

Lignin Characterization from Two Different Pretreatments in Bioethanol Production Processes from Olive Tree Pruning

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Biomass generated from pruning of olive trees is an abundant lignocellulosic residue that has been proposed for bioethanol production by means of a process including pretreatment, enzymatic hydrolysis, and fermentation. The pretreatment is critical step in the process, which allows the fractionation of the biomass. Lignin is the main byproduct of the process, and then its valorization is a key factor for the economical viability of the bioethanol production from olive tree pruning. In this work, two types of pretreatments have been compared steam explosion and organosolv in terms of the produced lignin suitability for further applications in the so-called new biorefinery concept.

Starting from this fact, special emphasis was given to determine the physico-chemical properties of lignins produced by the two processes in order to determine their suitability for the development of added value products (e.g. materials, building blocks, additives). Overall, the lignin obtained from organosolv pretreatment had better properties for its further valorization as it presents more suitable qualities for higher-added value applications like fine phenolic chemicals production.

1. Introduction

Olive tree pruning biomass (OTB) is a widely available lignocellulose residue, especially in Mediterranean countries. Spain has an area of 2.5 million olive trees, most of them concentrated in Andalusia. The pruning operation required to remove the old branches generates around 2 tonnes of OTB per hectare. Today, this waste has no industrial application. It is usually burned or grinded and scattered on the field. As an alternative, several recent research studies have been carried out to valorize OTB as solid (Volpe et al., 2016) or liquid (Martínez-Patiño et al., 2017) biofuels. Otherwise, it is also desirable to produce other high-value compounds from biomass under a biorefinery approach to achieve viability of the overall process (González-García et al., 2016). In this context, the production of compounds derived from lignin is being investigated extensively (Fernández-Rodríguez et al., 2017).

The production of bioethanol from structural sugars has been proposed by a process involving three main stages: pretreatment, enzymatic hydrolysis and fermentation. Steam explosion is one of the most effective pretreatment techniques for the disruption of lignocellulosic biomass structure (Zhao et al., 2017). It has been applied for the production of ethanol from OTB with promising results (Cara et al., 2008). The characterization of the residual solid from 2nd generation ethanol production is of great interest for the valorization of lignin (de Menezes et al., 2016). On the other hand, organosolv treatment has also been used for the extraction of lignin from OTB (Toledano et al., 2013). The main objective of this work was the characterization of the final lignin-rich solid by-product resulting from steam explosion and enzymatic hydrolysis of OTB and its comparison with lignin from OTB organosolv treatment. The main parameters studied were the Klason lignin content, impurities concentration (sugar and ashes), molecular weight and polydispersity index and chemical structure. All these, parameters are essential for tracing the final valorization path of the lignins and also to design the bioethanol production biorefinery plant.

2. Material and methods

The olive tree pruning biomass (OTB) was collected in the field and then dried at room temperature in the laboratory. Afterwards, they were prepared for being characterized using the procedures described in the standards developed by Technical Association of Pulp and Paper Industries (TAPPI) and other methods for polysaccharides content. Each procedure was made by triplicate, referring results as the arithmetic average value and the standard deviation. The results were: ash content $3.1\% \pm 0.4$ (TAPPI T211 om-93); ethanol-toluene extractives $9.5\% \pm 0.4$ (TAPPI T204-cm97), acid-insoluble lignin $23.2\% \pm 0.7$ (TAPPI T-222-om98) and holocellulose $66.8\% \pm 1.2$ (Wise et al. 1946); α -cellulose $58.4\% \pm 0.5$ (Rowell et al. 1983) and hemicelluloses $8.4\% \pm 0.3$ (obtained by difference between holocellulose and α -cellulose).

2.1 Steam explosion and enzymatic hydrolysis

OTB was ground to 1 cm particle size prior the steam explosion pretreatment. This pretreatment was performed in a batch pilot unit based on Masonite technology (Galbe and Zacchi, 2012) with a 4.5-L reaction vessel. 400 g of OTB (dry basis) were introduced into the reactor and heated to 210 °C for 5 minutes with saturated steam. The exploded material was discarded in a recovery vessel and filtered. The pretreated solid was washed and then enzymatically hydrolyzed by a cellulolytic complex (CellicCTec2, Novozymes A/S) with 15 FPU/g of solid and β -Glucosidase (Novozyme 50010). The tests were performed in Erlenmeyer flasks on a rotatory shaker for 72 h at 150 rpm, 50 °C and a solids concentration of 5 % (w/v). The remaining solid (SE-OTB) was recovered for lignin characterization.

