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Lignin from an integrated process consisting of liquid hot water and ethanol organosolv: Physicochemical and antioxidant properties

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ABSTRACT

Corncob was successively pretreated by liquid hot water (LHW) and ethanol organosolv (EO) in an integrated process. LHW was performed at 200 °C for 30 min, and EO was performed using uncatalyzed ethanol–water solutions, according to a design of experiments. The effects of the most influential operational variables (ethanol concentration, temperature and time) on yield and chemical composition of the fractions were assessed. Results showed the factor with the greatest effect was ethanol concentration ($p < 0.05$), leading to a high-purity lignin (86.7%–93.1%) with a total phenolic content of around 25 mg GAE/g. Moreover, the solids recovered from the high ethanol concentration conditions presented the lowest lignin contents (15.4%–17.2%) with good preservation of cellulose (82.5%–88.6% of glucans). The lignin antioxidant capacity showed that all lignin samples presented radical scavenging activity (IC_{50} of 0.17 mg/mL and 0.016 mg/mL on DPPH (2,2 diphenyl 1 picrylhydrazyl) and ABTS (2,2′ azino bis(3 ethylbenzothiazoline 6 sulphonic acid) assays, respectively) with values close to the commercial antioxidant BHT. Moreover, the chemical (FTIR) and thermal (DSC and TGA) characterization showed lignins with similar properties that were compared with lignin from direct ethanol organosolv process. Results showed that the integrated process of LHW-EO was the most effective way to obtain lignin with high purity and antioxidant capacity.

1. Introduction

Lignin, accounting for 20–30% in lignocellulosic materials (LCMs), is the second most abundant naturally synthesized polymer in the world. It is an amorphous and highly branched polyphenolic (aromatic) macromolecule, known to bind to cellulose and hemicelluloses [1].

During the last decades, the growing interest for green and bio-based materials from LCMs has promoted an increased use of lignin in various applications, e.g. as antioxidants [2]. Since 2011, the field of antioxidants was distributed in: rubbers (and latex) – 53%; plastics – 36%; food and nutrition – 8%, and oil fuels – 3% [3]. In the food industry, the demand for natural antioxidants is increasing mainly due to the questionable safety of synthetic antioxidant compounds that are related to their possible toxic effects when considering long-term intake. Besides, consumers prefer to select natural antioxidants in their products, which are considered safer and healthier [4].

Several studies have showed that lignin can serve as a renewable source of aromatic compounds with antioxidant capacity [5–10]. However, the antioxidant capacity of lignins, as well as their physicochemical properties, significantly depends on the source of lignocellulosic material and extraction method employed. Nevertheless, the differences between lignin from various plant sources are reported to be minor when compared to the differences that can be observed from different pretreatments [11,12].

Organosolv is an interesting process able to produce a high purity (low inorganic impurities and sulfur-free) and low molecular weight (Mw) lignin, offering new possibilities for high quality applications, and recovers a solid residue rich in cellulose, which is also suitable for enzymatic saccharification [12,13]. For some LCMs the combination of hydrothermal pretreatment and organosolv can be used to increase the effective fractionation of the materials [12]. Currently, liquid hot water (also known as autohydrolysis) has been used prior the organosolv process to selectively remove hemicelluloses and obtain cellulose and lignin [6,13–16]. Under this process, lignin can be degraded or de-polymerized into small molecular fragments, which need to be fully characterized in order to be applied as bio-based chemicals and materials [16,17].

However, the works that explored the integrated process focused mainly on the structural characterization of lignin [16,18,19], or only described the study of extraction of lignin, sometimes added of the enzymatic hydrolysis of the cellulose fraction [13–15,20]. Still, there are few studies, involving the integrated process, that have explored the biological properties of lignin, such as antioxidant activity [6,21]. In

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addition, all of these works have not successful achieved the complete removal of hemicellulose during hydrothermal pretreatment. Therefore, the present study presents an integrated fractionation of LCMs from corncob using a liquid hot water (LHW) step for the complete removal of hemicelluloses, and ethanol organosolv (EO) for the separation of cellulose and lignin. The EO conditions were evaluated using an experimental design (2^3 with a central point) and the lignins obtained were characterized regarding their in vitro antioxidant activity, total phenolics, structural features (FTIR) and thermal properties (TGA and DSC). These lignin samples were also compared with the lignin from the direct EO process.

2. Materials and methods

2.1. Materials

Corncob (CC) was supplied by a local farmer (Ponte da Barca, Northern Portugal). This feedstock was dried at 40 °C for 12 h. After that, it was milled and sieved to obtain particles sizes from 1 to 2.5 mm (Retsch SM 2000 cutting mill, Germany), and stored at room temperature.

The chemicals 2,2 diphenyl 1 picrylhydrazyl (DPPH), 2,2′ azino bis (3 ethylbenzothiazoline 6 sulphonic acid) (ABTS), 6 hydroxy 2,5,7,8 tetramethylchroman 2 carboxylic acid (Trolox), butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), Folin–Ciocalteu reagent and gallic acid were purchased from Sigma-Aldrich. Ethanol was obtained from Panreac (Spain).

2.2. Extraction of lignin

A two-step pretreatment, involving LHW and EO, was applied to corncob in an integrated process in order to extract the lignin. Lignin from the direct EO process was used as control to compare the effectiveness of the integrated process.

2.2.1. Liquid hot water pretreatment

LHW pretreatment condition was selected based on previous results obtained in a study of hydrothermal pretreatment [22]. Untreated corncob was mixed with distilled water at solid loading of 10% (w/v) in a 2.0 L stainless steel cylindrical reactor (Parr Instruments Company, Moline Illinois, USA) equipped with a Parr PID temperature controller (model 4848). The reaction media was stirred at 150 rpm and heated by an external jacket until a final temperature of 200 °C that was maintained for 30 min (these conditions correspond to a log $R_0 = 4.42$). After that, the reactor was rapidly cooled-down through water recirculation by an internal loop.

The insoluble solids were separated from the liquid fraction (slurry) by filtration using Whatman® N° 1 filter paper. These were washed with distilled water at 70 °C and dried at 50 °C. The hydrolysate obtained in the filtrate by the liquid hot water process was used to determine the compounds derived from hemicellulose, by-products and soluble lignin.

2.2.2. Organosolv process

The EO of the LHW-pretreated and untreated corncobs was carried out using a solid loading of 10% (w/v) in 160 mL stainless steel cylindrical reactors (4.0 cm internal diameter and 12.4 cm internal height), with a working volume of 50 mL. LHW-pretreated corncob was delignified using uncatalyzed ethanol-water solutions according to the DOE described in the next section. Untreated corncob was delignified in the optimal organosolv condition. The reactors were submerged into the oil bath with an open heating circulator (Julabo Labortechnik GmbH, Seelbath, Germany) and PID temperature control at the desired temperature. After the reaction time, the reactors were cooled in an ice bath to stop the reaction.

The hydrothermally pretreated ethanol organosolv residue (HP-EOR) and the ethanol organosolv residue (EOR) were obtained by filtration using Whatman® N° 1 filter paper. These were washed with ethanol (at the same concentration of the pretreatment) to remove any adsorbed lignin on the cellulosic fibers. Afterwards, the solid residues were washed with distilled water at 70 °C until a neutral pH was obtained. Finally, this material was dried at 100 °C, and the yields of organosolv process were determined by weight. The liquid fraction was combined with the ethanol washing volume and the lignin (hydrothermally pretreated ethanol organosolv lignin (HP-EOL) and ethanol organosolv lignin (EOL)) was recovered through solvent evaporation.

