

Article

Lignin Valorization for Added-Value Chemicals: Kraft Lignin versus Lignin Fractions

Carina A. E. Costa ^{1,2,*} , Filipa M. Casimiro ^{1,2} , Carlos Vega-Aguilar ^{1,2,3}  and Alírio E. Rodrigues ^{1,2} 

¹ LSRE-LCM—Laboratory of Separation and Reaction Engineering—Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

² ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

³ Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

* Correspondence: carina.costa@fe.up.pt

Abstract: Lignin is a raw material that can potentially be converted into valuable compounds through depolymerization reactions in addition to its use as a polymer or material. However, the chemical recalcitrance and the heterogeneous composition and structure of lignin make it challenging to establish processes that add value to this complex aromatic biopolymer. In this work, solvent fractionation was applied to obtain lignin fractions with a narrowed molecular weight and specific structural characteristics, improving its homogeneity and purity. A kraft lignin was submitted to fractionation using different ratios of acetone, ranging from 60 to 15% *v/v*, in aqueous mixtures. The composition, structure, and molecular weight of each fraction were studied and their potential applications were evaluated. The most water-soluble fraction has more phenolic OH, less aliphatic OH groups, and shows the lowest content of aryl-ether linkages, which is in accordance with its highest degree of condensation. On the other hand, the insoluble fraction from the mixture with 60% of acetone has the lowest molecular weight and the highest content of inorganic material. Radar plots were applied for lignin fractions evaluation and the fraction with the highest potential (IF 30:70) was submitted to alkaline oxidation with O₂. The results were compared with the products yielded from kraft lignin. An increase of about 13 and 19% was found for vanillin and syringaldehyde, respectively, when the fraction IF 30:70 was submitted to oxidation. In conclusion, the proposed fractionation process showed to be an effective method to obtain lignin fractions with specific composition and structural characteristics that could improve its potential as a source of high added-value monomeric phenolic compounds.

Keywords: kraft lignin; solvent fractionation; homogeneous fractions; structural characterization; oxidative depolymerization



Citation: Costa, C.A.E.; Casimiro, F.M.; Vega-Aguilar, C.; Rodrigues, A.E. Lignin Valorization for Added-Value Chemicals: Kraft Lignin versus Lignin Fractions. *ChemEngineering* **2023**, *7*, 42. <https://doi.org/10.3390/chemengineering7030042>

Academic Editors: Roberto Rosa, Anna Maria Ferrari, Consuelo Mugoni, Grazia Maria Cappucci and Vasile Lavric

Received: 20 March 2023

Revised: 13 April 2023

Accepted: 21 April 2023

Published: 1 May 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Lignin, along with cellulose and hemicellulose, is one of the main components of lignocellulosic materials. This renewable aromatic feedstock is abundantly present in terrestrial plants, typically constituting 10–40% of the dry weight and 40% of the energy [1,2]. Lignin's chemistry and availability makes it an interesting raw material for a wide variety of applications as well as a potential source of renewable fuels and high added-value chemicals [3]. However, the complexity and heterogeneous structure of lignin, its broad molecular weight distribution, and high reactivity lead to undesirable properties, such as low solubility, an amorphous structure, and a tendency to condensation reactions, that make its direct utilization and valorization difficult [4,5].

Lignin is a three-dimensional phenolic macromolecule composed of three types of phenyl propane units, *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S). These units differ between them in the methoxylation of the aromatic nuclei and are combined through

different types of complex linkages, including C-O-C bonds (β -O-4, α -O-4, 4-O-5', etc.) and C-C bonds (5-5', β - β , β -1, β -5, etc.) [6,7]. Usually, lignin structure (linkages and functional groups) and composition depend on the different feedstocks (hardwood, softwood, and non-wood crops), extraction, and depolymerization methods [8]. Consequently, an understanding of lignin chemical structure is essential for developing robust and effective valorization processes since the potential utilization of lignins remains underdeveloped. Nowadays, most of the produced lignin is used as fuel for heat generation in the recovery boilers at pulp and paper mills and only a small percentage, approximately 1–2%, is used commercially as binders, dispersants, emulsifiers, surfactants, etc. [1,9].

In the literature, there is an increased effort to improve homogeneity and understand the complexity of lignin to achieve its complete valorization. Fractionation is one of the most effective methods that can be easily tailored to produce lignin fractions with low polydispersity and well-defined properties [10,11]. Solvent fractionation and membrane ultrafiltration [10,12] are the main methods studied in the literature for lignin fractionation [13,14]. Solvent fractionation can be performed using a variety of organic solvents such as methanol [15], ethanol [16], acetone [17,18], diethyl ether [18], dioxane [15], tetrahydrofuran [19], propyleneglycol monomethyl ether [16], butanone [15], and hexane [19] as pure solvents or as mixtures [20]. Acetone is often used as a fractionation solvent to produce pure and homogeneous lignin fractions regardless of the lignin source. This solvent is safe, non-toxic, environmentally friendly, and commonly used in the industry over other organic solvents by technoeconomic assessment and health and safety issues. Most commonly, pure organic solvents are used to obtain lignin fractions with specific properties [21,22]. However, the dissolution of lignin followed by fractional precipitation using an antisolvent, such as hexane [19] or water [14], can also be found in the literature.

In this study, a solvent fractionation by precipitation, using mixtures of acetone–water, was applied to lignin isolated from industrial hardwood kraft liquor. Lignin fractions were studied in detail considering its composition, molecular weight, and structural characteristics. The selected fraction with more potential for phenolic compound production was submitted to oxidative depolymerization. The results will allow achieving a comprehensive study of the fractionation process efficiency, considering the evaluation of lignin potential.

2. Materials and Methods

2.1. Isolation of Kraft Lignin

Lignin used in the fractionation process was isolated from industrial *Eucalyptus globulus* kraft liquor, collected in a Portuguese pulp mill, by slow acidification until pH 5 using 5 M H₂SO₄, as previously described [23]. During acidification, the mixture was continuously stirred at low temperature. The precipitated lignin was recovered after centrifugation, washing with distilled water, and freeze-drying.

2.2. Lignin Solubility

The study of kraft lignin solubility was performed with the objective of selecting the optimal acetone aqueous solution concentration to be used in the selective fractionation process. An amount of 5.0 g of kraft lignin was mixed with 50 mL of pure acetone, water, and acetone–water mixtures with different proportions ranging from 20 to 80% *v/v*. The mixture of lignin and solvent was stirred for 90 min, at room temperature. Then, it was centrifuged at 3500 rpm for 12 min to separate the dissolved fraction of lignin from the insoluble one. The insoluble fraction was dried at 40 °C overnight to constant weight.

2.3. Fractionation Process of Kraft Lignin

For the fractionation process, about 10 g of kraft lignin were mixed with 100 mL of 60:40 (% *v/v*) acetone aqueous solution. The mixture was kept under stirring for 90 min and centrifuged for 12 min at 3500 rpm. After centrifugation, the insoluble lignin fraction obtained (IF 60:40) was dried at 40 °C overnight to constant weight. Then, water was added to the supernatant to decrease the acetone concentration from 60% to 30% and after that

to 15%, by volume. The resulting solutions were mixed and centrifuged using the same conditions previously described. The insoluble fractions obtained, IF 30:70 and IF 15:85, were separated from the soluble ones by centrifugation, dried at 40 °C, and weighed. The final fraction of soluble lignin in 15% of the acetone aqueous solution (SF 15:85) was separated by solvent evaporation under reduced pressure. The selective fractionation process of kraft lignin is schematized in Figure 1.

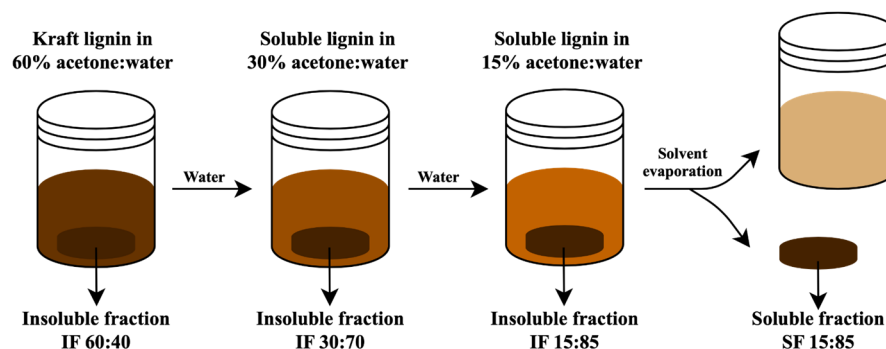


Figure 1. Fractionation process of kraft lignin with acetone aqueous mixtures.

