



ELSEVIER

Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

## Chemical Engineering Research and Design

journal homepage: [www.elsevier.com/locate/cherd](http://www.elsevier.com/locate/cherd)


# Separation process optimisation and characterisation of lignin from black carob tree sawdust into a biorefinery

Eliana P. Dagnino<sup>a,\*</sup>, Lucio R. Chiappero<sup>b</sup>, Verónica V. Nicolau<sup>b</sup>, Ester R. Chamorro<sup>a</sup>

<sup>a</sup> Centro de Investigación en Química Orgánica Biológica (QUIMOBIO, FRRe, UTN), Instituto de Modelado e Innovación Tecnológica (IMIT, CONICET, UNNE), French 414, 3500, Resistencia, Chaco, Argentina

<sup>b</sup> Grupo de Polímeros, Departamento de Ingeniería Química, Facultad Regional San Francisco, Universidad Tecnológica Nacional, Av. de la Universidad 501, 2400, San Francisco, Córdoba, Argentina

## ARTICLE INFO

## Article history:

Received 27 February 2019

Received in revised form 27 August 2019

Accepted 30 December 2019

Available online 9 January 2020

## Keywords:

Soda-ethanosolv

Lignin

Black carob tree sawdust

Biorefinery

## ABSTRACT

Biorefineries are meant to play a key role as regards both the complementation of energy sources and the integral exploitation of biomass. Wood residues left over from industrialisation have a high potential to obtain numerous bio-based products. The aim of this work was to obtain high quality and high purity lignin as well as a solid product rich in carbohydrates with not more than 10%ODW of the remaining acid-insoluble lignin. To achieve this goal, a soda-ethanosolv process was optimised for the delignification of washed black carob tree sawdust (WBCTS) using a central composite experimental design; afterwards, the time of reaction was adjusted. The optimum process (19.9% NaOH (ODW of WBCTS) and 58:42 EtOH:H<sub>2</sub>O) was found, and then the reaction time was adjusted to 80 min for a higher delignification (pretreated WBCTS with less than 10%ODW). Therefore, the lignin recovered from the liquor of the SodEt process is of high quality and good purity and, the IR spectrum of the lignin is consistent with GS lignins. The results found could be used to establish sustainable chains of high value of bio-based products obtained from black carob tree sawdust and by using low-impact technologies with low environmental impact which could be applied to biorefineries.

© 2020 Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

## 1. Introduction

At present, the refinery of non-renewable fuels is the main energy and worldwide downstream products supplier. Renewable alternatives must be promoted in order to mitigate climate change and to reduce the use of fossil fuels. The biorefinery of lignocellulosic residues is analogous to petroleum refinery, since it allows the separation of structural components for the subsequent exploitation of different biochemical and biofuel products (Cherubini, 2010; Alvira et al., 2010). Biorefineries are meant to play a key role concerning both, the complementation of energy sources and the integral exploitation of biomass (Plaza and Wandzich, 2016), industrial agroforestry residue. This is an important

development opportunity for Argentina which should aim at the diversification of animal and vegetable products as well as at the quality increase and the added value of agro-industrial activity (Salazar and Cárdenas, 2012). Because of this, optimised conversion technologies should be applied to the treatment of the raw material under study and to the desired end products.

The *Prosopis* genre can be found, mainly, in arid and semi-arid, tropical and subtropical countries (Burkart, 1976); it is a huge area that covers from the Southwest part of USA down to Patagonia, in Argentina, and which is typical of desert areas of the Chaco forest in Argentina (Cardozo et al., 2010). Black carob (*Prosopis nigra*) is a leguminous tree from South America that can be found in the Gran Chaco ecoregion

\* Corresponding author.

E-mail address: [pdagnino@frre.utn.edu.ar](mailto:pdagnino@frre.utn.edu.ar) (E.P. Dagnino).

<https://doi.org/10.1016/j.cherd.2019.12.027>

0263-8762/© 2020 Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

(particularly in the Southeast Chaco), in Argentina, Bolivia, Paraguay and Uruguay. It is known as black carob, sweet carob, purple carob and yellow carob. In Argentina, there are 29 *Prosopis* species with 14 endemic taxons. This is the main area of diversity in America (Pometti et al., 2009). *Prosopis* genres are highly resistant to heat, drought, alkalinity and salinity. The black carob wood is widely used in the furniture industry due to its structure and its high richness in tannin, characteristics which ensure resistance to environmental exposition and even to water immersion. In the province of Chaco (Argentina), the black carob tree is widely exploited, being used mainly in the furniture industry and indoors and windows manufacturing. Much residue remains after using only 20%–30% of the tree in this industry. It is estimated that in Chaco, 28,000 tons of wood are processed every month and the production of residue is of 70 tons per day (Cuadra, 2012). The accumulation of residues entails dangers and problems such as fire risk, air contamination and parasite proliferation. At present, these residues are burnt, buried or stored for a period of time, which is sometimes rather long.

Wood residues left over from industrialisation have high potential for being used as raw material for bio-based products, like in bioethanol production. As shown in a previous study, black carob tree sawdust is composed of 34.1% of glucans, 12.6% of xylans, 0.7% of arabinans, 31.2% of acid-insoluble lignin, 2.0% of acid-soluble lignin and 14.6% of water-soluble and ethanol-soluble extractive substances (Dagnino et al., 2013). This composition can be compared with other similar raw materials like *Prosopis juliflora* (49.4% cellulose (glucans), 18.0% hemicellulose (xylans), 4.3%, of inorganics, 28.3% lignin and extractive substance) (Naseeruddin et al., 2013). The possibility of using sawdust under the concept of biorefinery can be a way of reducing the waste generated by furniture manufacturing and of obtaining value-added products from for those residues.

In the processes of the biorefinery of lignocellulosic materials, lignin provides an opportunity to value them. Lignin is an amorphous and aromatic biopolymer that provides structure, resistance to chemical and biological degradation, and it also acts as an adhesive to the cell wall of the plant (Himmel et al., 2007). About 20–25% of the cell wall mass is composed of lignin. This high proportion makes it become the main renewable source of aromatic compounds (Azadi et al., 2013). Lignin based products will depend on the raw material of origin and on the process of fractionation or separation of it. Pretreatment technologies should be adjusted according to the different physico-chemical characteristics of the lignocellulosic biomasses. Furthermore, the pretreatment choice has a great impact on the following biorefinery stage/s, as regards cellulose digestibility, quality of the byproducts, generation of toxic compounds, which could be potential microorganism inhibitors, agitation power requirements, energy demand for the following process and demands of wastewater treatment, among others (Alvira et al., 2010).

In order to produce higher value products, the selected process to separate lignin from the cellulosic matrix, regarding a biorefinery, should be efficient, able to produce a high quality and high purity lignin and able to avoid the degradation of the carbohydrates present in the matrix. The most used delignification processes at industrial scale are found in paper industry; for example, the Kraft process which is an industrially mature technology for wood delignification, in which the produced lignin has many disadvantages such as the high content of sulphur; a fact which reduces its quality for some applications. Furthermore, the hemicellulosic fraction is widely degraded in saccharinic acids, which contaminate the lignin. On the other hand, there are alternative methods for lignocellulosic biomass processing in the biorefinery context, such as the alkaline and/or organosolv processes, which are very promising methods to obtain high value lignin (Koo et al., 2011). Besides, the ethanosolv processes have certain advantages such as low cost and low toxicity, the recovery of alcohol for its further reuse, the production of high quality lignin and the capacity to maintain most of the cellulose fraction in the pretreated solid (Zhou et al., 2018). The delignification capacity of ethanol might be improved with the use of sodium hydroxide as a catalyst during the ethanosolv pretreatment which could greatly increase the results in an enzymatic digestibility improvement (Dagnino et al., 2017; Marton and Granzow, 1982).

Acid treatments can be used for the separation of hemicellulose such as monomers in solution (Eisenhuber et al., 2013). The organosolv process combined with a previous stage of hydrolysis with diluted acid enables the efficient separation of hemicelluloses and the lignin from rice husk, carried out in a two-stage fractionation (Dagnino et al., 2017). However, in a subsequent study, it was shown that the acid treatment used to obtain black carob sawdust free from hemicellulose developed some type of resistance to subsequent lignin release, and, at the same time, that the removal of water-soluble extractive substances can be beneficial (Dagnino et al., 2018). Besides, in the same study, the range of the variables of sodium hydroxide and ethanol:water proportion was defined for the subsequent optimisation of soda-ethanosolv delignification process without acid pretreatment (Dagnino et al., 2018). Moreover, there are some researches that demonstrate that, in some cases, humins (or pseudo-lignin) are formed due to lignin and furan condensation on the degradation of monosaccharides in acid reaction conditions (Yoon and Shin, 2014). Besides, in the same study, the range of the variables of sodium hydroxide and ethanol:water proportion was defined for the subsequent optimisation of soda-ethanosolv delignification process without acid pretreatment (Dagnino et al., 2018).

Li and Lundquist state that lignin is difficult to eliminate after intensive heat treatments because of lignin re-polymerisation by a carbonium ion intermediate that fosters the formation of new bonds (Li et al., 2000; Li and Gellerstedt, 2008; Lora and Wayman, 1978; Sturgeon et al., 2013).

Possible uses of lignin in high value-added applications include cement additives, biofuel, BTX (benzene, toluene, xylenes), vanillin synthesis, carbon fibers, and other materials. However, these applications are restricted due to the complex and non-uniform structure, the uncertain reactivity and the presence of impurities of the lignins (Park et al., 2018; Boeriu et al., 2004). Despite the structural limitations of lignin, its highly functional nature makes it an excellent candidate for chemical modifications and the development of new materials. The chemical modification of lignins has prompted numerous efforts and research (Laurichesse and Avérous, 2014). The synthesis of thermostable polymers such as epoxy resins, phenol-formaldehyde resins, benzoxazines, represents an alternative for the use of modified lignins (Gioia et al., 2018). In the area of thermoplastics, the chemical modification of lignin consists mainly in functionalising the hydroxyl groups by esterification, etherification, and by grafting and copolymerisation reactions. It seeks to reduce the value of the glass transition temperature of lignin (T<sub>g</sub>) and to improve the thermal, chemical, physical stability and miscibility/compatibility with polymers. Currently, another area of interest is the synthesis of micro- and nano-scale materials (Beisl et al., 2017).

The chemical composition of lignin varies depending on its biological origin and the isolation procedure and it determines its reactivity and its potential applications. The reactivity of lignins is essentially influenced by the number of phenolic hydroxyls. The characterisation and the potential uses of organosolv lignin from black carob tree sawdust have not been widely reported.

The aim of this work was to obtain lignin of high quality and high purity as well as a solid product with a high content of carbohydrates with not more than 10%ODW of remaining acid-insoluble lignin. That was why the conditions of the industrial process for the separation of water-soluble substances (tannins) were replicated. Subsequently, based on previous studies, the soda-ethanosolv process optimisation was carried out on black carob sawdust free from extractives, and lastly, the required reaction time was adjusted to remove about 80% of lignin. The results found will create future sustainable production chains of high-value biobased products from lignocellulosic residues by using low environmental impact technologies applied to biorefineries.

