Characterization of isolated lignin from eucalyptus wood chip obtained by liquid hot water process

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Abstract

In this study, we propose a novel optimization for lignin isolation from the eucalyptus wood chip (EWC) under hydrothermal liquid hot water (LWH) process in the presence of alkaline catalyst (NaOH). The optimal conditions were obtained employing response surface methodology (RSM). The optimal condition was 160°C for 30 min in the presence of alkaline catalyst of 0.020 M. This condition demonstrated the highest lignin extraction with solid fraction of 77.3% and 76.1%. In addition, the physicochemical structure of isolated lignin was characterized with diverse techniques. The lignin recovery from liquid phase's GPC analysis illustrated a high average Mw/Mn (17500/7450 g/mol) while polydispersity index (2.34) was lower in comparison with the commercial organosolv lignin. The TGA analysis showed the maximum decomposition of lignin recovery at 140 to 350°C.

Furthermore, the Py/GCMS analysis showed a predominance of 57.63% of syringyl units (S) over 37.37% of guaiacyl units (G) under optimal conditions. The results revealed that the integrated process was a potential approach to add more value in the employment of the agricultural waste material.

Keywords: Eucalyptus wood chip, hydrothermal liquid hot water process, lignocellulose material, lignin fractionation, optimization.

Introduction

In the last decade, the production of biofuels from alternative renewable feedstocks i.e. lignocellulosic agricultural biomass has attracted considerable attention worldwide. Lignocellulosic components can be used as raw materials to produce biofuels, value-added products and reduced impact on the environmental problems[20].

In general, lignocellulosic biomass consists of cellulose, hemicellulose, lignin and others. Cellulose structure is that of a guaiacol linear polysaccharide including monomer glucose linked by β-1,4-glycosidic bond[6]. Hemicellulose is amorphous branched heteropolymer including six-carbon sugars (i.e. glucose, hexose etc.), pentose-carbon sugar i.e. xylose and arabinose[1]. Lignin is a heteropolymer of propyl phenol units or phenolic compounds consisting of p-hydroxyl phenyl alcohol (H-unit), coniferyl alcohol (G-unit) and sinapyl alcohol (S-unit). Typically, the chemical content of lignin is of softwood type (24–33%), hardwood type (19–28%) and grass type 15–25%[3].

Lignin structure has various cross-links i.e. aryglycerol-β-ether dimer (β-O-4), aryglycerol-α-ether dimer (α-O-4), siaryl ether (4-O-5), resinol (β-5), diphenylethane (β-1), phenylcoumaran (β-β′), phenylcoumaran (β-β) and biphenyl (5–5)[19]. However, lignin chemical composition is able to be converted into diverse high-value products namely phenolic acid, aromatic compounds and biofuel etc[9].

Liquid hot water (LHW) is considered as one of the most potential technologies for lignocellulosic biomass component fractionation-based biorefineries. LHW is attractive because of no additional chemicals, less energy input and lower environmental impact[25]. LHW has been employed to various lignocellulosic residues. The hydrolysis reaction of the lignocellulose with water (autohydrolysis process) implements through hydronium-catalyzed reactions[12]. Hydronium ions produced from water ionization cause the hemicellulose's depolymerization via the selective hydrolysis of heterocyclic ether bonds and cleavage of acetyl groups[4].

During the stages of reaction, the kinetic reaction was enhanced due to the hydronium ions generated from the autoionization process which acts as catalyst as hydronium ions produced from acetyl groups having an essential role in the reaction. The mentioned process is more appropriate for lignocellulosic materials which has a substantial content of acetyl groups. The previously-studied autohydrolysis process was on eucommia ulmoides oliver (EU) wood. The results showed that the lignin chemical composition consists of klason and acid-soluble lignin. The klason lignin increased from 21.1% to 31.5% at the temperature of 180°C within 30 min. However, the low lignin isolation resulted in decreased separation efficiency[26].

This research aims to isolate lignin from EWC using LHW in addition to alkaline catalyst (NaOH) with optimization of various parameters: catalyst concentration (0.010–0.030 M),

temperature (120–160 °C) and residue tine (15–45 min). The changes in solid fraction and isolated lignin were characterized using various techniques to analyze the basic structure and physical and chemical characteristics based on the qualitative and quantitative analyzes of the isolated product. In this work, we provide the optimized LHW to isolate EWC for integrated biorefineries applications.

Material and Methods

Raw materials: EWC was obtained from Ban so, Phayao, Thailand. EWC was dried at the temperature of 70 °C within 24 h in a hot oven and sieved to particle size around 0.50–0.85 cm by Retsch ZM200 cutting mill. The final moisture content of the milled EWC was 10% estimated by the weight loss after being oven dried at 105ºC to constant weight for 4 h. The sealed plastic bags were used to store the processed eucalyptus wood chip and these samples were kept at room temperature for further experimentation.

