Alkaline and Organosolv Lignins from Furfural Residue: Structural Features and Antioxidant Activity

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Furfural residue (FR), composed mainly of cellulose and lignin, is an industrial waste produced during furfural manufacture. In this study, dioxane, alkali, ethanol, alkali-ethanol, and alkaline hydrogen peroxide (AHP) were used to extract lignins from FR. The structural features of these lignins obtained were characterized by sugar analysis, GPC, UV, FT-IR, and HSQC spectra. As compared to dioxane lignin (DL), other lignins showed lower molecular weights (M_w) owing to the partial cleavage of the linkages between lignin units. Results from HSQC spectra revealed that *β-O-4′* and *β-5′* were still the major linkages of the FR lignin. Moreover, *p*-coumaric and ferulic acids were released and co-precipitated in the lignin preparations extracted with alkali and AHP, whereas part of the esters in DL were preserved during the dioxane extraction. Antioxidant activity investigation indicated that the antioxidant property of the alkali and alkali-ethanol lignins was higher than that of the commercial antioxidant, butylated hydroxytoluene.

Keywords: Furfural residue; Lignin; Structural features; Thermogravimetric analysis; Antioxidant property

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INTRODUCTION

Furfural residue (FR), an industrial waste, is produced during the manufacture of furfural from corncob, during which hemicelluloses are hydrolyzed to produce the furfural by acid catalysis, while the residue (mainly cellulose and lignin) is usually burnt. It has been estimated that the annual production of FR is about 23 million tons (Sun *et al.* 2008). With the rapidly increasing demand for energy and the declining supply of fossil resources, bioconversion of FR into biofuels, such as bioethanol, has received much attention. Ethanol production from FR would not only reduce environmental pollution, but also efficiently use the corncob material. It has been reported that the furfural production process decreases the degree of polymerization of cellulose, which is beneficial for the enzymatic hydrolysis (Zhang *et al.* 2009). However, lignin plays a vital role as an inter- and intra-molecular glue, strengthening plant cell walls, which leads to its inability to undergo rapid biotransformation into useful biofuels (Elumalai *et al.* 2012). Therefore, pretreatment of FR before its biotransformation is needed. The treatment approach is achieved by fractionation of FR into cellulose and lignin with good selectivity and high yield. Thus FR can be fully exploited for the subsequent conversion into biofuels, commodities (*i.e.*, alkali lignin, organosolv lignin, *etc*.), and other high value-added products.

Lignin is an amorphous polymer consisting of phenylpropane units, and their precursors are three monolignols, namely *p*-coumaryl, coniferyl, and sinapyl alcohols (Terashima and Fukushima 1989). The respective aromatic constituents of these alcohols in the polymers are called *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units (Buranov and Mazza 2008). During the lignification process, these monolignols form various interunit bonds, such as *β-O-4′*, *α-O-4′*, *4-O-5′*, *β-5′*, *β-1′*, *β-β′*, and *5-5′* (Buranov and Mazza 2008; Chakar and Ragauskas, 2004; Ralph et *al.* 2004; Wen *et al.* 2013). Besides the linkages within the lignin itself, lignin is always associated with carbohydrates via covalent bonds such as benzyl-ether, benzyl-ester, and phenyl-glycoside bonds, forming lignin-carbohydrate complexes (LCC) (Baucher *et al.* 1998). As an environmentally friendly and natural compound, lignin can be applied in many fields, such as biomaterials, fuels, biostabilisers, *etc.* (Lora and Glasser 2002). For example, it can be utilized to produce vanillin as a food and beverage flavouring agent in food industry and also may be an obvious candidate in resin industry (Buranov and Mazza 2008; Lora and Glasser 2002). In addition, lignin isolated from different treatment processes has antioxidant properties and can be used as an effective free radical scavenger.

In the past decades, a multitude of different treatment technologies have been developed to separate lignin from biomass. According to the different forces or energy used in the treatment processes, they can be classified as physical, chemical, physico-chemical, and biological methods (Alvira *et al.* 2010). Among them, chemical treatment is currently the most attractive technology for industrial applications. A series of lignin-dissolving agents have been used to disrupt the tight packing arrangement of plant cell walls, such as alkali, organosolv, and alkaline hydrogen peroxide (AHP). It has been reported that about 60 to 70% of wheat straw lignin could be removed when cooking in 1.5% NaOH at 100 ºC for 1 h, and this increased to 90% at 160 ºC (Cheng 1993). Furthermore, AHP treatment has been applied to many biomass feedstocks. It was used to improve the chemical and physical properties of dietary fiber, removing more that 50% of hemicelluloses and lignin from rice straw (Sangnark and Noomhorm 2004; Sun *et al.* 2000). In addition, organosolv treatment is also a promising strategy to treat lignocellulosic materials (Brudecki *et al.* 2013; Cybulska *et al.* 2012). Numerous organic or aqueous solvent mixtures can be utilized, including methanol, ethanol, acetone, ethylene glycol, and tetrahydrofurfuryl alcohol, in order to solubilize lignin and prepare cellulosic substrate for enzymatic hydrolysis. The main advantage of organosolv process is the recovery of relatively pure lignin as a by-product compared to other chemical treatments. In our laboratory, organosolv process has been suggested to be combined with NaOH to separate lignin with a high yield. For instance, when *Caragana sinica* was subjected to 70% ethanol containing 1% NaOH treatment at 75 ºC, 35% of the original lignins was dissolved, which is equivalent to 41% of the total dissolved lignin (Xiao *et al.* 2011). However, to our knowledge, the treatments with alkali, organosolv, and alkaline hydrogen peroxide aimed at isolation and structural characterization of lignins from FR have not been reported.

