

STRUCTURAL ALTERATION OF LIGNIN AFTER SOLID-STATE FERMENTATION OF WHEAT STRAW BY LIGNOCELLULOSE-DEGRADING FUNGI

G. Almendros¹, A.T. Martínez², R. Fründ,³ and F.J. González-Vila⁴

- 1 Instituto de Edafología y Biología Vegetal, (CSIC). Serrano 115. 28006-Madrid, Spain.
- 2 Centro de Investigaciones Biológicas, (CSIC), Velázquez 144, 28006-Madrid, Spain
- 3 Institut für Biophysik und physikalische Biochemie. Regensburg Uni, P.O. Box 397, D-8400 Regensburg, West Germany.
- 4 I.R.N.A., (CSIC). P.O. Box 1052, 41080-Sevilla, Spain.

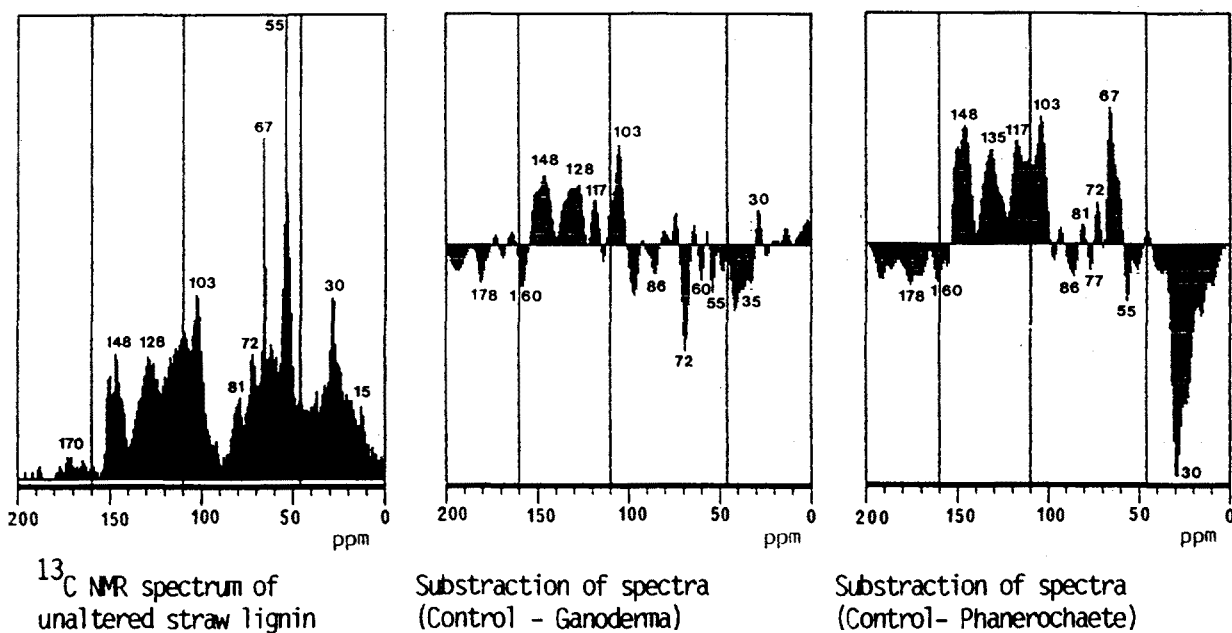
This study is about the effect of five species of lignin and cellulose-degrading fungi on the chemical characteristics of lignin after 45-day solid-state fermentation of wheat straw. The species inoculated were *Chaetomium virescens*, *Trichoderma reesei*, *Phanerochaete chrysosporium*, *Fomes fomentarius* and *Ganoderma applanatum*. The degraded straw yielded between 3.6% (*F. fomentarius*) and 7% (*C. virescens*) lignin upon extraction with dioxane-water-hydrochloric acid. This lignin-fraction was studied by chemical methods and ¹³C NMR spectroscopy under quantitative acquisition conditions.

