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Original article

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Facile fractionation of bamboo hydrolysate and characterization of isolated lignin and lignin-carbohydrate complexes

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Abstract: An efficient separation technology for hydrolysates towards a full valorization of bamboo is still a tough challenge, especially regarding the lignin and lignin-carbohydrate complexes (LCCs). The present study aimed to develop a facile approach using organic solvent extraction for efficiently fractionating the main components of bamboo hydrolysates. The high-purity lignin with only a trace of carbohydrates was first obtained by precipitation of the bamboo hydrolysate. The water-soluble lignin (WSL) fraction was extracted in organic solvent through a three-stage organic solvent extraction process, and the hemicellulosic sugars with increased purity were also collected. Furthermore, a thorough characterization including various NMR techniques (^{31}P, ^{13}C, and 2D-HSQC), GPC, and GC-MS was conducted to the obtained lignin-rich-fractions. It was found that the WSL fraction contained abundant functional groups and tremendous amount of LCC structures. As compared to native LCC of bamboo, the WSL fraction exhibited more typical LCC linkages, i.e. phenyl glycoside linkage, which is the main type of chemical linkage between lignin and carbohydrate in both LCC samples. The results demonstrate that organic phase extraction is a highly efficient protocol for the fractionation of hydrolysate and the isolation of LCC-rich streams possessing great potential applications.

Keywords: hydrolysate; lignin; lignin-carbohydrate complexes (LCCs); organic solvent fractionation.

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1 Introduction

Lignocellulosic biomass is the most abundant natural resource on our earth, and has been recognized as an increasingly well-known alternative to petroleum-based products on the basis of its renewability and sustainability. Recently, the integrated biorefinery concept provides a well-defined pathway to build a promising platform towards the whole valorization of carbohydrates and lignin in lignocellulosic biomass (Cao et al. 2019; Galkin and Samec 2016; Rivas et al. 2019). To achieve an integrated biorefinery process, a green and efficient fractionation approach is essential for breaking the recalcitrance of biomass. Autohydrolysis, in which raw material is pretreated with pure water, may be applied to selectively remove hemicelluloses prior to pulping processes (Koo et al. 2019; Korotkova et al. 2015; Santos et al. 2018; Ullah et al. 2018). Most of hemicelluloses could be extracted in the form of oligomers from lignocellulosic biomass through the hot-water extraction at a high temperature range (160–240 °C) (Alvira et al. 2010; Ballesteros et al. 2017). The purified hemicellulosic sugars are a widely available raw material for producing high-value-added products (Avanthi et al. 2017; Jeong et al. 2018; Mechmech

et al. 2015). However, other non-saccharide compounds, including lignin and lignin-carbohydrate complexes (LCCs), could also be partially depolymerized (Dieste et al. 2016; Mohtar et al. 2015). The dissolved lignin and LCCs have been proven to be promising potential products with added values (Huang et al. 2019; Narron et al. 2017; Zhang et al. 2018b). Lignin has been recognized as an appropriate feedstock for producing low molar mass chemicals, such as vanillin, hydroxylated aromatics, and aliphatic acids (Huber et al. 2006; Wang et al. 2013). There has particularly been an emerging interest in the use of LCCs in various fields, such as medicine and cosmetic formulations, due to their diverse unique pharmacological activities and novel natural antioxidants (Min et al. 2014; Niu et al. 2016; Sakagami and Matsuta 2013; You et al. 2015).

Most of the recovery and purification processes for the hydrolysates only focused on producing pure hemicellulosic sugars, while the dissolved lignin and LCCs were not valorized. Hence an effective separation method is critical to ensure an efficient separation of the main components in the hydrolysate, including lignin-rich compounds. Various treatment processes have been developed, such as precipitation (Mohtar et al. 2015), membrane filtration (Arkell et al. 2014), and adsorption (Chen et al. 2016). The previous study demonstrated that Amberlite XAD-4 resin could remove 90% of acid-soluble lignin from hemicelluloses-rich hydrolysates, and 85% of the adsorbed lignin could be further recovered (Schwartz and Lawoko 2010). It was found that the solubilized lignin fraction from the auto-hydrolysate contains a certain amount of LCCs, accounting 10 linkages per 100 aromatic rings (Narron et al. 2017). However, these processes were observed to be nonselective between lignin and LCCs. Lignin and LCCs are simply considered as a complex mixture of aromatic compounds in the hydrolysate and thus ignored their high-value potential. To overcome the challenges in separation and subsequent applications, more efforts should be contributed to the selective fractionation and structural characterization of the aromatic compounds, including lignin and LCCs, obtained from the hydrolysates.

