Characterization of Lignins Isolated from Industrial Residues and their Beneficial Uses

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The physico-chemical properties of lignin isolated from lignocellulosic bioethanol residues and hardwood kraft black liquor were compared with two commercial lignins, kraft softwood lignin, and soda non-wood lignin. Lignin from the industrial residues was isolated through the acid precipitation method. The amount of lignin isolated was approximately 38% of the dry weight of lignocellulosic bioethanol residues and approximately 27% of the black liquor solids. The numbers of methoxyl groups and phenolic and aliphatic hydroxyls were determined to derive a molecular formula for each of the four lignins. The molecular weights of the lignins were measured by high performance size exclusion chromatography. Potential value-added applications of the lignins were summarized based on their molecular weights and physico-chemical characteristics.

Keywords: Physico-chemical properties; Lignin; Kraft black liquor; Lignocellulosic bioethanol residues; Potential application

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INTRODUCTION

Industrial lignin is being produced in greater quantities as pulp mills look to co-products such as lignin to add new value stream to their businesses (Clancy 2013; Hughes 2014). The pulp industry currently produces approximately 50 million tons of lignin per year (Varanasi *et al.* 2013). Cellulosic bioethanol refineries have quickly expanded in the last 10 years due to biofuel policies in a number of countries. It is estimated that 225 million tons of lignin will be produced by lignocellulosic ethanol biorefineries by 2030 (Sahoo *et al.* 2011b). The majority of lignin produced today is used as bioenergy, with only a small portion used in the production of value-added materials (Doherty *et al.* 2011).

Lignin is an aromatic biopolymer and a prominent constituent of the plant cell wall. It is comprised of three phenylpropanoid units: syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H), which are covalently bonded through carbon-carbon and ether linkages to form a three dimensional structure (Derkacheva and Sukhov 2008). The ratio of syringyl, guaiacyl, and p-hydroxyphenyl units vary based on the type of plant, with softwoods containing mainly guaiacyl, hardwoods containing both syringyl and guaiacyl, and non-wood plants containing all three units. The exact molecular structure of lignin varies based on its extraction method as well as its plant source, varying with age and growing environment (Sahoo *et al.* 2011b).

The structure of native lignin is altered when it is subjected to chemical or biological treatment. For instance, during the kraft pulping process, β-O-4 and α-O-4 linkages are cleaved and produce a high proportion of non-etherified phenolic hydroxyl groups (Tejado *et al.* 2007). The same does not hold true for soda pulping of non-woody plants where only small quantities of phenolic hydroxyls are produced (Gellerstedt and Lindfors 1984; Tejado *et al.* 2007). In the steam explosion process (a pretreatment process used on lignocellulose for the production of bioethanol), biomass is exposed to high steam pressure followed by rapid pressure release. This process results in the hydrolysis of the hemicelluloses and the cleavage of lignin-hemicellulose bonds, facilitating enzyme access to the cellulose. In their work on lignin's structural changes during steam explosion, Li *et al.* (2007) demonstrated that while there is a significant cleavage of the β-O-4 linkages in the lignin structure, there is also substantial repolymerization occurring that actually results in an increase in the molecular weight and heterogeneity of the molecule (Li *et al.* 2007).

Differences in the molecular weight, elemental composition, and type and proportion of functional groups in the lignin molecule provide the opportunity to utilize lignin in various value-added applications. For example, lignins containing a higher proportion of guaiacyl units possess a higher reactivity with formaldehyde in the synthesis reaction of phenol formaldehyde resin because of the free C5 position on the phenolic ring (Sarkar and Adhikari 2001a; Alonso *et al.* 2004; Khan *et al.* 2004; Tejado *et al.* 2007; Abdelwahab and Nassar 2011; Mankar *et al.* 2012; Zhang *et al.* 2013). In applications where lignin serves as a filler and reinforcing phase for polymer blends, such as in polyethylenes, polypropylenes, and polylactides, the surface energy and compatibility of lignin with the matrices is the critical factor (Sahoo *et al.* 2011a). Increasing content of polar groups in lignin make it more compatible with polar polymers such as polyesters or polyvinylchloride, while any processes leading that decrease polar groups cause it to be more compatible with polyolefins (Kadla *et al.* 2002; Lora and Glasser 2002; Cazacu *et al.* 2004; Gosselink *et al.* 2004b; Hatakeyama *et al.* 2005; Barzegari *et al.* 2012; Schorr *et al.* 2014). Lignin that has a large amount of hydroxyl content is also suitable for the production of polyol (either direct utilization or after chemical modification), which can subsequently be used in polyurethane polymer production (Sarkar and Adhikari 2001b; Huang and Zhang 2002; Cateto *et al.* 2008). The purity and molecular weight of the lignin precursor are critical factors during the production of biocarbon fiber (Norberg 2012; Awal and Sain 2013; Mainka *et al.* 2015).

This work focuses on the fundamental chemical characteristics of lignins isolated from two industrial sources and their potential value-added applications. The knowledge about the chemical properties of isolated lignins from different sources provides useful information for industries interested in using lignin for product development and processing. Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) was used to elucidate the structure of the industrial and commercially available lignins. ^{31}P-nuclear magnetic resonance (^{31}P-NMR) and ^{1}H-nuclear magnetic resonance (^{1}H-NMR) were employed to investigate the structural properties and functional groups, and high performance size exclusion chromatography (HPSEC) was used to determine the molecular weight of the lignin samples. Finally, the potential value-added applications of lignins from various industrial sources were summarized based on their molecular weight and physico-chemical characteristics.

EXPERIMENTAL

Materials

Industrial lignin samples were obtained from a bioethanol plant in U.S. that uses hardwood and non-wood species, and from a kraft pulp mill in Botucatu, Brazil, where the main species is eucalyptus. Protobind (Lignin 4, or L4) is a commercial non-wood based soda lignin (ALM, Chandigarh, India) and Indulin AT (Lignin 3, or L3) is a highly purified lignin (Westvaco Company, Richmond, USA) (Table 1). In this paper, the term "lignin" is used to describe the materials isolated from the industrial residues (Lignin 1 [L1] and Lignin 2 [L2]), as well as the commercially available lignins (L3 and L4).

