Original Research Article

Physicochemical properties of Soda and Kraft lignin extracted from *Gmelina arborea* wood

Comment [O.I.1]: SUGGESTED TITLE: Determination of Functional Groups and Surface Morphology of Soda and Kraft lignin extracts of *Gmelina arborea* wood

Abstract

Lignin, an amorphous biopolymer is one of the major components of wood. In this study, lignin was extracted from *Gmelina arborea* wood using Soda and Kraft pulping processes. The lignin was characterized using Fourier Transformed Infrared Spectrometer (FTIR), UV/visible spectrometer, electrospray ionization mass spectrometer and scanning electron microscope (SEM). Results revealed that *G. arborea* wood lignins contained several chemical functional groups. Kraft lignin contain carboxyl and thiol group in addition to other functional groups. UV/ visible spectroscopy results revealed that Soda lignin absorbed at higher wavelength than Kraft lignin. The concentrations of both conjugated and non-conjugated phenolic group were higher in Kraft lignin than Soda lignin. ESI-MS spectra revealed that the composition of the monomers was higher in Kraft lignin while dimers composition was higher in Soda lignin. The surface morphology of both lignins were heterogeneous with uneven particle size.

Comment [O.I.2]: Please mention all the functional groups for Soda lignin and Kraft lignin.

Comment [O.I.3]: Please add a brief contribution to knowledge of this work here as a conclusion.

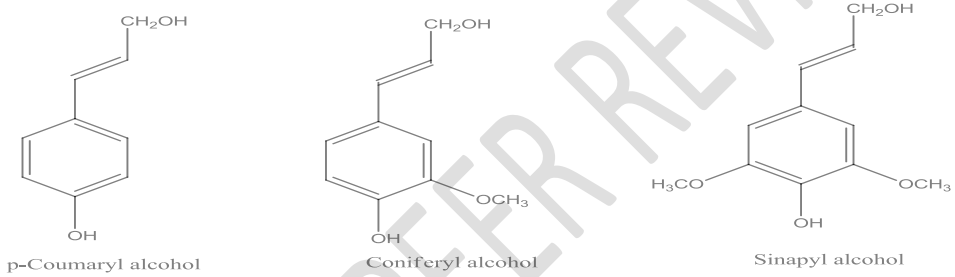
Keywords: *Gmelina arborea*, Kraft lignin, Soda lignin, physicochemical properties.

Introduction

Lignin is an abundant naturally occurring polyphenolic biopolymer present in lignocellulosic biomass cell wall and serves as binding agent for celluloses and hemicelluloses. Apart from providing mechanical support to the plant, it also plays a vital role in plant defense against various biotic and abiotic stresses as well as in seed dispersal (Boyce *et al*., 2004; Bhuiyan, 2009). Plants contain about 15 – 30 % by weight of lignin, together with cellulose and hemicelluloses (Thakur *et al*., 2014). Unlike most natural polymers such as cellulose, that consist of single inter monomeric linkages, lignin is a branched biopolymer consisting of many carbon-to-carbon and ether linkages. It is a heterogeneous polymer that composed of three types of

phenyl propane monomeric unit, namely; p-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol (Fig. 1) (Zakzeski *et al*., 2016; Upton and Kasko, 2016). The structure of these monomeric units differs in their degree of methoxylation of the aromatic ring at the *ortho*-, *meta*- and *para*-positions (Ragauskas, 2014).

The composition of the monomeric units in lignin is affected by the plant species, growth duration and lignin extraction method (Vanholme, *et al*., 2008; Gosselink *et al.*, 2010). Equal amounts of coniferyl and sinapyl units are present in hardwood lignin, while softwood lignin contains a greater percentage of coniferyl units (92-95%).



p-hydroxyphenol (H) Guaiacyl (G) Syringyl (S)