In the present study, the lignins were isolated from FR by treating with alkali, ethanol, alkali-ethanol, and alkaline hydrogen peroxide, respectively. Their structural features were characterized in terms of sugar analysis, molecular weights, ultraviolet, Fourier transform infrared (FT-IR), and heteronuclear single quantum coherence (HSQC) NMR spectroscopies, as well as thermal gravimetric analysis (TGA) and derivative thermogravimetric analysis (DTA). Additionally, antioxidant activity was also

investigated, since it is closely related to the chemical structure. The lignin extraction procedures and comprehensive structural features will afford information to explore its utilization and to further achieve comprehensive application of FR.

EXPERIMENTAL

Materials

Furfural residue, produced by hydrolysis of hemicelluloses from corncob using 5% H_2SO_4 as a catalyst at 180 ºC for 2 to 3 h, was kindly supplied by Xinxiang Yuyuan Chemical CO., LTD. (Xinxiang, China). The content of Klason (41.07%) and acid-soluble (0.89%) lignin was determined by National Renewable Energy laboratory's (NREL) standard analytical procedure (Sluiter *et al.* 2011). All chemicals purchased were of analytical or reagent grade and used without further purification.

Treatment of Furfural Residue

Furfural residue was first washed with distilled water until neutral and dried at 50 ºC for 12 h. Then 5.0 g of FR was treated with 70% ethanol, 70% ethanol containing 0.5% NaOH, 70% ethanol containing 1.0% NaOH, 1.0% NaOH, and aqueous 1.0% H_2O_2 containing 1.0% NaOH with a solid to liquor ratio of 1:20 (g/mL) at 80 ºC for 3 h, respectively. After that, the insoluble residue was collected by filtration with a Buchner funnel, and washed with distilled water (200 mL) until the pH of filtrates was neutral. The spent liquor and washing liquor were combined and concentrated with a rotatory evaporator under reduced pressure at 60 °C to about 50 mL. Then the concentrated liquor was acidified to pH 2 with HCl. Subsequently, the lignin was obtained by centrifugation and then freeze-drying. The acid-insoluble lignin fractions extracted with 70% ethanol, 70% ethanol containing 0.5% NaOH, 70% ethanol containing 1.0% NaOH, 1.0% NaOH, and aqueous 1.0% H_2O_2 containing 1.0% NaOH were labeled as EL, $AEL_{0.5}$, $AEL_{1.0}$, AL, and HAL, respectively. In addition, dioxane lignin (DL) was also isolated as follows. FR (20 g) was firstly ball-milled (5 h) and then was suspended in 96% dioxane with a solid-to-liquid ratio of 1:10 (g/mL) at room temperature for 24 h in darkness under nitrogen atmosphere. The mixture was filtered and washed with the same solvents until the filtrate was clear. Such operations were conducted twice. Then the combined filtrates were concentrated to about 50 mL with a rotary evaporator under reduced pressure and then transferred into 150 mL of 95% ethanol to precipitate hemicelluloses. The hemicelluloses were removed by filtration. The combined filtrates were then concentrated to about 30 mL and and adjusted to pH 2.0 under stirring. After washing with about 300 mL acidified water (pH 2.0) and freeze-drying, the fraction DL was obtained.

Chemical Analysis and Structural Characterization

Sugar analysis (neutral sugars and uronic acids) was conducted by using high-performance anion exchange chromatography (HPAEC). The neutral sugars and uronic acids in the lignin fractions (5 mg) were liberated by hydrolysis with 1.475 mL of 6% sulphuric acid at 105 ºC for 2.5 h. After this, the samples were diluted 50-fold, and analyzed by a HPAEC system (Dionex ISC 3000) with an amperometric detector, an AS50 autosample, 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 eluent (carbonate free and purged with nitrogen) for 20 min, followed by a 0.75 mM NaAc gradient in 5 mM NaOH for 15 min with a flow rate of 0.4 mL/min. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-glucose, D-mannose, D-galactose, glucuronic acid, and galacturonic acid, and the relative standard deviation of the results was below 5%.

Molecular weights of the lignin fractions were determined by gel permeation chromatography (GPC, Agilent 1200, USA) with a UV detector on a PL-gel 10 mm Mixed-B 7.5 mm i.d. column calibrated with PL polystyrene standards. About 4 mg sample was dissolved in 2 mL tetrahyrofuran (THF) and 20 μL lignin solutions were injected. The column was operated at ambient temperature and eluted with THF at a flow rate of 1.0 mL/min. The measurements were conducted in triplicate.

UV spectra were recorded on an ultraviolet/visible spectrophotometer (Tec comp, UV 2300). Each lignin sample (5 mg) was dissolved in dimethyl sulfoxide (DMSO) (10 mL). Then 1 mL aliquot was diluted to 10 mL DMSO, and the absorbances between 260 and 400 nm were recorded.

FT-IR spectra of lignin fractions were conducted using a Thermo Scientific Nicolet iN10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI) equipped with a liquid nitrogen cooled MCT detector. The dried samples were ground and palletized using BaF_2, and their spectra were recorded in the range from 4000 to 800 cm^{-1} at 4 cm^{-1} resolution and 128 scans per sample. The fingerprint region was baseline corrected between 1900 and 800 cm^{-1}. Before data collection, a background scanning was performed for correction.