The EWC's chemical composition (cellulose, hemicellulose, lignin, ash content and other compounds) was analyzed using the standard laboratory analytical procedures of the national renewable energy laboratory (NREL analysis)[18]. Major chemical composition of EWC was of cellulose (43.4%), hemicellulose (20.3%), lignin (31.1%) and 5.2% other components (e.g. ash and extractive).

Hydrothermal liquid hot water process for lignin fractionation: Hydrothermal LHW process was implemented in a stainless-steel reactor in which the volume was 600 ml. The reactor was heated in vertical shaking system in which the temperature was controlled with the purpose of offering the optimal mixing and a thermocouple for internal temperature measurement (Parr Reactor 4560, Parr instrument, Moline, IL, USA). The beginning standard reactor consisted of EWC (10 g) in distilled water (100 mL).

The reaction was performed by alkali (NaOH) as a promoter. Sodium hydroxide concentration of 0.010–0.030 M was selected with temperatures of 120, 140 and 160°C for 15, 30 and 45 min with stirring at 100 rpm for keeping the catalyst system homogeneous. In order to purge and adjust the initial pressure to 20 bars, nitrogen was delivered to the reactor. After the desired conditions, the reactor was drenched in water bath for 20 min.

The solid fraction was removed employing the filtration process of filter paper, together with a Buchner funnel. Next, it was washed by employing distilled water. Then dry at the temperature of 80 °C. Afterwards, the liquid fraction was obtained with the purpose of analysing lignin and hemicellulose and inhibitory by-products by HPLC analysis.

Thereafter, isolated, solid and native EWC's chemical composition was determined with NREL method. Cellulose yield, cellulose purity, hemicellulose removal, lignin removal and lignin recovery were calculated using the following equations:

$$\text{Cellulose yield (\%)} = \frac{(\text{cellulose remaining in solid pulp})}{(\text{cellulose content in raw corn stover})} \times 100 \quad (1)$$

$$\text{Lignin removal (\%)} = \frac{(\text{lignin content in raw corn stover}) - (\text{lignin remaining in solid pulp})}{(\text{lignin content in raw corn stover})} \times 100 \quad (2)$$

$$\text{Recovered lignin (\%)} = \frac{(\text{weight of recovered lignin from organic phase})}{(\text{lignin content in raw corn stover})} \times 100 \quad (3)$$

Lignin extraction: Lignin extraction method was in a modified from. The surface lignin's removal and recovery were implemented with exhaustive extraction in a Soxhlet apparatus with the solvent (ethanol or acetone). The pre-treated biomass (approximate 4 g) was attained applying continuous recirculation of solvent (250 ml) until there is no colour in the extract flow. After the extraction process, the recovered biomass solid was air dried with the purpose of evaporating solvent traces as well as utilizing for other experiments. Solvent removal was employed to recover the extracted solubilized lignin using a rotary evaporator, then air dried.

Experimental design and optimum parameter for lignin recovery using response surface methodology (RSM): The design of the experiment and statistical analysis were performed according to the Box–response surface design method using the Design Expert software (version 10.0.1). RSM and statistical analysis were selected to identify the optimization parameter of three variables (i.e. concentration, temperature and time) on cellulose yield, lignin removal and lignin recovery. To estimate the model coefficients, three modified Box-Behnken design was carried out (15 experiments). The three factors comprised of reaction concentration of NaOH base promoter (X_1, 0.010–0.030 M), temperature (X_2, 120–160°C) and residue time (X_3, 20–45 min) with three coded levels of each factor (-1, 0, 1).

The variance for each factor assessed was partitioned into offset term, linear, interaction and quadratic components and all predictive parameters were written as a second-order polynomial equation as follows:

$$Y = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 12X1X2 + \beta 13X1X3 + \beta 23X2X3 + \beta 11X21 + \beta 22X22 + \beta 33 \quad (4)$$

where Y is the predicted response, X1, X2 and X3 are the independent variables, β0 is a constant term, β1, β2 and β3 are the linear coefficients term, β12, β13 and β23 are the interaction coefficients and β11, β22 and β33 are the quadratic coefficients. Fitted quadratic polynomial was used to generate 3D surface plots of the correlation between independent variables and the response.

Characterization of isolated solid fractions after fractionated by hydrothermal liquid hot water

X-ray diffraction Spectroscopy (XRD analysis): The native EWC and isolated solid fractions' crystallinities were

examined by X-ray diffraction (XRD) using an X'Pert PRO diffractometer (Panalytical, Almelo, the Netherlands). The samples were scanned in a range of 2θ=10°–30° with a step size of 0.02°at 500 kV, 30 mA and radiation at Cu Kα (λ=1.54 Å). Crystallinity was quantified employing the following equation.

$$\mathrm{CrI} = \frac{\mathrm{I_{002}} - \mathrm{I_{amorphous}}}{\mathrm{I_{002}}} \times 100 \quad (5)$$

where I_{002} is the intensity for the crystalline portion of biomass (i.e. cellulose) at 2Θ = 22.4 and $I_{amorphous}$ is the peak for the amorphous portion (i.e. cellulose, hemicellulose and lignin) at 2Θ = 18.0

Scanning electron microscope (SEM): The analysis of the native EWC's microstructure together with the isolated solid fraction was implemented utilizing scanning electron microscopy (JSM-6301F, JEOL and Japan) with 5–20 kV of electron beam energy. In order to proceed SEM analysis, the EWC samples were dried before being covered with gold.

