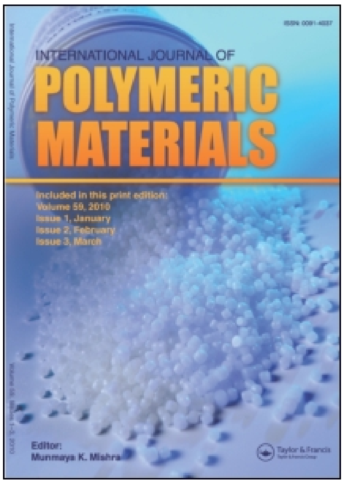

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CHARACTERIZATION BY INFRARED SPECTROMETRY OF LIGNINS OF THREE EUCALYPTUS SPECIES

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CHARACTERIZATION BY INFRARED SPECTROMETRY OF LIGNINS OF THREE EUCALYPTUS SPECIES

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Lignin is, after cellulose, the main component of most vegetable fibers. The term lignin is very widely used and it is considered as a mixture of ramified natural polymers, of great structural complexity, with prevalence of aromatic structures, particularly phenolics where any defined repeated unit does not exist [1].

Eucalyptus is the common name of a gender of trees of the Mirtáceas family. The species Eucalyptus pellet F. Muell, Eucalyptus citriodora Hook and Eucalyptus saligna Smith are very diffused in Pinar del Río county.

In the Center of Studies of the Forest Biomass of the Pinar del Río University man works in the chemical characterization of these species, for their best use.

In this work, lignin IR spectra from the wood of these three eucalyptuses species, obtained by means of treatment with H_2SO_4 and from three heights of the tree trunk, are studied and compared.

Keywords: lignin, eucalyptus, infrared spectrum

INTRODUCTION

Lignin function in the vegetable organisms is complex. Its better known characteristic is to impart rigidity to the cellular walls and to conform the wood fibrous structure (that covers the cells of the xylem), where it carries out the function of physical support. But lignin also participates in the water, nutrients and metabolites transport through the plant and in the protection of the vegetable tissue against the microorganisms attack [2].

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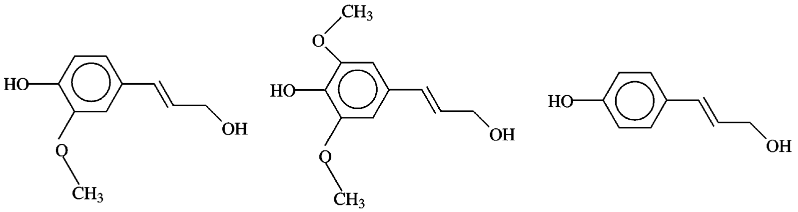
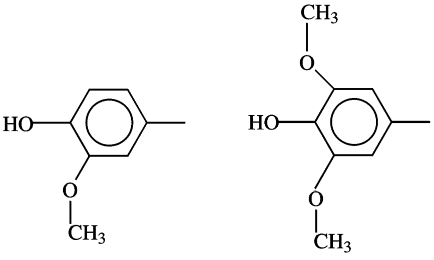
Lignin is known to be a mixture of polymeric natural products that are formed from random parahydroxycinamic alcohols deshydrogenative polymerization, catalyzed by enzymes *via* free radicals, mainly of three primary precursors: the coniferilic, sinapilic and p-coumarilic alcohols (see Scheme 1).



CONIFERILIC ALCOHOL SINAPILIC ALCOHOL *P*-COUMARILIC ALCOHOL

SCHEME 1

Different types of primary structures such as the guayacilic, the siringilic and the p-hydroxybencilic [3] constitute the lignin (see Scheme 2). These structures can be connected to other functional groups as carbonyl, methylene and methyl, alcoholic OH and ethylene double bonds, in dependence of the vegetable lignin origin and the treatment for their isolation.



GUAYACIL RADICAL SIRINGIL RADICAL

SCHEME 2

Lignin composition depends on many factors, among them, the method used for its separation, the studied species, age, part of the tree, environmental conditions where the plant has been developed, *etc.* [4]. Because of its abundance in the vegetable fibers and their structural complexity, lignin has been a product of great interest for the chemists [5]. Lignins and their derivatives have been studied, by means of IR spectroscopy, by several researches [6–14].

