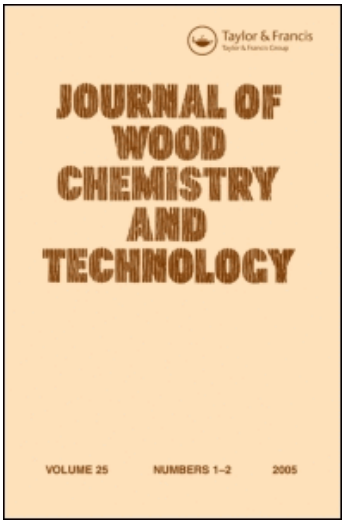

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SOLID STATE ANALYSIS OF PLANT POLYMERS BY FTIR

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ABSTRACT

Wheat straw, kenaf, oak, and pine were extracted to give samples with various contents of lignin, hemicellulose, and cellulose. Precise amounts of these samples were blended with KBr and pressed into discs, and their FTIR spectra were determined. Two-spectra subtraction, and combination of multiple spectra by matrix inversion and least squares matrix methods were used to give spectra of individual lignin, hemicellulose, and cellulose components in all 4 plant types. They are the most complete IR spectra available for lignin in plant matrices.

INTRODUCTION

Several techniques have been developed in the last 10 years for solid state analysis of plant materials. Solid state NMR[1-3], reflectance FTIR with the DRIFT cell[4], photoacoustic UV[5], photoacoustic FTIR[6] and NIR spectroscopy[7] are

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[1] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

examples. Information obtained from some of these techniques is quantitative but dependent on other primary chemical analyses. Some suffer problems of resolution, sensitivity and interpretability; thus, only limited structural information can be obtained.

Multicomponent analysis of mixtures also has become available recently on FTIR instruments.[8] Known mixtures or pure components can be used to calibrate an instrument. Then mixtures with unknown compositions can be analyzed, or pure component spectra can be derived from mixtures of known composition.[8-10] Multicomponent analysis has been largely limited to gases, liquids, and solutions consisting of four or fewer components. Multicomponent analysis correlates NIR absorbances and wide-line NMR resonances with concentrations of components in solids based on chemical analysis of the solids. These methods do not provide useful spectra of pure components for structural interpretation. Making mixtures of pure components that accurately represent solid biomass samples is not feasible, because it is not possible to isolate some plant components without significant structural changes. The major structural constituents of plants are cellulose, hemicellulose, and lignin. Lignin does not exist unchanged outside of plant matrixes, because lignin is a three-dimensional network polymer linked to other biomass components. Milled wood lignin, often considered the closest standard for lignin from biomass, represents no more than 50% of

total lignin in a plant and must have been degraded physically to become soluble. The isolation procedure cleaves interunit ether linkages and may also oxidize the lignin to a limited extent.

To study potential reactions of lignocellulosic biomass with chemicals and organisms for the purpose of creating new industrial products, a project was undertaken to obtain spectra of in situ lignin. Solid state analysis was chosen as the preferred approach. Several mixtures of varying composition were prepared for solid state analysis. The mixtures were obtained by selectively solubilizing portions of each component. The residual solids remained relatively unchanged structurally. In this paper, we show how infrared can be used to obtain spectra of individual plant components in situ from chemically extracted plants. We used quantitative mixtures of KBr and biomass samples to make KBr discs from which spectra of individual components in their native condition could be determined.

EXPERIMENTAL

Pretreatments

Four plant materials were chosen for study--wheat straw, kenaf stalks, oak and pine. Wheat straw contained a typical grass-type lignin (syringyl-guaiacyl-coumaryl structure); kenaf and oak contained typical angiosperm lignins (syringyl-guaiacyl structure); and pine contained typical gymnosperm lignin (guaiacyl structure). Oak and kenaf were both chosen, because oak, a perennial hardwood, and kenaf, an annual angiosperm, have

markedly different morphologies. Samples were Soxhlet extracted with benzene:ethanol, 2:1, to remove soluble organics. The samples were also extracted with an acetate buffer, pH 5, by stirring for 4 hr at 23°C, and then rinsing with water to equilibrate acid-base groups to a standard pH and to extract mineral components.

Chemical treatments to remove various components included extraction with pH 5 acetate buffer, 1N trifluoroacetic acid (TFA) for 7 hr at reflux,[11] sodium chlorite in acetic acid solution at 90°C, 12% NaOH at 80°C for 4 hr, or various combinations of these treatments. Compositions of the solid residues after chemical extraction were determined by several methods.