Table 1. Original Sources of Lignin Samples

Lignin	Lignin Isolation Treatment	Raw Material	Origin	Industry	Process
L1	Isolated (Abächerli and Doppenberg 2001)	Lignocellulosic bioethanol residues	Hardwood/ Non-wood	Bioethanol	Steam explosion/ Enzymatic hydrolysis
L2	Isolated (Tejado *et al.* 2007)	Kraft black liquor	Hardwood	Pulp	Kraft
L3	As received	Commercial lignin (Indulin AT)	Softwood	Pulp	Kraft
L4	As received	Commercial lignin (Protobind)	Non-wood	Pulp	Soda

Lignin Preparation

Lignin 1 was isolated from the remaining part of a mixture of hardwood and non-wood species after steam explosion pretreatment, using an isolation method for alkaline lignin described by Abächerli and Doppenberg (2001). In this method, the solid residue was ground and sieved to pass a 40-mesh screen, and 20 g of the powder was stirred in 100 mL of 0.5 M sodium hydroxide for 30 min. The solution was filtered with a Buchner funnel (1 µm pore filter paper). The filtrate was precipitated by reducing the pH to 2 by adding H_2SO_4 (1 M) and subsequently heated to 70 °C for 2 to 3 min. The resulting precipitate was separated by filtration, washed with water between 50 °C and 60 °C to remove degraded sugars and unreacted compounds, and then dried overnight at 50 °C in an oven.

Lignin 2 was isolated from black liquor following the method described by Tejado *et al.* (2007). The pH of the black liquor was lowered to a pH of 2 from an initial pH of 12.9 using 1 M H_2SO_4. The precipitated lignin was filtered on a Buchner funnel, washed with water between 50 °C and 60 °C, and oven-dried overnight at 50 °C.

FT-IR Analysis

Fourier transform infrared analysis was carried out on an FT-IR Varian 600-IR (Varian Resolutions, Varian Inc., Victoria, Australia), equipped with a mercury cadmium telluride (MCT) detector and an ATR unit (PIKE MIRacle, Pike Technologies, Madison, USA). Air-dried samples (in powder form) were placed in the sample compartment and pressed against the diamond crystal. Similar pressure was applied for all measurements by using the pressure applicator equipped with a torque knob. The wavenumber range was between 4000 cm^{-1} and 600 cm^{-1} with 150 scans and a resolution of 4 cm^{-1}. The spectra were detected in absorbance mode. Background scanning and correction was performed before running each sample. The relative peak absorbance was normalized (the intensity of highest absorbance peak normalized to unity) for all IR bands in each sample.

Ash Content

The ash content of the lignin samples were gravimetrically determined according to TAPPI T211 om-93 (1993). Approximately 0.5 g of the oven-dried lignin sample was weighed into a tared ceramic dish and placed in a muffle furnace at 525 ± 25 °C for 4 h. The samples were white/gray in color at the end of the heating cycle. Samples were weighed on an analytical balance to the nearest 0.1 mg. The ash content was determined as follows,

$$Ash\ Content\ (\%) = (A/B)\ \times 100 \tag{1}$$

where A is the mass of ash (g) and B is the mass of test specimen (oven-dried) (g).

Chemical Characterization

Klason lignin is defined as the solid residual material when a sample is subjected to hydrolysis treatment with 72% sulfuric acid. Klason lignin was determined according to TAPPI T222 om-98 (1998). Lignin samples were treated with 72% H_2SO_4 for 1 h in a water bath (30 °C), then diluted to 4% and autoclaved at 121 °C for 1 h. The hydrolyzed solution was vacuum filtered on a tared Gooch filtering crucible (medium pore size) and dried in oven at 105 °C for 3 h. The Klason lignin was calculated as a percentage of the weight of the oven-dried lignin sample.

The acid-soluble lignin was measured using UV spectroscopy (Maekawa *et al.* 1989; Zhu *et al.* 2014). The filtrate collected from the Klason lignin procedure was neutralized with calcium carbonate and filtered through a 0.2 μm syringe filter. The quantity of acid-soluble lignin was determined from the absorbance at 205 nm, according to the following equation,

$$Acid\ soluble\ lignin\ (\%) = \frac{D\times V\times A}{a\times W} \times 100 \tag{2}$$

where D is the dilution ratio, V is the filtrate volume (L), A is the absolute absorbance of the sample, a is absorptivity of the lignin (L $g^{-1}cm^{-1}$), and W is the oven-dried weight of the sample (g). The value of a was taken to be 110 L $g^{-1}cm^{-1}$ at 205 nm.

Elemental Analysis

The relative proportions of carbon (C), hydrogen (H), and nitrogen (N) in the air-dried samples were determined with a Perkin Elmer Model 2400II CHN analyzer (Waltham, MA, USA). Calibration was done with acetanilide before each test. The air-dried sample weight was corrected for moisture. The total sulfur content of the oven-dried lignin samples were evaluated by the microwave acid digestion, and inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis. The percentage of oxygen was obtained by subtracting the sum of C, H, and N contents from 100 percent (including the ash).

Sample Preparation for ^{31}P-NMR Analysis

Total aliphatic hydroxyl, phenolic hydroxyl, and carboxyl groups, as well as the G/S/H ratio of lignin samples, were determined by quantitative ^{31}P-NMR as has been described (Granata and Argyropoulos 1995; Cateto *et al.* 2008). A solvent solution of pyridine and $CDCl_3$ (1.6/1, v/v) was prepared for dissolving lignin. This amount of deuterium in $CDCl_3$ provided a strong deuterium signal for locking the NMR instrument. Phosphitylation of the lignin samples was performed using 2-chloro-4,4,5,5-tetramethyl-

1,3,2-dioxaphospholane (TMDP). An internal standard solution (cholesterol, 85 mg/mL) and relaxation reagent solution (chromium (III) acetylacetonate, 5.6 mg/mL) were prepared with the solvent solution of pyridine and $CDCl_3$ (1.6/1, v/v). Forty mg of oven-dried lignin was dissolved in 500 µL of the solvent solution in a sealed vial followed by the addition of 100 µL of internal standard and 50 µL of relaxation solution. Next, 100 µL of the phosphitylation reagent was added, and the vial was shaken to ensure a homogeneous mixture. After derivatization, the resulting solution was transferred to a 3-mm tube, and the ^{31}P-NMR spectrum was recorded using a Varian Unity Plus 600 MHz spectrometer (San Francisco, USA).

Sample Preparation for ^{1}H-NMR Analysis

The ^{1}H-NMR of acetylated lignins was carried out by a Varian Unity Plus 500 MHz spectrometer (San Francisco, USA) as described by Li and Lundquist (1994). Typical ^{1}H-NMR data points were recorded by accordance with the acquisition time of 4.0 s, the number of scans at 126, and the relaxation time of 1.0 s at room temperature. The lignin was acetylated by a 1:1 pyridine and acetic anhydride solution at 90 °C for 3 h in a sealed flask.

