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ORIGINAL ARTICLE

Fractionation of *Pinus radiata* by ethanol-based organosolv process

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Abstract

The lignocellulosic materials are promising feedstock to produce biofuels and bioproducts in the biorefinery framework. However, a pretreatment step is required to disrupt lignin-carbohydrate complex. In this work, the fractionation of *Pinus radiata* wood into its main components, cellulose-rich delignified solid, recovered lignin after precipitation, and soluble-in-black liquor hemicellulose, was studied. For this purpose, an organosolv process employing ethanol/water mixture as solvent in absence of a catalyst was carried out. The effects of operating conditions on delignified solid were evaluated by using a 2^3 central composite experimental design, being the responses delignified solid yield, delignification degree, hemicellulose content, and glucan content. The variables studied were temperature (170–200 °C), time (50–100 min), and ethanol concentration (40–60%). The increase of organosolv severity (temperature and time) and reduction of ethanol concentration favor the glucan enrichment of delignified solid, due to lignin removal and hemicellulose solubilization. A glucan content of more than 66% is obtained by applying temperature higher than 195 °C and time longer than 90 min, when 40 wt% alcohol concentration is used. The liquid fraction obtained during the organosolv process (black liquors) was used to recover lignin and hemicellulosic fractions solubilized. Furthermore, hemicellulose and lignin content of delignified solid was correlated with the thermal stability measured as $T_{10\%}$.

Keywords Organosolv process · Softwood · Fractionation · Central composite design · TGA

1 Introduction

The lignocellulosic materials are considered as a promising feedstock to be utilized in the sustainable production of liquid fuels and chemical products in the biorefinery framework [1–3]. The main advantages of these materials are that they do not compete with food, low cost, and availability. They are mainly composed of cellulose, hemicellulose, lignin, and extractives. These components form the lignocellulosic matrix, being strongly bonded through covalent or non-covalent bonds [2]. To overcome the strong recalcitrant structure of these lignocellulosic biomasses, the pretreatment is crucial step to get the partial destruction of complex formed by lignin-carbohydrates [4]. Many methods have been proposed as pretreatment of lignocellulosic materials, being classified into physical, physicochemical, chemical, and biological [2, 5, 6]. Among the chemical pretreatments, organosolv is considered as one of the most promising method [5, 7–9].

Organosolv process was originally developed as an alternative to the Kraft process within the context of the pulp and paper industry [10]. This process can be performed using different organic solvents, such as alcohols, organics acids, or other solvents, with or without catalyst [7, 11]. The sulfuric acid, hydrochloric acid, and phosphoric acid can be used as catalysts [1, 3, 4, 12, 13]. Some of these solvents are ethanol [3, 13–18], methanol [19], butanol [20, 21], ethylene glycol [22], glycerol [1, 23, 24], acetic acid [12, 25], formic acid [26, 27], acetone [4, 28, 29], or γ-Valerolactone [30]. Among them, ethanol is considered a successful solvent for pretreatment at low boiling point. This alcohol presents some advantages, such as low cost, ease of recovery, and lack of toxicity [31–33].

After organosolv process, three main components from lignocellulosic biomass can be efficiently fractioned: high purity cellulose, hemicellulose-derived compounds, and high quality lignin, low in sulfur [9, 31]. The cellulose

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remains in the solid fraction after pretreatment, while most lignin and hemicellulose are solubilized into solvent [34]. The organosolv lignin is recovered from black liquor by precipitation, and hemicellulose-derived compounds left in the liquid phase [8, 35, 36]. After the separation of components achieved in organosolv process, these fractions can be used to produce value-added chemicals.

The cellulose-rich fraction can be employed as substrate in enzymatic hydrolysis to be depolymerized to glucose [11, 14, 18, 29], or in simultaneous saccharification and fermentation step to produce biofuels [6, 17, 37]. After enzymatic hydrolysis step, the biohydrogen or acetone–butanol–ethanol (ABE) production has been studied [38, 39]. The biomethane production by anaerobic digestion has been also considered [13, 40]. An integrated process composed of organosolv and fast pyrolysis, in which this cellulose-rich fraction is used to produce a platform chemical, levoglucosan, has been also proposed [41]. In addition, the organosolv pretreated solid rich in cellulose has been also employed for nanocellulose crystal (NCC) production, after bleaching step [42]. This cellulose-rich solid has been used for omega-3 fatty acids and lactic acid production, employing hydrolysis and microbial fermentation steps. For this purpose, an acid-free delignification process called Oxiorganosolv which employs oxygen gas was used to remove lignin and obtain the cellulosic fraction [43].

This delignified solid rich in cellulose has been characterized to determine its thermal stability, and confirm the removal of other components, such as lignin and hemicellulose, occurred during organosolv process [23, 41, 44, 45]. From the other side, through the Fourier transform infrared spectroscopy, the effect of organosolv process on this delignified solid is studied, and the modification of its chemical composition is confirmed [15, 23, 32, 37, 39, 41, 45]

The lignin obtained from organosolv process can be employed for multiple applications, such as precursor for carbon fiber [46], antioxidant [47], or substitute of the phenol in a phenolic adhesive formulations for plywood [48]. The organosolv lignin has been also used for hydrogel synthesis, showing suitable antifungal properties [49]. The hemicellulose-derived compounds, which remain in the liquid stream, have potential in the production of valuable products, such as isobutanol [50], ethanol [21], or furfural and hydroxymethylfurfural [20, 51]. However, further steps are required, such as acid hydrolysis or the use of nanofiltration membrane and fermentation with transgenic *S. cerevisiae* [21, 51]. In addition, its use as a carbon source for the ligninolytic enzyme production or even its possible utilization as prebiotics has been also proposed [43, 52]. Thus, the organosolv pretreatment can be considered as a fractionation process of lignocellulosic feedstock. It not only gets the increment of enzymatic digestibility of cellulose by the removal the lignin and hemicellulose which act as barriers, but it also gets the obtainment of co-products from lignin and non-cellulosic polymer, fulfilling the requirements for the biorefinery concept [8, 26].

Several lignocellulosic materials have been employed as feedstocks for organosolv process, such as agricultural residues, crops, and woody biomass: poplar [18, 53], empty palm fruit bunch [54], *Liriodendron tulipifera* wood [14], *Eucalyptus globulus* wood [24], wheat straw [25], rice straw [1], date palm [34], almond and walnut shell [49], corn stover [22], sugarcane bagasse [19], barley straw [4], potato peel wastes [13], vine shoot [20], *Cynara cardunculus* [3], *Eucalyptus pellita* wood [51], *Eucalyptus nitens* bark [17], mustard biomass [29], rubber wood [40], beech wood [12], or *Pinus taeda* [33]. Among these materials, the woody biomasses are more resistant to organosolv compared to herbaceous, and softwoods present more recalcitrance than hardwood [41]. Softwoods exhibit more difficulty to be delignified than hardwoods due to higher lignin content, and higher content of guaiacyl units in lignin (G) [8].

The aim of the present work is to study the fractionation in its main components of a softwood, *Pinus radiata*, by an autocatalyzed organosolv process. The influence of operating conditions (ethanol concentration, temperature, and time) on delignified solid is evaluated by applying a 2^3 central composite experimental design. The delignified solids are characterized by chemical composition, thermal stability, and chemical structure, and hemicellulose-derived compounds in black liquors are quantified by HPLC. The relationship between thermal stability, measured as $T_{10\%}$, and chemical composition of delignified solid is established. The results allow to determine the effectiveness of autocatalyzed organosolv process to get the fractionation of *P. radiata* wood in its components, which can be valorized in following steps within biorefinery concept.

