Functionality of Wheat Straw Lignin Extracted in Organic Acid Media

Guo-Hua Delmas,[1,2] Bouchra Benjelloun-Mlayah,[1] Yves Le Bigot,[2] Michel Delmas[1,2]

[1]*Compagnie Industrielle de la Matière Végétale (CIMV), 134-142 rue Danton, 92593 Levallois Perret, France*
[2]*Université de Toulouse, Inp-Ensiacet, Laboratoire de Génie Chimique (LGC), 4 allée Emile Monso-BP 44362, 31030 Toulouse Cedex 4, France*

ABSTRACT: Wheat straw lignin was extracted by the CIMV process using organic acid media at pilot plant scale. The product was analyzed by gel permeation chromatography (GPC), ^{1}H and ^{13}C NMR spectroscopy, infrared attenuated total reflectance-Fourier transform infrared analysis (ATR-FTIR), and gas chromatography (GC) to clarify its structure and functionality. In most cases, lignin was esterified before analysis. Control of the esterification was conducted via ATR-FTIR and NMR. GC analysis was used to quantify total hydroxyl group of lignin by saponification of propionylated lignin and was also used to quantify phenolic hydroxyl groups of lignin by amino- lysis of propionylated lignin. Acetylated lignin was analyzed by GPC. Carboxylic group of lignin was determined by pH metric titration. Lignin extracted from the CIMV process was observed as a low molecular weight polymer with a low polydispersity index and high free hydroxyl content. The potential of lignin as a natural polyphenol was confirmed by the analytical results obtained. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 491–501, 2011

Key words: biopolymers; NMR; ATR-FTIR; gel permeation chromatography; lignin

INTRODUCTION

The industrial paper technology has not evolved for one century since chemical, mechanical, and thermo-mechanical pulps are still considered. Considerable progress was made on the modern production facilities for treating hard and soft wood in term of effectiveness and environment but the cellulose remains in both cases the most valued. Regarding most pulp extraction processes, hemicelluloses and lignin are strongly degraded and are generally used as source of energy.

The CIMV[1–4] process offers a new alternative of paper industry as it allows the separation of cellulose, lignin, and hemicelluloses that are the main components of lignocellulosic biomass.

Lignin is an abundant macromolecular material that is biosynthesized by a random coupling of radical phenylpropane units of coniferyl, sinapyl, and coumaryl alcohol. Lignin has been a challenge in structural research for a long time, regarding well known industrial pulp process because of its heterogeneous and complex molecular structure.[5] The most important functional groups affecting the functionality of lignin include free aliphatic and phenolic hydroxyl. To consider chemical reactions on lignin, it is necessary to quantify these reactive functions (aliphatic, phenolic, and carboxylic hydroxyl). Most of the lignins studied nowadays are extracted by the Kraft pulping process[6] in basic condition, whereas CIMV process extracts the lignin in acidic condition.

Several techniques have been developed to quantify hydroxyl function of Kraft lignin. Whiting and Goring[7] analyzed lignin by pyrolitic gas chromatography (GC). Mansson[8] determined quantitatively phenolic and total hydroxyl groups by aminolysis and GC. Roberts and Brunow[9] estimated quantitatively hydroxyl groups by ^{13}C NMR spectroscopy. Liu et al.[10] used conductometric titration in organic solvent to determine phenolic hydroxyl and carboxylic groups. Faix et al.[11] used aminolysis, Fourier transform infrared analysis (FTIR), ^{1}H NMR, and UV spectroscopy to analyze phenolic hydroxyl group and Barelle[12] used ^{19}F NMR spectroscopy to analyze hydroxyl groups in lignin. The method used to determine the hydroxyl groups has to be selective, sensible, and reproducible. Recent works of Banoub et al.[13,14] showed the stereoregular structure of CIMV wheat straw lignin. Following their works, we report in this article a complete analysis and comprehension of the structure and functionality of wheat straw lignin extracted in acidic condition by the CIMV process. Various techniques such as gel permeation chromatography (GPC), ^{1}H and ^{13}C NMR
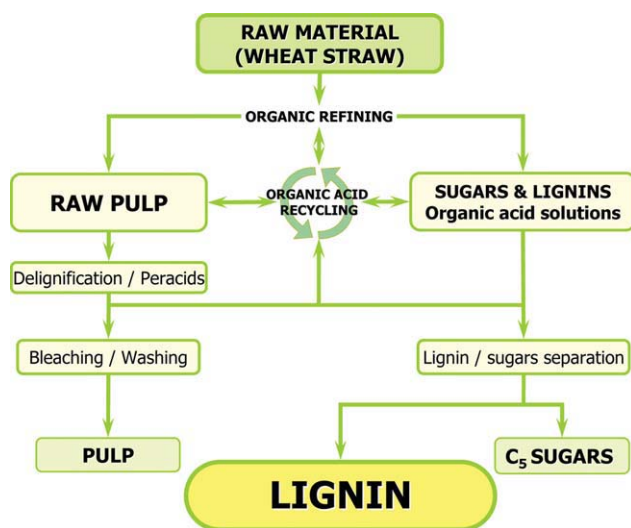
Figure 1 The CIMV organosolv process. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

spectroscopy, attenuated total reflectance-FTIR (ATR-FTIR), and GC were used.

MATERIALS AND METHODS

Lignin extraction

CIMV process is designed for the manufacture of whitened paper pulp, lignin, and hemicelluloses mainly from cereal straw. This new technology allows the separation without degradation of the three main components of the vegetable matter and represents the first biomass refinery technology (Fig. 1).

After an appropriate mechanical conditioning, wheat straw is treated at atmospheric pressure with a mixture of acetic acid/formic acid/water 30/55/15 (v/v/v), for 3.5 h of reaction time at 105°C. In these conditions, wheat straw lignin is dissolved and hemicelluloses are hydrolyzed in oligosaccharides and monosaccharides with high xylose content. The raw pulp is pressed, delignified, and bleached. Organic acids are then recycled by concentration of the extraction liquor containing lignin and hemicelluloses. The concentrated extraction liquor is treated with water to precipitate lignin, which is easily obtained by high pressure filtration. The analyzed wheat straw lignin was extracted at pilot scale at the CIMV pilot plant (Pomacle 51110, France). Around 1000 kg of wheat straw was treated to extract at the end of the process nearly: 500 kg of pulp, 250 kg of C5 sugars syrup, and 250 kg of lignin. Purity of the lignin fraction was ∼ 95%.

