CELLULOSE CHEMISTRY AND TECHNOLOGY

CHARACTERIZATION OF KRAFT LIGNIN FROM *PINUS PINASTER*

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This paper presents the results about the lignin from *Pinus pinaster*, the only softwood used in Portuguese pulp and paper industries. Six samples of lignin were collected and studied. The first three samples were precipitated from kraft black liquors at different H-factors, using conventional batch pulping. The other three corresponded to the lignin obtained from black liquors of flow-through reactor at the same H-factors. The residual sugars from the lignin obtained were hydrolysed according to the trifluoroacetic acid method and quantified by HPLC. The main residual sugars on the lignin samples from both reactors were found to be xylose, galactose and arabinose, which indicates that the lignin-carbohydrate linkages are similar to other common softwoods. The elemental analyses of these samples showed that, in the initial stage of delignification, the HS^- anions are strongly involved in the delignification mechanism. It was also shown that the C/H ratio was relatively constant, which indicated the weak participation of condensation reactions. The infrared spectra of these samples evidenced the presence of the main peaks corresponding to different functional groups of softwood lignin structure. They also showed that the content of conjugated carbonyl groups decreased with the reaction time. Finally, the permanganate potassium oxidation of the lignin samples showed the presence of the principal fragments present in softwood lignin. It was also shown that, when the time of delignification increased, the amount of non-condensed units decreased, whereas that corresponding to condensed ones increased.

Key words: *Pinus pinaster*, lignin structure, flow-through reactor, permanganate oxidation, residual sugars.

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INTRODUCTION

Portugal pulp and paper industries use mainly two vegetal species, namely: (i) *Eucalyptus globulus*, a hardwood exploited in the production of bleached kraft pulps and converted, mostly, to photocopies and written papers, and (ii) *Pinus pinaster*, a softwood from which unbleached kraft pulps are obtained and converted, essentially to kraft liner packaging paper. The pulps obtained from *Pinus pinaster* are coloured and display bad bleachability, to compare with those obtained from other common pines. This fact limits seriously the production of bleached pulps from Portuguese pine and lowers the properties of those produced presently.

Recently, our laboratory has been involved in a large program of research dealing with both fundamental and applied aspects related to *Pinus pinaster* species. In fact, studies devoted to establishing the chemical composition, as well as the identification of components constituting our pine (sugars, extractives, etc.) are currently under investigation and will be soon published. Then works devoted to the extraction of the residual lignin from kraft pulps and its characterization by elemental analysis, infrared spectroscopy and permanganate oxidation, followed by gas-chromatography coupled with mass spectrometry, were carried out and reported.[1] More recently, modified kraft pulping was carried out in order to establish whether the extractives play any role on colouring the kraft pulps from Portuguese pine. These results were communicated and showed that, indeed, the extractives are probably involved, but a clear-cut answer was not possible to establish.[2,3]

Lignin is a natural reactive cross-linked polymer very difficult to characterize. Indeed, the fact that this polymer is a network, excludes very useful techniques of characterisation. Moreover, it varies from a vegetal species to another (hardwood, softwood, etc.) and is very sensitive to cooking conditions (self-condensation, etc.) which induces drastic changes in its structure. The number of books devoted to the methods of characterization of this macromolecule testifies its complex character.[4-6]

The flow-through reactor was introduced by Ylner et al. in order to improve the quality of the pulps and was found to be a very useful technique to characterize black liquors lignin of pine wood.[7] This type of cooking presents the advantage that dissolved lignin fragments are continuously removed from the reaction medium, which reduces considerably self-condensation reactions and avoids their adsorption onto the pulp's surface.[8]

The present paper deals with the isolation and characterisation of *Pinus pinaster* lignin as precipitated from black liquors, using batch and flow-through reactors. Three lignin samples were obtained from black liquors of each reactor. The first one corresponds to the initial stage of delignification (an H-factor of about 70), the second one represents an intermediate cooking time (an H-factor of 950), whereas the third sample coincided with the final stage of pulping (an H-factor of ca. 1800). To the best of our knowledge, except our studies, there is no report dealing with the study of the structure of lignin from *Pinus pinaster*.

EXPERIMENTAL

Material

Pinus pinaster wood chips were a kind gift from Portucel Tejo Company. They were used without any further treatment or classification for BR pulping, whereas the size of wood chips selected for FTR cooking was of about 25×5×3 mm.

