Separation of phenols and ketones from bio-oil produced from ethanolysis of wheat stalk

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ABSTRACT

To realize a high-efficient utilization of bio-oil, extraction-column chromatography was employed to separate bio-oil produced by ethanolysis of wheat stalk. Extraction was employed to separate bio-oil roughly by acid, alkaline solutions as well as organic solvents, and column chromatography was used to enrich chemicals with petroleum ether, or mixture of acetone/carbon disulfide as elution solvent. Bio-oil and all sub-fractions were determined by gas chromatography-mass spectrometry. C₈-C₃₀ alkanes were enriched in petroleum elution sub-fractions, with a concentration of 97.6 %. Phenols were efficiently enriched in a high concentration ~100 % by elution of acetone/carbon disulfide. What's more, ketones were also separated efficiently from bio-oil in this study.

Acetophenone was enriched with a concentration of 71.2 % in a sub-fraction (ES_{b1}) eluted by mixture of acetone/carbon disulfide. 2-hydroxy-cyclopentenones was enriched in a sub-fraction (ES_{d4}) with a concentration of 52.6 %.

Keywords: bio-oil; extraction; column chromatography; phenols; acetophenone; cyclopentenones.

1. Introduction

Biomass has been walking into our life and industries as an interesting renewable resources used to provide second generation biofuels or chemicals [1,2]. Abundant amount and CO₂ neutrality with low sulfur and nitrogen content make biomass a sustainable and eco-friendly energy source [3]. Bio-oil, a liquid product from thermal conversion of biomass, has been paid attention to provide fuels and chemicals [4-6]. The bio-oil is a complicated liquid mixture consisting of hundreds of compounds (especially oxy-compounds), and exhibits a high water content, high oxygen content, low heating value, and high acidity [4,7-9]. The poor fuel quality of bio-oil makes it unsuitable to be used directly as vehicle fuel, and the compounds complexity makes it difficult to upgrade the fuel qualities of bio-oil [7]. Recently, increasing attention has been paid to the recovery of chemicals from bio-oil. Providing chemicals compared to fuels could make recovery of even small concentrations viable [5]. As we all know, most of the oxy-compounds such as phenols, ketones, and alcohols are value-added chemicals used in industry and life. Until now, most of oxygen-containing chemical stocks are mainly produced from fossil fuels via oxidation or hydration of olefins to introduce oxygen-containing functional groups. [Fortunately, these functional groups have already existed in bio-oil.](#) Hence, [providing value-added chemicals from bio-oil](#) is a potential

approach to efficiently utilize biomass energy.

Distillation, extraction and column chromatography are three common methods for separation. Distillation separates the bio-oil in different fractions by the boiling-point. [Amen-Chen *et al.* \[10\]](#) have summarized the literatures of the fractional distillation and flash distillation of bio-oil. The thermal and chemical instability of bio-oil leads to a low distillation yield and coke formation during distillation [7,10]. Recently, molecular distillation was introduced to separate bio-oil, which can increase the distillates yields and lower the formation of coke [11-13]. Bio-oil was mainly separated in light fraction (main compounds: acetic acid and 1-hydroxy-2-propanone), middle fraction (main compounds: phenols, aldehyde, and ketones), and heavy fraction (main compounds: levoglucosan) by molecular distillation [11,14,15]. Considering the mild condition, extraction and column chromatography has been employed to separate bio-oil. Table 1 summarizes the literatures which focused on the separation of bio-oil by extraction and column chromatography [2,7,8,16-26]. Extraction is always used as bench-scale separation technique of bio-oil and has been widely investigated to produce phenols and sugars (such as levoglucosan and glycolaldehyde) from bio-oil as Table 1 lists. Acid and alkaline solutions are always employed to separate phenols from bio-oil. Wang *et al.* [7] obtained a phenolic fraction with a high concentration of 94.35% through liquid-liquid extraction with acid and alkaline solutions and carbon dichloride. Zilnik *et al.* [20] tried to recover phenolic fractions from various bio-oil by two schemes and found that methyl isobutyl ketone combined with alkali solution is efficient for extraction of phenolic compounds. Recently, organic solvent liquid-liquid extraction without acid and alkaline solutions was

investigated by Wei *et al.* [21] and a fraction with 85% of phenols was obtained. Different from extraction, column chromatography is efficient in chemical purification. As Table 1 shows, separation of bio-oil by single column chromatography always gives three fractions (aliphatic, aromatic and polar fractions) [23-26]. The aliphatic fraction is determined as mixture of alkane and alkene [25], while aromatic and polar sub-fractions are paid less attention. By multi-steps column chromatography, Das *et al.* [22] separated cardanol, cardol, di-n-octyl phthalate, bis (2-ethylhexyl) phthalate, and di-n-decyl phthalate from pyrolysis oil, and Zeng *et al.* [2] obtained five fractions, mainly containing phthalate esters. Considering the high consumption of solvents, column chromatography was suggested as a final purification step of chemicals from bio-oil [10].

