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# Fractionation and Characterization of Alkali-Soluble Lignins from Wheat Straw

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The chemical compositions of seven alkali-soluble lignin fractions isolated from partly dewaxed wheat straw with sodium hydroxide at successively increasing strength from 0.125 to 1.50 M are reported. The physico-chemical properties of the lignin fractions were characterized by using GPC-, UV-, IR-, <sup>13</sup>C-NMR- spectroscopy and alkaline nitrobenzene oxidation. No significant difference in monomeric composition was found in the case of wheat straw lignin fractions. However, more condensed fractions were found to be LA 4–7 and less condensed fractions appeared between fractions LA-1 and LA-3.

A method of two steps of precipitation was proposed in this study to obtain the lignin fractions relatively free of polysaccharides. The isolated lignin fractions contained rather low amount of polysaccharides (1.7–1.9%), and had low molecular-average weight between 1281 and 1796. It was found that p-coumaric acid is ester-linked to lignin at C- $\alpha$  and C- $\gamma$ , while most of the ferulic acids are ether-linked to lignin at C- $\alpha$  and C- $\gamma$ .

**Keywords:** Wheat straw; lignin; phenolic acids and aldehydes; alkaline nitrobenzene oxidation; molecular weight; polysaccharides; infrared spectra; <sup>13</sup>C-NMR spectroscopy

## INTRODUCTION

Based on our previous study [1], wheat straw lignin accounted for about fourteen percent of the dry matter, which is one of the three main components of wheat straw after cellulose and hemicelluloses. Depending on its composition of guaiacyl (G), syringyl (S) and p-hydroxyphenylpropane (H) units, wheat straw lignin has been justified

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as GSH-lignins (Gramineae lignins from grasses), which are known to be different from those of softwood (G-lignins) or hardwood (GS-lignins) and compression wood (GH-lignins) lignins. Though there are always small amounts of p-hydroxyphenyl (H) residues present in softwood as well as in hardwood lignins, substantial amounts of all three aromatic nuclei (G, S and H) are only found in glumiflorate (Gramineae and Cyperaceae) lignins [2]. Furthermore, phenolic acids, mainly p-coumaric and ferulic acids, have been investigated to cross-links between lignins and polysaccharides [3]. Due to above complex nature of the straw lignin, the study of its structure has been found to be more difficult. Meanwhile, the difficulty of isolation of relatively pure lignin from grasses or cereal straw has made progress in obtaining structural information on grass or straw lignins slower than progress on wood lignins. The Björkman procedure [4] has been extensively employed in the isolation of a milled wood lignin from plant tissue, and has been proved to be a successful method of isolation of relatively pure wood lignins. However, when it is applied to other plant material having a lower lignin content such as wheat straw, the results have not been as successful [5].

Isolation of lignins through alkaline extraction has been recommended as a particularly suited method for straw or grass samples. Straw or grass lignins can be solubilized with dilute aqueous alkali under mild conditions that should not cause chemical modifications beyond the saponification of esterified hydroxycinnamic acids [6]. In this study we present a procedure to isolate lignin relatively free of polysaccharides from wheat straw using the two steps of precipitation. The extractions were carried out using different alkaline solutions at successive increase of alkaline concentrations from 0.125 to 1.50 M. The infrared (IR) and nuclear magnetic resonance (NMR) spectroscopic characterizations of these lignin fractions are reported.

## EXPERIMENTAL

### General Methods

All chemicals used in this study were of reagent grade. UV spectra were recorded on a Hewlett-Packard 8452A Diode Array spectro-photometer. Lignin sample (5 mg) was dissolved in 95% (v/v) dioxanewater (10 ml). A

1 ml aliquot was diluted to 10 ml with 50% (v/v) dioxanewater, 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 5 $\mu$  Mixed-D column. The samples were dissolved with tetrahydrofuran (THF) with a concentration of 0.2% and 200  $\mu$ l sample in solution was injected. The columns were operated at 40°C and eluted with tetrahydrofuran at a flow rate of 1 ml min<sup>-1</sup>. 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 <sup>13</sup>C-NMR spectra were obtained on a Bruker 250 AC operating in the FT mode at 62.4 MHz under total proton decoupled conditions. They were recorded at 25°C from 250 mg samples dissolved in 1.0 ml DMSO-d<sub>6</sub> after 30000 scans. A 40° pulse flipping angle, a 3.0  $\mu$ s 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 [1]. 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%).

### Fractionation and Isolation of Alkali-Soluble Lignins

The wheat straw (winter) was obtained from Silsoe Research Institute (Silsoe, Bedfordshire). Finely powdered straw was extracted (Soxhlet) with chloroform: methanol (2:1, v/v) for 5 hr to remove waxes. The air-dried dewaxed straw (48 g) was extracted sequentially in a thermostated reactor under nitrogen atmosphere and stirring with the extraction conditions below (Tab. I): (1) 0.125 M NaOH, 37°C, 2 hr, 48 g/2000 ml; (2) 0.25 M NaOH, 37°C, 2 hr, 39 g/2000 ml; (3) 0.50 M NaOH, 37°C, 2 hr, 34 g/2000 ml; (4) 0.75 M NaOH, 37°C, 2 hr, 31 g/2000 ml; (5) 1.00 M NaOH, 37°C, 6 hr, 28 g/2000 ml; (6) 1.25 M NaOH, 60°C, 6 hr, 26 g/2000 ml; (7) 1.50 M NaOH, 75°C, 6 hr, 22 g/2000 ml.

