Fractionation of Soda Pulp Lignin in Aqueous Solvent through Membrane-Assisted Ultrafiltration

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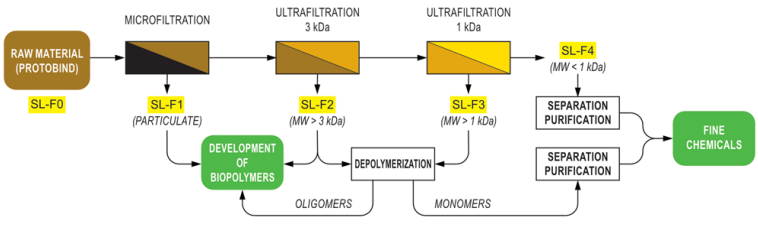
ABSTRACT: An industrial wheat straw lignin was fractionated by a multistep process involving microfiltration followed by two membrane-assisted ultrafiltration steps starting from an aqueous solvent solution. The parent lignin and the different fractions were fully characterized in terms of chemical composition and physicochemical properties by gel perme-ation chromatography, gas chromatography−mass spectrometry, high-performance liquid chromatography, ther--mogravimetric analysis, differential scanning calorimetry analysis, and Fourier transform infrared spectroscopy. The results show that the proposed process allows us to selectively control the molar mass distribution of the fractions and the related dependent properties. This strategy offers a better understanding of the structural complexity of soda pulp raw lignin and emerges as an essential tool for lignin valorization in the context of material science and preparative organic chemistry.



KEYWORDS: Biomass valorization, Lignin, Ultrafiltration, Membranes, Fractionation

INTRODUCTION

Lignin is a biobased aromatic amorphous polymer that acts as the natural glue providing structural integrity to plants.[1,2] It is one of the most abundant biopolymers in nature only following cellulose and represents about 15%−40% (w/w) of woody plants' dry matter and 17%−24% (w/w) of herbaceous plants.[3] It is one of the most recalcitrant biopolymers found in the cell wall of plants due to its inherent resistance to the degradation induced by chemical and biological methodologies.[4] Lignin consists of a complex methoxylated phenylpropane skeleton whose structure and physicochemical properties are strongly dependent on its natural origin and on the extraction method used to obtain it.[5] Although lignin represents a highly abundant aromatic feedstock, thermovalorization seems currently to be the most convenient exploitation strategy for this cheap and highly available type of biomass, and up to now, around 95% of the lignin produced worldwide every year is burned for energy recovery.[6]

However, considering its aromatic nature, other higher-added-value applications of lignin are highly desirable and are currently extensively investigated.[7−11] The lack of real effective strategies to add value to lignin processing can be mainly attributed to its chemical recalcitrance resulting from its structural complexity. Within this framework, depolymerization is currently at the basis of lignin valorization due to the inherent interest in obtaining low molecular weight aromatic building blocks for further incorporation in higher added value biobased compounds.[12−14] This process can be typically realized by chemical or thermochemical methods such as pyrolysis, chemical oxidation, hydrogenolysis, gasification, and hydrolysis under supercritical conditions. All of these processes are energy consuming and environmentally unfriendly and generate complex mixtures of products due to the repolymerization of small components present in the starting raw material.[15,16] As a suitable alternative, biocatalytic methods have been studied because they allow the use of mild conditions and greener reagents.[17] Unfortunately, the main interesting results of enzymatic degradation of lignin have been obtained on small model substrates, while the screening of

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these methods on real feedstocks is far from being close to real applications.[18−22]

As a result, the most interesting routes to profitable lignin valorization are related to its use as a precursor of biobased polymeric materials or as a biosourced platform of chemical intermediates of interest for a wide range of industrial sectors.[23] In particular, its use as a macromonomer or filler for incorporation into biobased polyurethanes, polyesters, epoxides, and other types of polymeric materials is currently being sought and intensely investigated.[24−31] Fractionation and/or depolymerization steps are typically required prior to direct valorization of such highly complex three-dimensional material in the attempt to improve the control over its chemical composition and functionality as well as its physical and structural properties. These preparatory steps are typically dictated by high chemical heterogeneity, difficult processability, and restricted solubility of most commercial lignins in common solvents. In this respect, lignin fractionation approaches conventionally rely on the independent use of chemical (pH-assisted precipitation, solvent extraction) and physical (membrane-based filtration) methods or on their sequential combination.[32−36] Among these, solvent extraction currently represents one of the most widely employed strategies to isolate lignin fractions of controlled molecular weight and homogeneous chemical characteristics.[37] In this respect, the common approach typically consists of treating sequentially the starting material with solvents of increasing hydrogen-bonding capability or favorable solubility parameter and in recovering the soluble or insoluble fractions for further use. Although effective, this method often relies on the repetitive use of large amounts of complex solvent systems[38−40] which poses some critical issues in terms of impact on process costs as well as on environmental sustainability of the overall approach.[41] As a result, more scalable and greener methods for lignin fractionation are generally preferable in order to widen further the industrial applicability of this material.