2 Materials and methods

2.1 Raw material and reagents

The raw material, *P. radiata* wood, was provided by CIFOR-INIA ("Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria"). Wood chips were milled and sieved to obtain a particle size between 0.30 and 2 mm. Ethanol was supplied by Alcoholes Aroca with a purity of 96% (v/v). Chemicals used as standard reagents are D-(+)-glucose (≥ 99.5%), D-(+)-xylose (≥ 99%), D-(+)-mannose (≥ 99.5%), D-(-)-arabinose (≥ 98%), D-(+)-galactose (≥ 99.5%), acetic acid (≥ 99.7%), formic acid (≥ 95%), furfural (≥ 99%), and 5-hydroxymethylfurfural (≥ 99%) and were purchased from Sigma-Aldrich. Sulfuric acid (72% w/w) from Fluka and calcium carbonate from Panreac were also employed.

2.2 Organosolv process

Organosolv process was carried out in a 450-mL Parr reactor with a 4848 reactor controller (Parr Instrument Company) equipped with cooling bath (MPC-K6, Huber) that pumped and recirculated silicone oil as the cooling fluid. The feedstock, 20 g of pine wood (dry weight), was treated with an ethanol/water mixture using a solid/liquid ratio of 1/8 (w/v). The reaction mixture was heated for 50 min to desired temperature, and kept during the reaction time. After this time, the reactor was cooled down to 40 °C, and the solid fraction (delignified solid) and liquid phase (black liquor) were separated by vacuum filtration in a Buchner and Kitasato system with a paper filter (1252 Filter-Lab). The delignified solid was washed with 160 mL of NaOH solution 0.4% (w/w) and water in order to remove the maximum amount of lignin and other impurities present in the surface. Finally, the solid fraction was dried in an oven (40 °C) before characterization. The delignified solid yield (Υ_{solid}) was determined according to the Eq. (1):

$$\Upsilon_{solid}(\%) = \frac{\text{Delignified solid(g)}}{\text{Raw material(g)}} \cdot 100 \qquad (1)$$

where delignified solid is the amount of solid obtained after organosolv process, and raw material is the initial mass of *P. radiata* introduced to the reactor, both expressed in oven-dry basis.

The effects of experimental conditions ethanol concentration (C), temperature (T), and time (t) of organosolv process on delignified solid were studied by using a 2^3 central composite experimental design. The ranges of these independent variables were 40–60% w/w, 170–200 °C, and 50–100 min. The total number of runs was 17, whose experimental conditions and severity factor (S_0) are summarized in Table 1. The software used to perform the multiple regression and analysis of variance (ANOVA) was Statgraphics Centurion XVII.

2.3 Analysis of raw material and delignified solids

The cellulose, hemicellulose, and lignin contents in the raw material and delignified solids were determined according to the NREL/TP-510–42,618 procedure [55]. The solid residue was determined gravimetrically and known as insoluble lignin (Klason lignin, IL). The soluble lignin (SL) was analyzed by a UV–Vis spectrophotometer (Cary 50, Varian) at 240 nm. The monomeric sugars were quantified by HPLC (Agilent, 1260) equipped with a Refractive Index Detector. For this purpose, a Carbo SepCHO 682 column (Transgenomic) with Micro-Guard cartridges (Bio-rad) was employed using the following conditions: ultrapure water as mobile-phase with a flow rate of 0.4 mL/min, and a column temperature of 80 °C. The sugar determination was performed after the samples were neutralized with calcium carbonate and filtered through membranes with a 0.22-µm pore size. In the present work, the cellulose is referred as glucan content (Gl), while the sum of xylan, galactan, and mannan

Table 1 Experimental conditions and results obtained for delignified solid yield (g/100 g raw material), chemical composition of delignified solids (g/100 g delignified solid), and delignification degree (%) of assays according to a 2^3 central composite design

Run	C_{EtOH} (wt%)	T (°C)	*t* (min)	S_0	Υ_{solid}	Gl	X	Ga	M	HMC	SL	IL	DD
1	33	185	75	4.38	59.0	58.6	3.9	0.4	6.1	10.4	3.9	24.4	45.9
2	60	200	50	4.64	66.0	56.6	4.5	0.7	12.4	17.6	3.2	19.0	52.8
3	50	210	75	5.11	43.5	71.0	4.1	0.4	7.2	11.7	4.3	11.1	81.8
4	60	170	50	3,76	79.6	48.9	4.7	2.2	13.3	20.2	3.6	22.5	32.6
5	60	170	100	4.06	77.4	47.7	4.5	2.0	13.5	20.0	4.9	21.2	38.2
6	50	185	75	4.38	63.7	55.2	4.2	0.9	12.5	17.6	4.4	20.1	51.8
7	50	185	75	4.38	65.9	56.2	4.1	0.7	11.7	16.5	4.6	17.7	56.1
8	50	185	33	4.02	71.5	50.2	4.4	1.4	12.4	18.2	4.6	20.3	45.4
9	50	185	75	4.38	65.7	54.3	4.2	0.9	12.0	17.1	4.3	18.9	53.3
10	40	200	50	4.64	52.5	72.0	4.1	0.5	5.1	9.7	3.6	17.9	64.6
11	67	185	75	4.38	74.9	52.1	4.8	1.9	12.5	19.2	5.3	19.7	44.5
12	50	160	75	3.64	81.3	47.8	4.7	2.2	11.1	18.0	4.6	23.8	27.2
13	40	200	100	4.94	44.6	72.4	3.7	0.4	4.7	8.8	4.8	14.9	75.0
14	60	200	100	4.94	59.9	60.5	4.6	0.3	11.3	16.2	5.6	17.1	61.5
15	40	170	50	3.76	75.8	51.8	4.8	2.0	11.9	18.7	4.1	22.3	36.4
16	50	185	117	4.57	60.9	59.0	4.1	0.6	11.5	16.2	4.5	14.4	67.0
17	40	170	100	4.06	69.5	53.0	4.5	1.0	10.1	15.6	4.7	18.7	51.1

C_{EtOH}, ethanol concentration; *S_0*, severity factor ($S_0 = \log(t \cdot \exp(T\text{-}100/14.75)$, *t*, time (min) and *T*, temperature (°C)); *Y_{solid}*, delignified solid yield; *Gl*, glucan; *X*, xylan; *Ga*, galactan; *M*, mannan; *HMC*, hemicellulose (sum of X, Ga, and M); *SL*, soluble lignin; *IL*, insoluble lignin; *DD*, delignification degree

is considered as the hemicellulose content (HMC). The delignification degree (DD) was calculated by the Eq. (2).

$$DD(\%) = \frac{m_{\mathrm{ILpine}} - m_{\mathrm{ILsolid}}}{m_{\mathrm{ILpine}}} \cdot 100 \tag{2}$$

where m_{ILpine} is the amount of insoluble lignin in the raw material (*P. radiata*), and m_{ILsolid} is the amount of insoluble lignin in the delignified solid. Both are determined from these equations: $m_{\mathrm{ILpine}} = \%IL_{\mathrm{pine}} \cdot m_{\mathrm{pine}}$ and $m_{\mathrm{ILsolid}} = \%IL_{\mathrm{solid}} \cdot m_{\mathrm{solid}}$, being m_{pine} the initial quantity of wood (20 g), m_{solid} the quantity of delignified solid after organosolv process, $\%IL_{\mathrm{pine}}$ the insoluble lignin content in the native wood, and $\%IL_{\mathrm{solid}}$ the insoluble lignin content in the delignified solid. The delignification degree is an important response in the study of organosolv process to evaluate the extension of reaction [17, 37].

