Characteristics of Lignin Extracted from Pararubber Wood Sawdust via Organosolv Fractionation

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Abstract: Among biomass-derived compounds, lignin is an underused component with potential for conversion to industrial-need products in biorefinery. In this study, organosolv fractionation of pararubber wood sawdust (PS) was studied using a ternary solvent mixture of methyl isobutyl ketone (MIBK), ethanol, and water in the presence of H_2SO_4 as an acid promoter aiming to separate high-purity lignin fraction. The optimized conditions were at 160°C for 40 min with MIBK/ethanol/water proportion of 0.25/0.42/0.33 and 0.025 M of H_2SO_4, which led to the highest lignin removal efficiency of 70.6% (w/w) from PS. Physicochemical characteristics of the fractionated lignin were determined by Klason lignin, X-ray fluorescence (XRF), and Fourier transform infrared spectroscopy (FT-IR). In addition, the lignin samples were then thermally depolymerized in MIBK to determine the content of specific lignin-derived chemicals. The main phenolic derivatives from PS-lignin were syringaldehyde, *cis*-isoeugenol and vanillin. This work demonstrates the potential of the fractionated lignin for production of valuable chemicals in biorefineries.

Keywords: Biomass; Organosolv fractionation; Lignin; Depolymerization.

1. Introduction

Nowadays, lignocellulosic biomass is a promising alternative feedstock for conversion to biofuels, commodity chemicals and biomaterials. Several biomasses such as agricultural residues, forest residues, energy crops, food wastes, and municipal and industrial wastes are composed of cellulose, hemicelluloses and lignin as the primary constituents. Utilization of a variety of agricultural residues as feedstocks in biorefinery is considered a great challenge with a key advantage on environmental sustainability. This allows less-dependence on the depleting fossil reserves, increasing energy security, reducing surplus carbon in the atmosphere, rising income of farmers, and enhancing overall economy of the country.

Recently, organosolv fractionation process has been developed in order to obtain high quantity and quality of cellulose, hemicelluloses and lignin. A wide range of organic solvents, including acetone, methanol, ethanol, organic acids (i.e., acetic acid, formic acid), inorganic and organic bases, ketones, ethylene glycol, esters and combined solvents with and without catalysts (acid and alkali) are used in biomass fractionation. These solvents provide selective solubilization of lignin from native lignocellulosic biomass. Short chain alcohols such as methanol and ethanol in form of single or solvent mixture with water are the most commonly used organic solvent in this process in presence and/or absence of catalytic promoters [1-4]. The Clean fractionation (CF) process has been recently introduced for one step separation of bio-polymers in lignocelluloses using a ternary miscible solvent mixture comprising a short chain alcohol (methanol or ethanol), methyl isobutyl ketone (MIBK) and water in the presence of an acid promoter [5]. After high-temperature treatment, the phase of the solvent mixture could be disturbed as addition of water, which resulted in separation of hemicellulose-derived products in the aqueous/alcohol phase, lignin in the MIBK phase, and cellulose in the the solid fraction. The CF-process has later been further studied for the use of heterogenous acid promoters and alternative solvent systems. This method has been applied for separation of lignocellulosic components from various hardwood, softwood and agricultural wastes, giving organosolv lignin fractions with high purity.

Lignin is considered an underused part in lignocellulosic biomass. It is a complex molecular structure containing cross-linked polymers of phenolic monomers mainly consisted of *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol [6-7].

In general, lignins are roughly classified into three major groups: softwood, hardwood, and grass lignins [8]. The effects of reaction parameters (temperature and time), types of catalytic promoter, and biomass sources have strong influences on obtaining different lignin products. Fractionation and utilization of organosolv lignin from various plant sources e.g. empty fruit bunch (EFB), rice husk, oil palm, rice straw spruce, flax, fiber hemp, wheat straw and eucalyptus wood have been reported [9-15]. Erdocia et al reported that lignin fraction of olive tree pruning fractionated from different organosolv treatments by mean of acetosolv, formosolv and acetosolv/formosolv processes had high purity with low sugar and other contaminations [16]. Depolymerization of commercial organosolv lignin in methanol with/without of homogeneous and heterogeneous acid catalysis was shown to result in the production of industrial-needed chemicals e.g. 4-ethylguaiacol, phenol, guaiacol, syringol and vanillin [17].

