Effects of Pretreatment Temperature and Alkali Concentration on the Composition of Alkali-Soluble Lignins from Wheat Straw

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SYNOPSIS

A new rapid method of two step precipitation for isolation of alkali-soluble lignins with relatively free of polysaccharides from wheat straw was proposed in this study. The isolated alkali-soluble lignin fractions LA contained rather low amounts of neutral sugars (0.7–1.0%) and had low average molecular weights (960–1440 Da). The isolated lignin fractions LA contained roughly equal amounts of guaiacyl (G) and syringyl (S) units with relatively fewer p-hydroxyphenyl (H) units, and appeared to be closely associated to phenolic acids and glucuronic acid or 4-O-methylglucuronic acid. The chemical compositions in each of the fractions are reported. The physico-chemical properties and structure features of these lignin fractions LA were characterized by UV-, IR-, ^{13}C-NMR-spectroscopy, and alkaline nitrobenzene oxidation. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

As part of our continuing study of wheat straw lignins, we have extensively investigated the phenolic monomers in the alkaline hydrolysates and alkaline nitrobenzene oxidation mixtures from wheat straw lignin, and fractionated the phenolic monomers into free phenolic monomers, loosely bound phenolic acids and aldehydes, and tightly bound phenolics. In our previous studies,[1,2] various types and concentrations of alkali as well as pretreatment time and temperature conditions have been used for the extraction of wheat straw lignins. In this study, we present a new rapid method for isolation of alkali-soluble lignins with relatively free of polysaccharides by two-step precipitation from alkali-treated wheat straw hydrolysates. The isolated lignin fractions LA were characterized by UV-, IR-, [13]-C-NMR- spectroscopy, and alkaline nitrobenzene oxidation.

EXPERIMENTAL

Extraction and Isolation of Alkali-Soluble Lignins

The wheat straw (winter) was obtained from Silsoe Research Institute (Silsoe, Bedfordshire). Finely powdered and dried straw (10 g, 2.5 g straw/100 mL solution) was treated under stirring with (a) 1.5% NaOH for 6 h at 0, 20, 40, 60, and 80°C, respectively, and (b) 0.5, 1.5, 3.0, 5.0, and 10.0% NaOH at 20°C for 6 h, respectively.

After filtration, the hydrolysates in each of the fractions were acidified to pH 6.5 with glacial acetic acid, concentrated with 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 fractions LA were then precipitated at pH 1.5 with 20% HCl from the supernatant solution (24 h, 20°C) and the isolated lignin fractions, after filtration, were freeze dried overnight.

Lignin Characterization

The methods for physico-chemical characterization of the isolated alkali-soluble lignins see our previous reports.[3,4]

RESULTS AND DISCUSSION

Lignin Yield

Lignin yield was calculated by comparing the amount of lignin precipitated (LA) with 20% HCl at pH 1.5 from the supernatant solution after isolation of crude hemicelluloses and coprecipitated (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).[3]

The recovery of alkali-soluble lignin in fractions LA and LB are shown in Table I. As expected, the yield of LA was much higher than for LB, as shown by LA/LB between 2.4 and 8.0. The very low yields were obtained in the extractions with 1.5% NaOH at 0°C for 6 h and 0.5% NaOH at 20°C for 6 h. However, these yields increased dramatically at temperature above 20°C or sodium hydroxide concentration above 3.0%. These results showed that high temperature and high concentration of sodium hydroxide tended to favor the lignin extraction. The foregoing data were accordance with our previous study[1] on lignin yield determined by gravimetric method from wheat straw. Vilpponen and co-workers[5] also stated that delignification can be increased substantially by extraction in alkaline condition at elevated temperature.

It is well known that alkaline treatments cleave the ester bond between p-coumaric acid and lignin or between ferulic acid and hemicelluloses. In our earlier report,[2,6] we indicated that the predominant phenolic monomers in the alkaline hydrolysates were found to be ferulic acid and p-coumaric acid, which together comprised about 80% of the total.