The thermal stability of delignified solids was determined by thermogravimetric analysis in a Mettler Toledo TGA/DSC1. The temperature range employed was 30 to 900 °C, using a heating rate of 10 °C/min, and a dry nitrogen flow of 30 mL/min. For each experiment, delignified solid (8–9 mg) was charged into an alumina crucible (70 µL). The parameters used to evaluate the effect of organosolv process on thermal stability of delignified solids were the temperature of 10% of the initial weight loss ($T_{10\%}$) and the ash content at 800 °C.

Fourier transform infrared spectroscopy (FTIR) of delignified solids was carried out in a spectrometer Jasco 4700 with Spectra Manager software to determine the structural changes in these solids after organosolv process. The solid samples in powder were mixed with KBr in a ratio 1:200 in an agate mortar. The resultant mixture was introduced in a manual hydraulic press at 7 ton and 30 s to obtain the pellet, which was analyzed in the absorbance range between 2000 and 800 cm^{-1}, using 16 scans, and a resolution of 4 cm^{-1}. In addition, a semiquantitive analysis was performed to determine the changes of bands. The ratio between absorbances was calculated using 1426 cm^{-1} as a reference band.

2.4 Black liquor compositional analysis

The liquid phase (black liquor) obtained after the organosolv process was analyzed by HPLC in order to quantify the hemicellulosic fraction of pine solubilized in form of sugars and degradation products. For this analysis, a ROA Organic Acid column (Phenomenex), 0.005 M H_2SO_4 as mobile-phase with a flow rate of 0.6 mL/min, and 60 °C for column compartment temperature were employed. Xylose (X), galactose (Ga), and mannose (M) were quantified as a single compound. The lignin dissolved in the black liquors was recovered by acid precipitation and characterized in a previous work [56].

3 Results and discussion

3.1 Composition of *Pinus radiata* and delignified solids

The raw material used in this study, *P. radiata* wood, presents the following chemical composition, expressed in oven-dry basis (wt%): 43.8% glucan, 5.4% xylan, 3.1% galactan, 10.4% mannan, 2.3% arabinan, 26.6% insoluble lignin, 5.4% soluble lignin, and 3.2% extractives.

The data of chemical composition and delignification degree of delignified solid obtained for each run are also shown in Table 1. The responses that have given significant models are delignified Y_{solid}, Gl, HMC, and DD. The mathematical models with *R*-squared value are shown in Table 2, in which the effects with a significance level lower than 95% were neglected for regression models ($F > 18.51$ and $P\text{-value} < 0.05$). The developed models for the responses present *R*-squared value between 94.39 and 97.86%, which involves these models explained adequately in the data variation.

The delignified solid yield varied between 44.6 and 79.6% in the studied condition range (Table 1). These results can be compared with values of organosolv process of lignocellulosic materials, such as 43–83% for wheat straw [28] or 51–81% for *E. globulus* wood [24]. Other authors obtained similar results in spite of using temperatures lower than those applied in this work, 36–81% for barley straw [4], or 50–72% for *C. Cardunculus* [3]. However, sulfuric acid was employed as catalyst in these works. The variation of yield depends on different solubilization of lignin and hemicellulose in the liquid phase in function of experimental conditions. The increment of temperature and time resulted in

Table 2 Predictive models for organosolv process responses

Mathematic model	R^2 (%)
$Y_{\mathrm{solid}}\ (\%) = 310.473 - 2.14389\mathrm{C} - 1.40994\ \mathrm{T} - 0.118115\mathrm{t} + 0.01425\mathrm{CT}$	97.86
$DD\ (\%) = -197.88 + 2.99284\mathrm{C} + 0.915013\ \mathrm{T} + 0.221769\mathrm{t} - 0.0331703\mathrm{C}^2$	94.39
$HMC\ (\%) = -24.7489 - 0.420504\mathrm{C} + 0.677257\ \mathrm{T} - 0.0262537\mathrm{t} - 0.0076506\mathrm{C}^2 + 0.00783333\mathrm{CT} - 0.00332166\mathrm{T}^2$	97.06
$Gl\ (\%) = 87.6443 + 2.60086\mathrm{C} - 1.48763\ \mathrm{T} + 0.0559885\mathrm{t} - 0.0159\mathrm{CT} + 0.00747852\mathrm{T}^2$	94.99

the reduction of delignified solid yield due to the delignification and hemicellulose hydrolysis of native wood produced during the process. In this way, the increment of the severity from mild conditions (170 °C and 50 min) to severe conditions (200 °C and 100 min) supposes a reduction of delignified solid yield from 79 to 52% (Fig. 1a). A reduction in alcohol concentration in solvent involves that solid yield decreases (Fig. 1b), because the higher water amount improves the hydrolysis of hemicellulose and, therefore, its loss of raw material [16]. Under 185 °C and 75 min of pretreatment, the increment of ethanol concentration from 40 to 60% supposes an increase in delignified solid yield from ca. 60 to 70%.

The low delignified solid yields are obtained at the expense of high DD, as is exhibited in Fig. 2, where the linear relationship between DD and delignified solid yield is exhibited for all runs carried out. This trend was also obtained by Romaní et al. [17] and Weerasai et al. [19], whose lowest solid yields were achieved with the highest delignification, showing the inverse relationship between these responses. However, the run 1 presents a different behavior in Fig. 2. The solid obtained presented a low yield (60%) with a low delignification degree (46%). The drop of delignified solid yield is due to the high loss of hemicellulose obtained in this run, which was carried out with low ethanol concentration (33%). Therefore, the hydrolysis of hemicellulose of pine is favored with the lower alcohol concentration and higher water concentration. The total HMC in delignified solid is 10.4% under these operating conditions (run no. 1). In the study of the HMC response, this trend is further detailed as shown the response surface plots.