Gel permeation chromatography

GPC analysis was conducted using a Waters (St-Quentin, France) 1515 isocratic HPLC pump and a

Waters 2414 Refractive detector. Two PolarGel-L (particle size: 8 μm) columns connected with one guard-column (Agilent Technologies, Les Ulis, France) were used. Tetrahydrofuran (THF) was used as eluent and sample solvent. Refractive detector and columns were constantly maintained at 30°C. Flow was regulated at 1 mL min^{-1} for all GPC analysis and the injection volume was 20 μL. Standard polymers used were polyethylene glycol (PEG): 7930, 3920, 3000, 2000, 1500, and 1010 g mol^{-1}. The concentration used was 100 mg mL^{-1} of sample in THF. Acetylated wheat straw lignin was totally soluble in THF and was representative of wheat straw lignin extracted from CIMV pilot plant.

Attenuated total reflectance-Fourier transform infrared analysis

Infrared analyses were conducted with an ATR system. This technique takes its advantage that no solvent or KBr pellets are used. The pure and dry product is directly analyzed on the crystal plate under high pressure so that no other interaction is involved.

Analyses were done on a PerkinElmer (Courtaboeuf, France) Spectrum 100 Universal ATR-FTIR instrument equipped with a diamond/ZnSe crystal single reflection. Few milligrams of dried samples were placed to the crystal plate with a constant pressure applied at 85 N mm^{-2} to all analyses during 5 scans at a resolution of 4 cm^{-1}. To avoid any traces of water on the FTIR spectrum, all samples were dried and stocked inside desiccators in dry atmosphere (pentoxide phosphate), and dry matter was measured at 99.9%.

Solid-state ^{13}C NMR analysis

The solid-state ^{13}C NMR spectrum of wheat straw lignin was recorded on a Bruker (Wissembourg, France) Advance 400 MHz instrument at 20°C.

^{1}H NMR analysis

The ^{1}H NMR spectrum was recorded on a Bruker Advance 500 MHz (coupled with cryoprobe) instrument at 25°C. Acetone d$_6$ was used as solvent. The tube was placed under ultrawave to have a complete solubilization of esterified lignin.

GC system

GC analyses were conducted using a PerkinElmer Autosystem XL equipped with a ionization flame detector and a DB-5 capillary column. Chromatographic conditions: gas vector is Helium at a pressure of 11 psi, injector temperature at 200°C, and injection volume at 0.5 μL. Temperature profile: 60°C during

1 min; 60–140°C at 15°C min^{-1}; 140–250°C at 5°C min^{-1}; isotherm at 250°C to evacuate possible undesired product, and reset of the oven to 60°C.

Propionylation of wheat straw lignin

Propionylation was conducted using propionic anhydride and pyridine. 200 mg of dry wheat straw lignin is dropped into small pillbox. Pyridine (2 mL) and propionic anhydride (2 mL) are added. Reaction is stirred and maintained at room temperature during 17 h and stopped by adding 16 mL of CH_2Cl_2 and 2 mL of methanol. Stirring is maintained at room temperature during 30 min. The mixture is transferred into a funnel and washed with: 2M HCl aqueous solution, $NaHCO_3$ aqueous saturated solution, and finally distilled water. The organic phase is collected and dried with $MgSO_4$. After filtration the solvent is evaporated under reduced pressure. Propionylated lignin is a solid and dark powder. It is kept in oven at 45°C during 48 h and stocked in dry atmosphere. ATR-FTIR analyses were done to confirm the complete propionylation reaction. Acetylation was conducted the same way with acetic anhydride at room temperature.

Total hydroxyl analysis of lignin by GC

Preparation of internal standard for GC calibration

Propionic acid solution (100 μL of 0.1M) is transferred in a 25 mL beaker with 100 μL of a 0.1M butyric acid solution. Mixture is then diluted with 15 mL of distilled water and adjusted to pH 8 with a 0.3M solution of tetrabutylammonium hydroxide. Final mixture is concentrated under reduced pressure to syrup and solubilized in 2 mL of acetone. Benzyl bromide (3 μL) is added and the mixture is kept to room temperature for 10 min. The procedure is the same for acetic acid and formic acid.

Preparation of benzyl alkylates from propionylated lignin

Dried propionylated wheat straw lignin (20 mg) is added in a 5 mL flask with 0.5 mL of 1,4-dioxane and 0.5 mL of a 1M solution of sodium methoxide in methanol. The flask is placed in an ultra wave bath at room temperature during 30 min. Solution of butyric acid (2 mL of 0.05M) is added to the mixture that is kept at room temperature during another 30 min to achieve saponification. The acidification of formed alkylates is assured by an ion-exchange column (40 × 10 mm^2) filled with a height of 2 cm of resin (Bayer-Katalysator hydrogen resin K2411) with a frit. The eluent used is a mixture 1/1/4 (v/v/v) of 1,4-dioxane/methanol/H_2O. The eluent (15 mL) is used to wash the resin. The eluent at the end of column must be colorless. The sample mixture is added to the column with 20 mL of eluent. The elution flow is regulated at 4 cm^3 min^{-1}. Collected effluent is adjusted with a pH-meter at 8–9 with a 0.03M aqueous solution of tetrabutylammonium hydroxide. Water is evaporated under reduced pressure to obtain a syrup at the end. Acetone (2 mL) is added to solubilize the syrup and transferred into a 5-mL flask. Benzyl bromide (40 μL) is added into the 5-mL flask. The mixture is kept at room temperature during 20 min to achieve the reaction.

Calibration method of GC system via internal standard (benzyl butyrate)

To process to the hydroxyl quantification, GC system was calibrated by determining the value of the response factors of benzyl propionate, acetate, and formiate. Benzyl butyrate was used as an internal standard. Each mixture of 0.5 μL was injected into the GC system. The areas of the benzyl alkyl ester A_a and benzyl butyrate A_e are determined by GC. Response factors of benzyl formiate, acetate, or propionate are determined by the expression:

$$f_{\text{tot}} = C_a \cdot A_e / C_e \cdot A_a$$

where f_{tot} = response factor of benzyl alkyl ester (formiate, acetate or propionate) related to benzyl butyrate, C_a = Concentration of alkyl acid (mol L^{-1}), C_e = Concentration of butyric acid (mol L^{-1}), A_a = Chromatographic area of benzyl alkyl ester, A_e = Chromatographic area of benzyl butyrate.

