EFFECT OF SOLVENT PRE-TREATMENT ON LIGNOPHENOL PRODUCTION FROM OIL PALM EMPTY FRUIT BUNCH FIBRES

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ABSTRACT

Oil palm empty fruit bunches (EFB) are a by-product in the palm oil industry, and represent an abundant, inexpensive and renewable resource. EFB can be categorized as a lignocellulosic material due to its cellulose, hemicellulose and lignin contents. The purpose of this research was to investigate the effect of solvent pre-treatment on lignophenol production from EFB. Two types of EFB were tested, i.e. *treated ground EFB (LP1) and non-treated ground EFB (LP2). EFB has potential as a starting material for lignophenol production using a two-step process involving a phase separation system at room temperature (~ 28℃). This process utilizes the phenol derivative (p-cresol) and concentrated acid (72% sulphuric acid) whereby the lignin is dissolved in the organic phase and the carbohydrates are present in the aqueous phase after 1 hr of stirring. LP1 and LP2 were further analysed and characterized by Proton nuclear magnetic resonance (^{1}H-NMR), Fourier transform infra-red spectroscopy (FTIR), gel permeation chromatography (GPC), ultraviolet spectroscopy (UV) and by thermomechanical analysis (TMA). The experiments gave sufficient information on the characteristics of the lignophenols from treated and untreated EFB. The lignophenols of LP1 and LP2 showed similar results in their characteristics while their molecular weights were 5759 and 5866, respectively. There was no significant difference in the amount of cresol attached to lignin in LP1 and LP2, both being 26±1%. The yields of lignophenols for LP1 and LP2 were almost similar at a value of 61±1%. TMA curves showed that LP1 and LP2 had an apparent change of phase at 166.4℃ and 160℃, respectively.*

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INTRODUCTION

Lignocellulosic biomass refers to plant biomass that is composed of cellulose, hemicellulose and lignin. It comprises more than 60% of plant biomass produced on earth (Tengerdy and Szakacs, 2003). In view of the expected depletion of fossil fuel resources in the future, the succeeding utilization of lignocellulosic resources is important. This vast resource is a potential source of biofuel, biofertilizers, animal feed, chemical feedstocks and raw material for the paper industry. The lignin incorporated in the plant cell wall complex should be extracted through chemical and/or biological processes to fully utilize the lignocellulose. Many products can be made from lignin, such as cresols, phenols, catechols and vanillin by a fragmentation process; dispersants and emulsion stabilizers from macromolecules in a solution system process; thermosetting resin, polyblends, antioxidants and rubber reinforced by macromolecules in material systems, as well as energy (Aziz *et al.*, 2002). A new application of lignin is to produce lignophenols through a phase separation treatment.

In Malaysia, there is a possibility to separate lignin from one of her main lignocellulosic residues, that is, oil palm empty fruit bunches (EFB). Empty fruit bunches is a by-product in the palm oil industry after the removal of the oil palm fruits for oil extraction, and EFB represents a very abundant, inexpensive and renewable resource. Approximately 15 million tonnes of EFB biomass waste are generated annually from palm oil mills (Rahman *et al.*, 2007). EFB contains about 77.7% holocellulose (which consists of 44.2% and 33.5% α-cellulose and hemicellulose, respectively) and 20.4% lignin (Aziz *et al.*, 2002). There is a huge potential for EFB to be exploited in the production of high value-added products, which not only complies with the zero-waste strategy but also generates additional profits for the palm oil industry. Currently, EFB are returned to the plantation for mulching, whereas in the old practice, they are burned in an incinerator to produce fertilizer (bunch ash).

A new phase-separation system originally designed by Funaoka *et al.* comprises a successive total utilization of lignocellulosics aiming for sustainable development (Funaoka *et al.*, 1995). Woody wastes are the preferred raw materials for the phase separation system. Through this system, hydrophobic lignin and hydrophilic carbohydrates are subjected individually to selective structural conversion at different phases, and then separated quantitatively as phenolic lignin-based polymers (lignophenols) and soluble sugars. In the organic phase, the lignin network is released through the cleavage of the benzyl aryl ethers, followed by phenolation to produce phenolic linear-type polymers, or lignophenols. On the other hand, carbohydrates are hydrolyzed into soluble poly-, oligo- and monosaccharides. The lignophenols produced have unique properties, such as high phenolic reactivity, obvious solid-liquid transition and recyclability, which can be applied to various industries. Various researches have been carried out on the exploitation of lignophenols for the production of high value-added products such as lead acid batteries, biodegradable polyester composites, fibre composites, photoresist for printed circuits and enzyme complexes.