The yield of lignin or solid residue was determined according to Eq. (1).

$$\text{Yield}\ (\%) = \left(\frac{m_{recovered}}{m_{corncob}}\right) \times 100 \quad (1)$$

where $m_{recovered}$ is the mass of lignin or solid residue recovered after the pretreatment (g), $m_{corncob}$ is the mass of the crude corncob used for the pretreatment (g).

The lignin extraction efficiency was determined according to Eq. (2).

$$\text{Extraction efficiency}\ (\%) = \left(\frac{m_{recovered}}{KL_{corncob}}\right) \times 100 \quad (2)$$

where $m_{recovered}$ is the mass of lignin recovered after the pretreatment (g) and $KL_{corncob}$ is the mass of Klason lignin in the crude corncob (g).

The delignification extent was determined by Eq. (3), according to Novo et al. [23].

$$\text{Delignification}\ (\%) = \left(\frac{KL_{corncob} - \left(KL_{pulp}\ \frac{Y_{pulp}}{100}\right)}{KL_{corncob}}\right) \times 100 \quad (3)$$

where $KL_{corncob}$ is the quantity of Klason lignin in the corncob (%), KL_{pulp} is the quantity of the Klason lignin in the pulp (%) and Y_{pulp} is the yield of the pulping reaction (%).

2.2.3. Design of experiments

The organosolv experiments were performed using a design of experiments (DOE) as a tool to evaluate the effect of temperature (T), time (t), and ethanol (EtOH) content on lignin extraction of the LHW-pretreated corncob.

The DOE used was a cubic experimental design procedure with three factors of two levels each (2^3) and using the central point for error evaluation. The independent variables were temperature (°C), [140(−1), 160(0), 180(+1)], time (min), [40(−1), 80(0), 120(+1)], and ethanol concentration (%), [20(−1), 40(0), 60(+1)]. The analyzed responses (dependent variables) were: solid residue and lignin yields, chemical composition, delignification percentage and lignin extraction efficiency. The results were assessed by Pareto diagram with STATISTICA 8.0 (Stat Soft Inc., USA) to verify the factors displaying a significance higher than 5%.

2.3. Analysis of raw and pretreated corncob

Aliquots of the solid material (untreated and pretreated feedstocks) were milled to a particle size <0.5 mm and subjected to chemical composition analyses according to the standard Laboratory Analytical Procedures (LAPs) for biomass analysis provided by the US National Renewable Energy Laboratory (NREL) [24]. All measurements were made in duplicate. Analyzed components were glucan, xylan, arabinan, acetyl groups, lignin, and ash. The protein content was calculated based on the nitrogen content estimated by the Kjeldahl method, multiplied by a factor of 6.25. The liquor from pretreated materials was analyzed for monomeric sugars, acetic acid, oligomeric sugars, acetyl groups and degradation products (hydroxymethylfurfural (HMF) and furfural). The oligomeric sugars were calculated after a quantitative posthydrolysis

with 4% sulfuric acid at 121 °C, during 60 min. The increase of monosaccharide (glucose, xylose and arabinose) and acetic acid concentrations caused by posthydrolysis provided a measure of the concentrations of oligomers and acetyl groups bound to oligosaccharides [25]. The monosaccharaides, organic acids and degradation products were determined by HPLC (JASCO Intelligent Sampler AS 2057 Plus) through a Metacarb 87H column (300 × 7.8 mm, Varian, USA). The mobile phase was 0.005 M H_2SO_4 in Milli-Q water, pumped at a flow rate of 0.6 mL·min^{-1}, at 60 °C. Sugars and acetic acid were analyzed with a refractive index (RI) detector and furfural and HMF with a UV detector.

2.4. Total phenolic content

Total phenolic content was estimated by the Folin–Ciocalteu colorimetric method, based on the procedure of Makkar et al. [26], using gallic acid as a standard phenolic compound. The absorbance at 725 nm was evaluated using glass cuvettes. The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in milligrams per gram by means of a calibration curve obtained with a standard of gallic acid.

2.5. Antioxidant activity

Lignin antioxidant capacity was studied by evaluating free radical scavenging effect through ABTS and DPPH methods that are common spectrophotometric procedures for determining the antioxidant capacities of components.

2.5.1. DPPH free radical scavenging activity

The DPPH radical scavenging activities of lignins (HP-EOL and EOL) were measured according to the slightly modified method of Blois [27]. Trolox, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were utilized as reference.

A 150 mM of DPPH solution was prepared in 80% ethanol and diluted to get an absorbance of 0.700 at 517 nm. Lignin samples were dissolved in 80% ethanol at concentrations from 0.05 mg/mL to 1 mg/mL and a volume of 25 μL was mixed with 200 μL of DPPH solution. These samples were incubated for 30 min at room temperature in dark. The decrease of the absorbance was measured using a UV–Vis spectrophotometer against a control sample (25 μL of 80% ethanol plus 200 μL of DPPH solution).

The percentage of radical scavenging activity (RSA) was calculated using the Eq. (4):

$$\text{RSA}\ (\%) = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \times 100 \quad (4)$$

where $A_{control}$ is the absorbance of the control sample and A_{sample} is the absorbance of the lignin sample.

The IC_{50} value was also calculated as the concentration of the compounds that causes 50% reduction in the DPPH color (also referred as inhibition). All experiments were carried out in duplicate.

2.5.2. ABTS radical scavenging activity

The ABTS radical scavenging activity of lignins (LHW-EOL and EOL) was determined according to the method of Re et al. [28], which is based on the ability of antioxidants to interact with the ABTS radical, decreasing its absorbance at 734 nm. Trolox, BHT and BHA were utilized as reference. The ABTS stock solution (7 mM ABTS and 2.45 mM potassium persulfate in ultrapure water, ratio of 1:1) was prepared and kept in the dark at room-temperature for 12–16 h before using. Before being used, the ABTS solution was diluted with ultra-pure water to get an absorbance of 0.700 at 734 nm. For the analysis, 100 μL of the diluted ABTS solution were mixed with 100 μL of the lignin sample dissolved in ethanol 60% at different concentrations (0.005–0.1 mg/mL). After 6 min the absorbance was measured and the percentage inhibition was calculated for each concentration against a blank sample (100 μL of diluted ABTS mixed with 100 μL of ultrapure water). The percentage of RSA was calculated as described previously.

2.6. Attenuated total reflectance - Fourier transform infrared spectroscopy (ATR-FTIR)

The ATR-FTIR spectra were obtained on a spectrophotometer model FT/IR-4100 type A (Origin, Jasco) of direct transmittance on the solid sample. Samples were analyzed from 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1} and 16 scans were recorded.

2.7. Thermogravimetric analysis (TGA)

Thermal degradation behavior of the lignin samples was determined by TGA using a thermogravimetric analyzer 4000 (Perkin Elmer, Waltham, Massachusetts, EUA) and a nitrogen atmosphere. Samples with approximately 10 mg of material were heated from 20 °C up to 800 °C at a rate of 10 °C/min. Analyzes were performed in duplicate.

2.8. Differential scanning calorimetry analysis (DSC)

The glass transition temperature was measured using a DSC analyzer 6000 (Perkin Elmer, Waltham, Massachusetts, EUA). Approximately 5 mg of sample was placed into aluminium pans, sealed with the aluminium covers (Perkin-Elmer, DSC, B0143016/B0143003, respectively) and heated between 0 and 200 °C at a scanning speed of 10 °C/min in a nitrogen atmosphere using a refrigerating cooling accessory. An empty aluminium pan was used as reference. Glass transition was calculated by using a half-height technique in the transition region. Calibration was performed using an indium sample. Analyzes were performed in duplicate.

3. Results and discussion

Corncob was subjected to the integrated process based on liquid hot water and ethanol organosolv process (Fig. 1). The main products obtained from the process included lignin, cellulose and compounds derived from hemicellulose. The next two subsections describe the chemical composition and yield of the pretreated corncob obtained with the integrated process and the organosolv process, as well as the crude corncob.

