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Aqueous acetone fractionation of kraft, organosolv and soda lignins

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ABSTRACT

Technical lignins are structurally heterogeneous and polydisperse. This work describes the use of a simple and green method for lignin fractionation, using different proportions of acetone (40 and 60%) in water. Lignins from three different sources (wheat straw organosolv lignin, wheat straw soda lignin and softwood kraft lignin) were used in this fractionation protocol. The obtained fractions showed different molar mass and functional groups. The lower molar mass fractions showed more phenolic hydroxyl groups and carboxylic acid moieties than higher molar mass fractions, which also possessed much higher amounts of carbohydrates. The chemical characterization of these fractionated lignins showed that the PREC fraction was exceptionally pure and homogenous lignin. Its total lignin content was >96% for all three lignins and it was practically free from carbohydrates and inorganics (ash). Furthermore, PREC fraction possessed the highest carbon content for the three lignin samples (63.05–69.26%). These results illustrate that the proposed aqueous acetone fractionation protocol could indeed produce pure and uniform lignin fraction and it was applicable for lignins from different sources.

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1. Introduction

Lignin is the second most abundant terrestrial polymer after cellulose. Lignin is a complex amorphous polymer synthesized mainly from three methoxylations: p-coumaryl, coniferyl, and sinapyl alcohols. During the lignification, each of these monolignols gives rise to a different type of lignin unit called p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively [1,2]. This biosynthesis process creates a unique lignin polymer in each plant species, including in different tissues of the same specimen [3]. Therefore, the amount and composition of lignins vary among plant origin, cell types, and also with environmental conditions. Indeed, softwood lignin is mainly composed of G units, together with a small amount of H units, whereas both G and S units are abundant in hardwood lignin. On the other hand, in grass lignins (e.g. in wheat straw), these three units, H, G and S are present [4].

Lignin is a potential bio-based raw material which is available in large quantities from wood pulping processes [5]. Unfortunately technical lignins are heterogeneous in different aspects. There is a significant variety of molar mass, functional groups as well as carbohydrate residues of different lignin molecules. Since lignin molecular structure reflects to its properties and the large heterogeneity of lignin feedstocks may impair its applicability in high-valued lignin applications [6,7].

Ultrafiltration [8–13] and solvent fractionation [14–22] are the most common technologies to separate lignin molecules into fractions with low polydispersity and well-defined properties. In ultrafiltration, membranes with selected cut-offs are applied for lignin solutions to separate the molecules based on their molar mass [8,9,11]. This separation method can be applied directly to spent liquors from pulp mill without a need to adjust pH or temperature [9]. On the other hand, the poor solubility of some lignins in common solvents suitable for ultrafiltration, fouling of the membranes and expensive instrumentation are major limitations for ultrafiltration [23].

Solvent fractionation can be performed using several different approaches. Most commonly, a variety of organic solvents are used successively to dissolve lignin fractions with specific properties [17–20,24–26]. An alternative approach to first dissolve lignin in
pure or aqueous organic solvent, followed by fractional precipitation of lignin using antisolvent, such as hexane or water [16,23,27]. The precipitation fractionation by antisolvent addition is a protocol that can be easily tailored to produce lignin fractions with specific properties. When using aqueous ethanol, acetone or propylene glycol monomethyl ether, the protocol includes only safe, non-toxic and environmentally sound solvents [23,27]. In addition, the presence of water in the lignin feedstock is not detrimental and simple technologies to recycle these chemicals exist [22].

In this paper, the recently developed solvent fractionation by precipitation protocol was tested for three different types of technical lignins: wheat straw organosolv lignin, wheat straw soda lignin and softwood Kraft lignin. The objective was to identify if lignin with constant quality can be obtained from lignins of different origin.

2. Materials and methods

2.1. Lignin sources

Lignin samples used in this study were obtained through three different pulping processes and all of them were extracted by precipitation from kraft, soda and organosolv liquors produced in these processes. Softwood Kraft lignin (SKL) was an industrial lignin precipitated using carbon dioxide from the black liquor of a pulp mill that produces paper-grade Kraft pulp and was kindly gifted by Metsä Fibre. The other two samples, wheat straw soda lignin (SSL) and wheat straw organosolv lignin (OSL) were acquired in the lab. SSL was recovered from the spent liquor of a soda pulping process performed at 100 °C for 150 min, containing 7% NaOH (o.d.m.) and the solid/liquid ratio was 1:10 [28]. This lignin sample was precipitated using sulphuric acid following the procedure by Domínguez-Robles et al. [28]. Organosolv process was performed at 210 °C for 60 min, using aqueous 60% ethanol as the reagent, being the solid/liquid ratio 1:10, as before [29]. Isolation of OSL was performed under a liquor-to-water ratio of 1:2 and after this step the solution was acidified with sulphuric acid to pH 2. The lignin sample was achieved after centrifugation at 9820g for 20 min and washed with water twice. Finally, the sample was dried at 60 °C in an oven for 48 h.