BET surface area measurement: The native EWC and the isolated solid fraction's total surface area were estimated employing the method of Brunauer, Emmett and Teller (BET) on a Belsorp-max TPD pro (BEL Japan, Tokyo, Japan), with a thermal conductivity detector (Semi-diffusion type, 4-element WeRe filament).

Analysis of aqueous phase: Soluble product profiles in the aqueous fraction were determined on a highly efficient liquid chromatograph (LDC Model 4100, Shimadzu, Kyoto, Japan). Moreover, a refractive index, a UV–Vis detector and an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA were equipped together. This analysis was operated at 65 °C with H_2SO_4 (5 mM) as the mobile phase and a flow rate was 0.5 mL/min. Next, the number of oligosaccharides were examined following the NREL method.

Characterization of lignin recovery after fractionation by hydrothermal liquid hot water

Elemental composition analysis: Elemental composition was determined by elemental analyzer CHNS-628 (LECO, Saint Joseph, MI, USA). Commercial organosolv lignin and lignin recovery were dried at the temperature of 60 °C using vacuum evaporator with 20 bars in order to eliminate moisture. Afterward, 0.1 g of commercial organosolv lignin and lignin recovery were placed into the container with the purpose of estimating carbon, hydrogen, oxygen and nitrogen in lignin sample. Regarding sulfur analysis, 0.2 g of commercial organosolv lignin and lignin recovery were put into a ceramic boat furnace. The incineration occurred at 1,350 °C employing sulfur IR cells to determine the amount of sulfur.

Fourier-transform infrared spectroscopy analysis FT-IR: The commercial organosolv lignin and lignin recovery's chemical structure were characterized using Fourier-transform infrared spectroscopy analysis. FTIR analysis was carried out using PerkinElmer, Waltham, MA, USA and the KBr pellet method was utilized to prepare the samples. The region between 4000 and 400 cm^{-1} with resolution of 4 cm^{-1} at 32 scans was recorded. Lignin sample's peaks and peaks of standard functional groups were compared.

Thermo gravimetric analysis TGA: The thermal stability by thermo gravimetry analysis of the commercial organosolv lignin and lignin recovery was measured by heating specimens with a rate of 7 ºC per minute in flowing nitrogen gas (20–35 ml/min). The specimen weight was controlled as a function of temperature employing TA Instruments Inc. (Model TA Q50). Approximately 30–50 mg of commercial organosolv lignin and lignin recovery was weighed in an aluminum pan. Afterward, the room temperature was heated at the rate of 5°C/min in order to reach at about 1,000°C with the purpose of determining total mass loss. A curve of weight loss against temperature was plotted from the data obtained. This curve (DTG)'s derivative was constructed to illustrate the temperatures which showed the maximum rates of weight loss.

Gel permeation chromatography GPC: The precipitated lignin fraction, together with the commercial organosolv lignin and lignin recovery were subjected to gel permeation chromatography to calculate the average molecular weight (Mw) of solutes and the polydispersity index (Mw/Mn) employing a Jasco instrument unit equipped with an interface (LC-NetII/ADC) together with a UV detector (254 nm). In the next step, 0.1 grams of lignin were dissolved in tetrahydrofuran solution and test carried out with two PolarGel-M columns 803 (300 mm ×7.5 mm) and PolarGel-M guard (50 mm ×7.5 mm) was used and tetrahydrofuran (THF) was selected as mobile phase. The flow rate was 0.5 ml/min at 40°C and the column temperature was 40 °C. Calibration was done utilizing polystyrene standards (Sigma-Aldrich), with the range of 55,000 - 266 g/mol.

Pyrolysis-gas chromatography mass spectrometry Py-GC-MS: Py-GC-MS was employed to identify chemical composition of the commercial organosolv lignin and lignin recovery. The commercial organosolv lignin and lignin recovery's pyrolysis were implemented in an EGA/PY-3030D microfurnace pyrolyzer (Frontier Laboratories Ltd., Fukushima, Japan) connected to an Agilent 5975 mass-selective detector (EI at 70 eV) and GC 7820A (Agilent Technologies, Inc., Santa Clara, CA). The dimensions of column were 30 m × 0.25 mm i.d., 0.25 μm in film thickness, DB-1701 (J and W Scientific, Folsom, CA). The oven's temperature was increased from 50°C (1 min) to 100°C at 20°C min^{-1} before the second increase to 280°C (5 min) at 6°C min^{-1} Helium was the carrier gas (1 mL min^{-1}).

The released compounds' identification was achieved by examining the differences between their mass spectra, the Wiley, NIST libraries and those reported in the literature[24]. Moreover, when possible, there could be a comparison with

the retention times and mass spectra of authentic standards. Molar peak areas were quantified for each lignin degradation product released. The total areas were normalized and two replicates' data were averaged and showed in percentages[21].