In this study, EFB were used to evaluate its potential as a starting material for lignophenol production by using the two-step process of the phase separation system. The effects of pre-treatment on EFB were investigated and characterized by Proton nuclear magnetic resonance (^{1}H-NMR), Fourier transform infra-red spectroscopy (FT-IR), gel permeation chromatography (GPC) and ultraviolet-vis spectrophotometer (UV).

MATERIALS AND METHODS

Raw Material

Shredded EFB were collected from a local palm oil mill (Dengkil, Selangor, Malaysia). The EFB were washed with water and dried in a forced-draft oven at 40ºC. The dried EFB were ground to pass through a 40-mesh screen, using a Wiley mill machine (for the non-treated ground EFB, LP2). Part of the ground EFB was then extracted with ethanol/benzene (1:2 v/v) for 48 hr using a soxhlet apparatus to remove oil residue and extractives (for the treated ground EFB, LP1).

Figure 1 shows the overall process flow chart for lignophenol production from EFB.
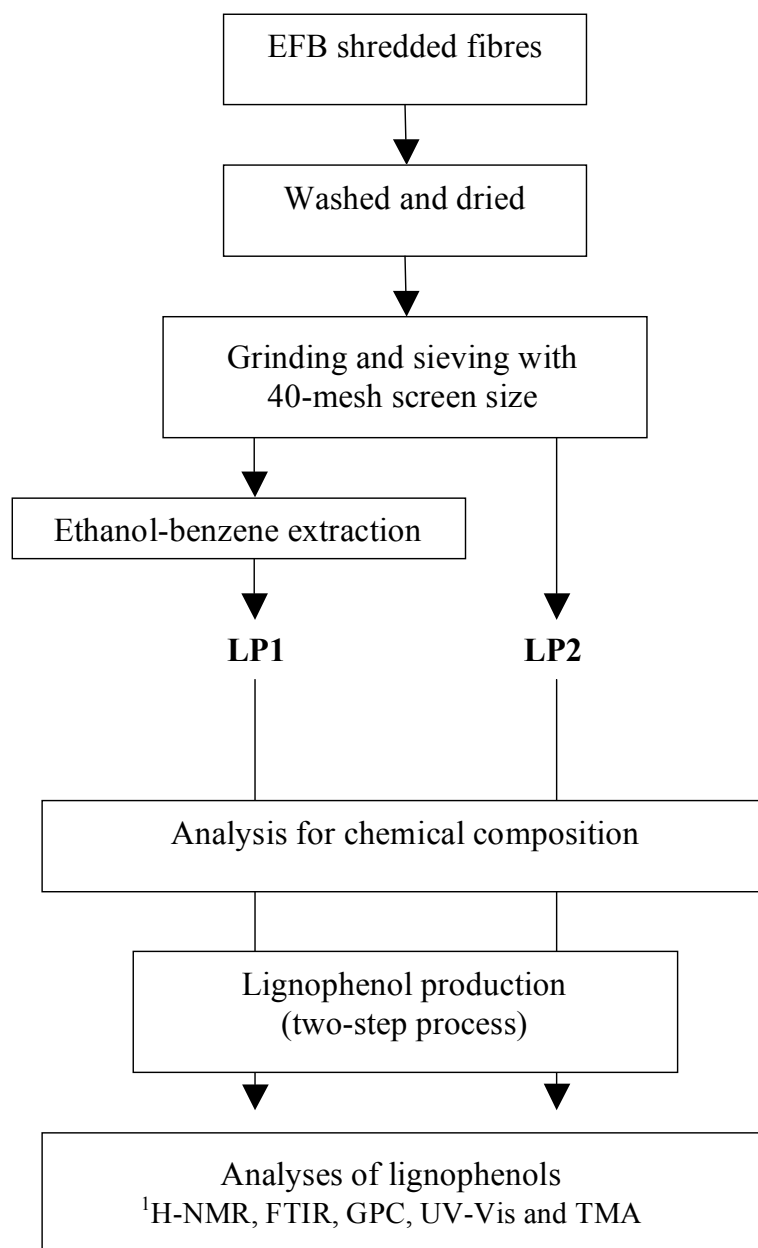
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┌─────────────────────────┐
│   EFB shredded fibres   │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│    Washed and dried     │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│  Grinding and sieving with │
│   40-mesh screen size   │
└─────────────────────────┘
      │                    │
      ▼                    │
┌──────────────────────┐   │
│ Ethanol-benzene      │   │
│ extraction           │   │
└──────────────────────┘   │
      │                    │
      ▼                    ▼
    LP1                  LP2
      │                    │
      ▼                    │
┌─────────────────────────────────┐
│  Analysis for chemical composition │
└─────────────────────────────────┘
             │
             ▼
┌─────────────────────────────────┐
│   Lignophenol production        │
│   (two-step process)            │
└─────────────────────────────────┘
      │                    │
      ▼                    ▼
┌─────────────────────────────────┐
│   Analyses of lignophenols      │
│ ¹H-NMR, FTIR, GPC, UV-Vis and TMA │
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Figure 1. Process flow chart for lignophenol production from empty fruit bunches (EFB).

