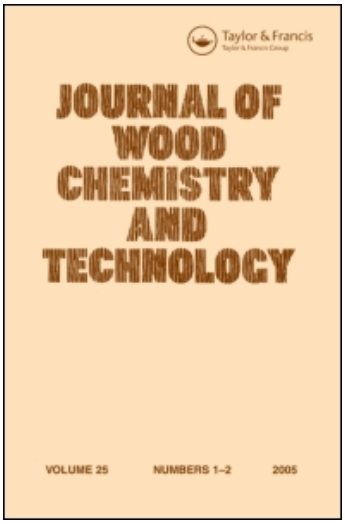

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CHARACTERIZATION OF DISSOLVED LIGNINS IN TWO-STAGE ORGANOSOLV DELIGNIFICATION OF WHEAT STRAW

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ABSTRACT

Wheat straw was pre-treated with 0.5 N NaOH at 75°C for 2 h and then post-delignified by 1.0 N H_2SO_4 catalyzed in ethanol-water (60/40, v/v) system at 75°C for 2 h (Alkaline Pretreatment) or pre-hydrolyzed with 1.0 N H_2SO_4 for partial release of hemicelluloses at reflux temperature for 2 h and then post-delignified in organosolv process (Acidic Pretreatment) through two-stage, respectively. Alkaline pretreatment yielded a higher release of lignin (51.2%) as compared to acidic pretreatment, in which 23.0% lignin was dissolved. On the other hand, acidic pretreatment appeared to be more effect on the rate of the organosolv delignification during the following stage. The isolated alkali lignin LA, acid-soluble lignin and organosolv lignin fractions were characterized by UV, IR and ^{13}C-NMR spectroscopy. The chemical composition of the four lignin fractions are reported.

INTRODUCTION

Delignification of wood in organic solvents, particularly in aqueous ethanol systems, is a promising method of pulp production that has undergone intensive

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development in recent years.[1] Non-solvent technologies such as chemi-thermomechanical pulping (CTMP), and alkaline sulfite anthraquinone (ASAQ) pulping processes are active competitors in this area, while biopulping has yet to emerge as a viable alternative.[2] The advantages of the organosolv pulping systems were found to have low capital and production costs, higher pulping yield, ease of breachability and diminished environmental constrains.[3] By acid catalyzed treatment in organosolv, it is possible to fractionate lignocellulosic biomass in its three major components: cellulosic fibres (remaining in solid phase), suitable for either papermaking or enzymatic conversion to glucose; solid low-molecular weight lignin (in solvent phase), usable either as fuel or as feedstock for chemical conversions; and hemicellulose sugars (in aqueous phase), which may be used for various fermentation processes or as chemical feedstocks.[4,5]

A number of organic solvents have been proposed for use in organosolv delignification, either as solvents or as combined with water, but so far only aqueous methanol and ethanol have been potential for practical application.[6] Kleinert[7] reported that aqueous solutions of ethyl alcohol were better delignifying agents than ethyl alcohol alone and that addition of a small amount of mineral acid increased the rate of delignification. Goyal et al.[3] confirmed that delignification increased with decreasing ethanol concentration over the range studied (50-70% by volume), and optimum selectivity in terms of delignification and pulp viscosity was obtained at 60% ethanol concentrations. Our previous studies,[8] however, showed that aqueous methanol, ethanol, 1-propanol, n-butanol, dioxane and acetone in a relatively high concentration (60/40, v/v) and low temperature (75°) for 2 h produced low yields of organosolv lignins (24-28%) as compared with alkali lignins obtained from wheat straw. In order to increase the rate of delignification during the organosolv and then physico-chemical characterization of the dissolved lignins, we have developed a two-stage acid catalyst method in aqueous ethanol systems at low temperature for delignification from wheat straw. Low temperature process was chosen as more advantageous for the protection of wheat straw against polysaccharide degradation and lignin condensation under the acidic reaction conditions.[9] In the first stage, ground wheat straw was pre-treated with either alkali or acid at moderate temperature for 2 h. The second stage involved the hydrolysis of

above residues with ethanol-water (60/40, v/v) under acid conditions (1.0 N H_2SO_4) at 75°C for 2 h. This paper presents the results of delignification during the two-stage treatments. A comparative characterization of the dissolved lignins in each of the fractions is reported.

EXPERIMENTAL

Material and Lignin Fractionation

The wheat straw (winter) was obtained from Silsoe Research Institute (Silsoe, Bedfordshire). It was ground using a Chrite Laboratory mill to pass a 60 mesh size screen. The ground and dried straw was extracted with toluene-methanol (2:1) for 5 h in a Soxhlet apparatus before treatments.