The purpose of this research is to isolate high-purity lignin from pararubber wood sawdust using the CF-based organosolv process. The effects of reaction temperature on lignin separation efficiency were studied. The isolated lignin samples were characterized by Klason lignin, X-ray fluorescence (XRF), and Fourier transform infrared spectroscopy (FT-IR). Product profiles from depolymerization of lignin fractionated were then compared. This study provides a platform for further study on value-added utilization of lignin in integrated biorefineries.

2. Experimental

2.1 Materials

Pararubber wood sawdust (PS) was provided from Prince of Songkla University, Songkla, Thailand. Local agricultural residues were milled and sieved to the size of <1 mm using Retsch Ultra Centrifugal Mill ZM200 (Retsch ZM2000, Haan, Germany). Chemical composition of the raw materials was analyzed by NREL method [18]. Chemicals and reagents were analytical grade and obtained from major chemical companies. The commercial grade organosolv lignin was purchased from Chemical Point UG, Germany.

2.2 Organosolv fractionation

The raw material (10 g) was fractionated using 100 ml ternary mixture solvent comprising methyl isobutyl ketone

(MIBK), ethanol, and water (25% : 42% : 33% v/v) in the presence of 0.025 M H_2SO_4 as acid promoter according to the method modified from Klamrassamee et al. 2013 [19]. The fractionation process was carried out in a 600 ml of stainless steel reactor heated by electric jacket with a thermocouple to measure temperature inside the reactor. The reaction was performed by different reaction temperatures (130, 150, and 170°C) for 40 min based on initial pressure of nitrogen (N_2) at 20 bars with stirring at 100 rpm for keeping the system homogeneous. After the organosolv treatment, the reaction was immediately stopped by quenching on ice for 10 min. The slurry was filtered through a 20-25 μm of the paper filter (Whatman No.4) using a Büchner funnel for separating liquid content (aqueous-organic fraction) from the solid. The solid was subsequently with MIBK and water (1:2 v/v) mixture. The liquid fraction of aqueous-organic mixture was separated into two phases by adding water in a separator funnel. The mixture was placed at room temperature for 20 min for complete phase separation. The aqueous phase contained hemicellulose content and a trace amount of soluble products e.g. soluble lignin, furfural, and 5-hydroxymethylfurfural (HMF) and organic phase MIBK contained lignin. The MIBK phase contained lignin fraction was evaporated on hotplate at 70°C. The lignin sample was then dried at 70°C for 2-3 day before further study.

Chemical composition of the solid fraction was determined using the NREL method [18]. The percentage of biomass composition (cellulose, hemicellulose, and lignin) was determined based on the remaining contents in the solid residues compared to their respective contents in the native biomass. Lignin removal efficiency was determined based on the amount of removed lignin calculated based on its residual content in the solid pulp compared to the initial lignin content in the raw material taking %solid recovery during the fractionation step into account.

2.3 Lignin depolymerization

Lignin sample (0.0875 g) from different sources was dissolved in 5 ml of MIBK in batch reactor that was sealed, evacuated and purged with N_2. The reaction was heated at 300°C for 1 hour with shaking at 40 rpm. After that, the reaction was quenched on ice and the mixture centrifuged at 5,000 rpm for 10 min in order to remove the un-dissolved solid. The liquid fraction was acidified to pH 2.0 with 1 M sulfuric acid to precipitate the residual lignin (RL). The separated RL was dried at 70°C for 24 hours and weighed. After that, the remaining liquid products were evaporated for removal of water and solvent by a rotary evaporator. Finally, the lignin products were dissolved in MIBK and filtered through a 0.45 μm syringe filter.