The solution-state heteronuclear single quantum correlation (HSQC) spectra of the samples were acquired on a Bruker AVIII 400 MHz spectrometer at 25 ºC. About 90 mg of lignin was dissolved in 0.5 mL of DMSO-d_6. The spectral widths were 5000 and 20000 Hz for the ^{1}H- and ^{13}C dimensions, respectively. The number of the collected complex points was 1024 for the ^{1}H dimension with a recycle delay of 1.5 s. The number of transients was 64 and 256 time increments were always recorded in the ^{13}C dimension. The $^1J_{CH}$ was set to 146 Hz. Before Fourier transformation, the data matrices were zero filled to 1024 points in the ^{13}C dimension. The central solvent (DMSO) peak was used as an internal chemical shift reference point (δ_C/δ_H 39.5/2.49).

Thermogravimetric analysis (TGA) was investigated with a simultaneous thermal analyzer (TGA Q200, TA, USA). Samples weighing 8 to 10 mg were heated in a platinum crucible from room temperature to 600 ºC at a heating rate of 20 ºC/min under N_2 atmosphere.

The antioxidant activity of the lignin samples and 2,6-di-tert-bntyl-p-cresol (BHT) was determined by a spectroscopic assay involving the consumption of the stable free radical originating from 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol solution. The colorimetric assay was performed according to the method described by Blois (1958) with minor modifications. Briefly, a lignin sample solution (0.05-2.5 mg/mL) in 0.1 mL dioxane-water (9/1, v/v) was added to 3.9 mL of a DPPH solution (25 mg/L in ethanol) as the free radical source and kept for 30 min at room temperature. The decrease of the solution absorbance, due to proton-donating activity, was measured at 517 nm using a UV 2300 spectrometer (Shanghai Tianmei Science and Technology Corporation, China). The DPPH radical-scavenging activity was calculated according to a previous paper (Lu *et al.* 2012).

RESULTS AND DISCUSSION

Lignin Isolation, Chemical Composition and Molecular Weight Analysis

The original FR contained 42.59% cellulose, 41.96% lignin (41.07% Klason lignin and 0.89% acid-soluble lignin), and small amounts of xylan (0.77%). The compositional analysis revealed that lignin and cellulose were the dominant constituents in the original FR, while hemicelluloses were mostly removed during the furfural production. Ethanol, sodium hydroxide, alkali ethanol, and alkaline hydrogen peroxide (AHP) solutions were selected as the delignification solvents for extracting lignins. The yields of all the lignin fractions (%, based on Klason lignin in FR) are given in Table 1.

Table 1. Lignin Yield and Chemical Composition of the Lignin Preparations

	Yield (%)[a]	TSC (%)[b]	Glucose	Xylose	Ash Content (%)	KLC (%)[c]
EL	18.87±0.24	0.77	0.60±0.00	0.17±0.01	0.88±0.10	92.18
$AEL_{0.5}$	25.17±0.32	0.69	0.45±0.04	0.24±0.00	2.74±0.34	92.05
$AEL_{1.0}$	28.35±0.19	0.74	0.53±0.06	0.21±0.02	5.10±0.88	92.09
AL	36.92±0.40	0.14	0.08±0.01	0.06±0.04	7.41±0.90	89.44
HAL	73.01±0.51	0.51	0.43±0.04	0.08±0.03	9.06±0.57	85.90
DL	ND[d]	0.26	0.17±0.02	0.09±0.04	1.92±0.23	93.05

[a] based on the Klason lignin content of FR
[b] TSC=Total sugar content
[c] KLC=Klason lignin content
[d] ND=not detected

Table 2. Weight-Average (M_w), Number-Average (M_n) Molecular Weights (g/mol) and Polydispersity (M_w/M_n) of the Lignin Preparations

	EL	$AEL_{0.5}$	$AEL_{1.0}$	AL	HAL	DL
M_w	1140	940	900	990	870	1160
M_n	780	670	660	750	690	750
M_w/M_n	1.46	1.40	1.36	1.32	1.26	1.55

The yields of all the lignin fractions increased in the order of EL < $AEL_{0.5}$ ≈ $AEL_{1.0}$ < AL < HAL (Table 1). The results showed that the yield of HAL prepared with 1.0% H_2O_2 containing 1.0% NaOH (AHP) was the highest among all the alkaline lignins. During the AHP treatment, hydroxyl radical and super-oxide radical anions produced by the decomposition of hydrogen peroxide can oxidize and degrade lignin, resulting in the depolymerization of lignins and the introduction of hydrophilic groups (Gärtner and Gellerstedt 2000). Then the oxidized lignin fragments are dissolved in the alkaline solution. Due to the relatively higher yields, AL and HAL were used for further structural and behavioral analyses. Additionally, the lignin fractions in this study contained lower levels of carbohydrates, as shown in Table 1. Specially, only glucose (0.08 to 0.60%) and xylose (0.06 to 0.24%) were observed as minor sugars. Besides, it can be seen that the

lignin fraction EL contained less ash content and more Klason lignin content than other lignin fractions, except DL.