Results and Discussion

The chemical composition of the raw material and solid fractions from LHW process: The optimization of experiments is designed to validate the results under specific experimental conditions. The influence of alkaline concentration, reaction temperature and residue time on cellulose yield, lignin removal and lignin recovery was investigated by RSM method. The optimization of the experiment with 15 experiments was given in table 1. The influence of each factor showed that it was found in the range of 52.1–73.2%, 55.5–75.4% and 55.1–74.5% of cellulose yield, lignin removal and lignin recovery respectively.

The results of the hydrothermal LHW process were analyzed using the variance analysis (ANOVA) as demonstrated in table 2. The optimization of the hydrothermal LHW conditions including alkaline promoter (0.01–0.03 M), reaction temperature (120–160°C) and residue time (15–45 min), was for maximizing cellulose yield, lignin removal and lignin recovery. The calculated regression equation for the optimization of hydrothermal LHW showed that the cellulose yield (Y_1,%), lignin removal (Y_2, %) and recovered lignin (Y_3, %) are the functions of alkaline promoter (X_1, M), reaction temperature (X_2, °C) and residue time (X_3, min). The predicted equation and experimental data showed that the second-order polynomial multiple regression equation was found to represent the cellulose yield, lignin removal and lignin recovery as defined in equations 5 to 7.

$$Y^1 = -108.7 + 1125\,X_1 + 1.788\,X_2 + 2.592\,X_3 - 42292\,X^2_1 - 0.006635\,X^2_2 - 0.02013\,X^2_3 + 7.25\,X_{12} - 13.17\,X_{13} \quad (5)$$

$$Y^2 = -12.27 - 781\,X_1 + 0.4945\,X_2 + 2.7433\,X_3 - 38008\,X^2_1 - 0.001633\,X^2_2 - 0.033059\,X^2_3 + 14.437\,X_{12} + 10.183X_{13} - 0.004750\,X_{23} \quad (6)$$

$$Y^3 = -12.9 - 927\,X_1 + 0.484X_2 + 2.869\,X_3 - 37828\,X^2_1 - 0.001520\,X^2_2 - 0.033299\,X^2_3 + 15.05\,X_{12} + 11.17\,X_{13} - 0.005667\,X_{23} \quad (7)$$

Optimization of the cellulose yield, lignin removal and lignin recovery from Eucalyptus wood chip: The target responses based on RSM method were illustrated in figure 1. It showed that all three criteria were combined to find the optimum conditions for biomass fraction. According to the program prediction, maximum cellulose yield of 74.0% was predicted to occur at 0.0175 M, 129.2929°C and 44.0909 min residue time. The maximum lignin removal and lignin recovery of 77.4% and 76.6% respectively were predicted at 0.0245M, 160.33 °C and 33.7879 min.

In the experimental stage under optimal conditions, all three criteria: (> 70%) of cellulose yield, (> 70%) of lignin removal and (>70%) of lignin recovery are well comparable to the predicted value. Results under this optimal condition give the maximum cellulose yield, lignin removal and lignin recovery efficiencies as 74.0%, 77.3% and 76.1% respectively of predicted value with alkaline promoter at 0.020 M at 160 ºC for 30 min.

Table 1
Effect of reaction factors on solid composition of hydrothermal liquid hot water process for isolated lignin with response surface method (RSM).

Factors			Responses (%)		
Concentration (M)	T (°C)	Time (Min)	Cellulose Yield (%)[a]	Lignin Removal (%)[b]	Lignin recovery (%)[c]
0.02	140	30	70.0	73.3	72.3
0.02	120	15	55.0	55.5	54.2
0.01	140	45	70.1	64.5	64.0
0.03	160	30	64.0	75.4	74.5
0.01	140	15	52.1	58.7	58.2
0.01	120	30	65.8	68.0	67.7
0.01	160	30	60.0	69.4	68.7
0.02	160	45	68.6	72.0	71.2
0.03	140	15	57.1	56.5	55.1
0.02	140	30	70.5	73.2	72.8
0.03	120	30	64.0	62.4	61.5
0.03	140	45	67.1	68.4	67.6
0.02	160	15	55.8	65.8	65.7
0.02	120	45	73.2	67.4	66.5
0.02	140	30	70.5	73.0	72.4

[a]Based on relative content of cellulose in remaining pulp
[b]Based on relative content of lignin in solid pulp compared with lignin content in raw material
[c]Based on the weight of lignin in organic phase

Table 2
Effect of regression models of responses based on ANOVA analysis

Source	p-value[a]		
	Cellulose yield	**Lignin removal**	**Lignin recovery**
Linear			
concentration	0.0055	0.0028	0.0901
temperature	0.0003	0.0000	0.0070
time	0.0000	0.0000	0.0000
Quadratic			
concentration*concentration	0.0000	0.0000	0.0000
temperature * temperature	0.0000	0.0003	0.0036
time*time	0.0000	0.0000	0.0000
2-Way Interaction			
concentration * temperature	0.0005	0.0000	0.0000
concentration *time	0.0001	0.0000	0.0000
temperature * time	0.0007	0.0000	0.0000