High Performance Size Exclusion Chromatography (HPSEC)

Molecular mass of the lignin samples were analyzed by alkaline aqueous phase HPSEC (González *et al.* 2000) with a DIONEX DX600 chromatograph (Bannockburn, USA) equipped with a UV detector and a PSS MCX column (1000 Å, 300 × 8 mm). The stationary phase of this column was a sulfonated styrene-divinylbenzene copolymer-network that was appropriate for carrying out HPSEC experiments in the pH 7 to 13 range. The UV detection was carried out at the wavelength of 280 nm at room temperature (25 °C). The injection volume was 25 µL. Eluent (0.1 M NaOH solution) was prepared with deionized water (Millipore water from an in-house purification system). Sodium polystyrene sulfonate, which exhibits similar behavior to lignin, was used to create a calibration curve for the column. Molecular weight standards were sodium polystyrene sulfonates (6520, 4230, 1830, and 1100 kilodaltons) supplied by Polymer Standard Services (Amherst, USA). The calibration curve was prepared by adding 10 mg of each polystyrene standard to 10 mL of water. Each lignin sample was prepared by dissolving 10 mg of the oven-dried lignin in 100 mL of 0.1 M sodium hydroxide solution. The number and average molecular weights were calculated based on the ASTM D5296 –11 (2011).

RESULTS AND DISCUSSION

Yield Percentage and Composition of Isolated Lignin

The amount of lignin isolated from the industrial residues was 38% of the dry weight of lignocellulosic bioethanol residues and 27% of the black liquor solids. The yield of lignin from the residues was of course an important consideration, as it affects the economics of the recovery process. The percentage of extracted lignin from the black liquor fell within the expected range of 20% to 40% lignin based on the dry content (Vishtal and Kraslawski 2011).

The composition of the lignin samples is shown in Table 2. There are some noticeable differences between the samples, with the industrial lignins (L3 and L4) possessing higher amounts of Klason (acid insoluble) lignin and lower amounts of acid-soluble lignin than the lignins isolated from the industrial residues (L1 and L2). It might be concluded from this comparison that the lignin isolation method used in this work resulted in lower yields of Klason lignin, but also lower acid-soluble lignin residues, than the method used for commercial lignin. Schorr *et al.* (2014) reported that the Klason lignin content for Indulin AT was 92, similar to that of the L3 sample. They found that the Klason lignin content of the two lignins isolated from the Kraft black liquors also had similarly high Klason lignin content (91%) (Schorr *et al.* 2014).

The amount of inorganics (ash) were quite low (< 2%) for all samples with the exception of L3, the commercial softwood kraft lignin that contained 4.25%. A similar ash content of 3.59% was reported by Schorr *et al.* (2014). Lignin 1 had the lowest ash content of the four samples. Unlike L2, L3, and L4 that were derived as residue from chemical pulping processes, L1 was derived from residue from a bioethanol process. In the bioethanol process, a steam explosion pretreatment was followed by enzymatic hydrolysis, detoxication, and fermentation, where there was some addition of chemicals/salts to the detoxication process. Hence, the low ash in L1 may be favorable for certain applications as ash is considered a contaminant and, depending on which elements are present, could cause negative effects during processing in the next stage.

Table 2. Composition of the Four Lignin Samples

Composition (%)	L1	L2	L3	L4
Insoluble Klason lignin	85.1 ± 3.9	86.3 ± 0.9	91.0 ± 0.45	91.2 ± 0.4
Acid-soluble lignin	5.4 ± 2.0	6.0 ± 1.8	2.1 ± 1.0	3.9 ± 1.1
Ash	0.45 ± 0.09	1.54 ± 0.13	4.25 ± 0.08	1.38 ± 0.11
Total	90.9	93.8	97.4	96.5

±, standard deviation

FT-IR Spectroscopy

FT-IR spectra of the two raw materials (lignocellulosic bioethanol residues and black liquor) and their isolated lignins (L1 and L2) are exhibited in Fig. 1. Noticeable differences were observed between the spectra of the two raw materials and their isolated lignins.

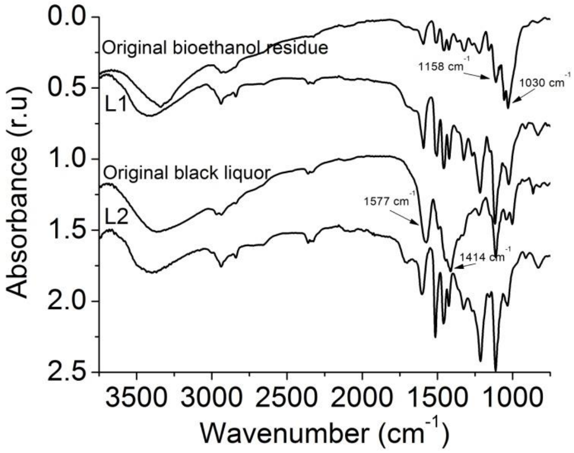

Fig. 1. Comparison between the raw materials and their isolated lignin; L1 (isolated from lignocellulosic bioethanol residues) and L2 (isolated from kraft black liquor)

In the L1 spectrum, a considerable decrease was observed in the absorption intensities at the bands 1158 cm^{-1}, 1055 cm^{-1}, and 1030 cm^{-1} after isolation from lignocellulosic bioethanol residues. The absorption peaks in the lignocellulosic bioethanol residues at 1158 cm^{-1} showed a C–O–C asymmetric vibration, and peaks at 1055 cm^{-1} and 1030 cm^{-1} indicated a C−O stretch of cellulose (glycosidic linkages) (Corredor *et al.* 2009; Adsul *et al.* 2011). In the steam explosion process, glycosidic bonds in the hemicelluloses (and to a lesser extent in the cellulose) were hydrolyzed. Hemicellulose-lignin bonds were also cleaved in this process. The solubility of hemicelluloses in water and the solubility of lignin in alkaline or organic solvents increased, leaving the cellulose with a reduced degree of polymerization (Li *et al.* 2007). Therefore, the appearance of these strong peaks could be attributed to cellulose and hemicellulose contaminates in the lignocellulosic bioethanol residues (She *et al.* 2010).

Differences in the kraft black liquor and L2 spectra were mostly observed in the area of aromatic ring vibrations (*e.g.*, bands at 1577 cm^{-1}, 1492 cm^{-1}, 1445 cm^{-1}, and 1414 cm^{-1}). The intensity of those bands was stronger than the intensity of the same absorption bands in the spectrum of isolated L2. This was due to the presence of low molecular weight phenolic compounds, which were created in the kraft process and subsequently removed during isolation process (precipitation and filtration of lignin by lowering the pH to 3 (Gellerstedt and Lindfors 1984).