The average molecular weights (M_w and M_n) and polydispersity index (M_w/M_n) of the six-lignin fractions were determined by GPC analysis (Table 2). As can be seen, all samples exhibited similar molecular weight distributions, ranging from 870 to 1160 g/mol. Specifically, the M_w and M_n of DL from FR were 1160 and 750 g/mol, whereas our previous work showed that the M_w and M_n values of dioxane lignin from corncob were around 2130 and 1000 g/mol, respectively (Sun *et al.* 2013). These results suggested that the degradation of lignin in the corncob took place during furfural production. In such a situation, the cleavage of the α-ether bond would reduce the molecular weight of the lignin macromolecules (Bu *et al.* 2011). Due to the lack of an effective nucleophilic reagent, the degradation fragments with lone pairs of electrons may connect to the carbocation for a low steric hindrance, forming a novel steady component (Bu *et al.* 2011). It was found that the molecular weight of HAL decreased as compared to the other five lignin samples, which was probably related to a substantial degradation of the lignin polymers by the cleavage of some amounts of *β-O-4′* linkages between the lignin precursors during the alkaline hydrogen peroxide treatment.

Spectroscopic Analysis of the Extracted Lignin

The UV/vis spectra of all lignin fractions are shown in Fig. 1. The maximum absorption at 280 nm originates from non-conjugated phenolic groups in a guaiacyl-rich lignin (syringyl units exhibit a band at 268 to 276 nm) (Xu *et al.* 2008). The absorption at about 320 nm, assigned to the $\pi \rightarrow \pi*$ transition in lignin units containing $C_\alpha=C_\beta$ linkages conjugated to the aromatic ring, is indicative of ferulic and *p*-coumaric acid (Oliveira *et al.* 2009; Scalbert *et al.* 1986). It was found that the intensity of the peak at 320 nm in AL and HAL was lower than those of other lignin fractions, which was probably due to the hydrolysis of the *p*-coumarate ester.

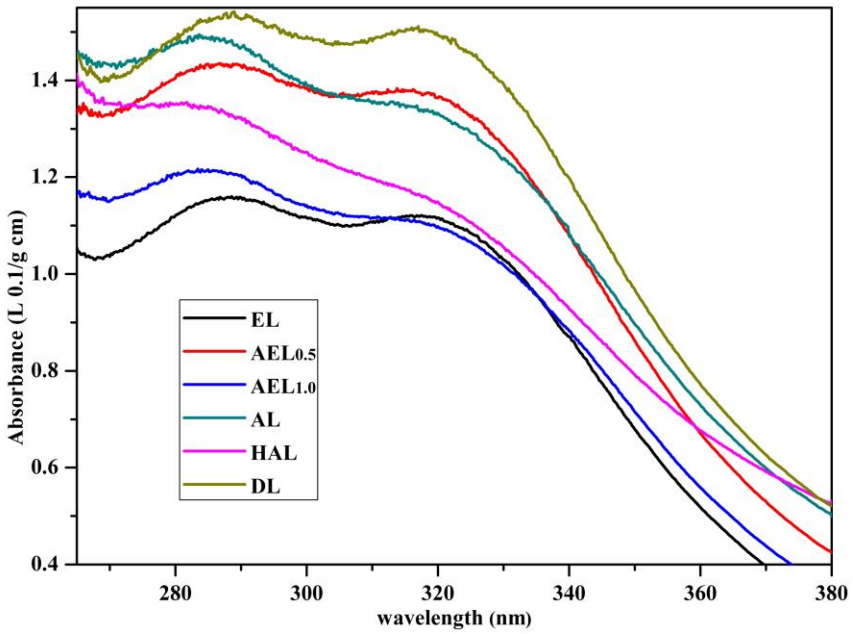

Fig. 1. UV spectra of the lignin fractions (DL, EL, $AEL_{0.5}$, $AEL_{1.0}$, AL, and HAL)

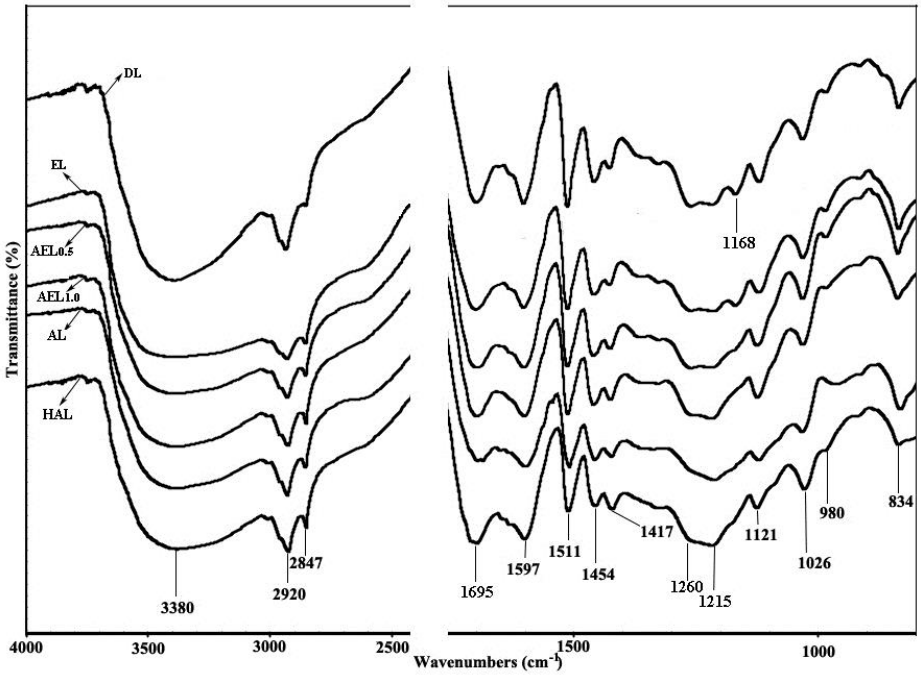

Fig. 2. FT-IR spectra of the lignin fractions (DL, EL, $AEL_{0.5}$, $AEL_{1.0}$, AL and HAL)