[a]The not significant p-value are highlighted

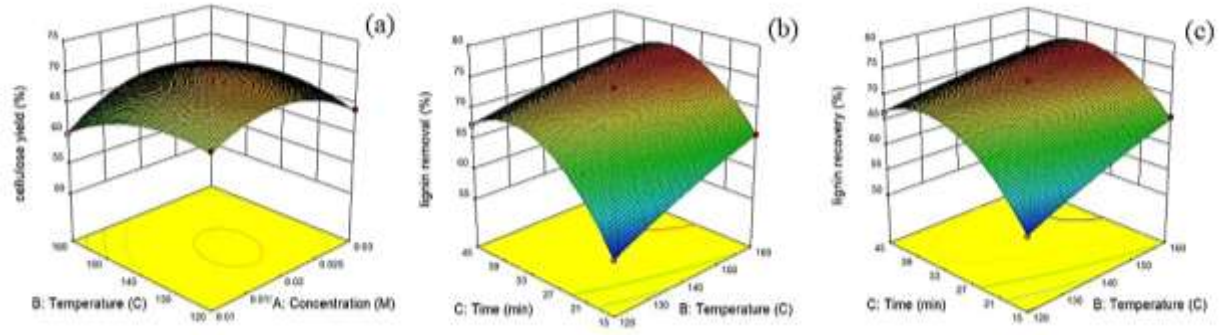

Figure 1: Response surface plot of LHW process: (a) Effect of alkaline promoter (0.010–0.030 M) and reaction temperature (120–160 ºC) on cellulose yield in solid fraction with varying alkaline promoter (0.010 M), (b) Effect of reaction time (15–45 min) and temperature (120–160 ºC) on lignin removal in solid fraction with varying alkaline promoter (0.020 M), (c) Effect of reaction time (15–45 min) and temperature (120–160 ºC) on lignin recovery in solid fraction with varying alkaline promoter (0.020 M)

Physicochemical characterization of native eucalyptus wood chip and solid remaining after LHW process by SEM and XRD pattern analysis: The structure analysis of native EWC compared with fractionated solid residues under optimal condition with LHW process using scanning electron microscopy is shown in figure 2. It showed that the native EWC displayed a smooth surface and intact cuticles of the biomass (Figure 2a). The fractionated solid residues under optimal condition after LHW process showed cracks, cavities and surface pores on the surface of solid residue as in figure 2b[15].

In addition, the crystallinity analysis (CrI) by XRD, before and after structural modifications of the native EWC with the LHW process are shown in table 3. It showed that the CrI of isolated solid showed decrease in CrI (69.3%) compared to the native EWC (78.5%) due to mostly removed hemicellulose and lignin on the surface. Moreover, the harsh conditions result in decrease in the CrI of solid residue.

Physicochemical characterization of lignin fraction by Fourier transform infrared spectroscopy analysis of commercial organosolv lignin and lignin recovery: In this present work, the physical and chemical properties of lignin recovery under stable condition were compared with commercial organosolv lignin. The FTIR spectroscopy analysis is shown in figure 3 after lignin fractionation under optimal condition with LHW process. The lignin recovery and commercial organosolv lignin showed the signal of hydroxyl group (O-H) in phenolic and aliphatic absorption region 3290 cm^{-1}[10]. The vibrations at 2921–2920 cm^{-1} are affiliated with C–H vibrations in the methyl absorption region[2]. The intensity at 1734 cm^{-1} is assigned to C=O stretching of carbonyl groups absorption region in commercial organosolv lignin[14].

The absorbance at 1715 cm^{-1} corresponded with C-O in aromatic group vibrations in lignin extraction while the intensity of 1688 cm^{-1} showed carbonyl/carboxyl group

absorption region; the absorption intensity of C-C vibrations was at intensity of 1464 to 1595 cm^{-1}. The intensity at 1499–1445 cm^{-1} is related to CH_3 vibrations in acetyl group. Similarly, the bands at 1327 cm^{-1} are assigned to C-H group bending of polysaccharides of syringyl (S) units and guaiacyl (G) units[7]. The signal at 1300 and 900 cm^{-1} corresponds to the C-O-C vibrations while the absorption intensity of 1200 cm^{-1} was associated with C-O vibrations in guaiacyl units. The absorbance at 1031–1030 cm^{-1} corresponds to the C–O group's bending vibrations[16]. In addition, the spectra of 833 to 834 cm^{-1} represented the C–H vibration group in guaiacyl units[8].