2.2 Organosolv pretreatment

OTB was ground to 0.4-0.6 cm particle size prior the organosolv pretreatment. The ethanol organosolv pretreatment was carried out in a 1.5 L pressure stainless steel reactor (Parr 4836) equipped with a heating mantle, mechanical stirrer and manometer. The digestion of the olive tree pruning was achieved using a mixture of ethanol-water (with a mass fraction of 70 % of ethanol) at 200 °C for 90 min in a pressure reactor with constant stirring. The solid to liquid mass ratio was 1:6. Solid and liquid fractions were separated by gravity filtration; the solid fraction was washed with ethanol/water mixtures with the same concentration than the one used for the pretreatment. The solid fraction was dried at 105 °C and the liquid fractions were stored in the fridge (5 °C) not to suffer alterations.

Prior to fermentation, delignified solid fraction was subjected to an enzymatic hydrolysis with cellulases for solubilizing polymeric sugars into their monomers. Dried samples were mixed in 50 cm³ erlenmeyer flasks with citrate buffer pH 4.8 (23 cm³ of acid citric solution 21.01 g/L and 27 cm³ of sodium citrate 29.41 g/L, in a 100 cm³ metric flask), in a proportion solid:liquid of approximately 15 % and were incubated in an orbital shaker (3 Hz) for 72 h at 50 °C. The hydrolytic enzymes used were Cellic Ctec2 (enzyme activity 72.55 FPU mL⁻¹) and HTec 2 (enzyme activity not available) (kindly supplied by Novozymes), with a doses of 9 % and 0.9 % respectively by weight of dry sample.

Organosolv liquid fraction was the result of the combination of the fresh liquid fraction from the organosolv treatment and washing liquors. Lignin was precipitated from the liquors by adding two volumes of acidified water (pH around 2). The precipitated lignin (OL) was then separated by centrifugation (4,000 rpm, 20 min) and dried at 50 °C. The flowsheet of the two processes is shown in Figure 1.

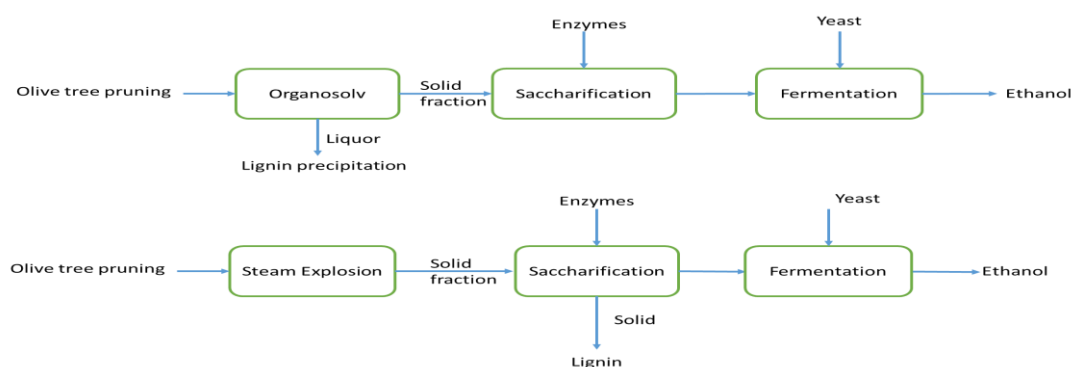


Figure 1: Flowsheet of ethanol and lignin by-product obtaining from organosolv and steam explosion pretreatments of olive tree pruning.

2.3 Analytical methods

The lignin derived from the organosolv pretreatment and after steam explosion pretreatment and further enzymatic hydrolysis (SE-OTB) were characterized following the procedures described in the standards developed by International Lignin Institute (ILI).

Extracted lignin was subjected to High Performance Size Exclusion Chromatography (HPSEC) to evaluate lignin molecular weight (MW) 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 + 0.1 % lithium bromide solution was used as eluent. The flow rate was 0.7 cm³ per minute and the analyses were carried out at 40 °C. Calibration was made using polystyrene standards (Sigma-Aldrich) ranging from 266 to 70,000 g mol⁻¹.