Within this framework, membrane-assisted ultrafiltration represents a particularly interesting technology due to its high separation efficiency, adaptability to different feeds and liquors, and relative ease of implementation, thus lending itself to potentially straightforward scalability at industrial level.[42,43] One major limitation of ultrafiltration is associated with the poor solubility of most commercial lignins, which typically leads to membrane fouling, reduction of processing throughput due to maintenance for cleaning cycles, and ultimately reduction of in-service membrane lifetime.[44] To overcome this issue, appropriate organic solvents or combinations thereof are very often employed to boost the solubility of the treated material and improve the efficiency of the fractionation process. In the context of commercial lignins, the majority of studies on the combined use of solvent extraction and membrane-assisted filtration have focused on the use of toxic or hazardous solvents and harsh conditions.[36,45] Only recently aqueous-based solvent systems have been proposed, mainly on commercial softwood lignin obtained from the Kraft pulping process.[46] Surprisingly, no examples of this approach appearing in the literature up to now have been applied to the other major class of alkaline lignin commercially available, namely, sulfur-free soda pulping lignin, despite the industrial relevance of such a type of lignin.

To bridge this gap, this work presents a study on the effect of membrane-assisted ultrafiltration on the physicochemical properties of a commercial wheat straw-Sarkanda grass soda pulp lignin. The parent material was first dissolved in a water−alcohol mixture at neutral pH, and the resulting solution was subjected to successive filtrations through membranes of decreasing cutoffs. The performance of the process was assessed on the basis of the characteristics of the resulting lignin fractions, which were analyzed in terms of their chemical, physical, thermal, and structural properties. The approach presented in this study demonstrates a straightforward sustainable process to obtain lignin fractions with tailored characteristics, without the use of hazardous or harmful solvents. Additionally, it provides important insights into the structure−property relationships of the resulting materials in view of their potential further exploitation for the development of biobased polymers and chemicals.

MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich. All solvents used for the extractions were of analytical grade, and in particular, acetone and methanol were HPLC grade (Sigma-Aldrich 34850 and Sigma-Aldrich 34860, respectively). All solutions were prepared in Milli-Q water (Elix Millipore Purification System, France).

The lignin used was Protobind 1000 provided by Green Value (Orbe, Switzerland). It is a mixed wheat straw/Sarkanda grass soda lignin coming from soda pulping of nonwoody biomass with a particle size below 200 μm.

Gas Chromatography−Mass Spectrometry. The identification and the quantification of low molecular weight compounds were carried out using gas chromatography coupled with mass spectrometry (GC-MS). The GC-MS apparatus used was an Agilent GC System 7890A, with an inert MSD with a Triple-Axis Detector 7975C. The gas carrier was helium at a flux of 1.18 mL/min. The separation was performed on a DB-5MS column (30 m × 250 μm × 0.25 μm, Phenomenex) with a temperature program of 50 °C (1 min) to 280 °C at 10 °C/min and 280 °C at 15 min (total run time 39 min). A solvent delay of 4 min was selected. The samples were dissolved in methanol or acetone in a concentration around 0.5−1 mg/mL.

In parallel, in order to analyze the less volatile compounds, samples were analyzed also as trimethylsilyl derivatives (TMS) with the following procedure. A mixture of 25 μL of pyridine, 250 μL of dioxane, and 75 μL of silylation mixture composed of *N,O*-bis(trimethylsilyl)trifluoroacetamide (with trimethylchlorosilane (Sigma T6381) was incubated with 1 mg of sample heated in a thermomixer (1.5 mL vial Eppendorf Thermomixer Confort) at 70 °C and 600 rpm for 30 min. At the end, 100 μL of the mixture were withdrawn and added to 100 μL of a standard solution of benzaldehyde (0.49 mM final concentration). Compound identification was preliminary performed by means of a NIST 2008 mass spectral library search, and then, selected peaks were confirmed with known standards (comparing both mass spectrum and chromatographic coordinate).

Gel Permeation Chromatography. Gel permeation chromatography (GPC) was used to determine the molecular weight of the lignin samples. A Waters 510 HPLC system was used equipped with a refractive index detector. Tetrahydrofuran (THF) was used as the luent. The sample to analyze (volume 200 μL, concentration 2 mg/mL in THF) was injected into a system of columns connected in series (Ultrastyragel HR, Waters), and the analysis was performed at 30 °C and at a flow rate of 0.5 mL/min. The GPC system was calibrated against polystyrene standards in the 10^2−10^4 g/mol molecular weight range. To allow complete solubility in the THF eluent, before the analysis, the parent lignin and the fractions were acetylated following a standard literature procedure.[47]

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) was performed on solid state samples (∼10−15 mg) by means of a Mettler-Toledo DSC/823e instrument at a scan rate of 20 °C/min under nitrogen flux.

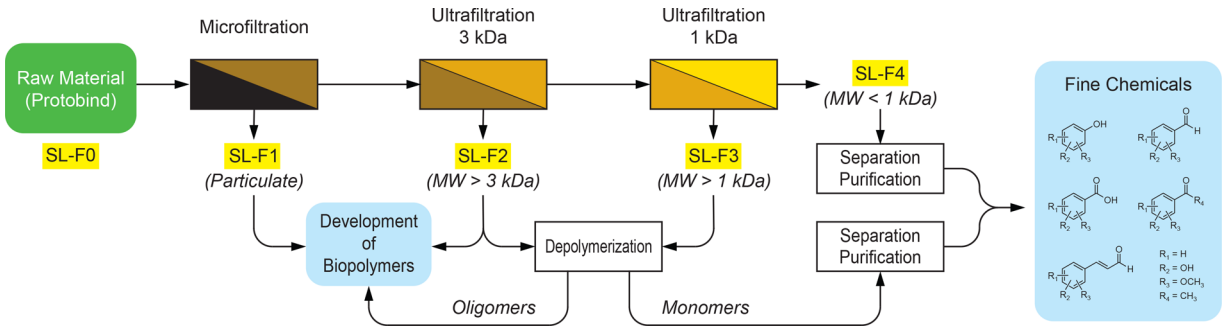

Figure 1. Schematic representation of the lignin fractionation process (SL-F0 is a solution of Protobind 1000 in ethanol/water at 15 g/L).

