

Comparative Study of Fractions from Alkaline Extraction of Wheat Straw through Chemical Degradation, Analytical Pyrolysis, and Spectroscopic Techniques

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A comparative assessment of spectroscopic and degradative techniques was carried out to characterize fractions from the sodium hydroxide extraction of wheat straw. CPMAS ¹³C NMR and IR spectra revealed lignin, lignin-carbohydrate complex, and hemicellulose in laboratory and industrial fractions. Preferential removal by alkali of syringyl lignin was observed, and lignin alteration during biological treatment of pulping effluents was indicated by enhanced carbonyl and alkyl signals. Relatively high syringyl/guaiacyl ratio values were obtained by cupric oxide degradation when compared with those from NMR and Py-GC-MS, the latter including ferulic acid. Additional information on straw polymer association and the role of cinnamic acids was obtained. The high guaiacyl content in lignin associated to the hemicellulose suggested bonds between the latter and guaiacyl lignin. Most linked *p*-coumaric acid was merely esterified, whereas ferulic acid was preferentially associated to lignin by nonsaponifiable bonds.

The limitations of present analytical methodologies for characterizing complex biopolymers represents an important drawback in the study of lignocellulosic materials. In the case of grass lignins, additional problems arise from their structural complexity due to the simultaneous presence of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, in chemical association with cinnamic acids (Higuchi, 1990). Analytical pyrolysis has proven to be a quick and useful technique to analyze lignin-containing materials since, although the side chain of lignin units is modified, the groups attached to the aromatic ring remain unaltered (Boon, 1989). On the other hand, CuO alkaline oxidation is a suitable degradative procedure to analyze grass lignins since, as opposed to pyrolysis, it allows the identification of cinnamic acids (Hedges and Ertel, 1982). Finally, spectroscopic techniques, such as NMR and IR, are complementary to the above degradative procedures (Martínez et al., 1991a) since they provide information on the whole structure of the polymer and avoid the possibility of degradation artifacts. Moreover, solid-state NMR of lignocellulosic materials using CPMAS ¹³C NMR (Fründ and Lüdemann, 1989a) eliminates methodological troubles related to previous extraction and solubilization for solution NMR.

Cereal straws represent alternative raw materials for the production of some types of paper pulp, especially in areas with limited forest resources (Marley, 1991). Some attempts to characterize straw lignins and lignin-carbohydrate complexes from soda pulping have been reported (Xiao-an et al., 1989). However, additional information concerning composition and distribution of straw polymers in fractions obtained during alkali treatment could permit

these pulping processes to be optimized. In the present study, carbohydrate and lignin-containing fractions from a laboratory extraction of wheat straw with sodium hydroxide and lignin recovered from an industrial effluent from straw soda pulping were characterized by the above analytical techniques to contrast the information provided on the chemical nature and association between cell-wall polymers.

EXPERIMENTAL PROCEDURES

Materials Studied. Wheat (*Triticum aestivum*) straw (WS), collected in Zaragoza, Spain, was extracted with 80% ethanol in a Soxhlet (16 h). Details on the subsequent fractionation were given by Terrón et al. (1992). Alkali-soluble material was obtained by treating the alcohol-extracted straw with 1 M NaOH (30 °C, 12 h). The so-called hemicellulose fraction (HC) was recovered from the above solution by methanol precipitation, and the alkali lignin 1 fraction (AL1) was then precipitated (pH 2) from the supernatant solution (Janshekar et al., 1982). An additional lignin-containing fraction, labeled alkali lignin 2 (AL2), was obtained using a lower NaOH concentration (0.25 M). The industrial effluent was collected after biological (anaerobic and aerobic) treatment of wastewater containing black and white liquors from semichemical soda pulping of the same straw in an atmospheric pulper. After elimination of suspended solids and methanol precipitation, the effluent alkali lignin (ALE) was precipitated at pH 2. Klason lignin content in different fractions and whole straw was estimated as the ash-free residue after Saeman's hydrolysis of straw polysaccharides (Effland, 1977). Neutral sugars in the hydrolysates were analyzed as alditol acetates by gas chromatography (TAPPI, 1975).