Composition Analysis

Cellulose and lignin contents were determined by treating 0.2 g plant material with 2 ml 72% H_2SO_4 for 1 hr at 30°C, then diluting the solution to 75 ml, and autoclaving at 160° for 2 hr. Solids were filtered on a medium porosity polypropylene funnel, washed, dried under vacuum at 70° for 16 hr, and weighed. The solids not soluble in 3% H_2SO_4 were weighed as lignin (Klason method), after correcting for ash content. The filtrate from the polypropylene funnel was neutralized with an alkaline ion-exchange resin and freeze-dried. The resulting sugars were taken up in 5 ml of water containing 0.4% erythritol as an internal standard, and their identity and concentrations were determined by HPLC.[12]

For quantitative comparisons, cellulose, glucose, mannose, xylose, and arabinose were hydrolyzed by the Klason method, and reaction loss factors were calculated based on these standards. Weight of glucans were obtained by multiplying recovered glucose by 0.9. All glucans were considered to be cellulose, except in pine and oak where glucomannans needed to be considered. Similarly, all non-glucose sugars were calculated as polymers and considered to be hemicellulose. Hemicellulose contents from the sugar analyses were increased by the amounts of acetate found. In pine, anhydroglucose units from glucomannan were estimated from the formula % hemicellulose glucan = 0.30 (% mannan). Percent cellulose in pine was determined by subtracting hemicellulose glucans from the total glucans. Hemicellulose glucan percentages were added to % hemicellulose derived from xylose, arabinose, mannose, and acetate values. Oak cellulose was similarly reduced by 0.50 (% mannan), and the balance was added to % hemicellulose to reflect anhydroglucose units in oak glucomannans.

Samples for acetyl contents were hydrolyzed with p-toluenesulfonic acid (0.1N) at 95^{o} for 2 hr. The solutions were cooled and filtered. Acetic acid contents were determined by HPLC on a Bio-Rad 87H column. Lignin contents were determined by the Klason method as described above and by the UV method[13] with absorptivities of 33.9 $g^{-1}1\ cm^{-1}$ for wheat straw and kenaf, 23.6 $g^{-1}1\ cm^{-1}$ for oak, and 22.9 $g^{-1}\ cm^{-1}$ for

pine. Ash contents were determined by pyrolysis for 45 min at 725°C in a platinum boat under flowing O_2.

FTIR Spectra Determination

KBr discs were prepared in a dry glove box by mixing 13.00 mg ± 1% of dry sample with 187 mg KBr and pulverizing the mixture in a Wig-L-Bug apparatus for 50 s with 2 stainless-steel balls in a stainless-steel container. Ten milligram of the mix was then blended with 290 mg KBr as above for 50 s, and the resulting 300 mg mix was pressed into a 13 mm (dia) X 1 mm disc.

Three discs were made from each sample, and their spectra were determined on a Mattson Cygnus 25 FTIR. One hundred scans were averaged at 2 cm^{-1} resolution. All discs were pressed and scanned within 24 hr of preparation. They were stored in a vacuum desiccator when not in use. The three spectra of each sample usually agreed within 5% of absorbance units at the maximum absorbing wavelengths. If the spectra were more divergent, three more discs were made. Although seldom necessary, a spectrum was rejected if transmittance was below 85% at 4000 cm^{-1} or below 90% at 2000 cm^{-1}. Spectra were baseline-corrected by measuring the lowest absorbance, which always occurred between 1800 cm^{-1} and 2200 cm^{-1}. Then the difference between the observed absorbance and the 0.00 line was subtracted from the entire spectrum such that the lowest point rested on zero absorbance. The spectrum from 4000 cm^{-1} to the observed low point was then baseline-corrected by using Mattson's

Bascor program. The Bascor program allows the operator to select spectral points which should rest on the baseline and connect them in a smooth baseline, and then appropriate absorbances can be subtracted from all data points to smoothly move the baseline to zero and retain true absorbances. The rationale for a two-step baseline correction method is that there are two common sources of baseline error, reflectance and scattering. Reflectance from the surface of the disc was assumed to be uniform across the spectrum. It was compensated for by moving the lowest point in the spectrum to zero absorbance. Imperfections in the disc and undispersed sample particles scattered shorter wavelengths more than longer wavelengths which accounted for the sloping baseline often seen from 4000 cm^{-1} to 2000 cm^{-1}. As stated earlier, this correction was always small for our samples and only necessary in the 4000 to 1800 cm^{-1} region. All "mixtures" were derived from one original sample foreach of the wheat straw, kenaf, oak, and pine series. The morphology or superstructure of each sample was only partially disrupted by chemical treatment. The products of chemical treatments were fibrous and similar in size to the starting material. Therefore, the dispersibility in KBr of the samples should be comparable.

FTIR Spectra Combination

An average FTIR spectrum of each sample was found by adding equal fractions of 3 to 6 individually determined spectra by

means of Mattson software. Spectra of samples with different compositions can be combined in several ways to obtain spectra of individual components.[14-16] For example, a simple method to obtain a spectrum of component A in a mixture is to subtract 20% of spectral absorbances of pure component B from the spectrum of a 80:20 mixture of A and B. Because components interact, a spectrum slightly different from pure component A will usually result from the subtraction.[14] Spectra of interacting components, not isolated pure components, are therefore best to represent their true state in the mixture and to reconstruct spectra of mixtures.

