Separation and Enrichment of Catechol and Sugars from Bio-oil Aqueous Phase

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Aiming at obtaining greater value from the complicated composition of the bio-oil aqueous phase, solvents of increasing polarity were employed to sequentially separate the bio-oil aqueous phase using column chromatography. This not only relieved the catalyst deactivation, but also made it possible to obtain fractions rich in different chemical families to produce high-grade liquid fuels and valuable chemicals. Gas chromatography was adopted as a monitoring technology, and 11 fractions rich in different chemical families were obtained. The phenolic compounds in the aqueous phase were primarily eluted using dichloromethane. The strong polar benzenediols were enriched gradually in a dichloromethane fraction, and a high catechol content of 62.81% was achieved with the subsequent combination of a pH control method. Ethyl acetate gave three fractions, and pyrolytic sugars were the predominant compounds, whose highest content reached 67.86% in the third fraction. Further separation of the sugar-rich fraction using column chromatography could remove the residual phenolic compounds and furans and acquire a sugar fraction suitable for fermentation.

Keywords: Bio-oil aqueous phase; Column chromatography; Separation; Catechol; Sugars

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INTRODUCTION

Renewable energy has drawn much attention due to fossil fuel depletion and environmental protection crisis. Biomass is considered as the only sustainable resource containing carbon, and it can be converted into different forms of energy and fuels *via* thermochemical, biological, and mechanical technologies (Yang *et al.* 2015). Fast pyrolysis can convert the solid biomass into crude liquid bio-oil (Mohan *et al.* 2006). However, bio-oil has to be upgraded before being usable for motor fuel because of inferior properties (Mortensen *et al.* 2011). The chemical composition of bio-oil reveals the presence of more than 300 compounds, whereas the content of each chemical family depends on the initial feedstock, the pyrolysis process, and the storage time (Kim *et al.* 2012; Chen *et al.* 2014). The large molecular compounds in the crude bio-oil easily lead to catalyst deactivation and reactor blockage during the direct upgrading process (Mortensen *et al.* 2011). As a result, the separation pretreatment of bio-oil to enrich the compounds into one fraction, which can be upgraded with the same upgrading technology, will improve the upgrading efficiency and retard catalyst deactivation.

Different separation technologies have to be adopted to divide bio-oil into various classes or to extract certain chemicals. Common separation technologies include column chromatography, extraction, and distillation. Traditional distillation often gives a low yield of distillates, and the thermally sensitive bio-oil is easy to deteriorate and coke during this

process (Lu *et al.* 2008). Molecular distillation can produce different fractions rich in different chemical families as long as there is control of pressure and temperature (Wang *et al.* 2009a; Guo *et al.* 2010a). Moreover, no coking problem occurs during the whole process. Extraction often uses one or several solvents of different polarities to obtain chemicals with similar structures or polarities. Wei *et al.* (2014) employed n-hexane, petroleum ether, and dichloromethane to extract the bio-oil aqueous phase, and found that dichloromethane had better extraction efficiency for ketones and phenols when compared with other solvents. The extractants of n-hexane and petroleum ether were similar, while petroleum ether extracted a higher content of guaiacyl phenolic compounds and a smaller content of furans and ketones.

The selectivity of extraction is one of the crucial factors that limit its application in the separation of bio-oil. Column chromatography is a common approach used to purify chemicals based on the polarity and structure of chemicals and solvents, as well as the properties of adsorbents. As such, this approach is more effective in the separation of bio-oil. Although the distribution and chemical polarity of chemical families in bio-oil are rather complex, the elution times for various compounds with different solvents from different adsorbents are also in sequence. The eluent with a single solvent to be concentrated as one fraction often gives undesirable results, and the bio-oil can only be divided into aliphatics, aromatics, and polar fractions (Ertaş and Alma 2010). Ma and Agblevor (2014) used different solvents of different polarities to extract the bio-oil in a certain order, after which column chromatography was adopted to elute the extracted fractions with solvent mixtures. Thin layer chromatography and ultraviolet detection were employed to monitor the eluents, and eluents of similar chemical reaction properties were combined and further separated using column chromatography. Although this method can produce several chemicals with high purity, it takes too long a time and consumes a large volume of solvents. As the compounds in bio-oil are very complex, simple thin layer chromatography has difficulty distinguishing the composition of eluents. Gas chromatography (GC), one of the most widely used detection technologies, can detect approximately 40 wt.% compounds in bio-oil. The primary identification of eluted chemicals through GC can produce fractions with certain compounds and simplify the subsequent purification procedures.

