreasing from 87,430 to 75,430 g/mol for GXI, and 86,570 to 71,310 g/mol for GXS. The same rule was also found at other H-factors. It is caused by the extensive cleavage of glycosidic bonds in hemicellulose and the formation of new reducing end-groups (Borrega & Sixta, 2013).

Table 5
Molecular weights analysis of the hemicellulose fractions GXS and GXI

Hemicellulose fractions		Molecular weights (g/mol)		
		M_n	M_w	PDI
H1-50	GXI	74,500	85,430	1.15
	GXS	68,610	82,540	1.20
H1-80	GXI	72,470	82,870	1.14
	GXS	63,790	75,150	1.18
H2-30	GXI	71,500	87,430	1.22
	GXS	66,270	86,570	1.31
H2-50	GXI	72,180	84,040	1.16
	GXS	66,560	81,040	1.22
H2-80	GXI	64,590	75,430	1.17
	GXS	58,050	71,310	1.23
H3-50	GXI	62,950	76,660	1.22
	GXS	62,460	78,170	1.25
H3-80	GXI	59,670	68,740	1.15
	GXS	56,520	69,989	1.24

The PDI (calculated by Mw/Mn) of GXI is range from 1.14 to 1.22, and that of GXS is 1.18 to 1.31, indicating that the molecular weight distribution of GXI is narrower than that of GXS. Furthermore, under the same extraction temperature, PDI was stable as the changing of H-factor compared with that influenced by extraction temperature, suggesting that the molecular weights and distribution of hemicellulose are largely related to the extraction temperature. In addition, it is worth to notice that the method of molecular weight determination here is relative and mainly depends on the measurement conditions used, such as the eluent, elution procedure and selection of standard samples et al.

3.3 FT-IR spectra analysis

The FT-IR spectra of the hemicellulose fractions GXI and GXS prepared from pulp with H2 and H3 at the same temperature is shown in Fig. 2. Generally, FT-IR spectra in the 1200 – 800 cm^{-1} region give the information about the main polysaccharides (Kacuráková, Ebringerová, & Hromádková, 1994). The spectra of GXI and GXS are similar, indicating that the chemical structure and conformational characteristics of them are basically same. The band at 3379 cm^{-1} and 2879 cm^{-1} are attributed to the -OH and C-H stretching vibration of hemicelluloses. The band at 1645 cm^{-1} corresponds to the water absorbed by hemicelluloses. The hemicelluloses have a strong affinity for water, and in the solid state these macromolecules may have disordered structures that can be easily hydrated (Kacuráková, Belton, & Wilson, 1998). Besides, the bands at 1596 and 1417 cm^{-1} are originated from the -COO^- symmetric stretching of the carboxyl group in MeGlcA. The bands at 1463 and 1384 cm^{-1} are correspond to the -CH_2 stretching and the C-H bending, respectively. In addition, the intensive absorption at 1041 cm^{-1} is due to the C-O-C stretching vibration of the glycosidic bond, which is the characteristic vibrations of xylans. Furthermore, a sharp band detected at 894 cm^{-1} is attributed to the C_1 group frequency, indicative of the β-configuration of the 1→4 glycosidic bond between the xylopyranose units in the xylan chains (Sun, Fang, & Tomkinson, 2001). These data are similar with those in the published literature (Peng et al., 2011; Sun et al., 2013).

These signals were observed in all the two components of different pulps, showing that the hemicelluloses extracted were mainly composed of glucuronoxylans, which was consistent with the HPAEC analysis. However, the spectral characteristic signal peaks of GXI component were slightly different. The signal at 1735 cm^{-1} in H3-GXI from protonated carbonyl stretching is generating a doublet in the spectrum. While the signal was not observed in GXS and H2-GXI components. After alkaline treatment, glucuronic acid residues and hexenuronic acid moieties from the pulp were deprotonated and converted to the sodium form (Bjarnestad & Dahlman, 2002). That indicated that the signal of carbonyl stretching in GXI extracted at higher temperatures were derived from structures other than uronic acids, which may be related to the binding linkages of lignin-carbohydrates (LCC). The LCC structure owns the characteristic peaks of stretching vibration from carbonyl group at 1720 cm^{-1} and an absorption band from substituted benzene at 1610 cm^{-1} (Guo, Xiu, & Liang, 2012). This means that the connected linkage of LCC structure is probably γ-ester which contains a carbonyl group. These results preliminary show that hemicellulose with LCC structure can be obtained under more stringent treatment conditions, and the position of the group connected with lignin is mainly along the xylan chains with high linearity.

3.4 NMR analysis

The main chain and the branching structure of the side chain distributed along the main chain of hemicellulose are investigated by NMR analysis. ^{1}H and ^{13}C spectrums of H2-GXS were shown in Fig. 3.