After filtration, the extracts in each of the fractions were acidified to pH 5 with glacial acetic acid, concentrated with a rotary evapora-

TABLE I Yield and extraction conditions of alkali-soluble lignin fractions

Fraction No.	NaOH concentration (M)	Temperature times (C hr)	Dry material extractant (g ml)	Yield (%)			
				Total	LA <sup>a</sup>	LA <sup>b</sup>	LA/LB
1	0.125	37 C 2 hr	48 2000	19.1	15.6	3.5	4.4
2	0.25	37 C 2 hr	39 2000	14.2	7.1	7.1	1.0
3	0.50	37 C 2 hr	34 2000	8.6	4.3	4.3	1.0
4	0.75	37 C 2 hr	31 2000	4.9	2.8	2.1	1.3
5	1.00	37 C 6 hr	28 2000	5.7	3.6	2.1	1.7
6	1.25	60 C 6 hr	26 2000	7.1	5.0	2.1	2.3
7	1.50	75 C 6 hr	22 2000	6.4	4.3	2.1	2.0

<sup>a</sup>obtained by re-precipitation of the supernatant solution with 20% HCl at pH 1.5 after isolation of hemicelluloses.

<sup>b</sup>co-precipitated in the hemicellulosic fractions.

tor under reduced pressure at 40 C to about 800 ml, and then mixed with 5 volumes of 95% ethanol (24 hr, 20 C) for isolation of crude hemicelluloses or hemicellulosic-lignin complexes. The alkali-soluble lignin fractions were then re-precipitated at pH 1.5 with 20% HCl from the supernatant solution (24 hr, 20 C), and the isolated lignin fractions, after filtration, were freeze-dried overnight.

## RESULTS AND DISCUSSION

### Lignin Yield

Lignin yield was calculated by comparing the amount of lignin re-precipitated (LA) by 20% HCl at pH 1.5 from the supernatant solution after isolation of hemicelluloses and co-precipitated in the hemicellulosic fractions (LB) with the total amount of acidic chlorite lignin present in the wheat straw (about 14.1% by weight).

Table I shows the yield of lignin and the extraction conditions. As can be seen, seven lignin fractions represented 66.0% of the acidic chlorite lignin from wheat straw, which indicated that about two thirds of the lignin were extracted sequentially with sodium hydroxide of increasing strength from 0.125 to 1.50 M at the conditions above. With the increase of sodium hydroxide concentrations from 0.125 to 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 M, the lignin yields of LA and LB in each of the fractions were 15.6 and 3.5, 7.1 and 7.1, 4.3 and 4.3, 2.8 and 2.1, 3.6 and 2.1, 5.0 and 2.1, and 4.3 and 2.1%, respectively. As

expected, the yields of LA appeared to be higher than LB in most of the extraction steps. With growth of sodium hydroxide concentration from 0.125 to 0.50 M, the fractional yield ratios of LA/LB decreased from over 4 to just 1, while when the extraction duration extended to 6 hr in fraction 5, the yield ratio raised to 1.7, and when extraction temperature increased from 37°C in fraction 5 to 75°C in fraction 7, the yield ratios also increased from 1.7 to 2.0. These data suggested that extension of extraction duration or high temperature tended to favour the lignin extraction both the total yield and the fractions (LA) enriched in lignin, particularly, between 37°C and 60°C. The results obtained here were in good agreement with Vipponen *et al.* [7] and Vázquez-Torres *et al.* [8], studies. The authors mentioned that the yield of lignin increased substantially by extraction in alkaline solutions at elevated temperature. Our previous study [1] showed that pre-treatment of wheat straw with 1.5% NaOH at 20°C released only 20.6% of the lignin, while at 80°C over 60% of the lignin was released. The foregoing data also showed that about 20% of lignin was released during the first step of extraction with 0.125 M NaOH. This high solubility of lignin at the first stage of alkaline extractions was due to partly cleavage the ester-bond between p-coumaric acid and lignin or between ferulic acid and hemicelluloses by dilute alkali. In our earlier report [9], we indicated that pre-treatment of wheat straw with 1.5% NaOH at 20°C for 6 hr released nearly all of the above ester bond in wheat straw cell walls, and the predominant free-phenolic monomers in alkaline hydrolysates were found to be ferulic and p-coumaric acid, which together comprised about 80% of the total.

It is of interest to note that, due to the lignin-hemicellulosic complexes in wheat straw cell walls, hemicelluloses impede the dissolution of lignin in other stages of alkaline extractions. Extractions with 0.25, 0.50 and 0.75 M NaOH at 37°C for 2 hr yielded, in descending order, 14.2, 8.6 and 4.9% of the total lignin, respectively. These results coincided with our assumption reported earlier [10]. In addition to the ester bond between ferulic acid and hemicelluloses or p-coumaric acid and lignin, and ether bond between ferulic acid and lignin, the majority of lignin in atmospheric refined and alkali pre-treated wheat straw cell walls is directly ether-linked to arabinose in hemicelluloses. Characterization of lignin-hemicellulosic complex fractions (LB) see our previous report [11].