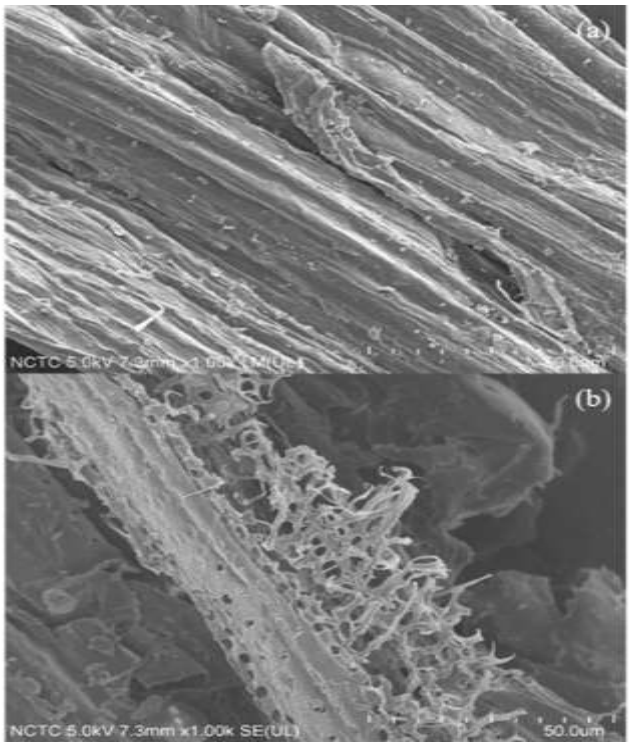

Figure 2: SEM of (a) native eucalyptus wood chip and (b) isolated solids after LHW process

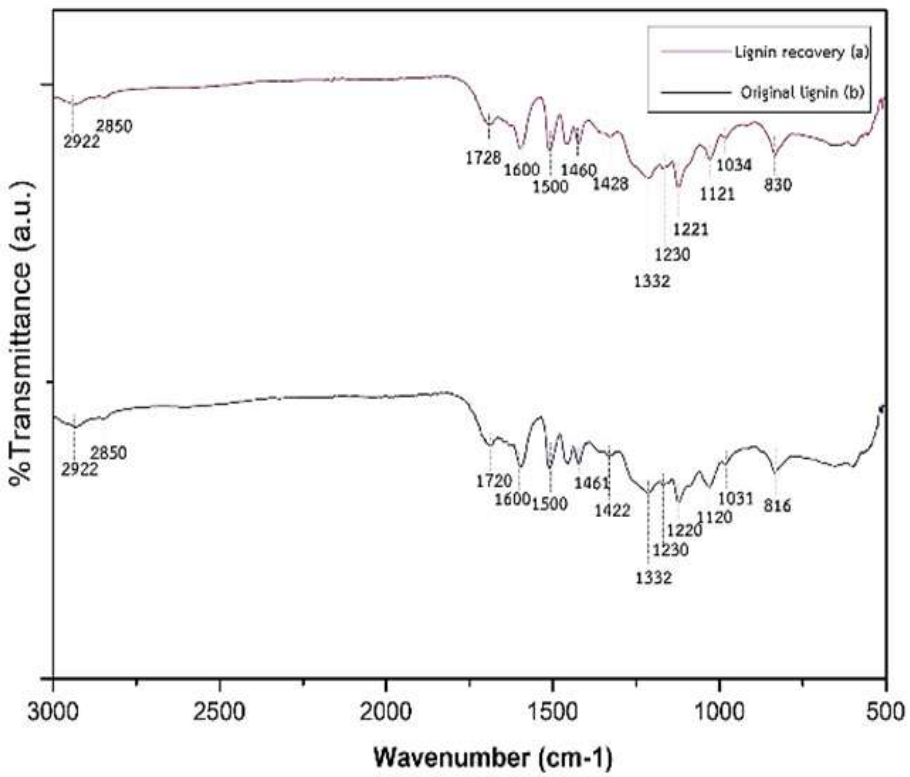

Figure 3: Assignment of FT-IR signals (a) Lignin recovery (b) commercial organosolv lignin

Table 3
BET surface area and crystallinity index of native eucalyptus wood chip and isolated solid after hydrothermal liquid hot water under optimized condition

Sample	Surface area (m^2/g)	Crystallinity index (%)
Native Eucalyptus wood chip	7.11	78.5
Isolated solid	11.5	69.3

Table 4
Weight-Average Molecular Weight (Mw) and polydispersity index (PDI) of lignin recovery and commercial organosolv lignin

Sample name	Mw[a] (g/mol)	Mn[b] (g/mol	PDI[c]
COL	17900	7550	2.37
LR	17500	7450	2.34

[a]Weight-average molecular weight (Mw)
[b]Number-average molecular weight (Mn)
[c]Polydispersity index (PDI)

Molecular weight distributions and polydispersity index (PDI) of the lignin recovery and commercial organosolv lignin: The lignin extraction's molecular weight distributions were employed to investigate the effect of lignin extraction after LHW process on the molecular weights of lignin recovery compared with commercial organosolv lignin as shown in table 4. The molecular weight distributions (Mw), number average (Mn) and PDI were estimated by GPC.

It was found that the Mw of lignin recovery showed high average Mw and Mn compared to commercial organosolv lignin. The average Mw and Mn of commercial organosoly lignin was 17900/7550 (g/mol) respectively. After lignin fraction with hot hydrothermal LHW process, the Mw/Mn of lignin recovery decreased to 17500/7450 (g/mol) which was marginally lower than that of the commercial organosolv lignin. It was found that low molecular weight lignin had a lower polydispersity index at 2.34 while that of the commercial organosolv lignin was 2.37.