Figure 2a compares the spectra of the four lignin samples. Only minor differences were observed between the samples due to similar functionalities; they differed from one another only in their percentages of functional groups. The absorption peaks were strongly consistent with the assigned peaks of the chemical components, as previously reported (Anglès *et al.* 2003; Derkacheva and Sukhov 2008; Zhou *et al.* 2011). The absorption peaks are described in Table 3. A wide peak was observed for all isolated lignins in the wavenumber range of 3500 cm^{-1} to 3100 cm^{-1}. This band typically refers to the presence of hydroxyl groups (alcoholic and phenolic) involved in hydrogen bonds. The two peaks at 2900 cm^{-1} and 2800 cm^{-1} correspond to methyl ($-CH_3$) and methylene ($-CH_2$) groups. The position of the bands at 1705 cm^{-1} and 1595 cm^{-1} can be attributed to non-conjugated and conjugated carbonyl groups, which were observed in all lignin spectra. The carboxyl groups in lignin samples were represented between 1750 cm^{-1} and 1550 cm^{-1} (Gosselink *et al.* 2004a). Absorption bands at 1595 cm^{-1} and 1513 cm^{-1} were assigned to aromatic skeletal vibration of lignins (Zhou *et al.* 2011; Camargo *et al.* 2012). In the 1460 cm^{-1} to 1420 cm^{-1} wavenumber range, two intense band peaks were observed. These bands corresponded to the C–H stretching band (methyl and methylene) and the C–H in-plane deformation with aromatic ring stretching (She *et al.* 2010; Awal and Sain 2011; Zhou *et al.* 2011).

To discern the differences in lignin structure, Fig. 2b exhibits the region between 1400 cm^{-1} and 1000 cm^{-1}. The phenolic OH groups in lignin (peak at 1365 cm^{-1}) were produced during a chemical process when β-O-4 linkages were cleaved and generated non-etherified hydroxyls. A low intensity peak in the L1 spectrum showed that a small portion of phenolic OH groups were generated (due to fewer β-O-4 linkage cleavages) during steam explosion. The stretching vibration of the C–O bonds in syringyl rings was observed at 1325 cm^{-1}. This peak did not appear in the L3 spectrum due to absence of syringyl units in softwood lignin (Anglès *et al.* 2003; Awal and Sain 2011).

Furthermore, the intensity of absorption bands at 1266 cm^{-1} (C–O stretching of guaiacyl ring) in L3 spectrum closely resembled those of the other lignins, while the absorbance band at 1213 cm^{-1} was observed with equal intensity (Pandey 1999). In addition, a peak was observed in the range of 1155 cm^{-1} to 1150 cm^{-1} in all spectra, which probably referred to the in-plane deformation vibration of both guaiacyl C–H and syringyl C–H. However, the intensity of the band at 1112 cm^{-1} (C–H deformation in syringyl ring) appeared with higher intensity for L2 when compared with L4, which suggests a higher percentage of syringyl in the hardwood sample than the non-wood sample.

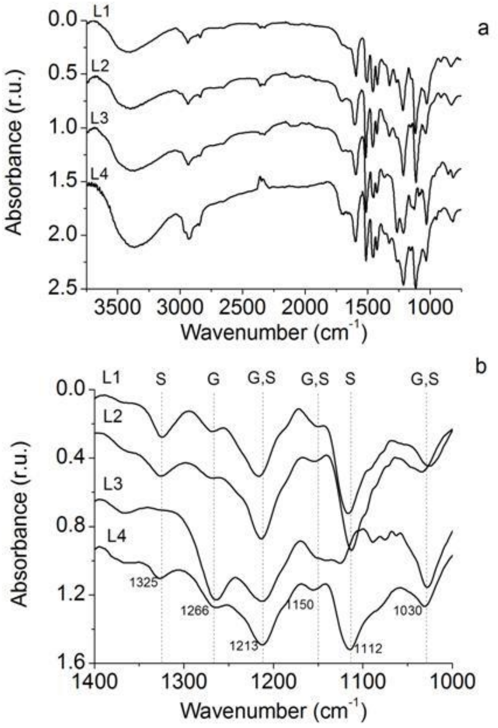

Fig. 2. FT-IR spectra of isolated lignin samples in the range of (a) 4000 cm^{-1} to 600 cm^{-1} and (b) 1400 cm^{-1} to 1000 cm^{-1}

Table 3. FT-IR Absorption Peaks in Four Lignin Samples

Band Position (cm^{-1})				Assignment
L1	L2	L3	L4	
3500-3100				OH-stretch (hydrogen bond)
2939	2939	2931	2923	C−H stretching
2833	2833	2833	2840	C−H stretching
1705	1705	1705	1705	C=O stretch (unconjugated)
1595	1595	1594	1595	Aromatic skeletal vibration, C=O stretching (conjugated)
1513	1513	1511	1513	Aromatic skeletal vibration
1458	1458	1455	1455	C−H deformation (methyl and methylene)
1426	1426	1422	1422	C−H in-plane deformation with aromatic ring stretching
1365	1365	1365	1365	In-plane deformation vibration of phenolic OH
1326	1326	-	1325	C−O stretching of syringyl ring
1268	1268	1266	1266	C−O stretching of guaiacyl ring
1213	1213	1213	1213	C−O stretching of guaiacyl and syringyl ring
1151	1151	1150	1155	In-plane deformation vibration of guaiacyl C−H and syringyl C−H
1112	1112	-	1112	In-plane deformation vibration of syringyl C-H
1030	1030	1027	1030	C−O of guaiacyl and syringyl ring, C−H bond in guaiacyl ring
-	-	851	-	C−H out-plane deformation in guaiacyl ring

The absorption band range between 1030 cm^{-1} and 1025 cm^{-1} was assigned to the deformation vibration of the C–H bonds in the guaiacyl ring, and also assigned to the C–O bonds in both syringyl and guaiacyl. This absorption band appeared with higher intensity in L3 compared to the others due to the higher content of guaiacyl-type lignin in softwoods.

Based on the discussion above, there was an obvious difference between the functional groups of the raw materials and their isolated lignins. The major chemical compounds in lignocellulosic bioethanol residues were cellulosic materials, while low molecular weight lignin-like materials were predominant chemical compounds in black liquor. Isolated lignins showed minor differences in their chemical structure that were mostly due to the absence of a syringyl unit.

Elemental Composition of Lignin

The elemental composition and empirical formula of the four lignin samples is shown in Table 4. The ratio of elements was calculated by the element percentage divided by the molar mass. L2 (hardwood lignin) had the lowest carbon and highest oxygen content, while L3 (softwood lignin) had the highest carbon and lowest oxygen content. This could have been related to the number of syringyl groups and consequently to the number of methoxyl groups in the lignin molecule (Schorr *et al.* 2014). Lignins with a higher number of methoxyl groups contain a lower percentage by weight of carbon and a higher percentage by weight of oxygen content.

L1 exhibited the lowest amounts of sulfur (0.03%) because sulfur was not involved in the bioethanol process nor in the isolation process. The highest amount of sulfur was exhibited by both kraft lignins, L2 (2.5%) and L3 (1.50%). The sulfur content for L3 has been reported as 1.05% (Cateto *et al.* 2008) and 2.1% (Schorr *et al.* 2014). L4 (commercial sulfur-free lignin) exhibited a low percentage of sulfur (0.37%). The amount of sulfur in L4 was reported as 0.41% (Sahoo *et al.* 2011b). Therefore, L1 could be also utilized as a sulfur-free lignin. A lower percentage of sulfur content in lignin is preferred for most of the value-added applications, such as moulded plastics, in which lower toxic gas emission during processing and disposal is produced (Sahoo *et al.* 2011b).