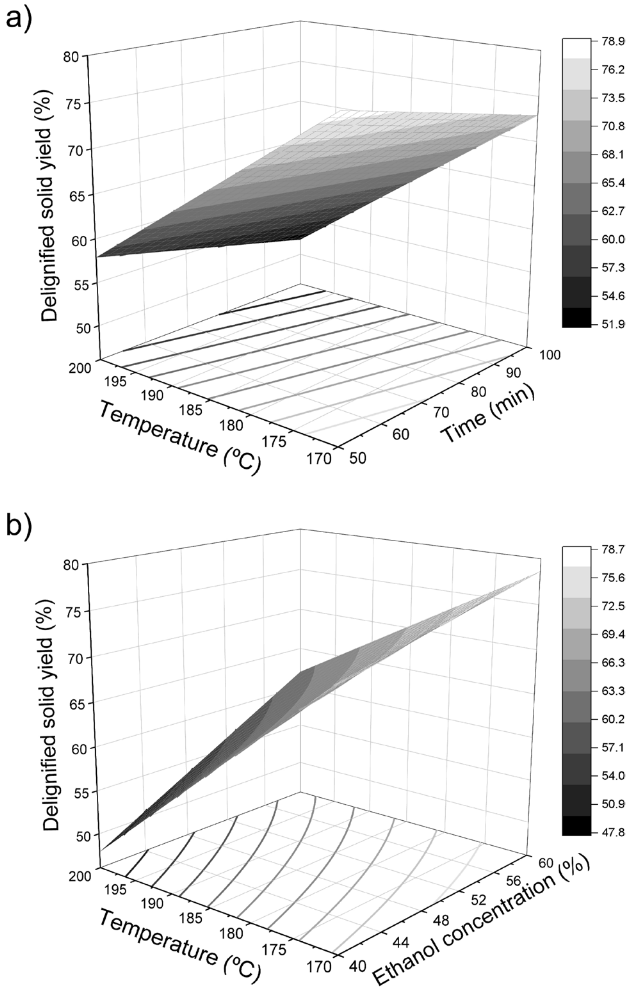

Fig. 1 The variation of delignified solid yield with two independent variables: **a** Temperature and time at $C_{EtOH}=50$ wt%, and **b** Temperature and ethanol concentration at $t=75$ min

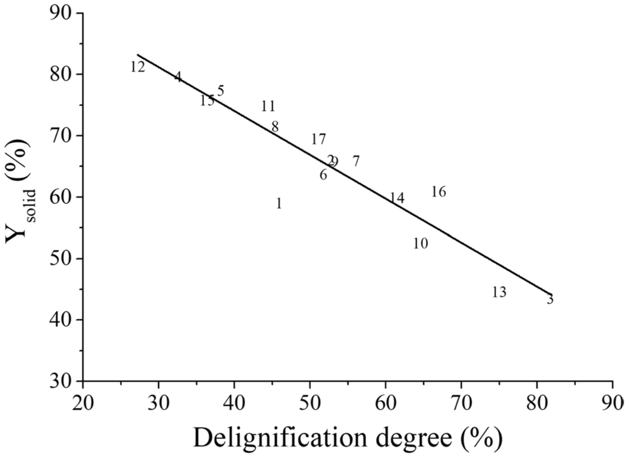

Fig. 2 Delignified solid yield vs delignified degree for each run from experimental design

The experimental results show that the DD values varied between 33 and 75% (Table 1). The increment of temperature and time has positive effect on the delignification of pine wood (Fig. 3a), which is in accordance with other lignocellulosic material results [4, 19]. Under middle time condition (75 min), an increment of 30 °C in organosolv process means a DD increase of ≈23%, from 41% at 170 °C to 68% at 200 °C, whereas, under 185 °C fixed temperature, an increase of 50 min involves a rise of ≈11% in DD response, from 49% at 50 min to 60% at 100 min (Fig. 3a). At fixed value of $C_{EtOH}=50$ wt%, the highest delignification degree ($DD=74\%$) is achieved at 200 °C and 100 min (Fig. 3a). These severe operating conditions for obtaining high delignification values are similar to those employed in others organosolv process carried out in absence of a catalyst, using ethanol/water as solvent and different raw materials. Romaní et al. [17] employed 200 °C, 90 min, and 50% of ethanol for reaching 62.2% of delignification in *E. nitens* bark, and Wei et al. [32] achieved a lignin removal of 52.4% in bagasse using 205 °C, 30 min, and 55% of ethanol. In this context, the use of severe conditions to achieve high lignin removal is also required in process without catalyst using

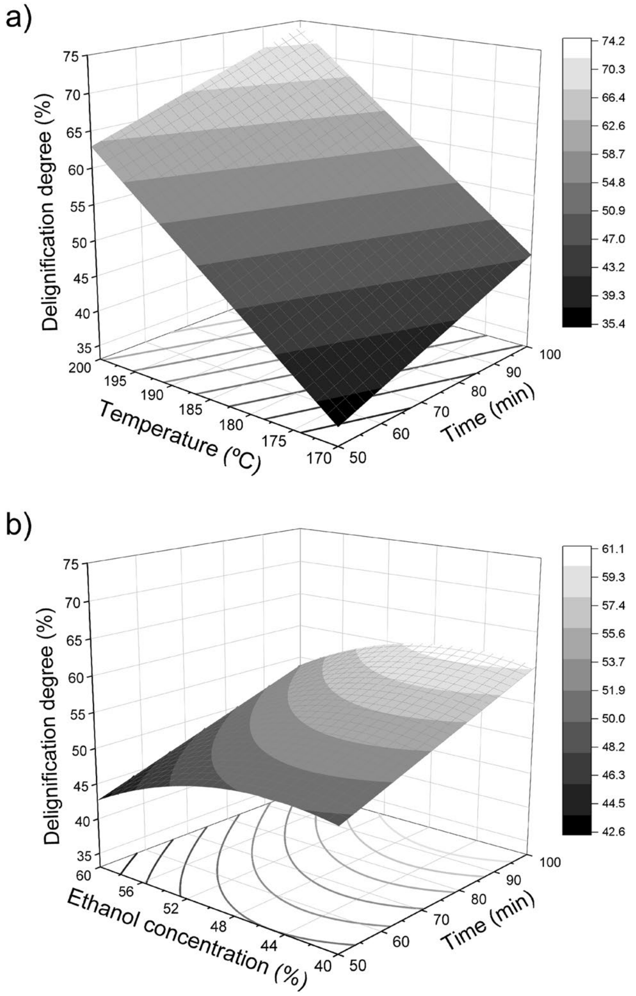

Fig. 3 The variation of delignification degree with two independent variables: **a** Temperature and time at $C_{EtOH} = 50$ wt%, and **b** Ethanol concentration and time at $T = 185$ °C

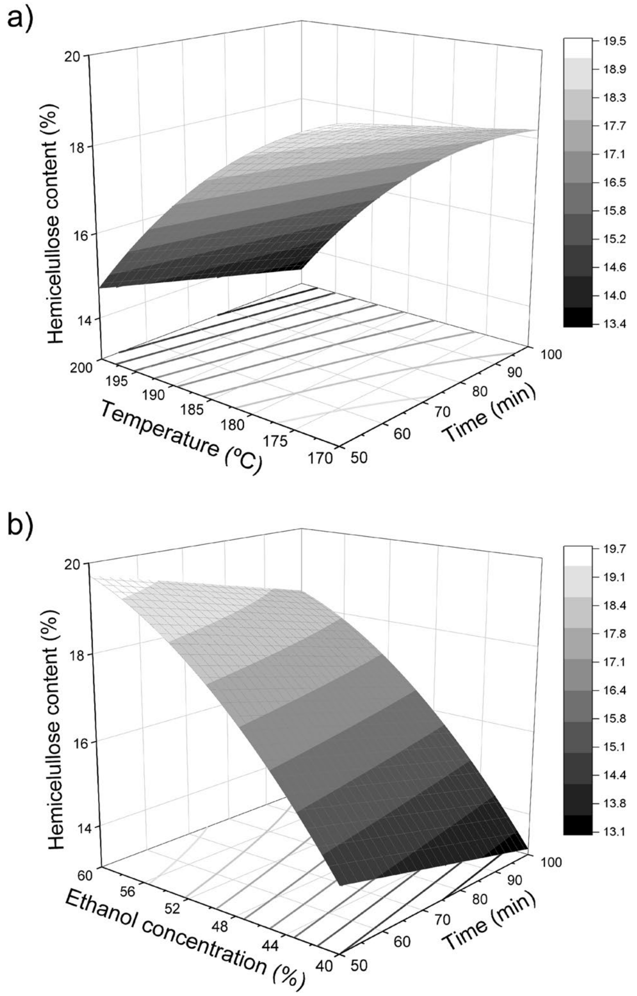

Fig. 4 The variation of hemicellulose content with two independent variables: **a** Temperature and time at $C_{EtOH} = 50$ wt%, and **b** Ethanol concentration and time at $T = 185$ °C

other solvents, such as acetone or glycerol. Huijgen et al. [28] applied 205 °C, 120 min, and 50% acetone to reach 79% of delignification in wheat straw, and Romaní et al. [24] reported 80% of delignification of *E. globulus* wood using 200 °C, > 58 min, and 80% of glycerol.