Pulping Conditions

The wood chips were cooked under conventional kraft conditions, namely: (i) alkali active of 21%, (ii) sulfidity of 30%, (iii) water to wood ratio of 4:1, and (iv) 90 minutes of heating from room temperature to 170°, on maintaining the reactor at this temperature for different times, depending on the desired H-factors.

Batch reactor (BR) kraft delignification was carried out using a 10 L equipment from Lowrentz & Wettre. The reaction was stopped at three levels of delignification, namely: at a H-factor of 85, 950 and 1830, respectively. An aliquot of black liquor was collected at each level. The kappa number of pulp in the final stage of cooking was 30.

Flow-through reactor (FTR) pulping was conducted on an industrial installation (Berghof, Germany), modified at the French School of Papermaking and Printing Industries.[9] The reaction conditions were the same as those described for BR, except the fact that, for FTR, a previously optimised flow of white liquor[10] was introduced at 25 mL/min. Three liquors were collected. Whereas the first concerns the end of the initial stage of delignification (H-factor-60), the two others are related to the bulk delignification process, e.g., H-factor-950 and H-factor-1820. In order to compare the two pulpings, the kappa number of the pulps in the end of cooking was determined and a value of 35 was found.

Lignin Precipitation and Drying

The collected liquors were acidified by 4M HCl, until the pH reached a value of about 2 and the resulting suspensions were then centrifuged at 4500 rpm for 15 min, washed first by diluted solution of HCl (0.01M) and then by distilled water. The resulting solid lignin was then recovered by removing the liquid phase and drying at 40°, under vacuum. Finally, the solid lignin was ground manually and stored in sealed capsules.

Lignin Characterization

Residual sugars were determined using the well-known trifluor-acetic acid (TFA) method,[11] after a slight modification consisting in the use of a diluted solution of TFA. They were then quantified by high performance liquid chromatography (HPLC). To this end, a Perkin Elmer 250 chromatograph equipped with a refractive index detector (HP 1074A, from Hewlett Packard) was used. For the separation of sugars, a commercial column ("Polysphere OH-PB" from Merck Co.) was connected to the HPLC. This column is filled by an organic support and is based on the cationic ion-exchange principle of separation (Pb^{++}). The flow rate of the eluent as well as the temperature of the column were commonly used for these types of analyses, e.g. 0.4 mL per minute of bi-distilled, degassed water and 80°, respectively. Calibration curves of D-glucose, L-arabinose, D-galactose, D-xylose and D-mannose were previously obtained using high purity commercial sugars (Merck Co).

The collected samples of lignin were also characterized by elemental analysis and infrared spectroscopy. The first analysis was made on Fisons Instruments – EA 1108 CHNS – O, whereas the infrared spectra were recorded on a Mattson 5000 FTIR spectrometer using KBr pellets.

Lignin was also submitted to a four-step degradation method consisting in its oxidation with potassium permanganate, as described by Gellerstedt.[12] However, two observations should be made: (i) dimethylsulfate was used as alkylation agent and (ii) the solvent was a mixture of dimetoxyethane-methanol-water. Thus, the oxidized fragments obtained were methyl derivatives. The GC-MS used was Fison GC-8000 series with an MS Trio 1000, equipped with a commercial silica-fused capillary column (DB-5). The separation conditions were the following: injection port temperature: 250°, initial oven temperature: 150°, temperature rises: 5°/min, final temperature: 270° for 7 min, while the ionization of potential source was of 70 eV, which is the most common potential.

Abbreviations

In this paper, the following abbreviations will be used:
L1: lignin from FTR isolated from black liquor at a H-factor of-60;
L2: lignin from FTR isolated from black liquor at a H-factor of-950;
L3: lignin from FTR isolated from black liquor at a H-factor of-1820;
I1: lignin from BR isolated from black liquor at a H-factor of-85;
I2: lignin from BR isolated from black liquor at a H-factor of-950;
I3: lignin from BR isolated from black liquor at a H-factor of-1830.