Bio-oil is a viscous liquid that often contains 13.5 to 27.7 wt.% pyrolytic lignin (Scholze and Meier 2001, Wang *et al.* 2015). The low reactivity of pyrolytic lignin leads to rapid catalyst deactivation during the upgrading of bio-oil. Hence, most research generally focuses on the small molecular compounds with a high reactivity in bio-oil (Remiro *et al.* 2013), while the separated pyrolytic lignin is decomposed to produce monophenols and carbon fibers or is used to synthesize phenolic resin and adhesive (Effendi *et al.* 2008; Zhao *et al.* 2010; Qin and Kadla 2012). The direct separation of crude bio-oil may lead to the co-extraction of some polymers, and pyrolytic lignin has difficulty passing through the adsorbent during the column chromatography of crude bio-oil because of its large molecular weight. The addition of water can separate the bio-oil into a hydrophobic fraction composed of lignin-derived compounds and a polar aqueous phase including sugars. The aqueous phase of bio-oil is often used to produce hydrogen by steam reforming (Liu *et al.* 2013). Although a high hydrogen yield can be obtained, the hydrophilic sugars easily form coke on the catalysts, and the phenolic compounds dissolved in this phase may also have negative effects on the hydrogen yield (Wang *et al.* 2013a). Consequently, further treatment of the bio-oil aqueous phase is a key procedure in solving these problems.

Silica gel and five solvents of various polarities were employed in this paper to separate the concentrated aqueous phase of lauan bio-oil using column chromatography. Gas chromatography was first used to identify the composition of each eluted fraction, and then the fractions with similar peak information were combined. They were then further detected using GC coupled with a mass spectrometer (GC/MS). Based on the chemical distribution of final fractions, catechol and sugar were further extracted from the selected fractions that contained the highest contents of the corresponding compounds.

EXPERIMENTAL

Water Extraction of Bio-oil

Bio-oil was produced *via* the fast pyrolysis of lauan sawdust in a fluidized bed reactor with a feeding rate of 5 kg/h, which was developed by Zhejiang University in China. The pyrolysis temperature was at 450 to 500 °C (Guo *et al.* 2010b). The composition of lauan sawdust was determined using ANKOM 200i fiber analyzer (Macedon, USA) based on the Van Soest method (Ru *et al.* 2015). The lignin, hemicelluloses, cellulose, extractives and acid-insoluble ash for lauan were 21.31, 16.28, 49.67, 12.41 and 0.33, respectively. Fine solid particles in the collected bio-oil were removed by suction filtration with Whatman qualitative filter papers before experiments. The crude bio-oil was then added dropwise into 10 volumes of water with the aid of high-speed stirring (5000 rpm) according to the report from Scholze and Meier (2001). The suspension was filtered through a 0.45 μm organic membrane to remove the pyrolytic lignin and acquire the aqueous phase. The precipitate (or pyrolytic lignin) was dried under vacuum at 40 °C and weighed. The aqueous phase was concentrated under vacuum distillation at 50 °C. The water content in the concentrated aqueous phase (CAP) was determined using a ZDJ-3S Karl Fischer titration equipment (Xianquweifeng Company, China).

Column Chromatography Separation of Bio-Oil Aqueous Phase

Eight grams of CAP was dissolved in about 10 g of ethanol and then stirred with 11 g of silica gel (100 to 200 mesh) that was pretreated at 110 °C for 72 h. The mixture was evaporated under vacuum at about 25 °C to remove the ethanol. The sample adsorbed on the silica gel was loaded on the top of the column (30 × 500 mm), filled with 100 g of silica gel (100 to 200 mesh), and washed with n-hexane before the sample was loaded. The sample was first eluted with 750 mL of n-hexane, then subsequently eluted with toluene (1750 mL), dichloromethane (1880 mL), ethyl acetate (1550 mL), and methanol (1180 mL) in sequence. Every 50-mL eluent was collected, and the collecting bottle was changed when the apparent color ribbon layer reached the end of the column. Each eluent sample and elution end of every solvent was monitored by 7890A GC (Agilent, USA). The eluents with similar characteristic peaks were combined and evaporated at a low temperature and under vacuum to remove the solvent. As shown in Fig. 1, the eluents of n-hexane were concentrated as a single fraction, referred to as HF, while those of toluene were divided into TF-1 (1350 mL) and TF-2 (400 mL). The eluents of dichloromethane and ethyl acetate, with medium polarities, were each divided into three fractions: DF-1 (150 mL), DF-2 (150 mL), DF-3 (1580 mL), EF-1 (200 mL), EF-2 (250 mL), and EF-3 (1100 mL), respectively. The final eluents of methanol, with strong polarities, were collected as MF-1 (75 mL) and MF-2 (1105 mL). The whole separation procedures, as described, were carried out two times for each condition.