Spectroscopic Analyses. For IR spectroscopy 2 mg of sample in 300 mg of KBr was used. Solid-state ¹³C NMR spectra were obtained in a Bruker MSL 300 spectrometer at 75.4 MHz with the CPMAS technique, under quantitative conditions (Fründ and Lüdemann, 1989b). The pulse repetition rate was 5 s, the cross polarization (CP) contact time was 1 ms, the sweep width was 31.25 kHz, the filter width was set to 37.5 kHz, and the acquisition time was 0.016 s. Magic-angle spinning (MAS) was performed at 4 kHz in double-bearing probes in phase-stabilized ZrO₂ rotors. The chemical shift was calibrated with glycine. Estimation of the S and G components in the 165-125 ppm

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Table I. Klason Lignin and Major Polysaccharide Sugars in the Wheat Straw Fractions

	Klason lignin ^a	polysaccharide ^a		
		glucose	xylose	arabinose
wheat straw (WS)	16.6	37.1	16.4	3.3
hemicellulose (HC)	4.6	4.9	56.7	6.3
alkali lignin 1 (AL1)	80.9	1.2	12.7	3.4
alkali lignin 2 (AL2)	56.1	3.8	24.7	4.4
effluent alkali lignin (ALE)	78.7	tr ^b	tr	tr

^a g/100 g of sample. ^b tr = traces.

aromatic region of the NMR spectra was carried out by digital subtraction of a softwood (G-lignin) spectrum (Manders, 1987; Martínez et al., 1991). After a zero subtraction value was obtained in the 146–148 ppm region, S and G components were obtained. The S/G ratio was calculated after area integration of both components and normalization to the same number of substituted aromatic carbons.

Analytical Pyrolysis (Py-GC-MS). Samples (approximately 1 mg) were pyrolyzed in a quartz sample holder at 600 °C for 5 s, using a platinum filament pyrolyzer (CDS pyrprobe 100) coupled to a gas chromatograph which was interfaced to an ion trap detector (ITD) mass spectrometer. The gas chromatograph was fitted with a SPB-5 column programmed from 50 to 250 °C at 5 °C/min, holding the initial temperature for 10 min. Mass spectra were recorded under electron impact at 70 eV from 40 to 350 *m/z* at 1 scan/s. The pyrolyzer-GC interface was at 180 °C and the GC-MS transfer line at 230 °C. Injection mode was split (approximately 1/100). Carrier gas was helium (1 mL/min). Product identification was performed on the basis of standards, the NBS library of spectra, and published spectra and gas chromatograms of lignocellulosic materials (Ralph and Hatfield, 1991). Relative molar abundances of the pyrolysis products were calculated by dividing peak areas by molecular weights.

CuO Degradation. One hundred milligrams of sample was degraded at 170 °C (3 h, N₂) in Teflon bombs with 2 g of CuO, 200 mg of Fe(NH₄)₂(SO₄)₂·6H₂O, and 14 mL of 2 M NaOH (Hedges and Ertel, 1982). The degradation products were recovered with ethyl ether, suspended in 100 μL of pyridine (ethylvanillin as internal standard), derivatized with BSTFA, and analyzed by GC-MS (30 m × 0.25 mm SP-2100 column, 100–270 °C at 4 °C/min, helium as carrier gas) using an ITD, under conditions described above. Quantitations were based on the area of the internal standard and the response factors obtained from standard compounds, and molar abundances of lignin degradation products were calculated.

