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Impact of Ionic Liquid 1-Ethyl-3-Methylimidazolium Acetate Mediated Extraction on Lignin Features

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Abstract

This study aims at investigating the impact of ionic liquid extraction on lignin structure by studying the mechanism of lignin depolymerization in 1-ethyl-3-methylimidazolium acetate EMIM[OAc] and comparing it with that of organosolv and milled wood methods. Ionic liquid mediated lignin (ILL) using EMIM[OAc], ethanol organosolv lignin (EOL) and milled wood lignin (MWL) were isolated from *Typha capensis* (TC) and subjected to several analytical characterizations. Experimental data shows that ILL exhibited a relatively lower degree of condensation, lower aromatic C-C structures and a higher aliphatic OH with values of 0.42/Ar, 1.94/Ar and 1.33/Ar moieties compared with EOL values of 0.92/Ar, 2.22/Ar and 0.51/Ar moieties respectively. The ILL was depolymerized under mild conditions giving relatively higher β -aryl ether linkages content, higher molecular mass, and exhibited closer structures and reactivity to native lignin than EOL. These insights on TC lignin depolymerization in EMIM[OAc] acetate may contribute to better value-addition of lignocellulosic biomass.

Keywords

1-Ethyl-3-Methylimidazolium Acetate, Ionic Liquid Lignin, Ethanol Organosolv Lignin, Lignin Depolymerization, Milled Wood Lignin

1. Introduction

Lignocellulose fractionation using organic solvents is receiving a growing inter-

est [1]. With organosolv pulping, a pulp with good enzymatic digestibility, but also high-quality lignin, can be obtained, allowing for multiple high value products [2] [3] [4]. More recently Ionic liquids (ILs) have also been used for biomass fractionation [5] [6] [7] [8] [9]. Among all the ILs used, EMIM [OAc] is particularly powerful, as the treatment conditions can be turned effectively to fractionate lignin and cellulose rich pulp from lignocellulosic biomass [5], and also collect intact cellulose nanocrystals [10] [11]. Additionally, EMIM [OAc] has interesting properties for a possible scale up: low toxicity ($LD_{50} > 2000 \text{ mg}\cdot\text{Kg}^{-1}$), low corrosiveness, a low melting point of less than -20°C , low viscosity (10 mPa s at 80°C) and recyclability [5]. Therefore, in view of a biorefinery deliverables of several high value products, EMIM [OAc] is very interesting. However, the delignification chemistry of EMIM [OAc] and the resulting lignin structure is not fully understood.

EMIM [OAc] treatment led to high lignin solubility but low wood flour solubility, and also enabled the disruption of the chemical integrity and provided a lignin extraction yield of 4.4 g/kg IL [6]. Furthermore, lignin extracted by EMIM [OAc] from poplar wood resulted in lower S/G ratio, reduction of molecular weight and more uniform fragments, compared to the MWL counterpart [12]. The functional-group, rather than the size of the anion of ILs, controls lignin fragmentation and the anion plays a much larger role in lignin depolymerization [13]. With EMIM [OAc] pretreatment of biomass, deacetylation of xylan and acetylation of lignin units occurs [14]; these processes contribute to swelling, followed by dissolution, disintegration and derivatization of wood [15].

While chemical and physico-chemical characterizations of ethanol organosolv lignin from different feedstocks have been widely reported, little work has been done in this regard for ionic liquids lignins from EMIM [OAc]. An earlier report by Kim *et al.* characterized EMIM [OAc] lignin from poplar, a wood plant, in comparison with the MWL [12]. Most other studies on ionic liquid for lignocellulosic fractionation emphasized identifying efficient ionic liquids and optimizing the conditions for increased delignification and improved enzymatic hydrolyzability of cellulose rich residues to fermentable sugar [5] [9] [16] [17] [18].

In the current report, the purpose is to study the chemical structure of lignin isolated by EMIM [OAc] IL from *Typha capensis* (an herbaceous plant) and compare it to the (1) well-known chemistry of ethanol organosolv lignin, and (2) milled wood lignin (MWL) structure, which is a reference point in lignin studies as lignin isolates closest to the intact lignin from the plant material. To that aim, lignins were extracted from *Typha capensis* using EMIM [OAc] and ethanol organosolv methods as well as the classical MWL method, and the isolated lignin samples were subjected to an array of analytical procedures, including high performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD), elemental analysis, Fourier transform infrared (FT-IR), ^{13}C and ^{31}P nuclear magnetic resonance (^{13}C & ^{31}P NMR), thermogravimetric analysis (TGA), gel permeation chromatography (GPC) and wet chemistry procedures for functional groups determination.

2. Experimental Methods

2.1. Materials

1-ethyl-3-methylimidazolium acetate (EMIM [OAc]), ethanol, sulfuric acid, dimethyl sulfoxide (DMSO), ethyl acetate, n-hexane, tetrahydrofuran (THF), dichloromethane (DCM), 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP), pyridine, chloroform, and chromium III acetylacetonate were all purchased from Sigma Aldrich. All the chemicals were analytical grade and used as supplied.

Typha capensis sample was uprooted from canals in Kura village, Kano State, Nigeria. The protocol edited by Hames *et al.* [19], was used for sample preparation. The freshly harvested TC was cut to ≤ 2 cm and sun dried for 4 days, packed in cellophane and brought to Germany. Samples were further dried in an oven at 40 °C for 48 h, milled to obtain particle size ≤ 0.4 mm and designated as raw TC (TC_{raw}). The TC_{raw} was sequentially and exhaustively extracted using a Soxhlet Extraction apparatus with water, ethanol and dichloromethane (DCM), refluxed for 16 h, 16 h and 8 h respectively to obtain extractive free TC (TC_{extracted}), and kept until further use [20]. 3 lignin isolation methods were performed, **Figure 1**.

2.2. Methods

2.2.1. MWL Isolation

The procedure by Bjorkman as modified by Obst and Kirk [21] and Rencoret *et al.* [22] was used to process MWL from TC [21] [22]. Details are provided in supplementary information.

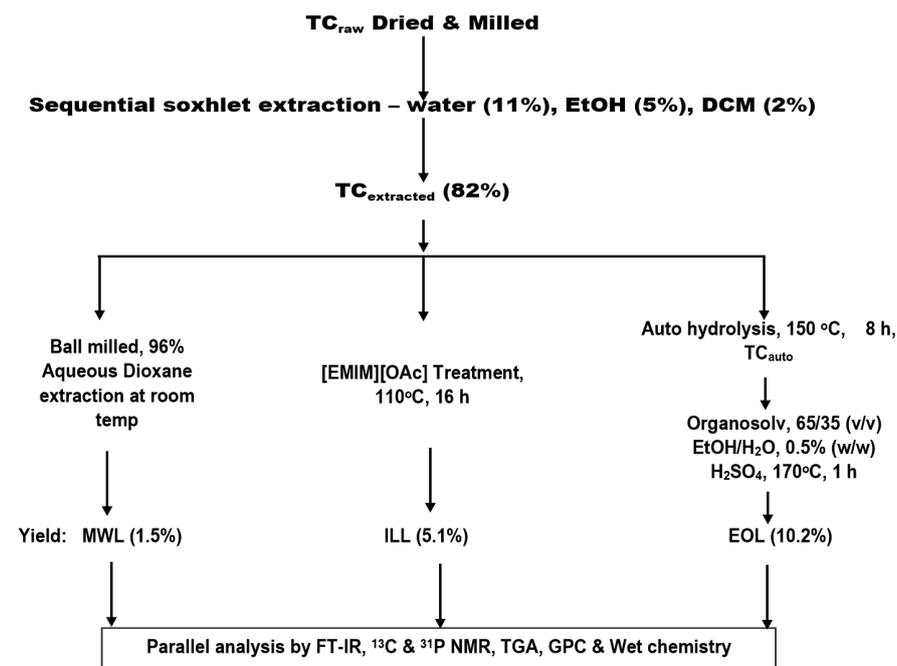


Figure 1. Experimental scheme for TC lignins isolation and characterization. Key: TC = *Typha capensis*; TC_{raw} = Raw TC, TC_{extracted} = Extractive free TC; TC_{auto} = Autohydrolyzed TC; MWL = milled wood lignin; EOL = ethanol organosolv lignin; ILL = ionic liquid lignin; [EMIM] [OAc] = 1-ethyl-3-methylimidazolium acetate; DCM = dichloromethane; EtOH = ethanol.