MATERIALS AND METHODS

Preparing of the Samples

Five trees of each named species, with similar morphologic characteristic and ages between 20 and 22 years, coming from the Integral Forest Company "Macurije", of Pinar del Río county in Cuba, where cut. The trees hat a average diameter of 18 cm at chest height, total height of 15 m and a commercial trunk longitude of 10 m, grown in a skeletal soil of quality II [15].

From each tree, pieces of 20 cm longitude at 25%, 55% and 85% of the height of the commercial trunk, that we denominate Base, Middle and Apex respectively, where cut. With the previous separation of the bark wood, the pieces were transformed in sawdust. This sawdust was dried off in the air. For each sample the separation of the extractable substances and the extraction of lignin was carried out in triplicate, according to the following procedures.

Obtaining the Extractable-Free Material

To each sample, the serial extraction with the following systems of solvents: toluene: ethanol = 2 : 1 during 8 hours, ethanol at 95% during 6 hours and water at 95°C during 3 hours [16] was carried out. In this procedure the hydrolizable compounds, like tannins, phenols, carbohydrates, *etc.*, and some salts, but not all the kinos were extracted [17, 18]. The kinos is the dark red phenolic perspired, incorrectly called rubber or resin that forms leuco polymerized or pro antocyanidins and whose presence are given by genetic factors and environmental conditions. Approximately 7% non-cellulose component can be in this material [18].

Obtaining Lignin

The separation of the acid insoluble lignin is carried out [16] in wood free of extractable substances; the sample is mixed with 15 ml of H_2SO_4 of 72% and it is frequently agitated at 15°C during 2 hours. The mixture is transferred to a 500 ml recipient to which 350 ml of distilled water is added, reflux during 4 hours and filtered; the residual dries off to constant mass.

The separation of lignin carry out its structural degradation, for this reason, it is difficult to obtain an identical lignin to which is in the original wood (native lignin), although the careful lignin separation is considered representative of the total lignin in the wood. Some authors suggest [17, 18] particularly for the gender Eucalyptus, a previous treatment to the extraction of lignin with NaOH, in order to eliminate the kinos.

The action of mineral acids in lignin, causes changes in its constitution that have been denominated "condensation reactions", where their resonant structures are stabilized with the formation of carbonium ions [3], that makes them very inadequate for structural studies. Although the condensation reaction extension depends on the acid used, the exposed treatment is frequently used, particularly in hard wood.

The study was carried out in the following samples:

(1) LPB: Pellita Lignin of the Base
(2) LPM: Pellita Lignin of the Middle
(3) LPA: Pellita Lignin of the Apex
(4) LCB: Citriodora Lignin of the Base
(5) LCM: Citriodora Lignin of the Middle
(6) LCA: Citriodora Lignin of the Apex
(7) LSB: Saligna Lignin of the Base
(8) LSM: Saligna Lignin of the Middle
(9) LSA: Saligna Lignin of the Apex

Infrared Spectrometry

The Infrared spectra (IR) of the samples in the range form 4000 to 400 cm^{-1} and in units of Absorbance, in an M-80 model spectrometer from Carl-Zeiss-Jena, Germany, were measured.

The intensity calculation, with a detection threshold for the change of slope of 0,005 units of Absorbance, by means of the program SOP 2,06 of search of maxim absorption, what gives a high fiability in the measurement, was carried out. The spectra baseline was fitted.

The KBr tablets of the samples, weighing exactly 1,5 mg of sample and 230 mg of KBr and applying a constant pressure of 15 Mpa during 2 min in the tablet conformer, were made.

The spectra measurement parameters are the following:

- Slit Program: 12(6 cm^{-1})
- Zero adjustment: 0
- Integration time: 1 seg
- Measurement time: 10 min
- X-axis scale: 1
- Y-axis scale: 1
- Threshold: 0,005

To be able to carry out comparisons, the absorption of the analytic interest intensity was normalized dividing the Absorbance of the band in question by the sample concentration, in order to correct the variations in the tablet width among the samples.

Procedure of the Normalized Intensity:

(1) For the measurement of their IR spectrum with air as reference, samples in KBr tablets, weighted with exactitude both the sample and the KBr, were prepared.

