Studies of *Eucalyptus Grandis* Lignin. Part I: Estimation of Lignin and Polyphenols Content in *Eucalyptus grandis* by Infrared Spectroscopy

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No presente artigo é apresenta uma nova técnica de determinação do conteúdo de lignina e polifenóis no *Eucalyptus grandis* por espectroscopia no IV convencional. Na calibração foram utilizados padrões nativos: ligninga de madeira moída e polifenóis. O conhecimento dos conteúdos de lignina de Klason e lignina solúvel tradicionais é necessário para a realização da analise. A exatidão desta técnica é boa.

This paper presents a new technique to determine the lignin and polyphenols contents in *Eucalyptus grandis* by conventional IR spectroscopy. The calibration standards are milled wood lignin (MW) and native polyphenols. For carrying out the analysis it is necessary to know the traditional Klason lignin and soluble lignin content. The accuracy of this method is good.

Key words: *lignin; polyphenols;* Eucalyptus*; IR spectroscopy.*

Introduction

The Klason method[1] for the determination of lignin including the soluble lignin[2] are not precise enough for woods which contain polyphenols, such as wood of *Eucalyptus* because polyphenols interfere with the analysis in two ways: through condensation with the lignin and through UV absorbance in the lignin range. To avoid these interferences, Bland and Men-shun[3] proposed that the polyphenols should be extracted from the wood with hot diluted alkaline solution before determina-tion of the Klason lignin. The soluble lignin and polyphenols are quantified by UV spectroscopy by Browning and Bublitz equation[2]. The calibration standards are the Klason lignin and the elagic acid as representative of all polyphenols.

In spite of being tedious, the Bland and Menshun method constitutes an improvement in analysis of Eucalyptus woods. However, the method is based on two assumptions, namely the klason lignin is representative of the protolignin and the elagic acid alone presents the absorptivities of all polyphenols. These assumptions do not correspond to reality when the method is applied to determination of the total content of lignin and polyphenols in wood of *Eucalyptus grandis.*

Another method to quantify the total content of lignin and polyphenols in *Eucalyptus grandis* was developed by Morais[4] who considered the hydrolytic lignin absorvity as reference for soluble lignin and polyphenols in the hydrolysate solution from which Klason lignin is removed. This method provides better results for the determination of the total contents of lignin and polyphenols than the procedure of Bland and Menshun. Nasimento[5] used the acetyl bromide technique by Johnson and co-workers[6] to obtain the total content of lignin and polyphenols directly from wood of *Eucalyptus grandis.* However, the last two methods can not provide separately the contents of lignin and polyphenols in spite of better accuracy.

The CP-MAS-^{13}C-NMR spectroscopy is also used to esti-mate the total lignin and tannin contents of woods[7]. However, the method is not very convincing because of the over simpli-fied assumptions and the relatively great complexity.

In the present work, a new method to estimate the total con-tent of lignin and polyphenols in wood of *Eucalyptus grandis* by IR spectroscopy is proposed.

Materials and Methods

Wood sample - Eucalyptus grandis wood (12 years old) was obtained from trees grown in the "cerrado" (savannah) near Uberlândia - Minas Gerais.

Milled wood lignin - The milled wood lignin (MWL) was isolated according to Björkmann's method[8] adapted for Euca-

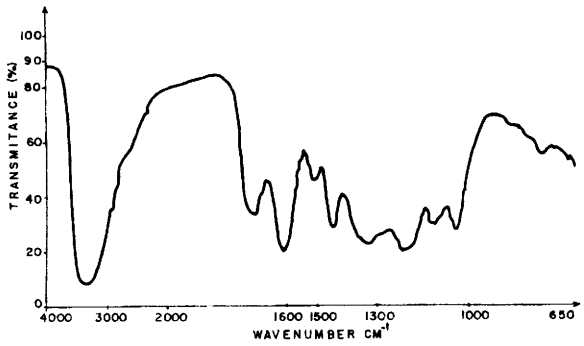

Figure 1. *IR spectrum of the "kino".*

lyptus wood by Bland and Menshun[9], Nascimento[5] and Morais[10]: 100 mesh ground wood was extracted successively with benzene-ethalon (2:1) and water. The wood meal was then treated with 0.1 M cold sodium hydroxide solution to remove the residual polyphenols. The resulting extract-free wood meal was treated with 1% acetic acid until pH = 4 and dried in an oven at 70°C for 12 hours. The wood meal was then extracted with chloroform for a week and with aceton:water (9:1) for two days. The acetone was removed from the solution and the residual wet mass was treated with 2% sodium hydroxide and filtered. To the resulting brown solution was added 2% acetic acid until pH = 4. The precipitated lignin was filtered and dried in a oven at 70°C for over night.