RESULTS AND DISCUSSION

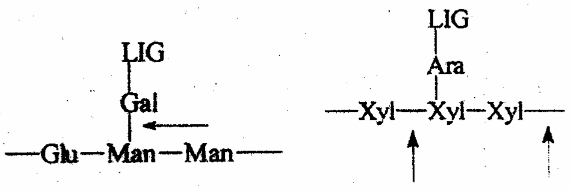
Determination of Residual Sugars

The first set of analyses concerned the quantification of the residual sugars in lignin samples. These analyses were carried out in order to establish the lignin-sugars linkages and to ascertain the selectivity of the cooking process. The nature, as well as the amount of residual sugars in the lignin samples were calculated from HPLC chromatograms. The data thus obtained are summarized in Table 1. From these results, some concluding remarks could be deduced. First, the most important sugars presented were D-xylose, D-galactose and L-arabinose, which corroborates quite well with the data available in the literature for softwoods.[13] In fact, the scheme commonly used to illustrate the lignin-polysaccharide linkages showed that lignin is linked to polysaccharides through galactose (Scheme 1a), and arabinose (Scheme 1b). Then, galactose was linked to the glucomannan chain, whereas arabinose was linked to xylan. If one examines scheme 1a, it could be concluded that the break of the linkage between the galactosic moiety and the mannosic one was more frequent than that along the glucomannan chain. On the other hand, the lignin-arabinose-xylose complex (Scheme 1b) seemed to be less sensitive to hydrolysis in lignin-arabinose and arabinose-xylose bridges, since the quantity of xylose was relatively high. This indicated that the break occurred probably along the xylan chain.

TABLE 1

Amounts (in % with respect to the quantity of lignin) of residual sugars

		Amounts of sugars in lignin samples, %					
		glucose	xylose	galactose	arabinose	mannose	total
FTR	L1	0.24	0.42	0.57	0.39	0.15	1.77
	L2	0.19	2.03	1.01	0.63	0.13	3.99
	L3	0.31	3.80	2.78	0.96	0.26	8.11
BR	l1	0.15	0.92	0.41	0.38	0.14	2.00
	l2	0.14	2.50	0.76	0.77	0.14	4.31
	l3	0.21	2.02	0.87	0.68	0.14	3.92



Scheme Ia

Scheme Ib

Scheme I

Then, the total quantity of residual sugars of FTR lignin samples was higher than that of those precipitated from BR trials, which could be attributed to the fact that, in FTR cooking, the fragments of dissolved lignin were continuously removed, which avoided further degradation of the sugars linked to them. Of course, this phenomenon occurred in the BR reactor, and subsequently decreased the quantity of residual sugars in the one dissolved along the cooking lignin samples.

Elemental Analyses

From the results of the elemental analyses on the lignin samples, summarized in Table 2, several remarks could be drawn. The first concerns the high content of sulphur atoms in the initial stage of cooking for both reactors; this phenomenon is well known in literature and it results from the fact that the hydrosulphide (HS^-) anions are involved mainly in the initial stage of reaction.[14,15] It is also well-known that, for bulk delignification, these anions do not intervene, which explains the drastic decrease of the sulphur atoms content for the other two stages. Then, the oxygen content, which is determined by difference, seems to increase with the time of cooking. It is worth mentioning that the values of oxygen content in the initial stage of delignification were underestimated, since the values of the amount of sulphur atoms were too high. Moreover, the fact that the quantity of residual sugars in the samples of lignin increased (see Table 1), induced an increase of the oxygen content. The content of carbon atoms did not show a clear tendency in the case of FTR cooking and increased for BR trials, which could indicate the occurrence of some condensation reactions. Unfortunately, this was not supported by the values of hydrogen atoms, since they should be decreased, if any condensation reactions occurred. This contradiction could be elucidated if one calculates the C/H ratio, which is constant in the case of BR experiments and for all cooking stages.