2.2.2. Ionic Liquid Mediated Lignin Extraction

The procedure published by Sun *et al.* with slight modification was used [5]. Briefly, 1 g of TC_{extracted} was weighed in a 100 mL conical flask and 20 g of 1-ethyl-3-methylimidazolium acetate was added and reacted at 110°C in an oil bath with magnetic stirring for 16 h. The reactant was cooled to room temperature and poured into a 400 mL beaker containing 150 mL acetone/water (1:1, v/v) solution and briskly stirred with a tuning fork. The precipitated cellulose rich residue was separated by filtration through Whatman filter paper using a Buchner funnel under reduced pressure. The residue was washed 3 times with 50 mL of acetone/water solution and filtered again. All the filtrates were joined together, placed in an open beaker and magnetic stirred overnight under the fume hood to evaporate the acetone. The liquid fraction containing lignin, water and IL was cooled overnight in a refrigerator at 4°C and lignin was separated by centrifugation at 4700 rpm for 20 minutes. The lignin was lyophilized and then oven dried overnight at 40°C. With the IL being recyclable, water in the liquid fraction could be evaporated to recover IL under reduced pressure for recycling. Purification step was performed by Soxhlet extraction of this lignin using ethanol, ethyl acetate and n-hexane refluxed for 8 h sequentially [23] but was not efficient. Washing with 0.1 M HCl was more effective in obtaining a relatively pure ILL.

2.2.3. Autohydrolysis Pretreatment and Sulfuric Acid Catalyzed Ethanol Organosolv Lignin Extraction

Autohydrolysis was performed as follows: 20 g dry matter of the TC_{extracted} sample was loaded into a 0.6 L stainless steel pressure Parr reactor equipped with Parr 4842 temperature controller (Parr Instrument Company, Moline IL) and was supplemented with deionized water to a solid to liquid ratio of 1:9. Content was reacted at 150°C with continuous stirring for 8 h, after which it was quenched in ice water. The liquid phase was separated by filtration using Whatman filter paper no. 4. The solid residue was washed with hot water, ca 70°C (3 × 50 mL). The recovered autohydrolyzed residue (TC_{auto}) was oven dried at 40°C.

For the ethanol organosolv lignin isolation, the oven dried TC_{auto} sample was weighed and loaded into the Parr reactor and an ethanol/water solution—65:35 (v/v) containing 0.5% sulfuric acid (w/w)—was added to obtain a solid to liquid ratio of 1:9. The mixture was then reacted at 170°C for 1 h. After cooling at the end of the reaction, the solid phase was recovered by filtration using Whatman filter paper number 4 and the residue was washed three times using warm (60°C) ethanol/water (4:1 ratio, v/v) at a volume of ca 2 mL per gram of pretreated sample. The filtrates were combined and deionized water added. The mixture was cooled overnight in a refrigerator at 4°C then centrifuged at 4700 rpm for 20 minutes to precipitate the ethanol organosolv lignin (EOL). The recovered EOL was further washed with deionized water and oven dried at 40°C for approximately 12 hours.

2.2.4. Lignin Content and Composition Analysis

A two-stage sulfuric acid hydrolysis procedure was performed to determine the

Klason lignin of TC_{raw} , $TC_{\text{extracted}}$ and that of the isolated lignins, followed by monosaccharide analysis of the hydrolysate. Monosaccharide contents were analyzed by HPAE-PAD (ICS-3000 Dionex) equipped with a Dionex CarboPac™ PA-20 guard column (3 × 30 mm) attached with a Dionex CarboPac™ PA-20 (3 × 150 mm) analytical column. Filtered samples (20 μL) were eluted at 35 °C and at 0.4 mL/min with the following composition: pure water 99.2%/250 mM NaOH 0.8%: 0 - 20 min; pure water 75%/250 mM NaOH 20%/NaOAc (1 M)-NaOH (20 mM) 5% 20 - 37 min; pure water 40%/250 mM NaOH 20%/NaOAc (1 M)-NaOH (20 mM) 40% 37 - 41 min; each elution was followed by a wash and subsequent equilibration time. External sugar and uronic acids standards were used for calibration (7 points per curve; fructose, glucose, xylose, galactose, mannose, rhamnose, arabinose, galacturonic acid and glucuronic acid -all purchased from Sigma-Aldrich). In addition to impurities identified in the sugar analysis of hydrolysates, nitrogen and sulfur from elemental analysis were also identified as impurity.

2.2.5. Fourier Transform Infrared Spectroscopy (FT-IR)

Infrared spectra were acquired from FT-IR spectrometer 65 (Perkin Elmer, USA) in transmittance mode of 64 scans, resolution of 4 cm^{-1} spanning 4000 to 400 cm^{-1} band using KBr pellets containing 1% of finely ground sample. Baseline correction and normalization was done with a zero baseline taken from a common point at 1900 cm^{-1} and with a maximum range of 4000 - 900 cm^{-1} . An internal standard was selected by having bands normalized as a ratio of each absorbance to the absorbance at 1510 cm^{-1} [24] [25]. The selected band at 1510 cm^{-1} is assigned as reference, being a typical stretching of aromatic rings which has relatively constant intensity [24].

2.2.6. TGA

Thermogravimetric analysis was conducted using Pyris 1 (Perkin Elmer, USA) and heated from 20 °C to 900 °C at the rate of 10 °C min^{-1} under constant air flow.

2.2.7. ^{13}C and ^{31}P NMR

Quantitative solution state ^{13}C and ^{31}P NMR analysis were conducted on a Bruker Avance-400 NMR spectrometer and the data was analyzed using 3.2 topspin Bruker software. 150 mg of the sample was dissolved with slight heating and stirring in 0.38 mL DMSO- d_6 and 20 μL of 0.25 mg/mL chromium acetylacetonate in DMSO- d_6 as a relaxation agent and placed in an NMR tube to acquire ^{13}C NMR data.

Phosphorus NMR used inverse-gated decoupling (Walzt-16) pulse sequence to avoid the Nuclear Overhauser effect (NOE). Sample preparation used 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) as a phosphitilating reagent. The method outlined by Granata and Argyropoulos was applied [26]. Between 24 - 25 mg of dried samples were each dissolved in 400 μL solvent mixture of pyridine/chloroform (1.6/1; v/v) and 150 μL of 3.6 mg/mL chromium III

acetylacetonate (relaxation agent), 4.0 mg/mL cyclohexanol (internal standard) solution in pyridine/chloroform solvent mixture was added and vigorously stirred to dissolve. The phosphitylation reaction has been observed to be unstable, therefore, addition of the TMDP (50 μ L) was done just at the start of the NMR experiment.

2.2.8. GPC

The molecular-average weights were determined by gel permeation chromatography (GPC). Samples were first acetylated using aceto-bromination [27]. Briefly, a 10 mg sample was weighed in a 50 mL round bottom flask and 2.3 mL of acetic anhydride was added. The content was reacted at room temperature with magnetic stirring for two hours. 0.25 mL of acetyl bromide was then added and stirring continued for two hours. Acetic anhydride and acetyl bromide were evaporated using rotary evaporator. The acetylated samples were then freeze dried then dissolved in tetrahydrofuran (THF) at concentration of 4 mg/mL. The solution was filtered using a syringe filter (PTFE, 0.45 μ m pore size). Samples were injected into the GPC system at a flow rate of 1 mL/min at 22.5°C and 280 nm wavelength using THF as an eluent. The GPC system used includes an isocratic pump, a preparative autosampler, a UV detector and an SDV column combination. Data was acquired using narrow standard polystyrene calibration standard [27].

2.2.9. Functional Groups Determination Using Wet Chemistry Procedure

To provide complementing information, established wet chemistry procedures for functional groups determination were applied to obtain the methoxy, carbonyl, carboxyl, phenolic and aliphatic hydroxyl contents of the lignin isolates [28] [29] [30] [31] [32]. As proof of the methoxy and carbonyl methods, vanillin was analyzed alongside each method. No significant differences between experimental and theoretical values were found for vanillin in each method, indicating the robustness of these procedures. Benzoic acid was used as a blank to prove the presence of phenolic OH and carboxyl group. A detailed procedure for determination of the functional groups is provided in supplementary documents.