Figure 2 shows the FT-IR spectra of all the six lignin fractions obtained from FR, and peaks were assigned according with the literature (Faix 2009; Fengel and Shao 1985; Wen *et al.* 2013). As can be seen, the spectra of all lignins were rather similar. The absorption at 1695 cm^{-1} is attributed to the carbonyl stretching in conjugated ketone and carboxylic acid groups, as indicated by the presence of *p*-coumaric acid in Fig. 3. The bands at 1597, 1511, and 1417 cm^{-1}, arising from aromatic skeletal vibrations and the C-H deformation combined with aromatic ring vibration at 1454 cm^{-1}, were significantly exhibited. The band at 1328 cm^{-1} is attributed to the syringyl and condensed guaiacyl units.

The fingerprint region of the FT-IR spectra of lignins showed typical grass lignin patterns, with the 1121 cm^{-1} band dominated as a signal of a GS lignin. Aromatic C-H in-plane deformation vibration was observed at 1026 cm^{-1}. Besides, the absorbances at 985 and 834 cm^{-1} are assigned to CH=CH bending and C-H bending of syringyl units, respectively.

It should be mentioned that the band at 1168 cm^{-1}, corresponding to the antisymmetric C-O stretching of ester groups, was observed in DL and EL, whereas it almost disappeared in other alkaline lignin fractions. This disappearance could be due to the saponification of hydroxycinnanates or acetate groups during the alkaline, alkali ethanol, and alkaline hydrogen peroxide treatments.

To further understand the structural features of the lignin fractions, the HSQC NMR spectra of lignins were acquired. The HSQC spectra of three lignin fractions (AL, HAL, and DL) and main substructures identified are shown in Fig. 3 and 4, respectively, and signal assignments based on the literature data (Yelle *et al.* 2008; Li *et al.* 2012) are listed in Table 3.

