FRACTIONAL AND STRUCTURAL CHARACTERIZATION OF ALKALINE LIGNINS FROM *CAREX MEYERIANA* KUNTH

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Successive extractions with distilled water, 0.25% NaOH-95% ethanol solution, 0.5%, 1%, 1.5% and 2% NaOH aqueous solutions at a solid-liquid ratio of 1:25 (g mL^{-1}) at 80 °C for 3 h from *Carex meyeriana* Kunth released 4.4%, 6.9%, 10.9%, 13.2%, 7.9% and 4.9% of the original lignin, respectively. The physico-chemical properties and structural features of these lignin fractions were comprehensively characterized by HPLC, GPC, NMR spectroscopy, and TGA. The alkali concentration increment had a positive influence on the purity of the lignin fractions, but a negative influence on the average molecular weight and thermal stability. The FT-IR spectra showed that the lignin fractions had similar structural features with those of HGS lignin. The ^{1}H, ^{13}C and 2D NMR spectra illustrated that typical lignin fractions had predominantly *β*-O-4 aryl ether linkages followed by *β*-*β* and *β*-5' ones.

Keywords: *Carex meyeriana* Kunth, alkaline lignin, fractionation, *β*-O-4 aryl ether linkages

INTRODUCTION

Plant biomass presents an important part of renewable resources for food, energy and useful chemicals, due to its abundance in nature. From an economic point of view, its efficient and rational utilization can achieve an ideal status, if various biomass components (cellulose, hemicelluloses and lignin) are fractionated completely and all used for production.[1] Rapidly growing grassy plants, such as sorghum, miscanthus, reed and switchgrass, belong to the promising sources of plant biomass.[2] Their abundant content and short rotation period allow good application possibilities. *Carex meyeriana* Kunth is widely distributed in the north-east of China, playing a significant role in water conservation.[3] It is a better alternative to increasingly running out fossil resources, in comparison with other grassy plants, which can be used as a nutritive energy source for ruminants. However, plant cell wall characteristics (lignin content and crosslinking through ferulates) limit the conversion of cell wall to energy.[4]

Lignin is the most important component after cellulose and hemicelluloses in grass biomass, which has been studied considerably less than cellulose and hemicelluloses. However, some of the applications, such as lignin-based polyesters and polyurethanes, carbon fibres, plastics, surfactants, elastomer-reinforcing agents, depolymerised low molecular weight chemicals, adhesives, and others, have shown the potential value of lignin exploitation.[5] As a three-dimensional natural polymer, lignin is widely present in the cell wall of vascular plants and is formed of polysaccharide gels, by the polymerization of monolignols, such as sinapyl, coniferyl and *p*-coumaryl alcohol,[6] linked through ether, aryl and carbon-carbon bonds, such as *β*-O-4', 4-O-5', *β*-*β*', *β*-1', *β*-5' and 5-5',[7] increasing the mechanical strength of the plant

and the resistance against microbial attack. Besides, lignin is covalently linked to polysaccharides, forming a lignin-hemicelluloses compound made by of benzyl-ether,[8-10] benzyl-ester[11-13] and phenyl-glycoside bonds.[14, 15]

The characterization of the structure of lignin fractions is carried out by the method of lignin separation – because of the complex structure, the lignin fractions isolated under various reaction conditions exhibit different structure features, which are due to the damage to the lignin fractions.[16] Many of the prior studies focusing on the pretreatment of lignocellulosic materials used corn stover, wood samples or other lignocellulosic materials for biomass sources, and enzymatic mild acidolysis lignin as raw material, while very few of these studies actually used grass as a biomass source.[17] An efficient procedure to isolate lignin and characterize its structural features in different fractions, such as ball-milled lignin, cellulolytic enzyme lignin and others, is still being looked for. Alkali conditions are generally effective in isolating relatively pure components *via* the cleavage of alkali-labile linkages between lignin and carbohydrates.[18] Therefore, in the present study, dewaxed *Carex meyeriana* Kunth was successively fractionated *via* water and alkali solutions with gradually increasing concentrations of alkali to obtain six lignin fractions. To explore the effect of the alkali concentration on the structural features, physico-chemical and thermal properties of lignin, the six lignin fractions were characterized by FT-IR, HPLC, ^{1}H, ^{13}C NMR, GPC and TG analysis.

EXPERIMENTAL

Material

Carex meyeriana Kunth was obtained from a farm in Heilongjiang province, China. It was dried under the sunshine, and then ground by a plant crusher passed through a 40-60 mesh sieve. The powder was oven-dried with air circulation for 16 h at 50 °C and dewaxed with 2:1 (v/v) toluene-EtOH in a Soxhlet apparatus for 6 h. Lignocellulose is the main component in *Carex meyeriana* Kunth, which has a compact structure of cellulose (32.2%) and hemicelluloses (31.2%), in close association with lignin (21.2%). All standard chemicals, such as monosaccharide, were used according to an internal standard, and were chromatographically pure, other chemical agents were analytically pure.

Fractional isolation of lignins

Prior to a mild alkali treatment, the dewaxed sample (10 g) was first treated with distilled water (250 mL) at 80 °C for 3 h. Then the brown mixture was filtrated with four layers of sterile gauze and the insoluble residue fraction remained on the gauze. The residue was subsequently washed with distilled water, and then oven-dried at 50 °C for 16 h. The supernatant from the hot-water treatment was concentrated to about 20 mL on a rotary evaporator under reduced pressure, at bath temperatures not exceeding 50 °C, and then the water-soluble hemicellulosic fraction was precipitated with 3 volumes of 95% ethanol. The pH of the filtrate was adjusted to 1.5 with HCl, and the filtrate was centrifuged and freeze-dried to obtain a lignin fraction, which was labeled L1. The mild alkali treatment was carried out in a 500 mL glass reactor at atmospheric pressure. The residue was successively extracted with 0.25% NaOH-ethanol (95%) solution, 0.5%, 1%, 1.5%, 2% and 3% NaOH aqueous solution at a solid-liquid ratio of 1:25 (g mL^{-1}) at 80 °C for 3 h, and before each extraction step the residue was oven-dried for 16 h. After filtration, the filter liquor was neutralized with a 6 M HCl solution to pH 5.5-6.0 and concentrated to a certain volume with no salt precipitation. The released hemicelluloses were precipitated by pouring the neutralized supernatant fluid into 3 volumes of 95% ethanol. After centrifugation, the pH of the filtrates was adjusted to 1.5 with HCl, and the lignin fractions were further precipitated by centrifugation. Subsequently, they were freeze-dried and labeled as L2, L3, L4, L5 and L6, respectively. The sequential treatments of *Carex meyeriana* Kunth and the isolation of lignin fractions are illustrated in Figure 1.