Purification of Catechol and Sugars

The fraction (DF-3) rich in benzenediols was alkalized to a pH of 13 using a 5 wt.% NaOH solution. Equivoluminal dichloromethane was used to extract the chemicals from this alkaline solution twice. Afterwards, the residual solution was acidized with 1 M HCl to a pH of 5.8, and it was extracted twice using equivoluminal dichloromethane and ethyl acetate in sequence, respectively. The extracted fractions were combined and evaporated to remove the solvents at 32 °C.

The fraction (EF-3) with a high content of sugars was first extracted using the appropriate amount of ethyl acetate. The residual sample was then dissolved in water and extracted with ethyl acetate. The raffinate was finally placed in the column (25 × 140 mm), which was filled with active carbon (20 to 40 mesh) and diatomite (200 to 300 mesh) (mass ratio = 1:1). The specific surface area and pore volume for active carbon were 7.21 m^2/g and 0.039 cm^3/g, respectively, while those for diatomite were 0.63 m^2/g and 0.0013 cm^3/g, respectively. The sample was eluted with 200 mL of water, and the eluent was concentrated to remove the water.

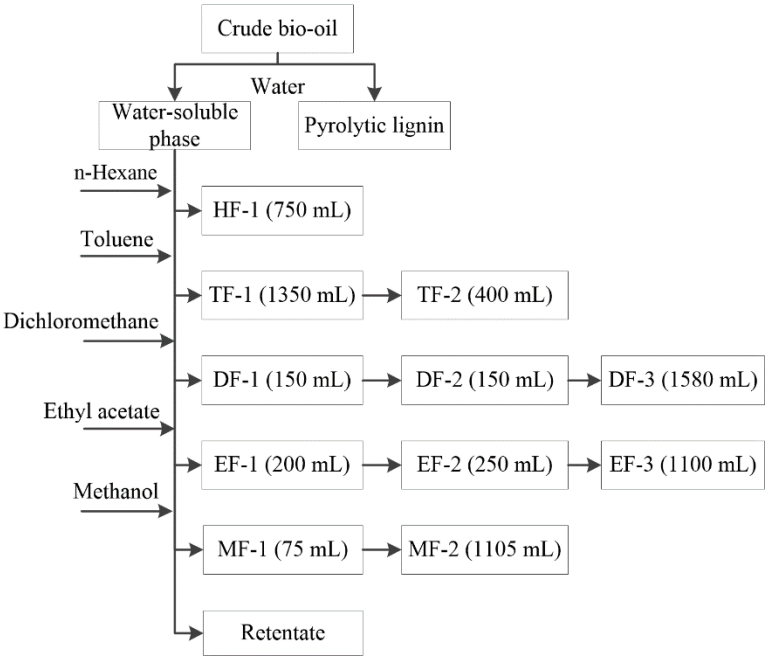

Fig. 1. Column-chromatography separation of bio-oil

Analysis of Samples

The identification of chemicals in the bio-oil and eluted fractions was carried out using Trace DSQ II GC/MS. A Db-Wax polar capillary column manufactured by the Agilent (USA) was used. The initial column temperature was kept at 40 °C for 1 min and was then heated to 240 °C at 8 °C/min and held for 10 min at that temperature. Each eluent before combination and concentration was detected using a 7890A GC (Agilent, USA). The column used was an Innowax capillary column, and its temperature program was in accordance with the GC/MS used.

RESULTS AND DISCUSSION

Phase Separation of Bio-oil

Bio-oil often acts as a homogeneous and viscous liquid when it has a low water content, and stratification occurs when the water content reaches approximately 45 wt.% (Bennett *et al*. 2009). The crude bio-oil had a water content of 21.73 wt.%. When the water content was increased, pyrolytic lignin with a large amount of hydrophobic functional groups precipitated, while the compounds with hydrophilic functional groups remained in the aqueous phase. As listed in Table 1, the pyrolytic lignin content was 35.58 wt.%, slightly higher than the 27.7 wt.% reported by Scholze and Meier (2001). The content of pyrolytic lignin is primarily affected by two factors. For one thing, pyrolytic lignin has some active functional groups and can react with some compounds like aldehydes and ketones that contain unsaturated bonds (Bayerbach and Meier 2009). As a result, the content and molecular weight of pyrolytic lignin will increase over time. Additionally, the raw biomass, the pyrolysis degree, and the extraction method also have an influence on the acquisition of pyrolytic lignin. The content of water-soluble organics in bio-oil was calculated by difference, and it amounted to 42.69 wt.%, about half of the crude bio-oil. Because the removal of pyrolytic lignin introduced a large amount of water in the aqueous phase, vacuum distillation was used to remove the majority of water to further separate the compounds of CAP using column chromatography.

