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Fine-tune of lignin properties by its fractionation with a sequential organic solvent extraction

Nagore Izaguirre^a, Eduardo Robles^b, Rodrigo Llano-Ponte^a, Jalel Labidi^a, Xabier Erdocia^{c,*}

^a Biorefinery Processes Research Group, Chemical & Environmental Engineering Department, Faculty of Engineering, Gipuzkoa, University of the Basque Country UPV/EHU, Plaza Europa 1, 20018 Donostia, Spain

^b University of Pau and the Adour Region, E2S UPPA, CNRS, Institute of Analytical and Physicochemical Sciences for the Environment and Materials (IPREM-UMR 5254), 371 Rue du Ruisseau, 40004 Mont de Marsan, France

^c Biorefinery Processes Research Group, Department of Applied Mathematics, University of the Basque Country UPV/EHU, Rafael Moreno "Pichichi" 3, 48013, Bilbao, Spain

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ABSTRACT

In this work, different lignins were obtained from two different extraction methods (kraft and organosolv) but from the same raw material (*Eucalyptus globulus* sp.). They were subsequently fractionated to determine the differences of each extraction method and their corresponding physicochemical properties found in fractionation sequence and obtained fractions. The goal of the fractionation was to obtain lignin fractions with narrower molecular weight distribution and lower polydispersity index (PI). The solvent sequence was designed based on the environmental friendly properties, health and safety assessments of the selected organic solvents: (methanol (MeOH), ethanol (EtOH), propan-2-one (DMK), ethyl acetate (EtOAc), propan-1-ol (nPrOH), propan-2-ol (iPrOH), butan-2-one (MEK), and butan-1-ol (tBuOH)). The different fractions obtained were characterised to determine their chemical structure by several analytical techniques, such as Fourier Transformed Infrared Spectroscopy (FTIR), Ultraviolet (UV), Phosphorus-31 Nuclear Magnetic Resonance (³¹P NMR), Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS), Thermogravimetric analysis (TGA), and Differential scanning calorimetry (DSC). In addition, Gel Permeation Chromatography (GPC) was used to obtain the molecular weight distribution. This study showed an effective method for obtaining homogeneous lignins with specific structures and properties depending on the solvent and molecular weight attained. Moreover, the method designed was found to be effective regardless of the lignin extraction process employed; besides, various lignin fractions were obtained which were different from each other, having specific target applications depending on their structure and chemical properties, ranging from small molecules with abundant reactive sites to act as active materials or copolymer reagents for many applications, to larger and more inactive molecules with higher thermal resistivity.

1. Introduction

The design of novel green methods for lignin extraction has gained a vast interest in recent years in contrast to the traditional delignification process used in the Pulp & Paper industry, such as the kraft process. In this frame, the organosolv method is the most widely used green method due to its simplicity and solvent recyclability. In addition, the obtained lignin reaches high phenolic content, good solubility in common organic solvents, and low glass transition temperature. Nevertheless, it is heterogeneous, with a high standard deviation in key parameters such as molecular weight and total phenolic content (Ramakoti et al., 2019).

The different technical lignins currently found in the market depend

on the feedstock and extraction method (Matsushita, 2015). In this sense, kraft lignin (KL) usually presents lower molecular weight than organosolv lignin (OL) and lower than original lignin found in nature, caused by the high-temperature alkaline conditions employed in the kraft pulping process (Melro et al., 2018a). As a result, KL appears with a molecular weight of 200–200000 g/mol, a considerable amount of carbohydrates, and 1–3% sulfur content (Lora and Glasser, 2002). Most of the KL produced, around 40–50 million tons per year, is used for energy recovery inside the pulp mill, while an excess remains unused (Wenger et al., 2020). This remaining liquor can be implemented in a wide range of applications such as dispersants, blends, and composites, as well as aromatic compounds precursor (Dessbesell et al., 2020),

* Corresponding author.

E-mail address: xabier.erdocia@ehu.eus (X. Erdocia).

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contributing to sustainable practices that their fossil-based analogs hamper. In the organosolv process, instead of alkaline conditions, organic solvents are used for lignin extraction. The use of organosolv fractionation in modern lignocellulosic biorefineries has been widely studied since it allows operating with green solvents (González et al., 2008). Moreover, it has the potential to become an optimised process for the obtaining of high-quality lignin with many attractive characteristics, such as high purity, due to the lack of sulfur content and low ash content (Mesa et al., 2011).

Different approaches have been developed to overcome the heterogeneity of lignin, a common drawback in both lignin extraction methods—the main ones being acid precipitation, solvent fractionation, and membrane fractionation processes (Gigli and Crestini, 2020). Fractionation using organic solvents is an utterly customizable method, where solvents (or solvent mixtures), fractionation step numbers, and anti-solvents used can be adjusted to specific needs or aims (Pang et al., 2021). Works where industrial lignins fractionated in single solvent processes (Li and McDonald, 2014a), spruce and eucalyptus kraft lignins fractionated by multiple organic solvents (Tagami et al., 2019), wheat straw organosolv lignin, wheat straw soda lignin, and softwood kraft lignin fractionated with different proportions of acetone/water mixtures (Domínguez-Robles et al., 2018) have been reported, for instance. The sequential organic solvent extraction (SOSE) method is the most suitable process for obtaining narrower molecular weight fractions (Park et al., 2018). The method designed by Park et al. used a sequence of different organic solvents with different polarities to separate lignin in different fractions with specific characteristics. The capacity of the organic solvent to solubilise lignin depends on the hydrogen bonding capacity, which increases with short aliphatic hydrocarbon side chains and more hydrogen bond donor sites (Melro et al., 2018b). Moreover, solvents mixed with water show better solubility than pure solvents, leading to increased hydrogen bonding capacity and facilitating the diffusion of organic solvents into lignin (Domínguez-Robles et al., 2018). Nevertheless, the addition of water represents another variable, affecting the properties of the fractions obtained and the unappealing formation of azeotropes, which may also affect the enticement of the process in the economic aspect (Ajao et al., 2019).

Consequently, to avoid the issues mentioned, it was decided that all the organic solvents chosen for this method would be used at high purity, having lower boiling points than water, and therefore, being more accessible to recycle (Alfonsi et al., 2008). Furthermore, these solvents were selected using the 'Environmental Health and Safety (EHS) method,' only choosing the solvents considered 'green,' as this work aims to obtain homogenous lignin fractions through a sustainable method (Dessbesell et al., 2020).

This work aimed to obtain fractions of different molecular weights and chemical characteristics, observing the impact of the lignin extraction method (KL and OL) and the solvent used in the final lignin properties obtained for each fraction. For designing an effective SOSE method, an initial screening of the green organic solvents was performed, where solubilities and obtained fraction yields of each solvent were analysed individually. After initial trials, a decision on which solvents would be implemented in the process was reached. The sequence was arranged according to the dissolution performance of the solvent, starting from the lowest dissolution performance and finishing with the highest capacity. Different physicochemical methods were employed to analyse the fractions obtained, and characteristic structures and properties of the different fractions were identified. While there are abundant works on lignin solubilisation, none of them addresses the influence of the delignification process on the SOSE method. For that reason, two types of lignin (kraft and organosolv) derived from the same wood species (*Eucalyptus sp.*) were compared, and the effects the extraction methods had on the SOSE process were identified and analysed to assess a method able to be implemented in a wide variety of lignins.

2. Materials and methods

2.1. Materials

Eucalyptus chips and kraft liquor were kindly supplied by Papelera Zikuñaga (Hernani, Gipuzkoa). Characterisation of the raw material was done using a mixture of toluene (108–88–3, 99%, Fisher) and ethanol (64–17–5, absolute, Scharlab) for the extractives. A solution of H₂SO₄ 72% (v/v) prepared from concentrated H₂SO₄ (7664–93–9, 96% technical grade, PanReac) was used for the quantitative acid hydrolysis (QAH); acetic acid glacial (64–19–7, technical grade, PanReac) and NaClO₂ (7758–19–2, 25% w/w, PanReac) were used for the holocellulose determination; NaOH (1310–73–2, for analysis, CS, ISO, PanReac) was used to prepare solutions with the concentrations 17.5% and 8.3% (w/w) and the acetic acid used before to prepare a solution of 10% (v/v) for cellulose determination.

