World Academy of Science, Engineering and Technology
International Journal of Materials and Metallurgical Engineering
Vol:7, No:4, 2013

Structural Analysis of Lignins from Different Sources

I. F. Fiţigău, F. Peter, and C. G. Boeriu

***Abstract*—**Five lignin samples were fractionated with Acetone/Water mixtures and the obtained fractions were subjected to extensive structural characterization, including Fourier Transform Infrared (FT-IR), Gel permeation Chromatography (GPC) and Phosphorus-31 NMR spectroscopy (^{31}P-NMR). The results showed that for all studied lignins the solubility increases with the increment of the acetone concentration. Wheat straw lignin has the highest solubility in 90/10 (v/v) Acetone/Water mixture, 400 mg lignin being dissolved in 1 mL mixture. The weight average molecular weight of the obtained fractions increased with the increment of acetone concentration and thus with solubility. ^{31}P-NMR analysis based on lignin modification by reactive phospholane into phosphitylated compounds was used to differentiate and quantify the different types of OH groups (aromatic, aliphatic, and carboxylic) found in the fractions obtained with 70/30 (v/v) Acetone/Water mixture.

***Keywords*—**Lignin, fractionation, FT-IR, GPC, ^{31}P-NMR.

Open Science Index, Materials and Metallurgical Engineering Vol:7, No:4, 2013 publications.waset.org/4184/pdf

I. Introduction

LIGNINS are polymeric aromatic constituents in plant cell walls. They are traditionally considered to be dehydrogenative polymers from three monolignols: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Lignins as they occur in nature (protolignins) have been grouped into several types, characteristic of hardwoods, softwoods and grasses. The hardwood (angiosperm) lignins are built from the guaiacyl propane units (the abbreviation is G) and the syringyl propane units (the abbreviation is S).

Softwood lignins are composed mostly of guaiacyl-type lignin. Lignins obtained from straws are built from the guaiacyl propane units, the syringyl propane units and 4-hydroxylphenyl propane units (the abbreviation is H) [1] – [5].

Lignin properties are much more influenced by the kind of lignocellulosic material and the pulping process. The structure of lignin varies depending on its origin and the extraction method used to obtain it. Lignin molecules are very heterogeneous; therefore, fractionation has become one of the best ways to obtain specific lignins. The aim of fractionation is to obtain specific molecular weight fractions with defined properties; so they can be then used as high added value products [6, 7].

Lignins are polar polymer (on average 1–2 hydroxyl groups per monomer) and thus present a poor solubility in nonpolar media such as polyolefins. Because of that their reactivity with the radicals responsible for the oxidation and subsequently their protecting effect compared to that of synthetic antioxidants are limited [8].

Fractionation can be used to separate the principal components of a fibrous raw material: cellulose, hemicelluloses and lignin. This study analyzed the structural changes of five technicals lignins from different sources during fractionation with acetone/water mixtures in order to elucidate the molecular basis of differences found between the obtained fractions. Due to the very complex constitution of lignins, it is difficult to find a single technique to characterize their structures.

The fractionated and unfractionated lignins were characterized using FT-IR spectroscopy, ^{31}P-NMR analysis and gel permeation chromatography (GPC).

Fourier Transformed-Infrared Spectroscopy (FT-IR) technique was applied to identify the characteristic adsorption bands of the chemical groups in lignin. The weight average molecular weight (Mw) was determined by size exclusion chromatography (SEC) and the ^{31}P-NMR analysis was used to determine the content of hydroxyl groups in lignin (aliphatic-OH, phenolic-OH, carboxyl-OH), as an indicator of their potential reactivity.

II. Materials and Methods

A. Materials

Soda wheat straw lignin and P1000 soda lignin from mixed Sarkanda grass and wheat straw were obtained from Granit SA (Lausanne, Switzerland). Alcell TM organosolv lignin from mixed hardwoods (maple, birch and poplar) was obtained from Repap Technologies Inc. (Valley Forge, PA, USA). Kraft lignin, Indulin AT, from softwood was obtained from MeadWestvaco (USA). Alkali-pretreated wheat straw lignin was purchased from TU Bergakademie (Freiberg, Germany) All other chemicals used were of analytical grade.