Table 1. Composition of Bio-Oil

Composition	Content (wt.%)
Water	21.73
Water-soluble organics	42.69
Pyrolytic lignin	35.58

Bio-oil contains various chemical families. The distribution of these compounds in bio-oil and CAP was determined based on the peak area normalization, as shown in Fig. 2. Because the water-soluble phase of bio-oil was concentrated at 50 °C and under vacuum, some small molecular volatiles, such as acetic acid and acetol, were distilled out. As a result, although they were hydrophilic, their contents in CAP decreased with respect to their original contents in crude bio-oil. The content of alcohols increased from 2.54% in the crude bio-oil to 31.10% in CAP, respectively. 2,2-Diethoxy-ethanol, the most abundant alcohol in CAP, had a content of 29.15%, much higher than that in crude bio-oil. Because the solubility of monophenols was not high, their content decreased from 57.50% in crude bio-oil to 32.6% in CAP. When compared with other chemical families, the water-soluble monophenols were mostly benzenediols, which were relatively hydrophilic because of their two phenolic hydroxyls, with a content of 55.87% based on total monophenols. However, for crude bio-oil, guaiacol and its derivatives occupied the highest content of 20.67%, while benzenediols had a relatively low content of 13.72%. Sugars, which were strongly hydrophilic, were present at 13.42%, which was higher than the 7.99% in crude bio-oil. Levoglucosan was the pyrolytic sugar that typically had the highest amount relative to other sugars. This anhydrosugar is the key chemical material in producing pesticide, growth regulators, surfactant, and ethanol (Czernik and Bridgwater 2004). Bennett *et al.* (2009) studied the influence of the water-to-oil ratio, extraction temperature, and extraction time on the acquisition of levoglucosan. They found that the optimal extraction concentration of levoglucosan was up to 87 g/L at 34 °C. Li *et al.* (2013) also carried out a

similar study to obtain a maximum levoglucosan yield of 12.7 wt.%. Although this method can relatively increase the levoglucosan content, the water-soluble phase still contains other abundant compounds. As such, only water extraction can be used as the primary pretreatment of bio-oil. The complexity of the aqueous phase and the reactivity of each compound require further separation to obtain valuable compounds and fractions that can be upgraded with suitable upgrading technologies.

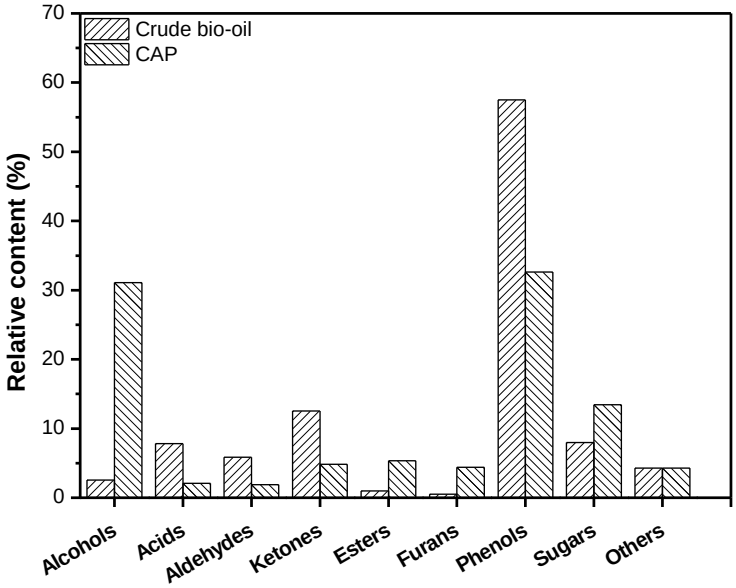

Fig. 2. Chemical distribution of crude bio-oil and CAP

Chemical Distribution of Eluted Fractions

The weight distribution of each fraction is displayed in Table 2. Although polar compounds are the predominant composition in CAP, a small amount of non-polar compounds (0.96 wt.%) still was present and was eluted using n-hexane. The polar compounds were eluted gradually with increasing solvent polarity. Fractions eluted with ethyl acetate had a weight content of 31.06%. Although methanol could dissolve the total aqueous phase, the final fractions eluted using methanol only occupied 19.26 wt.% based on the feedstock. This is because most of the compounds were already eluted with ethyl acetate. The final recovery of organic compounds was 78.61 wt.%, with respect to the total loaded sample.