Characterization of lignin fractions

The FT-IR spectra of the lignin fractions were recorded on a FT-IR microscope (Thermo Scientific IN 10) under liquid nitrogen conditions with a sample holder, which allowed the measurement of IR spectra on solid samples without KBr preparation. Thirty-two scans were taken of each sample, recorded from 4000 to 700 cm^{-1} at a resolution of 2 cm^{-1} in the transmittance mode.

For the determination of lignin purity, the lignin samples (5 mg) were hydrolyzed using 1.48 mL of 6% H_2SO_4 for 2.5 h at 105 °C. After filtration with 0.22 μm filters, the samples were diluted 50-fold and injected into the HPAEC system (Dionex ISC 3000, USA) with an amperometric detector, an AS50 autosampler, a CarbopacTM PA-20 column (4×250 mm, Dionex) and a guard PA-20 column (3×30 mm, Dionex). Neutral sugars and uronic acids were separated in a 5 mM NaOH isocratic (carbonate-free and purged with

nitrogen) for 20 min, followed by a 0-75 mM NaAc gradient in 5 mM NaOH for 15 min. Then the columns were washed with 200 mM NaOH to remove the carbonate for 10 min, and followed by a 5 min elution with 5 mM NaOH to re-equilibrate the column before the next injection. The total analysis time was of 50 min and the flow rate was of 0.4 mL min^{-1}. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-rhamnose, D-mannose, D-galactose, glucuronic acid and galacturonic acid. The experiments were performed at least in duplicate, and the average values were calculated for all of the lignin fractions

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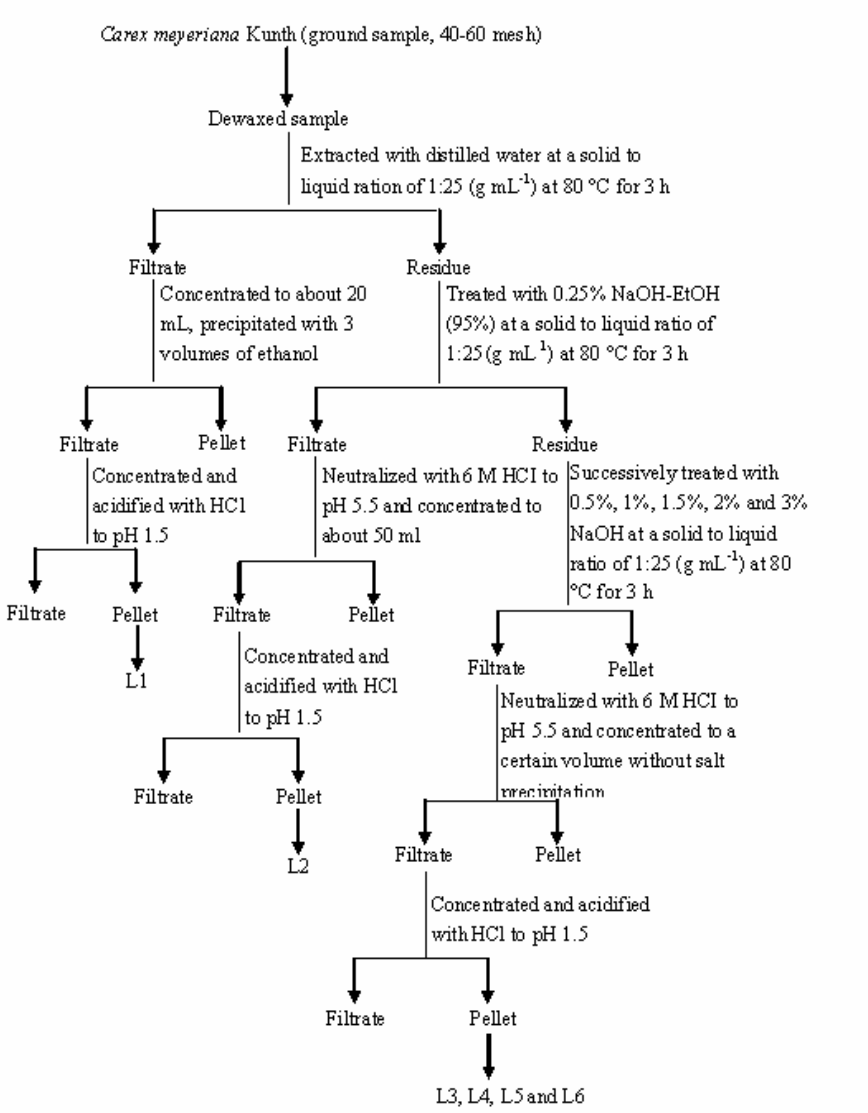

Figure 1: Scheme for isolation of lignins from *Carex meyeriana* Kunth

The molecular weights of the lignin fractions were determined by Gel Permeation Chromatography (GPC, Agilent 1200, USA) with a refraction index detector (RID) on a PL-gel 10 μm Mixed-B 7.5 mm ID column, calibrated with PL pullulan polystyrene standards (peak average molecular weights of 435500, 66000, 9200, and 1320 g mol^{-1}, Polymer Laboratories Ltd.). A 2 mg sample was dissolved in 1 mL tetrahydrofuran, and 20 μL samples were injected. The column was operated at ambient temperature and eluted with tetrahydrofuran at a flow rate of 1 mL/min. The chemical composition of phenolic aldehydes and acids were analyzed by alkaline nitrobenzene oxidation method, which was performed at 170 °C for 3 h, and determined by high-performance liquid chromatiography (HPLC) on a ZORBAX Eclipse

XDB-C18 HPLC column of dimensions 250×4.6 mm (1200 series, Agilent Technologies, USA).

The solution-state ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AVIII 400 MHz spectrometer operating in the FT mode at 100.6 MHz. The lignin sample (25 mg for ^{1}H NMR, 90 mg for ^{13}C NMR) was dissolved in 0.5 mL d_6-DMSO. The ^{1}H spectrum was recorded at 25 °C after 128 scans. A 30° pulse flipping angle, a 13.6 μs pulse width, a 3.98 s acquisition time, and 1 s relaxation delay time were used. The ^{13}C NMR 2D NMR spectrum was recorded at 25 °C after 30000 scans. A 30° pulse flipping angle, a 9.2 μs pulse width, a 1.67 s acquisition time, and 2 s relaxation delay time were used. The Heteronuclear Single Quantum Coherence (HSQC) experiment was performed on the same spectrometer after 128 scans with a 25 mg sample dissolved in 0.5 mL d_6-DMSO. The spectral widths were of 2200 and 15400 Hz for the ^{1}H- and ^{13}C-dimensions, respectively. The number of collected complex points was 1024 for the ^{1}H-dimension with a relaxation delay of 1.5 s. The number of scans was 128, and 256 time increments were always recorded in the ^{13}C-dimension. The $^{1}J_{C\text{-}H}$ used was of 146 Hz. Prior to Fourier transformation, the data matrixes were zero-filled to 1024 points in the ^{13}C-dimension.