B. Methods

1. Lignin Fractionation

4 g of lignin was added to 10 ml acetone/water in different ratios. The reaction mixture was stirred for 24 hours at room temperature. The undissolved fractions were separated on a glass filter and dried to constant weight in a vacuum oven at 105°C. The lignin concentration in the filtrate was analysed spectrophotometrically by dilution of the soluble fraction with 1% NaOH solution. Lignin concentration was calculated based on a calibration curve established with a series of lignin solutions with differing concentrations in 1% NaOH solution. The UV light absorption was determined in the range from 200 to 600 nm in the double-beam Lambda

Firuţa I. Fiţigău is PhD student at Politehnica University of Timisoara, Faculty of Industrial Chemistry and Environmental Engineering, C. Telbisz 6, 300001 Timisoara, Romania (Corresponding author: phone: +40-256-404216; fax: +40-256-403060, e-mail: fitigau_firuta@yahoo.com).

Francisc Peter is Professor of Industrial Biotechnologies at Politehnica University of Timisoara, Faculty of Industrial Chemistry and Environmental Engineering, Timisoara, Romania (e-mail: Francisc.Peter@chim.upt.ro).

Carmen G. Boeriu is Senior Scientist at Wageningen UR Food & Biobased Research, Wageningen, The Netherlands (e-mail: Carmen.Boeriu@wur.nl).

Open Science Index, Materials and Metallurgical Engineering Vol:7, No:4, 2013 publications.waset.org/4184/pdf

spectrophotometer. The maximum absorbance was 290 nm for Kraft lignin and Alcell, 287 nm for alkali pre-treated wheat straw lignin and P1000 Soda lignin, and 285 nm for wheat straw lignin. Each fraction was evaporated under reduced pressure and the final remaining residue dried to constant weight.

2. FT-IR Spectroscopy

Fourier Transform Infrared (FT-IR) spectra of the solid lignin samples were obtained in attenuated total reflectance (ATR) mode on a Varian Scimitar 1000 FT-IR spectrometer equipped with a DTSG-detector. The measurement resolution was set at 4 cm-1, and the spectra were collected in the range 4000-650 cm-1 with 128 co-added scans. The sample chamber was purged with N2 gas before scanning was started. ATR was performed on a PIKE MIRACLE ATR equipped with a diamond w/ZnSe lens single reflection plate.

3.Alkaline SEC Analysis

SEC analysis of samples was performed using a Waters size exclusion chromatographic system equipped with UV detection and differential refractive index (RI) detection.

Lignin samples of 1 mg/ml dissolved in 0.5 M NaOH were injected onto a manually packed column (4.6 mm x 30 cm) of ethylene glycol methacrylate copolymer TSK gel ToyopearlHW-55F. The analyses were carried out at 30°C using 0.5 M NaOH in water as the eluent, at a flow rate of 1 mL min^{-1} and UV-detection at 280 nm.

The GPC system was calibrated with polystyrene standards in the molecular weight range of 891 – 1.02 $\times 10^6$ Da. Empower Pro software (Waters) was used for data processing.

4. Quantitative ^{31}P-NMR

In a 1 mL vial, 30 mg of lignin was mixed with 100 µl N, N-Dimethylformamide (DMF)/pyridine (1:1 v/v) and 100 µl internal standard solution containing 15 mg/ml cyclohexanol (internal standard) and 2.5 mg/ml chromium (III) acetylacetonate in pyridine. This suspension was stirred for 4-16 hours at room temperature. One hundred µl derivatization reagent (2-chloro-4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaphospholane) was mixed with 400 µl of CDCl3 prior to addition to the lignin solution. After 10 minutes mixing, the mixture was analyzed by NMR (Bruker 400 MHz), with 30 degree pulse angle, inverse gated proton decoupling, a delay time of 5 seconds and 256 co-added scans. Signal was performed as described by Granata and Agryropoulus [9]. Lignin weight was corrected for moisture content.