## UV Spectra

The UV spectra of seven lignin fractions (LA) exhibited the basic UV spectrum typical of lignins, with a maximum near 280 nm and a second maximum at 316–320 nm. Free and etherified hydroxyl group contribute significantly to the characteristic absorption maximum of lignins near 280 nm [12], while the second maximum is undoubtedly associated with esterified *p*-coumaric acid or etherified ferulic acid [13]. The effect of extraction conditions on the wheat straw lignin in different fractions is shown in Figure 1. As can be seen, with growth of sodium hydroxide concentration from 0.125 to 0.75 M, the UV absorbance coefficient decreased from 1.88 to 0.49 (278–280 nm) and 1.73 to 0.36 (316–320 nm), respectively. This decreasing absorbance was probably due to more non-lignin material in extracted lignin fractions. As mentioned earlier, extension of extraction duration from 2 hr to 6 hr at 37°C between fractions 4 and 5 resulted in high yield of lignin, and the absorbance coefficient raised dramatically from 0.49 to 0.81 (278–280 nm) and 0.36 to 0.59 (316–320 nm). Interestingly, increase of temperature from 37°C to 75°C and sodium hydroxide

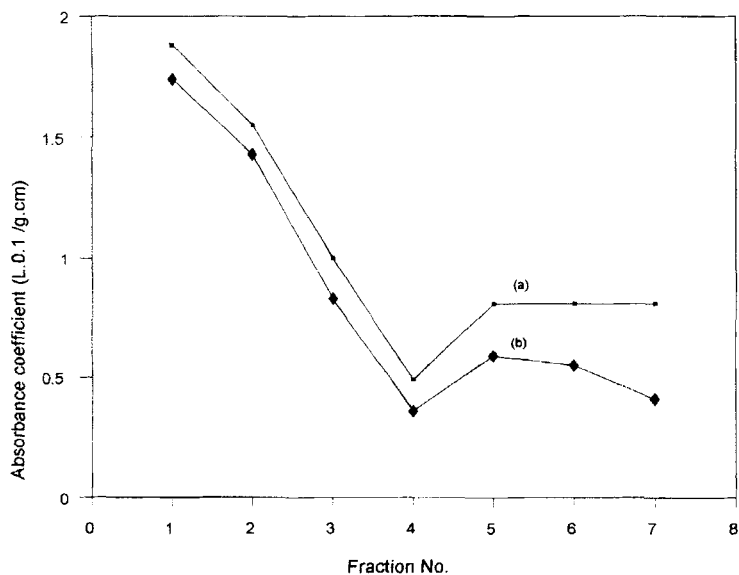


FIGURE 1 Effect of extraction conditions on the alkali-soluble wheat straw lignin LA in different fractions: (a) measurement at 278–280 nm; (b) measurement at 316–320 nm.

concentration from 1.00 to 1.50 M produced a constant value of absorbance coefficient at 278–280 nm and a slight drop at 316–320 nm, which was due to the reduce of p-coumaric acid or ferulic acid in the extracted alkali-soluble lignin fractions.

### Composition of Bound Polysaccharides

The content of polysaccharide sugars and uronic acids in the wheat straw alkali-soluble lignin fractions (LA) is given in Table II. As expected, compared to a number of previous reports [5, 14–16] on polysaccharide content in alkali-soluble lignin preparations, all the lignin fractions obtained by two steps of precipitation in this study contained much lower polysaccharides, 1.7–1.9%. Scalbert and co-workers [14] have been attempted on reduce of polysaccharide content using liquid-liquid extraction, phenyl-sepharose chromatography and enzymatic hydrolysis with commercial Driselase in the purification procedures. But none of these methods allowed to obtain the lignin fractions with lower polysaccharide content. The lignin preparations 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 about tenfold higher in alkali-soluble lignin preparation LA than that of the lignin fractions obtained in this study. Similar difficulties to obtain a pure lignin fraction from cereal straw or grasses were also reported by Himmelsbach and Barton [5], Liu *et al.* [15], Fidalgo *et al.* [3], and Ben-Ghedalia and Yosef [16]. By comparative study of lignin fractions from alkaline extraction of wheat

TABLE II The content (% lignin sample w/w) of Polysaccharide sugars and uronic acids in the wheat straw lignin fractions

Fraction No.	Sugars					Uronic acids	Total
	Total	Ara	Xyl	Gal	Glc		
LA-1	0.7	0.1	0.2	0.3	0.1	1.0	1.7
LA-2	0.7	0.1	0.3	0.2	0.1	1.0	1.7
LA-3	0.7	0.1	0.4	0.1	0.1	1.1	1.8
LA-4	0.8	0.2	0.4	0.1	0.1	1.1	1.9
LA-5	0.8	0.2	0.3	0.2	0.1	1.0	1.8
LA-6	0.9	0.2	0.3	0.2	0.2	1.0	1.9
LA-7	1.0	0.2	0.3	0.3	0.2	0.7	1.7



straw through chemical degradation, analytical pyrolysis and spectroscopic techniques. Fidalgo and co-workers [3] mentioned that the alkali-soluble lignin fractions 1 and 2 contained 17.3 and 32.9% of polysaccharide sugars, respectively. Ben-Ghedalia and Yosef [16] have fractionated alkali-soluble lignin fractions with 1 M NaOH from ball-milled of the neutral detergent fiber of wheat straw for 7, 14 and 21 days in a porcelain rotary ball-mill, and mentioned that the lignin preparations still contained 13.1, 6.3 and 5.2% of polysaccharides. This high content of polysaccharides in lignin fractions has been considered due to specific structural patterns of association between lignin and polysaccharides in wheat straw cell walls [14]. However, it is easy to obtain the lignin fractions relatively free of polysaccharides by using the two steps of precipitation proposed in this paper.