The thermal behavior of the lignins was studied using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (DTG-60, Shimadzu). After drying to a constant weight in a cabinet oven at 105 °C for 2 h, the samples (5-10 mg) were heated from 40 °C to 600 °C at a heating rate of 10 °C/min under flowing nitrogen (at a flow rate of 30 mL/min) protection.

RESULTS AND DISSCUSSION

Yield of lignin fractions

The isolation conditions and the yields of the lignin fractions are given in Table 1. As can be seen, the successive treatments with distilled water, 0.25% NaOH-EtOH (95%) solution, 0.5%, 1%, 1.5%, and 2% NaOH aqueous solution at a solid-liquid ratio of 1:25 (g mL^{-1}) at 80 °C for 3 h permitted to obtain lignin fractions with yields of 4.4%, 6.9%, 10.9%, 13.2%, 7.9% and 4.9% of the original lignin (% based on the amount of Klason lignin in the starting material, w/w), respectively. This result indicates that the sequential treatments resulted in up to 48.2% of the original lignins being fractionated. In addition, the 1% NaOH-soluble fraction had the highest yield – of 13.2%, while the water-soluble fraction released only 4.4% of the original lignin. The phenomenon shows that the method used, two-step precipitation for isolation of alkali lignin, is rapid and efficient, as reported.[19] In comparison, the effect of various alkali concentrations from 0.25% to 2% on the yield showed that the alkali concentration was a more important factor than the extraction solvent, and it indicated the increase in alkali concentration favored the release of lignin. It is worth noting that the beneficial effect of the alkali concentration increment on the yield of the lignin fractions of *Carex meyeriana* Kunth, a similar result being reported by a previous study, which showed that higher alkali concentration increment resulted in higher yield of hemicellulosic fractions.[20] Therefore, these two points illustrate better that higher alkali concentration is more beneficial to the cleavage of the ester bonds between hemicelluloses and hydroxycinnamic acids, such as *p*-coumaric and ferulic acids, and to the α-benzyl ether linkages between lignin and hemicelluloses.[21]

Table 1
Fractionation conditions and yield of lignin (% based on amount of Klason lignin in starting material, w/w) solubilized from extractive-free *Carex meyeriana* Kunth

Lignin fraction	Reaction solvent	Temperature (°C)	Extraction time (h)	Yield (%)
L1	Distilled water	80	3	4.4
L2	0.25% NaOH-EtOH	80	3	6.9
L3	0.5% NaOH	80	3	10.9
L4	1% NaOH	80	3	13.2
L5	1.5% NaOH	80	3	7.9
L6	2% NaOH	80	3	4.9

Table 2
Yield (% lignin sample, w/w) of neutral sugars and uronic acids in isolated lignin fractions

Neutral sugars/uronic acids	Lignin fraction					
	L1	L2	L3	L4	L5	L6
Arabinose	0.74	1.74	0.55	1.90	1.03	0.37
Rhamnose	ND	ND	ND	ND	ND	ND
Galactose	0.91	1.29	0.38	0.88	0.61	0.23
Glucose	6.85	0.89	0.13	0.17	0.18	0.18
Manose	ND	ND	ND	ND	ND	ND
Xylose	1.16	3.48	1.04	3.93	2.88	1.97
Uronic acids	2.02	1.11	0.74	0.58	0.52	0.71
Total	11.68	8.52	2.84	7.45	5.23	3.46

*ND, not detected

Purity of lignin fractions

The composition of associated polysaccharides in the six isolated lignin fractions was determined by their neutral sugar and uronic acid contents, the analytical results being given in Table 2. Obviously, the water-soluble lignin fraction contained much higher amounts of bound polysaccharides (11.68%) than the alkali-extracted lignin fractions (2.84-8.52%), and it is worth mentioning that the lignin fractions extracted with 0.25% NaOH-EtOH (95%) contained relatively higher amounts of bound polysaccharides above the other alkali-soluble lignin fractions as shown by 8.52%, in which the glucose accounted for 6.85%. The source of glucose could be understood as following two pathways, one was the branch position of glucuronic acid of hemicelluloses, and the other was the cellulose in a crosslink structure with lignin. A previous study confirmed the presence of this structure in coniferous and non-coniferous wood.[22] Table 2 also shows that xylose, arabinose, and uronic acids were the three major components, while mannose and rhamnose were not found in six lignin fractions. The total contents of neutral sugars and uronic acids in the six lignin fractions were decreased, while the alkali concentration was increased. This result indicates that the alkaline treatment under the conditions used significantly cleaved the ether bonds between lignin and hemicelluloses in the cell walls of *Carex meyeriana* Kunth, in addition to partial saponification of hydroxycinnamic esters, such as between *p*-coumaric acid and lignin/polysaccharides or between ferulic acid and hemicelluloses.[23]

FT-IR spectra

The FT-IR spectra of lignin fractions L1, L2, L3, L4, L5 and L6 (extracted with distilled water, 0.25% NaOH-EtOH (95%), 0.5% NaOH, 1% NaOH, 1.5% NaOH and 2% NaOH) are illustrated in Figures 2 and 3. As can be seen, the relative intensities of the bands for aromatic skeleton vibrations, assigned at 1600, 1510, 1460 and 1420 cm^{-1} are quite similar, indicating a similar structure of the six lignin fractions.[24] An intensive band at 1590 cm^{-1} in the six lignin fractions spectra is assigned to the carbon-carbon bonds of the aromatic ring.[25] Depending on the alkali concentration, the spectra showed a difference in the 1711-1647 cm^{-1} region. In the case of spectra a, e and f, an intensive band at 1708 cm^{-1} assigned to non-conjugated carbonyl groups was observed, while it was weak in spectra b, c and d, indicating that the presence of non-conjugated ketone and/or aldehyde groups in the lignin fractions partially degraded during the corresponding treatments. The bands due to conjugated carbonyl groups (α-carbonyl groups) were observed at 1660, 1651 and 1647 cm^{-1} in the spectra of the six lignin fractions.[26] More bands were located at 1325 cm^{-1} (syringyl ring breathing with C-O stretching), 1262 cm^{-1} (guaiacyl ring breathing with C=O stretching), 1123 cm^{-1} (aromatic C-H in-plane deformation, syringyl type), 1067 cm^{-1} (C-O deformation, secondary alcohol and aliphatic ethers), 1030 cm^{-1} (aromatic C-H in-plane deformation plus C-O in primary alcohol, guaiacyl type), and 915 cm^{-1} (C-H out of

plane deformation).[27] Other weak signal at 1158 cm^{-1} showed the presence of a *p*-coumaric ester group, typical of HGS lignins.[28,29] Aromatic C-H out-of-plane bending appeared at 835 cm^{-1}.[30]