Fig.1 : Monomeric units in lignin

These monomeric units undergo free radical polymerization to form lignin macro structure (Khaldi-Hansen and. Kamm, 2016). According to Terashima *et al*., (2012), the phenoxy radical stabilization occurs when monolignol radicals are randomly coupled to one another in any of the positions of the unpaired electron within the plant cell wall, leading to the formation of lignin polymer. Lignin composition and structure depend on the availability, and nature of monolignol present and the type of bonds formed during the free radical polymerization of monolignols. This radical polymerization led to the formation of condensed (5-5′, β-5′, β-β, β-1′) or non-condensed bonds (β-*O*-4′, 4-*O*-5′, α-*O*-4′) linkages in lignin macro structure (Terashima *et al*., 2012). The most common lignin bond is the β-O-4 linkage, which constitutes about 50% in softwood and 60% in hardwood (Sjostrom, 1993; Henriksson, 2007). In hardwood, these β-O-4 linkages consist of about 40% guaiacyl type and 60% syringyl type (Sjostrom, 1993). The carbon-carbon bonds in lignin are generally more stable compared to the ether bonds and often resist to

processes such as chemical pulping (Henriksson, 2007). The presence of several functional groups in lignin makes it is possible to modify lignin into different industrial products such as bioplastics, binders, adsorbent, dispersant, corrosion inhibitors, epoxy resins as well as some industrial chemicals such as phenol, catechol, vanillin, benzene etc., (Thakur *et al.*, 2014; Kadla, *et al*.,2002). The utilization of lignin and its modified products is worth considering since lignin is a waste material and does not compete with food (Laurichesse and Averous, 2014).

Cherif *et al*., (2020) reported the physicochemical properties of Organosolv and Kraft lignins from selected hard and soft woods. Fingerprint analysis by FTIR showed unique peaks corresponding to lignin, such as C=C and C-O in aromatic rings, however, there was no significant differences in the fingerprint result between both lignin. Corn stover, rice straw and softwood Kraft lignin samples were characterized by Fox and McDonald, (2010) using pyrolysis GC-MS, ^{13}C CP/MAS NMR spectroscopy, and permanganate oxidation degradation. Pyrolysis GC-MS showed the softwood Kraft, corn stover, and rice straw lignins to be *G* – type, *H*/*G*/*S* – type, and *G*/*S* – type, respectively. The Kraft and rice straw lignins were found to have high degrees of condensation. Watkins *et al*., (2014) extracted and characterized lignin from wheat straw, flax fiber, pine straw, and alfalfa. Also, Yang *et al*., (2016) carried out the comparative study of lignin from wheat straw obtained using soda-anthraquinone or kraft pretreatment and reported that the main lignin linkages were β -aryl ether substructures (β-O-4), phenylcoumaran (β -5) and resinol (β - β) substructures, while minor content of spirodienone (β -1), dibenzodioxocin (5-50) and α, β -diaryl ether linkages were detected as well. The physicochemical properties of lignin from *Chromolaena odorata* and *Tithonia diversifolia* have also been reported (Nwosu and Muzakir, 2015).

Numerous reports exist on the biological activities of *G. arborea* organs. These include the phytochemical constituent, antioxidant, antihelminthic, antidiabetic, antimicrobial, diuretic, antipyretic and analgesic activities (Khare, 2004; Unnikrishnan, 2007; Satyanarayana, 1986; Patil *et al*., 2009; Rohith *et al*., 2012; Ambujakshi and Thakkar, 2009; Pattanayak, *et al*., 2011; El-Mahmood *et al*., 2010; Sravani *et al.* 2011).

To the best of our knowledge, reports on the physicochemical properties of *G. arfborea* lignin is scarce. This present study aimed at extracting lignin from *Gmelina arborea* wood using Soda and Kraft pulping processes, and also evaluating the physicochemical properties of the extracted lignin in order to ascertain their industrial potentials.

Comment [O.I.4]: There is paucity of information on the physicochemical properties of *G arborea* lignin, hence the present study, aimed at

Comment [O.I.5]: The physicochemical properties should be functional groups and surface morphology

Material and methods

2.1 Sample Collection and Preparation

The sample, *Gmelina arborea* was collected from Ikot Obio Inyang, in Etinan Local Government Area of Akwa Ibom State. The tree was cut into logs and further processed into chips of about 1cm by 1cm; air dried for three months, and stored in plastic bags pending pulping.

2.2 Extraction of lignin

Lignin was extracted from the *Gmelina arborea* wood chips using the Soda and Kraft pulping processes.

