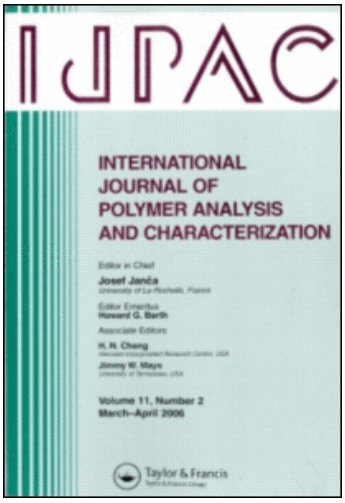

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Analysis of Lignins Solubilized in Two-Stage Organosolv and Alkaline Peroxide Treatments from Haloxylon ammodendron and Elaeagnus angustifolia

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Analysis of Lignins Solubilized in Two-Stage Organosolv and Alkaline Peroxide Treatments from Haloxylon ammodendron *and* Elaeagnus angustifolia

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The chemistry of acidic organosolv and alkaline peroxide delignification of dewaxed Haloxylon ammodendron *and* Elaeagnus angustifolia *was studied by analysis of lignin fractions solubilized during pretreatment with ethanol-H_2O (60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h and post-treatment with 2% H_2O_2 at pH 11.5 for 16 h at 45°C, respectively. It was found that alkaline peroxide post-treatment gave higher solubility and degradation of lignin (8.0% from* Haloxylon ammodendron *and 7.8% from* Elaeagnus angustifolia*) than that of acidic organosolv pretreatment (5.5% from* Haloxylon ammodendron *and* Elaeagnus angustifolia*, respectively). The two-stage treatments together solubilized or degraded over 80% of the original lignins from* Haloxylon ammodendron *or* Elaeagnus angustifolia*. For lignins isolated from acidic organosolv pre-treatment, it was shown that the*

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treatment gave lignin fractions that are chemically different from those released during the alkaline peroxide post-treatment by having enriched guaiacyl units, and slightly higher molecular weight and thermal stability. The depolymerization of lignins from the acidic organosolv pretreatment was lower as compared to lignins from alkaline peroxide post-treatment. Particularly, lignin preparations solubilized during the alkaline peroxide post-treatment contained carboxyl groups, indicating substantial oxidation of the lignins during post-treatment.

Keywords: *Haloxylon ammodendron*; *Elaeagnus angustifolia*; Lignin; Acidic organosolv pretreatment; Alkaline peroxide post-treatment

Haloxylon ammodendron and *Elaeagnus angustifolia* are the main two shrubs of man-made forests in the desert region of Gansu Province in China, planted to prevent wind erosion and control desertification. In the early stages of plantation they grow well and fix shifting sand dunes, but certain problems appear after years. These include decline in growth or the plants even dying[1]. Finding solutions to this problem include the identification of the chemical composition of *Haloxylon ammodendron* and *Elaeagnus angustifolia*. This is particularly true for their lignin characterization since lignin in wood acts as an adhesive that binds the cellulose fiber structure and imparts stiffness to the wood matrix[2]. It originates from phenylpropanoid precursors such as coumaryl, coniferyl, and sinapyl alcohol C_6-C_3 and is present in vascular plants[2]. At present, it has not been possible to determine exactly the inter- and intramolecular bonds involving lignin and other polymers in the cell wall[3]. In addition, its heterogeneity has been a struggle for all wood researchers worldwide.

Delignification in pulping processes consists of the degradation and dissolution of lignin polymers into smaller fragments[4]. Although a number of organosolv pulping processes during the past 15 years have been investigated, the chemistry of delignification during organosolv pulping is still not fully understand. This is particularly true for processes under acidic catalyst conditions[5]. It is known that acids catalyze the cleavage of β-*O*-4 linkages and depolymerize lignin[6]. In addition, alkaline peroxide, as a chlorine-free bleaching and delignifying agent, has become increasingly important in bleaching technology as the pulp and paper industry is moving toward minimization of environmental impact[7]. Hydroperoxide anion (HOO^-), formed in alkaline media, is the principal active species in hydrogen peroxide bleaching processes. This

anion is a strong nucleophile that, during bleaching, preferentially attacks ethylenic and carbonyl groups present in lignin. As a consequence, such chromophores as quinones, cinnamaldehyde, and ring-conjugated ketones are converted to nonchromophoric species under the alkaline conditions. However, hydrogen peroxide is unstable in alkaline conditions and readily decompose to more active radicals such as hydroxyl and superoxide anion radicals ($HO\cdot$, $O_2^{-}\cdot$), which participate in the delignifying mechanism[8]. To minimize the extent of hydrogen peroxide decomposition, low temperature, in general, has been used. The advantages of delignification with hydrogen peroxide are low investment cost and the accompanying strong bleaching effect. In addition, the degraded or solubilized lignins during the alkaline peroxide treatment under mild conditions are theoretically interesting for lignin structural studies to assist in understanding the plants' decline in growth.

The aim of this work was to analyze the chemical composition of the lignin and characterize its structural features during acidic organosolv pre- and alkaline peroxide post-treatments of *Haloxylon ammodendron* and *Elaeagnus angustifolia*, respectively. Lignin samples were characterized by fractional yield, associated polysaccharides, alkaline nitrobenzene oxidation and thermal analysis, ultraviolet (UV), Fourier transform infrared (FT-IR), and carbon-13 magnetic resonance spectroscopy (^{13}C-NMR), as well as gel permeation chromatography (GPC), and the results discussed.

EXPERIMENTAL

Materials

Haloxylon ammodendron and *Elaeagnus angustifolia*, eight years old, were harvested in July 1999, in the desert region of Gansu Province, China. It was dried in sunlight and then chipped into small pieces. The chips were then ground to pass a 1.0-mm size screen. Crude lipids were removed by extraction with toluene-ethanol (2:1,v/v) in a Soxhlet for 6 h. The extractives-free sample was dried in an oven with air circulation at 60°C for 16 h and then kept at 5°C before treatment. All weights and calculations were made on an oven-dried (60°C, 16 h) basis.