2.2. Lignin solubility in acetone/water

Lignin solubility is an important parameter to select the optimal solvent concentration in the fractionation process. 2 g of lignin was mixed with 20 mL of acetone solutions of 30–100 vol%. The solution was mixed with magnetic stirrer for 60 min at room temperature and then the dispersion was centrifuged at 3226g for 20 min to separate the dissolved fraction from the insoluble one. Lignin content was measured by UV absorption at 280 nm in the dissolved fraction and the insoluble one was dried under vacuum at 40 °C for overnight.

UV absorption coefficient for the unfractionated lignin samples was determined by dissolving 10, 20, and 30 mg of lignin (o.d.) in 50 mL 0.1 M NaOH solution stirring overnight at room temperature. Three different concentrations (0.2, 0.4, and 0.6 g/L) were used to achieve this coefficient for each sample. The absorbance at 280 nm (the average of the three concentrations) designates the absorptivity values of 27.15, 19.76 and 25.91 L·g⁻¹·cm⁻¹ for OSL, SSL and SKL, respectively. These values were calculated for the total lignin (the sum of Klasson lignin and acid soluble lignin) and assuming that ash or carbohydrates residues do not affect the absorbance. Finally, these coefficients were used to determine the concentration of dissolved lignin samples.

2.3. Aqueous acetone fractionation

20 g (o.d.) of lignin was dispersed in 200 mL of aqueous acetone (60% by volume) solution. The dispersion was mixed in an Erlenmeyer flask under magnetic stirring for 60 min. The solution was centrifuged for 20 min at 15,180g and the supernatant was recovered. The insoluble lignin fraction (INS) was dried in a vacuum oven at 40 °C for overnight and weighed. Water was then added to the supernatant to reduce the acetone concentration from 60% to 40%. This solution was mixed for 60 min to allow lignin to precipitate followed by centrifugation at 15,180g for 20 min and the insoluble fraction (PREC) was separated from the soluble one. Once again, the precipitate was dried in a vacuum oven at 40 °C for overnight and then weighed. The final remaining soluble lignin fraction (SOL) in 40% acetone solution was separated by evaporating the solvent under vacuum at 40 °C. The scheme of this fractionation process is showed in Fig. 1.

2.4. Lignin characterization

Lignin fractions were analysed with the purpose of establishing their physicochemical characteristics. For lignin composition, the ash content was determined gravimetrically in a muffle furnace. Samples were heated to 103 °C and maintained at this temperature for 240 min, after this first stage, samples were heated to 550 °C and the temperature was maintained for 420 min.

To determine Klasson lignin and soluble lignin content, an acid hydrolysis was performed [30] defining the Klasson lignin by gravimetric yield, acid soluble lignin from the UV-absorption of the hydrolysate at 215 and 280 nm [31] and carbohydrates from the hydrolysate using HPAEC-PAD [32].

The Fourier Transform Infrared (FTIR) spectra of the lignin samples were recorded using a Spectrum Two™ instrument (Perkin Elmer, Waltham, MA, USA) by the attenuated total reflectance (ATR) technique. The spectra were recorded from 4000 cm⁻¹ to 450 cm⁻¹ with a resolution of 4 cm⁻¹ and performing 20 scans.

Elemental analysis (C, H, N and S content) was performed using a FLASH 2000 series elemental analyser (Thermo Fisher Scientific, Waltham, USA).
Waltham, MA, USA). Before performing the analyses 30 mg of the samples were ground and dried at 105°C for overnight.

The molar mass distributions (MMD), number average molar mass (Mn), weight average molar mass (Mw) and polydispersity (PD) of the lignin samples were determined by size exclusion chromatography (SEC) with UV detection at 280 nm as explained elsewhere [23]. Samples were dissolved in 0.1 M NaOH solution (1 mg/ml) and filtered (0.45 µm). SEC analysis was performed using 0.1 M NaOH eluent (pH 13, using a flow of 0.5 mL/min and a temperature of 25°C) and PSS MCX 1000 & 100,000 Å columns. The molar mass calculations were performed based on external calibration of the method using polystyrene sulphonate standards (eight standards with a range of 3420–148500 g/mol) and the Waters Empower 3 software (Milford, MA, USA).