## 2. Materials and methods

### 2.1. Raw materials and supplies

Black carob tree sawdust (*Prosopis nigra*; BCTS) was provided by local industry, from Machagay, Chaco, Argentina. The size

of the raw material was determined by the feeding, rejection and sieving method. Then, it was washed thoroughly with distilled boiling water, stirred for two minutes and filtered over a screw of 100  $\mu\text{m}$ . The washed sample was dried at room temperature for 48 h. Finally, the washed black carob tree sawdust (WBCTS) and the non-washed material were characterised by the techniques described in 2.2 Section and stored in a closed container at room temperature until further use.

## 2.2. Characterisation of raw material and reaction products

### 2.2.1. Pretreated solid

The characterisation of the lignocellulosic biomass was carried out, principally, according to the standards of the Laboratory Analytical Procedure, Technical Reports (NREL/TP). It consisted in the determination of the:

- Moisture content and/or volatile substances, using an analytical balance prepared to determine moisture content.
- Extractive substances content soluble in water and ethanol, according to the NREL/TP 510-42621 standard norm. The amount of the material removed by the Soxhlet extraction method was quantified on a percent dry weight basis.
- Glucans and xylans content, according to NREL/TP-510-42618 standard. The solid sample was hydrolysed in two steps, using 72% and 4% of sulphuric acid solution for the 1 h at 30 °C and 121 °C, for the first and second steps, respectively. The hydrolysis liquid fraction was analysed by HPLC (Shimadzu), with a Rezex<sup>TM</sup> RHM-Monosaccharide H + column (8%) (phenomenex) with 4mM H<sub>2</sub>SO<sub>4</sub> as mobile phase, a flow rate of 0.6 ml/min, 55 °C and refractive index detector. Anhydrous correction factors (0.88 for pentoses and 0.90 for hexoses) were applied to the monomeric sugar concentrations in order to obtain the concentration of the corresponding polymeric sugars.
- Lignin content, according to standard NREL/TP-510-42618. For this purpose, the solid fraction of the method detailed in the previous point was used and calcined in a muffle at 575  $\pm$  5 °C for 2 h. The acid-insoluble lignin (AIL) was calculated as the difference between the mass of retained residue and the mass of inorganic acid insoluble obtained after the muffle. The acid-soluble lignin content was determined on the liquid phase by spectrophotometric measurement at 320 nm.
- Determination of ash content (inorganic substances), according to NREL/TP 510-2622 standard. The muffle furnace was used at 575  $\pm$  25 °C for 3 h. This determination is performed by constant weight measurement, that is, it is placed in a flask for one hour at 575 °C, cooled in a desiccator and weighed. Then, the procedure is repeated until two successive weights have no significant difference.

All the results were expressed as a percentage on dry basis (%ODW). The pretreated solid was characterised in the same way, excluding the determination of extractive substances in water and alcohol.

### 2.2.2. Pretreatment liquid

The pretreatment liquid was characterised by the determination of the sugar content (glucose, xylose, arabinose) and breakdown products (organic acids, furfural and 5-hydroxymethylfurfural (HMF)). The quantification was done by HPLC (Shimadzu), with a Rezex<sup>TM</sup> RHM-Monosaccharide H +

column (8%) (phenomenex) with 4mM H<sub>2</sub>SO<sub>4</sub> as mobile phase, flow rate of 0.6 ml/min, 55 °C and refractive index and UV detectors.

### 2.2.3. Lignin extracted

- Guayacyl (G) and Syringyl (S) units of lignin were determined by differential UV-spectrophotometric method as described in the literature (Gartner and Gellerstedt, 1999; Liitiä and Tamminen, 2007). This method is based on the difference in absorption at 300 and 360 nm between phenolic units in neutral and alkaline solutions. A Perkin Elmer Lambda 365 UV-vis spectrophotometer was used to measure the absorbance of the alkaline solutions at 300 and 360 nm employing the pH 6 buffer as reference (Zakis, 1994). The content of ionising phenol hydroxyl groups can be quantitatively evaluated by comparing the ionisation spectrum ( $\Delta\epsilon$ ) values of the substance studied at 300 and 360 nm to the values of  $\Delta\epsilon$  of the respective model compounds (I, II, III, IV types) shown in Fig. 1.
- Moisture content was gravimetrically determined by drying samples at 105 °C to constant weight.
- Ash content was gravimetrically determined after furnace calcination at 575 °C for 3 h. The determination is made by measuring constant weight, in the same way as in determining the ash content in the pretreated solid.
- Simple carbohydrates were measured by high performance liquid chromatography (HPLC) after a two-step acid hydrolysis treatment with 72 wt% H<sub>2</sub>SO<sub>4</sub> solution (Kaar et al., 1991). The quantification was done by HPLC (Shimadzu), with a Rezex<sup>TM</sup> RHM-Monosaccharide H + column (8%) (phenomenex) with 4mM H<sub>2</sub>SO<sub>4</sub> as mobile phase, a flow rate of 0.6 ml/min, 55 °C and refractive index and UV detectors. The purity was calculated as follows: Purity (wt%) = 100 - %ash (%ODW) - %carbohydrates (%ODW).
- The solubility of lignin in distilled water and solutions of 1 N NaOH and 1 N HCl was qualitatively determined. About 1 g of lignin was dissolved in 10 ml of distilled water, 1 N NaOH and 1 N HCl respectively. The absence of suspended material indicated a total dissolution of the sample.
- Functional groups were analysed by Fourier transform spectroscopy (FTIR). About 3  $\times$  10<sup>-3</sup> g of dry lignin were mixed with 0.3 g of dried KBr and pressed into a self-supporting pellet. Measurements were performed using a Shimadzu FTIR-8201 PC between 4000 and 400cm<sup>-1</sup>.
- Thermal stability was studied by thermogravimetric analysis (TGA) on a Toledo 812e instrument under N<sub>2</sub> atmosphere using air as a purge. The samples were placed in aluminum crucibles and heated from 30 to 900 °C at 10 °C/min.
- Glass transition temperature of lignin was determined by Mettler Toledo DSC 822 differential scanning calorimetry. The sample of lignin was heated from 0 °C to 200 °C at 10 °C/min. The sample was first run from 0 to 200 °C and cooled down to 25 °C to eliminate any interference of water. The obtained result is from the second heating thermogram.

Phenolic hydroxyls and moisture were determined in all of the lignins, and the remaining determinations were made on the final lignin (obtained from the optimised process).

## 2.3. Process

### 2.3.1. Optimisation of the soda-ethanol process

Central Composite Experimental Designs with two replicas of the centre point were used to take care of the optimi-

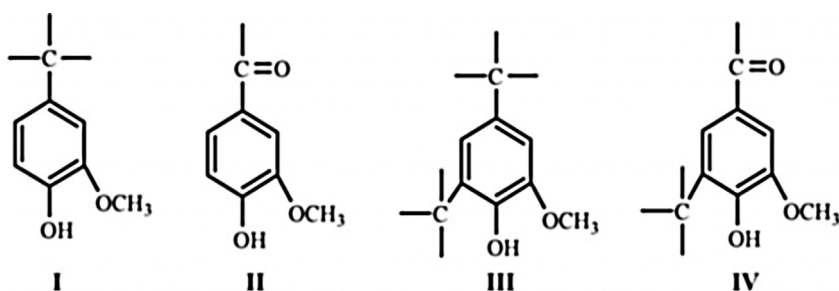


Fig. 1 – (I and II) Guayacyl units; (III and IV) Syringyl units.

**Table 1 – Conditions of Central Composite Experimental Designs (CCD), assays and conditions, real and coded values.**

Conditions of CCD		
Test Number	%NaOH <sup>a</sup> (coded value)	EtOH:H <sub>2</sub> O (coded value)
1	10 (–1)	50:50 (–1)
2	20 (1)	50:50 (–1)
3	10 (–1)	60:40 (1)
4	20 (1)	60:40 (1)
5	7.9 (–1.41)	55:45 (0)
6	22.1 (1.41)	55:45 (0)
7	15 (0)	48:52 (–1.41)
8	15 (0)	62:38 (1.41)
9	15 (0)	55:45 (0)
10	15 (0)	55:45 (0)

<sup>a</sup> (%ODW): over dry weight of WBCTS.

sation of the soda-ethanol process (from now on SodEt) applied, using the sodium hydroxide concentrations and the ethanol:water (v/v) proportion (EtOH:H<sub>2</sub>O) as independent variables. The study intervals were determined in previous works (Dagnino et al., 2018). Table 1 shows assays and conditions and coded and real values. Results were analysed using multivariate analysis of variance (ANOVA). Statistical analysis was performed at a 95% level of significance.

The SodEt fractionations were carried out in a 180 ml AISI 316 stainless steel reactor. The WBCTS solid was mixed with the soda-ethanol-water solution in a proportion of 10% solids. The reactor was submerged in a bath of silicon at 160 °C during 60 min, without agitation. After the reaction time, the reactor was placed in a cold water bath. The liquid-solid mixtures were filtered by a 150 mesh screen, the liquor was recovered and the pretreated solids were washed repeatedly with water to remove the remaining solution. The solids were cooled to 4 °C until characterisation. Lignin, carbohydrates, and inorganics concentrations were determined in the solid fraction and glucose, xylose, arabinose and degradation products in the liquid fraction. The mass yield solid recovered was determined by the relation of the mass of the solid pretreated recovered (weighted after the process) and the mass of the initial raw material (15 g dry of WBCTS).

### 2.3.2. Lignin separation from the liquor

Lignin was isolated from the black liquor by precipitation after acidification. The liquor was diluted with water (dilution factor 1:2), then heated to 50 °C in a water bath, and stirred at 10 rpm. Subsequently, the mixture was acidified with 5%v/v of a sulphuric acid solution until pH 3, and it was immediately cooled in a water bath at room temperature. The precipitated lignin was filtered with vacuum in a Buchner funnel, washed with 0.1N of NaOH and then with deionized water. Finally,

it was dried at room temperature in a desiccator for 24 h. The percentage of recovered lignin was determined by gravimetric analysis, according to the equation: %AIL rec: (gAIL recovered/gAIL extracted from WBCTS)\*100. The AIL extracted from WBCTS is the difference of %AIL in WBCTS, and the %AIL remained in the pretreated solid.

### 2.3.3. Reaction time setting

Once the optimal conditions of sodium hydroxide concentrations and the ethanol:water ratio for the delignification of SodEt process were obtained at 160 °C, a study of the reaction time was carried out. Tests were carried out at 15, 60, 80, 100 and 120 min, evaluating the results by the composition of the pretreated solid and the loss of carbohydrates in the final liquor.

### 2.3.4. Mass balance

For the validation of the results obtained by means of the statistical model, a Batch process was performed under the optimal conditions thrown by the CCD and the setted reaction time, all the inputs and outputs of SodEt process were evaluated.

## 3. Results and discussion

### 3.1. Raw material

The size distribution of the raw material was 0.1%, 35.7%, 43.7%, 13.3% and 3.0% detained by ASTM 20 Mesh, 35 Mesh, 60 Mesh, 80 Mesh, 100 Mesh, respectively and 4.3% pass by a 100 Mesh. The mixture of particles with the original particle size distribution was used. The composition of the original BCTS and the WBCTS was determined. The results are expressed in percentage of dry weight (%ODW) of original black carob tree sawdust (BCTS) and the washed black carob tree sawdust (WBCTS).