Characterization of the Raw Material

Cellulose, hemicellulose, lignin and ash contents of the samples were determined according to methods for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) (Gorring and Van Soest, 1970). Moisture content was determined by drying at 105°C a 1-g sample to constant weight in a forced-draft oven. The amount of extractives in the EFB samples was determined by solvent extraction (ethanol benzene, 1:2 v/v) carried out for 48 hr, while the amount of protein present in all the samples was determined

using the Kjeldahl method according to the AOAC Official Method 984.13 (Anon., 1998).

Preparation of Lignophenol

The preparation of lignophenol was carried out based on a two-step process in the phase separation system (Funaoka *et al.*, 1995). The EFB samples (LP1 and LP2) were completely soaked in *p*-cresol (3 mol equivalent/C_9) –acetone solution, and after 24 hr, the acetone was totally evaporated off by continuous stirring. A 72% sulphuric acid was added to the phenol-sorped EFB and the mixture was stirred

vigorously for 1 hr. This treatment was carried out by immersing the beaker in water to maintain it at room temperature (~28°C). The reaction mixture was then carefully poured into an excess amount of water and stirred vigorously for 1 hr. The mixture was left overnight to let it sediment before discarding the clear supernatant. The precipitates were subjected to repeated washing and centrifuging to remove the acid. After drying completely over phosphorus pentaoxide (P_2O_5), the precipitates were extracted with acetone. The acetone-soluble products were concentrated and added drop-wise into an excess amount of cooled diethyl ether with vigorous stirring for 1 hr. The resultant precipitates (lignophenols) were collected by centrifugation, and dried over P_2O_5 after evaporating off the solvent.

Characterization of Lignophenols

^{1}H-NMR spectroscopy. This analysis was carried out to quantify the amount of cresol which was introduced into the lignophenols. The lignophenol samples were prepared as the original material. The ^{1}H-NMR spectra were recorded on a JEOL JNM-A500 FT-NMR spectrometer. The amount of cresol introduced was calculated based on the signal intensity of the cresolic methyl protons (1.6-2.4 ppm) against aromatic protons (7.8-8.4 ppm) of *p*-nitrobenzaldehyde (internal standard) in the ^{1}H-NMR spectra (Funaoka and Fukatsu, 1996).

Infra-red spectroscopy. FTIR spectra of the lignophenols were recorded on a Perkin Elmer Spectrum GX FTIR spectrophotometer after the preparation of KBr discs. About 1 mg of a lignophenol sample was mixed and ground with 200 mg of KBr to reduce particle size and to obtain uniform dispersion of the sample in the disks. The spectra were recorded at a wavelength of 370 to 4000 cm^{-1} (Funaoka and Fukatsu, 1996).

Ultraviolet spectroscopy. UV spectra were recorded on a Jasco UV-Vis spectrophotometer at a range of wavelengths from 200 to 600 nm. The method is based on the difference in absorption between lignophenol in alkaline solution and lignophenol in neutral solution.

Thermal analysis. The phase transition points of the lignophenol samples were analysed on a thermomechanical analyser (TMA), model SS6000

SII (Seiko Instruments Inc.). Under a 5-g loading, the solid-liquid transition of lignophenol was measured by heating the samples from room temperature (23°C-25°C) to 250°C at a rate of 2°C min^{-1} under N_2 flow.

Average molecular weight. The average molecular weights were measured by gel permeation chromatography (GPC) on a Shimadzu Class LC-10 system, with four shodex columns (KF-801, KF-802, KF-803 and KF-804). Tetrahydofuran (THF) was used as an eluent and polystyrene as a standard.

RESULTS AND DISCUSSION

Yield of Lignophenols

The yields of lignophenols for both samples LP1 and LP2 were not much different (61%) (*Table 1*), and the values were low compared to previous results for hardwood and softwood lignin (101%-112%) (Funaoka, 1998). The low yields might be due to the nature of the native lignin in EFB which is more flexible and consists of small subunits which make it soluble in diethylether during the purification step. The physical appearances of the lignophenols from LP1 and LP2 were similar (pinkish white).