3.1. Chemical composition of the raw and LHW-pretreated corncob

The chemical composition of untreated and LHW-pretreated corncob is presented in Table 1. Corncob is mainly composed by cellulose (≈36%), hemicellulose (≈31%) and lignin (≈21%) and its composition was similar to the ones previously reported by other authors [22,29–32].

The LHW pretreatment condition was established based on previous results [22], which showed the effect of temperature and residence time of LHW-pretreatment on hemicellulose-derived products' solubilization, such as XOS, xylose and furfural, as well as on the recovery of cellulose and lignin from corncob. In the current study, the selected condition was 200 °C and 30 min. Under this condition, 51% of spent solid was recovered and all hemicellulose (30.5%) was solubilized in the hydrolysate mainly as free xylose (5.7 g/L) and furfural (7.7 g/L). The hemicellulose removal increased the cellulose and lignin contents in pretreated corncob through a concentration effect (Table 1). The content in cellulose, expressed as glucan, increased from 35.8% to 60.4% in the pretreated material, whereas the lignin content (Klason lignin and acid-soluble lignin) increased from 20.7% to 34.8%. Delignification (6.56%) is low due to the limited solubility of lignin in acid medium, which is caused by the solubilization of acetic acid from hemicellulose [33,34].

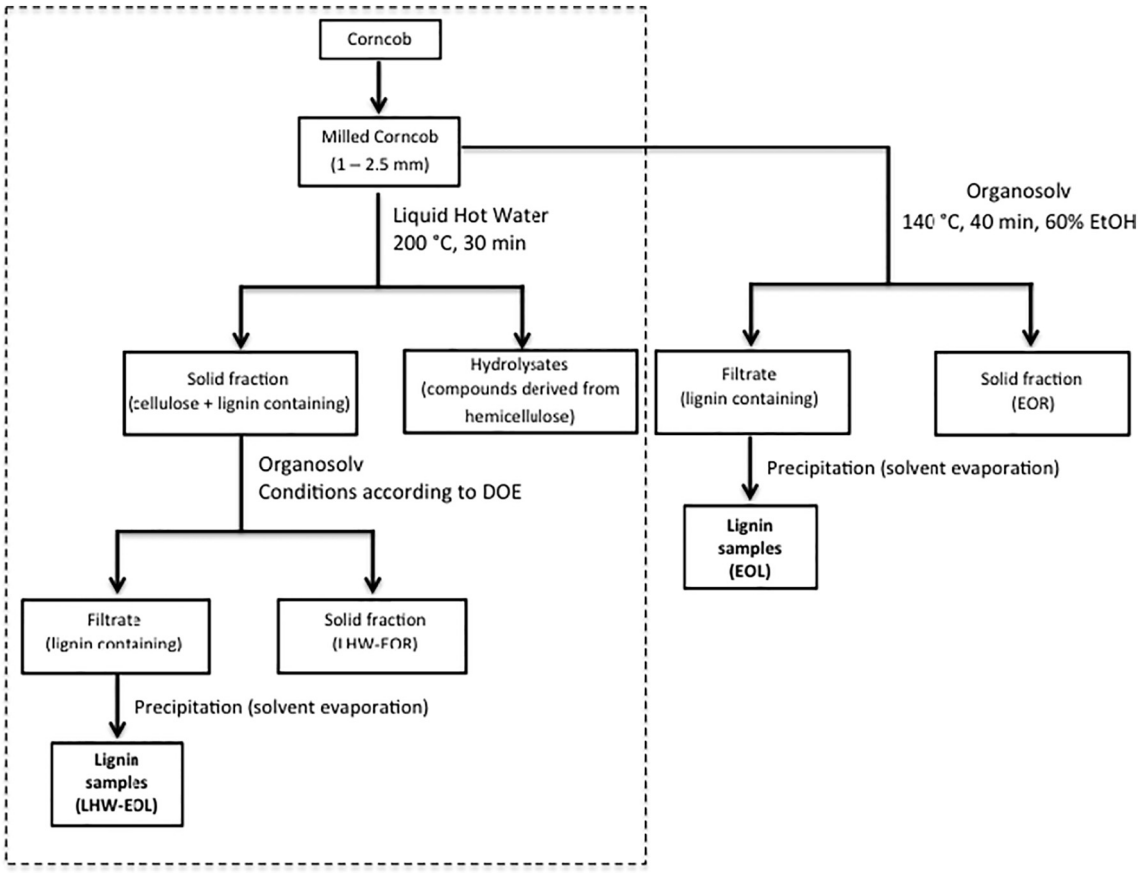

Fig. 1. The overall scheme of the integrated process (HP-EOL: hydrothermally pretreated ethanol organosolv residue; EOR: ethanol organosolv residue, HP-EOL: hydrothermally pretreated ethanol organosolv lignin; EOL: ethanol organosolv lignin).

Table 1
Chemical composition of raw and LHW-pretreated corncob, expressed as percentage on a dry weight basis and reported as average values (±standard deviation).

Components	Raw corncob % (w/w)	LHW-pretreated corncob % (w/w)
Solids (%)		
Cellulose[a]	35.84 ± 0.01	60.42 ± 0.85
Hemicellulose		
Xylan	22.51 ± 1.62	n.d.
Arabinan	5.60 ± 0.01	n.d.
Acetyl group	2.42 ± 0.21	n.d.
Lignin		
Klason lignin	18.00 ± 0.98	32.98 ± 0.22
Soluble lignin	2.66 ± 0.20	1.82 ± 0.04
Ashes	7.70 ± 0.30	0.78 ± 0.10
Protein	3.46 ± 0.01	
Hydrolysates (g/L)		
Oligosaccharides		
Gluco-oligosaccharides		1.24 ± 0.05
Xylo-oligosaccharides		n.d.
Arabino-oligosaccharides		n.d.
Acetyl groups-oligosaccharides		n.d.
Monosaccharides		
Glucose		1.32 ± 0.05
Xylose		5.65 ± 0.29
Arabinose		1.63 ± 0.11
Acetic acid		4.81 ± 0.23
Degradation products		
Hydroxymethylfurfural		0.53 ± 0.01
Furfural		7.69 ± 0.06

n.d.: not detected.
[a] Estimated from glucan content.

Gurgel et al. [20] have reported that soluble lignin tends to condensate in acid–catalyzed reactions, such as autocatalyzed pretreatments with longer times, leading to a more condensed lignin in the solid fraction. In addition, it has also been suggested that dissolved lignin-derived products with high molecular mass precipitate on the surface of the lignocellulosic residue during reactor cooling. Their results also showed increased glucan (from 43.43% to 66.53%) and lignin (from 22.00% to 30.02%) contents in the LHW-pretreated material, and a residual of hemicellulose in the solid fraction. Overall, delignification in LHW at 180 °C for 20 min reached 12.83%.

Romaní et al. [14] have also reported that increasing severity (*So*) and/or temperature results in lignin repolymerization. Therefore, the longer pretreatment time of our work may have led to more condensed lignin in the solid fraction and, consequently, to less delignification. The lower delignification in our work is interesting because the aim in this step (LHW pretreatment) was removing hemicellulose, that was successfully achieved, while preserving the cellulose and lignin fractions to be separated in the next step (organosolv pretreatment).

