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 pulp effluents was indicated by enhanced carboxyl 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.

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 (Jansebak 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 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 160–125 ppm
Table 1. Klasson Lignin and Major Polysaccharide Sugars in the Wheat Straw Fractions

<table>
<thead>
<tr>
<th></th>
<th>Klasson lignin</th>
<th>polysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>wheat straw (WS)</td>
<td>16.6</td>
<td>37.1</td>
</tr>
<tr>
<td>hemicellulose (HC)</td>
<td>4.6</td>
<td>49</td>
</tr>
<tr>
<td>alkali lignin 1 (AL1)</td>
<td>80.9</td>
<td>1.2</td>
</tr>
<tr>
<td>alkali lignin 2 (AL2)</td>
<td>56.1</td>
<td>3.8</td>
</tr>
<tr>
<td>effluent alkali lignin (ALE)</td>
<td>78.7</td>
<td>tr</td>
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*a g/100 g of sample, *tr = traces.

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 Klasson 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 ALE fractions was carbohydrate-free due to biological degradation. 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 (1800, 1510, and 1460, 1420, 1335, 1270, 1230, 1130, 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 1650 cm⁻¹.

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 signals.
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dehydro (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 Klassen 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 in 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 (Saclabert 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 vinylguaiaacol 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-Jimenez 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-(2H)-pyran-2-one; 7 = 2-OH-3-Me-2-cyclopenten-1-one; 8 = diMe-dihydropyranone; 9 = p-cresol; 10 = guaiacol; 11 = levoglucosenone; 12 = 3,4-diOH-benzaldehyde; 13 = 4-Me-guaiacol; 14 = 4-vinylphenol; 15 = 3-MeO-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; I1 = 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 C-C 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 11), 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,
The nature of whole samples. More details were obtained as an important drawback of the degradative techniques. Partial depolymerization seemed to be a suitable technique for analyzing straw lignin.

Table 11. H/Q/S Molar Ratio in the Straw Fractions As Revealed by CuO Degradation

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|---------------------------------|----------------|----------------|----------------|
| Wheat straw (WS)               | 8.41:51        | 3.93:2.2       | 0.9:0.8        |
| Hemicellulose (HC)             | 3.46:49        | 1.3:2.2        | 0.5:1.2        |
| Alkali lignin 1 (AL1)          | 4.34:32        | 5.8:2.5        | 5.8:1.2        |
| Alkali lignin 2 (AL2)          | 4.31:35        | 5.9:2.4        | 24.3:1.2       |
| Effluent alkali lignin (ALE)   | 9.33:57        | 5.5:1.0        | 12.1:1.2       |

* 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 C8 and Cs in phenolic S-units and overlapped with the 146 ppm signal of C7 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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LITERATURE CITED


Effland, M. J. Modified procedure to determine acid-insoluble lignin in wood and pulp. Tappi 1977, 60, 143-144.


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