

FT-IR Determination of Lignin Degradation in Wheat Straw by White Rot Fungus *Stropharia rugosoannulata* with Different Oxygen Concentrations

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FT-IR spectra were obtained of a series of wheat straws subjected to different degrees of degradation by the white rot fungus *Stropharia rugosoannulata*. Spectra showed significant differences in the region (1400-1750 cm^{-1}) assigned to aromatic absorptions of lignin. The spectrum of the most degraded lignin-containing straw resembles closely the spectrum of lignin prepared by 72% H_2SO_4 extraction. The fungal degradation of lignins in straw can be monitored by comparison of the FT-IR spectra of the different straw preparations.

Lignocellulosic materials, such as straws, are a major economic concern, and attempts are being made to convert the lignin-containing materials into useful forages for ruminants (Sundstol and Owen, 1984). However, lignins in straw and their constituent phenolics affect carbohydrate availability such that low utilization of straws as forages for ruminant animals has resulted (Jung and Fahey, 1983).

The amount and structural types of lignin influence the digestibility of the forages (Akin, 1979); therefore, various methods of lignin degradation have been investigated. These methods are mainly chemical hydrolysis using basic media (dilute NaOH, ammonia, urea) (Bolsen et al., 1983) or acidic media (SO_2) (Van Soest and McBurney, 1985) or microbiological treatments employing fungi (Kamra and Zadrazil, 1986).

Lignin appears to be defined by the methods used to isolate the material, and gravimetric measurements have generally been used by forage scientists (Goering and Van Soest, 1970). Inconsistent results are obtained when digestibility is related to lignin content determined by chemical methods (Barton et al., 1976).

Infrared spectroscopy (IR) has been useful in studying lignin structure and in ascertaining differences in lignins prepared by different procedures (Hergert, 1971). However, the use of dispersive IR instruments has provided only broad and ill-defined bands in spectra of complex natural products (Gerasimowicz et al., 1986). Since Fourier transform infrared spectroscopy (FT-IR) provides a much higher signal to noise ratio and improved accuracy of assignments of frequencies, studies have been made of lignin polymer models and of wheat lignin (Faix, 1986; Jung and Himmelsbach, 1989). Now, this technique is to be applied to a study of lignin in straw treated with fungi compared to lignin obtained by standard techniques. FT-IR techniques will be used to monitor relative changes in lignin in straw caused by different degrees of fungal degradation. The amount of information obtained by FT-IR measurements will be increased by use of second-derivative spectroscopy (Lee et al., 1985).

MATERIALS AND METHODS

Straw Treatment. Cultures of *Stropharia rugosoannulata* Farlow ex. Murrill were maintained on malt agar at 25 °C. Fifteen

grams of wheat straw (particle size <1 mm) and 20 mL of water were added to 500-mL Erlenmeyer flasks, and the mixture was autoclaved for 30 min at 121 °C. A 6-day submerged culture of fungus in 2.5% malt extract medium was diluted two times with autoclaved, distilled water, and 25 mL of this diluted culture was added aseptically to each flask. Thirty flasks were connected in a chain with polyethylene tubing so that gas could be admitted at one end of the chain and exhausted at the other end (Figure 1). Before the start of incubation, air was displaced from the chain of flasks by CO_2 . Oxygen (2 L/day) was passed continuously through the chain of 30 flasks. The loss of organic matter (LOM) and of lignin (LL), lignin content (LC), and in vitro digestibility (Tilley and Terry, 1963) were estimated after 40 days of incubation at 25 °C following Zadrazil and Brunnert (1980). In vitro digestibility and lignin content of the control (LCC) were 28.10 and 20.87%, respectively. LL was calculated as follows: $\text{LL} = 100 - (\text{LC}/\text{LCC})(100 - \text{LOM})$. Where slight increases were obtained (samples 13 and 21), LL was assumed zero.

Sample Preparation. Straw lignin was prepared according to the Goering and Van Soest procedure by treatment with 72% H_2SO_4 for 3 h. Samples were ground in a UDY cyclone mill to 40 mesh.