III. Results and Discussion

A. Lignin Fractionation with Acetone: Water Mixture

The five selected technical lignins showed different solubility in acetone/water mixture (Fig. 1). It can be seen that the solubility of lignins was highly dependent on the acetone concentration, increasing strongly with the increase of acetone concentration. At small acetone concentrations (10%, 30%) the solubility of all five chosen lignins was very low, only a small quantity of lignin was dissolved in solution.

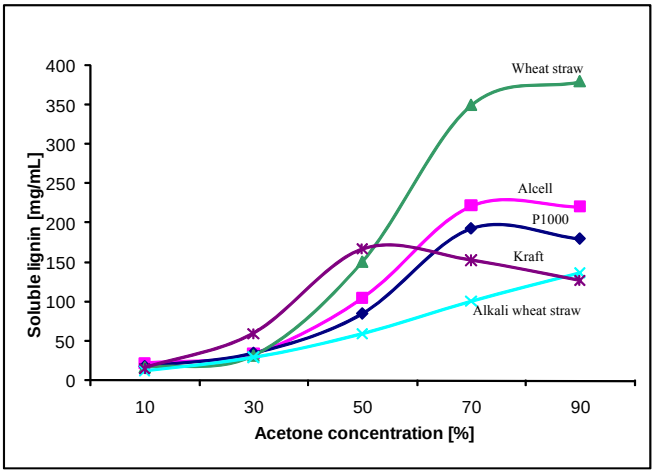


Fig. 1 Effect of acetone concentration on lignin solubility (at 23°C). Solubility expressed as fraction dissolved of initial 400 mg lignin/mL in the solvent mixture

According to data from Fig. 1, on can expect that the mixtures above 70% acetone concentration should result in the highest solubility, thing that was true for wheat straw lignin and alkali pretreated wheat straw lignin.

For Alcell, P1000 and Kraft lignin, the solubility increased with the increase of the acetone concentration till the maximum of solubility was reached (when 70 % acetone was used in the reaction mixture) after that a slightly decrease was observed when the acetone concentration was increased at 90 vol. %. The observed solubility trends are similar to those reported for ethanol-water mixtures by Ni et al. [10].

In the case of Kraft lignin the maximum of solubility was achieved when the acetone concentration was of 50% in the reaction mixture. Among the studied lignins, Kraft lignin was the only lignin that shows a maximum of solubility in 50:50 (v/v) Acetone/Water. About 165 mg / ml of Kraft lignin can be dissolved in this mixture and the solubility also decreased when higher acetone concentrations were used.

The wheat straw lignin has the highest solubility, being almost completely dissolved when the acetone concentration was 90%. At a concentration higher than 400 mg/mL of wheat straw lignin in 90:10 (v/v) Acetone/Water mixture the solution became very viscous. In this case, it becomes difficult to determine lignin concentration in the filtrate. Alkali pretreated wheat straw lignin has the lowest solubility in acetone water mixtures among the five lignins showing a maximum of solubility of 130 mg/mL lignin in Acetone:Water 90/10 (v/v).

B. Fourier Transform Infrared Spectroscopy

FT-IR spectroscopy, which is a complementary and extensively used method, yields information about the molecular conformation and hydrogen bonding patterns. The analysis of the characteristic bands of the lignin skeleton and its functional groups was carried out according to literature data [11, 12]. Because of the amorphous character of lignins and the complicated and diverse structure of their structural units, use of theoretical methods to interpret the spectra is almost impossible. Therefore, the bands are assigned based on

Open Science Index, Materials and Metallurgical Engineering Vol:7, No:4, 2013 publications.waset.org/4184/pdf

spectral data of model compounds and specially produced lignin preparations. The spectra of lignins are complicated and most of the bands observed are produced by imposition of various types of vibrations of different functional groups. Therefore, assignment of many bands is possible only in the approximation of the predominant contribution of certain atomic groups.