When n components are present, at least n mixture spectra need to be combined to obtain individual component spectra. If the weight fractions of cellulose (G), hemicellulose (H), lignin (L), and ash (S) in wheat straw are given by g_j, h_j, l_j, and s_j, respectively, where j = 1,2,3...n, then for 4 samples, equations can be written where

$$g_1 + h_1 + l_1 + s_1 = 1$$
$$g_2 + h_2 + l_2 + s_2 = 1$$
$$g_3 + h_3 + l_3 + s_3 = 1$$
$$g_4 + h_4 + l_4 + s_4 = 1$$

Weight fraction terms may be used to represent the concentrations of individual components, even if the weight fractions do not add up to 1.0 in all samples. This is so because of the well-known

proportionality between absorbance a and concentration c given by the Beer-Lambert relation

$$a = kc$$

where k is the proportionality or absorption coefficient. Because absorbances are additive, the above equations can be rewritten

$$k_G g_1 + k_H h_1 + k_L l_1 + k_s s_1 = A_1$$
$$k_G g_2 + k_H h_2 + k_L l_2 + k_s s_2 = A_2$$
$$k_G g_3 + k_H h_3 + k_L l_3 + k_s s_3 = A_3$$
$$k_G g_4 + k_H h_4 + k_L l_4 + k_s s_4 = A_4$$

where the A's are the sums of the absorbances of the individual components. That is, the A's are the absorbances of the sample mixtures. In matrix form these equations are

$$\left|k_G\ k_H\ k_L\ k_S\right| \times \begin{vmatrix} g_1 & g_2 & g_3 & g_4 \\ h_1 & h_2 & h_3 & h_4 \\ l_1 & l_2 & l_3 & l_4 \\ s_1 & s_2 & s_3 & s_4 \end{vmatrix} = \left|A_1\ A_2\ A_3\ A_4\right|$$

or more simple denoted $KC = A$

At unit concentration k = a so that the individual component spectra are the K's given by

$$K = AC^{-1}$$

where C^{-1} is the inverse of the concentration matrix.

If the elements of C^{-1} are represented by $\hat{g}_j$, $\hat{h}_j$, $\hat{l}_j$, and $\hat{s}_j$, then

$$C^{-1} = \begin{vmatrix} \hat{g}_1 & \hat{h}_1 & \hat{l}_1 & \hat{s}_1 \\ \hat{g}_2 & \hat{h}_2 & \hat{l}_2 & \hat{s}_2 \\ \hat{g}_3 & \hat{h}_3 & \hat{l}_3 & \hat{s}_3 \\ \hat{g}_4 & \hat{h}_4 & \hat{l}_4 & \hat{s}_4 \end{vmatrix}$$

such that $CC^{-1} = I$, the identity matrix, and C^{-1} can be calculated on most personal computers (PC's).

Thus, from $AC^{-1} = K$ the individual component spectra are obtained as follows:

$$A_1\hat{g}_1 + A_2\hat{g}_2 + A_3\hat{g}_3 + A_4\hat{g}_4 = k_G \quad \text{spectrum of G}$$
$$A_1\hat{h}_1 + A_2\hat{h}_2 + A_3\hat{h}_3 + A_4\hat{h}_4 = k_H \quad \text{spectrum of H}$$
$$A_1\hat{l}_1 + A_2\hat{l}_2 + A_3\hat{l}_3 + A_4\hat{l}_4 = k_L \quad \text{spectrum of L}$$
$$A_1\hat{s}_1 + A_2\hat{s}_2 + A_3\hat{s}_3 + A_4\hat{s}_4 = k_S \quad \text{spectrum of S}$$

Therefore, it can be seen that once the elements of the inverse matrix C^{-1} are known, the individual component spectra can be calculated manually. On most modern FTIR spectrometers the mixture spectra A_1, A_2, A_3, and A_4 are simply multiplied

(scaled) by the corresponding elements (scaling factors) from the inverse matrix and then summed. Spectra of pure components can thus be obtained on instruments without multicomponent analysis software. The factors for combining spectra depend only on the concentration of individual components, which means that the same spectral combinations can be made on any spectral data as long as spectral intensities are directly proportional to concentrations. If spectra of more than four different mixtures are obtained, then least squares absorbance coefficients can be calculated to obtain the "best" spectra of individual components that fit all available data. In the matrix system A =KC, the least squares fit is $AC^T(CC^T)^{-1}$ =K, so that factors for manually multiplying spectra are acquired from the least squares inverse matrix $C^T(CC^T)^{-1}$.

More recently, many manufacturers have added multicomponent analysis software to FTIR spectrophotometers, which saves time over using a separate PC to combine spectra by data manipulation. In this paper we show the results of simple two-spectra subtraction, PC-calculated spectral data combinations, and the CALPURE program developed by Jay W. Powell at Mattson Instruments, Inc.

RESULTS AND DISCUSSION

Pretreatment and Analysis

Tables 1-4 show the compositions of samples by weight fraction and by weight percent based on 100 g of

TABLE 1
Composition of Extracted Wheat Straw as Function of Treatment

	Treatment	Weight loss (%)	Weight fraction (weight % of A[1]) Cellulose	Hemicellulose	Lignin	Ash
A.	Benzene-ethanol	---	0.348 (34.8)	0.274 (27.4)	0.176 (17.6)	0.0680 (6.8)
B.	pH 5 buffer of A	12.1	0.356 (31.3)	0.316 (27.7)	0.200 (17.6)	0.0769 (6.8)
C.	$NaClO_2$ of A	35.2	0.498 (32.2)	0.332 (21.5)	0.0085 (0.6)	0.0677 (4.4)
D.	TFA of C	42.4	0.728 (27.2)	0.071 (2.6)	0.0065 (0.2)	0.0792 (3.0)
E.	TFA of B	39.4	0.518 (27.6)	0.0589 (3.1)	0.275 (14.6)	0.0567 (3.0)
F.	$NaClO_2$ of B	30.9	0.521 (31.6)	0.398 (24.2)	0.017 (1.0)	0.0671 (4.1)
G.	NaOH of D	49.3	0.919 (17.4)	0.0637 (1.2)	0.010 (0.2)	0.0025 (0.1)
H.	$NaClO_2$ of E	29.7	0.802 (30.0)	0.0992 (3.7)	0.025 (0.9)	0.680 (2.6)

[1]For example, treating 100 g of A with buffer (12.1% loss) then TFA (39.4% loss of B) leaves 53.3 g of E. E is 0.518 weight fraction cellulose or 27.6 g cellulose, i.e. 27.6% of A.