3. Results

3.1. Process Efficiency and Samples Compositions

Table 1 depicts the compositions of raw TC (TC_{raw}), extractive free TC ($TC_{extracted}$) and the lignins - MWL, ILL and EOL, determined by two stage sulfuric acid hydrolysis and sugar analysis of the resulting hydrolysates using HPAE-PAD. TC_{raw} consists of 18% extractives and structural components include 39% glucan, 19% hemicellulose sugars (xylan, arabinose, fructose, rhamnose, mannose) and 23% lignin, (**Table 1(a)**). Other components in small quantities include galacturonic and glucuronic acids. Carbon, hydrogen, nitrogen and sulfur content were determined using elemental analysis procedure, oxygen values were obtained by differences, results in supplementary document as **Table S1**. EOL had

Table 1. Composition of raw & extractive free TC and lignin isolates. Values in g/100g biomass (%), dry basis. (a) Composition of raw TC; (b) Extractive free and lignin isolates composition.

(a)

Extractives			Structural components		
H ₂ O Soluble	EtOH Soluble	DCM Soluble	Glucan	Hemicellulose	Lignin
11.0 ± 0.5	5.0 ± 0.2	2.0 ± 0.1	39.0 ± 3.4	19.0 ± 1.1	23.0 ± 1.2

(b)

Parameter	TC _{extracted}	MWL	ILL	EOL
Lignin Yield	^a 24.0 ± 1.4	1.5 ± 0.4	5.1 ± 0.5	10.2 ± 0.2
Lignin content (KL)	24.0 ± 1.4	89.0 ± 1.6	93.0 ± 1.5	90.0 ± 2.2
Glucose	49.0 ± 1.6	2.0 ± 0.2	0.6 ± 0.1	0.2 ± 0.0
HS	22.6 ± 1.4	5.5 ± 0.6	6.1 ± 0.1	NG
GalU	1.8 ± 0.1	0.1 ± 0.0	NG	NG
GlcU	2.1 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	NG

Key: TC_{raw} = Raw TC, TC_{extracted} = Extractive free TC, KL = Klason lignin, ^ayield indicates total lignin in TC_{extracted}, HS = Hemicellulose sugars, GalU = Galacturonic acid, GlcU = Glucuronic acid, NG = negligible.

the least values of impurities compared to ILL and MWL, **Table 1(b)**. Elemental analysis of the lignin samples reveals that there are no significant differences in values of carbon and hydrogen for the 3 samples.

However, ILL had significantly high values of nitrogen, while 2% sulfur was detected in EOL compared to 0% in MWL and ILL and 0.4% in the extractive free TC.

Yields of lignin extraction in this study reflect the extent of the disruption of the chemical integrity of the TC system and solubilisation of lignin, with the highest yield obtained from a more severe reaction conditions (temperature and acid catalysis) [33]. The mild reaction condition of MWL had the lowest yield of 1.5% followed by the ILL with a value of 5%, extracted at 110°C, while the highest yield of 10% was obtained from the EOL process, extracted at 170°C under dilute sulfuric acid catalyst, **Table 1(b)**. Low yield of the MWL has been an issue, especially with herbaceous plant materials like TC, so the values agree with literature [34].

3.2. Structural Characterization of Lignin Fractions

3.2.1. FT-IR Spectra

FT-IR full spectra are presented in **Figure 2(a)** and band assignments are presented as supplementary documents in **Table S2** based on previous publications [24] [35] [36]. The expanded region of interest of the FT-IR spectra for the 3 lignin isolates is portrayed in **Figure 2(b)**, highlighting key differences revealing changes in isolation mechanisms.

The band at 1729 cm⁻¹ assigned to alcoholic acetate carbonyl was observed in the MWL but absent in both EOL and ILL, indicating extensive ester hydrolysis

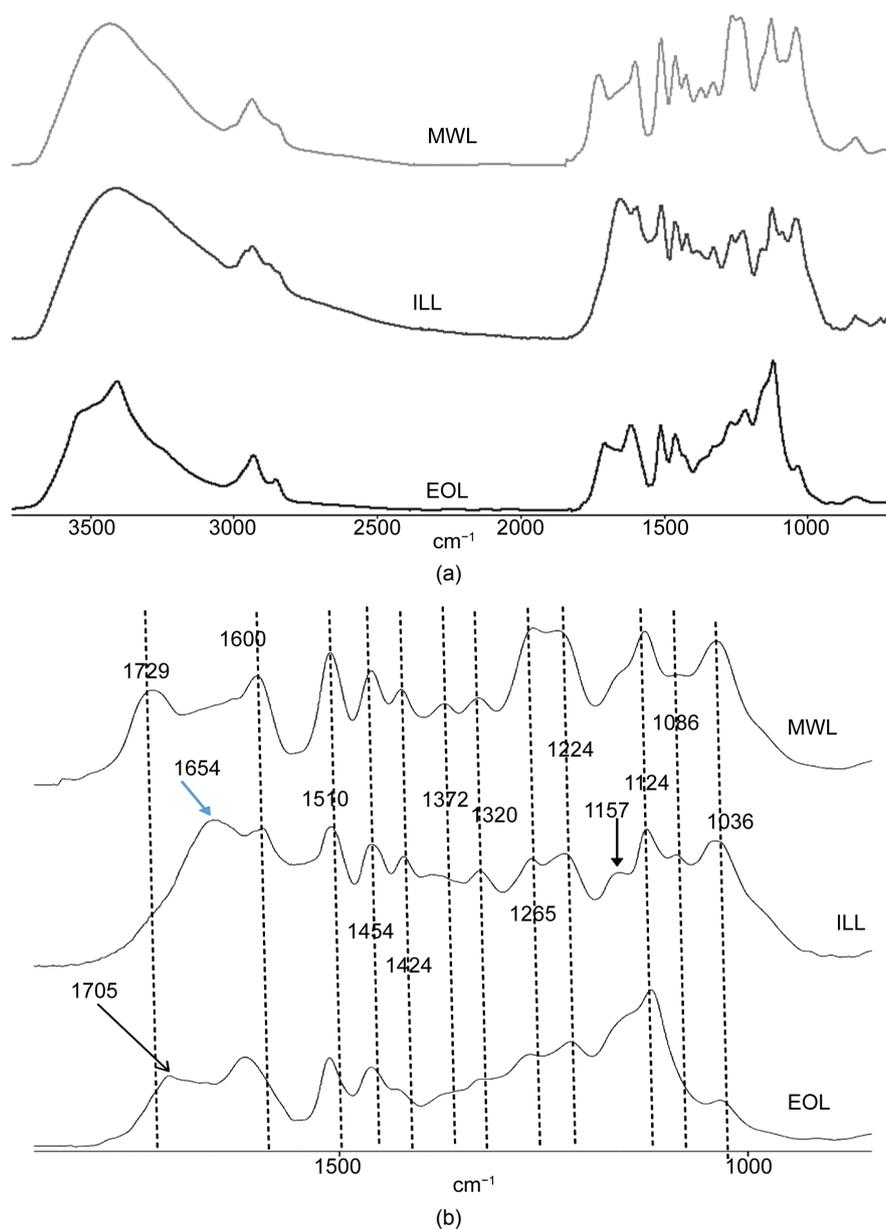


Figure 2. FTIR Spectra: (a) Full spectra of the three lignin isolates, (b) Exploded view of the lignin isolates from *Typha capensis* (MWL, ILL and EOL), revealing key structural differences.

in EOL and ILL. The presence of acetate carbonyl in MWL also reveals that natural acetylation of TC was preserved. This was confirmed by the same absorbance in the extractive free TC (result not presented). Natural acetylation of TC agrees with literature, as lignins and hemicelluloses from herbaceous plants have been observed to be naturally acetylated [37] [38]. An absorbance at 1705 cm^{-1} observed in EOL reveals carbonyl, a hydrolysis product of the β -O-4 ether cleavage. This band is assigned to ketone groups arising from dehydration reactions occurring at the lateral chain of lignin under harsh conditions, and indeed a clear indication of the occurrence of Hibbert's ketones [33] [39]. At bands 1036

cm^{-1} , associated to C-O stretching of primary alcohols, and 1086 cm^{-1} , assigned to C-O deformation in secondary alcohols and aliphatic ethers, higher intensities were observed for $\text{MWL} > \text{ILL} > \text{EOL}$, indicating more primary and secondary alcohols in that order. These bands are also indicative of the differences in carbohydrate contents or polysaccharide impurities, with EOL having the least value [40] [41].