(2) The I_k absorption intensity, that should be in the range from 0,1 to 0,6 in Absorbance units, was measured.
(3) The normalized intensity NI_k is calculated, according to the expression:

$$NI_k = I_k/Cm$$

where,

NI_k: normalized intensity band in $k\,cm^{-1}$
I_k: Absorbance band in $k\,cm^{-1}$
$Cm = pm/pKBr$: sample concentration
pm: sample weigh
pKBr: KBr weigh

RESULTS AND DISCUSSION

Lignin chemical composition varies in dependence of the plant type from which it comes. Gibbs [19] classified in a very simple way the vegetable Kingdom lignins in 2 classes, the "guayacilic lignins", present in most of the gymnosperms, and the "guayacil-siringilic lignins" existing in all angiosperms.

Several eucalyptus species lignins have been reported with high contents of siringil radical [20].

In the vegetable cell, lignin is always strongly associated to the hemicelluloses, as much from the physical point of view as chemical, and to certain aromatic carboxylic acids by esters bonds.

Qualitative Analysis

The IR spectra of the samples were assigned using the ASIR v2,0 software [21–23]. It is an automated system for the characteristic absorptions assignment of the more relevant chemical functional groups in the Infrared spectrum and specialized literature [6–8, 14].

The IR spectrum of sample, if it is not pure compound, offers an integral vision of all individual molecular types present in the same one, mainly those that are in more quantities.

In Table 1, the assignment of the most important characteristic group frequencies in the Infrared spectra of the samples of eucalyptus lignin is shown, that do not exhibit differences from the qualitative point of view. When a band is assigned to more than a vibration type, they are equally probable and are superimposed.

The assigned characteristic absorptions can be classified in the following way:

- OH group (bands Nrs. 1, 12, 17b)
- CH_2 group (bands Nrs. 3, 4, 9, 11)

TABLE 1 Eucalyptus lignin infrared spectra assignment

Nr.	*Assignment*	*cm*$^{-1}$
1.	ν OH (hydrogen bond)	3423 ± 14
2.	ν = C—H aromatic ring	3010 h
3.	ν_{as} CH_2 (guayacil–siringil)	2936 ± 1
4.	ν_s CH_2 (guayacil–siringil)	2840 ± 1
5.	ν C = O non conjugated ketone and aromatic ester	1712 ± 3
6.	ν C = O aryl ketone *p*-substituted (guayacil)	1670 h
7.	ν C = C aromatic ring	1610 ± 2
8.	ν C = C aromatic ring (guayacil–siringil)	1501 ± 3
9.	δ_{as} CH_2	1457 ± 1
10.	ν C = C aromatic ring (guayacil–siringil)	1424 ± 1
11.	δ_s CH_2	1370 h
12.	δ C—OH phenolic	1340 h
13.	ν_s N (siringil ring) and ν C—O	1314 ± 1
14.	a) ν_s N(guayacil ring) and ν C—O b) ν_{as} C—O—C aromatic ester	1285 ± 3
15.	ν_s N(siringil ring) and ν C—O	1225 ± 3
16.	ν_s C—O—C aromatic ester	1107 ± 1
17.	a) δ C—H guayacil aromatic ring b) δ C—OH primary alcohol	1024 ± 1
18.	γ = CH aromatic ring (guayacil–siringil)	913 ± 1
19.	γ = CH aromatic ring (guayacil–siringil)	866 ± 6

ν: Bond valence vibration.
δ: Bending in-plane vibration.
γ: Bending out-of-plane vibration.
N: Cycle.
as: Asymmetric.
s: Symmetric.

- aromatic rings (bands Nrs. 2, 7, 8, 10, 13, 14a, 15, 17a, 18, 19)
- carbonyl group (bands Nrs. 5 and 6)
- aromatic esters (bands Nrs. 14b and 16)

As can be noted in Table 1, the absorptions showed in the IR spectra agree with the "guayacil–siringilic" structures, present in all angiosperms lignins. Hemicelluloses characteristic bands are not appreciated, if they are present, their concentration is low with regard of lignin one.

Comparative Study

To compare lignin contents in the samples, the normalized intensities of the band Nr. 8 of Table 1 is calculated, in the lignin IR spectra from 3 species of eucalyptuses to 3 heights of the commercial trunk. The absorption Nr. 8 is assigned to an aromatic ring valence vibration, very characteristic from the spectroscopic point of view. This band is very particular of lignin [8, 14, 24], especially of the guayacil–siringil system that is predominant in the studied gender.