TABLE 2

Elemental analyses of lignin samples, in % with respect to the total quantity

		Elements, %			
		C	H	O	S
FTR	L1	55.1	5.6	21.4	17.4
	L2	58.0	5.4	29.2	7.3
	L3	54.8	5.3	37.8	2.1
BR	11	54.0	5.1	22.8	17.9
	12	56.0	5.4	35.3	3.2
	13	57.2	5.5	34.7	2.5

Infrared Spectroscopy

The infrared spectra of the lignin samples were also recorded and showed the presence of the main bands characterizing the lignin structure, namely:

(i) band at around 3415 cm^{-1}, attributed to the OH groups;
(ii) band between 2850 and 3000 cm^{-1}, representing CH, CH_2 and CH_3 moieties;
(iii) two bands at about 1710 and 1690 cm^{-1}, characterizing conjugated and non-conjugated carbonyl functions;
(iv) band at 1600 cm^{-1}, corresponding to aromatic ring vibrations;
(v) bands at 1514 cm^{-1}, a proof of the presence of guaiacyl (G) groups;
(vi) band at 1460 cm^{-1}, representing OCH_3 groups;

(vii) band at 1425 cm^{-1}, corresponding to the CH vibration of aromatic rings and/or representing methoxy functions;

(viii) band at 1220 cm^{-1}, which corresponded to C-O bonds and/or C-O-C bridges;

(ix) band at 1140 cm^{-1}, which represented the G groups;

(x) band at 1080 showing the presence of C-O from aliphatic secondary alcohols and/or aliphatic ethers;

(xi) band at 1030 cm^{-1}, corresponding to aromatic cycle "breathing";

(xii) bands at 860 and 815 cm^{-1}, specific to C-H stretching from the 2,5,6-substituted aromatic ring.

Then, it is very complicated to establish a quantitative study from FTIR spectra in general and from those reported here in particular. Indeed, the spectra recorded for all six samples presented very similar patterns, except for the band intensities at 1710 and 1685 cm^{-1}, which tended to reverse. In fact, as shown in Figure 1, the intensity of the band at 1710 cm^{-1} was high in the initial stage of cooking and lowered with the increasing time, whereas the intensity of the peak at 1790 cm^{-1} showed the inverse trend. This phenomenon indicated that the quantity

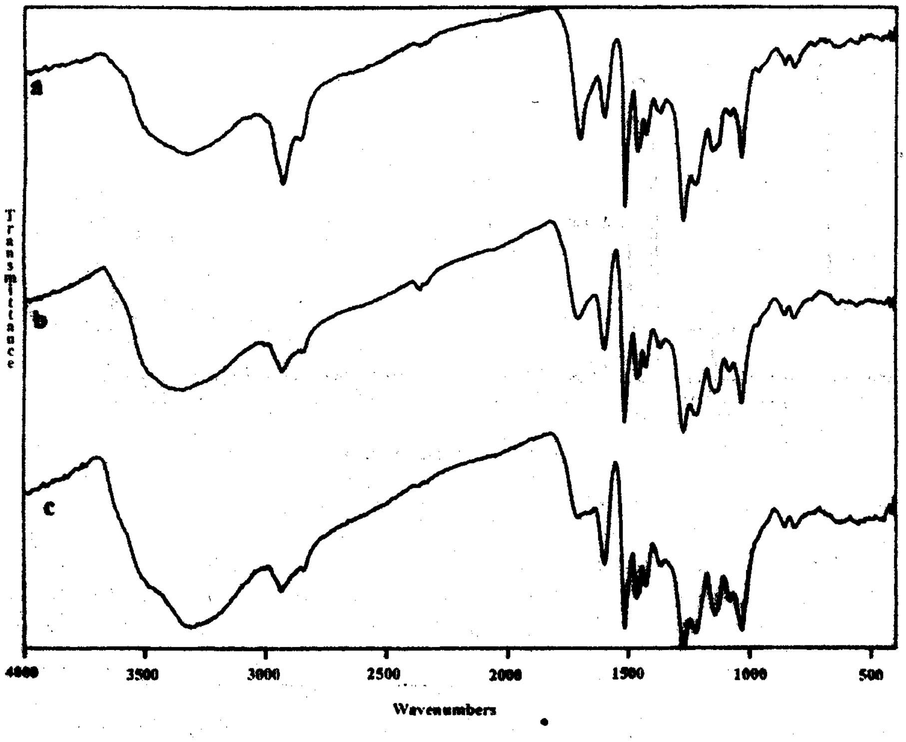


Fig. 1 – FTIR spectra of (a) L1, (b) L2 and (c) L3.

of conjugated carbonyl groups decreased as the time of delignification increased, which was not surprising.