TABLE 2
Extracted Kenaf Weight Fraction Composition

Treatment	Weight loss (%)	Weight fraction (weight % of A)			
		Cellulose	Hemicellulose	Lignin	Ash
A. Benzene-ethanol	---	0.350 (35.0)	0.133 (13.3)	0.146 (14.6)	0.0305 (3.1)
B. pH 5 buffer of A	14.4	0.395 (33.8)	0.163 (14.0)	0.150 (12.8)	0.0061 (0.5)
C. $NaClO_2$ of A	31.1	0.559 (38.5)	0.219 (15.1)	0.0150 (1.0)	0.0036 (0.3)
D. TFA of C	39.2	0.916 (38.3)	0.0692 (2.9)	0.0293 (1.2)	0 (0)
E. TFA of B	40.5	0.706 (35.9)	0.0607 (3.1)	0.222 (11.3)	0 (0)
F. $NaClO_2$ of B	19.7	0.580 (39.8)	0.216 (14.8)	0.0064 (0.4)	0.0075 (0.5)
G. NaOH of B	42.5	0.703 (33.3)	0.0624 (3.0)	0.171 (9.0)	0.0050 (0.3)
H. $NaClO_2$ of E	15.3	0.913 (39.4)	0.0960 (4.1)	0.0129 (0.6)	0 (0)

TABLE 3
Extracted Oak-Weight Fraction Composition

	Treatment	Weight loss (%)	Weight fraction (weight % of A)		
			Cellulose	Hemicellulose	Lignin
A.	Benzene-ethanol	---	0.369 (36.9)	0.229 (22.9)	0.189 (18.9)
B.	pH 5 buffer of A	8.01	0.385 (35.4)	0.238 (21.9)	0.222 (20.4)
C.	$NaClO_2$ of A	33.6	0.561 (37.3)	0.294 (19.5)	0.0192 (1.3)
D.	TFA of C	44.0	0.869 (32.3)	0.143 (5.3)	0.0081 (0.3)
E.	TFA of B	38.3	0.613 (34.8)	0.0646 (3.7)	0.320 (18.1)
F.	$NaClO_2$ of B	29.7	0.548 (35.4)	0.303 (19.6)	0.0308 (2.0)
G.	NaOH of B	35.3	0.537 (32.0)	0.115 (6.8)	0.269 (16.0)
H.	$NaClO_2$ of E	37.7	0.853 (30.2)	0.105 (3.7)	0.0287 (1.0)

TABLE 4
Extracted Pine-Weight Fraction Composition

	Treatment	Weight loss (%)	Weight fraction (weight % of A) Cellulose	Hemicellulose	Lignin
A.	Benzene-ethanol	---	0.375 (37.5)	0.268 (26.8)	0.263 (26.3)
B.	pH 5 buffer of A	9.2	0.406 (36.9)	0.288 (26.2)	0.315 (28.6)
C.	$NaClO_2$ of A	35.8	0.536 (34.4)	0.280 (18.0)	0.0724 (4.7)
D.	TFA of F	48.5	0.956 (32.0)	0.0428 (1.4)	0 (0)
E.	TFA of B	36.8	0.589 (33.8)	0.0998 (5.7)	0.366 (21.0)
F.	$NaClO_2$ of B	28.4	0.570 (37.1)	0.290 (18.9)	0.0872 (5.7)
G.	NaOH of B	13.9	0.460 (36.0)	0.0219 (17.8)	0.339 (26.5)
H.	$NaClO_2$ of E	35.6	0.971 (35.9)	0.0460 (1.7)	0.0353 (1.3)

benzene-ethanol-extracted plant material (A in Table 1). The weight percent of A listed in Tables 1-4 was calculated by multiplying the weight fraction composition with the sample weight recovered after a series of treatments. The benefit of the second set of data is that the amount of each component lost in a given treatment can be readily seen. Also, if only a small portion of a component remained, it may not have been representative of the whole component. In Table 1, for example, the cellulose contents in both G and H were high. The second part of the table showed that only half of the cellulose was retained in sample G, but most of it was retained in sample H. Therefore, H would likely be a better choice than G for a high cellulose-containing sample. The analysis for cellulose depends on its conversion to glucose. Because $NaClO_2$-treated samples C, F and H in Table 1 retained cellulose in a relatively unchanged form (readily hydrolyzed to glucose) compared to sample B which had not been $NaClO_2$ treated, we concluded that the cellulose in $NaClO_2$-treated samples was representative of whole cellulose in wheat straw.