Acidic Organosolv Pre- and Alkaline Peroxide Post-Treatments

In acidic organosolv pretreatment, the extractive-free powder (9.75 g) was treated with ethanol-H_2O (195 mL, 60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h. After filtration on a nylon cloth, the residue was subsequently washed with ethanol and distilled water,

and then oven dried at 60°C for 16 h. Ethanol in the combined supernatant was removed with a rotary vacuum evaporator at 40°C. Then the supernatant was neutralized to pH 5.5 with 1 M NaOH, concentrated on a rotary evaporator under reduced pressure to about 100 mL, and then mixed with 3 volumes of 95% ethanol (12 h, 25°C) for isolation of hemicellulose-lignin complexes. After filtration and evaporation of ethanol, the organosolv lignins were obtained by reprecipitation at pH 1.5 with 6 M HCl from the corresponding supernatants. The isolated lignin preparations were washed with acidified water (pH 1.5–2.0), freeze-dried overnight and kept at 5°C until analysis. To obtain alkaline peroxide soluble hemicellulose-lignin complexes and lignin preparations, the above residues were successively extracted by post-treatment with 2% H_2O_2 (residue:extractant, 1:25) at pH 11.5 for 16 h at 45°C. The dissolved hemicellulose-lignin complexes and lignin fractions were isolated as in the method mentioned above. No further adjustments in pH were made during the course of the post-treatment. Under these conditions, the reaction pH remained nearly constant for 2 h before slowly rising to a final value of ca. 12.9. Note that the fractions 1 (F_1) and 2 (F_2) represent the acid-insoluble lignin fractions extracted successively with acidic organosolv and alkaline peroxide from *Haloxylon ammodendron*, whereas the fractions 3 (F_3) and 4 (F_4) represent the acid-insoluble lignin fractions extracted successively with acidic organosolv and alkaline peroxide from *Elaeagnus angustifolia*. A scheme for pre- and post-treatments of *Haloxylon ammodendron* or *Elaeagnus angustifolia* is shown in Figure 1. Both of the treatments were repeated twice, giving very reproducible yields.

Lignin Analysis

The four acid-insoluble lignin preparations were subjected to alkaline nitrobenzene oxidation at 175°C for 2.5 h. The phenolic acids and aldehydes liberated were separated on a Hichrom H5ODS HPLC column of dimensions 250 × 4.6 mm (Phenomenex Co., Beijing). The identification of the individual compounds were detected at 280 nm by computer comparison of the retention times and peak areas with authentic phenolics[9]. The hemicellulosic moieties associated with lignin preparations were hydrolyzed with 2 M trifluoroacetic acid for 2 h at 120°C. Liberated neutral sugars were analyzed as their alditol-acetate derivatives by gas chromatography (GC)[10,11]. Methods for recording UV spectra and determination of molecular-average weights of the acid-insoluble lignin fractions are described in previous papers[9,10].

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet 510) using a KBr disc containing 1% finely ground lignin

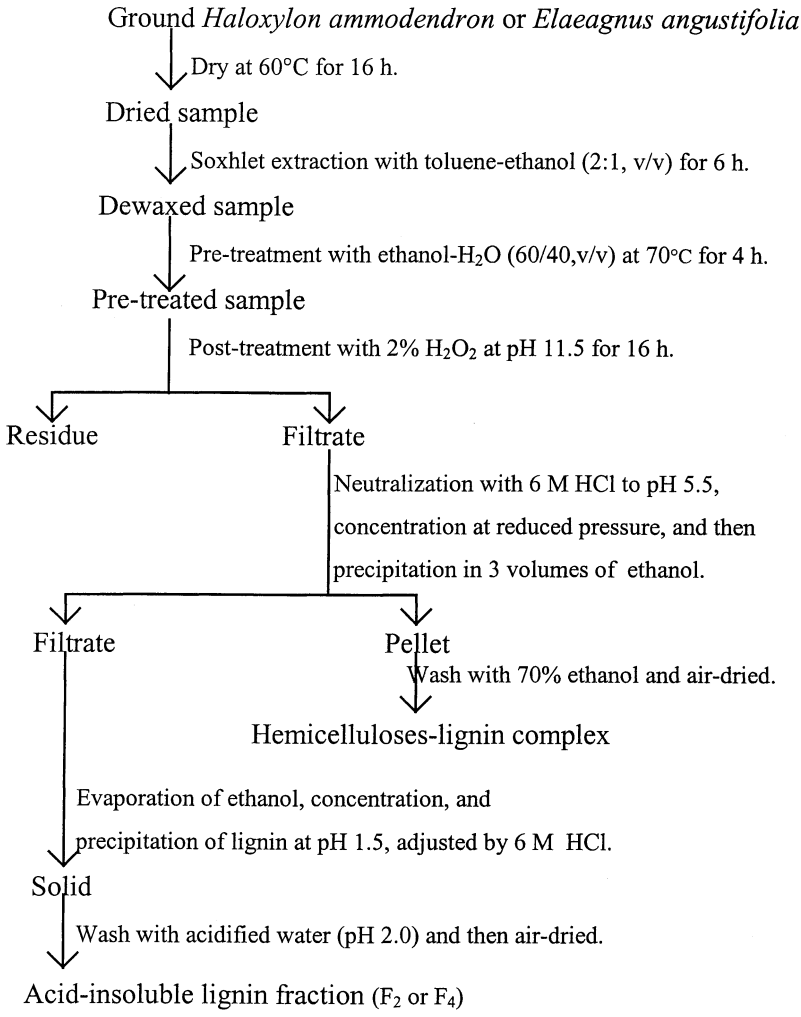

FIGURE 1 Scheme for isolation of acid-insoluble lignin fraction from the hydrolysates of 2% H_2O_2 post-treatment of the ethanol-H_2O pretreated *Haloxylon ammodendron* or *Elaeagnus angustifolia*.

samples. The solution ^{13}C-NMR spectrum was recorded on a Bruker MSI-300 spectrometer at 74.5 MHz from 200 mg of sample dissolved in 1.0 mL DMSO-d_6 after 20,000 scans. A 70° pulse flipping angle, a 10 μs pulse width and a 15 s delay time between scans were used.

