

Extraction of Lignin from Empty Fruit Bunch Fiber via Microwave-Assisted Deep-Eutectic Solvent Heating

I.S. Muryadi^{1,*}, M.N.A. Yaakob¹, R. Roslan¹

¹Faculty of Industrial Science and Technology, Universiti Malaysia Pahang, 26600 Pahang, Malaysia.

ABSTRACT – This work study about the extraction of lignin from Empty Fruit Bunch (EFB). It is a type of lignocellulosic waste produced during the palm oil extraction process. There are three main components of lignocellulosic, which is one of them is lignin. A deep eutectic solvent (DES) with microwave-assisted heating has been used as a process to extract the lignin from EFB and turn it into a value-product. This convenient method was started with the mixing of EFB and DES. After that, the mixture was heated via microwave synthesis reactor at different temperature and time parameters. The extracted lignin yield was dried and ground into a powder form. The highest lignin yield recovered is 30 % by the highest time and temperature. Interestingly, the purity of all lignin yields are above than 80 %. The highest yield of lignin was characterized. According to Fourier-Transform Infrared (FTIR) spectra, there was a significant functional group of phenolic and aliphatic hydroxyl in lignin. Besides, the methoxy group was also configured in lignin spectra. The presence of conjugated alkene also conveyed the characteristic of lignin. The FTIR spectra were intensified with ¹H Nuclear Magnetic Resonance (NMR) spectra where there was a chemical shift in lignin and raw EFB which was designated to aliphatic and aromatic protons bonded to a carbon atom. Three regions of decomposition occur in the Thermogravimetric Analysis (TGA) spectra. The initial decompose temperature of lignin was lower compare to raw EFB. Next, second-stage lignin decomposed at 434.14 °C with weight loss of 36.21 %. Lastly, for the final stage, lignin decomposes at 552.54 °C. Moreover, Differential Scanning Calorimetry (DSC) spectra demonstrate that the T_g value of lignin managed to be identified. However, the T_g value of raw EFB cannot be well defined. As for the characterization in residual fractions of EFB, the lowest crystallinity index (CrI) value of raw EFB has proven the presence of lignocellulosic in its structure. The residual fractions that reacted at higher temperatures have an inflated value of CrI as they contain abundant left out cellulose.

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INTRODUCTION

The major solid waste in palm oil mills processing is an empty fruit bunch (EFB), fibers, and oil palm shells. Palm oil mills produce about 14% fibers, 7% shells, and 23% EFB per ton of fresh fruit bunch (FFB) [1]. Annually, approximately 15 million tons of EFB waste is produced [2], resulting in an EFB dump that created a major disposal problem. The short path to eliminate the wastes is by burned off, which can cause harm to the environment and social aspect. To achieve environmentally friendly production while maximizing profit, EFB must be utilized to generate high value-added products. EFB is a kind of lignocellulosic waste generated during the palm oil extraction process and is a renewable raw material resource. There are three main components of lignocellulose, i.e. (i) cellulose, (ii) hemicellulose, and (iii) lignin. Lignin is usually the least utilized among lignocellulose major constituents [2].

Extraction technology is a critical step to isolate the main component of lignocellulose before upgrading onto high value-added products. There are varieties of lignin extraction methods, such as physical, chemical, biological, or the combination of those methods implemented and published [3]. Before this, traditional extraction methods such as kraft, lignosulfonates, soda, organosolv, and ionic liquid have been widely practiced and shown their advantage. Nevertheless, those methods was also showed some deficiencies, such as required long reaction time during the lignin extraction process, ineffective cost, and low amount of lignin extracted. In this modern era, DES was able to replace ionic liquids (ILs) by achieving green solvents that have similar physiochemical like its. Recently, DESs have established their powerful abilities in the pretreatment of lignocellulose. Many groups have applied DESs into the extraction trials and came out with good results [4]. In this work, DESs are used to enhance the extraction of lignin from EFB by mixing dicarboxylic acid (oxalic acid) with choline chloride via microwave-assisted heating.