Thermogravimetric Analysis. Thermogravimetric analysis (TGA) was carried out on solid state samples (~10−15 mg) with a Q500 TGA system (TA Instruments) from ambient temperature to 800 °C at a scan rate of 10 °C/min under nitrogen flux.

Fourier transform infrared spectroscopy. Fourier transform infrared (FTIR) spectra of all lignin fractions were recorded in transmission mode on films spin-cast onto KBr disks. The analysis was performed by means of a Nicolet 760-FTIR spectrophotometer at room temperature in air in the 4000−700 cm^{-1} wavenumber range with 64 accumulated scans and a resolution of 2 cm^{-1}.

High-Performance Liquid Chromatography (HPLC). The chromatographic sample analyses were carried out with an AZURA analytical HPLC system (KNAUER) composed of a 10 mL/min analytical pump (HPG P6.1L), a UV−vis diode array detector (DAD6-1L), an autosampler (3950), and a column oven (CT 2.1). High sensitivity (50 mm/6 μL) and standard (10 mm/2 μL) light guide flow cells were used. Data recording and analysis were performed with ClarityChrom software. Three different reversed phase column types (KNAUER RP) were used, namely, a C18A (250 mm × 4 mm, 5 μm), a phenyl phase (250 mm × 4 mm, 5 μm) and a C8A (250 mm × 4 mm, 5 μm). The optimized method consists of a 10 μL sample injection (full loop) and a column temperature set to 40 °C. Sample elution was carried out by binary gradient composed of eluent A (H_2O + 0.05% trifluoroacetic acid) and eluent B (CH_3CN/trifluoroacetic acid): 80/0.1 v/v at the a flow rate of 1 mL/min. The mobile phase composition was maintained at 90% eluent A for 4 min, changed linearly to 100% B at 25 min and held 3 min, and then followed by a return to the initial conditions within 1 min and kept 8 min for the chromatograph column equilibrium. The UV/vis detection was performed at λ of 280 nm. A 50 Hz sample rate with 0.02 s time constant was used for data acquisition.

Before the HPLC analyses, the calibration of compounds 1−5 had been performed by determining their molar extinction in CH_3CN/H_2O 8/2 (v/v) at 280 nm wavelength (Supporting Information, Table S6).

Membrane-Assisted Fractionation. Lignin fractionation was performed by means of an ultrafiltration setup equipped with flowmeters and pressure sensors to control permeate flow, transmembrane pressure, and filtration time. The membranes in poly(ether sulfone) (PES) were purchased from Pall Life Science and had molecular-weight cutoffs of 3 and 1 kDa. The new PES membranes were activated by successive flushing of water and an ethanol/water solution through them in order to assess and ensure their mechanical integrity.

Fraction Extraction Procedure. The recovered fractions were evaporated under reduced pressure to remove ethanol. The aqueous solutions were acidified to pH close to 1 with 37% HCl and extracted three times with ethyl acetate. The combined organic phases were dried over sodium sulfate and evaporated under reduced pressure. The final solid residues were dried until a constant weight was achieved prior to analysis and quantification.

RESULTS AND DISCUSSION

Fractionation Process. In this work, a commercially available wheat straw/Sarkanda grass lignin (Protobind 1000) was used as the starting raw material. The fractionation process was setup using neutral water/ethanol solutions. Three lignin solutions were prepared for the fractionation process using a mixture of water/ethanol (60/40, v/v) as a solvent. The dissolution of lignin occurs with a decrease in pH from neutrality to slightly acidic conditions (pH ~ 4). Three concentrations of the samples were tested because of the limited solubility of lignin in water/ethanol: 15, 10, and 5 g/L. In these conditions, three starting black solutions were obtained, characterized by the presence of a non-negligible amount of insoluble material manifested as suspended solid particles. Each of these starting solutions was submitted at first to a microfiltration process through a 0.7 μm membrane under vacuum in order to eliminate the insoluble particles. During this step, the amount of dry matter of insoluble recovered solid lignin was found to be 2.34, 1.58, and 0.68 g for a 1 L water/ethanol dilution of 15, 10, and 5 g of starting material, respectively. This highlights the fact that in all three cases around 13−16 wt % of starting lignin resulted to be insoluble in these acidic conditions.

Following the microfiltration process, two steps of ultrafiltrations (cross-flow filtrations) were performed with two successive membranes in PES with hydrophilic characteristics and a cutoff of 3 and 1 kDa, respectively. These membranes are compatible with high pressure, high temperature, and a wide range of pH values from 1 to 13. A schematic representation of the performed filtration steps is summarized in Figure 1 (see Supporting Information for details on filtration apparatus and filtration conditions).