The present study aimed at the simultaneous separation and recovery of the main components in a bamboo hydrolysate, especially for the dissolved lignin and LCCs. First, the non-soluble lignin in the hydrolysate was recovered by precipitation, and then a combined organic solvent extraction process, consisting of two-stage dioxane and one-stage ethanol extraction, was performed for separation of hemicellulosic sugars and water-soluble lignin (WSL) (Figure 1). The chemical composition and structural features of the obtained aromatic compounds were comprehensively revealed using different analytical technologies, such as gas chromatography (GC), pyrolysis gas chromatography-mass spectrometry (Py-GC-MS), high-performance size exclusion chromatography (HPSEC), and two-dimensional heteronuclear single quantum coherence (2D HSQC). The successful separation of auto-hydrolysate and the thorough characterization of the obtained aromatic compounds could facilitate their further valorization and improve the economics of overall biorefining of lignocellulosic biomass.

2 Materials and methods

2.1 Materials

Bamboo chips with less than 3 mm in thickness were obtained from the Sichuan province of China. The contents of Klason lignin, acid-soluble lignin, and ash in the bamboo chips were 25.56, 1.87, and 2.20%, respectively (Zhang et al. 2018a). The carbohydrate composition included glucose (44.03%), xylose (24.95%), arabinose (1.57%), galactose (0.48%), and mannose (0.29%). All chemical reagents were of analytical grade.

2.2 Autohydrolysis process

The mixture of 200 g of dry bamboo chips and 1.2 L of distilled water were added in a 15 L electrically heated rotary digester (Xianyang Tongda Light Industrial Equipment Co. Ltd., China), which was heated to 170 °C for 60 min. After the autohydrolysis treatment, the mixture was rapidly cooled down to about 80 °C and then separated by filtration. The obtained solid residue was fully washed with distilled water and oven-dried at 50 °C.

2.3 Separation of hydrolysate

As shown schematically in Figure 1, the obtained hydrolysates were centrifuged to remove the non-soluble solid. The obtained solid was precipitated lignin (PL). The filtrate was then evaporated to near dryness by using a rotary evaporator at 45 °C. The concentrated solid was extracted by a three-stage organic solvent extraction, including a two-stage dioxane extraction and one-stage ethanol extraction, at boiling for 3 h using soxhlet extractor, respectively. After each extraction stage, the residue solid, namely hemicellulosic sugars, was collected and further dried using a vacuum oven at 50 °C. The aromatic compounds were dissolved into organic phase during organic solvent extraction. The obtained organic solutions from the three-stage solvent extraction process were combined. Then the aromatic compounds-rich solution was evaporated to recover the organic solvents for reuse. The concentrated aromatic compounds were further dried using a vacuum oven at 50 °C, referred as WSL.

2.4 Preparation of milled wood lignin (MWL) and native LCC

Milled wood lignin (MWL) and native LCC from bamboo were isolated for comparison. The MWL and native LCC from the bamboo chips were prepared based on the Björkman method (Björkman 1954). Briefly,

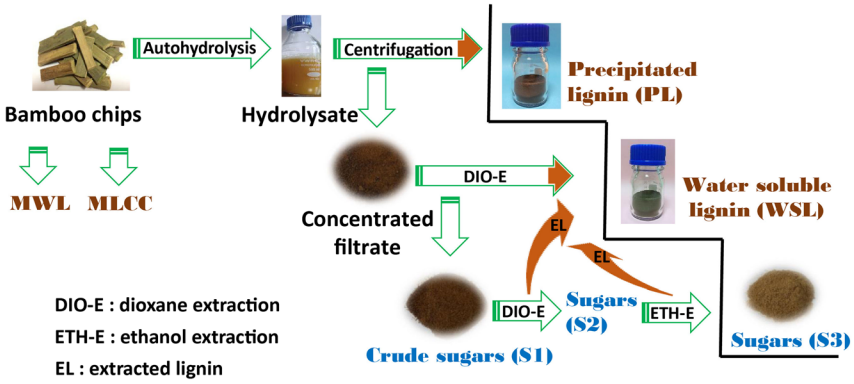

Figure 1: Flowchart for the fractionation of bamboo hydrolysate.

after the ball milling of bamboo, the obtained solid carried out a two-stage extraction process with dioxane/water (96:4, v/v). After centrifugation of the mixture, the supernatant was crude MWL solution, while the residue was reserved to extract LCC. The organic solvent in crude MWL solution was removed by evaporation. The obtained crude MWL solid was further purified by using acetic acid/water (9:1, v/v) and 1,2-dichloroethane/ethanol (2:1, v/v), respectively. The above reserved residue was dissolved into dimethyl sulfoxide and continuously purifying with 1,2-dichloroethane-ethanol (2:1, v/v) and acetone-acetic acid (99:1, v/v). The isolated Björkman LCC from the raw materials was termed as MLCC.