RESULTS AND DISCUSSION

The major constituents of the wheat straw fractions studied are presented in Table I. Additional information on these samples was reported elsewhere (Terrón et al., 1992). As expected, the greatest Klason lignin content was found in the alkali lignin 1 (AL1) and effluent alkali lignin (ALE) fractions, but some amount of lignin was present in the hemicellulose (HC) fraction. Different amounts of carbohydrate were present in the lignin-containing fractions AL1 and AL2. On the other hand, the ALE fraction was carbohydrate-free due to biological treatment of the industrial effluent. The straw hemicellulose appeared as an arabinoxylan (xylose/arabinose ratio of 9:1), and the high pentose/glucose ratio in the AL1 and AL2 fractions, compared to that in whole straw, suggested association of lignin with the arabinoxylan. Moreover, the xylose/arabinose ratio (10:3) in the alkali lignin fraction AL1 revealed that the percentage of arabinose units linked to lignin (as percent of total arabinose) was higher than the percentage of linked xylose, in accordance with the findings of Xiao-an et al. (1989).

Spectroscopic Analyses. The IR spectra of the four straw fractions are shown in Figure 1. The most prominent bands in these spectra correspond to (i) lignin (1600, 1510,

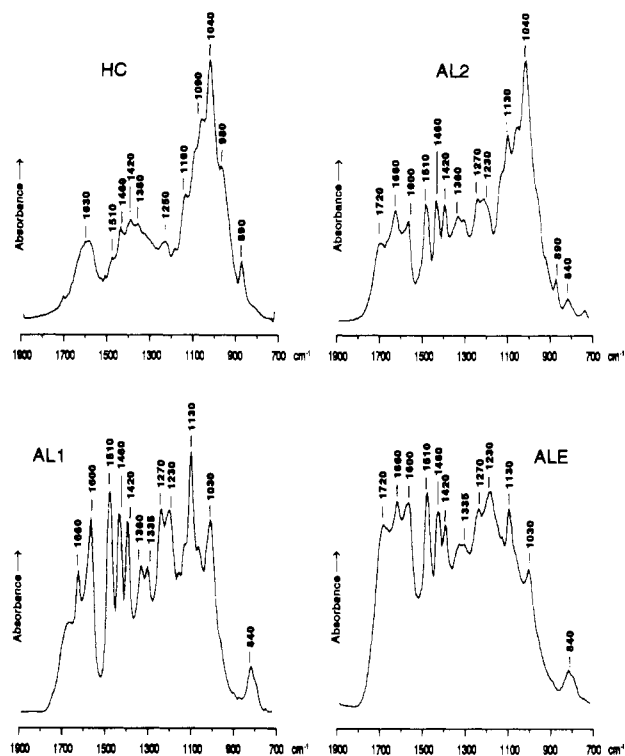


Figure 1. IR spectra of the wheat straw fractions (HC = hemicellulose, AL1 = alkali lignin 1, AL2 = alkali lignin 2, ALE = effluent alkali lignin).

1460, 1420, 1335, 1270, 1230, 1130, 1030, and 840 cm⁻¹) and (ii) carbohydrates (1380, 890 cm⁻¹ and the broad band around 1040 cm⁻¹. Bands from carbonyl groups (e.g., in cinnamic acids or lipids) were also present at 1720 and 1660 cm⁻¹.

The NMR spectra of the four fractions are shown in Figure 2. Most of the signals corresponded to carbons in the different lignin units: 161 ppm for C₄ in H; 153 ppm for C₃ and C₅ in S (etherified); 148 ppm for C₃ and C₄ in G (etherified) and for C₃ and C₅ in S (phenolic); 146 ppm for C₄ in G (phenolic); 134 ppm for C₁ and C₄ in S and for C₁ in G; 128 ppm for C₂ and C₆ in H; 114 ppm for C₅ in G; 105 ppm for C₂ and C₆ in S; 84 ppm for C_β in β-O-4; 74 ppm for C_α-OH in β-O-4; and 56 ppm for methoxy C. Signals from phenolic units are more evident in lignin from wheat straw than from hardwoods, as a consequence of its relatively high (*ca.* 20%) phenolic content (Kühn et al., 1992). Several signals corresponded to polysaccharide unit carbons: 103 ppm for C₁; 75 ppm for C₂, C₃, and C₅; 65 ppm for C₆. Finally, two other signals were present: 172 ppm for carbonyl C and 31 ppm for nonsubstituted alkyl C (e.g., in lipids).