The objective of this study is to isolate possible chemicals such as phenols and ketones from bio-oil at a laboratory scale. Extraction and column chromatography were combined to separate bio-oil, and provide possible chemicals. The bio-oil was produced by ethanolysis of wheat stalk. Liquid-liquid extraction was employed to separate bio-oil in different chemical classes. Column chromatography was used to enrich chemicals into sub-fractions with petroleum ether, or mixture of acetone/carbon disulfide as elution solvent. C₈-C₃₀ alkanes, phenols, acetophenone and cyclopentenones were enriched efficiently. Different from the separation of phenols from bio-oil, publications on separation of ketones from bio-oil are very limited. Acetophenone and cyclopentenones were also separated with high content from bio-oil in this study.

2. Experimental

2.1. Wheat stalk and reagents

The wheat stalk used in this study was collected from Xuzhou, Jiangsu Province, China. It was pulverized and passed through an 80-mesh sieve and dried in a vacuum oven at 105 °C for 3.5 h. Proximate, ultimate, and chemical analyses of the sample was reported previously [27]. All the organic solvents, including benzene, petroleum ether (PE; bp. 30–60 °C), acetone, carbon disulfide (CS₂), and diethyl ether (DEE), used in this study were analytical reagents and distilled prior to use. Adsorbent silica gel (SG, 100–200 mesh) was activated in a vacuum oven at 145 °C for 6 h.

2.2. Production of bio-oil from wheat stalk

The bio-oil used in this study was produced from wheat stalk depolymerization in supercritical ethanol. Detailed information about the production of bio-oil has been published elsewhere [27]. 10 g wheat stalk and 300 mL ethanol were placed into a 1 L magnetically stirred autoclave. After drawing air out from the autoclave, the autoclave was heated to 300 °C and kept at 300 °C for 0.5 h, and then immediately cooled to room temperature in an ice-water bath. The reaction mixture was taken out from the autoclave as exhaustively as possible with added ethanol and filtrate through a polytetrafluoroethylene membrane filter with 0.8 μ m of pore size. The filter cake was repeatedly washed with ethanol and the solution was also filtrated through the filter. Bio-oil (named as SEB) was obtained by evaporating the filtrate with a rotary evaporator.

2.3. Extraction of bio-oil

As exhibited in Fig. 1, 4 g SEB was added into 50 mL benzene and stirred well under ultrasonication. Benzene insoluble phase (BIP) was removed by filtration, and was washed by 50 mL benzene, repeatedly three times. The benzene soluble phase was subsequently mixed with 200

mL water, stirring constantly for 20 min. After 60 minutes' standing, two layers were obtained: an upper aqueous phase (WP_I) and a bottom benzene phase (BSP_I). WP_I was added to 200 mL DEE, stirring for 20 min, and standing 60 minutes. DEE soluble phase and insoluble phase were separated and distilled to support DEE soluble phase I (DEEP_I) and DEE insoluble phase I (DEEIP_I). The benzene phase (BSP_I) was extracted by 200 mL 5% NaOH aqueous solution to afford upper phase (BSP_{II}), middle phase (precipitate), and lower phase (NaOH aqueous phase). The precipitate was washed by acetone to afford acetone-extractable portion (ASP), and the residue was named acetone-insoluble portion (AIP). The NaOH aqueous phase was neutralized with 5% HCl aqueous solution to pH 6-7, and then extracted with 200 mL DEE. Two layers (DEE soluble phase and insoluble phase) were separated and distilled to support DEE soluble phase II (DEEP_{II}) and DEE insoluble phase II (DEEIP_{II}).

2.4. Column chromatography of extracted fractions

Separation of column chromatography was carried out for the extracted fractions BSP_{II}, DEEP_I, and DEEP_{II}. (1) 0.2 g BSP_{II} was dissolved in 1 mL benzene, and then adsorbed by 0.5 g SG. After dried at room temperature, the mixture was transferred into a 2 cm inner diameter, SG-packed column (Height of SG-packed column of 50 cm). The BSP_{II} was sequentially eluted with 7.5 L and 9.3 L PE to afford sub-fractions ES_{c1} and ES_{c2}. (2) 0.2 g DEEP_I was dissolved in 1 mL CS₂, and then adsorbed by 0.5 mg SG. After dried at room temperature, the mixture was transferred into a 1 cm inner diameter, SG-packed column (Height of SG-packed column of 27 cm). Firstly, the DEEP_I was eluted with 300 mL isometric CS₂/acetone mixed solvent to collect the first color bar in the

column (namely ES_{b1}). The residue was eluted with 500 mL isometric CS₂/acetone mixed solvent to afford the second sub-fractions (ES_{b2}). (3) 0.2 g DEEP_{II} was dissolved in 1 mL CS₂, and then adsorbed by 0.5 mg SG. After dried at room temperature, the mixture was transferred into a 1 cm inner diameter, SG-packed column (Height of SG-packed column of 27 cm). Different from DEEP_I, there was no color bar observed for DEEP_{II} separation. The DEEP_{II} was successively eluted with 200 mL, 300 mL, 300 mL, and 500 mL isometric CS₂/acetone mixed solvent to afford four sub-fractions (ES_{d1} - ES_{d4}).