UV Spectra

The UV absorption spectra of the alkali-soluble lignin fractions LA, extracted with 0.5, 3.0, and 5.0% NaOH at 20°C for 6 h, respectively, are shown in Figure 1. All lignin fractions exhibited the basic UV spectrum typical of lignins with maxima at 278–280 nm and 314–316 nm.[7] The second maxima at 314–316 nm is undoubtedly associated with the ferulic or p-coumaric acid,[8] because it disappears following further alkaline (1M NaOH, 25°C, 16 h) treatment.[6]

Composition of Bound Polysaccharides

As sodium hydroxide treatment of wheat straw would be expected to saponify the hydroxycinnamic esters that have been hypothesized to crosslink lignin to a cell wall hemicelluloses,[7,8] all the alkali-soluble lignin fractions LA contained rather low levels of polysaccharides (1.4–1.8%) (Table II). Wheat straw lignin preparations reported earlier, however, appeared significantly richer in polysaccharides.[7,9–11] Various isolation and purification procedures have been previously attempted to reduce the polysaccharide content of extracted lignin preparations, but none of these methods allowed obtaining the lignin preparations with lower polysaccharide content. Scalbert and co-workers[7] purified lignin fractions by means of liquid–liquid extraction, chromatography, and enzymatic hydrolysis. The lignin fractions of milled wheat straw lignin LM, enzyme lignin LE, and alkali-soluble lignin LA still contained 7.1, 16.8, and 18.2% polysaccharides, respectively, which was more than 10 times higher in alkali-soluble lignin LA than that of the lignin fractions obtained in this study. Fidalgo et al.[9]

Table I   The Yield (%) of Alkali-Soluble Lignins Released at the Various Pretreatment (1.5% NaOH, 6 h) Temperatures and Different Sodium Hydroxide Concentrations

Pretreatment Conditions	Total	LA[a]	LB[b]	LA/LB
1.5% NaOH, 6 h, 0°C	5.1	3.6	1.5	2.4
1.5% NaOH, 6 h, 20°C	20.1	16.3	3.8	4.3
1.5% NaOH, 6 h, 40°C	33.9	28.9	5.0	5.8
1.5% NaOH, 6 h, 60°C	53.2	46.6	6.6	7.1
1.5% NaOH, 6 h, 80°C	62.9	55.9	7.0	8.0
0.5% NaOH, 20°C, 6 h	4.0	3.3	0.7	4.7
3.0% NaOH, 20°C, 6 h	43.8	35.3	8.5	4.2
5.0% NaOH, 20°C, 6 h	46.0	35.6	10.4	3.4
10.0% NaOH, 20°C, 6 h	49.2	39.0	10.2	3.8

[a] Obtained by precipitation of the supernatant solution with 20% HCl at pH 1.5 after isolation of hemicelluloses.
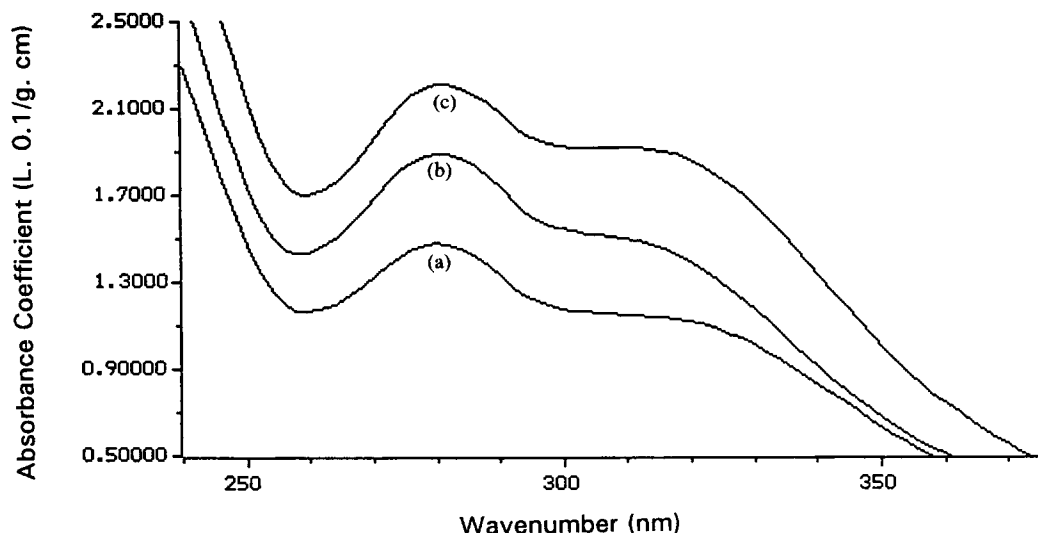[b] Coprecipitated in the hemicelluloses.