2.5 Characterization

GC was used for the analysis of carbohydrate content by according to a previous publication (Sundberg et al. 1996). Briefly, after the acid methanolysis process of the dried samples, sorbitol and resorcinol were added into the mixture. Then the mixture was evaporated by nitrogen flow. After further oven drying, the samples were silylated by adding pyridine, HDMS, and TMCS reagents, respectively, and then the mixture was transferred to GC vials for analyzing by GC-FID. Molar mass and dispersity of the obtained lignin fractions were analyzed by a HPLC instrument (Agilent 1100 Series) equipped with UV detector (Shimadzu Corp., Tokyo, Japan). In brief, the acetylated lignin was first dissolved in tetrahydrofuran (THF) with a concentration of 1 mg/mL, and then the molar mass and dispersity index were determined using a Shimadzu HPLC system, including system controller SCL-10AVP on-line degasser DGU-14A, low-pressure gradient valve FCV-10ALVP, HPLC pump LC-10ATVP, autosampler SIL-20AHT and column oven CTO-10ACVP, equipped with a sequentially connected guard column (50 × 7.8 mm) and two Jordi Gel DVB 500A (300 × 7.8 mm) columns in series. The column was operated at 40 °C and the eluent was THF with 1% acetic acid at a flow rate of 0.8 min^{-1}. Fifty-microliter solution was injected by the autosampler.

A 500 MHz Bruker Avance instrument was used for the 2D HSQC-NMR analysis. 80 mg of sample dissolved in 0.75 mL of DMSO-d_6 was placed into a 5 mm NMR tube. A standard Bruker HSQC pulse sequence, "hsqcedegpsisp2.3," was used. Quantitative ^{31}P NMR spectra were performed according to published method (Zhang et al. 2017).

Py-GC-MS analysis of the samples was conducted by a filament pulse resistance-heated pyrolyser Pyrola 2000 (Pyrol AB, Lund, Sweden) connected to an HP 6890–5973 GC-quadrupole-MSD instrument (Hewlett-Packard, Palo Alto, CA) equipped with a ZB-35 column of 30 m × 0.25 mm inside diameter according to published method (Zhang et al. 2018b).

Table 1: Characteristics of bamboo hydrolysate (mg/mL).

Components	
Hemicellulosic sugars	9.5
Oligosaccharides	8.5
Xylo-oligosaccharides	7.3
Monosaccharides	1.0
Furfural	0.6
Acetic acid	2.9
Precipitated lignin	1.01
Water soluble lignin	2.13
Total solid	39.4
pH	4.1

3 Results and discussion

3.1 Separation of the main components in the hydrolysate

The bamboo chips were extracted using autohydrolysis to obtain hydrolysates with a complex composition. As

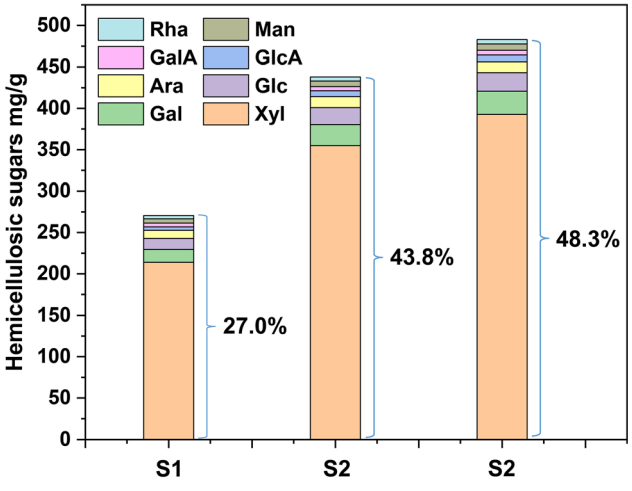

Figure 2: Carbohydrate components of recovered and purified hemicellulosic sugars from hydrolysate.

Table 2: Composition analysis of milled wood lignin (MWL), MLCC and the isolated lignin fractions from hydrolysate.

Sample	Ara	Xyl	Rha	GlcA	GalA (mg/g)	Man	Gal	Glc	Total
MWL	3.6	34.6	1.8	8.4	9.9	7.4	1.3	3.6	70.5
MLCC	36.9	401.6	4.2	5.1	6.6	5.2	26.7	61.3	547.6
PL	3.6	9.3	0.2	3.4	4.0	0.4	1.2	8.5	30.5
WSL	51.5	97.7	6.90	3.0	4.7	2.6	14.2	37.5	218.1

Arabinose (Ara), xylose (Xyl), rhamnose (Rha), glucuronic acid (GlcA), galacturonic acid (GalA), mannose (Man), galactose (Gal), glucose (Glc).