2.4. Composition and Structural Characterization of Kraft Lignin and Lignin Fractions

2.4.1. Inorganics Content Determination

The inorganic content of kraft lignin and lignin fractions was determined by gravimetric quantification after incineration at 600 °C for 6 h.

2.4.2. Gel Permeation Chromatography (GPC)

The molecular weight of kraft lignin and lignin fractions was determined through GPC analyses using the equipment, the columns, and the operating conditions already detailed in previous works by the authors [24]. The procedure for GPC analyses was performed according to the literature [24]; briefly, analyses were performed by employing a mobile phase solvent dimethylformamide (DMF) with 0.5% *w/v* of lithium chloride (LiCl) at a temperature of 70 °C and with a flow rate of 0.8 mL·min^{−1}. Prior to analyses, the GPC system was calibrated using polystyrene standards with a molecular weight ranging from 162 to 55,000 g·mol^{−1}. Polystyrene standards, kraft lignin, and lignin fractions were dissolved in the mobile phase solvent with a final concentration of about 5 mg·mL^{−1}. Before injection, the resulting solutions were filtered through a 0.2 µm syringe filter.

2.4.3. Nitrobenzene Oxidation (NO)

Kraft lignin and lignin fractions were submitted to alkaline NO following the method and conditions described in previous works [25]. Briefly, about 30 mg of each lignin was dissolved in 7.0 mL of an aqueous solution NaOH 2M with 0.45 mL of nitrobenzene, and after stirring, the mixture was heated for 4 h to a temperature of 170 °C. The oxidized mixture was submitted to a first extraction with chloroform and after that, the aqueous phase was acidified with H₂SO₄ and extracted again with chloroform. The resulting organic phase was evaporated and re-dissolved in 10 mL of methanol. The products from NO were analyzed using the equipment, the operating conditions, the column, the eluents, and the gradient already described [26].

2.4.4. ¹³C NMR

The ¹³C NMR analyses were performed using a spectrometer Bruker AVANCE III 400 operating at 400 MHz, with a temperature of 45 °C, for 72 h. Kraft lignin and lignin fractions, about 170 mg of dry weight, were dissolved in 0.5 mL deuterated dimethyl sulfoxide (DMSO-*d*₆) and quantitatively measured using the NMR conditions described in [8]. The quantitative analyses were carried out using Bruker TopSpin NMR software.

2.4.5. ^{31}P NMR

Lignin preparation for ^{31}P NMR analyses was performed according to the procedure previously published [8]. Phosphitylated lignins were analyzed using a Bruker AVANCE III 400 spectrometer operating at 400 MHz and 25 °C. The ^{31}P NMR spectrum was acquired for 30 min with 10 s relaxation time, 45° pulse angle, and 4 s pulse delay. The cholesterol was used as the internal reference (δ 145 ppm) and the quantitative analyses were carried out using Bruker TopSpin 4.1.1 NMR software.

2.4.6. Oxidative Depolymerization

Kraft lignin and lignin fraction IF 30:70 were submitted to oxidative depolymerization using O_2 as the oxidant in an alkaline medium. Lignin oxidation was performed in an autoclave Büchi AG (model BEP280 type II) with a capacity of 1 L. The information about the equipment set-up and the reaction conditions used for the oxidative depolymerization were described in detail in the literature [27]. About 30 g of kraft lignin and lignin fraction IF 30:70 were dissolved in 500 mL of 2 M NaOH and, after stirring, were introduced into the reactor preheated (120 °C) and pressurized with nitrogen until 6.8 bar. The oxidation reaction begins with the admission of O_2 and the start of the data acquisition. During oxidation, the total pressure in the reactor was kept at 9.8 bar with a partial pressure of O_2 of 3.0 bar by a continuous supply of O_2 over time. Samples from the reaction mixture were collected at regular time intervals. The oxidation products in the collected samples were analyzed following the procedure, equipment, and conditions as previously published in the literature [26,28].

3. Results and Discussion

3.1. Kraft Lignin Solubility in Acetone Aqueous Solutions

The selective fractionation started dissolving kraft lignin in acetone, water, and acetone–water mixtures with different proportions ranging from 20 to 80% v/v —Figure 2. The objective of the solubility tests was to find the ideal acetone percentage (v/v) in an aqueous solution where lignin dissolves as completely as possible but still close to the tipping point where a small increase in water or acetone content decreases its solubility, causing lignin precipitation. Moreover, it is important to state that lignin's physical and chemical properties can vary depending on the wood species and its isolation process. Consequently, some characteristics, namely the solubility in a specific solvent, could be very different between lignins and depend on their structural characteristics.

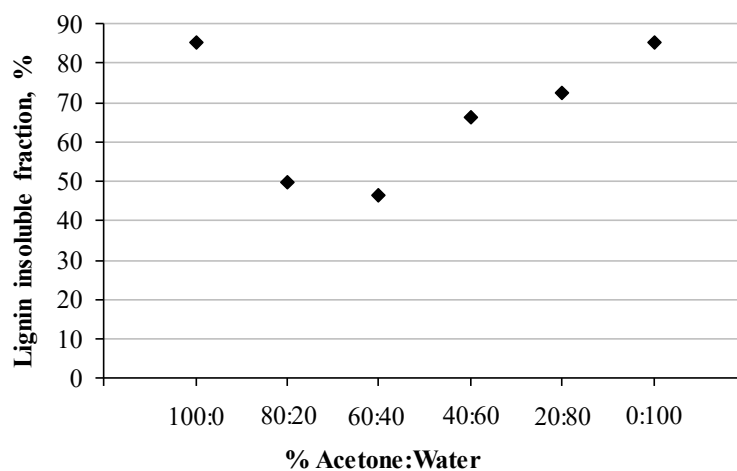


Figure 2. Percentage of insoluble fraction of lignin in different acetone aqueous solutions.

From Figure 2, it is observed that kraft lignin solubility depends strongly on acetone concentration in the mixture, and the highest solubility was achieved when the percentage of acetone in the aqueous solution was 60% v/v . Higher or lower volumes of acetone

or water in the mixture result in a higher percentage of insoluble lignin fraction. The results agree with those found in other solvent fractionation studies using acetone aqueous solutions [14,29].

3.2. Fractionation Yields of Kraft Lignin Fractions

After the selection of the acetone concentration that leads to the highest solubility of kraft lignin, the fractionation process was performed following the scheme of Figure 1. The fractionation with acetone aqueous mixtures shows that kraft lignin is composed of 45%, 39%, and 13% of the insoluble fractions IF 60:40, IF 30:70, and IF 15:85, respectively, and only 3% of the soluble fraction SF 15:85, as illustrated in Figure 3. The sum of all the insoluble and soluble fractions was close to 100%.

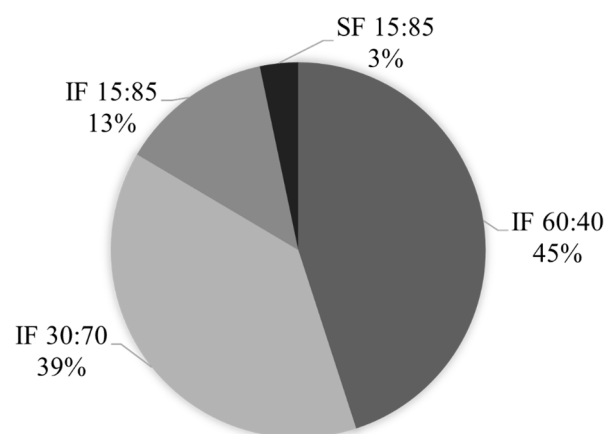


Figure 3. Fractionation yields (%) for IF 60:40, IF 30:70, IF 15:85, and SF 15:85, relative to the initial content of kraft lignin (LKL).

3.3. Inorganics Content of Kraft Lignin and Lignin Fractions

The content of inorganics determined in the kraft lignin (LKL) and lignin fractions is depicted in Table 1 as component weight per 100 g of lignin.

Table 1. Inorganics content of kraft lignin and lignin fractions.