Molecular mass

GPC is effective in estimating the molecular weight of unknown polymers of similar or identical chemical structures with those used to calibrate columns.[31] The macromolecular properties of the lignin fractions were determined with a GPC. The weight-average weights (*Mw*), number-average weight (*Mn*), and polydispersity (*Mw*/*Mn*) of the lignin fractions are given in Table 3. The data show that the six lignin fractions had weight-average molecular weights between 1240 and 4590 g mol^{-1}, and the polydispersity values fluctuated between 1.07 and 1.26. It was found that the extraction of *Carex meyeriana* Kunth with alkali-alcohol solution resulted in the lignin fraction with the lowest average molecular weight (1240) and a higher polydispersity (1.22). In addition, with increasing sodium hydroxide concentration from 0.5% to 2% in the alkali treatments at 80 °C for 3 h, the *Mw* and *Mn* reduced fast and then slowed down from 4590 to 2280 g mol^{-1}, from 4120 to 1940 g mol^{-1}, respectively. No significant regularities of *Mw*/*Mn* were found from the 0.5% to 2% sodium hydroxide treatments at 80 °C for 3 h. The decrease of the molecular weight was related to the relative decrease of associated polysaccharides contents, because most of the dissolved lignin in alkaline treatments was free of linkages with polysaccharide. In other words, the high alkali concentration could cleave the bonds between hemicelluloses and lignin.

^{1}H, ^{13}C and 2D NMR spectra

In order to study the structural differences between lignin fractions L2 and L3, the chemical structure of the lignin fractions were comparatively investigated with ^{1}H, ^{13}C and 2D NMR spectroscopy. In Figures 4 and 5 presenting the ^{1}H NMR spectra of lignin fractions L2 and L3, the signals between 8.00 and 6.00 ppm are assigned to the aromatic protons in G and S units.[32] The signals at 6.80 and 6.71 ppm are assigned to the aromatic protons in G and S units, respectively. Two strong signals at 3.73 and 3.36 ppm are assigned to methoxyl protons, a quite sharp signal at 2.51 ppm is derived from the solvent DMSO. The signals between 1.51 and 0.86 ppm are attributed to aliphatic moiety in the lignin fractions.

The difficulty of analyzing the lignin structure by ^{1}H NMR was mainly caused by the overlapping signals; therefore, the ^{13}C and 2D NMR were used for further understanding the lignin structure. Figures 6 and 7 show that the signals for carbons in polysaccharides from 104.1 to 61.4 ppm are rather weak, indicating the relatively lower content for bond polysaccharides.[33] The weak signals at 174.9 and 60.3 ppm, present only in Figure 6 and absent in Figure 7, are assigned to the carbonyl of a small amount of uronic acids and esters of lignin fraction L2, which represented C-6 in methyl uronates and the 4-O-methoxyl group of glucuronic acid residue.[34] This result is consistent with the sugar content data of the lignin fractions. Similarly, the signals at 152.4 (C-3/C-5, S) and 104.6 (C-2/C-6) ppm attributed to syringyl (S) units were present in both spectra of lignin fractions L2 and L3, while the signals at 138.4 (C-5, S etherified) and 134.4 (C-1, S etherified) ppm attributed to etherified syringyl (S) units were only present in the spectrum of lignin fraction L3. The G units were identified by signals at 149.4 and 148.2 (C-3, G etherified), 144.8 (C-4, G nonetherified), 119.6 (C-6, G), 115.9 (C-5, G) and 111.5 ppm (C-2, G). The H units were verified by the signal at 130.3 ppm (C-2/C-6, H). The *β*-O-4 structure was detected by the signals at 86.3, 71.6 and 60.2 ppm for C-*β*, C-*α* and C-*γ* in *β*-O-4 linkages, indicating that the isolation method could reserve a certain amount of *β*-aryl ether structure.

2D HSQC is a powerful tool for the qualitative analysis of the lignin structure.[35,36] The HSQC spectra of lignin fractions L2 and L3 are given in Figure 8. Most of the observed signals are assigned according to the wood lignin spectra previously reported.[37-41] As can be seen, the S units give signals at 104.3/6.68 ppm for $C_{2,6}$-$H_{2,6}$, while the signals at 104.7/7.32 ppm represent

$C_{2,6}$-$H_{2,6}$ in C_{α}-oxidized syringyl units (S' and S'', respectively) in Figure 8 (a). G units were observed at C_2-H_2 (111.5/7.28 ppm), C_5-H_5 (115.5/6.31 ppm) and C_6-H_6 (119.6/6.71 ppm). H units were observed at $C_{2,6}$-$H_{2,6}$ (128.1/7.16 ppm). A signal at 123.0/7.08 ppm corresponds to the C_6-H_6 correlation for ferulic acid ether structure. Aromatic ring signals corresponding to correlations $C_{2',6'}$-$H_{2',6'}$ and $C_{3',5'}$-$H_{3'5'}$ were observed at 130.3/7.50 and 116.1/6.8 ppm, respectively. In addition, signals of the unsaturated $C_{\alpha'}$-$H_{\alpha'}$ unit and of $C_{2',6'}$-$H_{2',6'}$ in the *p*-coumarate structure were also observed at 144.8/7.47 and 130.3/7.50 ppm. As may be noted in Figure 8 (b), the methoxy groups give an overlapped signal at 56.0/3.73 ppm, and the signals of C_{β}-H_{β} in β-O-4 substructures linked to the G unit and S unit were observed at 86.3/4.10 and 84.1/4.28 ppm, respectively.