2.5 Analysis of SEB and sub-fractions

SEB and all the sub-fractions were analyzed with gas chromatography–mass spectrometry (GC-MS, Hewlett–Packard 6890/5973) equipped with a capillary column coated with HP-5MS (cross-link 5% PH ME siloxane, 60 m length, 0.25 mm inner diameter, 0.25 μ m film thickness) and a quadrupole analyzer in electron-impact mode at 70 eV. The column temperature was held at 50 °C for 2 min, raised to 200 °C at a rate of 3 °C min⁻¹ and then to 300 °C at a rate of 10 °C min⁻¹ followed by holding the temperature for 8 min. Mass spectra were scanned from 30 to 500 amu. In addition, ASP and AIP were also analyzed with a Nicolet Magna IR-560 Fourier transform infrared (FTIR) spectrometer by collecting 50 scans at a resolution of 8 cm⁻¹ in reflectance mode with measuring region of 4000-400 cm⁻¹.

3. Results and discussion

3.1. Chemical composition of SEB

The SEB is a dark and sticky liquid. The total ion chromatogram (TIC) of SEB is shown in Fig.

SI1 (Supporting Information), and all of the organic compounds of SEB detected by GC-MS are listed in Table SI1. Main compounds in SEB are listed in Table 2, including 2 hydrocarbons, 9 phenols, 3 ethers, 4 tetrahydrofurans, 6 esters, and 1 acid. In total, 121 compounds were detected in the SEB, and classified into nine chemical families: hydrocarbons, alcohols, phenols, ethers, esters, tetrahydrofurans, aldehydes, ketones, carboxylic acids, and others. Total area % of each chemical families decrease in the order: esters (33.4%) > phenols (28.4%) > ketones (10.6%) > tetrahydrofurans (10.3%) > hydrocarbons (6.9%) > ethers (5.3%) > alcohols (2.4%) > acids (1.3%) > aldehydes (0.8%) > others (0.7%). In SEB, esters, phenols, ketones, and tetrahydrofurans are major components. Hereafter, the percentage value refers to area percent (area %), if there is no special explain.

3.2. Extraction of SEB

The detailed extraction procedure has been illustrated in Fig.1. SEB was extracted into eight fractions, and the yield of each extracted fractions are given in Table 3. Totally, 92.8 % were recovered after the extraction. All the fractions were analyzed by GC-MS. There are no peaks observed in the TICs of benzene insoluble phase (BIP), DEE insoluble phase I and II (DEEIP_I and DEEIP_{II}), acetone soluble phase (ASP), and acetone insoluble phase (AIP). This would be caused by the limitation of GC-MS for the detection of polar compounds or heavy molecular compounds. The TICs of benzene soluble phase II (BSP_{II}), DEE soluble phase I (DEEP_I), and DEE soluble phase II (DEEP_{II}) are given in Fig. SI-2, Fig. SI-3, and Fig. SI-4. The main compounds (area% ≥ 1%) in BSP_{II}, DEEP_I, and DEEP_{II} are listed in Table 4, Table 5, and Table 6.

Extraction can enrich phenols from SEB in DEEP_I and DEEP_{II}, with an area % of 29.7 % and 73.0 %, respectively. Phenols in DEEP_I are mainly polyhydroxyl benzenes (PHBs), while dominant phenols in DEEP_{II} are monohydroxybenzenes (MHBs) with alkyl and/or methoxyl substituents. Except phenols in DEEP_I, ketones are another main chemical family with an area % of 31.6 %. Ketones in DEEP_I are mainly acetophenone and its derivatives, and hydroxycyclopentenone (HCP) and its derivatives (8.5 % and 15.9 %, respectively). The composition of BSP_{II} is more complex than that of DEEP_I and DEEP_{II}. Ketones (mainly alkyl-cyclopentenones) and esters (mainly diesters) are two main families in BSP_{II}, with an area % of 31.7 % and 31.0 %, respectively.

Ester is one of main chemical families in SEB. And our unpublished data shows that C₈-C₂₈ fatty acid esters can be separated from SEB by column chromatography. However, esters were not detected in eluted sub-fractions which will be discussed in next section. FTIR was employed to find the trace of esters. Fig. 2 shows the FTIR spectra of ASP and AIP. The peaks at 3425 and 910 cm⁻¹, 1702 cm⁻¹, 1183 cm⁻¹ were due to vibrations of -OH, C=O, and C-O, respectively. Thus, the compounds in this fraction were mainly acids. Moreover, the peaks at 2974, 2933, 1464, and 1367 cm⁻¹ were attributed to vibrations of -CH₂ and -CH₃. The peak at 721 cm⁻¹ was due to the rocking vibration of -(CH₂)_n- (n > 4). It was inferred that the compounds in ASP were mainly fatty acid. The spectrum of AIP in Fig. 2 is simple, only four peaks at 2929, 2871, 1584, and 1403 cm⁻¹, which are typical peaks for fatty acid salt. In this study, NaOH solution was employed to basify BSP_I. It can be inferred that the main compounds in AIP should be fatty acid sodium.

