STRUCTURAL CHARACTERIZATION OF ISOLATED LIGNINS FROM *CARAGANA KORSHINSKII KOM.*

K. WANG,[*] H. Y. YANG,[*] X. YAO,[**] R. C. SUN[*,***] and G. L. JONES

[*]*Institute of Biomass Chemistry and Technology, Beijing Forestry University, Beijing 100083, China*
[**]*International Centre of Bamboo and Rattan, Beijing, 100102, China*
[***]*State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China*
The BioComposites Centre, University of Wales, Bangor LL57 2UW, United Kingdom

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Lignin fractions, isolated from *Caragana korshinskii* Kom., were subjected to extensive structural characterization, including chemical component analysis, gel permeation chromatography (GPC), Fourier transform infrared (FT-IR), ultraviolet (UV), thermo gravimetric/differential thermal analysis (TGA/DTA), and nuclear magnetic resonance (NMR). The structural differences caused by different solvent treatments (water, ethanol and NaOH alkaline solution in concentrations of 1%, 3%, 5%, 8% and 10%) were studied comparatively. The results indicated that 84.1% lignin was fractionated with the successive treatments. The weight-average molecular weights of all lignin fractions ranged from 926 to 2686 g/mol. Noticeable amounts of esterified hydroxycinnamic acids were identified in the lignin fractions obtained from water and ethanol treatments. The cleavage of ester and other types of lignin-carbohydrate interaction was obviously induced under basic conditions, and the purity of the isolated lignin was consequently improved. However, the thermal stability of the lignin fraction was slightly enhanced. Some substructures, such as *β-O-4*, *β-β*', were also detected in *C. korshinskii* Kom. lignin by NMR technology.

Keywords: *Caragana korshinskii* Kom., lignin, fractionation, structural characterization

INTRODUCTION

Caragana belongs to the Papilionoideae (Leguminosae) and comprises about 70 species.[1] Korshinsk peashrub (*C. korshinskii* Kom.) is a perennial sandy grassland and desert deciduous shrub species, mainly distributed in the northwest of China and Mongolia.[2] It is a diploid (2n = 2x = 16) with advantages in drought and cold resistance, vegetation succession from shifting dunes to sandy grasslands, restoring degraded land by fixing atmospheric nitrogen, and forming shrub belts for crops or artificial grassland.[3] Generally, this shrub generates a large amount of lignocellulosic residues, as it is cut once every 3 years to make it flourish, and only a small amount of the residues is used for the production of fiberboard.[4] It is believed that a reasonable and comprehensive utilization of this abundant resource will facilitate the growing shortage of wood resources in pulp, paper and other wood-based industries. Recently, the second-generation bioethanol from lignocellulosic materials has been attracting more and more interest, for fulfilling the high-speed development of social economy and meeting the shortage of mineral resources.[5] Taking into account the high production cost, however, the single utilization of the cellulosic component could not satisfy the objectives of industrial production. Thereby, the concept of biorefinery was proposed, being expected to generate a variety of products, through a combination of multi-step processes.[6-8]

The first and key step in a biorefinery approach is the fractionation process, in which the main lignocellulosic components – cellulose, hemicelluloses and lignin – are separated with a yield and purity as high as possible. Lignin is one of the main components of woody cell walls, accounting for up to 30% of the organic carbon in the biosphere.[9] The lignin macromolecule is biosynthetically polymerized *via* carbon-carbon

and carbon-oxygen (ether) bond connection of phenyl propanoid building blocks.[10-11] Three precursors, coniferyl, sinapyl and *p*-coumaryl alcohols, are called monolignols and contribute to guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) propane units in lignin, respectively. With the increase in biomass utilization, lignin is considered as a potential starting material for the manufacture of adhesives, epoxi- and phenolic-resin, and polyolefins, due to its polyphenolic chemical structure.[12] However, it is necessary to characterize the complex structure of lignin, providing basic and valuable information for further modification and application.