The main identified compounds enriched in each fraction are listed in Table 3. n-Hexane and toluene are common solvents with a low polarity. The aqueous phase was first eluted using these two solvents. When the eluents from linear n-hexane were concentrated, white aliphatic chemicals appeared. The GC/MS results show that 96.70% of compounds in this fraction were non-polar. However, this fraction had the lowest weight proportion because these non-polar compounds and water were incompatible. As for the following fraction, TF-1 still had a non-polar compound content of 36.84%, among which the content of compounds with a similar structure, like xylene and ethylbenzene, increased to 5.63%. Subsequently, TF-2 only had a hydrocarbon content of 0.33% and was in abundance of phenolic compounds with a content of 68.7%. As shown in Table 3, 1,2,4-trimethoxybenzene (17.59%) and syringol (22.15%) were the most abundant compounds in TF-2. These phenomena suggest that toluene had a good elution effect on the non-polar compounds with similar structures, or the monophenols with low polarity. Ertaş and Alma

(2010) also found that toluene was a good solvent to elute the aromatic compounds in the separation of bio-oil.

Table 2. Fraction Distribution of CAP Separated using Column Chromatography

Fraction	Content (wt.%)
Water	14.78
Organic phase	85.22
HF	0.96
TF-1	7.67
TF-2	2.66
DF-1	2.98
DF-2	2.64
DF-3	11.38
EF-1	12.25
EF-2	12.13
EF-3	6.68
MF-1	10.35
MF-2	8.91
Recovery	78.61

Phenolic compounds are the main pyrolytic products from the pyrolysis of lignin in biomass (Wang *et al.* 2009b) and they often occupy a high content in bio-oil. Phenolic compounds are classified into five categories based on typical lignin structural types. Guaiacol and its alkyl derivatives are grouped into guaiacols, syringols include syringol and its alkyl derivatives, phenols comprise phenol and its derivatives, and aromatic compounds bearing two phenolic hydroxyl groups belong to benzenediols. Other phenolic compounds not belonging to the aforementioned categories are defined as others. The distribution of phenolic compounds in CAP and its fractions is presented in Fig. 3. At the beginning of toluene elution, phenols were eluted first, and reached 31.56% in TF-1. After which, the contents of guaiacols, syringols, and benzenediols became elevated respectively in TF-2. It is worth noting that the contents of phenolic compounds in the dichloromethane-eluted fractions were all above 65%, especially for DF-3 (89.13%). The elution of benzenediols was more difficult than other phenolic compounds. In the initial dichloromethane-eluted fraction, guaiacols and syringols occupied a relatively high proportion, and their contents decreased as the elution time increased, whereas the content of benzenediols reached 79.98% in DF-3. This is because the chemicals with strong polar bonds had strong intermolecular interactions with the silica gel, resulting in delayed retention times when compared with the weak polar chemicals. Because the alkoxyl group had a stronger electron-donating effect than the alkyl group, the polarity of alkoxyl-monophenol was weaker than that of alkyl-monophenol. Therefore, alkoxyl-monophenols like guaiacols and syringols were more easily eluted, resulting in total high contents of guaiacols and syringols in TF-2 and DF-1 (Wang *et al.* 2014a). Subsequently, the benzenediols were eluted gradually, and a fraction rich in benzenediols was obtained. Afterwards, the contents of phenolic compounds in fractions eluted using ethyl acetate and methanol were decreased. DF-3, which was the most abundant in phenolic compounds, was selected for further purification of phenolic compounds.

Table 3. Main Compounds Enriched in Each Fraction (Relative Content, %)

Compound	CAP	TF-2	DF-1	DF-2	DF-3	EF-1	EF-2	EF-3	MF-1	MF-2
2,5-Diethoxytetrahydro-furan	4.41	0.11	1.34	1.30	2.01	3.30	/	2.51	5.06	3.27
1,3,5-Trioxane	/ [a]	/	/	/	/	9.97	8.62	1.63	2.72	/
Acetic acid	1.65	/	0.24	/	0.31	/	/	0.43	3.49	6.74
2,2-Diethoxy-ethanol	29.15	/	/	0.22	0.12	31.09	32.59	9.62	1.33	1.11
Methyl cyclopentenolone	1.44	0.42	3.68	5.56	1.01	/	/	/	/	1.29
Butanoic acid-propyl ester	/	/	/	/	/	/	0.69	/	9.29	11.74
Methyl pyruvate dimethyl acetal	/	/	/	/	/	/	17.29	/	1.76	1.61
1,2,4-Trimethoxybenzene	3.42	17.59	17.07	11.72	0.28	/	/	/	/	/
5-Hydroxymethylfurfural	1.33	/	/	/	/	5.12	0.81	0.30	1.45	1.63
Vanillin	1.46	9.13	7.58	4.86	0.22	/	/	/	0.66	0.87
Acetovanillone	1.11	5.63	6.45	5.24	0.21	/	/	/	/	/
Syringol	4.37	22.15	17.30	10.93	0.43	/	/	/	/	0.77
4-Allyl-syringol	0.61	5.02	2.87	1.61	/	/	/	0.27	0.59	/
Syringaldehyde	1.53	0.46	0.49	1.01	1.41	3.32	/	/	1.87	1.16
Acetosyringone	1.19	0.42	0.68	0.83	0.77	3.26	0.54	/	/	/
Catechol	10.86	2.75	5.22	8.25	46.94	5.23	/	/	/	4.08
3-Methyl-catechol	0.84	6.80	10.79	14.26	0.07	/	/	/	/	/
4-Methyl-catechol	4.99	2.03	3.66	5.64	24.80	2.63	/	/	/	2.41
3-Methoxy-catechol	0.82	5.66	5.05	4.22	0.73	/	/	/	/	/
4-Ethylcatechol	1.55	4.26	3.52	2.92	7.31	/	/	/	/	/
Levoglucosan	10.84	/	/	/	/	1.27	6.23	65.91	29.58	20.93