The extractives components are of interest; they have commercial value as tannins, so they are important in the biorefinery of black carob tree sawdust. Moreover, by separating them, their possible degradation is prevented, and this also avoids incorporating unnecessary material into the reactor. The washing process significantly diminishes the concentration of water-soluble extractive substances that could hamper the following reactions: from 17.6% to 3.3%. This decrease of extractive concentration produces a relative increase from 31.2% (30.7% acid-insoluble lignin and 0.5% acid-soluble lignin) to 36.6% (36.2% acid-insoluble lignin and 0.4% acid-soluble lignin) in lignin concentration and of 48.4% (34.1% glucans, 12.6% xylans and 1.7% arabinans) to 53.8% (37.5% glucans, 14.8% xylans and 1.7% arabinans) in total carbohydrates. However, the inorganic concentration slightly decreases from

2.8% to 1.8, probably due to the solubilisation of some minerals in the dirt that could be present in the sample.

Very similar data regarding sawdust of black carob tree from the northeast of Argentina were found by [Dagnino et al. \(2013\)](#): 34.1% of glucans, 12.6% of xylans, 0.7% of arabinans, 31.2% of acid-insoluble lignin, 2.0% of acid-soluble lignin and 14.6% of water-soluble and ethanol-soluble extractive substances. This composition could be compared to other hardwood such as *Eucalyptus viminalis* (42.5% glucans, 15.2% of xylans, 1.2% of arabinans, 25.7% de acid-insoluble lignin, 3.3% of extractive substances and 0.5% of inorganics) ([Ramos et al., 2000](#)), Southern hardwood mixture (SHM) contained sweet and black gum (35%), oak (35%), maple (15%), poplar and sycamore (12%) and southern magnolia (3%) (43.7% glucans, 15.5% xylans, 0.5% arabinans, 28.6% of acid-insoluble lignin of 0.4% of inorganics) ([Tunc et al., 2010](#)) and *Prosopis Juliflora* (49.4% cellulose (glucans), 18.0% hemicellulose (xylans), 4.3%, of inorganics, 28.3% lignin and extractive substance) ([Naseeruddin et al., 2013](#)).

### 3.2. Optimisation

The aims of soda-ethanosolv pretreatment as regards biorefinery are to extract the maximum amount of lignin or to minimise its residual proportion in the pretreated solid, to retain the highest proportion of cellulose and hemicellulose in the pretreated solid, and to obtain good quality lignin. The pretreatment with alkaline solutions and ethanol generates two products, a porous solid, with a large surface area and a high concentration of glucans and xylans, and a fluid that contains solubilised lignin in the form of phenolates, as well as small proportions of solubilised carbohydrates and sugar degradation products, 5-hydroxy-methyl-furfural and furfural. The raw materials used to obtain different products are derived from this pretreated solid and/or liquid. For example, when it is hydrolysed by cellulase enzymes it produces glucose which is then fermented to produce bioethanol. Besides, the lignin obtained in this way is of better quality than the one obtained by conventional processes because of the mild conditions used compared to a Kraft or sulfite process, and it could be used for different products ([Nitsos et al., 2016](#); [Zhou et al., 2018](#)). Therefore, an efficient pretreatment process is the one that maximises the concentration of glucans and xylans and which minimises lignin concentration in the pretreated solid, and which also maximises the reactivity of the recovered lignin in the pretreated liquid phase (% total OH phenolics).

#### 3.2.1. Study of the pretreated solid

Based on previous studies ([Dagnino et al., 2018](#)) the SodEt process was selected over WBCTS as a delignification process to be optimised and to consider the soda concentration (%ODW NaOH) and the ethanol:water proportion as independent variables.

The organosolv process uses an organic solvent as a delignification agent, and it is one of the most promising alternatives to the existing pulping technologies. This process provides a solid with high proportions of cellulose and a liquor solution which contains dissolved lignin. According to the process conditions, this liquor could contain degrading products of hemicellulose and cellulose such as furfural and 5-hydroxy-methyl-furfural. Structurally, the organosolv lignin contains more phenolic hydroxyls and carbonyl groups which lead to more oxidised forms (Hibbert ketones) ([De la Torre et al., 2013](#)), than the more aggressive processes such

as Kraft or sulphite. Additionally, the positive effect of alcohol in the delignification process is normally attributed to the reduction of the superficial tension of cooking liquor which better the solubility of lignin fragments and/or the alkylation of benzylic alcohol compounds, which reduces the tendency to condensation reactions of lignin ([Shatalov and Pereira, 2013](#)).

The concentration of lignin, glucans and xylans in the pretreated solid, expressed as percentage on dry basis (%ODW) of WBCTS, total phenolic hydroxyls in the extracted lignin and the mass yield, according to the different conditions of the SodEt process established in CCD ([Table 1](#)) are shown in [Table 2](#).

The %AIL in the solid pretreated with different concentrations of soda and the different relationships of ethanol:water varied between 14.2%ODW (pretreatment with 22.1%NaOH and 55:55 EtOH:H<sub>2</sub>O) and 25.5%ODW (pretreatment with 10%NaOH and 60:40 EtOH:H<sub>2</sub>O). The standard deviation of replicas at the central point for %AIL was of 0.068%. The soda concentration was shown as a significant variable with a *p*-value of 0.0001 with a reliability level of 95.0% by the statistical analysis of the variance of %ODW AIL, and it was also shown that the relationship between lignin concentration in the pretreated solid with the soda variable (%NaOH) is linear. This model, adjusted in this way, explains the 87.6% of the variability in %AIL. The relationship between ethanol:water was not relevant within the study interval (*p* = 0.9300). The regression equation that adjusts to these data is Eq. (1):

$$\%AIL = 32.6 - 0.9NaOHR^2 = 0.88 \quad (1)$$

According to the model, the minimum values that can be reached, in the conditions that have been tested, are around 13.0%AIL and can be obtained with high values of soda concentrations ([Fig. 2a](#)). In this figure, it is shown the present linear dependence between %AIL and the soda concentration used. Therefore, the maximum delignification was obtained under the most severe treatment by removing 60.9% of the lignin present in WBCTS. The ANOVA analysis shows the combination of variables in order to minimise the concentration of lignin and it establishes a process with 22.1% of soda and with a 55:55 ethanol:water relationship. As expected, the alkali concentration is relevant regarding the pulp delignification degree. [Shatalov and Pereira \(2013\)](#) used an experimental design for optimisation, and they found that the soda concentration and its relationship with the reaction temperature were the most significant effects in the range of evaluation.

As regards the characteristics of the obtained lignins, the phenolic hydroxyl concentration, according to the variance analysis, indicated a positive dependence with soda concentration (*p* = 0.0338) and ethanol:water proportion (*p* = 0.0169) within the studied range ([Fig. 2b](#)). The concentration of total OH phenolics varied between 3.5% and 1.5%, in the processes with 20% and 7.9% of NaOH, and both of them with 55:45 of ethanol:water, respectively. The regression equation that adjusts to the range of the data studied with a regression coefficient of 70.4% is Eq. (2):

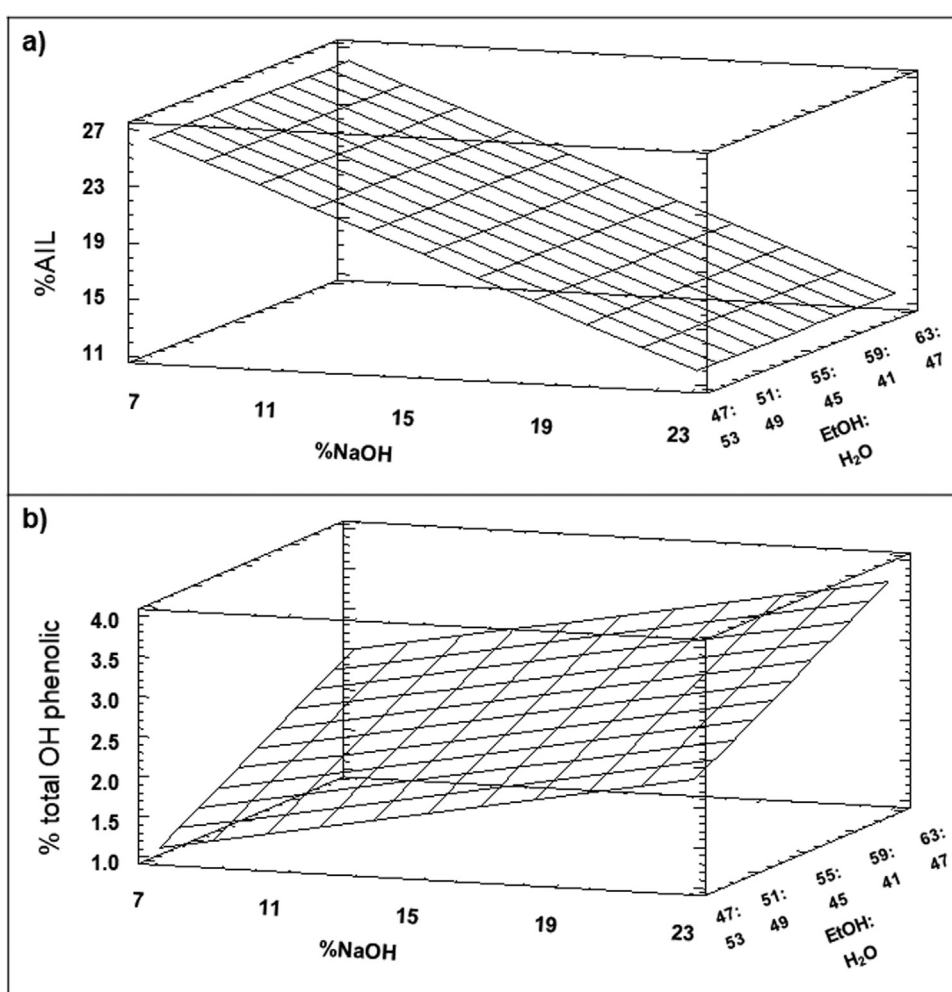
$$\%totalOHphenolics = -3.8NaOH + 0.09EtOH : H_2OR^2 = 0.70 \quad (2)$$

The combination of the level of the factors that maximise the percentage of total OH phenolic according to the model is of 22.1% of soda and 61:39 of ethanol:water proportion, from which it could be expected to obtain a lignin with 3.6% of a percentage of total OH phenolic.

**Table 2 – Insoluble-acid lignin (AIL), glucans and xylans in the pretreated solid, total phenolic hydroxyls in the extracted lignin and process mass yield percentages, according to the conditions of the design of central composite experiments for the optimisation of SodEt applied to WBCTS.**

Test N°	In pretreated solid			In extracted lignin	Process
	%AIL (%ODW) <sup>a</sup>	%glucans (%ODW) <sup>a</sup>	%xylans (%ODW) <sup>a</sup>	% total OH phenolic	%mass yield
1	25.9	29.9	13.1	1.9	72.5
2	15.2	25.1	9.4	1.9	59.0
3	25.5	27.6	11.6	2.3	72.0
4	14.8	26.9	12.0	3.5	55.3
5	24.4	29.4	14.5	1.5	69.7
6	14.2	24.2	12.3	2.8	55.8
7	17.8	26.6	9.9	2.2	56.3
8	18.7	30.4	11.4	3.4	61.3
9	17.1	29.5	12.6	1.9	60.7
10	17.0	30.1	12.3	2.7	62.1

<sup>a</sup> (%ODW of WBCTS): over dry weight on WBCTS basis.