Chemical Compositions of Raw Material

Table 2 shows the chemical compositions of LP1 and LP2, respectively. It was found that the cellulose and lignin contents in the EFB treated with ethanol-benzene (1:2 v/v) extraction was not much affected compared to the untreated EFB. There was almost no difference in cellulose and lignin contents between LP1 and LP2, both of which have 44% cellulose and 20% lignin. These results were similar to those given in previous reports (Basiron and Husin, 1996; Ramli *et al.*, 2002).

The extraction process was used to remove the extractives in the EFB samples and this had the potential of interfering with the lignophenol production. Historically, ethanol-benzene has been used to extract waxes, fats, some resins, and portions of wood gums (Erhman, 1994). The amounts of extractives in LP1 and LP2 were 0.05% and 3.90%, respectively. Ethanol-benzene extraction also affected the ash content which was lower in LP1 (0.66%) as compared to LP2 (0.87%). It is believed that some of the ash had been removed during the ethanol-benzene

TABLE 1. AMOUNT OF INTRODUCED CRESOL IN LIGNOPHENOL AND YIELD OF LIGNOPHENOL

Sample	Amount of introduced cresol		Yield of lignophenol (%)	
	wt %	mol/C$_9$	Cresol-free	Original
LP1	26.49±1	0.72	44.96±1	61.16±1
LP2	26.76±1	0.73	44.53±1	60.57±1

TABLE 2. CHEMICAL COMPOSITION OF OIL PALM
EMPTY FRUIT BUNCHES

Chemical composition (%)	Type of oil palm empty fruit bunches	
	LP1	LP2
Cellulose	44.36	44.09
Hemicellulose	32.98	28.06
Lignin	20.32	20.78
Ash	0.66	0.87
Extractives	0.05	3.90
Protein	3.03	2.53
Moisture	6.58	6.01

Note: LP1 : After 48-hr extraction using ethanol-benzene (1:2, v/v), sieved with 40-mesh screen size, brown colour.
LP2 : Without extraction, sieved with 40-mesh screen size, brown colour.

extraction. It is reported that ethanol-benzene (1:2 v/v) extraction is the best method for removing extractives in rice straw, giving the highest yield compared to other solvents like hexanes, ethyl acetate, chloroform and ethanol-toluene (Inglesby *et al.*, 2005). With benzene being hazardous, ethanol-toluene has been suggested as an alternative (Inglesby *et al.*, 2005).

Characterization of Lignophenols

¹H-NMR spectroscopy. The ¹H-NMR spectra for both of the original lignophenols (LP1 and LP2) had an intensive signal of the cresolic methyl protons at 2.1 ppm (*Figures 2a* and *2b*), similar to what had been reported by other researchers (Funaoka *et al.*, 1995). The amount of cresol introduced was calculated based on the signal intensity of the cresolic methyl protons (1.6-2.4 ppm) against aromatic protons (7.8-8.4 ppm) of *p*-nitrobenzaldehyde (internal standard) in the ¹H-NMR spectra. From the results obtained, it was clear that there was almost no difference in the amounts of cresol attached to the EFB lignin in the LP1 and LP2 samples.