Permanganate Oxidation

Before oxidation with permanganate, the phenolic hydroxy functions contained in lignin samples were protected by alkylation with dimethyl sulphate, in order to avoid their oxidation. The fragments which resulted from the oxidation procedure were derivatized before their quantification by GC-MS. This was achieved by esterification with diazomethane. The mixture was then injected into gas chromatography and different products were separated, as shown in Figure 2 for the L3 sample. From the chromatograms obtained for all samples, the main peaks were identified using mass spectrometry.

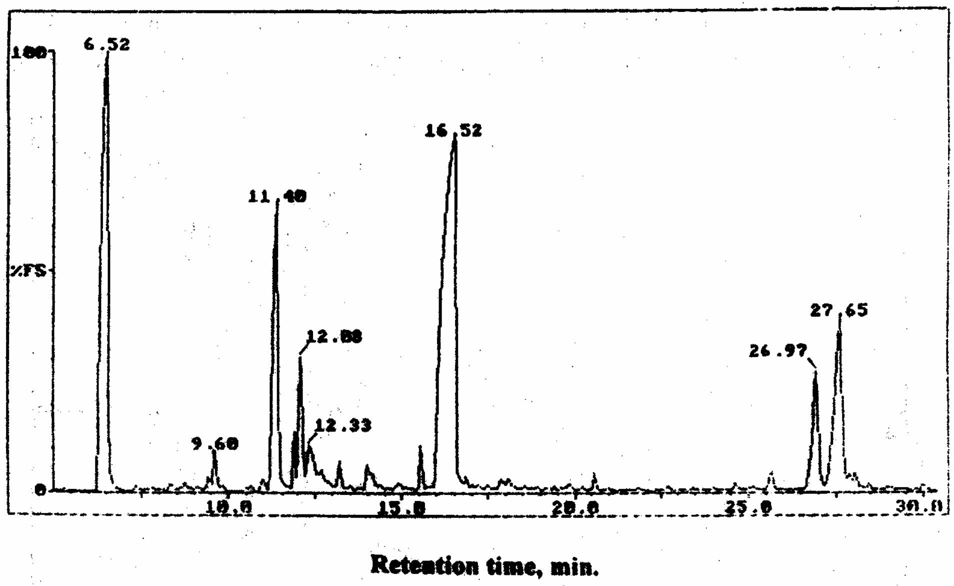


Fig. 2 – Chromatogram of the permanganate oxidation products of L3.

The chemical structure of the principal fragments obtained were given in Scheme II, which showed two types of molecules. The first family dealt with non-condensed guaiacyl structure (Structure I), which resulted from the degradative oxidation of the end groups linked to the lignin macromolecules through the propyl chain. The second family (Structures II-V) concerns the so-called condensed moieties, linked to lignin through the C-5 or C-6 position. These structures were in total agreement with those given by Gellerstedt[12] for softwoods,

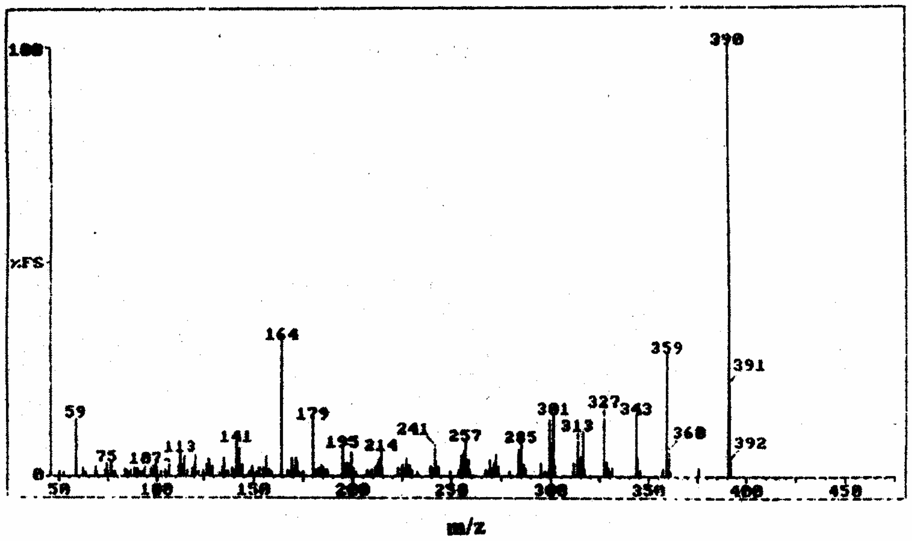


Fig. 3 – Mass spectra of the structure V, resulting from the permanganate oxidation products of L3.