It is well known that, in lignin-hemicellulosic complexes, arabinose is ether-linked to lignin, whereas uronic acids are ester-linked to lignin. In alkaline solution, however, no uronic acids are released even though the ester bond is easily broken. The reason for this is that the glycoside bond between uronic acids and xylose is not affected and uronic acids still exist in hemicelluloses which are associated with lignin through arabinose residues [15]. Chemical studies on linkages between lignin and hemicelluloses, especially arabinoxylans, have emphasized the important role of arabinose residues in forming the linkages [17]. Chesson *et al.* [18] suggested the presence of a covalent association between arabinose side-chains of xylan and phenolic substances including lignin in forage species. Furthermore, Table II showed that the content of uronic acids in most of the alkali-soluble lignin fractions was slightly higher than that of the neutral sugars. This relatively high concentration of uronic acids in alkali-soluble lignin fractions obtained in this study was probably due to the ester bonds between lignin and glucuronic acid residue of hemicelluloses or between lignin and galacturonic acid residue of pectic polysaccharides 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 [19] indicated 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. Our previous study showed that the extracted pectic polysaccharide from wheat straw contained 44.8% of galacturonic acid,

28.4% of neutral sugars and about 3.0% lignin [20]. The detailed study of lignin-pectic polysaccharide complexes is presently underway in our laboratory.

### Components of Phenolic Acids and Aldehydes

Yields of phenolic acids and aldehydes obtained by nitrobenzene oxidation of seven lignin fractions LA are given in Table III. The compounds resulting from nitrobenzene oxidation were identified by comparison with the retention times of standards [21]. The retention times of compounds observed in the oxidation mixture showed a close correspondence with those of the standards. The main products of alkaline nitrobenzene oxidation were vanillin and syringaldehyde, resulting from the degradation of guaiacyl and syringyl non-condensed monomers within the core, respectively. *p*-Hydroxybenzaldehyde, syringic acid, gallic acid, vanillic acid and *p*-hydroxybenzoic acid appeared to be as minor amounts. The presence of small quantities of *p*-hydroxyphenyl unit indicated the incorporation of *p*-hydroxycinnamoyl alcohol in lignin fractions [22]. Meanwhile, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid also result partly from *p*-coumaric acid oxidation [14]. The data in Table III showed that fractions LA 1–2 were slightly enriched in guaiacyl unit whereas fractions LA 3–7 were

TABLE III The concentrations (% lignin sample w/w) of phenolic acids and aldehydes in the nitrobenzene oxidation products of lignin fractions

Phenolic acids and aldehydes	Lignin fraction No.						
	LA-1	LA-2	LA-3	LA-4	LA-5	LA-6	LA-7
Gallic acid	2.22	2.04	1.88	1.08	1.22	1.56	1.86
Protocatechuic acid	0.10	0.49	0.20	0.20	tr <sup>a</sup>	tr	tr
<i>p</i> -Hydroxybenzoic acid	1.00	1.00	0.48	0.24	0.26	0.28	0.28
<i>p</i> -Hydroxybenzaldehyde	1.68	1.65	1.44	0.74	0.78	0.72	0.50
Vanillic acid	0.53	1.06	1.06	0.82	0.96	1.00	0.76
Syringic acid	2.77	2.51	2.00	1.24	1.14	1.52	1.70
Vanillin	14.80	11.74	10.08	4.64	5.76	6.64	6.86
Syringaldehyde	14.34	11.69	11.62	5.80	7.64	7.76	7.06
<i>p</i> -Coumaric acid	0.42	0.40	0.20	0.14	0.12	0.10	0.10
Ferulic acid	0.78	0.67	0.50	0.40	0.20	0.10	tr
Total	38.64	33.25	29.46	15.30	18.08	19.68	19.12

<sup>a</sup> = traces.

enriched in syringyl unit. The guaiacyl to syringyl ratios in the first three fractions decreased from 1.03 to 0.87, while they increased from 0.80 to 0.97 between fractions LA-4 and LA-7, which indicated that the guaiacyl to syringyl ratios did not differ significantly in the alkali-soluble lignin fractions. Scalbert *et al.* [14] showed the isolated alkaline lignin from wheat straw contained roughly equal amounts of guaiacyl and syringyl units. With the study of molecular variability of lignin fractions isolated from wheat straw, Billa and Monties [22] mentioned that lignin fractions from leaves are relatively richer in guaiacyl units whereas lignin from internodes are generally enriched in syringyl units, suggesting different types of lignification in these wheat plant organs. Furthermore, relatively high syringyl/guaiacyl ratio values were obtained by cupric oxide degradation of wheat straw lignin [3]. These different ratios of G/S was probably due to the various experimental conditions such as reaction mixture, reaction time and temperature used, and factors such as soil, climate, collection time, botanical variety etc. can also affect the contents of guaiacyl and syringyl units in wheat straw.