The pyrolysis of the lignin samples was carried out using a CDS analytical Pyroprobe 5150. The pyrolysis temperature was set at 500 °C for 15 sec with a heating rate of 2 K msec⁻¹. Then the products were analyzed in a GC (7890A)-MS (5975C inert MSD with Triple-Axis Detector) Agilent equipped with a capillary column HP-5MS ((5 %-Phenyl)-methylpolysiloxane, 30 m x 0.25 mm). The oven program started at 50 °C and was held 2 min at this temperature. Then it was raised to 120 °C at K min⁻¹ and held 5 min, raised to 280 °C at K min⁻¹, held 8 min and finally raised to 300 °C at K min⁻¹ and held 10 min.

3. Results and discussion

3.1 Chemical composition

The samples after steam explosion pretreatment and further enzymatic hydrolysis (SE-OTB) were fractionated in three different fractions depending on the particles size: Fraction 1 > 2 mm, Fraction 2 between 0.25 mm and 2 mm, Fraction 3 < 0.25 mm (Table 1).

Table 1: Weight percentage of the different fractions obtained after sieving of SE-OTB.

	Fraction 1	Fraction 2	Fraction 3
% of sample (% w/w)	54.63	37.17	8.20

Table 2 shows the chemical composition of the different fractions. It can be noticed the high content in inorganics, mainly in the biggest size fraction. The acid insoluble lignin content was moderately high and it increased while the size of the particles decreased. Fraction 3 was the most pure one while Fraction 1 had the lowest quantity of acid insoluble lignin. However, the difference was not very high. The acid soluble lignin content was very low and similar in all the fractions and in this case it behaved contrary to the insoluble lignin content: the acid soluble lignin content decreased while the size of the particles decreased. The main impurities in the samples were sugars. Glucose was the main sugar in all the samples which comes from cellulose. The other sugar present in the samples was xylose which derives from hemicelluloses impurities. It has to be also mentioned the high content of ash in the first fraction of the sample which suggest that inorganic compounds like Na, Ca, S, etc. were more abundant in the big size particles. The organosolv lignin had lower ash and sugar content comparing to the SE-OTB lignins.

Table 2: Chemical composition of the different lignins obtained (% in weight).

	Organosolv	SE-OTB		
	Lignin	Fraction 1	Fraction 2	Fraction 3
Moisture (105 °C)	3.42 ± 0.09	4.81 ± 0.07	4.72 ± 0.27	4.89 ± 0.17
Ash (525 °C)	0.39 ± 0.01	7.81 ± 0.09	4.44 ± 0.10	4.84 ± 0.30
Acid insoluble lignin (on dry basis)	71.90 ± 0.79	66.92 ± 0.43	67.90 ± 0.21	68.24 ± 0.07
Acid soluble lignin (on dry basis)	1.63 ± 0.08	3.02 ± 0.13	2.63 ± 0.17	2.58 ± 0.13
Glucose (on dry basis)	1.75 ± 0.12	9.07 ± 0.91	12.89 ± 0.52	11.09 ± 0.16
Xylose (on dry basis)	1.10 ± 0.03	2.33 ± 0.29	2.85 ± 0.30	2.55 ± 0.22

3.2 Chemical structure

The chemical structure of the samples was analyzed by two different techniques.

The FTIR spectra of all the fractions (Figure 2a) showed no differences between them. As it can be observed in Table 3 all the identified peaks are assigned to characteristic vibrational modes of lignin samples. The most intense peaks which are located at 1,605, 1,322, 1,120, 1,032 and 780 cm⁻¹, are very significant in any lignin sample as they are related to aromatic skeletal, C=O and OCH₃ stretching and vibrations. Figure 2.b shows the FTIR spectrum of organosolv olive tree pruning lignin. The obtained spectrum corresponded to a high quality lignin and it is similar to the SE-OTB lignin samples being the peak at 1,120 cm⁻¹ the most intense one.

Otherwise, in all lignin spectra could be observed that the peak at $1,322\text{ cm}^{-1}$ is very intense which indicates that the obtained lignin samples had high percentage of syringyl units. This suggest, that the lignin from olive tree pruning its S/G type with higher amount of S (syringyl) units which is common for hardwoods.