2.2.1 Soda pulping processes

Briefly, 100g of sample chips were soaked in 1000 mL of 20 % NaOH solution in a 10 L laboratory digester. The pulping was done for three hours at 30 ^{o}C. After the pulping, the pulps were separated from the black liquor and beaten using mortar and pestle. It was quantitatively transferred to the digester and pulped again for another three hours using the same pulping liquor in order to extract greater quantity of lignin. Thereafter, the pulps were separated from black liquor and washed with tap water. The black liquor was kept pending precipitation of lignin.

2.2.2 Kraft pulping process

In this process, wood chips (100g) were pulped with Kraft pulping liquor at 30 % sulphidity. The liquor was prepared by dissolving NaOH and Na_2S at a ratio of 3:1 in 1000 mL of water. The wood chips were soaked in the Kraft pulping liquor at the ratio of 1: 10 (wt/v). Pulping was done using the same procedure described previously for Soda pulping.

3.4 Precipitation of lignin from black and spent liquors

The filtrate obtained during Soda pulping is known as black liquor, whereas the one obtained during Kraft pulping is known as spent liquor. Each of this liquor was precipitated using 3 M

H_2SO_4. The acid was added gradually and lignin was obtained as a precipitate at a pH of 2 (Li and Ge, 2011). The obtained lignin was filtered, washed with distilled water and dried in the oven at 70 ^oC for 24 hours.

The percentage yield of the lignin based on oven dry weight was calculated as follows:

$$\text{Percentage yield (\%)} = \frac{W_2}{W_1} \text{ X } 100$$

Where w_1= weight of the sample (g)

w_2 = weight of the lignin (g)

2.2 Characterization of Lignin

2.2.1 Determination of functional groups

The functional group of Soda and Kraft lignin were determined using Fourier Transform Infrared spectroscopy (FTIR). KBr disks containing 1 % finely ground lignin samples were used.

2.2.2 Determination of wavelength of maximum absorption (λ_{max}) and absorbance

The wavelength of maximum absorption (λ_{max}) and absorbance of Sand Kraft lignin were determined using Ultra Violet/ visible spectroscopy in accordance with Surina *et al.* (2015). Briefly, stock solutions of lignin were prepared by dissolving 4 mg of lignin in 50 ml of 0.2 M NaOH, the mixture was shaken in a mechanical shaker for 30 min to achieve complete dissolution. UV-VIS spectrophotometer was used to determine the absorbance and λ_{max} at the absorption region of 190 to 450 nm, scan speed 5 nm/s and 1nm resolution.

2.2.3 Determination of types of phenolic structure present in Kraft and Soda lignin

UV- spectroscopy offers a simple and reliable way of determining phenolic hydroxyl groups present in lignin. This technique is based on the difference in the absorption between lignin in

alkaline and in neutral solution. 5 mg of lignin was dissolved separately in 5 ml dioxane and 5 ml of 0.2 M NaOH was added. From each lignin solution, 2 ml of lignin solution in dioxane was diluted to 25 ml using a buffer solution of pH 6, (citrate /NaOH) and 5 mL of lignin solution in 0.2 M NaOH was also diluted to 25 mL using 0.2 M NaOH (Gartner and Gellerstedt, 1999). This gives each lignin solution a concentration of about 0.04 g/L. The difference UV- VIS spectra were obtained using Genesys spectrophotometer in the absorption region of 200 to 450 nm, scan speed 5 nm/s and 1nm resolution. The lignin solution in dioxane was used as a reference and the lignin solution in 0.2 M NaOH was measured against it. From the difference spectra, the absorbance values at 300 and 350 nm were recorded.

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Concentration of each type of phenolic structure of lignin were calculated as follows:

a) Non conjugated phenolic structures (I and III)

$$\text{OH (I and III)} = [\,(0.250 \text{ x A300 nm (NaOH)} + 0.0595 \text{ x A350nm (NaOH)}]$$

$$\text{x}\frac{1}{(c\ x\ d)}\ \text{mmol/g}$$

b) Conjugated phenolic structures (II and IV)

$$\text{OH (II and IV)} = [\,0.0476 \text{ x A350 nm (NaOH)}] \text{ x } \frac{1}{(c\ x\ d)}\ \text{mmol/g}$$

c) Total amount of phenolic hydroxyl groups

$$\text{OH (I + II + III + IV)} = [\,0.250 \text{ x A300nm (NaOH)} + 0.107 \text{ x A350nm (NaOH)}]$$

$$\text{x } \frac{1}{(c\ x\ d)}\ \text{mmol/g}$$

Where c = The concentration of lignin in g/L and

d = The path length through the sample in cm.