TABLE 3

Main mass fragments of structure V

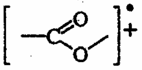
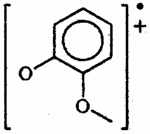
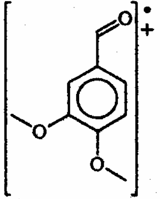
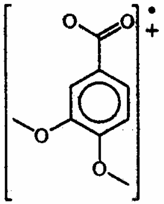
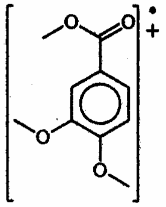
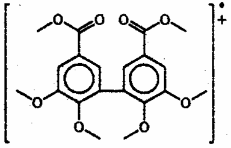
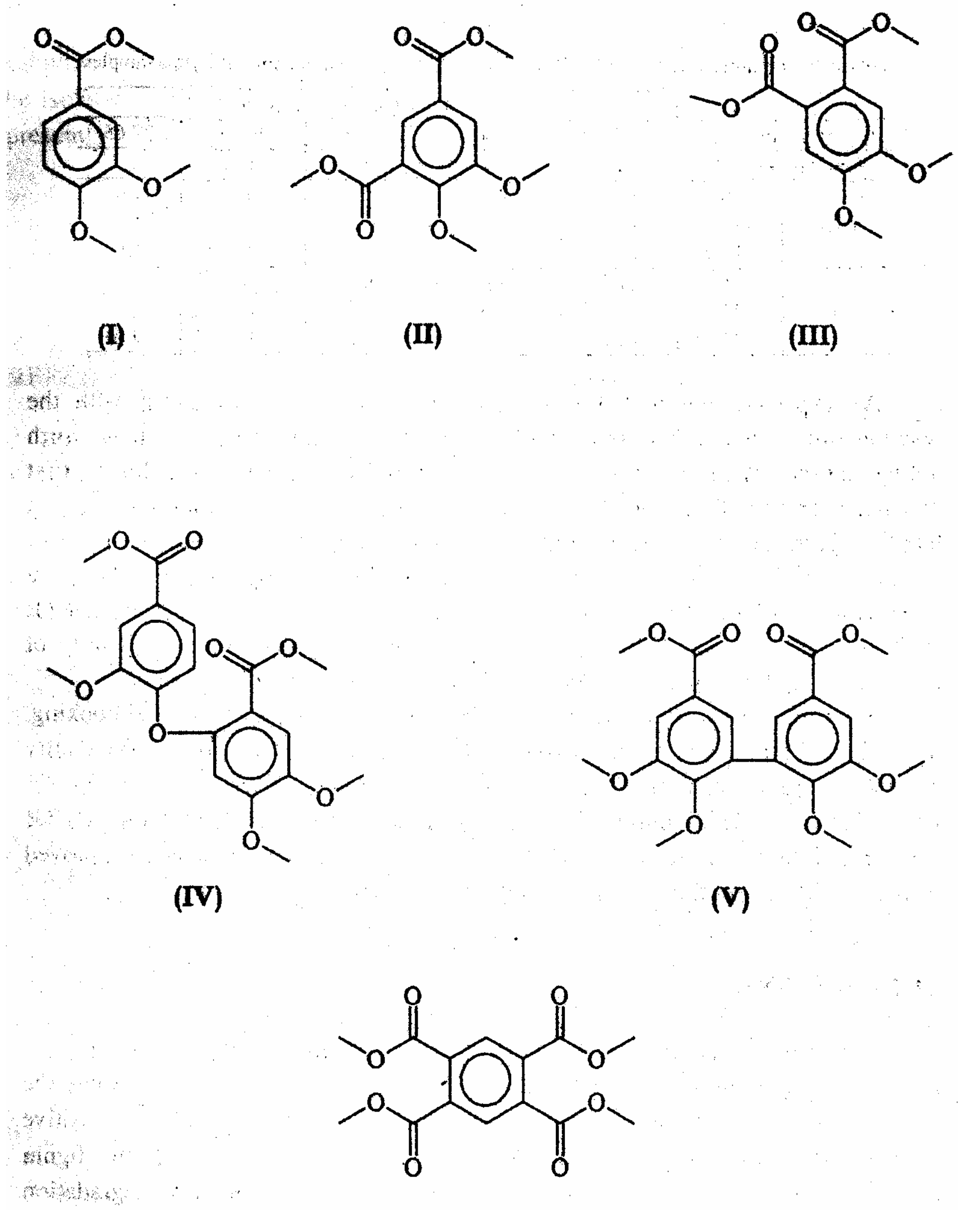
m/z	Mass Fragment
59	
121	
164	
180	