3.2.2. ^{13}C , ^{31}P NMR

Further structural properties of the lignin isolates were studied via ^{13}C and ^{31}P NMR. The broad acquisition spectrum and quantitative option of ^{13}C NMR presents the opportunity to acquire more structural components and bonding information, and the quantification of the various functional groups. **Figure 3** and **Figure 4** show ^{13}C NMR spectra of the acetylated lignin isolates and the expanded aromatic region. In the aromatic region (162 - 103 ppm), the signals of the residues of syringyl (153.8 ppm, 105 ppm), p-hydroxyphenyl (130 ppm, 121 ppm) and guaiacyl (151, 136, 120, 119, 112 ppm) indicate that the lignin species are SGH type, as expected. However, compared to ILL and EOL, higher concentration of G-residue was observed in the MWL implied by more intense signals of the guaiacyl residue. The presence of ferulic acid was reflected by signals at 169.0 (CO) and 127.3 ppm (C-1) in all the 3 lignin isolates with a signal at 111.6 (C-2), peculiar to MWL and ILL, while signals at 148.8 (C-3) and 150 (C-4) ppm were only observed in MWL. The various structural groups were estimated by integration of the regions of interest, referenced to the aromatic region (160 - 103 ppm), where a value of 6.12 was taken to represent the six aromatic carbons and a contribution of 0.12 per 100 aromatic units from side-chain carbons of coniferic and coumaric moieties. Thus, the integral value divided by 6.12 is equivalent to

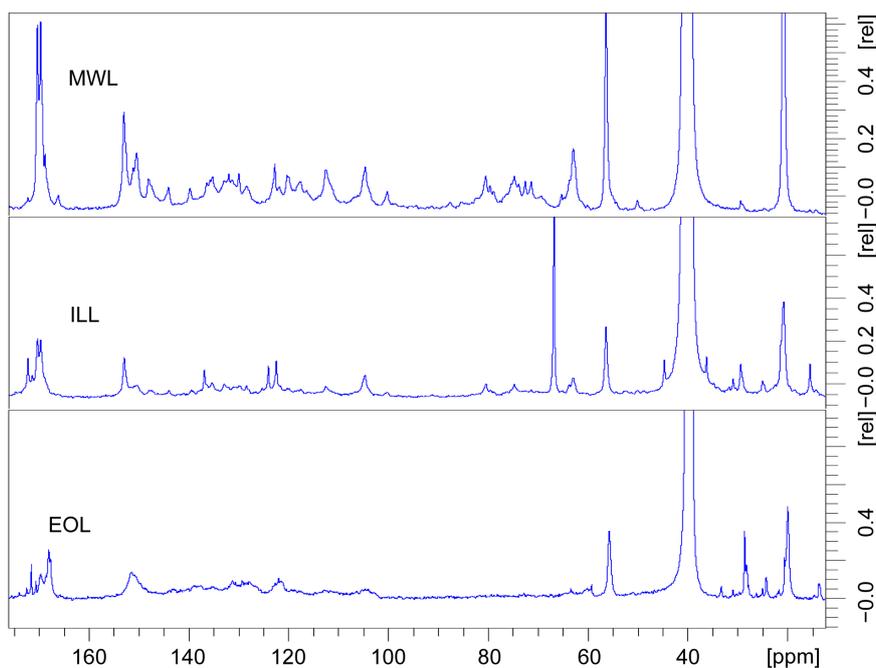


Figure 3. ^{13}C Spectra of acetylated lignin isolates.

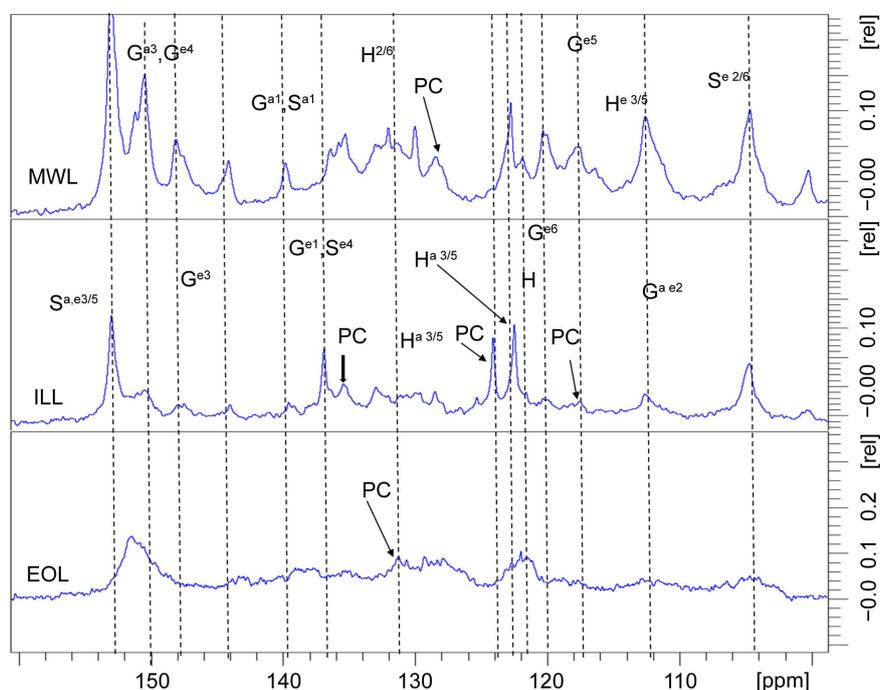


Figure 4. Expanded aromatic region of ^{13}C spectra of acetylated lignin isolates: S = syringyl, G = guaiacyl, H = p-hydroxyphenyl, PC = p-coumaric acid, e = etherified, a = acetylated.

one aromatic ring (Ar) [42] [43]. Values in **Table 2** indicate higher values of aromatic C-C structures (2.22/Ar) and phenolic OH (0.59/Ar), but lower aliphatic OH (0.51) moieties in EOL compared to the corresponding values for ILL (1.94/Ar, 0.38/Ar and 1.34/Ar) and MWL (1.78/Ar, 0.31/Ar and 0.97/Ar). A lower C-O bond was observed in ILL compared to EOL, which also correlates with lower G units in ILL since the S units normally have a higher number of C-O bonds than H and G units. A higher S unit in EOL was further validated in the S/G ratio determined by ^{31}P NMR. The degree of condensation was estimated using the integrals in the region 125 - 103 ppm, assuming that uncondensed herbaceous plant lignin will contain three protonated aromatic carbons per aromatic ring. Therefore, three minus the integration of the region (125 - 203 ppm) yields the degree of condensation [43]. A significant difference in the degree of condensation was observed, with EOL having a leading value of 0.92/Ar compared to 0.42/Ar in ILL and 0.58/Ar in MWL. Further information on the carbon-carbon bond structures were observed in the intense signals of MWL > ILL at 72.0 ppm (C- α in β -O-4' G and S erythro), 70.8 ppm (C- α in β -O-4' G and S threo) and 61.5 ppm (C- γ in β -O-4' G and S threo and erythro) associated with resonances of C- α and C- γ in β -O-4 linkages, [44]. This reveals that a higher proportion of β -aryl ether structures were preserved in the order of mild conditions.

Quantitative ^{31}P NMR enabled the determination of the hydroxyl functional groups, spectra in **Figure 5**. Values of the hydroxyl functional groups obtained from the integration of the respective regions using the cyclohexanol peak as

Table 2. Functional groups data. (a) Determined from spectral regions of ^{13}C NMR; (b) Determined from spectral regions of ^{31}P NMR; (c) Determined by wet chemistry procedure.

(a)				
Structure	Chemical shift range (ppm)	No. of Moieties/Aromatic Ring		
		MWL	ILL	EOL
Methoxy content	57 - 54	0.83	0.74	0.73
Aromatic methine carbons	125 - 103	2.42	2.58	2.08
Aromatic C-C structures	141 - 125	1.78	1.94	2.22
Oxygenated aromatic carbons	160 - 141	1.86	1.67	1.78
Phenolic OH, conjugated COOR	168.6 - 166	0.31	0.38	0.59
Secondary aliphatic OH	169.6 - 168.6	0.61	0.67	0.24
Primary aliphatic OH	172 - 169.6	0.36	0.67	0.27
Degree of condensation ^a	125 - 103	0.58	0.42	0.92

(b)				
Structure	Chemical shift range (ppm)	mmol/g lignin		
		MWL	ILL	EOL
Aliphatic OH	150 - 145	3.59	1.55	0.98
Syringyl OH	144.5 - 140.5	0.39	0.31	1.24
Guaiacyl OH	140.5 - 138.5	0.48	0.2	0.73
p-hydroxyphenyl OH	138.5 - 136.5	0.45	0.2	0.38
Carboxylic	135 - 133	0.16	0.12	0.21

(c)			
Functional group	MWL	mmol/g lignin	
		ILL	EOL
Methoxy	4.3 ± 0.0	2.9 ± 0.0	4.2 ± 0.0
Carbonyl	1.5 ± 0.0	0.2 ± 0.1	3.0 ± 0.1
Carboxyl	0.8 ± 0.0	0.5 ± 0.0	1.1 ± 0.1
Phenolic OH	1.5 ± 0.9	1.5 ± 0.1	2.4 ± 0.1
Aliphatic OH	2.5 ± 0.5	5.1 ± 0.1	2.2 ± 0.1

^aCalculated from $3\text{-I}_{125-103}$.