A= Absorbance

2.2.5 Determination of surface morphology

Surface morphology of lignin was determined using scanning electrons microscope (SEM).

2.4.6 Determination of lignin monomers and dimers

The monomeric and dimeric unit of the kraft degraded from kraft and soda lignin were determined by Electron spray ionization - mass spectrometer (ESI-MS). The molecular formula of the lignin monomers and dimers were determined using high resolution ESI-MS at the range of 100 to 500 m/e.

3.0 Results and Discussion

3.1 Functional groups present in Soda and Kraft lignin

FTIR spectra (Table 1) showed a broad intense absorption band at 3000 – 3500 cm^{-1} in both Soda and Kraft lignin corresponding to the stretching vibration of the free aliphatic and aromatic hydroxyl group and bonded hydroxyl of carboxylic acids ((Faix, 1992; Namasivayam and Kavith, 2006). The peaks around 1595 – 1510 cm^{-1} found in both samples are attributed to the presence of the C-C stretching of aromatic ring (Han *et al*., 2010). The absorption peak occurring at 1595cm^{-1} and 1599cm^{-1} in Kraft and Soda respectively are characteristics of aromatic C-O stretching of phenolic hydroxyl groups. There was aliphatic OH bending vibrations at 1036 and 1077 cm^{-1} in KL and 1036 and 1088 cm^{-1} in SL. There were C-O vibration band of phenol around 1200 – 1215 cm^{-1}, in both lignin samples. The band at 1300 cm^{-1} and 1200 cm^{-1} indicate presence of both syringyl and guaiacyl groups in lignin (Chen, 2014). At 1600 and 1510 cm^{-1}, aromatic skeletal vibration bands were seen for both Soda and Kraft lignins. Symmetric aryl stretching was found at 1595.3 cm^{-1} and 1599.0 cm^{-1} in Kraft and Soda lignin respectively, while asymmetric aryl stretching was found at 1513 cm^{-1} and 1509 cm^{-1} in Kraft and Soda lignin respectively. Stark et al., reported 1594 cm^{-1} (Symmetric aryl stretching) and 1512 cm^{-1} (asymmetric aryl stretching) for softwood (P. ponderosa).

The major structural difference between Soda lignin and Kraft lignin is the presence of C-S vibration peak at 2650 -2600 cm^{-1} found in Kraft lignin (Fig. 2 and 3). This is as a result of the presence of sulphur in Kraft pulping liquor which reacts with lignin to form lignosulpahate and lignothiol (Sjostrom, 1993). Also, there was no carbonyl absorption band in Soda lignin. The C=O absorption band (1703 cm^{-1}) present in Kraft lignin, may be due to oxidation reaction that takes place during Kraft pulping.

Table 1: FTIR Spectra of Soda and Kraft lignin

Band (cm^{-1})	Assignment	Sample present
3700 - 3000	O-H stretching alcohol	All samples
3000 – 2840	C-H stretching alkane	All samples
2850 – 2815	C-H stretch (methoxy, methyl ether)	All samples
2600 – 2650	S-H stretching thiol	Kraft lignin
1982 – 1833	C-H bending aromatic	All samples
1595 – 1510	C-C stretching aromatic	All samples
1465 – 1450	C-H bending methylene and methyl group	All samples
1427 – 1423	O-H bending alcohol	All samples
1215 – 1200	C-O stretching phenol	All samples
1160 - 1085	C-O-C stretching ether	All samples

