Xabier Erdocia, Raquel Prado, M. Ángeles Corcuera, Jalel Labidi, Effect of different organosolv treatments on the structure and properties of olive tree pruning lignin, Journal of Industrial and Engineering Chemistry, Volume 20, 2014, Pages 1103-1108, ISSN 1226-086X https://doi.org/10.1016/j.jiec.2013.06.048

(https://www.sciencedirect.com/science/article/pii/S1226086X1300292X)

Abstract: Different organosolv processes (acetosolv, formosolv and acetosolv/formosolv) were applied to extract lignin from olive tree pruning. Obtained lignins were characterized by several methods to determine their composition, structure and functional groups with the aim of evaluating their potential to be used for obtaining added value compounds. All lignins had very high purity and low sugar and inorganic contamination, especially in the case of lignin obtained from formosolv treatment. Hydroxyl groups were the main functional groups in all lignin samples while the carbonyl groups were the lowest. Finally, the main difference between the lignins was the average molecular weight.

Keywords: Organosolv treatment; Organic acids; Lignin; Olive tree pruning

Effect of different organosolv treatments on the structure and properties of olive tree pruning lignin

Xabier Erdocia, Raquel Prado, M. Ángeles Corcuera, Jalel Labidi*

Chemical and Environmental Engineering Department. University of the Basque Country.

Plaza Europa 1, 20018 San Sebastian (Spain)

E-mail: jalel.labidi@ehu.es; Tel: +34 943017125

ABSTRACT

Different organosolv processes (acetosolv, formosolv and acetosolv/formosolv) were applied to extract lignin from olive tree pruning. Obtained lignins were characterized by several methods to determine their composition, structure and functional groups with the aim of evaluating their potential to be used for obtaining added value compounds. All lignins had very high purity and low sugar and inorganic contamination, especially in the case of lignin obtained from formosolv treatment. Hydroxyl groups were the main functional groups in all lignin samples while the carbonyl groups were the lowest. Finally, the main difference between the lignins was the average molecular weight.

Keywords

Organosolv treatment; Organic acids; Lignin; Olive tree pruning.

1. Introduction

Biomass is considered an appropriate raw material to replace fossil sources to produce chemical products or energy. Among the different types of biomass, lignocellulosic biomass is the most abundant one with 200 billion metric tons available worldwide [1]. Conversion of lignocellulosic biomass into fuels or chemical products is a rapidly growing research area. This type of biomass is primarily composed of three polymers: cellulose, hemicellulose and lignin.

Lignin is a phenolic biopolymer composed by combination of three phenylpropanoid units: p-coumaryl alcohol, guaiacyl alcohol and syringil alcohol. Several interunit linkages including β-O-4, α-O-4, β-5, β-β, 4-O-5, 5-5 are formed by dehydrogenation, cross-coupling, and dehydrodimerization reactions during the biosysthesis process of macromolecular lignin. In plant cell walls, lignin fills the spaces between cellulose and hemicellulose, and it acts like a resin that holds the lignocellulose matrix together. About 25–35% of the organic matrix of wood is composed by lignin, moreover, it is a major component of grasses, leaves, and needles [2]. Depending on its origin lignin structure presents differences concerning its monolignol composition. In softwood lignin, usually referred to guaiacyl lignin, the structural elements are derived principally from coniferyl alcohol (G) and trace amounts of synapyl alcohol-derived units (S). On the other hand, normal hardwood lignin, termed guaiacyl-syringyl lignins, is comprised of coniferyl alcohol and sinapyl alcohol-derived units in varying ratios. Nowadays, lignin is used as low heating fuel to generate energy in the pulp and paper industry. However, the aromatic chemical structure of lignin makes it unique and very promising source of renewable products and commodity chemicals [3].

Several different lignin sources, derived from a specific form of biomass pretreatment, could be potentially used as feedstocks for lignin valorization in a biorefinery. These sources could originate either from pretreatments in the pulp and paper industries (i.e., kraft or lignosulfonate) or new feedstocks specific to the biorefinery scheme (i.e., organosolv). The organosolv treatment is of interest as an alternative to conventional pulping processes due to many advantages such as; low boiling points, simplicity of process, non-sulfur formulas, and easy recycling possibilities with some organic chemicals [4]. Among organosolvents, acetic acid and formic acid have received considerable attention and many studies have been done employing these two organic acids as delignification agents [5-8].

The extraction of lignin from lignocellulosic materials is conducted under conditions where lignin is progressively broken down to lower molecular weight fragments, resulting in changes to its physicochemical properties. Thus, apart from the source of the lignin, the method of extraction will have a significant influence on composition and properties of lignin.

Among lignocellulosic materials olive tree pruning is a lignocellulosic residue obtained from an operation realized to eliminate old branches and prepare trees for the next crop. This agricultural residue is usually left on the cropland (with the consequent risk of plagues) or used as firewood, representing both activities and economic misuse or devaluation of their potential exploitation. In Spain, the current annual production is more than $7x10^9$ kg with an estimation per year of 3000 kg/ha [9].