Polyphenols ("kino") - The "kino" was obtained from wood meal after extraction with benzene-ethanol (2:1). The dried wood meal was extracted in a soxhlet with water until the extracted solution became clear.

Infrared spectroscopy - The IR spectra were run with a Shimadzu spectrophotometer (IR-408). The KBr pellets were quantitatively prepared from 1.00 mg of the sample tritured with 99.00 mg of KBr. The powder was oven-dried for 1 hour at 105°C and then pressed. A pure and dried KBr pellet was use as reference.

Result and Discussion

The elemental analysis of the "kino" is: C = 48.63%; H = 4.5%; O = 47.15% and N = 0.17% and a metoxyl content of approximately 2%. Figure 1 shows the IR spectrum of "kino". The absorbance at 1500 cm^{-1} and the methoxyl content suggest the possible presence of a small amount of lignin as a contami-nant in the "kino". The ^{13}C-NMR spectrum showed in figure 2, however, indicates that the "kino" contains practically no lignin. In addition, the intensive signal at δ 144 (Figure 2a) is characteristic of gallotannins[7]. Thus, it is evident that the "kino" is free of lignin.

Figure 3 shows the IR spectrum (wave number runge: 1800 - 1500 cm^{-1}) of MVL and "kinos" extracted by two different ways: by water and ethanol-water. It is well established that the water-ethanol extractives contain some lignin from Euca-lyptus. The band ant 1600 cm^{-1} has the same intensity in figu-res 1 and 3a; on the other hand, the band at around 1500 cm^{-1} increases the intensity with increasing lignin content in the sample. Thus, the intensity ration of the bands at 1600 and 1500 cm^{-1}

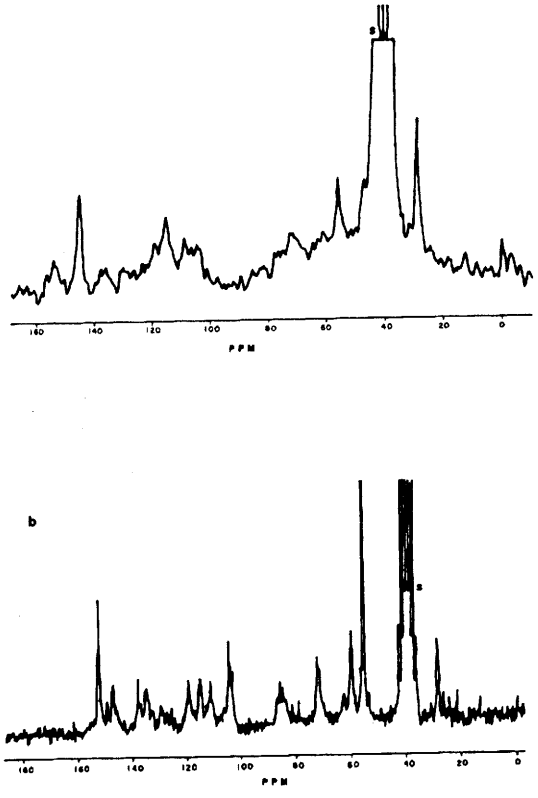

Figure 2. *^{13}C NMR spectra of: a) "kino" and b) MWL*

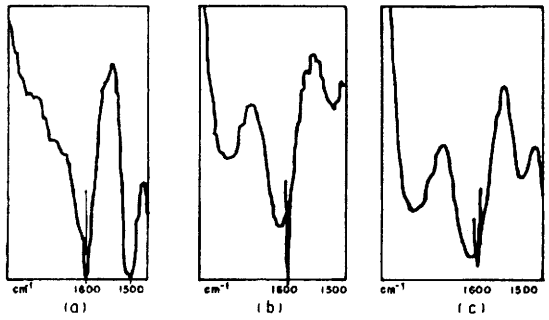

Figure 3. *IR spectrum segments of: a) MWL; b) "Kino" (water extrat); and c) "kino" (ethanol-water extract).*