The percentage of nitrogen in each lignin varied due to the differences in the plant source and in the additives of commercial lignins (Sahoo *et al.* 2011b). The hardwood kraft lignin had the lowest amount of nitrogen content (0.13%), while the softwood kraft lignin contained the highest nitrogen content (0.67%), in comparison with other lignins. The nitrogen content of L3 was reported as 0.48% (Cateto *et al.* 2008). The commercial non-wood soda lignin contained 0.59% nitrogen. Sahoo *et al.* (2011b) reported a 0.66% nitrogen content in L4 and stated that the high percentage of nitrogen might be due to some added amino compounds to make the lignin compatible with different polymeric systems (Sahoo *et al.* 2011b). Whether this nitrogen was present as part of the macromolecule structure of lignin cannot, however, be confirmed from this work.

Table 4. Elemental Composition and Empirical Formulas of Lignin Samples

Lignin	C (%)	H (%)	N (%)	S (%)	O (%)	Empirical Formula
L1	62.36	5.89	0.49	0.03	31.22	$C_{5.20}H_{5.89}N_{0.035}S_{0.001}O_{1.95}$
L2	59.51	5.79	0.13	2.54	32.03	$C_{4.96}H_{5.79}N_{0.010}S_{0.079}O_{2.00}$
L3	66.10	6.37	0.67	1.57	25.30	$C_{5.51}H_{6.37}N_{0.048}S_{0.049}O_{1.58}$
L4	65.41	6.53	0.59	0.38	27.09	$C_{5.45}H_{6.53}N_{0.042}S_{0.012}O_{1.69}$

Total Hydroxyl and Carboxyl Content

Table 5 shows the phenolic, aliphatic, total hydroxyl, and carboxyl content in the lignin samples, as determined by ^{31}P-NMR spectroscopy (Fig. 3). In comparison with other lignin samples, L1 contained the lowest and L2 the highest hydroxyl content. The total hydroxyl content of the commercial L3 lignin closely agreed with the value found in the literature, which was 6.85 mmol/g (Cateto *et al.* 2008).

^{31}P-NMR results also revealed a noticeable difference between the number of phenolic hydroxyl groups and a similar number of aliphatic hydroxyl for all lignin samples. The differences in the number of phenolic hydroxyl related to the severity of the extraction method. In the kraft and soda processes, β-O-4 and α-O-4 linkages were cleaved and produced non-etherified phenolic hydroxyl groups in lignin. In steam explosion separation, less chemicals were utilized during the process and, as a result, the lignin underwent less bond cleavage. In result, L1 showed less phenolic OH groups in comparison with kraft and soda lignins. The number of hydroxyl groups is one of the most characteristic functions in lignin that shows the reactivity in lignin macromolecular chemistry (Cateto *et al.* 2008).

Table 5. Total Hydroxyl, Carboxyl, Phenolic, and Aliphatic Hydroxyl Contents with ^{31}P-NMR (mmol/g)

Lignin	Phenolic OH	Aliphatic OH	Total OH	Total COOH
L1	1.51	2.22	3.73	0.54
L2	4.91	2.35	7.26	0.82
L3	4.00	2.59	6.59	0.20
L4	2.28	2.47	4.73	0.22

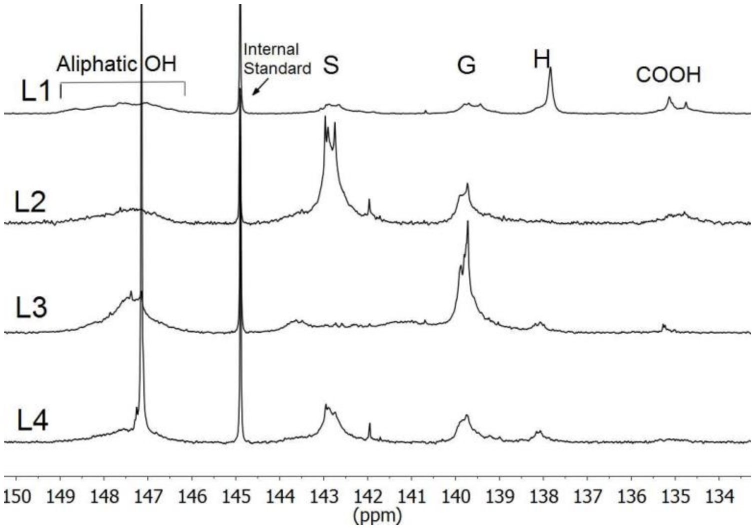

Fig. 3. ^{31}P-NMR spectra of the four lignin samples; lignin units are syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H)

The carboxyl content of L2 was the highest (0.82 mmol/g). Oxidation reactions that may have occurred during the pulping process caused the lignin structure to acquire carboxyl groups. Low dosages of oxidants increases the content of carboxyl in alkali lignin, and high dosages of oxidant cleave the C–C bond in the side chain of the phenylpropane unit to be oxidized into carboxyl (Zhao and Ouyang 2012). Therefore, the carboxyl content was an estimation of the degradation degree of the lignin macromolecule. Thus, of the four lignins tested in this study, L2 was the most degraded from its original form. Lignins with a fair amount of hydroxyl (phenolic and aliphatic) and carboxyl groups can potentially replace polyols in polyurethane production (Pan and Saddler 2013).

Determination of G/H/S ratio by ^{31}PNMR

The ^{31}PNMR analysis of lignin samples was carried out to determine the ratio of lignin units of the four lignin samples. Table 6 represents the molar ratio of each phenylpropanoid type (G, S, and H) in the four samples. The ratio of each unit was measured by calculating the area under each band and the internal standard. The signals at 143.1 ppm to 142.4 ppm, 140.0 ppm to 138.8 ppm, and 138.2 ppm to 137.2 ppm were attributed to syringyl, guaiacyl, and p-phenylpropane units, respectively.

Table 6. Contents of Lignin (%) in Lignin Samples Based on ^{31}PNMR Spectra

Lignin	Syringyl (%)	Guaiacyl (%)	p-Hydroxyphenyl (%)
L1	26	31	44
L2	68	31	1
L3	0	95	5
L4	51	40	9

The percentage of phenylpropanoid units in the lignin structure is different based on the plant type (softwood, hardwood, and non-wood) (Telmo and Lousada 2011). The ratio of phenylpropanoid units in L1 and L4 lignins varied, containing all three precursors. In general, the content of p-hydroxyphenyl in annual crops was higher than in softwoods and hardwoods. L2 (hardwood lignin) was primarily comprised of guaiacyl and syringyl lignins with minor hydroxyphenyl lignin. L3 (softwood lignin) contained guaiacyl with only small amounts of hydroxyphenyl units.