	Lignins, % w/w lignin (Dry Weight)				
	LKL	IF 60:40	IF 30:70	IF 15:85	SF 15:85
Inorganics content	17.5	74.1	0.94	0.37	1.40

The unfractionated LKL contains 17.5% of inorganics. Concerning lignin fractions, the highest inorganics content was found in IF 60:40 (74.1%). This fraction shows a higher percentage of inorganic contaminants than the initial kraft lignin and consequently has a low purity degree. The inorganic material accumulates in this insoluble fraction (IF 60:40), confirming the hydrophobic behavior of ashes and contributing to the low molecular weight of this fraction.

All the other fractions, IF 30:70, IF 15:85, and SF 15:85, are relatively pure with a total content of inorganic material below 1.5%. It could be noted that the fractionation process using acetone aqueous mixtures allows obtaining purified lignin fractions from inorganic materials.

3.4. Molecular Weight Distribution of Lignins

The molecular weight distribution of kraft lignin and lignin fractions was determined using GPC. The chromatograms of lignins, normalized and overlaid, are presented in Figure 4.

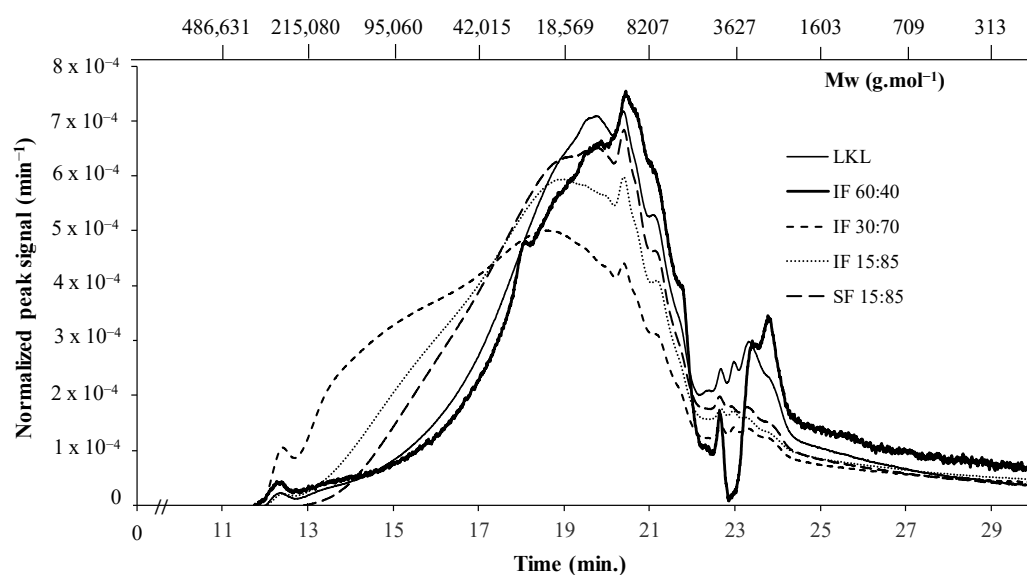


Figure 4. Molecular weight distribution curves obtained from GPC analyses of kraft lignin and lignin fractions.

The molecular weight (Mw) and polydispersity index (Mw/Mn) determined for LKL and each lignin fraction are shown in Table 2. It is important to point out that the GPC calibration was made with standards of polystyrene, thus, the obtained values of Mw are relative to these polymers and the analysis of lignin samples only provides the relative values of molecular weight. Moreover, structural differences between the polystyrene standards (linear polymers) and the branched lignin macromolecules are a known source of error in the determination of molecular weight [30]. The lack of standardization and the difficulties associated with accurate lignin molecular weight determination were already stated in previous studies [11,31].

Table 2. Molecular weight (Mw) and polydispersity (Mw/Mn) of kraft lignin and lignin fractions analyzed by GPC.

Lignin	Mw (g.mol ⁻¹)	Mw/Mn
LKL	69,233	1.17
IF 60:40	48,966	1.23
IF 30:70	121,989	1.27
IF 15:85	78,538	1.21
SF 15:85	54,476	1.15

Considering the samples IF 30:70, IF 15:85, and SF 15:85, it is possible to observe that their chromatographic profiles (Figure 4) progressively shift from low elution volumes to higher ones as the fractionation proceeds, which corresponds to an increase in the lignin fragments of lower molecular weight. Moreover, the observed decrease in molecular weight and polydispersity (Table 2) from IF 30:70 to SF 15:85 indicates that more homogeneous lignins with narrower molecular weight distributions were obtained, as already found by other authors [10,12].

Considering IF 60:40, the lowest molecular mass obtained for this fraction is a result of the low molecular weight of inorganic contaminants in its composition, mainly due to the presence of pulping residual chemicals, especially Na and S, which remain with lignin after the isolation process from kraft liquor. However, as already stated, it is difficult to compare the Mw and Mw/Mn values obtained for kraft lignin fractions with the available data found in the literature. This is a consequence of the strong dependence of the molecular weight distribution with the isolation and fractionation procedure, the nature of the lignin samples, and the uncertainties related to the GPC analysis itself [10,31].

3.5. Nitrobenzene Oxidation

The relative proportion between the aromatic moieties H, G, and S in the non-condensed structures of kraft lignin and lignin fractions was accessed by NO. Oxidation using nitrobenzene in an alkaline medium allows an evaluation of the yields and types of simple phenolic monomers: p-hydroxybenzaldehyde (Hy), vanillin (V), syringaldehyde (Sy), vanillic acid (VA), and syringic acid (SA). In Table 3, the product yields are reported on the total lignin weight free of inorganics.

Table 3. Yields of phenolic monomers obtained by NO of kraft lignin and lignin fractions.

Lignin	Products, % w/w_{lignin} ¹					
	Hy	VA	SA	V	Sy	η NO _{total}
LKL	0.13	0.34	2.79	2.58	10.5	16.3
IF 60:40	0.00	0.07	1.41	1.02	3.66	6.15
IF 30:70	0.03	0.44	1.30	3.34	8.82	13.9
IF 15:85	0.11	0.39	2.42	2.85	8.46	14.2
SF 15:85	0.08	0.32	1.24	2.32	8.16	12.1

¹ the product yields are reported to nonvolatile solids weight free of inorganics.

The main oxidation product is Sy, evidencing that all the lignin fractions show a predominance of S units, a consequence of the origin of kraft lignin—hardwood biomass. Significant contents of V and SA were also obtained. Total yields on a lignin basis are in the range of 6.15–16.3%. IF 60:40 shows the lowest fraction of non-condensed structures, confirmed by the lowest NO yield; moreover, as already stated before, this fraction also denotes a low degree of purity due to the high content of contaminants. IF 30:70 and IF 15:85 show the highest yield of monomeric phenolic products among all samples, around 14% w/w_{lignin} , confirming the lowest content of condensed fragments in these fractions.

3.6. ¹³C NMR

¹³C NMR allows obtaining valuable information about the chemical structure of lignin. The assignments and the quantification of the structures/linkages and functional groups of kraft lignin and lignin fractions were made based on reference spectra and data from this spectroscopic method available in the literature [8,32,33]. Table 4 depicts the main structures, linkages, and functional groups identified and later quantified in lignin fractions. The ¹³C NMR spectrum of each fraction is shown in Figure 5. Fraction IF 60:40 was not analyzed by ¹³C NMR since the high amount of inorganic contaminants made its dissolution in the analysis solvent difficult (DMSO-*d*₆).

Table 4. Assignments and quantification (number per aromatic ring) of the structures/linkages and functional groups identified by ¹³C NMR for kraft lignin and lignin fractions.