[a] Not detected.

As some small molecular carboxylic acids were distilled out during the concentration of the aqueous phase, those polar compounds did not present an obvious enrichment trend and only gave a relatively high content of 10.10% in MF-2. The amount of aldehydes and furans were low, and they also did not show special enrichment in any fraction. Because alcohols, esters, ketones, and sugars were also the main chemical families, the distribution of these compounds in CAP and its fractions is displayed in Fig. 4. Alcohols with aliphatic hydroxyl groups often had a strong intermolecular force with the silica gel, and eluted gradually when ethyl acetate was used, as it had a higher polarity than dichloromethane and toluene. As listed in Table 3, the most abundant alcohol containing an ether structure, 2,2-diethoxy-ethanol, had concentrated contents of 31.09%, 32.59%, and 9.62% in EF-1, EF-2, and EF-3, respectively. Its contents decreased in methanol fractions because most of this alcohol was eluted using ethyl acetate. Although the amount of esters in CAP was only 5.33%, they performed at a relatively high enrichment tendency in EF-2 (27.41%) and MF-2 (29.64%). Meanwhile, the category of esters in MF-2 was more abundant than it was in other fractions, and its most prevalent compound was butanoic acid-propyl ester with a content of 11.74% in MF-2. Esters had similar ester group with ethyl acetate, and methanol had stronger intermolecular forces with silica gel than esters. Thus, methanol further eluted the compounds remaining on the surface of silica gel, including esters. However, the enrichment of compounds in MFs was not apparent when compared with other fractions. Ketones involved furanones and cyclopentanones, as well

as linear chain ketones, leading to the wide polarity of this chemical family. Ketones containing rings were eluted first using dichloromethane, while large molecular ketones were eluted using methanol. Because of their low content in CAP, they did not show a notable enrichment tendency in any fraction. Pyrolytic sugars had relatively strong polarities compared to other compounds. Solvents with low and medium polarities such as n-hexane, toluene, and dichloromethane had poor elution efficiency for sugars. This led to its low contents in these fractions. Its content increased gradually in the ethyl acetate eluted fractions and achieved 67.86% in EF-3, in which the levoglucosan content was up to 65.91%, thereby providing a material suitable for the enrichment of levoglucosan. During the initial elution stage of methanol, the sugars were eluted subsequently, and levoglucosan was still the main component.