Phenolic hydroxyl analysis of lignin by GC

Preparation of internal standard for GC calibration

In a 250-mL reactor, 0.1 mol of pyrrolidine (C_4H_9N) is added to 90 mL of dichloromethane. The solution is cooled in an ice bath. Propionylated chloride (C_2H_5COCl) of 0.05 mol is slowly added to have a soft reflux. After complete addition, the mixture is heated to reflux during 1.5 h, cooled to room temperature, and washed with a 2M HCl aqueous solution followed by $NaHCO_3$ aqueous solution and water. Organic phase is dried with $MgSO_4$ and concentrated under reduced pressure. The final product (1-propionylpyrrolidine) is obtained by distillation under reduced pressure. The boiling point of 1-propionylpyrrolidine was determined at 105–111°C under 11 mmHg.

Preparation of aminolysis samples from propionylated lignin

The samples of propionylated wheat straw lignin and the aminolysis reactant are separately prepared

in two flasks (A and B). The reaction starts at time zero ($t = 0$), when A is added to B. Solution A is prepared as follows: 2.5 mL of pyrrolidine added to 2.5 mL of 1,4-dioxane. Solution B is prepared as follows: 250 mg of propionylated wheat straw lignin, 25 mg of 1-methylnaphtalene, and 5 mL of 1,4-dioxane.

Aminolysis sample is obtained by mixing 0.5 mL of A with 0.5 mL of B in a flask and the reaction time $t = 0$ starts. The pyrrolidine begins the aminolysis reaction. At the time $t_i = t_0 + \Delta t$, 0.5 μL of the sample is injected in the GC system.

Calibration method of GC system via internal standard (1-methylnaphatlene)

The response factor of 1-propionylpyrrolidine relative to 1-methylnaphtalene, f_{PP} is determined by the expression:

$$f_{PP} = C_a \cdot A_e / C_e \cdot A_a$$

where f_{PP} = response factor of 1-propionylpyrrolidine relative to 1-methylnaphtalene, C_a = Concentration of 1-propionylpyrrolidine (mol L^{-1}), C_e = Concentration of 1-methylnaphtalene (mol L^{-1}), A_a = Chromatographic area of 1-propionylpyrrolidine, A_e = Chromatographic area of internal standard (1-methylnaphtalene).

Aminolysis shows the content of 1-propionyl-pyrrolidine formation following the time of the reaction. The curve shows from 0 to 10 min, the aminolysis of phenolic hydroxyl groups, and from 10 to 60 min, the aminolysis of aliphatic hydroxyl (Fig. 8).

Wheat straw lignin carboxylic acid quantification

Two mixtures (C and D) are prepared as follows: Mixture C: 200 mg of dried wheat straw lignin is solubilized in 2 mL of N,N'-dimethylformamide (DMF) in a 125-mL beaker. Mixture D: 2 mL of DMF, 40 mL of water, and 10 mL of a $1M$ solution of calcium acetate are, respectively, added in a beaker of 125 mL. The analysis sample is obtained by mixing C and D.

A blank solution, without lignin, is also prepared for reference. Both the samples (mixture and blank) are placed under N_2 flux during 24 h under stirring and are titrated by a NaOH ($0.01M$) solution with a pH meter until pH 9.

RESULTS AND DISCUSSION

Wheat straw lignin extracted from CIMV pilot plant was first analyzed by a solid-state ^{13}C NMR to identify its main functions. To study wheat straw
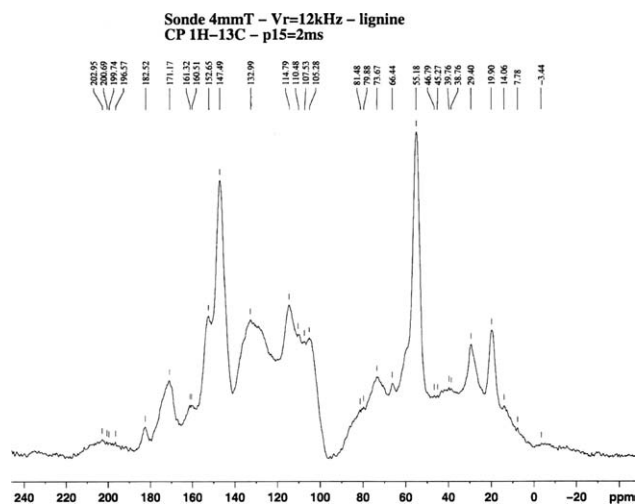

Figure 2 Solid state ^{13}C NMR spectrum of CIMV wheat straw lignin.

lignin's functionality, the complete solubilization in organic solvent is necessary. The solubilization process goes necessarily through transformation of polar hydroxyls functions to less polar functions. We decided to transform hydroxyl groups into ester groups. The reversible nature of the reaction allows their quantification.

Solid-state ^{13}C NMR analysis

Wheat straw lignin extracted by CIMV process was analyzed by solid-state ^{13}C NMR (Fig. 2). Functional attribution of solid-state ^{13}C NMR analysis of wheat straw lignin was done following referenced works. The resonance at 55.1 ppm was attributed to methoxyl groups linked to a phenyl structure.[14] Aliphatic hydroxyl functions substituted to carbons in lignin side chains were assigned at 66.4 and 73.6 ppm.[15] The region between 100 and 160 ppm was assigned to aromatic carbons of lignin's coniferyl units.[14] Resonances at 152.6 and 147.5 ppm were assigned to etherified and free phenolic unit, respectively.[16] Carboxylic group linked to a phenyl structure resonance was observed at 171.1 ppm.[14] This first analysis of wheat straw lignin gives precise information about its main structure and functionality. The polymer is composed of carboxylic groups and mainly aliphatic hydroxyls and phenolic hydroxyls groups.