The β-(1→4) linked D-Xyl_p units were characterized by the signals at 3.05, 3.17, 3.26, 3.45, 3.88 and 4.27 ppm, corresponding to H-2, H-5a, H-3, H-4, H-5e and H-1, respectively. The two signals at 5.04 and 5.16 ppm originated from the protons of the hydroxyl groups were assigned to -OH at C-3 and C-2 positions, respectively. The strong signal at 3.42 ppm was ascribed to the residual solvent. The signal of acetyl groups at 2.11 ppm was not detected in the samples (Teleman et al., 2000), indicating the removal of acetyl groups under alkaline conditions. The ^{13}C NMR spectrum is displayed in Fig. 3b. The major signals at 102.25 ppm assigned to the anomeric region of C-1 in a β-configuration, and the signals at 63.73, 73.12, 74.49 and 75.92 ppm corresponded to C-5, C-2, C-3 and C-4, respectively. They are in agreement with those of literature (Habibi & Vignon, 2005; Sun et al., 2013). A weak signal peak of -OCH3 of MeGlcA residues could be observed at 59.54 ppm, but the intensity of the peak was not obvious due to the low frenquency as opposed to xylans (Vignon & Gey, 1998). There is no obvious distinction between the ^{13}C and ^{1}H NMR spectra of GXS and GXI (data not displayed), and 2D analysis is required for further structural characterization.

The 2D HSQC spectra of GXI and GXS in pulp with H2 and H3 extracted at 50°C are presented in Fig. 4a-4d. Obviously, the dominant characteristic cross-peaks of **β-(1→4) linked D-Xyl_p** units were detected at 102.2/4.27, 73.10/3.06, 74.51/3.27, 75.86/3.51, 63.71/3.88 and 63.77/3.17 ppm. Similarly, the corresponding signals of xylan in GXS are at 101.6/4.36, 72.58/3.16, 73.57/3.43, 76.22/3.66, 62.85/3.98 and 62.86/3.25 ppm. Moreover, the signals of uronic acid with less intensity could be observed in GXS (Fig. 4a,4b) at 98.04/5.23, 71.29/3.51, 73.62/3.66, 82.41/3.09, 72.05/4.22 and 59.80/3.34 ppm, which are assigned to the C_1-H_1, C_2-H_2, C_3-H_3, C_4-H_4, C_5-H_5 and $-OCH_3$ of **4-O-Me-α-D-GlcpA (MeGlcA)** units. These data are consistent with the literature published (Ebringerová et al., 2000; Arumugam et al., 2018).

Furthermore, there were more apparent characteristic signals of polysaccharide which were observed in GXS fraction (Fig. 4a,4b). While there were no obvious characteristic signals of glucose and arabinose in GXI (Fig. 4c,4d) showing that the GXI was consisted of glucuronoxylan with less glycosyl branched chains. Particularly, the intense cross-peak at 100.9/5.36 ppm was corresponded to terminal and →4-α-D-Glcp residues (Gullón et al., 2011), and similar signal was observed showing the linkage bond of (1→4)-α-D-Glcp. The characteristic signals of galactose and arabinose were identified partially in the Fig. 4a,4b, suggesting the presence of heteropolysaccharides in the multi-branched chain samples.

As previously analyzed, xylan chains attached with LCC structure is extracted under more stringent treatment, and similar characteristics were observed in 2D HSQC spectrum. The signal at position 56.25/3.73 ppm came from the $-OCH_3$ group on benzene ring of LCC complex structure (Yuan, Sun & Xu, 2011), and the signal was more obvious in GXI component, which indicated that LCC were mainly connected with xylan chains with higher linearity. This conclusion was consistent with FT-IR analysis.

The chemical shifts corresponding to more detailed structures of hemicellulose components separated are summarized in Table 6. The signals of anomeric proton resonances originating from these groups of different xylose residues could be divided into five types: (i) unsubstituted backbone D-xylose units corresponding to the major signals (**→4)-β-D-Xylp-(1→**); (ii) D-Xyl units substituted with 4-O-Me-D-GlcA or

α-D-Galp (**→4,2)-β-D-Xylp-(1→**); (iii) terminal (non-reducing end) D-Xyl residues **β-D-Xylp-(4→**); (iv) 4-O-Me-D-GlcA residues **4-O-Me-α-D-GlcpA-(1→**); (v) substituted α-D-Galp residues (**→4)-α-D-Glcp-(1→**). The chemical shifts of xylose residues reported here were in agreement with the published literature values earlier (Peng et al., 2011; Moghaddam et al., 2017). The peaks in the fingerprint region could be separated well from the other resonances.

According to the cross-peaks characteristic of the structural element of 2D HSQC diagram and combined with FT-IR analyses, a relatively possible structure of the extracted hemicellulose can be obtained. The Xylp units are attached with MeGlcA mainly substituted at the C_2 position, and glucose may be linked to the main or branch chains of Xylp in a 1→4 linkage. There are LCC complex structures connected by γ-ester on xylan backbone chains with higher linearity.