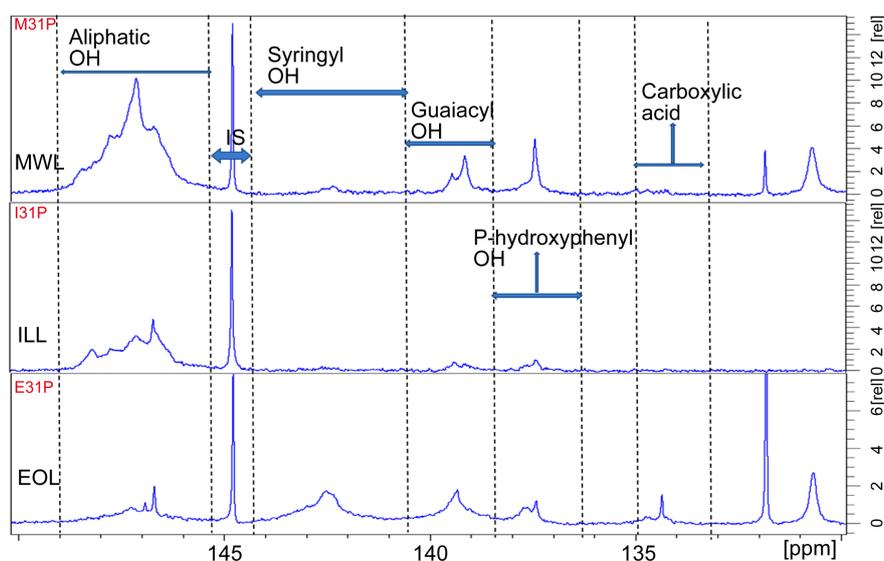


Figure 5. ^{31}P NMR spectra of the lignin isolates.

reference is in **Table 2**. Further evidence of the lateral chain dehydration reaction was seen in values of aliphatic hydroxyl, where EOL had the lowest values, implying that it was more depolymerized. An intense syringyl OH signal around 142.8 ppm was observed in EOL, giving a value of 1.08 mmol/g lignin, compared to values of 0.86 mmol/g and 0.50 mmol/g lignin in MWL and ILL respectively. The S:G:H abundance determined by this method are 30:36:34 (MWL), 43:28:28 (ILL) and 53:31:16 (EOL), giving S/G ratios of 0.87, 1.19 and 1.24 for the MWL, ILL and EOL in that order. The H unit values in the order MWL > ILL > EOL imply increasing hydrolysis of p-coumarate, which is in the order of harsh conditions.

3.2.3. Functional Groups by Wet Chemistry Process: Comparison with Data from NMR

Table 2(c) shows values of the functional groups obtained from wet chemistry procedures. In comparison to ^{13}C and ^{31}P NMR, the phenolic OH yielded the closest absolute values between the methods in each sample. However, significant variation was observed for aliphatic OH, especially for ILL, where wet chemistry procedures gave a value of 5.1 g/mol, compared to the value of 1.55 g/mol by quantitative ^{31}P NMR. Carboxyl values were generally higher in wet chemistry compared to NMR. Carboxyl groups have the lowest value of all the functional groups determined in all the 3 lignin species, examined in both wet and ^{31}P NMR methods. This agrees with literature, as several structural studies indicate that carboxyl groups in native lignin exist in extremely low concentration [30] [45]. Significant variation in carbonyl content was observed in this order EOL > MWL > ILL, with values of 3.0, 1.5 and 0.2 m-mol-g $^{-1}$ lignin respectively, obtained only by wet chemistry procedures. This result indicates significant cleavage of α - and β -aryl ether bonds in EOL much more than in MWL & ILL. Geiger and Fuggerer demonstrated the formation of C=O containing structures from etherified p-hydrocinnamyl alcohol substructures during lignin treatment with phloroglucinol in concentrated HCl acid [46]. In addition to cinnamaldehyde type conjugated carbonyls, spruce MWL was found to contain conjugated carbonyl and possibly non-conjugated (α -carbonyl) groups as well as quinone keta structures [47].

We observed obvious differences in aliphatic OH between the NMR and the wet the chemistry procedure for ILL. The acetylation and hydrolysis reactions involved in the wet chemistry used to determine aliphatic OH may have proceeded in more than one way due to the numerous types of structural elements in ILL [44].

3.2.4. Molecular Weight Distribution Analysis GPC

Table 3 shows the weight average (M_w), the number average (M_n) molecular weights and the polydispersity index (M_w/M_n) of the 3 lignin isolates estimated from the GPC curves using polystyrene as a calibration standard and observed by the UV detector. The GPC curves are presented under supplementary documents as **Figure S1**. Based on the results, the EOL species is more depolymerized

with molecular weights, in the order EOL < ILL < MWL. Also, EOL had a more narrowed molecular distribution, with the lowest polydispersity index value of 1.7, followed by the ILL, with a value of 1.9, while the MWL recorded a broader molecular distribution, with a value of 2.8.

3.2.5. Thermo-Physical Properties

Thermo-physical properties were analyzed by the thermal degradation of lignin isolates, conducted under air atmospheres to achieve maximum degradation, **Figure 6(a)** and **Figure 6(b)**. Results revealed a peak at the range of 80°C - 180°C corresponding to moisture elimination. This was followed by two broad peaks: between 200°C - 400°C, during which cleavage of the functional groups and formation of lower molecular weight products occur, then at the temperature range of 440°C - 630°C, where the structural arrangement of products occur, followed by a long tail beyond 700°C. Various oxygen functional groups and many aromatic rings, with various branches and activity of chemical bonds in lignins exhibit differing thermal stability, thereby scission occurs at different temperatures [48] [49]. Hence the broad temperature ranges (200°C - 700°C) for thermal degradation of these lignins. The TG curves of the lignin decomposition

Table 3. Effects of the processing method on molecular weight distribution in TC lignins.

Sample	M_w (g/mol)	M_n (g/mol)	M_w/M_n
MWL	10959 ± 82	3892 ± 14	2.8 ± 0.0
ILL	4157 ± 19	2238 ± 15	1.9 ± 0.0
EOL	2084 ± 9	1205 ± 12	1.7 ± 0.0

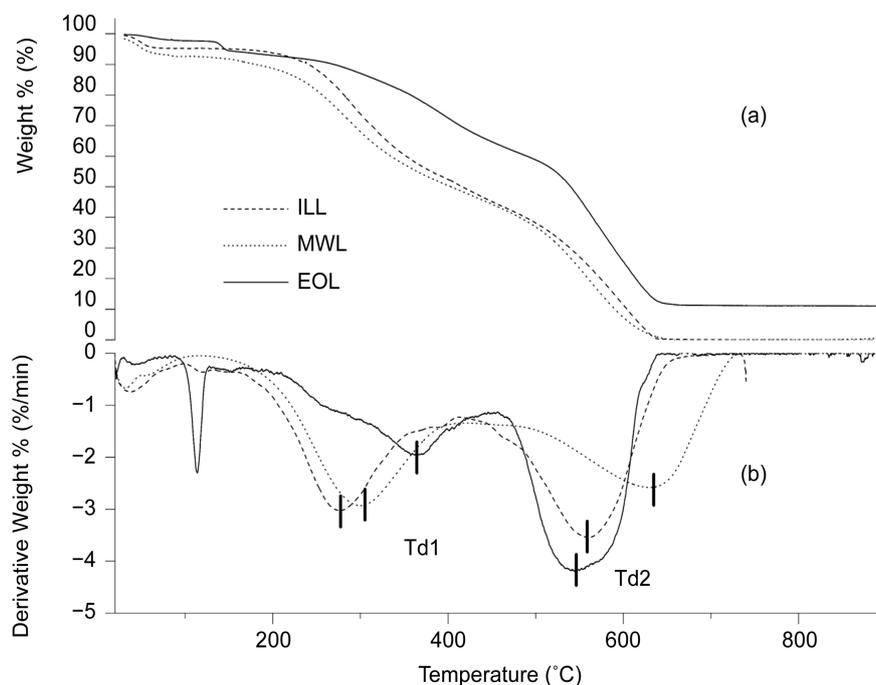


Figure 6. (a) Thermogravimetric curves (TG), (b) Thermal decomposition temperature curves (DTG).

reveal a gentle sloping baseline and flat peaks that makes it difficult to determine an activation energy for the reaction. ILL exhibited very close thermal properties to the MWL, revealed in the two decomposition stages (Td1: 270°C - 365°C and Td2: 545°C - 630°C), **Table 4**. Further similarity between the MWL and ILL is revealed, as both decomposed completely with zero residues whereas 11% residue remained in EOL from 700°C to the final temperature of 900°C.