Table 1 shows the amount of cresol introduced into both LP1 and LP2 was 27% (about 0.73 mol/C_9). Hardwood lignophenol has more attached cresol than softwood lignophenol, indicating that hardwood lignin is more reactive towards cresol than softwood lignin. The amount of cresol introduced into softwood lignocresol was about 25% (0.65 mol/C9) while the amount is about 30% (0.9 mol/C9) in hardwood lignocresol (Funaoka, 1998). The difference between softwood and hardwood lignins is probably due to both the flexibility of the molecules and the quantity of reactive functional groups in the side-chains. The EFB lignin can be categorized as a
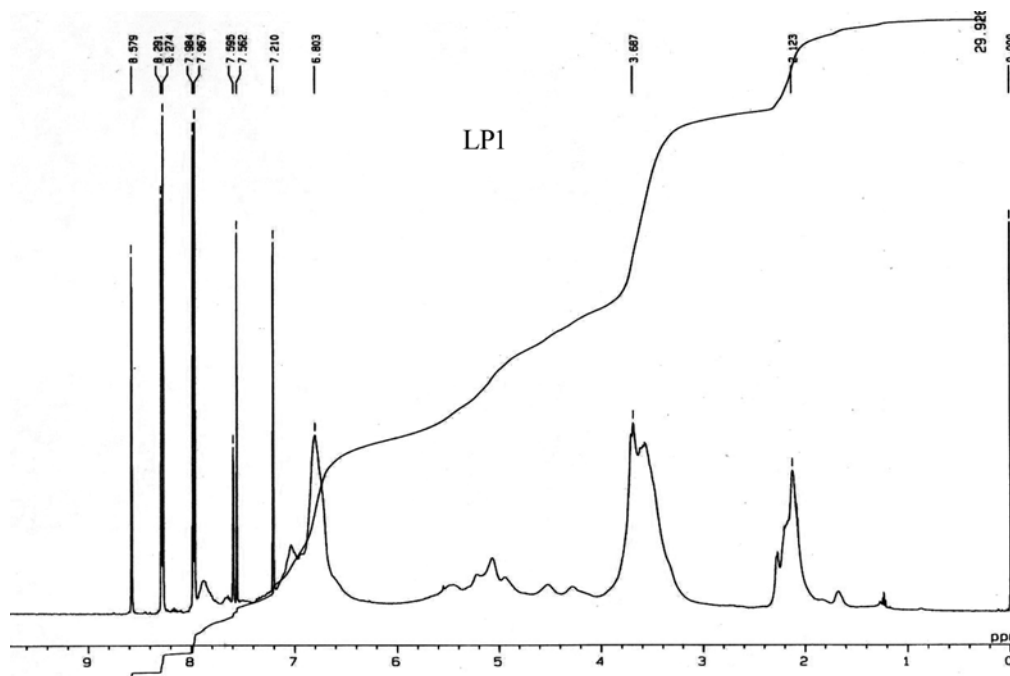

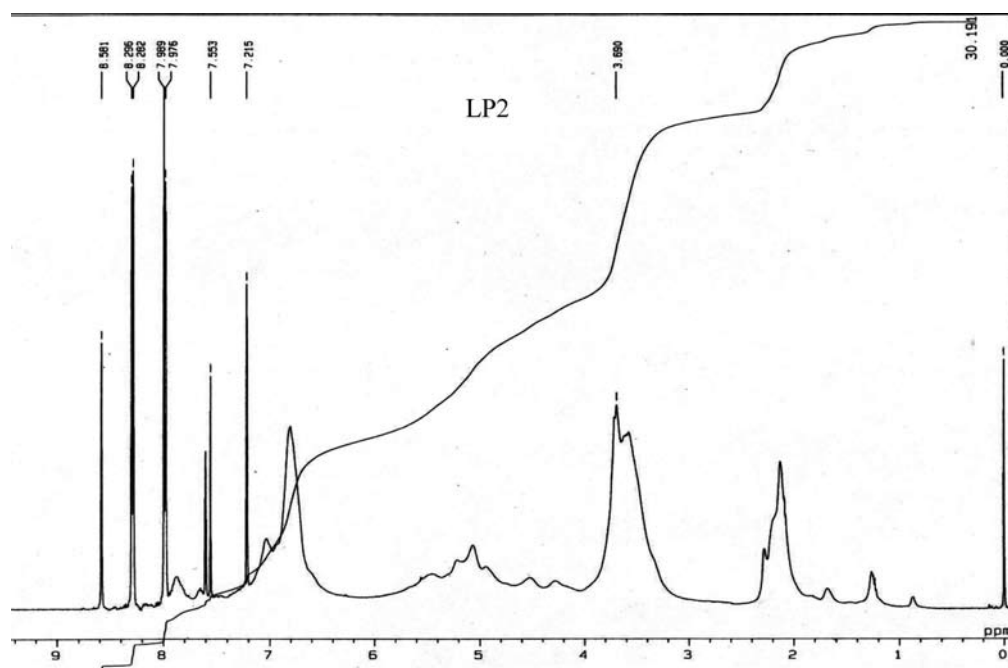*Figure 2a. The Proton nuclear magnetic resonance (¹H-NMR) spectrum of lignophenol from LP1.*

Figure 2b. The Proton nuclear magnetic resonance (^{1}H-NMR) spectrum of lignophenol from LP2.

grass-type lignin due to the syringyl, guaiacyl and small amounts of *p*-hydroxyphenyl in its structure (Sun and Bolton, 1999; Ibrahim *et al.*, 2004).