A scheme for treatments of wheat straw is shown in Figure 1. In alkaline pretreatment, the extractive free powder was firstly pre-treated with 0.5 N sodium hydroxide solution (15 g straw/ 400 mL solution) at 75°C for 2 h (stage 1). After filtration, the supernatant was acidified to pH 6.5 with dilute acetic acid, concentrated on a rotary evaporator under reduced pressure at 40°C, and then mixed with 5 volumes of 95% ethanol (24 h, 20°C) for isolation of crude hemicelluloses or hemicellulose-lignin complexes. The alkali-soluble lignin fraction LA (1a) was then re-precipitated at pH 1.5 with 20% HCl from the supernatant solution (24 h, 20°C). The isolated lignin fraction, after filtration, was freeze-dried overnight. The residue from stage 1 was delignified with ethanol-water (60/40, v/v) in 400 mL 1.0 N H_2SO_4 at 75°C for another 2 h (stage 2). The residue, after filtration, was then subsequently washed three times with ethanol-water (60/40, v/v) 200 mL and finally washed with 300 mL of distilled water. Washed residues were dried in oven at 50°C for 16 h. Ethanol was removed with a rotary vacuum evaporator at 40°C, the lignin from the black liquor was precipitated at pH 1.5 (24 h, 20°C), filtered, washed, and then air dried.

In acidic pretreatment, the extractive free powder (10 g) was conducted in a 400 mL batch stirred reactor at reflux temperature (99.0±1.0°C) for 2 h in

which the hemicellulose hydrolysis was catalyzed by 1.0 N H_2SO_4 (stage 1). The reaction mixture after hydrolysis was vacuum-filtered to obtain a solid residue and a acid-soluble lignin-/sugar- containing filtrate. The lignin fraction in the filtrate was precipitated at pH 1.5 (24 h, 20°C), then filtered, washed with pH 2.0 distilled water, and air dried. The second stage of organosolv delignification was performed from the residue of stage 1 with the method same as in alkaline pretreatment stage 2.

Lignin Characterization

UV spectra were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer. Lignin sample (5 mg) was dissolved in 95% (v/v) dioxane-water (10 mL). A 1 mL aliquot was diluted to 10 mL with 50% (v/v) dioxane-water, and the absorbances between 240 and 400 nm were measured.

The molecular-average weight of lignin fractions were determined by gel permeation chromatography on a PLgel 5μ Mixed-D column. The samples were dissolved with tetrahydrofuran (THF) with a concentration of 0.2% and 200 μL sample in solution was injected. The column was operated at 40°C and eluted with tetrahydrofuran at a flow rate of 1 mL min^{-1}. The column was calibrated using polystyrene standards.

IR spectra were obtained on an IR spectrophotometer (Mattson cygnus 100) using a KBr disc containing 1% finely ground samples. The solution of ^{13}C-NMR spectrum was obtained on a Brucker 250 AC operating in the FT mode at 62.4 MHz under total proton decoupled conditions. It is recorded at 25°C from 250 mg samples dissolved in 1.0 mL DMSO-d_6 after 30000 scans. A 40° pulse flipping angle , a 3.0 μs pulse width and 0.85 s acquisition time were used.

The methods of neutral sugar and uronic acid analyses, alkaline nitrobenzene oxidation of lignin and determination of phenolic acids and aldehydes with HPLC in extracted lignin fractions were described in previous papers.[10,11] All nitrobenzene oxidation results represent the mean of at least triplicate, and each oxidation mixture was chromatographed twice. Other experiments were performed in duplicate. The standard errors or deviations were always observed to be lower than 5% except for the variations among triplicate nitrobenzene oxidation (8-16%).