The aim of this study is to characterize olive tree pruning lignin obtained by different organosolv treatments in order to assess the possible changes occurred in lignin structure and properties. Acetosolv, formosolv and combination of acetosolv and formosolv treatments were used to obtain different types of lignin from olive tree pruning. The obtained lignins were deeply characterized employing several analytical techniques. Thus, the possibility of using these lignins for further applications is evaluated as an integral part of future biorefineries.

2. Experimental

2.1. Conditioning and analysis of the raw material

Olive tree pruning (Olea europaea, Arroniz variety) used in this work was obtained from a private cultivation in Estella (Navarra, Spain). Olive tree pruning (branches and wood) was accondition up to constant moisture and was milled in a Retsch 2000 hammer mill to obtain 4-6 cm size fraction chips free of impurities such as small stones, sand and dust. Characterization of olive tree pruning chips was done according to standard methods [10]. Moisture content (6.50 ± 0.2 wt.%) was determined after drying the samples at 105 ºC for 24 h (TAPPI T264 cm-97). Chemical composition, given on dry weight basis, was the following: 3.54 ± 0.32% ash (TAPPI T211 om-93), 15.53 ± 0.12% hot water soluble matter (TAPPI T207 om-93), 31.26 ± 0.46% aqueous NaOH soluble matter (TAPPI T212 om-98), 11.72 ± 0.66% ethanol-toluene extractives (TAPPI T204 cm-97), 22.84 ± 0.67% lignin (TAPPI T222 om-98), 64.63 ± 1.32% holocellulose [11] and 51.16 ± 0.83% α-cellulose [12].

2.2. Lignin obtaining by different organosolv pulping

Acetosolv treatment was carried out based on the conditions used in previous works [13,14]. The olive tree pruning was treated with 90 wt.% acetic acid solution with 0.2% of HCl as catalyst. The solid:liquid ratio was 1:10 and was carried out at 130 ºC for 90 minutes in a 4 L pressure stainless steel batch reactor (EL0723 Iberfluid) controlled by Adkir software. The black liquor, where the lignin was dissolved, was treated with 5 volumes of water and the precipitated lignin (AL) was isolated by centrifugation (5500 rpm, 15 minutes).

Formosolv treatment was carried out varying only the acid nature and concentration from acetosolv treatment and based on the conditions used in a previous work [6]. Thus, the olive tree pruning was treated with 80 wt.% formic acid solution with 0.2% of HCl as catalyst. All the other parameters were the same as in the acetosolv pulping. The obtained lignin will be named FL from now on.

Acetosolv and formosolv treatments were carried out together. The concentration and nature of pulping media was chosen from a previous work [15]. The olive tree pruning was treated with a solution of formic acid-acetic acid-water (30/60/10, v/v/v) and 0.2% of HCl as catalyst. The other parameters of the treatment and separation of lignin (AFL) were the same as in the previous pulping.

2.3. Lignin characterization

Chemical structure composition of the different lignins was characterized by attenuated-total reflection infrared spectroscopy (ATR-IR) by direct transmittance in a single-reflection ATR System (ATR top plate fixed to an optical beam condensing unit

with ZnSe lens) with an MKII Golden Gate SPECAC instrument. Transmittance spectra were recorded over 32 scans in the wavenumber range from 4000 to 600 cm^{-1}, with a resolution of 4 cm^{-1}.

NMR spectra were recorded at 30 ºC on a Bruker Avance 500 MHz equipped with a z-gradient BBI probe. Tipically, 40 mg of sample were dissolved in DMSO-d6. The spectral widths were 25000 Hz for the ^{13}C dimensions.

The chemical phenolic compositions were determined by alkaline nitrobenzene oxidation. 50 mg of lignin were placed in a tube with sodium hydroxide solution and nitrobenzene and left at 175 ºC for 2.5 hours. The oxidation products were analyzed by HPLC JASCO instrument equipped with an interface (LC-NetII/ADC) and a photodiode array detector (MD-2018). A Teknokroma Mediterranean sea TR-010006 column (25 x 0.46 cm) was used for the experiments and a solution of acetonitrile:water in a ratio of 1:8 with 1% of acetic acid was used as mobile phase. The flow rate was 0.5 mL/min and the analyses were carried out at 40 ºC. Calibration was made using compounds standards (Sigma-Aldrich) –vanillic acid, syringic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, vanillin, syringaldehyde, acetovanillone and ferulic acid-.

Lignins were subjected to High Performance Size Exclusion Chromatography (HPSEC) to evaluate lignin average molecular weight (M_W) and molecular weight distribution (MWD) using a JASCO instrument equipped with an interface (LC-NetII/ADC) and a reflex index detector (RI-2031Plus). Two PolarGel-M columns (300 x 7.5 mm) and PolarGel-M guard (50 x 7.5 mm) were employed. Dimethylformamide with 0.1% lithium bromide was the eluent. The flow rate was 0.7 mL/min and the analyses were carried out at 40

ºC. Calibration was made using polystyrene standards (Sigma-Aldrich) ranging from 70000 to 266 g/mol.