The ethanol concentration in this autocatalyzed process has a relevant influence on delignification degree. The response surface plot of delignification degree for a constant temperature of 185 °C is shown in Fig. 3b, where maximum delignification degree of *P. radiata* is achieved at $C_{EtOH} = 45$ wt%, and high times (> 90 min). This result is likely a consequence of lignin degradation and lignin solubilization produced during organosolv process, which depend on the ethanol concentration. Lower ethanol concentration promotes the cleavage of α- and β-ether linkages in the lignin due to higher hydronium ion concentration; whereas, high alcohol concentration involves major solubilization of lignin [16, 35, 53]. In this work, the studied organosolv is an autocatalyzed process and, therefore, these effects only depend on ethanol concentration from initial mixture due to the absence of ions provided by catalyst. The maximum delignification is reached at 45 wt% of ethanol concentration, which is similar to the ones obtained by other authors studying solvent proportion in autocatalyzed process, such as 40 wt% required of acetone for wheat straw [28] or 40% (v/v) of ethanol for bagasse [32].

The elimination of lignin of *P. radiata* wood during the organosolv process is not exclusive, due to the hemicellulose, which is covalently linked to lignin and acts as the connection between lignin and cellulose, also being extracted [7]. The response surface plots of hemicellulose content (the sum of xylan, galactan, and mannan) are exhibited in Fig. 4. The hemicellulose content in delignified solids is in the range between 8.8 and 20.2% (Table 1). The temperature and time favor the hydrolysis and extraction of hemicellulose of *P. radiata* wood. Under $C_{EtOH} = 50$ wt%, reducing HMC percentage from 19.5 (170 °C and 50 min) to 13.4% (200 °C and 100 min) was achieved (Fig. 4a). The ethanol concentration in the reaction medium has also great influence on HMC content of delignified solid, related to the water concentration. A higher hydronium ion concentration promotes the cleavage of hemicellulose bonds and, therefore, its loss from raw material. For this reason, under constant temperature of 185 °C and 100 min, the reduction of alcohol concentration from 60 to 40% involves a drop from 18.4 to 13% in HMC content (Fig. 4b). This hemicellulose hydrolysis reduction with organic solvent proportion has also been reported for organosolv of wheat straw employing acetone/water mixture [28].

In the studied range, the extraction of initial hemicellulose of *P. radiata* varied between 24 and 81%. The high hemicellulose removal is also attributed to its amorphicity in comparison to cellulose [57]. A wide range of hemicellulose removal (16–88%) was also found for ethanol-based organosolv of cardoon [3].

As a result of solubilization of lignin and hemicellulose, the delignified solids are enriched in cellulose (glucan). This response varied between 48 and 72% (Table 1). The response surface plots of glucan content at a constant ethanol concentration of 50 wt% and time of 75 min are exhibited in Fig. 5a and b, respectively. The pattern in previous studied responses (DD or HMC) is similar to those of glucan. The increment of time and temperature supposes the increase in glucan of delignified solid (Fig. 4a), reaching ≈66% in glucan content under operating conditions of 200 °C and 100 min ($C_{\mathrm{EtOH}} = 50$ wt%). The effect of high temperature is remarkable from 190 °C, when the isolines are closer; a minor increment of temperature supposes the same increase of glucan content. At low ethanol concentrations, the glucan content is higher than of obtained at high ethanol concentration, due to the hydronium ion concentration as mentioned before. At fixed time of 75 min and 185 °C, 59% of glucan content is achieved under 40 wt% alcohol concentration, while this content is reduced to 52% when the initial ethanol concentration is 60 wt% (Fig. 4b). At $C_{\mathrm{EtOH}} = 40$ wt%, a delignified solid with a glucan content > 66% is predicted by applying temperatures > 195 °C and times > 90 min, supposing an enrichment of at least 50% respect to initial glucan content in *P. radiata* wood.

The amount of initial glucan of pine wood remains in delignified solid after organosolv process varying between 70 and 90%. These values are close to other obtained woods under organosolv treatment, such as 75% obtained for *Pinus contorta* [35], 88% for *Populus nigra x P. maximowiczii* [53], and 94% for *E. globulus* [24]. The average amount of glucan lost during organosolv process is lower than 18%. This fact is associated with amorphous regions of this polymer, which are more easily hydrolyzable [58]. Therefore, the organosolv process allows obtaining a cellulose-rich delignified solid under high temperatures and times, and low ethanol concentrations due to the extraction of lignin and hemicellulose fractions. A high glucan content (72%) is achieved under operating conditions equal to 200 °C, 100 min ($S_0 = 4.94$) and 40 wt% (run no. 13), involving a 1.65-fold increase of glucan content respect to initial pine wood. Note that the glucan recovery obtained under these severe conditions can be considered low (74%). However, a low content of hemicellulose (8.8%) and high delignification degree (75%) are reached. This removal of hemicellulose and lignin of lignocellulosic materials is related to the improvement of the enzymatic hydrolysis [7, 15, 45]. Therefore, this solid rich in cellulose could be employed to obtain glucose or other added-value products, such as bioethanol, biohydrogen, or nanocellulose crystal [17, 38, 42].

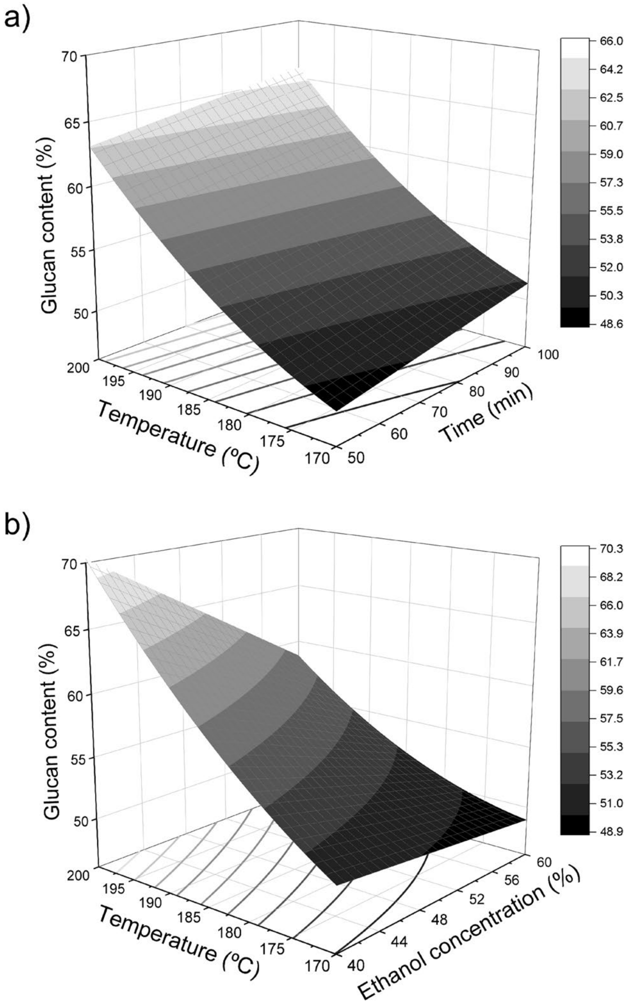

Fig. 5 The variation of glucan content with two independent variables: **a** temperature and time at $C_{\mathrm{EtOH}} = 50$ wt%, and **b** temperature and ethanol concentration at $t = 75$ min