FT-IR Spectroscopy. The ground samples were incorporated into KBr (2:50) and pressed into a 3-mm pellet with use of a Qwik Handi-Press. The infrared spectrum from 4000 to 700 cm^{-1} was obtained on a Nicolet Model 60-SX FT-IR spectrometer operating at a nominal resolution of 4 cm^{-1} . The spectrometer was equipped with a globar source and a liquid nitrogen cooled Hg/Cd/Te (MCT-A) detector. A total of 32 interferograms were collected, phase-corrected, and apodized by the Happ-Genzel function prior to Fourier transformation. Duplicate experiments were performed. Second-derivative spectra were obtained with use of the Nicolet software DR2.

HPLC Analysis. Ground samples (200 mg) were extracted for free phenolics with methanol (5 mL) overnight at room temperature. The extract was diluted with water (1:1), filtered through a 0.45- μm filter, and injected (0.1 mL) into a Varian 5060 liquid chromatograph equipped with a Waters 450 variable-wavelength detector set at 280 nm, a Rheodyne 7125 sample injector, a Hewlett-Packard 3390A reporting integrator, and a Polymer Laboratories Ltd. PLRP-S styrenedivinylbenzene (150 \times 4.6 mm (i.d.), 5 μm) column (Buta, 1984). A gradient elution with 6 \times 10⁻⁵ M HCl/ CH_3CN (from 95:5 (0-5 min) to 80:20 (5-30 min) and held for 10 min) was used at 1.0 mL min^{-1} . Peaks were quantified by external standard.

RESULTS AND DISCUSSION

The chain of flasks connected in series under oxygen stream was used as a model to simulate conditions within a solid-state fermentor.

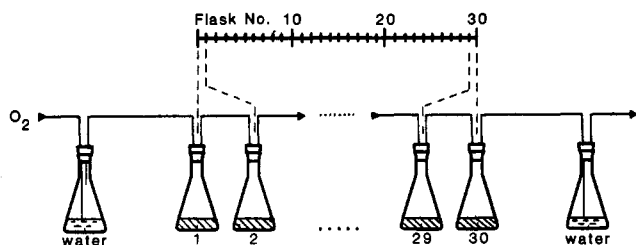
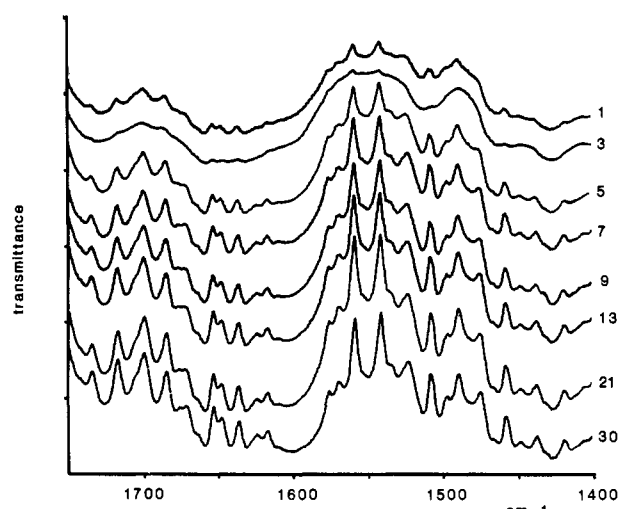
Eight samples of degraded wheat straw were selected from the chain of flasks to best show the changes in lignin content that had occurred during the fungal treatment. The degradation of lignin by the fungus was highest in the first 10 flasks (Table I). Further down the chain, no loss of lignin was observed and there was a decrease in digestibility. Growth of *S. rugosoannulata* on wheat straw and

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Table I. Degradation of Wheat Straw by White Rot Fungus *S. rugosoannulata* and Oxygen Treatment (2 L/day)