Ruiz et al. [15] reported a similar effect of autohydrolysis in wheat straw residue. Cellulose and lignin contents increased from 37.4% to 63.7% and 19.4 to 26.9%, respectively, due to the hemicellulose removal. However, unlike our work, not all hemicellulose has been removed, with 7.8% of the 33.8% of the raw material remaining at the end of the process. Romaní et al. [14] reported contents of cellulose and lignin for *Eucalyptus globulus* wood samples in the ranges 54.3–61.4% and 26.3–32.0%, respectively, after autohydrolysis. However, a small hemicellulose fraction also remained in the solid fraction (2.7–10.9%) and xylooligomers were the major hemicellulose-derived soluble components, with minor amounts of xylose and furfural.

Zhu et al. [16] reported that cellulose content increased from 42.7% to 54.1% after an autohydrolysis process, whereas the content of

hemicelluloses decreased from 19.8% to 3.2%, to form XOS and other byproducts. In that work, the autohydrolysis also resulted in an increase of the content of lignin (Klason lignin and acid-soluble lignin) from 21.1% (dewaxed residue) to 31.5% (hydrothermally treated residue). Finally, Wen et al. [18] studied bamboo culm. The carbohydrate analysis revealed that glucan (40.1%) and xylan (29.0%) were the dominant constituents in crude bamboo, while arabinans are only present in minor amounts. Lignin content was 26.7%. After autohydrolysis, the glucan and lignin contents increased to 53.0% and 37.0%, respectively, while the content of xylan decreased to 8.8%.

Therefore, the LHW condition of our work allowed a complete hemicellulose removal and good preservation of cellulose and lignin in the solid fraction to be treated in the next step.

3.2. Extraction of lignin

The spent solid recovered from LHW was submitted to an EO, where different variables were studied according to an experimental design. The results showed a significant delignification (around 57–62%) with a good glucan preservation (83–89%) for the solid residues (or cellulose pulp) of the samples 2, 4, 6 and 8 (Table 2). The high glucan contents of these solids are an advantage for their use as potential substrates for biofuel production after enzymatic hydrolysis. Under these conditions, the lowest amount of residual Klason lignin (around 15–17%) was observed. It is important to note that higher values of cellulose content on solid residues were attained for the highest ethanol concentrations (60%, v/v).

Regarding lignin (liquid samples), most of it was depolymerized and removed after the EO process. Maximum delignification of LHW-pretreated corncob (obtained for samples 2, 4, 6 and 8) led to a solubilization (lignin yield) of 16.7 to 18% of lignin (relative to the LHW-solids mass), that was recovered by precipitation (Table 3). Under these conditions, high purity lignin was recovered (lignin content of 87–93%), with an extraction efficiency between 50.6 and 54.6%.

Results showed that the extraction yield and the efficiency of extraction of lignin, as well as the yield and delignification of cellulose pulp, were significantly affected by ethanol concentration ($p < 0.05$). Therefore, the best compromise between lignin removal and cellulose preservation was obtained at a high ethanol concentration. This effect is supported by Pareto charts (Fig. 2) drawn from the experimental design. In the case of delignification (Fig. 2B), temperature (T) is also affecting the results.

Romaní et al. [14] recovered a solid phase from the integrated process of autohydrolysis and EO containing 70.3–86.9% cellulose and 10.2–22.7% lignin. However, a value ranging between 3.0 and 8.3% of xylan remained in the solid fraction, since autohydrolysis did not solubilize all hemicellulose, as mentioned previously. The authors also observed that higher severities (*So* and/or temperature) improved delignification up to a minimum of 10.2% of lignin in the solid residue, and that increasing *So* and/or temperature beyond these limits resulted in increased lignin, a fact ascribed to lignin repolymerization. Although Romaní et al. [14] reported a slightly smaller lignin content in the solid fraction, a small content of hemicellulose still remained in these fractions. Furthermore, no indicative of repolimerization of lignin was observed in our work under the studied organosolv conditions, that indicates that delignification can be improved. Ruiz et al. [15] also used the integrated process of autohydrolysis and EO (using 0.1% (w/v) NaOH as catalyst) and recovered solids with 75.9% cellulose, 16.2% lignin and 6.6% of residual hemicellulose. Therefore, even using a catalyst, a significant amount of lignin and hemicellulose remained in the solids, compared with our work.

An et al. [35] obtained similar yields (13.4–22.5%) for three lignin fractions obtained from a residue pretreated by steam explosion and enzymatic hydrolysis, and that was purified using a dioxane extraction process and dichloromethane fractionation. However, the concentration of Klason lignin in these fractions was lowered (73.6–83.7%), and showed a small carbohydrate content (6.6–14.1%) derived from hemicellulose and cellulose.

In order to evaluate the effect of LHW in the process, EO was applied to the crude corncob without using the hydrothermal process. Since the ethanol concentration was the main parameter that affected the evaluated responses, this variable was maximized for the new experiment, using the lowest temperature and time and thus reducing the extraction costs and making the process economically more feasible. So, the EO process of the crude corncob was performed at 140 °C, 40 min and 60% ethanol.

Organosolv applied to crude corncob recovered a solid residue (EOR) containing 51.47% cellulose, 31.86% hemicellulose, and 11.61% of residual Klason lignin. Therefore, mainly lignin was extracted from solids according to the chemical composition of the crude corncob. The delignification degree (42.26%) and the solid yield (89.52%) were similar to some samples from HP-EOR (except for samples 2, 4, 6 and 8). Regarding the EOL solubilized into the solvent, the lignin extraction yield was 10.48%, i.e. similar to some HP-EOL samples (except for samples 2, 4, 6 and 8). However, the extraction efficiency (58.22%) was as high as HP-EOL 2, 4, 6 and 8 samples, that were recovered by using high ethanol concentration. The solubilized lignin contained 10.32% of carbohydrates, while the lignin content (approx. 55%) was lower than HP-EOR (87–93%). In the LHW pretreatment the hemicellulose fraction of the corncob is removed, which makes the resulting lignocellulosic biomass

Table 2
Chemical composition of residual solids after delignification of the LHW-pretreated corncob.

Sample	Organosolv process conditions			Results organosolv process			
	T (°C)[a]	t (min)[a]	EtOH (%)[a]	Solid residue yield (%)	Cellulose (%)[b]	Residual lignin (%)[c]	D (%)[d]
1	140 (−1)	40 (−1)	20 (−1)	93.77	75.66 ± 2.46	23.43 ± 2.05	33.38
2	140 (−1)	40 (−1)	60 (+1)	82.52	82.72 ± 0.96	17.21 ± 1.76	56.94
3	140 (−1)	120 (+1)	20 (−1)	93.70	76.99 ± 3.27	22.67 ± 2.10	35.59
4	140 (−1)	120 (+1)	60 (+1)	83.30	83.00 ± 3.00	16.73 ± 1.21	57.74
5	180 (+1)	40 (−1)	20 (−1)	92.35	74.46 ± 0.76	21.29 ± 0.34	40.38
6	180 (+1)	40 (−1)	60 (+1)	81.99	82.53 ± 0.64	15.37 ± 1.34	61.79
7	180 (+1)	120 (+1)	20 (−1)	91.93	74.54 ± 1.50	22.51 ± 0.59	37.25
8	180 (+1)	120 (+1)	60 (+1)	82.19	88.62 ± 2.04	15.82 ± 0.20	60.57
9	160 (0)	80 (0)	40 (0)	89.59	78.95 ± 2.50	19.82 ± 2.99	46.16
10	160 (0)	80 (0)	40 (0)	89.63	77.69 ± 1.90	19.94 ± 3.78	45.81
11	160 (0)	80 (0)	40 (0)	89.23	77.89 ± 2.85	19.71 ± 3.49	46.67
EOL	140 (−1)	40 (−1)	60 (+1)	89.52	51.47 ± 3.50	11.61 ± 0.10	42.26

n.d.: not detected.
[a] Value of coded variable levels in parenthesis.
[b] Estimated from glucan content.
[c] Acid insoluble lignin.
[d] D: delignification.