Infra-red spectroscopy. *Figure 3* shows that all the samples gave almost similar spectra and were comparable to a typical lignophenol. The strong
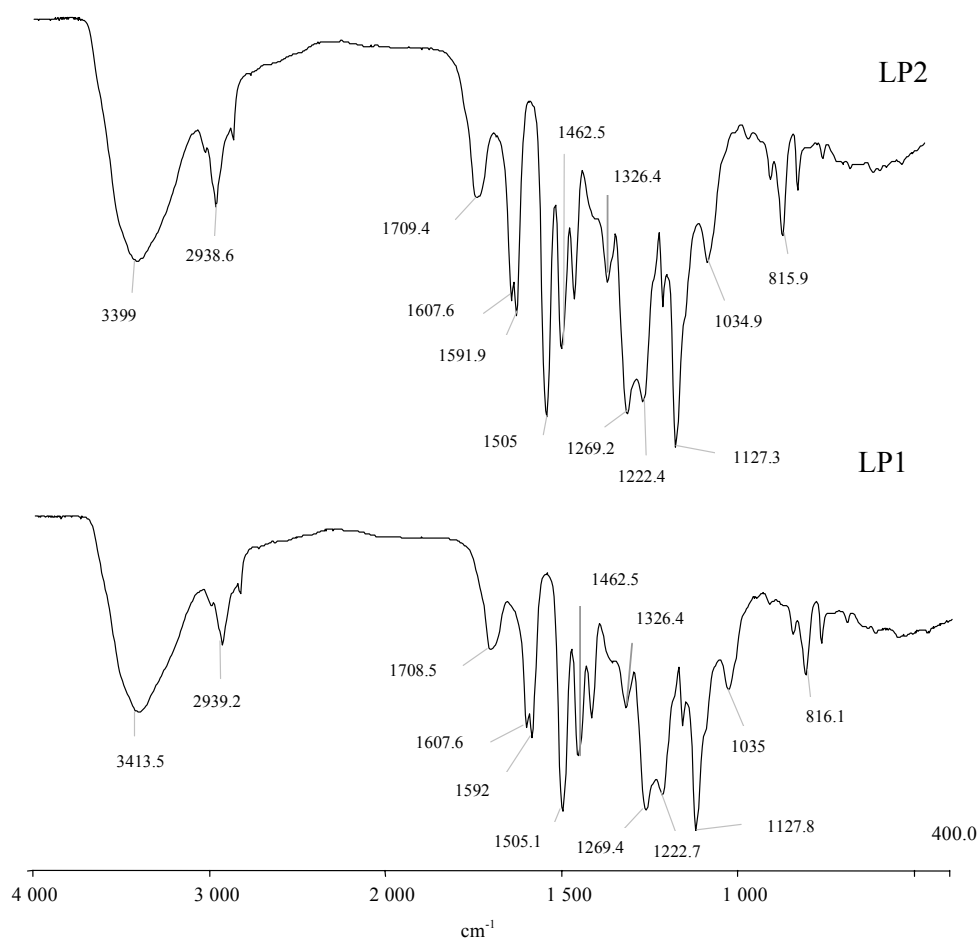

Figure 3. Infra-red (IR) spectra of lignophenols from oil palm empty fruit bunches (EFB) (LP1 produced from ethanol-benzene extraction of EFB, LP2 produced from EFB without extraction).

705

and broad bands at 3395 cm⁻¹ and 3414 cm⁻¹ are characteristic of the aromatic hydroxyl groups of lignin. Absorption around 2939 cm⁻¹ and the band at 1463 cm⁻¹ were assigned to CH stretching of the methyl or methylene group (Ibrahim *et al.*, 2004). Broad medium bands at 1709 cm⁻¹ and at 1713 cm⁻¹ were due to the appearance of ester groups of the hydroxyphenyl unit in EFB lignin. This group gives flexibility to the lignophenols. A band at 1505 cm⁻¹ for all the samples was due to the aromatic skeletal vibrations. Moreover, two small bands at 1608 cm⁻¹ and 1592 cm⁻¹ for all the samples revealed the presence of other aromatic skeletal vibrations plus a C=O stretch in the *p*-hydroxyphenyl unit in EFB lignin. The bands at 1326 cm⁻¹, 1222 cm⁻¹ and a strong band at 1126-1128 cm⁻¹ corresponded to the syringyl unit, whereas small bands at 1269 cm⁻¹ and 1035 cm⁻¹ were assigned to the guaiacyl unit of the lignin molecules (Ibrahim *et al.*, 2004). Furthermore, the lignophenols had an intensive band around 816 cm⁻¹ assigned to C-H deformations (two adjacent H) of the cresol (Funaoka and Fukatsu, 1996). These results confirmed that the EFB lignin can be classified as a grass lignin and that cresol attached to the lignin unit.

