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Analysis of Lignin Isolated from Alkaline Leaching of Alfa Grass

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The analysis of Alfa grass lignin is examined, isolated from industrial black liquor of the Algerian Pulp and Paper Company using the soda process. The used experimental techniques are: UV/visible, microscopy (MEB), FTIR, spectroscopy, EDAX and for the first time, HPLC (high performance liquid chromatography), MS (mass spectrometry) and GC/MS (coupled gas chromatography/mass spectrometry) analysis that permit us the valorization of this product.

Key Words: Alfa grass lignin, Polyphenolic polymer, Vanillin.

INTRODUCTION

Lignins are phenolic polymers. They are known for their function of reinforcement of the terrestrial plants. The word lignin indicates polyphenolic polymer with high molecular weight. Their compositions and structures are variable and complex too. The lignin is the majority product of black liquor of paper mill. Its composition differs from a type of plant to another one. In present case, the paper mill of Algiers (Celpap Company) treats the Alfa grass (*Stipa tenacissima* L). The properties of lignin are a function of the method of extraction. The analysis of the lignin is important for the researchers and users in response to a specified need. Gaïtner *et al.*[1] determined the phenolic hydroxyl groups in residual lignin using a modified UV-method. The molecular structures of the lignins were analyzed by Hatalli[2]. FT-IR absorption spectrum of straw and wood lignin were presented by Faix[3] and Jahan[4]. Using pyrolysis, gas chromatography and mass spectrometry, Shiyu[5] characterized the residual lignins from pulps and effluents. However, few works mentioned the valorization of the lignin isolated from alkaline leaching of Alfa grass, particularly the high performance liquid chromatography and mass spectrometry analysis of vanillin.

EXPERIMENTAL

Leaching of alfa fiber: The leaching has been carried out in stainless steel tank of 3 L capacity. The operation conditions are: feed fiber (Esparto) of 3 cm long, leaching time 1 h, leaching temperature 90 °C, pressure 1 atm., reagent 45 g/L of

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NaOH, solid/liquid ratio 0.25. The obtained pulp is filtered in a vacuum büchner. The filtrate is called black liquor whose composition is given in Table-1. This solution is acidified by H_2SO_4 (1 M) until pH = 2; after setting during 24 h, the supernatant is eliminated and the residual is dissolved a second time with an alkaline solution (pH = 10). The second precipitation is carried out under agitation (250 rpm) with a sulphuric acid solution (0.5 M) until pH = 4. The precipitate undergoes a centrifugation under 3000 rpm for 10 min. The solid product obtained is dried during 12 h at 70 °C.

TABLE-1
BLACK LIQUOR CHARACTERISTICS

Parameters	pH	Conductivity (mS/cm)	Suspension matter (mg/L)	Chemical demand for oxygen (ppm)	Turbidity (NTU)
Values	12	11.7	1155	15900	770

FT-IR was recorded on FT-IR spectrometry using KBr pellets. UV-Visible spectra was recorded on Lambda spectrophotometer. For SEM of powdered lignin, Jeol 6360 equipped by EDAX analyzer was used.

High performance liquid chromatography analysis: refinement of lignin: The oxidation with nitrobenzene is followed by an extraction with chloroform: 50 mg of dried lignin is added to 7 mL of NaOH solution (2 M) and 0.4 mL of nitrobenzene ($C_6H_5NO_2$ 99 % purum). The mixture is stirred during 2 h 50 min at 155 °C. After cooling, the solution is transferred in a separating bulb. Then we add 10 mL of chloroform. After an agitation of 1 h, the solution is let elutriated. The extraction is carried out in several stages. Chloroform is then evaporated and the residue recovered in 5 mL of dichloromethane and an excess of chloroform (10 mL) is added. The result of present sample is compared with those of vanillin standard (50 mg) and mixture (25 mg lignin + vanillin 25 mg) standard. The apparatus used is from Shimadzu equipped with an universal UV/Visible detector and a C-R6A type integrator. The chromatographic solvents are marketed by Prolabo. We use CH_3CN, CH_3COOH like eluant, a nucleosil column C18, 5 µm, a flow of 1 mL/min and a wavelength λ = 280 nm.

Gas chromatography/mass spectrometry (GC/MS) analysis: The analysis was carried out using Nermag R10-10C mass spectrometer 10C with a variation in temperature from 140 to 260 °C (8 °C/mn) and from 260 to 280 °C (3 °C/mn). Two capillary columns are tested, one of 60 m (Supelco) with non-polar phase, other of 25 m (CP-SIL5CB). Four standards (vanillin, *p*-cresol, phenol and pyrocatechin) are dissolved in dichloromethane then injected in column for GC-MS analysis. The vanillin and lignin extracts were also analyzed.