For the organosolv lignin extraction, the same ethanol absolute and oxalic acid (144–62–7, 98%, anhydrous, Acros Organics) were used. For the precipitation of kraft and organosolv lignin, H₂SO₄ 96% was used. Dimethylformamide (68–12–2, 99.5%, HPLC grade, Fisher) and lithium bromide (7550–35–8, PanReac) were used for the GPC; Pyridine (613–002–00–7, anhydrous, 99.8%, Sigma-Aldrich, Germany), chloroform-d (865–49–6, "100%", 99.96 atom % D, Aldrich, USA), chromium (III) acetylacetonate (Cr(acac)₃) (21679–31–2, 97%, Aldrich, USA), N-hydroxy-5-norbornene-2,3-dicarboxylic acid imide (NHND) (21715–90–2, 97%, Aldrich, China) and 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) (14812–59–0, 95%, Sigma-Aldrich, India) were used for the ³¹P NMR. Dimethyl sulfoxide (67–68–5, ACS, PanReac), Na₂CO₃ (497–19–8, 99.8%, Scharlab), Folin-Ciocalteu reagent (12111–13–6, Scharlab) and dioxane (123–91–1, >99.8%, Fisher) were used for the ultraviolet measurements. The organic solvents used for the fractionation of lignin were methanol (67–56–1, >=99.8%, Fisher), ethanol (64–17–5, absolute, Scharlab), propan-2-one (67–64–1, technical, Scharlab), ethyl acetate (141–78–6, >=99.5%, Fisher), propan-1-ol (71–23–8, >99.5%, Alfa Aesar), propan-2-ol (67–63–0, extra pure, Scharlab), butan-2-one (78–93–3, 99 + %, Alfa Aesar) and butan-1-ol (71–36–3, 99% extra pure, Acros Organics).

2.2. Characterisation of raw material

Eucalyptus chips were characterised according to the TAPPI standardised methods. Moisture (T 412 om-16, 2016), ash (TAPPI T 211 om-16, 2016), solvent extractives (TAPPI T 204 cm-07, 2007) (using toluene instead of benzene), acid-insoluble and soluble lignin (TAPPI T 222 om-11, 2011), and hemicellulose and cellulose (Wise et al., 1946) content determinations were carried out. Briefly, for the moisture content 2.00 ± 0.01 g of sample were weighed and dried at 105 °C for 24 h. For the ash content, 1.00 ± 0.01 g of sample were heated at 525 °C for 3 h. For the extractives, 4.00 ± 0.1 g of sample were introduced in a cellulose cartridge, assembled in a Soxhlet extraction apparatus, and the extractives were solubilised in 150 mL toluene/ethanol mixture (2:1 v/v ratio), in multiple cycles for a maximum of 6 h period. The extractives were dried at 105 °C for 24 h.

QAH determined acid-insoluble lignin. 0.25 ± 0.001 g of sample were heated at 30 °C for 1 h with 72% H₂SO₄. The solution was transferred to autoclave tubes and diluted to around 3% H₂SO₄. The bottles were autoclaved at 121 °C for 1 h, and the solid was filtered and washed until neutral pH. The liquid obtained was used for the acid-soluble lignin determination. The liquid was diluted using 1 M H₂SO₄, and absorbance values obtained at 205 nm by UV–vis spectrophotometry were recorded. For the holocellulose determination, 2.5 ± 0.1 g of extractive free sample were dissolved in 80 mL hot water and introduced in a bath at 70 °C. Hourly additions of 0.5 mL acetic acid glacial and 2.6 mL of 25% (w/w) NaClO₂ were completed during 6–8 h, and the solution was maintained on the bath overnight before filtering it. 2.0 ± 0.1 g of the holocellulose obtained was used for the cellulose determination. 10 mL

of 17.5% (w/w) NaOH were added, and 3 more additions of 5 mL of the same solutions were made every 5 min. Finally, 33 mL of deionised water were added, and the solution was left to settle for 1 h before filtering. The solid was washed by adding 100 mL of 8.3% (w/w) NaOH and 2 more additions of 100 mL of hot deionised water. The fibres were then covered using 15 mL of 10% (v/v) acetic acid for 3 min and washed with water until neutral pH. The hemicellulose content was determined by the subtraction of cellulose from the holocellulose.

2.3. Organosolv lignin extraction

Organosolv lignin was extracted from eucalyptus chips using an organosolv method, as described in previous works (Alfonsi et al., 2008; Gordobil et al., 2016). The reaction conditions were selected as optimal, being 90 min at 180 °C, with a solid to liquid ratio of 1:7 (w/w), using ethanol/H₂O 50/50 (v/v) solvent and 50 mmol/L of oxalic acid as catalyst. Once the reaction was finished, the liquor was separated from the delignified solid by filtration. Then, this delignified material was washed first with the same ethanol/water mixture employed in the lignin extraction and next with deionised water until neutral pH, and it was further characterised. The extraction was conducted inside a 1.5 L stainless steel Parr Series 5100 pressure reactor with temperature and pressure control (4836 Controller).

2.4. Characterisation of kraft and organosolv liquor

Kraft liquor provided by Papelera Zikuñaga and organosolv liquor were characterised before carrying out the lignin precipitation. pH was determined using a PH BASIC 20 m. Density was determined gravimetrically. Total solid content and inorganic content were determined using TAPPI T650 (T 650 om-15, 2015) and TAPPI T211 (TAPPI T 211 om-16, 2016) standards, respectively, while organic content was determined as the difference between the total solid content and inorganic content.

2.5. Obtaining of kraft and organosolv lignins

Kraft and organosolv lignins were obtained by reducing the pH of the black liquors. Kraft lignin was precipitated by adding dropwise concentrated H₂SO₄ (96%) until the pH of the liquor reached a value of 2. Organosolv lignin was obtained by adding twice as much water to the liquor, acidified at pH 2 with H₂SO₄ (96%). Precipitated lignins settled overnight before being filtrated with a 0.45 µm nylon filter paper and neutralised. The filtrated lignins were dried at room temperature.

2.6. Selection of the organic solvents and order for the fractionation

Methanol (MeOH), ethanol (EtOH), propan-2-one (DMK), ethyl acetate (EtOAc), propan-1-ol (nPrOH), propan-2-ol (iPrOH), butan-2-one (MEK), and butan-1-ol (tBuOH) were selected as suitable green solvents for the process (Ajao et al., 2019). These eight solvents were tried separately, and solubilisation yield of lignins and molecular weights of the solubilised lignin fractions were determined. 1 g of lignin was dissolved in 10 mL of solvent and left stirring for 2 h. After that, the mixture was filtered using a nylon filter with a pore size of 0.22 µm. The insoluble fraction was dried at 50 °C overnight, and the soluble fraction was concentrated with a rotary evaporator below atmospheric pressure and dried in an oven at 50 °C for two days.

The selection and order of the organic solvents were decided according to the results obtained in this section. The solvents selected provided lignin fractions with different molecular weights and yields. In the case where various fractions had tangible similarities (similar solubilisation yield and molecular weight), the most environmentally friendly solvent was chosen. Therefore, only four solvents were retained. For the decision of the order, the solvents were employed in increasing order of dissolving capacity, so the solvent with lowest lignin dissolving

capacity was employed first and the solvent with the highest capacity was used the last.

2.7. Fractionation of lignins

The sequential organic solvent extraction started with the solvent with the lowest solubility capacity. It was followed using solvents that sequentially increased their solubility capacity. 5 g of lignin was solubilised with 100 mL of solvent, left stirring for 2 h, and filtered using a nylon filter of 0.22 µm. The insoluble fraction was dried in the oven at 50 °C overnight, the soluble fraction was rotavaporated to remove the solvent in 75%, and the remaining was precipitated using acidified water (H₂SO₄ at pH 2). The precipitated lignin (the soluble fraction) was filtered the following day using a 0.22 µm nylon filter membrane. Fig. 1.

2.8. Characterisation of obtained lignin fractions

2.8.1. Quantitative acid hydrolysis (QAH), char and ash content

Quantitative acid hydrolysis (Sluiter et al., 2012) was performed on all lignin samples to determine the acid-insoluble lignin, also known as klason lignin. The procedure followed was the same as for the raw material characterisation.

TGA was used to determine the char content. A TGA/SDTA RSI analyser of Mettler Toledo was used, heating 5–10 mg of lignin sample from 25 °C to 800 °C, at a rate of 10 °C/min in N₂ atmosphere. Once the sample reached that temperature, the atmosphere was changed to air, and ash content was calculated.