After depolymerization process, all phenolic products obtained from lignin sample were analyzed by GC-MS (GC-MS 7890A 5975 C MSD, GenTech Scientific, New York, United States) on a Mega column (30m x 0.25 mm; 0.25 μm) (MEGA-WAX Plus, Mega s.n.c., Legnano, Italy) in helium (He) with a temperature program staring for 5 min at 50°C to 120°C for 5 min, heated up to 280°C and kept at this temperature for 10 min. The final temperature was then heated at 300°C for 10 min at the same heating rate. The compound products were identified by comparison of mass spectra data to the NIST library.

2.4 Lignin characterization
2.4.1 Klason lignin

The composition of lignin recovered from the organic phase was determined based on the Klason lignin according to the laboratory analytical procedure provided by the National Renewable Energy Laboratory (NREL) [18].

2.4.2 Fourier transform infrared spectroscopy (FTIR)

The functional groups on the lignin samples were analyzed by Fourier-Transformed Infrared Spectroscopy (FT-IR) (Perkin-Elmer System 2000, Waltham, United States) with infrared spectra collected in the wave number range of 400–4000 cm^{-1}.

2.4.3 X-ray fluorescence (XRF)

Moisture was removed from organosolv lignin samples by drying at 70°C. The samples were analyzed using Orbis PC Micro-EDXRF elemental analyzer (EDAX Inc., New Jersey, USA). The condition was set at 35 KV/1mA for 2 min in area 2 millimeter. Identification of elements was performed by comparison to FP nostds data. The percentage of each element was calculated based on a weight basis.

3. Results and Discussion

3.1 Organosolv fractionation

The composition of raw materials as pararubber wood sawdust consist of cellulose, hemicellulose, lignin, ash and extractive. It is listed in Table 1. It can be seen that it contained cellulose as the major composition (37.8±1.3% (w/w) on a dry weight basis). The ash content was 1.3±0.0% (w/w). The extractive fraction contained starch, wax, syrup, organic acids, and soluble protein was 16.0±2.0% (w/w).

Firstly, the effect of reaction temperature on organosolv fractionation process of pararubber wood sawdust was studied at different temperature from 160°C to 180°C for 40 min using solvent mixture comprising methyl isobutyl ketone (MIBK), ethanol, and water (25% : 42% : 33% v/v) with and without the presence of 0.025 M H_2SO_4 as acid promoter. Addition of acid promoter was found to result in solubilization of hemicellulose and lignin into the aqueous/alcohol and MIBK phases, respectively, resulting in enrichment of cellulose in the solid fractions. (Figure 1). Increasing on reaction temperature from 140°C to 180°C led to decline of pulp yield in range of 37.6-84.7% due to removal of the non-cellulosic (hemicellulose and lignin) components. According to the results, the cellulose content in solid fraction was main component in range of 57.4±2.0% to 81.0±2.3%. The content of hemicellulose in the solid was in the range of 0.4±1.0% to 12.4±0.3% whereas the remaining lignin was highly varied in the range of 10.4±0.5% to 23.8±1.1%. The %pulp yield was determined based on the remaining weight of solid pulp after fractionation process compared to the initial weight of raw material and the %yield of composition (cellulose, hemicellulose, and lignin) is based on the remaining contents of cellulose, hemicellulose, and lignin in the solid residues compared to their respective contents in the native biomass on a dry weight basis.

Table 1. Chemical composition of lignocellulosic biomass on a dry weight basis.