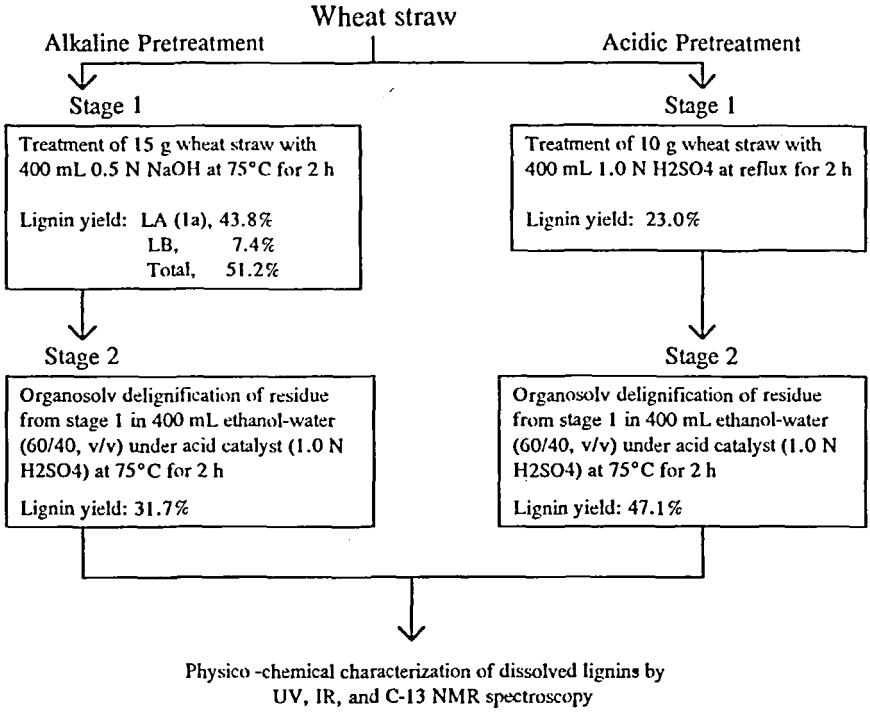

FIGURE 1. Scheme for organosolv delignification from alkaline and acidic pretreatments of wheat straw (LA, re-precipitated with 20% HCl at pH 1.5 after isolation of hemicelluloses; LB, co-precipitated in the hemicellulosic fraction).

RESULTS AND DISCUSSION

Lignin Yield

In the alkaline pretreatment, lignin yield was calculated by comparing the amount of lignin re-precipitated (LA) with 20% HCl at pH 1.5 from the supernatant solution after isolation of crude hemicelluloses and co-precipitated (LB) in the hemicelluloses or hemicellulose-lignin complexes with the total amount of acidic chlorite lignin present in the wheat straw (about 14.1% by

weight)[10,11]. Other three lignin fraction yields were calculated by comparing the amount of lignin directly precipitated at pH 1.5 from the filtrates with the total amount of acidic chlorite lignin present in the wheat straw.

The yields of four lignin fractions are shown in Figure 1. In alkaline pretreatment, the yields of alkali lignin fractions LA (1a) enriched in lignin and LB enriched in hemicelluloses were 43.8 and 7.4%, respectively. As expected, the yield of LA was much higher than for LB as shown by LA/LB, 5.9. The total alkali-soluble lignin accounted for over 50% of the acidic chlorite lignin from wheat straw. This high yield of alkali lignin is probable that alkaline treatment cleaves the ester bond between p-coumaric acid and lignin or between ferulic acid and hemicelluloses. These results were in a good agreement with our previous study on wheat straw alkali-soluble lignin fractions.[12] We showed that the predominant phenolic monomers in the alkaline hydrolysates were found to be ferulic and p-coumaric acids, which together comprised about 80% of the total.

The second stage (1b) after alkaline pretreatment yielded 31.7% of organosolv lignin by treatment with ethanol-water (60/40, v/v) in 1.0 N H_2SO_4 at 75°C for 2 h. Cleavage of ether linkages is a major factor in lignin breakdown during the organosolv pulping. Under acidic conditions, this is particularly true for α-ethers, but β-ether cleavage also plays a role, especially in hardwood pulping.[13] Model-compounds studies[14] have shown that α-ether linkages are more easily split than β-aryl ether linkages, especially when they occur in a lignin structural unit containing a free phenolic hydroxyl group in the para position. The likelihood of β-ether cleavages is greater in more strongly acidic system such as 1.0 N H_2SO_4 system. One reason is that these systems bear a greater resemblance to the classical acidolysis systems, in which the breakage of β-ethers has been established.[13] The effective role of acid catalyst also may be due to rapid hydrolysis of hemicelluloses resulting in increased porosity and accessibility of the solvent to the lignin.[5] Together in two-stage, about 83% of lignin was released and 81% of hemicelluloses were removed, which was an expected result and much better than the one-stage alkaline treatment without organosolv process. In our previous studies,[11] treatment of wheat straw with 1.5% NaOH at 80°C for 6 h released 63.2% of lignin and 75.5% of hemicelluloses, indicating that organosolv process (stage 2) is more favourable to delignification.

Some of the lignin in wood can be removed simply by heating the wood in water. For example, Nimz[15] dissolved 40% of the lignin in beech and 10% of that in spruce by treatment with water at 100°C. The responsible reactions, which were very likely also occur in the hydrolysis of wheat straw with 1.0 N sulfuric acid. As can be observed in Figure 1, about 23% of the lignin was released during the acidic pretreatment of wheat straw with 1.0 N H_2SO_4 solution at 75°C for 2 h. This finding was in accordance with the studies of Papatheofanous et al.[9] and González et al.,[16] in which wheat straw was fractionated into cellulosic fibres, hemicellulose sugars and solid lignin fractions through a two-stage, acid catalyzed process. During the first stage, raw material was treated with dilute H_2SO_4 (1.43 N) at reflux temperature for 3 h, selective hydrolysis of about 89% of the straw hemicelluloses converted to water-soluble sugars and 24% of lignin removal. By hydrolysis of wheat straw with sulfuric acid at 34-90°C, González et al.[16] mentioned that the treatment at 90°C yielded completed solubilization of hemicelluloses to xylose and arabinose without significant amounts of furfural. With the prehydrolysis of hardwood with dilute sulfuric acid, Springer[17] indicated that the reaction temperature, reaction time, and hydrogen ion concentration greatly influenced the rates of prehydrolysis. A maximum potential xylose increased with increasing temperature and acid concentration; 87% was obtained at 190°C with 0.8% H_2SO_4.