Table 3
Chemical composition of precipitated lignin after organosolv process of the LHW-pretreated corncob.

Sample	Organosolv process conditions			Results organosolv process			
	T (°C)[a]	t (min)[a]	EtOH (%)[a]	Lignin yield (%)[b]	Lignin (%)[c]	Carbohydrate (%)[d]	Extraction efficiency (%)
1	140 (−1)	40 (−1)	20 (−1)	6.23	79.44 ± 2.46	n.d.	18.88
2	140 (−1)	40 (−1)	60 (+1)	17.48	91.79 ± 0.19	n.d.	53.00
3	140 (−1)	120 (+1)	20 (−1)	6.3	80.84 ± 3.27	n.d.	19.11
4	140 (−1)	120 (+1)	60 (+1)	16.7	87.15 ± 3.00	n.d.	50.62
5	180 (+1)	40 (−1)	20 (−1)	7.65	78.18 ± 0.76	1.66 ± 0.34	23.21
6	180 (+1)	40 (−1)	60 (+1)	18.01	86.66 ± 0.64	1.66 ± 1.34	54.61
7	180 (+1)	120 (+1)	20 (−1)	8.07	78.27 ± 1.50	2.22 ± 0.59	24.46
8	180 (+1)	120 (+1)	60 (+1)	17.81	93.05 ± 2.04	n.d.	53.99
9	160 (0)	80 (0)	40 (0)	10.41	82.90 ± 2.50	n.d.	31.56
10	160 (0)	80 (0)	40 (0)	10.37	81.57 ± 1.90	n.d.	31.43
11	160 (0)	80 (0)	40 (0)	10.77	81.78 ± 2.85	n.d.	32.64
EOL	140 (−1)	40 (−1)	60 (+1)	10.48	54.90 ± 11.18	10.51 ± 0.10	58.22

n.d.: not detected.
[a] Value of coded variable levels in parenthesis.
[b] Calculated by difference.
[c] Acid insoluble lignin.
[d] Only glucose was detected, with exception of EOL.

more accessible for delignifying solvents at mild conditions. However, in case of direct delignification, a certain pretreatment severity is required to remove hemicellulose before an effective delignification can occur.

Therefore, these results proved the effectiveness of an integrated process, mainly because the LHW process promoted the solubilization of hemicelluloses and cleavage of lignin–carbohydrate, improving lignin extraction and cellulose-rich residue preservation. Wen et al. [18] recovered lignin by an integrated process (hydrothermal treatment plus organosolv, HT-OL) and obtained an extraction efficiency of 62.2% (mentioned by the authors as lignin yield based on the Klason lignin), which was higher than the direct OL (31.5%), showing the advantages of the integrated process. After the organosolv process, glucan content on the solid residue significantly increased to 73.2%. However, if the organosolv process was directly applied to residue without autohydrolysis, the content of glucan increased slightly to 44.6%, while almost all xylan remained on solids, showing again advantage of the integrated process. Regarding the residual lignin on pretreated solids, if direct organosolv was applied to raw material, 21.7% from 26.7% of Klason lignin still remained in the pretreated material. However, if autohydrolysis was applied to residue prior to organosolv process, only 12.1% of Klason lignin was found in the pretreated material. According to the lignin yield obtained, it was found that autohydrolysis improved the organosolv process enhancing the delignification and recovering most of lignin (62.2%). Instead, the direct organosolv process recovered only a smaller part of lignin (31.5%).

Gurgel et al. [20] reported a significant delignification (≈82.46%) with a good preservation of glucan (≈84.58%) with the delignification of the LHW pretreated sugarcane bagasse (SCB) at 140 °C for 45 min. They compared their results to that reported by Pasquini et al. [36],

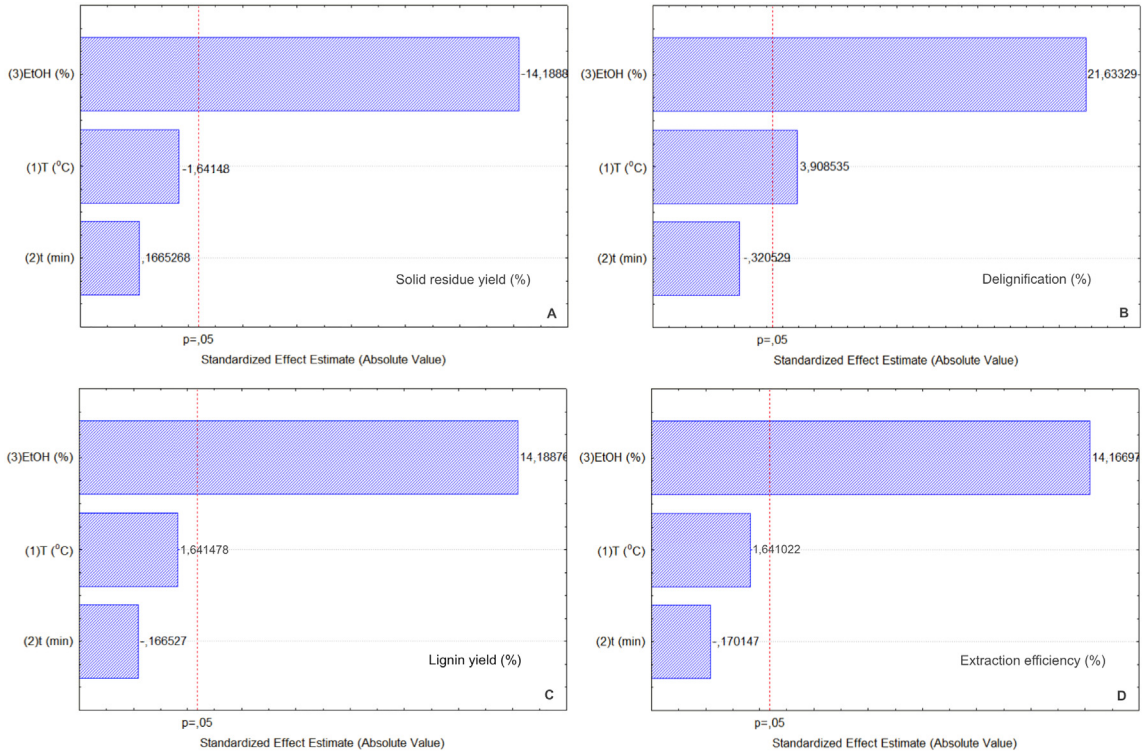

Fig. 2. Pareto diagrams of the responses from the cubic experimental design (2^3). *T* denotes the temperature, *t* the time and *EtOH* the ethanol concentration.

which studied the delignification of untreated SCB, and concluded that LHW pretreated SCB presented higher delignification at a lower temperature and a shorter reaction time than unpretreated SCB. This result shows the impact of LHW pretreatment conditions on the structure of lignin. In general, the results reported by other authors were similar to those of the present work, with the advantage of the complete removal of hemicellulose in our work.

3.3. Total phenolic content and antioxidant activities of lignins

Phenolic hydroxyl groups of lignin are the main responsible for its antioxidant activity [35]. The presence of phenolics and antioxidant activity can be evaluated by the total phenolic content and the DPPH and ABTS methods.