RESULTS AND DISCUSSION

FT-IR spectrum showed the typical peaks of lignin, same as literature reported elsewhere[6,7] and summarized in Table-2.

TABLE-2
FT-IR PEAKS (cm^{-1}) ASSIGNMENTS FOR LIGNIN

cm^{-1}	Assignments
3447	Hydroxyl stretching
2924	CH_2 stretching
2850	$O\text{-}CH_3$ stretching
1648	C=C aromatic ring vibration
1465	C-H in plane deformation with the aromatic ring stretching
1384	CH_2 waggings
1100	Guaiacyl ring deformation with C=O stretching
1050	C-O-C ether bending allot to conyferilic alcohol
840	Synapilic and/or conyferilic alcohol groups vibration
618	CS connection vibration

The absorption band at 3460-3400 cm^{-1} indicates that OH groups are not free but enters in various modes of hydrogen bonds O-H stretching. The absorption at 1648 cm^{-1} is a lignin characteristic peak due to the C=C vibrations of the aromatic rings with ethylic connection. The 2850 cm^{-1} vibration corresponding to $O\text{-}CH_3$ group can be allot to vanillin. The 1442 cm^{-1} vibration is attributed to the C-H bond of CH_3 and CH_2 groups bonded to aromatic ring[9]. The absence of bands in area 2600 cm^{-1} indicates the absence of thial (HS) in soda lignin. The absence of absorption in the domain 1750-1659 cm^{-1} confirms the hemicellulose inexistence in soda lignin.

The UV/visible spectrum (Fig. 1) indicates a wavelength of maximum absorption of 239.9 nm. The UV/visible and FT-IR spectra show the presence of phenolic groups, hydroxylic and carboxylic functions and presence of double bonds. Thus the lignin can be used as basic commodity for the resin manufacturing. It replaces partially phenol of the pure resin and can play the dual part of binder and load.

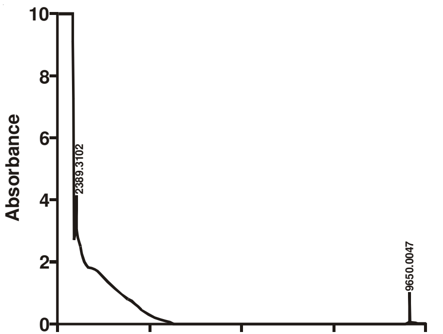


Fig. 1. Lignin UV/visible spectroscopy spectrum

Scanning electron microscopy (SEM) and EDAX analysis: SEM photographs (Fig. 2a) shows an heterogeneous granulometry of lignin powder. The carbon and oxygen constitute more than 95 % of the atomic distribution (Fig. 2b). The low sulphur content shows that the process of alkaline firing is carried out without sulphide addition (Table-3).

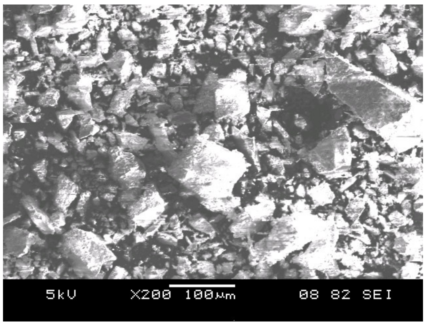


Fig. 2a. Lignin scanning electron microscopy

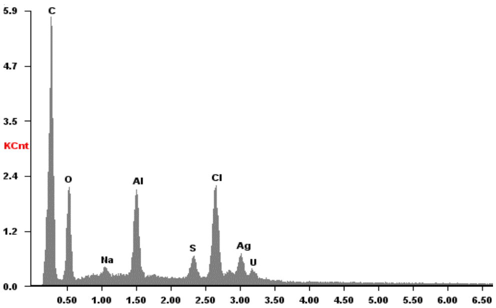


Fig. 2b. EDAX lignin

TABLE-3
EDAX LIGNIN ELEMENTARY ANALYSIS

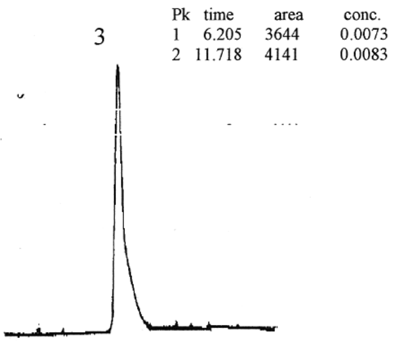
Element	Wt%	At%
C K	66.05	75.92
O K	23.19	20.01
NaK	00.66	00.40
AlK	03.27	01.67
S K	00.71	00.30
ClK	03.48	01.36
AgL	02.63	00.34
U M	00.00	00.00

HPL Chromatography analysis: The analysis of the resulting products from lignin oxidation and some standard products, suitable to be formed during the decomposition of lignin. Standard mixture analysis by high performance liquid chromatography gives a chromatogram where all the standards are separated. Retention times are given in Table-4.