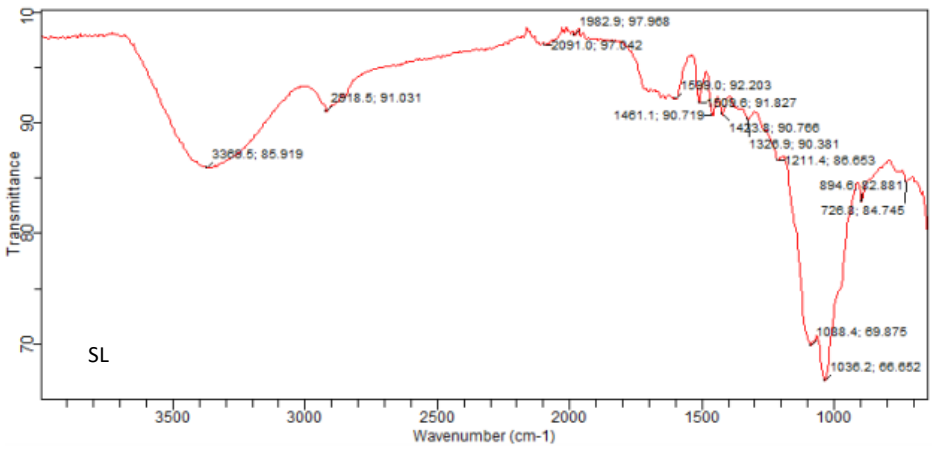

Fig. 2: FTIR spectrum of Soda lignin

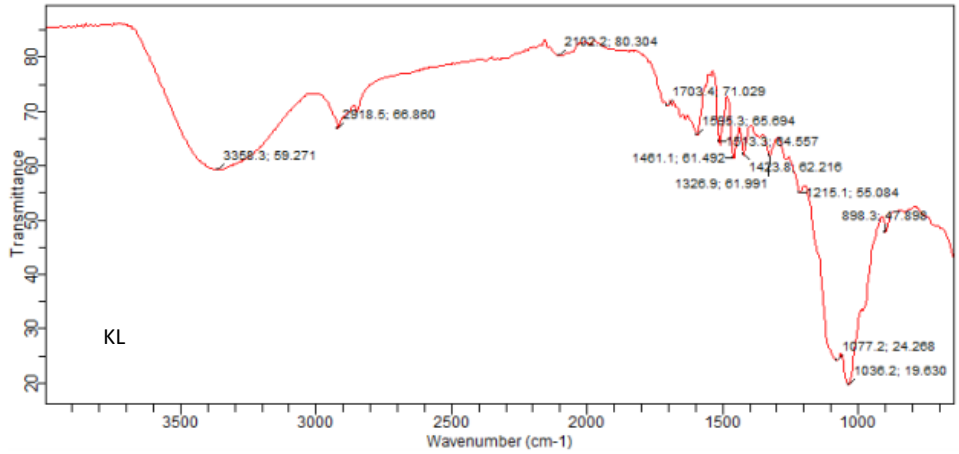

Fig. 3: FTIR spectrum of Kraft lignin

4.2 Absorbance and wavelength of maximum absorption

The wavelength of maximum absorption (λ_{max}) and absorbance of lignin are presented in Table 2. Wavelength of maximum absorption (λ_{max}) of Kraft lignin was 266 nm at absorbance of 4.538, while that of Soda lignin was 280 nm at absorbance of 3.758. The increase in the absorbance of Kraft lignin is due to the presence of carbonyl functional group on the lignin moiety. The

increase in λ_{max} of Soda lignin is as a result of degradation of lignin that usually occurs during Soda pulping, which results in the formation of greater amount of lignin monomers and dimers. This also causes a decrease in absorbance (Sjostrom, 1993). The decrease in the λ_{max} of Kraft lignin implies that it contains greater percentage of S-type monomeric unit, and G-type lignin or H-type lignin was probably degraded to some extent during pulping (Pei and Yang, 2014). Absorption band of non-conjugated guaiacyl and 3, 4 – dimethoxy-phenyl occurred around at 277 to 282 nm, and this absorption band was found in Soda lignin.

Table 2: Absorbance and (λ_{max}) of lignin and lignin esters

Sample	Absorbance	λ_{max} (nm)
Kraft lignin	4.538	266
Soda lignin	3.758	280

2.3 Types of phenolic structure present in lignin

UV spectroscopic technique was also used to determine the amount of the different types of phenolic hydroxyl group present in Kraft and Soda lignin. The results are presented in Table 3.