The total phenolic content of the HP-EOL samples was highly influenced by ethanol concentration, increasing for higher ethanol concentrations. This was the only condition tested that statistically influenced the total phenolic content values ($p < 0.05$). Ethanol leads to the depolymerization of lignin structure, that in turn enhances its solubilization into the solvent and the formation of new phenolic hydroxyl groups. Among all HP-EOL samples, samples 2, 4, 6 and 8 showed the highest total phenolic content which was up to 25.6 mg GAE/g, indicating that the total phenolic content of lignin was remarkably improved by ethanol concentration (60% w/v). Qazi et al. [37] reported total phenolic content values of pyrolytic Kraft lignins between 0.42 and 50 mg GAE/g. On the other hand, An et al. [35] achieved up to 246.13 mg GAE/g for lignin from corn straw recovered from a residue pretreated by steam explosion and enzymatic hydrolysis, and purified using a dioxane extraction process and dichloromethane fractionation. This higher phenolic content can be associated with the lignin fractionation that promotes the formation of new phenolic hydroxyl groups [38].

The antioxidant activity of the lignin samples was investigated in comparison with the commercial antioxidants BHT, BHA and Trolox, which were used as positive controls. The results of the DPPH• and ABTS•$^+$ assays are presented in Table 4 in terms of IC_{50} (the concentration of the tested antioxidant samples required for a 50% inhibition of radical species). The lower the IC_{50} value, the higher the RSA of the compounds tested.

All lignin samples presented antioxidant activity, with IC_{50} values ranging from 0.17 to 0.26 mg/mL in DPPH assay for HP-EOL samples and IC_{50} values of 0.91 mg/mL for EOL sample. This result confirmed that the lignin recovered by the integrated process achieved a remarkable improvement in its radical scavenging capacity. The commercial antioxidants Trolox, BHA and BHT present values of 0.07 mg/mL, 0.04 mg/mL and 0.16 mg/mL, respectively, showing a higher antioxidant potential than lignin samples. Nevertheless, some HP-EOL samples (7 and 10) presented IC_{50} values closer to the values obtained for BHT.

Table 4
Total phenolic and IC_{50} obtained by the DPPH and ABTS method of lignins, BHT, BHA and Trolox.

Assay number	Total phenolic (mg GAE/g)	IC_{50} (DPPH) (mg/mL)	IC_{50} (ABTS) (mg/mL)
1	7.2 ± 0.04	0.21	0.027
2	25.6 ± 0.22	0.21	0.022
3	7.8 ± 0.01	0.26	0.028
4	23.0 ± 0.36	0.20	0.022
5	8.7 ± 0.01	0.25	0.016
6	23.2 ± 0.06	0.22	0.016
7	9.6 ± 0.08	0.18	0.020
8	24.0 ± 0.12	0.23	0.023
9	14.7 ± 0.12	0.20	0.020
10	15.2 ± 0.19	0.17	0.018
11	17.0 ± 0.10	0.19	0.018
EOL	7.5 ± 0.04	0.91	0.092
BHT	–	0.16	0.019
BHA	–	0.04	0.005
Trolox	–	0.07	0.007

The antioxidant activity evaluated using ABTS presents a similar behavior.

Arshanitsa et al. [8] reported IC_{50} values of 0.04–0.05 mg/mL in DPPH assay and 0.009–0.01 mg/mL in ABTS assay for BIOLIGNIN™ (lignin extracted from wheat straw using a mixture of acetic acid/formic acid/water and further fractionation by successive extraction with dichloromethane, methanol and the mixture of methanol with dichloromethane). Lu et al. [7] reported IC_{50} values of 0.66 mg/mL (DPPH assay) for lignin from *Acanthopanax senticosus* residue, which was extracted using acetic acid–water organosolv pulping method; while Aguié-Béghin et al. [39] reported IC_{50} around 0.33 mg/mL (DPPH assay) for organosolv and alkali lignin samples. These differences in the antioxidant capacity of the lignin samples is related to the extraction method, as well as to the source of lignocellulosic material, as commented previously [11,12].

Fig. 3 shows DPPH and ABTS radical scavenging activities of commercial antioxidants (BHT, BHA and Trolox), EOL and a sample of HP-EOL, as example, since the behavior of all HP-EOL was similar. BHA and Trolox were the most potent antioxidants for both assays. However, HP-EOL (sample 7) showed similar RSA to BHT in DPPH assay and higher RSA than BHT in ABTS assay, which is often used as a standard. The same behavior was observed by Sun et al. [40] that also obtained lignins with antioxidant activity higher than BHT. The RSA against stable free ABTS•$^+$ radicals of HP-EOL (sample 7) was rather close to that of antioxidant BHA, indicating that the lignin sample has potential applications as commercial antioxidants.

EOL showed the lowest antioxidant potential, achieving maximal RSA of 49.3% and 58.8% for DPPH and ABTS assays using lignin concentrations of 1.0 and 0.1 mg/mL, respectively. This can be explained by the presence of carbohydrates (around 10.5% in the EOL sample), which can decrease antioxidant activity of lignin since their polar groups may form hydrogen bonds with lignin phenolic groups [41].

Michelin et al. [22] in a previous work, studied LHW and enzymatic hydrolysis (EH) as an integrated process to obtain lignocellulose derived products, including lignin. The IC_{50} of the EH lignins were 0.5–0.75 mg/mL (DPPH assay), i.e. higher than the HP-EOL of the current work and thus with lower antioxidant capacity; this was probably due to the carbohydrate content usually present in EH lignin. An et al. [35] described IC_{50} values from 0.06 to 0.15 mg/mL (DPPH assay) for lignin obtained from an integrated process, described previously. These lignins presented phenolics content of 155.4–246.3 mg GAE/g. However, in our study no relationship was found between total phenolic content and RSA among the lignin samples.

Despite several works on scavenging effects of lignin related compounds have shown that the free phenolic hydroxyl groups are crucial for lignin antioxidant activity, there are other groups that contribute to this activity (e.g. aliphatic hydroxyl group and methoxyl group). Methoxyl groups contained in lignin have been reported to act as a stabilizer for phenoxyl radicals formed during scavenging of free radicals [42,43]. On the other hand, the conjugated carbonyl group in the side chain has a negative effect on the antioxidant activity [5,8]. Moreover, it has been reported that the lignins with high molecular weight and polydispersity had low antioxidant activity [5,8,40,42], as well as the presence of carbohydrate may influence the potential of lignins as antioxidants, as commented previously, by decreasing the concentration of the reactive phenolic functions and by changing the polarity [8]. Therefore, several factors could be influencing the RSA of lignins.

3.4. Chemical structure of lignins

Lignins recovered from the integrated process of LHW-EO and the direct EO process were characterized by ATR-FTIR spectroscopy, being the corresponding spectra presented in Fig. 4. The spectra and the relative intensities of the bands were very similar, which confirmed that the "core" of the structure of lignin did not change significantly for the

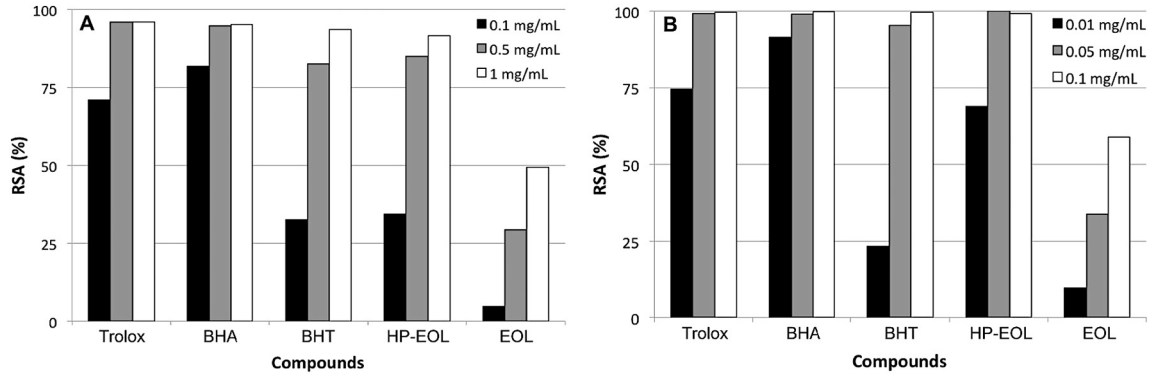

Fig. 3. Radical scavenging activities of lignin samples (HP-EOL – sample 7 and EOL) compared with commercial antioxidants (BHA, BHT and Trolox) for DPPH assay (A) and ABTS assay (B).