4. Discussion

IL treatment results in some chemical breakdown of the extracted lignin. In the present study, the chemical modification of lignin during IL extraction was studied and we compared with EOL and MWL.

Compared to MWL, the ILL had a higher extraction yield and purity, but lower molecular weight. The EOL exhibited less impurities, with hemicelluloses sugars and nitrogen as the major contributors to ILL impurities. The origin of nitrogen impurity in ILL was likely from the imidazole component of the 1-ethyl-3-methylimidazolium acetate. A purification effort was performed and although significant reduction in nitrogen impurities was achieved, it was not completely removed from the ILL. Elemental analysis data showed a value of 0.34% nitrogen in the cellulose rich fraction of ILL extraction, compared to 0.71% in TC_{extracted} and 2.78% in the ILL fraction after the purification step, indicating preferential attachment of imidazole to the lignin. Strong affinity of the imidazole to lignin could be attributed to attractive forces between the cation component of the ionic liquid and the OH groups of the lignin, leading to strong bonding. A similar contaminant that originated from 1-butylimidazolium hydrogen sulfate, which was used for lignin extraction was observed, and despite purification steps, it was never completely removed from the lignin [50].

In this study, the spectroscopic and chromatographic data obtained from the analysis of lignins demonstrated the following features:

- phenolic OH content : EOL >> ILL ≈ MWL
- molecular masses: EOL < ILL < MWL

A lower proportion of β -O-4 structures in EOL was observed by ¹³C NMR. Scission of β -O-4 linkages and deconstruction of β - β and β -5' linkages at higher temperatures may have led to the lower molecular weight in EOL. In addition, the higher carboxyl content detected in EOL in wet chemistry, quantitative NMR and FTIR methods, is in accordance with the formation of Hibbert's ketones, due to the acid-catalyzed solvolytic cleavage of aryl-ether linkages. Reduction in

Table 4. Thermal decomposition profiles of the 3 lignin isolates.

Sample	T5%	T10%	Td1	Td2	Ash
	T (°C)				
MWL	130	218	298	630	715
ILL	141	235	270	553	650
EOL	120	235	365	545	701

the aliphatic hydroxyl groups observed in EOL could be attributed to dehydration reactions on the lateral chain that occurred in view of the acidic conditions. All these observations are rationalized by lower aryl-ether bond cleavage rate in ILL and MWL, due to milder conditions. According to George *et al.*, when using imidazolium-based ionic liquid, the α -aryl ether linkages cleavage is due to dehydration reaction, catalyzed by the EMIM anion basicity and its affinity towards water [13]. Furthermore, rather than acting as nucleophiles or a catalyst to cleave β -O-4 linkages, acetate anion in EMIM acts as weak nucleophile to remove OH groups from guaiacyl- β -guaiacyl ether-like linkages to form more stable vinyl ether linkages.

The ^{13}C NMR and thermo-physical analysis also revealed that the ILL is less condensed than EOL. This observation is based on the degree of condensation value of 0.42/Ar for ILL compared to 0.92/Ar in EOL, and the higher thermal stability of EOL. This is consistent with literature, as severe acidic conditions should lead to higher condensation [51]. Although EOL processing was catalyzed by mild dilute sulfuric acid - at high temperatures, cleavage of acetyl groups from hemicelluloses leads to the formation of acetic acid and carboxylic acids, thereby making the condition more acidic [52]. Similar observations of the high value of the degree of condensation was made by Granata and Argyropoulos in steam exploded lignins and was attributed to the scission of β -O-4 bonds, leading to the release of the syringyl monomer [26]. This is in consonant with the fact that guaiacyl is more involved in the recondensation reaction, therefore while G decreases, S is increased. This also correlates with the highest value of the syringyl unit exhibited by EOL as reflected in ^{31}P NMR data. At the temperature range of 440°C - 630°C, where DTGmax occurred for the lignin samples, thermal degradation in this zone involved fragmentation of inter-unit linkages, releasing monomeric phenols into the vapor phase and decomposition of some aromatic rings [53]. Beyond this temperature range, the ILL and MWL revealed differing behavior, compared to EOL, by exhibiting complete decomposition, while EOL had about 11% of material remaining un-volatilized, indicating the formation of highly condensed aromatic structures [53] in EOL. Conversely, there were very low C-C linkages at this zone for ILL and MWL, resulting in more thermal degradation, where volatilized monomeric fragments were released into vapor phase, leading to complete decomposition. Moreover, inorganic salts and acids are known to act as flame retardants in pyrolysis and combustion, leading to an increase in ash yields [54] [55]. The presence of sulfates from any of the metallic substances earlier observed in EOL may have influenced the ash yield exhibited.

With a relatively lower degree of condensation, lower aromatic C-C structures and higher aliphatic OH than EOL, the ILL displays a chemical structure closer to that of MWL. Consequently, ILL could be useful in areas requiring high quality lignin with high ether contents and low C-C structures. Consequently, ILL could be useful in areas requiring high quality lignin with high ether contents and low C-C structures, for instant, for further depolymerization by chemical,

enzymatic or thermal methods for the production of aromatic monomers.

5. Conclusion

The cleavage of ether linkages is primarily responsible for lignin breakdown in both ionic liquid and organosolv methods. The solvent's properties and treatment conditions influenced the depolymerization and functionalization of the lignin isolates from TC. The ILL from TC demonstrated higher aliphatic OH but low condensation because of low dehydration reactions and low C-C bonds but higher β -O-4 structures compared to EOL. By and large, ILL exhibited closer structures and reactivity to the MWL counterpart than EOL. At the moderate condition of 110°C, with no acid catalysis for ILL treatment, compared to the more severe condition of 170°C, under dilute acid catalysis in EOL, lignin extraction using EMIMOAc IL looks promising as a greener technology, especially in view of the low energy requirement in its isolation and the possibility of recovery and reuse of the IL. In addition, there are possibilities to use ILL in applications requiring high quality lignin with high ether contents and low C-C structures. In addition, there are possibilities to use ILL in applications requiring high quality lignin with high ether contents and low C-C structures, for example, aromatic monomers could be produced from ILL by further depolymerization using chemical, enzymatic or thermal methods. Findings revealed in the current study may provide valuable insights on the impact of ionic liquid mediated extraction on lignin depolymerization and may instigate further investigations in this direction towards identifying better techniques for utilizing lignocellulosic biomass.

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Supplementary Materials

Procedure for MWL Isolation

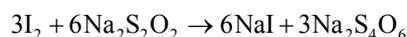
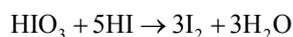
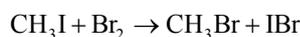
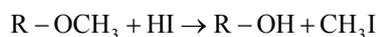
The procedure by Bjorkman as modified by Obst and Kirk [1] and Rencoret *et al.* [2], was used to process MWL from TC. The EFTC was ball milled in batches of about 40 g in 500 mL grinding jars with 20 mL steel balls using a Retsch Planetary Ball Mill PM 100 for 50 h. The mill was programmed to operate at intervals of 10 minutes active milling followed by 5 minutes rest and reverse rotation after each 5 minutes milling to avoid overheating and agglomeration. A total of 100 g of ball milled TC was placed in 2 L round bottom flask and 1 L of 96% aqueous dioxane was added and magnetic stirred at room temperature for 24 h. The mixture was centrifuged to separate insoluble material while the lignin contained in the supernatant was recovered by evaporating the liquids to dryness under reduced pressure at 40°C using rotary evaporator - Buchi rotavapo R-210 (equipped with vacuum controller V-850 and heating bath B-491). The process was repeated 2 times and lignin joined together. The dry lignin residue was dissolved by pouring 30 mL of acetic acid/water (9:1, v/v) into the round bottom flask containing the lignin. Lignin was precipitated by adding the acetic acid water lignin mixture dropwise to 300 mL of stirred water and separated by centrifuging for 30 minutes at 4700 rpm. The precipitated lignin was re-dissolved in 30 ml solution of dichloromethane: ethanol (2:1, v/v) and the mixture was then centrifuged to remove insoluble material. Lignin was precipitated using 300 mL of diethyl ether separated by centrifuge.