2.8.2. Fourier transformed infrared spectroscopy (FTIR)

FTIR spectra were recorded to observe the chemical structure of the lignins and identify their characteristic functional groups. The wavenumber range was set from 4000 to 600 cm⁻¹, with a resolution of 4 cm⁻¹ and 64 scans.

2.8.3. Gel permeation chromatography (GPC)

Gel Permeation Chromatography determined molecular weight and polydispersity index of lignins. A Jasco LC-Net II/ADC device is equipped with a RI-2031 Plus Intelligent refractive index detector, two PolarGel-M columns (300 mm × 7.5 mm) placed in series PolarGel-M guard (50 mm 7.5 mm) was used. 20 µL of 50 ppm lignin solution was injected for each measurement. The solvent of the solution was dimethylformamide with 0.1% of lithium bromide, also used as the mobile phase of the column. The column eluded the mobile phase at 40 °C and a flow rate of 0.7 mL/min. With molecular weights ranging from 266 to 70,000 g/mol, polystyrene was used as the standard for the calibration curve.

2.8.4. ³¹P NMR spectroscopy

³¹P NMR was used for the quantitative determination of hydroxyl groups and the S/G ratio. Pyridine and deuterated chloroform were used as a solvent in a ratio of 1.6:1 (v/v). 50 mg of each lignin sample were solubilised in 0.5 mL of the solvent, and 0.1 mL of internal standard was added to each sample. The Internal Standard solution was prepared, adding 5 mg of Cr(acac)₃ and 18 mg of NHND per 1 mL. Once the samples were dissolved, 0.1 mL of TMDP was added to carry on the phosphorylation. ³¹P NMR spectra were recorded in a Bruker AVANCE 500 MHz, using the parameters recommended by Meng et al. (2019), and MestReNova 11.0 was used for the data processing.

Specific spectral regions can be associated with different hydroxyl types: 144–137 ppm corresponds to the total phenolic OH content (from 144 to 141.5 ppm are C5-substituted OH groups, that are considered equivalent to S unit OH groups, from 140.5 to 139 ppm are G unit OH groups, and from 139 to 137 ppm are H unit OH groups), 150–145 ppm range corresponds to aliphatic OH groups and 136–134 ppm to carboxylic acid OH groups. The integrated areas of these regions were compared with the standard internal signal, and the concentrations of

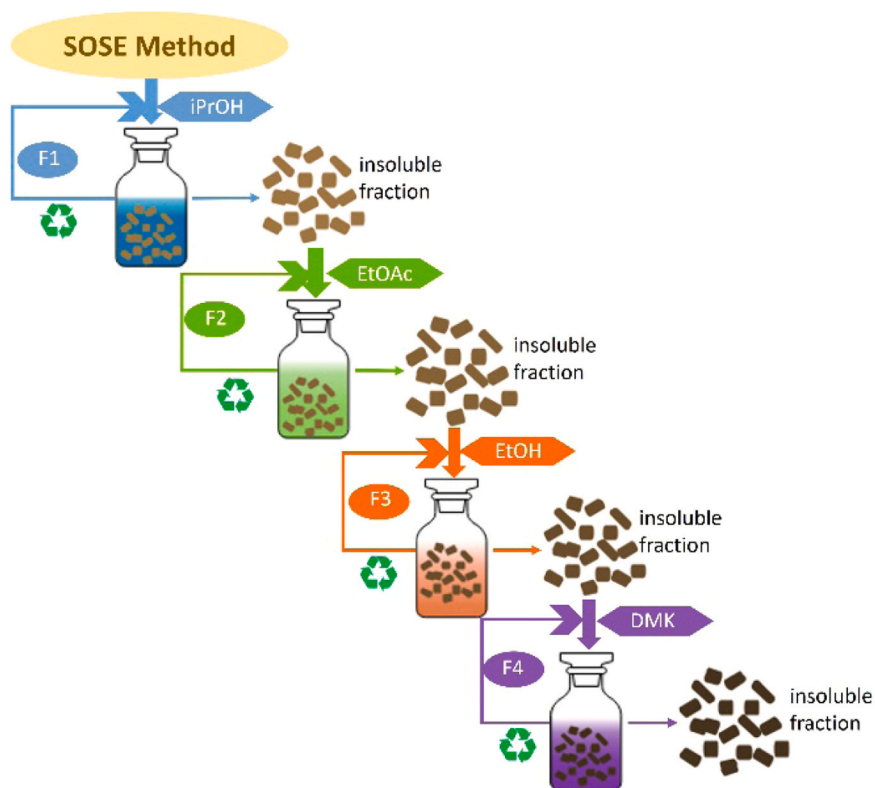


Fig. 1. SOSE method for both kraft and organosolv lignins.

the OH groups were quantified.

2.8.5. UV-vis spectrophotometry

UV-vis spectrophotometry (Jasco V-630 spectrophotometer) was used to determine the conjugated and non-conjugated OH groups following the method proposed by Yuan et al. (2009). Briefly, 5 mg of lignin was dissolved in 10 mL of dioxane/H₂O 95% (v/v). Next, 1 mL of that solution was diluted in 10 mL of dioxane/H₂O 50% (v/v). The final solvent was analysed by measuring the absorbance values in the range of 400–260 nm.

2.8.6. Analysis of pyrolysis products

The gases from the pyrolysis of the original lignin samples and their fractions were analysed. For this, a gas chromatograph coupled to a mass spectrometer (Agilent Techs. Inc. 6890 GC/5973 MSD), a 5150 Pyroprobe filament pyrolyser (CDS Analytical Inc., Oxford, PA), and an Equity-1701 (30 m × 0.20 mm × 0.25 μm) fused-silica capillary column were used. The method followed was described by Herrera et al. (2014). The samples were introduced in a quartz crucible and heated with a 20 °C/ms (ramp-off) heating rate until reaching 600 °C and maintained for 15 s while keeping the interface at 260 °C. Helium gas was used to provide inert conditions for the purge of the pyrolysates from the pyrolysis interface to the gas chromatograph injector. The oven program started at 50 °C, was held for 2 min, and raised to 120 °C at 10 °C/min. Next, it was held for 5 min. After that, it was raised to 280 °C at 10 °C/min, held for 8 min before finally raising it to 300 °C at 10 °C/min and held for 10 min. For identifying the compounds, the obtained mass spectra were compared to those reported by the National Institute of Standards Library (NIST Number 69) and other compounds reported in the literature (Del Río et al., 2005; Fernández-Rodríguez et al., 2017; Li and McDonald, 2014a; Vane, 2003).

2.8.7. Thermal properties

Kraft lignin, organosolv lignin, and their fractions were thermally

analysed using TGA and DSC. For the first, a TGA/SDTA RSI analyser (Mettler Toledo) was used, heating 5–10 mg of lignin sample from 25 °C to 800 °C, at a rate of 10 °C/min in an inert atmosphere, from which the main thermal degradation parameters were identified.

Differential Scanning Calorimetry was performed using a DSC822e (Mettler Toledo). The process consisted of heating from –25–225 °C at 10 °C/min, followed by cooling to –25 °C at the same rate. Finally, a second heating to 225 °C was done with the same parameters as the first one. This scan was used for the determination of the glass transition temperature (T_g) of each sample.

2.8.8. Correlation between different properties

The relationship between known interconnected properties of lignin was assessed by the Pearson correlation coefficient between two variables, and linear or asymptotic fits where required by using OriginPro v2020 software.

3. Results and discussion

3.1. Characterisation of eucalyptus (E) and delignified eucalyptus (DE)

Eucalyptus chips were characterised both before and after the organosolv delignification process. In the results obtained, a considerable reduction of the lignin content can be observed, this being the objective of the process. The lignin loss resulted in a variation of the remaining compounds, notably the cellulose, which was enriched considerably. The results are illustrated in Fig. 2; percentages of each compound are represented on a dry base (KL and OL moisture contents are 7.72% and 7.45%, respectively) and are comparable to the ones obtained by other authors in similar conditions (Pan et al., 2005; Dong et al., 2019).