The total yields of aldehydes and acids from alkaline nitrobenzene oxidation were low in the fractions between LA-4 and LA-7, while values of 29.46–38.64% were found in fractions LA 1–3. These different yields of products were found to depend on the degree of condensation of the lignin aromatic nuclei. When compared to the corresponding yields of hardwoods, the low yields of alkaline nitrobenzene oxidation of wheat straw alkali-soluble lignin fractions LA 4–7 confirmed the higher degree of condensation of gymnosperm lignins which have fewer  $\beta$ -aryl ether linkages than hardwood or softwood [14, 22]. Fractions LA 1–3 gave higher yields in monomeric units suggested that less condensed fractions, and fraction LA-1 was the least condensed fraction as shown by the nitrobenzene oxidation results.

In wheat straw cell walls, *p*-coumaric acid appears to be esterified to lignin and to polysaccharides [13, 23] whereas ferulic acid is ether-linked to lignin and ester-linked to arabinose residues of hemicelluloses. Based on the model test, Kosiková [24] illustrated that phenolic acid ester and phenolic  $\alpha$ -O-4 ether were easily hydrolysed in alkaline solution (1 M NaOH, 25 C) and non-phenolic- $\alpha$ -benzylaryl ether bonds might be hydrolysed in the temperature conditions of acidolysis [15]. Therefore, *p*-coumaric and ferulic acids released suc-

cessively by alkaline hydrolysis were due to break either ester or phenolic  $\alpha$ -O-4 ether bond. As can be seen in Table III, the contents of p-coumaric and ferulic acids decreased with the increase of sodium hydroxide concentrations from 0.125 to 1.50 M, and it appeared the ferulic acid diminished more fast than that of p-coumaric acid even though the content of ferulic acid was higher than that of p-coumaric acid in most of the lignin fractions. In fraction LA-7 the content of ferulic acid was too low to be detected. These results observed here suggested that some amounts of p-coumaric acid in alkali-soluble lignins appeared to be non-phenolic  $\alpha$ -aryl ether-linked to lignin. Ferulic acid, on the other hand, might be linked to lignin by phenolic  $\alpha$ -aryl ether bond and to hemicelluloses by ester bond, subsequently resulting in release in the alkali solutions. However, as can be seen in Table I, over 30% of lignin was still bound with polysaccharides in the seven successively treated wheat straw residue, which suggested that these amounts of lignin were directly linked to hemicelluloses with ether bond or to p-coumaric acid by non-phenolic  $\alpha$ -aryl ether bond. It is concluded that both ferulic acid and p-coumaric acid had a significant effect on dissolution of lignin during the alkaline solution. This result coincided with our previous reports [9–10], but disagreed with Liu and co-workers' study [15]. The authors mentioned that the dissolving of p-coumaric acid ester linkage did not significantly promote the solubility of lignin, while the contribution of ferulic acid was much more important in the alkaline treatment of wheat straw. This difference was probably due to only few samples tested and negation of the undissolved lignin content in the alkaline treated straw residue in the study of Liu and co-workers [15].

### Molecular Weight Distribution

The data in Table IV indicated that molecular-average weights of wheat straw alkali-soluble lignin fractions appeared between 1281 and 1796. These low molecular weight indicated the extensive cleavage of interunit linkages under the conditions chosen. It was obvious that the molecular-average weight of fraction LA-1 extracted with 0.125 M NaOH was higher than those of the second, third and fourth stage extractions with 0.25, 0.50 and 0.75 M NaOH. This particular finding was in accord with the results obtained in our previous study on

TABLE IV Molecular-average weight of lignin fractions

<i>Fraction No.</i>	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w / \overline{M}_n$
LA-1	1539	554	2.78
LA-2	1296	491	2.64
LA-3	1289	492	2.62
LA-4	1281	503	2.55
LA-5	1796	654	2.74
LA-6	1437	582	2.47
LA-7	1358	673	2.02

hemicellulosic-lignin complexes from wheat straw [11], but did not coincide with the data of Kosiková *et al.* [25] showing an opposite trend. Extension of extraction duration from 2 hr to 6 hr at 37°C and increase of sodium hydroxide concentration from 0.75 to 1.00 M between fractions LA-4 and LA-5, the average molecular weights raised from 1281 to 1796, and then decreased to 1358 with the growth of temperature from 37°C to 75°C and sodium hydroxide concentration from 1.00 to 1.50 M. This decrease of molecular weight was probably due to the degradation of lignin at high temperature and high sodium hydroxide concentration during the extraction procedures.

