International Journal of Biological Macromolecules 215 (2022) 272–279

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Hardwood and softwood lignins from sulfite liquors: Structural characterization and valorization through depolymerization

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ARTICLE INFO

Keywords:
Sulfite liquor
Lignin
Structural characterization
Oxidative depolymerization
Phenolic monomers

ABSTRACT

This work aims to evaluate the structural characteristics and study the oxidative depolymerization of lignins obtained from hardwood and softwood sulfite liquors. Lignins were obtained after ultrafiltration and freeze-drying of the sulfite liquors and characterized based on inorganic content, nitrobenzene oxidation, ^{13}C NMR, and molecular weight determination. The structural characteristics achieved allow evaluating the potential of each lignin through oxidative depolymerization to produce added-value phenolic monomers. Hardwood and softwood lignins were submitted to alkaline oxidation with oxygen and the reaction conditions optimized to obtain a final oxidation mixture with the maximum yield of phenolic monomers. Through oxidation with O_2, hardwood lignin generates mostly syringaldehyde while lignin from softwood biomass mainly produces vanillin; moreover, a lower reaction time and the interruption of O_2 admission avoid the degradation of the oxidation products in the final mixture for both lignins, more evidenced to hardwood lignin due to its higher reactivity. From the results, it is possible to conclude that a phenolic aldehyde-rich oxidation mixture could be obtained, confirming the viability of lignin as raw material to produce added-value products as vanillin and syringaldehyde.

1. Introduction

Lignin is the most abundant natural phenolic polymer in the world. It is an amorphous, three-dimensional biopolymer arising from polymerization of three basic units: *p*-hydroxycinnamyl, coniferyl, and sinapyl alcohol. These monomers differ in the degree of substitution at the phenolic ring and are the precursors of the main moieties present in lignin structure: H (*p*-hydroxyphenyl), G (guaiacyl), and S (syringyl) units. The ratio between G, S and H units, type of structures, linkages, and functional groups in lignin differ with the biomass species, processing, and isolation conditions [1–3].

Nowadays, technical lignins can be generated as a byproduct of the pulping process. The major commercial lignins are obtained from kraft and sulfite processes however, other lignins such as organosolv are obtained from pulping processes using organic solvents. Kraft pulping is the main process for production of pulp and paper and originates kraft lignin while sulfite pulping is the predominant lignin-producing process [4]. Lignins from sulfite pulping process are denoted as lignosulfonates and are produced using sulfites (SO_3^{2-}) or bisulfites (HSO_3^-) as the pulping agents, at different pH levels [3]. The most common counter ion of sulfite and bisulfite salts is magnesium (Mg^{2+}), but calcium (Ca^{2+}), sodium (Na^+) or ammonium (NH_4^+) have been employed as well in sulfite process [5].

Currently, lignosulfonates account for 90 % of the total market of commercial lignin, and its total annual worldwide production is approximately 1.8 million tons [6]. Lignosulfonates are water-soluble and have quite high inorganic content [7,8]. Moreover, these types of lignins are typically highly cross-linked lignins and its weight-average molecular weight is higher than kraft lignins, values in the range 10–60 kDa have been reported in literature, with a corresponding broad polydispersity [9,10]. Lignins from sulfite process have an established market being used mainly as dispersants in cement admixtures, additives, surfactants, pesticides, stabilizers, flocculants, and binder for pelleting animal feed [3,6,7].

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https://doi.org/10.1016/j.ijbiomac.2022.06.067
Received 21 April 2022; Received in revised form 7 June 2022; Accepted 10 June 2022
Available online 16 June 2022

The variability of lignin structure and composition is one of the main challenges considering lignin valorization through the production of value-added products. Vanillin (V), syringaldehyde (Sy), vanillic acid (VA), syringic acid (SA), acetovanillone (VO), and acetosyringone (SO) are main reaction products derived from lignin depolymerization through alkaline oxidation using oxygen as oxidant. V is one of the most widely used flavoring agents, it is known for its vanilla scent and flavor and it is used as a precursor for the synthesis of several second-generation fine chemicals and pharmaceuticals, and in the food and fragrances industries as well [8,11]. Nowadays, 85 % of vanillin supply is synthesized from oil derived guaiacol and glyoxylic acid, attaining around 15,000 t per year at the price 6–15 USD/kg, the remaining 15 % come from softwood lignin [12]. Only, about 1 % of the global demand for vanillin is assured by natural origin (vanilla beans) [5]. VA, an oxidized form of V, is used as a flavoring agent and is often applied as preservative in argan oil and as acidity regulator in wine and vinegar. VO, also known as apocynin, is structurally related to V, and has been studied due to their important pharmacological properties [13,14]. Sy is an organic compound occurring, in small quantities, in nature. This phenolic is important in pharmaceutical applications due to their anti-oxidant, anti-inflammatory, antimicrobial and antifungal properties [15]. SA is extensively used as therapeutic agent, antioxidant, antimicrobial, anti-inflammatory and anticancer [16,17], while SO is a chemical compound related to acetophenone and is used as a plant hormone and insect attractor [18].

The depolymerization of lignosulfonates by alkaline oxidation has been a commercial process since at least 1945 [19–21]. Moreover, oxidation in alkaline medium with oxygen was the main method studied in literature and by our research group, in associate laboratory LSRE-LCM, for lignin valorization through depolymerization to produce high-added-value chemicals [22–30]. In this work, lignins from hardwood and softwood sulfite liquors, LHSL and LSSL, were evaluated as potential sources of phenolic monomers, mainly Sy and V, through oxidation with O_2 in alkaline medium. The profiles of Sy, V, and other minor products were established, and some reaction conditions optimized. Characterization of both lignins was also achieved.

2. Material and methods

2.1. Sulfite liquors and lignins

Two spent sulfite liquors from different origins were studied. A hardwood sulfite liquor (HSL, total solids, 15.0 % w/w_{liquor}) from *Eucalyptus globulus*, gently provided by Caima (Constância, Portugal), and a softwood sulfite liquor (SSL, total solids, 11.3 % w/w_{liquor}), supplied by a pulp mill of Company A, from a mixture of 90 % of spruce (softwood material) and 10 % of beech (hardwood biomass).

HSL and SSL were submitted to ultrafiltration performed in a G.E. Osmonics SEPA CF II crossflow filtration system using a Synder MT membrane with a molecular weight cutoff (MWCO) of 5 kDa. The experiment was carried out with a total recirculation of the feed solution. The operating conditions of the ultrafiltration of each liquor are presented in Table 1.

The resulting retentates were freeze-dried (−80 °C and 0.05 bar) and the yields on lignin recovered from this isolation procedure were 39 % for LHSL and 53 % for LSSL, calculated with reference to the total nonvolatile solids on the respective liquors. The obtained lignins, LHSL and LSSL, were characterized and submitted to oxidation using the methods and techniques described in the following sections.

Table 1
SSL and HSL ultrafiltration operating conditions.