Thermal analysis of pure lignin preparations was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (NETZSCH STA-409). The apparatus was continually flushed with nitrogen. The sample weighed between 8 and 12 mg. Each sample was heated from room temperature to 600°C at a rate of 10°C per minute.

RESULTS AND DISCUSSION

In organosolv pretreatment a mixture of organic solvent, such as ethanol in this study, and water is used as treatment liquor. The solvent primarily acts on the promotion of vegetal tissue impregnation and the solubilization of the lignin fragments so produced[12]. In noncatalyzed treatment (autocatalyzed) the treating liquor becomes acidified due to the acetic acid released from the wood. However, during organosolv acid delignification, lignin is dissolved essentially by acid-catalyzed cleavage of such bonds as α-aryl ether and arylglycerol-β-aryl ether in the lignin macromolecule[4,13]. However, in alkaline peroxide post-treatment, dissociated hydrogen peroxide, that is hydroperoxide anion, reacts with lignins in wood mainly as a nucleophile, although it also acts as an oxidant. The hydroperoxide anion attacks carbonyls conjugated with aromatic rings in the lignin, such as α-carbonyl in *β-O*-4 type substructures. Additionally, the lignin in woods also undergoes alkaline catalyzed oxidation with hydrogen peroxide oxidizing the α-hydroxyl groups and C-C double bonds conjugated with the aromatic rings in the lignin[14].

As shown in table I, the pretreatment with ethanol-H_2O (60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h and sequential post-treatment with 2% H_2O_2 at pH 11.5 for 16 h resulted in a dissolution of 34.4% and 50.0% of the original lignin and 15.5% and 24.7% hemicellulose-lignin complexes (% dry starting material) from dewaxed *Haloxylon ammodendron*, and 34.0% and 48.1% of the original lignin and 17.1% and 27.4% hemicellulose-lignin complexes (% dry starting material) from dewaxed *Elaeagnus angustifolia*, respectively. Obviously, the two-stage treatment together dissolved over 80% of the original lignin from dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia*. As expected, the acid-insoluble lignin fraction (precipitated at pH 1.5 aqueous solution) was the major lignin preparation, comprising 58.2–66.3% of the total solubilized lignins, while the lignin fraction, associated in the solubilized hemicelluloses, accounted for a small amount, 23.8–29.1% of total degraded lignins. This result implied that both ethanol-H_2O pretreatment and 2% H_2O_2 post-treatment substantially cleaved the α-ether linkages between lignin and hemicelluloses from the cell walls of *Haloxylon ammodendron* and *Elaeagnus angustifolia*. In addition, as can

TABLE I The yield of lignin (% dry matter) solubilized in pretreatment of dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia* with ethanol-H_2O (60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h and post-treatment with 2% H_2O_2 at pH 11.5 for 16 h.

Yield (%)	*Haloxylon ammodendron*	*Elaeagnus angustifolia*
Total solubilized lignin in pre- and post-treatment	13.5	13.3
Total solubilized lignin in pretreatment[a]	5.5	5.5
Acid-insoluble lignin[b]	3.5	3.2
Lignin associated in the isolated hemicelluloses	1.4	1.6
Acid-soluble lignin[c]	0.6	0.7
Total solubilized lignin in post-treatment[d]	8.0	7.8
Acid-insoluble lignin[b]	5.3	4.8
Lignin associated in the isolated hemicelluloses	1.9	2.1
Acid-soluble lignin[c]	0.8	0.9
Lignin in residues (crude cellulose)[e]	2.5	2.9

[a]The lignin fraction obtained by pretreatment of dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia* with ethanol-H_2O (60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h.

[b]Acid-insoluble lignin fraction obtained by precipitation of the supernatant solution at pH 1.5 after isolation of the solubilized hemicelluloses.

[c]Acid-soluble lignin fraction that is still solubilized in the supernatant (pH 1.5) after precipitation of the acid-insoluble lignins.

[d]The lignin fraction solubilized in the post-treatment.

[e]The lignin fraction in the two-stage treated residues.

be seen from Table I, the acid-soluble lignin fraction (solubilized in pH 1.5 supernatant) yielded 10.0–12.7% of total released lignins, indicating that a noticeable degradation reaction occurred during the ethanol-H_2O pretreatment and 2% H_2O_2 post-treatment under the conditions given.

To determine the purity of the isolated lignin preparations, the four acid-insoluble lignin fractions were studied by UV spectroscopy at 240–370 nm. As shown in Figure 2, the four lignin fractions exhibited the basic UV spectrum typical of lignins with a maximum at 276–282 nm, originating from the nonconjugated phenolic groups in the lignin[15].

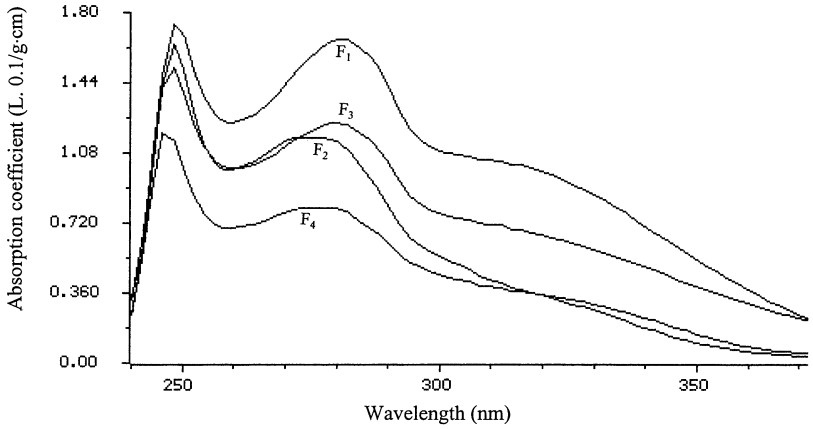

FIGURE 2 UV spectra of acid-insoluble lignin fractions of F_1, F_2, F_3, and F_4.