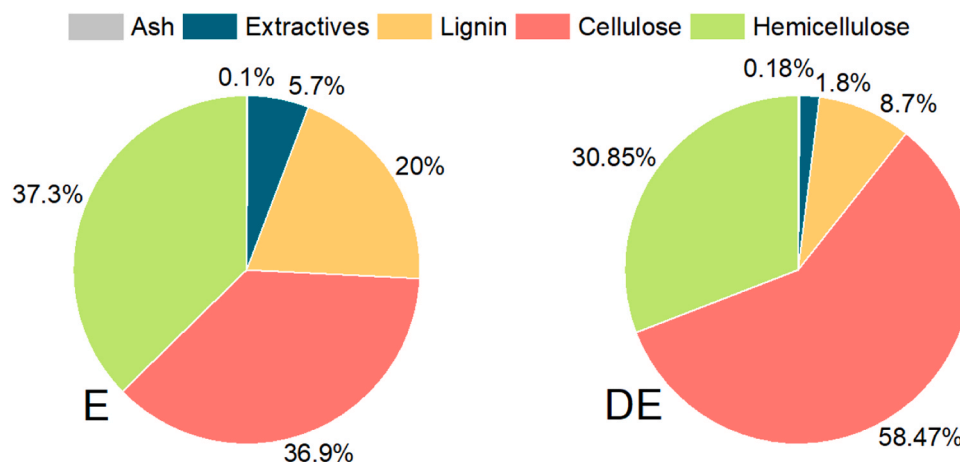


Fig. 2. Composition of the eucalyptus fibres before and after organosolv treatment.

3.2. Characterisation of kraft (K) and organosolv (O) liquors

The properties of each liquor are listed in Table 1. Significant differences between the two liquors could be observed. Overall, kraft liquor was richer in solid content, especially in the inorganic content. This is due to the harsh conditions in which the paper industry extracts lignin, using compounds like NaOH and Na₂S, forming different residual inorganic compounds. The main difference of the delignification processes is the solvent used, consequently varying the pH of the liquors: kraft liquor has a basic medium, while organosolv lignin has a slightly acidic medium. For this reason, the extracted lignins present structural and chemical differences.

3.3. Yield and molecular weight determination of the initial screening of the solvents for kraft and organosolv lignin solubilisations

Before the SOSE design, different green organic solvents were tested to observe how the molecular structures of these solvents influenced lignin solubilisation. For that, eight different solvents were chosen: one ester, two ketones, and five alcohols (three primaries, two secondaries, and one tertiary); the selected solvents, as well as some of their main properties, are shown in Table S1.

Fig. 3 presents the solubilisation yield and the molecular weight of the solubilised fraction for all the selected solvents. It can be observed that the solubility yields obtained with kraft lignin were higher in all solvents than those for organosolv lignin. This is probably due to the lower molecular weight of the lignin, which was nearly half as big as the organosolv lignin (3167 and 5800 g/mol). The molecular weights of the solvent fractions maintained a similar tendency, especially in the case of EtOH, EtOAc, nPrOH, iPrOH, MEK, and tBuOH, were Mw values fluctuated identically in KL and OL.

The contrary happened with the yields obtained from those solvents. The quantities recovered from KL were significant, while those values diminished drastically for OL fractions. That might be attributed to the PI of the lignins. KL was smaller and more homogeneous than OL (PI of 2.91 and 4.44, respectively), making it more soluble in different solvents. OL, however, was larger and more diverse, leading to molecules varying in size and making those bigger molecules more selective to be solubilised. That was the reason of obtaining lower yields with OL than

with KL for the solvents with lower lignin solubility capacity. MeOH and DMK were the solvents with the highest solubility yields and highest molecular weights in both lignin types. Even though these two solvents are not constituted by the same functional group (MeOH is a primary alcohol while MEK is a ketone), they are formed by a similar branch, a methyl group. Since it is the smallest alkane branch, the functional groups of the solvents were highly accessible to dissolve small lignin molecules and the bigger ones.

EtOH and MEK presented a similar behaviour and composition as to MeOH and DMK. EtOH is a primary alcohol and MEK a ketone, and they are branched with ethyl groups. Since EtOH and MEK have longer branches than MeOH and DMK, it was slightly harder to solubilise the bigger lignin molecules, so the yields and Mw obtained were considerably lower. The remaining solvents were EtOAc, nPrOH, iPrOH, and tBuOH. These are more impeded than the solvent mentioned before. Apart from being more branched, the functional groups are also different. Even though three of them are alcohols (nPrOH, iPrOH, and tBuOH), they are not the same type of alcohol since nPrOH is primary, iPrOH is secondary tBuOH is tertiary.

Furthermore, EtOAc is an ester, so the polarities of these four solvents are different, and the order goes nPrOH>iPrOH>tBuOH>EtOAc. However, there was no apparent relation between the polarity of the solvent and the yield or molecular weight of the fraction obtained. Therefore, it can be concluded that instead of the polarity, the factor affecting most lignin solubilisation is the molecular size of the solvent, being the most effective the smallest in size, which is more easily accessed to the large macromolecule that is lignin. Therefore, the solvents and their order in the sequential fractioning were decided to perform the greenest method possible and to obtain the most different but homogeneous fractions by considering the yields and molecular weights. Consequently, it was decided that iPrOH, EtOAc, EtOH, and DMK were the most suitable solvents for the process, due to the good yields obtained that minimise the loss of material in the process, as well as for their different properties, that ensure the obtaining of different lignin fractions.

3.4. Yields of the sequential organic solvent extraction

Fig. 4 presents the yields of the sequential fractioning. It can be seen

Table 1

Properties of the industrial kraft liquor (K) and laboratory organosolv liquor (O).

	Density (g/cm ³)	pH	Total solid content (%)	Organic content (%)	Inorganic content (%)	Dry lignin content (g/L)
K	1.09 ± 0.01	13.02 ± 0.03	15.49 ± 2.94	8.55 ± 0.82	8.74 ± 0.00	41.67 ± 5.65
O	0.95 ± 0.01	4.02 ± 0.08	5.57 ± 0.21	5.54 ± 1.17	0.04 ± 0.15	29.11 ± 2.92

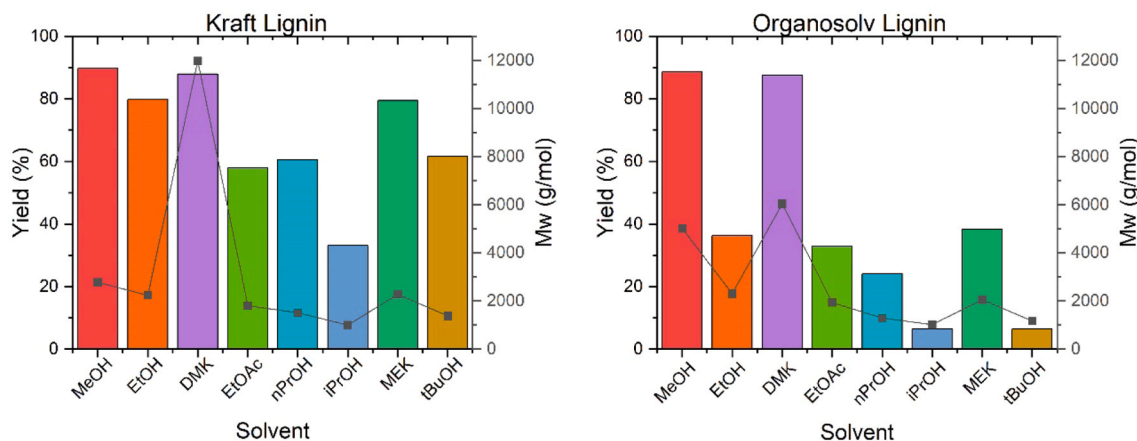


Fig. 3. Solubilisation yields and molecular weight for each lignin type.