3.3. Enrichment of chemicals from sub-fractions

BSP_{II}, DEEP_I, and DEEP_{II} were further separated by SG-packed column with elution solvent PE for BSP_{II} and isometric CS₂/acetone mixture for DEEP_I and DEEP_{II}. The TICs of eluted sub-fractions are illustrated in Fig. 3 and Fig. 4, and compounds identified by GC-MS are summarized in Table 7, Table 8, and Table 9. C₈-C₃₀ alkanes, MHBs, benzenediols, acetophenone, and HCPs are enriched efficiently as discussed below.

3.3.1. Enrichment of C₈-C₃₀ alkanes

As Fig. 3 and Table 7 show, alkanes with C₈-C₃₀ (except C₁₁) and C₁₈-C₃₀ are predominant in ES_{c1} and ES_{c2} with an area % of 55.9% and 97.2%, respectively. After stored in shade for 10 days, milk-white solids (ES_{c1-w} and ES_{c2-w}) were obtained from ES_{c1} and ES_{c2}. Reprecipitated by PE, the milk-white solids were analyzed with GC-MS as alkanes with C₁₂-C₃₀ in ES_{c1-w} and C₁₈-C₃₀ in ES_{c2-w}.

3.3.2. Enrichment of phenolic compounds

As a necessary industry chemical, the main resource of phenols is coal tar. With the large depletion of coal, the production of phenols from bio-oil is being paid attention [7,14,16,17]. In SEB employed in this study, phenols are second dominant compounds. Producing phenols from SEB is very attractive. 35 phenols are collected with extraction-column chromatography, which are expected to be the promising resource to meet the demand of phenol market. As Fig. 4 and Fig. 5 show, phenols were efficiently enriched in ES_{d1}, ES_{d2} and ES_{d3}, with an area % of 82.3%, 100% and 64%, respectively. ES_{d1} is a mixture of MHBs (69.8%) and PHBs (12.5%) with hydroxyl-acetophenone (15.8%). ES_{d2} mainly consists of 4 guaiacols with an area % of 95.7%, including

guaiacol (18.6%), 4-methylguaiacol (8.3%), 4-ethylguaiacol (49.4%), and 4-propylguaiacol (14.4%). The dominant compound in ES_{d3} is syringol (2,6-dimethoxyphenol) with an area % of 57.2%. PHBs were mainly collected in ES_{b2} with an area % of 55.6%, mainly including catechol (16.3%), 3-methylcatechol (11.4%), 4-methylcatechol (10.7%), and 4-ethylcatechol (16.4%).

3.3.3. Enrichment of ketones

In contrast with the extensive studies of phenols in bio-oil, ketones have been paid less attention. There are few reports on the production of ketones from bio-oil. Ketones in SEB are mainly benzene ring-containing ketones (BRCKs), cyclopentenones (CPs) and furanones (DHF). As shown in Fig. 4 and Fig. 5, BRCKs were mainly enriched in ES_{b1} and ES_{d1}. Acetophenone was isolated in ES_{b1}, with an area % of 71.2%. After two weeks, a faint yellow crystalline solid appeared on the wall of the container but it was failed to analyze the crystalline. Acetophenone is widely used as an industrial solvent for fiber/resin and an important stock used to produce chemicals such as benzoic acid, benzoylformic acid, dibenzoylfuran 2-oxide, phenylethanol, phenol, and ethylbenzene, which have well-established industrial applications. Different from the BRCKs in ES_{b1}, BRCKs in ES_{d1} were mainly hydroxyl- acetophenones, with an area % of 15.8%.

CPs is another type of ketones in SEB, which possibly generated from cellulose and hemicellulose[28]. CPs can be used as chemical intermediates and food additives for flavor enhancement. In this study, CPs was mainly collected in ES_{b2} and ES_{d4} with an area % of 25.9% and 52.6%, respectively. As shown in Fig. 4, most of CPs collected were HCPs with alkyl substituents. Two main HCPs in ES_{d4} were 2-hydroxy-3,4-dimethylcyclopentenone and

2-hydroxy-3-ethylcyclopentenone with an area % of 12.2% and 25.8%, respectively.