It is interest to note that the increase in yield was associated with a more extensive extraction of high molecular weight lignins (Tab. I, Tab. IV). This result was in good agreement with the study of Ben-Ghedalia and Yosef [16]. Moreover, due to much higher content of associated polysaccharides in extracted wheat straw lignin preparations in a number of previous studies [15–16, 25–26], the lignin fractions reported earlier gave high molecular-average weights. Kosiková *et al.* [25] fractionated wheat straw lignins into one acetone, two alkali-extractable and one dioxane-extractable lignin preparations, and showed that the molecular-average weights of these lignin fractions appeared at 1700, 4700, 2600 and 4000, respectively. By comparison of characteristics of soluble lignins from untreated and ammonia treated wheat straw, Kondo and co-workers [26] showed that the average molecular weights of untreated wheat straw and ammonia treated wheat straw lignins were 2170 and 2200, respectively. Furthermore, with successive extraction of wheat straw with 1 M NaOH at 25°C for 0.5 hr, 2 hr and 48 hr, the obtained three alkali-soluble lignin fractions had molecular-average weights appeared at 6624, 13148 and

19854, respectively [15]. Ben-Ghedalia and Yosef [16] extracted four alkali-soluble lignin fractions from 7, 14, 21 and 28 days ball-milled wheat straw and stated that the molecular-average weights ranged between 16459 and 19689. In our earlier report, the lignin-hemicellulosic complexes isolated by successively increase of sodium hydroxide strength from 0.125 to 1.00 M appeared to have the molecular-average weights between 27800 and 45500 [11]. Therefore, the lignin fractions obtained by Liu *et al.* [15] and Ben-Ghedalia and Yosef [16] might be attributed to a class of lignin-hemicellulosic complexes.

The elution curve of the lignin fraction LA-5 on PLgel 5 $\mu$  Mixed-D column is shown in Figure 2. The first elution maximum peak corresponding to polystyrene molecular weight appeared at 1076, and the second peak attributed to low molecular components, probably dimers or trimers. As shown in Figure 2, the elution profile illustrated a wide polymolecularity and ranged from monomer or dimer to polystyrene of molecular weight over 40000. As mentioned earlier, due to high content of associated polysaccharides (18.2%) in alkali-soluble lignin preparation obtained by Scalbert and Monties [23], the major peak appeared at molecular weight 1450, which was high than that of

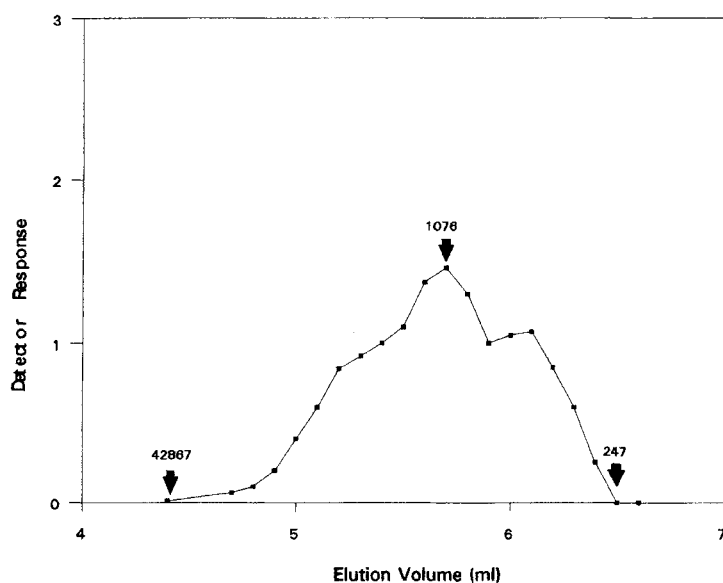


FIGURE 2 The GPC range of molecular weight of alkali-soluble lignin fraction LA-5.

lignin fraction LA-5 contained only few amount of polysaccharides (1.8%) in this study.

### IR Spectra

The IR spectra (Fig. 3) of the three alkali-soluble lignin fractions showed typical bands due to guaiacyl (1020, 1165, 1225 and 1270  $\text{cm}^{-1}$ ) and syringyl (1120, and 1325  $\text{cm}^{-1}$ ) units of lignin-building units [26]. The aromatic skeletal vibrations in alkali-soluble lignin fractions showed absorption at 1598, 1505 and 1420  $\text{cm}^{-1}$  and the aromatic methyl group vibrations appeared at 1465  $\text{cm}^{-1}$ . The most striking characteristic of the IR spectra was the presence of different intensity peaks at 1710 and 1655  $\text{cm}^{-1}$ . These two bands were due to carbonyls. The band at 1710  $\text{cm}^{-1}$  has been assigned to carbonyl stretching in unconjugated ketone and carboxyl groups [27], and this absorbance band was greatest for fraction LA-7. The 1655  $\text{cm}^{-1}$  band in fraction LA-1 was a carbonyl stretching band due to C=O groups conjugated to the aromatic ring (para-substituted ketones or aryl aldehydes) [28], while in fractions LA-3 and LA-7, this band was partly