^{1}H-NMR Spectrometry

Acetylated lignins were analyzed with ^{1}H-NMR to determine the content of methoxyl groups, the number of aliphatic hydrogens, the aromatic hydrogens (free positions on the aromatic ring), and the aliphatic and phenolic hydroxyl groups in lignin samples by the integration of the hydrogen signal of the related region. The ^{1}H-NMR spectrum of acetylated lignins is exhibited in Fig. 4.

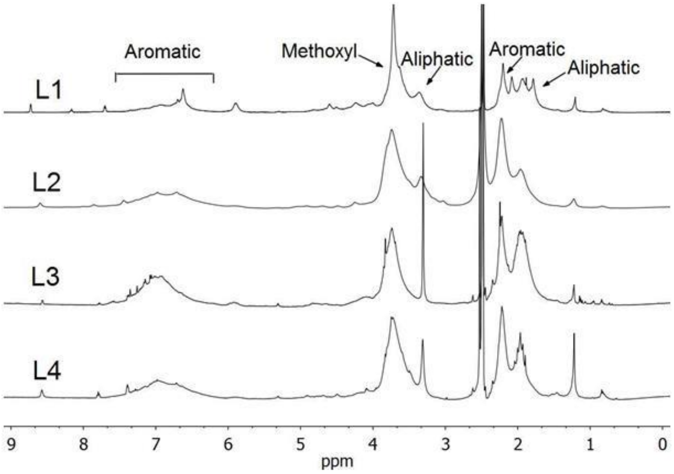

Fig. 4. ^{1}H-NMR spectra of four acetylated lignins

The area of each hydrogen type was obtained by the integration of the hydrogen signals in the ^{1}H-NMR spectrum (Gonçalves *et al.* 2000). From the elemental analysis (Table 4), the total atom ratio of protons was 5.89, 5.79, 6.37, and 6.53 in L1, L2, L3, and L4, respectively. The integration of the hydrogen signals in Table 7 resulted in a total area of 100 arbitrary units, which corresponded to the number of protons in the above ratio.

The number of methoxyl groups was calculated from the number of hydrogen atoms divided by three hydrogen atoms in the methoxyl region (δ 3.40 to 3.95). Therefore, the number of methoxyl groups were calculated to be 0.69, 0.69, 0.56, and 0.74 for L1, L2, L3, and L4, respectively. Theoretically, the molecular structure of lignin was composed of hydroxyphenyl structural units with a phenol ring attached to a propyl side-chain. Depending on the number of methoxyl groups on the phenol ring, the structure was called guaiacyl (1 methoxyl), syringyl (2 methoxyl) or p-hydroxyphenyl (0 methoxyl). The C9-formula of lignin was a combination of these three units. Table 8 represents the approximate C9-formula derived from the ratio of OCH_3 for each lignin sample. L2 (hardwood lignin) contained the highest amount of methoxyl groups and L3 (softwood lignin) contained the lowest. The C9-formula for L3 can be compared with results reported in the literature: $C_9H_{8.74}N_{0.064}S_{0.062}O_{2.56}(OCH_3)_{0.77}$ (Cateto *et al.* 2008) and $C_9H_{8.13}N_{0.05}S_{0.11}O_{2.1}(OCH_3)_{0.66}$ (Schorr *et al.* 2014). However, these values were an approximation of the C9-formula because technical lignins may contain some impurities such as ash and carbohydrates. Based on the empirical formula, the molecular weight (M_w) of the C9 unit was also determined for each lignin sample.

Table 7. Area and Number of Hydrogen in Lignin Samples Obtained from ^{1}H-NMR Spectra

δ (ppm)	Attribution	L1		L2		L3		L4	
		Area	H	Area	H	Area	H	Area	H
9.00-12.0	Carboxylic acids and aldehydes	0.08	0.00	0.05	0.00	0.69	0.04	0.02	0.00
6.20-7.90	Aromatics	13.86	0.82	18.78	1.09	22.69	1.45	17.78	1.16
5.75-6.20	Noncyclic benzylic	2.06	0.12	0.46	0.03	1.34	0.09	0.30	0.02
5.20-5.75	Cyclic benzylic	0.07	0.00	0.65	0.04	3.45	0.22	1.11	0.07
3.95-5.20	Aliphatics	10.10	0.59	1.84	0.11	2.01	0.13	3.47	0.23
3.40-3.95	Methoxyl	35.00	2.06	35.59	2.06	26.40	1.68	33.88	2.21
2.50-3.20	Aliphatics	2.47	0.15	5.91	0.34	0.16	0.01	4.77	0.31
2.10-2.45	Aromatic acetoxyl	11.67	0.69	21.23	1.23	17.31	1.10	18.59	1.21
1.60-2.10	Aliphatic acetoxyl	21.65	1.28	14.46	0.84	23.80	1.52	16.97	1.11
0.00-1.60	Non-oxygenated aliphatic	3.05	0.18	1.03	0.06	2.15	0.14	3.12	0.20
	Sum	100.00	5.89	100.00	5.79	100.00	6.37	100.00	6.53

δ, chemical shift; H, number of hydrogens

The ratio of aromatic hydrogens (H^{ar}) and aliphatic hydrogens (H^{al}), as well as the ratio of phenolic hydroxyl (OH^{ph}) and aliphatic hydroxyl (OH^{al}) groups, were also found through integration of ^{1}H-NMR bands from different regions. Aromatic and aliphatic acetoxyl groups were divided by three to find the number of aromatic and aliphatic hydroxyl groups. Thus, an expanded C9-formula for lignin samples was established as presented in Table 8.

In the expanded C9-formula, the atom ratio of acidic and aldehyde protons, as well as sulfur and nitrogen, were not presented because they were negligible in comparison to other groups or atoms.

Double Bonds Equivalent (DBE)

From the C9-formula, the double bonds equivalent (DBE) was also found for each lignin sample. The degree of unsaturation was estimated by the following equation (Robert *et al.* 1984),

$$\mathrm{DBE} = \frac{(2a+2)-b}{2} \tag{3}$$

where a is the atom ratio of carbon and b is atom ratio of hydrogen in the C9-fromula

The DBE values revealed the number of double bonds and the presence of the aromatic ring together with the degree of inter-unit linkages in the phenylpropanoid lignin units. The calculated DBE in Table 8 showed that isolated lignin from lignocellulosic bioethanol residues (L1) contained more unsaturated bonds than the kraft and soda lignin samples. The lower DBE of lignin was attributed to the cleavage of β-aryl ether bonds and the formation of free phenolic hydroxyl groups (Robert *et al.* 1984; Mansouri and Salvadó 2006).

The DBE value for milled wood lignin from spruce was reported as 5.36 by Mansouri and Salvadó (2006). The cracking reactions may have occurred during pulping of the kraft and soda, which caused double bonds in the lignin structure (Anglès *et al.* 2003).