Figure 1 UV Spectra of alkali-soluble lignin fractions LA extracted with (a) 0.5% NaOH at 20°C for 6 h, (b) 3.0% NaOH at 20°C for 6 h, and (c) 5.0% NaOH at 20°C for 6 h.

fractionated the alkali-soluble wheat straw lignins into alkali lignins 1 and 2, which contained 17.3 and 32.9% of polysaccharide sugars, respectively. This high content of polysaccharides in alkali-soluble lignin fractions was regarded to be due to specific structural patterns of association between lignin and polysaccharides in wheat straw cell walls.[7] However, it is easy to obtain the lignin fractions relatively free of polysaccharides using the method of two step precipitation proposed in this article.

It is of interest to note that there are roughly equal amounts of polysaccharide sugars and uronic acids in each of the isolated alkali-soluble lignin fractions LA. This relatively high concentration of uronic acids in alkali-soluble lignin fractions LA was

probably due to the ester bonds between lignin and glucuronic acid residue of hemicelluloses in wheat straw cell walls. Based on the conjugate acid DDQ-oxidation of a water-soluble lignin–carbohydrate complex (LCC-WE) from the beech wood, Imamura and co-workers[12] calculated that the frequency of the ester bond between the lignin and glucuronic acid residue of glucuronoxylan was determined to be 1.6 per molecule of LCC-WE.

Components of Phenolic Monomers

The phenolic acids and aldehydes resulted from oxidation of the "core" of alkali-soluble lignin fractions LA are given in Table III. The major products of

Table II   The Content of Polysaccharide Sugars and Uronic Acids in Alkali-Soluble Lignin Fractions LA Extracted at Different Pretreatment Temperatures and Various Sodium Hydroxide Concentrations (% Lignin Sample w/w)

Pretreatment Conditions	Sugars					Uronic Acids	Total
	Total	Ara	Xyl	Gal	Glc		
1.5% NaOH, 6 h, 0°C	0.7	0.1	0.2	0.2	0.2	0.7	1.4
1.5% NaOH, 6 h, 20°C	0.8	0.1	0.2	0.3	0.2	0.7	1.5
1.5% NaOH, 6 h, 40°C	0.9	0.2	0.2	0.3	0.2	0.8	1.7
1.5% NaOH, 6 h, 60°C	1.0	0.2	0.3	0.3	0.2	0.8	1.8
1.5% NaOH, 6 h, 80°C	0.8	0.2	0.2	0.2	0.2	0.8	1.6
0.5% NaOH, 20°C, 6 h	0.7	0.1	0.2	0.2	0.2	0.7	1.4
3.0% NaOH, 20°C, 6 h	0.9	0.2	0.3	0.2	0.2	0.7	1.6
5.0% NaOH, 20°C, 6 h	0.9	0.2	0.2	0.3	0.2	0.8	1.7
10.0% NaOH, 20°C, 6 h	1.0	0.2	0.3	0.3	0.2	0.8	1.8

Table III The Content (% Lignin Sample w/w) of Phenolic Acids and Aldehydes in the Alkaline Nitrobenzene Oxidation Products of Alkali-Soluble Lignin Fractions LA

Phenolic Acids and Aldehydes	1.5% NaOH (6 h) Treatment Temperature (°C)					NaOH (20°C, 6 h) Treatment Concentration (%)			
	0	20	40	60	80	0.5	3.0	5.0	10.0
Gallic acid	0.92	0.91	0.86	1.00	0.95	0.63	0.75	0.80	0.60
Protocatechuic acid	0.033	0.060	0.033	0.033	0.034	0.028	0.032	0.034	0.020
p-Hydroxybenzoic acid	0.45	0.21	0.45	0.57	0.46	0.17	0.68	0.47	0.50
p-Hydroxybenzaldehyde	0.74	0.68	1.26	1.61	1.46	0.49	0.88	1.20	1.33
Vanillic acid	0.94	0.78	1.06	1.04	0.96	0.64	0.94	0.87	0.80
Syringic acid	2.07	1.58	2.07	2.47	2.46	1.25	1.78	2.21	1.83
Vanillin	8.77	8.86	9.73	10.57	10.71	8.49	9.26	9.43	9.23
Syringaldehyde	8.07	8.78	9.50	10.50	10.65	8.06	8.84	9.34	9.21
p-Coumaric acid	0.32	0.23	0.36	0.36	0.36	0.24	0.24	0.38	0.34
Acetovanillone	0.12	0.090	0.18	0.16	0.091	0.070	0.090	0.093	0.085
Ferulic acid	0.80	0.76	0.81	0.80	0.61	0.61	0.80	0.83	0.76
Cinnamic acid	0.027	0.044	0.040	0.035	tr	0.037	0.040	tr	tr
Total	23.26	22.99	26.35	29.45	28.75	20.72	24.33	25.65	24.71

tr = traces.