Lignins extracted from fermented straw showed increased values for molecular size, O/C atomic ratio (excepting *C. virescens* and *T. reesei*) and specific extinction. A direct comparison between the NMR spectral patterns was achieved from the differential spectra, which was obtained by subtracting the spectra of the lignins altered by the fungi from the spectrum of the unaltered straw lignin.

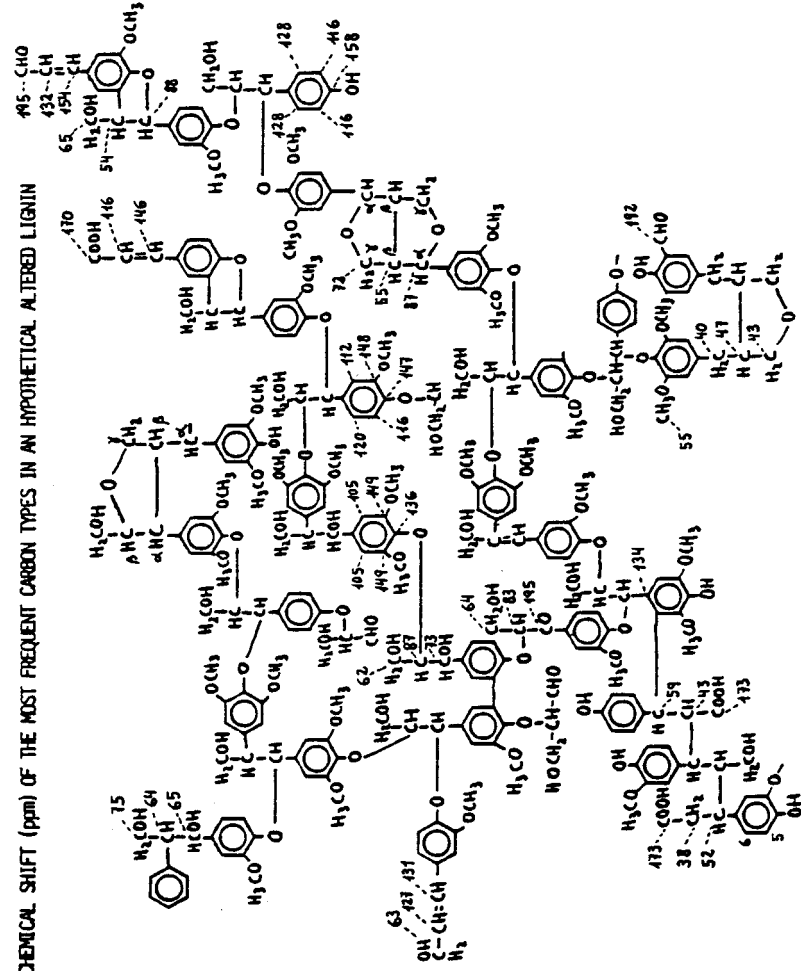
The altered lignins presented a relative increase in the amounts of carboxyl C's (200-160 ppm) and alkyl C's (46-5 ppm), suggesting respectively the oxidative alteration of lignin and the incorporation or coextraction of fungal lipids in the lignin fraction.

The lignins degraded by the white-rot fungi *G. applanatum* and *P. chrysosporium* presented decreased amounts of aryl C's, and the latter species caused an additional decrease in the intensity of the resonances produced by the side-chain phenylpropane units and the lignin-hemicellulose links. *Fomes fomentarius* produced simultaneous modification in the spectral regions corresponding to the aromatic and the O-alkyl structures.

The cellulolytic species *C. virescens* and *T. reesei* were responsible for a significant carboxylation of the lignin fraction, but did not cause significant changes in the 160-110 ppm aromatic region. These species produced the most important demethoxylation and selective decrease in the intensity of the resonances assigned to residual carbohydrate linked to lignin.