Table 8. C9-Formula, Expanded C9-Formula, Molecular Weight, and Double Bond Equivalent (DBE) for Lignin Samples

Lignin	C9 formula	Extended C9 formula	M_w	DBE
L1	$C_9H_{7.64}N_{0.061}S_{0.002}O_{2.52}(OCH_3)_{1.37}$	$C_9H^{ar}_{1.63}H^{al}_{4.70}O_{1.21}(OH)^{ph}_{0.46}(OH)^{al}_{0.85}(OCH_3)_{1.37}$	198.4	5.50
L2	$C_9H_{7.85}N_{0.017}S_{0.144}O_{2.77}(OCH_3)_{1.45}$	$C_9H^{ar}_{2.29}H^{al}_{4.11}O_{1.31}(OH)^{ph}_{0.86}(OH)^{al}_{0.59}(OCH_3)_{1.45}$	205.0	5.35
L3	$C_9H_{8.53}N_{0.078}S_{0.080}O_{1.85}(OCH_3)_{1.02}$	$C_9H^{ar}_{2.63}H^{al}_{4.31}O_{0.27}(OH)^{ph}_{0.67}(OH)^{al}_{0.92}(OCH_3)_{1.02}$	177.8	5.23
L4	$C_9H_{8.25}N_{0.070}S_{0.019}O_{1.82}(OCH_3)_{1.41}$	$C_9H^{ar}_{2.22}H^{al}_{4.55}O_{0.34}(OH)^{ph}_{0.77}(OH)^{al}_{0.71}(OCH_3)_{1.41}$	189.0	5.17

Determination of Molecular Mass Distribution (MMD) of Lignin

Figures 5a and 5b exhibit the chromatographs of HPSEC and the molecular weight distribution of four lignin samples.

All chromatographs showed that the molecular weight distributions of the lignins had normal curves, while L1 had a bimodal curve, which showed that there were two lignin weight fractions.

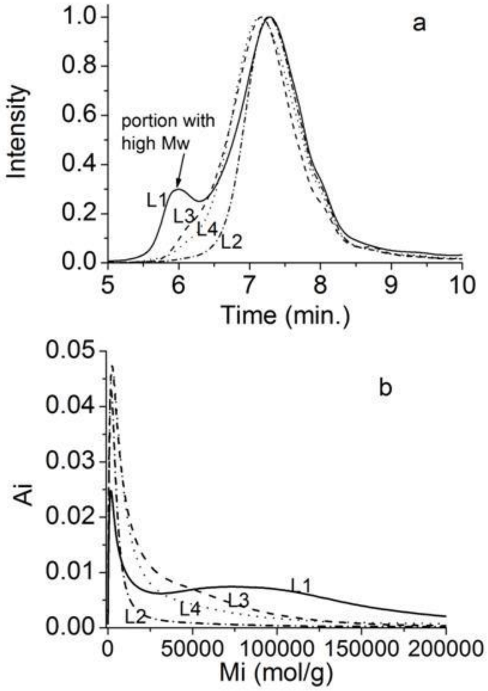

Fig. 5. Molecular weight distributions of the lignin samples: a) Intensity *vs.* Time, b) A_i (mass fraction) *vs.* M_i (Molecular weight)

The number and weight average of the molecular weights, and the molecular weight distribution, were calculated according to the following equations and based on the ASTM D5296 –11 (2011),

$$Mn = \frac{\sum n_i M_i}{\sum n_i} = \frac{\sum_{i=1}^{N} A_i}{\sum_{i}^{N} \frac{A_i}{M_i}} = \frac{\sum h_i}{\sum \frac{h_i}{M_i}} \tag{4}$$

$$Mw = \frac{\sum n_i M_i^2}{\sum n_i M_i} = \frac{\sum_{i=1}^{N} A_i M_i}{\sum_{i=1}^{N} A_i} = \frac{\sum_{i=1}^{N} h_i M_i}{\sum_{i=1}^{N} h_i} \tag{5}$$

$$PD = \frac{M_w}{M_n} \tag{6}$$

where M_n is the number average molecular weight: M_w is the weight average molecular weight, n_i is the number of molecules in the molecular weight M_i, M_i is the molecular weight, A_i is the slice area at each interval of the molecular weight M_i, h_i is the peak height at each interval of molecular weight M_i, and *PD* is the polydispersity (a measure for the heterogeneity of molecules sizes).

Table 9 indicates the number-average (M_n), weight-average (M_w), molecular weights, and polydispersity (*PD*) for the four lignins. The molecular weight of lignin may be dependent on the apparatus and protocol used for its isolation, and is thus difficult to compare (Baumberger *et al.* 2007). The isolated lignin from lignocellulosic bioethanol residues (L1) had a higher molecular weight than other isolated lignins, which was due to less cleavage of ether bonds in the steam explosion process. This higher weight agreed with the higher number of phenolic hydroxyl groups in kraft and soda lignins (Table 5), in which the cleavage of β-O-4 bonds occurred during the alkaline pulping process.

The degree of fragmentation during the alkaline pulping process may have affected the molar mass of lignin. In the kraft process, α-aryl and β-aryl linkages are cleaved. In the soda process of non-woody plants, primarily α-aryl linkages are cleaved, and only small quantities of β-aryl linkages are cleaved (Tejado *et al.* 2007). In this study, β-aryl cleavage occurred during the kraft method due to the more severe conditions, which also caused a lower M_w than soda. Therefore, L2 was found to have a lower average molecular weight than L4, which makes it more valuable in certain applications.

The molecular weight of lignin depends not only on the isolation process of each sample, but on plant species (percentage of G/S/H units) (Cazacu *et al.* 2013). After β-O-4, the most common bonds between lignin units, C–C bonds are the most important linkages between the structural units. Among C–C bonds, the most abundant bond is C5, which makes a linkage between aromatic rings ((Brunow *et al.* 1999). These bonds are formed only between guaiacyl units, as it is not possible for them to form between syringyl units because methoxyl groups are substituted at this position. During pulping processes, C–C bonds are not cleaved due to their higher stability. Therefore, lignins with higher guaiacyl units (softwoods) are expected to have a higher molecular weight than syringyl units (hardwoods and non-woods). Therefore, as expected, the molecular weight of L3 was higher than the L2 and L4 lignin samples.

Table 9. The Number Average (M_n), Weight Average (M_w), Molecular Weight, and Polydispersity (*PD*) for the Four Lignin Samples

Lignin	M_n (g/mol)	M_w (g/mol)	*PD*
L1	1093	13488	12.34
L2	866	2565	2.96
L3	1191	6096	5.12
L4	1084	5008	4.62

Potential Applications for Lignin from Different Sources

Industrial applications for two isolated lignins and two commercial lignins are summarized in Table 10 based on their molecular weights and their relevant physico-chemical properties.