Aliphatic hydroxyl functions are commonly used for organic synthesis. The high potential of wheat straw lignin lies in its phenolic content as a bio polyphenol material. The quantification of those hydroxyls function becomes very interesting and essential on further lignin based materials. All methods used to quantify total hydroxyl groups were adapted to CIMV lignin regarding works of Chen[17] and
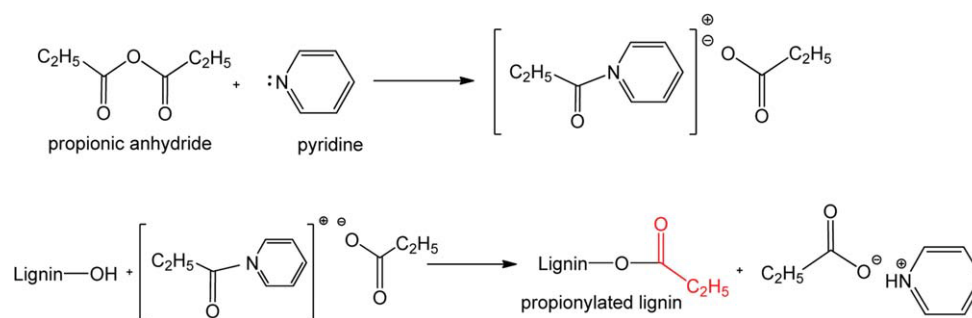
Figure 3 Propionylation of wheat straw lignin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Mansson[8] who proceeded to acetylation of hydroxyl functions. Regarding CIMV process, hydroxyl groups can not be quantified by following those methods[8,17] because:

- Free acetic and formic acid might be slightly present after extraction regarding the refining process of wheat straw.
- Partial formylated and acetylated hydroxyl functions of extracted lignin may lead to formic and acetic acid during Step 3 (Fig. 6).

Those observations mean that the hydroxyl quantification gathers not only free acetic and formic acid from treatment but also acetic and formic acid from extracted lignin. Thus, propionic anhydride was chosen to quantify by GC, the derivative form of propionic acid that was not initially present during CIMV process. The chromatographic study allows determination of:

- Hydroxyl groups partially esterified during wheat straw treatment by formic and acetic acid.
- Hydroxyl groups completely esterified by propionic anhydride.

To quantify hydroxyl functions of wheat straw lignin, it is essential to check that all hydroxyls (aliphatic and phenolic) have been propionylated. The control technique used was ATR-FTIR to observe the vanishing of hydroxyls bands and the formation of carbonyl ester band. Using this technique, the possibility to analyze directly the pure product avoids the use of KBr pellets or solvents so impurities bands from water or CO_2 can not be observed.

Propionylation of wheat straw lignin

Lignin was propionylated using propionic anhydride and pyridine (Fig. 3).

Figure 4 shows the superposition of ATR-FTIR spectra from wheat straw lignin and propionylated wheat straw lignin. A specific extension was applied on the spectrum from 4000 to 2600 cm^{-1} to highlight the esterification of lignin hydroxyl function. It is clearly observed that all hydroxyl functions were totally propionylated because the absorption band of hydroxyl (3412 cm^{-1}) was replaced by the absorption band of carbonyl ester (1738 cm^{-1}).

Regarding lignin spectrum (black line), hydroxyl absorption bands (ν_{OH} aliphatic and ν_{C-O} phenolic)
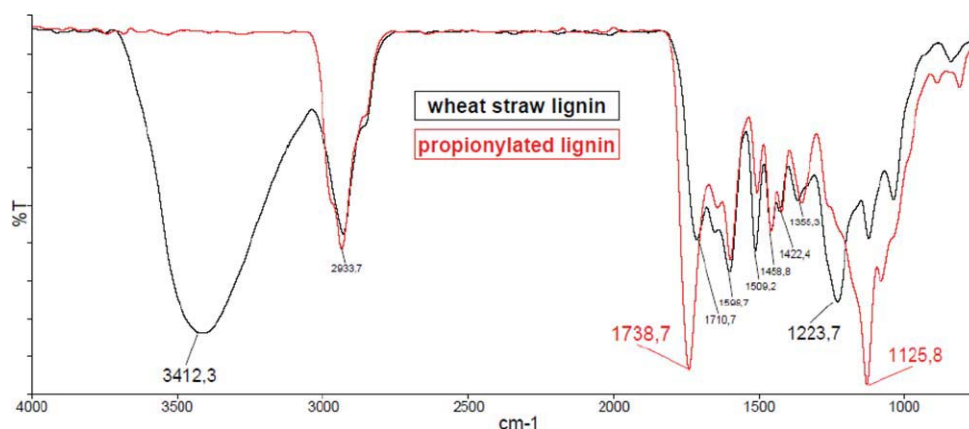

Figure 4 ATR-FTIR spectra of wheat straw lignin and propionylated lignin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
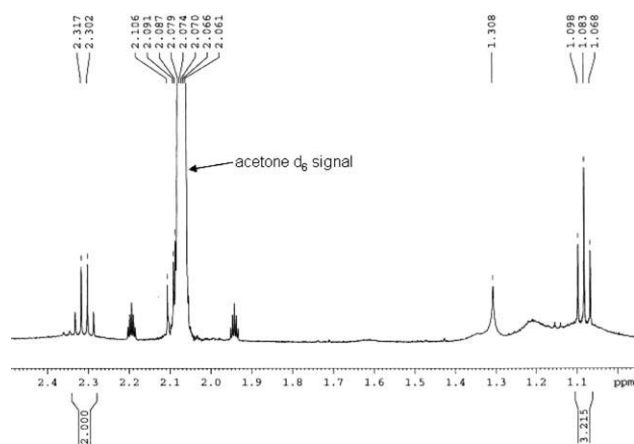
Figure 5 Partial ^{1}H NMR spectrum of propionylated lignin in acetone d$_6$.

are observed near 3412 and 1223 cm^{-1}, respectively. Carboxylic acid function absorption band is observed with the $v_{C=O}$ near 1710 cm^{-1}. The three absorption bands (1598, 1509, and 1422 cm^{-1}) are the characteristics of aromatic skeletal vibration, and the absorption band near 1458 cm^{-1} is attributed to aromatic methyl group vibrations. Propionylated lignin (red line) has no hydroxyl bands but a strong presence of carbonyl ester band $v_{C=O}$ near 1738 cm^{-1}. The v_{C-O} phenol absorption band disappeared, leading to formation of v_{C-O} ester aliphatic and aromatic absorption band near 1125 cm^{-1}.