Fig. 2 shows the infrared spectra of the studied lignins and their fractions. FT-IR spectra reflect the chemical structure as well as the purity of lignins. Common features as well as particular vibrations, specific to each lignin, are found in the spectra. Below we present an analysis of the recorded spectra of the lignin samples used in this study, based on the assignments given by Faix (1992) [11].

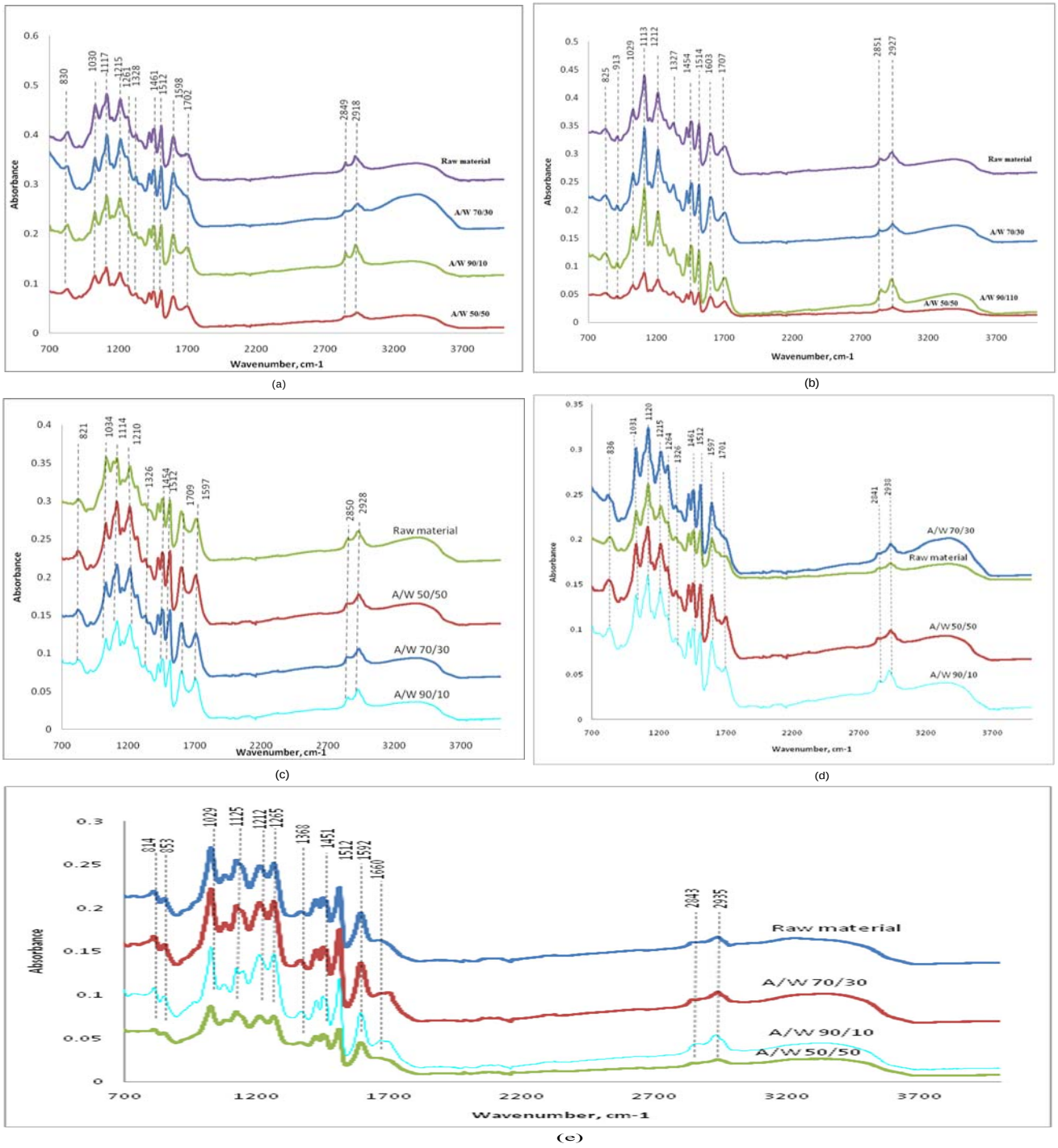


Fig. 2 FT-IR spectra of (a) P1000, (b) Alcell, (c) Alkali wheat straw, (d) Wheat straw and (e) Kraft fractionated lignins

