Sequential Fractionation of Lignin-derived Pyrolysis Oil via Extraction with a Combination of Water and Organic Solvents

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Alkali lignin pyrolysis oil (PO) was subjected to a sequential extraction method in which PO was separated into water soluble (WS), water insoluble (WI), methanol soluble (MS), methanol insoluble (MI), dichloromethane soluble (DS), and dichloromethane insoluble (DI) subfractions via combinations of water and organic solvents (water, methanol, and dichloromethane). Several analytical techniques were used to characterize PO and its subfractions. Elemental analysis showed that the higher heating value (HHV) of DS was highest, and there was higher aromaticity in the MI fraction, while the DI fraction had lower aromaticity. Interestingly, gas chromatography-mass spectrometry (GC-MS) and positive ion electrospray high resolution mass spectrometry (ESI+-HRMS) analyses showed that both the MI and DI subfractions were lignin-derived oligomers, which accounted for 25.0% of the total mass of the original PO. Additionally, the oligomers in the PO were mainly composed of dimers to pentamers. The secondary tandem MS/MS (MS²) experiments revealed that the m/z 360 is a dimer linked by coniferyl alcohol and a ferulate linkage, and no further fragmentation was observed in both the m/z 475 and 701 due to the rigidity of these structural motifs.

Keywords: Alkali lignin; Pyrolysis oil; Sequential fractionation; Solvents extraction; Oligomers

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INTRODUCTION

Lignocellulosic biomass is a vital raw material for the sustainable production of fuels, chemicals, and energy. It consists of three major biopolymers that are ubiquitous in nature (Jiang *et al.* 2016). Among those biopolymers, lignin is the second largest component of lignocellulosic biomass, accounting for 15% to 30% of the mass, and it is generally considered to be composed of three phenylpropane units. These monomers include guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) units that are connected by carbon-carbon and ether bonds (Liu *et al.* 2011; Parthasarathi *et al.* 2011; Zeng *et al.* 2015). Currently, the thermochemical conversion of lignin into high value-added aromatic monomers has attracted appreciable attention due to the rapid depletion of fossil resources (Shu *et al.* 2015). Thermochemical conversion of biomass uses gasification, liquefaction, and pyrolysis processes. Pyrolysis is a promising thermochemical conversion method that produces solid char, liquid fuel/bio-oil, and non-condensed gas through heating lignin materials in the absence of oxygen (Goyal *et al.* 2008). However, widespread commercial application of bio-oil is restricted by its several undesired factors, which include its high water content, corrosiveness, and poor stability and miscibility when blended with

petroleum fuels (Meng *et al.* 2015; Li *et al.* 2017). The poor fuel quality of bio-oil makes it unsuitable to be used directly as fuels for vehicles (gasoline and diesel) (Wang *et al.* 2014). Additionally, bio-oil is partially water-soluble; the water-insoluble bio-oil fraction is sometimes called pyrolytic lignin (PL). The insoluble fraction mainly consists of ligninderived phenolic oligomers with molecular weight (MW) up to 2500 Da, as well as some phenolic monomers (Scholze and Meier 2001). Phenolic monomers are important commodity chemicals and biofuels. In contrast, phenolic oligomers in bio-oil are non-volatile, and are considered the main factor contributing to bio-oil instability (Scholze and Meier 2001; Bayerbach and Meier 2009). Therefore, fractionation techniques must be used to extract valuable chemicals and to improve the quality of bio-oils to upgrade them to higher-valued products.