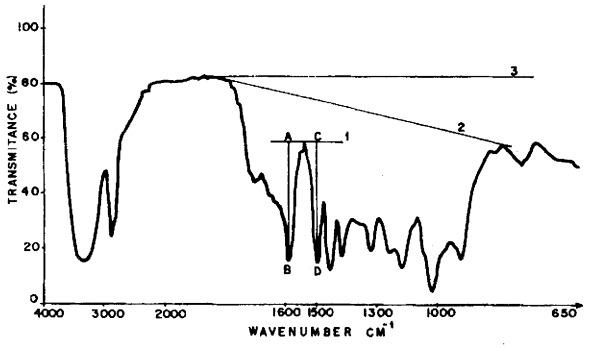

Figure 4. *IR spectrum of the MWL with the baselines (see text).*

can provide a way to determine lignin content in "kinos" and even in woods.

In order to investigate this possibility, six parameters were selected for determining the band ratios: three were based on the baselines 1, 2 and 3 as shown in figure 4 and the another three are based on the absorbances of each maximum relative to the same baselines. For instance, parameter 1 is the ratio r1 = segment AB / segment CD (figure 4); parameter 4 is the ratio r_4 = Δ absorbance corresponding to segment AB / Δ absorbance corresponding to segment CD.

Table 2. *Lignin content in some* Eucalyptus grandis *"kinos" by IR spectroscopy.*

Sample	extractive	bands ratio	% MWL
1	ethanol:benzene (2:1)	1.81	15.0
2	ethanol:water (2:1)	1.81	15.0
3	NaOH 0.1N	1.92	11.0
4	dioxane:water (9:1)	1.05	62.0

Table 1 list these six parameters with increasing lignin con-tent in "kino", while figure 5 depicts these six parameters as function of lignin concentration in the sample.

All curves are exponential and can, in principle, be used as standards. The small deviations observed in various points are due to experimental error. However, the curve of parameter 1 vs lignin concentration is more uniform and will be taken as a standard in the estimations

Table 2 shows the lignin contents in some *E.grandis* "kinos" estimated by this technique. The dioxane:water (9:1), v/v) solvent system is more effective than 0.1 N sodium hydroxide solution in extracting lignin from wood meal. The free phenolic hydroxyl content in lignin is very low and does not intervene significantly in the extraction process.

The advantage of this technique is to provide the lignin and polyphenols contents in *E. Grandis* wood directly. It is well established that these contents vary with age and geographic location where the wood grows. It is necessary, however, to determine the Klason lignin content, and this must be done without any previous extraction. Furthermore, the soluble lignin and polyphenols must also be determined by UV spectroscopy. The sum of both values give the total lignin and polyphenols content. It is 39.8% for *Eucalyptus grandis*[5].

Table 1. *Ratios of band intensities at 1600 and 1500 cm^{-1}* versus *lignin content for the different procedure.s*

	Parameters					
%MWL	1	2	3	4	5	6
0	3.28	1.83	1.92	4.93	2.43	2.13
1	2.63	1.67	1.47	4.11	2.31	2.11
5	2.17	1.49	1.37	3.51	2.23	2.08
10	1.95	1.44	1.30	3.10	2.08	1.91
15	1.80	1.38	1.25	2.60	1.98	1.85
20	1.64	1.32	1.22	2.48	1.92	1.79
28	1.47	1.28	1.18	1.87	1.56	1.47
39	1.28	1.20	1.10	1.60	1.43	1.36
50	1.13	1.13	1.06	1.28	1.22	1.18
60	1.04	1.07	1.04	1.13	1.11	1.04
63	1.03	1.05	1.03	1.06	1.07	1.02
70	1.01	1.03	1.02	1.03	1.04	0.98
80	0.95	1.02	0.98	0.97	0.97	0.95
85	0.92	0.98	0.96	0.88	0.88	0.87
100	0.90	0.96	0.93	0.87	0.87	0.87