**Fig. 2 – Estimated response surface for: (a) Proportion of acid-insoluble lignin (AIL) expressed as a percentage on dry basis (% ODW) referred to WBCTS remaining in the pretreated solid, (b) % total OH phenolic of the extracted lignin from WBCTS.**

In the processes of delignification, the splitting of ether bonds produces new hydroxyls phenolic groups in the lignin and it increases the solubility of lignin fragments in water. These changes affect the chemical reaction of the recovered lignin and its potential usage. The organosolv processes produce lignin structures that present a higher proportion of hydroxyl phenolic groups and oxidised groups that favour their incorporation in polymer formulations and its chemical modification (Kubo and Kadla, 2004). Nitsos et al. (2016) have proven that organosolv lignins are of high purity (93%), an infe-

rior range of molecular weight between 600 Da and 1600 Da and an increase in the hydroxyl phenolic content, depending on the source and the conditions previous to treatment.

Generally, the organosolv treatments have little effect on the degradation of carbohydrates and a good selectivity in the delignification reaction. The predominant degradation reaction, in alkaline conditions, is a peeling reaction in which, through this mechanism, the glucose units are removed from the cellulose molecule, and the polymerisation degree (PD) is reduced. When using protic solvents, like alcohols, in the

absence of acids and bases, the main reactions that are produced in the process of paste production are hydrolysis and, to a lesser extent, solvolysis (Ziaie-Shirkolaee et al., 2007). Due to this, in the combination of organosolv processes with the addition of alkaline solutions, the partial hydrolysis of the cellulose or hemicelluloses could be produced. Some studies have proven the minimum impact of the organosolv pretreatment with aqueous ethanol on the fibre structure (using sweeping electron microscopy) and on the *Miscanthus* biomass composition (as regards sugar and polyphenols contents) (Obama et al., 2012).

Xylans are the main components of the hemicelluloses of hardwood. These polymers of amorphous structure, as it was previously mentioned, could undergo hydrolysis according to process conditions used. Under the conditions of CCD (Table 1), xylans varied between 14.5% (in test N°5, 7.9% of NaOH and 55:45 of EtOH:H<sub>2</sub>O) and 9.4% (in test N°2, 20% of NaOH and 50:50 of EtOH:H<sub>2</sub>O). The variance analysis of the xylans proportion in the pretreated solid indicated that this proportion was significantly influenced by both variables studied, soda concentration ( $p = 0.0013$ ) and ethanol:water proportion ( $p = 0.0305$ ). Furthermore, both variables show a significant interaction ( $p = 0.0038$ ). The standard deviation of the replicas in the central point for the xylans concentration was of 0.2%. The regression equation, which explains the 96.0% of the variability in the %xylans and which has been adjusted to the data of the model is the Eq. (3):

$$\%xylans = -88.6 - 2.4NaOH + 4.3EtOH : H_2O + 0.04NaOHEtOH : H_2O - 0.04(EtOH : H_2O)^2 R^2 = 0.96 \quad (3)$$

In the surface graphic of estimated response for %xylans in the pretreated solid (Fig. 3a) minimum values are observed when using low proportions of ethanol:water and high concentrations of soda. High concentrations of soda favour the hydrolysis of the xylans, but at the same time, ethanol, in some way, also protects xylans since when tests with 20% of soda and 40:60 and 60:40 of ethanol are compared, 9.4% and 12.0% of xylans are respectively obtained. Also, Shatalov and Pereira (2002) in their work with alkaline pulping of *Arundo donax* have proven that the addition of organic solvents has a positive effect on the protection of carbohydrates. It is likely that alcohols act as radical collectors that can eliminate radical degradation processes during the pulping process. Besides, the speeding up of stopping reactions or the inhibition of the conversion of terminal aldehyde groups into ketones favour the elimination of polysaccharide peeling reaction.

The best process that could maximise the retention of xylans in the pretreated solid (14.7%ODW), according to the model, would be the one that uses the lowest concentration of soda (7.9%ODW) and intermediate values of ethanol:water (53:47).

The proportion of glucans presented some variances regarding the application of the different pretreatments within the studied range. A reduction of glucans of 37.5% to 30.4%, as regards the proportion contained in the starting material, was observed in the process with 15% of soda and 30.4% of ethanol:water; whereas, when the concentration of NaOH was increased, a loss of glucans up to 13.3% took place. The variance analysis indicated that, as regards the concentration of glucans, both variables are of statistical significance. The concentration of soda with  $p = 0.0007$ , the

proportion of ethanol:water (in quadratic form) and an interaction between both variables with  $p = 0.0196$ . The standard deviation of the replicas in the central point for the concentration of glucans was of 0.4%. The regression equation, which explains the 95.1% of the variability of the %glucans, and that has been adjusted for the model data, is Eq. (4):

$$\%glucans = -84.6 - 0.9NaOH + 4.5EtOH : H_2O - 0.06(NaOH)^2 + 0.04NaOHEtOH : H_2O - 0.05(EtOH : H_2O)^2 R^2 = 0.95 \quad (4)$$

The surface graphic for %glucans in the pretreated solid could be observed in Fig. 3b. The proportion of glucans in the pretreated solid reaches minimum values when low concentrations of ethanol:water and high concentrations of soda are used. Consequently, similarly as for xylans, the process that maximises the concentration of glucans in the pretreated solid is the one that uses low concentrations of soda (11.6%) and intermediate values of ethanol:water (54:46).

In the % arabinans none of the effects has a  $p$ -value lower than 0.0500, which indicates that they are not significantly different from zero, with a reliability level of 95.0%.

Finally, the percentage of mass yield is related to the extraction of lignin and the hydrolysis of carbohydrates. The ideal process would be the one that could extract all the lignin, and in which carbohydrates can remain unaltered. However, with 10%NaOH and 50:50 ethanol:water, the recovered solids exceed this value (72.5%) due to the lignin retention of 25.9% with the loss of 10% of carbohydrates and 8% of glucans and hydrolysed xylans, respectively. Besides, the minimum values of solids recovery, 53.3% and 55.8%, were obtained with the treatment of 20%NaOH and 60:40 of ethanol:water, respectively, in which about 60% of lignin was removed (retention of approximately 14.5% of AIL) with a loss of carbohydrates similar to the previous case. The proportion of recovered solids decreases with the concentration of soda ( $p = 0.0002$ ), which coincides with the increase of lignin extraction. The regression equation that is adjusted for data is the equation data (5). The R-squared R statistics indicate that the model, in the way it was adjusted, explains the 88.3% of the variability of the percentage of mass yield.

$$\%massyield = 81.8 - 1.2NaOHR^2 = 0.88 \quad (5)$$

### 3.2.2. Study of the pretreatment liquid

There are two liquid fractions of the SodEt process; the first one is the concentrated black liquor present in solubilised lignin, and the second liquid is the water used in the washing of pretreated solids.

Lignin was separated from black liquor by a pH decrease, from an initial pH that depends on the conditions of the process of about 12 to a pH 3. The recovery percentage varied approximately between 60% and 90% of effectively solubilised lignin. Lower recovery percentages were obtained with high concentrations of NaOH and/or high relationships of ethanol:water, and the maximum recoveries were obtained with low concentrations of %NaOH and intermediate relationships of ethanol:water. Mussatto et al. (2007) in their study of precipitation, at different pH of the lignin present in the black liquor of the Soda process, have recovered between 68.5% and 80.3% taking the pH to 4.3 and 2.3 respectively.

Furthermore, the highest concentration of hydrolysed glucose and xylose in the treatment liquor was of 0.2%ODW, both

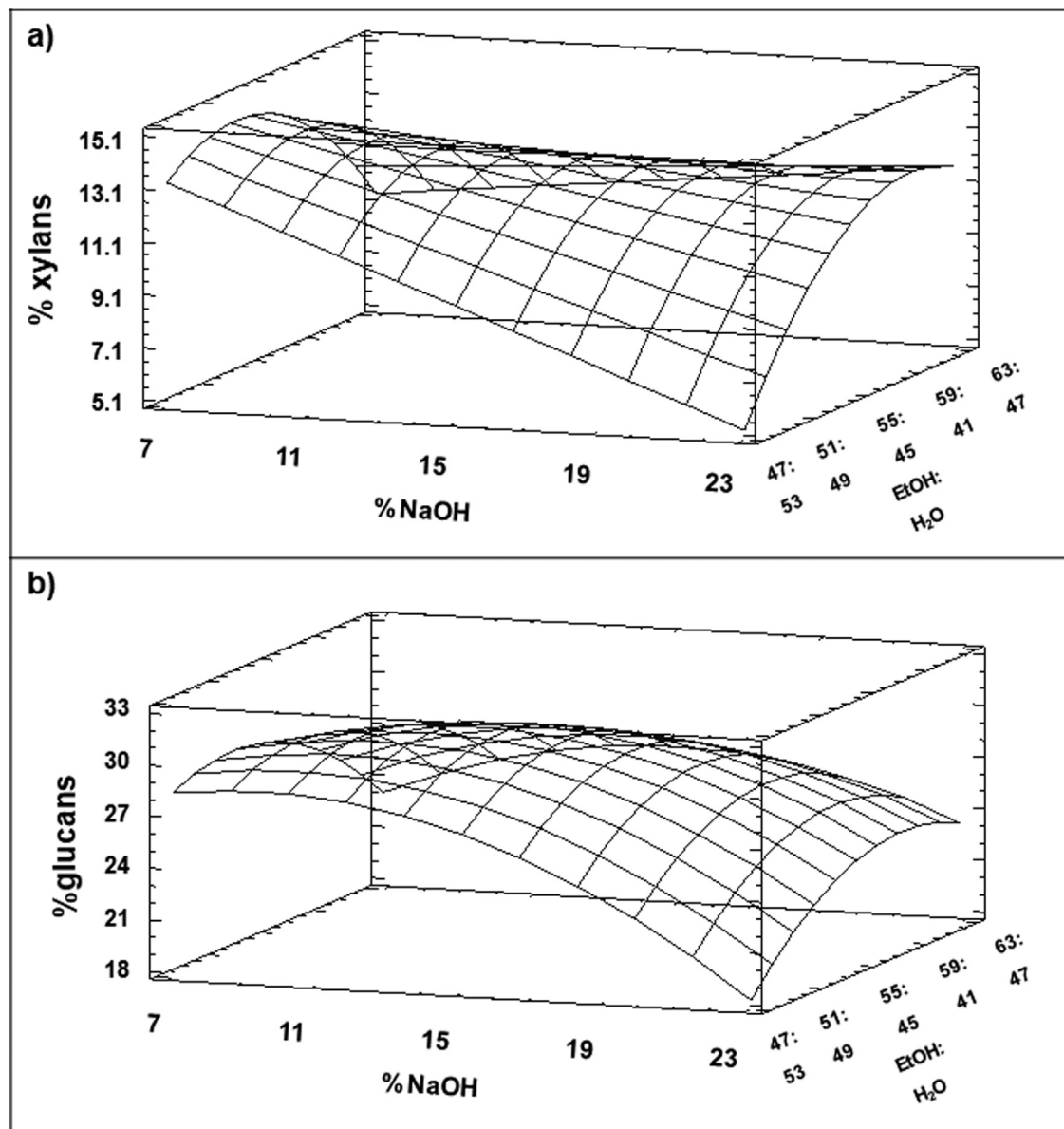


Fig. 3 – Estimated response surface for: (a) proportion of xylans and, (b) proportion of glucans, both expressed as a percentage on dry basis (% ODW) referred to WBCTS.

of them for the treatment with the highest concentration of NaOH. Afterwards, in the washing liquid of the pretreated solid, the xylose concentration increased up to 0.4% and the glucose concentration up to 0.3%. These results indicate that the sugars resulting from the loss of carbohydrates (glucans and xylans) observed in the pretreated solid, as regards the amount contained in the starting WBCTS, are distributed in the liquor, the washing liquid, and the extracted lignin. As the concentration of solubilised sugars in the treatments is small, the concentration of sugars in these three fractions is low.