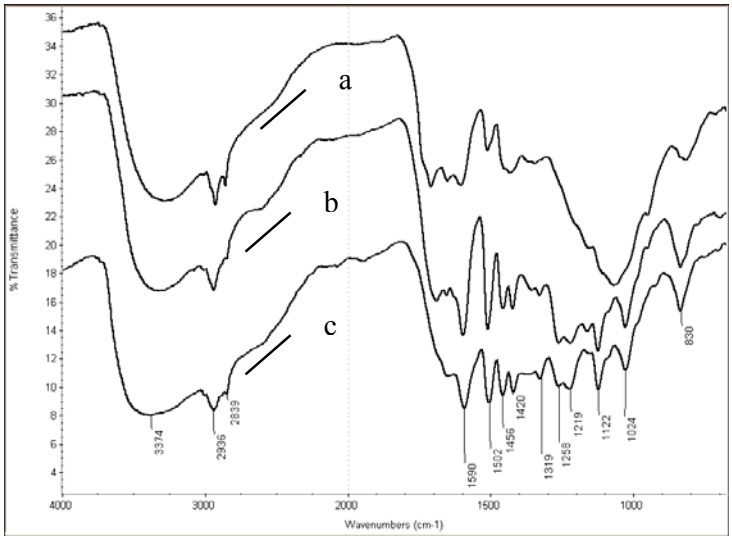

Figure 2: FT-IR spectra of *Carex meyeriana* Kunth lignin fractions isolated with distilled water (a), 0.25% NaOH-EtOH (95%) (b) and 0.5% NaOH (c) at 80 °C for 3 h

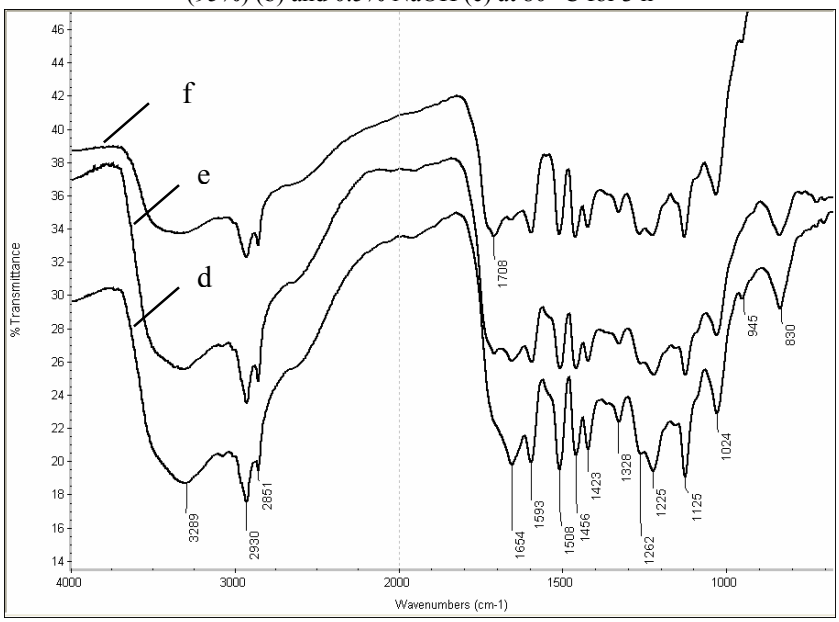

Figure 3: FT-IR spectra of *Carex meyeriana* Kunth lignin fractions isolated with 1% NaOH (d), 1.5% NaOH (e) and 2% NaOH (f) at 80 °C for 3 h

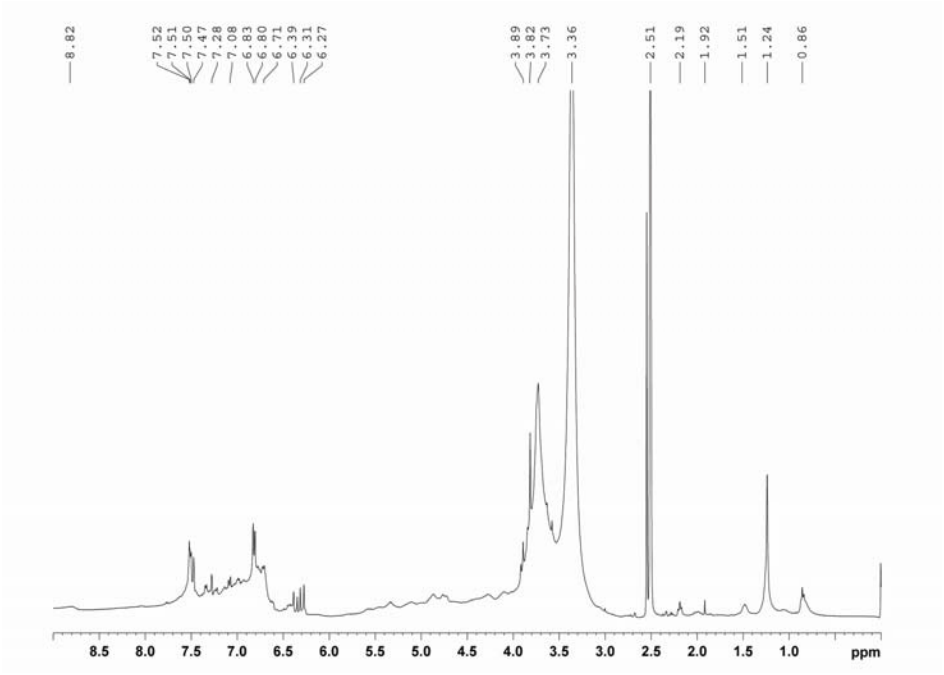

Figure 4: ^{1}H NMR spectrum of lignin fraction L2 isolated with 0.25% NaOH-EtOH (95%) at 80 °C for 3 h

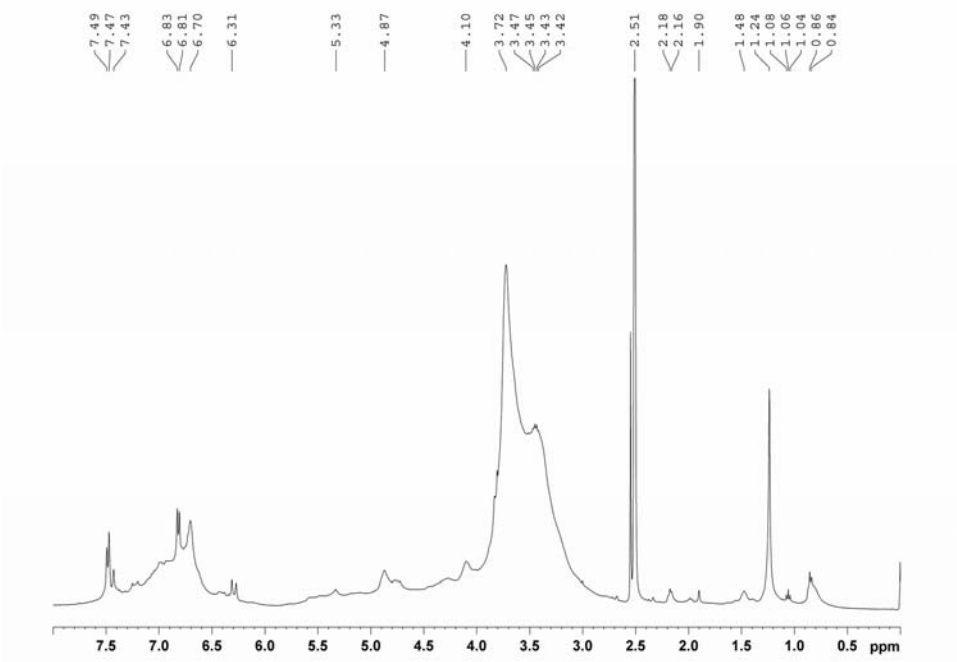

Figure 5: ^{1}H NMR spectrum of lignin fraction L3 isolated with 0.5% NaOH at 80 °C for 3 h