To date, investigators have examined numerous conventional fractionation and purification techniques to separate bio-oil; these techniques including distillation (Ibrahim et al. 2017; Ledezma-Martínez et al. 2017), centrifugation (Zheng et al. 2017), column chromatography (Borisova et al. 2017), membrane separation processes (O'Loughlin et al. 2018), and solvent extraction (Naik et al. 2017; Pinheiro et al. 2018) to name a few. Although distillation can separate the bio-oil into different fractions by the different boiling-points, there are a lot of chemical instability and thermosensitive compounds in bio-oil, and these lead to a low distillation yield, polymerization reactions, and easy production of coke from the residual fraction (Amen *et al.* 1997). Centrifugation also can be used to separate bio-oil into fractions; however each fraction obtained in that way tends to be heterogeneous. Separation by column chromatography is based on the different adsorption capabilities of compounds on a stationary phase. However, because of the huge range of polarities and the large fraction of oxygenated compounds, it is very difficult to separate the bio-oil, and a high consumption of organic solvents and silica gel can be expected. Membrane-based technologies in separation of natural products have been investigated extensively in the past three decades. However, membranes are usually easy to plug, so there is a need for regular cleaning in the separation process, and there is also the problem of treatment and disposal of concentrates. A promising separation method is solvent extraction, which is based on the principle of taking advantages of differences in the solubility of different components of the solute into the solvent. It has advantages that include its relatively low cost and high technological maturity. Additionally, unlike many other conventional separation methods, it can be performed at room temperature and atmospheric pressure to stabilize the bio-oil and separate the bio-oil into different target chemicals. So far, solvent extraction methods mainly have focused on water extraction (Scholze and Meier 2001; Vitasari et al. 2011), inorganic salt extraction (Kang et al. 2011; Žilnik and Jazbinšek 2012), and organic solvent extraction. Compared with water extraction or inorganic salt extraction alone, the combination of water extraction and organic solvent extraction has attracted more and more interest (Nsimba et al. 2013; Wang et al. 2014; Chua et al. 2017). Nevertheless, these studies cannot achieve higher purity of monomers and oligomers with higher separation efficiency. In addition, the steps required for the fractionation via a combination of water and organic solvents extraction are cumbersome, and some organic solvents used have toxicity problems, which are harmful to human health and cause environmental problems.

In this investigation, PO was extracted and fractionated *via* a combination of water and organic solvents extraction, and separated into subfractions. These subfractions were characterized by elemental analysis and GC-MS. In addition to these traditional analytical procedures, positive ion electrospray high resolution mass spectrometry (ESI⁺–HRMS) coupled with secondary tandem MS/MS (MS²) was used to obtain structural information of the main oligomers in the PO and its subfractions. This information can then be used to help in the processing and purification of bio-oils in a bio-refinery system.

EXPERIMENTAL

Materials

The chemical reagents, including sulfuric acid (98%, w/w), *p*-dioxane, sodium bicarbonate, ethyl acetate, methanol, and dichloromethane that were used in this work were all of analytical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The water used was distilled. Monophenols, such as *o*-cresol (> 99.7% purity), guaiacol (> 99.0% purity), 2,4-dimethylphenol (98.0% purity), and 4-ethylguaiacol (99.0% purity), were purchased from Shanghai Aladdin Reagent Co., Ltd. (Shanghai, China), 4-Methylguaiacol (99% purity) was purchased from (Chemical Reagent Co., Ltd., Beijing, China). The 4-propylguaiacol (\geq 99% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All of the purchased chemicals were used as received without further purification. Alkali lignin (AL) was derived from the black liquors that were obtained from the soda black liquor using sulfuric acid. The AL was then vacuum-dried over phosphorus pentoxide (P₂O₅) at 60 °C.

Liquid samples production

The AL pyrolysis was performed in a high purity nitrogen (99.99%) atmosphere using a pyrolysis furnace (OTF-1200X, Crystal Material Technology Co., Ltd., Hefei, China) that was preprogrammed with a specific temperature profile. The furnace contained two reactors connected in tandem whereby each can be independently controlled in the temperature range of 40 to 1200°C. The reactor consisted of two heating jackets (Ø 55 mm × 200 mm), a Ø 50 mm × 3 mm × 800 mm quartz tube, two program temperature controllers, a power control system, and a Ø 36 mm × 3 mm × 23 (H) mm quartz crucible. Nitrogen gas is fed as an inert gas to reduce oxygen input into the reactor. An electric heater can preheat the nitrogen gas to the temperature (600 °C) prior to being introduced into the reactor. Initially, the reactor was stabilized to the desired temperature of 600 °C. Approximately 1 g of each sample was loaded into the quartz crucible and then inserted into one of the heating zones for a residence time of 2 min. The generated volatiles were purged by nitrogen gas (400 mL min⁻¹), which was then passed through two condensers that were immersed in liquid nitrogen. Afterwards, the liquid products were collected for subsequent chemical analysis.