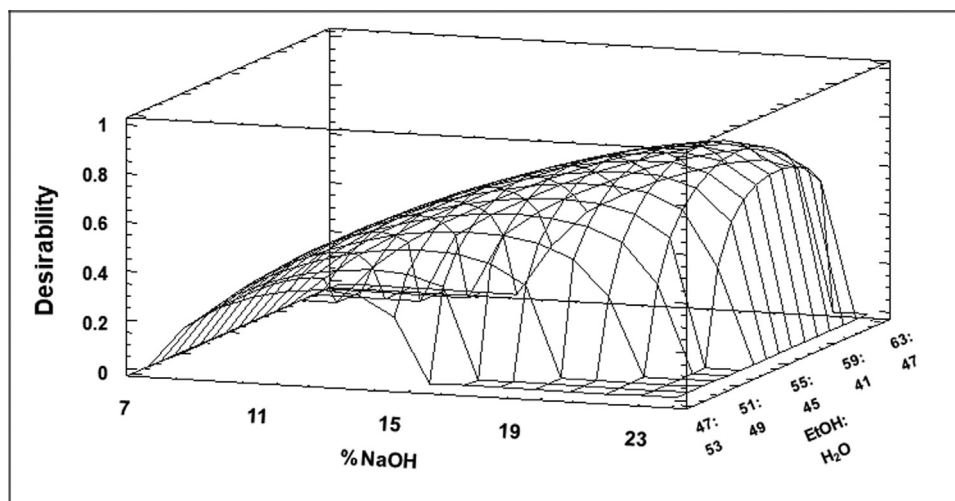
### 3.2.3. Optimal treatment

The desirability function is the most popular method to solve the optimisation problem of multiple responses. This approach, which is used to simultaneously optimise multiple equations, translates the functions to a common scale  $([0, 1])$ , and it combines them using the geometric median and optimising the metrics. The minimisation of the %AIL remained in the pretreated solid, the maximisation of the %glucans, %xylans in the pretreated solid and %total OH phenolics in the extracted lignin were considered. In addition, the min-

imisation of lignin concentration in the pretreated solid was considered the most significant one at the moment of optimising the response in order to obtain a reduction of the remaining proportion of AIL lower to 10%ODW.

The estimated surface graphic of the desirability function, which was obtained from the response variables preset, is shown in Fig. 4. This graphic was obtained using the Multiple Response Optimisation of the Statgraphics software. In Fig. 4, it could be observed that the optimum value does not appear among the tested conditions (the desired function does not reach value 1). The highest value of the desirability function that could be observed in the graphic (0.7–0.8) could be obtained with values of ethanol:water between 54:46 and 61:39 and 16.5% and 21.5% of NaOH. The optimum value lies at point 58:42 of the ethanol:water relationship and 19.9% of NaOH. The ethanol:water proportion, given by the model, agrees with other authors' observations, which indicate that the optimum ethanol concentration is found in the range 50–60 wt% (Zhao et al., 2017). Shatalov and Pereira (2013) have found the optimal conditions of ethanol-alkali pulping of Giant reed, which





**Fig. 4 – Estimated response surface for the desirability function. Considering the minimisation of acid-insoluble lignin (AIL), the maximisation of glucans and xylans in the pretreated solid, and the maximisation of the %total OH phenolic in the extracted lignin, according to a pre-established adjustment.**

are rather similar to the ones found in this work ( $T^{\circ}$ :158.8 °C; ethanol concentration: 45.2%; concentration of alkali: 33.2%).

According to this model, in the optimal pretreatment process conditions, it could be obtained a solid with 14.6% of AIL, 27.0% of glucans and 12.3%ODW of xylans regarding the starting WBCTS, and lignin extracted with 3.1% of %total OH phenolics. The mass yield in these conditions, according to Eq. 5, would be of 57.9%.

Additional experiments concerning pretreatment with SodEt under the specified optimum conditions in order to determine the estimated value of response variables were conducted. The experimental value for the concentration of glucans in the pretreated solid was of 33.0% and of xylans of 15.1%ODW regarding WBCTS; whereas, the pre-stated values for the model were of 28.7% and 12.2%, respectively. They correspond to 47.9% of glucans and 21.9% of xylans in the delignified solid. Therefore, the experimental residual lignin value in the pretreated solid was of 15.5% (24.3% in the delignified solid) whereas, the pre-stated value is of 14.5%. Other studies were conducted in this field; for example, [Michelin et al. \(2018\)](#) evaluated an organosolv process applied to corn cobs with high concentrations of ethanol without a catalyst, and they found a solid with 51.47% of cellulose (glucans), 31.86% of hemicellulose (xylans) and 11.61% of Klason lignin (AIL).

### 3.2.4. Adjustment of reaction time

Previous results have shown that, even in optimal conditions, a solid with approximately 15% of residual lignin is obtained, which is why it was decided to continue with the adjustment of the reaction time to improve the extraction of lignin, not in disfavour of carbohydrates present in the pretreated solid.

Different tests at different time sequences (15, 60, 80, 120 min) were conducted. The concentration of remaining lignin in the pretreated solid, expressed as a percentage on dry basis, concerning the starting material, and as regards the reaction time, could be observed in [Fig. 5](#). In this figure, it can be observed that the extraction of lignin stops at about the 80 min of reaction time and that the concentration of the remaining lignin could be reduced below 10%ODW. At 60 min, a delignification of 51.3% is obtained, whereas at 80 min it increases up

to 78.4%, and then, at 100 and 120 min it increases up to 81.0% and 82.7% respectively.

The reaction time used to obtain a good delignification depends on the aim of the process, on the other reaction conditions (soda concentration, ethanol, solid charge, among others) and on the raw material used. For example, [Tang et al. \(2017\)](#) carried out the pretreatment of corn stalks with ethanol and NaOH as a catalyst at different reaction times, and they found that 90 min is the optimal time to obtain a delignification higher than 80%. Whereas, [Shatalov and Pereira \(2002\)](#) evaluated the reaction time among other process parameters, and they found that the minimum time, 150 min, was appropriate for a good delignification of *Arundo donax* and that 199.7 min was appropriate for ethanol-alkali pulping of Giant reed ([Shatalov and Pereira, 2013](#)).

### 3.2.5. Mass balance

In order to validate the results obtained through the Statistical Model and the analysis of the adjustment of reaction time, a batch process, in the optimum conditions given by CCD (19.9% NaOH and 58:42 EtOH:H<sub>2</sub>O) and the reaction time adjusted (80 min), was carried out. The complete process, including the separation of water-soluble extractive substances, the delignification of WBCTS through an optimised SodEt process and, finally, lignin recovery from the liquor process, was evaluated. Results are shown in [Fig. 6](#).

The mass balance of the BCTS biorefinery process starts with 100 g of untreated dry biomass. The first part of the diagram considers the separation of water-soluble extractive substances, in which 88% of them are separated, and 84 g of WBCTS ready to continue the process are thus obtained. The following stage corresponds to SodEt fractionation for the delignification of WBCTS, which is carried out according to the conditions that were optimised in the previous stages. The resulting solid (46.2 g) is rich in carbohydrates (55.8% glucans, 24.7% xylans and 0.6% arabinans) and which contains 7.4 g of the remaining AIL. The black liquor presents a high proportion of lignin in the form of phenolates (23 g), which is then acidulated to pH 3, and which recovers lignin with a performance of 72% (16.6 g).

This mass balance shows that it is possible to separate about 80% of lignin present in WBCTS using the optimised

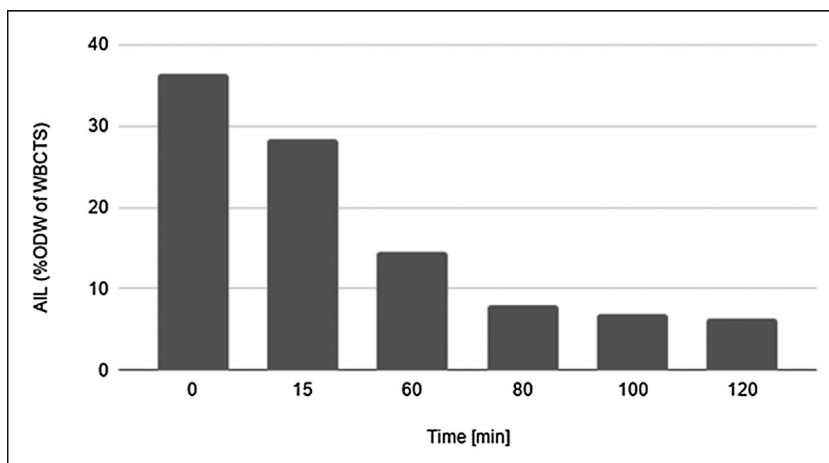


Fig. 5 – Remaining acid-insoluble lignin (AIL) in the pretreated solid, regarding the reaction time.