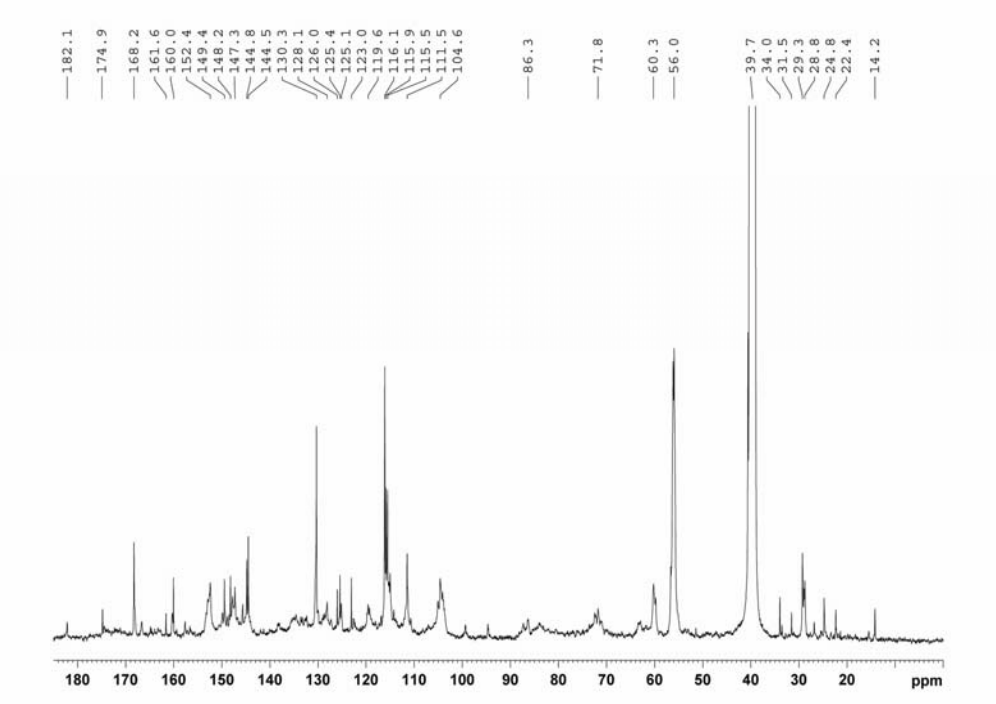

Figure 6: ^{13}C NMR spectrum of lignin fraction L2 isolated with 0.25% NaOH-EtOH (95%) at 80 °C for 3 h

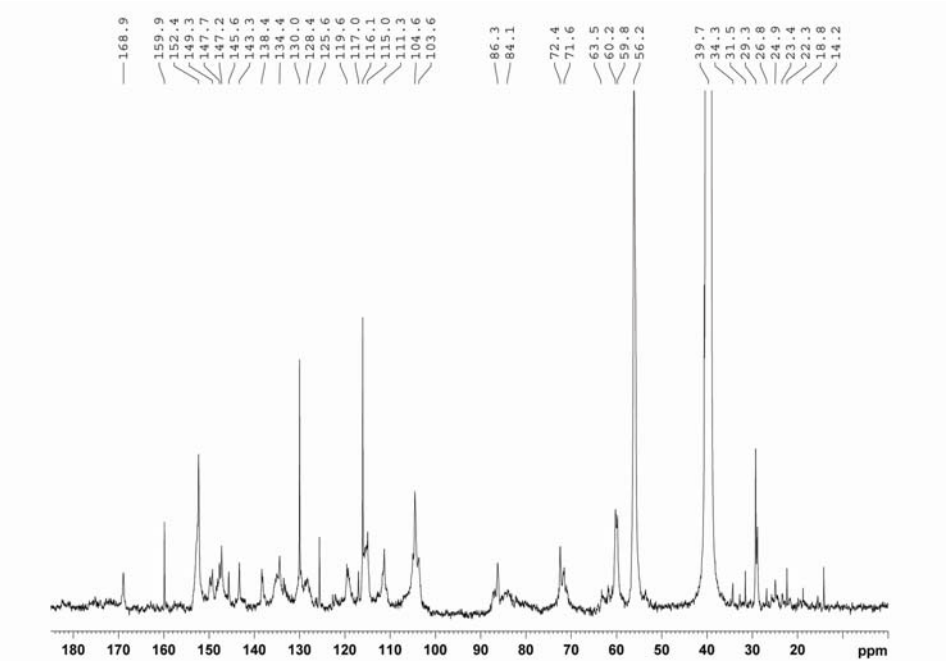

Figure 7: ^{13}C NMR spectrum of lignin fraction L3 isolated with 0.5% NaOH at 80 °C for 3 h