Open Science Index, Materials and Metallurgical Engineering Vol:7, No:4, 2013 publications.waset.org/4184/pdf

TABLE I
BANDS ASSIGNMENTS OF THE FRACTIONATED LIGNINS

Peak wavenumber (cm^{-1})					
P 1000	Alcell	Wheat straw	Alkali wheat straw	Kraft	Band assignment
3445	3444	3450	3440	3415	Stretching vibrations in O-H
2927	2918	2938	2928	2935	CH stretching vibrations
2851	2840	2841	2850	2843	Tertiary CH groups
1707	1702	1701	1709	1660	Carbonyl–carboxyl stretching
1603	1598	1597	1597	1592	Aromatic stretching band
1454	1461	1461	1454	1451	C-H deformation
1212	1117	1120	1114	1265	C-O stretching, aromatic (phenyl)
1030	1030	1031	1030	1029	C-O deformation (methoxyl group)
825	830	836	821	814	C–H out-of-plane vibrations in position 2, 5 and 6 of guaiacyl units

Table I presents the wavenumbers and the important band identifications found. All lignins show a broad band at 3410–3460 cm^{-1}, attributed to the hydroxyl groups in phenolic and aliphatic structures, and the bands centered around 2910 and 2850 cm^{-1}, predominantly arising from CH stretching in aromatic methoxyl groups and in methyl and methylene groups of side chains. In the carbonyl/carboxyl region, is found o band around 1700 cm^{-1}, originating from unconjugated carbonyl/carboxyl stretching, bands that are more pronounced in the alkali pretreated wheat straw lignin fractions. Weak absorptions around 1660 cm^{-1} in the case of Kraft lignin, resulting in the asymmetry and broadening of the more intense bands at 1592 cm^{-1} may originate from both protein impurity and water associated with lignin.

Aromatic skeleton vibrations at 1600, 1512 and 1425 cm^{-1} and the C–H deformation combined with aromatic ring vibration at around 1450 cm^{-1} are common for all lignins, although the intensity of the bands may differ. The spectral region below 1400 cm^{-1} is more difficult to analyze, since most bands are complex, with contribution from various vibration modes. However, this region contains vibrations that are specific to the different monolignol units and allows the structural characterization of lignins. The spectra of all lignin samples show the vibrations characteristic for the guaiacyl unit (at around 1260 cm^{-1}, G ring and C=O stretch; 1150 cm^{-1}, CH in-plane deformation; 830 and 915 cm^{-1}, C–H out-of-plane vibrations in position 2, 5 and 6 of guaiacyl units) but the intensity of the bands vary significantly between samples. Syringyl (S) and guaiacyl (G) units are detected by aromatic skeleton vibrations at 1600 and 1328 cm^{-1} (S), 1512 and 1261 cm^{-1} (G), and aromatic in plane C−H vibrations at 1117 (S) and 1030 cm^{-1} (G).

Common for the spectra of all lignin samples are a weak band at 1370–1375 cm^{-1} originating from phenolic OH and aliphatic C–H in methyl groups and a strong vibration at 1215–1220 cm^{-1} that can be associated with C–C plus C–O plus C=O stretching. The aromatic C–H deformation at 1030 cm^{-1} appears as a complex vibration associated with the C–O, C–C stretching and C–OH bending in polysaccharides. Carbohydrate originating vibrations are associated also with other vibrations in the spectral region 1000–1300 cm^{-1}.

C. Molecular Weight Distribution

Size-Exclusion chromatography (SEC) offers many advantages, such as wide availability, short analysis time, low sample demand, and determination of molar mass distribution over a wide range. The molecular weight distributions from the GPC results are mainly from the phenolic compounds derived from lignin, because these compounds have high sensitivity in the UV detector. The molecular weight changes of the fractionated and unfractionated lignins as shown in Fig. 4.