Taking into account the industrial relevance of the process, only a 15 g/L stock solution was considered for further fractionation due to its higher solid concentration and thus its higher interest from a process standpoint. As a result, only fractions obtained from the 15 g/L starting solution will be discussed in the following sections.

Quantification of Different Fractions. The different lignin fractions coming from successive fractionation treatments were processed through four main steps: evaporation of ethanol, acidification of aqueous solutions at a pH close to 1, extraction in organic solvent and at the end the evaporation of the solvent, and final recovery and drying of the solids.

Before analyzing the physicochemical properties of the different fractions resulting from the fractionation process, a quantification of the yield of each fractionation step was performed (Table 1).

Table 1. Yield of Main Fractions Obtained during the Fractionation Process ([a])

Fraction	Mass recovery in terms of % of starting material (fraction mass/lignin mass)
SL-F1	16
SL-F2	50
SL-F3	4.4
SL-F4	2.5

[a]Starting solution of 15 g/L of Protobind 1000 in ethanol/water 60/40 v/v.

As expected, starting from the parent SL-F0 material, the retentate fraction of the highest cutoff membrane (SL-F2) exhibits the highest lignin concentration, accounting for approximately 50% of the overall initial input mass. On the contrary, very diluted solutions accounting for about 2.5% of the initial mass are recovered after ultrafiltration through the 1 kDa membrane (SL-F4), thus suggesting a relatively limited concentration of low molecular weight species in this type of commercial lignin. This aspect will be discussed in detail in the following paragraphs, where a thorough physicochemical characterization of all fractions after acidification to pH 3, extraction with organic solvent, and solvent evaporation will be presented.

Gel Permeation Chromatography (GPC). Irrespective of their source (softwood, hardwood, herbaceous), commercial lignins are typically characterized by a relatively broad molecular weight distribution that directly reflects the great variability in the relative abundance of their main repeating units (p-hydroxyphenyl, guaiacyl, and syringyl) and in the formation of the linkages between them via random radical polymerization. Indeed, values of polydispersity index ($Đ$) larger than 2−3 are routinely encountered in lignins obtained from consolidated industrial processes such as the alkaline and the organosolv process.[48]

Within this framework, in order to evaluate the effect of the filtration steps on the molecular weight distribution of the soda pulp lignin considered in this work, all fractions were analyzed by means of gel permeation chromatography (GPC), and the values of number-average molecular weight (M_n), weight-average molecular weight (M_w), and $Đ$ were estimated against monodispersed polystyrene standards. The results are presented in Figure 2, and data are summarized in Table 2.

The first membrane-assisted microfiltration process allows us to eliminate the insoluble material (SL-F1) present as suspended solid particles in the starting water/ethanol solution, which is found to exhibit slightly higher molecular weight and broader molecular weight distribution compared with pristine lignin (SL-F0). Upon ultrafiltration, the molecular weight of the fractions recovered after permeation through increasingly finer membranes is found to decrease progressively. In particular, the filtration through a 3 kDa cutoff membrane leads to SL-F2 (retentate fraction) that exhibits a slightly lower M_n than the insoluble fraction SL-F1 obtained from the microfiltration process (1390 vs 1430 g/mol, respectively). On the other hand, a broader polydispersity is observed in SL-F2 (8.5) compared with SL-F1 (4.4), which originates from the much higher value of M_w (11870 and 6350 g/mol for SL-F2 and SL-F1, respectively) likely attributable to an enrichment in higher molecular weight material experienced as a result of the first ultrafiltration step.

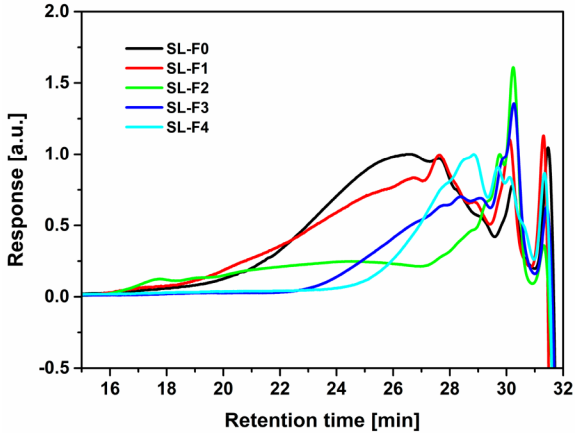

Figure 2. GPC chromatograms of all extracted lignin fractions (the parent material SL-F0 is also reported as reference). Samples were acetylated prior to analysis.

Table 2. Molecular Weights (M_n and M_w) and Polydispersity Index ($Đ$) of All Examined Soluble Lignin Fractions[a]

Fraction	M_n (Da)	M_w (Da)	Đ
SL-F0	1390	4660	3.3
SL-F1	1430	6350	4.4
SL-F2	1390	11870	8.5
SL-F3	860	1330	1.5
SL-F4	720	900	1.2

[a]Parent material SL-F0 is also reported as reference. Samples have been eluted after acetylation. Reported values for molecular weights are relative to polystyrene standards.

On the basis of these considerations, the ultrafiltration step through the 3 kDa membrane appears to provide a 2-fold effect on the input lignin material. On the one side, it allows us to recover a high-molecular-weight soluble lignin fraction that can be potentially employed as macromonomer for the development of lignin-based polymeric materials. On the other side, it allows us to narrow down the molecular weight distribution of the permeate stream, which is therefore enriched in low molecular weight species to be potentially recovered downstream as valuable fine chemicals (as discussed further on in reference to the GC/MS analysis).