In the present work, lignin from *C. korshinskii* Kom. was fractionated by successive treatments with water, ethanol and alkaline solutions in different concentrations. The lignin fractions obtained were characterized by component analysis, gel permeation chromatography (GPC), UV, FT-IR, NMR and thermal analysis. In spite of a considerable amount of existing data on the physico-chemical properties of the lignin component, we aimed at enriching the structural knowledge of this abundant resource and expected to take full advantage of it.

EXPERIMENTAL

Materials

C. korshinskii Kom. (3 years old) was harvested at the Shalin arboretum of Yikezhao League of Inner Mongolia, China. After the leaves and capitula were removed, the stalks were ground and screened to obtain a uniform particle size (40-60 mesh size). Before the fractionation process, the wood powder was dewaxed by refluxing with toluene-ethanol (2:1, V/V) for 6 h in a Soxhlet apparatus. Its composition was determined as follows: cellulose – 45.1%, hemicelluloses – 23.2%, and lignin – 18.3%.[13]

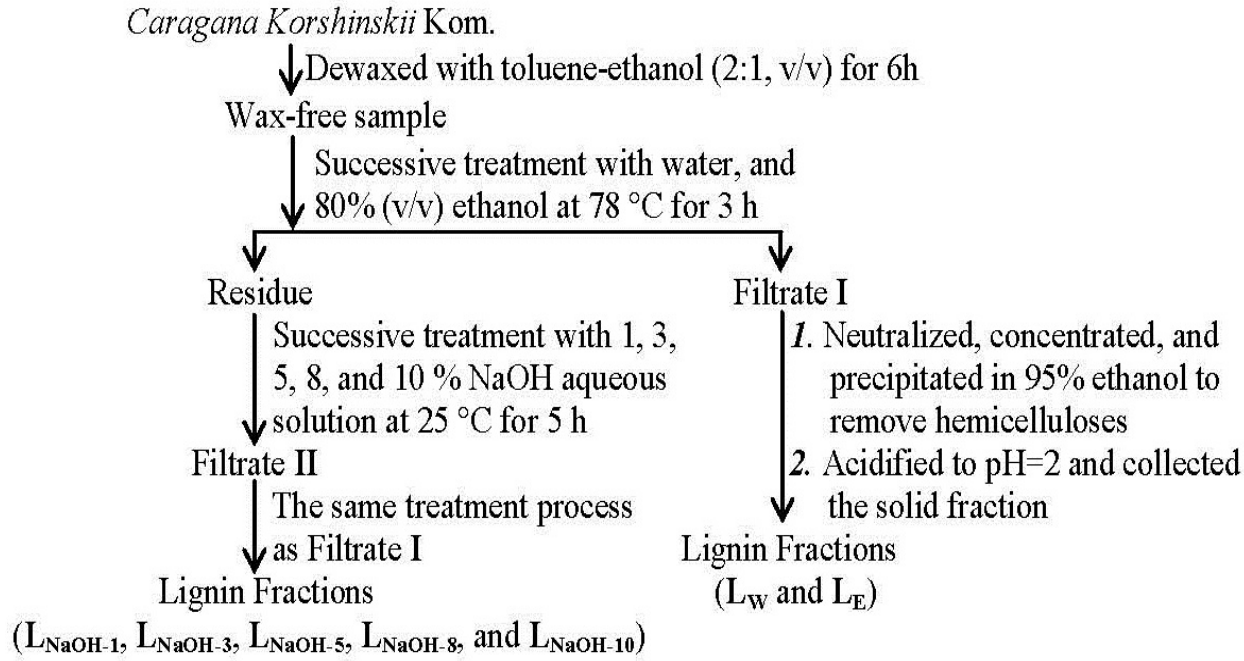

Figure 1: Scheme for isolation of lignin fractions

Fractionation process

The scheme in Figure 1 describes the fractionation process of lignin from *C. korshinskii* Kom. Briefly, the dewaxed material was firstly soaked in distilled water and 80% (V/V) ethanol aqueous solution at 78 °C for 3 h, the lignin fractions (L_W and L_E) isolated under neutral circumstances were obtained by precipitation in acidic medium after the removal of hemicelluloses in ethanol. Then the insoluble residues were successively treated with NaOH aqueous solutions in concentrations of 1, 3, 5, 8 and 10% for 5 h. The liquid was collected by filtration, adjusted to pH 5.5 with 6 M HCl and concentrated under vacuum. The lignin fractions were isolated by the procedure described above, and labeled as L_{NaOH-1}, L_{NaOH-3}, L_{NaOH-5}, L_{NaOH-8}, and $L_{NaOH-10}$, respectively, corresponding to the alkaline concentrations afore-mentioned.