Interestingly, as shown in the spectra, the two lignin fractions solubilized during the acidic organosolv pretreatment gave higher absorption coefficients than those released during the 2% H_2O_2 post-treatment, indicating that the former two lignin preparations (F_1 and F_3) may contain relatively higher amounts of guaiacyl units (G) than syringyl units (S), whereas the reverse composition occurred in the latter two lignin fractions. This was confirmed by the results obtained by alkaline nitrobenzene oxidation. Similar results from wood lignins have been reported by Faix and Schweers[16]. The authors stated that all of the lignin structural moieties give different absorption maximum and extinction coefficients. The extinction coefficient of guaiacyl unit at 280 nm is 3.5 times of that of syringyl unit, and the extinction coefficient of *p*-hydroxyphenyl unit is lower than that of guaiacyl unit, but higher than that of syringyl unit. In comparison with the two lignin fractions (F_1 and F_3) obtained from *Haloxylon ammodendron*, the relatively lower absorption coefficients of the lignin fractions F_2 and F_4 obtained from *Elaeagnus angustifolia* were also partially due to the more co-precipitated nonlignin materials such as ash and salts.

Sugar analysis showed that the four acid-insoluble lignin preparations contained rather low amounts of bound polysaccharides as indicated by 0.36–0.74% neutral sugar content (Table II). This revealed that the acidic organosolv pretreatment and alkaline peroxide post-treatment of the dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia* under the conditions given can peel more lignin from most of the neighboring hemicellulosic moieties. Xylose, glucose, and galactose were identified as the main sugar components. Arabinose in F_1 and F_3 fractions solubilized

TABLE II The content of neutral sugars (% dry weight, w/w) in isolated acid-insoluble lignin fractions released in pretreatment of dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia* with ethanol-H_2O (60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h and post-treatment with 2% H_2O_2 at pH 11.6 for 16 h.

	Acid-insoluble lignin fractions			
Sugars (%)	F_1[a]	F_2[a]	F_3[b]	F_4[b]
Arabinose	0.14	ND[d]	0.10	ND
Xylose	0.23	0.18	0.18	0.14
Mannose	Tr[c]	Tr	0.12	Tr
Glucose	0.15	0.14	0.22	0.12
Galactose	0.10	0.12	0.12	0.10
Total	0.62	0.44	0.74	0.36

[a]Fractions 1 (F_1) and 2 (F_2) represent the acid-insoluble lignin fractions extracted successively with ethanol-H_2O (60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h and 2% H_2O_2 at pH 11.5 for 16 h at 45°C from *Haloxylon ammodendron*.

[b]Fractions 3 (F_3) and 4 (F_4) represent the acid-insoluble lignin fractions extracted successively with ethanol-H_2O (60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h and 2% H_2O_2 at pH 11.5 for 16 h at 45°C from *Elaeagnus angustifolia*.

[c]Tr = trace.

[d]ND = not detected.

during the acidic organosolv pretreatment accounted for 0.14% and 0.10% of the dry lignin samples, although it was not detected in F_2 and F_4 fractions released during the 2% H_2O_2 post-treatment. In addition, the content of total sugars in F_1 and F_3 fractions (0.62%, 0.74%) was higher than that in F_2 and F_4 fractions (0.44%, 0.36%). This data together with the yield of lignin released during the pre- and post-treatments as shown in Table I indicated that 2% H_2O_2 post-treatment under the alkaline condition given was more severe than ethanol-H_2O pretreatment under the acid condition used. In other words, as delignification agents, alkaline peroxide was stronger than the organosolv used in this study under the condition given.

Table III shows the yields of the monomeric products obtained from alkaline nitrobenzene oxidation of the four acid-insoluble lignin preparations. Comparing the four lignin fractions, the two acidic organosolv lignin preparations were found quite different from the two alkaline peroxide lignin fractions. The F_1 and F_3 lignin fractions, obtained by

TABLE III The yield (% lignin sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of the isolated four acid-insoluble lignin fractions.

Phenolic acids and aldehydes	Acid-insoluble lignin fractions[a]			
	F_1	F_2	F_3	F_4
p-Hydroxybenzoic acid	0.36	0.96	1.80	0.97
p-Hydroxybenzaldehyde	0.80	0.45	0.42	0.75
Vanillic acid	0.72	0.82	0.73	1.06
Syringic acid	1.22	2.52	0.88	2.82
Vanillin	14.08	6.26	9.05	10.80
Syringaldehyde	8.12	10.08	5.88	14.84
Acetovanillin	0.13	0.10	0.35	0.23
p-Coumaric acid	2.02	1.00	0.98	0.56
Acetosyringone	0.10	0.16	0.12	0.10
Ferulic acid	0.25	0.10	0.32	0.34
Total	27.80	22.45	20.53	32.47
Molar ratio (G:S:H)[b]	62:32:6	37:55:8	55:31:14	42:51:7

[a]Corresponding to acid-insoluble lignin fractions in Table II.