Proceeding through the fractionation process, significantly lower values of molecular weight are observed for the 1 kDa retentate fraction SL-F3 (M_n = 860 g/mol, M_w = 1330 g/mol) and for the corresponding permeate fraction SL-F4 which is also accompanied by a sharp reduction of polydispersity index from 1.5 to 1.2, respectively. These results confirm that a fine control over the molecular weight of the starting material can be obtained by means of the presented method.

Differential Scanning Calorimetry (DSC). To investigate the effect of membrane-assisted ultrafiltration on the thermal properties of the resulting lignin fractions, DSC analysis was performed on all materials. The results are presented in Figure 3a, where the DSC trace of the parent lignin SL-F0 is also reported for reference. Compared to the parent material, a slight increase in glass transition temperature (T_g) is observed for the fractions recovered from the first microfiltration and from the first ultracentrifugation step, with T_g values of 166° and 209 °C for SL-F1 and SL-F2, respectively. Conversely, lower T_g values are found in both retentate (SL-F3, T_g = 131 °C) and permeate (SL-F4, T_g = 40 °C) fractions recovered

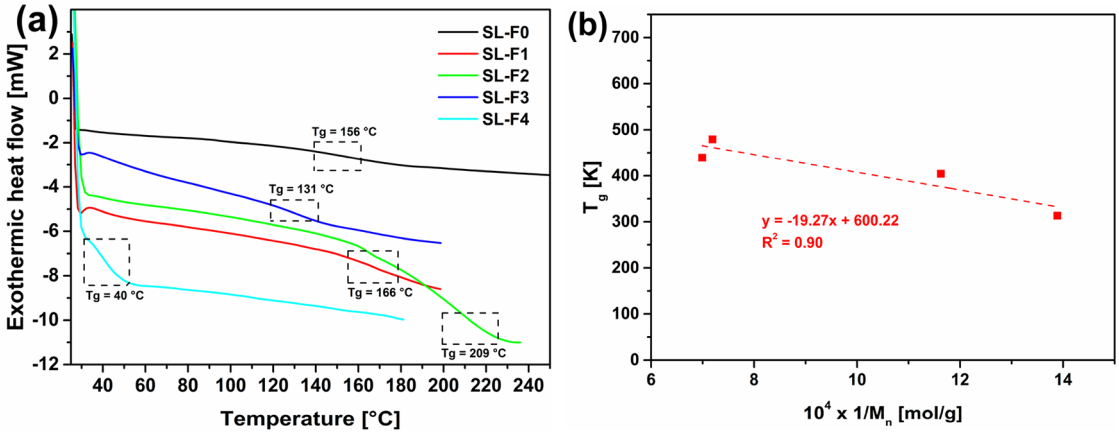

Figure 3. (a) DSC traces of analyzed lignin fractions with the corresponding T_g value. (b) T_g of analyzed fractions as a function of M_n (experimental data were fitted by means of the Flory–Fox model, and the fitting equation is also reported).

after ultracentrifugation by means of the finer 1 kDa membrane. Considering the results obtained by means of GPC analysis (Figure 2), these trends clearly highlight a major dependence of T_g of the resulting fractions on their corresponding molecular weight, as expected. This relationship can be more systematically described by resorting to the well-known Flory–Fox model[49]

$$T_g = T_g^{\infty} - \frac{K}{M_n} \quad (1)$$

in which T_g^{∞} is the glass transition temperature of a polymer with high molecular weight in which the effect of chain ends is negligible, and K is an empirical constant correlated with the free volume of the material through the following expression:

$$K = \frac{2\vartheta N_A \rho}{\Delta a_f} \quad (2)$$

with ϑ being the excess free volume of macromolecular chain ends, N_A Avogadro's number, ρ the density of the material, and $\Delta\alpha_f$ the free volume thermal expansion coefficient. In this respect, eq 1 can provide some further insights in the correlation between excess free volume and molecular weight of the lignin fraction, notwithstanding the uncertainties associated with the evaluation of the molecular weight by GPC analysis. As observed in Figure 3b, where a plot of the T_g values for all lignin fractions as a function of the reciprocal of M_n is presented, the linear fit of the experimental data by means of the Flory–Fox equation gave numerical values for K and T_g^{∞} of 19.27 × 10^4 and 600.22, respectively. It is interesting to observe that the K value obtained here is slightly higher than what reported in recent fractionation studies employing kraft lignin as input material (~11–14 × 10^4).[36,45] Because of the direct dependence of K on ϑ, this evidence indicates that in the lignin material analyzed here the amount of excess free volume of chain ends is comparatively higher and thus plays a more relevant role in the three-dimensional structure of the macromolecular network. Considering that a higher number of chain ends per unit volume (thus, a higher amount of excess free volume of chain ends) is expected in polymers with relatively short macromolecular chains compared to polymers consisting of longer chains, these observations are consistent with the lower T_g and molecular weights found in the lignin material analyzed in this work with respect to kraft lignin.