	HSL	SSL
Membrane MWCO, Da	5000	
Pressure, bar	9	
Initial volume, mL	4000	
Liquor pH	2.3	3.4
Temperature[a], °C	28	27
Retentate volume, mL	800	900

[a] Average value.

2.2. Inorganic content of lignins

The inorganic content of LSSL and LHSL was determined by gravimetric quantification after incineration of about 100 mg of each lignin in a muffle furnace at 600 °C during 6 h [2,27]. Inorganics determination was performed in duplicated. The results, as component weigh per 100 g of lignin, are depicted in Table 2.

The inorganic content found for LHSL and LSSL are in accordance with other studies in literature concerning lignosulfonates characterization, where values of inorganics in the range 4–10 % w/w_{lignin} were reported [31–34].

2.3. Nitrobenzene oxidation (NO)

Lignins, LHSL and LSSL, were submitted to alkaline NO as already described in the literature [2,35]. Briefly, 30 mg of each lignin was dissolved in 7 mL of 2 M NaOH aqueous solution in a teflon vessel and, after adding 450 μL of nitrobenzene the vessel was placed into a stainless steel reactor and heated up to 170 °C for 4 h. The oxidized material was extracted twice with chloroform. The organic phase collected was evaporated under reduce pressure, the dried sample was dissolved in methanol and made up to 10 mL with methanol. Quantification of reaction products was made by high-performance liquid chromatography (HPLC), using the equipment, column, eluents, and conditions described elsewhere [35]. NO and HPLC analyses were performed in duplicated.

2.4. Carbon-13 nuclear magnetic resonance (^{13}C NMR)

Quantitative ^{13}C NMR procedure was reported elsewhere [29]. ^{13}C NMR spectra were recorded using a Bruker AVANCE III 400 spectrometer operating at 400 MHz, with a temperature of 45 °C, during 72 h. Approximately 160 mg of each lignin was dissolved in 0.5 mL deuterated dimethyl sulfoxide (DMSO-d_6). Conditions for ^{13}C NMR measurements: simple 1D pulse sequence, recycling time of 12 s, 17000 scans, and 1D sequence with power-gated coupling using 90° flip angle.

2.5. Gel permeation chromatography (GPC)

Molecular weight analyses were performed using a Shimadzu UFLC, equipped with a diode array detector (operating at 268 nm wavelength) and a refraction index detector (RI). Two Agilent gel columns were used: an OligoPore column 300 × 7.5 mm and a MesoPore column 300 × 7.5 mm. A guard column Oligopore 50 × 7.5 mm was assembled prior to the columns. GPC analyses were performed according to the procedure used in the literature [36]. Briefly, GPC analysis were performed at 70 °C with a flowrate of 0.8 mL.min^{-1} and using an isocratic mobile phase of dimethylformamide (DMF) with 0.5 %w/v of lithium chloride (LiCl) to avoid lignin agglomeration. The system was calibrated with polystyrene molecular weight standards ranging from 162 to 55,000 g.mol^{-1}, before the analysis. Solutions of about 5 mg.mL^{-1} of each polystyrene standard and lignin samples were dissolved in the mobile phase solvent. Prior to analysis, lignin samples were stirred and filtered through a 0.2 μm syringe filter.

Table 2
Inorganic content of LHSL and LSSL, presented in %w/w_{lignin} (dry weight).

	LHSL	LSSL
Inorganic content, %w/w_{lignin}	7.2 ± 0.2	10.1 ± 0.3

2.6. Alkaline oxidation with O_2

Oxidation of lignins in alkaline medium using O_2 was performed in a Büchi AG laboratory autoclave with a capacity of 1 L (model BEP280 type II, Switzerland). The heating and temperature control was assured by a Haake thermostatic bath (model N2-B, Karlsruhe, Germany). Temperature and total pressure inside the reactor were measured using a thermocouple type K and a pressure transducer (Kulite model XYME-190 M G, Leonia, USA), respectively. The O_2 flow rate was measured by means a mass flow meter EL FLOW (Bronkhorst High-Tech B.V., model F-201C-FAC-11-V, Ruurlo, Netherlands). The reaction samples were collected at preset time intervals.

For each oxidation reaction, about 30 g of lignin was dissolved in 500 mL of NaOH (80 $g.L^{-1}$) solution, introduced into the reactor, heated to 120 °C, and pressurized. The reaction time started when the initial temperature reached 120 °C. At this time, O_2 was introduced starting the data acquisition. The total pressure in the reactor was kept at 9.8 bar with a partial pressure of O_2 of 3.0 bar by continuous supply of O_2 along the time.

The low molecular weight phenolic compounds produced during LHSL and LSSL oxidation were extracted by solid phase extraction (SPE), as detailed described in previous studies of the same research group [35]. The HPLC analysis, for quantification of phenolic monomers, were performed as described in Section 2.3.

3. Results

3.1. Nitrobenzene oxidation of LSSL and LHSL

Nitrobenzene oxidation in alkaline medium represents an important method for qualitatively and quantitatively determining lignin composition, providing information about the phenolic monomers in the noncondensed fraction of lignin [37]. Studies on the nitrobenzene oxidation of lignin model compounds indicated that phenolic monomers are derived from oxidative degradation of the corresponding 4-hydroxyphenylpropane units and their esters, in particular the corresponding 4-*O*-alkylated and α-*O*-4 and β-*O*-4 lignin structures [38].

The yields and types of phenolic aldehydes, acids, and ketones obtained for LHSL and LSSL by nitrobenzene oxidation are depicted in Table 3. The quantification of the maximum individual yield of the referred phenolic monomers represents an important parameter for the evaluation of lignin potential through lignin oxidative depolymerization [29,35].

As shown in Table 3, V and Sy are the main products, accounting about 90 % of the total phenolic monomers identified in both lignins. Minor contents of their corresponding acids (VA and SA, respectively) and Hy were also obtained. As expected, the softwood lignin (LSSL) has mostly G units, with V and VA accounting 91 % of the total yield, with a lower content of S units. On the other hand, LHSL have a percentage of Sy higher than 18 % w/w_{lignin}, accounting about 68 % of the total yield of the phenolic monomers identified.

From a perspective of lignins valorization to added-value products, the results from NO allow anticipate the maximum yields that could be achieve through lignin depolymerization however, it is reported that the oxidation of lignin in alkaline medium using O_2 as oxidant only can reach up to 50 % of the total yields obtained by NO [2,39]. Moreover, it is well known that high aldehyde/acid ratios are also an advantage in the perspective of lignin valorization. In this study, both lignins, LSSL and LHSL, show a value of Sy/SA and V/VA ratio in the range from 8.0 to 12. The obtained values are higher than the ones obtained for hardwood kraft liquors and lignins, that showed ratios between 3 and 4.6 [26], confirming the advantage of lignins from sulfite liquors from the point of view of selectivity for phenolic aldehydes.

Table 3
Yields of monomeric phenolics obtained by NO of LHSL and LSSL lignins.

	Products, % w/w_{lignin} [a]					
	Hy	VA	SA	V	Sy	Total yield
LHSL	0.07	0.52	1.90	6.20	18.7	27.4
LSSL	0.27	1.96	0.19	19.3	1.55	23.3

[a] Reported to nonvolatile solids weight after removing inorganic content.