To complete the analysis, ^{1}H NMR spectroscopy of propionylated lignin was done (Fig. 5). The characteristics signals of CH$_3$ and CH$_2$ linked to the carbonyl function of propionylated lignin are clearly observed on the ^{1}H NMR spectrum. The triplet around 1.08 ppm is corresponding to a CH$_3$—C—C=O signal and the quadruplet around 2.3 ppm to a —C—CH$_2$—CO—OR signal. The integration of the signals confirmed the correct attribution. As lignin polymer has a complex molecular structure, with a high proton density, the mobility of chemical groups is reduced, giving large peaks on spectra and difficulties on a precise attribution. In the case of propionylated lignin, it was very surprising to observe such clean signals of CH$_3$ and CH$_2$, which mean that the propionyl ester groups may have enough mobility regarding steric hindrance of lignin.

ATR-FTIR and NMR analyses clearly proves that propionylation of wheat straw lignin is complete. All free hydroxyls functions of wheat straw lignin were propionylated, so quantification of all bonded (formylated and acetylated hydroxyl) and free hydroxyl group (propionylated hydroxyl) regrouped total hydroxyl groups present on the macromolecule.

Quantification of total hydroxyl groups of wheat straw lignin

Principle of the method

The quantification of total hydroxyl groups of wheat straw lignin (aliphatic + phenolic) is presented in Figure 6 and may be determined according to Mansson's method[8] in 4 steps:

- Step 1–Propionylation of wheat straw lignin.
- Step 2–Transesterification in organic solvent with sodium methoxide.
- Step 3–Saponification.
- Step 4–GC analysis.

In Step 3, water is added to the mixture and an immediate reaction with methoxide anion occurs to form hydroxyls ions that are active in methyl propionate saponification. Acidification of the mixture leads to propionic acid. In Step 4, tetrabutylammonium hydroxide is used to transform propionic acid into tetrabutylammonium propionate, which reacts with benzyl bromide to form benzyl propionate. The final product is quantitatively determined by GC.

Quantification of total esterified hydroxyl groups of wheat straw lignin by GC

Total esterified hydroxyl groups of wheat straw lignin are quantified by the expression[18]:

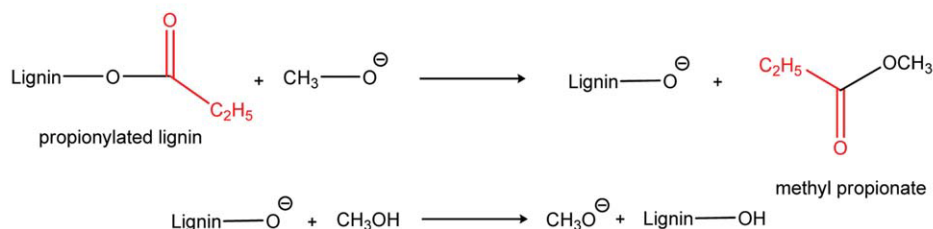$$T_{tot} = (f_{tot} \cdot n_e \cdot 1000/P_S) \cdot (A_a/A_e)$$

where T_{tot} = total esterified hydroxyl group content (mmol g^{-1} of lignin), f_{tot} = response factor of benzyl alkyl ester relative to benzyl butyrate, n_e = quantity of butyric acid used (mmol), P_s = sample weight (mg), A_a = chromatographic area of benzyl alkylate, A_e = chromatographic area of benzyl butyrate.

Regarding extraction process, GC analysis chromatogram showed quantitative formic, acetic, propionic, and butyric acids signals. Three types of total esterified hydroxyls groups (T_{tot}) in wheat straw lignin were quantified by using the following method: T_{tot} propionic, T_{tot} acetic, and T_{tot} formic. Total hydroxyl groups (free and bonded) of wheat straw lignin extracted after process were determined by regrouping the sum of the three different types of quantified esterified hydroxyls groups. The different factor values f_{tot} were verified at various concentration of the analyzed product for benzyl propionate, acetate, and formiate.
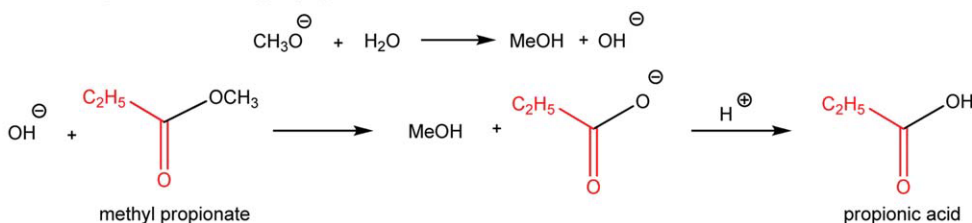
Table I shows that f_{tot} is nearly the same in different concentration ratios for each of the three benzyl esters. Average values of f_{tot} were used for the quantification of hydroxyl group of wheat straw lignin.
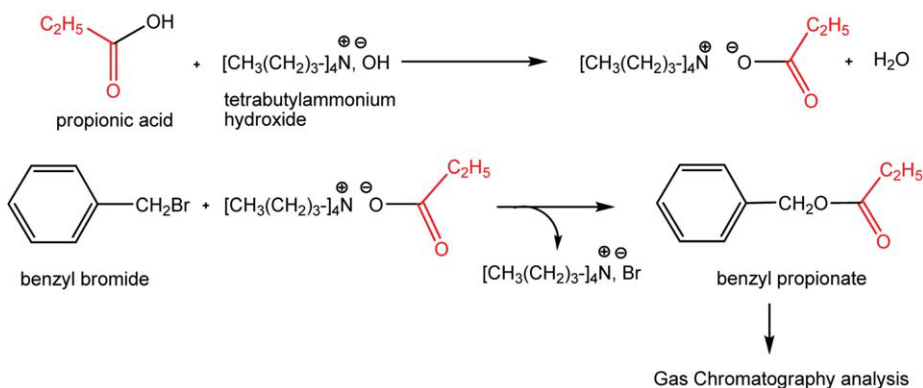
Figure 6 Total hydroxyl quantification of CIMV wheat straw lignin following Mansson's method.[8] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Total esterified hydroxyl groups content (Table II) of lignin is calculated with the previous established expression:

$$T_{tot} = (f_{tot} \cdot n_e \cdot 1000/P_S) \cdot (A_a/A_e)$$

Ester groups quantified by GC correspond to the total hydroxyl function groups initially present on wheat straw lignin. Results show that wheat straw lignin, in its undegraded structure, has nearly 5 mmol g^{-1} of total hydroxyl functions. Wheat straw lignin is partially formylated and acetylated during refining process with formic and acetic acids. These ester groups represent bonded hydroxyl functions. The functionality of wheat straw lignin is represented by the free total hydroxyl groups content; they are represented by propionyl ester group quantified at 3.404 mmol g^{-1}.