TFA hydrolysis of hemicelluloses has been described in the literature for wheat straw[11] but not for the other major plant types. Data for samples B and E in Table 1 showed that more than 80% of the hemicellulose, principally xylans, had been removed by TFA hydrolysis. All xylose was not recovered. Some was converted to furfuraldehyde as reported earlier.[11] The TFA

extracts of kenaf, oak and pine were passed through an ion exchange column or distilled to remove TFA and then analyzed for sugars by HPLC or as aldononitriles by gas chromatography-mass spectroscopy. Kenaf extracts consisted of approximately 70% xylose, 15% glucose, 5% mannose, with traces of arabinose and galactose. Oak extracts consisted of approximately 50% xylose, 20% mannose, 15% glucose, 6% arabinose, and 3% galactose. Pine extracts consisted of approximately 40% mannose, 23% xylose, 15% glucose, 10% galactose, and 9% arabinose. Sugars were the only significant products found after TFA was removed. Compositional data summarized in Tables 1-4 show good variation for solid state analysis. Because chemically derived residues were from the same starting sample, the morphologies of the residues were quite similar within each plant group.

FTIR Spectra of In Situ Plant Polymers

Simple subtraction of two spectra has often been useful to observe functional group changes in a degraded material.[17] It is also useful for obtaining an approximate spectrum of the major component in a mixture. For example, wheat straw spectrum B (Fig. 1) of a sample with weight fraction composition 0.521 cellulose, 0.398 hemicellulose, 0.0170 lignin, and 0.0671 ash was subtracted from spectrum A (Fig. 1) of a sample with composition 0.356 cellulose, 0.316 hemicellulose, 0.200 lignin, and 0.0769 ash. An arbitrary factor was applied to A to maximize known lignin absorbances. The resulting difference spectrum was

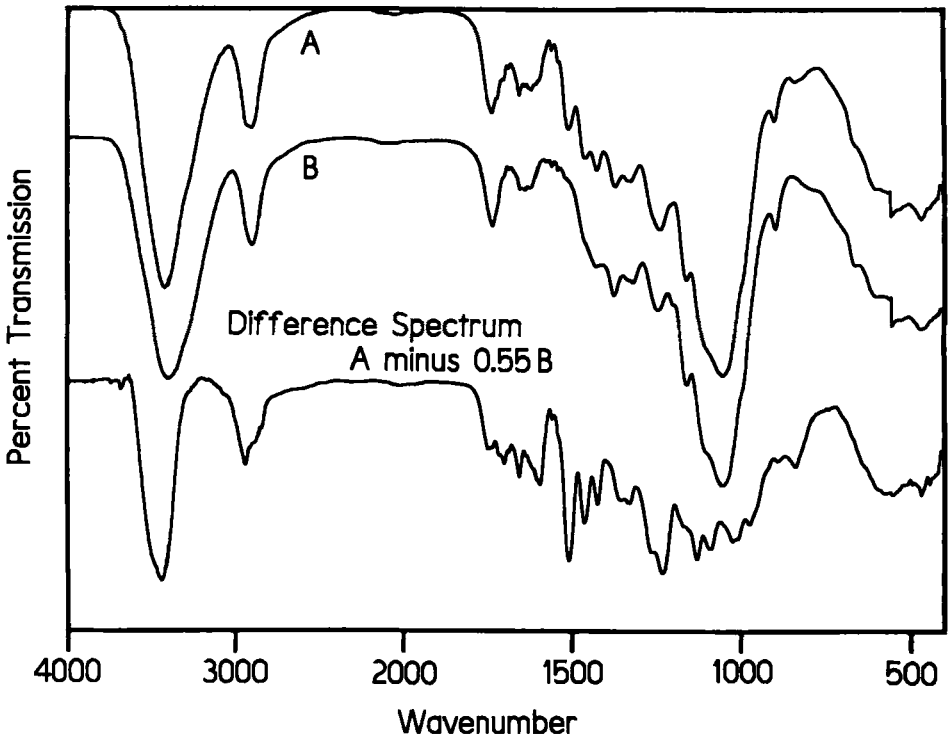

Figure 1. Buffer-extracted wheat straw (A) minus $NaClO_2$-extracted wheat straw (B) to yield an estimated spectrum of lignin (A-.55XB).

principally that of lignin (Fig. 1). The difference spectrum contains contributions from components other than lignin, but this method is useful as a first approximation of the in situ wheat straw lignin spectrum.

A more objective and cleaner spectrum of wheat straw lignin can be obtained by combining spectra of four samples of different