Table 3: Types of phenolic hydroxyl present in soda and kraft lignin

	Kraft lignin	Soda lignin
Absorbance at 300	0.417	0.150
Absorbance at 350	0.345	0.241
Types of phenolic hydroxyl group		
i) Non conjugated phenolic OH (I+III) (mmol/g)	3.120	1.295
ii) Conjugated phenolic OH (II + IV)) (mmol/g)	0.410	0.287
Total phenolic (mmol/g)	3.530	1.582

According to Gartner and Gellerstedt (1999), there are six types of phenolic lignin structures (Figure 4). The UV- absorption maxima at 300 nm and 350 – 360 nm were assigned to the unconjugated phenolic structures (I and III), and those at 350 to 370 nm was assigned to conjugated structures (II and IV). The absorption maxima at 360 nm was attributed only to

phenolic structural type IIa and IVa in lignin (Zakis, 1994). The UV results (Table 3) revealed that the concentration of non-conjugated phenolic group and conjugated phenolic group in Kraft lignin were 3.120 mmol/g and 0.410 mmol/g respectively, and the total amount of phenolic hydroxyl group was 3.530 mmol/g, while the concentration of non-conjugated phenolic hydroxyl group and conjugated phenolic hydroxyl group in Soda lignin were 1.295 mmol/g and 0.287 mmol/g respectively and the total amount of phenolic hydroxyl was 1.582 mmol/g. The decrease in the amount of both conjugated and non- conjugated phenolic group in Soda lignin may be attributed to the high rate of degradation of Soda lignin during Soda pulping in which most of the lignin fragments are solubilized in the pulping liquor (Sjostrom, 1993). Surina *et al*., 2015 reported 1.621 mmol/g, o.229 mmol/g and 1.850 mmol/g for non-conjugated phenolic hydroxyl group, conjugated phenolic hydroxyl group and total amount of phenolic hydroxyl respectively in non-wood Soda-anthraquinone lignin.

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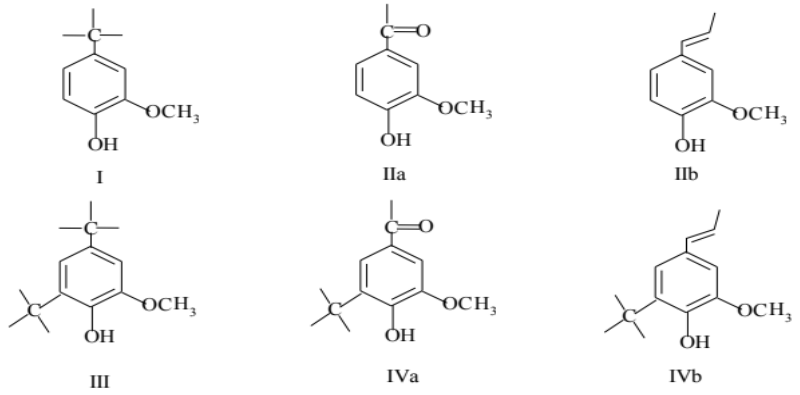

Figure 4: Types of phenolic structures of lignin (Zakis, 1994; Gartner and Gellerstedt, 1999)

4.4 Surface morphology

Surface morphological structure of Kraft and Soda lignin are presented in Figure 5. The structure revealed that the surface of both Kraft and Soda lignin are rough heterogeneous

surfaces with uneven particle size and pore spaces. The particle sizes of Kraft lignin are larger than that of Soda lignin.

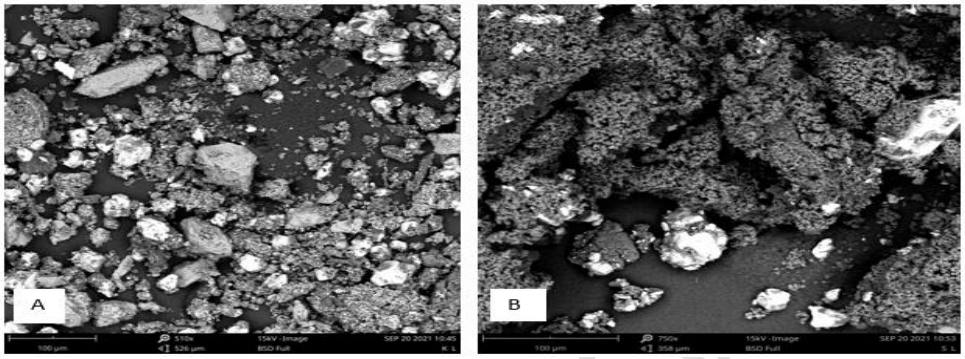

Figure 5: Surface morphology of Kraft lignin (A) and Soda lignin (B)