TABLE 2 Normalized intensities of 1500 cm^{-1} band

Nr.	*Sample*	*NI*
1.	Lignin Pellita Base	104.46
2.	Lignin Pellita Middle	104.12
3.	Lignin Pellita Apex	106.34
4.	Lignin Citriodora Base	112.21
5.	Lignin Citriodora Middle	103.68
6.	Lignin Citriodora Apex	94.90
7.	Lignin Saligna Base	110.67
8.	Lignin Saligna Middle	104.54
9.	Lignin Saligna Apex	94.61
	Average	103.95
	Standard Deviation	5.67

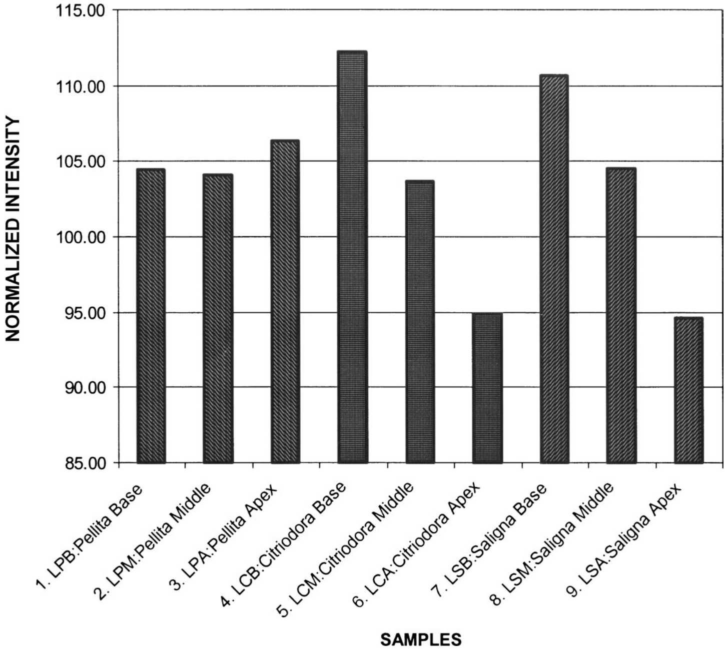


FIGURE 1 Eucalyptus lignin IR spectra. Band in 1501 cm^{-1} intensities comparation.

In Table 2 and in Figure 1 the normalized intensities of these measurements and their graphic representation are shown, respectively. This data indicate that in the species Eucalyptus citriodora Hook and Eucalyptus saligna Smith, lignin contents are similar and they diminish toward the apex.

This behavior is clearly related with the formation process of wood cellular wall, because the lignification is the last stage. For this reason in the apex, whose wood is the youngest of those studied, lignin is the fewer ramified and the quantity of aromatic systems they conform is smaller. In the same way, in the base, where the wood is more aged, the content or aromatic rings in lignin is higher.

In the species Eucalyptus pellet F. Muell the intensity of the absorption at 1500 cm^{-1} along the trunk is very similar, with a slight increment toward the apex. This eucalyptus species could be particularly abundant in kinos, that is a material rich in phenolic compounds for that, in this case, the intensity of the measured band reflects the presence not only of lignin, but also of the residual kinos presents in the sample. It happens because in the process of lignin extraction it was not carried out the recommended [17, 18] treatment with NaOH.

CONCLUSIONS

(1) The infrared absorption spectra of lignin samples, of three species of eucalyptuses from three heights of the commercial trunk, were assigned.
(2) In the IR spectra of all samples, the characteristic absorptions of lignin standard structures are identified and no significant qualitative differences among the samples are observed.
(3) Lignin content was evaluated in the samples by means of the band at 1501 cm^{-1} intensity that is very characteristic in lignin.
(4) In the species Eucalyptus citriodora Hook and Eucalyptus saligna Smith, it is observed that lignin content decreasing as the height of the trunk increased. This reflects the formation process of the wood cellular wall, because in the base, where the wood is more aged, lignin content is higher.
(5) It was not possible to evaluate lignin content in the species Eucalyptus Pellita F. Muell, due to their high kinos content.
(6) It is possible to estimate lignin content in Eucalyptus wood by means of the band at 1501 cm^{-1} intensity if the kinos content in the sample is taken off.

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