Thermogravimetric Analysis (TGA). The investigation of the effect of membrane-assisted ultrafiltration on the thermolytic stability of the retentate and the permeate lignin fractions was carried out through TGA analysis in N_2. The results are presented in Figure 4 and Table 3. In the low temperature range (T < 300 °C), the highest stabilities are experienced by SL-F1 and SL-F2, which exhibit comparable values of temperatures at which 10% weight loss occurs ($T_{10\%}$) with respect to the parent material ($T_{10\%}$ = 245, 254, and 243 °C for SL-F0, SL-F1, and SL-F2, respectively). This trend may be correlated with the higher molecular weight registered for these materials, as previously discussed (Figure 2, Table 2).

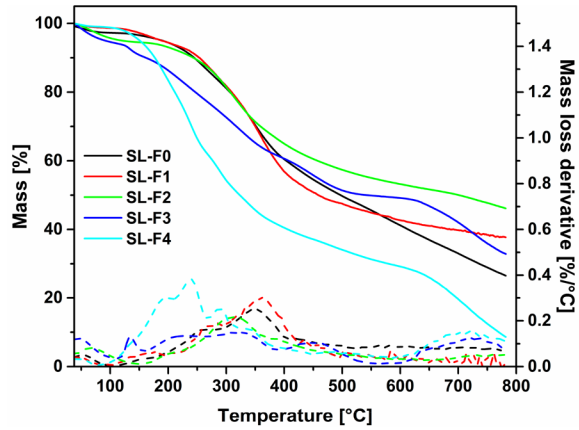

Figure 4. TGA/DTGA thermograms of analyzed soluble lignin fractions.

Table 3. Thermal Degradation Temperatures at 10% ($T_{10\%}$) and 50% ($T_{50\%}$) Mass Loss and Final Char Residue at 780 °C (R_{780}) for All Lignin Fractions Examined in This Work[a]

	$T_{10\%}$ [°C]	$T_{50\%}$ [°C]	R_{780} [%]
SL-F0	245	497	26.7
SL-F1	254	459	37.8
SL-F2	243	701	46.2
SL-F3	162	542	33.1
SL-F4	177	323	8.8

[a]Measured by TGA analysis under N_2 stream. For reference, data for the parent material (SL-F0) are also presented.

As temperature increases, SL-F2 appears to be the most stable fraction, with a char mass residue at 780 °C ($R_{780\ °C}$) of 46.2% and no significant mass loss events observed for $T > 300$ °C. This behavior likely indicates that the high T_g and molecular weight of this fraction (Figure 3a and Figure 4, respectively) positively affect its thermolytic response.[36,43,50] As opposed to this, SL-F3 and SL-F4 possess lower thermal stability in the entire temperature range investigated, with a more pronounced mass loss event observed for SL-F4 at low temperature. Indeed, a very low $T_{10\%}$ is observed for this material (177 °C), accompanied by the lowest value of $R_{780\ °C}$ (8.8%). These trends can be directly correlated with the low molecular weight of this fraction, which directly affects its thermal stability also at relatively low operating temperatures.

Fourier Transform Infrared Spectroscopy (FTIR). To gain further insights into the chemical composition of each resulting lignin fraction, FTIR spectra were collected on all materials recovered upon membrane-assisted filtration treatment. FTIR spectra of all lignin fractions are presented in Figure 5, where also the spectrum of pristine material SL-F0 is

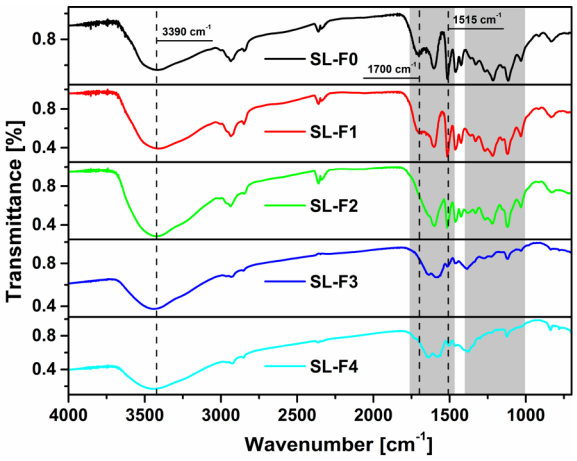

Figure 5. FTIR spectra of recovered lignin fractions.

reported for easy reference. All samples present a broad absorption band centered at 3390 cm^{-1} of variable intensity that can be attributed to stretching vibrations of phenolic and aliphatic O−H groups present in lignin. Differences between the fractions appear in the 1800−1500 and 1400−1000 cm^{-1} wavenumber ranges (please refer to the shaded areas in the plot shown in Figure 5).

Vibrations attributable to C═O bonds in conjugated aldehydes and carboxylic acids are observed at around 1700 cm^{-1}. Such a signal is only observed in SL-F1, while its relative intensity is found to decrease significantly and ultimately disappear for the fractionated samples from SL-F2 to SL-F4. These trends suggest that the extraction process leads to a decreased concentration of carbonyl and carboxyl groups compared to the parent material. A similar trend is observed for the intense peak found at 1515 cm^{-1} which is typical of pure aromatic skeletal vibrations in lignin. In the 1400−1000 cm^{-1} spectral region, several signals are observed which can be associated with vibrations of phenolic O−H and aliphatic C−H in methyl groups (1365−1370 cm^{-1}), C−O, C−C, and C═O stretching vibrations (1270 and 1210 cm^{-1}), C−H in plane deformations (1120 cm^{-1}), adn C−O deformations in primary (1030 cm^{-1}) alcohols. Also for this spectral region, a general decrease in relative signal intensity is observed for filtrated fractions (SL-F3 and SL-F4) as compared to the pristine materials.