Characteristic analysis

The analysis of the polysaccharides and aromatic compounds of the lignin fractions, described in a previous paper, was conducted by high-performance anion-exchange chromatography (HPAEC), with pulsed amperometric detector (PAD), an ion exchange Carbopac PA-1 column, a HPLC system with diode array detector (DAD) and ZORBAX Eclipse XDB-C_{18} column.[14] The molecular-average weights were determined by gel permeation chromatography (GPC) on a 5 mm PLgel Mixed-D column. The samples were dissolved in tetrahydrofuran (THF) at a concentration of 0.2%, and eluted with THF at a flow rate of 0.5 mL/min. To calibrate the column, monodisperse polystyrene of known weight-average molecular weight was used as the standard.

Fourier transform infrared (FT-IR) spectra were recorded from an FT-IR spectrophotometer (Tensor 27, Bruker, Germany) using KBr discs containing 1% finely ground samples. UV spectra were obtained on an ultraviolet/visible spectrophotometer (Tec Company, UV 2300). Lignin fractions, dissolving in dioxane-water mixture, were scanned between 260-400 nm to acquire the absorption spectra. ^{1}H NMR spectra were recorded on a Bruker AVIII NMR spectrometer at 400 MHz using 10 mg of lignin in 0.5 mL DMSO-d_6. Data processing was performed using standard Bruker Topspin-NMR software. The thermal stability of each sample was determined using a simultaneous thermal analyzer (DTG-60, Shimadzu, Japan) from room temperature to 600 °C, at a rate of 10 °C/min under a constant nitrogen flow atmosphere.

RESULTS AND DISCUSSION

Fractional yield and purity

Table 1 gives the yields of all the lignin fractions obtained from the various solvents (water, ethanol and alkaline solutions with different concentrations). Altogether, 84.1% lignin was isolated from the raw material after successive treatments. These data, in conjunction with those on sugar composition (Table 1), permit the conclusion that the lignin fractions obtained under neutral conditions (water and ethanol) contained a certain amount of polysaccharides (2.53% for L_W and 1.77% for L_E, respectively). As known, covalent linkages between lignin and carbohydrates complexes (LCC) have been extensively accepted,[15, 16] and the main types of LCC bonds have been suggested to be benzyl esters, benzyl ethers and phenyl glycosides.[17] Under alkaline conditions, a large amount of ester bonds between hydroxycinnamic acid and lignin or carbohydrates were significantly saponified, together with the cleavage of ether bonds. Thereby, the contents of associated sugars decreased correspondingly to 0.72% (L_{NaOH-1}), and finally to 0.02% ($L_{NaOH-10}$), as alkaline concentration increased to 10%. Starch could be efficiently extracted in a neutral solution, resulting in a high content of glucose in L_W and L_E. In alkaline-soluble lignins, xylose was determined as the major sugar, along with small amounts of arabinose and galactose, which indicates that the xylan-lignin linkage, taking arabinose and galactose as bridges, was the most important connection in these fractions.

Table 1
Yield (wt%, related to the starting material), molecular weight and monosaccharide components (wt%, related to the lignin sample) of the isolated lignin fractions

	Lignin fractions [a]						
	L_W	L_E	L_{NaOH-1}	L_{NaOH-3}	L_{NaOH-5}	L_{NaOH-8}	$L_{NaOH-10}$
Yield	1.0	2.1	1.8	1.5	2.6	3.5	2.9
M_w	1128	1595	1426	2686	1673	1021	926
M_n	708	785	939	637	469	439	514
M_w/M_n	1.6	2.0	2.4	4.1	3.6	2.3	1.8
Rha	0.22	0.02	0.01	ND [b]	ND	ND	ND
Ara	0.28	0.33	0.12	0.11	0.04	0.06	ND
Gal	0.45	0.85	0.34	0.26	0.24	ND	ND
Glu	1.43	0.46	0.08	0.10	0.06	0.09	0.01
Man	0.02	ND	ND	ND	ND	ND	ND
Xyl	0.13	0.12	0.21	0.16	0.07	0.03	0.01
Total	2.53	1.77	0.72	0.63	0.41	0.18	0.02