Procedure for Determination of Functional Groups Using Wet Chemistry Methods

Detail procedure for determination of each functional groups are described as follows:

Determination of methoxy contents

The procedure by Vieböck and Schwappach [3] detailed by Zakis [4] was used to determine the methoxy contents. The mechanism of action of the procedure is based on the following series of reactions:



First 20 - 25 mg of dried sample was weighed in the reaction chamber, then 0.2 g red phosphorus, 0.5 g phenol, 6 drops of acetic anhydride and 5 mL hydroiodic acid were added. The Vieböck Schwappach apparatus was then coupled allowing carbon dioxide gas to flow through the gas inlet into the reaction chamber at about 1 bubble per 2 seconds flow rate. The mixture was boiled for 1

hour by heating to 140°C in an oil bath. At the end of 1 hr boiling, the apparatus was decoupled and content of the reaction chamber quantitatively transferred into an Erlenmeyer flask containing 1.5 g sodium acetate dissolved in a little water. The reaction chamber was rinsed into the Erlenmeyer flask several times with total of about 150 mL deionized water. Then formic acid was added dropwise until the solution became fully discolored. The content was kept for about 5 minutes and 3 drops of methyl red added followed by 10 mL of solution of potassium iodide (10 g in 100 mL deionized water) and 5 mL dilute H₂SO₄ acid (50% v/v) added. The solution was then titrated using sodium thiosulphate standard solution with 1% starch solution as an indicator.

Methoxy content was calculated by the relationship:

$$\text{OCH}_3 \left[\text{mmol} \cdot \text{g}^{-1} \right] = \frac{c * (v/6) * \tau}{m}$$

where c is the concentration of Na₂S₂O₃ [mol·L⁻¹], V is the volume of Na₂S₂O₃ required [mL], 6 is the ratio of n(OCH₃)/n(Na₂S₂O₃), τ is the factor of Na₂S₂O₃, and m is the sample's weight [g].

Determination of total hydroxyl contents

Hydroxyl content consisting of phenolic and aliphatic hydroxyl was determined using acetylated lignin samples based on procedure by Chen [5]. The technic is based on acetylation and cleavage of the acetate to produce acetic acid, obtained acetic acid is then titrated using NaOH.

60 mg of samples were weighed in a 50 mL double necked flask, 4 mL H₂SO₄ acid (50%, v/v) added and magnetic stirring rod inserted. The flask was coupled to reflux condenser and second neck closed with stopper, all joints sealed with grease that have been tested to be blank. The mixture was heated with stirring to its boiling point in an oil bath at 120°C for 1 hour. The mixture was then relieved from the oil bath and 10 mL deionized water added from the top of the reflux condenser. The mixture was then boiled again for 10 minutes, relieved from the oil bath and left to cool down for 10 minutes. The reflux condenser was rinsed with deionized water into the flask and the flask was decoupled from the reflux condenser and coupled to a short path distillation apparatus with a thermometer and a 100 mL round-bottom flask as receiver flask. The second neck was rinsed and replaced with dropping funnel without pressure compensator. The mixture was heated to begin distillation and deionized water gradually added using the dropping funnel each time the temperature drops below 100°C. When about 70 mL distillate is obtained, the distillation is stopped and distillate quantitatively transferred into an Erlenmeyer flask and 3 - 5 drops of phenolphthalein added followed by titration with 0.1 N NaOH. Blank is performed with unacetylated lignin. Total hydroxyl content was calculated using the formula below.

$$[\text{OH}] \% = \frac{\tau * V * 170}{m - 4.2 * \tau * V}$$

where τ is the concentration factor of the standard solutions, V is the titer value obtained by the difference between the sample and blank titer values, 170 is a factor ($c[\text{NaOH}][\text{mol/L}] * M(\text{OH})[\text{g/mol}] * 100[\%]$), m is weight of sample and 4.2 is the factor from ($c[\text{NaOH}][\text{mol/L}] * (M\{\text{OC-CH}_3\}[\text{g/mol}]-M(\text{H})[\text{g/mol}])$)

Determination of carboxyl and phenolic contents

Carboxyl groups are determined based on acidity of this group by a neutralization process of the carboxylic acid using a potentiometry to detect the titrimetric end point [6]. Concurrently, the weakly acidic phenolic hydroxyl groups can be determined. The procedure entails non-aqueous potentiometric titration of lignin with tetra-*n*-butylammoniumhydroxide in the presence of p-hydroxybenzoic acid as internal standard. The procedure outlined by Dence [6] with slight modification was used to determine the phenolic and carboxylic groups. A specialized equipment for this is the automated Titroline Alpha by Schott with Pt 6280 electrode consisting of an input keyboard, a pumping device connected to the standard solution tank for titration, a magnetic stirrer and a PC for data acquisition. Titer values for lignin samples, blank and internal standard have been optimized, so specified range of titer values for stopping the titration have been documented during which the required inflexion points for calculations may have been acquired. First, standard solution was prepared by weighing 62.5 g tetra-*n*-butylammonium hydroxide (TnBAH) into a 3 L chemical bottle and 2 L isopropyl alcohol was added and mixed thoroughly to obtain 0.05 M of TnBAH standard solution.

To obtain the concentration of TnBAH, 0.15 g benzoic acid was weighed into a titration vessel and 30 mL DMF added and stirred for 30 minutes. This was followed by titration using the standard solution to stop at about 30 mL titer value from which TnBAH concentration was determined using equation:

$$N\left(\frac{\text{mol}}{\text{L}}\right) = \frac{M(\text{benzoic acid})[\text{g}]}{V(\text{TnBAH})[\text{mL}] 0.12212}$$

where 0.12212 benzoic acid factor (molar mass of benzoic acid [g/mol]/1000).

At least two replications of each sample was analyzed by weighing 0.35 g of the sample and 0.07 g p-hydroxybenzoic acid in Erlenmeyer flask, then 30 mL DMF added and stirred for 30 minutes. This was followed by titration using the standard solution to about 65 mL titer value when the inflection points were recorded.

Theoretical consumption a , assigned to the internal standard (p-hydroxybenzoic acid) in mL is given by equation:

$$a = \frac{m}{0.13812 N(\text{TnBAH})}$$

where m is the weight of p-hydroxybenzoic acid, 0.13812 is the molar mass of p-hydroxybenzoic acid [$\text{g}\cdot\text{mol}^{-1}/1000$], $N(\text{TnBAH})$ is the concentration of TnBAH solution as calculated in equation earlier.

Carboxyl content in $\text{mmol}\cdot\text{g}^{-1}$ is obtained by:

$$\text{COOH} = \frac{(V_x - a) * N(\text{TnBAH})}{m}$$

where V_x is titer value up to the first inflection point (mL), a is the theoretical consumption as calculated, $N(\text{TnBAH})$ is the concentration of TnBAH in mol/L, and m is lignin sample weight in g.

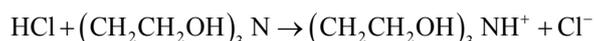
The phenolic hydroxyl content in $\text{mmol}\cdot\text{g}^{-1}$ is calculated using the equation:

$$\text{OH} = \frac{(V_y - V_x - a) * N(\text{TnBAH})}{m}$$

where V_x and V_y are the titer values up to the first and second inflection points (mL) respectively, a is the theoretical consumption of p-hydroxybenzoic acid, $N(\text{TnBAH})$ is the concentration of TnBAH in mol/L, m is the lignin sample weight in g.