[b]G represents the relatively total moles of vanillin, vanillic acid, and acetovanillin; S represents the relatively total moles of syringaldehyde, syringic acid, and acetosyringone; H represents the relatively total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

pretreatment of dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia* with ethanol-H_2O (60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h, had much higher guaiacyl units but fewer syringyl units than the F_2 and F_4 lignin fractions, obtained by post-treatment with 2% H_2O_2 at pH 11.5 for 16 h. The molar ratio of G (relatively total moles of vanillin, vanillic acid, and acetovanillin) : S (relatively total moles of syringaldehyde, syringic acid, and acetosyringone) : H (relatively total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) was 62:32:6 in F_1 and 55:31:14 in F_3, while it dropped to 37:55:8 in F_2 and 42:51:7 in F_4 fraction, respectively. That is, as compared to the two alkaline peroxide lignins, the two acidic organosolv lignins had a similar higher ratio of guaiacyl to syringyl units, but different content of *p*-hydroxyphenyl units. The current results suggested that guaiacyl units engaged in *β-O*-4 lignin structures were more easily degraded compared to syringyl units by acidic organosolv under the condition used, whereas the reverse trend appeared during the alkaline peroxide extraction process. This result seems contradictory to the literature data, as it is generally considered

that the cleavage of β-aryl syringyl ether bonds is easier for the syringyl structure[17–19]. The reason for this different behavior is that the presence of free hydroxyls bared principally by the guaiacyl units is at the origin of the higher reactivity of guaiacyl monomers than the syringyl ones[20]. However, the β-aryl syringyl ether bonds are more easily oxidized than the guaiacyl structures, which resulted in a higher molar ratio of S/G units in lignin fractions F_2 and F_4 obtained during the post-treatment with alkaline peroxide[21,22]. The total yields of oxidation products decreased in the order of F_4, F_1, F_2, and F_3, indicating that condensation degree of the acid-insoluble lignin fractions increased in the same order. Based on the total yield of phenolic acids and aldehydes obtained by alkaline nitrobenzene oxidation, the extent of condensation of the four lignin preparations increased from F_4 to F_1, to F_2, and to F_3, and the lignin in F_2 and F_3 is richer in condensed structure than the lignins in F_1 and F_4 fractions.

As shown in Table IV, the values of the weight-average molecular weight M_w and number-average molecular weight M_n of acidic organosolv lignins F_1 and F_3 (M_w 1350–1420 g mol^{-1}, M_n 1110–1170 g mol^{-1}) exhibited slightly higher than those of the alkaline lignins F_2 and F_4 (M_w 1260–1290 g mol^{-1}, M_n 1050–1080 g mol^{-1}). This is an expected result since a more intense treatment, such as post-treatment with 2% H_2O_2 at pH 11.5 for 16 h, led to more extensive depolymerization of lignin molecules. Therefore, the molecular weight of the lignin samples, released during the post-treatment, was slightly lower, indicating once more that pretreatment with organosolv in acid media induced the cleavage of β-*O*-4 structure, and the subsequent treatment with hydrogen peroxide in alkali media significantly cleaved the remaining β-*O*-4 structures from the cell walls of *Haloxylon ammodendron* and *Elaeagnus angustifolia*. The

TABLE IV Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of acid-insoluble lignin fractions isolated sequentially with ethanol-H_2O (60/40, v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h and 2% H_2O_2 at pH 11.5 for 16 h at 45°C from dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia*.

	Acid-insoluble lignin fractions[a]			
	F_1	F_2	F_3	F_4
M_w	1350	1260	1420	1290
M_n	1110	1050	1170	1080
M_w/M_n	1.22	1.20	1.21	1.19

[a]Corresponding to acid-insoluble lignin fractions in Table II.

range of the molecular weights measured (158–9300) was in the same range as reported in literature for organosolv pulping of wood and annual plants[4].

The polydispersity of the molecular distribution of lignin samples (M_w/M_n) also decreased slightly for alkaline peroxide treatment. This parameter had a value of 1.22 and 1.21 for the lignin fractions F_1 and F_3, respectively. Similar values were found between F_2 and F_4 lignin preparations. Therefore, it can be concluded that severe treatment gave rise to higher lignin fragmentation and a narrower molecular weight distribution. This phenomenon was also reported by Gilarranz et al.[4] and Tirtowidjojo et al.[23] with increasing intensity of cooking conditions (higher temperature, longer time, and lower methanol concentration) and acid catalyst concentration in the pulping liquor.

FT-IR spectra of the four acid-insoluble lignin samples are given in Figure 3. The most striking feature of the two acidic organosolv lignin fractions F_1 (spectrum a) and F_3 (spectrum c) is the extremely low level of carbonyl groups at 1725 and 1646 cm^{-1} as compared to the two alkaline peroxide-soluble lignin preparations F_2 (spectrum b) and F_4 (spectrum d), indicating a very low degree of oxidation of the organosolv lignins. The

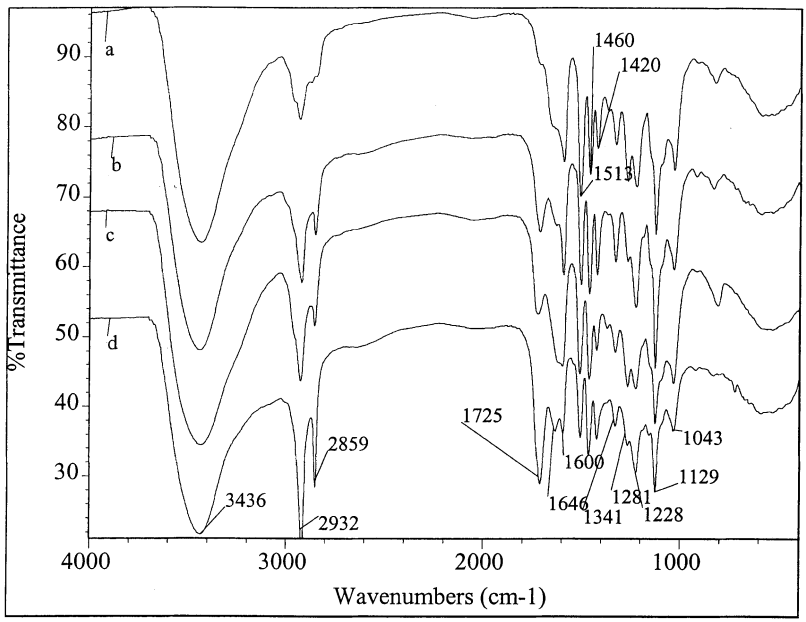

FIGURE 3 FT-IR spectra of acid-insoluble lignin fractions F_1 (spectrum a), F_2 (spectrum b), F_3 (spectrum c), and F_4 (spectrum d).