variable conditions of fractionation. However, there were some significant changes on functional groups. Bands were assigned as follows:

A wide absorption band around 3300 cm^{-1} is originated from the O—H stretch. The bands at 2940 and 2840 cm^{-1} arise from the C—H stretch in methyl and methylene groups [44]. These bands were less intense in 3, 4, 7, 8 and 9/10/11 lignin samples, in which the extraction time was longer (120 min), suggesting that methyl and methylene groups were removed or transformed into other chemical groups (for example —C— or C═O) in these lignins [45]. These bands occurred at 2924 cm^{-1} and 2850 cm^{-1} for 1, 2, 5, 6 and 12 lignin samples that were extracted with short extraction time (40 min). According to Boeriu et al. [46], bands in this region arise from the C—H stretch in aliphatic methylene groups that can originate from fatty acids present in the lignin samples.

The absorption at 1695 cm^{-1} is due to the C═O stretch in conjugated aldehydes and carboxylic acid groups [44]. Additionally, a small shoulder was observed at 1650 cm^{-1} for EOL, attributed to a C═O stretch in the conjugated carbonyl group [47]. Dizhbite et al. [5] reported that α carbonyl substitution in the propanoid chain decreases drastically the RSA of lignin, as commented previously. Thus, the conjugated carbonyl group in EOL was likely to cause the reduction in its antioxidant activity, discussed in the previous section.

The bands at around 1595, 1509, and 1427 cm^{-1}, corresponding to aromatic skeletal vibrations [44], indicate that the basic aromatic structure of the lignin was not severely disrupted during pretreatment. In EOL a more intense band at 1595 cm^{-1} was observed than in HP-EOL, indicating a lower disruption during the pretreatment. This is explained by the single pretreatment step used on EOL sample, whereas in HP-EOL the two pretreatment steps could have increased lignin disruption. Moreover, the vibration at around 1455 cm^{-1} in —CH_3 and —CH_2— groups was caused by asymmetric C—H deformations.

An aliphatic C—H stretch in CH_3 groups was observed at 1365 cm^{-1} [44]. Abdelkafi et al. [48] observed a stronger band at 1365 cm^{-1} in acetylated lignin preparations, suggesting that natural acetylation occurs in lignin samples extracted with neutral solvent. Phenolic OH groups are also shown by absorption bands at 1365 cm^{-1}, 1330 cm^{-1} and 1218 cm^{-1} [49]. The absence of some of these bands for EOL indicates a small phenolic OH groups content, that is consistent with the results of total phenolic and antioxidant activity. In the organosolv process β-O-4-linkages are cleaved generating both phenolic hydroxyl (1365 cm^{-1}) and carbonyl groups (around 1700 cm^{-1}) [49,50].

Several bands were attributed to syringyl (S) and guaiacyl (G) structures. Syringyl ring breathing with C—O stretching are seen at around 1328 cm^{-1}. Additionally, a typical infrared band of S structure at 1115 cm^{-1} was originated from aromatic C—H in-plane deformation. These bands were absent or less evident in EOL, suggesting a lower percentage of S unit in this lignin sample.

A typical G bands was observed at around 1260 cm^{-1} (G ring plus C═O stretch) for HP-EOL [44]. However, for EOL a more accentuated band was detected at 1250 cm^{-1}. The band at around 1030 cm^{-1} is attributed to aromatic C—H in-plane deformation vibrations (G > S), and this was bigger for EOL [44]. Bands in the region between 1000 and 1300 cm^{-1} are related to the carbohydrate content in lignin [46]. So, the bands at around 1250, 1160, 1115, 1030 cm^{-1} are an indicative of

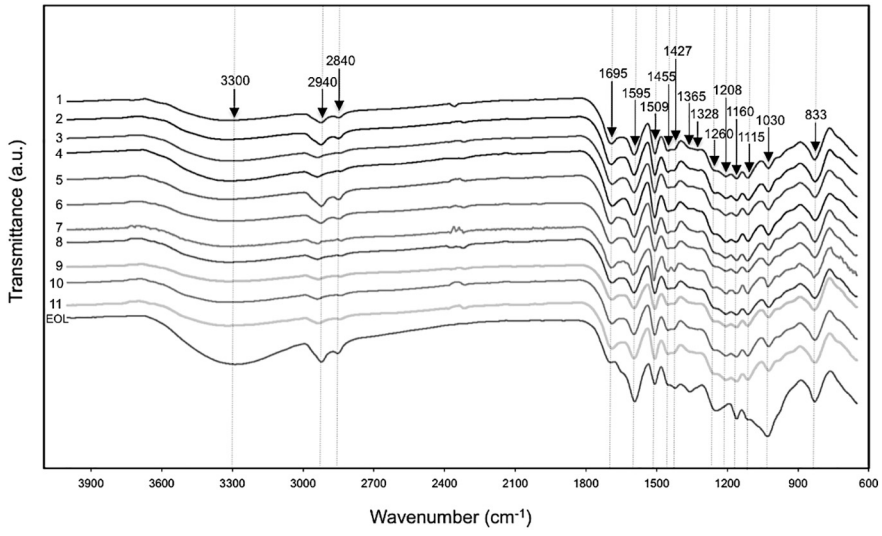

Fig. 4. ATR-FTIR spectra of hydrothermally pretreated ethanol organosolv lignin (HP-EOL - samples 1 to 11) and ethanol organosolv lignin (EOL) from corncob.

hemicelluloses [51–53]. Therefore, the influence of hemicellulose impurities on the spectral profile was more visible for EOL and is in agreement with the results of chemical composition of lignin on Table 3.

Another important spectral feature of HGS lignin is the intense band at around 833 cm^{-1} (C—H out-of-plane in positions 2 and 6 of S units, and in all position of H units) [44]. Moreover, the presence of the band at 1160 cm^{-1} (p coumaryl unit, H) always allows a clear assignment to the HGS type. This band was more evident in EOL than HP-EOL, confirming that the studied lignins are of the HGS type. On the other hand, An et al. [35] reported that the increasing intensity of signal at 1160 cm^{-1} revealed that the lignin from corn straw with higher molecular weight had a high content of H unit. They suggested that during the pretreatment, lignin fragments with H units were easier to condense and to form lignin with high molecular weight. So, the higher intensity band at 1160 cm^{-1} for EOL suggests higher molecular weight for EOL than HP-EOL. Additionally, the band at around 1160 cm^{-1} indicates C═O vibrations in conjugated ester groups [44].

3.5. Thermal degradation of lignins

The thermal degradation of lignin can be affected by its inherent structure, degrees of branching and condensation and functional groups [54]. So, in order to understand the relationship between the structural and thermal properties of the extracted lignins, TGA was applied to study the thermal properties of HP-EOL, as well as to EOL.

The thermal decomposition of lignin is a complex process and happens slowly over a wide temperature range, with the most extensive mass loss (40–50% of the dried sample) in temperatures ranging from 230 to 500 °C for LWH-EOL and from 175 to 540 °C for EOL. This occurs because the complex structure of lignin, composed of phenolic hydroxyl, benzylic hydroxyl, and carbonyl groups, have different thermal stability, and then events occurring at different temperatures [55].