3.2. Lignins characterization by ^{13}C NMR

^{13}C NMR provides important information about the carbons in different structural and chemical environments in the condensed and noncondensed fractions of lignin structure. The carbon chemical shifts of each functional group, linkage or structure identified in lignins was made based on reference spectra and data available on literature [29,40,41]. The ^{13}C NMR spectra of LSSL and LHSL, with the main assignments identified, are shown in Fig. 1.

The assignments and the corresponding chemical shifts of the main structures, linkages, and functional groups, as well as the resulting content, reported as the ratio of the integral of a given carbon signal to one-sixth of the integral of aromatic carbons (Ar), are presented in Table 4. The integral of the δ 103–162 ppm region was set as the reference, assuming that it includes six aromatic carbons [38,40].

From the quantitative data from ^{13}C NMR it is possible to calculate basic parameters that summarizes the main structural characteristics of lignins: the content of β-*O*-4 structures, the degree of condensation (DC), and the S/G ratio. The content of β-*O*-4 structures and the DC value of each lignin was estimated as described in detail in a previous work [29]. The referred parameters, calculated for LHSL and LSSL, are depicted in Table 5.

The most significant difference in LHSL and LSSL structure, besides the proportion of S:G:H units, is the highest content of condensed structures in LSSL, reflected by the DC value found for this lignin. The higher DC is related with the high proportion of G units in LSSL, also confirmed by S/G ratio. G units have more ability to form C-C linkages, present in the most common condensed moieties in lignin structure such as 5-5′, β-5, and 4-O-5′, due to the availability of the C_5 position in the aromatic ring [9,42]. For hardwood lignins, as LHSL, the higher proportion of S units makes it difficult the formation of these type of linkages, since they have both C_3 and C_5 positions substituted by a methoxyl group [43].

Considering the lowest value of DC and the highest number of β-*O*-4 structures obtained for LHSL, it is expected that this lignin give origin to higher yields of phenolic monomers through depolymerization, which are also confirmed by the results obtained by NO (Table 3).

3.3. Molecular weight distribution

Gel permeation chromatography (GPC) allow to obtain the molecular weight distribution of the lignins from ultrafiltration of hardwood and softwood sulfite liquors. The chromatograms obtained for LHSL and LSSL were normalized, overlaid, and presented in Fig. 2. The values of weight-average molecular weight (Mw) and polydispersity index (Mw/Mn) found are shown in Table 6. As already stated in Section 2.5, the GPC calibration was performed with polystyrene standards thus, the obtained values of Mw are relative to this polymer and the analysis of lignin only provides relative molecular weight values [36].

The molecular weight distribution curve obtained for LSSL lignin shows two peaks in the high molecular weight region, not visible for LHSL, that correspond to a low elution time. This lignin has the highest value of molecular weight, which is coherent with the described peaks and the presence of more condensed moieties in its structure (Table 5). In literature other authors already stated that lignins mostly composed by G units are expected to show higher Mw than those presenting high contents of S units due to the higher probability to form condensed structures [44]. In the low molecular weight region, LSSL and LHSL show the presence of peaks, albeit in different intensities, indicated that

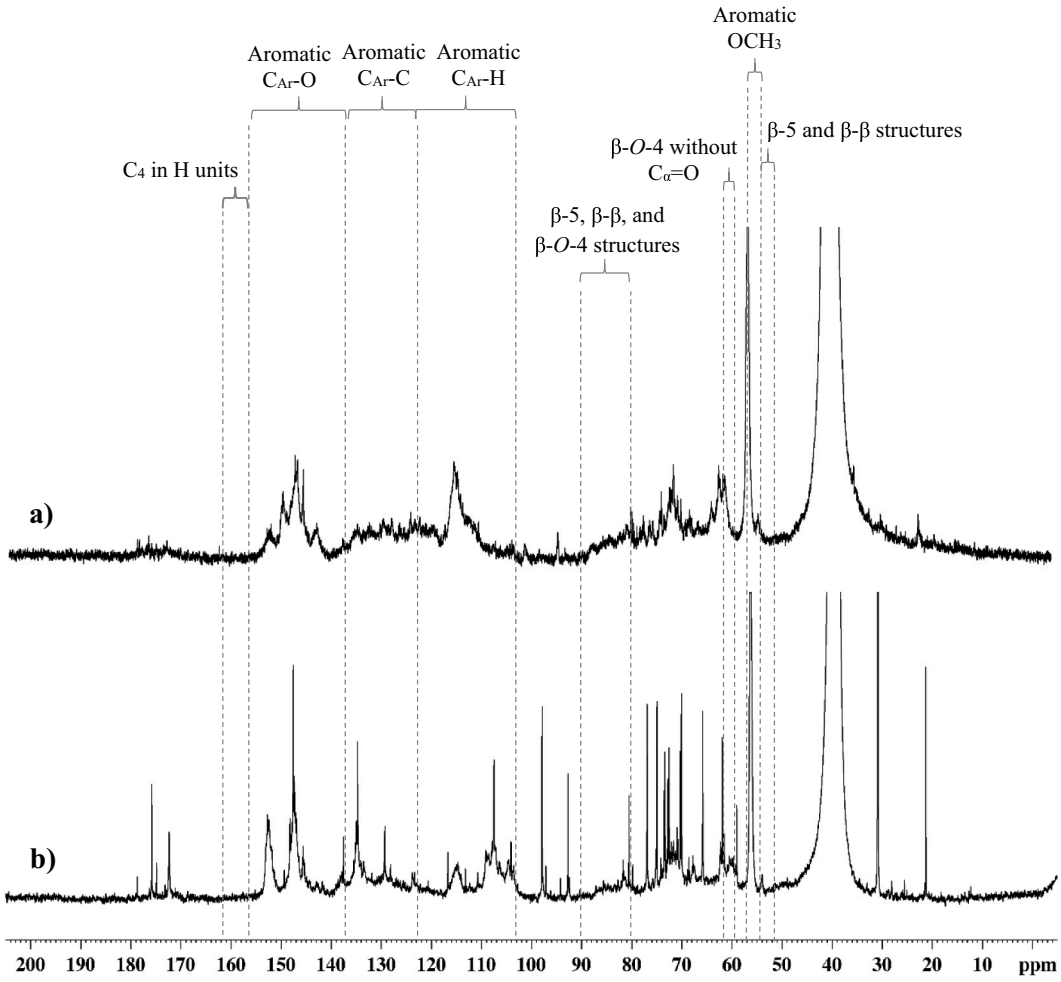

Fig. 1. Quantitative ^{13}C NMR spectra of lignins: a) LSSL and b) LHSL, in DMSO-d_6.

Table 4
Assignments and quantification (number per aryl unit) of the structures/linkages and functional groups identified by ^{13}C NMR.