3.2 Thermal stability of delignified solids

The TGA and DTG curves of four delignified solids (runs no. 3, 8, 12, and 16) are represented in Fig. 6. These solids were obtained under an ethanol concentration of 50 wt%, and grouped as a function of fixed conditions, either 75 min of time (Fig. 6a) or 185 °C of temperature (Fig. 6b). In these thermograms, the temperature at which 10% initial weight is lost ($T_{10\%}$) and the ash content at 800 °C are indicated. The higher $T_{10\%}$ values and lower ash contents are obtained under higher severity factors of organosolv process ($S_0=5.11$ and $S_0=4.57$), as shown in Fig. 6. This fact means that the delignified solids obtained under severe conditions of organosolv process present higher thermal stability.

Regarding to the influence of temperature and time on thermal stability (Fig. 6), the increment of temperature pretreatment from 160 to 210 °C supposed an increase of ≈30 °C in $T_{10\%}$ value (no. 3 and no. 12, $t=75$ min), whereas, under 185 °C fixed temperature, the difference of $T_{10\%}$ values was ≈15 °C when the organosolv time was increased (no. 8 and no. 16). Thus, the TGA results show that the temperature and time have a positive effect on thermal stability of delignified solids because the higher these conditions are, the higher $T_{10\%}$ values are. Besides, the influence of temperature is greater than of time. On the other hand, the low residual contents of delignified solids (19–25%) can be attributed to low lignin content remained after organosolv process [23, 41]. This fact is according to high delignification degree values obtained for these runs (Section 3.1). For example, run no. 3 achieved a DD equal of 82% and presented the lowest ash content (19%), while run no. 12, whose DD was 27%, presented an ash content of 25%.

Respect to DTG curves, run no. 3 presents a clear peak, which can be related to high cellulose content in delignified solid (71%), while the rest of DTG curves exhibit an unsymmetrical peak due to the presence of hemicellulose and lignin [41]. The degradation of three polymers presented in delignified solids is overlapped in this unsymmetrical peak shown by runs no. 8, 12, and 16 (Fig. 6). The degradation of hemicellulose takes place between 220 and 315 °C, while

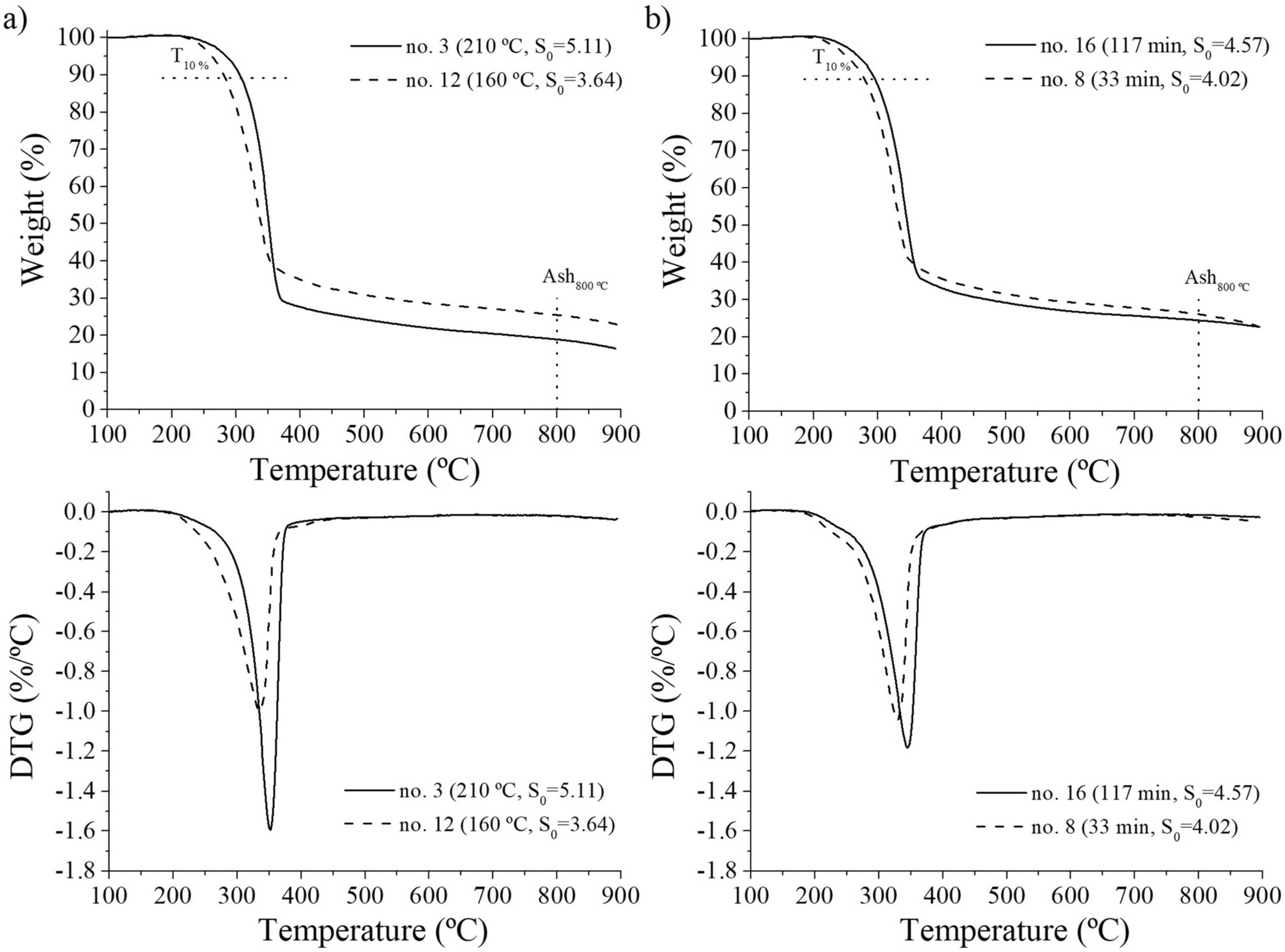

Fig. 6 TGA and DTG curves of the delignified solids obtained at $C_{EtOH}=50$ wt%: **a** $t=75$ min, and **b** $T=185$ °C