Solvent selection and fractionation process design

The PO was separated into various fractions in accordance to the solubility of its components in various solvents. A cost-effective sequential fractionation method, which was environmentally compatible, was employed to fractionate the PO. Precipitation in cold water is the most common method used for PO fractionation (Scholze and Meier 2001). The PO was separated into the WS and WI phases. The upper WS fraction was enriched with MW organic acids, ketones, and aldehydes, as well as a small proportion of the monophenols, all of which have high to moderate polarity. However, some monophenols

and less polar phenolic oligomers, which have high MWs and multiple functional groups (and are possibly cross-linked), remained in the WI phase. Further fractionation of this phase can be achieved by disrupting the intermolecular interactions of the water insoluble components. Methanol is nontoxic to low toxic, and dichloromethane is a low toxic organic solvent; sequential extraction by these two organic solvents removed solutes in WI that were less polar because both solvents had lower polarities than deionized water. In this study, the fractionation scheme started with ice-cooled distilled water extraction of the PO. Then, the WI phase was extracted with methanol that resulted in methanol soluble (MS) and insoluble (MI) phases. Finally, the MS phase was extracted with dichloromethane to result in dichloromethane soluble (DS) and insoluble (DI) phases. In these cases, the distribution coefficients and recovery efficiencies of the main monophenols between the various phases are presented.

Sequential fractionation of pyrolysis oil

An appropriate amount of PO was added dropwise into distilled water (ice-chilled). The dropwise added components were stirred in the water at a speed of 1700 rpm for 30 min. Then, the mixture was allowed to settle for 30 min to ensure good phase separation. An upper WS fraction and a bottom WI fraction were formed; the two phases were separated from one another *via* decanting. The WS fraction was extracted with ethyl acetate and was allowed to stand for 30 min. The aqueous and ethyl acetate phases were separated from one another by decanting. The ethyl acetate phase was evaporated under a vacuum at 45 °C to remove the organic solvent. Subsequent steps focused on the fractionation of the WI phase by various organic solvent extractions. The WI fraction was extracted with methanol under shaking, then allowed to stand still for 30 min, and then filtered through Whatman filter paper (Ø 7 cm).



Fig. 1. Flow chart for the fractionation of PO by sequential solvent extraction

The precipitate was denoted as the methanol insoluble (MI) fraction, whereas the

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saved supernate was denoted as the methanol soluble (MS) fraction. The MS fraction was concentrated by rotary evaporation under a vacuum at 45°C to remove the methanol. Finally, the MS fraction was extracted with dichloromethane under shaking, then allowed to stand still for 30 min, and then filtered through Whatman filter paper (Ø 7 cm). The precipitate was denoted as dichloromethane insoluble (DI), whereas the supernatant solution was denoted as dichloromethane soluble (DS). The DS fraction was concentrated by rotary evaporation under a vacuum at 45 °C. All the isolated subfractions were dried overnight under a vacuum at 35 °C using P₂O₅ as a desiccant. The dried solids from fractionation were then weighed to determine their masses. In each of the extraction steps, the process was repeated three times. An extraction solvent-to-oil ratio of 20:1 was used. The sequential solvent fractionation scheme is shown in Fig. 1.

Methods

Physiochemical characterization

Physicochemical properties of the PO, such as higher heating values (HHV), H/C atomic ratio, and O/C atomic ratio were determined. The contents of C, H, N, and S in PO and its subfractions were carried out using a Vario-EL CUBE elemental analyzer (Karlsruhe, Germany), whereas the content of O was calculated by the difference. The results of elemental analyses are presented later in the paper (Table 3). The HHV of the PO and its subfractions were estimated by the expression (Chu *et al.* 2017),

$$HHV = 35.2C + 116.2H + 6.3N + 10.5S - 11.1O$$
(1)

where *HHV* is in MJ·(kg dry basis)⁻¹, and *C*, *H*, *O*, *N*, and *S* are the mass percentages (%, dry basis) of these elements contained in PO or its subfractions according to the elemental analysis.

GC-MS analysis

The chemical composition of PO and its subfractions was analyzed using a 7890A gas chromatograph coupled with a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The liquid samples that were dissolved in the ethyl acetate solvent were used for GC-MS analysis. The injection volume was 1 µL. Helium (99.99%) was used as the carrier gas, and the flow rate was 1.20 mL·min⁻¹. The injector, detector, and interface temperatures were all set at 260 °C. The capillary column HP-5MS (30 m \times 0.25 mm \times 0.25 µm) was used as the separation column. All of the samples were filtered through a 0.45-µm syringe-driven filter prior to injection. The sample injection was made in the split ratio of 1:1. The oven temperature profile was programmed from 40 °C (kept for 2 min) to 80 °C (kept for 5 min) with a 20 °C·min⁻¹ heating rate, then increased to 150 °C (kept for 3 min) with a 3 °C·min⁻¹ heating rate, and then increased to 260 °C (kept for 2 min) with a 20 °C·min⁻¹ heating rate. The mass spectrometer was set at an ionizing voltage of 70 eV, and the mass range (m/z) was set from 30 to 700 using a scan speed of 1.0 s/decade. Data processing was performed using Perkin Elmer NIST Spectral software (Version 08, Santa Clara, CA, USA). The amount of the various chemical compounds in the sample was calculated based on the relative areas (%) of the respective GC peaks. The main monophenols with higher content in the liquid products were further quantitated by using an external standard method with the corresponding standards on a gas chromatograph coupled with a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The detecting conditions were the same as those used for the GC-MS qualitative analysis.