Among the technologies used, the microwave-assisted process has proven to be promising to pretreat biomass due to less time required for the process. This technology also lowers the severity of ionic liquid and alkaline pretreatments. The ability of ease of control the reaction conditions, fast and selective heating, low reaction temperature,

*CORRESPONDING AUTHOR | R. Roslan | ✉ rasidi@ump.edu.my

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and low energy requirements are additional advantages of using microwave radiations[3]. Previous research has proved that microwave-assisted heating is successfully applied to wood pretreatment. Compared to conventional heating, it was able to extract a large amount of lignin. Microwave-assisted DES pretreatment has allowed 80 % lignin extracted from wood in just 3 min reaction time [5]. However, using conventional heating, choline chloride: oxalic acid needs at least 9 h at 110 °C to achieve the same amount of lignin. In this work, the EFB extraction will be conducted with a microwave to extract a high amount of lignin like wood. This approach valorizes the available biomass, for example, modified lignin into UV-curable coating since lignin has an ultraviolet (UV)-absorbing property which is suitable to be used as coatings.

DES are used via microwave-assisted heating which can reduce time and energy due to the microwave radiations during the extraction process. Therefore, this study aims to extract the lignin yield utilizing DES via microwave-assisted heating. Then, the yield and purity of lignin will be calculated according to TAPPI and NREL standards. The residual EFB was also investigated based on their crystallinity index. The scope of this work is to find the amount of lignin yield for each sample and characterize the highest lignin yield recovered using FTIR, TGA, DSC, and NMR. As for the residual fractions of EFB for all samples was characterized using XRD.

MATERIALS AND METHODS

Materials

Materials used in this study are EFB fibers (Szetech Sdn Bhd), deionized water, toluene (ACS reagent 99.5%), absolute ethanol, acetone (ACS reagent 99.5%), choline chloride (Reagent grade 98%), and oxalic acid (Reagent grade 98%).

Methodology

Pre-treatment of EFB Fibers

Initially, the EFB fibers wash regularly with deionized water to ensure its free from dirt, dust, and impurities on the fiber surface and dry under direct sunlight. The dried fibers were grounded into a powder with a commercial cutting mill (Retsch SM100). The oil and wax components from the EFB were removed using soxhlet extraction with toluene: ethanol (2:1) mixture for 7h. Then, the extractive-free dewaxed fibers were washed using deionized water to remove the excess soxhlet solvent, dry at 105 °C, and store in a dry cabinet for further use. The EFB powder was characterized to identify alpha-cellulose, extractives/fat, ash, moisture, and lignin, based on TAPPI T203, T204, T211, T421, and T222.

Lignin Extraction from EFB Fiber Using Microwave-Assisted Heating

DES process was utilized in the extraction of lignin from EFB fibers. The DES was prepared by mixed chlorine chloride as a hydrogen bond acceptor (HBA) and oxalic acid as a hydrogen bond donor (HBD) with a molar ratio of 1:1. The mixture was mixed under reduce pressure at 90 °C until a transparent solvent form. The lignin extraction from EFB fiber was performed by using microwave-assisted heating. The EFB fiber with an amount of 20 g will be mixed with 200 g DES. The mixture was reacted in a microwave synthesis reactor. The temperature will be raised from room temperature to 80 °C in between 2 min and held at the temperature for 5 min. Once the reaction is completed, the dark-brown liquid was mixed with 200mL acetone: water (1:1 v/v) and filtered by vacuum filtration to isolate the soluble and insoluble DES fractions. The acetone in the filtrate was evaporated using a rotary evaporator and form black liquor, while the lignin was precipitated and collected through centrifugation. Lastly, the lignin sample was dried at 45 °C in an oven for 12 h and placed in a glass vial at room temperature. For the optimization, the reaction was repeated with different temperatures (80 °C, 90 °C, and 100 °C) and times (10 min, 20 min, and 30 min).

Percentage of Yield and Purification Lignin

$$L(\%) = \frac{m_{\text{isolated}}}{m_{\text{TAPPI}}} \times 100\% \quad (1)$$

Where L is crude lignin yield (%); m_{isolated} (g) is the mass of lignin isolated in microwave-assisted heating and m_{TAPPI} (g) is the mass of lignin calculated using TAPPI method.

The yield of lignin produced after microwave-assisted heating was calculated using TAPPI T222. In short, 0.03 g of lignin was treated with 1.2 mL of H₂SO₄ (67 wt %) at room temperature for 24 h. The mixture was placed in an ultrasonic water bath for 1 h at 30 °C. The mixture was located in the autoclave at 1 h at 102 °C. By using vacuum filtration, the acid-insoluble excess was isolated, and the excess was washed with deionized water until it reaches neutral

pH followed by 24 hour drying at temperature 105 °C. The acid-soluble lignin was determined using the first filtrate acquired in the lignin that is soluble in acid. The filtrate would be 4 wt % diluted. H₂SO₄ with a ratio of 1:10 (v / v) and acid-soluble lignin were counted at 240 nm from the UV absorbance. The overall amount of acid-insoluble and acid-soluble lignin was decided as the amount of the sample purity lignin content.