4. Conclusions

In total, 121 compounds were detected in SEB produced by ethanolysis of wheat stalk. Oxy-compounds especially esters and phenols were dominant. The combination of extraction and liquid column chromatography was employed to separate SEB systematically. C₈-C₃₀ alkanes were separated in PE eluted fractions. Phenols were divided into four fractions, with the phenol concentration up to ~100%. Catechols, guaiacols and syringol were isolated in sub-fractions ES_{b2} (55.6 %), ES_{d2} (95.7%), and ES_{d3} (57.2%), respectively. Acetophenone was successfully isolated in ES_{b1} (71.2 %), while HCPs were also efficiently collected in ES_{d4} (52.6%). Future work is going to improve the recovery efficiency of ketones and quantify the recovered chemicals from SEB.

Abbreviations

Abbreviation	Full name/description
SEB	Bio-oil produced from supercritical ethanol
CS ₂	Carbon disulfide
DEE	Diethyl ether
PE	Petroleum ether
BRCKs	Benzene ring-containing ketones
CPs	Cyclopentenones
HCPs	Hydroxy-cyclopentenones
DHF	Furanones
NaOH	Sodium hydroxide
HCl	Hydrogen chloride
PHBs	Polyhydroxyl benzenes
MHBs	Monohydroxyl benzenes
PE	Petroleum ether
SG	Silica gel
GC-MS	Gas chromatography–mass spectrometry
TIC	Total ion chromatography
FTIR	Fourier transform infrared spectroscopy

BIP	Benzene insoluble phase
WP _I	Water phase I
BSP _I	Benzene phase I
DEEP _I	DEE soluble phase I extracted from WP _I
DEEIP _I	DEE insoluble phase I extracted from acidified aqueous by HCl
BSP _{II}	Benzene phase II
DEEP _{II}	DEE soluble phase II extracted from acidified aqueous by HCl
DEEIP _{II}	DEE insoluble phase II extracted from acidified aqueous by HCl
ES _{bn}	Fractions eluted from DEEP _I
ES _{dn}	Fractions eluted from DEEP _{II}
ES _{cn}	Fractions eluted from BSP _{II}
ES _{cn-w}	Milk-white solids obtained from ES _{cn}
ASP	Acetone soluble phase
AIP	Acetone insoluble phase

Acknowledgements

This work was subsidized by National Basic Research Program of China (Grant 2012CB215302), the Fund from Natural Science Foundation of China for Innovative Research Group (Grant 51221462), the Fundamental Research Funds for Central Universities (China University of Mining & Technology; Grant 2013XK05), and [a Project Funded by](#) the Priority Academic Program Development of Jiangsu Higher Education Institutions. WZ and KN acknowledge the support by MOST-JST, Strategic International Collaborative Research Program, SICORP.

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Fig. 1. Schematic diagram for the separation of SEB.

Fig. 2. FTIR spectra of ASP and AIP.

Fig. 3. TICs of ES_{c1} and ES_{c1-w} .

Fig. 4. TICs of ES_{bn} and ES_{dn} .

Fig. 5. Distribution of phenols and ketones in each ES_{bn} and ES_{dn}.

Table 1. Summary of Bio-oil Separation by Extraction and Column Chromatography

	Bio-oil	Solvent	Separation fraction
Extraction			
Rasrendra <i>et al.</i> [16]	An aqueous pyrolysis oil from forest residue	Batch liquid-liquid reactive extraction and continuous reactive extractions with Tri-n-octylamine in 2-ethyl-hexanol	86% acetic acid was recovered by batch extraction, while acetic acid recoveries of 51% and 71% were obtained in a single CCS device and a two stage cross cascade.
Vitasari <i>et al.</i> [17]	Aqueous model mixture for aqueous fast pyrolysis oil	Bio-oil was extracted by tri-n-ctylamine/2-ethyl-1-hexanol	Co-extraction of glycolaldehyde and acetic acid was tried with tri-n-ctylamine/2-ethyl-1-hexanol.
Bennett <i>et al.</i> [18]	Pyrolysis oil prepared by VTT processes from scots pine feedstock	Bio-oil was extracted by water with different water/oil ratio.	Optimal extraction conditions (41 wt% water, 34 °C) produced an aqueous extract with a concentration of up to 88 g/L levoglucosan.
Wang <i>et al.</i> [7]	Bio-oil from lauan sawdust via fluidized bed pyrolysis	Extraction by water, and NaOH solution (pH=14), dichloromethane (CH ₂ Cl ₂). HCl solution to adjust pH.	Fraction with a high concentration of 94.35% phenolic compounds was obtained. Pyrolytic lignins were analyzed in the raffinate after extraction.
Amen-Chen <i>et al.</i> [10]	Bio-oil converted from Eucalyptus wood tar	Dissolution in ethyl acetate, extraction by NaOH solution, acidification of aqueous phase to pH 6, and then extraction of ethyl acetate.	Complete recovery of phenols was achieved at a pH of 12-13 by using a concentrated alkaline solution.
Li et al. [19]	Bio-oil from corn stalk directly deoxy-liquefied	Distillation under vacuum. Extraction of 180-260 °C fraction by mixing with NaOH solution and HCl solution.	The high-purity phenol derivatives, such as phenol, cresols, guaiacol, 4-methyl guaiacol and syringol were obtained.
Zilnik et al. [20]	Bio-oils produced by fast pyrolysis process of wood and forest residues	Two schemes were considered: one is starting with an aqueous extraction (water, aqueous NaHSO ₃ , or alkali solution), another one with simultaneous use of a hydrophobic-polar solvent (such as MIKB or ethyl acetate) and anti-solvent in the extraction (water, aqueous NaHSO ₃ , or	Phenolics were recovered from pyrolysis oil. The distribution coefficients and extraction factors of phenolic compounds were compared for different solvents. MIBK was the most efficient solvent for extraction of phenolics from bio-oil in combination with aqueous NaOH solution.