Table 6
Chemical shift assignments for glucuronoxylan in ^{1}H-^{13}C 2D-NMR

Monosaccharide unit		**1**	**2**	**3**	**4**	**5_{ex}[a]**	**5_{aq}**	**6**	**O-CH_3**
→4)-β-D-Xylp-(1→	^{1}H	4.36	3.16	3.43	3.66	3.98	3.25	-	-
	^{13}C	101.6	72.58	73.57	76.22	62.85	62.86	-	-
→4,2)-β-D-Xylp-(1→	^{1}H	4.52	3.30	3.42	3.66	3.98	3.25	-	-
	^{13}C	101.2	75.45	72.72	76.22	62.85	62.86	-	-
β-D-Xylp-(4→	^{1}H	4.52	3.16	3.43	3.48	3.85	3.18	-	-
	^{13}C	101.2	73.53	74.88	72.05	65.10	65.26	-	-
4-O-Me-α-D-GlcpA-(1→	^{1}H	5.17	3.51	3.66	3.09	4.22		-	3.34
	^{13}C	97.38	71.29	73.62	82.41	72.05		-	59.80
→4)-α-D-Glcp − (1→	^{1}H	4.58	3.56	3.69	3.61	3.73		4.24	-
	^{13}C	101.2	69.19	72.21	77.91	69.99		65.86	-

[a] Ax = axial, eq = equatorial

4. Conclusions

It is feasible to separate hemicellulose from pulp by alkali extraction and ethanol purification according to the solubility of different hemicellulose fractions. The glucuronoxylans extracted are divided into water-insoluble (GXI) and water-soluble hemicellulose fractions (GXS). It is found that GXI is dominated by straight xylan chain with high linearity, and GXS is composed by multi-branches xylan chain. At the same time, the cooking H-factor and extraction temperature affected seriously on the composition and structure of the two components. The higher H-factor and extraction temperature will reduce the contents of glycosyl side chains especially glucose (0.13–4.47% in GXI, 0.16–5.43% in GXS) and glucuronic acid (2.28–5.48% in GXI, 6.04–11.26% in GXS), and it also affects the molecular weight and distribution of hemicellulose fractions. Generally, the molecular weight of GXI (68,700 − 85,400 g/mol) was higher than that of GXS (70,000–82,500 g/mol). Hemicellulose with narrow distribution could be obtained under mild conditions (PDI = 1.15 for GXI, 1.20 for GXS), and more oligosaccharides were generated under harsher conditions, resulting more widely molecular weight distributed (PDI = 1.22 for GXI, 1.25 for GXS), Moreover, there is conversion of glucuronic acid (UA) to hexenuronic acid (HexA) accompanied with degradation of them. Glycosyl analysis showed that the UA in the straight xylan chains is more likely to convert into HexA, and the UA and HexA generated in the multi-branch xylan chains are tend to shed during the cooking and extracting. In addition, FT-IR and NMR analysis indicated the lignin-carbohydrates structures containing ester groups were mainly connected with high linearity xylan chains. These findings will be of great significance for the study of wood chemistry and further improve the pulping efficiency during the alkaline cooking.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Written informed consent for publication was obtained from all participants

Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files

Competing interests

All authors disclosed no relevant relationships.

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Authors' contributions

Min Luo performed the experiment, contributed significantly to data analyses and wrote the manuscript.

Shenlong Tian helped perform the analysis with constructive discussions.

Xingyu Lan helped perform the preparation of samples.

Shiyu Fu contributed to the conception of the study and manuscript revision.

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References

1. Arumugam, N., Biely, P., Puchart, V., Singh, S., & Pillai, S. (2018). Structure of peanut shell xylan and its conversion to oligosaccharides. Process Biochemistry, 72, 124–129.
2. Borrega, M., & Sixta, H. (2013). Purification of cellulosic pulp by hot water extraction. Cellulose, 20(6), 2803–2812.
3. Borrega, M., Tolonen, L. K., Bardot, F., Testova, L., & Sixta, H. (2013). Potential of hot water extraction of birch wood to produce high-purity dissolving pulp after alkaline pulping. Bioresource Technology, 135(Complete), 665–671.
4. Bjarnestad, S., & Dahlman, O. (2002). Chemical compositions of hardwood and softwood pulps employing photoacoustic fourier transform infrared spectroscopy in combination with partial least-squares analysis. Analytical Chemistry, 74(22), 5851.
5. Blackwood, A. D., Salter, J., Dettmar, P. W., & Chaplin, M. F. (2000). Dietary fibre, physicochemical properties and their relationship to health. Journal of the Royal Society for the Promotion of Health, 120(4), 242.
6. Cyran, M., Courtin, C. M., & Delcour, J. A. (2004). Heterogeneity in the fine structure of alkali-extractable arabinoxylans isolated from two rye flours with high and low breadmaking quality and their coexistence with other cell wall components. Journal of Agricultural and Food Chemistry, 52(9), 2671–2680.
7. Ebringerová, A., Alföldi, J., Hromádková, Z., Pavlov, G. M., & Harding, S. E. (2000). Water-soluble p-carboxybenzylated beechwood 4-O-methylglucuronoxylan: Structural features and properties. Carbohydrate Polymers, 42(2), 123–131.