Substrate	Cellulose (%w/w)	Hemicellulose (%w/w)	Lignin (%w/w)	Ash (%w/w)	Extractive* (%w/w)
Pararubber wood sawdust	37.8±1.3%	16.6±1.9%	27.6±0.4%	1.3±0.0%	16.0±2.0%

*Extractive: starch, wax, syrup, organic acids, and soluble protein
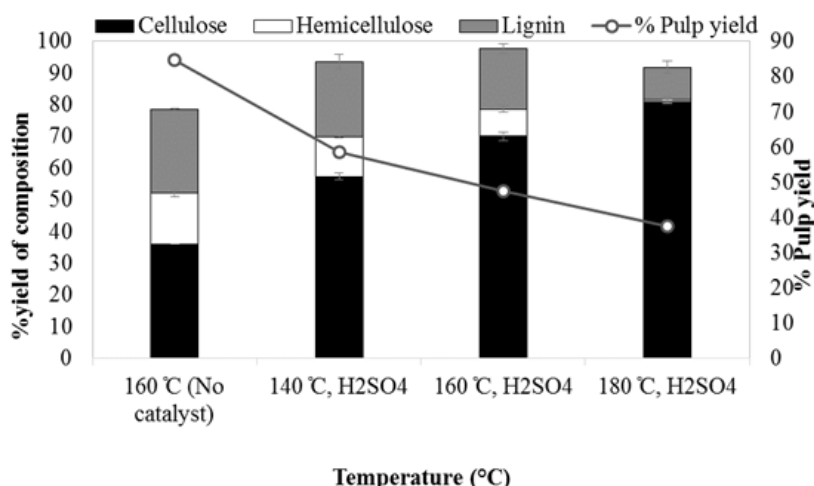
Figure 1. Effect of reaction temperature on biomass composition of the solid residues obtained from organosolv fractionation of pararubber wood sawdust. The reaction contained 10% (w/v) biomass in ternary solvent mixture comprising MIBK, ethanol, and water (25% : 42% : 33% v/v) in the presence of 0.025 M H_2SO_4 as an acid catalyst and heated at 160°C for 40 min compared to the control reaction with no catalyst.

The effects of temperature on lignin removal efficiency was studied by varying the temperature from 140-180°C (Figure 2). The lignin removal was lignin that was extracted from raw biomass. The temperature was found to have strong influence on lignin removal with a marked increase on the removal efficiency when the temperature increased from 140-180°C in the presence of acid promoter. The lignin removal efficiency was determined based on the amount of removed lignin calculated based on its residual content in the solid pulp compared to the initial lignin content in the raw material taking %solid recovery during the fractionation step into account. The highest removal efficiency of 78.3% was obtained for PS at 180°C. Influence of temperature on organosolv fractionation of biomass has been recently investigated by several researchers [19-21]. Huijgen et al (2010) revealed that increase of temperature in the range of 160-220°C in an acetone-based organosolv pretreatment of wheat straw resulted in enhancement of delignification, solubilization of hemicellulose, and enzymatic digestibility of the solid fraction. The addition of acid catalyst such as H_2SO_4 on ethanosolv fractionation of wheat straw led to similar results in decrease of pulp yield, increase of delignification, xylan hydrolysis, and enzymatic digestibility of cellulose after rising the reaction temperature from 160 to 210°C [20]. In addition, Klamrassamee et al (2013) reported the organosolv fractionation of eucalyptus wood sawdust using solvent mixture of MIBK, methanol, and water in the presence of homogeneous acid promoters in range of temperature (130-250°C). It was revealed that the total yield of soluble hemicellulose products and lignin yield increased when the reaction temperature increased from 140 to 180°C; further increase in temperature to 200-220°C led to decreases in the total soluble hemicellulose yield with increasing hydrolysis of cellulose to glucose and cello-oligosaccharides [19].

3.2 Characterization of isolated lignins

The purity of lignin recovered from the organic phase at 160°C was determined by Klason lignin method and compared to the commercial organosolv lignin. As presented in Table 2, the purities of lignin from pararubber wood sawdust are 87.6±1.5%. Trace amount of sugar and ash was 1.22% and 0.4% relatively. It should be noted that the purity of lignin obtained in this study were relatively higher those previously reported using different organosolv processes, leading to Klason lignin between 65.9-79.0% from various raw materials e.g. prairie cordgrass, switchgrass, corn stover, hybrid poplar and olive tree [16, 22, 23]. This therefore suggested that organosolv lignins pararubber