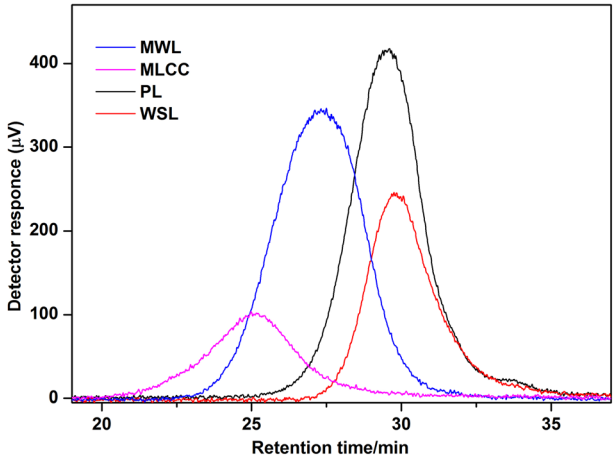

Figure 3: High-performance size exclusion chromatography (HPSEC) chromatogram of the isolated fractions (PL and WSL) from hydrolysate as compared to the milled wood lignin (MWL) and MLCC from bamboo.

shown in Table 1, the main composition in the hydrolysate included hemicellulosic sugars, lignin, and other degraded products from carbohydrates. The oligosaccharides and monosaccharides were the major products obtained from the extracted hemicelluloses with a concentration of 8.5 and 1.0 mg/mL, respectively, as expressed by sugar monomers. It is indicated that 89.4% of the hemicelluloses were extracted in the form of oligomers during autohydrolysis. Besides hemicellulosic sugars, lignin was also abundant in the hydrolysate, including precipitated lignin (1.01 mg/mL) and water soluble lignin (2.13 mg/mL). The dissolved and colloidal lignin in hydrolysate makes the separation and purification of hemicellulosic sugars extremely difficult due to its special physical chemistry characteristics (Koivula et al. 2013; Norgren and Edlund 2014). However, the dissolved lignin-rich fractions are also expected to be recovered as value-added products from hydrolysate.

Dioxane has been widely applied as an effective solvent for extracting lignin with relatively low structural modification (Evtuguin et al. 2001). In this work, organic solvent extraction including two-stage dioxane and one-stage ethanol extraction, was explored to extract the solubilized lignin while purifying the hemicellulosic sugars. As it can be seen from Figure 2, an increased content of hemicellulosic sugars from 27.0 to 43.8% could be achieved through the second-stage dioxane extraction, and the final purity of 48.3% was reached after the final ethanol extraction. The main constituent of the recovered hemicellulosic sugars was xylose, but other sugars were also present, such as arabinose, rhamnnose, and glucose. It can also be found that the brightness of the hemicellulosic sugar-rich fractions (S1, S2, and S3) obtained via organic solvent extraction was increased due to that a certain amount of lignin was removed.

As shown in Table 2, the carbohydrate analysis of the separated lignin-rich fractions from the hydrolysate indicated that xylose/xylan was the predominant sugar component. It is also noticed that a significant amount of carbohydrates existed in the WSL, reaching approximately 21.8%, indicating that these carbohydrates might be covalently linked to lignin structures, namely LCC. Narron et al. (2017) found that the obtained soluble lignin in a prehydrolysate was full of LCC substructures. Meanwhile for precipitated lignin, only 3.0% of carbohydrates could be found, indicating a relatively high purity, which is beneficial for their subsequent application. Compared to other separation processes of hydrolysates, such as polyaluminium chloride precipitation and XAD resin adsorption, organic solvent extraction presents a facile and efficient process to achieve the whole recovery of carbohydrates and lignin-rich fractions in the hydrolysate (Chen et al. 2014; Narron et al. 2017).

3.2 Molar mass

The molar mass distribution of the isolated PL and WSL fractions from the hydrolysate, as well as their reference MWL and MLCC, are shown in Figure 3. As can be seen, the MWL and MLCC showed a higher molar mass and a wider molar mass distribution than PL and WSL, indicating that the degradation of the lignin occurred during the