band at 1725 cm^{-1} is assigned to unconjugated ketone and carboxylic acid, while the band at 1646 cm^{-1} is attributed to C=O stretch in conjugated p-substituted aryl ketone[24]. In other words, two rather weak peaks at 1646 and 1725 cm^{-1} in two organosolv lignin fractions revealed that the lignins obtained from the acidic organosolv pretreatment may be significantly less oxidized than the lignins obtained from the alkaline peroxide post-treatment. This was confirmed by another stronger band at 1228 cm^{-1} in the two alkaline peroxide-soluble lignins (spectra b and d) than in the two organosolv lignins (spectra a and c) since this peak is originated from C-O plus C=O stretch of guaiacyl units in lignin. Thus, it is very likely that the residual lignin in alkaline peroxide post-treatment undergoes alkaline catalyzed oxidation with hydrogen peroxide oxidizing the α-hydroxyl groups and C-C double bonds conjugated with the aromatic rings or that the side chain of lignin could be cleaved either between the α, β double bond or between the β, γ bond by alkaline peroxide oxidation. This is particularly true for guaiacyl units in lignin molecules. Aromatic skeleton vibrations in four lignin fractions give bands at 1600, 1513, and 1420 cm^{-1}. Absorption at 1460 cm^{-1} is indicative of the C-H deformations and aromatic ring vibrations. The bands at 1341 and 1281 cm^{-1} in the spectra correspond to syringyl and guaiacyl ring breathing with C-O stretching, respectively. The intensive bands at 1129 and 1043 cm^{-1} relate to the aromatic C-H in-plain deformation for syringyl type and guaiacyl type, respectively. This great similarity in the aromatic ring skeleton between the spectra of acidic organosolv lignins and alkaline peroxide-soluble lignins implied that alkaline peroxide post-treatment under the condition given did not affect the overall structure of lignin from *Haloxylon ammodendron* and *Elaeagnus angustifolia* except for the remarkable increases of carboxyl and carbonyl groups in lignin side chains.

The changes in acid-insoluble lignin structure during the alkaline peroxide post-treatment were also studied by ^{13}C-NMR spectroscopy. Figure 4 illustrates the alkaline peroxide-soluble lignin fraction F_2 obtained from *Haloxylon ammodendron*. Most of the observed signals have been previously assigned in straw and wood lignin spectra[15,25–27]. As can be seen from Figure 4, the most striking characteristic of the ^{13}C-NMR spectrum is the almost absence of typical polysaccharide signals between 57 and 103 ppm. The spectrum does show two signals at 81.9 ppm (data not shown, C-α etherified to polysaccharides) and 63.3 ppm (data not shown, C-5, Xyl internal unit) for the associated hemicelluloses. However, the peak intensity is rather weak, indicating a trace amount of associated polysaccharides in the lignin fraction. This observation supported the data obtained by sugar analysis. Similar results were reported by Xie et al.[28] from the study of ginkgo lignin-carbohydrate complexes (LCC). The authors stated that three lignin-carbohydrate linkages (i.e., ether type, ester type, ketal type) were found at the C-α position of the

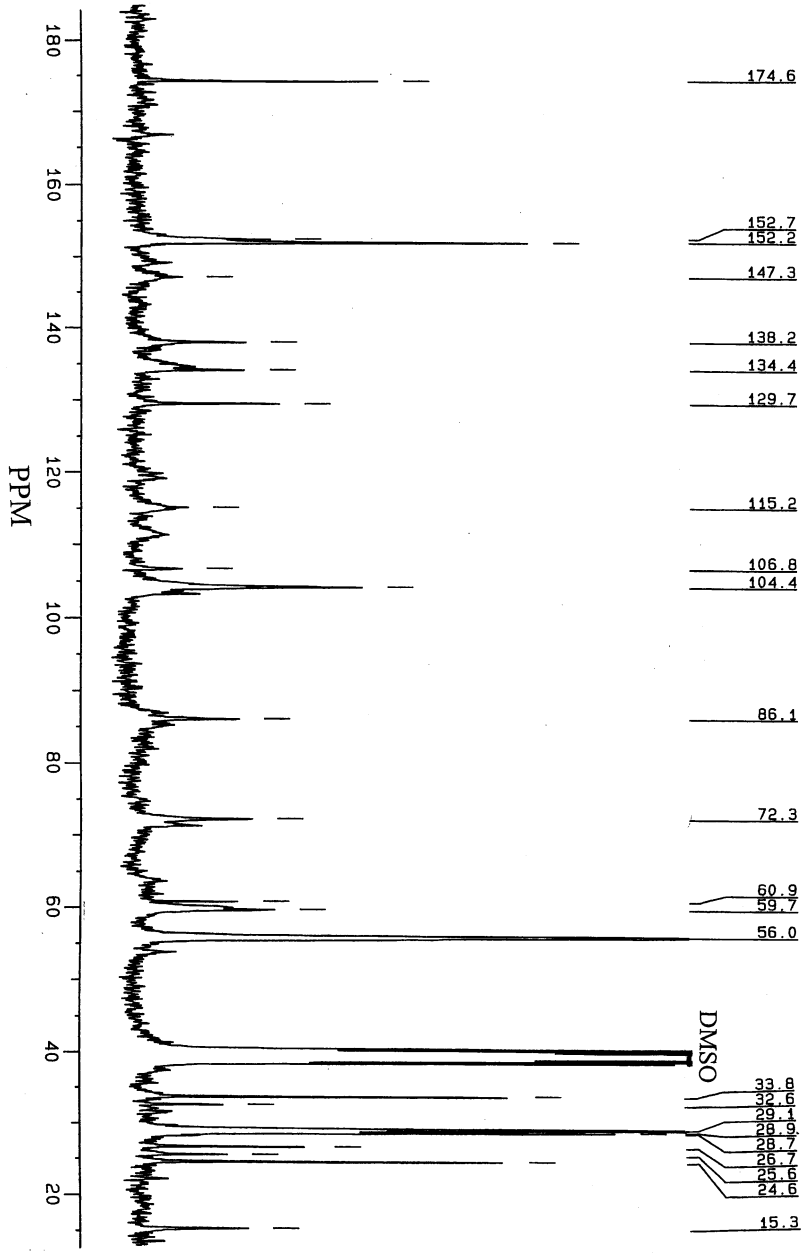

FIGURE 4 ^{13}C-NMR spectrum of acid-insoluble lignin fraction F_2 extracted with 2% H_2O_2 at 45°C for 16 h at pH 11.5 from the pretreated *Haloxylon ammodendron*.

side chain of phenylpropane units in ginkgo LCC, and no lignin-carbohydrate bond at the C-β or C-γ position of the lignin side chain was observed in the ^{13}C-NMR spectra of the ^{13}C-enriched LCCS.