Acid insoluble lignin (AIL) was determined by subjecting lignin to an acid hydrolysis process consisting in two stages. The first acidic hydrolysis was carried out adding 3.75 mL of sulphuric acid 72% to 0.375 g of lignin. The mixture was left for 1 hour at 30 ºC. Then it was diluted with 36.25 mL of deionized water for 3 hours at 100 ºC. After this time, the solution was cooled for 15 minutes and then filtered using filters over G4 glass filter crucible. The remaining solid is the acid insoluble lignin. Acid soluble lignin (ASL) was determined by spectrophotometry (UV absorption at 205 nm). Filtrate samples had to be diluted with 1M H_2SO_4 until absorption was between 0.1 to 0.8. (TAPPI UM250 um-83).

Sugars content was determined injecting the obtained filtrate from AIL analysis into a high performance liquid chromatography (Jasco LC Net II /ADC with a ROA Organic Acid (00H-0138-K0) column (Phenomenex) equipped with a refractive index detector (RI-2031Plus) and a photodiode array detector (MD-2018Plus)). 0.005 N H_2SO_4 prepared with 100% deionized and degassed water was used as mobile phase (0.35 mL/min flow, 40 ºC and injection volume 20 µL). High purity standards of D-(+)-glucose, D-(+)-xylose and D-(-)-arabinose (provided by Fluka, with ≥99% of purity) were used for calibration.

A Thermogravimetric Analysis (TGA) was carried out in a TGA/SDTA RSI analyzer of Mettler Toledo to determine the ash content. Samples of approximately 7 mg were heated from 25 ºC up to 800 ºC at a rate of 10 ºC/min in air atmosphere.

Carboxyl groups were studied by aqueous titration. A weight of lignin sample (0.25 g) was suspended in 12.5 mL of sodium hydroxide 0.05 M. After stirring for approximately 3 hours until complete dissolution of the lignin sample, the solution was potentiometrically titrated with 0.1 M hydrochloride acid until reaching a pH 7. Carboxyl groups were calculated as the difference between the added NaOH and the consumed. Oximation reaction was used to determine lignin carbonyl content. Lignin was dissolved in 2 ml of DMSO. Once dissolved, 5 mL of oximating mixture were added to the solution and the mixture was heated at 80 ºC for two hours. After that time, the solution was potenciometrically titrated with hydrochloric acid.

The total phenolic content in the analyzed lignin samples was determined by the Folin-Ciocalteau spectrophotometric method [16] using gallic acid as reference compound and dimethyl sulfoxide as solvent. Each lignin solution (2 g/L in DMSO) was prepared for total phenolic content assay (0.5 mL of sample with 2.5 mL of Folin-Ciocalteau reagent, 5 mL of Na_2CO_3 20% and distillate water up to 50 mL). The preparations were kept in a thermostatic bath at 40 ºC for 30 min, and afterwards the absorbance of the samples at 750 nm was registered, and the percentage of total phenolics in the lignin, as % of gallic acid equivalent, was calculated.

3. Results and discussion

3.1. Lignin composition and functional groups

Chemical composition of the obtained three lignin samples is shown in Table 1.

AL and AFL had almost the same quantity of Klason lignin; around 69%. These results are in agreement with other ones obtained by other organosolv extraction methods [16-18]. On the other hand, FL purity was highest one with 79.01% of Klason lignin. In accordance

with these purity results, the most contaminated sample by carbohydrates was AFL sample while FL lignin presented very low sugars contamination. Among carbohydrates, glucose was the major sugar present in all samples, especially in AFL. Otherwise, arabinose content was very low and remained constant for all lignin samples due to the low selectivity of acetic and formic acid in the extraction of arabinoxylans [16]. Lignin ash content was in concordance with other organosolv lignins [16,18]. In addition, this ash percentage was very low compared with soda or Kraft lignins [16,17] which indicated that the application of organic acids in the extraction method produced lignins with low inorganic matter. Finally, acid soluble lignin (ASL) contents were low in all extracted lignins and were in agreement with the results obtained in other woks [18].

In Table 2 are represented the elemental compositions of the different lignins. It can be observed that the amount of the different elements was almost the same except in the case of AFL where carbon percentage was higher and oxygen was lower. The main constituent of the lignin samples were carbon and oxygen with less quantity of hydrogen and even less of nitrogen. However, in molar basis, hydrogen was the main element in all samples especially in AL. It was also noticeable, the high amount of nitrogen present in all the samples.

The content of different functional groups in lignin structure is essential to know its reactivity. In this work carbonyl, carboxyl and hydroxyl content were measured in order to identify the effect of extraction method in the obtained lignins. AFL had the highest content in hydroxyl groups while AL and FL presented similar quantity. Otherwise, carboxylic groups percentage were similar in AL and AFL and higher than FL indicating that acetic acid addition in the extraction process promoted oxidation reactions. On the other hand, carbonyl content was very low in all cases, but proportionally higher in FL.