alkaline nitrobenzene oxidation were identified to be vanillin, syringaldehyde, p-hydroxybenzaldehyde, and syringic acid. The main degradation products, vanillin and syringaldehyde, result from the degradation of noncondensed guaiacyl and syringyl units, respectively. The presence of small quantities of p-hydroxybenzaldehyde is generally considered indicative of p-hydroxyphenyl units within the "core." Occurrence of noncondensed units of G S and relatively fewer H was demonstrated in each of the lignin fractions LA, which indicated that these alkali-soluble lignin fractions LA can be justified as GSH-lignins.

The slightly high G content in each of the lignin fractions LA evidenced that the less condensed G-lignin is readily extracted by sodium hydroxide. Preferential removal of G-lignin during alkali treatments from wheat straw was also reported by Scalbert et al.[7] and Liu et al.[13] In our previous studies,[1,14] we showed that wheat straw yielded slightly higher vanillin than syringaldehyde, while the opposite trend appeared in the alkaline nitrobenzene oxidation of alkali treated wheat straw residues.

When compared to the corresponding yields of hardwoods, the low yields of alkaline nitrobenzene oxidation of alkali-soluble lignin fractions LA indicated a higher degree of condensation of these lignins. Fractions LA, extracted with 1.5% NaOH for 6 h at 60 and 80°C, giving higher yields in monomers

indicated the less condensation fractions. All other alkali-soluble lignin fractions LA had an equivalent high degree of condensation, as shown by the roughly same corresponding low amounts of alkaline nitrobenzene oxidation.

As mentioned earlier, alkaline treatments would be expected to saponify the ester bonds between p-coumaric acid and lignin or ferulic acid and hemicelluloses in wheat straw cell walls. In Table III, a relatively high content of ferulic acid in the alkali-soluble lignin fractions LA indicated significant amounts of nonsaponifiable linkages such as ether bonds between ferulic acid and lignin. The low constant amount of p-coumaric acid in these fractions LA suggested that a considerable proportion of this compound is esterified with lignin. These results agreed with our previous study[2] on wheat straw lignin. The authors showed 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 ferulic acid is ether-linked to lignin.

Molecular Weight Distribution

The effects of extraction temperature on the weight-average (M_w), number-average (M_n) molecular weights and polydispersity (M_w/M_n) of each fraction LA are given in Table IV. As can be seen, increase of the pretreatment temperature resulted in a decrease of molecular-average weight from 1440 to 960.

Table IV   Weight and Number Average Molecular Weight and Polydispersity of Alkali-Soluble Lignin Fractions LA

Pretreatment Conditions	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
1.5% NaOH, 6 h, 0°C	1440	460	3.1
1.5% NaOH, 6 h, 20°C	1340	480	2.8
1.5% NaOH, 6 h, 40°C	1320	490	2.7
1.5% NaOH, 6 h, 60°C	1230	420	2.9
1.5% NaOH, 6 h, 80°C	960	400	2.4

These results were in good agreement with Vilpponen et al.[5]'s studies. The authors showed that elevated treatment temperature and alkali addition reduced the average molar mass of the lignins extracted from well-washed pulp. This decrease of molecular weight was probably due to the degradation or extensive cleavage to interunit linkages of lignin at high temperature during the alkaline treatments.