The IR and NMR spectra of the HC and AL1 fractions were characteristic of polysaccharide and lignin, respectively (Martínez et al., 1991b; Almendros et al., 1992), including the diagnostic peaks listed above. The AL1 spectra were nearly identical to the spectrum of milled-straw lignin extracted according to the Björkman (1956) method (not shown). In the HC fraction, the broad band at 1630 cm⁻¹ was probably due to linked water, and the small bands at 1460, 1420, and 1510 cm⁻¹ showed low lignin content (Table I) in this fraction. The absence of carbonyl and methyl C signals in the NMR spectrum suggested a low amount of acetyl groups in the HC fraction.

On the other hand, the spectra of the AL2 fraction evidenced a mixed pattern, which can be described in terms of the overlapping of polysaccharide and lignin bands. The NMR spectrum included all of the above carbohydrate

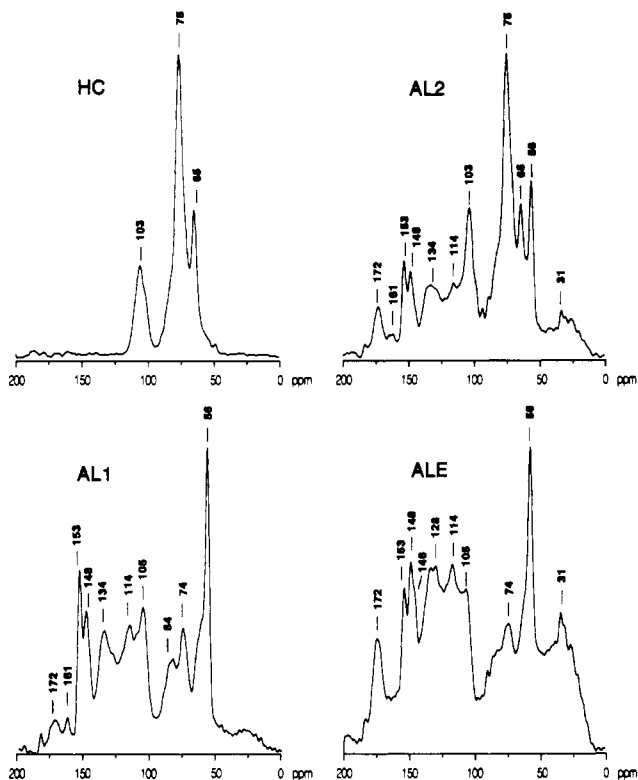


Figure 2. CPMAS ^{13}C NMR spectra of the wheat straw fractions (HC = hemicellulose, AL1 = alkali lignin 1, AL2 = alkali lignin 2, ALE = effluent alkali lignin).

and lignin signals, whereas the IR spectrum showed most lignin bands in addition to those of carbohydrates at 1040 and 890 cm^{-1} .

Finally, the spectra of the ALE fraction were similar to those of the AL1 fraction, but they evidence modifications of the lignin polymer due to the anaerobic and aerobic treatments of the industrial effluent. The most significant features showed by the IR spectrum were the strong bands of unconjugated and conjugated carbonyls at 1720 and 1660 cm^{-1} , respectively (Hergert, 1971). The former band could correspond to microbial lipids, whereas that at 1660 cm^{-1} probably reflects oxidative alteration (formation of α -carbonyl groups with or without side-chain breakdown) during biological treatment (Kirk and Farrell, 1987). The increased amount of carbonyl groups was also evidenced by the NMR spectrum, exhibiting an intense 172 ppm signal, and the broad signal around 31 ppm suggested microbial lipids. Some differences in aromatic C signals were observed when ALE was compared with the AL1 fraction. The increase of the 128 ppm signal could be due to a high proportion of H units (see Table II and Figure 5), whereas an increased phenolic content could contribute to the 148 ppm signal, including the 146 ppm shoulder.