Finally, it is very remarkable that phenolic hydroxyl content in lignins obtained by organosolv methods employing an organic acid as solvent, like in this study, were significantly higher than other lignins obtained by alakali treatment, autohydrolysis process or organosolv process with ethanol [19].

3.2. Lignin structure and molecular weight

FTIR spectra of different olive tree pruning lignin samples (Figure 1) showed weak changes in the peak intensities and form. The wide band observed around 3350 cm^{-1} represented the stretching vibration of hydroxyl group and hydrogen bonding. The bands at 2920 and 2850 cm^{-1} corresponded with C-H stretching vibration in methyl and methylene groups while the band at 1460 cm^{-1} was related to C–H bending vibrations in the same groups. Typical aromatic skeletal vibrations were detected at 1600, 1515 and 1425 cm^{-1}. The following structure signals were also found in all samples: stretching of non-conjugated carbonyl groups with the aromatic ring (1710 cm^{-1}), syringil ring C-H in-plane and out of plane deformations (1118 and 833 cm^{-1} respectively) and guaiacyl ring C-H in plane and out of plane bending at (1031 and 912 cm^{-1}). All these concordances between the spectra suggested that the extraction method did not entail significant changes in the main structure of lignin and all the bands were in good agreement with the reported data in previous studies of lignin [20].

However, there were some slight differences between the three lignin spectra detailed in the Figure 1. The first one was located at 1365 cm^{-1}. This peak was more intense in the specter of AL, it was less intense in the specter of AFL and it did not appear in the specter of FL. This band was attributed to aliphatic C-H stretching in CH_3 [21]. The band at 1270 cm^{-1} only appeared in the FL spectrum and was associated to guaiacyl ring breathing with C-O stretching [22]. Other differences between the spectra were located

at 1220 and 1158 cm^{-1}. The first band was associated to ether bridges while the second one was attributed to C-O stretch in ester group [23]. In FL these bands were very wide and came together in a single peak while in AFL the band at 1158 cm^{-1} was less intense and it could be observed as a shoulder. Finally, these bands were very narrow and they were clearly separated in AL.

Nitrobenze oxidation of obtained lignins was performed in order to establish the ratio between guaiacyl, syringyl and p-coumaryl units present in the samples. As it is exposed in Table 4 the oxidation products obtained after nitrobenzene oxidation treatment came only from guaiacyl and syringyl units and there was not any product coming from p-hydroxyphenyl units. These results suggested that olive tree pruning lignin was GS type, as it is common for hardwood.

The obtained products in all lignin samples were the same except in the case of AFL were vanillic acid was formed. In addition, in all cases vanillin and syringaldehyde were the main products. Otherwise, AL, FL and AFL presented very similar S/G ratio, around 2. This confirmed that lignin extraction method did not affect to the nature of lignin.

Molecular weight distribution of the obtained olive tree pruning lignins and weight-average (M_w), number-average (M_n) molecular weight and polydispersity (M_w/M_n) of the different extracted lignins are shown in Figure 2 and Table 5. AL and AFL presented very high M_w and high polydispersity. On the other hand, FL had both, the lowest M_w and the lowest polydispersity.

Organosolv treatments are said to produce low molecular weight lignins [24] but under an acidic condition, condensation reactions can occur besides acidic depolymerization reactions. As proposed by Li et al. [25], the Cα of the side chain is prone to form a carbon

cation, which can then bind with an electron-rich carbon atom in the aromatic ring of another lignin unit. On the other hand, extraction conditions also affect to lignin size. Zhang et al. [26], claimed that higher temperatures and longer residence times increase the degree of cleavage of ether bonds in the lignin macromolecule, and therefore lower size lignins could be obtained when the extraction process becomes severer. In this work, the extraction process was not very severe and not only the temperature (130 ºC) was low but also the residence time (90 min) was very short. This way obtained lignins had high M_w; however there were some differences between the samples. Formic acid is stronger than acetic acid so the lignin extraction was more severe in the case of the first acid and consequently FL M_w was the lowest one. In addition, the effect of formic acid could be observed comparing AL and AFL; the first one had higher M_w than the second one because of the strength of formic acid. Otherwise, the use of formic acid in the organosolv pulping decreased the polidispersity of lignin. In FL the polidispersity was of 5.54, while in AFL was of 9.28 and in AL where only acetic acid was used as solvent the polidispersity was the highest one (10.75). This high polidispersity indicated the presence of very different molecular weight fractions in the lignins. The formation of new carbon−carbon bonds, thereby, gives an increase in the heterogeneity of the resulting lignin.

In Figure 2, the molecular weight distribution of extracted different lignins could be observed. It can be noticed that the peaks were very wide which indicated a high heterogeneity on lignin molecular weight and therefore a high polidispersity. The narrowest peak was related to formosolv lignin which had the lowest polidispersity and in this case the lowest average molecular weight. Moreover, some shoulders were

identified at higher retention times which indicated the presence of low molecular weight fractions of lignin.