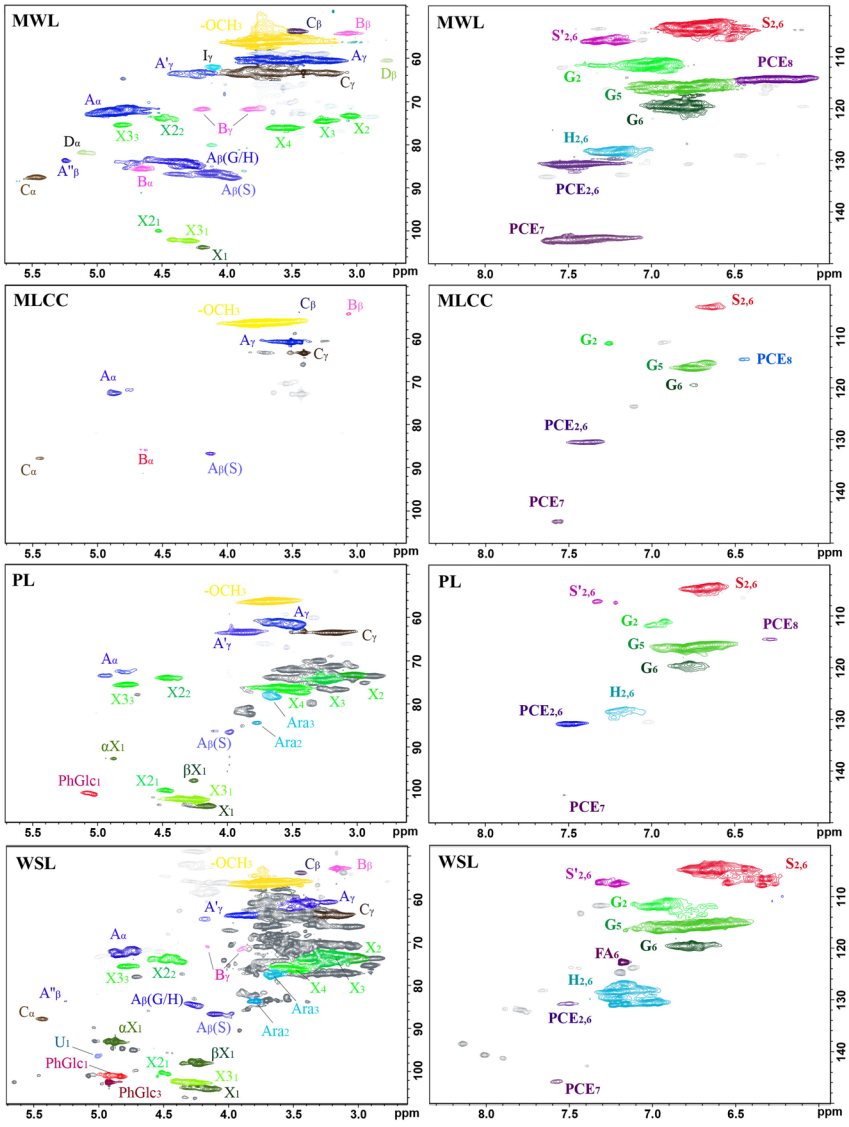

Figure 4: Side-chain regions (left column) and aromatic regions (right column) in the 2D HSQC NMR spectra of the isolated fractions (PL and WSL) from hydrolysate as compared to the MWL and MLCC from bamboo.

autohydrolysis process. It has been demonstrated that the lignin with high water solubility could be attributed to a high carbohydrate content and low molar mass (Capanema et al. 2004; Nakagame et al. 2010). In this work, although the WSL showed a significant higher carbohydrate content, its chromatogram of the molar mass showed a similar main peak as compared to that of PL. This suggested that a certain amount of carbohydrates in the WSL might link to lignin and form LCC structures, thus possessing high molar mass that is comparable to PL.

3.3 Structural analysis by 2D-HQSC

The PL and WSL, as well as the isolated MWL and MLCC from their original whole cell walls, were analyzed by 2D HSQC NMR. The obtained spectra and the main substructures are depicted in Figures 4 and 5, respectively. The main signal assignments are listed in Supplementary Table S1 based on published literature (Huang et al. 2018; You et al. 2015; Zhang et al. 2018a,b). As it can be seen from Figure 4A, MWL showed a predominance of lignin signals, including β-*O*-4 alkyl-aryl ether linkages (A, A′, and A″), resinol (β–β, B) and the phenyl coumaran substructures (β-5, C). These three prominent signals could also be observed in the HSQC spectra of PL, WSL, and MLCC. It is noted that the weak signals corresponding to β-*O*-4, β–β, and β-5 in the spectrum of the PL were also observed. The prominent signals in the aromatic region corresponding to the syringyl (S) unit, guaiacyl (G) unit, and pendent moieties *p*-coumarte (PCE), could be observed in the spectra of all the samples, while *p*-hydroxyphenyl (H) lignin units was missing in MLCC.