Assignments (Spectroscopic Range)	Amount (Number per Aromatic Ring)			
	LKL	IF 30:70	IF 15:85	SF 15:85
Cβ in β-5 and β-β structures (δ 51.0–53.8 ppm)	0.12	0.12	0.13	0.11
Aromatic OCH ₃ (δ 54.3–57.3 ppm)	1.29	1.40	1.44	1.43
Cγ in β-O-4 structures without Cα=O (δ 59.3–60.8 ppm)	0.14	0.19	0.16	0.13
Cγ in β-5 and β-O-4 structures with Cα=O; Cγ in β-1 (δ 62.5–63.8 ppm)	0.12	0.07	0.07	0.05
Cα in β-O-4 structures; Cγ in pinioresinol/syringaresinol and β-β structures (δ 70.0–76.0 ppm)	0.65	0.40	0.35	0.30
Cβ in β-O-4 structures; Cα in β-5 and β-β structures (δ 80.0–90.0 ppm)	0.38	0.46	0.40	0.32
Aromatic C _{Ar} -H (δ 103.0–123.0 ppm)	1.93	1.94	1.87	1.88
Aromatic C _{Ar} -C (δ 123.0–137.0 ppm)	1.64	1.76	1.70	1.76
Aromatic C _{Ar} -O (δ 137.0–156.0 ppm)	2.34	2.40	2.36	2.32

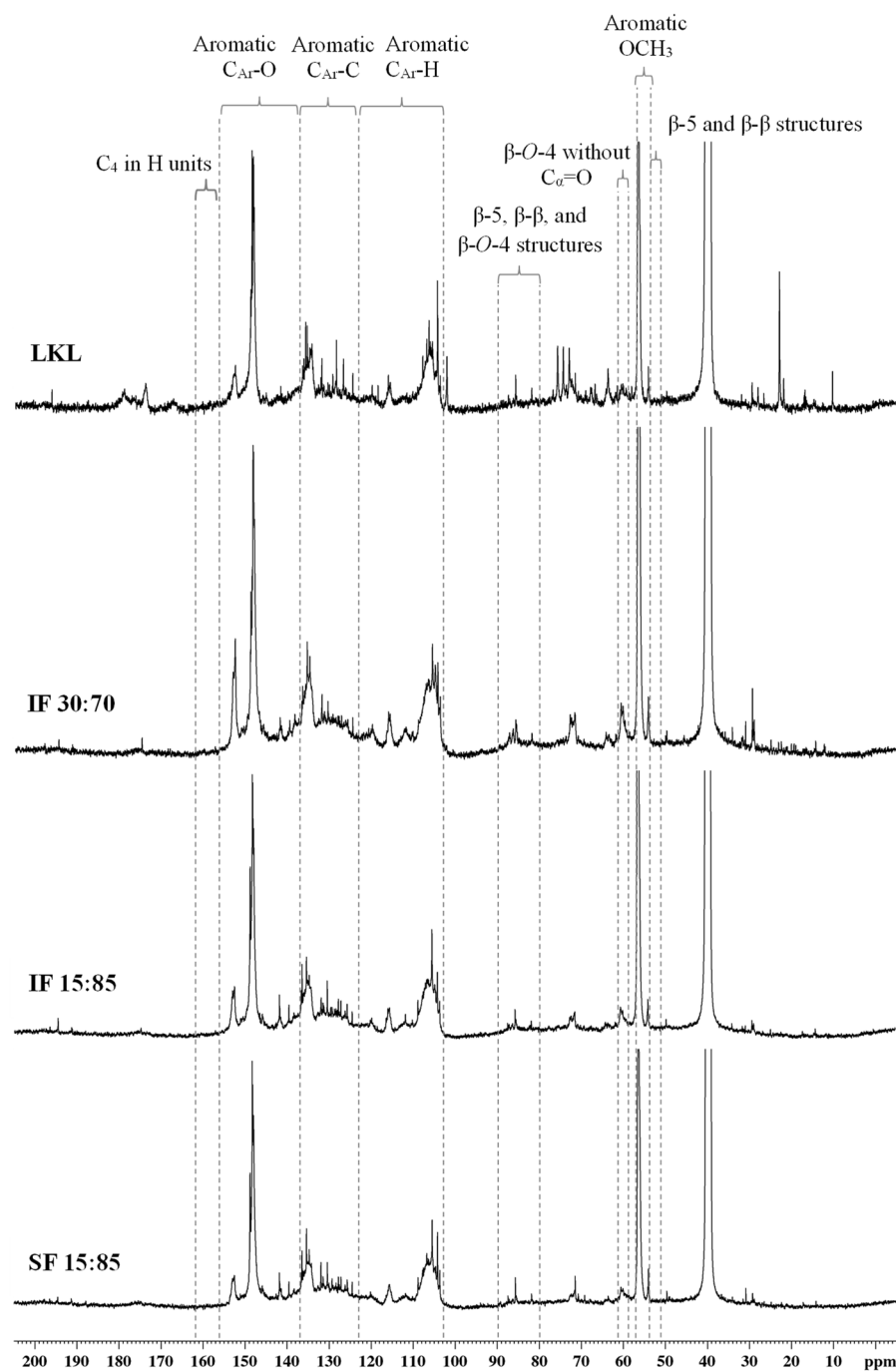


Figure 5. Quantitative ^{13}C NMR spectra of LKL, IF 30:70, IF 15:85, and SF 15:85.

The determination of the main structural characteristics of kraft lignin and lignin fractions was attained using the quantitative data from ^{13}C NMR (Table 5): the content of β -O-4 structures (number per 100 aromatic rings), the S:G:H, S/G ratio, and the DC (degree of condensation, in percentage). The content of β -O-4 structures was determined from the integral at δ 80–90 ppm; however, this region of integration also includes β -5 and β - β structures. Thus, the amount of total β -O-4 is calculated from the region that includes the integral at δ 51–53.8 ppm subtracted from the value obtained by the integral at δ 80–90 ppm. The DC value was achieved from the difference between the theoretical and the experimental values of $\text{C}_{\text{Ar-H}}$, resulting in the percentage of condensed moieties in lignin structure, as described previously in the literature [8].

Table 5. β -O-4 structures content (number per 100 aromatic rings), DC, S:G:H, and S/G ratio found for LKL and lignin fractions.

Lignins	β -O-4 Structures	DC (%)	S:G:H	S/G
LKL	26	34	62:26:12	2.35
IF 30:70	33	36	63:30:07	2.10
IF 15:85	27	39	66:27:07	2.46
SF 15:85	20	40	68:27:05	2.49

The ^{13}C NMR data show that lignin fraction SF 15:85 has the lowest content of non-condensed β -O-4 structures (Table 5), which is in accordance with the lowest total yield of phenolic products in this sample, determined by NO (Table 3). Moreover, this fraction also presents the highest degree of condensation. The inversely proportional relationship between condensation degree and NO total yield was already stated in the literature [8], and it is related to the resistance of aryl–aryl covalent linkages to the depolymerization induced by nitrobenzene oxidation in an alkaline medium. From Table 5, it is also possible to observe that S units and S/G ratio increase as the fractionation proceeds. The fraction IF 30:70 shows the lowest content of S units and S/G ratio and the highest values of Mw and polydispersity (Table 2). On the other hand, the proportion of S units, and consequently the S/G ratio, slightly increases in the most water-soluble fraction, SF 15:85, which shows the lowest value of molecular weight.

3.7. ^{31}P NMR

The phenolic hydroxyl group is one of the most important functional groups affecting the physical and chemical properties of lignin. Its quantitative measurement provides relevant information about the structure and reactivity of each lignin [11]. ^{31}P NMR analyses were performed for all the lignin fractions except for IF 60:40. As already verified for ^{13}C NMR analyses, due to the high content of inorganics, this fraction did not dissolve in the NMR solvents and the analysis by ^{31}P NMR was not possible. The ^{31}P NMR spectrum of lignin fractions and kraft lignin is presented in Figure 6. Quantitative ^{31}P NMR data of lignins are detailed in Table 6. Identification and quantification were performed as detailed in the literature [4,8,34].

Table 6. Assignments and quantification of phenolic and aliphatic hydroxyl groups and carboxylic acids in kraft lignin and lignin fractions by ^{31}P NMR.