Assignments (spectroscopic range)	Amount (number per Ar)	
	LHSL	LSSL
C_β in β-5 and β-β structures (δ 51.0–53.8 ppm)	0.15	0.26
Aromatic OCH_3 (δ 54.3–57.3 ppm)	1.35	1.44
Cγ in β-*O*-4 structures without Cα = O (δ 59.3–60.8 ppm)	0.26	0.30
Cγ in β-5, β-*O*-4 structures with Cα = O and β-1 (δ 62.5–63.8 ppm)	0.14	0.24
Cα in β-*O*-4 structures; Cγ in pinoresinol/syringaresinol and β-β structures (δ 70.0–76.0 ppm)	1.24	1.00
C_β in β-*O*-4 structures; Cα in β-5 and β-β structures (δ 80.0–90.0 ppm)	0.57	0.66
Aromatic C_{Ar}-H (δ 103.0–123.0 ppm)	2.06	2.37
Aromatic C_{Ar}-C (δ 123.0–137.0 ppm)	1.61	1.57
Aromatic C_{Ar}-O (δ 137.0–156.0 ppm)	2.28	2.00
C_4 in H units (δ 157.0–162.0 ppm)	0.05	0.07
CHO in benzaldehyde structures (δ 191.0–192.0 ppm)	0.00	0.02
CHO in cinnamaldehyde structures (δ 193.5–194.5 ppm)	0.01	0.03
CO in aldehydes and ketones (δ 195.0–210.0 ppm)	0.59	0.40

low molecular weight fragments are present in both lignins. The lower polydispersity found for LSSL confirms that this lignin has a structure slightly more homogeneous, with narrower molecular weight distribution.

Table 5
β-*O*-4 structures content (number per 100 Ar), DC, and S/G ratio calculated for LHSL and LSSL.

	β-*O*-4, n/100 Ar	DC, %	S/G
LHSL	42	24	2.1
LSSL	40	38	0.24

3.4. Oxidation of LSSL and LHSL with O_2 in alkaline medium

The oxidation experiments of LHSL and LSSL lignins were carried out in 0.5 L of a 2 M NaOH solution (80 $g.L^{-1}$) with a lignin initial concentration of 60 $g.L^{-1}$, an initial temperature of 120 °C, and a partial pressure of O_2 of 3 bar (total pressure of 9.8 bar), as already described in Section 2.6. The first oxidation reaction of both lignins was performed for 120 min, to evaluate the complete profile of all the oxidation phenolic products, including their formation and consequent degradation. The second oxidation experiment was interrupted just before the maximum yield of the main phenolic monomers is achieved, the addition of O_2 was stopped and the temperature of the reactor decreased. These oxidation reactions were over after 26 and 8 min, for LSSL and LHSL, respectively. The objective is to avoid further degradation of the oxidation products and consequently achieve a final mixture with the maximum yields of the valuable phenolics of interest, namely V and Sy.

The profiles of the phenolic monomers identified in oxidation experiment 1, for 120 min, and experiment 2, for 8 and 26 min, for LHSL and LSSL, respectively, are presented in Figs. 3 and 4.

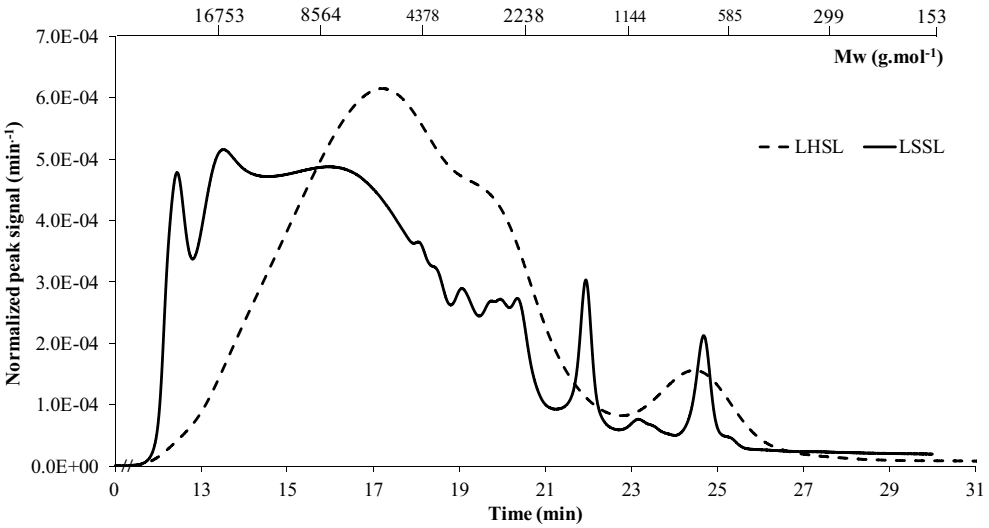

Fig. 2. Molecular weight distribution curves obtained from GPC analyses of LHSL and LSSL lignins.

Table 6
Molecular weight (Mw) and polydispersity (Mw/Mn) of LHSL and LSSL lignins analyzed by GPC.

	Mw ($g.mol^{-1}$)	Mw/Mn
LHSL	31,347	1.64
LSSL	48,566	1.57

As expected, V and VA are the main products from the oxidation of LSSL, with minor yields of Sy and VO (Fig. 4). In the case of LHSL, the main phenolic products identified in the oxidation mixture were Sy and V; however, the carboxylic acids SA and VA were also identified, as the phenolic ketones VO and SO (Fig. 3). During the hardwood lignin oxidation Sy reaches to a maximum yield of 2.5 % w/w_{lignin} at a reaction time between 10 and 15 min followed by an accentuated decrease for longer reaction times. For V, the time to maximum is higher, about 20 min, and, after the maximum (1.2 % w/w_{lignin}), a smooth drop occurs when compared with Sy. In the case of LSSL lignin V reaches the maximum yield of 4.7 % w/w_{lignin} after 30 min and Sy reaches 0.23 % w/w_{lignin} between 15 and 20 min of oxidation reaction. The degradation profile of both phenolic aldehydes in this lignin is like the described for LHSL.

The lower reaction times to reach the maximum yield of the phenolic aldehydes observed for LHSL confirms the higher reactivity of this lignin comparatively to LSSL, related with the presence of high contents of S units. Sy is more sensitive than V to oxygen pressure; it forms quickly but it readily degrades under a high oxygen pressure. A similar trend in the reactivity of the S nuclei comparatively to the G nuclei was also found by