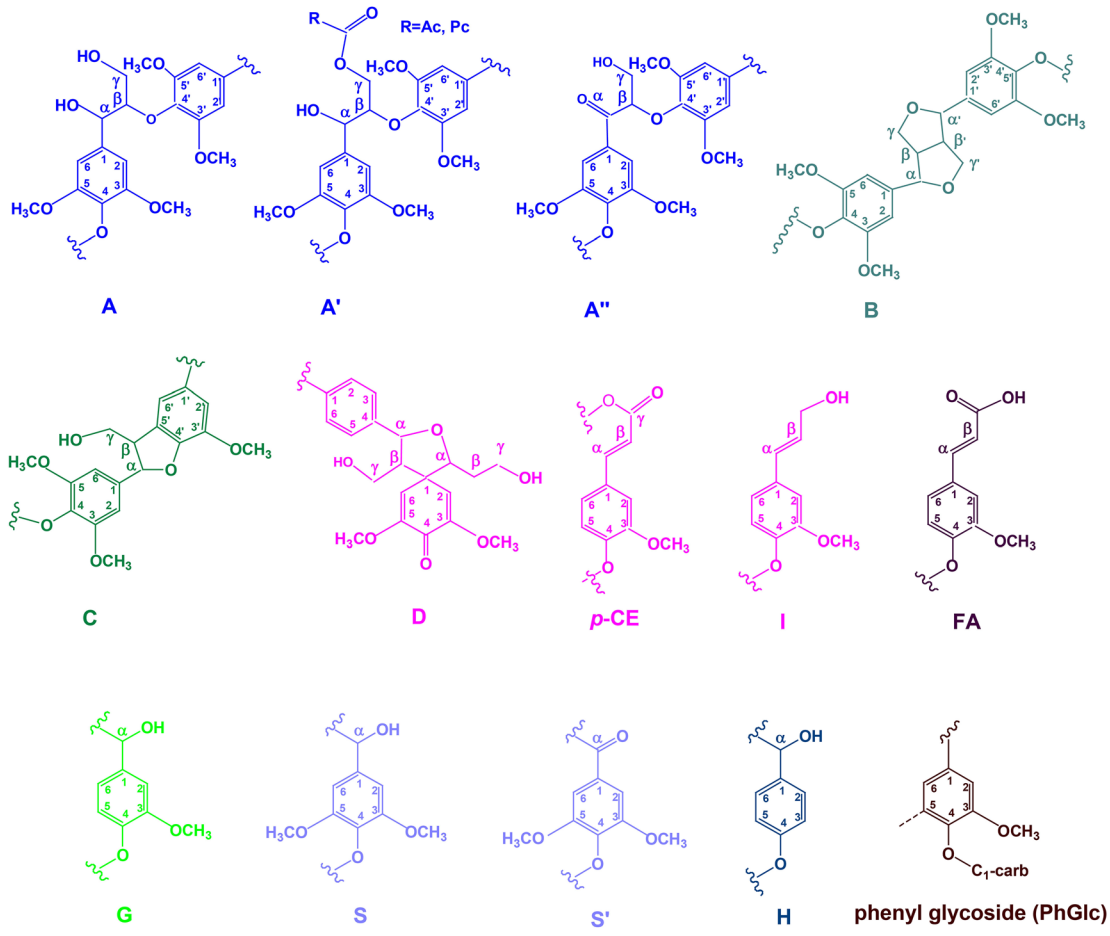

Figure 5: Main substructures and linkages of isolated lignin and LCC involving different side-chain linkages and aromatic units identified by 2D HSQC NMR.

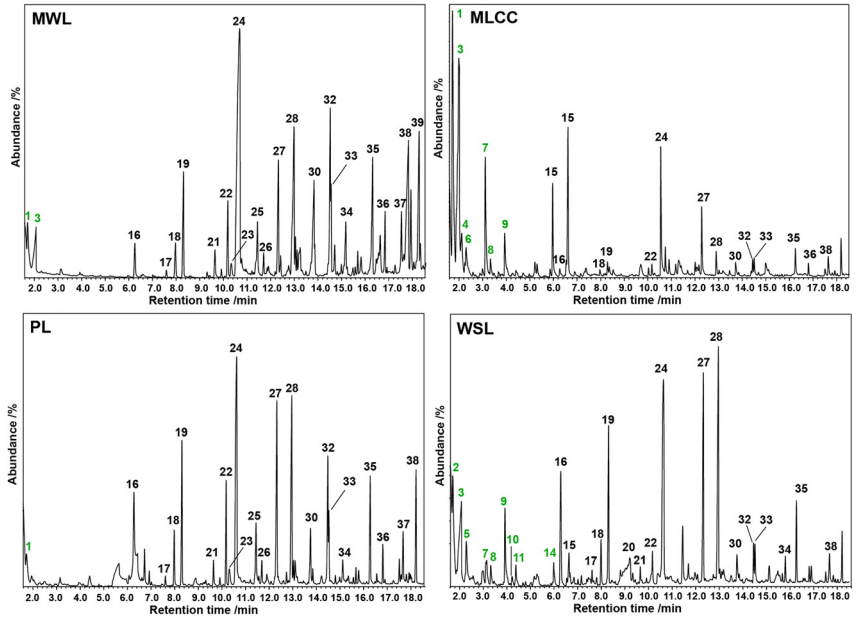

Figure 6: Pyrogram of Py-GC-MS of different lignin fractions.