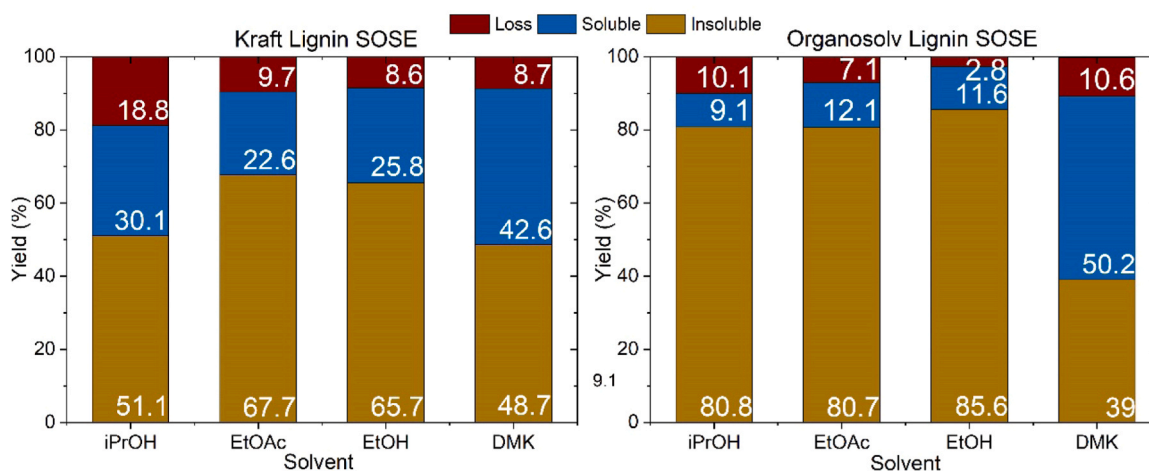


Fig. 4. Sequential solvent extraction yields.

that kraft lignin was more solubilised than organosolv lignin, as it was also observed during the solvent screening, probably due to the lower molecular weights. However, the loss during the process was also more significant with kraft lignin. Overall, the solubilisation percentages remained similar in the first three steps of the sequential extraction.

Nevertheless, the solubilised yield considerably increased in the last step, where DMK was used. Similar to the results from the solvent screening, DMK solubilised the most considerable amount of lignin.

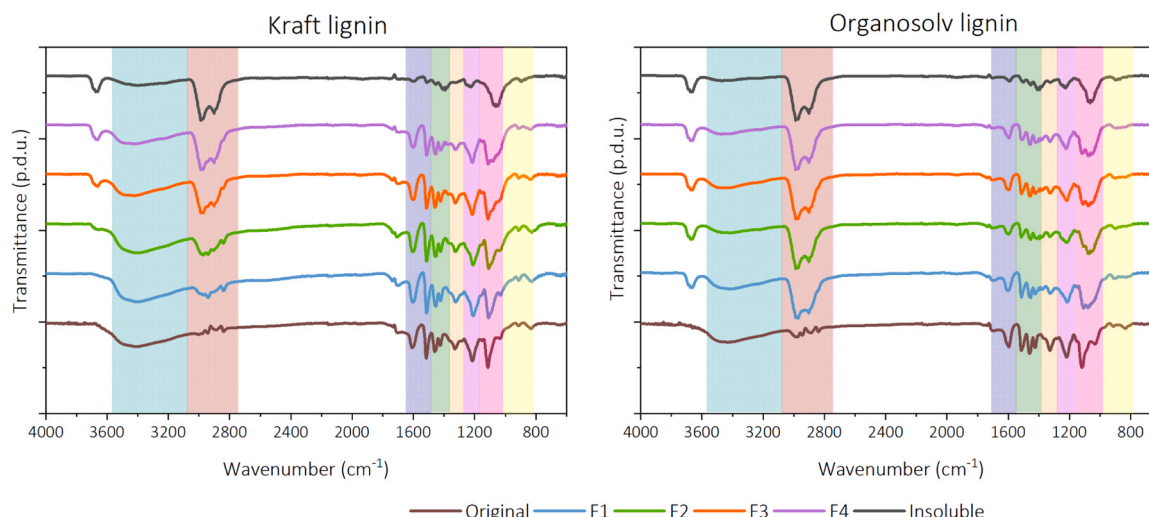


Fig. 5. Infrared spectra of kraft and organosolv lignins and their fractions.

3.5. FTIR spectra

The infrared spectra of kraft lignin, organosolv lignin, and their fractions are shown in Fig. 5, reflecting the common and particular structural features and functional groups of the molecules. The first band observed in the spectra is the broad band at 3400 cm^{-1} , attributed to the O-H stretching of aliphatic hydroxyl groups. It can be observed in both lignin types that the sequential fractionation process reduces the amount of this functional group, being nearly inexistent in the insoluble fraction. The band at 2900 cm^{-1} is attributed to the C-H stretching of methoxyl, methyl and methylene groups. In the original kraft and organosolv lignins, these bands are considerably less intense than in their fractions. In fractionated kraft lignin, the intensity increases as the fraction number increases, while for the fractionated organosolv lignin, this difference, although visible, is not as severe. The increase in the intensity of this band is that as the fractionation process advances, the solubilised molecules have greater molecular weight as a consequence of the more abundant molecules with side chains with methyl and methylene groups (Alekhina et al., 2015). This is also visible from the results obtained in Py-GC/MS, where the abundance of pyrolysis-derived products from branched molecules followed the same trend. Bands between 1600 and 1420 cm^{-1} are attributed to the vibration and deformation bands of the C-H groups forming the aromatic rings. Bands at 1325 cm^{-1} correspond to the S ring, while bands at 1260 cm^{-1} correspond to the G ring. It can be seen that these bands decrease at each step of the cascade fractioning, even though the fluctuation of the S/G ratio is not evident.

3.6. Molecular weight and chemical characterisation of SOSE fractions

Molecular weight and polydispersity index were also calculated for the fractions obtained in the SOSE. These values, along with their chemical characterisation values, are collected in Table 2. Some noticeable differences could be observed from the values in Table S2. For example, the first fraction, soluble in iPrOH, had similar Mw and PI values (Table S2). However, from the second step forward, molecular weight values differed due to the removals of the fractions solubilised in the previous steps, which affected the overall values. In this sense, the second fraction (EtOAc) had a decrease of $\approx 25\%$ in the Mw for KL and $\approx 30\%$ for OL, the third fraction (EtOH) had a decrease of $\approx 9\%$ for KL and $\approx 15\%$ for OL, and the fourth fraction (DMK) had a decrease $\approx 60\%$ for KL and $\approx 24\%$ for OL. However, the insoluble fractions were similar in both lignins (14514 KL and 14255 for OL).

Overall, it can be observed that regardless of the origin of the lignins, where kraft lignin had a considerably smaller molecular weight than organosolv lignin (3167 g/mol against 5800 g/mol), the fractions obtained in each fractionation step were very similar. K1 and O1 fractions obtained from the solubilisation of original lignins with iPrOH, the simplest secondary alcohol, were very similar, 951 and 1036 g/mol. The second fractions obtained were solubilised with EtOAc, the ester of ethanol and acetic acid, had molecular weights between 1310 and 1341 g/mol, respectively. The trend of having similar molecular weights

Table 2
Molecular weights and purity assessment of the different lignins.

	Mw (g/mol)	PI	AIL (%)	ASL (%)	Ash (%)
KL	3167	2.91	73.99 ± 2.24	19.00 ± 0.77	7.01
KL F1	951	1.85	81.64 ± 1.93	14.04 ± 2.95	4.32
KL F2	1310	2.14	75.75 ± 5.95	18.44 ± 2.49	5.81
KL F3	1893	2.39	87.72 ± 0.63	9.14 ± 0.34	3.14
KL F4	4682	3.50	88.68 ± 0.73	8.24 ± 0.25	3.08
KL FI	14514	4.43	83.54 ± 2.59	12.66 ± 0.61	3.80
OL	5800	4.44	90.91 ± 0.82	6.23 ± 0.67	2.86
OL F1	1036	1.61	84.01 ± 2.05	13.79 ± 0.56	2.20
OL F2	1341	1.81	81.42 ± 0.06	17.23 ± 4.01	1.35
OL F3	2096	2.47	85.38 ± 2.00	12.26 ± 0.96	2.36
OL F4	4569	3.08	82.00 ± 2.45	14.71 ± 1.31	3.29
OL FI	14255	3.82	89.02 ± 6.00	7.90 ± 0.54	3.08

in the same fractions followed throughout the whole process. The fractions obtained in the third step (EtOH, primary alcohol) had molecular weights of 1893 and 2096 g/mol, and the fourth step fractions corresponding to DMK had molecular weights of 4682 and 4569 g/mol. Consequently, the SOSE process successfully obtained homogeneous fractions, whether these were from kraft or organosolv lignin. The polydispersity index increased as the Mw of the fraction raised because the solvent used had higher solubility capacities and, therefore, could solubilise a broader range of molecules.