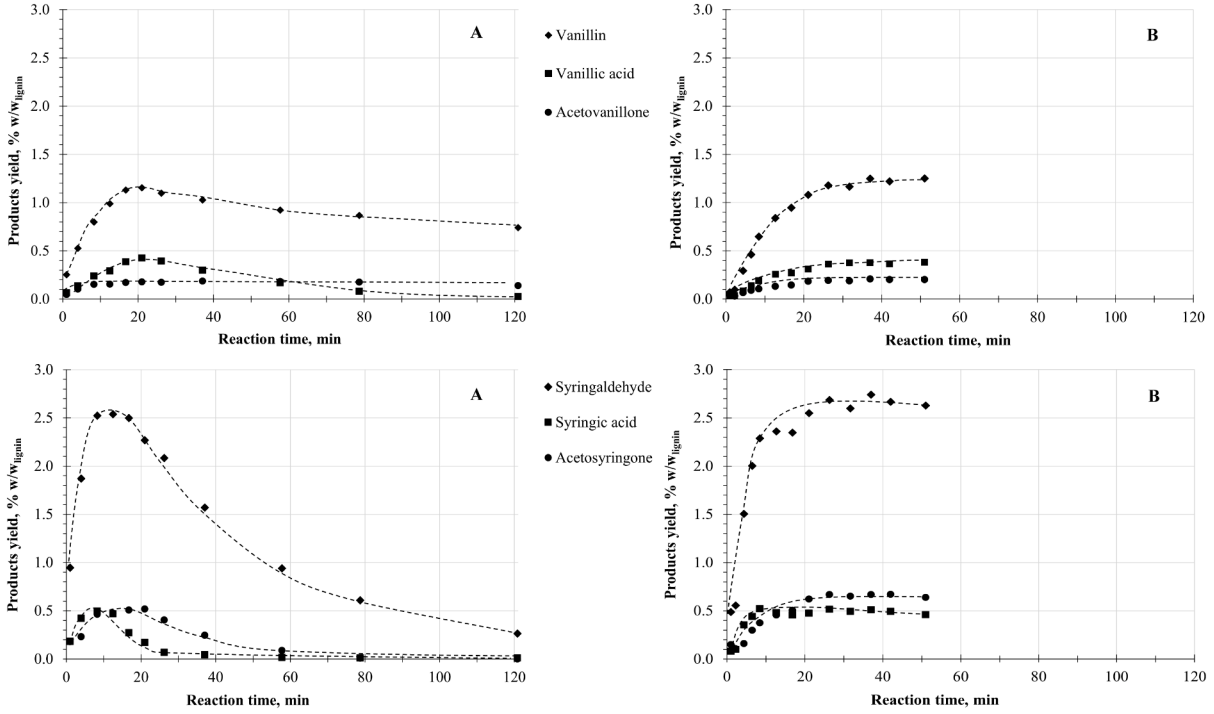

Fig. 3. Profiles of monomeric products: V, VA, VO, Sy, SA, and SO during the oxidation experiment 1 (A) and experiment 2 (B) of LHSL lignin. General conditions: lignin concentration 60 $g.L^{-1}$, [NaOH] = 80 $g.L^{-1}$, $pH_{initial} \geq 13.8$, $pO_2 = 3$ bar, $P_{total} = 9.8$ bar, $T_i = 120$ °C.

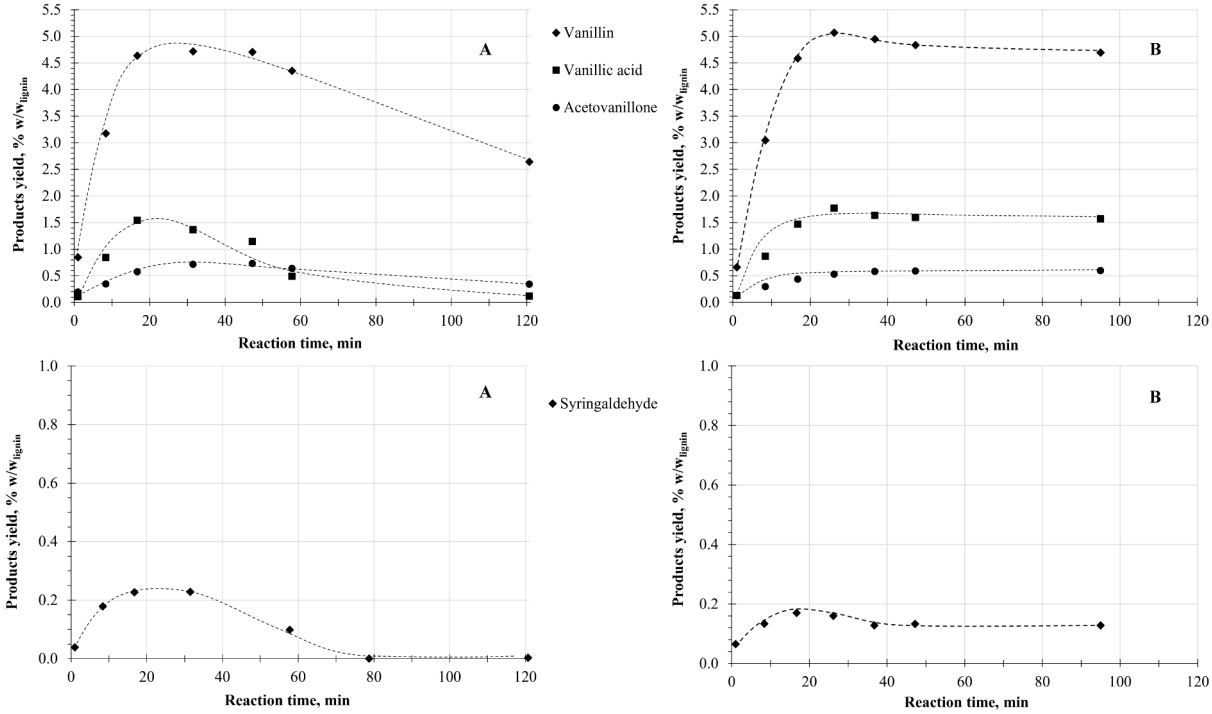

Fig. 4. Profiles of monomeric products: V, VA, VO, and Sy during the oxidation experiment 1 (A) and experiment 2 (B) of LSSL lignin. General conditions: lignin concentration 60 g.L^{-1}, [NaOH] = 80 g.L^{-1}, $pH_{initial} \geq 13.8$, $pO_2 = 3$ bar, $P_{total} = 9.8$ bar, $T_i = 120$ °C.

other authors in studies about the effect of O_2 pressure in lignin oxidation [26,45,46].

When the time of the oxidation reaction of LSSL and LHSL is reduced, and the admission of O_2 is interrupted just before the maximum yield of the main products is reached, it is possible to see a decrease in V and Sy degradation, resulting in a final oxidation mixture with higher yield of these phenolics (Fig. 5). For LHSL, the influence in the degradation of V and Sy is more evidenced since in the case of this lignin, the oxidation reaction is interrupted after 8 min, and it is not observed any decrease in the yield of these oxidation products after this time. Considering V, the yield in the final oxidation mixture is about 40 % higher in the reaction stopped at 8 and 26 min, for LHSL and LSSL, respectively, comparatively to the content of this phenolic compound in the final mixture obtained after the oxidation reaction for 120 min (Fig. 5). For Sy, due to its higher reactivity, an increase of 95 % in the final yield of this aldehyde was obtained when the oxidation is performed only for 8 min. These results indicate that an initial high pressure of oxygen rapidly breaks down the lignin and then a decrease in the oxygen pressure allows proceeding the oxidation of the lignin fragments but prevents the degradation of the phenolic monomers. Since the main objective of lignin depolymerization is to attain an oxidation mixture with the maximum yield of the phenolic compounds of interest, it is essential to select the experimental conditions that allow reaching the best compromise between the formation and the degradation of the oxidation products.