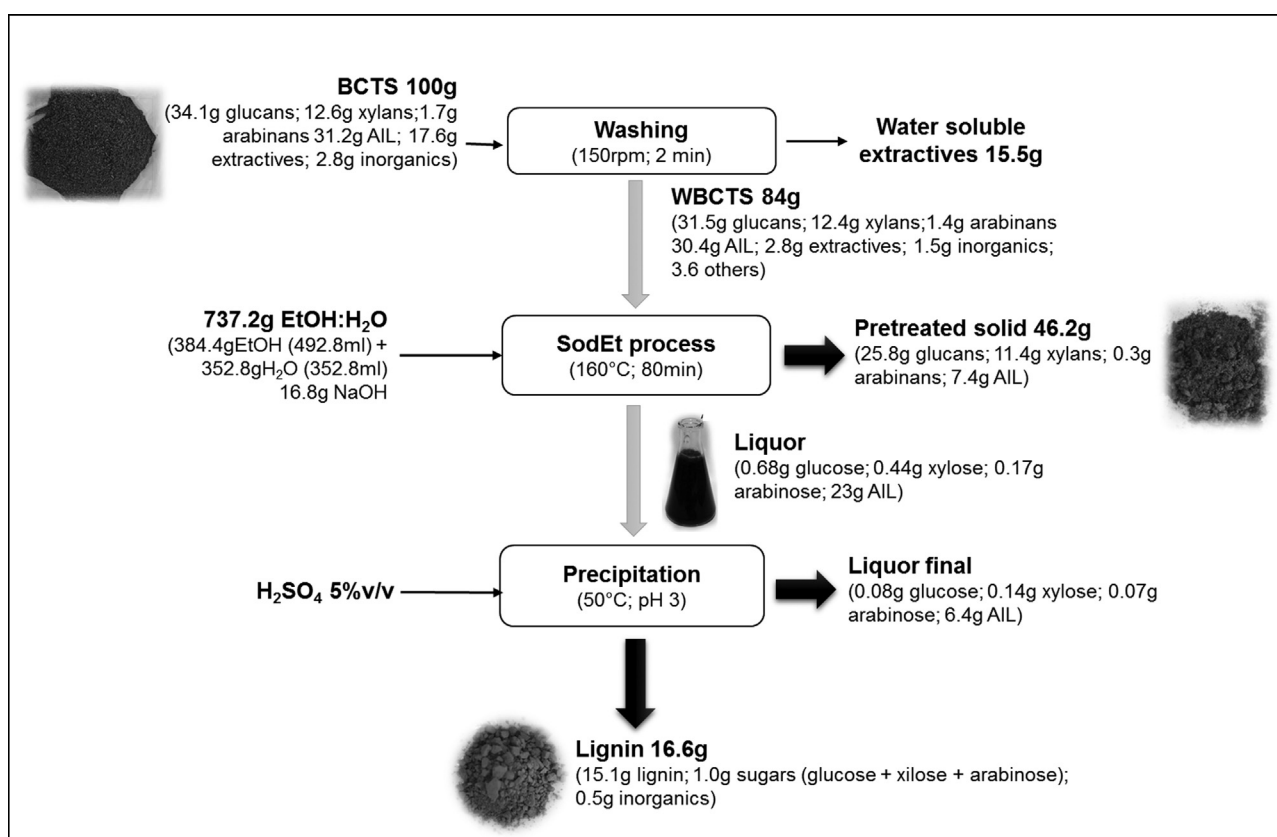


Fig. 6 – Mass balance flow diagram of black carob tree sawdust (BCTS) biorefinery process.

soda-ethanosolv process. Besides, a pretreated solid rich in carbohydrates and high quality and high purity lignin are obtained.

### 3.2.6. Characterisation of extracted lignin

The final lignin obtained from the optimised treatment according to the CCD and the adjusted reaction time was characterised to evaluate its quality and structural characteristics. Table 3 shows the composition of lignin. Moisture and ash values are similar to the ones reported by El Mansouri and Salvadó (2006) for organosolv lignin obtained from *Miscanthus sinensis*, but carbohydrates are higher. Total phenolic hydroxyl content is similar to that reported by Podschun et al. (2015) for organosolv lignins by ethanol water pulping of beech wood chips, but lower than what was reported by El Mansouri et al. (2006) (Podschun et al., 2015).

The FTIR spectrum of lignin is shown in Fig. 7. The FTIR spectrum shows a broad band at 3410–3460  $\text{cm}^{-1}$  (peak 1), attributed to the hydroxyl groups in phenolic and aliphatic structures, and bands that are centred around 2938 (peak 2) and 2842  $\text{cm}^{-1}$  (peak 3), predominantly from the stretching of CH in groups methoxyl aromatics and in the side chain methyl and methylene groups (Boeriu et al., 2004). Fig. 7 shows strong bands in this region, with peaks at 2916 and 2842  $\text{cm}^{-1}$ .

In addition, the FTIR spectrum shows a very weak or null signal between 1709–1738  $\text{cm}^{-1}$ , attributable to unconjugated carbonyl/carboxyl stretching and around 1680  $\text{cm}^{-1}$  (peak 4), a signal that can be associated with stretching of carbonyl-carboxyl conjugated groups. The vibrations of the aromatic skeleton at 1600 (peak 5), 1515 (peak 6), and 1426  $\text{cm}^{-1}$  (peak 8), and the deformation C–H combined with the vibration of

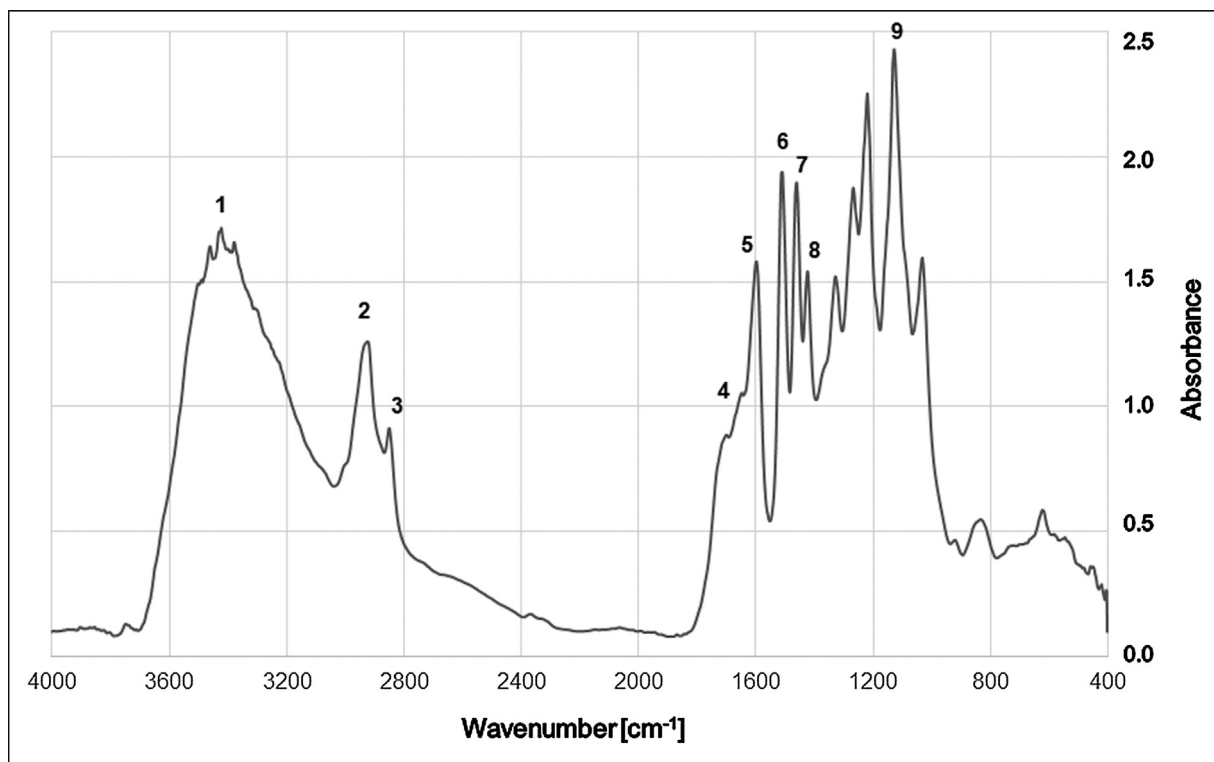


Fig. 7 – FTIR spectrum of lignin recovered from the liquor of SodEt process optimized.

**Table 3 – Characterisation of extracted lignin: Moisture, inorganics, carbohydrates, purity, phenolic hydroxyls (structures I, II, III, IV according to Fig. 1 and Syringyl (S) and Guaiacyl (G) units) and solubility. Percentages expressed on dry basis (%ODW).**

Determination		Value
Moisture		8.99
Inorganics <sup>a</sup>		3.18
Carbohydrates <sup>a</sup>		6.29
Purity		90.53
	I	0.65
	II	0.21
	III	1.66
Phenolic hydroxyls*	IV	0.12
	G	0.86
	S	1.78
	Total	2.62
	Water	No
Solubility	NaOH 0.1 N	Yes
	HCl 0.1 N	No

<sup>a</sup> Percentages expressed on dry basis (%ODW).

the aromatic ring at  $1462\text{ cm}^{-1}$  (peak 7) are common for all lignins, with different bands intensities.

On the other hand, the spectral region below  $1400\text{ cm}^{-1}$  is more difficult to analyze, due to the complexity of the bands caused by the contribution of several vibrational modes. However, this region contains vibrations that are specific to the different units of monolignol, and it allows the structural characterization of the lignins (Boeriu et al., 2004).

Faix (1991) classified the lignins into two types based on the analysis of the bands of the IR spectrum, those giving rise mainly to vanillin (G type), and those yielding vanillin and syringaldehyde (GS type). In G-type lignins, the composition of the Banding System between  $1175$  and  $1065\text{ cm}^{-1}$  shows a maximum at  $1140\text{ cm}^{-1}$ ; this feature is typical only

for G lignins. In GS type lignins, the same band system shows a maximum between  $1128$  and  $1124\text{ cm}^{-1}$ . This feature is a very sensitive criterion for GS lignins because a few percent S units in lignin are enough to change the maximum absorption from  $1140$  to a wavenumber below  $1128\text{ cm}^{-1}$ .

The IR spectrum of the lignin under study (Fig. 7) shows a maximum at  $1122\text{ cm}^{-1}$  (peak 9) in the range between  $1175$  and  $1065\text{ cm}^{-1}$  consistent with a GS-type lignin of tropical hardwood origin (Faix, 1991). This result is in accordance with the UV-spectrophotometric measurements where  $G/S = 0.48$  (Table 3).

Three degradation stages can be identified in the lignin thermogram given in Fig. 8 a. The initial weight loss of 9%ODW below  $200\text{ }^{\circ}\text{C}$  was due to the volatilization of moisture present in the lignin as well as to some decomposition products with low molecular weight, such as carbon dioxide, carbon monoxide, and methane. It is relevant to note that this value is consistent with the moisture content (Table 3). The second degradation stage can be attributed to the violent degradation of lignin (Dávila et al., 2017) which takes place between  $200$  and  $400\text{ }^{\circ}\text{C}$ . A third degradation stage occurs in the temperature range between  $400$  and  $800\text{ }^{\circ}\text{C}$  at which decomposition reactions and condensation reactions of aromatic rings take place (Shi et al., 2012). At the end of the test, char is 14% due to the condensed structure of the lignin (Dávila et al., 2019).

The first derivative of TGA curve (Fig. 8 b) shows the rate of weight loss. The peak of this curve (DTGmax) may be expressed as a single thermal decomposition temperature and can be used to compare the thermal stability characteristics of different materials. The DTGmax appears at  $343\text{ }^{\circ}\text{C}$  at which pyrolytic degradation is expected to occur. This degradation process involves fragmentation of inter-unit linkages and the releasing of monomers and derivatives of phenol into the vapor phase (Watkins et al., 2015).