Washing the Residual Fractions

The residual obtained after isolation of lignin was wash repeatedly using deionized water and acetone with a ratio 1:10 (v/v) by using a hot plate. Then, the residual fractions were dried by using the oven at 90 °C. The dried residual was ground by using mortar and pestle until it turns into powder form.

Characterization of Lignin and Residue EFB.

The 1D- ¹H-NMR were recorded with Nuclear Magnetic Resonance (NMR) spectrometer (Bruker, 500MHz), using deuterated dimethylsulfoxide (DMSO-d₆) as a solvent. The lignin was dissolved in 1.0 mL of DMSO-d₆ and the solvent peak was used as an internal reference (DMSO H 2.50). The spectra were processed for their coherent alignment, spectral phasing, calibration, baseline correction, and integration through TopSpin software. Meanwhile, Computer-Assisted Structure Elucidation in MestreNova 14.0.0 was used to illustrated the NMR spectra. FTIR analysis was used the powder of lignin by put in the cassette and placed in the light path. Spectra analysis was performed on a Perkin Elmer Spectrum 100 FTIR Spectrometer with Potassium Bromide (KBr) disk method and 4 cm⁻¹ resolution at the 4000-700 cm⁻¹ wavenumber range [6]. TGA was performed using the TGA Q500 TA instrument. Approximately 15 mg of the samples was weighed and heated from 25 to 600 °C at a heating rate of 10 °C/min under a nitrogen flow of 40 mL/min. DSC were performed on a NETZSCH DSC 214-Polyma, under the N₂ atmosphere with a flow rate of 50 mL/min. Approximately 10 mg samples were weighed in an aluminum crucible and heated from 25-300 °C at a heating rate of 10 °C/min. The residual fractions were characterized by X-ray Diffractometer (XRD) (Rigaku Miniflex II). The x-ray diffractometer was utilized Cu K α radiation with wavelength 1.5460 Å and Nickel, Ni filter; with an accelerating voltage of 30 kV and cathodic current of 15 mA under a wide range of 2 θ Bragg angles (10° ≤ 2 θ ≤ 60°) at a scanning rate of 2 °/min.

RESULT AND DISCUSSION

Percentage of yield and purity of lignin

As shown in Table 1 and Figure 1, the highest yield of isolated lignin extracted using DES via microwave-assisted heating is 30 %. The presence of acidic strength from oxalic acid in DES pretreatment has enhanced hydrogen accepting bond. As the temperature ramp, the yield recovered was also increase. This indicates that there was a breakage of hydrogen bonds in EFB fibers via microwave-assisted at elevated temperature during the extraction. However, the excessive temperature will result in less isolation lignin due to condensation that influences less solubility lignin in DES. In terms of reaction time, the prolonged reaction time has raised lignin's yield, strengthening the plant cell wall, allowing the lignin macromolecules in EFB to split into tiny lignin fractions in the DES solution [7]. The DES complied with acid hydrogen bond donor (HBD) which succeeded in the extraction of lignin from EFB fibers. Moreover, microwave irradiation may increase the ion characteristic, thus raising the molecular polarity of DES [8]. The purity of lignin different regarding their temperature and time of reaction. Overall purity of lignin yield achieves higher than 80 %.