TABLE I
Response Factor f_{tot} at Different Concentrations in Benzyl Esters Using Benzyl Butyrate as Internal Standard

	f_{tot}		
C_a/C_e [a]	Benzyl formiate	Benzyl acetate	Benzyl propionate
1	1.58	1.19	1.09
0.8	1.62	1.23	1.13
0.6	1.49	1.20	1.12
0.4	1.51	1.24	1.08
0.2	1.53	1.22	1.09
f_{tot} Average value	1.54	1.22	1.10
Standard deviation	0.05	0.02	0.02

[a] C_a/C_e, fraction of concentration of propionic acid (or formic, acetic acid) and internal standard concentration (butyric acid).

TABLE II
Total Esterified Group Contents of Propionylated Wheat
Straw Lignin

Lignin	Total ester group content of propionylated lignin (mmol g^{-1})		
	Formyl	Acetyl	Propionyl
Wheat straw	0.503	1.010	3.404

Quantification of phenolic hydroxyl groups in wheat straw lignin

Principle of the method

Phenolic group is one of the most important function affecting physical and chemical properties of lignin.[19] It is among the most reactive site in lignin. Selective quantification was done via aminolysis of propionylated lignin by following the formation of 1-propionylpyrrolidine by GC.

Aminolysis is a direct method based on the fact that deacetylation of aromatic acetates in pyrrolidine, under softs conditions, is faster than deacetylation of aliphatic acetates.[8] Then, the phenolic propionyl group of propionylated lignin may be selectively depropionylated in pyrrolidine before aliphatic propionyl group (Fig. 7).

Quantification of phenolic hydroxyl groups in wheat straw lignin by GC

Determination of 1-propionylpyrrolidine was done by GC using 1-methylnaphtalene as internal standard. Several aminolysis of propionylated lignins with different times were analyzed by GC. The phenolic propionyl content is determined by extrapolation of the kinetic curve of aminolysis to origin. The phenolic hydroxyl groups (T_{phenol}) relative to 1-propionylpyrrolidine content from propionylated lignin are determined at t_i from the expression[18]:

$$T_{\text{phenol}} = \left(f_{\text{pp}} \cdot P_e \cdot 1000/M_e P_S\right) \times (A_a/A_e)$$

where T_{phenol} = 1-propionylpyrrolidine content at t_i, mmol g^{-1}, of lignin, f_{pp} = response factor of 1-propionylpyrrolidine relative to internal standard, P_e = internal standard (1-methylnaphtalene), mg, M_e = molecular weight of internal standard, g mol^{-1}, P_s = sample weight, mg, A_a = chromatographic area of 1-propionylpyrrolidine, A_e = chromatographic area of 1-methylnaphtalene.

Response factor of 1-propionylpyrrolidine (f_{PP}) was determined by GC at $f_{\text{pp}} = 2.28$.

The extrapolation of the linear region between 12 and 72 min of the kinetic curve is conducted by the method of the lesser square from the last six experimental points. The content of 1-propionylpyrrolidine by extrapolation of the linear region to time zero is directly relative to phenolic hydroxyl content of wheat straw lignin (Fig. 8).

The phenolic hydroxyl content is determined near 1 mmol g^{-1} of lignin meaning that the free aliphatic hydroxyl content of wheat straw lignin is 2.404 mmol g^{-1}. During CIMV wheat straw refining, aliphatic hydroxyl groups were partially esterified, whereas in acidic condition, esterification of phenols is more difficult. No formylated or acetylated phenolic hydroxyl during wheat straw lignin extraction was observed. This conclusion means that the quantification of phenolic hydroxyl regroups only free phenolic functions of wheat straw lignin.
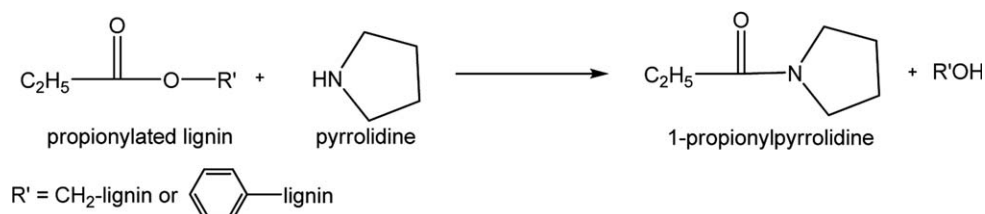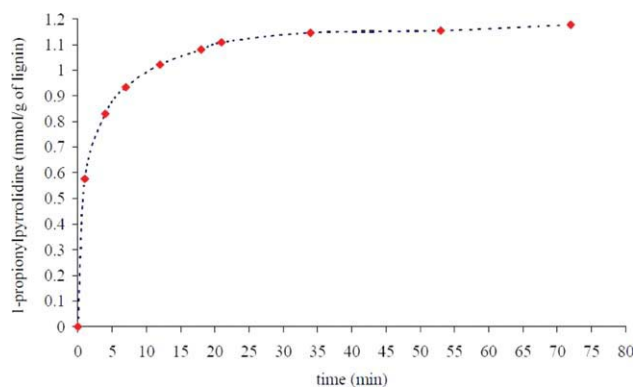
Figure 8 Aminolysis of propionylated wheat straw lignin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 7 Aminolysis of propionylated lignin.

Wheat straw lignin esterified groups	Total hydroxyl mmol g^{-1} of lignin	Phenolic hydroxyl mmol g^{-1} of lignin	Aliphatic hydroxyl mmol g^{-1} of lignin
Formyl	0.503	0	0.503
Acetyl	1.010	0	1.010
Propionyl	3.404	1	2.404
Total	4.917	1	3.917

Overall results for hydroxyl quantification of wheat straw lignin

The final results for the quantification of hydroxyl content of wheat straw lignin[20] are regrouped in Table III. The chemical functionality of wheat straw lignin is represented by the free aliphatic hydroxyl and also phenolic hydroxyl groups. One gram of wheat straw lignin has an approximate ratio of 1 mmol of phenolic hydroxyl for 4 mmol of free aliphatic hydroxyl. Regarding those results, a large scope of chemical reaction on wheat straw lignin is possible.