[a] Corresponding to the lignin fractions in Figure 1
[b] ND = not detectable

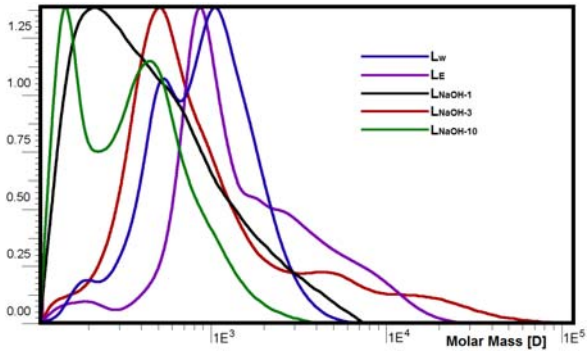

Figure 2: Molecular weight distribution curves of lignin fractions

Table 2
Yield of phenolic acids and aldehydes (relative mole %) obtained by alkaline nitrobenzene oxidation

	Lignin fractions [a]						
	L_W	L_E	L_{NaOH-1}	L_{NaOH-3}	L_{NaOH-5}	L_{NaOH-8}	$L_{NaOH-10}$
p-Hydroxybenzaldehyde	28.1	26.3	20.2	20.7	21.5	14.3	14.8
p-Hydroxybenzonic acid	1.1	0.9	0.3	0.4	0.3	ND [b]	ND
Syringaldehyde	30.6	20.8	15.2	16	18.1	21.2	26.1
Syringic acid	2.9	8.2	3.5	3.9	3.6	2.6	2.8
Vanillin	33.4	38.1	52.5	50.6	49.2	48.9	50.6
Vanillic acid	3.9	5.7	8.3	8.4	7.3	13	5.7
V/S [c]	1.1	1.5	3.3	3.0	2.6	2.6	2.0

[a] Corresponding to the lignin fractions in Figure 1
[b] ND = not detectable
[c] S – the total amount of syringaldehyde and syringic acid, V – the total amount of vanillin and vanillic acid

Molecular distribution

The effects of the fractionation process with different solvents on the lignin macromolecular structures were studied by weight/number-average molecular weight (Table 1) and its corresponding distribution (Figure 2). Surprisingly, the molecular weights of L_W and L_E were similar to the other lignin fractions, which was not consistent with previous results.[18] The high content of sugar was the probable reason. Lawoko *et al*.[19] concluded that the presence of poly(oligo-)saccharide chains bonded to lignin led the molecular mass distribution shift to shorter retention time by comparing the SEC curves of MWL, MWL-impure and EMWL-impure. Overall, the weight-average molecular weights of the lignin fractions from *C. korshinskii* Kom. were low, reflecting the inherent structure of lignin in this plant species. As can be seen, L_{NaOH-3} had the highest value (2686 g/mol), corresponding to the highest polydispersity (4.1). It could be probably due to the existence of a trace amount of huge lignin macromolecules in this fraction, almost reaching 1×10^6 g/mol (Figure 2). As further increasing the alkaline concentration, the values of molecular weight gradually decreased, as well as the polydispersity, which was probably due to the partial degradation of lignin macromolecules under strong basic conditions.