The molecular weight of L1 was found to be much higher than other lignin samples. Lignins with high molecular weight are preferred as matrices in polyolefins composites, as the higher molecular mass of lignin has a better affinity with the matrix (Schorr *et al.* 2014). Lignins with lower molecular weights, such as L2, L3, and L4, are preferred for phenol-formaldehyde resin due to higher reactivity than lignins with high molecular weight (Mansouri and Salvadó 2006). Moreover, lignins with lower molecular weights can be utilized in other applications like antioxidants, adhesives, and paints (Hussin *et al.* 2014). Lignins with high molecular weight show poor antioxidant activity (El Hage *et al.* 2012).

Lignins with lower percentages of sulfur and ash content are preferred for most value-added applications. For instance, sulfur-free lignins are preferable for use in moulded plastics, and as filler for the polymers that produce lower toxic gas emission during processing and disposal (Lora and Glasser 2002; Sahoo *et al.* 2011b). Furthermore, sulfur-free lignins can be also used in thermosetting formulations for the preparation of resins such as phenolic, epoxy, and isocyanate (Kelley *et al.* 1989; World and Glasser 1989). In addition, low sulfur and ash content is more favorable for the synthesis of polymers and low molecular weight substances (Vishtal and Kraslawski 2011). Therefore, L1 and L4 (sulfur-free lignins) have great potential application in the plastic and resin industries where sulfonated lignins have limited use.

Nitrogen in lignin samples is present as proteins or amino acids (organic materials) that are generated from the original source (Niemelä 1990; Veverka and Nichols 1992). Nitrogen-containing substances from lignin can be removed by treatment with proteolytic enzymes such as proteases (Vishtal and Kraslawski 2011). The high percentage of nitrogen in commercial lignins might be due to some amino compounds that are added to the lignin to make it compatible with different polymeric systems (Sahoo *et al.* 2011b). The percentage of nitrogen in L1 is high, which might be due to the remaining enzymes in the hydrolysis process.

It is important to note that lignin with a fair amount of phenolic hydroxyl, aliphatic hydroxyl, and carboxyl groups has great potential to replace polyols in polyurethane production (Pan and Saddler 2013). For instance, polyurethane foam was prepared from organosolv lignin and kraft lignin that contain 5.64 mmol/g and 8.41 mmol/g hydroxyl, respectively (Pan and Saddler 2013). Polyurethane film was prepared with consistent properties from 50% organosolv lignin (total hydroxyl content of 5.38 mmol/g) and polyethylene glycol as co-polyol (total hydroxyl content of 5 mmol/g), using a catalyst for polymerization (Ni and Thring 2003). Therefore, L2 and L3, with a high number of hydroxyl groups, can be used as polyol in synthesis of polyurethane and epoxy resins. Chemical modification, such as oxypropylation with alkylene oxide, improve the accessibility of hydroxyl groups in the lignin macromolecule (Cateto *et al.* 2009; Lora and Glasser 2002). L1, with its relatively high M_w, may not be suitable for this application.

The polymerization reaction of formaldehyde occurs at ortho- and para-free positions of phenol through electrophilic substitution during the synthesis of phenol-formaldehyde resin. In lignin, both C3 and C5 positions are free in p-hydroxyphenyl units, while guaiacyl units have a free C5 position in the ring. In syringyl units, both C3 and C5 are linked to a methoxy group, resulting in lignin’s low reactivity with formaldehyde. Therefore, lignin with p-hydroxyphenyl or guaiacyl units must be more suitable for phenol-formaldehyde formulations. However, it is very important to note that the quantity of phenolic hydroxyls in the lignin molecule activate the free ring positions, while these phenolic hydroxyls can also promote non-covalent interaction between lignin units, making lignin a stiff macromolecule and thus decreasing its final properties (Tejado *et al.* 2007). L1 contained a high number of p-hydroxyphenyl and guaiacyl units, however, the number of phenolic hydroxyls was lower than in other lignin samples. On the other hand, the number of phenolic groups in L2 was higher than in other lignins, but the syringyl was the dominant unit. Thus, the reactivity of both L1 and L2 was low with formaldehyde. L3 (composed primarily of guaiacyl units) presented high numbers of free positions and phenolic hydroxyls in comparison with other lignin samples. Therefore, L3 may have higher reactivity towards electrophilic substitution reactions, rendering it more appropriate for phenolic resins than the other lignin samples (Tejado *et al.* 2007).

Conversion of lignin to high performance carbon fiber requires high purity and a narrow distribution of molecular weight (Baker and Rials 2013). L4 could be considered a precursor source for lignin carbon fiber because it contains low impurities and a narrower molecular weight distribution in comparison with other lignins.

Table 10. Summarized Physico-Chemical Properties of Lignin Samples with Potential Applications

	Lignin			
Property	L1	L2	L3	L4
M_w (g/mol)	13488	2565	6096	5008
Ash (%)	0.45	1.54	4.25	1.38
Impurities in the raw material	Cellulosic materials	Low M_w lignin-like materials	N/A	N/A
S:G:H	26:31:44	68:31:1	0:95:5	51:40:9
OH^{ph} (mmol/g)	1.51	4.91	4.00	2.28
OH^{al} (mmol/g)	2.22	2.35	2.59	2.47
Sulfur (%)	0.03	2.54	1.57	0.38
Nitrogen (%)	0.49	0.13	0.67	0.59
Applications	Filler for plastics and polymer, composites	Phenolic resins, polyurethane, epoxy resins, antioxidants, adhesives and paints	Phenolic resins, polyurethane, epoxy resins, antioxidants, adhesives and paints	Filler for plastics and polymers, Phenolic resins, antioxidants, adhesives and paints, carbon fiber

OH^{al} aliphatic hydroxyl
OH^{ph} phenolic hydroxyl

CONCLUSIONS

1. Lignin from two industrial residues (lignocellulosic bioethanol residues and kraft hardwood black liquor) was isolated, characterized, and compared with two commercial lignins; kraft softwood lignin and soda non-wood lignin. The amount of lignin isolated from the industrial residues was 38% of the dry weight of lignocellulosic bioethanol residues and 27% of the black liquor solids. The amount of lignin isolated from the industrial residues was 38% of the dry weight of lignocellulosic bioethanol residues and 27% of the black liquor solids.
2. The physico-chemical properties of isolated lignins from different sources are not the same for all lignins, which depends on the extraction and isolation processes.
3. Isolated lignin from lignocellulosic bioethanol residues was found to contain the lowest sulfur content, highest molecular weight, and lowest phenolic OH groups compared to the other lignin samples in this study, including hardwood and softwood kraft lignins and non-wood soda lignin.
4. Lignin isolated from hardwood black liquor was found to contain the highest sulfur content, lowest molecular weight, and highest content of phenolic OH groups compared to the other lignin samples in this study, including isolated lignin from lignocellulosic bioethanol residues, softwood kraft lignin and non-wood soda lignin.

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