Hydroxyl content analyses were determined with a quantitative 31P NMR by using the procedure by Granata and Argyropoulos [33]. NMR spectra were acquired using a Bruker 500 MHz spectrometer. The NMR parameters used were 1024 scans with pulse delay of 5s, 90°C pulse and and line broadening of 2 and default base line correction. For 31P NMR analyses, 25 mg of each lignin sample was accurately weighed and dissolved in 150 µL N,N-Dimethylformamide in 4 mL vial. After total dissolution, 100 µL of pyridine, internal standard solution (ISTD) (200 µL) endo-NHydroxy-5-norborne-2,3-dicarboximide (e-HNDI, 0.005 mmol) in pyridine/(CDCl3 1.6/1, v/v) and chromium (III) acetylacetonate solution (50 µL) (11.4 mg/1 mL) in pyridine/(CDCl3 1.6/1, v/v) were added. Then, 150 µL of the phosphorylating reagent (2-chloro-4,4,5,5-tetramethyl 1,2,3dioxaphospholane) was added. Finally, CDCl3 (300 µL) was added to the solution and clear brown to black solution was achieved.

3. Results and discussion

3.1. Lignin solubility

The initial acetone concentration for this protocol was defined based on lignin solubility in aqueous acetone (Fig. 2). Lignin had the highest solubility when acetone concentration was 60–80% whereas higher or lower water content decreased the solubility significantly. These results are according with those found in other solvent fractionation methods using ethanol [19] or acetone [27] as solvents. SSL had a remarkably lower solubility compared with the other two samples, and the highest solubility of this lignin sample was achieved with a solvent concentration of 60%. Thus, this concentration was finally selected to perform the fractionation process.

Fig. 2. Lignin solubility in aqueous acetone based on the UV absorption of the solution.

3.2. Fractionation yield

Lignin solvent fractionation produces fractions with narrow molar mass distribution [21] and more uniform structure. As defined above, three fractions called INS, PREC and SOL were recovered from each lignin sample, performing this aqueous acetone fractionation (Fig. 1). The yields obtained in each of these fractions were measured and the results are presented in Fig. 3. The PREC fraction of OSL and SKL showed the same value (64.2) and it was the highest one achieved.

However, SSL, after performing the fractionation process was composed of 38.0%, 23.8% and 34.8% of INS, PREC and SOL fractions, respectively, as is illustrated in Fig. 3. This lignin sample presented a greater distribution of its fractions. The yield of the INS fraction of this SSL was much higher than those obtained in the other two samples, because of its lower solubility in aqueous acetone solutions (Fig. 2). The SOL fractions were recovered by evaporating the aqueous solvent and the sum of the different fractions was close to 100% in the three lignin samples.

3.3. Chemical composition

The chemical composition of the distinct lignin fractions after the aqueous acetone fractionation process is presented in Fig. 4. Unfractionated lignins showed different values of total lignin content, which is commonly considered the sum of Klasson lignin and acid-soluble lignin. SSL had the lowest Klasson lignin content (64.9%) compared to the OSL and SKL (91.4% and 92.3%, respectively). In contrast, this SSL registered a great amount of inorganic particles (29.3%), and therefore the purity of this sample was very low. These
Thus, the analytical results of lignin fractions obtained from wheat straw lignins showed that the fractionation process produced fractions that possessed extremely high purity.

The SOL fractions possessed more ash and carbohydrates than the PREC fractions and higher proportion of acid-soluble lignin (ASL) compared to other fractions (Fig. 4). Acid soluble lignin is formed by low-molecular degradation products and hydrophilic derivatives of lignin [35] and therefore it is reasonable that it accumulated in this most soluble lignin fraction.

Carbohydrates were enriched mostly in the INS fraction, even if notable amount was also detected in the SOL fractions (Table 1). This result agrees with our previous results [22,23] but is different than has been observed for lignin fractionation by ultrafiltration [9] or sequential solvent fractionation with several organic solvents [17] in which the carbohydrates have been found to accumulate in the high molecular mass fractions. The carbohydrate compositions in Table 1 illustrate that the INS possessed 11–34% of carbohydrates on sample weight, and these carbohydrates were mostly glucose (samples OSL-INS and SKL-INS), xylose (samples SSL-INS and SKL-INS) and galactose (SKL-INS). For all three lignins, most of glucose remained in the insoluble fraction, which could indicate that if there is any cellulose residue in lignin, they are retained in the INS fraction. The SOL fractions contained 0.8–4.9% of carbohydrates, most of which were xylose (OSL-SOL, SSL-SOL and SKL-SOL), arabinose (SSL-SOL) and galactose (SKL-SOL). The distribution of carbohydrates in the SKL fractions were corresponding to those reported for acetone-based solvent fractionation also elsewhere [22].

![Fig. 4. Chemical composition of the unfractinated lignin samples and the fractions. Some of the inorganic substances may be present both in ash and in Klasson lignin and thus may add up the chemical composition above 100%.](image-url)
The PREC fractions possessed only minor content of carbohydrates. This result is an important observation which confirms that this fractionation process can be applied as a method to purify lignin samples from carbohydrates. In addition, this result indicates that some of the lignin molecules dissolved in pulping liquors are free from carbohydrates.