Py-GC-MS. The products obtained after pyrolysis of the straw fractions (Figure 3) were ascribed to the thermal decomposition of the main plant polymers, according to Martín et al. (1979), Mulder et al. (1991), and Ralph and Hatfield (1991). A series of pyrolysis products from phenylpropanoid structures (i.e., lignin and cinnamic acids), including phenol, guaiacol, and syringol and their *para* methyl, ethyl, vinyl, and propenyl derivatives, were also identified. The origin of most of them can be straightforwardly deduced as derived from H (peaks 5, 9, and 14 in Figure 3), G (peaks 10, 13, 16, 17, 19, 20, 22, 23, 25, and 27), and S (peaks 18, 21, 24, 26, and 28–34) lignin units or cinnamic acids, and Py-GC-MS molar H/G/S ratios were calculated (Figure 5). 3,4-Dihydroxybenzal-

dehyde (peak 12) was only found in the HC fraction, whereas 3-methoxycatechol (peak 15) appeared in the three lignin-containing fractions (AL1, AL2, and ALE) and could be derived from lignin demethylation during alkaline treatment. The lignin pyrolysis peaks were characteristic of the latter fractions, but some of them (peaks 10, 17, 18, and 26) were identified also in the HC fraction. In addition, different products from polysaccharide pyrolysis (peaks 1–4, 6–8, and 11) were found mainly in pyrograms of the HC and AL2 fractions. In fact, after pyrolysis and spectroscopic analyses, the latter fraction can be considered as a lignin-polysaccharide complex.

When the relative abundances of the peaks corresponding to lignin pyrolysis products were compared with the Klason lignin content or with the low intensities of the NMR and IR lignin bands (160–110 ppm aromatic C region; and 1510, 1460, and 1420 cm^{-1}), it appeared that pyrolysis led to some overestimation of lignin in the HC fraction. This was probably due to the higher yield of lignin-derived compounds under pyrolysis, when compared to the complex isomeric mixtures of dehydration products from carbohydrates.

CuO Degradation. Cinnamic acids (peaks XI and XII) and products derived from H (peaks I, II, and VI), G (peaks III, V, and IX), and S lignin units (peaks VII, VIII, and X) were identified after CuO alkaline degradation (Figure 4). Three types of aromatic compounds with different degrees of oxidation (i.e., aldehydes, ketones, and acids) were released after CuO degradation of each lignin unit. The small lignin amount in the HC fraction already suggested by the Py-GC-MS and the IR spectrum was confirmed after CuO degradation. The cinnamic acids were nearly absent in the HC fraction, but their *trans* isomers were among the most abundant compounds in the three lignin-containing fractions. Minor peaks for *cis* isomers are indicated on the chromatograms.

The above results suggested that cinnamic acids were linked to hemicellulose only by ester bonds, which were saponified by cold sodium hydroxide. On the other hand, their constant presence in the three lignin-containing fractions indicated significant amounts of nonsaponifiable linkages (such as ethers) between cinnamic acids and lignin. The low amount of *p*-coumaric acid in these fractions, compared with that in the whole straw, suggested that a considerable proportion of this compound was esterified with lignin. These results agree with models involving bridges between wheat straw hemicellulose and lignin through ferulic acid (Scalbert et al., 1985; Iiyama et al., 1990; Lam et al., 1992; Ralph et al., 1992).

Lignin Composition As Revealed by the Different Analytical Techniques. The molar H/G/S ratios in lignin and the amounts of cinnamic acids relative to lignin units were calculated from the results of CuO degradation (Table II). The high S content in the three lignin-containing fractions evidenced that the less condensed S-lignin is readily extracted by sodium hydroxide. Preferential removal of S-lignin during alkali extraction from wheat straw was also reported by Lapierre et al. (1989). By contrast, the lignin included in the HC fraction presented the lowest S/G ratio, especially when analyzed by pyrolysis, as expected after preferential removal by alkali of S-lignin. It is known that vinylphenol and vinylguaiacol are formed by pyrolytical decarboxylation of *p*-coumaric and ferulic acids, respectively (Boon, 1989; Galletti et al., 1991). However, they have also been found after pyrolysis of cinnamic-free lignin preparations (Saiz-Jiménez and de Leeuw, 1984), as a result of the breakdown of H and G lignin units. To compare with pyrolysis, the