Glass transition temperature ( $T_g$ ) of lignins is often very difficult to determine due to the heterogeneity of the lignin

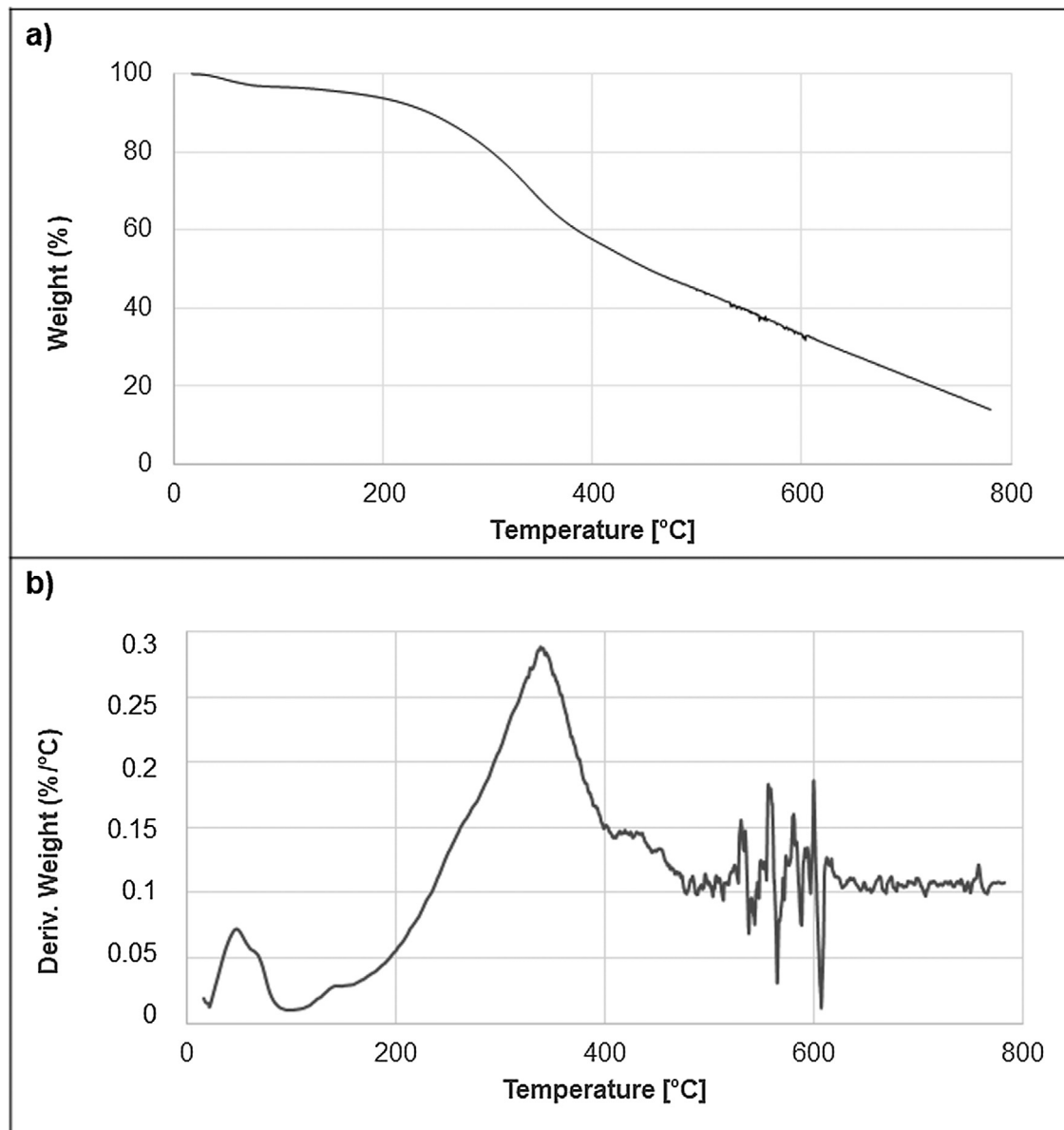


Fig. 8 – Thermal properties of lignin, (a) Thermogravimetric analysis (TGA) and (b) First derivative thermogravimetry (DTG) of lignin recovered from the liquor of SodEt process optimised.

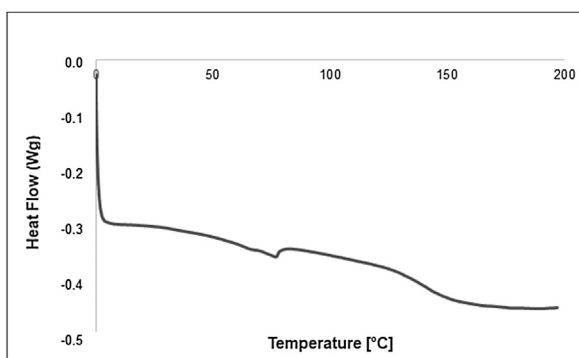


Fig. 9 – Differential scanning calorimetry (DSC) of lignin recovered from the liquor of SodEt.

chemistry, as well as to the broad molecular weight distribution (Gordobil et al., 2014). The differential scanning calorimetry (DSC) curve of lignin is presented in Fig. 9. Lignin shows a  $T_g$  value around 142 °C, similar to that obtained

by Gordobil et al. (2014) for lignin organosolv extracted from almond shells through organosolv process.

#### 4. Conclusions

The Soda-ethanosolv process was optimised for the delignification of washed black carob tree sawdust (WBCTS) using a central composite experimental design. It was found that the optimum process is the one that uses 19.9% NaOH (ODW of WBCTS) and 58:42 ethanol:water proportion. The result of this process is a solid composed of 33.0%ODW glucans, 15.1%ODW xylans and 15.5%ODW of AIL residual, concerning the starting material used.

The reaction time was adjusted to achieve a high delignification, obtaining a pretreated WBCTS with less than 10%ODW which led to conclude that 80 min are enough to remove approximately 80% of the original lignin present in the starting material.

The mass balance indicated that it is possible to separate approximately 80% of the lignin present in the WBCTS using an optimised soda-ethanosolv process. Besides, a pretreated

solid rich in carbohydrates and a lignin of high quality and purity were obtained.

Finally, it was found that the lignin recovered from the liquor of SodEt process is of high quality and good purity, a fact which makes it a promising raw material for the production of a product of higher value. The IR spectrum of the lignin is consistent with GS lignins.

The results found could be used to establish sustainable chains of high value bio-based products from BCTS using low-impact technologies of low environmental impact which could be applied to biorefineries.

## Acknowledgement

The authors gratefully acknowledge to UTN (National Technological University) for their facilities.

## References

- Alvira, P., Tomás-Pejó, E., Ballesteros, M.J., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresour. Technol.* 101 (13), 4851–4861, <http://dx.doi.org/10.1016/j.biortech.2009.11.093>.
- Azadi, P., Inderwildi, O.R., Farnood, R., King, D.A., 2013. Liquid fuels, hydrogen and chemicals from lignin: a critical review. *Renew. Enew. Sust. Energ. Rev.* 21, 506–523, <http://dx.doi.org/10.1016/j.rser.2012.12.022>.
- Beisl, S., Friedl, A., Miltner, A., 2017. Lignin from micro-to nanosize: applications. *Int. J. Mol. Sci.* 18 (11), 2367, <http://dx.doi.org/10.3390/ijms18112367>.
- Boeriu, C.G., Bravo, D., Gosselink, R.J., van Dam, J.E., 2004. Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy. *Ind. Crops Prod.* 20 (2), 205–218, <http://dx.doi.org/10.1016/j.indcrop.2004.04.022>.
- Burkart, A., 1976. A monograph of the genus *Prosopis* (Leguminosae subfam. Mimosoideae). *J. Arnold Arbor.* 57 (4), 450–525 <https://www.jstor.org/stable/43781999>.
- Cardozo, M.L., Ordoñez, R.M., Zampini, I.C., Cuello, A.S., Dibenedetto, G., Isla, M.I., 2010. Evaluation of antioxidant capacity, genotoxicity and polyphenol content of non-conventional foods: *Prosopis* flour. *Food Res. Int.* 43 (5), 1505–1510, <http://dx.doi.org/10.1016/j.foodres.2010.04.004>.
- Cuadra, D.E., 2012. Industria maderera y vulnerabilidad socioambiental: el caso de machagai en el centro del chaco. In: Foschiatti, E.M.H. (Ed.), *Escenarios Vulnerables Del Nordeste Argentino. Resistencia, Chaco. UNNE - CONICET*, pp. 315–336 <http://repositorio.unne.edu.ar/handle/123456789/499>.
- Cherubini, F., 2010. The biorefinery concept: using biomass instead of oil for producing energy and chemicals. *Energy Convers. Manage.* 51 (7), 1412–1421, <http://dx.doi.org/10.1016/j.enconman.2010.01.015>.
- Dagnino, E.P., Chamorro, E.R., Romano, S.D., Felissia, F.E., Area, M.C., 2013. Optimization of the pretreatment of *Prosopis nigra* sawdust for the production of fermentable sugars. *BioResour.* 8 (1), 499–514 <https://bioresources.cnr.ncsu.edu/resources/optimization-of-the-pretreatment-of-prosopis-nigra-sawdust-for-the-production-of-fermentable-sugars/>.
- Dagnino, E.P., Felissia, F.E., Chamorro, E., Area, M.C., 2017. Optimization of the soda-ethanol delignification stage for a rice husk biorefinery. *Ind. Crop. Prod.* 97, 156–165, <http://dx.doi.org/10.1016/j.indcrop.2016.12.016>.
- Dagnino, E.P., Ruiz, C., Chamorro, E., 2018. Ensayos preliminares de deslignificación de aserrín de algarrobo negro, en vistas a la producción eficiente de azúcares fermentables. *AVERMA* 22 (6), 61–66 <http://www.exporenovables.com.ar/2018/descargas/actas/asades-tema-6-131.pdf>.
- Dávila, I., Gullón, B., Labidi, J., Gullón, P., 2019. Multiproduct biorefinery from vine shoots: bio-ethanol and lignin production. *Renew. Ener.* 142, 612–623, <http://dx.doi.org/10.1016/j.renene.2019.04.131> (C).
- Dávila, I., Gullón, P., Andrés, M.A., Labidi, J., 2017. Coproduction of lignin and glucose from vine shoots by eco-friendly strategies: toward the development of an integrated biorefinery. *Bioresour. Technol.* 244, 328–337, <http://dx.doi.org/10.1016/j.biortech.2017.07.104>.
- de la Torre, M.J., Moral, A., Hernández, M.D., Cabeza, E., Tijero, A., 2013. Organosolv lignin for biofuel. *Ind. Crop. Prod.* 45, 58–63, <http://dx.doi.org/10.1016/j.indcrop.2012.12.002>.
- Eisenhuber, K., Krennhuber, K., Steinmüller, V., Jäger, A., 2013. Comparison of different pre-treatment methods for separating hemicellulose from straw during lignocellulose bioethanol production. *Ener. Procedia* 40, 172–181, <http://dx.doi.org/10.1016/j.egypro.2013.08.021>.
- El Mansouri, N.E., Salvadó, J., 2006. Structural characterization of technical lignins for the production of adhesives: application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins. *Ind. Crop. Prod.* 24 (1), 8–16, <http://dx.doi.org/10.1016/j.indcrop.2005.10.002>.
- Faix, O., 1991. Classification of lignins from different botanical origins by FT-IR spectroscopy. *Holzforschung-Inter. J. Biol., Chem., Physics Technol. Wood* 45 (1), 21–28, <http://dx.doi.org/10.1515/hfsg.1991.45.s1.21>.
- Gartner, A., Gellerstedt, G., 1999. *Determination of phenolic hydroxyl groups in residual lignin using a modified UV-method.* *Nord. Pulp Pap. Res. J.* 14 (2), 163–170.
- Gioia, C., Lo Re, G., Lawoko, M., Berglund, L., 2018. Tunable thermosetting epoxies based on fractionated and well-characterized lignins. *J. Am. Chem. Soc.* 140 (11), 4054–4061, <http://dx.doi.org/10.1021/jacs.7b13620>.
- Gordobil, O., Egués, I., Llano-Ponte, R., Labidi, J., 2014. Physicochemical properties of PLA lignin blends. *Polym. Degrad. Stab.* 108 (2), 330–370, <http://dx.doi.org/10.1016/j.polymdegradstab.2014.01.002>.
- Himmel, M.E., Ding, S.Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D., 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Sci.* 315, 804–807, <http://dx.doi.org/10.1126/science.1137016>.
- Kaar, W.E., Cool, L.G., Merriman, M.M., Brink, D.L., 1991. The complete analysis of wood polysaccharides using HPLC. *J. Wood Chem. Technol.* 11 (4), 447–463, <http://dx.doi.org/10.1080/02773819108051086>.
- Koo, B.W., Park, N., Jeong, H.S., Choi, J.W., Yeo, H., Choi, I.G., 2011. Characterization of by-products from organosolv pretreatments of yellow poplar wood (*Liriodendron tulipifera*) in the presence of acid and alkali catalysts. *J. Ind. Eng. Chem.* 17 (1), 18–24, <http://dx.doi.org/10.1016/j.jiec.2010.10.003>.
- Kubo, S., Kadla, J.F., 2004. Poly(ethylene oxide)/organosolv lignin blends: relationship between thermal properties chemical structure, and blend behavior. *Macromol.* 37, 6904–6911, <http://dx.doi.org/10.1021/ma0490552>.
- Laurichesse, S., Avérous, L., 2014. Chemical modification of lignins: towards biobased polymers. *Prog. Polym. Sci.* 39 (7), 1266–1290, <http://dx.doi.org/10.1016/j.progpolymsci.2013.11.004>.
- Li, S., Lundquist, K., Westermark, U., 2000. Cleavage of arylglycerol  $\beta$ -aryl ethers under neutral and acid conditions. *Nord. Pulp Pap. Res. J.* 15 (4), 292–299, <http://dx.doi.org/10.3183/NPPRJ-2000-15-04-p292-299>.
- Li, J., Gellerstedt, G., 2008. Improved lignin properties and reactivity by modifications in the autohydrolysis process of aspen wood. *Ind. Crops Prod.* 27 (2), 175–181, <http://dx.doi.org/10.1016/j.indcrop.2007.07.022>.
- Liitiä, T., Tamminen, T., 2007. Direct method for the determination of phenolic hydroxyl groups in pulp. *Holzforschung* 61 (6), 623–627, <http://dx.doi.org/10.1515/HF.2007.101>.
- Lora, J.H., Wayman, M., 1978. *Delignification of hardwoods by autohydrolysis and extraction.* *J. Tech. Assoc. Pulp Pap. Ind.* 61 (6), 47–50.
- Marton, R., Granzow, S., 1982. Ethanol-alkali pulping. *J. Tech. Assoc. Pulp Pap. Ind.* 65 (6), 103–106.