Another of the most striking characteristics of the ^{13}C-NMR spectrum is the increase in the carboxylic groups in the prominent intensities at 174.6 ppm, assigned to the carboxyl carbons attached to the aromatic/vinylic and aliphatic moieties, respectively[29]. Therefore, it is also very likely that the alkaline peroxide favored oxidation of the carbons linked by hydroxyl, aldehyde, and ketone groups and aryl ethers, in the lignin side chains. As these carbons were oxidized to carboxyl, aldehyde groups or ketone groups, the contiguous aryl ethers could be easily cleaved simultaneously and be subjected to further degradation. Such an oxidation may be the main reason for the substantial degradation of lignins by alkaline peroxide post-treatment from *Haloxylon ammodendron*[30].

It is worthwhile to comment on the signals in the aromatic region (153–104 ppm). The syringyl residues were detected by signals at 152.2 and 152.7 (C-3/C-5 in syringyl units), 147.3 (C-3/C-5 in non-etherified syringyl units), 138.2 (C-4 in etherified syringyl units), 134.4 (C-1 in etherified syringyl units), 106.8 (C-2/C-6 in syringyl units with α-CO) and 104.4 ppm (C-2/C-6 in syringyl units). The guaiacyl units were identified with signals at 147.3 (C-4 in etherified guaiacyl units), 134.4 (C-1 in etherified guaiacyl units), 119.0 (C-6 in guaiacyl units, data not shown in the spectrum), and 115.2 ppm (C-5 in guaiacyl units). These signals indicated that the lignin fraction F_2 could be justified as hardwood lignins. The signal at 129.7 ppm (C-2/C-6 in esterified *p*-coumaric acid) is originated from the esterified *p*-coumaric acid. Etherified ferulic acid gave a signal at 167.2 ppm (C-γ FE ether, data not shown in the spectrum). It seems clear that *p*-coumaric acid is linked to lignin by ester bonds, while the ferulic acid is linked to lignin by ether bonds.

Interestingly, the ^{13}C-NMR spectrum of the alkaline peroxide-soluble lignin isolated from *Haloxylon ammodendron* shows three strong resonances at 86.1, 72.3, and 59.7 ppm, assigned to C-β in β-*O*-4, C-α in β-*O*-4 and C-γ in β-*O*-4, respectively. This observation suggested that β-*O*-4 linkages are still the major bonds between the lignin structural units. In addition, some common carbon-carbon linkages such as β-β (C-γ in β-β units, 71.6 ppm, C-β in β-β, 55.5 ppm, data not shown in the spectrum) and β-5 (C-4 in β-5 units, 144.8 ppm, data not shown in the spectrum) were also identified in the lignin fraction. These signals confirmed that the linkages in the alkaline peroxide-soluble lignin are mainly composed of β-*O*-4 ether bonds together with small amounts of β-β and β-5 carbon-carbon linkages. The current results revealed that alkaline peroxide post-treatment under the conditions given does not attack the β-aryl ether structure to a significant extent. The signals representing the γ-methyl,

α and β-methylene groups in *n*-propyl side chains of the lignin fraction occurred in the spectrum between 15.3 and 33.8 ppm. A very strong signal at 56.0 ppm is due to the OCH_3 in syringyl and guaiacyl units.

The thermal properties of the two acidic organosolv lignin fractions and two alkaline peroxide-soluble lignin preparations were investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), and their thermograms are illustrated in Figures 5 and 6. Remarkably, some small differences in the thermal stability were found between the lignin samples although they had a similar starting decomposition temperature. As shown in Figures 5 and 6, all the lignin decomposition temperature started around 200°C (thermogram of F_3 not shown). At 10% weight loss the decomposition temperature of the lignins was observed at 253°C for F_1, 257°C for F_2, 256°C for F_3, and 263°C for F_4, respectively. However, at 50% weight loss the decomposition temperature decreased substantially from F_1 (516°C) to F_2 (443°C), and from F_3 (508°C) to F_4 (428°C). This phenomenon indicated that the two acidic organosolv lignin fractions F_1 and F_3 had a slightly higher thermal stability than that of the two alkaline peroxide-soluble lignin preparations F_2 and F_4, which corresponded to a slight decrease in molecular weight from acidic organosolv lignin to alkaline peroxide-soluble lignin. This confirmed once again that alkaline peroxide post-treatment had more effect on the degradation of lignins from *Haloxylon ammodendron* and *Elaeagnus angustifolia* than acidic organosolv pretreatment under the condition used.