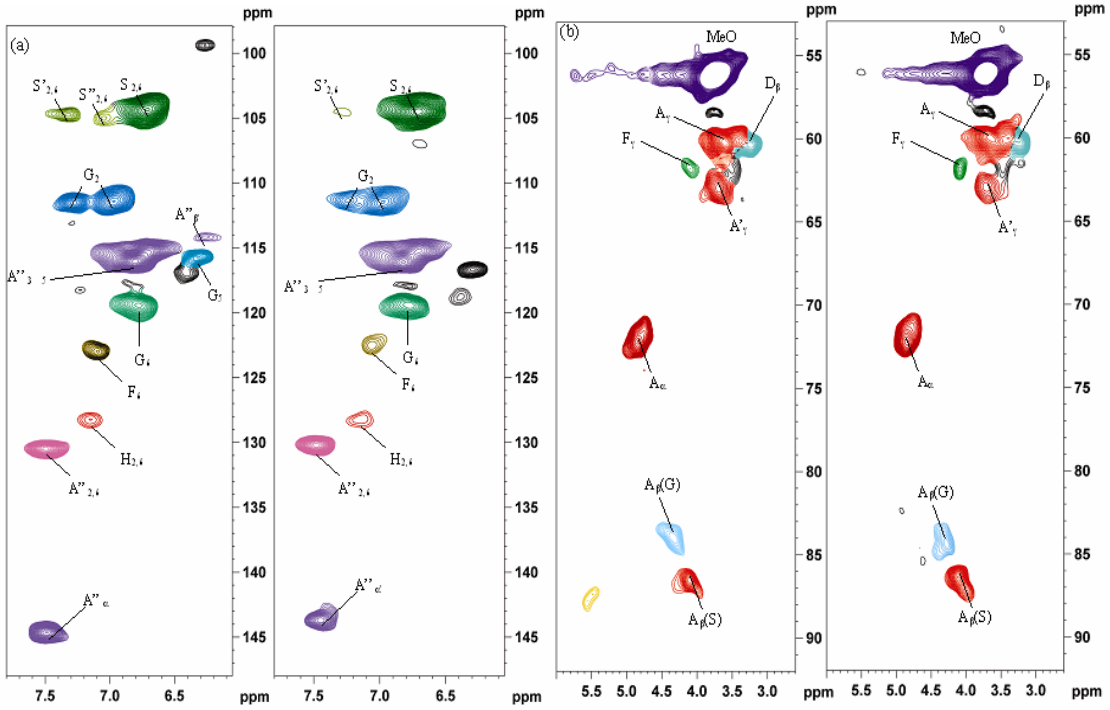

Figure 8: HSQC NMR spectra of lignin fractions L2 (left) and L3 (right) isolated with 0.25% NaOH-EtOH (95%) and 0.5% NaOH at 80 °C for 3 h from *Carex meyeriana* Kunth: (a) oxygenated aliphatic region δ_C/δ_H 53-92/2.6-6 ppm (b) aromatic region δ_C/δ_H 98-148/6-7.8 ppm

Table 3
Weight-average (*Mw*) and number-average (*Mn*) molecular weights and polydispersity (*Mw*/*Mn*) of lignin fractions

	Lignin fraction					
	L1	L2	L3	L4	L5	L6
Mw	1390	1240	4590	2640	2120	2280
Mn	1300	1020	4120	2100	1750	1940
Mw/*Mn*	1.07	1.22	1.12	1.26	1.21	1.18

Table 4
Assignments of main lignin ^{13}C-^{1}H correlation signals in HSQC spectra shown in Figure 9

Labels	δC/δH (ppm)	Assignment
-OCH3	56.0/3.73	C_β-H_β in methoxyls (MeO)
A_γ	60.3/3.68	C_γ-H_γ in *β*-O-4' substructures (A)
F_γ	59.8/3.89	C_γ-H_γ in *p*-hydroxycinnamyl alcohol end groups (F)
A'_γ	63.0/3.69	C_γ-H_γ in acylated *β*-O-4' substructures (A' and A'')
A_α	71.8/4.85	C_α-H_α in *β*-O-4' substructures
A_β(G)	84.1/4.28	C_β-H_β in *β*-O-4' substructures linked to G unit (A)
A_β(S)	86.3/4.10	C_β-H_β in *β*-O-4' substructures linked to S unit (A) (erythro)
$S_{2,6}$	104.3/6.68	$C_{2,6}$-$H_{2,6}$ in etherified syringyl units (S)
$S'_{2,6}$	104.7/7.32	$C_{2,6}$-$H_{2,6}$ in oxidized (C_αOOH) syringyl units (S')
$S''_{2,6}$	104.9/7.05	$C_{2,6}$-$H_{2,6}$ in oxidized (C_α=O) phenolic syringyl units (S'')
G_2	111.5/7.28	C_2-H_2 in guaiacyl units (G)
G_5	115.5/6.31	C_5-H_5 in guaiacyl units (G)

$A''_{3,5}$	116.1/6.80	$C_{3,5}$-$H_{3,5}$, *p*-coumaroylated substructures (A'')
G_6	119.6/6.71	C_6-H_6, G units (G)
F_6	123.0/7.08	C_6-H_6 in FE ester (F)
$H_{2,6}$	128.1/7.16	C_6-H_6 in H units
$A''_{2,6}$	130.3/7.50	$C_{2,6}$-$H_{2,6}$, *p*-coumaroylated substructures (A'')
A''_{α}'	144.8/7.47	C_α-H_α, *p*-coumaroylated substructures (A'')