wood sawdust show a potent for subsequent conversion to value added chemical products.
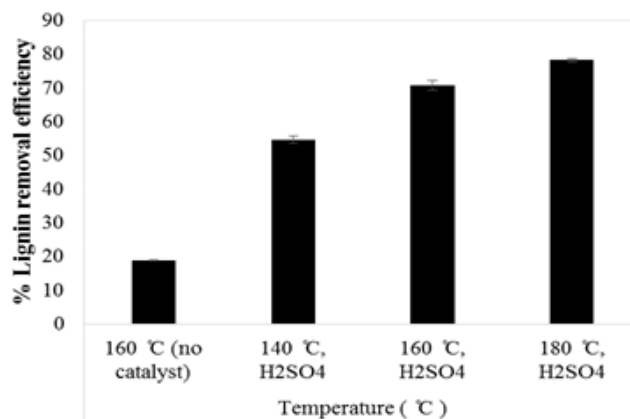

Figure 2. Lignin removal efficiency from organosolv fractionation of pararubber wood sawdust at different temperatures. The reaction contained 10% (w/v) biomass in ternary solvent mixture comprising MIBK, ethanol, and water (25% : 42% : 33% v/v) in the presence of 0.025 M H_2SO_4 as an acid catalyst and heated at 160-180°C for 40 min compared to the control reaction with no catalyst.

Table 2. Characteristics of organosolv lignin fraction recovered from the organic phase.

	Sources	
	Organosolv commercial lignin	**Pararubber wood sawdust**
Klason lignin (%)	91.7±1.3	87.6±1.5
Sugar (%):		
Glucose	0.92±0.2	0.2±0.1
Xylose	0.12±0.2	1.0±0.2
Arabinose	0.12±0.02	0.02±0.02
Ash (%)	0.86±0.1	0.4±0.3

The mineral contents of lignin recovered from organic phase were analyzed by XRF, as listed in **Table 3**. It was found that isolated lignin samples contained sulfur (S) (76.52%w/w),

and iron (Fe) (23.48%w/w); these mineral elements were also detected in commercial organosolv lignin. It should be noted that the presence of sulfur could be due to the use of sulfuric acid as acid promoter as well as from the amino acids (contained in elemental sulfur) as cysteine and methionine transported around the plant [24]. High level of iron component in lignin fraction might come from soil as reported by Kumar et al (2013) [25]. This iron ion could interact with methoxyl structure of lignin, leading to the protection of lignin decomposition [26].

FT-IR spectra of these isolated lignin and the commercial organosolv lignin were almost identical as shown in **Figure 3**. The functional groups of these lignin samples were corresponded to assignment of FT-IR spectra of lignin reported earlier [14, 15, 27]. It presented the vibration of O-H stretching at 3423 cm^{-1} while the FT-IR absorption at 2937 and 2844 cm^{-1} was CH sketch in CH$_2$ and CH$_3$ groups, respectively. The C=O group stretching of carbonyl group showed peaks at 1711 cm^{-1} while the peaks of aromatic skeleton vibrations were at 1600 cm^{-1}, 1514 cm^{-1}, and 1426 cm^{-1}. The presence of absorption band at 1462 cm^{-1} was corresponded to CH formation and banding of aromatic ring vibration in lignin. In addition, the peaks of (-OH) phenolic group from all lignin samples are at 1329cm^{-1}, and the aromatic ether aryl group showed peaks at 1269 and 1219 cm^{-1}. The absorption of syringyl ring in form of C-H in plane and out of plane deformations appeared at 1119 and 832 cm^{-1}, respectively while peaks of guaiacyl group were observed at 1034 and 912 cm^{-1}. These results suggested no chemical modification in the lignin structure during the organosolv fractionation of lignocellulosic material under the experimental conditions using sulfuric acid as the promoter.

3.3 Depolymerization of isolated lignin

After fractionation and characterization, the isolated lignin was thermally depolymerized in MIBK at 300°C for 1 h without addition of an extra catalyst in order to compare profiles of lignin-derived chemical compounds from different raw material.