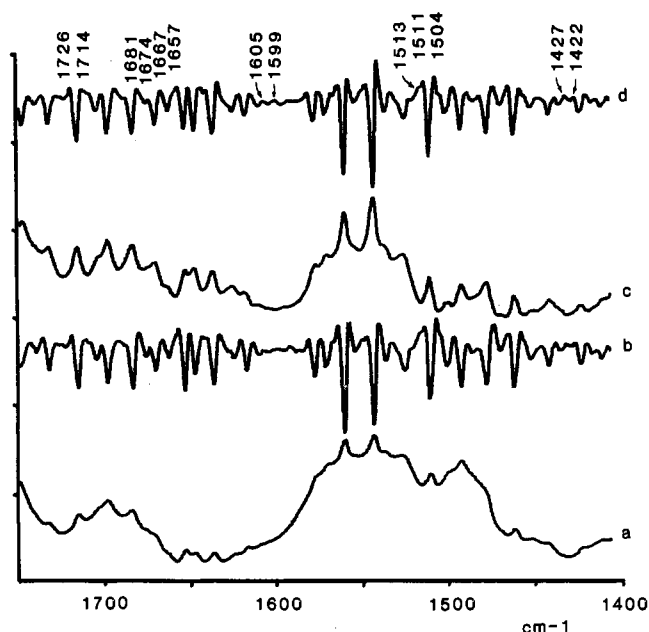
sample	loss of org matter ^a	in vitro digestibility ^a	change of in vitro digestibility ^{a,b}	lignin content ^a	loss of lignin ^{a,c}
1	28.67	54.69	26.59	16.97	42.01
3	28.00	49.10	21.00	19.11	34.05
5	24.00	39.29	11.19	20.64	24.84
7	18.00	31.74	3.64	21.38	16.04
9	15.33	20.06	-8.04	24.08	2.31
13	13.33	10.18	-17.92	24.96	0.00
21	12.67	8.54	-19.56	25.07	0.00
30	12.00	14.56	-13.54	23.61	0.00

^a Percent dry matter. ^b Control without fungal treatment is assigned the value 0 (actual value 28.10%). ^c Control without fungal treatment is assigned the value 0 (actual value 20.87%).

**Figure 1.** Diagram of experimental apparatus.**Figure 2.** FT-IR spectra of wheat straw degraded by fungal treatment with white rot fungus *S. rugosoannulata*. Treatment no. 1 is the most degraded, and treatment no. 30 is the least degraded. Spectra were placed in this format to allow comparison of relative absorbances.

degradation of organic matter decreased with increasing chain length from the first flasks in the chain. There was an increase in digestibility of wheat straw fermented with *S. rugosoannulata* only in the first 10 flasks of the chain at the low rate of aeration. However, when either 9.5 L of oxygen/day or 9.9 L of air/day (Zadrazil and Kamra, 1988) was passed through the chain, there was an increase in digestibility of wheat straw in all flasks of the chain. In this case the air flow through the substrate was sufficiently high to displace enough of the gaseous metabolites to achieve this increased digestibility.

The FT-IR spectra of these eight samples selected from the chain of flasks are shown in Figure 2. The region from 1800 to 4000 cm^{-1} did not show any useful information other than broad hydroxyl and aliphatic C-H absorption, which will not be considered further. The aromatic absorption region of the spectra contains bands assigned to lignin (Hergert, 1971). The relative absorbances of the spectra of the samples are changed significantly, apparently dependent upon the degree of degradation caused by the different degrees of oxygenation where the most

**Figure 3.** (a) FT-IR spectrum of wheat straw treatment no. 1. (b) Second derivative of spectrum a. (c) FT-IR spectrum of 72% H_2SO_4 lignin. (d) Second derivative of spectrum c.**Table II. Assignments of FT-IR Absorption Bands (cm^{-1})**

lit. ^a	72% H_2SO_4 lignin ^b	treatment no. 1 straw ^b	assignment
1720	1726	1729	C=O aliph carboxyl str
1710-1715	1714	1712	C=O sat open-chain ketone str
1660-1680	1657, 1667	1657, 1666	C=O conj ketone str
1595-1605	1674, 1681	1674, 1681	
	1599, 1605	1597, 1606	aromatic skeletal vibrations
1505-1515	1504, 1511	1504, 1511	aromatic skeletal vibrations
1425-1430	1513		
	1422, 1427	1422, 1427	aromatic skeletal vibrations

^a See Hergert (1971). ^b Absorption maxima from the second derivative of the spectra.

degraded sample (no. 1) has the smallest absorbance and the least degraded sample (no. 30) has the highest absorbance. The duplicate experiments gave superimposable spectra. Since a commonly used method of lignin preparation is the treatment with 72% H_2SO_4 (Goering and Van Soest, 1970), the spectra of this lignin (Figure 3) were used as a comparison to the various lignin-containing straws resulting from the fungal treatments. The spectrum of treatment no. 1 (Figure 3) resembled that of the reference lignin most closely.