As mentioned earlier, the ethanol content of the solvent system favoured its selectivity towards delignification. With a concentration of ethanol-water (60/40,v/v) delignification in 1.0 N H_2SO_4 solution at 75°C for 2 h produced 47.1% of the lignin release (Acidic Pretreatment, stage 2). This effect of increasing delignification appeared to be more efficient for the two-stage process as compared to one stage of organosolv delignification in our previous study.[8] We showed that delignification of wheat straw directly with aqueous ethanol 60%, v/v) in the presence of acid catalyst (1.0 N H_2SO_4) at low temperature (75°C) for 2 h yielded 43.4% of the lignin removal based on the total amount of acidic chlorite lignin present in wheat straw. When some amounts of the original hemicelluloses were previously hydrolyzed (stage 1) the action of the acid during the second stage of the organosolv process appeared to concentrated on the lignin breakdown,[9] resulting in high yield of lignin released (47.1%), and limiting further degradation of polysaccharides, particularly cellulose.

UV Spectra

The UV absorption spectra of the lignin fractions are shown in Figure 2. The four lignins showed similar UV spectra having a absorption maximum at 280 nm originating from non-conjugated phenolic groups (aromatic ring) in lignin.[18] The fractions (1a, 2a) appeared to have a second maximum at 314-316 nm originating from conjugated phenolic groups in p-coumaric and ferulic acids. The absorption coefficient of 1a at 314-316 nm was much higher than of 2a; this difference is probably due to a higher content of ferulic and p-coumaric acids in isolated lignin fraction 1a and a lower content of p-coumaric and ferulic acids in isolated lignin fraction 2a, which was confirmed by the data of alkaline nitrobenzene oxidation. The low absorptions of 2a, 2b and 1b might be due to non-lignin material such as protein, silica or polysaccharide sugars co-precipitated in the isolated lignin fractions.

Content of Polysaccharide Sugars and Uronic Acids

As sodium hydroxide treatment of wheat straw would be expected to saponify the hydroxycinnamic esters that have been hypothesized to cross-link to a cell wall hemicelluloses.[18] The alkali-soluble lignin fraction LA (1a), obtained by two steps of precipitation, contained rather low levels of polysaccharide sugars (1.2%) and uronic acids (0.9%) (Table 1). Wheat straw alkali lignin fractions reported earlier, however, appeared significantly richer in polysaccharides.[18-21] The reason for this relatively free of polysaccharides in alkali lignin LA (1a) obtained in this study was explained previously.[22,23] When compared with milled-straw lignin LM and enzyme lignin LE,[18] the organosolv lignin fractions 1b and 2b, and acid-soluble lignin fraction 2a showed a low polysaccharide content (3.2-3.4%), suggesting that the bonds anchoring lignin to hemicelluloses in wheat straw are readily hydrolyzed under acidic conditions. These data supported the hypothesis that these bonds consist of ether linkages between the polysaccharide and the α-carbon atoms of lignin sidechains.[13] Therefore, release of lignin in the organosolv process or acidic aqueous solution resulted from the hydrolysis of α-aryl ether bonds and lignin-hemicellulose ether

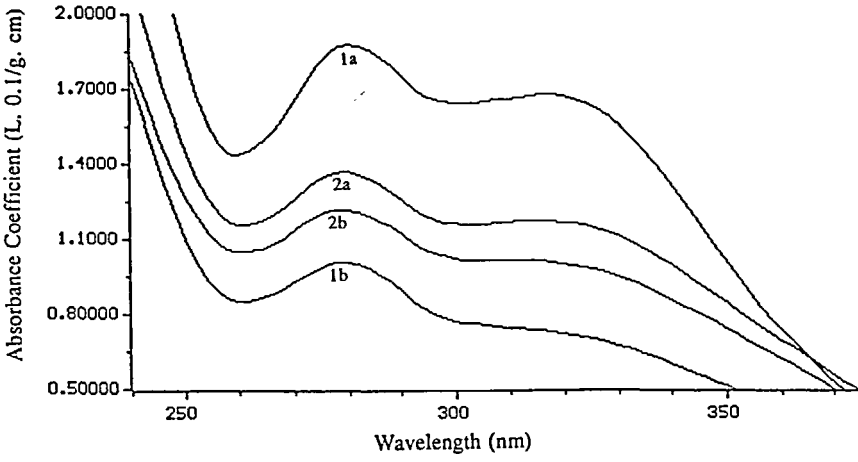

FIGURE 2. UV Spectra of the organosolv lignin fractions extracted from alkaline pretreatment, stage 1 (1a) and stage 2 (1b), and acidic pretreatment, stage 1 (2a) and stage 2 (2b).