Assignments (Spectroscopic Range)	Amount (mmol/g lignin)			
	LKL	IF 30:70	IF 15:85	SF 15:85
Aliphatic OH (δ 150.2–145.8 ppm)	3.59	1.84	1.38	1.03
Carboxylic acids (δ 135.0–133.0 ppm)	2.34	0.18	0.20	0.14
Total phenolic units	7.72	3.98	4.11	4.33
Condensed phenolic units (δ 144.4–143.2 ppm; δ 141.6–139.6 ppm)	2.04	0.79	0.83	0.81
Non-condensed phenolic units				
S phenolic units (δ 143.2–141.6 ppm)	4.59	2.31	2.43	2.64
G phenolic units (δ 139.6–136.9 ppm)	1.04	0.86	0.84	0.87
H phenolic units (δ 136.8–136.3 ppm)	0.04	0.02	0.01	0.01

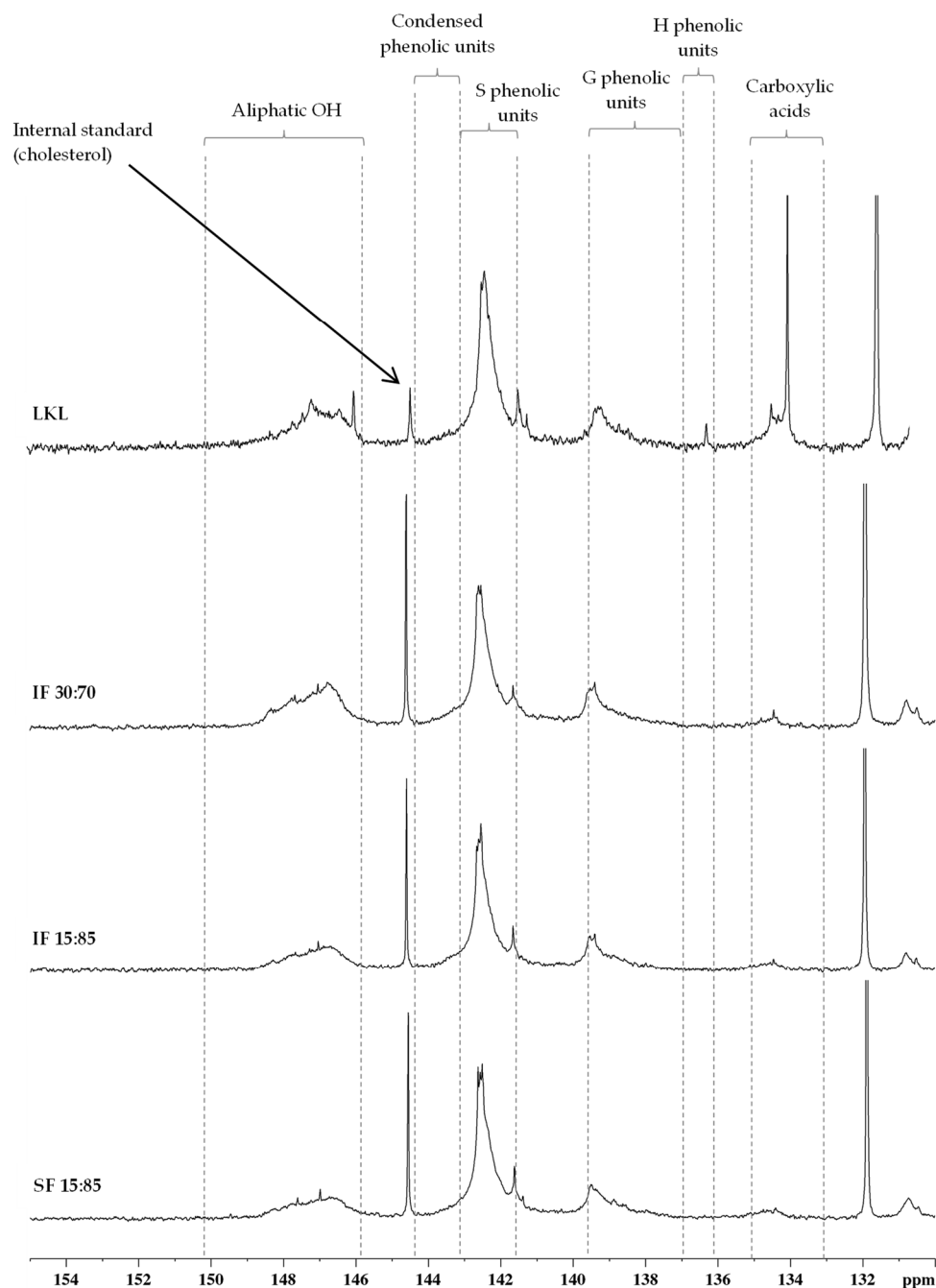


Figure 6. Quantitative ^{31}P NMR spectra (δ 155–130 ppm) of phosphitylated lignins: LKL, IF 30:70, IF 15:85, and SF 15:85 (in CDCl_3).

From ^{31}P NMR results, it is possible to observe differences in the integrated regions of each lignin fraction; however, phenolic OH units were predominant in all the lignin fractions. Phenolic OH units enhance the hydrophilicity of lignins, and consequently, fractions with lower molecular weights but a higher phenolic OH content were separated in a large stage of the fractionation process, resulting in the most water-soluble fractions [15,35]. SF 15:85 has the highest amount of total phenolic units and is the most soluble fraction along the sequential fractionation process, confirming the relationship stated. It is also noticeable that IF 30:70, the insoluble lignin fraction from the solution with a higher percentage of acetone, considering that IF 60:40 was not analyzed, contains a higher content of aliphatic OH groups and a lower content of phenolic OH groups. This fraction also has the highest Mw, proving that the aliphatic OH groups are present in the fractions with higher

molecular weight. The increase in phenolic OH groups and the simultaneous decrease in aliphatic OH with the decrease in molecular weight has been reported in several studies of kraft lignin fractionation using organic solvents [13,16,19].

As already stated, β -O-4 structures are the most common in lignin structure and their cleavage lead to an increase in phenolic OH-group content and a decrease in lignin molecular mass. The decrease in aryl-ether linkages and the increase in phenolic OH groups during the sequential fractionation process studied is observed in Figure 7, where the correlation between the molar mass, the content of phenolic OH, and the content of β -O-4 structures is represented. It can be observed that the fraction with the lower Mw, SF 15:85, shows the lowest content of β -O-4 structures and presents an increase in the content of phenolic OH comparatively to the other fractions. Results from ^{31}P NMR, namely the content of total phenolic OH groups, clearly demonstrate that the obtained lignin fractions present significant differences considering its functionality. A higher quantity of phenolic groups in lignin implies a high reactivity and enhances the hydrophilicity of lignins, thus consequently making the lignin more attractive for utilization in specific applications.

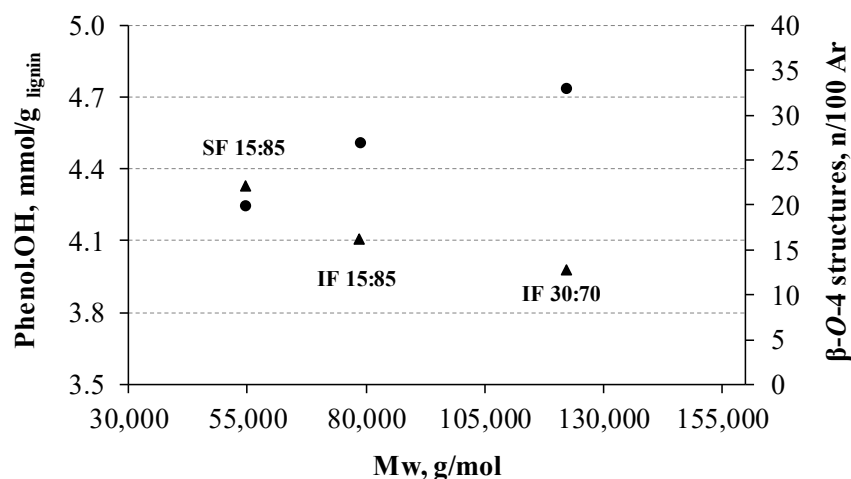


Figure 7. Graphical representation of phenolic OH (▲) and β -O-4 structures (●) versus Mw ($\text{g}\cdot\text{mol}^{-1}$) obtained for each lignin fraction.

3.8. Radar Classification of Kraft Lignin and Lignin Fractions

Since one of the objectives of this work is to find the fraction with the highest potential to produce phenolic compounds, mainly V and Sy, radar plots were used as classification tools, allowing a direct comparison between the structural characteristics of each lignin fraction and LKL. For radar representation, the descriptors selected are β -O-4 structures content, number of noncondensed structures ($\text{NCS} = 100 - \text{DC}$), S and G units, and V and Sy yields obtained from NO. The radar plots of LKL and lignin fractions are presented in Figure 8.