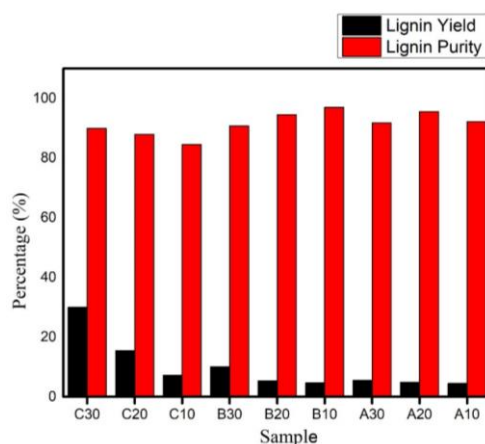


Figure 1. Yield and purity of lignin under divergent temperature and time

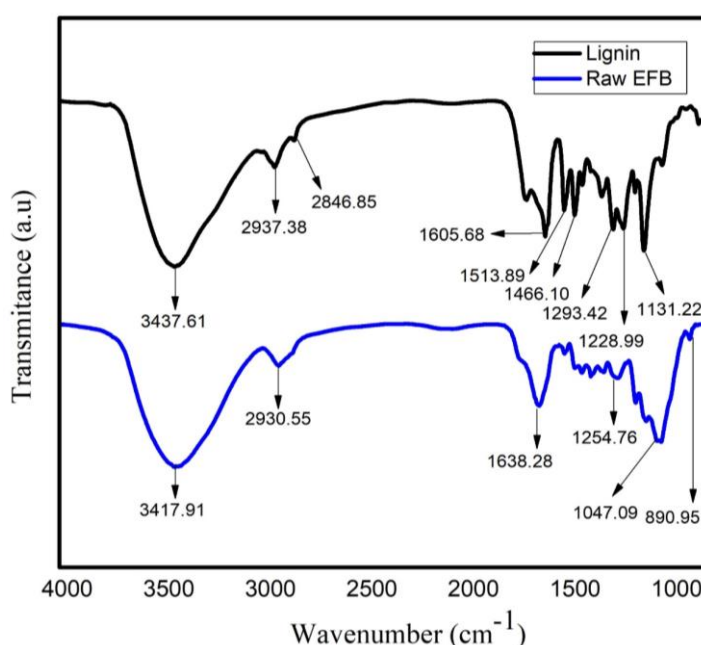
Table 1. Yield and purity of lignin recovered

Sample	Reaction Time (min)	Reaction Temperature (°C)	Lignin yield (%)	Purity of lignin (%)
C30	30	100	30	89.94
C20	20	100	15.44	87.96
C10	10	100	7.17	84.52
B30	30	90	10.07	90.74
B20	20	90	5.44	94.53
B10	10	90	4.64	96.93
A30	30	80	5.51	91.84
A20	20	80	4.93	95.55
A10	10	80	4.44	92.17

Structural Characterization of Highest Lignin Yield

Fourier-Transform Infrared Spectroscopy (FTIR)

According to the FTIR spectra displayed in Figure 2, a broadband at 3417.91 cm^{-1} and 3437.61 cm^{-1} was detected representing strong intermolecular force bonding of a stretching vibration between phenolic and aliphatic hydroxyl which abundant in the extracted lignin and raw EFB. At 2930.55 cm^{-1} and 2846.85 cm^{-1} on lignin spectra while at 2937.38 cm^{-1} in raw EFB spectra which both attributes to C-H stretching vibrations of methyl groups. The presence of stretching vibration of alkene group at 1638.28 cm^{-1} in raw EFB. Moving to lignin fractions, at 1605.68 cm^{-1} there was presence of stretching vibration of C=C conjugated alkene, which generally in lignin functionalities [9]. The presence of aromatic skeletal vibrations was shown from 1605.68 cm^{-1} to 1466.10 cm^{-1} . In addition, there were phenylpropane signals detected in the lignin structure at 1293.42 cm^{-1} stretching vibrations of C-O aromatic ester. Hardwood lignin contains a mixture of guaiacyl (G) and syringyl (S) where lignin spectra in Figure 3 show the C-O stretching vibrations in syringyl units (S) approximately at 1293.42 cm^{-1} while raw EFB at 1254.76 cm^{-1} [10], Two adjacent signals at 1228.99 cm^{-1} and 1131.22 cm^{-1} were attributed to the deformation of ester bonds. On the other hand, the existence of guaiacyl structure (G) also presents on that signal. Raw EFB spectra contain broadband at 1047.09 cm^{-1} meanwhile in the lignin spectra at peak 1131.22 cm^{-1} shown the primary alcohol. Lastly, at 890.95 cm^{-1} of raw EFB spectra, there are bending vibrations of alkene.