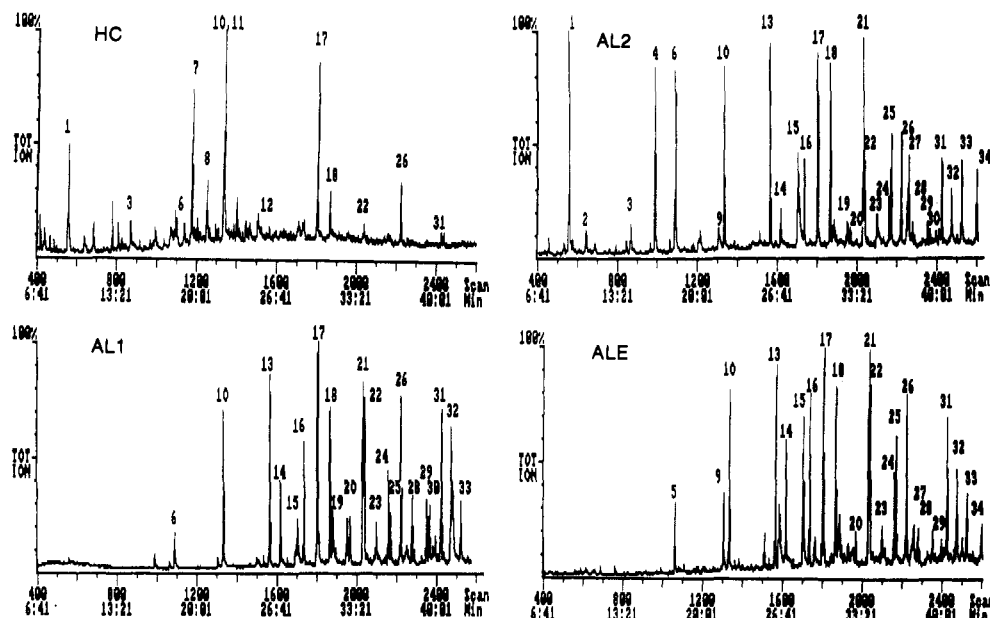


Figure 3. Py-GC-MS of the wheat straw fractions (HC = hemicellulose, AL1 = alkali lignin 1, AL2 = alkali lignin 2, ALE = effluent alkali lignin). 1 = 2-Furaldehyde; 2 = 2-OH-Me-furan; 3 = 2,3-dihydro-5-Me-furan-2-one; 4 = *N*-Et-furanone; 5 = phenol; 6 = 4-OH-5,6-dihydro-(2*H*)-pyran-2-one; 7 = 2-OH-3-Me-2-cyclopenten-1-one; 8 = diMe-dihdropyranone; 9 = *p*-cresol; 10 = guaiacol; 11 = levoglucosone; 12 = 3,4-diOH-benzaldehyde; 13 = 4-Me-guaiacol; 14 = 4-vinylphenol; 15 = 3-Me-O-catechol; 16 = 4-Et-guaiacol; 17 = 4-vinylguaiacol; 18 = syringol; 19 = vanillin; 20 = *cis*-isoeugenol; 21 = 4-Me-syringol; 22 = *trans*-isoeugenol; 23 = acetovanillone; 24 = 4-Et-syringol; 25 = guaiacylacetone; 26 = 4-vinylsyringol; 27 = guaiacyl vinyl ketone; 28 = 4-allylsyringol; 29 = *cis*-4-propenylsyringol; 30 = syringaldehyde; 31 = *trans*-4-propenylsyringol; 32 = acetosyringone; 33 = syringylacetone; 34 = propiosyringone.