Mass spectrometry

The MW distribution of the PO and its subfractions were characterized using a high resolution mass spectrometer (HRMS, Agilent 1290/maxis impact, Karlsruhe, Germany) that was equipped with an electrospray ionization (ESI) source that was working in positive-ion mode. Positive ion ESI mass spectra were recorded over the mass range (m/z)of 50 to 2000. A 0.6 mL·min⁻¹ flow of ultrahigh purity methanol was used as the mobile phase. Nitrogen gas (99.99%) was used as the nebulizer gas and was maintained at a flow rate of 4.0 mL·min⁻¹. The nebulizer gas pressure was 0.3 bar and the temperature was 180° C. The charging voltage and capillary voltage were set at 3500 and 2000 V, respectively. The secondary tandem MS/MS (MS²) experiments involved the separation and fragmentation of selected ions formed during the positive ion mode ESI. The ion abundance cutoff for performing collision-activated dissociation (CAD) was determined by tripling the average abundance of background ions in the total ion current (TIC) collected. The ions were kinetically excited and allowed to undergo collisions with nitrogen target gas for 30 ms at a q-value of 0.25 at a normalized collision energy of 40%. The fragmented product had an abundance of at least 5% relative to the most abundant product ion reported in this work. Data analysis was performed using customized software that has been described elsewhere (Liu et al. 2010).

Definitions

Extraction capabilities were expressed as distribution coefficients and recovery efficiencies. The distribution coefficients for a particular component (D_i) were defined as the ratio of the equilibrium mass fraction of that component in the aqueous extract phase $(X_{i,aq})$ and its equilibrium mass fraction in the residual phase $(X_{i,org})$:

$$D_{i} = \frac{X_{i,aq}}{X_{i,org}}$$
(2)

The total recovery efficiencies for a certain compound (Y_i) was calculated by dividing the mass of that compound in the total of aqueous phase $(m_{i, aq})$ and residual phase $(m_{i, org})$ at equilibrium with its initial mass in the feed $(m_{i, f})$:

$$Y_{i} = \frac{m_{i,aq} + m_{i,org}}{m_{i,f}} \times 100\%$$
(3)

RESULTS AND DISCUSSION

Extraction Capability

To explore the feasibility of PO fractionation using sequential solvent extractions (Fig. 1), the recovery efficiencies and distribution coefficients for the six main monophenols in the PO, *o*-cresol, guaiacol, 2,4-dimethylphenol, 4-methylguaiacol, 4-ethylguaiacol, and 4-propylguaiacol were examined.

The distribution coefficients of the main phenolics in the PO are shown in Fig. 2. The distribution coefficients for *o*-cresol were the highest, which was followed by guaiacol, 4-methylguaiacol, 2,4-dimethylphenol, 4-ethylguaiacol, and 4-propylguaiacol.



Fig. 2. The distribution coefficients (a) and recovery efficiencies (b) of the main monophenolics from PO

It was inferred that water was more effective for the extraction of *o*-cresol and guaiacol in comparison to 4-methylguaiacol, 2,4-dimethylphenol, 4-ethylguaiacol, and 4-propylguaiacol. The distribution coefficients for *o*-cresol exceeded 2.8 times higher than the partitioning for 4-methylguaiacol or 2,4-dimethylphenol and exceeded 5.8 times higher than the partitioning for 4-ethylguaiacol. The results presented here were consistent with those reported by Doerr and Fiddler (1970). Higher distribution coefficients for *o*-cresol and guaiacol were most likely the result of a methyl or methoxyl group that was ortho to the hydroxyl group in these phenolics. Moreover, for 4-methylguaiacol, 2,4-dimethylphenol, 4-ethylguaiacol, and 4-propylguaiacol, increasing the chain length of the *p*-substituent on guaiacol or *o*-cresol from C₁ to C₃ resulted in a lower distribution coefficient value because the alkyl group (methyl, ethyl, or propyl) is a hydrophobic group that hinders the interactions of water with the hydroxyl group, thus reducing the solubility of the compound (Cesari *et al.* 2017).