Structural characterization

Firstly, alkaline nitrobenzene oxidation was employed as a degradation method, to investigate the main phenolic compounds in the lignin fractions (Table 2). Clearly, the lignin component in *C. korshinskii* Kom. was identified as G-S-H type lignin. The predominant degradation products, vanillin and syringaldehyde, resulted from the degradation of non-condensed guaiacyl and syringyl units, respectively. A certain amount of *p*-hydrobenzaldehyde and *p*-hydrobenzonic acid was also detected, suggesting the presence of non-condensed *p*-hydroxycinnamoyl units in *C. korshinskii* Kom. lignin. The V/S ratio (guaiacyl to syringyl) of the lignin fraction is considered as a significant parameter in delignification processes. Interestingly, a gradual decrease of the ratio from 3.3 (L_{NaOH-1}) to 2.0 (L_{NaOH-1}) with an increase in alkaline concentration from 1% to 10% demonstrated that the guaiacyl units engaged in lignin macromolecules were more easily degraded compared to syringyl units during the

successive alkaline treatments.

The six lignin fractions exhibited the basic UV spectrum of typical lignin with two obvious peaks at around 280 and 320 nm. The former originates from non-conjugated phenolic groups (aromatic ring), while the latter is assigned to the esters of *p*-coumaric and ferulic acids.[20] The obviously higher intensity of the adsorption at 320 nm in L_E indicated that the ester linkages between hydroxycinnamic acids and lignin remained after ethanol extraction. Besides, the relatively lower adsorptions of the other five lignin fractions were presumed to be due to the slightly higher amounts of non-lignin materials, such as carbohydrates, ash and salt.

To further investigate the heterogenous structure and functional groups in the released lignins, FT-IR spectra of lignin fractions L_W, L_E, L_{NaOH-1} and L_{NaOH-5} were recorded (Figure 4). The characteristic bands at 1600, 1500 and 1420 cm^{-1} for the aromatic ring vibrations of the phenylpropane skeleton were clearly observed. The C=O in unconjugated ketone (*β*-carbonyl) and carboxylic acid, and the C=O stretch in conjugated *p*-substituted aryl ketone (*α*-carbonyl) were also observed in all the spectra at 1713 and 1650 cm^{-1}, respectively.[21] However, the most significant differences were clearly observed regarding the absorption at 1740 cm^{-1}. The absence of this peak in the lignin fractions L_{NaOH-1} and L_{NaOH-5} obtained from the alkaline aqueous solution revealed that the ester bonds between *p*-hydroxybenzoic acid or hydroxycinnamic acids and lignins were completely cleaved or decreased below the detection limit. Besides, the value of $A_{1328}^{-1}/A_{1270}^{-1}$ was employed to estimate the S/G ratio.[22] As may be observed in Fig. 4, all the lignin fractions contained a higher amount of guaiacyl units than syringyl units, further confirming the results of alkaline nitrobenzene oxidation.

^{1}H NMR technology was then used to provide more information about the lignin macromolecule (Figure 5). Most of the observed signals were previously assigned in straw and wood lignin spectra.[23, 24] The obvious signals between 7.3 and 7.5 ppm are assigned to aromatic protons of ester-linked *p*-coumaric and ether-linked ferulic acid. The remarkable peaks between 6.2 and 6.5 ppm are attributed to the 3, 5 position and *β*-position of hydroxycinnamic acids. These phenomena were closely related to the noticeable amounts of cross-linked *p*-coumaric and ferulic acids, corresponding to the results obtained by the chemical analysis and by the UV spectroscopic method. All signals between 6.0 and 8.0 ppm belong to aromatic protons, in which the signals at 7.0 and 6.7 ppm are attributed to the aromatic protons in G and S units, respectively. The rather weak signal at 7.0 ppm revealed the relatively small amounts of guaiacyl units as discussed above. It is worth noting an obvious peak at 5.3 ppm in the ^{1}H NMR spectrum of L_E, which supports the presence of a phenylcoumaran substructure in the lignin fraction. Other substructures, such as *β-O-4* and *β-β*', were identified by the signals between 3.7 and 4.1 ppm. The associated polysaccharides also give a strong signal at 3.3 ppm, assigned to H_3 in the 1→4 linked *β*–D-Xyl residues. An intense signal at 2.49 ppm is indicative of residual proton in DMSO-d_6. The peaks between 2.2 and 1.9 ppm relate to the methyl or methylene protons adjacent to double bonds or carbonyl groups. Proton in aliphatic groups exhibit signals between 1.5 and 0.8 ppm.