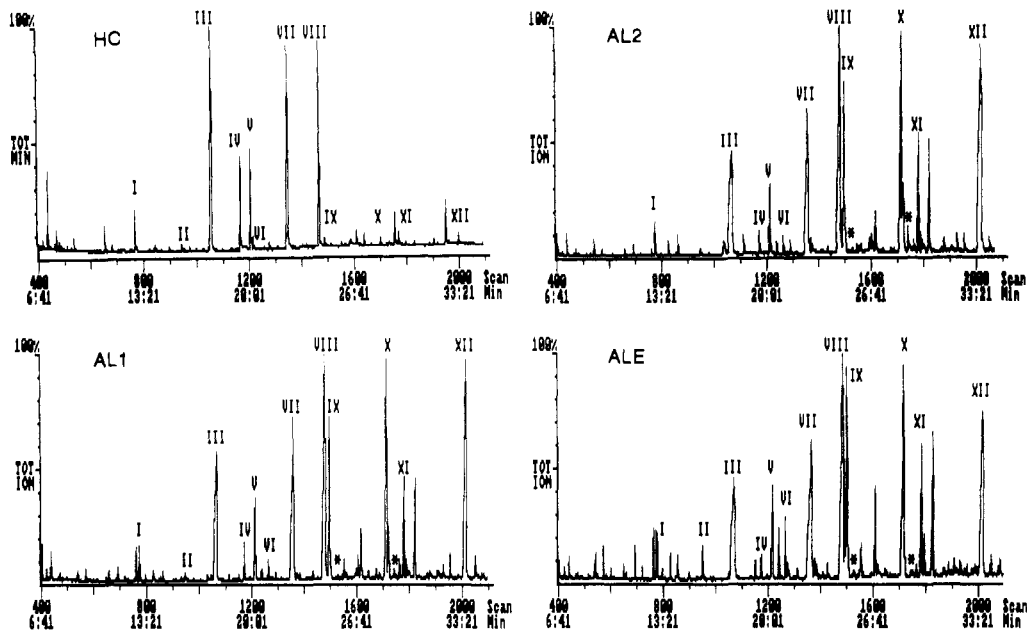


Figure 4. GC-MS analysis of lignin and cinnamic acids after CuO degradation of the straw fractions (HC = hemicellulose, AL1 = alkali lignin 1, AL2 = alkali lignin 2, ALE = effluent alkali lignin). I = *p*-OH-benzaldehyde; II = *p*-OH-acetophenone; III = vanillin; IV = ethylvanillin (standard); V = acetovanillone; VI = *p*-OH-benzoic acid; VII = syringaldehyde; VIII = acetosyringone; IX = vanillic acid; X = syringic acid; XI = *trans*-*p*-coumaric acid; XII = *trans*-ferulic acid; * = *cis* isomers of XI and XII.

H/G/S ratios after CuO degradation were recalculated including cinnamic acids. Some differences between the lignin compositions deduced from both techniques were observed (Figure 5).

In general, it is assumed that CuO degradation breaks predominantly ether linkages in lignin, whereas different C-C bonds can be cleaved by pyrolysis (Galletti et al., 1991). The highest G yield after pyrolysis of the straw fractions pointed to a more extensive lignin depolymerization, affecting also C₅-C₈ bonds in condensed G-lignin. Among the different fractions, the lowest S/G ratio was found in HC. Since cinnamic acids were nearly absent

from this fraction (Table II), the G-type compounds identified after pyrolysis were considered to derive from lignin, suggesting specific association between hemicellulose and G-lignin through nonsaponifiable bonds. The earlier deposition of G-lignin during monocotyledons lignification (He and Terashima, 1991) could facilitate linkages between G-units and hemicellulose in the cell wall. These results also suggest that some C-C linkages, cleaved by pyrolysis but resistant to CuO degradation, were present in lignin associated to hemicellulose.