The average recovery efficiencies on GC-eluted phenolics by using water, methanol, and dichloromethane extractions were approximately 79.8%, 88.4%, and 82.6%, respectively (Fig. 2). On the basis of these results, it was concluded that either water, methanol, or dichloromethane were suitable as extraction solvents for the recovery of phenolics from PO. Thus, fractionation of PO *via* a combination of water and organic solvents as extraction media was a feasible method.

Volatile Compounds Identified by GC–MS

The main compounds identified in the PO and its subfractions are summarized in Table 1. Based on the results from GC-MS, the PO obtained from AL pyrolysis at 600°C was mainly comprised of phenolic, aromatic, and ketone compounds. It was found that the chemical species in WS clearly decreased compared to the PO, and it was mainly composed of small molecular compounds with strong water hydrophilicity, such as phenols and ketones. Although aromatic compounds were almost unaffected by water extraction, the relative content of phenols and ketones in the aqueous phase was different from that of PO. These observations illustrated that simple water extraction of the PO can strip from the crude PO organic compounds that are of low molecular weight and that exhibit high water solubility.

RT ^a	Compoundo	Relative Area (%)							
(min)	Compounds	PO	WS	WI	MS	MI	DS	DI	
9.292	o-Cresol	1.72	1.76	0.72	0.41	-	0.81	-	
9.920	4-Methylphenol	1.82	1.65	-	-	-	-	-	
10.366	Guaiacol	3.59	3.87	2.00	0.95	-	1.81	-	
11.301	Ethanone,1-(2-hydroxy-6- methoxyphenyl)-	4.12	4.78	-	-	-	-	-	
12.380	2,4-Dimethylphenol	3.51	2.13	2.68	1.65	-	3.21	-	
13.948	4-Methylguaiacol	5.83	3.77	4.66	2.38	-	4.77	-	
14.216	9H-Fluorene, 2-methyl-	2.94	3.39	0.58	0.36	-	0.67	-	
15.033	(E)-Stilbene	4.39	5.00	1.08	0.66	-	1.70	-	
15.715	Benzene,1-ethyl-4-methoxy-	1.46	-	1.64	0.96	-	2.08	-	
17.229	4-Ethylguaiacol	3.14	1.31	3.34	1.96	-	3.87	-	
18.024	Benzene,1,4-dimethoxy-2,3,5,6- tetramethyl-	1.28	1.28	-	-	-	-	-	
18.571	4-t-Butylcatechol, dimethyl ether	1.84	1.32	0.91	0.65	-	1.20	-	
18.700	4-Vinylguaiacol	0.94	-	1.02	0.61	-	1.17	-	
20.789	4-Propylguaiacol	0.51	-	0.56	0.35	-	0.72	-	
33.521	Heptadecane	0.27	0.71	-	-	-	-	-	
36.845	3,6-Dimethylphenanthrene	0.99	-	1.28	0.79	-	1.46	-	
37.952	Phenanthrene,1-methyl-7-(1- methylethyl)-	1.13	-	1.70	1.08	-	2.33	-	

Table 1. Main Compounds Identified in Pyrolysis Oil and Its Subfractions by GC-MS

^a Retention time

It should be pointed out that the WI fraction will contain small molecular organic compounds such as monophenols. This may be explained as being the result of their weak hydrophilicity and polyfunctional and complex chemical interactions that exist in PO (Chen *et al.* 2011). Completely breaking the intermolecular interactions between PL and other chemical compounds just by using water extraction was very difficult. As a result,

some small molecular mass compounds need to be further separated out from the WI phase with methanol and dichloromethane to break the intermolecular interactions. What was surprising was that there were no compounds that were identified in the MI and DI subfractions by GC-MS. Based on these observations, it was assumed that both the MI and DI subfractions contained lignin-derived oligomers. To verify whether this hypothesis was correct or not, further experiments were performed using HRMS/ESI⁺-MS and MS² analyses.