The result indicates a similar particle size of lignin recovery after hydrothermal LHW. As previously reported, lignin was isolated from Eucalyptus grandis under optimal conditions with the hydrothermal treatment process. It showed that the PDI of isolated lignin after hydrothermal treatment process was 2.13 slightly lower than that of the control at 2.25[23].

Effect on proximate analysis and ultimate analysis of the lignin recovery and commercial organosolv lignin: The lignin composition of lignin recovery characterized by proximate and ultimate analysis under optimal condition was compared with commercial organosolv lignin. The lignin recovery and commercial organosolv lignin's proximate analysis showed high contents of volatile matter (70.1–65.1 wt%), fixed carbon (33.72–28.9 wt%), moisture content (0.48–0.44 wt%) and ash content (0.70–0.56 wt%). The ultimate analysis of lignin recovery consists of carbon content in the range of 63.5–62.9 wt%, hydrogen content 5.60–5.14 wt%, oxygen content 31.3–30.7 wt% and nitrogen 0.80–0.01 wt%.

The results revealed that lignin recovery has a higher carbon content than oxygen, hydrogen and nitrogen content because of the removal of cellulose and hemicellulose out of the lignin structure[11].

As a result, the amount of oxygen is reduced compared to before the treatment. However, the lignin recovery showed higher nitrogen content compared to commercial organosolv lignin because high amounts of nitrogen are used as base catalyst reaction (NAOH) under the hydrothermal LHW[5].

Thermogravimetric analysis of lignin recovery and commercial organosolv lignin: After lignin extraction, the thermal stability of lignin recovery was analyzed and compared to that of commercial organosolv lignin and lignin recovery under optimal conditions. TGA curves for the lignin sample of commercial organosolv lignin and lignin recovery were analyzed via thermogravimetric analysis as shown in figure 4.

Thermal decomposition of the lignin samples can be divided into four stages. Initially, the stage one occurs at ~30–140 °C due to the degradation of moisture loss under dehydration process[13].

The second stage at 140–350 ˚C shows the breaking of the volatile compound and decomposition of bonds between phenolic groups such as CO, CO_2, CH_4 and b'-O-4' linkages[17]. These results correspond to the many products derived from the lignin sample such as phenolic and aldehyde acids in the final stage from 450 to 550 °C showing the decomposition of pyrolytic degradation, aromatic rings and methoxyl groups[22].

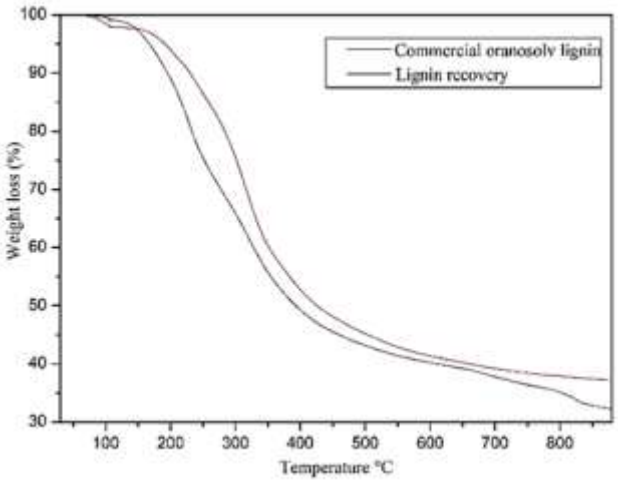

Figure 4: TGA curves of lignin recovery and commercial organosolv lignin

Py-GC-MS of commercial organosolv lignin and lignin recovery: The identification and relative molar abundance (%) of the commercial organosolv lignin and lignin recovery were identified in the Py-GC-MS. The relative abundance of the pyrolysis is indicative of the relative amount of many volatile compounds present in the lignin sample. The result showed that lignin recovery contained mainly aromatic hydrocarbons group, alkoxys type and phenolic compounds. Lignin sample comprised mainly of syringol unit (S) compounds such as syringol group (12), 4-Ethylsyringol (19), 4-Vinylsyringol (22), syringaldehyde (28) and trans-Sinapyl alcohol (39) or products in the group of guaiacol units (G) i.e. Guaiacol (2), Eugenol (10), 4-Methylguaiacol (5), 4-Ethylguaiacol (6), 4-Vinylguaiacol (7), cis-Isoeugenol (13) and trans-Coniferyl alcohol (33).

However, significant amounts of compounds were derived from p-hydroxylphenyl units (H) i.e. phenol (1), 2-Methylphenol (3) and 4-Methylphenol (4) etc. In addition, the commercial organosolv lignin and lignin recovery contain almost exclusively S-type and G-type compounds. S/G ratio is 1.77 and 1.54 for both commercial organosolv lignin and lignin recovery respectively.