Table 3: Peak assignment and relative molar abundances of the phenolic compounds released from MWL, MLCC and the isolated lignin fractions from hydrolysate after py-GC-MS.

Peak no.	Phenolic compounds	Relative content (%)			
		MWL	PL	MLCC	WSL
1	Acetone	1.66	1.87	10.62	
2	1-Methyl acetate				5.56
3	Acetic acid	1.86		9.56	5.41
4	Methylcyclopentane			2.18	
5	Hydroxyacetone				1.73
6	Methyl acetate			1.72	
7	3-Hydroxypropanal			4.30	0.57
8	Methyl 2-oxopropanoate			0.73	0.90
9	Furfural			1.60	3.29
10	2-Furanmethanol				0.45
11	Acetoxyacetone				1.00
12	2(5h)-Furanoneone				0.50
13	1,2-Cyclopentanedione				0.93
14	5-Methylfurfural				0.94
15	4-Hydroxy-5,6-dihydro-(2h)-pyran-2-one			7.12	1.21
16	Phenol	0.91	5.26	0.27	4.62
17	*o*-Cresol	0.15	0.27		0.36
18	*p*-Cresol	0.89	1.74	0.17	1.48
19	Guaiacol	2.41	4.69	0.40	5.01
20	3-Pyridinol				4.18
21	*p*-Ethylphenol	0.70	0.83		0.45
22	3-Methylguaiacol	0.17	0.23		0.16
23	4-Methylguaiacol	1.62	2.98	0.30	0.10
24	Catechol	0.49	0.97		0.68
25	4-Vinylphenol	17.00	13.76	3.89	12.52
26	3-Methoxycatechol	1.69	2.66	0.34	2.44
27	4-Ethylguaiacol	0.45	0.78	0.12	0.70
28	4-Vinylguaiacol	2.92	7.01	1.87	7.12
29	Syringol	5.41	7.69	0.68	9.21
30	Eugenol	0.75	0.71	0.12	0.31
31	3,4-Dimethoxyphenol	0.44	0.97		0.33
32	4-Propylguaiacol	0.53	0.25		
33	Vanillin	3.43	2.19	0.51	1.18
34	*Cis*-isoeugenol	0.35	0.41	0.10	0.28
35	4-Methylsyringol	3.85	4.16	0.41	1.05
36	*Trans*-isoeugenol	1.17	1.85	0.46	1.15
37	Acetovanillone	1.35	0.88	0.23	0.82
38	D-Allose	0.38	0.44	0.57	0.78
39	4-Vinylsyringol	3.17	3.42	0.74	2.32
40	4-Prop-2-enyl syringol	1.17	1.03	0.30	0.40
41	4-Allylsyringol	1.46	0.76	0.22	0.30
42	Syringaldehyde	5.90	1.91	0.58	1.19
43	*Cis*-coniferyl alcohol	1.72	0.34		0.26
	Phenol units (P)	18.72	16.33	4.06	14.60
	Guaiacol units (G)	28.79	26.64	5.69	21.83
	Syringol (S)	38.85	27.83	5.82	19.84

The HSQC spectra of the WSL and MLCC exhibited relatively strong signals from the associated carbohydrates, including β-$_D$-xylopyranoside units (X) and arabinofuranoside units (Ara). The prominent cross-signals of C_1–H_1 (X_1), C_2–H_2 (X_2), C_3–H_3 (X_3), and C_4–H_4 (X_4) correlations from X were clearly observed. It can be concluded that β-$_D$-xylopyranoside was the prominent polysaccharide linked with lignin in both the WSL and MLCC. However, no signals from carbohydrates could be found in the HSQC spectrum of the PL, which is in accordance with the carbohydrate analysis results. It is also further proved that the PL exhibited a relatively high purity. Previous studies reported that the predominant linkages in LCCs, including phenyl glycoside (PhGlc), benzyl ether, and benzyl ester linkages, can be identified from 2D HSQC cross-signals. It could now be found that the relatively strong signals corresponding to PhGlc linkages, including $PhGlc_1$ and $PhGlc_3$ linkages, were present in the WSL, while only a weak signal from $PhGlc_1$ linkages could be detected in the MLCC. Hence, it can be concluded that the isolated WSL was a LCC-rich lignin fraction, which contained more PhGlc LCCs structures than the prepared native bamboo LCC (MLCC). It also proved to be highly selective for the separation between lignin and LCCs using the presented pathway.