TABLE 1

The Content of Polysaccharide Sugars and Uronic Acids in Lignin Fractions Isolated from Alkaline Pretreatment and Acidic Pretreatment in Two Stages (% Lignin Sample, w/w).

Sugars or uronic acids	Alkaline Pretreatment		Acidic Pretreatment	
	1a (stage 1)	1b (stage 2)	2a (stage 1)	2b (stage 2)
Arabinose	0.1	0.1	0.2	0.3
Xylose	0.3	0.5	0.7	0.8
Galactose	0.3	0.4	0.5	0.5
Glucose	0.5	1.3	1.1	0.9
Uronic Acids	0.9	0.9	0.7	0.9
Total	2.1	3.2	3.2	3.4

bonds. A higher content of uronic acids in isolated alkali lignin LA (1a) , acid-soluble lignin (2a), and organosolv lignin fractions (1b, 2b) suggested that the appearance of ester bonds between glucuronic acid or 4-*O*-methylglucuronic acid and lignin units, which was confirmed by ^{13}C-NMR spectra.[22]

TABLE 2

The Content of Phenolic Acids and Aldehydes in Nitrobenzene Oxidation Products of Lignin Fractions Obtained from Alkaline Pretreatment and Acidic Pretreatment in Two Stages (% Lignin Sample, w/w).

Phenolic monomers	Alkaline Pretreatment		Acidic Pretreatment	
	1a (stage 1)	1b (stage 2)	2a (stage 1)	2b (stage 2)
Gallic acid	1.2	1.2	1.0	1.4
Protocatechuic acid	0.04	0	0.01	0
p-Hydroxybenzoic acid	0.1	0.2	0.08	0.1
p-Hydroxybenzaldehyde	1.3	0.6	0.8	0.8
Vanillic acid	1.4	0.5	0.7	0.6
Syringic acid	1.9	1.2	1.1	0.7
Vanillin	9.4	5.6	4.1	4.4
Syringaldehyde	9.6	6.5	4.3	4.7
p-Coumaric acid	0.4	0.2	0.4	0.3
Ferulic acid	0.8	0.2	0.2	0.2
Total	26.1	16.2	12.7	13.2

Alkaline Nitrobenzene Oxidation

For comparison purposes, four lignin fractions were also investigated by alkaline nitrobenzene oxidation in order to gain insight into the lignin structure. The phenolic acids and aldehydes resulted from oxidation of the "core" of alkali lignin LA (1a), acid-soluble lignin (2a) and organosolv lignins (1b, 2b) are summarized in Table 2. The major products of alkaline nitrobenzene oxidation were identified to be vanillin and syringaldehyde, resulted from the degradation of non-condensed guaiacyl and syringyl units, respectively. The presence of small quantities of p-hydroxybenzaldehyde is generally considerated indicative of p-hydroxyphenyl units within the "core". Occurrence of non-condensed units, guaiacyl (G), syringyl (S) and relatively fewer p-hydroxyphenyl (H) was demonstrated in each of the lignin fractions, indicating that the isolated alkali lignin LA (1a), acid-soluble lignin (2a) and organosolv lignins (1b, 2b) all can be justified as GSH-lignins. A higher ratio of S/V in lignin fraction (1b) than for other three fractions (Table 2) evidenced that non condensed syringyl units is readily extracted by organosolv after the straw was pre-treated with alkali. Other three lignin fractions (1a, 2a, 2b) appeared to have roughly equal amounts of

non condensed guaiacyl and syringyl units as shown by the relatively same amounts of syringaldehyde and vanillin in each of the products of alkaline nitrobenzene oxidation.

When compared to the corresponding yields of alkaline nitrobenzene oxidation products from hardwood or softwood lignins, the lower yields of alkaline nitrobenzene oxidation of alkali lignin LA (1a), acid-soluble lignin (2a) and organosolv lignins (1b, 2b) indicated a higher degree of condensation of these lignins. Alkali lignin LA (1a) gave a relatively high yield of alkaline nitrobenzene oxidation products, indicating a higher purity of the lignin fraction. Whereas the acid-soluble lignin (2a) and organosolv lignin (1b, 2b) yielded low amounts of alkaline nitrobenzene oxidation products, suggesting that more contents of non-lignin material might be co-precipitated in these lignin fractions.