- Michelin, M., Liebentritt, S., Vicente, A.A., Teixeira, J.A., 2018. Lignin from an integrated process consisting of liquid hot water and ethanol organosolv: physicochemical and antioxidant properties. *Int. J. Biol. Macromol.* 120, 159–169, <http://dx.doi.org/10.1016/j.ijbiomac.2018.08.046>.
- Mussatto, S.I., Fernandes, M., Roberto, I.C., 2007. Lignin recovery from brewer's spent grain black liquor. *Carbohydr. Polym.* 70 (2), 218–223, <http://dx.doi.org/10.1016/j.carbpol.2007.03.021>.
- Naseeruddin, S., Yadav, K.S., Sateesh, L., Manikyam, A., Desai, S., Rao, L.V., 2013. Selection of the best chemical pretreatment for lignocellulosic substrate *Prosopis juliflora*. *Bioresour. Technol.* 136, 542–549, <http://dx.doi.org/10.1016/j.biortech.2013.03.053>.
- Nitsos, C., Stoklosa, R., Karnaouri, A., Vörös, D., Lange, H., Hodge, D., Crestini, C., Rova, U., Christakopoulos, P., 2016. Isolation and characterization of organosolv and alkaline lignins from hardwood and softwood biomass. *ACS Sustainable Chem. Eng.* 4 (10), 5181–5193, <http://dx.doi.org/10.1021/acssuschemeng.6b01205>.
- Obama, P., Ricochon, G., Muniglia, L., Brosse, N., 2012. Combination of enzymatic hydrolysis and ethanol organosolv pretreatments: effect on lignin structures, delignification yields and cellulose-to-glucose conversion. *Bioresour. Technol.* 112, 156–163, <http://dx.doi.org/10.1016/j.biortech.2012.02.080>.
- Park, S.Y., Kim, J.-Y., Youn, H.J., Choi, J.W., 2018. Fractionation of lignin macromolecules by sequential organic solvents systems and their characterization for further valuable applications. *Int. J. Biol. Macromol.* 106, 793–802, <http://dx.doi.org/10.1016/j.ijbiomac.2017.08.069>.
- Plaza, G.A., Wandzich, W., 2016. Biorefineries-new green strategy for development of smart and innovative industry. *Manage. Syst. Prod. Eng.* 3, 150–155, <http://dx.doi.org/10.2478/mspe-02-03-2016>.
- Podschun, J., Saake, B., Lehnen, R., 2015. Reactivity enhancement of organosolv lignin by phenolation for improved bio-based thermostets. *Eur. Polym. J.* 67 (4), 1–11, <http://dx.doi.org/10.1016/j.eurpolymj.2015.03.029>.
- Pometti, C.L., Pizzo, B., Brunetti, M., Macchioni, N., Ewens, M., Saidman, B.O., 2009. Argentinean native wood species: physical and mechanical characterization of some *Prosopis* species and *Acacia* aroma (Leguminosae; mimosoideae). *Bioresour. Technol.* 100 (6), 1999–2004, <http://dx.doi.org/10.1016/j.biortech.2008.09.061>.
- Ramos, L.P., Carpes, S.T., Silva, F.T., Ganter, J.L.M., 2000. Comparison of the susceptibility of two hardwood species, *Mimosa scabrella* benth and *Eucalyptus viminalis* Labill, to steam explosion and enzymatic hydrolysis. *Braz. Arch. Biol. Technol.* 43 (2), 195–206, <http://dx.doi.org/10.1590/S1516-89132000000200009>.
- Salazar, R., Cárdenas, G., 2012. *Bioeconomía y biorrefinerías. Avances agroindustriales* 34 (3), 31–34, ISSN 0326-113. Available in [www.eaac.org.ar/upload/publicaciones/archivos/356/20131204115302000000.pdf](http://www.eaac.org.ar/upload/publicaciones/archivos/356/20131204115302000000.pdf).
- Shatalov, A.A., Pereira, H., 2002. Carbohydrate behaviour of *Arundo donax* L. in ethanol-alkali medium of variable composition during organosolv delignification. *Carbohydr. Polym.* 49 (3), 331–336, [http://dx.doi.org/10.1016/S0144-8617\(01\)00340-X](http://dx.doi.org/10.1016/S0144-8617(01)00340-X).
- Shatalov, A.A., Pereira, H., 2013. High-grade sulfur-free cellulose fibers by pre-hydrolysis and ethanol-alkali delignification of giant reed (*Arundo donax* L.) stems. *Ind. Crop. Prod.* 43, 623–630.
- Shi, Z., Xiao, L., Xu, F., Sun, R., 2012. Physicochemical characterization of lignin fractions sequentially isolated from bamboo (*Dendrocalamus brandisii*) with hot water and alkaline ethanol solution. *J. Appl. Polym. Sci.*, <http://dx.doi.org/10.1002/app>.
- Sturgeon, M.R., Kim, S., Lawrence, K., Paton, R.S., Chmely, S.C., Nimlos, M., Foust, T., Beckham, G.T., 2013. A mechanistic investigation of acid-catalyzed cleavage of aryl-ether linkages: implications for lignin depolymerization in acidic environments. *ACS Sust. Chem. Eng.* 2 (3), 472–485, <http://dx.doi.org/10.1021/sc400384w>.
- Tang, C., Chen, Y., Liu, J., Shen, T., Cao, Z., Shan, J., Zhu, C., Ying, H., 2017. Sustainable biobutanol production using alkali-catalyzed organosolv pretreated cornstalks. *Ind. Crop. Prod.* 95, 383–392, <http://dx.doi.org/10.1016/j.indcrop.2016.10.048>.
- Tunc, M.S., Lawoko, M., van Heiningen, A.R., 2010. Understanding the limitations of removal of hemicelluloses during autohydrolysis of a mixture of southern hardwoods. *BioResour.* 5 (1), 356–371.
- Yoon, S.Y., Shin, S.J., 2014. The effect of hemicelluloses and lignin on acid hydrolysis of cellulose. *Energy* 77, 19–24, <http://dx.doi.org/10.1016/j.energy.2014.01.104>.
- Watkins, D., Nuruddin, M., Hosur, M., Tcherbi-Narteh, A., Jeelani, S., 2015. Extraction and characterization of lignin from different biomass resources. *J. Mater. Res. Technol.* 4 (1), 26–32, <http://dx.doi.org/10.1016/j.jmrt.2014.10.009>.
- Zakis, G.F., 1994. *Functional Analysis of lignins and their derivatives*. J. Tech. Assoc. Pulp Pap. Ind., Atlanta.
- Ziaie-Shirkolaei, Y., Mohammadi-Rovshandeh, J., Rezayati Charani, P., Khajeheian, M.B., 2007. Study on cellulose degradation during organosolv-delignification of wheat straw and evaluation of pulp properties. *Iran. Polym. J.* 16 (2), 83–96.
- Zhao, X., Li, S., Wu, R., Liu, D., 2017. Organosolv fractionating pre-treatment of lignocellulosic biomass for efficient enzymatic saccharification: chemistry, kinetics, and substrate structures. *Biofuels, Bioprod. Biorefin.* 11 (3), 567–590, <http://dx.doi.org/10.1002/bbb.1768>.
- Zhou, Z., Lei, F., Li, P., Jiang, J., 2018. Lignocellulosic biomass to biofuels and biochemicals: a comprehensive review with a focus on ethanol organosolv pretreatment technology. *Biotechnol. Bioeng.* 115 (11), 2683–2702, <http://dx.doi.org/10.1002/bit.26788>.