The intensities of 1335- and 1270-cm⁻¹ IR bands, assigned to S- and G-ring breathing respectively (Hergert,

Table II. H/G/S Molar Ratio in Lignin and Cinnamic Acid Content in the Wheat Straw Fractions As Revealed by CuO Degradation

	H/G/S ratio ^b	cinnamic acids ^a	
		<i>p</i> -coumaric	ferulic
wheat straw (WS)	8:41:51	28.9	37.4
hemicellulose (HC)	5:45:49	0.9	0.8
alkali lignin 1 (AL1)	4:34:62	5.8	25.0
alkali lignin 2 (AL2)	4:31:65	5.9	24.3
effluent alkali lignin (ALE)	9:33:57	5.5	12.1

^a mol/100 mol of etherified lignin (=H + G + S). ^b H = *p*-hydroxyphenyl; G = guaiacyl; S = syringyl.

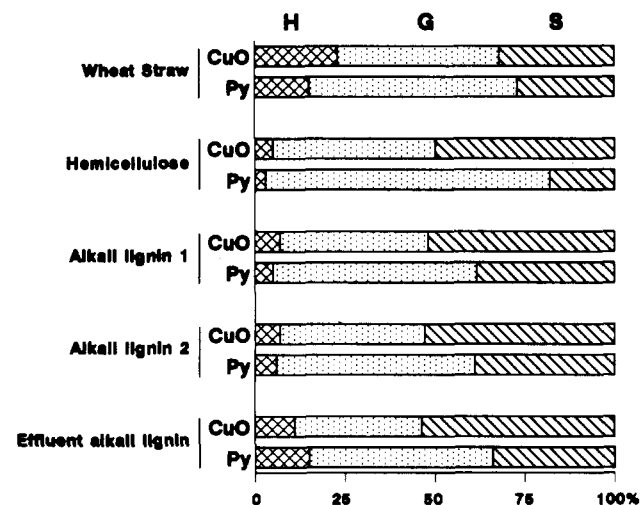


Figure 5. Quantitative comparison of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) molar ratios (cinnamic acids included) through alkaline degradation (CuO) and pyrolysis (Py).

1971), suggested high G content in wheat straw lignin, when compared with lignin from hardwoods. This was supported by the relative intensities of 153 and 148 ppm NMR signals. However, accurate determination of S/G ratios according to the method described by Manders (1987) (based on the assumption that the 148 ppm signal in wood can be entirely attributed to G-lignin) was difficult due to the structural complexity of grass lignins. In fact, the 148 ppm signal also included C₃ and C₅ in phenolic S-units and overlapped with the 146 ppm signal of C₄ in phenolic G-structures. In spite of this limitation, an S/G ratio of 0.5 was estimated for laboratory alkali lignins (AL1 and AL2 fractions) by obtaining the S and G components of the aromatic region of the NMR spectra. The comparatively low 153 ppm signal in the ALE fraction could suggest the lowest S/G ratio among the alkali lignin fractions, but this assumption was not supported by the S/G values from the degradative techniques. A more consistent explanation could be that the high 146–148 ppm band in the ALE spectrum (Figure 2) was the sum of different signals, including those due to phenolic units in lignin.

In general, the four techniques used gave complementary information on chemical composition of plant polymers in the straw fractions. CPMAS ¹³C NMR and IR provided generic, but significant and unequivocal, information on the nature of whole samples. More details were obtained after polymer degradation, but partial depolymerization was an important drawback of the degradative techniques. This fact was evidenced by differences in S/G ratios obtained by three techniques (CuO oxidation > pyrolysis > NMR), due to the more recalcitrant nature of G-lignin toward depolymerization. Cupric oxide degradation seemed to be a suitable technique for analyzing straw lignin

since it (as opposed to other chemical degradation procedures, e.g., nitrobenzene oxidation) led to separate estimation of lignin units and cinnamic acids. This distinction, which represents an important point when grass materials are analyzed, cannot be achieved by pyrolysis, but the latter technique provided a more extensive degradation of the lignin polymer and gave information on other plant constituents.

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