It is well established that alkali treatment appeared to hydrolysis the ester bonds between p-coumaric acid and lignin or between ferulic acid and hemicelluloses, and acidic aqueous solution or acidic organosolv treatments would be expected to break the ether bonds between ferulic acid and lignin or directly between lignin and hemicelluloses. As seen in Table 2, a higher content of ferulic acid in the nitrobenzene oxidation products of alkali lignin LA (1a) indicated that there is a significant amount of non-saponifiable linkages such as ether bonds between ferulic acid and lignin in the lignin fraction (1a). In contrast, the relatively low quantity of p-coumaric acid produced in the oxidation of alkaline lignin fraction LA (1a) suggested that a fewer proportion of this compound is etherified with lignin, because most of the esterified bonds disappeared during the above alkaline pretreatment. These results were good in accordance with our previous study.[12] The authors stated that about 90% of p-coumaric acid in wheat straw cell walls is present in the ester-linked form to lignin, while more than 60% of the ferulic acid is ether-linked to lignin.

Molecular Weight Distribution

The weight-average (M_W), number-average (M_n) molecular weights and polydispersity (M_W/M_n) of each lignin fraction are given in Table 3. As can be seen the alkali lignin LA (a), acid-soluble lignin (2a) and organosolv lignin (1b) appeared to have low molecular-average weight raging between 1240 and 1320.

TABLE 3

Molecular Weight of Lignin Fractions Isolated from Alkaline Pretreatment and Acidic Pretreatment in Two Stages.

	Alkaline Pretreatment		Acidic Pretreatment	
	1a (stage 1)	1b (stage 2)	2a (stage 1)	2b (stage 2)
$\overline{M}_w$	1240	1300	1320	2160
$\overline{M}_n$	510	440	460	580
$\overline{M}_w/\overline{M}_n$	2.4	2.9	2.9	3.7

These data accorded with our previous study on ball-milled straw lignin LM, enzyme lignin LE and one-stage organosolv lignin fractions from wheat straw.[8,24] Interestingly, the molecular-average weight of organosolv lignin fraction (2b) was about twofold higher than alkali lignin LA (1a), which was probably due to lignin condensation and reprecipitation of dissolved lignin during a higher acidic organosolv delignification stage (2b) after the straw was pre-treated with acid.

Based on the study of organosolv lignins from softwood or hardwood samples, Sarkanen[4] mentioned that the isolated solid organosolv lignins had relatively low molecular weights M_n=800-1200; M_w=1700-2000. On the other hand, with the study of organosolv delignification of black cottonwood in aqueous methanol at 150-170°C using 0.02-0.10 N H_2SO_4 as catalyst, Tirtowidjojo et al.[25] reported that the recovered lignin had considerable higher molecular-average weight (M_w) ranging from 3300 to 50200. These different molecular weights of organosolv lignins were probably due to the nature of sample and the various organosolv pulping conditions such as reaction system, reaction temperature and time used.

The GPC range of molecular weight of organosolv lignin fraction (2b) isolated from the acidic pre-treated straw sample is illustrated in Figure 3. Elution profile of the lignin showed a wide polymolecularity, ranging from monomer up to polystyrene of molecular weight 21300. The elution maximum appeared at 2080, and the second peak corresponded to very low molecular components, probably dimers.

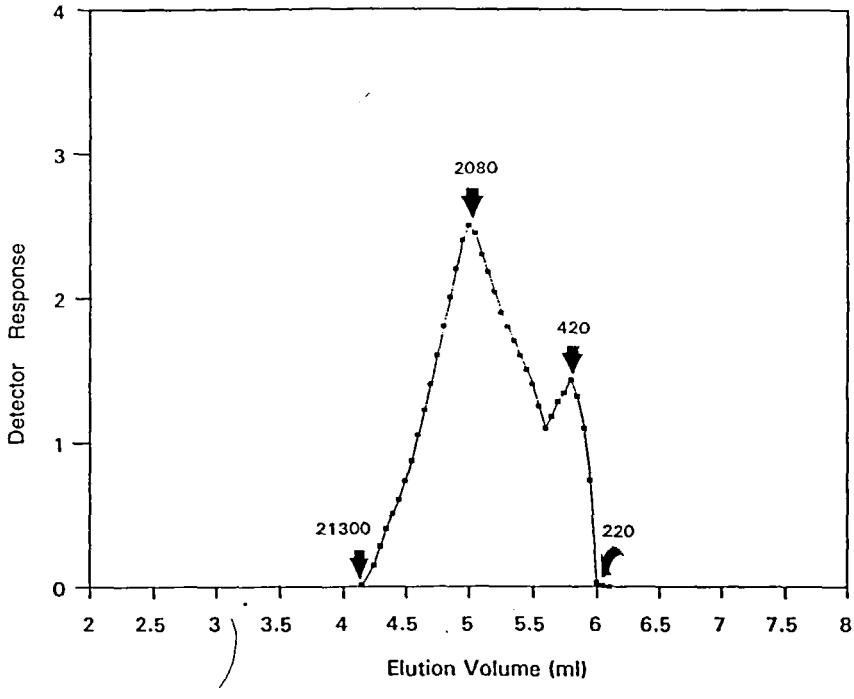

FIGURE 3. The GPC range of molecular weight of organosolv lignin fraction (2b) isolated from acidic pretreatment of wheat straw.