As for the Acid Soluble and Insoluble Lignin (ASL and AIL), OL was highly pure due to the much milder isolation process of the lignin (AIL, also known as Klason lignin, content of 90.91%) compared to the KL (AIL of 73.99%). As for the ASL content, it had considerably less content than KL due to the purer process from which it was obtained (Gordobil et al., 2016). The higher purity on the initial lignin affected to the fractions obtained and their yield. The very high purity of OL (90.91%) prevents its purification and all the obtained OL fractions have similar or lower purity than the initial lignin. However, the SOSE process led to purer fractions of KL, increasing around 10% the purity of most fractions.

Regarding the yields of the fractions recovered which were depicted in Fig. 4, it could be observed that they were considerably smaller with OL, especially on the initial fractions, which is related to the higher molecular weight of this type of lignin. The lignins with high molecular weight have lower solubility so, at first, OL was less soluble than KL. Nonetheless, the fractionation led to more homogeneous Mw lignins with lower PI which occurred for both types of lignin confirming that the SOSE process acts equally regardless of the initial lignin nature.

3.7. ^{31}P Nuclear magnetic resonance

One of the most prominent lignin properties is associated with its hydroxyl content, affecting solubility and reactivity. Consequently, the quantitative determination of the different OH groups present in the molecules is of great importance. To do so, one of the most precise and advanced methods is the quantitative analysis of ^{31}P NMR spectra. Different peaks are associated with different hydroxyl groups, and their integration, compared with the integration of the internal standards, provided their concentrations. Concentrations of aliphatic OH, C5-substituted OH, guaiacyl OH, p-hydroxyphenyl OH, and carboxylic acid OH groups were obtained in mmol/L.

Moreover, the S/G ratio was also calculated by dividing C5-substituted OH groups (considered syringyl OH groups due to signal overlap issues) by guaiacyl OH groups. The results are listed in Table 3, and the spectra are represented in Fig. S1 and Fig. S2.

It was confirmed that the lignins came from hardwood due to the lack of p-hydroxyphenyl OH units, only present in softwoods and grasses. S/G ratios obtained were above one, which is also another characteristic of hardwood lignin (Del Río et al., 2005). It can be observed that even though the raw material used for both lignin types was the same, there were significant differences in the concentrations of hydroxyl groups.

The overall hydroxyl content of organosolv lignin was more abundant, especially in aliphatic OH groups, three times bigger than in kraft lignin. However, the concentration of C5-substituted OH groups was smaller while maintaining similar guaiacyl OH content and not finding any carboxylic acid hydroxyl groups. Consequently, the S/G ratio was also smaller. In terms of the influence exerted by the extraction method on the fractions obtained, a different trend was detected for kraft and organosolv lignin.

The first soluble kraft lignin fraction (iPrOH) had more than twice hydroxyl groups than the original lignin. However, the most noticeable difference happened with C5-substituted OH groups, which increased almost three times and reached a high concentration. Nevertheless, the S/G ratio decreased due to the increase of G units. The second fraction obtained (EtOAc), on the contrary, had a fewer amount of hydroxyl

Table 3
Hydroxyl types and S/G ratio as obtained from ³¹P NMR.

	OH type					S/G ratio
	Aliphatic	C5-substituted	Guaiacyl	p-Hydroxyphenyl	Carboxylic acid	
KL	0.43	1.37	0.25	–	0.21	5.39
KL F1	0.63	3.73	0.82	–	0.55	4.55
KL F2	0.19	1.38	0.40	–	0.19	3.44
KL F3	1.76	3.73	1.28	–	0.34	2.92
KL F4	1.42	2.86	1.24	–	0.31	2.30
KL F1	1.37	0.94	0.43	–	0.10	2.18
OL	1.44	0.98	0.32	–	–	3.08
OL F1	1.41	3.03	1.34	–	0.09	2.27
OL F2	1.52	2.90	0.81	0.05	0.10	3.59
OL F3	1.76	2.69	0.85	–	0.12	3.17
OL F4	2.50	1.89	0.85	–	0.06	2.21
OL F1	3.56	1.11	0.36	–	0.04	1.70

groups than kraft lignin itself. This might be because EtOAC does not have a high solubility capacity, but another reason might be that since being an ester, it does not have free OH groups, and consequently, the fractions solubilised do present fewer OH groups accessible. The fraction obtained with the third solvent (EtOH) followed a similar trend to the first fraction, where all types of OH increased in their abundance. The most significant rise was with the guaiacyl OH groups, reaching four times its initial concentration, followed by aliphatic OH groups. C5-substituted OH groups reached the maximum amount of all the fractions obtained, 3.73 mmol/L, yet the S/G ratio decreased since G units increased more than S units in proportion.

The last fraction obtained (DMK) also increased its OH content. Apart from slightly increasing the carboxylic acid content and increasing the aliphatic content, S and G units also increased. G units increased four

times their initial content, while S units doubled, so the S/G ratio decreased, obtaining the smallest ratio of all the soluble fractions. In terms of the insoluble fraction remaining after the sequential extraction, it was noticeable that it was most abundant in aliphatic hydroxyl content, even though the phenolic hydroxyl content was not that far from that of the initial lignin.

In terms of the organosolv lignin and its solubilised fractions, it could be seen that overall, the total OH content was higher than in kraft lignin. However, the phenolic OH content was similar or even lower than in kraft lignin, except for the second soluble fractions, since with kraft lignin, phenolic hydroxyl content was the lowest out of all the kraft fractions, and with organosolv lignin, the content maintained similarly to its other fractions.

The difference in aliphatic OH groups in organosolv lignin fractions

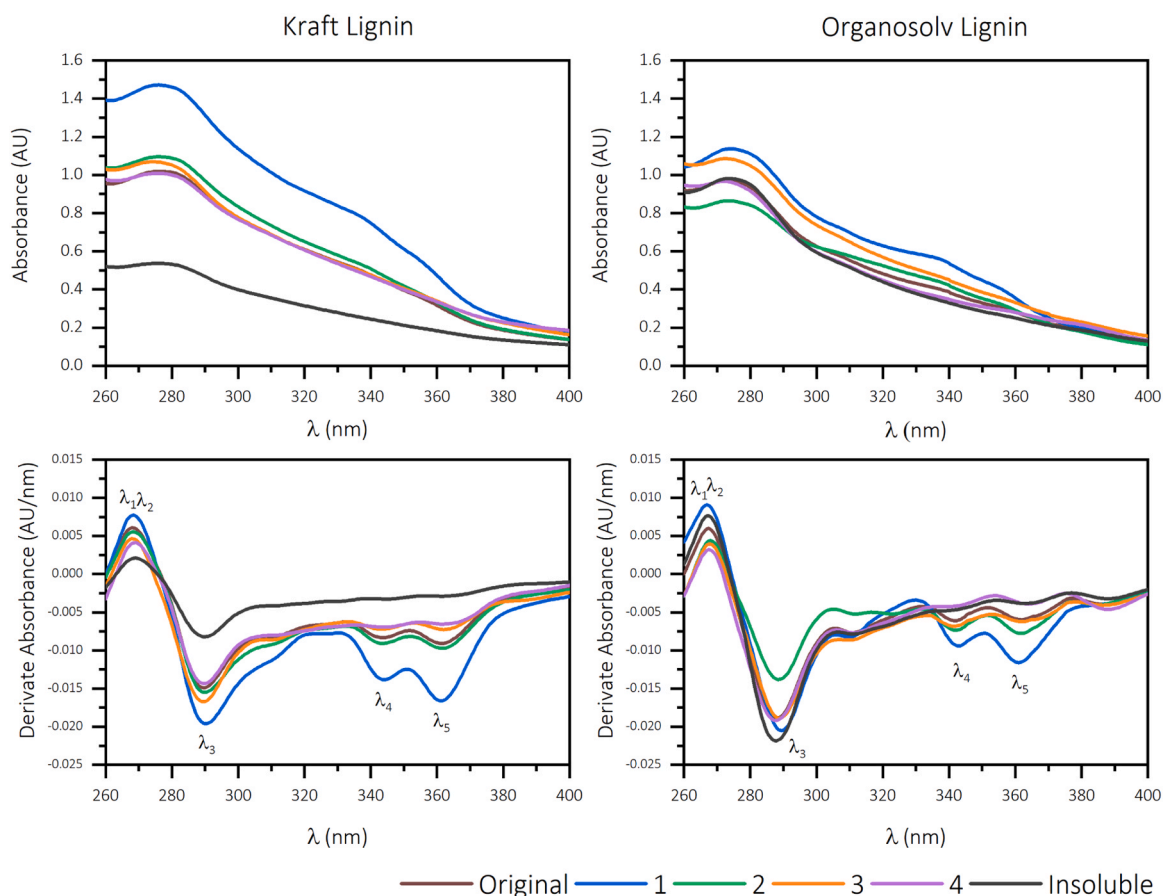


Fig. 6. UV-vis spectra of the different lignins, above absorption spectra; below, derivate absorption spectra.

was not significant, except for the last soluble fraction and the insoluble fraction. In terms of the phenolic OH content, soluble fractions more than doubled the amount of those groups compared to the original lignin, the first fraction having the highest concentration of both the C5-substituted OH groups but especially guaiacyl OH groups.