The vanillin extract provides the chromatogram related in Fig. 3. The retention time of 23.38 min is observed. The chromatogram shown in Fig. 4 gives the analysis of the vanillin/lignin mixture. The a retention time is 26.3 min. The lignin analysis (Fig. 5) presents 3 main peaks (17.00, 22.46 and 28.05 min); time equal to 22.46 min corresponds to the vanillin chromatogram.

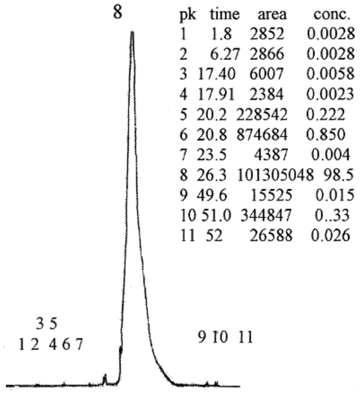
TABLE-4
INJECTED STANDARD. RETENTION TIMES (HPLC)

Standard products	Retention time (min)
Gallic acid	4.090
Resorcinol	7.508
Pyrocatechin	9.558
Acid P-OH-benzoic	11.828
Phenol	20.410
Vanillin	22.772
p-Cresol	50.092



Pk	time	area	conc.
1	6.205	3644	0.0073
2	11.718	4141	0.0083

Fig. 3.



pk	time	area	conc.
1	1.8	2852	0.0028
2	6.27	2866	0.0028
3	17.40	6007	0.0058
4	17.91	2384	0.0023
5	20.2	228542	0.222
6	20.8	874684	0.850
7	23.5	4387	0.004
8	26.3	101305048	98.5
9	49.6	15525	0.015
10	51.0	344847	0..33
11	52	26588	0.026

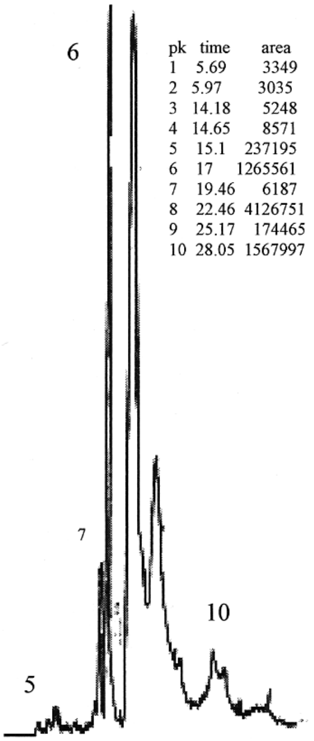
Fig. 4. Extract of mixture lignine/vanilline

Mass spectrometry (MS) analysis: The mass spectrometry analysis it is possible to check the structure and to estimate the purity of the standards, in order to identify the components of lignin (Fig. 6).

The spectra of the various standards show the fragmentation of each compound giving the abundance of the ions according to their m/z ratio; m and z are respectively weight and charge. The presence of the molecular peak of each standard and of other fragmentations also noticed. Concerning vanillin (Fig. 6a), it is noted one important peak of molecular mass equal to 152. For lignin spectrum (Fig. 6b), 3 main peaks which molecular mass is higher than 150 are noticed (151, 182 and 198).

GC-MS analysis: Good separation is obtained for the mixture of the 4 soluble standards in CH_2Cl_2 injected into a column of 25 m. Coupling GC/MS gives the chromatogram as well as the spectra with generation of the various peaks of ionization of each product.