To assist in determining the absorption maxima of the straw spectra that would correspond to maxima of functional groups found in lignin polymer, the second-deriva-

Table III. Quantitation of Free Phenolics from Degraded Wheat Straw by HPLC^a

compound	treatment no.				
	1	5	9	21	30
syringic acid	0.0221	0.0150	0.0071	0.0024	<0.0005
vanillic acid	0.0130	0.0102	0.0043	0.0024	0.0020
<i>p</i> -coumaric acid	0.0098	0.0098	0.0070	0.0063	0.0063
ferulic acid	0.0048	0.0039	0.0015	0.0015	0.0020
vanillin	0.0056	0.0047	0.0031	0.0031	0.0031
<i>p</i> -hydroxybenzaldehyde	0.0017	0.0015	0.0010	0.0010	0.0010

^a Values expressed as percent of dry matter.

tive spectra were obtained. A very close correspondence of the bands (second derivatives) in the 1400–1750-cm⁻¹ region can be seen in the spectra of the reference lignin and treatment no. 1 lignin-containing straw (Figure 3). Bands assignable to lignin moieties are shown in Table II. Bands at ca. 1715 cm⁻¹ are assignable to carbonyl stretching of saturated open-chain ketones while the series of bands at 1660–1680 cm⁻¹ are assigned to conjugated ketone carbonyl stretching. Aromatic skeleton vibrations in lignin are assigned at 1425–1430, 1505–1515, and 1595–1605 cm⁻¹. The band at 1725 cm⁻¹ may be assigned to aliphatic carboxyl groups such as free acids present in lignin.

The correspondence of bands and absorbance in the spectra of the treatment no. 1 lignin-containing straw and the reference 72% H₂SO₄ lignin indicate that similar lignins have been produced by the different processes, at least in terms of the FT-IR spectra. It is very difficult to describe lignin as a well-defined chemical product (Sarkanen and Ludwig, 1971). The 72% H₂SO₄ lignin is considered to be composed of a large number of condensed aromatic moieties, and a similar spectrum was obtained for the most degraded lignin-containing straw (no. 1). A major loss of aromatic absorbance was seen in the FT-IR spectrum of the most degraded treatment (no. 1) compared to that of the least degraded treatment (no. 30).

To attempt to determine whether these changes in aromatic absorbance were associated with cleavage of lignin polymers in straws, FT-IR spectra of the degraded straws were obtained after methanol extraction. These spectra were identical with those of the unextracted materials. Also, HPLC analysis of the free phenolics of these various samples was done, and the results are shown in Table III. The free phenolics are readily extracted with methanol from the insoluble polymerized matrix. The largest quantities of free phenolic acids and aldehydes were extracted from the most degraded lignin-containing straw (treatment no. 1), and the smallest quantities of these compounds were extracted from the least degraded material (treatment no. 30). In the most degraded straw lignin, the percentages of extractable phenolic acids have increased much more than the percentage of those compounds extracted from the least degraded treatment. Although the absolute quantities of free phenolics are small compared to what should be obtained from the catabolic degradation of lignin, the results of the analysis of free phenolics corroborate the interpretation of FT-IR spectra, where it is concluded that fungal degradation of the straw has diminished the quantities of aromatic moieties in the lignin polymer portion of the straw.

Registry No. Lignin, 9005-53-2; syringic acid, 530-57-4; vanillic acid, 121-34-6; *p*-coumaric acid, 7400-08-0; ferulic acid, 1135-24-6; vanillin, 121-33-5; *p*-hydroxybenzaldehyde, 123-08-0.

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