Table 3 (continued)

m/z	Mass Fragment
195	
242	$[M - 3CH_3 - 3OCH_3]^{\cdot+}$
268	$[M - 4CH_3 - 2OCH_3]^{\cdot+}$
284	$[M - 5CH_3 - OCH_3]^{\cdot+}$
300	$[M - 6CH_3]^{\cdot+}$
315	$[M - 5CH_3]^{\cdot+}$
330	$[M - 4CH_3]^{\cdot+}$
345	$[M - 3CH_3]^{\cdot+}$
360	$[M - 2CH_3]^{\cdot+}$
375	$[M - CH_3]^{\cdot+}$
390	 OR $[M]^{\cdot+}$

and Figure 3 shows an example of the mass spectra of Structure V. The main mass fragment of this compound was identified as summarized in Table 3.

The quantification of these fragments was carried out using 1,2,4,5-benzenetetracarboxylic acid tetramethyl ester as an internal standard (see Scheme II). The calibration curves of compound I were established and showed that its response factor was close to unity, as expected from literature.[12] For the other compounds, the same correlation between the ratio of masses and that of surface areas measured by GC was assumed. From the results summarized in Table 4, the main information concerned the fact that the quantity of non-condensed groups in dissolved lignin was much higher than the sum of condensed units, in all stages studied. This indicated that residual lignin could be very rich with highly condensed structures, which could explain the dark colour of the pulps obtained.


(I) (II) (III)

(IV) (V)

Internal Standard

Scheme II

TABLE 4

Amounts of oxidised fragments, in % with respect to the initial quantity of lignin samples

		Amount of lignin fragments, %				
Retention time, min.		6.52	11.40	12.08	26.97	27.65
Structure number		I	II	III	IV	V
FTR	L1	17.9	4.4	0.7	0.2	1.0
	L2	13.1	6.2	1.2	1.0	1.0
	L3	14.5	7.3	2.7	3.0	5.9
BR	11	24.9	4.0	traces	-	-
	12	18.0	5.9	1.0	1.0	2.1
	13	11.1	6.3	2.7	1.4	4.0

As expected, the quantity of non-condensed groups decreased with the reaction time, whereas the amount of condensed moieties increased. It is worth noting that the amount of non-condensed Structure I was lower in FTR for the first two stages of delignification. This unexpected trend could be attributed to the fact that the lignin samples corresponding to the initial stage of delignification reactors were quite different for both reactors. Indeed, the lignin sample were collected at an H-factor of 85 for BR experiments, whereas the H-factor corresponding to FTR trials was only 60, as mentioned above. This was confirmed by the quantity of precipitated lignin, which was much higher in the case of BR black liquor.

Then, the quantities of structures II to IV increased with the time of cooking, indicating that the bulk delignification started to be deep. Finally, the total quantity of oxidized fragments in the final stage of cooking was higher in FTR than that for BR, which is probably due to the fact that the self-condensation reaction in FTR was negligible compared with BR, since the fragments dissolved were removed from the reaction medium and immediately cooled.

CONCLUSIONS

This study constitutes only a part of our global strategy of studying the structure of the lignin from *Pinus pinaster*. Nevertheless, it showed once more the suitability of a flow-through reactor in studying such a complicated and sensitive to degradation macromolecule as lignin. In fact, it was shown that the lignin dissolved during different stages of pulping was submitted to less degradation when using FTR instead of the classical batch reactor. It was also shown that the quantity of non-condensed groups in dissolved lignin was much higher than the sum of condensed units, which suggests that the residual lignin is rich in highly-condensed structures, which could explain the colour of the kraft pulps obtained.

In order to get deeper knowledge on their structure, the lignin isolated will be submitted to other analyses, such NMR, GPC. Moreover, the residual lignin will be isolated from the corresponding pulps and will be characterized. This work is presently under investigation.

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