Lignin samples chemical structure was also analyzed by ^{13}C-NMR showed in the Figure 3. AL and AFL spectra were very similar while FL spectrum was the most different one. Among the common signals presented in all the samples there was one at 55.5 ppm that could be attributed to C-H in methoxyl groups linked to aromatic rings [27]. The signals of the organic dissolvent used in the deligninfication process of the olive tree pruning were also visible in the three spectra. In AL spectrum, signal at 28.7 ppm and 172.0 ppm corresponded to methyl and carboxylic groups respectively [28] derived from the acetic acid used in the extraction process. Moreover, in FL spectrum the signal attributed to the employed solvent (formic acid) was located at 163.0 ppm [29]. Finally, in AFL the signals related to both, acetic and formic acid, were present but with lower abundance. As it was mentioned before, AL and AFL spectra were very similar and apart from the signals described above both of them presented some signals in the region of 29-39 ppm that could be attributed to CH_2 in aliphatic chains [30]. Furthermore, in FL spectrum more signals that could be related to the aromatic structure of the lignin were found which is consistent with the lignin composition discussed before as FL was the purest lignin. The integral of signals between 61.0 and 69.0 could be related to C-γ in β-O-4 and β-5 structures [31], while those between 70.0 and 77.0 ppm could be attributed to C-α in β-O-4 guiacyl and syringil units [30]. In addition, there is signal at 147.9 ppm which corresponded to C3/C5 in syringyl β-O-4 non etherified structures [30].

4. Conclusions

In this work different organosolv treatments were used in order to extract lignin from olive tree pruning. The obtained lignins (AL, Fl and AFL) were analyzed by different techniques to elucidate their structure and chemical composition and this way relate the extraction method with the nature of lignin. Olive tree pruning lignin was GS type and both S/G ratio (around 2) and structure did not change due to the extraction method.

The purity of all lignin samples was high (more than 69% of Klason lignin) with low content of inorganics and sugars. Otherwise, all lignin samples had very high percentage of phenolic hydroxyl groups in their structure which allows their use for polymer formulation and their chemical modification. The average molecular weight was high in all cases which was consistent with applied weak pulping conditions and the utilization of organic acids as solvents. However, the average molecular weight was very different between the three lignin samples. The polidispersity values were in general very high so the lignin samples had very different molecular weight fractions.

Acknowledgments

The authors would like to thank the Department of Education, Universities and Investigation of the Basque Government (pre-doctoral scholarship programs of young researchers training) and Spanish Ministry of Economy and Competitiveness (CTQ2010-19844-C02-02) and the University of the Basque Country (GIU11/10) for supporting this work.

References

[1] A. García, A. Toledano, L. Serrano, I. Egüés, M. González, F. Marín, J. Labidi, Sep. Purif. Technol. 68 (2009) 193-198.

[2] M. Kleinert, T. Barth, Chem. Eng. Technol. 31 (5) (2008) 736-745.

[3] R.J.A. Gosselink, PhD Thesis. Wageningen University. (2011)

[4] H.T. Sahin, Appl. Biol. Sci. 1 (1) (2003) 63-67.

[5] P. Ligero, A. Vega, M. Bao, Ind. Crops Prod. 21 (2005) 235-240.

[6] S. Dapía, V. Santos, J.C. Parajó, Biomass Bioenergy 22 (2002) 213-221.

[7] J.J. Villaverde, P. Ligero, A. Vega, J. Cleaner Prod. 18 (2010) 395-401.

[8] H.T. Sahin, R.A. Young, Ind. Crops Prod. 28 (2008) 24-28.

[9] A. Toledano, L. Serrano, J. Labidi, Chem. Eng. J. 193-194 (2012) 396-403.

[10] TAPPI Standards, (2007). TAPPI Test Methods, Atlanta.

[11] L.E. Wise, M. Murphy, A.A. D'Adieco, Paper Trade J. 122 (2) (1946) 35-43.

[12] R. Rowell, The Chemistry of Solid Wood: Based on Short Course and Symposium Sponsored by the Division of Cellulose Paper and Textile Chemistry at the 185th meeting of the American Chemical Society. Seattle, Washington, 1983, pp. 70-72.

[13] J.C. Parajó, J.L. Alonso, V. Santos, Bioresour. Technol. 51 (1995) 153-162.

[14] C. Vila, V. Santos, J.C. Parajó, Bioresour. Technol. 90 (2003) 339-344.

[15] F. Xu, J.X. Sun, R.C. Sun, P. Fowler, M.S. Baird, Ind. Crops Prod. 23 (2006) 180-193.

[16] A. Garcia, M. González Alriols, G. Spigno, J. Labidi, Biochem. Eng. J. 67 (2012) 173-185.

[17] C. Mancera, F. Ferrando, J. Salvadó, N.E. El Mansouri, Biomass bioenergy 35 (2011) 2072-2079.

[18] A.U. Buranov, K.A. Ross, G. Mazza, Bioresour. Technol. 101 (2010) 7446-7455.