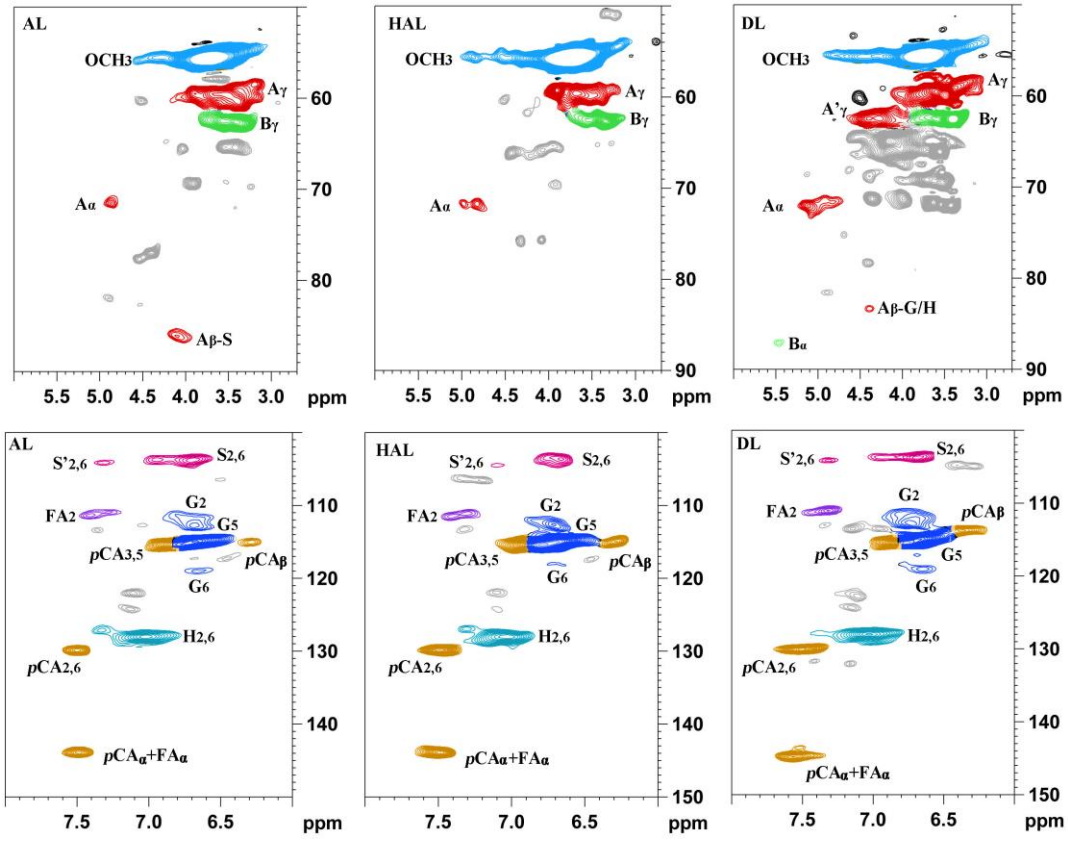

Fig. 3. HSQC spectra of the lignin fractions AL, HAL, and DL

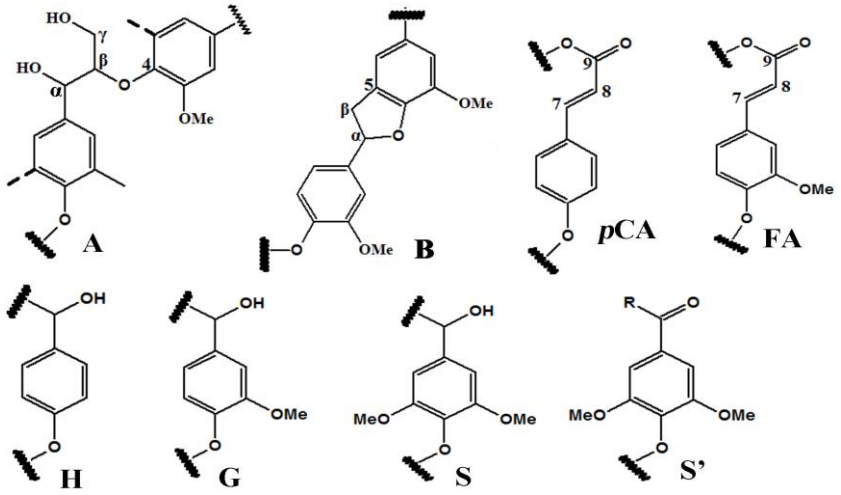

Fig. 4. Main structures present in furfural residue lignins: (A) *β*-aryl-ether units (*β-O-4'*); (B) phenylcoumaran substructures (*β-5'*); (*p*CA) *p*-coumaric acid; (FA) ferulic acid; (H) *p*-hydroxyphenyl units; (G) guaiacyl units; (S) syringyl units; (S') oxidized syringyl units bearing a carbonyl at C_α

As can be seen, the major inter-units of lignin, such as *β*-aryl ether (A), phenyl coumaran (B), and methoxyl are readily characterized in the aliphatic side-chain region. Methoxyl groups showed signals at δ_C/δ_H 55.6/3.71. The spectra show predominant signals corresponding to *β-O-4'* alkyl-aryl ether linkages A. The C_α-H_α and C_γ-H_γ

correlations in *β-O-4′* substructures A were observed at δ_C/δ_H 72.0/4.98 and 59.9/3.49, respectively, whereas the C_β-H_β correlations were observed at δ_C/δ_H 83.4/4.38 for the substructures linked to G and H units in DL and at δ_C/δ_H 86.1/4.09 for substructures linked to H units in AL, respectively. Moreover, *γ*-acylated *β-O-4′* alkyl-aryl ether linkages (A′/A″/A‴) in DL were observed with their correlations at δ_C/δ_H 62.5/4.27. Phenyl coumaran substructures B were also identified with the signals for C_α-H_α and C_γ-H_γ at δ_C/δ_H 87.2/5.49 and 62.6/3.40, respectively.

Table 3. Assignments of ^{13}C-^{1}H Cross-Signals in the HSQC Spectra of the Lignin from Furfural Residue

Label	δ_C/δ_H (ppm)	Assignments
-OCH_3	55.6/3.71	C-H in methoxyls
A_γ	59.9/3.49	C_γ-H_γ in *β-O-4′* substructures (A)
A'_γ	62.5/4.27	C_γ-H_γ in *γ*-acylated *β-O-4′* substructures (A′ and A″)
B_γ	62.6/3.40	C_γ-H_γ in phenylcoumaran substructures (B)
A_α	72.0/4.98	C_α-H_α in *β-O-4′* units (A)
$A_{\beta(G/H)}$	83.4/4.38	C_β-H_β in *β-O-4′* linked to G/H unit (A)
$A_{\beta(S)}$	86.1/4.09	C_β-H_β in *β-O-4′* linked to a S unit (A)
B_α	87.2/5.49	C_α-H_α in phenylcoumaran substructures (B)
$S_{2,6}$	103.7/6.69	$C_{2,6}$-$H_{2,6}$ in syringyl units (S)
$S'_{2,6}$	104.1/7.31	$C_{2,6}$-$H_{2,6}$ in oxidized S units (S′)
G_2	112.7/6.73	C_2-H_2 in guaiacyl units (G)
$G_5/H_{3,5}$	115.4/6.77	C_5-H_5 in guaiacyl units (G) and $C_{3,5}$-$H_{3,5}$ in H units (H)
G_6	119.0/6.65	C_6-H_6 in guaiacyl units (G)
$H_{2,6}$	128.0/7.02	$C_{2,6}$-$H_{2,6}$ in H units (H)
p$CA_{2,6}$	129.9/7.50	$C_{2,6}$-$H_{2,6}$ in *p*-coumaric acid (*p*CA)
pCA_α/FA_α	144.8/7.56	C_α-H_α in *p*-coumaric acid (*p*CA) and ferulic acid (FA)
pCA_β	115.0/6.29	C_β-H_β in *p*-coumaric acid (*p*CA)
FA_2	111.0/7.32	C_2-H_2 in ferulic acid (FA)
FA_6	122.7/7.11	C_6-H_6 in ferulic acid (FA)