IR Spectra

Infrared spectra were also taken of the lignin fraction in order to see whether additional information could be obtained. These spectra are shown in Figure 4. The most striking characteristic of the IR spectra of the four lignin fractions from wheat straw is the presence of peaks at 1715 and 1650 cm^{-1} which are assigned to carbonyl stretching in unconjugated ketone and conjugated ketone, respectively. As shown in Figure 4, the spectrum of alkali lignin LA (1a) appeared to similar with the spectrum of organosolv lignin (1b) by a strong absorbance at 1220 cm^{-1} , while the acid-soluble lignin (2a) and organosolv lignin (2b) showed similar spectra by the very weak absorptions at 1325, 1265, 1220 and 1020 cm^{-1} , suggesting that the two lignin fractions (1a, 1b) have a

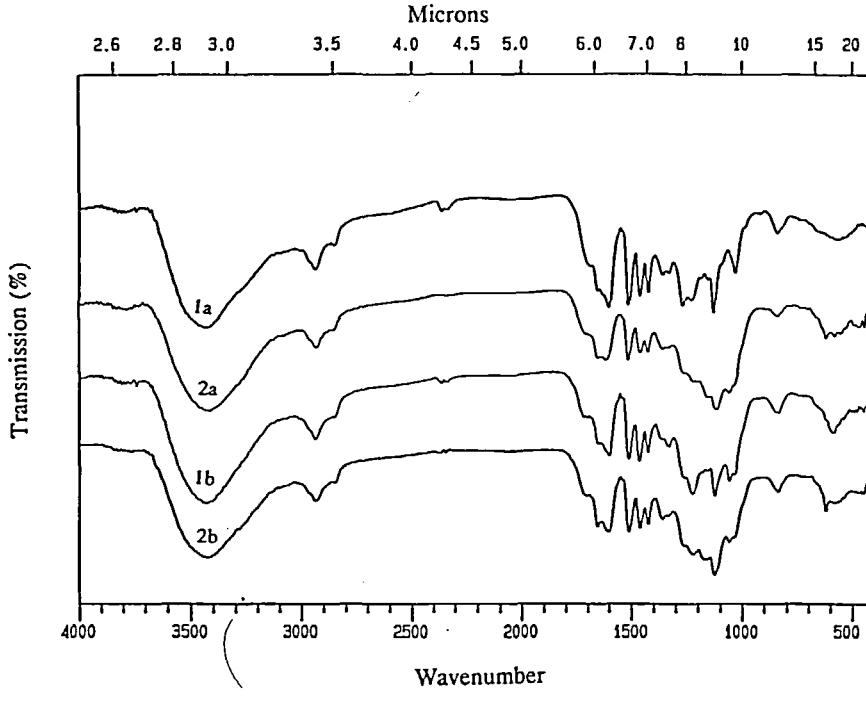

FIGURE 4. IR Spectra of the organosolv lignin fractions extracted from alkaline pretreatment, stage 1 (1a) and stage 2 (1b), and acidic pretreatment, stage 1 (2a) and stage 2 (2b).

relatively same "core" of lignin structure, whereas another two lignin fractions (2a, 2b) show the roughly equal lignin structure. Aromatic skeleton vibrations in these lignin fractions are assigned at 1420, 1503, and 1596 cm^{-1}[1,26,27] . The 1325, 1265 and 1220 cm^{-1} bands have been assigned to ring breathing with C-O stretch. The 1325 cm^{-1} band has been associated with sinapyl units, and 1265 and 1220 cm^{-1} bands with coniferyl units.[28] The bands at 1150, 1120 and 1020 cm^{-1} indicate the aromatic CH in-plane deformation. The alkali lignin (1a) appeared greatest absorbance at 1020 cm^{-1} while partial disappearance of this band was found in the other three lignin fractions. Aromatic C-H out of plane bending appears at 836 cm^{-1}. The very weak absorption at 1040 cm^{-1} in three lignin fractions (2a, 1b, 2b) indicated the small amounts of associated polysaccharides, which was corresponded with the chemical analyses. The alkali