3.5. Evaluation of the S:G:H units proportion

The proportion of S:G:H in LSSL and LHSL was determined from the results of NO, oxidation with O_2, and ^{13}C NMR (Table 7). Through NO only uncondensed lignin structural units are accessible, consequently the proportion of G, S, and H in the uncondensed fraction of lignins were calculated using the phenolic monomers yields obtained from this method. G units were determined from the contribution of V and VA, while for S units only Sy and SA were considered. Hy yield allow the calculation of H units. The same procedure was performed to achieve the ratio S:G:H from oxidation with O_2, with the difference that this method

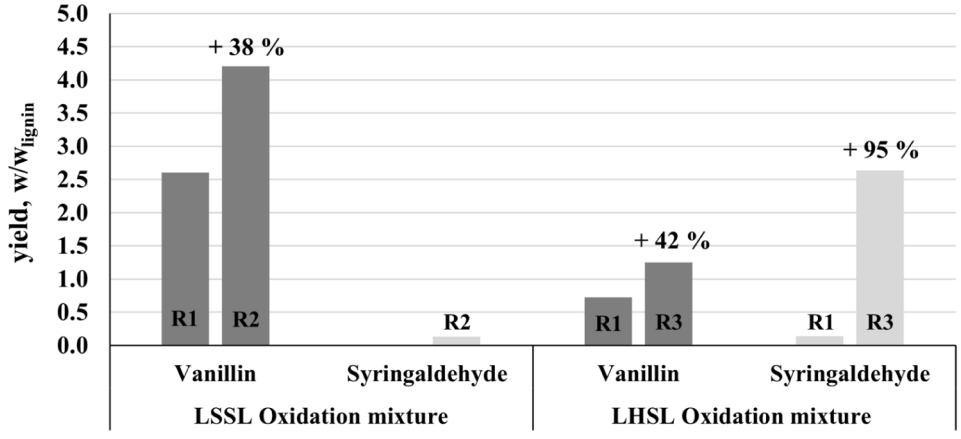

Fig. 5. Yield of V and Sy in the final solution obtained from the oxidation for 120 min (R1), 26 min (R2), and 8 min (R3) for LSSL and LHSL lignins.

Table 7
Proportion of S:G:H units obtained from the results of ^{13}C NMR, NO, and alkaline oxidation with O_2.

		^{13}C NMR	NO	Oxid. O_2
LSSL	S units	18	8	7
	G units	75	91	92
	H units	7	1	1
LHSL	S units	64	75	65
	G units	30	24	32
	H units	6	1	2

is not restrict only to the noncondensed fraction of lignin. ^{13}C NMR results reflect the structural features of the whole lignin, accessing both condensed and noncondensed moieties, and the proportion of S, G, and H structures obtained from this technique was also achieved.

For LSSL lignin it is observed that the proportion of S:G:H units obtained from the results of NO is similar to that obtained from the results from alkaline oxidation with O_2. This behavior suggests that alkaline oxidation with O_2 mainly attacks the noncondensed structures of this lignin. Moreover, the proportion of S:G:H from ^{13}C NMR, that reflects the structural features of the whole lignin, shows a higher content of S units, suggesting that a significant part of these units are involved in condensed structures. Since the main condensed structures present in lignin are 5-5′, β-5, and 4-O-5′ it could be concluded that in this lignin, obtained from softwood biomass, S units are mainly involved in condensed structures through C_β in β-5 structures (phenylcoumarin structure), since this type of units have the C_5 position already substituted by a methoxyl group. This statement is also evidenced by the data from quantitative ^{13}C NMR, (first row of Table 4), that shows higher amounts of C_β in β-5 and β-β structures in LSSL lignin. In literature, other authors also noted the higher content of β-5 linkages in softwood lignins [47,48]. In opposition, LHSL shows that the proportion of S:G:H units obtained from the results of ^{13}C NMR is similar to that obtained from the results from alkaline oxidation with O_2. This indicate that for this lignin the alkaline oxidation using O_2 as oxidant attacks the whole lignin structure (condensed and uncondensed structures) in the same proportion, instead of showing more propensity to cleave the structures in the noncondensed fraction of lignin, as observed in the case of LSSL. This type of structural information about lignin is essential to evaluate its reactivity and to understand its efficient utilization either through depolymerization reactions or in material applications.

3.6. Lignosulfonates valorization: separation and recovery of the phenolic compounds from the oxidized mixture

Since the oxidative depolymerization in alkaline medium generates a complex mixture of lignin fragments and low molecular weight compounds (V, Sy, among others), a sequence of separation and purification processes will be required concerning lignin valorization through the recovery of the added-value phenolic monomers produced. Considering that, the complex oxidized mixture will be fractionated and concentrated, through ultra and nanofiltration, into different streams: partially depolymerized lignin, intermediate molecular weight oligomers, and phenolate monomers. The aim of this two-stage filtration is to concentrate the low molecular weight phenolic compounds taking advantage of its solubility in alkaline medium and produce a stream containing phenolate monomers to proceed for separation and conversion. This process will be performed based on our research team results in ultra and nanofiltration that demonstrated the effectiveness of phenolate compounds recovery from model solutions [49,50]. After that, the permeate from nanofiltration will be submitted to a final purification by adsorption and desorption cycles, with a selected nonpolar adsorbent, allowing to efficiently eliminate the most polar compounds and to recover, at higher concentration levels, V, Sy, and the other low molecular weight phenolics resulting from lignin oxidized mixture. Adsorption/desorption is one of the capabilities of our research team at LSRE-LCM (FEUP) and polymeric resins were previously tested to recover V, studying the effects of operational and feed conditions [51,52]. The recovery of Sy and V (from other products of oxidation mixture) was also studied through an analytical application developed by the team, showing adsorbing and desorbing stages with high recoveries of both compounds [3,53,54]. After the evaluation of the final solution rich in phenolic aldehydes, the extraction, through a step of liquid-liquid extraction, and crystallization of V and Sy would be possible with low energy requirements. The combination of all the described steps will result in an integrated process that confirms the viability of lignin exploitation for Sy and V production and represents a key role in the economic competitiveness of lignin-based products.

4. Conclusions

The hardwood and softwood lignins obtained from the ultrafiltration of the sulfite liquors were characterized and the results showed that LHSL has the lowest value of DC and the highest number of β-*O*-4 structures, consequently this lignin give origin to higher yields of phenolic monomers through depolymerization by NO. Concerning LSSL, lignin from softwood biomass, the GPC analysis confirmed its highest value of molecular weight, which is coherent with presence of more condensed moieties in its structure related with the higher availability of G units in this lignin. Both lignins were subjected to alkaline oxidation with O_2 and the yield of aromatic monomers and the selectivity of the target products were evaluated. When the O_2 pressure is maintained constant, a higher rate of oxygen is transferred from the gas phase into the liquid phase, the conversion takes place and the yields of phenolic monomers increase in a very short time, followed by their accentuated degradation. It was found that it is preferable to keep an initial high pressure of oxygen and then decrease it to continue the oxidation of the lignin fragments but prevent the degradation of the phenolic monomers. With the experimental conditions optimized, LSSL showed to be the raw material with better performance toward oxidation, producing a maximum of 2.7 $g.L^{-1}$ of V in the final oxidation mixture. In the end of this work, it is possible to conclude that alkaline oxidation of the studied lignins allow obtaining a final oxidized mixture rich in the produced phenolic monomers what is an extremely important point from a techno-economic approach.