[19] A. Toledano, X. Erdocia, L. Serrano, J. Labidi, Environ. Prog. Sustainable Energy DOI: 10.1002/ep.11725

[20] L. Serrano, I. Egües, M. González Alriols, R. Llano-Ponte, J. Labidi, Chem. Eng. J. 156 (2010) 49-55.

[21] R.E. Hage, N. Brosse, L. Chrusciel, C. Sanchez, P. Sannigrahi, A. Ragauskas, Polym. Degrad. Stab. 94 (2009) 1632-1638.

[22] M. González Alriols, A. García, R. Llano-Ponte, J. Labidi, Chem. Eng. J. 157 (2010) 113-120.

[23] S.N. Sun, M.F. Li, T.Q. Yuan, F. Xu, R.C. Sun, Ind. Crops Prod. 37 (2012) 51-60.

[24] J. Lora, in: N.M. Belgacem, A. Gandini (Eds.), Monomer, polymers and composites from renewable resources, Elsevier Science. 2008, pp. 225-241.

[25] J. Li, G. Henriksson, G. Gellerstedt, Bioresour. Technol. 98 (2007) 3061-3068.

[26] J. Zhang, H. Deng, L. Lin, Y. Sun, C. Pan, S. Liu, Bioresour. Technol. 101 (2010) 2311-2316.

[27] J. Rencoret, A. Gutiérrez, L. Nieto, J. Jiménez-Barbero, C.B. Faulds, H. Kim, J. Ralph, A.T. Martínez, J.C. del Río, Plant Physiol. 55 (2010) 667-682.

[28] H.E. Gottlieb, V. Kotlyar, A. Nudelman, J. Org. Chem. 62 (1997) 7512-7515.

[29] X. Wei, L. Ye, Y. Yuan, J. Nat. Gas Chem. 18 (3) (2009) 295-299.

[30] D. Robert, in: S.Y. Lin, C.W. Dence, (Eds.), Methods in Lignin Chemistry, Springer-Verlag. Berlin, 1992, pp. 250-273.

[31] D.V. Evtuguin, C.P. Neto, A.M.S. Silva, P.M. Domingues, F.M.L. Amado, D. Robert, O. Faix, J. Agric. Food Chem. 49 (2001) 4252-4261.

Figure Captions

Figure 1. FTIR spectra of obtained different lignins. a) Wave number from 4000 to 700 cm^{-1} and b) magnification of 1800-700 cm^{-1} region.