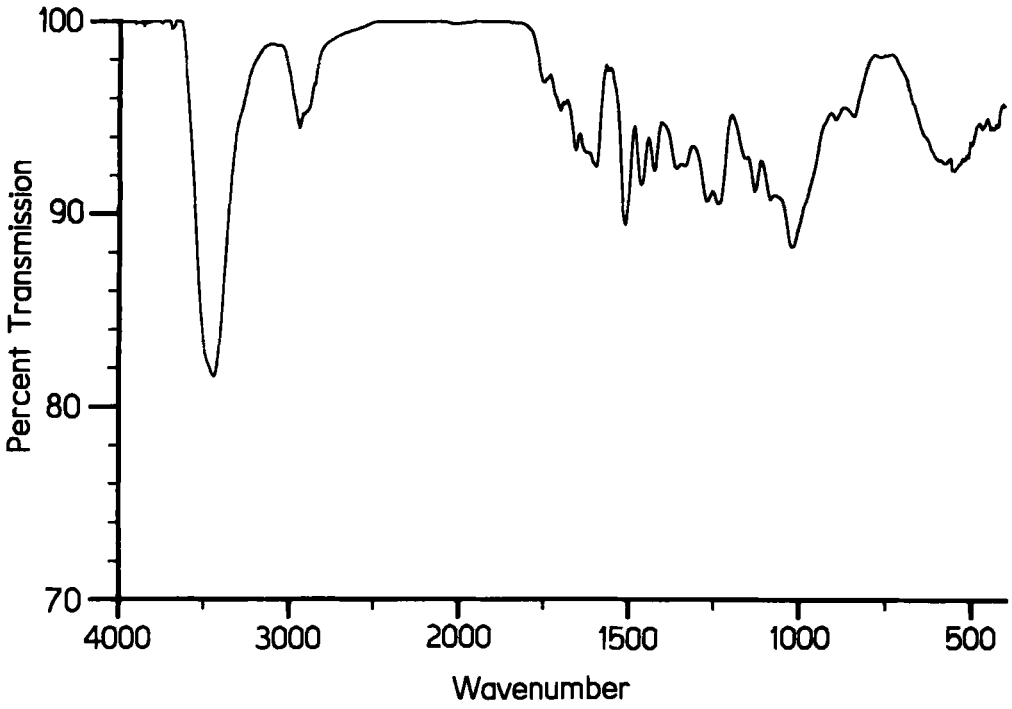

Figure 2. A calculated wheat straw lignin FTIR spectrum by combining 4 spectra with coefficients found in the inverse of the concentration matrix.

compositions, and by using factors determined by the inverse concentration matrix. Figure 2 shows the spectrum of wheat straw lignin obtained by this method. Least squares matrix methods have no advantage over simpler inverse matrix determinations for four components in four samples.

Least squares combination of eight wheat straw samples described in the concentration matrix in Figure 3 gave equation 1

Composition Matrix for Spectra

E	0.498	0.3320	0.0085	0.0677
C	0.728	0.0710	0.0065	0.0792
A	0.356	0.3160	0.2000	0.0769
F	0.518	0.0589	0.2750	0.0567
B	0.521	0.3980	0.0170	0.0671
D	0.919	0.0637	0.0100	0.0025
G	0.802	0.0992	0.0250	0.0680
H	0.348	0.2740	0.1760	0.0680

$AC^T(CC^T)^{-1} = K$

$C^T(CC^T)^{-1}$ Matrix

-3.6406E-2	0.8711	-0.9583	0.8350
-2.5165E-2	-1.6366	-1.4526	9.8007
-0.1708	0.6052	1.1043	0.1416
0.1475	-0.7828	2.3678	-0.2581
2.6931E-2	1.4743	-0.7433	-1.9069
0.9727	0.8615	0.6990	-11.2535
0.1923	-0.9810	-0.8491	4.8327
-0.1189	0.5017	0.9796	-2.2753E-2

2.368 F - 1.453 C + 1.104 A - 0.9584 E + 0.9797 H - 0.8492 G + 0.6991 D - 0.7433 B = Lignin

Figure 3. Concentration (weight fraction composition) matrix used to determine least squares coefficient matrix and the equation for combining the eight spectra to obtain a spectrum of wheat straw lignin.

for combining spectra based on factors taken from the least squares matrix $C^T(CC^T)^{-1}$.

However, the combination of eight spectra by such tedious data manipulation gave the same spectrum for wheat straw lignin as was obtained in 15 min with the CALPURE program.

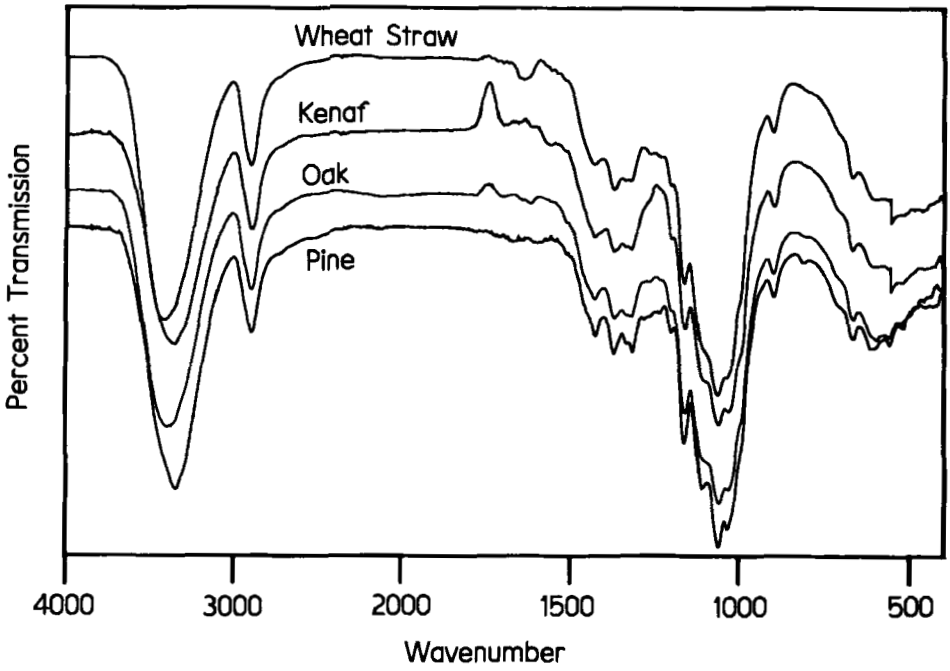

Figure 4. FTIR spectra of in situ celluloses in plants.