3.8. Conjugated and non-conjugated OH groups

A UV scan between 400 nm and 260 nm was performed to identify conjugated and non-conjugated OH groups. Phenolic structures can be divided into six structural types, as suggested by Gärtner et al. (1999a). The unconjugated phenolic structures are estimated using the maxima at 300 and 350–360 nm and conjugated structures obtained from the maximum at 350–370 nm (Gärtner et al., 1999b).

Fig. 6 presents the UV–vis absorption spectra (top) and the derivative absorption spectra (down), used to identify the real position of peaks and resolve the overlapping peaks, as derivative curves are more structured than the original spectra (Bosch Ojeda and Sanchez Rojas, 2013). In this sense, there are no striking differences between the original kraft and organosolv lignins, with most differences occurring in their fractions. The identified derivative peaks, which correspond with the main inflections of the spectra, are listed in Tables S4 and S5. The peaks, present in all samples, were identified by their λ_{\max} , and the main absorption shifts are discussed below.

There are two phenomena to take into account, the first is the intensity of the peaks at the regions mentioned above, and the second is the difference in the absorption wavelengths, as absorption at a longer wavelength (bathochromic shift) may be due to the effect of an increased degree of conjugation (Faleva et al., 2021). In kraft lignin, the higher intensities are those of K1, both in non-conjugated peaks (λ_1 and λ_2) and in the conjugated peak (λ_5). In the conjugated spectrum (350–370 nm), it can be observed that most fractions from kraft lignins were isochromic, except the K3 fraction, which had a bathochromic shift. Nevertheless, also a hyperchromic shift, with the hyperchromic trend followed the pattern KLF1 >KLF2 >KL>KLF3 >KLF4 >KLF1, thus meaning that the conjugated absorption was hypochromic for KLF3, KLF4, and KLF1.

In organosolv lignin, the OLF1 fraction had a hypochromic shift while the insoluble fraction had a bathochromic shift, the rest having the conjugated spectrum isochromic. On the other hand, the intensity of the absorption at the conjugated spectrum followed the trend OLF1 >OLF2 >OLF3 >OL>OLF4 >OLF1.

3.9. Py-GC/MS

The pyrolysis of samples was performed, and the gaseous products were analysed to quantify the syringyl, guaiacyl, hydroxyphenyl units of lignin, and the carbohydrate derivatives of each lignin sample and its fractions. The percentages of identified lignin, S, G, and H units and S/G ratios are listed in Table 4.

Table 4
Percentages of identified lignin, S, G, and H units and S/G ratio.

	Lignin identified (%)	S units (%)	G units (%)	H units (%)	S/G ratio
KL	74.55	51.00	44.90	4.11	1.14
KL F1	92.39	50.89	44.88	4.23	1.13
KLF2	98.25	55.07	42.11	2.82	1.31
KL F3	92.59	53.74	42.31	3.95	1.27
KL F4	92.79	48.15	45.62	6.24	1.06
KL F1	69.09	31.94	43.06	25.00	0.74
OL	91.50	54.88	42.33	2.80	1.30
OL F1	91.97	50.83	44.59	4.58	1.14
OLF2	92.69	48.75	46.39	4.86	1.05
OL F3	87.48	62.94	34.02	3.04	1.85
OL F4	89.35	57.61	39.50	2.88	1.46
OL F1	90.81	47.61	46.17	6.22	1.03

There are some differences between the results obtained by Py-GC/MS and those of ^{31}P NMR. The most noticeable difference was the content of the H groups. From the ^{31}P NMR spectra obtained, only O2 contained H units. Nevertheless, in the results from Py-GC/MS, all the fractions show some H groups, even though this was the least abundant unit. This can be explained by the nature of the analysis, as being Py-GC/MS a destructive method, potential confusion on the identification of the pyrolysis products might lead to an increase of H units. In addition, there are identifiable differences regarding the S and G units, even though they are subtle. For kraft fractions, the content of S units followed the trend KLF2 >KLF3 >KLF1 >KLF4 >KL>KLF1. The content of G units followed the trend KLF4 >KLF2 >KLF3 >KL>KLF1 >KLF1. For organosolv fractions, in the case of S units, the trend was OL>OLF3 >OLF4 >OLF1 >OLF2 >OLF1, while in terms of G units, the trend followed was OLF2 >OLF1>OLF1 >OLF4 >OLF3 >OL.

S/G ratios also varied, following trends of KLF2 >KLF3 >KL>KLF1 >KLF4 >KLF1 and OLF2 >OLF3 >OL>OLF1 >OLF4 >OLF1. A correlation analysis from the S/G ratios obtained from ^{31}P NMR and Py-GC/MS throws a Pearson coefficient of 0.4008 for kraft lignins and 0.2649 for organosolv lignins (correlation curves can be consulted in Fig. S3). It has been addressed previously (Chen et al., 2020; Tagami et al., 2019) that fractions with higher molecular weights tend to have less content of S units, so the S content reduces as the fractionation process carries on the S/G ratio also decreases.

This is a trend visible from the results obtained in the ^{31}P NMR analysis (with trends of KL>KLF1 >KLF2 >KLF3 >KLF4 >KLF1 and OLF2 >OLF3 >OL>OLF1 >OLF4 >OLF1). The relationship between S/G ratio and the Mw can be appreciated in detail in Fig. S4, where it is possible to observe that without taking into account the original lignins but their fractions, there is a drastic decrease in the S/G ratio as the Mw is increased, which is more pronounced after ≈ 4000 g/mol, as reported by (Wang et al., 2018).

3.10. Thermal analysis

The thermogravimetric curves obtained from the samples reveal the thermal stability of the lignins and their fractions. Stability was calculated by the mass loss of the sample at high temperatures. The temperature at which 5% and 50% of the mass was degraded was calculated ($T_{5\%}$ and $T_{50\%}$, respectively) and the final char residue. Fig. 7 shows TG and DTG curves of kraft (left) and organosolv (right) lignin and their fractions. Moreover, all the degradation stages and their temperatures are listed in Table S6.

For most samples, the 5% mass degradation occurred in temperatures below or near 100 °C, corresponding to moisture loss. However, the temperature in which 50% of the mass degradation occurred differed. Even though all the $T_{50\%}$ ranged from 320° to 400°C, similar trends on the fractions obtained in the fractionation process could be observed. K1 and O1 fractions had lower $T_{50\%}$, probably because these fractions are the ones with the smallest molecular weights, and consequently, degrade more easily. However, as the SOSE process continued and the fractions obtained had higher molecular weights, the $T_{50\%}$ also increased. The only exception to the rule was for K3, where $T_{50\%}$ was lower despite having a higher Mw than K2, K1, or KL. The reason might be the higher content of aliphatic OH groups in this K3 fraction, as they are more degradable than other groups (Tejado et al., 2007). However, some fractions have a lower $T_{50\%}$ not connected with the easier degradability. The lower amount of char residue means less carbonaceous content, which is not degraded by heating in an inert atmosphere.

As for the DTGA, the different fractions had different numbers of degradation stages and different temperatures. It has been reported that degradation steps between 100 and 150 °C correspond to the moisture loss, between 200 and 300 °C is the degradation of polysaccharides and aliphatic alcohols and acids, and at temperatures above 300 °C degrade aromatic rings and syringyl and guaiacyl compounds into phenols, aldehydes, alcohols, and acids (Ramakoti et al., 2019).