Radar plots validate the structural information found in the previous sections for each lignin fraction and kraft lignin. Among all the lignin fractions, IF 30:70 benefits from its higher intensity of β -O-4 linkages and NCS when compared with the other fractions. The high content of these types of structures leads to the higher reactivity of this lignin, standing out as the fraction with a higher potential to produce phenolic aldehydes through oxidative depolymerization. Besides these structural advantages, it is important to state that the main difference found between all the lignin samples is the low content of inorganic contaminants in fractions IF 30:70, IF 15:85, and SF 15:85 comparatively to LKL and IF 60:40, leading to lignin fractions with high purity.

For the selection of the most suitable fraction to proceed with oxidative depolymerization, a balance between the viability and cost of the fractionation process and the structural features of the obtained fractions is essential. Considering these factors, the insoluble fraction from the aqueous mixture with 30% of acetone (IF 30:70) has been selected as

the more advantageous fraction to proceed for oxidation with O₂ in an alkaline medium, contemplating a route for lignin valorization.

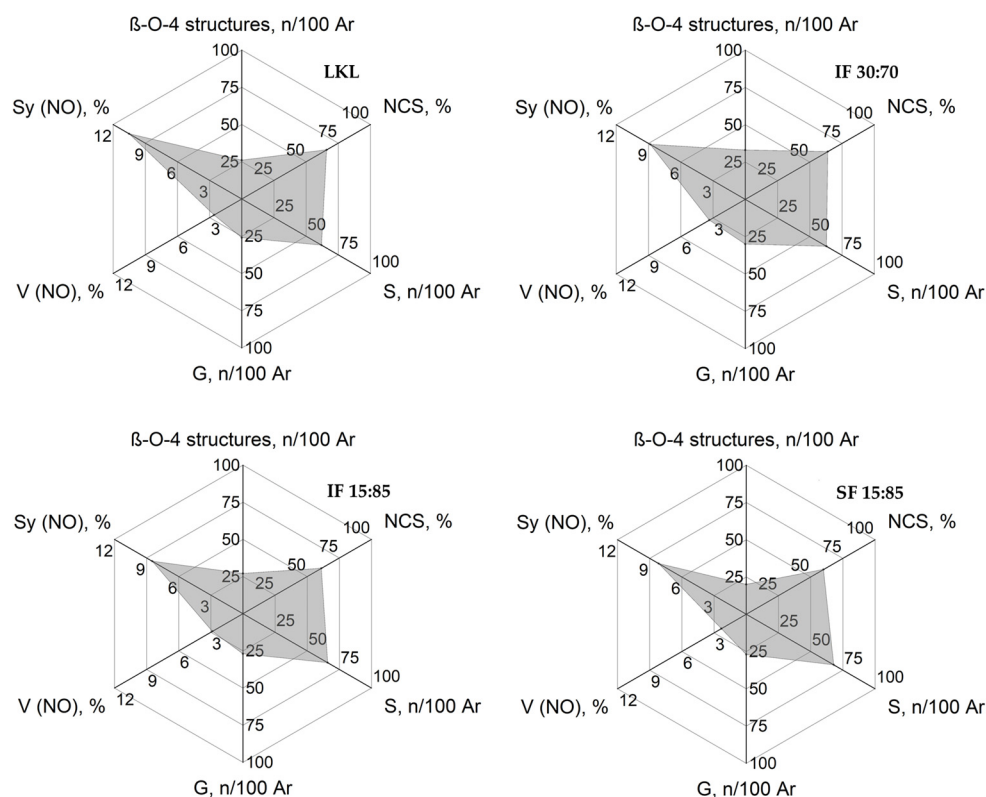


Figure 8. Radar classification of kraft lignin and lignin fractions obtained from the fractionation process using acetone aqueous mixtures.

3.9. Oxidative Depolymerization of Kraft Lignin and Fraction IF 30:70

After the validation from the radar plots, batch oxidation in an alkaline medium with O₂ of LKL and IF 30:70 was performed following the procedure described in Section 2.4.6. The product profiles are presented in Figure 9. V, VA, Sy, and SA have been reported as the main phenolic compounds found in the reaction mixture. Besides the phenolic aldehydes and their respective acids, acetovanillone (VO) and acetosyringone (SO) were also found as oxidation products in lower yields.

The profiles of V and Sy show their maximum yields (% w/w_{lignin}) when the fraction IF 30:70 was submitted to oxidation. An increase of about 13 and 19% was found for V and Sy, respectively, relative to the maximum yield obtained for LKL. Considering the phenolic ketones, SO shows a significant increase of about 50% of the maximum yield in IF 30:70 comparatively to the value found for LKL. The higher yield of this product in the oxidation mixture could be related to the occurrence of specific structures/linkages whose cleavage could originate this type of phenolic products. In the literature, it could be found that the mechanism for the lignin oxidation that expresses the formation of aceto-derivative products, such as VO and SO, is based on the competing addition of OH to the α -position of quinone methide and its subsequent oxidation [6,36,37]. Considering this assumption and based on ¹³C NMR results (Table 4), it could be noticed that the lignin fraction IF 30:70 show the highest content of C α in β -5 and β - β structures (Figure 10) that justifies the higher content of SO found as the oxidation product from this fraction. Moreover, the increase in SO in IF 30:70 comparatively with LKL was remarkably high when compared to the behavior of VO. This result could be related to the different reaction rate of SO and to the higher content S units in the structure of the kraft lignin. The S units have higher reactivity than guaiacyl counterparts (G units) in an alkaline medium and under conditions of O₂ oxidation [6,38,39]. Thus, the oxidation of S units is faster and shows higher yields

than G units for both production and degradation of phenolic products in an oxidation mixture. Concerning the profile of G units, there is an increase in VA content when the fraction IF 30:70 is submitted to oxidation. The increase of this phenolic acid yield could be indicative of a high content of structures with a carbonyl group at C α (benzyl carbon) in the lignin fraction as in the literature it is stated that structures carrying C=O at C α lead preferentially to the carboxylic acids VA and SA [25,40]. However, the estimation of this structural feature in lignin (β -O-4 structures without C α =O), by ^{13}C NMR, is based on C γ in a very narrow chemical shift interval (Table 4) with a high probability of interference of signals from adjacent chemical shifts (for example, C γ in cinnamyl alcohol units) [25]. This reason could justify the fact that the described tendency was not observed in the results from the structural characterization of LKL and the correspondent fractions from acetone aqueous mixtures.