It is noted that MIBK was chosen as solvent due to its high lignin solubility (more than 90%) compared to other organic solvents (e.g. alcohols, ethyl acetate, tetrahydrofuran) and water. From our study, relatively higher lignin conversion and total phenolic product yield could be obtained from depolymerization of lignin using MIBK as solvent (compared to the uses of water, tetrahydrofuran, dioxane, acetone, acetic acid, methanol, ethanol, ethyl acetate, methanol, ethanol, propanol, acetic acid, triethylamide and hexane as solvents, from which the conversion and yield were in the range of 71.3-82.9% and 3.6-12.8%, respectively). From the analysis using GC-MS, the pyrograms of products from the depolymerization of lignin-derived from different lignocellulosic materials are shown in **Figure. 4.** which corresponded to different lignin products in term of identity and relative molar abundances as listed in **Table 4**.

Table 3. Inorganic element composition of lignin fraction from different sources.

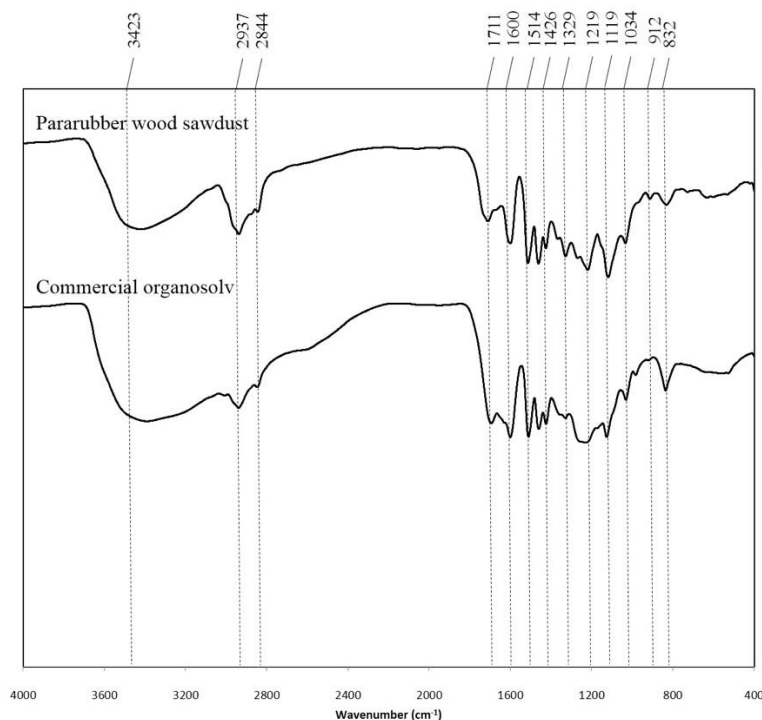|	Commercial organosolv lignin (% wt)	Pararubber wood sawdust (% wt)
Si	30.30	-
S	50.93	76.52
K	-	-
Ca	3.18	-
Ti	2.34	-
Cr	-	-
Fe	13.26	23.48
Cu	-	-
Al	-	-
P	-	-
Mn	-	-
Zn	-	-
Na	-	-
Total	100.01	100