Determination of Carbonyl content

The procedure based on oximation of carbonyl groups using hydroxylamine hydrochloride was applied in this process [4] [7]. Triethanolamine is added which reacts with the released hydrochloric acid to shift the equilibrium of the reaction to oxime formed and the excess base is titrated with standard solution of hydrochloric acid. Reactions involved are as below:



Two solutions were prepared. Solution one - 1.2 g of triethanolamine was weighed in 50 mL volumetric flask and volume made up to the mark using un-denatured 96% (v/v) ethanol and stirred. Solution two was prepared by weighing 0.7 g hydroxyammonium chloride into 50 mL volumetric flask and dissolved by adding 5 mL deionized water then 25 mL of solution one added and volume made up using un-denatured 96% (v/v) ethanol.

Sample analysis was carried out by weighing 80 mg of dry lignin in a test tube, then 2 mL DMSO was added and mixed thoroughly to dissolve. 5 mL of solution two was added and thoroughly mixed. The tube was placed in water bath at 80°C for 5 minutes then sealed with rubber stopper and heating continues for 2 h. This was followed by quantitatively transferring the solution into a beaker and titrated using 0.05 N HCl to pH 3.3. The procedure was repeated for blank without lignin sample.

Carbonyl content in $\text{mmol}\cdot\text{g}^{-1}$ was calculated using the relation:

$$\text{CO} [\text{mmol} \cdot \text{g}^{-1}] = \frac{c * V * \tau}{m}$$

where c is the concentration of HCl ($\text{mol}\cdot\text{L}^{-1}$), V is the volume of HCl obtained from the difference of titer values of samples and that of the blank, τ is the HCl concentration factor, and m is weight of sample.

Table S1. Elemental composition of TC_{extracted}, lignin isolates and IL CR, values in %.

Sample	TC _{extracted}	MWL	ILL	EOL	IL CR
Carbon	45.02 ± 1.24	57.32 ± 0.84	56.11 ± 1.22	56.31 ± 0.91	46.21 ± 0.72
Hydrogen	5.88 ± 0.04	5.89 ± 0.08	6.6 ± 0.14	5.61 ± 0.23	6.2 ± 0.15
Nitrogen	0.71 ± 0.02	0.39 ± 0.00	5.8 ± 0.25, ^a 2.78 ± 0.12	0.84 ± 0.03	0.3 ± 0.05
Oxygen	47.97 ± 0.56	36.4 ± 0.38	31.91 ± 1.04	35.23 ± 0.34	47.29 ± 1.68
Sulfur	0.42 ± 0.24	ND	ND	2.01 ± 0.08	ND

TC_{extracted} - Extractive free TC; IL CR - cellulose rich fraction from ionic liquid procedure; ^avalue after purification steps.

Table S2. Absorbance and assignment of IR spectra of the 3 lignins (MWL, ILL & EOL).

Band range (cm ⁻¹)	MWL	ILL	EOL	Assignment
	Intensity			
3500 - 3300	1.13	1.12	1.54	Presence of alcoholic and phenolic OH groups stretching vibrations
3000 - 2842	0.52	0.69	0.66	C-H stretching in methyl and methylene groups
	0.00	0.00	0.36	
1740 - 1720	0.71			Alcoholic Acetate carbonyl
1719 - 1705			0.80	C=O stretch in unconjugated ketones, carbonyl and ester groups
1654		1.04		C=O stretch in unconjugated p-sybst. aryl ketones
1616 - 1600	0.82	0.00	1.02	C=C stretching of aromatic ring in lignin
1567		0.99		C=C stretching (ionic compounds only)
1515 - 1505	4.00	1.00	1.00	C=C stretching of aromatic ring in lignin
1470 - 1460	0.87	0.87	0.89	C-H deformation; asym, in -CH ₃ and -CH ₂ -
1460 - 1422	0.73	0.78		Aromatic skeletal vibrations combined with C-H in plane deformation
1386 - 1371	0.62	0.65		C-H deformation symmetric
1330 - 1323	0.65	0.69		S ring plus G ring condensed; <i>i.e.</i> G ring substituted in pos. 5
1270 - 1260	1.19	0.76	1.05	G ring plus C=O stretch
1230 - 1217	1.17	0.80	1.20	C-C, C-O AND C=O stretching of G
1166 - 1163		0.66		C-N stretch in aliphatics
1128 - 1118	1.17	0.98	1.79	aromatic C-H deformation of S, plus secondary alcohols + C=O stretch
1088		0.80		C-O deformation in secondary alcohols and aliphatic ethers
1040 - 1030	1.10	0.90	0.52	C-O stretching of primary alcohols
835 - 834	0.23	0.17	0.16	C-H out of plane in positions 2, and 6 of S, and in all positions of H units

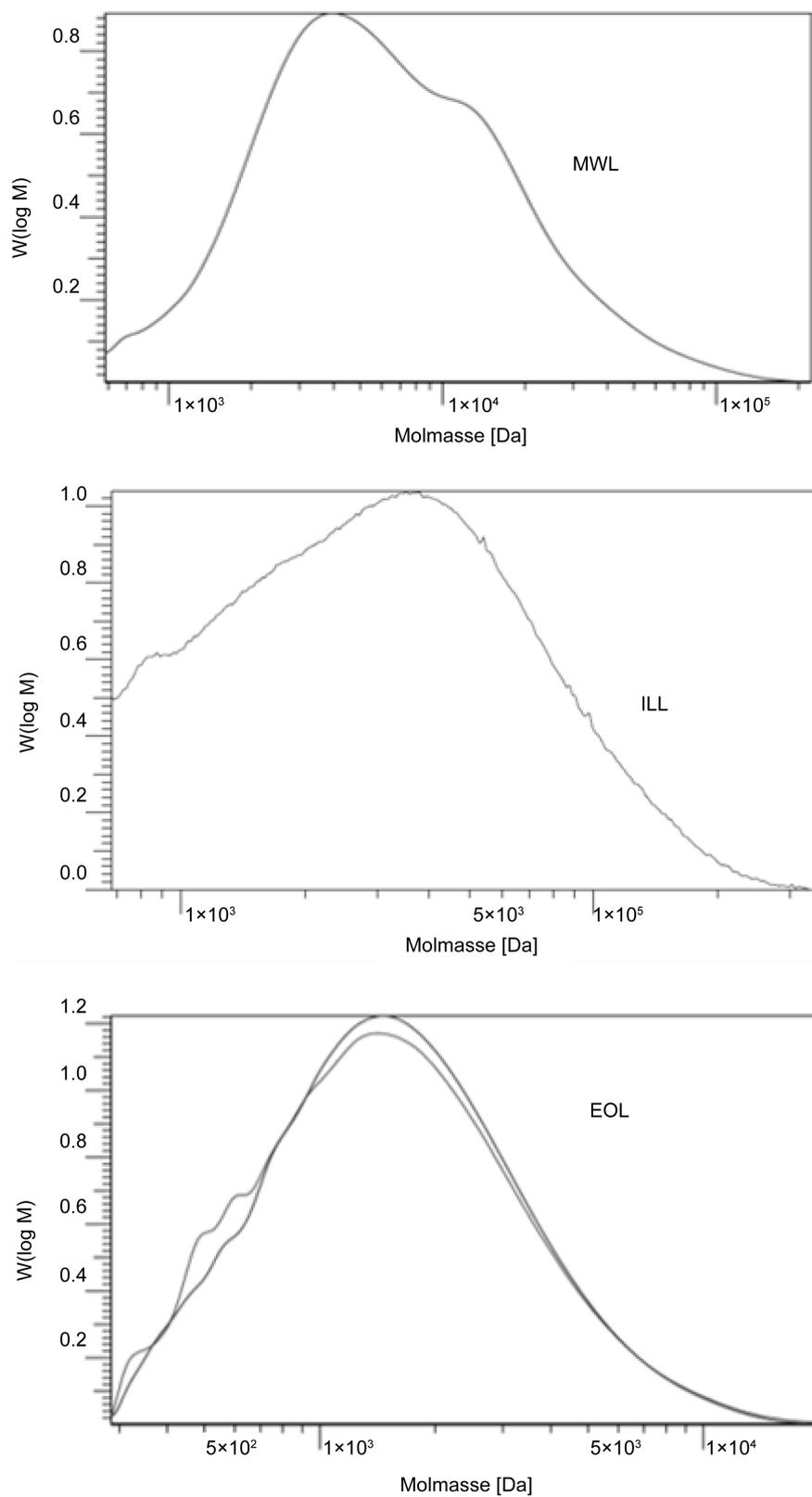


Figure S1. Gel permeation chromatography (GPC) curves of MWL, ILL and EOL.

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