**Figure 2.** FTIR spectra of lignin yield and raw EFB

Thermogravimetric Analysis (TGA)

According to Figure 3, there are three regions of weight loss in which the sample started to decompose. The initial temperature was the evaporation of water and moisture from each sample. Then, the decomposition initiated in the sample detected around 230.72 °C in lignin and 256.28 °C in raw EFB where there was a cleavage of α - or β -aryl-alkyl ether bonds [10]. In the first stage, the weight loss approximately drops from 99 % to 92.80 % in lignin, slightly different from raw EFB (86.16 %). It was obvious a broad and smooth shoulder was observed instead of a peak due to the low degradation temperature fragmented by the hydrogen bonding present in DES that causing low degradation. Up to 150 °C, DES has influenced the stability of lignin which proves DES suitable for the EFB extraction method [3]. Besides, the thermogravimetric revealed that acetone and water extracted lignin dominate excessive stability below 300 °C were known as one of lignin factor. Move to the second stage in lignin at 434.14 °C with approximately 36.21% weight loss of lignin contradict to raw EFB where the weight loss is 41.53% at 359.29 °C. The lignin particle required a high amount of energy to decompose at a high temperature compared to raw EFB. As for the third stage, the lignin decomposes at 552.54°C meanwhile raw EFB at 546.41°C. However, the degradation continues until both samples turn into ash.

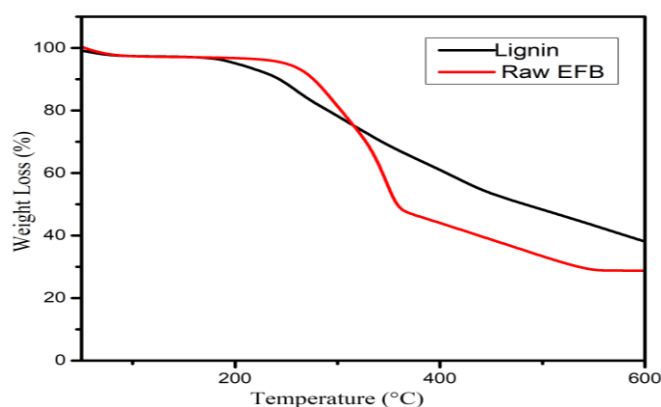


Figure 3. TGA graph of lignin yield and raw EFB

Differential Scanning Calorimetry (DSC)

DSC was utilized to detect the glass transition (T_g) temperature of lignin and raw EFB. T_g can be defined as the temperature where the polymer substrate transforms from a rigid glassy into a soft substance. According to the thermogram in Figure 4, T_g value, the lignin substance is in a glassy state where the structure is amorphous while between the T_g value, its turn into a rubbery state [11]. The removal of moisture and water occurs below 30 °C. When temperature ramp, reaction initiates approximately at 100 °C. Normally the value of T_g begins from 110 °C to 200 °C. T_g value of lignin is 195 °C while there is no difference in residue EFB thermogram due to the strong intermolecular force of the molecule in EFB structure consist of cellulose, hemicellulose, and lignin [12]. Lignin typically different comparing the other two substitutes in EFB due to its packed propane units interconnected in a complex amorphous matrix. Other than that, the amorphous state has caused the T_g not analyzed in well defined [13].

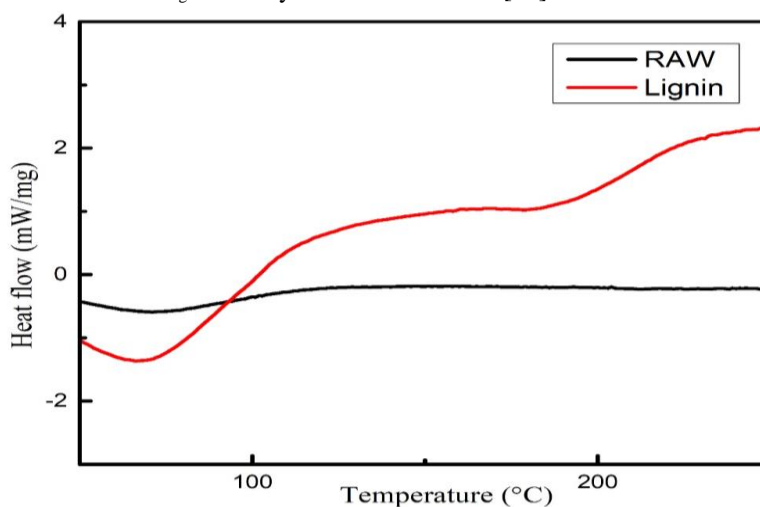


Figure 4. DSC thermogram of lignin yield and EFB raw

Nuclear Magnetic Resonance (NMR)