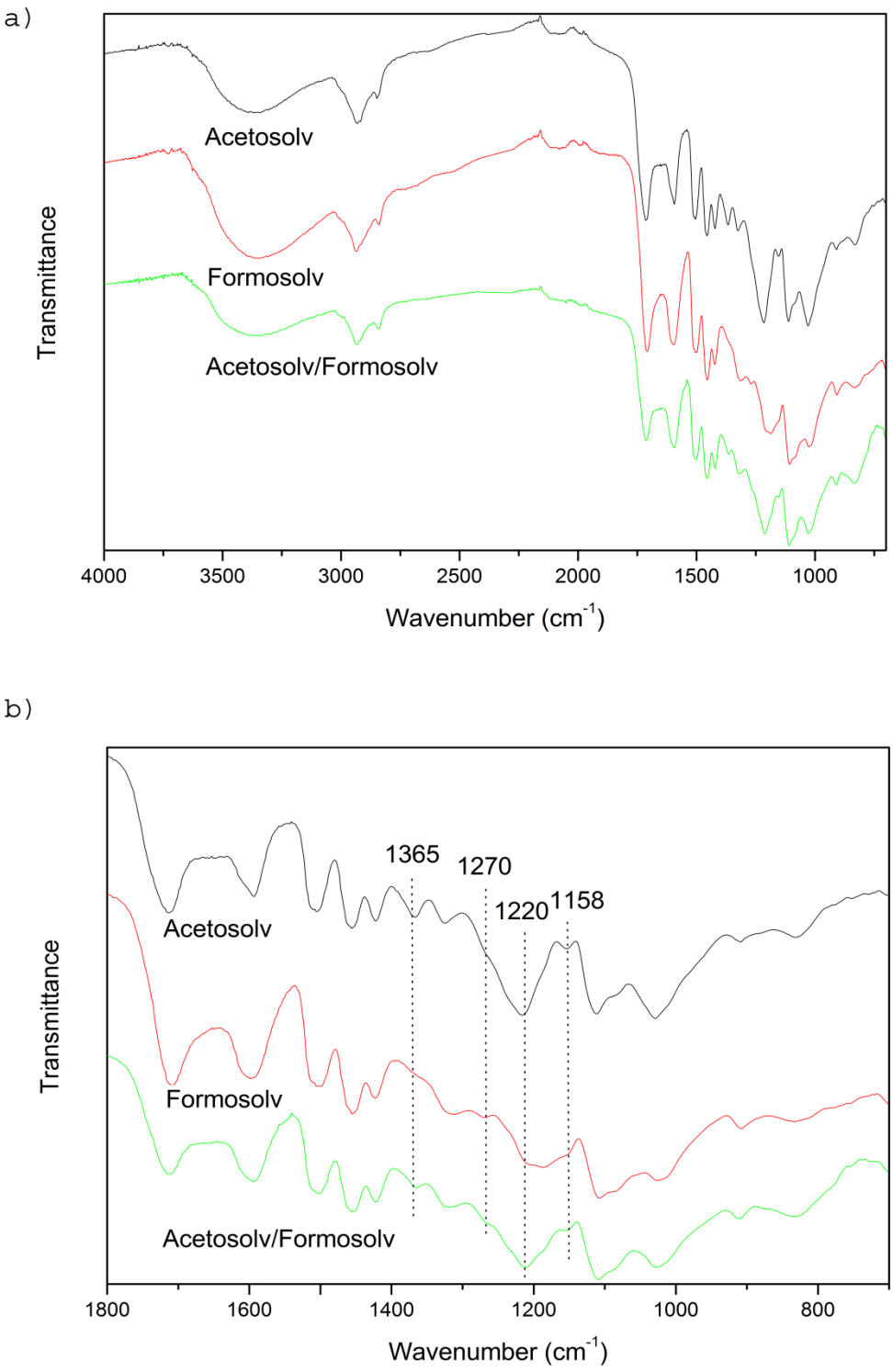


Figure 2. Molecular weight distribution of extracted lignins HPSEC analysis.

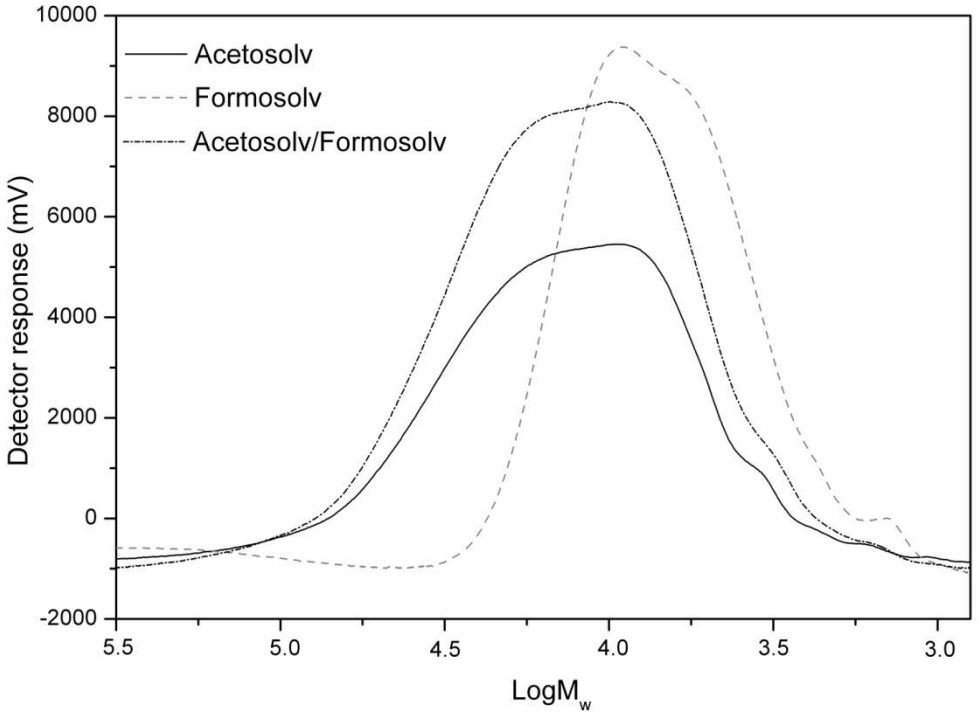
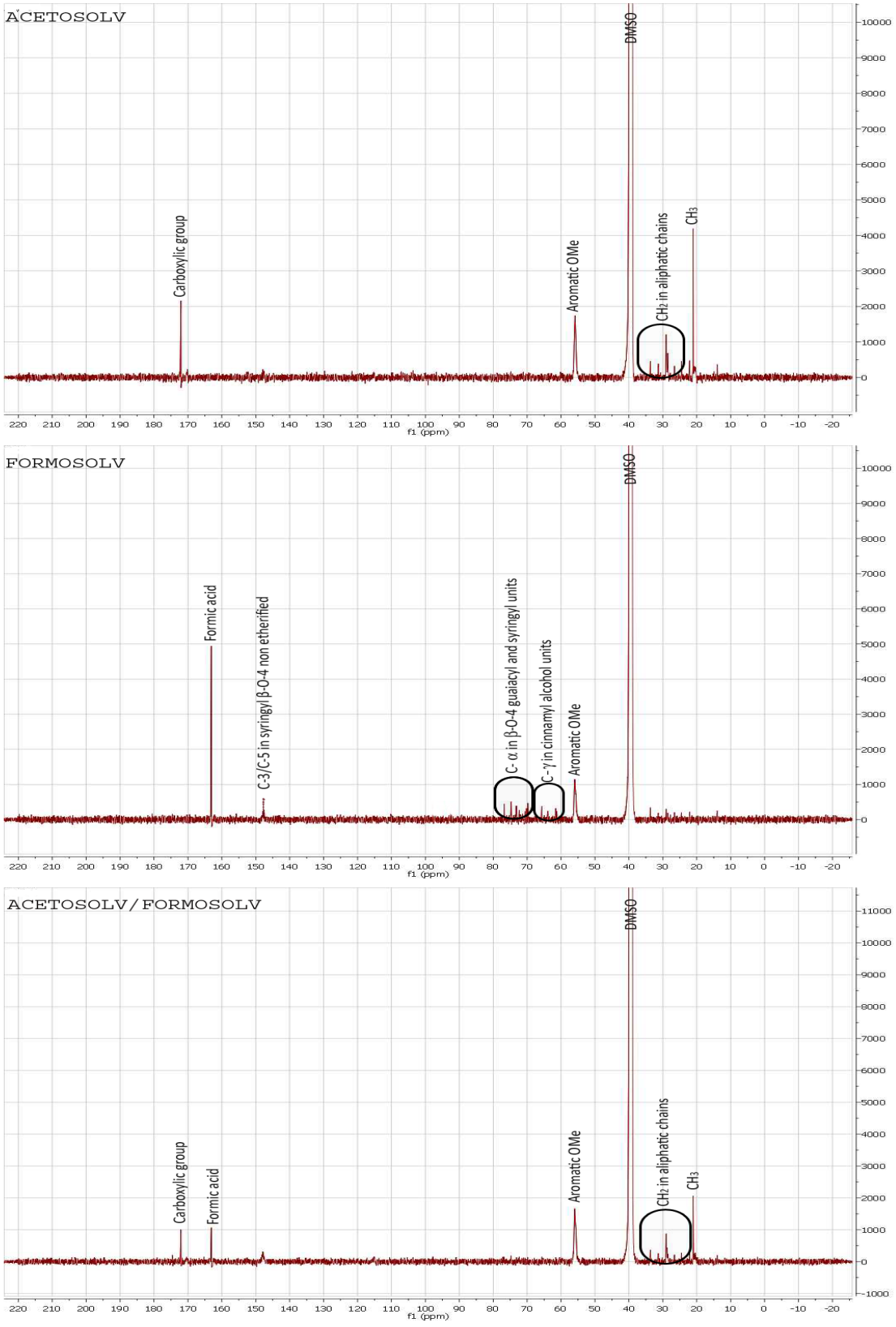