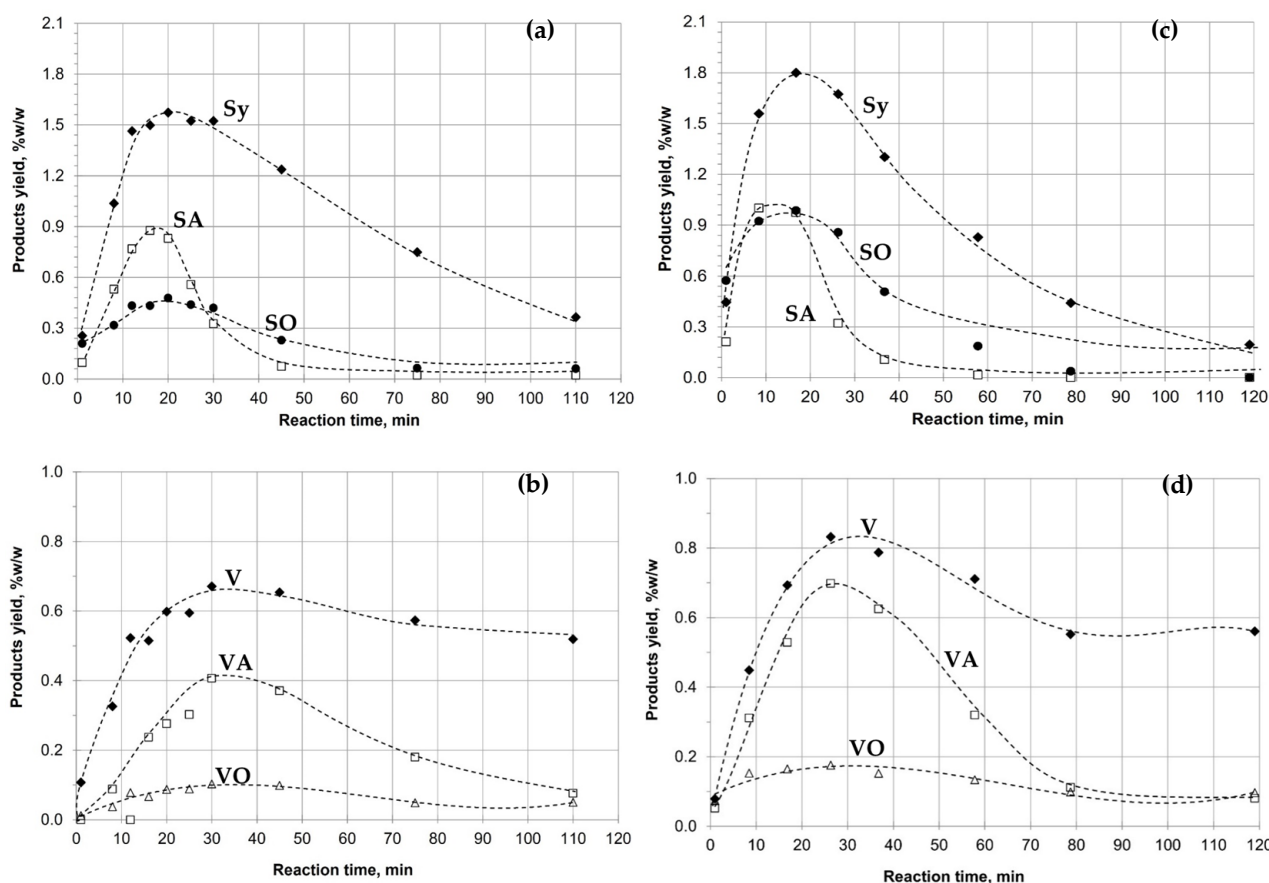


Figure 9. Time evolution of monomeric products: V, Sy, VA, SA, VO, and SO during the oxidation of LKL (a,b) and IF 30:70 (c,d). Lignin concentration = 60 g/L; NaOH concentration = 80 g/L; pH initial ≥ 13.8 , $p\text{O}_2 = 3$ bar; mixture volume = 0.5 L; $P_{\text{total}} = 9.8$ bar; $T_{\text{initial}} = 120$ °C.

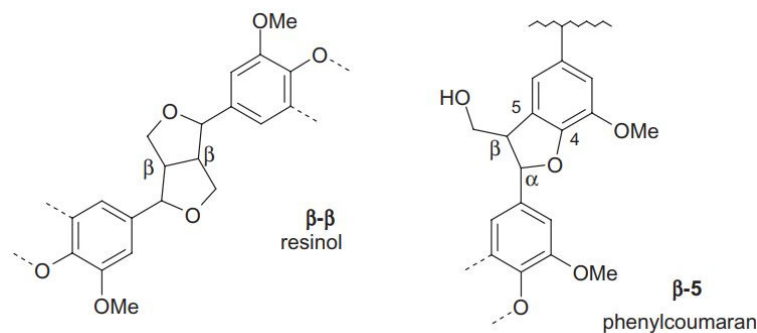


Figure 10. Representation of β -5 and β - β structures present in lignin structure.

4. Conclusions

Sequential solvent fractionation of kraft lignin using acetone aqueous mixtures showed to be an effective method to obtain lignin fractions with specific structural characteristics and low content of impurities. GPC analyses allow the observation of a decrease in molecular weight and polydispersity as the fractionation proceeds, which indicates that lower molecular weight fractions lead to a more homogeneous structure with a narrower molecular weight distribution. The most water-soluble fraction, SF 15:85, shows the lowest molecular weight and a higher phenolic OH content, being the lignin with high reactivity, and is the most suitable to be used as an extender or as a resins component. On the other hand, the fraction IF 30:70 shows the highest values of Mw and content of aliphatic OH groups and the lowest content of phenolic OH groups, proving that this type of OH units is more present in the structures and/or fragments with high molecular weight. Fraction IF 60:40 is the one with a lower degree of purity, containing more than 70% of inorganics, and could be considered for usage as a dispersant or as a chelating agent. Through oxidative depolymerization in an alkaline medium with O₂, fraction IF 30:70 showed to be a potential source of added-value phenolic monomers, with an increase of about 13 and 19% found for V and Sy, respectively, relative to the maximum yield obtained for LKL. Lignin depolymerization and valorization remains a challenge; however, this work allows for corroborating that lignin fractions with different molecular weight and specific structural characteristics could be used for different valuable industrial applications.

Author Contributions: Conceptualization, C.A.E.C. and A.E.R.; methodology, C.A.E.C., F.M.C. and C.V.-A.; validation, C.A.E.C. and A.E.R.; investigation, C.A.E.C.; resources, A.E.R.; writing—original draft, C.A.E.C.; writing—review and editing, C.A.E.C., F.M.C., C.V.-A. and A.E.R.; supervision, A.E.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by LA/P/0045/2020 (ALiCE), UIDB/50020/2020 and UIDP/50020/2020 (LSRE-LCM), funded by national funds through FCT/MCTES (PIDDAC) and Base Funding—UIDB/00690/2020 of CIMO—Centro de Investigação de Montanha, funded by national funds through FCT/MCTES (PIDDAC). COST Action LignoCOST (CA17128). Carlos Vega-Aguilar thanks the Costa Rican Science, Technology and Telecommunications Ministry for the PhD. Scholarship MICITT-PINN-CON-2-1-4-17-1-002.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rodrigues, A.E.; Pinto, P.C.R.; Barreiro, M.F.; Costa, C.A.E.; Mota, M.I.F.; Fernandes, I. *An Integrated Approach for Added-Value Products from Lignocellulosic Biorefineries: Vanillin, Syringaldehyde, Polyphenols and Polyurethane*; Springer International Publishing: Berlin/Heidelberg, Germany, 2018.
2. Yan, J.; Tan, E.C.D.; Katahira, R.; Pray, T.R.; Sun, N. Fractionation of lignin streams using tangential flow filtration. *Ind. Eng. Chem. Res.* **2022**, *61*, 4407–4417. [[CrossRef](#)]
3. Azadi, P.; Inderwildi, O.R.; Farnood, R.; King, D.A. Liquid fuels, hydrogen and chemicals from lignin: A critical review. *Renew. Sustain. Energy Rev.* **2013**, *21*, 506–523. [[CrossRef](#)]
4. Heitner, C.; Dimmel, D.; Schmidt, J. *Lignin and Lignans: Advances in Chemistry*; Taylor & Francis: Oxfordshire, UK, 2010.
5. Sethupathy, S.; Murillo Morales, G.; Gao, L.; Wang, H.; Yang, B.; Jiang, J.; Sun, J.; Zhu, D. Lignin valorization: Status, challenges and opportunities. *Bioresour. Technol.* **2022**, *347*, 126696. [[CrossRef](#)]
6. Pinto, P.C.R.; Borges da Silva, E.A.; Rodrigues, A.E. Lignin as source of fine chemicals: Vanillin and syringaldehyde. In *Biomass Conversion*; Baskar, C., Baskar, S., Dhillon, R.S., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 381–420.
7. Berlin, A.; Balakshin, M. Chapter 18—Industrial lignins: Analysis, properties, and applications. In *Bioenergy Research: Advances and Applications*; Gupta, V.K., Tuohy, M.G., Kubicek, C.P., Saddler, J., Xu, F., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; pp. 315–336. [[CrossRef](#)]
8. Costa, C.A.E.; Pinto, P.C.R.; Rodrigues, A.E. Evaluation of chemical processing impact on E. globulus wood lignin and comparison with bark lignin. *Ind. Crops Prod.* **2014**, *61*, 479–491. [[CrossRef](#)]
9. Rodrigues, J.S.; Lima, V.; Araújo, L.C.P.; Botaro, V.R. Lignin fractionation methods: Can lignin fractions be separated in a true industrial process? *Ind. Eng. Chem. Res.* **2021**, *60*, 10863–10881. [[CrossRef](#)]