^1H NMR was studied by using DMSO as a solvent. However, the lignin was not completely dissolved. The ^1H NMR spectra, illustrated in Figure 5 was used to identify the lignin structure and prove the linkage presence in FTIR spectra. The signal observed in lignin spectra as shown in Figure 5(iii) around 6.5 ppm represented an aromatic proton in guaiacyl and syringyl shifts while there is no peak in raw EFB spectra [14]. Moreover, the chemical shifts at 1.26 ppm assigned to aliphatic and aromatic protons bonded to a carbon atom which present in raw EFB spectra, while lignin only has aliphatic protons [15]. The same aliphatic protons were represented in lignin structure in EFB fibers, Figure 5 (i) [16]. However, raw EFB has a lower peak compare to lignin because of the limited amount of lignin in the EFB fiber. The chemical shifts in Figure 5 (ii) between 5.0 ppm represent benzylic H in lignin structure. Next, at 2.52 ppm both spectra have peaks constituted to solvent peak in Figure 5 (iv).

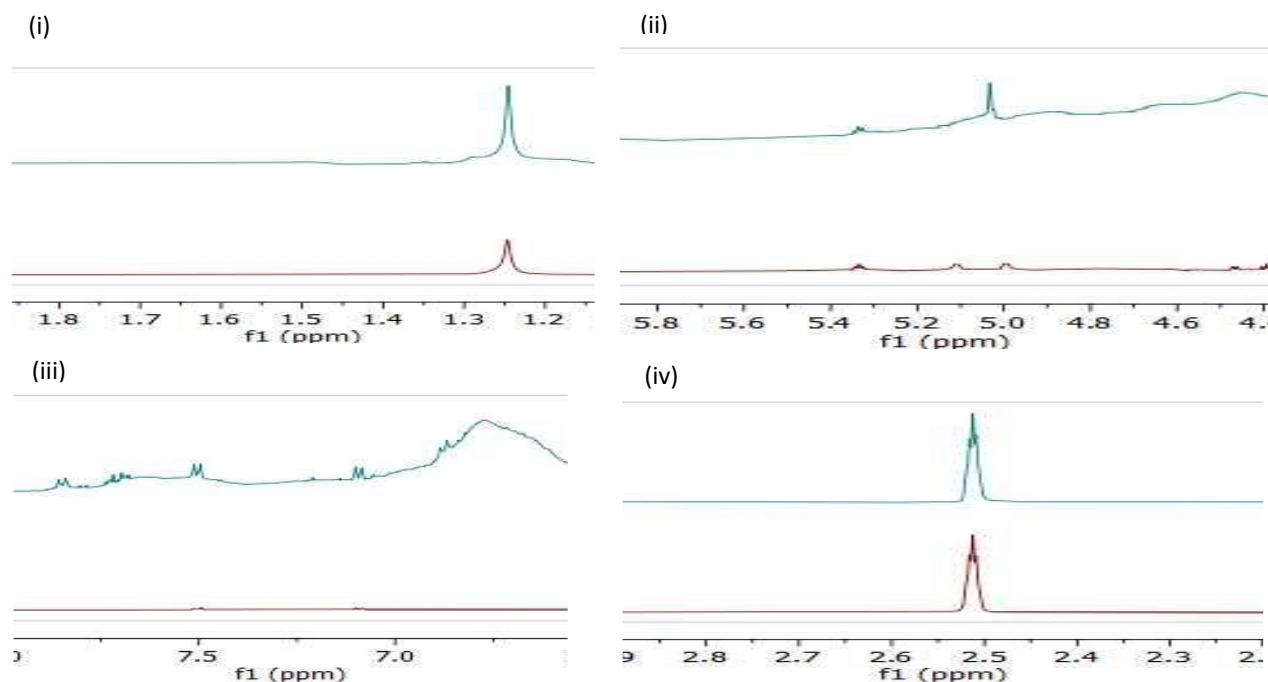


Figure 5. 1D- ^1H NMR spectra of raw EFB and Lignin

Characterization of Residual Fractions

X-ray Diffraction (XRD)

The crystallinity Index (CrI) of all samples was calculated based on XRD spectra. According to plane 200 at $2\theta = 21^\circ$ in all sample asymmetric, the CrI of each sample was escalated as the temperature increase. The raw EFB has the lowest value, which is 54.83 %, influenced by the presence of lignocellulose in the EFB structure. Residue fiber obtained after lignin extraction was characterized, as depicted in Figures 6,7 and 8. The sample that undergoes reaction at the highest temperature has a high degree of crystallinity, which is 70 %. Based on Table 2, the crystallinity index of each samples different due to the structure of amorphous or crystalline. Consequently, it indicated that there was a high amount of removal of lignin. Meanwhile, CrI of the sample extracted at 80°C decreasing continuously from 64.28% to 55.42%. The lowest compared to others proven there was cellulose removal, which contributes to a low crystalline degree of residue EFB. The XRD pattern exhibit in a broad peak describes the amorphous structure [17].