Figure 3. ^{13}C NMR spectra of acetosolv, formosolv and acetosolv/formosolv lignins.



Tables

Table 1. Lignin composition.

	AL	FL	AFL
Klason lignin (%)	69.05 ± 0.55	79.01 ± 0.35	69.09 ± 0.52
ASL (%)	1.97 ± 0.01	2.47 ± 0.07	1.76 ± 0.02
Sugars (%)	5.01 ± 0.41	1.88 ± 0.10	5.87 ± 0.32
Glucose (%)	2.61 ± 0.30	1.00 ± 0.04	3.30 ± 0.22
Xilose (%)	2.26 ± 0.12	0.75 ± 0.07	2.42 ± 0.11
Arabinose (%)	0.14 ± 0.00	0.14 ± 0.02	0.15 ± 0.02
Ash (%)	2.57 ± 0.11	2.71 ± 0.09	2.95 ± 0.14

Table 2. Elemental composition of lignin.

	AL	FL	AFL
C (wt %)	59.33	61.79	63.13
H (wt %)	6.09	5.54	5.66
N (wt %)	1.02	0.81	0.91
O (wt %)	30.99	29.15	27.35
H/C molar ratio	1.22	1.07	1.07
O/C molar ratio	0.39	0.35	0.33

Table 3. Functional groups (% w/w) present in lignin.

	AL	FL	AFL
Functional groups			
Hydroxyl groups	18.13 ± 0.27	18.55 ± 0.23	21.32 ± 0.39
Carbonyl groups	0.40 ± 0.02	0.62 ± 0.02	0.52 ± 0.01
Carboxylic groups	10.50 ± 0.17	9.70 ± 0.11	9.84 ± 0.09

Table 4. Yields (%, w/w) of the detected phenolic acids and aldehydes compounds formed from different lignins alkaline nitrobenzene oxidation.

	AL	FL	AFL
Vanillic acid	-	-	0.27
Syringic acid	0.29	0.23	0.36
Vanillin	10.37	4.61	7.55
Syringaldehyde	21.40	9.16	15.63
Acetovanillone	0.23	0.18	0.14
Total	32.29	14.18	23.95
S/G ratio	2.05	1.96	2.01

Table 5. Weight-average (*Mw*), number-average (*Mn*) molar mass and polydispersity (*Mw*/*Mn*) of the different lignins.

	AL	**FL**	**AFL**
M_w	16416	7924	15088
M_n	1528	1430	1626
M_w/M_n	10.75	5.54	9.28