8. Evtuguin, D. V., Daniel, A., & Neto, C. P. (2002). Determination of hexenuronic acid and residual lignin in pulps by UV spectroscopy in cadoxen solutions. Journal of Pulp and Paper Science, 28(6), 189–192.
9. Fundador, N., Enomoto-Rogers, Y., Takemura, A., & Iwata, T. (2012). Acetylation and characterization of xylan from hardwood kraft pulp. Carbohydrate Polymers, 87(1), 170–176.
10. Gellerstedt, G. (1996). An HPLC method for the quantitative determination of hexeneuronic acid groups in chemical pulps. Carbohydrate Research, 294(1–4), 41–51.
11. Goldmann, W. M., Ahola, J., Mikola, M., & Tanskanen, J. (2017). Formic acid aided hot water extraction of hemicellulose from european silver birch (*betula pendula*) sawdust. Bioresource technology, 232, 176–182.
12. Gullón, P., González-Munoz, M. J., Gool, M. P., Schols, H. A., & Hirsch, J., et al. (2011). Structural features and properties of soluble products derived from eucalyptus globulus hemicelluloses. Food Chemistry, 127(4), 1798–1807.
13. Guo, F., Xiu, Z. L., & Liang, Z. X. (2012). Synthesis of biodiesel from acidified soybean soapstock using a lignin-derived carbonaceous catalyst. Applied Energy, 98, 47–52.
14. Habibi, Y., & Vignon, M. R. (2005). Isolation and characterization of xylans from seed pericarp of Argania spinosa fruit. Carbohydrate Research, 340(7), 1431–1436
15. Hakala, T. K., Liitiä, T., & Suurnäkki, A. (2013). Enzyme-aided alkaline extraction of oligosaccharides and polymeric xylan from hardwood kraft pulp. Carbohydrate Polymers, 93(1), 102–108.
16. Himmel, M. E., Tatsumoto, K., Grohmann, K., Johnson, D. K., & Chum, H. L. (1990). Molecular weight distribution of aspen lignins from conventional gel permeation chromatography, universal calibration and sedimentation equilibrium. Journal of Chromatography A, 498(1), 93–104.
17. Hoffmann, R. A., Roza, M., Maat, J., Kamerling, J. P., Vliegenthart, J. FG. (1991). Structural characteristics of the cold-water-soluble arabinoxylans from the white flour of the soft wheat variety Kadet. Carbohydrate Polymers, 15, 415–430.
18. Höije, A., Gröndahl, M., Tømmeraas, K., & Gatenholm, P. (2005). Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks. Carbohydrate Polymers, 61(3), 266–275.
19. Jacobs, A., & Dahlman, O. (2001). Characterization of the molar masses of hemicelluloses from wood and pulps employing size exclusion chromatography and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Biomacromolecules, 2(3), 894–905.
20. Kacuráková, M., Belton, P. S., Wilson, R. H., Hirsch, J., & Ebringerová, A. (1998). Hydration properties of xylan-type structures: an ftir study of xylooligosaccharides. Journal of the science of Food and Agriculture, 77(1), 38–44.
21. Kacuráková, M., Ebringerová, A., Hirsch, J., & Hromádková, Z. (1994). Infrared study of arabinoxylans. Journal of the Science of Food and Agriculture, 66(3), 423–427.
22. Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H. V., & Auer, M., et al. (2010). Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and

enzymatic saccharification. Bioresource Technology, 101(13), 4900–4906.