GC-MS Analysis. The distribution of small molecules such as aromatic monomers and aliphatic carboxylic acids isolated from the different fractions was determined by GC-MS (Table 4 and Figure 6). The samples submitted to GC-MS analysis

Table 4. Summary of GC-MS Results of Fractions (SL-F1−SL-F4) Illustrating the Estimated Amount of Volatile Compounds, Divided in Three Main Groups, Reported as Total Mass of Compounds/Mass of Initial Fraction × 100[a]

Compounds	SL-F1 (%)	SL-F2 (%)	SL-F3 (%)	SL-F4 (%)
ArCHO, ArCOR[b]	0.32	0.21	7.7	13
ArCOOH + ArCHCHCOOH[b]	0.22	0.13	11	29
RCOOH[c]	0.10	0.14	2.1	8.7
Total monomers	0.64	0.48	21	51

[a]Values were rounded up to the nearest two significant figures with an estimated error of ±0.01. [b]Ar = aromatic residue. [c]R = aliphatic chain.

were obtained after small scale chromatography on silica gel in order to eliminate all the polymeric/oligomeric fractions and to recover only the fraction suitable for GC-MS analysis. The identified compounds found in greatest abundance can be divided into three main classes (Table 4): benzaldehyde and acetophenone derivatives (ArCHO, ArCOR), benzoic and coumaric acids (ArCOOH, ArCHCHCOOH), and aliphatic long chain carboxylic acids (RCOOH). The GC/MS chromatograms are reported in Figure 6.

As it is appears in Table 4, the estimated percentage of monomers in each of the SL-F1 and SL-F2 fractions is near 0.5%−0.6% of the fraction, in SL-F3 the monomers account for 21%, and in the final permeate SL-F4 the quantity of monomers is 51% as it was predictable going downstream in the process. The chromatogram profiles of the different fractions are reported in Figure 6 and show very clearly that going from SL-F1 to SL-F4 the number of peaks becomes much more relevant, attesting to the increasing presence of small products going through the fractionation process. The complete characterization of all peaks is reported in the Supporting Information.

HPLC Analysis of Fraction SL-F4. HPLC analysis was performed on the lowest molecular weight fraction SL-F4. In order to find the best separation conditions, different experiments have been performed by using three different stationary phases: phenyl phase, C18A, and C8A. For a better comparison of the separation profile of the three tested columns, an overlay of the chromatograms recorded at 280 nm from the three columns was made (Figure 7). The identified compounds in the individual chromatograms are indicated with numbers from 1 to 5. The compounds associated with the group of peaks 1−4 were found to elute from the C8A (blue line) and phenyl phase (red line) at similar elution times, namely, between 12 and 13 min. Their elution time was faster on the C18A column (Figure 7, green line and Table 5). These observations are consistent with the polar charge of the isolated compounds that interact more strongly with the C8A and phenyl stationary phases than with the less polar C18A phase.

The phenyl phase provided the best separation profile from the three tested columns affording a full separation of syringic

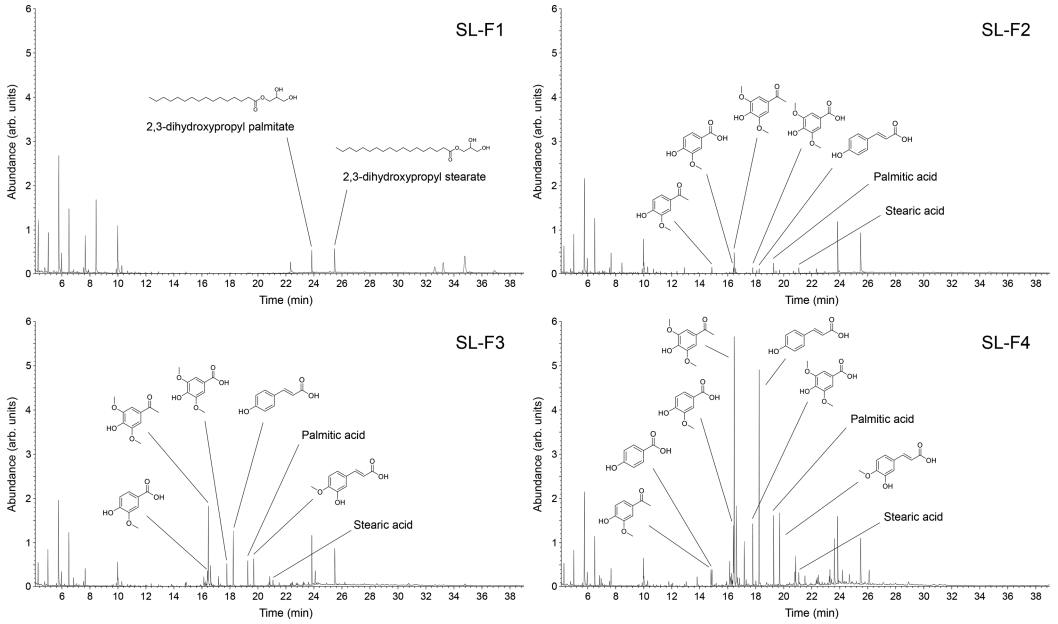

Figure 6. GC/MS chromatograms for the fractions SL-F1, SL-F2, SL-F3, and SL-F4 with the assignment of the main compounds signals.