CRediT authorship contribution statement

F.M.C. contributed to conceptualization, methodology, investigation, formal analysis, and writing-original draft. C.A.E.C. contributed to conceptualization, methodology, investigation, formal analysis, and writing-review & editing. C.V.-A. involved in methodology, investigation, and writing-review & editing. A.E.R. contributed to conceptualization, resources, writing-review & editing, and supervision.

Acknowledgments

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); 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. Authors are grateful to Company A and Caima - Indústria de Celulose, S.A. (Portugal) for the supply of the samples of sulfite liquor.

References

[1] Y. Pu, D. Zhang, P.M. Singh, A.J. Ragauskas, The new forestry biofuels sector, Biofuels Bioprod. Biorefin. 2 (2008) 58–73.

[2] C.A.E. Costa, P.C.R. Pinto, A.E. Rodrigues, Radar tool for lignin classification on the perspective of its valorization, Ind. Eng. Chem. Res. 54 (2015) 7580–7590.
[3] A. Rodrigues, P. Pinto, F. Barreiro, C.A.E. Costa, M.I.F. Mota, I. Fernandes, An Integrated Approach for Added-value Products From Lignocellulosic Biorefineries, 1 ed., Springer International Publishing, Springer Nature Switzerland AG, 2018.
[4] Z. Strassberger, S. Tanase, G. Rothenberg, The pros and cons of lignin valorisation in an integrated biorefinery, RSC Adv. 4 (2014) 25310–25318.
[5] P.C.R. Pinto, E.A.B. Silva, A.E. Rodrigues, Lignin as source of fine chemicals: vanillin and syringaldehyde, in: C. Baskar, S. Baskar, S.R. Dhillon (Eds.), Biomass Conversion: The Interface of Biotechnology, Chemistry and Materials Science, Springer Berlin Heidelberg, Berlin, Heidelberg, 2012, pp. 381–420.
[6] T. Aro, P. Fatehi, Production and application of lignosulfonates and sulfonated lignin, ChemSusChem 10 (2017) 1861–1877.
[7] J. Fernández-Rodríguez, X. Erdocia, F. Hernández-Ramos, M.G. Alriols, J. Labidi, Chapter 7 - lignin separation and fractionation by ultrafiltration, in: C.M. Galanakis (Ed.), Separation of Functional Molecules in Food by Membrane Technology, Academic Press, 2019, pp. 229–265.
[8] F.G. Calvo-Flores, J.A. Dobado, Lignin as renewable raw material, ChemSusChem 3 (2010) 1227–1235.
[9] A. Berlin, M. Balakshin, Chapter 18 - industrial lignins: analysis, properties, and applications, in: V.K. Gupta, M.G. Tuohy, C.P. Kubicek, J. Saddler, F. Xu (Eds.), Bioenergy Research: Advances and Applications, Elsevier, Amsterdam, 2014, pp. 315–336.
[10] M.V. Galkin, J.S.M. Samec, Lignin valorization through catalytic lignocellulose fractionation: a fundamental platform for the future biorefinery, ChemSusChem 9 (2016) 1544–1558.
[11] D.S. Munavalli, S.A. Chimatadar, S.T. Nandibewoor, Oxidation of vanillin by a new oxidant diperiodatoargentate(III) in aqueous alkaline medium, Ind. Eng. Chem. Res. 46 (2007) 1459–1464.
[12] V.E. Tarabanko, N. Tarabanko, Catalytic oxidation of lignins into the aromatic aldehydes: general process trends and development prospects, Int. J. Mol. Sci. 18 (2017).
[13] B.A.T. Hart, J.M. Simons, K.S. Shoshan, N.P.M. Bakker, R.P. Labadie, Antiarthritic activity of the newly developed neutrophil oxidative burst antagonist apocynin, Free Radic. Biol. Med. 9 (1990) 127–131.
[14] J. Stefanska, A. Sarniak, A. Wlodarczyk, M. Sokolowska, Z. Doniec, P. Bialasiewicz, D. Nowak, R. Pawliczak, Hydrogen peroxide and nitrite reduction in exhaled breath condensate of COPD patients, Pulm. Pharmacol. Ther. 25 (2012) 343–348.
[15] D. Yancheva, E. Velcheva, Z. Glavcheva, B. Stamboliyska, A. Smelcerovic, Insights in the radical scavenging mechanism of syringaldehyde and generation of its anion, J. Mol. Struct. 1108 (2016) 552–559.
[16] G. Karthik, M. Angappan, A. VijayaKumar, S. Natarajapillai, Syringic acid exerts antiangiogenic activity by downregulation of VEGF in zebrafish embryos, Biomed. Prev.Nut. 4 (2014) 203–208.
[17] C. Srinivasulu, M. Ramgopal, G. Ramanjaneyulu, C.M. Anuradha, C. Suresh Kumar, Syringic acid (SA) – a review of its occurrence, biosynthesis, pharmacological and industrial importance, Biomed. Pharmacother. 108 (2018) 547–557.
[18] C.J. Baker, N.M. Mock, B.D. Whitaker, D.P. Roberts, C.P. Rice, K.L. Deahl, A. A. Aver'yanov, Involvement of acetosyringone in plant–pathogen recognition, Biochem. Biophys. Res. Commun. 328 (2005) 130–136.
[19] C. David C.D. Logan Method of producing vanillin and other useful products from lignosulfonic acid compounds. Patent US 3,054,659. 1958.
[20] E.W. Schoeffel Manufacture of vanillin. Patent US 2,598,311. 1950.
[21] J.R. Salvesen D.L. Brink D.G. Diddams O. Peter Process for making vanillin. Patent US 2,434,626. 1945.
[22] A.L. Mathias, Produção de vanilina a partir da lenhina: estudo cinético e do processo, Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, 1993.
[23] A.L. Mathias, A.E. Rodrigues, Production of vanillin by oxidation of pine kraft lignins with oxygen, Holzforschung 49 (1995) 273–278.
[24] C. Fargues, Á. Mathias, A. Rodrigues, Kinetics of vanillin production from kraft lignin oxidation, Ind. Eng. Chem. Res. 35 (1996) 28–36.
[25] J.D.P. Araújo, Production of Vanillin From Lignin Present in the Kraft Black Liquor of the Pulp and Paper Industry, LSRE, Department of Chemical Engineering, Faculty of Engineering University of Porto, Porto, 2008.
[26] P.C.R. Pinto, C.E. Costa, A.E. Rodrigues, Oxidation of lignin from Eucalyptus globulus pulping liquors to produce syringaldehyde and vanillin, Ind. Eng. Chem. Res. 52 (2013) 4421–4428.