23. Ma, M. G., Jia, N., Zhu, J. F., Li, S. M., Peng, F., & Sun, R. C. (2012). Isolation and characterization of hemicelluloses extracted by hydrothermal pretreatment. Bioresource Technology, 114(3), 677–683.
24. Marques, G., Gutiérrez, A., del Río, J. C., & Evtuguin, D. V. (2010). Acetylated heteroxylan from *Agave sisalana* and its behavior in alkaline pulping and TCF/ECT bleaching. Carbohydrate Polymers, 81(3), 517–523.
25. Mašura, V. (1998). A mathematical model for neutral sulfite pulping of various broadleaved wood species. Wood Science & Technology, 32(1), 1–13.
26. Miller, J. G., & Fry, S. C. (2001). Characteristics of xyloglucan after attack by hydroxyl radicals. Carbohydrate Research, 332(4), 389–403.
27. Moghaddam, L., Rencoret, J., Maliger, V. R., & Rackemann, D. W., et al. (2017). Structural characteristics of bagasse furfural residue and its lignin component. an NMR, PY-GC/MS, and FT-IR Study. ACS Sustainable Chem. Eng., 5(6), 4846–4855.
28. Peng, P., Peng, F., Bian, J., & Xu, F. (2011). Isolation and structural characterization of hemicelluloses from the bamboo species *Phyllostachys incarnata Wen*. Carbohydrate Polymers, 86(2), 883–890.
29. Silva, T., Colodette, J. L., Lucia, L. A., Oliveira, R. C., Oliveira, F. N., & Silva, L. (2011). Adsorption of chemically modified xylans on eucalyptus pulp and its effect on the pulp physical properties. Industrial & Engineering Chemistry Research, 50(2), 1138–1145.
30. Sun, S. L., Wen, J. L., Ma, M. G., & Sun, R. C. (2013). Successive alkali extraction and structural characterization of hemicelluloses from sweet sorghum stem. Carbohydrate Polymers, 92(2), 2224–2231.
31. Teleman, A., Lundqvist, J., Tjerneld, F., Stalbrand, H., & Dahlman, O. (2000). Characterization of acetylated 4-o-methylglucuronoxylan isolated from aspen employing H-1 and C-13 NMR spectroscopy. Carbohydrate Research, 329(4), 807–815.
32. Valoppi, F., Lahtinen, M. H., Bhattarai, M., Kirjoranta, S. J., Juntti, V. K., & Peltonen, L. J., et al. (2019) Centrifugal fractionation of softwood extracts improves the biorefinery workflow and yields functional emulsifiers. Green Chemistry, 21.
33. Vignon, M. R., & Gey, C. (1998). Isolation, 1H and 13C NMR studies of (4-O-methyld-glucurono)-d-xylans from luffa fruit fibres, jute bast fibres and mucilage of quince tree seeds. Carbohydrate Research, 307(1–2), 107–111
34. Wang, S. R., Guo, X. J., Liang, T., Zhou, Y., & Luo, Z. Y. (2012) Mechanism research on cellulose pyrolysis by Py-GC/MS and subsequent density functional theory studies. Bioresource Technology, 104, 722–728.
35. Wang, S. R., Ru, B., Lin, H. Z., & Luo, Z. Y. (2013). Degradation mechanism of monosaccharides and xylan under pyrolytic conditions with theoretic modeling on the energy profiles. Bioresource Technology, 143, 378–383.
36. Wen, J. L., Sun, Y. C., Xu, F., & Sun, R. C. (2010). Fractional isolation and chemical structure of hemicellulose polymers obtained from bambusa rigida species. Journal of Agricultural and Food

Chemistry, 58(21), 11372–11383.

37. Xu, F., Sun, J. X., Geng, Z. C., Liu, C. F., Ren, J. L., Sun, R. C., Fowler, P., & Baird, M. S., 2007. Comparative study of water-soluble and alkali-soluble hemicelluloses from perennial ryegrass leaves (*Lolium peree*). Carbohydrate. Polymers, 67, 56–65.
38. Yuan, T. Q., Sun, S. N., Xu, F., & Sun R. C. (2011). Characterization of lignin structures and lignin-carbohydrate complex (LCC) linkages by quantitative C-13 and 2D HSQC NMR spectroscopy. Journal of Agricultural and Food Chemistry, 59(19), 10604–10614.
39. Yuan, Y., Zou, P., & Zhou, J. H., Geng, Y. T., Fan, J. J., & Li, Y. Q., et al. (2019). Microwave-assisted hydrothermal extraction of non-structural carbohydrates and hemicelluloses from tobacco biomass. Carbohydrate Polymers, 223.
40. Zhang, H., Nie, S., Qin, C., & Wang, S. (2019). Removal of hexenuronic acid to reduce AOX formation in hot chlorine dioxide bleaching of bagasse pulp. Industrial Crops and Products, 128, 338–345.