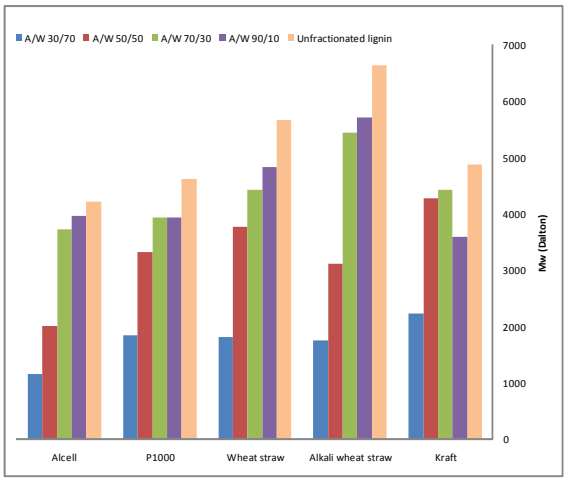


Fig. 4 Molecular mass distribution for Acetone/Water fractionated and unfractionated technical lignins

A steady increment of the molecular weight was observed with the increment of acetone concentration and thus with solubility. Due to the good solubilisation of the Allcell, P1000, Wheat straw and Alkali pretreated wheat straw lignins in 70/30 Acetone/Water mixture the Mw values obtained for these fractions are very close to those of the unfractionated lingins.

For the Kraft lignin the maximum of solubility was reached when the acetone concentration in the reaction mixture was of 50% and obviously this fraction had the highest weight average molecular weight.

D. ^{31}P-NMR Characterization

In order to quantify the different hydroxyl groups present in the lignin fractions a ^{31}P-NMR study of the phosphitylated polymer was carried out. The results found for Indulin AT and Alcell lignin are in agreement with those reported by Granata and Argyropoulos (1995) [9]. A comprehensive compilation of hydroxyl groups in lignin and their typical chemical shifts/integration ranges using ^{31}P-NMR analysis is summarized in Table II [13, 14].

TABLE II
INTEGRATION REGIONS USED FOR ^{31}P–NMR ANALYSIS OF FRACTIONATED LIGNINS [13, 14]

Structure	δ(ppm)
Aliphatic OH	149.0 – 145.5
Cyclohexanol (Internal standard)	144.7 – 145.5
β–5	143.118
Syringyl	142.117
5–5	140.2 – 141.7
Guaiacyl	139.0 – 140.0
Para–hydroxy–phenyl	137.3 – 138.2
Carboxylic acid OH	133.6 – 136.6

A typical ^{31}P-NMR spectrum of the P1000 lignin is shown in Fig. 5. Because of the good solubilization of all studied lignins in 70/30 acetone/water mixture we compared the hydroxyl group content of these fractions with the raw material ones.

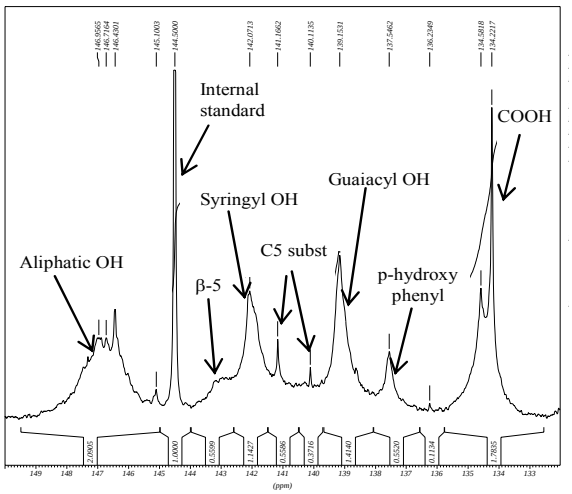


Fig. 5 Quantitative ^{31}P-NMR spectrum of P1000 Soda lignin

Table III summarizes the results of quantitative 31P NMR analysis of lignin fractions.

Among the various hydroxyl groups, the aliphatic hydroxyl signal is typically the dominant in lignin.