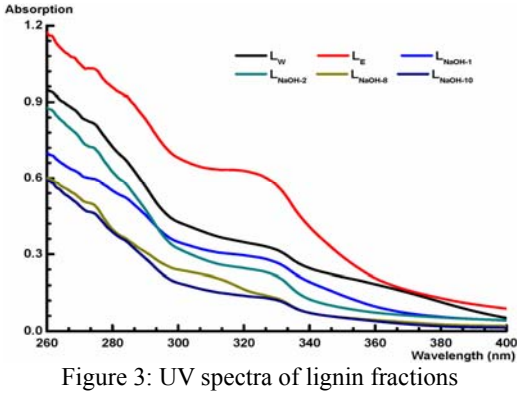

Figure 3: UV spectra of lignin fractions

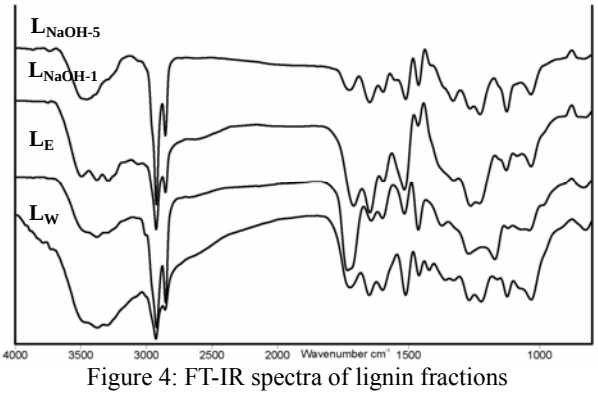

Figure 4: FT-IR spectra of lignin fractions

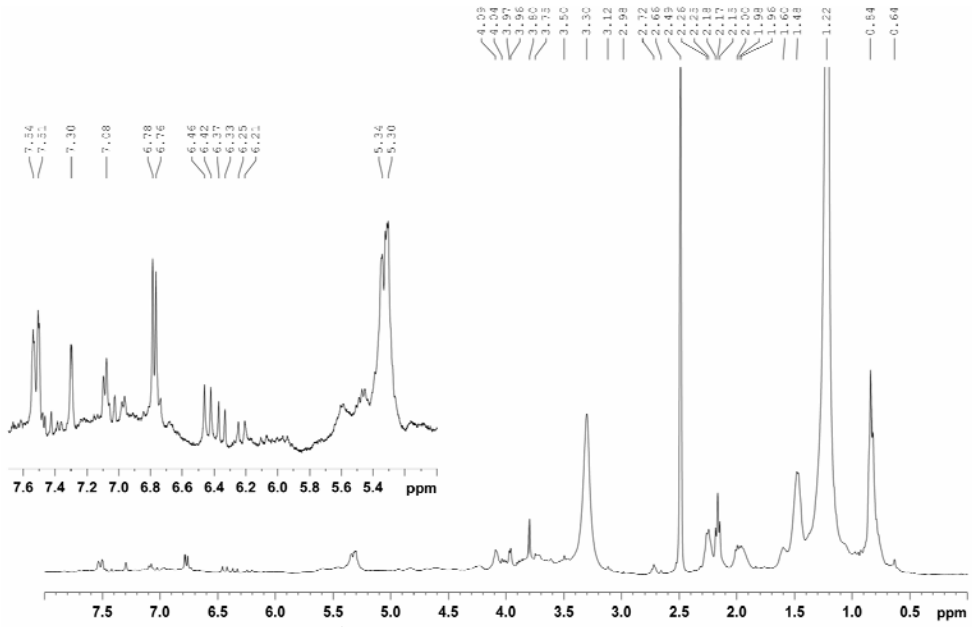

Figure 5: ^{1}H NMR spectrum of lignin fraction L_E

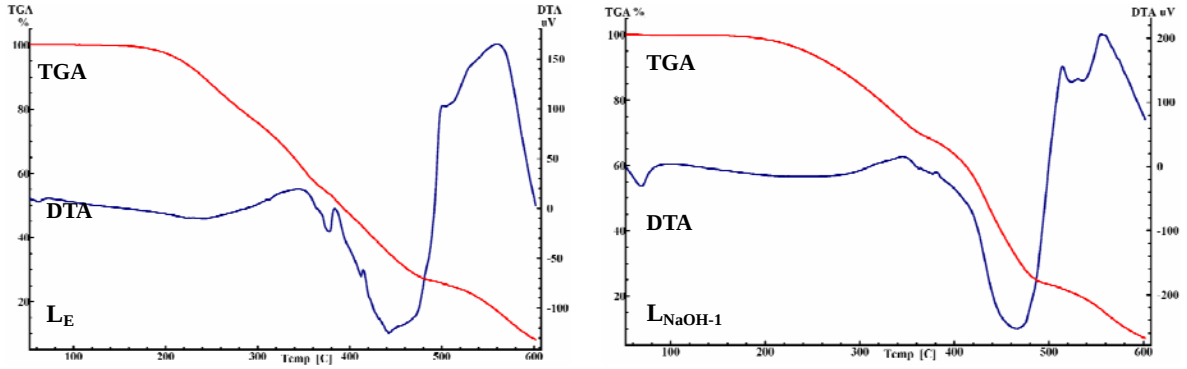

Figure 6: TGA/DTA curves of lignin fractions L_E and L_{NaOH-1}

Thermal stability

The thermal degradation patterns of lignin fractions L_E and L_{NaOH-1} are shown in Figure 6. Due to its inherent structure, consisting of aromatic rings with various branches, lignin is considered as the most thermo-stable component in lignocellulosic material, being degraded within a wide temperature range. From the DTA curves, a remarkable exothermic peak can be seen at temperatures lower than 500 °C, which is related to the cleavage of linkages between aromatic rings, releasing CO_2 and a tiny amount of CH_4.[25] When the temperature was higher than 500 °C, the DTA values turned to positive, indicating the occurrence of endothermic reactions. It was attributed to the decompostion or condensation of aromatic rings,[26] releasing the highest amount of CH_4.[25] Unlike commercial lignin preparation,[25] the weight of the solid residues after the pyrolysis process were rather low and no significant differences were noted between L_E (8.47%) and L_{NaOH-1} (7.58%). However, the onset temperature of thermal degradation obviously increased from 199.3 °C (L_E) to 229.4 °C (L_{NaOH-1}), since the solvent system in the fractionation process was transformed from neutral ethanol to 1% alkaline aqueous solution. It is probably due to the efficient removal of hemicellulosic components (which is in good agreement with the sugar analysis mentioned above), as hemicelluloses are the most thermolabile component. The notable peak at 370 °C in the DTA curve of L_E was also relative to the hemicelluloses degradation.