The Molecular Weight Distribution and MS² Analysis

The GC-MS analysis has detection limitations that make it difficult to identify oligomeric structures, such as lignin-derived oligomers, which have higher MWs and lower volatilities. Hence, additional analyses were performed using HRMS/ESI⁺-MS. The ESI is a soft ionization technique that enables ionization of higher mass compounds than ionizable with GC-EI-MS. It further encourages degradation of the compounds via fragmentation, but the soft ionization of ESI overcomes the challenges associated with complete characterization and the current literature indicates that the probability of fragmentation is very low or no fragmentation of the molecules (Piccolo et al. 2010). In addition, the ionization efficiencies of all compounds, both of low and high MWs, were similar. Thus, the results from ESI analysis can be considered semi-quantitative. Relative abundance of per analyste is defined as the abundance of each peak divided by the highest abundance peak, which is scaled to 100% in the mass spectra. Because compounds below m/z 150 are easily analyzed by GC-MS, the authors adjusted the m/z of the detected precursor ions peaks to scan from 150 to 2000. The low mass end of the spectra (m/z < 150) was not visible, whereas the high mass end of the distribution spectra trailed off in intensity at approximately m/z 800 (*i.e.*, relative abundances less than 5% were not taken into account). Thus, dimeric and higher MW compounds were defined as oligomeric compounds.

It was observed that the highest relative abundance peak in the spectra of PO and its subfractions (except for DS fraction) was at $[M]^+$ = 360 (Fig. 3). The DS fraction of PO had the highest relative abundance peak at $[M]^+$ = 609. The precursor ions peaks detected in WS and WI subfractions were higher when compared to that of PO, particularly for the WI fraction. This observation may have been explained by the presence of a large number of complex components and a few low concentration components in PO, making them difficult to be detected by the ESI+-HRMS. The relative abundances of monomers and dimers in the WI subfraction were higher than those in the WS subfraction. This suggested that some of the components in the PO were partially water-soluble, whereas the majority of the components were water-insoluble. Although the MS and MI subfractions were obtained from the WI subfraction, they have different relative abundance distributions for the precursor ions peaks due to the compounds having different solubilities in methanol. The same observation was noted for the DS and DI subfractions from MS. In contrast, it was found that a high dispersity of DS indicated it had a broad MW distribution, which might mean that the degrees of polymerization for oligomers were scattered. This phenomenon resulted in reduced relative abundances of low-mass precursor ions because only a few small molecular compounds were isolated during the separation process of MS. One anticipated finding was the absence of monomeric compounds (Fig. 3). The MW values of the precursor ions peaks were greater than 300 Da in both of the MI and DI subfractions.





Fig. 3. HRMS of pyrolysis oil and its subfractions from pyrolysis of alkali lignin ($T = 600^{\circ}$ C, t = 2 min) analyzed by ESI⁺-MS method

These findings suggested that both MI and DI subfractions contained lignin-derived oligomers, which provided evidence to support the aforementioned hypothesis. It was worthwhile to note that no signals above the noise level beyond m/z 800 in the spectra were observed. This indicated that no heavy oligomeric structures were detected in the PO. In addition, according to the m/z size range in the spectra, it could be inferred that the main oligomers in all the subfractions included plenty of dimers to pentamers regardless of which fraction was separated (Mullen and Boateng 2011).

To expand the general understanding of main oligomers, the authors adopted the positive-ion mode ESI coupled with secondary tandem MS/MS to identify and count many different types of functionalities in analytes. Three main precursor ions peaks (m/z 360, 475, and 701) were selected from the MS experiment and subjected to CAD, which caused additional fragmentation that resulted in a different pattern in the MS² spectra. Five major product ions peaks (m/z 363, 358, 340, 300, and 256) were formed upon CAD of the precursor ion of m/z 360.3238 (Fig. 4). The product ions (m/z 363 and 358) were isotropic peaks or adducts formed from the precursor ion at m/z 360. The loss of water at m/z 340 (*i.e.*, $[M]^+ - H_2O$) was initiated by the cleavage of a hydroxide ion in coniferval alcohol from the precursor ion at m/z 358 (Haupert *et al.* 2012). The possible fragmentation pathway from the product ions formed at m/z 300 was the combined loss of methoxy groups and CO occurring from the ion at m/z 358. The product ion at m/z 256 formed from an ion at m/z 300 via the loss of CO₂ at m/z 44; this was indicative of the presence of a carboxylic functional group (Jarrell et al. 2014). Based on the observations, it was concluded that the precursor ion at m/z 360 was a dimer (not a trimer) that was connected by coniferyl alcohol and ferulate linkage. Tandem mass spectrometry (MS/MS) might distinguish some structural isomers; however, most of them have several nearby peaks, and the effective isolation of precursor ions may be difficult, so it was difficult to determine the molecular structural compositions of this trimer.