Vanillin extract spectrum is given on Fig. 7. The presence of the peak characteristic of mass equal to 152 is observed. The same results are obtained with a shorter separation time using second column CP-SIL5CB (25 m).



pk	time	area	conc.
1	5.69	3349	6.04
2	5.97	3035	0 .04
3	14.18	5248	0.071
4	14.65	8571	0.115
5	15.1	237195	3.20
6	17	1265561	17.10
7	19.46	6187	0.083
8	22.46	4126751	55.78
9	25.17	174465	2.35
10	28.05	1567997	21.19

Fig. 5. Extract of mixture lignine/vanilline

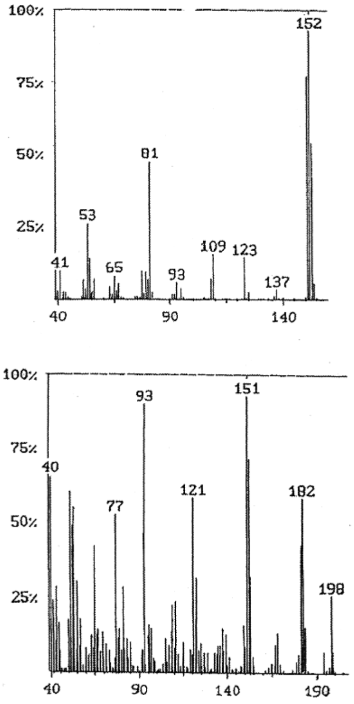


Fig. 6. Masse spectroscopy spectra (a) lignin extract (b) vanillin extract

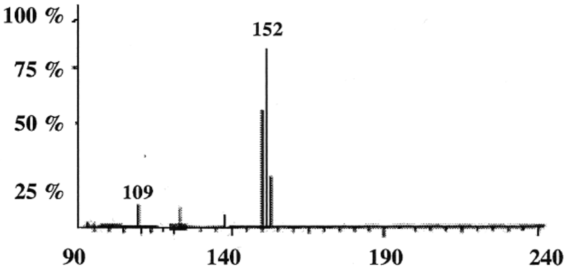


Fig. 7. Vanillin extract spectrum (GC/MS)

The spectrum of the vanillin/lignin mixture reveals the presence of three bulk products: 152, 182 and 198 (Fig. 8). The peak appearing to 152 is identified to the vanillin standard. The two other peaks could not be identified.

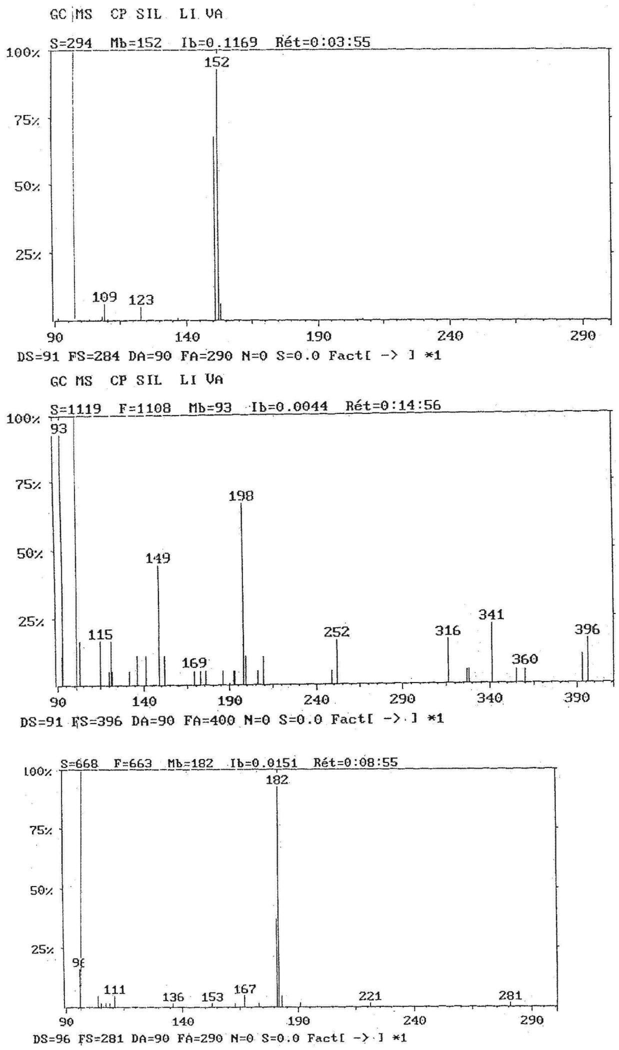


Fig. 8. Vanillin/lignin mixture extract spectrum (GC-MS) spectrum (GC-MS)

Conclusion

UV/visible spectroscopy has shown a presence of aromatic structure. According to FT-IR results, intensities of absorption bands at 3447, 1648 and 1465 cm^{-1} are characteristic peaks of lignin. Carbon and oxygen constitute more than 95 % of the atomic distribution. The low sulphur content shows that the process of alkaline leaching is carried out without sulphide addition. The results obtained in high performance liquid chromatography and GC/MS showed that the lignin contains vanillin. The peak appearing to 152 is identified to the vanillin standard. The two other peaks could not be identified.

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