Figures

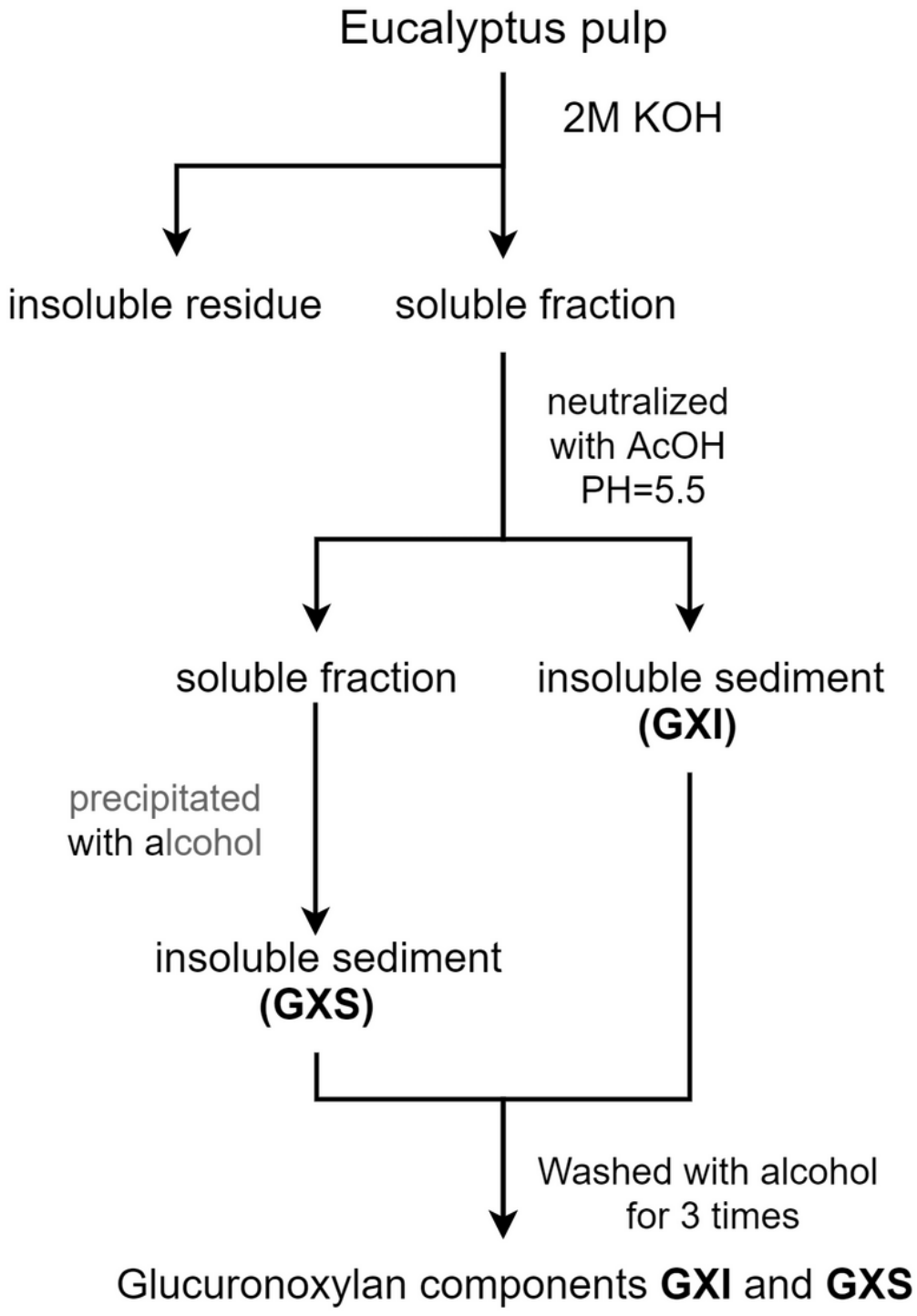

Figure 1

Sequential extraction and fractionation of hemicelluloses in Eucalyptus pulp

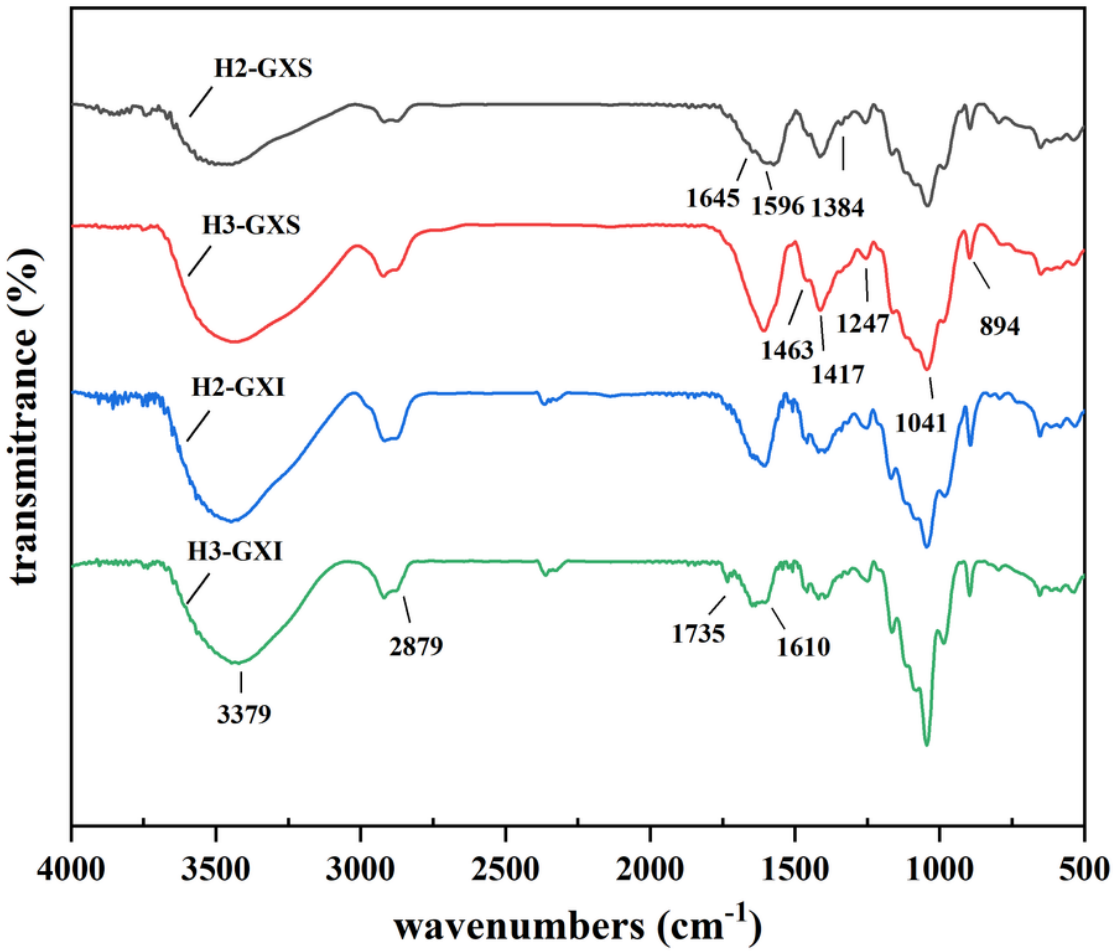

Figure 2

FT-IR spectrum of hemicellulose fractions GXI and GXS isolated