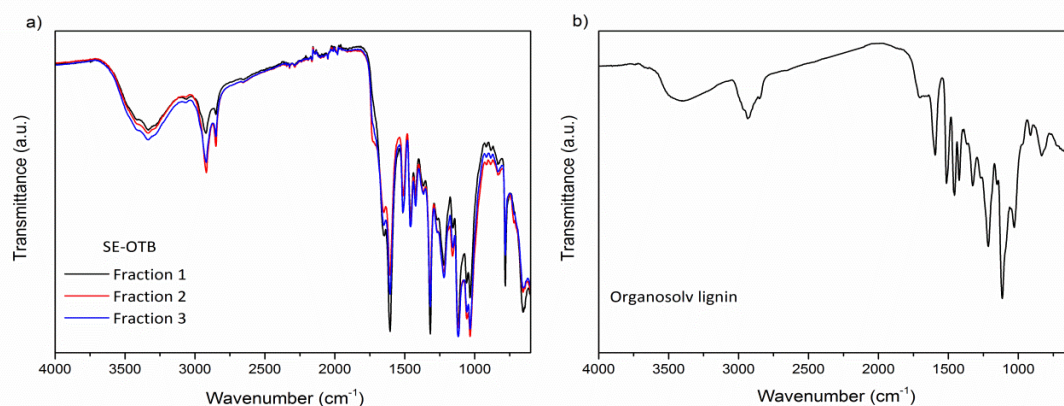


Figure 2: FTIR spectra of obtained different lignins. a) Lignins from SE-OTB after sieving and b) Organosolv lignin.

Table 3: Vibrational modes identified in the FTIR spectra of the lignins.

Wavenumber (cm^{-1})	Assignment
3420	O-H stretching vibration in aromatic and aliphatic OH groups
2922	Out-of-plane asymmetric CH stretch
2845	CH stretch in OCH_3 , symmetric
1656	Lignin C=C stretch of coniferyl alcohol and sinapyl alcohol
1605	lignin aromatic skeletal vibrations
1513	Aryl ring stretch, asymmetric
1460	Lignin methoxy deformation, CH_2 scissoring
1420	lignin methoxy deformation, methyl bending, aromatic skeletal vibrations
1322	C=O stretching in syringyl unit
1218	Aryl-O of aryl-OH and aryl- OCH_3 ; ring deformation
1159	C=O in ester groups (conjugated)
1120	lignin methoxy vibrations; aryl CH bend
1032	lignin CH_3 wagging; CH_3 out-of-plane rock; aromatic skeletal vibrations, methoxy vibrations
920	Ring deformation; in-plane CC stretch; COC stretch
834	aromatic C-H out-of-plane deformation
780	CO stretch; lignin aromatic skeletal vibrations
650	Ring and skeletal deformation

The samples were subjected to a pyrolysis-GC/MS analysis in order to elucidate their structure. It could be observed in Figure 3 that the obtained pyrograms were very similar and the majority of the compounds identified were assigned to lignin molecule. However, there are some differences between the fractions and especially between the SE-OTB lignins and organosolv lignins. The fraction 1 had some compounds derived from sugars pyrolysis (Furfural, 1,2-Cyclopentanedione and 5-methyl- 2-Furancarboxaldehyde) that were not significant in the rest of the fractions. In addition, the organosolv lignin had higher percentages of lignin derivative compounds particularly in the case of syringol, vanillin and vanillic acid compounds. These results were in accordance with previous lignin chemical characterization where organosolv lignin has been found to be the most pure and with the lowest sugar impurities. Furthermore, the quantity of syringol is much higher than guaiacol in all the samples which validates the results from FTIR analysis and confirms that the S/G ratio of olive tree lignin samples is higher than 1.

In Table 4 the compounds that have more than 1 % of area the whole pyrogram are listed.