Several stages of weight-loss can be observed in the DTG curves. Initially, a small weight loss (<3% of weight loss) was firstly observed below 100 °C, which is justified by the residual moisture present in the lignin samples. The weight loss related to lignin degradation started at around 230–253 °C (T_{onset}) for HP-EOL and 175 °C for EOL. This fact, in the latter case, can be related to a small presence of hemicelluloses that decomposes at lower temperatures (see Table 3). Two main degradation events (DTG curve) were detected during the lignin decomposition, that occurred at 301–319 °C (peak 1) and 344–378 °C (peak 2) for HP-EOL; and at 198 °C (peak 1) and 282 (peak 2) for EOL.

According to Laurichesse and Avérous [56], the decomposition of the lignin structure starts at relatively low temperatures, i.e., 150–275 °C, as observed in our work. It is thought that the first decomposition step is due to the dehydration of the hydroxyl groups located in the benzyl group. Between 150 and 300 °C occurs the cleavage of α- and β aryl alkyl ether linkages. Aliphatic side chains start splitting off from the aromatic ring at around 300 °C, while at 370–400 °C occurs the carbon carbon cleavage between lignin structural units.

Finally, at temperatures >500 °C, the typical weight losses in TG curves flatten out, with a slow release of the volatile products (such as CO, CO_2, CH_4, H_2), before the formation of (30–50%) char [56,57]. In our study, 40% lignin samples remained unvolatilized at 800 °C (char residue), due to the formation of highly condensed aromatic structures. The decomposition temperature strongly depends on the molecular structure of lignin. In general, the pretreatment has a significant effect on the thermal stability of the produced lignin products, which can explain the main differences between HP-EOL and EOL. Table 5 summarizes the main events occurred during thermogravimetric analysis.

Representative TG and DTG curves of HP-EOL and EOL are presented on Fig. 5, which are in good agreement with the thermogravimetric behavior verified in other works [51,58–60].

3.6. Glass transition of lignins

DSC is the most common technique used to determine glass transition temperatures (T_g), as well as the thermal behavior of polymers. The T_g is correlated to the viscoelastic behavior of amorphous polymers [61,62]. At temperatures below the T_g the materials are hard and glassy. This hardness decreases in the region of transition and the material shows a viscous or rubbery state as the temperature increases [49].

Lignin behaves as an amorphous thermoplastic material, exhibiting a T_g that varies widely depending on the extraction method, water content, molecular weight, chemical modification, and thermal history [56,63]. It is an important parameter to know when considering the use of lignins in polymer applications, but it is often difficult to determine due to the broad heterogeneity of the lignin structure and molecular weight [64].

Different underivatized lignin preparations are reported to have T_g values between 90 and 180 °C [65,66], corresponding the lower values to organosolv lignins and the higher ones usually to softwood kraft lignins [58,65,67]. HP-EOL have shown T_g values in the range from 60 to 90 °C (Table 5), i.e. in general, the HP-EOL presented lower T_g values than the ones reported for organosolv lignins.

According to Kubo and Kadla [68], the variations in T_g values for the lignins illustrate mainly the variations in their chemical structure, which can be correlated to the degree of crosslinking, the variations in the flexibility of the polymeric chains, and the amount of impurities. Structurally, it has been reported that lignin samples with a high S and low G content would exhibit low T_g; and lignin samples with low T_g have less charred residues [69]. Still, organosolv lignins have a more oxidized structure, a relatively higher amount of phenolic hydroxyl groups, high purity, low T_g and are easy to thermally process [64,67].

Additionally, the extraction methods also have shown a great influence on the final structure and properties of lignin [64]. For example,

Table 5
Values of glass transition temperature (T_g), maximum thermal decomposition temperature at DTG curve (T_{peak}), extrapolated initial decomposition temperature (T_{onset}) and unvolatilized weight fraction at 800 °C (char residue) for HP-EOL and EOL.

Sample	T_g (°C)	T_{peak1} (°C)	T_{peak2} (°C)	T_{onset} (°C)	Maximal weigh loss (%)	$Char_{800}$ (%)
1	75.3 ± 1.25	301.0	343.6	234.6	44.9	41.2
2	90.3 ± 4.33	306.6	348.3	241.0	42.2	42.6
3	86.3 ± 0.29	301.4	361.9	240.1	41.4	42.2
4	60.3 ± 1.74	306.4	362.1	238.9	43.1	40.0
5	64.1 ± 0.33	302.2	367.8	227.5	44.8	39.3
6	75.2 ± 0.76	304.7	361.5	250.6	48.7	39.3
7	68.0 ± 0.62	313.8	366.4	241.4	47.4	39.1
8	78.2 ± 0.30	318.5	374.2	252.7	46.0	41.3
9	62.9 ± 1.17	306.4	377.5	239.1	44.5	40.9
10	63.9 ± 1.34	311.5	366.4	243.6	44.1	39.9
11	60.3 ± 1.74	309.1	381.0	241.0	44.6	41.2
EOL	n.d.	198.6	281.9	175.0	51.2	39.7

n.d. not detected.

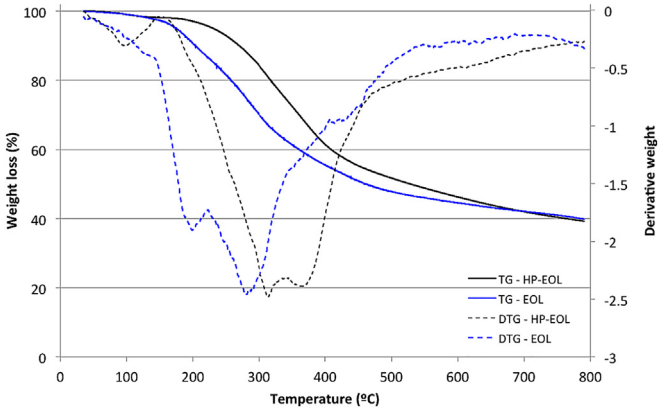

Fig. 5. TG (solid lines) and DTG (dotted lines) curves of hydrothermally pretreated ethanol organosolv lignin (HP-EOL – sample 7) and ethanol organosolv lignin (EOL) from corncob.

Huijgen et al. [70] have reported organosolv lignins with T_g between 87 and 133 °C, where the high process temperature (i.e. high severity) seems to reduce the T_g. Therefore, the lower T_g observed for HP-EOL can be related to the integrated process, since a high severity HP pretreatment step was performed previously the organosolv process.

Other authors also reported low T_g for the studied lignins. For example, Hansen et al. [49] reported T_g value of 75 °C for LigSteam (resulted from an acid steam explosion process using wheat straw as raw material); and Luong et al. [59] have reported T_g values 46 °C for lignin-SKKU. It was not possible to determine the T_g value for EOL in DSC curve. So, it was not possible to correlate the structure and extraction methods of HP-EOL and EOL based on the T_g values.

4. Conclusions

The combination of the LHW and EO as an integrated process, allowed the complete removal of hemicellulose in the first step and the recovery of solids with a high cellulose content (60.4%) and lignin (33%). The use of organosolv process on LHW-pretreated corncob recovered fractions with >60% delignification, good glucan preservation (almost 90%) in the solid residues (or cellulose pulp) and lignin with high purity (>90%). On the other hand, the EO process of untreated corncob recovered a lignin sample (approx. 55%) with 10.32% carbohydrate, and a solid fraction with a high hemicellulose content (51.47% cellulose, 31.86% hemicellulose, and 11.61% of Klason lignin). Therefore, an effective separation of the three components was achieved on the integrated process. FTIR and TGA analyses proved that the different extraction strategies had great influence on lignin structure and cellulose pulp. Lignin antioxidant capacity indicated the lignin obtained by integrated process had the highest antioxidant activity, that was similar to some commercial antioxidants. This finding opens the possibility for lignin application in several industrial sectors, including food and pharmaceutical.

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