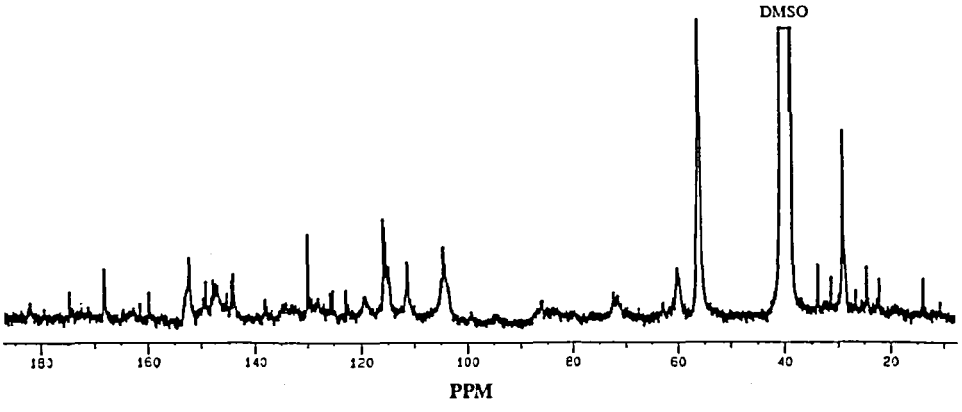

FIGURE 5. ^{13}C-NMR Spectrum of alkali lignin fraction (1a) isolated from the stage of alkaline pretreatment.

lignin (1a) is relatively free of polysaccharides, as indicated by the partial disappearance of this band.

^{13}C-NMR Spectrum.

Figure 5 represents ^{13}C-NMR spectrum of alkali lignin LA (1a) isolated from the alkaline pretreatment stage. Most of the observed signals have been previously assigned in straw and other lignin spectra.[20,29-35] The most striking characteristic of the ^{13}C-NMR spectrum is the absence of typical polysaccharide signals. This is due to the relatively free of sugars in the isolated alkali-soluble lignin fraction LA (1a), which was corresponded with the chemical analysis. On the other hand, due to a large amount of polysaccharides associated in the alkali lignin samples from wheat straw in a number of previous studies,[18,29,31,36] all of the lignin spectra reported earlier showed rather large resonances in the 57-103 ppm regions which made the assignments more difficult and overlap.

The region from δ 104.4 to 160.0 is amenable assignments as the aromatic part of lignin. The syringyl residues were indicated by signals at δ 152.2, 138.1 and 104.4, and guaiacyl and p-hydroxyphenyl residues gave signals at δ 149.3, 148.0, 147.5, 145.3, 119.4. 114.6, 111.3 (G) and 128.0 (H), respectively. The signals at δ 159.6, 144.6, 130.1, 125.8, 125.3, and 115.4 indicated the

esterified p-coumaric acid. Etherified ferulic acid was observed with signals at δ 168.1, 144.3, 122.3, 115.9, and 115.7. The side chain carbon atoms in p-coumarate residues gave signals at δ 144.6 (C-α). A signal at δ 168.1 corresponded to etherified ferulic acid (C-γ). The signal at δ 144.3 represented for etherified ferulic acid (C-α). Therefore, it seems that p-coumaric acid is linked to alkali lignin by ester bond at C-α, while the ferulic acid is linked to alkali lignin by ether bond at C-α and C-γ. A very strong signal at δ 56.0 corresponds to OCH_3 in syringyl and guaiacyl units. The carbonyl resonances from uronic acids and ester may contribute to signals at δ 174.6 and 60.1. The signal at δ 174.6 indicates for C-6 in methyl uronates, and the signal at δ 60.1 originates from the 4-*O*-methoxyl group of glucuronic acid residue in the xylan.[20,30]

CONCLUSIONS

The results presented in this study showed that the isolated alkali lignin (1a), acid-soluble lignin (2a) and organosolv lignins (1b, 2b) did not appear significant difference in their chemical composition and physico-chemical properties except for a relatively high ferulic acid content and low amounts of associated polysaccharides in alkali lignin fraction LA (1a). Cleavage of ether linkages in syrinyl-type lignin was found to be just faster than their guaiacyl counterparts during the acidic organosolv delignification stage (1b) after the straw was pre-treated with alkali. All other three lignin fractions (1a, 2a, 2b) contained roughly equal amounts of non condensed guaiacyl and syringyl units with fewer p-hyroxyphenyl units. They are more condensed than typical hardwood or softwood lignins. As compared to ball-milled wheat straw lignin and enzyme lignin, the isolated lignin fractions including alkali lignin LA (1a) acid-soluble lignin (2a) and organosolv lignins (1b, 2b) showed much lower content of polysaccharides (2.1-3.4%) and low molecular weight. Alkali lignin LA (1a) appeared to be very closely associated with p-coumaric acid (esterified to lignin at C-α), ferulic acid (etherified to lignin at C-α, C-γ), and glucuronic acid or 4-O-methylglucuronic acid (esterified).

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