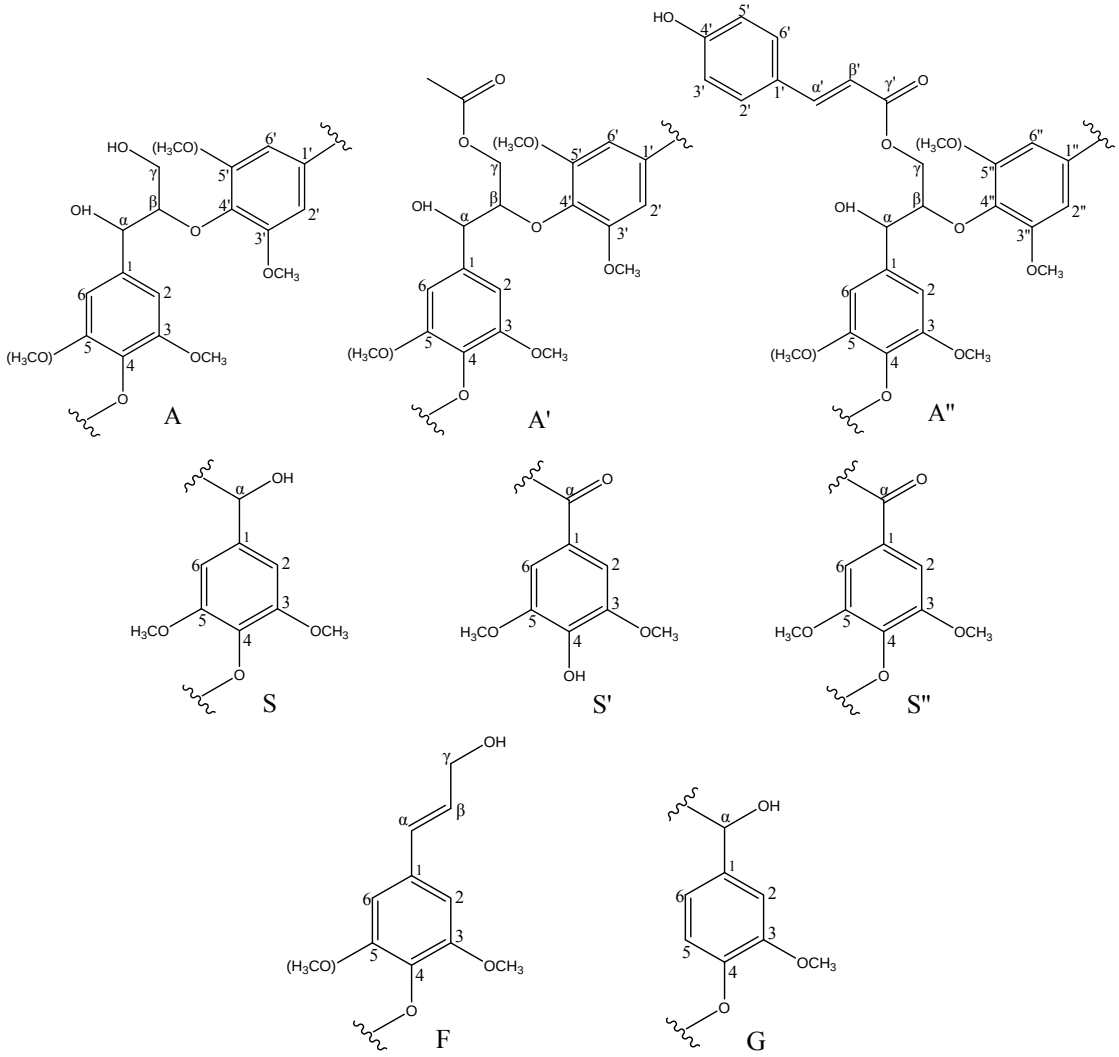

Figure 9: Main structures present in *Carex meyeriana* Kunth lignin: (A) *β*-O-4' aryl ether linkages with a free –OH at C-*γ*; (A') *β*-O-4' aryl ether linkages with acetylated –OH at C-*γ*; (A'') *β*-O-4' aryl ether linkages with *p*-coumaroylated –OH at C-*γ*; (S) syringyl unit and (S' and S'') oxidized syringyl units with a C*α* ketone or a C*α* carboxyl group; (F) cinnamyl alcohol end-groups; (G) guaiacyl unit

Besides, the signal of C_α-H_α was observed at 71.8/4.85 ppm, and C_γ-H_γ in the *β*-O-4' substructure presented a signal at 63.0/3.69 ppm. The assignments of the main lignin ^{13}C-^{1}H correlations of the HSQC spectra are listed in Table 4, and the main substructures found in fractions L2 and L3 are presented in Figure 9.

Thermal stability

Lignin thermally decomposes over a broader temperature range than the cellulose and hemicelluloses of biomass, because various oxygen functional groups from its structure have different thermal stability, their scission occurring at different temperatures.[42] The TGA and DTA

were used to study the thermal stability of lignin fractions L2, L3 and L5, isolated with 0.25% NaOH-EtOH (95%), 0.5% NaOH and 1.5% NaOH, respectively (Figure 10). As can be seen from the curves, the decomposition process of lignin fractions L2, L3 and L5 covered a broad temperature range from 200 to 500 °C. The DTA peak temperatures were of 385.3, 366.5 and 387.3 °C, indicating the maximum heat absorption rates of three lignin fractions. According to the curves, the weight loss rates of the three lignin fractions were of 51.7%, 47.4%, and 41.7%, indicating that the alkali concentration increment had an adverse effect on the thermal stability of the lignin fractions.

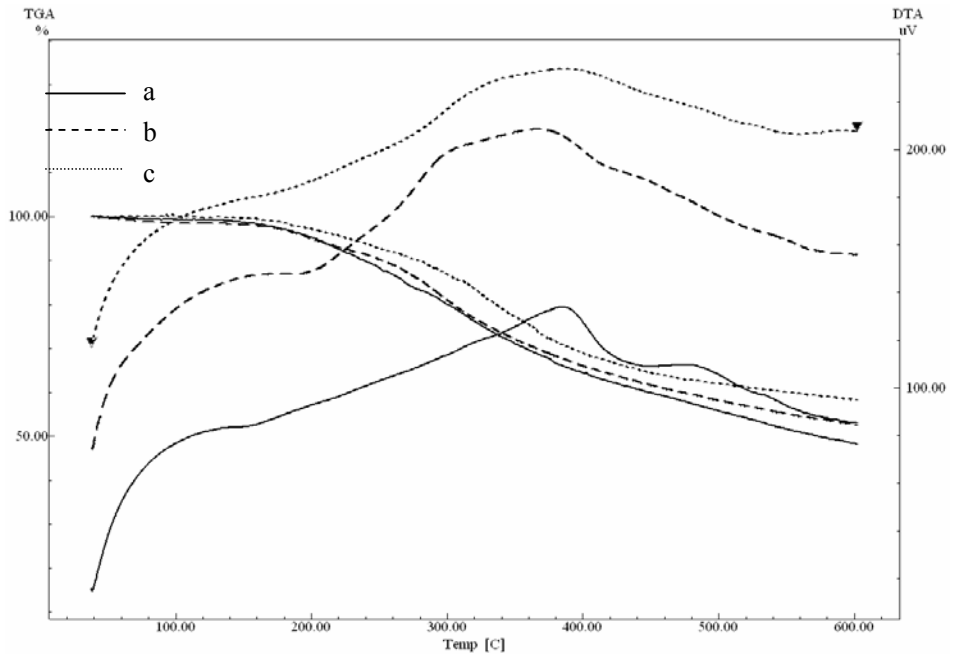

Figure 10: TGA/DTA curves of lignin fractions L2 (a), L3 (b) and L5 (c) isolated with 0.25% NaOH-EtOH (95%), 0.5% NaOH and 1.5% NaOH at 80 °C for 3 h, respectively

CONCLUSIONS

The sequential six-step extraction procedures of *Carex meyeriana* Kunth, carried on with distilled water, 0.25% NaOH-ethanol (95%) solution, 0.5%, 1%, 1.5% and 2% NaOH aqueous solutions resulted in a yield of 48.2% of the original lignins, with the 1% NaOH-soluble fraction having the highest yield, of 13.2% of the original lignin. The alkali concentration increment had a positive influence on the purity of the lignin fractions, but a negative influence on the average molecular weight and thermal stability. The characterization of six lignin fractions indicated that they were all HGS-type lignin. Furthermore, the bonds between the lignin monomers in the alkali lignin fractions were mainly composed of *β*-O-4 linkages and small quantities of *β*-*β*, *β*-5' carbon-carbon linkages.

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