Conclusion

Lignin extraction from EWC was subjected to hydrothermal LHW process under the optimized variables. The optimal condition was made of 0.020 M of alkaline catalyst (NaOH) at 160°C and residue time of 30 min resulting in high lignin removal from solid phase up to 77.3% and optimal condition of lignin recovery in liquid phase (76.1%). In addition, cellulose yield of 74% was obtained in solid phase. The relative Mw/Mn of isolated lignin was 17500/7450 g/mol and lower polydispersity index of 2.34 was obtained. This result emphasized that the molecular weight of lignin product- based molecules possessed a non-uniform distribution.

TGA analysis indicated that the maximum degradation of lignin recovery was at 140–350 ^{o}C due to degradation of phenolic hydroxyl linkages. The study illustrated the promising method for EWC components' fractionation in the process of an integrated biorefinery.

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References

1. Bajpai P., *Elsevier*, 1974 **(2018)**

2. Balan V., Mihai C.T., Cojocaru F.D., Uritu C.M., Dodi G., Botezat D. and Gardikiotis I., *Materials*, **12(18)**, 2884 **(2019)**

3. Bujanovic B., Ralph S., Reiner R., Hirth K. and Atalla R., *Materials*, **3(3)**, 18881903 **(2010)**

4. Carvalheiro F., Duarte L.C., Gírio F. and Moniz P., In Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery, Amsterdam, The Netherlands, 315-347 **(2016)**

5. Cheng S., Wei L., Alsowij M., Corbin F., Boakye E., Gu Z. and Raynie D., *AIMS Environ. Sci.*, **4(3)**, 417430 **(2017)**

6. Crichton R., Academic Press, 3579 (**2018)**

7. Esteves B., Marques A.V., Domingos I. and Pereira H., *Maderas. Cienc. Tecnol.*, **15**, 245258 **(2013)**

8. Galletti A.M.R., Alessio A., Licursi D., Antonetti C., Valentini G., Galia A. and Di Nasso N.N., *J Spectrosc.*, 112 **(2015)**

9. Ge X., Chang C., Zhang L., Cui S., Luo X., Hu S. and Li Y., *Elsevier*, **3**, 161213 **(2018)**

10. Gonultas O. and Candan Z., *Maderas. Cienc. Tecnol*., **20(3)**, 431 **(2018)**

11. Guo H., Zhang B., Qi Z., Li C., Ji J., Dai T., Wang A. and Zhang T., *Chem Sus Chem.*, **10(3)**, 523532 **(2017)**

12. Jeong S. and Lee J.W., *Elsevier*, 619 **(2015)**

13. Lazzari L., Domingos E., Silva L., Kuznetsov A., Romão W. and Araujo J., *Polímeros*, **29(4)**, 16785169 **(2019)**

14. Oliveira L., Evtuguin D., Cordeiro N. and Silvestre A.J.D., *Ind Crops Prod*., **29(1)**, 8695 **(2009)**

15. Rehman N., Alam S., Amin N.U., Mian I. and Ullah H., *Int. J. Polym. Sci.*, https://doi.org/10.1155/2018/8381501 **(2018)**

16. Sang Y.O., Dong Y., Younsook S. and Gon S., *Carbohydr. Res.*, **304**, 417428 **(2005)**

17. Schutyser W., Renders T., Van Den Bosch S., Koelewijn S.F., Beckham G.T. and Sels B.F., *Chem Soc Rev.*, **47(3)**, 852908 **(2018)**

18. Sluiter A., Hames B., Ruiz R., Scarlata C. and Sluiter J., Laboratory Analytical Procedure **(2008)**

19. Suseela V., Academic Press, 103124 **(2019)**

20. Takkellapati S., Li T. and Gonzalez M.A., *Clean Technol. Environ. Policy*, **20(7)**, 16151630 **(2018)**

21. Wanmolee W., Laosiripojana N., Daorattanachai P., Moghaddam L., Rencoret J., Del Río J.C. and Doherty W.O.S., *ACS Sustain. Chem. Eng*., **6(3)**, 30103018 **(2018)**

22. Watkins D., Nuruddin Md., Hosu M., Tcherbi-Narteh A. and Jeelani S., *J. Mater. Res. Technol.*, **4(1)**, 2632 **(2015)**

23. Xiao L.P., Lin Z., Peng W.X., Yuan T.Q., Xu F., Li N.C., Tao Q.S., Xiang H. and Sun R.C., *Sustain. Chem. Process*, **2(9)**, 212 **(2014)**

24. Zhan S., Chenguang W., Kang B., Xinghua Z., Chiling Y., Renjie D. and Changle P., *Int. J. Agric. Biol. Eng*., **10(5)**, 214225 **(2017)**

25. Zhang Li.J., Zhang J., Gao S., Li Q. J. and Zhang W., *Ind Crops Prod*., **120**, 2533 **(2018)**

26. Zhu M.Q., Wen J.L., Su Y.Q., Wei Q. and Sun R.C., *Bioresour. Technol.*, **185**, 378385 **(2015)**.

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