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4.1.6 Lignin monomers and dimers composition

Macro structure of lignin degrades during pulping. The main degradation reactions of lignin in alkaline medium are the cleavage of β-aryl ether bonds. (Sjostrom, 1993). The monomeric and dimeric composition of lignin was determined using electrospray ionization mass spectrometry (ESI-MS). The results obtained (Fig. 6 and 7) showed that Soda lignin contained a mixture of 27 monomers and dimers, while Kraft lignin contained a mixture of 15 monomers and dimers. High resolution ESI-MS identified 2 monomeric and 18 and dimeric components in Soda lignin, while 5 monomeric and 15 and dimeric components were present in Kraft lignin. The high level monomeric and dimeric constituent in Soda lignin was due to the fact that the Soda liquor had greater tendency to attack ether linkage (β-O- 4 linkages) in lignin during pulping and cause cleavage of ether-lignin bond (Sjostrom, 1993). This cleavage promotes lignin solubilization by decreasing the molecular weight and increasing the phenolic hydroxyl group content (Balakshin, 2013). The reaction of phenolic β-*O*-4 unit in guaiacyl structures begins with the reversible

formation of an unstable quinone methide (Sjostrom, 1993). The quinone methide structure may undergo several reactions depending on the severity of the alkaline medium. In the absence of hydrogen sulfide, the degradation of quinone methide proceeds mainly via elimination of formaldehyde unit, and as a result, the enol ether is formed, this led to the absence of carbonyl group in Soda lignin as already shown in FTIR spectra. In the presence of nucleophiles, lignin condensation products may be formed resulting in the formation of lignin dimers (Gilarranz, 2000). The non-phenolic lignin units are difficult to degrade, however, Soda liquor effectively depolymerised both phenolic and non-phenolic fractions of lignin β-O-4 bonds resulting in greater degradation products found in soda lignin (Sjostrom, 1993, Sannigrahi and Ragauskas, 2010).

Table 4: Degradation products of soda and kraft lignin identified by high resolution ESI

	Molecular formula	Molecular weight	Sample found	Type
1	$C_7H_{10}O_3$	142	Kraft lignin	Monomer
2	$C_8H_{11}O_3$	155	Kraft lignin	Monomer
3	$C_9H_{12}O_3$	164	Soda lignin	Monomer
4	$C_8H_{13}O_4$	173	Kraft lignin	Monomer
5	$C_{10}H_{11}O_4$	195	Kraft lignin	Monomer
6	$C_{11}H_{13}O_4$	209	Both samples	Monomer
7	$C_{12}H_{10}O_3$	202	Soda lignin	Dimer
8	$C_{13}H_{12}O_3$	216	Soda lignin	Dimer
9	$C_{14}H_{14}O_3$	230	Soda lignin	Dimer
10	$C_{13}H_{16}O_3$	244	Soda lignin	Dimer
11	$C_{14}H_{13}O_4$	245	Both samples	Dimer
12	$C_{16}H_{16}O_3$	256	Soda lignin	Dimer
13	$C_{15}H_{19}O_5$	274	Both samples	Dimer
14	$C_{17}H_{17}O_5$	301	Soda lignin	Dimer
15	$C_{15}H_{19}O_5$	304	Kraft lignin	Dimer
16	$C_{18}H_{17}O_6$	318	Soda lignin	Dimer
17	$C_{18}H_{21}O_6$	334	Both samples	Dimer
18	$C_{18}H_{19}O_7$	349	Soda lignin	Dimer
19	$C_{22}H_{30}O_7$	352	Both samples	Dimer
20	$C_{20}H_{26}O_6$	362	Soda lignin	Dimer
21	$C_{21}H_{29}O_7$	381	Both samples	Dimer
22	$C_{21}H_{29}O_7$	393	Soda lignin	Dimer