Ultraviolet spectroscopy. Ionization of phenolic hydroxyl groups in lignin with alkali causes a bathocromic shift (the shift of absorption to a longer wavelength due to substituent or solvent effects) and a hyperchromic effect (an increase in absorption intensity) in the absorption spectrum (Lin, 1992). An alkaline ionization difference spectrum is obtained by subtracting the spectrum of the solute in a neutral solution from the corresponding spectrum measured in an alkaline medium.

The ultraviolet portion of the electromagnetic spectrum suitable for lignin spectroscopy extends from 200 to 380 nm (Lin, 1992). The higher the absorbance value, the purer is the lignin compound (Ibrahim *et al.*, 2004). *Figures 4a* and *4b* show UV-Vis spectra of LP1 and LP2. The UV-Vis spectra of both lignophenol samples had a sharp band only at 300 nm with no shoulder or peak at a larger wavelength. These findings imply that selective and effective phenolation had taken place at the reactive sites in the side chains, leading to the disappearance of a conjugated system (Funaoka *et al.*, 1995).

Thermal analysis. The TMA curves for LP1 and LP2 in *Figure 5* indicate an apparent change of phase at about 166.4°C and 160.0°C, respectively. The curves reveal that the volume decreased at 150°C-195°C due to transformation of the solid state to a clear liquid state. The TMA curve of lignocresol indicates an apparent change of phase at about 130°C in hardwood, and at about 170°C in softwood (Funaoka, 1998). From the results obtained, the lignophenols from EFB (grass lignin) had an apparent change of phase at around 160°C which is closer to softwood lignin. The plasticization behaviour is very important in polymer application. Native lignin cannot be transformed into a fluid liquid under this condition due to the three-dimensional network polymers. Therefore, in the treatment, the native lignin is converted into a linear-type polymer in the phase separation system which makes it flow easily when heat is applied (150°C-195°C) (Funaoka, 1998).
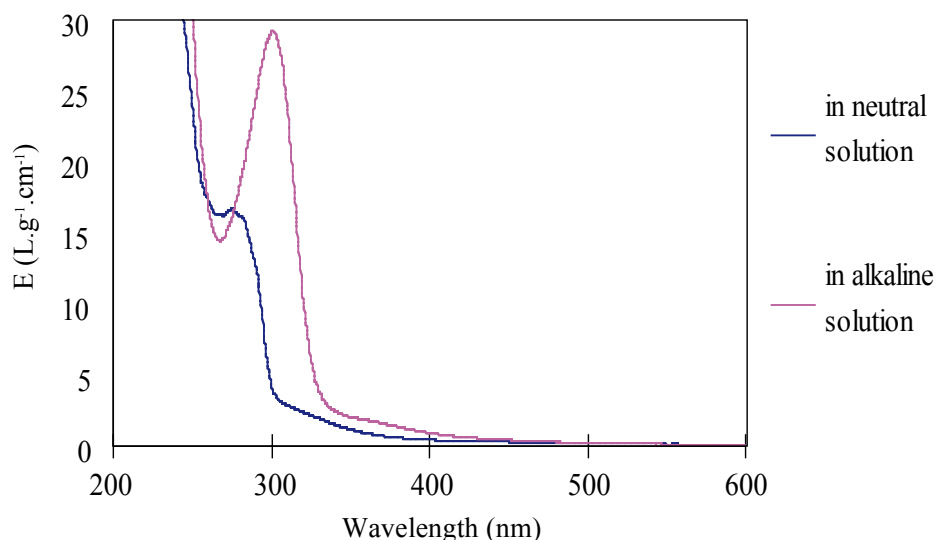

Figure 4a. Ultraviolet-vis spectrophotometer (UV-Vis) spectra of lignophenol from LP1.
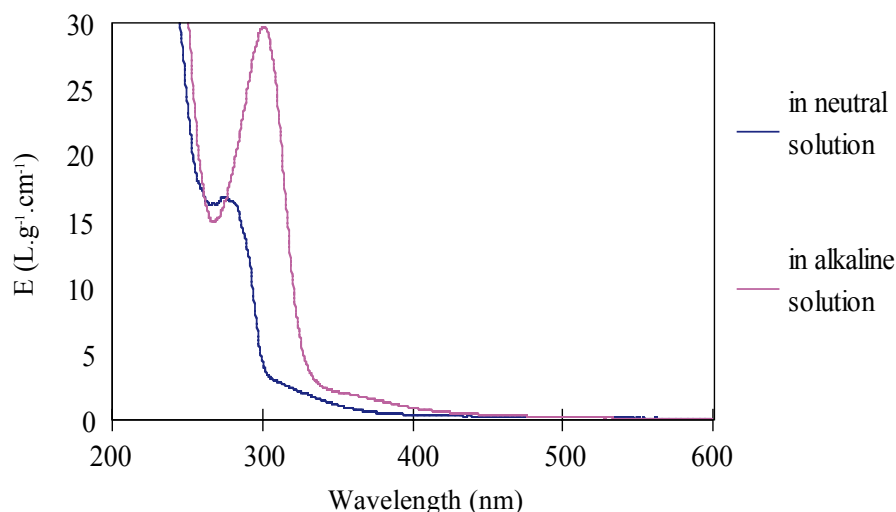
706