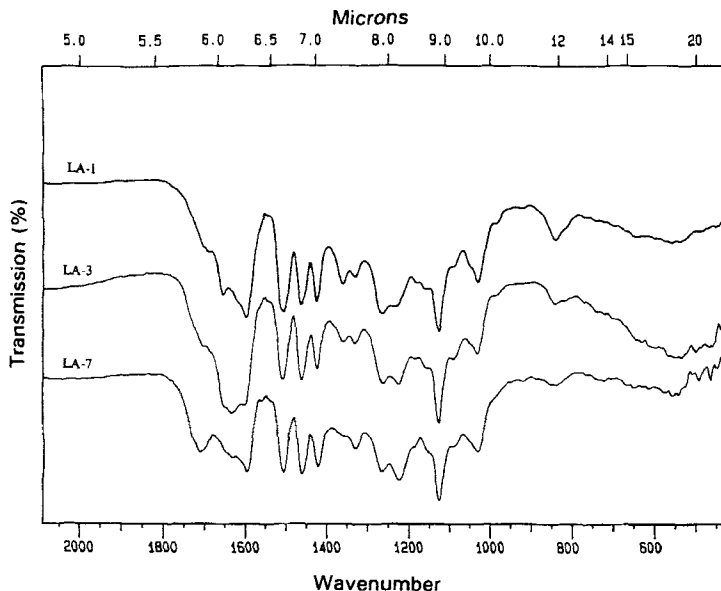


FIGURE 3 IR spectra of alkali-soluble lignin fractions LA-1, LA-3 and LA-7.

disappeared. Other than this, the infrared spectra confirmed that the "core" lignin structure did not change dramatically in the above successive extractions.

### <sup>13</sup>C-NMR Spectrum

Figure 4 shows the <sup>13</sup>C-NMR spectrum of alkali-soluble lignin fraction LA-2. The corresponding  $\delta$  (ppm) values, signal intensities and signal assignments are listed in Table V. Most of the observed signals have been previously assigned in other spectra [12, 5, 14, 25, 27, 29–30]. The most striking characteristic of the <sup>13</sup>C-NMR spectrum is the nearly disappearance of polysaccharide signals between 57 and 103 ppm. However, due to a large amount of polysaccharides associated in the extracted wheat straw lignin samples in previous studies [5, 14, 25, 27], all of the lignin spectra reported earlier showed rather large resonances between 57 and 103 ppm which made the assignments more difficult.

The region from 104.4 to 161.4 ppm is amenable assignment as the aromatic part of lignin. The syringyl residues are indicated by signals at 152.2, 138.2 and 104.4 ppm, and guaiacyl and p-hydroxyphenyl residues give signals at 119.4, 114.8 and 111.3 ppm (G), and 128.7 and 128.1 ppm (H), respectively. The side chain carbon atoms in p-coumarate residues are expected to give signals 144.6 ( $\alpha$ ) and 168.1 ppm ( $\gamma$ ) which are, however, overlapped by the signals (C- $\gamma$ , FE ether). From Figure 4 it can be seen very clearly that all eight signals at 168.1, 159.9, 144.6, 130.1, 125.8, 125.2, 115.9 and 115.3 ppm are the prevailing signals for p-coumaric acid in the aromatic part of the <sup>13</sup>C-NMR spectrum of lignin fraction LA-2. Ferulic acid gives signals at 168.1 (C- $\gamma$ , ether), 144.3 (C- $\alpha$ , ether), 122.9 (C-6, ester) and 122.4 ppm (C-6, ether). Therefore, it seems very likely that p-coumaric acid is ester-linked to lignin, while most of the ferulic acids are ether-linked to lignin in fraction LA-2.

The intensive signals assigned to  $\gamma$ -methyl,  $\alpha$ - and  $\beta$ -methylene groups in n-propyl side chains are observed in the spectrum between 13.6 and 33.8 ppm. Additional signals detected for lipid or waxes also appear at 14 and 22.2–33.8 ppm. Very strong signal at 56.0 ppm corresponds to OCH<sub>3</sub> in guaiacyl and syringyl units. The carbonyl resonances from uronic acids and esters, in addition to cinnamic acids