		alkali solution).	
Wei <i>et al.</i> [21]	Bio-oil from microwave pyrolysis	Water, and then organic solvent (hexane, petroleum ether, chloroform).	The phenols and guaiacols were recovered at efficiency of 85% in the extracted phase by water-chloroform extraction.
Column chromatography			
Das <i>et al.</i> [22]	Bio-oil from Cashew nut shell via vacuum fluidized bed pyrolysis	Elution by hexane–ethyl acetate mixture and chloroform–methanol mixtures.	Five purified compounds (cardanol, cardol, di-n-octyl phthalate, bis (2-ethylhexyl) phthalate, and di-n-decyl phthalate) were obtained.
Acikgoz <i>et al.</i> [23]	Bio-oil from linseed via slow pyrolysis	1. Extraction by n-pentane, 2. Elution with pentane, toluene, and methanol.	88 wt% of bio-oil was extracted in n-pentane soluble. Aliphatic (C ₁₃ -C ₂₉), aromatic and polar fractions account for 30, 34 and 36 wt% of the n-pentane soluble, respectively.
Onay <i>et al.</i> [24]	Bio-oil from rapeseed, linseed and hazelnut shell via slow hydrolysis	Elution by n-hexane, toluene and dichloromethane/methanol (1:1, v/v)	Aliphatic (predominantly C ₁₃ -C ₃₀ n-alkanes), aromatic and polar fractions were obtained.
Zeng <i>et al.</i> [2]	Bio-oil from rice husk via fluidized bed fast pyrolysis	Extraction by saturated NaOH solution, and then acidified by HCl solution to get solid precipitate. The solid precipitate was separated by column eluted by ethyl acetate (EA) and ethanol or mixture of EA/petroleum ether or EA/ethanol.	Solid precipitate was obtained from bio-oil after basification–acidification. Five fractions main containing phthalate esters were obtained.
Putun <i>et al.</i> [25]	Bio-oil from soybean-cake via pyrolysis and steam pyrolysis	Elution with pentane, toluene, ether and methanol.	Aliphatic (alkanes, alkenes, and branched hydrocarbons), aromatic, ester and polar fractions were obtained.
Putun <i>et al.</i> [26]	Bio-oil from rice straw via pyrolysis and steam pyrolysis	1. Extraction by n-pentane, 2. Elution with pentane, toluene, ether and methanol.	Elution of pentane soluble materials gave aliphatic, aromatic, ester and polar fractions.

Table 2. Main Components in SEB (Area% $\geq 1\%$).

Peak ^a	Compound	Area %
<i>Hydrocarbons</i>		
43	2,5-Dimethylhexa-1,5-diene	2.8
110	4-Propyl-1,1'-biphenyl	1.5
<i>Phenols</i>		
40	Phenol	1.7
51	3-Cresol	1.3
55	4-Methoxyphenol	4.1
73	4-Ethylphenol	3.2
78	4-Methylguaiacol	1.7
84	1,2,3-Benzenetriol	2.9
89	4-Ethylguaiacol	4.2
99	Syringol	2.6
102	4-Propylguaiacol	1.6
<i>Ethers</i>		
41	2-Ethoxy-2-methylbutane	1.2
68	Ethoxybenzene	1.6
109	1,2,3-Trimethoxy-5-toluene	1.4
<i>Furan derivatives</i>		
3	Tetrahydrofuran	3.8
16	2-Ethoxytetrahydrofuran	4.0
23	Furan-2-ylmethanol	1.1
31	Dihydrofuran-2(3H)-one	1.6
<i>Esters</i>		
1	Ethyl acetate	1.0
15	Ethyl 2-hydroxypropanoate	2.8
29	Ethyl 2-hydroxybutanoate	5.2
54	Pentan-2-yl butyrate	11.5
88	Diethyl glutarate	1.3
115	Ethyl palmitate	1.2
<i>Alkanoic Acid</i>		
2	Acetic acid	1.1

^a refers to the number marked in Fig. SI1.