23	$C_{20}H_{29}O_8$	397	Both samples	Dimer
24	$C_{22}H_{29}O_9$	437	Both samples	Dimer
25	$C_{22}H_{32}O_9$	440	Both samples	Dimer

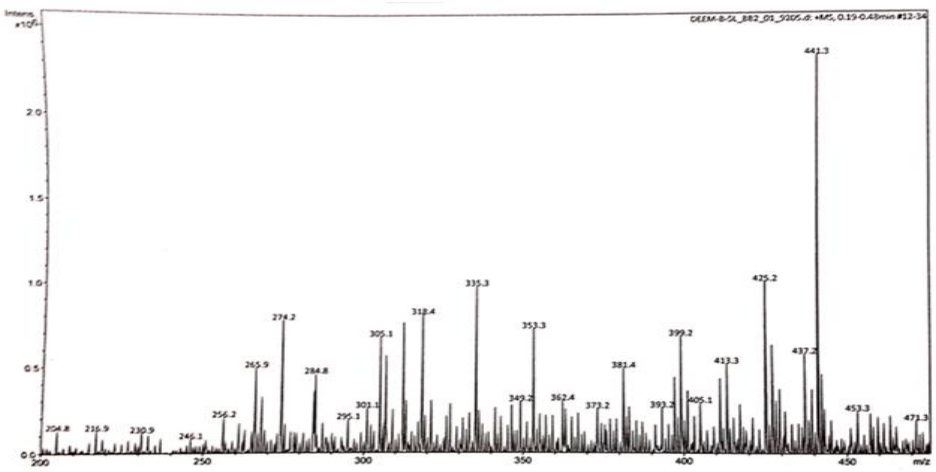

Figure 6: ESI-MS Spectra of Soda lignin

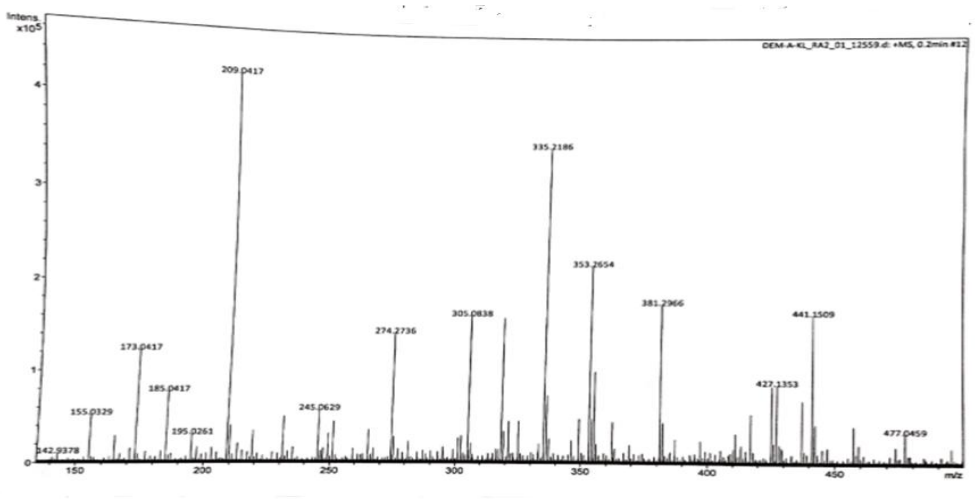

Figure 7: ESI-MS Spectra of kraft lignin

Conclusion

Lignin was successfully extracted from *Gmelina arborea* wood. The physicochemical analysis of lignin revealed that *G. arborea* wood lignin extracted by Soda and Kraft pulping processes had several functional groups such as hydroxyl, phenolic, carboxylic, methoxy, thiols and ethers in their moiety. Kraft lignin had greater concentration of both conjugated and non-conjugated phenolic groups than Soda lignin. The monomeric composition in Kraft lignin was higher than that of the Soda lignin, while lignin dimer composition was higher in Soda lignin. The surface of both Kraft and Soda lignins was rough and heterogeneous with uneven pore sizes. Since *G. arborea* lignin has several functional groups, it can be modified and utilized in several industrial purposes.

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