Figure 3. FTIR spectra of commercial organosolv lignin and recovered lignin from pararubber wood sawdust
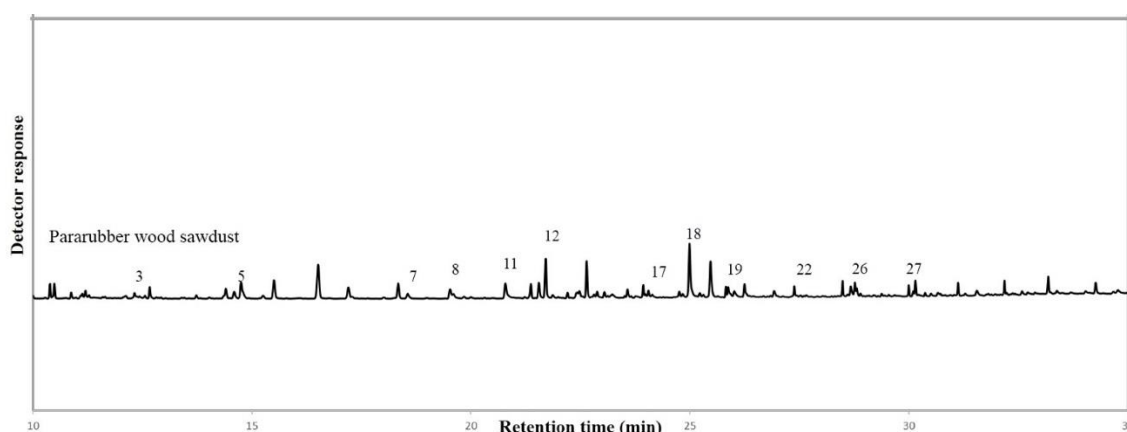
Figure 4. GC-MS chromatogram of fractionated lignin from pararubber wood sawdust. The depolymerization reaction was performed in MIBK at 300°C for 1 hour with no catalyst.

Table 4. Identities and relative molar abundances of the lignin-derived compounds from different raw material

No.	Compound	Pararubber wood sawdust
1	Phenol	-
2	p-Cresol	-
3	Guaiacol	0.92
4	4-ethylphenol	
5	Creosol	0.99
6	4-Ethylguaiacol	-
7	4-Vinylguaiacol	0.91
8	Syringol	1.47
9	Ethyl b-d-riboside	-
10	4-Propylguaiacol	-
11	Vanillin	2.96
12	cis-Isoeugenol	4.36
13	trans-Isoeugenol	-
14	Homovanillyl alcohol	0.37
15	Methylparaben	-
16	Ethylparaben	-
17	4-allylsyringol	0.82
18	Syringaldehyde	6.87
19	Acetosyringone	1.48
20	Coniferyl aldehyde	0.99
21	p-Hydroxycinnamic acid ethyl ester	-
22	Methyl hexadecanoate	1.11
23	Ferulic acid methyl ester	-
24	Hexadecanoic acid	-
25	Ethyl hexadecanoate	-
26	Sinapicaldehyde	1.02
27	Ethyl Oleate	1.83
28	Ethyl octadecanoate	-
29	Bis(2-ethylhexyl)phthalate	-

The pyrogram compounds derived from guaiacyl (G) and syringyl (S) lignin units. The most abundant phenolic compounds were guaiacol (3), 4-vinylguaiacol (7), syringol (8), vanillin (11), cis-isoeugenol (12), syringaldehyde (18), and acetosyringone (19). It was found that pararubber wood sawdust is categorized in angiosperm hardwood which contained lignin structure both guaiacyl (G) and syringyl (S) units [14, 28]. The depolymerized products from PS- lignin mostly contained syringaldehyde, *cis*-isoeugenol, vanillin, acetosyringone, and syringol corresponding to the relative molar abundances with 6.87, 4.36, 2.96, 1.48, and 1.47, respectively. From this study, it can be revealed that syringaldehyde, *cis*-isoeugenol, syringol, guaiacol, and vanillin are important phenolic compounds from the depolymerization of organosolv lignins. These compounds are potent precursors for the production of industrial commodity chemicals and specialty such as synthetic flavors and fragrances, food, chemical and pharmaceutical industry [29-31].

4. Conclusions

Isolation of lignin from pararubber wood sawdust using the CF-based organosolv process was demonstrated in this study. The fractionated lignin showed a potent for subsequent conversion to value added chemical products. Depolymerization of the isolated lignin showed variation in the products profiles and their relative ratios. The work demonstrates an efficient sequential organosolv fractionation and depolymerization of isolated lignin as promising approach for the production of industrial-need chemicals, leading to application of synthetic flavors and fragrances, food, chemical and pharmaceutical industry.

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