Table 3. Extract Yields (*wt%*) from SEB

BIP	DEEP _I	DEEIP _I	DEEP _{II}	DEEIP _{II}	BSP _{II}	ASP	AIP
42.5	5.0	0.3	10.0	5.0	17.5	2.5	10.0

Table 4. Main Compounds Detected in BSP_{II} (Area % $\geq 1\%$).

Peak ^b	Compounds	Area %
2	Ethylbenzene	2.0
3	2-Methylcyclopentenone	2.6
5	1,2-Dimethylcyclopentenone	1.1
10	3,4-Dimethylcyclopentenone	1.3
11	2,5-Dimethylcyclopentenone	3.9
14	Acetophenone	1.2
17	2,3-Dimethylcyclopentenone	3.2
19	2,3,4-Trimethylcyclopentenone	1.3
20	3-Ethylcyclopentenone	2.0
22	Hydrindane	2.0
23	4-Methoxyphenol	1.2
27	Propyl cyclohexene	1.7
31	2-Methyl-4-octyne	1.5
37	Ethyl benzoate	1.3
39	2-Methyl-diethyl succinate	4.0
40	Diethyl glutarate	1.1
41	Decalin	1.9
42	Furyl-2- butanone	2.7
43	Diethyl 3-methyl glutarate	1.8
44	1,4-Dimethoxyl-2-methylbenzene	1.4
45	2-Ethylfurfural	1.4
46	3-Methyl-2-vinylcyclohexanone	1.1
47	Ethyl 4-methoxyl benzoate	1.6
51	1-Methoxyl-3,5-dimethylbenzene	1.7
52	Diethyl adipate	3.6
53	Ethyl 3-methyl benzoate	1.1
55	Ethyl 4-methyl benzoate	1.1
58	3-Methyl-2,3-indenone	1.5
59	4-Methylthioacetophenone	1.4
61	1-Propyl-4-methyl-2-methoxylbenzene	1.2
64	4- <i>tert</i> -Butylbenzyl alcohol	1.3
67	1,3-Dimethyl-5-vinylbenzene	1.1
68	4- <i>tert</i> -Butylacetophenone	1.1
69	3,4-Dimethoxylacetophenone	1.4
73	Ethyl tetradecanoate	1.3
77	Ethyl palmitate	2.1

^b refers to the number marked in Fig. SI2.

Table 5. Main Compounds Detected in DEEP_I (Area % $\geq 1\%$).

Peak ^c	Compound	Area %
1	Butan-2-ol	1.2
3	Tetrahydrofuran	1.3
10	2-Ethoxybutane	1.5
11	Ethyl 2-hydroxypropanoate	3.1
12	2-Ethoxypentane	1.8
14	4-Hydroxy-4-methylpentan-2-one	1.3
15	But-2-enoic acid	7.1
16	Ethyl 2-hydroxybutanoate	4.9
20	Phenol	3.7
21	Ethyl 2-hydroxypentanoate	1.2
22	3-Methylcyclopentane-1,2-dione	4.4
24	2-Hydroxy-3,4-dimethylcyclopent-2-enone	2.4
25	Acetophenone	15.4
26	(Tetrahydrofuran-2-yl)methyl acetate	7.1
27	2-Methoxyphenol	2.6
28	3-Ethyl-2-hydroxycyclopent-2-enone	4.2
29	2-(Methylthio)phenol	1.1
30	6-Oxo-6 <i>H</i> -pyran-3-carboxylic acid	1.5
31	Isopropyl butyl ether	1.2
32	4-Ethylphenol	1.4
34	Catechol	5.3
36	3-Ethylcatechol	3.2
38	4-Methylcatechol	2.3
40	4-Ethylcatechol	1.7
41	Syringol	3.6
42	4-Ethylcatechol	3.3

^c refers to the number marked in Fig. SI3.

Table 6. Main Compounds Detected in DEEP_{II} (Area % $\geq 1\%$).

Peak ^d	Compound	Area %
4	Ethyl acetate	1.8
7	2-Ethoxybutane	9.1
11	Phenol	2.6
13	2,3-Xylenol	1.5
14	2-Hydroxy-3,4-dimethylcyclopent-2-enone	1.5
15	<i>p</i> -Cresol	2.1
16	Tetrahydrofuran-2-ol	1.7
17	Guaiacol	12.8
18	3-Ethyl-2-hydroxycyclopent-2-enone	3.4
19	2-Ethylphenol	1.7
20	3,5-Dimethoxyphenol	1.7
21	2-Ethoxyphenol	2.6
22	6-Oxo-6 <i>H</i> -pyran-3-carboxylic acid	2.5
23	4-Ethoxyphenol	11.0
25	4-Methylcatechol	1.3
27	4-Methylguaiacol	4.0
31	3-Ethyl- 5-cresol	4.9
35	4-Ethylguaiacol	15.2
36	5-Isopropyl -2-cresol	1.5
42	Syringol	9.6
44	3,4-Xylenol	4.2

^d refers to the number marked in Fig. SI4

Table 7. Alkanes Separated in ES_{c1} and ES_{c2} from the SEB.