GPC of acetylated wheat straw lignin

CIMV wheat straw lignin was acetylated to analyze its molecular weight and polydispersity by GPC. The Varian GPC column used (Polargel-L) has moderated polarity by mixed-bed packing technique, comparing with well known styrene-divinylbenzene GPC column. Polar molecule such as lignin would be easily bonded to column phases, so acetylated sample was the best formulation. The molecular mass difference of acetylated lignin with the lignin is around 10%, so to determine the molecular mass and polydispersity of wheat straw lignin, acetylated sample was entirely solubilized in THF and analyzed by GPC. Calibration of GPC system was made with PEG standards (Fig. 9).

The polydispersity index (PDI) obtained for acetylated lignin, representative of extracted lignin by CIMV process, is really low and shows that wheat straw lignin polymer chains approach uniform chain length. The results obtained by mass spectrometry[13,14] showed a stereoregular molecular structure of wheat straw lignin and regarding the PDI determined, there is a correlation between those two different analyses. Thus, the CIMV organosolv process is able to extract lignin with slight degradation. This observation is important because lignin is extremely sensitive during paper pulp processes, while, nowadays, most of them use hard chemical conditions altering the structure of the biopolymer leading to its fractionation and recondensation after extraction.[21] Most GPC analysis of lignins gave results with either very high molecular weight due to recombination, low molecular weight with fractionation, or high PDI value.[22,23]

Quantification of carboxylic acid group in wheat straw lignin

The determination of the carboxylic groups in wheat straw lignin is based on ionic exchange with calcium acetate.[24] The method consists of mixing wheat straw lignin and excess of a calcium acetate solution in an organic solvent to finally quantify acetic acid freed by titration (Fig. 10).

Excess of acetate is added to move the reaction to the (1) side. The method has been applied to a solution of lignin in a mixture of water and DMF. Quantification of carboxylic group (T_{COOH}) in wheat straw lignin is determined by the expression.

$$T_{COOH} = \left[\left(V_{sample} - V_{blank} \right) \right] \cdot C \cdot 1000 / m_{sample}$$

where V_{sample} = equivalent volume of NaOH consumed of sample titration (mL), V_{blank} = equivalent volume of NaOH consumed of blank titration (mL), C = concentration of NaOH solution (mol L^{-1}), m_{sample} = sample mass (mg).

After several experiments, titrations results gave $T_{COOH} = 1.07$ mmol g^{-1} of lignin. This result shows that there are as many carboxylic groups in wheat straw lignin as phenolic groups.[20] The presence of carboxylic group may be interesting for other chemical reaction based on lignin.
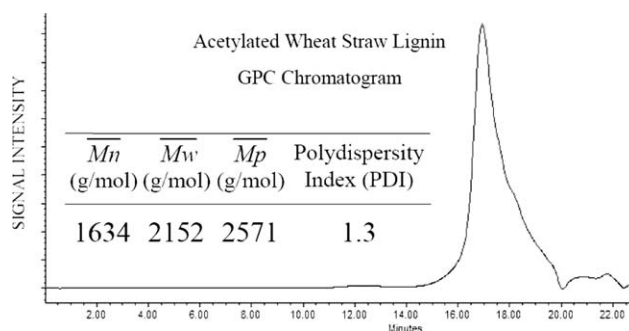

Figure 9 Results obtained of acetylated wheat straw lignin GPC analysis in THF.[20]
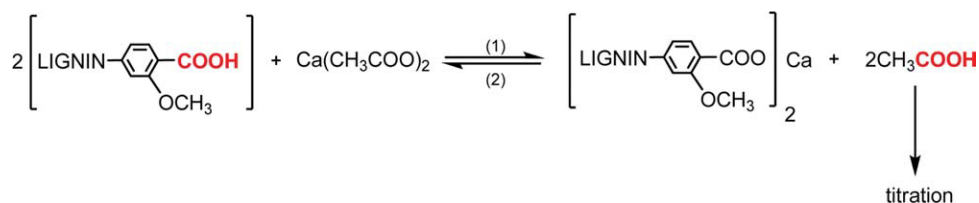
Figure 10 Ionic exchange between lignin's carboxylic function and calcium acetate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Proposed structures of CIMV wheat straw lignin's fragments

Previous structures of CIMV wheat straw lignin's fragments by APPI-TOF (Atmospheric Pressure Photo Ionization - Time Of Flight) mass spectrometry were proposed[13,14] (Fig. 11). The etherified phenols observed in Figure 11 may be in a different form without the possibility to highlight it. Solid-state ^{13}C NMR analysis showed the presence of phenolic hydroxyl (free or etherified), and they were quantified by GC. Under basic condition, they would be all opened under the form of phenates, but at neutral or acidic condition they would be in one of those two forms (Fig. 12).

CONCLUSIONS

A complete chemical analysis of wheat straw lignin extracted in acidic conditions by the CIMV process was reported in this work. Wheat straw was refined in a mixture of water, formic acid, and acetic acids at low temperature and atmospheric pressure. During CIMV refining, wheat straw lignin was partially formylated and acetylated. Lignin analyses were done on samples issued from the pilot plant.

Solid-state ^{13}C NMR of wheat straw lignin was an important tool to elucidate the main chemical functions of the polymer. ATR-FTIR analysis was done to verify the complete esterification of lignin. Soluble acetylated lignin was analyzed by GPC to determine its molecular weight and PDI. Total aliphatic and phenolic hydroxyl groups of CIMV wheat straw lignin were precisely quantified by GC using propionylated lignin.