The GPC molecular weight distribution of alkali-soluble lignin fraction LA extracted with 3.0% NaOH at 20°C for 6 h is illustrated in Figure 2. The elution maximum corresponded to polystyrene molecular weight 1130. Elution profile showed a wide polymolecularity from monomer up to polystyrene of molecular weight 21,700. The small second peak corresponded to very low molecular components, probably dimers or trimers. This phenomenon observed in this study showed that the two-step precipitation is an important factor not only to the lignin fractions LA relatively free of polysaccharides but also to the lignin fractions LA having low molecular weight. However, due to the high content of associated polysaccharides (18.2%) in the alkaline
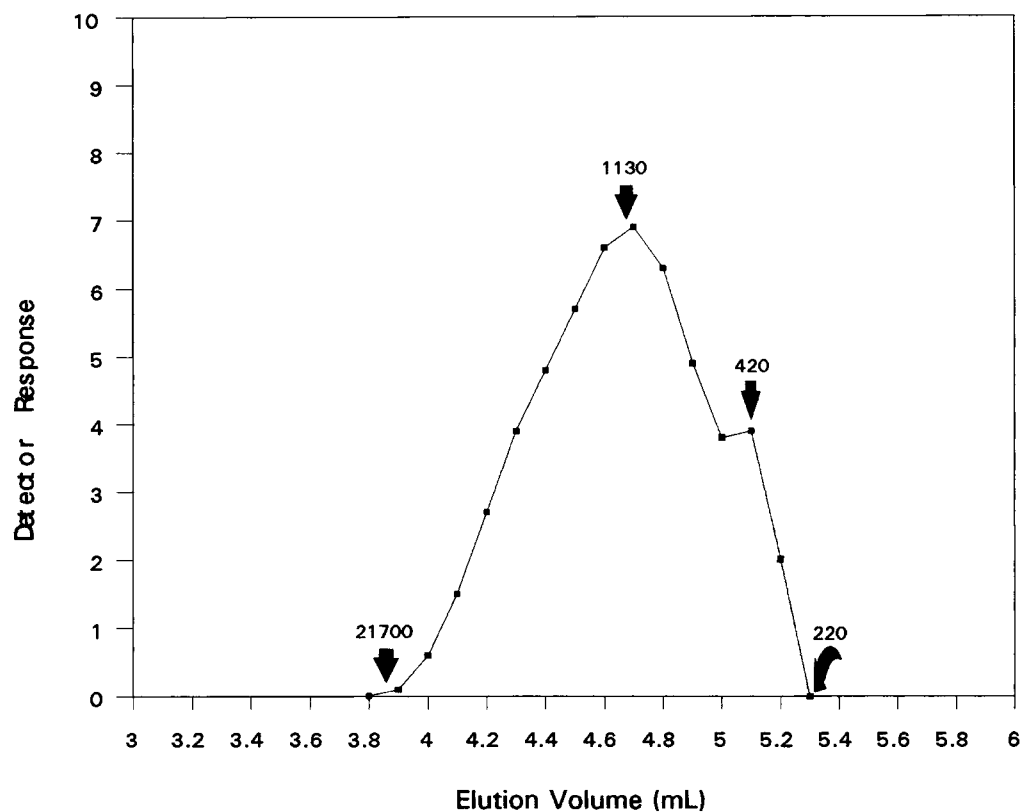

Figure 2   The GPC molecular weight range of alkali-soluble lignin fraction LA extracted with 3.0% NaOH at 20°C for 6 h.

lignin fraction isolated by Scalbert and Monties,[15] the elution maximum of alkaline lignin LA was higher and corresponded to polystyrene molecular weight 1450.

IR Spectra

Infrared spectra were also taken of some of the lignin fractions LA in order to see whether additional information could be obtained. These spectra are shown in Figures 3 and 4. The most striking characteristic of the IR spectra of alkali-soluble lignin fractions LA from wheat straw is the present of peaks at 1708 and 1646 cm^{-1}, which are assigned to $C=O$ stretch in unconjugated ketone, carbonyl, and in ester groups or $C=O$ stretch in conjugated p-substituted aryl ketones, respectively.[16] Aromatic skeleton vibrations in these lignin fractions LA are assigned at 1420, 1505, and 1598 cm^{-1}, and showed minor changes in the peak intensities that confirmed that the "core" of lignin structure does not change dramatically during the alkali treatment conditions chosen in this study. The 1218, 1270, and 1320 cm^{-1} bands have been assigned to ring breathing with CO

stretch. The 1218 and 1270 cm^{-1} have been associated with coniferyl units and the 1320 cm^{-1} band with sinapyl units.[17] The 1365 cm^{-1} band attributes the aliphatic CH stretch in CH_3. The bands at 1020, 1118, and 1150 cm^{-1} contribute the aromatic CH in plane deformation. 1165 cm^{-1} Band corresponds to $C=O$ ester groups (conj.). Aromatic $C-H$ out of plane bending appears at 830 cm^{-1}.