[27] P.C.R. Pinto, E.A.B.D. Silva, A.E. Rodrigues, Insights into oxidative conversion of lignin to high-added-value phenolic aldehydes, Ind. Eng. Chem. Res. 50 (2011) 741–748.
[28] E.D. Gomes, Development of a Continuous Process for the Production of Vanillin and Syringaldehyde From Kraft Black Liquor, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, 2019.
[29] C.A.E. Costa, P.C.R. Pinto, A.E. Rodrigues, Evaluation of chemical processing impact on E. globulus wood lignin and comparison with bark lignin, Ind. Crop. Prod. 61 (2014) 479–491.
[30] C.A.E. Costa, Vanillin and Syringaldehyde From Side Streams of Pulp and Paper Industries and Biorefineries, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, 2017.
[31] A. Vishtal, A. Kraslawski, Challenges in industrial applications of technical lignins, Bioresources 6 (2011) 3547–3568.
[32] A. Tribot, G. Amer, M. Abdou Alio, H. de Baynast, C. Delattre, A. Pons, J.-D. Mathias, J.-M. Callois, C. Vial, P. Michaud, C.-G. Dussap, Wood-lignin: supply, extraction processes and use as bio-based material, Eur. Polym. J. 112 (2019) 228–240.
[33] N.E.E. Mansouri, J. Salvadó, Structural characterization of technical lignins for the production of adhesives: application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins, Ind. Crop. Prod. 24 (2006) 8–16.
[34] S.E. Lebo Jr., J.D. Gargulak, T.J. McNally, Lignin, in: Kirk-Othmer Encyclopedia of Chemical Technology, 2001.
[35] P.R. Pinto, E.B.D. Silva, A.E. Rodrigues, Comparative study of solid-phase extraction and liquid−liquid extraction for the reliable quantification of high value added compounds from oxidation processes of wood-derived lignin, Ind. Eng. Chem. Res. 49 (2010) 12311–12318.
[36] C.A.E. Costa, P.C.R. Pinto, A.E. Rodrigues, Lignin fractionation from E.globulus kraft liquor by ultrafiltration in a three stage membrane sequence, Sep. Purif. Technol. 192 (2018) 140–151.
[37] R. Sun, J.M. Lawther, W.B. Banks, The effect of alkaline nitrobenzene oxidation conditions on the yield and components of phenolic monomers in wheat straw lignin and compared to cupric(II) oxidation, Ind. Crop. Prod. 4 (1995) 241–254.
[38] S.Y. Lin, C.W. Dence, Methods in Lignin Chemistry, Springer-Verlag, 1992.
[39] V.E. Tarabanko, N.A. Fomova, B.N. Kuznetsov, N.M. Ivanchenko, A.V. Kudryashev, On the mechanism of vanillin formation in the catalytic oxidation of lignin with oxygen, React. Kinet. Catal. Lett. 55 (1995) 161–170.
[40] E.A. Capanema, M.Y. Balakshin, J.F. Kadla, A comprehensive approach for quantitative lignin characterization by NMR spectroscopy, J. Agric. Food Chem. 52 (2004) 1850–1860.
[41] D.V. Evtuguin, C.P. Neto, A.M.S. Silva, P.M. Domingues, F.M.L. Amado, D. Robert, O. Faix, Comprehensive study on the chemical structure of dioxane lignin from plantation Eucalyptus globulus wood, J. Agric. Food Chem. 49 (2001) 4252–4261.
[42] C. Fernández-Costas, S. Gouveia, M.A. Sanromán, D. Moldes, Structural characterization of kraft lignins from different spent cooking liquors by 1D and 2D nuclear magnetic resonance spectroscopy, Biomass Bioenergy 63 (2014) 156–166.
[43] M.G. Alriols, A. García, R. Llano-Ponte, J. Labidi, Combined organosolv and ultrafiltration lignocellulosic biorefinery process, Chem. Eng. J. 157 (2010) 113–120.
[44] A. Toledano, L. Serrano, A. Garcia, I. Mondragon, J. Labidi, Comparative study of lignin fractionation by ultrafiltration and selective precipitation, Chem. Eng. J. 157 (2010) 93–99.
[45] G. Wu, M. Heitz, E. Chornet, Improved alkaline oxidation process for the production of aldehydes (vanillin and syringaldehyde) from steam-explosion hardwood lignin, Ind. Eng. Chem. Res. 33 (1994) 718–723.
[46] V.S. Sultanov, A.F.A. Wallis, Reactivities of guaiacyl and syringyl lignin model phenols towards oxidation with oxygen-alkali, J.Wood Chem.Technol. 11 (1991) 291–305.
[47] S. Constant, H.L.J. Wienk, A.E. Frissen, R. Boelens, D.S. van Es, R.J.H. Grisel, B. M. Weckhuysen, W.J.J. Huijgen, R.J.A. Gosselink, P.C.A. Bruijnincx, P.D. Peinder, New insights into the structure and composition of technical lignins: a comparative characterisation study, Green Chem. 18 (2016) 2651–2665.
[48] C.W. Lahive, C.S. Lancefield, A. Codina, P.C.J. Kamer, N.J. Westwood, Revealing the fate of the phenylcoumaran linkage during lignin oxidation reactions, Org. Biomol.Chem. 16 (2018) 1976–1982.
[49] M. Žabková, E.A.B. Silva, A.E. Rodrigues, Recovery of vanillin from lignin/vanillin mixture by using tubular ceramic ultrafiltration membranes, J. Membr. Sci. 301 (2007) 221–237.
[50] I.F. Mota, P.R. Pinto, A.M. Ribeiro, J.M. Loureiro, A.E. Rodrigues, Downstream processing of an oxidized industrial kraft liquor by membrane fractionation for vanillin and syringaldehyde recovery, Sep. Purif. Technol. 197 (2018) 360–371.
[51] M. Zabkova, M. Otero, M. Minceva, M. Zabka, A.E. Rodrigues, Separation of synthetic vanillin at different pH onto polymeric adsorbent Sephabeads SP206, Chem. Eng. Process. Process Intensif. 45 (2006) 598–607.
[52] M.I.F. Mota, P.C.R. Pinto, J.M. Loureiro, A.E. Rodrigues, Successful recovery and concentration of vanillin and syringaldehyde onto a polymeric adsorbent with ethanol/water solution, Chem. Eng. J. 294 (2016) 73–82.
[53] E.D. Gomes, M.I. Mota, A.E. Rodrigues, Fractionation of acids, ketones and aldehydes from alkaline lignin oxidation solution with SP700 resin, Sep. Purif. Technol. 194 (2018) 256–264.
[54] I.F. Mota, P.R. Pinto, J.M. Loureiro, A.E. Rodrigues, Purification of syringaldehyde and vanillin from an oxidized industrial kraft liquor by chromatographic processes, Sep. Purif. Technol. 234 (2020), 116083.