In the aromatic region, the main cross-signals of *p*-hydroxyphenyl (H), syringyl (S), and guaiacyl (G) lignin units were observed. The S-lignin units showed a prominent signal for the $C_{2,6}$-$H_{2,6}$ correlations at δ_C/δ_H 103.7/6.69, whereas the G units presented C_2-H_2, C_5-H_5, and C_6-H_6 correlations at δ_C/δ_H 112.7/6.73, 115.4/6.77, and 119.0/6.65, respectively. The oxidized structure of syringyl lignin (*α*-ketone, S′) was identified at δ_C/δ_H 104.1/7.31 corresponding to $C_{2,6}$-$H_{2,6}$ correlations. The $C_{2,6}$-$H_{2,6}$ aromatic correlations of H units were detected at δ_C/δ_H 128.0/7.02, but the $C_{3,5}$-$H_{3,5}$ correlations were overlapped with those from 5-position of G units. In addition, the assignments of *p*-coumarate (*p*CA) and ferulate (FA) were also detected in these lignins. It was found that the cross-signals of *p*CA_β in AL and HAL were at δ_C/δ_H 115.0/6.29, while they shifted to δ_C/δ_H 113.8/6.35 in DL. The result suggested that DL extracted with neutral solvent preserved the part of the esterified linkages while these linkages were mostly cleaved

during the alkali treatments (AL and HAL). In addition, the cross-signals at δ_C/δ_H 129.9/7.50 belong to *p*$CA_{2,6}$, and δ_C/δ_H 115.4/6.77 correspond to *p*$CA_{3,5}$. These signals overlapped with those from G units. The *p*CA_α correlation appears at δ_C/δ_H 144.8/7.56. For FA, the correlations of 2- and 6-positions were observed at δ_C/δ_H 111.0/7.32 and 122.7/7.11, respectively. Furthermore, FA_α correlation coincides with that of *p*CA_α at δ_C/δ_H 144.8/7.56.

Thermal Degradation and Antioxidant Activity Characteristics

Thermal stability, closely relating to the structural characteristics and aggregation status of polymer, was measured to provide further information. The thermogravimetric analysis (TGA) and differential thermal analysis (DTA) curves of the lignin fractions AL, HAL, and DL are shown in Fig. 5, and their degradation behaviour is greatly influenced by their complicated structures.

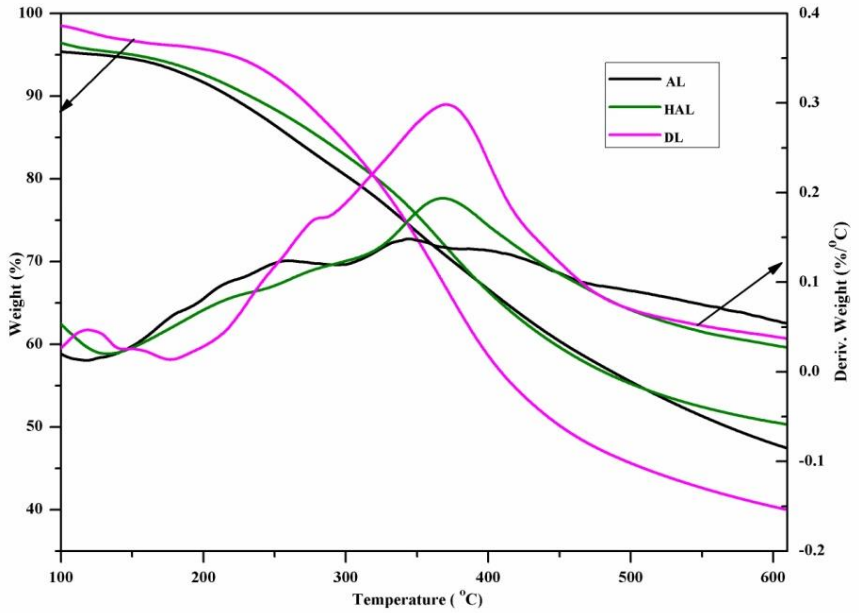

Fig. 5. Thermogravimetric analyses of the lignin fractions AL, HAL, and DL

The main mass loss occurred between 200 and 500 ºC, corresponding to condensation of the thermally excited degradation lignin fragments, in which water splitting off reactions were dominant. Beside water, other products, such as CO, CO_2, CH_3CHO, and HCHO, have been recorded as a result of re-polymerization through various substitution sites of the aromatic ring in this stage (Shukry *et al.* 2008). Whether from the onset temperature or the percent of weight loss at 600 ºC, HAL was proven to be more thermal-stable than AL in the TGA curves, probably relating to the AHP treatment. When the temperature reached ~600 ºC, about 48%, 50%, and 40% weight of the residual was preserved for AL, HAL, and DL, respectively. These high contents of the residues mainly resulted from the functional groups present in the associated lignin, which formed complex cross-linked structures that did not decompose at such a high temperature. Previous studies reported that condensation of lignin and formation of char would occur during the pyrolysis of lignin (Monteil-Riveran *et al.* 2006; Vasile and Brebu 2006). It seems that the HAL required a higher temperature to reach complete degradation. In addition, DTA curves were measured to reflect the energy consumption property in

pyrolysis, and the whole process of lignin degradation was endothermic. The maximum decomposition rates were recorded at 345, 368, and 370 ºC for AL, HAL, and DL, respectively.