Figure 4b. Ultraviolet-vis spectrophotometer (UV-Vis) spectra of lignophenol from LP2.
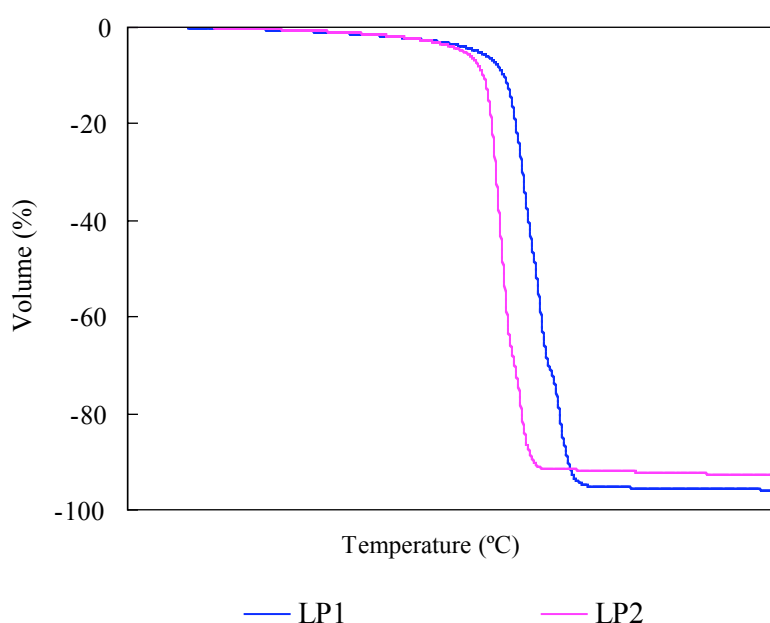

Figure 5. Thermomechanical analysis (TMA) profiles of lignophenols from LP1 and LP2.

Average Molecular Weight

The weight average (*Mw*) and number average (*Mn*) molecular weight and polydispersity (*Mw/Mn*) of lignophenols from EFB are given in *Table 3*. From the GPC results, no significant difference was found in the weight average of the lignophenols from LP1 and LP2, which were 5759 and 5866, respectively. These values for the original lignophenols are considered low as compared to spruce and birch lignophenols which are 12900 and 8200 in average molecular weight (Mikame and Funaoka, 2006). The EFB lignophenols had relatively low polydispersity which indicated that a high fraction of low molecular weight lignophenols was present. This is a good

indicator that the lignophenols can be applied as an extender or as a component of phenol-formaldehyde resins because of their high reactivity (Alriols *et al.*, 2009).

TABLE 3. WEIGHT AVERAGE ($\overline{Mw}$) AND NUMBER AVERAGE ($\overline{Mn}$) MOLECULAR WEIGHTS AND POLYDISPERSITY ($\overline{Mw}/\overline{Mn}$) OF LIGNOPHENOLS FROM EMPTY FRUIT BUNCH (EFB)

Sample	Molecular weight		
	$\overline{Mw}$	$\overline{Mn}$	$\overline{Mw}/\overline{Mn}$
LP1	5 759	3 686	1.56
LP2	5 866	3 555	1.64

CONCLUSION

EFB can be classified as a grass lignin and has the potential of being a starting material in lignophenol production. Ethanol-benzene extraction did not affect the cellulose and lignin contents but affected the contents of protein and extractives in EFB. Ethanol-benzene extraction also did not affect the characteristics of the lignophenols as the results showed that there was no difference between the lignophenols produced from extractive-free and from non-extractive-free EFB as revealed by ^{1}H-NMR, GPC, FTIR, UV-spectroscopy and TMA analysis. It is suggested that extraction-free EFB (LP2) be used for lignophenol production on an industrial scale in order to reduce cost and to avoid the use of harmful chemicals.

ACKNOWLEDGEMENT

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