By using the CALPURE program with the weight fractions of each component as obtained earlier, the spectra of the individual components of wheat straw, kenaf, oak, and pine were determined and are shown in Figures 4-6. Cellulose spectra (Fig. 4) derived from the four plant materials are quite similar. Pine cellulose absorption bands are better resolved and may be the result of a more ordered environment (i.e. more crystalline cellulose). A negative absorbance was observed for kenaf cellulose (Fig. 4) at 1741 cm^{-1}. Hemicellulose (10%) remained in samples treated

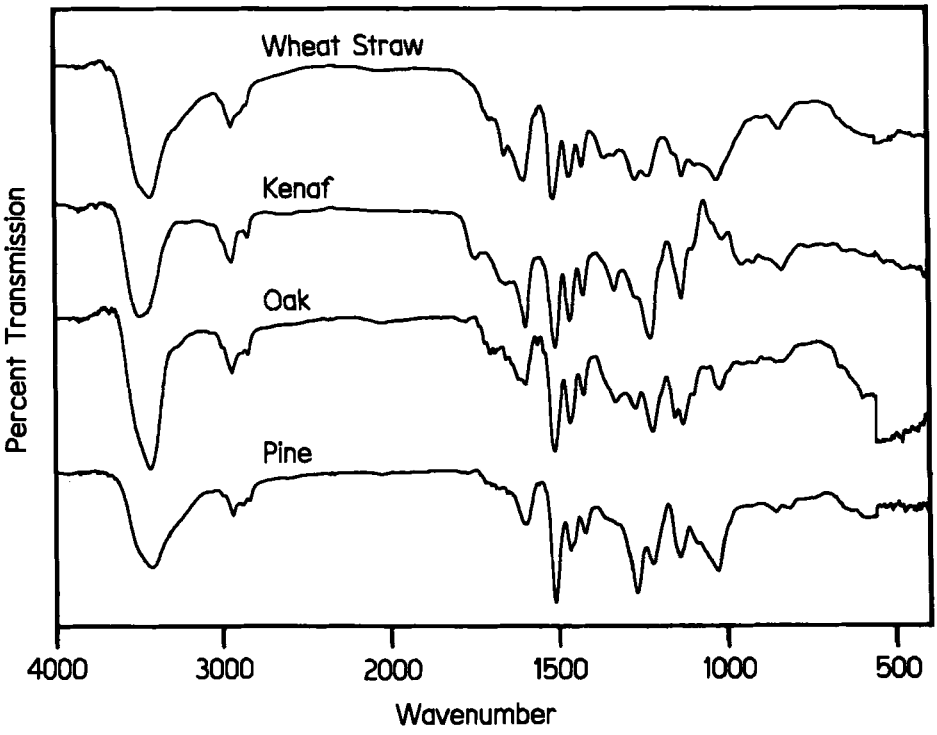

Figure 5. FTIR spectra of in situ lignins in plants.

with $NaClO_2$ and TFA, but no ester absorbance at 1740 cm^{-1} was observed. Initially, kenaf hemicellulose was highly esterified (Fig. 6). Apparently, some ester groups had been hydrolyzed during treatments. The method of combining spectra required a consistent spectrum of hemicellulose in all samples. Because residual hemicellulose had no ester absorbance, the cellulose difference spectrum showed a negative band which exactly compensated for the hemicellulose ester carbonyl.