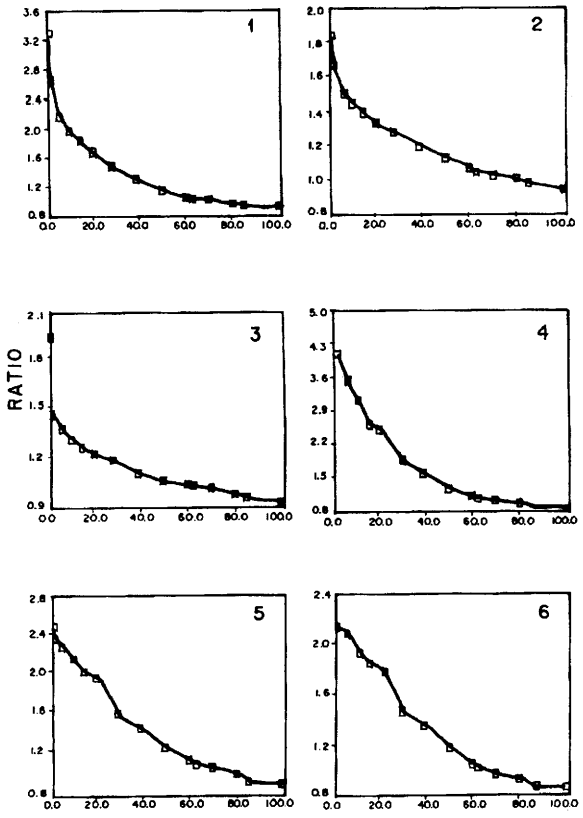

Figure 5. *Ratio of band intensities (1600/1500cm^{-1}) versus lignin content in "kino".*

Figure 6 presents the IR spectrum of *E. grandis* wood obtained under the same conditions described for obtaining the IR spectrum of "kino" (figure 5). Parameter 1, the ration of band intensities at 1600 and 1500 cm^{-1}, is 1.0. This value cor-responds to 69.0% of lignin in "kino". Thus, the contents of lignin and polyphenols in *Eucalyptus grandis* are: 38.9% x 0,69 + 27.5% and 39.8% - 27.5% + 12.3%, respectively.

The determination of soluble and insoluble lignins as proposed by Bland and Menshun, that is, determination of the Klason lignin and the soluble lignin by spectroscopy, after extraction of the wood with benzene:ethanol (2:1), water, then sodium 0.1 M hydroxide, gave a total lignin of 29.5%. It must be mentioned that this result does not include the benzene-ethanol extractive (1,2%) which is mainly composed of lignin and polyphenols.

The different lignin contents found by means of the two techniques indicate the necessity of refining the current methods of lignin determination.

The method presented in this work has the advantage of be-ing based on pre-determined calibration using polyphenols and lignin isolated from the wood to be investigated as standards.

Conclusions

Better accuracy for the determination of lignin and polyphe-nols contents in *Eucalyptus grandis* wood is obtained if the total lignin and polyphenols (soluble and insoluble) are determined without any previous extraction, and the proportion of each one is estimated by IR spectroscopy.

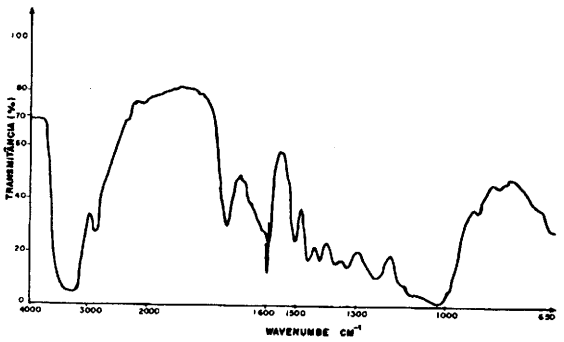

Figure 6. *IR spectrum of Eucalyptus grandis wood.*

Acknowledgments

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