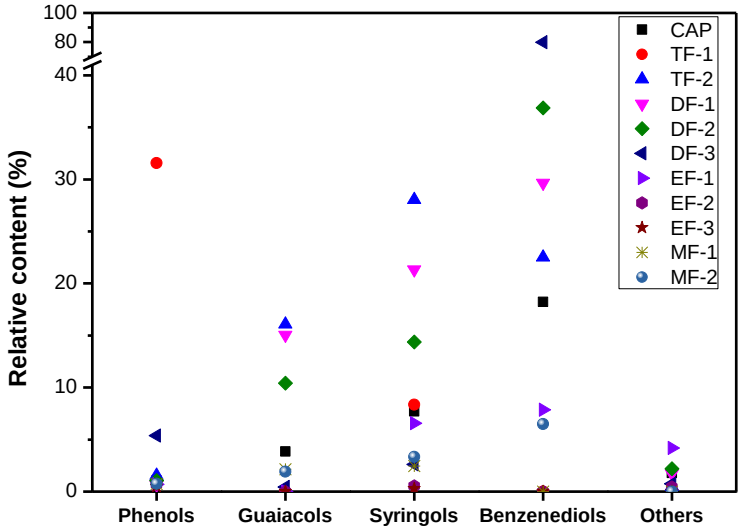

Fig. 3. Distribution of phenolic compounds in CAP and its fractions

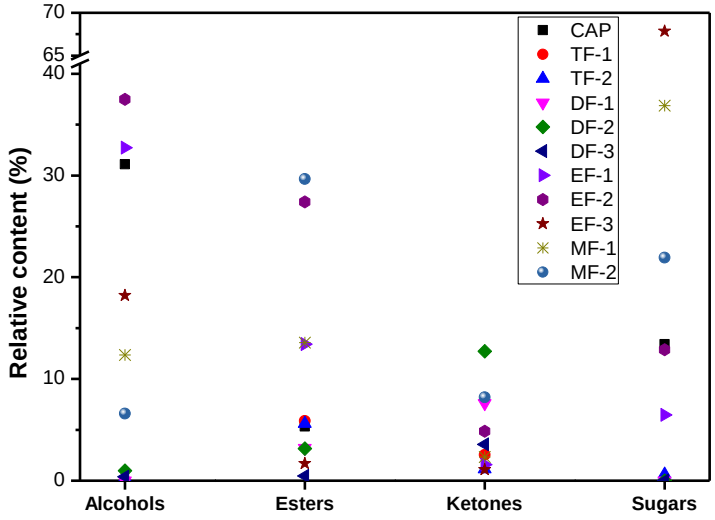

Fig. 4. Distribution of several chemical families in CAP and its fractions

The chemical distribution of bio-oil fractions indicates that the elution efficiency of a certain solvent was related to both the chemical polarity, and the structure information. Esters and sugars, which had strong intermolecular forces with silica gel, were eluted

gradually using strong polar solvents, while hydrocarbons could be eluted using non-polar and weak polar solvents. With increasing solvent polarity, compounds of different polarities in the bio-oil aqueous phase were eluted off the column at different elution times, providing a convenient route for the collection of different fractions rich in different chemicals or chemical families. The pre-detection of elution using GC could effectively distinguish the composition of each eluent, resulting in the high-efficiency enrichment of certain compounds in certain fractions. Based on the analysis of each fraction, DF-3 and EF-3 were selected to further extract the catechol and sugars.

Extraction of Catechol

Catechol can be used as an antiseptic, photographic developer, antioxidant, and so forth (Murwanashyaka *et al.* 2001). As it is soluble in water, a majority of catechol in bio-oil remained in the aqueous phase. The catechol content in DF-3 was the highest compared to other fractions, and this chemical was enriched using the pH control method. Because phenolic compounds often show weak acidity, they can react with strong alkali to form phenolates that are freely soluble in water. These phenolates can then be acidified to form the original phenols again and can be extracted subsequently by solvents (Wang *et al.* 2014b). When pH value was adjusted to 13, the extracted fraction using dichloromethane still had a high content of phenolic compounds (71.80%), among which syringol occupied the highest content at 25.04%, followed by benzenediols (22.15%). Under the alkaline condition, the extraction of phenolic compounds was still not dominant (Amen-Chen *et al.* 1997; Wang *et al.* 2014b). However, some phenolic compounds with low reactivity still remained in the water phase in the form of their initial structure, and these compounds could be extracted even under the alkaline condition. After the alkaline solution was further acidized to pH 5.8, the fraction that was extracted using dichloromethane and ethyl acetate occupied about 2.60 wt.% of DF-3, and had a high content of catechol, reaching 62.81%. As shown in Fig. 5, a tall sharp peak representing catechol at 25.99 min appeared in GC analysis. Thus, the acidity of catechol was stronger than that of other monophenols and alkyl derivatives of catechol, resulting in the re-formation of catechol at a low pH value.

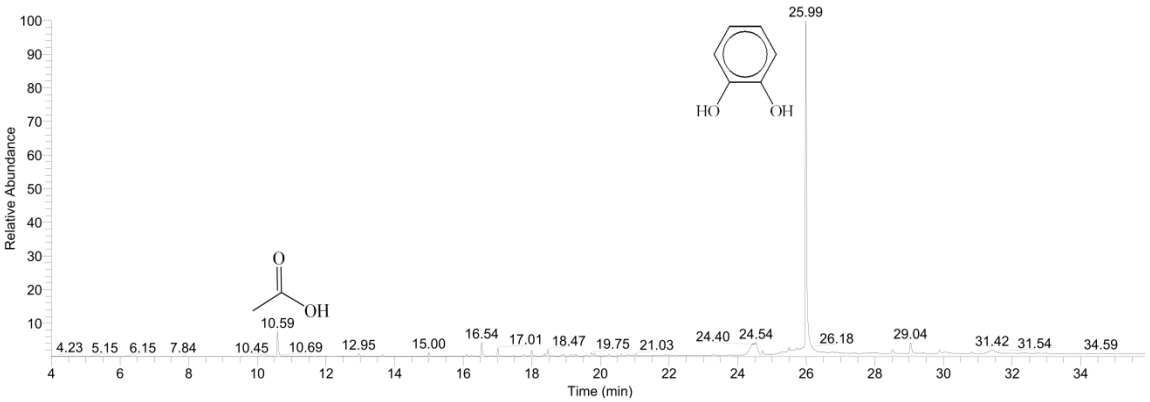

Fig. 5. Ion chromatogram of catechol-rich fraction

Inhibitor Removal from Sugar-Rich Fraction

Pyrolytic sugars in bio-oil could remain in the aqueous phase by water extraction and be enriched in the eluted fraction of ethyl acetate using column chromatography. During the processing of sugar-rich fraction to produce some chemicals, fermentation pretreatment is often demanded. However, the hindrance of sugar fermentation is due to