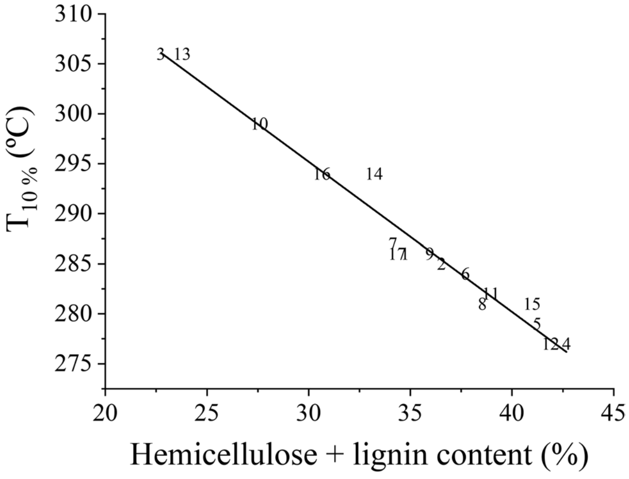

Fig. 7 Relationship between $T_{10\%}$ and hemicellulose + lignin content (%) in delignified solids

cellulose does between 315 and 400 °C. Lignin presents a broader range of degradation between 200 and 480 °C [59]. For this reason, the weight loss of delignified solids with higher hemicellulose content begins at lower temperatures than those solids with lower hemicellulose content (Fig. 6). Therefore, DTG curves show the dependence of the thermal stability of delignified solids on their chemical compositions.

$T_{10\%}$ of all samples from experimental design has been represented as a function of the sum of hemicellulose and lignin content in delignified solids (Fig. 7). There is a clear trend between these responses; $T_{10\%}$ increased linearly with decreasing the hemicellulose and lignin content. Thus, the hemicellulose + lignin content of delignified solid under mild conditions (no. 4, 60 wt%, $S_0 = 3.76$) was 42.6% corresponding to $T_{10\%}$ of 277 °C, whereas, this content was reduced until 23.7%, increasing $T_{10\%}$ until 306 °C, by applying severe conditions (no. 13, 40 wt%, $S_0 = 4.94$). The increment of thermal stability of delignified solids can be attributed to the removal of these polymers and, therefore, the increase of cellulose (glucan) content occurred during the process [45]. This is similar to the results obtained from the other lignocellulosic materials, such as wheat straw and sugarcane bagasse, in which the degradation 20 and 50% of samples treated by organosolv was achieved at higher temperatures than that of raw materials. For instance, the temperature at which wheat straw degraded 20% was increased from 264 to 337 °C when an organosolv was carried out by employing 220 °C, 3 h, and 70% aqueous glycerol [23, 44]. Thus, the TGA and DTG results confirm that cellulose remains in delignified solids after organosolv process, while other fractions of *P. radiata* wood are removed, and reveal the relationship between the thermal stability of this cellulose-rich delignified solid and its lignin and hemicellulose content.

3.3 Structural changes of delignified solids

The FTIR spectra of the delignified solids obtained at different severity factors and ethanol concentrations, corresponding to maximum and minimum values of experimental design, are exhibited in Fig. 8. The main changes observed in the spectra are related to hemicellulose and lignin bands, which are the polymers removed during organosolv process. The band 1735 cm^{-1} corresponds to acetyl groups in hemicellulose [60]. This band is weaker when severity factor is increased due to the reduction of hemicellulose content in delignified solids, as confirmed in compositional analysis (Section 3.1). For instance, in the delignified solid from run no. 4, which presents a HMC content of 20.2%, the band at 1735 cm^{-1} is more defined than that of the delignified solid from run no. 13, whose HMC content is 8.8% and, therefore, this band is not appreciated. The semicuantitative analysis confirms this trend; a reduction in the ratio A_{1735}/A_{1426} from 0.9208 to 0.8061 with the increment from $S_0 = 3.76$ to $S_0 = 4.94$ was observed, at $C_{EtOH} = 60$ wt% (no. 4 and no. 14).

The characteristic bands of lignin also reduced its intensity gradually as a consequence of the severity factor increment in organosolv process. These bands correspond to aromatic skeletal vibration breathing with C = O stretching (1596 cm^{-1}), aromatic skeletal vibration (1512 cm^{-1}), and guaiacyl ring breathing with C = O stretching (1267 cm^{-1}) [61]. The reduction of these bands is due to the delignification occurred in the process, and can be related to delignification degree. For example, the ratio A_{1512}/A_{1426} in samples no. 13 and no. 15 was reduced from 0.3085 to 0.2322, corresponding to the increment of DD from 36.4 to 75%.

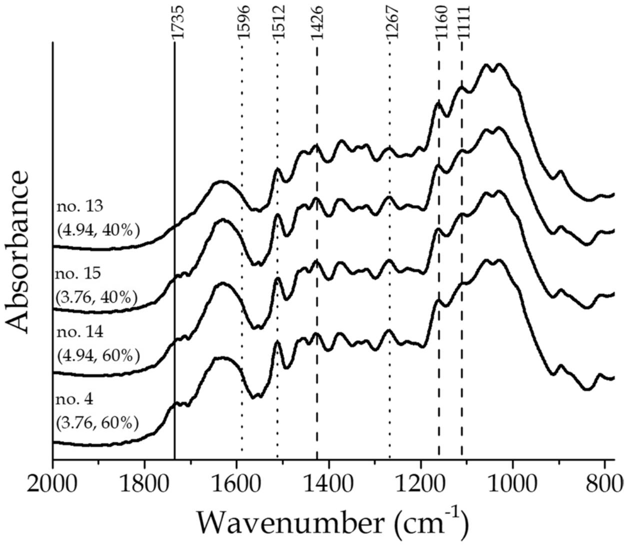

Fig. 8 FTIR spectra of delignified solids obtained from runs no 4, 13, 14, and 15

According to the enrichment in cellulose (glucan) of delignified solids, the cellulose bands incremented its intensity with the increase of the severity factor of organosolv process. These bands correspond with C–O–C stretching asymmetric (1160 cm^{-1}), and glucose ring stretching asymmetric (1111 cm^{-1}) [62]. Other authors have observed similar changes in FTIR spectra after organosolv process, employing different solvents and raw materials, such as bagasse [15, 29, 32], wheat straw [23, 37], corn stover [22], or hybrid *Pennisetum* grass [45]. Therefore, the FTIR analysis confirms the loss of hemicellulose and lignin of pine wood produced during ethanol-based organosolv process, depending on experimental conditions. This trend is according to the delignified solid composition and delignification degree previously calculated (Section 3.1).

3.4 Black liquor composition

The pH value and sugar and degradation product concentration of the black liquor of the seventeen experiments carried out in the experimental design are shown in Table 3.

The pH values of the black liquors ranged between 3.8 and 5.1. These values are similar to the achieved organosolv liquid phase of other lignocellulosic materials, such as *E. globulus* or wheat straw, using also an ethanol/water mixture as solvent [10, 16]. Under the alcohol concentration $C_{EtOH} = 40$ wt%, the lowest pH value corresponds to the highest severity factor of organosolv process (pH = 3.78, $S_0 = 4.94$). This behavior can be explained by the hydrolysis and solubilization of hemicellulose in the black liquors. A higher hemicellulose hydrolysis involves the higher formation of acetic acid from acetyl groups. The run no. 13 ($S_0 = 4.94$), which presented a low HMC content in delignified solid (8.8%), reached an acetic acid concentration in the black liquor of 2.66 g/L, and the lowest pH value (3.78). In addition, the solubilized hemicellulose can be degraded into organic acid, such as formic acid (FA), as shown in Table 3. The release of both acids contributes to the pH value drop of the reaction medium.