The functionality of CIMV wheat straw lignin extracted in organic acid media at pilot scale presented in this work is a valuable tool for future modification of lignin as a natural polyphenol. Results obtained show the important potential of lignin in polymers manufacturing. The industry of epoxy resins[25] and phenol–formol particle boards[26] is based on the use of phenol material. The high toxicity of the industrial products encourages researchers to use natural chemical products without any impact on health such as lignin and poor toxic epoxide agent for epoxy resins,[27] and lignin with
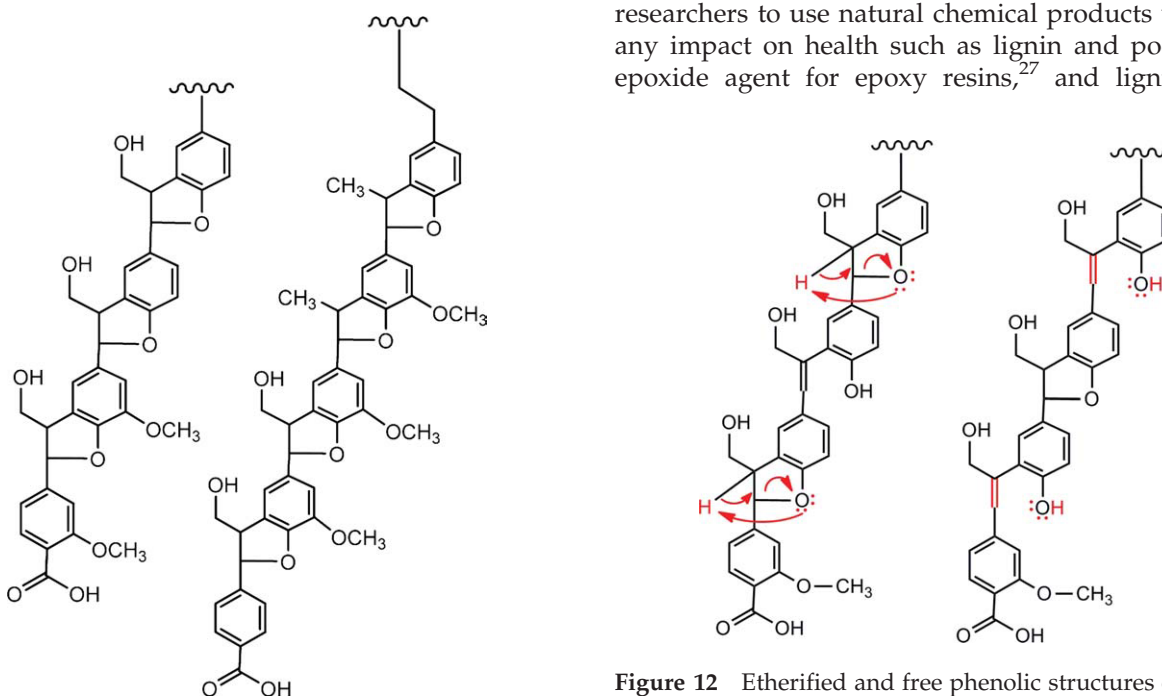

Figure 11 Structures of wheat straw lignin's fragments.[13,14]

Figure 12 Etherified and free phenolic structures of wheat straw lignin fragments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

glyoxal for particle board production. Those techniques are to be presented in a future work.

References

 1. Delmas, M.; Avignon, G. WO0,222,945 (2001).
 2. Lam, H. Q.; Le Bigot, Y.; Delmas, M.; Avignon, G. Ind Crops Prod 2001, 14, 139.
 3. Benjelloun-Mlayah, B.; Delmas, M.; Avignon, G. WO2,006,117,295 (2006).
 4. Delmas, M. Chem Eng Technol 2008, 31, 792.
 5. Tiainen, E.; Drakenberg, T.; Tamminen, T.; Kataja, K.; Hase, A. Holzforschung 1999, 5, 529.
 6. Steltenkamp, M. S. U.S. Pat.4,507,172 (1985).
 7. Whiting, P.; Goring, D. A. I. Paperri ja puu 1982, 10, 592.
 8. Mansson, P. Holzforschung 1983, 37, 143.
 9. Robert, D. R; Brunow, G. Holzforschung 1984, 38, 85.
10. Liu, H. W.; Yu, J. I.; Chen, J. X. Cell Chem Tech 1992, 26, 6.
11. Faix, O.; Grünwald, C.; Beinhoff, O. Holzforschung 1992, 46, 425.
12. Barelle, M. Holzforschung 1993, 47, 261.
13. Banoub, J. H.; Delmas, M. J Mass Spectrom 2003, 38, 900.
14. Banoub, J. H.; Benjelloun-Mlayah, B.; Ziarelli, F.; Joly, N.; Delmas, M. Rapid Commun Mass Spectrom 2007, 21, 2867.
15. Almendros, G.; Martinez, A. T.; Gonzales, A. E.; Gonzales-Vila, F. J.; Frund, R.; Ludemann, H. D. J Agric Food Chem 1992, 40, 1297.
16. Jung, H. J. G.; Himmelsbach D. S. J Agric Food Chem 1989, 37, 81.
17. Chen, C. L.; In Methods in Lignin Chemistry; Lin, S. Y., Dence, C. W., Eds.; Springer: Berlin, 1992; pp 409.
18. Vo-Huu, T.; Ph.D. Thesis, University of Montpellier - France, 2005.
19. Adler, E. Wood Sci Techol 1977, 11, 169.
20. Delmas, G. H.; Benjelloun-Mlayah, B.; Le Bigot, Y.; Delmas, M. 18th European Biomass Conference, Visual Poster 4.1.1, Lyon - France, May 3rd-7th; 2010.
21. Baumberger, S.; Lapierre, C.; Monties, B. J Agric Food Chem 1998, 46, 2234.
22. Majcherczyk, A.; Hüttermann, A. J Chromatogr A 1997, 764, 183.
23. Sun, X. F.; Xu, F.; Sun, R. C.; Wang, Y. X.; Fowler, .P; Baird, M. S. Polym Degrad Stab 2003, 86, 245.
24. Schnitzer, M.; Gupta, U. C. Soil Sci Soc Am J 1965, 29, 274.
25. Vargiu, S.; Pitzalis, M.; Cresponili, G.; Giulani, G. U.S. Pat.4,132,718 (1979).
26. Clarke, M. R.; Steiner, P. R.; Anderson, A. W. U.S. Pat.4,897,314 (1990).
27. Delmas G. H., Ph.D. Thesis, Ecole Nationale Supérieure des Ingénieurs en Arts Chimiques et Technologiques (ENSIACET), Toulouse, France (expected to 2011).