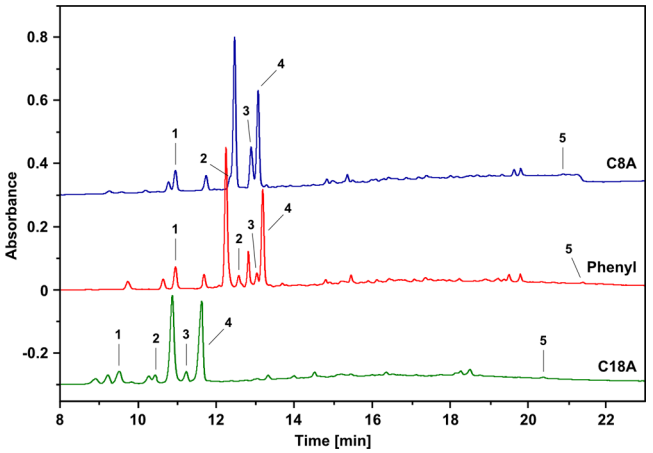

Figure 7. Overlay of SL-F4 HPLC chromatograms from three columns, view of relevant area: blue, C8A; red, phenyl phase; green, C18A; 1, syringic acid; 2, vanillin; 3, acetovanillone; 4, acetosyringone; 5, anthraquinone. All chromatograms were recorded at 280 nm and plotted in the same scale (C8A and C18A are vertically displaced for clarity).

acid, vanillin, acetovanillone, and acetosyringone. Three additional peaks were detected between the two larger peaks. Compound identities were determined by comparison of the retention time of commercial standards analyzed in the same conditions. At an approximately 25 min retention time, anthraquinone 5 was also detected.

Several other so far unidentified peaks were detected. The concentration of the identified substances was calculated over the peak height of the standards and the peaks in the sample (Table 6). Acetosyringone had the highest concentration in the sample with about 26 μg/mL, followed by syringic acid with about 5 μg/mL. The three additional compounds were present in lower concentrations. From 5 μg of the injected SL-F4 sample, 5% of the analyzed sample was acetosyringone. The other substances were less than 1% in the sample.

Table 6. Estimation of Concentration of Identified Compounds from 10 μL Injection of 0.5 μg/μL SL-F4 Sample[a]

Compound	Concentration in SL-F4 (μg/mL)
Syringic acid	4.67
Vanillin	1.67
Acetovanillone	2.96
Acetosyringone	26.0
Anthraquinone	2.39

[a]Determination by peak height.

CONCLUSIONS

Fractionation of an industrial wheat straw/Sarkanda grass lignin obtained from the soda pulp process was successfully

Table 5. Retention Times of Identified Compounds in SL-F4 Sample Obtained by HPLC Analysis Operating with a Detector Set at 280 nm on Three Different Columns (C18A, phenyl, and C8A)

Compound number	Compound	IUPAC name of the compound [CAS Number]	t_R in min (C18A)	t_R in min (phenyl)	t_R in min (C8A)
1	Syringic acid	4-hydroxy-3,5-dimethoxybenzoic acid [530-57-4]	9.52	10.959	10.957
2	Vanillin	4-hydroxy-3-methoxybenzaldehyde [121-33-5]	10.430	12.580	12.474
3	Acetovanillone	1-(4-hydroxy-3-methoxyphenyl)ethan-1-one [498-02-2]	11.235	13.051	12.905
4	Acetosyringone	1-(4-hydroxy-3,5-dimethoxyphenyl)ethan-1-one [2478-38-8]	11.625	13.199	13.082
5	Anthraquinone	Anthracene-9,10-dione [84-65-1]	20.405	21.418	20.910

carried out by means of a scalable membrane-assisted ultrafiltration approach starting from an aqueous solvent lignin solution. This process consists of four steps, namely, a dissolution step in an ethanolic aqueous solution followed by a microfiltration in order to eliminate all the insoluble parts of the lignin suspension and two subsequent ultrafiltrations. Four different fractions were obtained exhibiting very different physicochemical properties. The described fractionation process represents a very valuable tool for the industrial valorization of lignin, as it allows well-defined fractions to be readily obtained and potentially employed for different applications. Indeed, while the lower molecular weight fractions could, in principle, be further separated into their monomeric components in order to become a source of natural platform chemicals, the higher molecular weight ones could be used both as starting material for depolymerization processes and as macromolecular building blocks in biobased polymeric materials. Particularly, the obtained results suggest that the proposed membrane-assisted ultrafiltration process may constitute a valuable strategy to produce lignin fractions tailored for applications in which highly specific material requirements are expected (e.g., high thermal stability, control over molecular weight). Furthermore, the selective separation of monomers from the biomass can lead to a full exploitation of lignin as a feedstock material.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.8b01410.

Ultrafiltration apparatus for lignin fractionation; parameters used for the membrane-assisted ultrafiltration process; GC/MS results of fractions SL-F1, SL-F2, SL-F3 and SL-F4; molar extinction coefficients measured at λ= 280 nm for compounds 1−5. (PDF)

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Notes

The authors declare no competing financial interest.

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