It should be noted that no further fragmentation of the molecules was observed for precursor ions at m/z 475 and 701 (Fig. 4), probably due to the lack of weak binding groups for these two precursor ions, which resulted in no additional fragmentation, indicating the rigidity of these structural motifs.



Fig. 4. Proposed mechanisms for fragmentation pathways observed in MS² spectra for product ions formed upon CAD of the precursor ion at: m/z 360.3238 (a), m/z 475.3280 (b), and m/z 701.4958 (c)

Physicochemical Properties and Fractions Yield

Table 2. Yields (wt %) and Physiochemical Properties of PO and Its Subfractions									
	Sample	PO	WS	WI	MS	MI	DS	DI	

Sample	PO	WS	WI	MS	MI	DS	DI
Yield ^b (%)	-	31.78	66.15	53.13	9.90	37.50	15.10
C (%)	66.32	63.96	70.57	70.80	58.71	72.12	67.89
H (%)	6.91	6.75	6.92	7.10	8.64	7.06	5.54
O ° (%)	23.52	28.91	21.34	20.76	32.27	19.66	24.57
N (%)	2.35	0.07	0.04	0.10	0.29	0.06	0.23
S (%)	0.90	0.31	1.13	1.24	0.09	1.20	1.77
H/C atomic ratio	1.25	1.27	1.18	1.20	1.77	1.17	0.98
O/C atomic ratio	0.27	0.34	0.23	0.22	0.41	0.20	0.27
HHV(MJ/kg)	29.01	27.19	30.63	31.00	27.15	31.53	27.81

^b Yield (%) = $\frac{m_{\text{fraction}}}{100\%}$

, mpyrolysis oil

^c Oxygen content calculated by difference

The yields and physicochemical properties of the PO and its subfractions are presented in Table 2. Compared to PO, water addition slightly reduced the hydrogen content and significantly increased the oxygen content in the WS, while the oxygen content in the WI decreased. This indicated that water facilitated the extraction of oxygenated chemical compounds (e.g., phenolic compounds and/or ketones) from PO. The carbon content of the MS subfraction was higher than that of the MI subfraction, whereas the oxygen and hydrogen contents of the MS subfraction were lower than that of the MI subfraction. The carbon and hydrogen contents of the DS subfraction were higher than the DI subfraction, while the oxygen content of the DS subfraction was lower than the DI subfraction. The calculated HHV of the DS subfraction was higher than that of the DI subfraction. Throughout the different subfractions, both the hydrogen and oxygen contents in the MI subfraction were higher than any other PO subfractions, whereas the carbon content was the lowest. In addition, the HHV was also the lowest. In contrast, the HHV of the DS subfraction was the highest. The H/C ratio of PO and its subfractions ranged from 0.98 to 1.77; these findings were consistent with the reports by Kind and Fiehn (2007). It was noted that the H/C and O/C values for the MI fraction were the highest in comparison to the other fractions, whereas the H/C ratio for the DI fraction was the lowest. According to the literature, the H/C and O/C values can be used to estimate the aromaticity and polarity of fractionated POs, respectively (Zhang and Wu 2018). As a result, the MI fraction had higher aromaticity and polarity than the other subfractions; however, the DI fraction had lower aromaticity. It should be noted that the total mass of the MI and DI fractions accounted for 25.0 wt% of the original pyrolysis oil, and these fractions were believed to contain lignin-derived oligomers.

CONCLUSIONS

- 1. A combination of water and organic solvents as extraction media was found to be a feasible method for the fractionation of pyrolysis oil. The average recovery efficiencies of phenolic components using this method were approximately 79.8, 88.4, and 82.6%, respectively.
- 2. Both the MI and DI subfractions were lignin-derived oligomers. The total mass of them accounted for 25.0 wt% of the pyrolysis oil. In addition, the pyrolysis oil mainly contained dimers to pentamers.
- 3. The precursor ion at m/z 360 was a dimer connected by coniferyl alcohol and a ferulate linkage. No further fragmentations were observed in the two precursor ions at m/z 475 and 701 due to the lack of weak binding groups.

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