A minor amount of carboxylic OH groups is observed in lignins from hardwoods (0.29 mmol g^{-1}) and softwoods (0.54 mmol g^{-1}) while lignins extracted from wheat straw contain higher amounts of carboxylic OH groups (0.9 – 1.2 mmol g^{-1}).

The carboxyl group content increased after the fractionation process in the case of Alcell, P1000 and Alkali pretreated wheat straw lignins indicating that the formation of oxidized functional groups could take place during processing.

The ^{31}P-NMR data (Table III) showed that Kraft softwood lignin has the highest aliphatic OH content and the major phenolic hydroxyl appeared to be guaiacyl phenolics with a minor amount of *p*-hydroxyphenyl (0.33 mmol g^{-1}). Syringyl unit it's not detected in raw Kraft lignin but a slightly increase of the syringyl OH content (0.33 mmol g^{-1}) could be observed in the obtained fractions after the fractionation with 70/30 Acetone/Water mixture. In wheat straw lignins and P1000 lignin all three types of phenolic OH groups were found (H-OH, G-OH, and S-OH)

Alcell lignin being a hardwood lignin it is typically composed of syringyl and guaiacyl units and small amounts of *p*- hydroxyphenyl groups. The results found for Kraft Indulin AT and Alcell lignins are in agreement with those reported by Granata and Argyropoulos (1995) [9].

From the Table III it could be also observed that the total phenolic hydroxyl content increased after the fractionation with 70/30 acetone/water mixture for all chosen lignins excepting wheat straw lignin.

TABLE III
HYDROXYL GROUP CONTENTS OF 70/30 ACETONE/WATER LIGNIN FRACTIONS AS DETERMINED BY ^{31}P-NMR ANALYSIS

	OH content (mmol g^{-1})					
Sample	S-OH	G-OH	H-OH	Total phenolic	Aliphatic	COOH
Alcell						
Raw	1.04	0.69	0.18	1.91	1.11	0.29
A/W 70/30	1.32	0.91	0.25	2.48	1.28	0.41
P1000						
Raw	0.60	0.75	0.50	1.85	1.79	1.11
A/W 70/30	0.67	0.88	0.59	2.14	1.56	1.20
Wheat straw						
Raw	0.81	0.98	0.50	2.29	2.10	1.10
A/W 70/30	0.68	0.81	0.40	1.89	1.91	0.92
Alkali wheat straw						
Raw	0.58	0.72	0.28	1.58	1.07	0.92
A/W 70/30	0.83	0.92	0.37	2.12	1.21	1.06
Kraft						
Raw	0	1.98	0.33	2.31	2.41	0.54
A/W 70/30	0.33	1.79	0.30	2.42	1.95	0.47

S-OH = syringyl phenolic hydroxyl; G-OH =guaiacyl phenolic hydroxyl; H-OH = p-hydroxyphenyl phenolic hydroxyl

Open Science Index, Materials and Metallurgical Engineering Vol:7, No:4, 2013 publications.waset.org/4184/pdf

Open Science Index, Materials and Metallurgical Engineering Vol:7, No:4, 2013 publications.waset.org/4184/pdf

IV. Conclusion

In summary, five different lignins were fractionated using Acetone/Water mixtures and the obtained fractions were characterized using FT-IR, HPSEC and ^{31}P-NMR analysis. Among the studied lignins, wheat straw lignin has the highest solubility in 90:10 (v/v) Acetone/Water about 400 mg/mL lignin can be dissolved in this mixture. Kraft lignin has the maximum of solubility when 50% of acetone was used in the reaction mixture. At higher acetone concentrations the solubility decreased slowly with the increase of acetone concentration.

FT-IR spectroscopy was applied to obtain the structural information and characterization of the five native lignins and the obtained fractions. From the FT-IR spectra the characteristic adsorptions bands of lignins were identified. FT-IR analysis showed some differences between the spectra for the five lignins studied but no significant changes were observed between the native lignins and the obtained fractions. All the lignins exhibited relatively low molecular weights after the fractionation process, indicating a lower condensation than the native lignin samples.