^{13}C-NMR Spectrum

^{13}C-NMR spectrum of alkali-soluble lignin fraction LA extracted with 3.0% NaOH at 20°C for 6 h is shown in Figure 5. Most of the observed signals have been previously assigned in straw and other lignin spectra.[10,12,13,18-22] The most striking characteristic of the ^{13}C-NMR spectrum is the absence of typical polysaccharide signals. This is due to the relatively free amount of sugars in the isolated alkali-soluble lignin fractions LA, which was corresponded with the chemical analyses. On the other hand, due to a large amount of polysaccharides associated in the alkaline lignin samples from wheat straw in a number of previous studies,[7,13,18,23] all of the lignin spectra
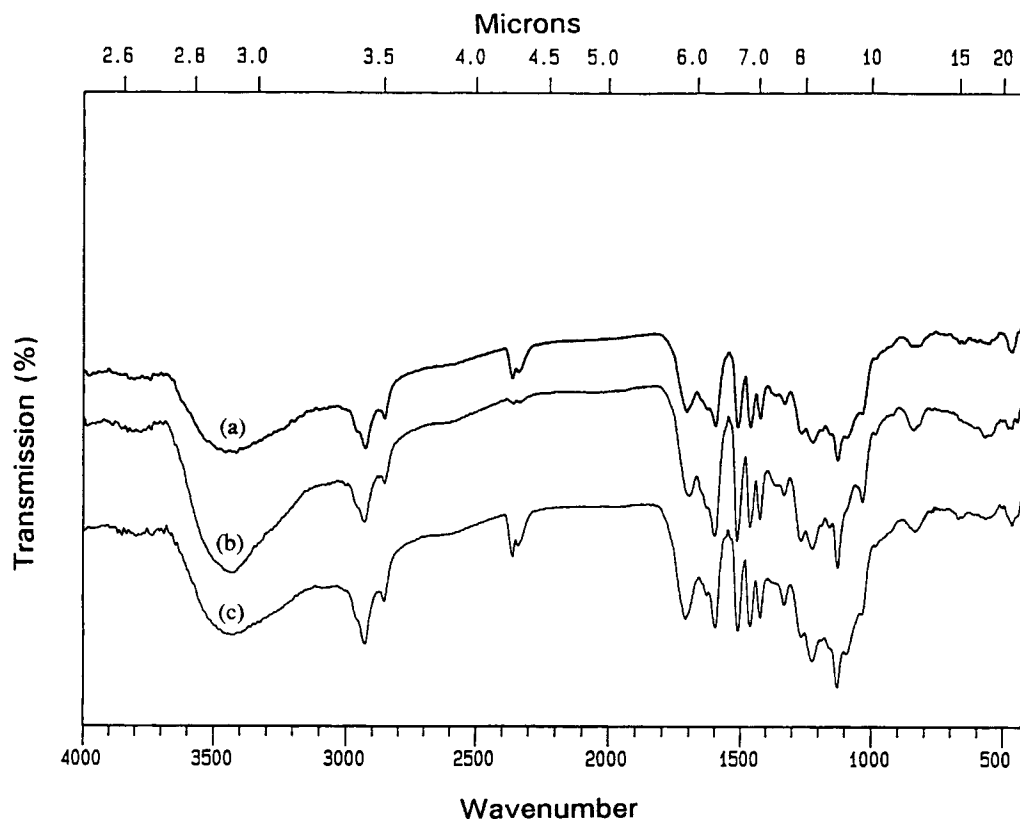