3.4 GC-MS analysis

After the organic phase extraction process, the structures of the obtained lignin-rich fractions, including PL and WSL, as well as their reference MWL and MLCC, were further investigated by using GC-MS. As it can be seen in Figure 6, the first part of the pyrograms in the range of 1–6 min originated from the pyrolysis products of carbohydrates, while the range of 6–19 min contained the complex mixture of phenolic lignin-derived products. The identified peaks and their relative abundances are listed in Table 2, according to previously published data (Liu et al. 2017; Łucejko et al. 2009).

As can be seen in Table 3, the typical lignin-derived products could be detected from the four samples, including phenol (P), guaiacol (G) and syringyl (S) unit fragments. By contrast, a slight decrease in the S/G ratio was noticed, being 1.35, 1.04, 1.02, and 0.91 in the MWL, PL, MLCC, and WSL, respectively. Among the pyrolytic products, their abundances derived from different samples were quite different (Table 3). As can be seen, the most abundant pyrolytic lignin fragments were 4-vinylphenol (17.00%), syringaldehyde (5.90%), and syringol (5.41%) for the MWL, 4-vinylphenol (13.76%), syringol (7.69%), and phenol (5.26%) for the PL, while the MLCC presented the abundant pyrolytic carbohydrate fragments, including acetone (10.62%), acetic acid (9.56%), and 4-hydroxy-

Table 4: Quantification of the lignin samples isolated from hydrolysate as compared to MWL by quantitative ^{31}P NMR method (mmol/g).

Lignin sample	Aliphatic OH	Phenolic OH					Carboxylic acid
		Syringyl OH		Guaiacyl OH		*p*-Hydroxyl	
		C[a]	NC[b]	C	NC	OH	
MWL	4.52	0.05	0.23	0.14	0.48	0.74	0.24
PL	3.07	0.10	0.80	0.33	0.92	0.80	0.62
WSL	92.67	0.64	4.53	0.83	4.55	4.19	0.11

[a]C, condensed.
[b]NC, non-condensed.

5,6-dihydro-(2h)-pyran-2-one (7.12%%). The large amounts of 4-vinylphenol may be explained by the presence of *p*-coumaric acid, since 4-vinylphenol originate from the decarboxylation of *p*-coumaric acid under pyrolytic conditions (Choi et al. 2001; del Río et al. 2012). The approximately sparse signals from pyrolytic carbohydrates of PL could be tracked, indicating the high purity of the obtained PL. For the WSL, pyrolytic fragments originated from both lignin and carbohydrates could be observed, including 4-vinylphenol (12.52%), syringol (9.21%) and 4-vinylguaiacol (7.12%) from the pyrolytic lignin and 1-methyl acetate (5.56%) acetic acid (5.41%) and furfural (3.29%) from the pyrolytic carbohydrates. This could be explained by the numerous LCC structures as revealed by the above-mentioned HSQC analysis.

3.5 Functional group analysis by ^{31}P NMR spectra

Quantitative ^{31}P NMR analysis of the obtained lignin samples from the hydrolysate and raw material is detailed in Table 4. The decrease of the content of aliphatic hydroxyl groups in the PL suggested that the autohydrolysis process resulted in the oxidation or modification of this kind of structure, as compared to MWL (Wen et al. 2013). Moreover, PL exhibited a higher content of phenolic hydroxyl groups than MWL, indicating that the cleavage of a certain number of β-*O*-4 linkages occurred during autohydrolysis (El Hage et al. 2009; Wen et al. 2013). The increasing of the content of carboxylic groups in the PL could be explained by the occurred oxidation reaction of lignin. It was reported that *p*-CE could be cleaved during autohydrolysis and became free *p*-CA and co-precipitated with lignin (Wen et al. 2013). Most impressively, the content of hydroxyl groups in the WSL was greater than that in the MWL and PL, especially for aliphatic hydroxyl groups, which might be attributed to the numerous hydroxyl-rich carbohydrates linked to lignin, possessing more functional groups. The abundant functional groups in the isolated lignin-based fractions made them more chemically reactive, which may open new opportunities for further applications.

4 Conclusions

This work demonstrated a facile pathway to separate the main components from bamboo hydrolysate, especially for the lignin and LCCs. The precipitated lignin exhibited a high-purity, as well as lower and narrowly distributed molar mass compared to MWL. Moreover, the three-stage extraction process using organic solvent fully recovered the LCC fraction (WSL) from the hydrolysate and significantly increased the purity of the resultant hemicellulosic sugars. The extracted LCC fraction contained abundant functional groups, including aliphatic and phenolic hydroxyl groups, and more typical phenyl glycoside linkage as compared to native bamboo LCC, which makes it hold a great application potential. The efficient selectivity of the present pathway may provide a breakthrough for improving the valorization of whole carbohydrate, lignin and LCCs in hydrolysate.

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