CHEMICAL SHIFT (ppm) OF THE MOST FREQUENT CARBON TYPES IN AN HYPOTHETICAL ALTERED LIGNIN



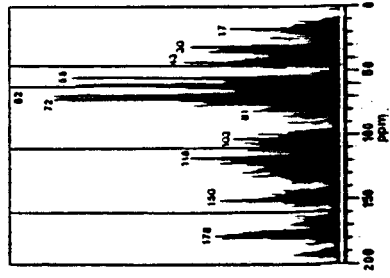
Assignment of lignins in ¹³C NMR spectra of lignin fractions degraded by fungi

137	OH, terminal
138	C-1 lignin
139	C-1 lignin and C-2 acetyl lignin
140	C-1 lignin in 4-OH-2,6-DiMe
141	C-1 lignin in 4-OH-2,6-DiMe
142	C-1 lignin in 4-OH-2,6-DiMe
143	C-1 lignin in 4-OH-2,6-DiMe
144	C-1 lignin in 4-OH-2,6-DiMe
145	C-1 lignin in 4-OH-2,6-DiMe
146	C-1 lignin in 4-OH-2,6-DiMe
147	C-1 lignin in 4-OH-2,6-DiMe
148	C-1 lignin in 4-OH-2,6-DiMe
149	C-1 lignin in 4-OH-2,6-DiMe
150	C-1 lignin in 4-OH-2,6-DiMe
151	C-1 lignin in 4-OH-2,6-DiMe
152	C-1 lignin in 4-OH-2,6-DiMe
153	C-1 lignin in 4-OH-2,6-DiMe
154	C-1 lignin in 4-OH-2,6-DiMe
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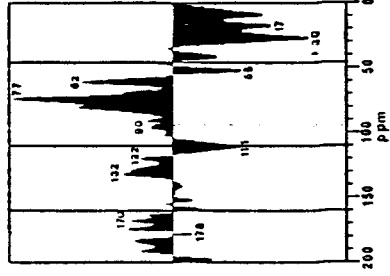
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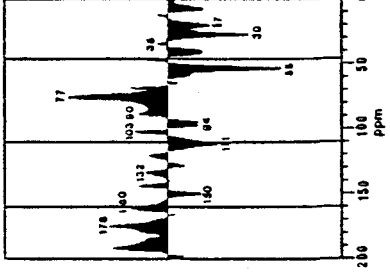
LMW - Products



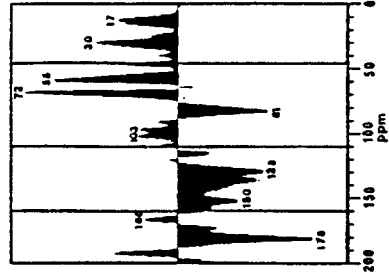
^{13}C NMR spectrum of acid-soluble straw lignin (low molecular weight fraction)



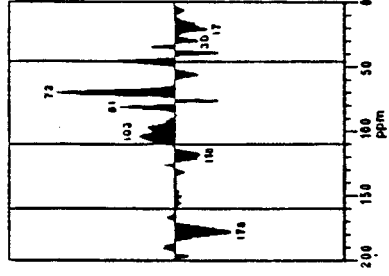
Subtraction of spectra (Control) - Trichoderma



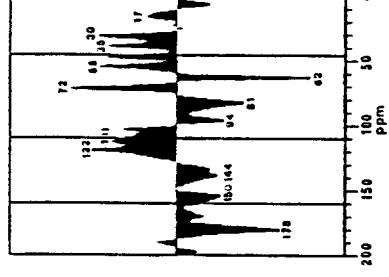
Subtraction of spectra (Control) - Chaetomium