Figure 3   IR Spectra of alkali-soluble lignin fractions extracted with (a) 1.5% NaOH for 6 h at 0°C, (b) 1.5% NaOH for 6 h at 40°C, and (c) 1.5% NaOH for 6 h at 80°C.
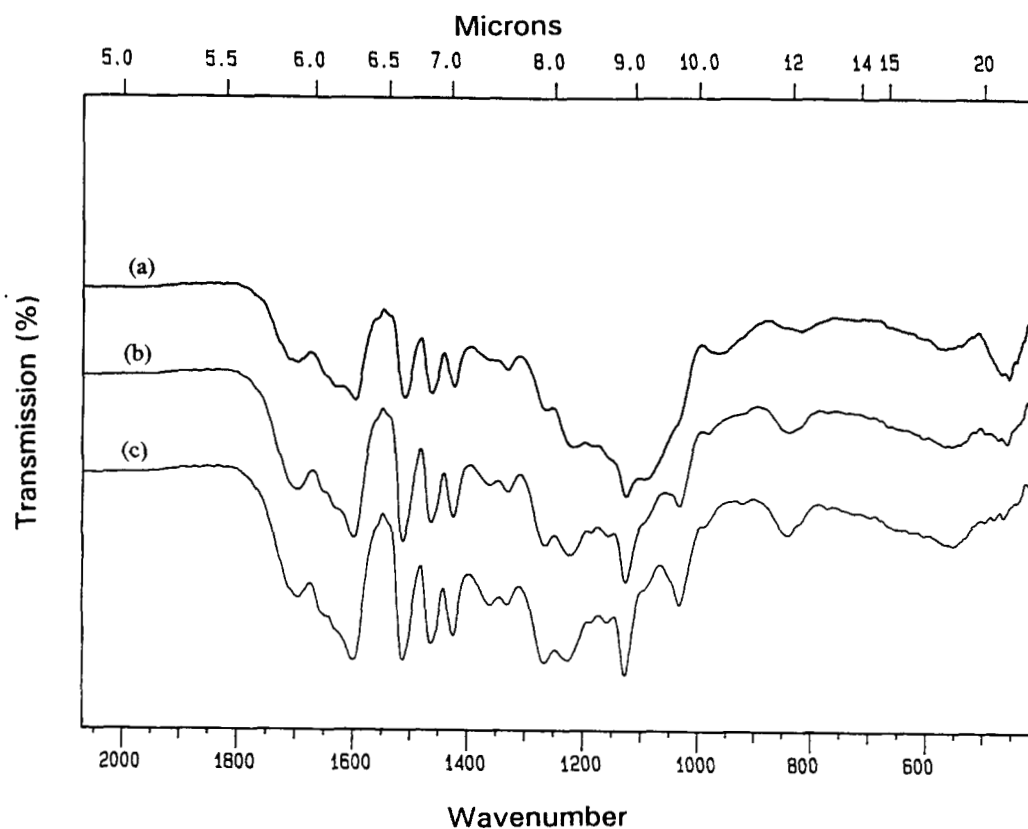
Figure 4   IR spectra of alkali-soluble lignin fractions extracted with (a) 0.5% NaOH for 6 h at 20°C, (b) 3.0% NaOH for 6 h at 20°C, and (c) 10.0% NaOH for 6 h at 20°C.
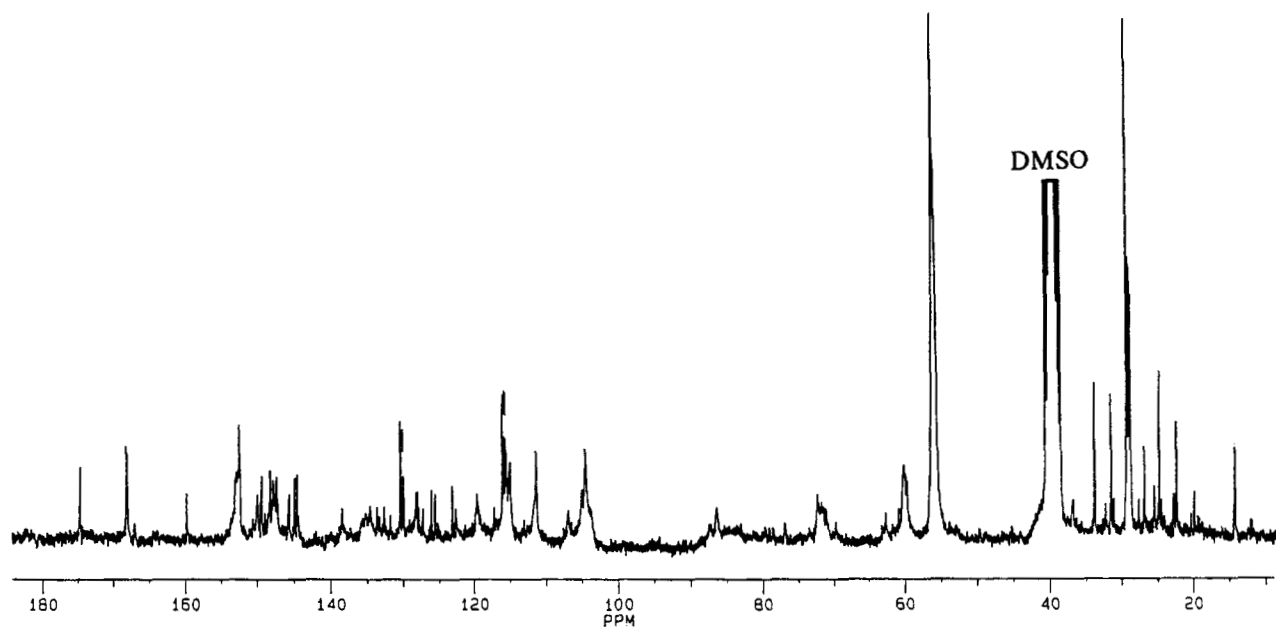