These results are in accordance with the concentration of sugars and degradation products quantified in the black liquors (Table 3). The release of glucose (Gl), xylose + mannose + galactose (X-M-Ga), and arabinose (A) agrees with the loss of hemicellulosic and cellulosic fraction of delignified solid. The glucose concentration is lower than that of the other sugars, due to the minor degradation of glucan of the raw material during the organosolv process, as commented before (Section 3.1). The maximum values of glucose concentration are 1.07 and 1.09 g/L, which are obtained under high organosolv temperature (200 °C). This limited glucan hydrolysis during the organosolv is consistent with the results obtained by other authors using diverse raw materials such as wheat straw [28], empty palm fruit bunch [54], bagasse [32], or *E. nitens* bark [17]. According to the extraction of hemicellulose of *P. radiata* during ethanol-based organosolv, the hemicellulose-derived sugars in black liquors increased with temperature and time (severity factor). These results are similar to the findings in the Oxiorganosolv of beech wood, in which the hemicellulose recovery

Table 3 Experimental results for pH, and sugars, and degradation product concentration of black liquors (g/L)

Run	pH	Gl	X-M-Ga	A	FA	AA	HMF	F
1	3.90	0.38	1.41	0.76	0.49	0.76	0.21	0.17
2	4.64	0.12	0.35	0.51	0.55	1.04	0.03	0.02
3	4.01	0.49	1.11	0.33	0.49	1.34	0.92	0.07
4	5.08	-	0.15	0.90	0.19	0.36	-	0.01
5	4.84	-	0.14	0.77	0.24	0.42	0.06	0.01
6	4.42	0.04	0.31	0.58	0.30	0.52	0.12	0.04
7	4.42	0.03	0.35	0.51	0.26	0.45	0.15	0.08
8	4.48	0.02	0.24	0.84	0.20	0.35	0.10	0.01
9	4.49	0.03	0.35	0.67	0.32	0.55	0.16	0.02
10	3.93	1.07	3.62	0.63	0.41	0.12	1.33	0.03
11	4.68	0.01	0.16	0.55	0.25	0.62	0.19	0.01
12	4.63	0.02	0.10	0.70	0.18	0.32	0.06	-
13	3.78	1.09	2.80	0.89	1.52	2.66	1.58	0.08
14	4.40	0.33	0.70	0.35	0.74	1.39	0.46	0.04
15	4.30	0.09	0.27	0.97	0.28	0.42	0.13	0.01
16	4.42	0.22	0.76	0.75	0.72	1.21	0.26	0.02
17	4.32	0.11	0.53	1.03	0.53	0.53	0.16	0.03

Gl, glucose; *X-M-Ga*, xylose + mannose + galactose; *A*, arabinose; *FA*, formic acid; *AA*, acetic acid; *HMF*, hydroxymethylfurfural; *F*, furfural in liquid fraction was higher when pretreatment temperature and cooking time increased. For example, the increment of time from 60 to 120 min supposed the increase of hemicellulose recovery in liquor from 16.0 to 33.4%, while the temperature increase from 150 to 175 °C involved a rise from 33.4 to 76.2% [43]. The hemicellulose hydrolysis is autocatalyzed by acetic acid (AA) released from acetyl groups [28]. Thus, the increase of severity factor from $S_0 = 3.76$ to $S_0 = 4.94$ supposed a rise from 0.15 to 0.70 g/L in X-M-Ga concentration (60 wt%, runs no. 4 and no. 14). However, the reduction of C_{EtOH} from 60 to 40 wt% involves an increment of sugar concentration from 0.70 to 2.80 g/L for the same severity factor ($S_0 = 4.94$, runs no. 13 and no. 14). This behavior is justified by the increment of hydronium ion concentration, which favors the hydrolysis of hemicellulose, when the alcohol concentration is lower, as mentioned before. The highest value of X-M-Ga concentration is detected in run no. 10 (3.62 g/L), when severe conditions were applied (200 °C, 50 min, and $C_{EtOH} = 40\%$). However, the increment of time from 50 to 100 min supposes the concentration reduction to 2.80 g/L (run no. 13), due to the degradation to furans. Thus, the hydroxymethylfurfural concentration was incremented from 1.33 to 1.58 g/L (runs no. 10 and 13).

Regarding the formation of furan byproducts, the hydroxymethylfurfural (HMF) concentration is higher than furfural (F) in all studied range. This fact is attributed to the HMF that comes from the degradation of the hexoses which mainly composed the raw material employed, a softwood (*P. radiata*). Unlike hardwood or herbaceous plants, whose major hemicelluloses are xylans, the main hemicelluloses in softwood are galactoglucomannans [8]. The effect of organosolv conditions on the furan formation is similar to other response, such as pH or monomeric sugars. The increase of temperature and time promotes the formation of furans from hemicellulose and cellulose fractions solubilized in the black liquor [54].The HMF and furfural concentrations are increased from 0.13 and 0.01 g/L to 1.58 and 0.08 g/L, respectively, when the severity factor increased from $S_0 = 3.76$ to $S_0 = 4.94$, at $C_{EtOH} = 40$ wt% (runs no. 13 and no. 15). In contrast, under the same severity factor ($S_0 = 4.06$, runs no. 4 and no. 17), when the alcohol concentration in solvent is increased from 40 to 60 wt%, these concentrations are reduced from 0.16 and 0.03 g/L to 0.06 and 0.01 g/L, respectively. These trends agree with those found by Wei et al. [32], who carried out an autocatalyzed organosolv process for bagasse. In this study, the increment of ethanol content from 25 to 70% (v/v) involved the decrease in HMF and furfural concentration from 1.38 and 0.41 g/L to 0.25 and 0.03 g/L, respectively. In the same way, the use of other mixture in autocatalyzed organosolv such as acetone/water achieved similar results, in which an increase of acetone content supposed the reduction of furan concentration in black liquors [28].

These results show the hemicellulosic fraction of *P. radiata* wood, solubilized during organosolv process, remains in black liquors after the recovery of lignin, carried out via precipitation in our previous work [56]. This stream with hemicellulose from lignocellulosic materials has shown its potential to be valorized in several applications [21, 50, 52]. For instance, furan (furfural and hydroxymethylfurfural) production has been recently proposed by acidic treatment of this stream [20].

4 Conclusions

An autocatalyzed organosolv process was investigated to fractionate a softwood, *P. radiata*, by employing a central composite design. The increment of temperature and time showed positive influence on the delignification and hemicellulose hydrolysis of pine and, therefore, improved the glucan content of delignified solid, but involved the solid yield reduction. However, the increase of ethanol concentration supposed the opposite trend of these responses, except on delignification, whose maximum was achieved at $C_{EtOH} = 45$ wt%. On average, more than 80% of initial cellulose of pine was recovered in delignified solid in studied operating conditions. At least 50% of glucan enrichment respect initial wood can be achieved by employing of T > 195 °C and t > 90 min, at $C_{EtOH} = 40$ wt%, involving a glucan content of more than 66% in delignified solid. The inverse relationship between the hemicellulose and lignin content of delignified solid and its thermal stability was revealed from TGA analysis. The lignin and hemicellulosic fractions solubilized during the organosolv process were recovered in black liquors. The efficient separation of compounds, which could be used as feedstock for value-added chemical production, allows the valorization of *P. radiata* wood.

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Declarations

Conflict of interest The authors declare no competing interests.

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