CONCLUSIONS

The results of the present study showed that the sequential treatments of *Caragana korshinskii* Kom. with water, ethanol and NaOH aqueous

solutions in concentrations of 1%, 3%, 5%, 8% and 10% under the specified conditions released most of the lignin from the raw material. Over 80% lignin was separated and differences in structural features were comparatively investigated. A certain amount of esterified hydroxycinnamic acids were identified in the lignin fractions obtained by water and ethanol treatments, and the addition of alkali significantly cleaved the lignin-carbohydrate interaction. The content of polysaccharides, thereby, decreased correspondingly from 2.53% to 0.02%. More guaiacyl units than syringyl units were present in all lignin fractions. The V/S ratio decreased gradually from 3.3 (L_{NaOH-1}) to 2.0 (L_{NaOH-1}) with an increase in alkaline concentration from 1% to 10%, indicating that the guaiacyl units were more easily degraded as compared to syringyl units during the successive alkaline treatments. Some substructures, such as *β-O-4* and *β-β*', were detected in *C. korshinskii* Kom. lignin by the NMR technology. The results here described represent the first comprehensive study on lignin extracted from *C. korshinskii* Kom. Lignin macromolecules could and should find a rational utilization, as a source of aromatic chemicals and materials. Further exploration of the main component this abundant plant resource will reveal its potential for a variety of applications.

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