Figure 5   ^{13}C-NMR Spectrum of alkali-soluble lignin fraction LA extracted with 3.0% NaOH for 6 h at 20°C (in DMSO-d_6).

reported earlier showed rather large resonances in the δ 70–90 and 95–110 regions, which made the assignments more difficult and overlap. Signals for C-α (PC ester), C-1 (PC ester), C-6 (FE ester) and C-2/C-6 in S with α-CO were identified at δ 144.7, 125.4, 123.0, and 106.8, respectively, in the spectrum of 3.0% NaOH at 20°C for 6 h extracted alkali-soluble lignin fraction LA obtained in this study, while none was found in the spectrum of alkaline lignin obtained by Scalbert and co-workers.[7]

The region from 104.4 to 160.0 is amenable assignments as the aromatic part of the lignin. The syringyl residues were indicated by signals at δ 152.3, 138.2, 134.4, 132.5, 106.8, and 104.4, and guaiacyl and p-hydroxyphenyl residues gave signals at δ 149.8, 148.0, 147.6, 145.4, 133.5, 132.5, 119.5. 114.8, 111.2 (G), and δ 128.0, 127.9 (H), respectively. These signals confirmed that alkali-soluble wheat straw lignin fraction LA could be justified as GSH-lignin. The signals at δ 166.9, 159.7, 144.7, 130.2, 129.8, 125.9, 125.4, 115.9, 115.6, and 115.4 indicated the esterified p-coumaric acid. Etherified ferulic acid was observed with signals at δ 168.2, 144.3, 122.5, and 117.0. A very weak signal at δ 123.0 is expected for esterified ferulic acid. The side-chain carbon atoms in p-coumarate residues gave signals at 144.7 (C-α) and 166.9 (C-γ). Therefore, it seems that p-coumaric acid is linked to lignin by an ester bond, while the majority of the ferulic acids are linked to lignin by an ether bond.

The intensive signals assigned to γ-methyl, α- and β-methylene groups in n-propyl side chains appeared in the spectrum between δ 14.1 and 36.7. Signals at δ 14.1 and 22.2–33.8 were also detected for lipid or waxes, which suggested that small amount of lipid or waxes was extracted in the isolated alkali-soluble lignin fractions LA. A very strong signal at δ 56.0 corresponds to OCH₃ in syringyl and guaiacyl units. The carbonyl resonances from uronic acids and ester, in addition to cinnamic acids and esters, acetyl groups, and other aliphatic esters, may contribute to signals at δ 174.7 and 60.1. A signal at δ 174.7 indicates for C-6 in methyl uronates, and signal at δ 60.1 originates from the 4-O-methoxyl group of glucuronic acid residue in the xylan.[10,12]

SUMMARY

The highest yield of alkali-soluble lignins, isolated by this new rapid method of two-step precipitation, accounted up to 62.9% during the treatment with 1.5% NaOH for 6 h at 80°C. Increase of the alkaline

treatment temperature and alkaline concentration resulted in raising yields of alkali-soluble lignins. No significant effect of pretreatment temperature and alkali concentration was found on the changes of the "core" of extracted lignin structure. All the lignin fractions LA contained roughly equal amounts of noncondensed guaiacyl and syringyl units with few p-hydroxyphenyl units. They seem more condensed than typical hardwood or softwood lignins. As expected, the isolated lignins LA were relatively free of polysaccharides (1.4–1.8%) and had low molecular weight. Lignin in wheat straw cell walls appears to be very closely associated to phenolic acids and glucuronic acid or 4-O-methylglucuronic acid. p-Coumaric acid and glucuronic acid or 4-O-methylglucuronic acid are ester linked to lignin, whereas the majority of the ferulic acids are ether linked to lignin.

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