The antioxidant activity of all the lignin fractions was investigated in comparison with a typical commercial antioxidant (BHT), and the DPPH inhibitory effects of different lignin solutions are shown in Fig. 6. There was a significant correlation between the inhibitory effect and the sample concentration. The DPPH inhibitory effect increased with the increment of sample concentration. The radical scavenging index (RSI) values of EL, $AEL_{0.5}$, $AEL_{1.0}$, AL, HAL, and DL were 0.86, 1.65, 1.54, 1.31, 0.70, and 1.86, as compared to 1.16 for BHT, respectively. The data indicated that $AEL_{0.5}$, $AEL_{1.0}$, AL, and DL had higher antioxidant activity than BHT, whereas EL and HAL had relatively lower antioxidant activity. In this work, the antioxidant activity decreased in the order DL > $AEL_{0.5} \approx AEL_{1.0}$ > AL > EL > HAL.

It has been reported that the lignin fractions with low molecular weight and polydispersity had high antioxidant activity (Pan *et al.* 2006). However, in this study there was no significant relationship found between molecular weight and lignin RSI among the six lignin fractions. This was probably due to the different contents of functional groups (*e.g.*, phenolic hydroxyl groups, aliphatic hydroxyl groups, and methoxyl groups) and structures of the lignins, isolated during the various treatments (Dizhbite *et al.* 2004; Pan *et al.* 2006). In this case, the relatively high RSI value of these lignins (DL, $AEL_{0.5}$, $AEL_{1.0}$, and AL) suggests that these lignins may be used as potential antioxidants in food industry. Additionally, the lignins provide a natural and relatively safe source for antioxidants as compared to the costly and less efficient synthetic antioxidants (*e.g.*, BHT) (Faustino *et al.* 2010).

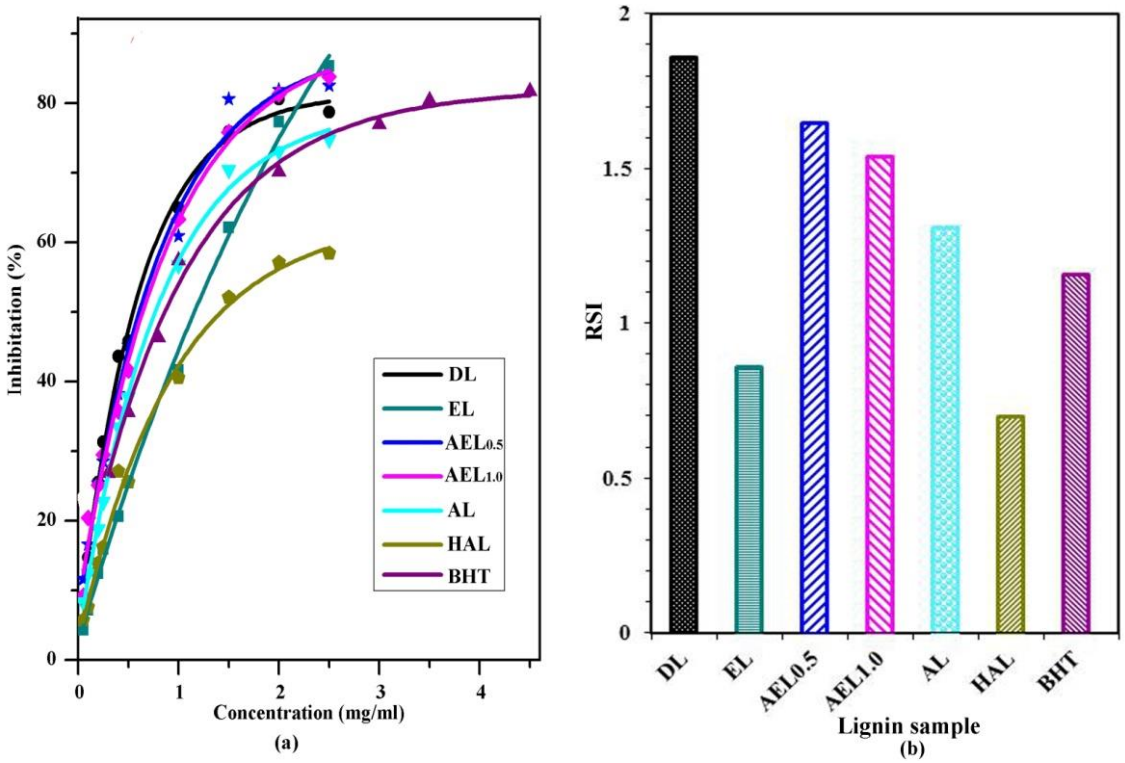

Fig. 6. Antioxidant activity against DPPH of all the lignin fractions (DL, EL, $AEL_{0.5}$, $AEL_{1.0}$, AL, and HAL) as compared to BHT. (a) DPPH inhibitory effect; (b) radical scavenging index (RSI) values

CONCLUSIONS

1. In the present study, alkali, ethanol, alkali-ethanol, and alkaline hydrogen peroxide solutions were applied to isolate lignin from furfural residue (FR) under mild temperature conditions.
2. All the lignin samples contained low levels of carbohydrates (0.14 to 0.77%) and showed low molecular weights (870 to 1160 g/mol).
3. Lignins extracted with alkali-ethanol and alkali solutions showed higher antioxidant activity than BHT. The results suggested that the alkali ethanol and alkali treatments provided promising ways to isolate lignin from furfural reside for potential applications in the food industry.

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