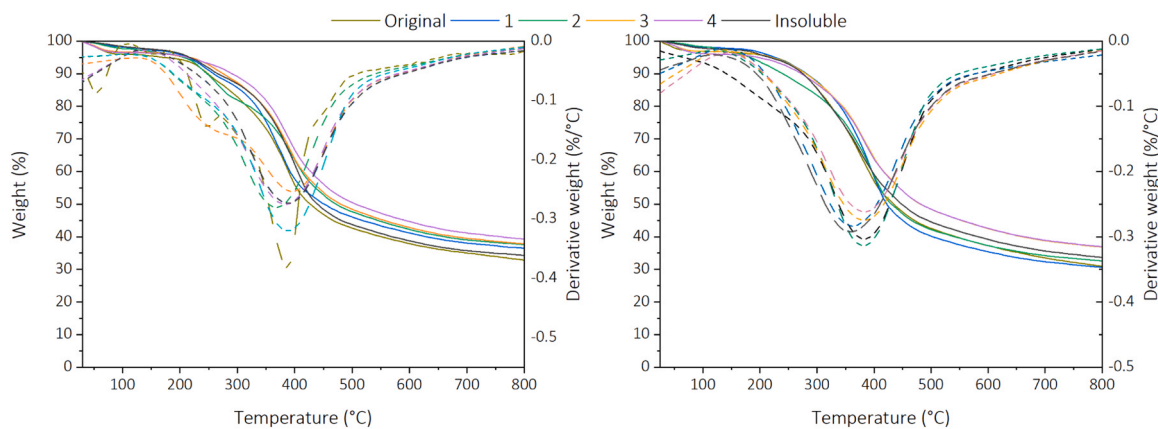


Fig. 7. TG and DTG curves of kraft (left) and organosolv (right) lignin and their fractions.

Fig. 8 shows the DSC curves of the different lignins and their fractions, the different T_g obtained from the derivative analysis of DSC curves are shown in Table S7. The T_g obtained followed the same trend for both KL and OL fractionations (K1>K4 >K3 >KL>K2 >K1 for kraft lignins, and OI>O4 >O3 >OL>O2 >O1 for organosolv lignins). It is known that at higher Mw, the T_g presents a directly proportional behaviour, which in the case of the obtained lignins presents a significant correlation at the 0.05 level, with a Pearson value of 0.7651 for kraft lignins and of 0.7844 for organosolv lignins (Fig. S5). The lignins that outlined the linear fit were the fractions extracted with EtOH (K3 and O3) and DMK (K4 and O4). These fractions were also the ones showing higher thermal stability in Fig. 7. The reason for this higher T_g is related to a higher lignin condensation (high Mn), as reported by Allegretti et al. (2018). Modelling the T_g as a function of the Mn of the different lignin fractions through the Flory–Fox model revealed that the K for kraft fractions was 4.41, while for organosolv fractions, it was 5.34 T_g^∞ was 472.66 for kraft fractions and 464.94 for organosolv fractions (Fig. S6). These values are close to those found by other works for solvolytically fractionated kraft lignins (Park et al., 2018; Passoni et al., 2016).

3.11. Applicability of the Sequential Organic Solvent Extraction Method

The SOSE method allowed the obtaining of lignin fractions with specific properties. Lignin reactivity is associated with its phenolic hydroxyl (PhOH) group content (Tan et al., 2020) so, since the phenolic hydroxyl content of the first soluble fraction of the lignins was notably increased, their reactivity was also improved. Therefore, lignin fractions

soluble in iPrOH can be used for the manufacturing of lignin-based epoxy resin where highly reactive lignins are required. Otherwise, these first fractions of lignin presented low molecular weight and low polydispersity index which can be utilised in the fabrication of polymer electrolytes (Baroncini et al., 2021).

Otherwise, the last insoluble lignin fractions which have a very high molecular weight should exhibit enhanced capacity in hydrophobicity and thermal stability (Li and McDonald, 2014b). Due to its properties, although they contain fewer OH groups than the first solubilised fractions, these insoluble lignins can be employed to prepare mechanically robust thermoplastic polyurethanes and lignin-based thermoplastic free-standing films, as well as, in the manufacturing of carbon fibres, nanospheres, adsorbents or tuneable thermosetting epoxies (Pang et al., 2021).

This method show is very versatile since can be applied to different types of lignins, easy to implement and the organic solvents used are considered green. However, the at this stage, the industrial scaling is still remote, and the recovery and reusability of the employed solvents should be further studied.”

4. Conclusions

The sequential Organic Solvent Extraction method is efficient for obtaining fractions with homogeneous molecular weights regardless of the extraction method (kraft or organosolv). The organic solvents are chosen to ease the separation of different molecular weight fractions, and the chosen order provides fractions with molecular weights in ascending order. It is also found that this difference in the molecular

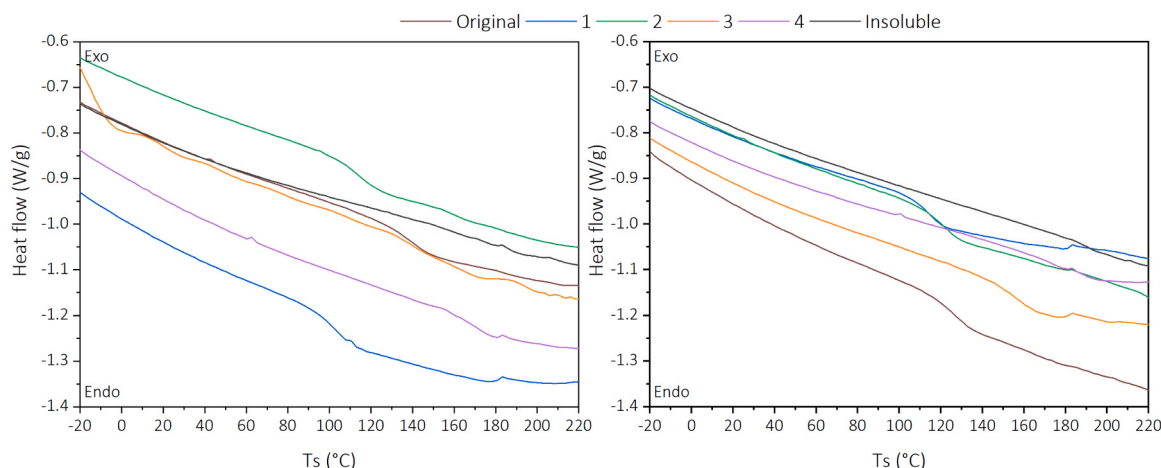


Fig. 8. DSC curves of kraft (left), organosolv (right) lignin, and their fractions.

weight influences the structural and thermochemical characteristics; the higher molecular weight the fraction has, the lower the abundance of S units and OH groups, and the higher the methyl and methoxyl groups. Consequently, the S/G ratio also varies, obtaining smaller values as the process progresses. This also influences the degradation parameters of the fractions, having less degradable lignin molecules with the last fractions obtained in the process. In conclusion, the different lignin fractions obtained had a concrete structure and properties, which are presumed to maintain even with lignins derived from different lignocellulosic materials. Even though it was observed that the type of lignin had minor effects on the final fractions characteristics, further work needs to be done to determine the applicability of the method with a broader type of feedstock. The smallest fractions, richer in hydroxyl groups, will be advantageous for processes where chemically active lignins are needed, while larger lignin fractions are interesting for their incorporation for enhanced thermochemical resistance materials. The smallest fractions, richer in hydroxyl groups, will be advantageous for processes where chemically active lignins are needed, while larger lignin fractions are interesting for their incorporation for enhanced thermochemical resistance materials.

CRedit authorship contribution statement

Nagore Izaguirre: Conceptualization, Methodology, Investigation, Data curation, Validation, Writing-Original Draft, Writing-review & editing. **Eduardo Robles:** Conceptualization, Methodology, Investigation, Visualization. **Rodrigo Llano-Ponte:** Supervision, Validation, Data curation, Visualization. **Jalel Labidi:** Validation, Formal analysis, Resources, Supervision, Writing-review & editing. **Xabier Erdocia:** Methodology, Formal analysis, Data curation, Validation, Supervision, Writing-review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.indcrop.2021.114251](https://doi.org/10.1016/j.indcrop.2021.114251).

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