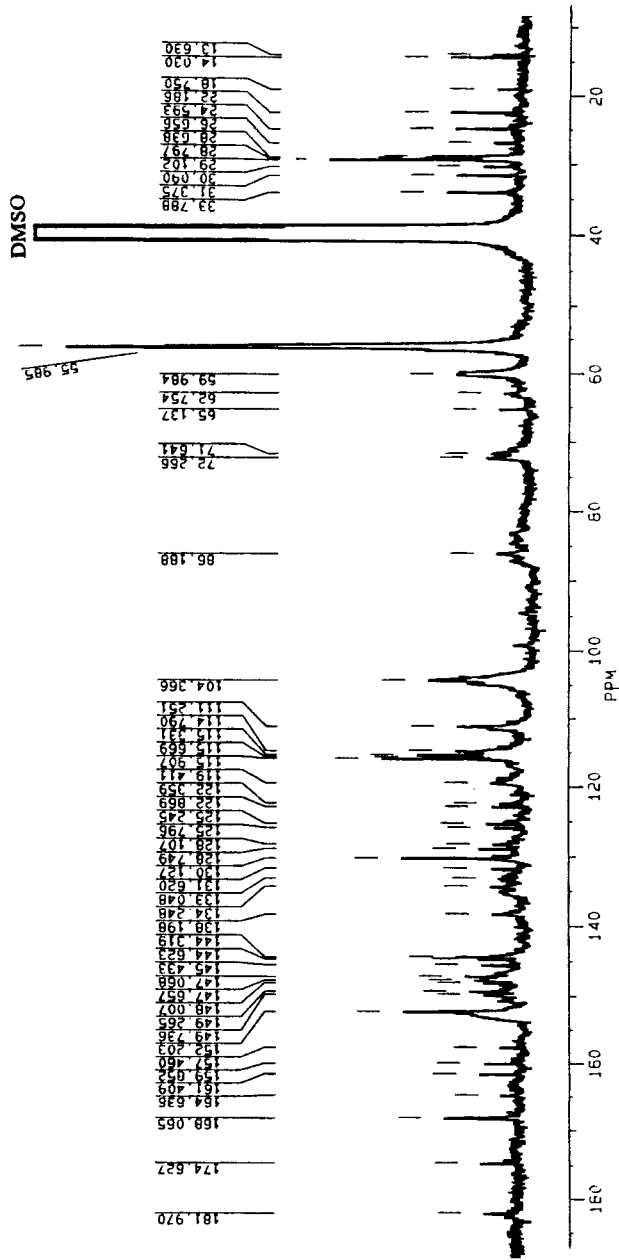


FIGURE 4 <sup>13</sup>C-NMR spectrum of alkali-soluble wheat straw lignin fraction LA-2 (in DMSO-d<sub>6</sub>).

TABLE V The chemical shift value ( $\delta$ , ppm), intensity and signal assignment of alkali-soluble lignin fraction LA-2 from wheat straw

<i>ppm</i>	<i>Intensity<sup>a</sup></i>	<i>Assignment<sup>b</sup></i>	<i>ppm</i>	<i>Intensity</i>	<i>Assignment</i>
182.0	W	C-6, GlcA and ester	122.4	VW	C-6, FE ether
174.6	W	-COOH, aliphatic	119.4	M	C-6, G; C-5, G
168.1	M	C- $\gamma$ , FE ether; C- $\gamma$ , PC ester	115.9	S	C-3/C-5, PC ester
164.6	VW		115.3	S	C-3/C-5, PC ester
161.4	W		114.8	M	C-5, G
159.9	W	C-4, PC ester	111.3	M	C-2, G
157.5	VW		104.4	S	C-2/C-6, S
152.2	S	C-3/C-5, S	86.2	W	C- $\beta$ , $\beta$ -aryl ether; C- $\alpha$ in $\beta$ - $\beta$
149.8	W	C-4, G etherified	72.3	W	C- $\gamma$ in $\beta$ - $\beta$ ; C- $\alpha$ , $\beta$ -aryl ether
149.3	M	C-4, G etherified	71.6	W	C- $\gamma$ in $\beta$ - $\beta$
148.0	W	C-3, G	65.1	W	C- $\gamma$ in $\beta$ -5; Xyl non reducing end unit
147.6	M	C-3, G	62.8	VW	C- $\gamma$ in $\beta$ -O-4; C-5, Xyl internal unit
147.0	M	C-3, G	60.0	M	C- $\gamma$ in $\beta$ -O-4; $\beta$ -aryl ether; C-6, Glc
145.4	W	C-4, G in $\beta$ -5 (non etherified)	56.0	VS	OCH <sub>3</sub> GS
144.6	M	C- $\alpha$ , PC ester	33.8	M	CH <sub>3</sub> group in ketones(conj.) or in aliphatic
144.3	M	C- $\alpha$ , FE ether	31.4	M	Same
138.2	W	C-4, S etherified	30.1	W	Same
134.2	W	C-1, S etherified; C-1, G etherified	29.1	S	CH <sub>2</sub> in aliphatic side chain
133.0	VW	C-1, S non etherified; C-1, G non etherified	28.8	M	Same
131.6	W		28.6	M	Same
130.1	S	C-2/C-6, H (PC ester)	26.7	W	CH <sub>3</sub> or CH <sub>2</sub> group in saturated side chains
128.7	W	C-2/C-6, H	24.6	M	Same
128.1	VW	C-2/C-6, H	22.2	M	CH <sub>3</sub> or CH <sub>2</sub> in saturated aliphatic chain
125.8	W	C-1, PC ester	18.8	W	Same
125.2	W	C-1, PC ester	14.0	M	$\gamma$ CH <sub>3</sub> in <i>n</i> -propyl side chain
122.9	VW	C-6, FE ether	13.6	W	Same

<sup>a</sup>Intensity abbreviations: S, strong; M, medium; W, weak; Vs, very strong; VW, very weak.

<sup>b</sup>Assignment abbreviations: G, guaiacyl unit; S, syringyl unit; H, p-hydroxyphenyl unit; PC, p-coumaric acid; FE, Ferulic acid; Xyl, xylose; Glc, glucose; GlcA, glucuronic acid.

and esters, acetyl groups and other aliphatic esters, contribute to signal at 182.0 ppm. This new finding was in good agreement with our previous study on uronic acids in hemicelluloses from wheat straw [11].

According to the above results, it is concluded that the alkali-soluble wheat straw lignin fractions, obtained by two steps of precipita-

tion, are composed of coniferyl and sinapyl lignin units with relatively fewer p-coumaryl units being incorporated into the structure. They seem more condensed than typical hardwood or softwood lignins. The isolated lignins contained rather low amount of polysaccharides (1.7–1.9%) and had low molecular-average weight. Lignin in the cell walls appears to be very closely associated to phenolic acids. p-Coumaric acid is ester-linked to lignin at C- $\alpha$  and C- $\gamma$ , while most of the ferulic acids are either-linked to lignin at C- $\alpha$  and C- $\gamma$ .

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