CONCLUSION

In this article, it is shown that pretreatment of dewaxed *Haloxylon ammodendron* and *Elaeagnus angustifolia* with ethanol-H_2O (60/40,v/v) under acid catalyst (0.2 N HCl) at 70°C for 4 h released the two acidic organosolv lignin preparations having a negligible amount of carboxylic acid groups together with slightly higher values of molecular weight (M_w 1350–1420 g/mol^{-1}) and associated polysaccharides (0.62–0.74%), indicating a very low degree of degradation and oxidation of the lignin, while the post-treatment with 2% H_2O_2 at pH 11.5 for 16 h solubilized the two alkaline peroxide-soluble lignin fractions having noticeable quantities of carboxylic acid groups together with a slightly lower molecular weight (M_w 1260–1290 g/mol^{-1}) and relatively free of associated polysaccharides (0.36–0.44%), indicating a remarkable degree of degradation and oxidation of the lignin. Moreover, the two acidic organosolv lignin fractions contained higher guaiacyl units as shown by molar ratio of G/S 62:32 in F_1 and 55:31 in F_3; the two alkaline peroxide-soluble lignin

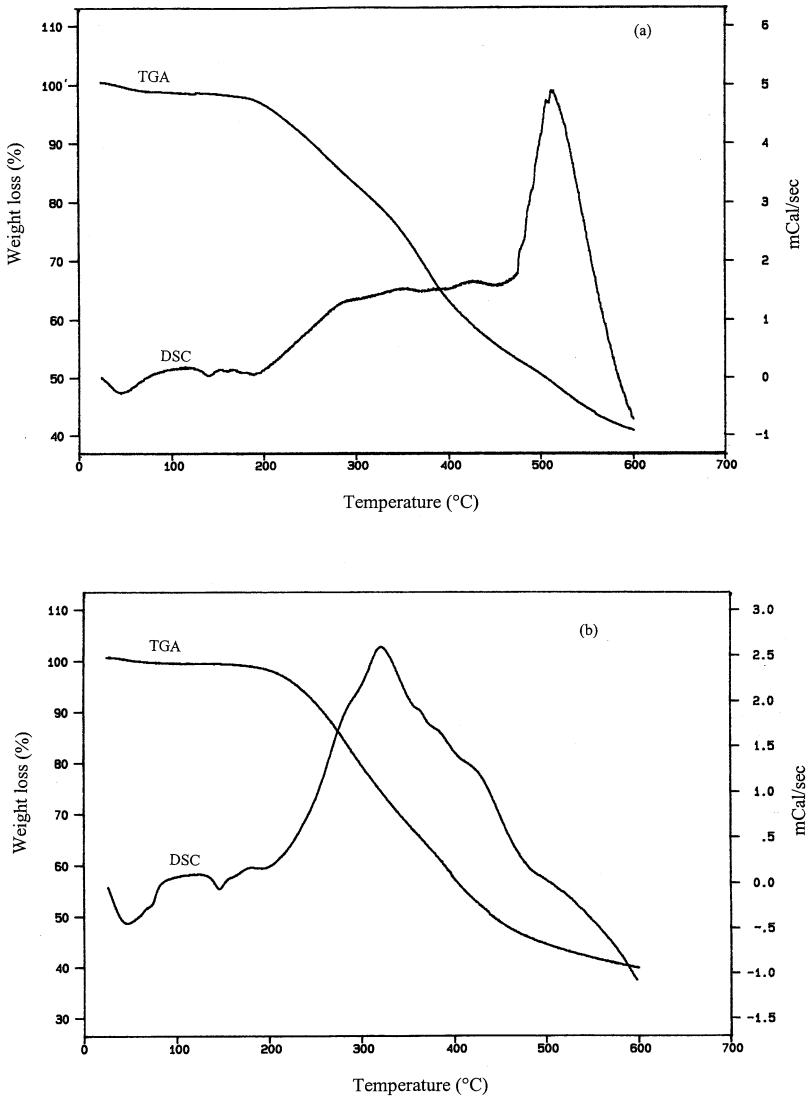

FIGURE 5 Thermograms of acid-insoluble lignin fractions F_1 (a) and F_2 (b) obtained from dewaxed *Haloxylon ammodendron*.

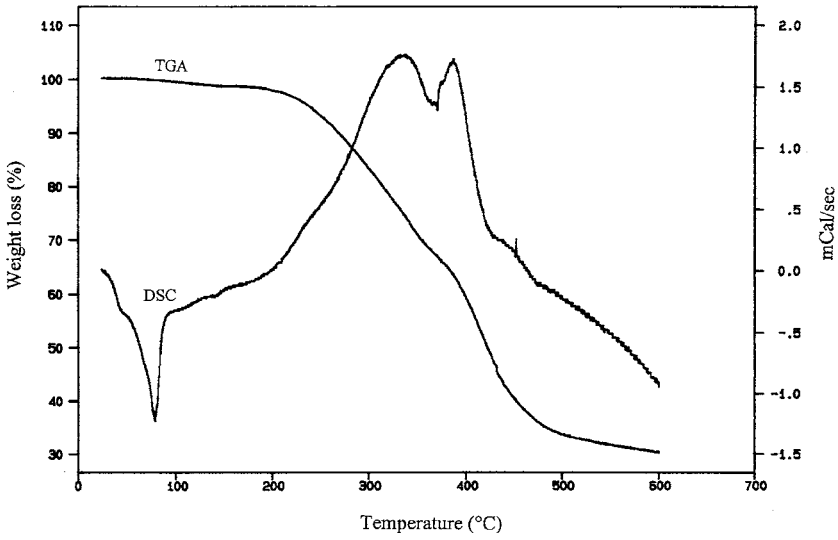

FIGURE 6 Thermogram of acid-insoluble lignin fraction F_4 (a) extracted with 2% H_2O_2 at 45°C for 16 h at pH 11.5 from the pretreated *Elaeagnus angustifolia*.

preparations had higher syringyl units as shown by molar ratio of G/S 37:55 in F_2 and 42:51 in F_4, indicating that the cleavage of β-aryl guaiacyl ether bonds is easier than that of β-aryl syringyl ether bonds in the cell walls of *Haloxylon ammodendron* and *Elaeagnus angustifolia*. These differences implied that lignins from *Haloxylon ammodendron* or *Elaeagnus angustifolia* may be chemically and/or structurally heterogeneous. Furthermore, thermal analysis demonstrated that the two acidic organosolv lignin fractions F_1 and F_3 had a slightly higher thermal stability than that of the two alkaline peroxide-soluble lignin preparations F_2 and F_4. Finally, the ^{13}C-NMR spectroscopy showed that *p*-coumaric acid is linked to lignin by ester bonds, whereas the ferulic acid is linked to lignin by ether bonds.

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