Peak ^e	Compound	Area %	
		ES _{c1}	ES _{c2}
1	Octane	1.5	0.6
2	Nonane	1.7	
3	Decane	0.7	
4	Dodecane	1.2	
5	Tridecane	1.7	
6	Tetradecane	2.8	
7	Pentadecane	5.1	
8	Hexadecane	6.3	
9	Heptadecane	3.9	
10	Octadecane	3.4	5.4
11	Nonadecane	3.1	5.1
12	Icosane	3.0	11.7
13	Henicosane	3.2	12.8
14	Docosane	3.0	11.7
15	Tricosane	3.0	8.5
16	Tetracosane	2.0	7.4
17	Pentacosane	1.6	6.3
18	Hexacosane	1.6	6.8
19	Heptacosane	0.9	6
20	Octacosane	1.2	6.1
21	Nonacosane	1.3	4.7
22	triacontane	3.7	4.1
Total		55.9	97.2

^e refers to the number marked in Fig. 4.

Table 8. Phenols Detected in ES_{bn} and ES_{dn}.

Peak ^f	Compound	Area %					
		ES _{b1}	ES _{b2}	ES _{d1}	ES _{d2}	ES _{d3}	ES _{d4}
<i>MHBs</i>							
11	Guaiacol				18.6		
16	2-(Methylthio)phenol		0.7				
18	2-Ethoxyphenol			7.3	2.4		
20	4-Ethylphenol	11.3					
22	6-Methyl guaiacol			7.9			
23	4-Ethyl-3-methoxyphenol			7.6	1.1		
24	4-Methyl guaiacol			3.9	8.3		
26	2,3,5-Trimethylphenol			0.7			
29	2,4,6-Trimethylphenol			1.0			
32	2-Ethoxy-4-cresol			2.4			
33	2-Isopropyl-4-cresol			8.2			
34	4-Ethyl guaiacol			13.2	49.4	2.4	
36	2-Propyl-4-cresol			2.1			
37	Ethylxlenol			5.9			
38	4-Ethyl-3-methoxyphenol			4.3			
39	2-Isopropyl-5-cresol			2.0			
40	4-Isopropyl-3-cresol				1.7		
47	Syringol		11.4			57.2	19.4
49	4-Allyl guaiacol				1.5		
52	4-Propyl guaiacol				14.4		
53	2-Allyl-4-cresol			1.8			
57	2-tert-Butyl-4-methoxyphenol			1.5			
<i>PHBs</i>							
8	Benzene-1,3,5-triol		0.8				
12	2-Methoxyhydroquinone					6.6	1.4
15	3-Methoxycatechol						3.6
19	2-Methoxyresorcinol						2.0
25	Catechol		16.3				
30	4-Methylcatechol		10.7	2.9			
35	3-Methylcatechol		11.4				
42	2-tert-Butylhydroquinone			1.9			
44	4-Ethylresorcinol						
50	4-tert-Butylcatechol			7.7			
54	4-Ethylcatechol		16.4				

^f refers to the number marked in Fig. SI5.

Table 9. Ketones Detected in ES_{bn} and ES_{dn}

Peak ^g	Compound	Area %					
		ES _{b1}	ES _{b2}	ES _{d1}	ES _{d2}	ES _{d3}	ES _{d4}
<i>DHF</i>							
1	5-Methyldihydrofuran-2(3 <i>H</i>)-one		0.4				
<i>CPs</i>							
2	3-Methylcyclopent-2-enone		1.1				
3	3-Methylcyclopentane-1,2-dione						1.7
4	2-Hydroxy-3-methylcyclopent-2-enone		11.2				
5	2-Hydroxy-3,4-dimethylcyclopent-2-enone					1.5	12.2
6	3-Hydroxy-2,4-dimethylcyclopent-2-enone		2.5				
9	2-Acetylcyclopentanone						1.6
13	3-Ethyl-2-hydroxycyclopent-2-enone					1.1	25.8
14	2-Ethyl-3-hydroxycyclopent-2-enone		7.6				
27	2-Hydroxy-3-propylcyclopent-2-enone						8.2
41	3-Butyl-2-hydroxycyclopent-2-enone						3.1
<i>BRCKs</i>							
7	Acetophenone	71.2					
45	1-(2-Hydroxy-6-methoxyphenyl)ethanone			5.8			
46	1-(2,6-Dihydroxy-3-tolyl)ethanone			4.1			
48	1-(2-Hydroxy-5-methoxyphenyl)ethanone			3.0			
51	1-(2,4-Dihydroxyphenyl)propan-1-one			2.9			
58	1-(4-Hydroxy-3-methoxyphenyl)ethanone		1.3				
59	1-(4-Hydroxy-3-methoxyphenyl)propan-2-one		1.8				

^g refers to the number marked in Fig. SI5.