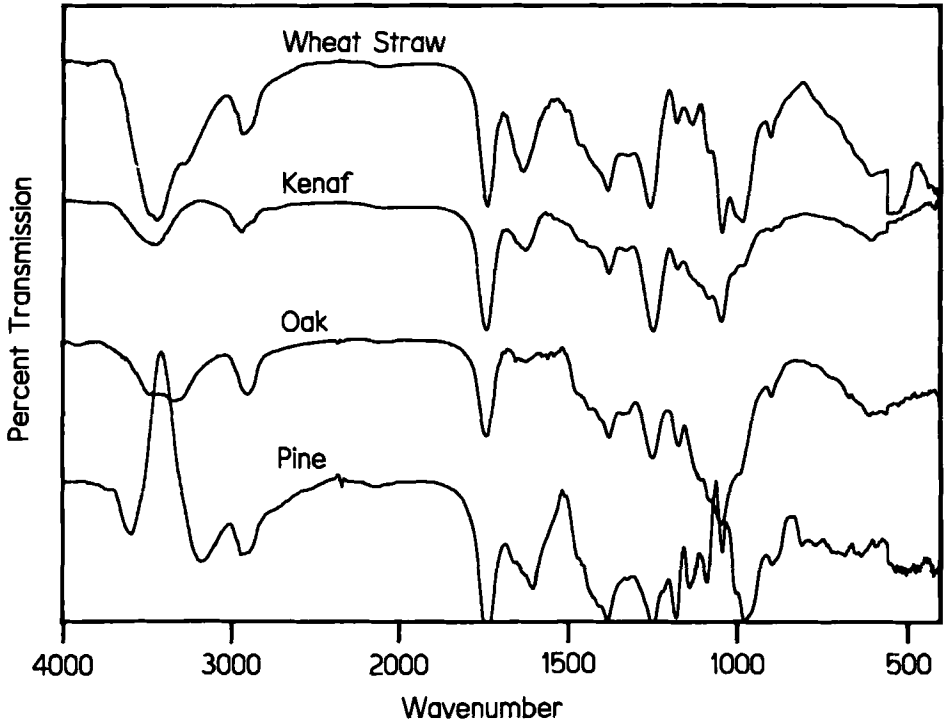

Figure 6. FTIR spectra of in situ hemicelluloses in plants.

The spectra of lignins (Fig. 5) agree well with literature spectra of milled wood lignins. The pine lignin was similar to published spectra of pine milled wood lignin[18, 19] and of lignin determined by difference spectra of thin films of whole wood and holocellulose.[20] The oak lignin spectrum in Figure 5 was similar to published spectra of milled wood lignin from various hardwoods,[21] but the absorbances in the 1000 cm^{-1} to 1250 cm^{-1} region were sharper and better defined than those reported in the literature. Kenaf lignin, although previously

shown to be a typical syringyl-guaiacyl lignin by chemical analysis,[22] had a spectrum somewhat different from that of oak lignin. Ester functionality (1741 cm^{-1}) in the kenaf lignin spectrum indicates more acetate or phenolic acid esterification than for oak. Absorbances in the 1710 cm^{-1} region have been attributed to both carboxylic acid groups and nonconjugated keto groups.[18]

In contrast to previous findings[23, 24] for grass milled wood lignins, wheat straw lignin had no ester absorbance at 1740 cm^{-1}. Milled wood lignins of grasses are less representative of true in situ lignins than are milled wood lignins from woody plants. Grasses contain less lignin than woody plants, and grass lignins have lower molecular weights. Consequently, in contrast with woody plants, the milled wood isolation procedure provides a lower percentage of native lignin from grasses. Such lignins are usually contaminated with hemicellulose and other plant components.[24] Thus, we feel that the spectrum shown in Figure 5 is more accurate in some aspects but still similar to published spectra[24] of wheat straw ligni..

Derived hemicellulose spectra were consistent with partially esterified polysaccharides in the 2000 to 700 cm^{-1} region (Fig. 6). Only pine hemicellulose is disappointingly negative in the 3400 cm^{-1} region. We have tested a number of hypotheses to improve our results, but the spectrum of pine hemicellulose remains anomalous.

One test for validity of the pure component spectra was reconstruction of mixture spectra from the three component spectra combined in appropriate ratios. Except for the NaOH-treated sample, spectra for all kenaf mixtures could be reconstructed from combinations of the three component spectra despite the small negative absorbance at 1741 cm^{-1} for cellulose. Sodium hydroxide (12%) extracted samples, in general, are chemically changed. Their spectra could not be reconstructed from component spectra. Pine, in particular, showed distinct increases in absorbances at 1593 cm^{-1} relative to other lignin absorbances.

As with all KBr disc techniques, the questions of undispersed sample, chemical changes of the sample in the disc and reproducibility of instrument variables arise. The small amount of scattering at short wavelengths, lack of scattering between 2000 cm^{-1} and 600 cm^{-1}, and reproducibility of our spectra give us confidence that large scattering particles are not present. Further, samples of oak mixed in the Wig-L-Bug for total times of 100, 150, and 220 s all gave the same spectrum within 5% and showed no trend toward increased absorbances with increased mixing time. Twenty-one spectra showed no significant qualitative changes upon remeasuring 6 weeks later. Absorption intensities increased in all spectra by about 7% due to variations in the source or detector response. For this reason, all spectra for a given set (all pine or all oak, etc.) were

taken over a short time (usually 2-3 days). The 25 pine spectra were acquired in less than 8 hr.

Because molar absorbances of cellulose for pine, oak, kenaf, and wheat straw were not identical, we can assume some difference in structure, conformation, and/or dispersibility in KBr among plant types. For the future, we suggest two possible improvements in our method. First, an internal standard of 0.3% or less of KCN could be incorporated in the KBr to allow spectra to be compensated for machine variables. After normalization the KCN spectrum can be subtracted from the total spectrum. KCN should interact very little with biomass samples in KBr in the less than 24 hr needed to accumulate spectra, but longer times may lead to reactions with the KCN.[25] We have found that KCN is acceptable for this use. The second suggestion is that an internal standard could be attached to the biomass to determine and compensate for dispersibility. We have had limited success with deuterotrimethyl silylating agents but believe that these offer great potential.

The FTIR spectra presented here are the most complete infrared spectra obtained for individual biomass components within the plant matrix. The method used is generally applicable to spectra of solids where absorbance is proportional to concentration, and can be used even if software is not available on the instrument for calculating pure component spectra. Spectral enhancement of the lignin spectra in Figure 5 by second

derivative, deconvolution, and Gaussian curve-fitting is a useful method for structural interpretation.[26]

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