The ^{31}P-NMR technique has been used for the quantitative assessment of various hydroxyl groups present in lignin in a relatively short time, including guaiacyl, syringyl, C5 substituted guaiacylphenolics, p–hydroxyphenyls, aliphatic and carboxylic hydroxyls.

After the fractionation with 70/30 acetone/water mixture the total phenolic hydroxyl content increased for all chosen lignins excepting wheat straw lignin.

Acknowledgment

The work of Firuța I. Fițigău was partially supported by the strategic grant POSDRU 107/1.5/S/77265, inside POSDRU Romania 2007-2013 co-financed by the European Social Fund – Investing in People.

Jacinta van der Putten, Richard Gosselink and Guus Frissen were kindly acknowledged for their help with the SEC, NMR and FT-IR analyses.

References

[1] K. V. Sarkanen, C. H. Ludwig, *Lignins: Occurrence, Formation, Structure and Reactions*, Wiley-Interscience, New-York, 1971.

[2] A.T Martinez, M. Speranza, F.J. Ruiz-Duenas, P. Ferreira, S. Camarero, F. Guillen, M.J. Martinez, A. Gutierrez, J.C. del Rio, "Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin", *Int Microbiol,* vol. 8, pp. 195-204, 2005.

[3] M. Dashtban, H. Schraft, T. A. Syed, W. Qin, "Fungal biodegradation and enzymatic modificationof lignin", *Int J Biochem Mol Biol,* vol. 1(1) pp. 36-50, 2010.

[4] E. A. Capanema, M. YU. Balakshin, J. F. Kadla, "Quantitative Characterization of a Hardwood Milled Wood Lignin by Nuclear Magnetic Resonance Spectroscopy", *J. Agric. Food Chem.* vol. 53, pp. 9639- 9649, 2005.

[5] J.R. Obst, " Guaiacyl and Syringyl Lignin Composition in Hardwood Cell Components", *Holzforschung*, vol. 36, pp. 143-152, 1982

[6] J.R. Rostrup-Nielsen, "Chemistry: making fuels from biomass", *Science* vol. 308, pp. 1421-1422, 2005.

[7] D. Yang, X. Qiu, M. Zhou, H. Lou, "Properties of sodium lignosulfonate as dispersant of coal water slurry", *Energy Convers. Manage.*, vol. 48 pp. 2433–2438, 2007.

[8] C. Pouteau, P. Dole, B. Cathala, L. Averous, N. Boquillon, "Antioxidant properties of lignin in polypropylene", *Polymer Degradation and Stability*, vol. 81, pp. 9–18, 2003.

[9] A. Granata, D. S. Argyropoulos, "2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, a reagent for the accurate determination of the uncondensed and condensed phenolic moieties in lignins", *J. Agric. Food Chem.,* vol. 43, pp. 1538-1544, 1995.

[10] Y. Ni, Q. Hu, "Alcell lignin solubility in ethanol-water mixtures" *J. Appl. Polym. Sci.*, vol. 57, pp. 1441–1446, 1995.

[11] O., Faix, *Fourier transformed infrared spectroscopy,* In: S.Y., Lin, C.W. Dence, (Eds.), *Methods in Lignin Chemistry*, Springer-Verlag, Berlin-Heidelberg, pp. 458–464, 1992.

[12] C. G. Boeriu, D. Bravo, R.J.A. Gosselink, J.E.G. van Dam "Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy", *Industrial Crops and Products,* vol. 20, pp. 205–218, 2004.

[13] Y. Pu ,S. Cao, A. J. Ragauskas, "Application of quantitative ^{31}P-NMR in biomass lignin and biofuel precursors characterization", *Energy Environ. Sci.*, vol. 4, pp. 3154 - 3166, 2011.

[14] F. Abdelkafi, H. Ammar, B. Rousseau, M. Tessier, R. El Gharbi, A. Fradet, "Structural analysis of alfa grass (Stipa tenacissima L.) lignin obtained by acetic acid/formic acid delignification", *Biomacromolecules*, vol. 12, pp. 3895−3902, 2011.