the inhibition of some compounds in bio-oil, such as aldehydes, acetic acid, and phenolic compounds. Phenolic compounds in particular have the strongest negative effects on the subsequent fermentation of pyrolytic sugars (Lian *et al.* 2010; Sukhbaatar *et al.* 2014). Hence, the elimination of inhibitors from sugar-rich fraction is the key procedure in producing alcohols with a high yield, or in extracting high-purity sugars. Rover *et al.* (2014) studied the removal of ketones, aldehydes, acids, and phenolic compounds from bio-oil through several approaches, including solvent extraction, ionic liquid extraction, ion exchange resin adsorption, and neutralization methods. They found that NaOH had good removal efficiency for acidic compounds, with almost no loss of sugars. However, many compounds remained in the aqueous phase in the form of organic salts. As such, this method did not fundamentally remove the inhibitors and was not beneficial for the further fermentation and extraction of sugar-rich fractions. Active carbon is a common adsorbent and can effectively remove the impurities in the aqueous phase. Because EF-3 had a high content of sugars, this fraction was further purified using column chromatography to eliminate the inhibitors and recovered 31.72 wt.% of chemicals. In the concentrated fraction, the total sugar content was up to 79.58%. The levoglucosan content amounted to 47.30% (as shown in Fig. 6), and 19.05% of sugar alcohols were also detected, while the content of phenolic compounds and furans declined to a negligible amount. Other alcohols were also reduced from 18.20% to 7.42%, whereas the contents of aldehydes and ketones held the same low level in those two fractions. These results imply that active carbon can effectively remove the phenolic compounds, furans, and some other alcohols from the aqueous phase and obtain a sugar fraction suitable for fermentation in the production of ethanol, butanol, acetone, 5-hydroxymethylfurfural, succinic acid, *etc*. (Huber and Dumesic 2006; Wang *et al.* 2013b).

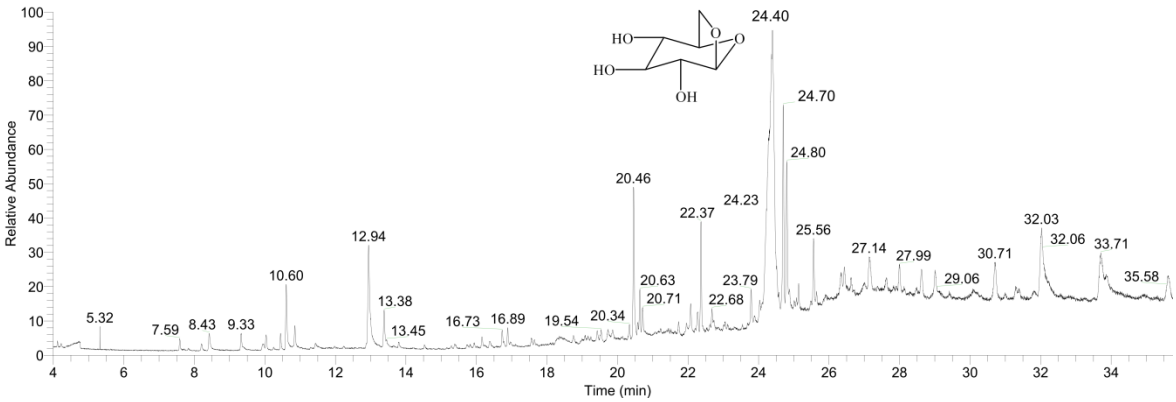

Fig. 6. Ion chromatogram of sugar fraction

CONCLUSIONS

1. Eleven fractions rich in different chemical families were obtained from the bio-oil aqueous phase by employing column chromatography and GC pre-detection.
2. Low-polarity phenolic compounds were enriched in the fractions eluted using dichloromethane, and their content in DF-3 was up to 89.13%, of which benzenediols amounted to 79.98%.

3. The pyrolytic sugars were gradually eluted and enriched in the fractions of ethyl acetate, and their content in EF-3 was 67.86%.
4. The further purification of DF-3 and EF-3 suggested that the pH control method could produce a catechol-rich fraction with a content of 62.81%, while impurities like phenolic compounds and furans in the sugar-rich fraction were eliminated using active carbon and diatomite.

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