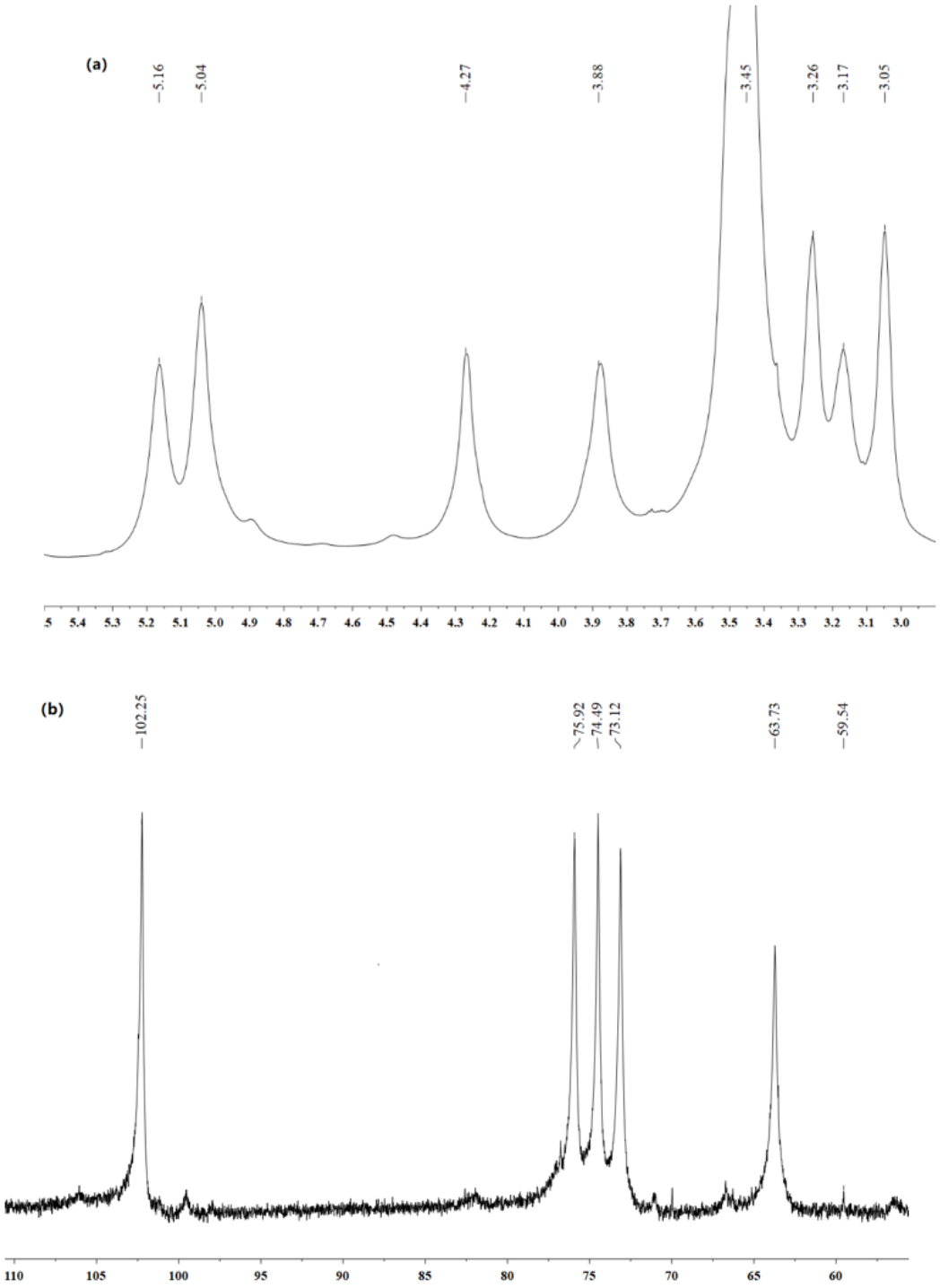

Figure 3

^{1}H (a) and ^{13}C (b) NMR spectra of hemicellulose fraction H2-GXS

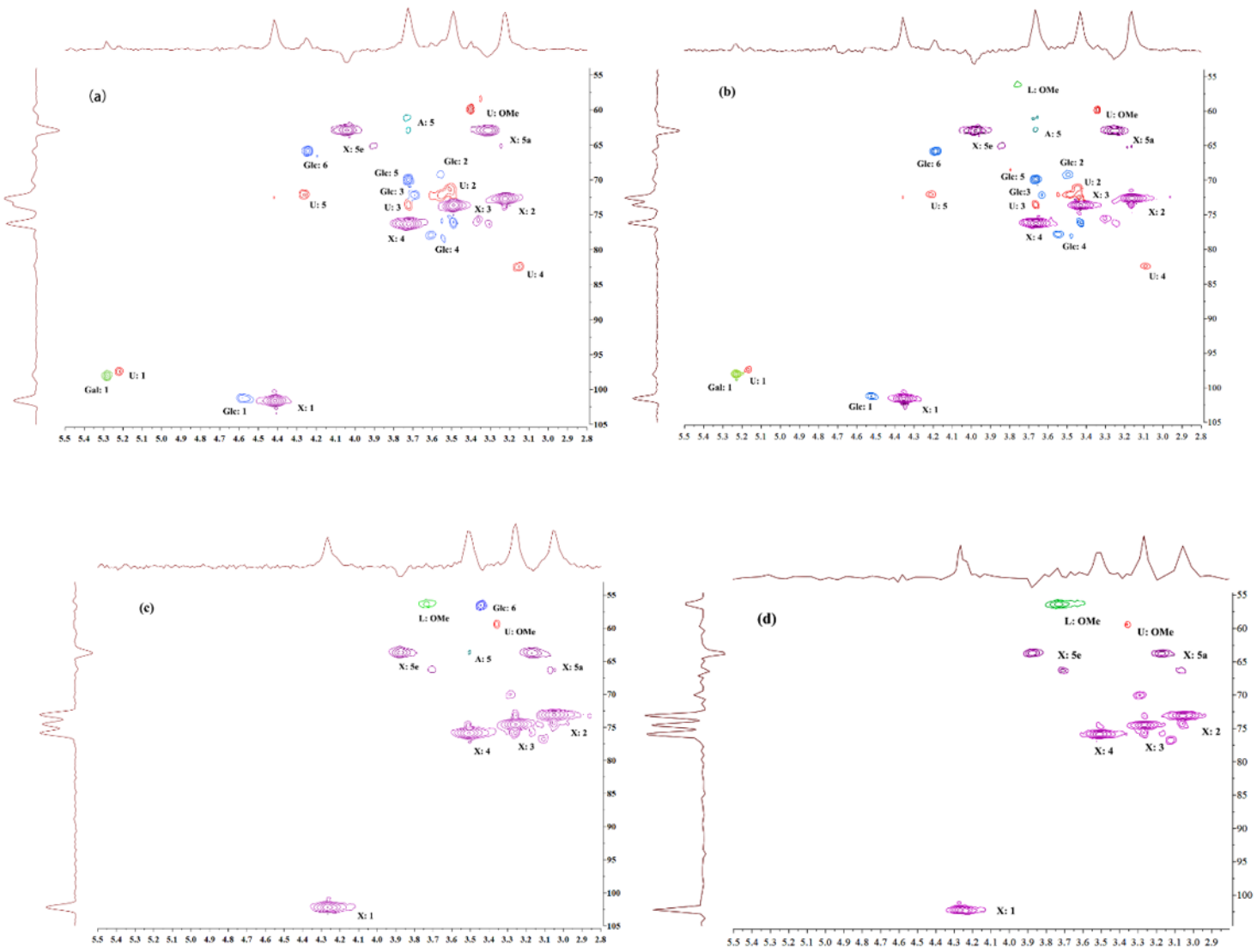

Figure 4

2D HSQC (GXS in D_2O, GXI in DMSO, 25°C) spectra of hemicellulose fractions (a).H2-GXS; (b).H3-GXS; (c).H2-GXI; (d).H3-GXI extracted at 50 °C from different H-factors pulp. Designations are as follows: X, Xylp unit; U, 4-O-Me-α-D-GlcpA (MeGlcA) unit; Glc, Glcp unit; Gal, Galp unit; L, lignin-carbohydrates.