Subtraction of spectra (Control) - Phanerochaete



Subtraction of spectra (Control) - Ganoderma

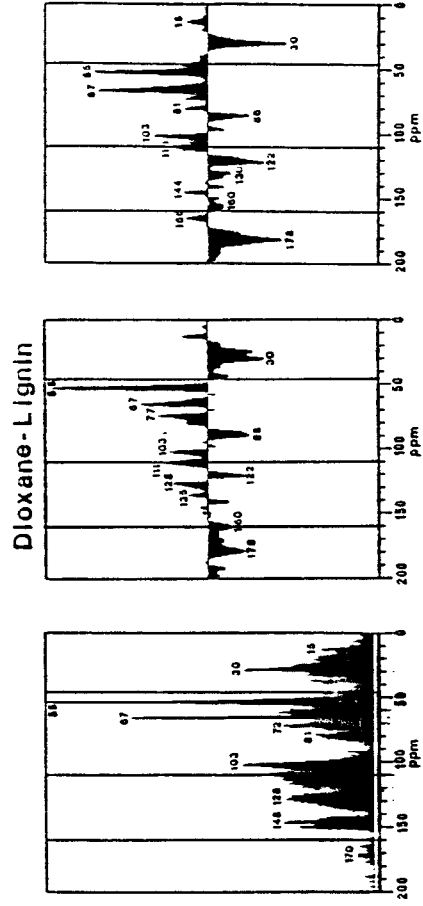


Subtraction of spectra (Control) - Fomes

An acid-soluble, low molecular weight lignin fraction was also isolated by adsorption chromatography on polyvinylpyrrolidone. This lignin fraction presumably includes a series of oligomer degradation products, in the case of the samples degraded by the fungi.

A relative increase in the amounts of polymethylene compounds in this lignin fraction was observed with the cellulolytic genus Trichoderma and Chaetomium (a probable accumulation of fungal lipids). The acid-soluble lignin fraction released by these species also presented a decreased carboxylation and comparatively low content in O-alkyl structures, mainly in the aryl ethers producing resonances at about 77 ppm. These lignin fractions also presented an increased content in methoxylated (55 ppm) and guaiacyl (111 ppm) structures.

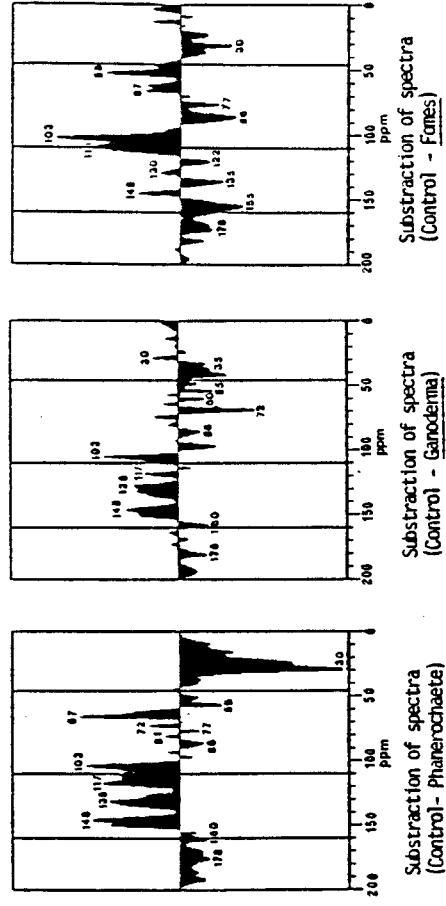
The contrary tendency was observed with the ligninolytic species. After degradation, the acid soluble lignin fractions presented an increased carboxylation and aromaticity and specific changes in the side-chain structures.



¹³C NMR spectrum of untreated straw lignin

Subtraction of spectra (Control) - Trichoderma

Subtraction of spectra (Control) - Chaetomium



Subtraction of spectra (Control) - Phanerochaete

Subtraction of spectra (Control) - Ganoderma

Subtraction of spectra (Control) - Fomes

The altered lignins presented a relative increase in the amounts of carboxyl C's (200-160 ppm) and alkyl C's (46-5 ppm), suggesting respectively the oxidative alteration of lignin and the incorporation or coextraction of fungal lipids in the lignin fraction.

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