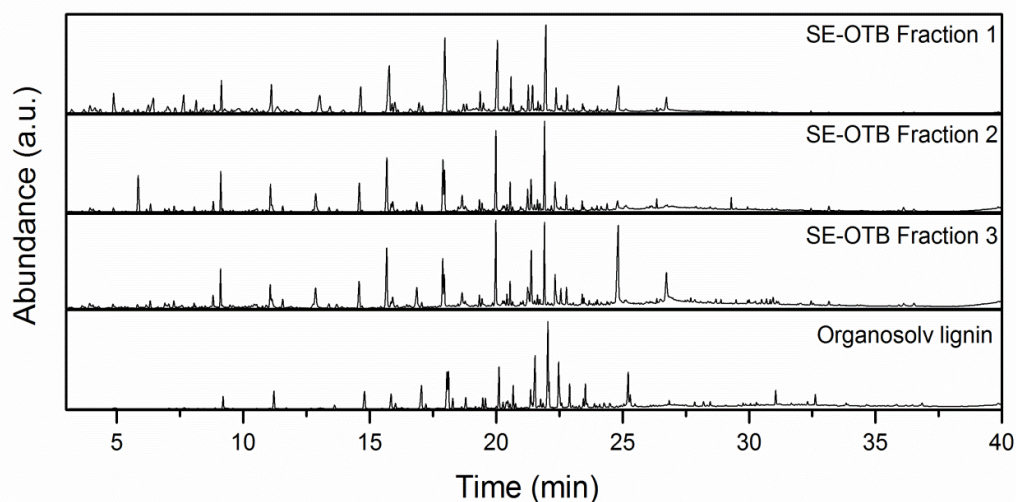


Figure 3: Pyrograms of the four different lignins

Table 4: Compounds with more than 1 % of the total chromatogram area identified in the pyrolysis of the lignins.

Compound	RT (min)	% Chromatogram area			
		Fraction 1	Fraction 2	Fraction 3	Organosolv lignin
Furfural	4.88	1.51	---	---	---
Styrene	5.84	---	3.70	---	---
1,2-Cyclopentanedione	6.44	1.56	---	---	---
5-methyl- 2-Furancarboxaldehyde	7.02	1.20	---	---	---
Guaiacol	9.11	1.45	2.56	1.66	2.08
2-methoxy-4-methylphenol	11.08	2.20	2.26	1.24	2.57
1,2-Benzenediol	11.21	1.30	1.00	0.89	0.18
3-methoxy-1,2-Benzenediol	12.92	2.45	2.69	1.78	2.29
2-Methoxy-4-vinylphenol	14.62	1.95	2.86	1.78	1.44
Syringol	15.69	4.30	5.32	3.84	5.76
3,4-dimethoxyphenol	15.94	0.87	1.22	0.84	1.79
Vanillin	16.88	0.85	1.12	1.81	2.18
Vanillic acid	17.93	5.98	4.04	2.50	7.09
Isoeugenol acetate (E)	17.98	---	2.89	1.59	---
Acetovanillone	18.66	0.74	2.25	1.41	0.67
5-tert-Butylpyrogallol	19.35	1.05	0.80	0.57	---
3,4-Dimethoxyacetophenone	20.01	4.78	5.70	4.28	4.08
Methoxyeugenol	20.57	3.10	3.51	2.81	1.12
2,6-Dimethoxy-4-allylphenol	21.24	---	---	---	1.65
Syringaldehyde	21.43	1.70	2.43	3.12	4.64
4-allylsyringol	21.93	5.38	5.79	3.49	4.10
Acetosyringone	22.35	1.65	3.02	2.31	1.72
Homosyringic acid	22.80	1.10	1.14	1.20	1.20
Palmitic acid	24.82	2.85	1.16	7.60	8.49
Stearic acid	26.74	1.94	---	4.54	6.96

3.3 Molecular weight

The molecular weight of all fractions is very similar and comparable to organosolv lignins (Table 5). However, the polydispersity index (Mw/Mn) is very high indicating many different molecular weight fractions inside the samples. The organosolv lignin has similar molecular weight than the SE-OTB samples with lower polydispersity index which indicates that this lignin is more homogeneous.

Table 5: Molecular weight and polydispersity index of the lignins.

	Mw (g/mol)	Mn	Mw/Mn
Organosolv	7232	2125	3.40
Fraction 1	8207	786	10.44
Fraction 2	7832	722	10.85
Fraction 3	8917	789	11.31

4. Conclusions

This work showed that olive tree pruning biomass could be converted in biorefinery processes into high-added value products by lignin valorization. On the one hand, the solid residue remaining after the studied fractionation processes (organosolv and steam explosion) was suitable for saccharification and gave high lignin extraction yields. On the other hand, the fractionation processes produced lignins with similar molecular weight. However, the low polydispersity index of organosolv lignin and less sugar impurities make it suitable for use in materials applications or in aromatic chemicals obtaining by its depolymerization. Otherwise, SE-OTB lignin could be used for the production of building blocks. An economical assessment of both processes is required to determine the processes for the entire valorisation of olive tree pruning biomass.

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