10. Costa, C.A.E.; Pinto, P.C.R.; Rodrigues, A.E. Lignin fractionation from E. globulus kraft liquor by ultrafiltration in a three stage membrane sequence. *Sep. Purif. Technol.* **2018**, *192*, 140–151. [\[CrossRef\]](#)
11. Lin, S.Y.; Dence, C.W. *Methods in Lignin Chemistry*; Springer: Berlin/Heidelberg, Germany, 1992.
12. Toledano, A.; Serrano, L.; Garcia, A.; Mondragon, I.; Labidi, J. Comparative study of lignin fractionation by ultrafiltration and selective precipitation. *Chem. Eng. J.* **2010**, *157*, 93–99. [\[CrossRef\]](#)
13. Jiang, X.; Savithri, D.; Du, X.; Pawar, S.; Jameel, H.; Chang, H.-m.; Zhou, X. Fractionation and characterization of kraft lignin by sequential precipitation with various organic solvents. *ACS Sustain. Chem. Eng.* **2017**, *5*, 835–842. [\[CrossRef\]](#)
14. Domínguez-Robles, J.; Tamminen, T.; Liitiä, T.; Peresin, M.S.; Rodríguez, A.; Jääskeläinen, A.-S. Aqueous acetone fractionation of kraft, organosolv and soda lignins. *Int. J. Biol. Macromol.* **2018**, *106*, 979–987. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Park, S.Y.; Kim, J.-Y.; Youn, H.J.; Choi, J.W. Fractionation of lignin macromolecules by sequential organic solvents systems and their characterization for further valuable applications. *Int. J. Biol. Macromol.* **2018**, *106*, 793–802. [\[CrossRef\]](#)
16. Jääskeläinen, A.S.; Liitiä, T.; Mikkelsen, A.; Tamminen, T. Aqueous organic solvent fractionation as means to improve lignin homogeneity and purity. *Ind. Crops Prod.* **2017**, *103*, 51–58. [\[CrossRef\]](#)
17. Boeriu, C.G.; Fițigău, F.I.; Gosselink, R.J.A.; Frissen, A.E.; Stoutjesdijk, J.; Peter, F. Fractionation of five technical lignins by selective extraction in green solvents and characterisation of isolated fractions. *Ind. Crops Prod.* **2014**, *62*, 481–490. [\[CrossRef\]](#)
18. Sadeghifar, H.; Ragauskas, A. Perspective on technical lignin fractionation. *ACS Sustain. Chem. Eng.* **2020**, *8*, 8086–8101. [\[CrossRef\]](#)
19. Wang, Y.Y.; Li, M.; Wyman, C.E.; Cai, C.M.; Ragauskas, A.J. Fast fractionation of technical lignins by organic cosolvents. *ACS Sustain. Chem. Eng.* **2018**, *6*, 6064–6072. [\[CrossRef\]](#)
20. Jin, H.; Shi, H.; Jia, W.; Sun, Y.; Sheng, X.; Guo, Y.; Li, H.; Sun, H. Green solvents-based molecular weight controllable fractionation process for industrial alkali lignin at room temperature. *Int. J. Biol. Macromol.* **2022**, *207*, 531–540. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Ponnuchamy, V.; Gordobil, O.; Diaz, R.H.; Sandak, A.; Sandak, J. Fractionation of lignin using organic solvents: A combined experimental and theoretical study. *Int. J. Biol. Macromol.* **2021**, *168*, 792–805. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Sivagurunathan, P.; Raj, T.; Mohanta, C.S.; Semwal, S.; Satlewal, A.; Gupta, R.P.; Puri, S.K.; Ramakumar, S.S.V.; Kumar, R. 2G waste lignin to fuel and high value-added chemicals: Approaches, challenges and future outlook for sustainable development. *Chemosphere* **2021**, *268*, 129326. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Pinto, P.C.R.; Costa, C.E.; Rodrigues, A.E. Oxidation of lignin from Eucalyptus globulus pulping liquors to produce syringaldehyde and vanillin. *Ind. Eng. Chem. Res.* **2013**, *52*, 4421–4428. [\[CrossRef\]](#)
24. Casimiro, F.M.; Costa, C.A.E.; Vega-Aguilar, C.; Rodrigues, A.E. Hardwood and softwood lignins from sulfite liquors: Structural characterization and valorization through depolymerization. *Int. J. Biol. Macromol.* **2022**, *215*, 272–279. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Costa, C.A.E.; Pinto, P.C.R.; Rodrigues, A.E. Radar tool for lignin classification on the perspective of its valorization. *Ind. Eng. Chem. Res.* **2015**, *54*, 7580–7590. [\[CrossRef\]](#)
26. Pinto, P.C.; Borges da Silva, E.A.; Rodrigues, A.E. Comparative study of SPE and liquid-liquid extraction of lignin oxidation products for HPLC-UV quantification. *Ind. Eng. Chem. Res.* **2010**, *49*, 12311–12318. [\[CrossRef\]](#)
27. Casimiro, F.M.; Costa, C.A.E.; Botelho, C.M.; Barreiro, M.F.; Rodrigues, A.E. Kinetics of oxidative degradation of lignin-based phenolic compounds in batch reactor. *Ind. Eng. Chem. Res.* **2019**, *58*, 16442–16449. [\[CrossRef\]](#)
28. Pinto, P.C.; Borges da Silva, E.A.; Rodrigues, A.E. Insights into oxidative conversion of lignin to high-added-value phenolic aldehydes. *Ind. Eng. Chem. Res.* **2011**, *50*, 741–748. [\[CrossRef\]](#)
29. Sadeghifar, H.; Wells, T.; Le, R.K.; Sadeghifar, F.; Yuan, J.S.; Jonas Ragauskas, A. Fractionation of organosolv lignin using acetone:water and properties of the obtained fractions. *ACS Sustain. Chem. Eng.* **2017**, *5*, 580–587. [\[CrossRef\]](#)
30. Ringena, O.; Lebiada, S.; Lehnen, R.; Saake, B. Size-exclusion chromatography of technical lignins in dimethyl sulfoxide/water and dimethylacetamide. *J. Chromatogr. A* **2006**, *1102*, 154–163. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Constant, S.; Wienk, H.L.J.; Frissen, A.E.; Peinder, P.d.; Boelens, R.; van Es, D.S.; Grisel, R.J.H.; Weckhuysen, B.M.; Huijgen, W.J.J.; Gosselink, R.J.A.; et al. New insights into the structure and composition of technical lignins: A comparative characterisation study. *Green Chem.* **2016**, *18*, 2651–2665. [\[CrossRef\]](#)
32. Balakshin, M.Y.; Capanema, E.A.; Santos, R.B.; Chang, H.; Jameel, H. Structural analysis of hardwood native lignins by quantitative ¹³C NMR spectroscopy. *Holzforschung* **2015**, *70*, 95–108. [\[CrossRef\]](#)
33. Capanema, E.A.; Balakshin, M.Y.; Kadla, J.F. A comprehensive approach for quantitative lignin characterization by NMR spectroscopy. *J. Agric. Food Chem.* **2004**, *52*, 1850–1860. [\[CrossRef\]](#)
34. Argyropoulos, D.S.; Abacherli, A.; Rincón, A.G.; Arx, U.V. Quantitative ³¹P nuclear magnetic resonance (NMR) spectra of lignin. In *Analytical Methods for Lignin Characterisation*; International Lignin Institute: Lausanne, Switzerland, 2009.
35. Araújo, L.C.P.; Yamaji, F.M.; Lima, V.H.; Botaro, V.R. Kraft lignin fractionation by organic solvents: Correlation between molar mass and higher heating value. *Bioresour. Technol.* **2020**, *314*, 123757. [\[CrossRef\]](#)
36. Tarabanko, V.E.; Tarabanko, N. Catalytic oxidation of lignins into the aromatic aldehydes: General process trends and development prospects. *Int. J. Mol. Sci.* **2017**, *18*, 2421. [\[CrossRef\]](#)
37. Tarabanko, V.E.; Petukhov, D.V.; Selyutin, G.E. New mechanism for the catalytic oxidation of lignin to vanillin. *Kinet. Catal.* **2004**, *45*, 569–577. [\[CrossRef\]](#)
38. Tsutsumi, Y.; Kondo, R.; Sakai, K.; Imamura, H. The difference of reactivity between syringyl lignin and guaiacyl lignin in alkaline systems. *Holzforschung* **1995**, *49*, 423–428. [\[CrossRef\]](#)

39. Sultanov, V.S.; Wallis, A.F.A. Reactivities of guaiacyl and syringyl lignin model phenols towards oxidation with oxygen-alkali. *J. Wood Chem. Technol.* **1991**, *11*, 291–305. [[CrossRef](#)]
40. Gierer, J.; Imsgard, F.; Norén, I. Studies on the degradation of phenolic lignin units of the β aryl ether type with oxygen in alkaline media. *Acta Chem. Scand. B* **1977**, *31*, 561–572. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.