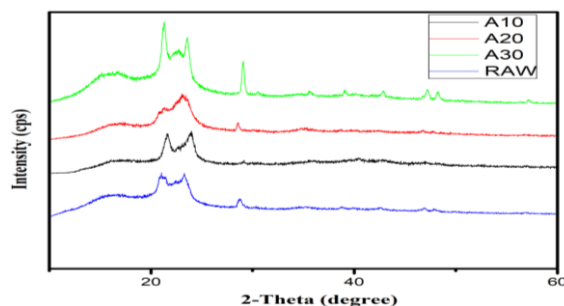


Figure 6. XRD graph of EFB residue (A10): 80°C 10 min (A20): 80°C 20 min (A30): 80°C 30 min RAW: Raw EFB

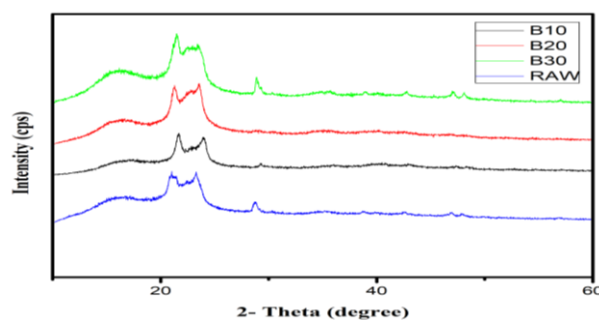


Figure 7. XRD graph of EFB residue (B10):90 °C 10 min (B20):90 °C 20 min (B30):90 °C 30 min RAW: Raw EFB

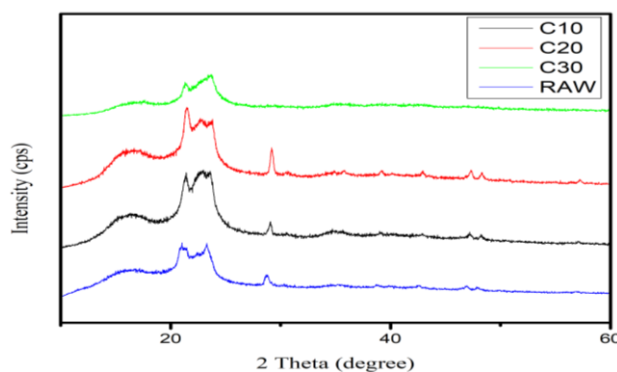


Figure 8. XRD graph of EFB residue (C10):100 °C 10 min (C20):100 °C 20 min (C30):100 °C 30 min RAW: Raw EFB

Table 2. Crystallinity index of raw EFB and residue fiber

Sample	I_{am}	I_{200}	CrI (%)
Raw EFB	787.26	1742.91	54.83
C30	243.53	830.40	70.00
C20	262.14	855.33	69.35
C10	255.60	823.10	68.95
B30	406.19	1272.59	67.92
B20	406.17	1247.54	67.44
B10	627.07	1871.11	66.49
A30	869.10	2433.15	64.28
A20	795.47	1949.69	59.20
A10	402.67	903.29	55.42

CONCLUSION

A convenient DES method via microwave-assisted heating is succeeded extract 30 % lignin from EFB fiber at the highest temperature and time. The amount of lignin yield percentage is affected by those parameters, while the lowest temperature, 80 °C, has a lower percentage of recovered lignin. Thus, the purity of lignin exceeds 80 %. Meanwhile, the FTIR spectra have shown the characteristic of lignin in comparison to raw EFB. Besides, on the XRD spectra, there are distinctions of raw EFB thermogram with lignin where consolidation detachment of cellulose and hemicellulose from the reaction. Hence, the amount of cellulose and hemicellulose, also high in the residual fraction based on its value of the crystallinity index has indicated the loss of lignin.

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