### 3.4. Elemental composition

Elemental composition results of the three lignin fractions (INS, PREC and SOL) of each lignin sample are shown in Table 2. SSL lignin possessed very low carbon content due to inorganics. The greater carbon content is directly related to the higher Klasson lignin content (Fig. 4), since lignin has a higher carbon content than carbohydrates. Precipitated fractions of the three lignin samples presented the largest percentage of carbon content, with the results between them being very similar (Table 2).

Organosolv lignin fractions were free from sulphur since this pulping and lignin recovery processes did not contain any sulphur-containing compounds. Sulphur content found in the three Kraft fractions is due to the used pulping process and it is according to those values shown in this study [36]. Also, for this lignin, sulphur content was higher in the most soluble lignin SOL fraction than in the acetone-insoluble INS fraction, which agrees the results in literature [9,24]. However, the sulphur content found in the SSL is due to the acid (H2SO4) used during the isolation of the lignin sample [37]. Interestingly, the PREC fraction of soda lignin showed minimum sulphur content (0.07%), compared to the other two fractions (2.26 and 2.32 for INS and SOL fractions respectively), which indicates that the fractionation process helped to remove this element.

No clear trend in the nitrogen content is observed in the lignin fractions studied. The origin of nitrogen in lignin samples is from cell proteins. Nevertheless, it can be observed that wheat straw lignin fractions have a higher amount of these proteins than softwood lignin fractions.

### 3.5. FTIR

The chemical structure and purity of the acquired lignin fractions were studied using FTIR spectroscopy. The obtained spectra for all the samples confirmed the results from the more detailed analyses. Signals assigned to guaiacyl (G) units were presented in the PREC and SOL lignin fractions, specifically the bands at 1260 and 1220 cm⁻¹, indicating G ring and C–O stretch [38]. The band at 1123 cm⁻¹ was assigned to vibration caused by deformation of C–H in S rings (at 1028 cm⁻¹ for G rings), while the band at 834 cm⁻¹ represented C–H out of plane in positions 2 and 6 of S units and in all positions of H units [3,39]. The latest two bands showed the features of HGS type lignin [3]. Finally, the band at 617 cm⁻¹ indicated the use of sulphuric acid to precipitate this wheat straw soda lignin (SSL) since this signal was attributed to C–S stretching [28]. The sulphur is present in the unfractuated lignin sample, as well as in the INS and SOL lignin fractions. However, this band is absent in the PREC lignin fraction, which is consistent with the minimum sulphur content (0.07%) found in this fraction (Table 2).

SSL fractions spectra (Fig. 5) provided further information on their lignin composition. These spectra of SSL fractions confirm that the INS fraction was enriched in silica, due to the strong wide band located at ca. 1096 cm⁻¹ found in this spectrum which can be assigned to the asymmetric stretching vibrations of the Si–O bonds [40]. Otherwise, these results are related to those found in the chemical composition (Fig. 4). It can be seen how the content of inorganic particles (50.8, 29.3, 10.8 and 0.3% for INS, unfractuated, SOL and PREC soda lignin fractions, respectively) is directly linked to the size and the form of this band and also the rest of the spectra presented in Fig. 5. Additionally, the aromatic skeleton in lignin is assigned at 1595, 1510 and 1422 cm⁻¹ [3,28,38,39]. A decrease in the intensity of these bands was observed in the unfractuated lignin sample compared to the PREC and SOL lignin fractions, and what is more remarkable is that these bands are practically inexist-ent in the INS lignin fraction. This fact is related to presence of high amounts of inorganic particles and therefore a lower amount of Klasson lignin in the INS fraction and in the unfractuated lignin sample (Fig. 5).

### 3.6. Molar mass fractions

For molar mass determination, the samples were dissolved in 0.1 M NaOH solution followed by size exclusion chromatographic separation. All PREC and INS samples dissolved rapidly in alkali solution whereas INS samples required more time to dissolve. In addition, a minute amount of lignin from unfractuated and INS fractions may have remained insoluble and hence removed by filtration prior to GPC analysis. Hence, the results describe accurately the molar mass distributions of PREC and SOL fractions and the alkali-soluble portion of INS fraction (Fig. 6).

The unfractuated OSL exhibited a lower molar mass and PDI compared to the other two unfractuated lignins, SSL and SKL. Organosolv lignins usually have a low molar mass and PDI compared to other type of lignins [41]. It could be explained because lignin coming from organosolv process is more depolymerised than lignin obtained from alkaline processes. Additionally, repolymerisation reactions could be induced during alkaline conditions, such as soda or kraft processes, affecting the MW of the samples [42]. All this could explain the results showed in Table 3.

The INS fraction of SSL and SKL showed very high molar mass and polydispersity (Table 3). It is known that the aggregation may occur in lignin solutions [43] and, furthermore, it has recently been reported that the acetone-insoluble fraction may possess significantly higher molar mass than the unfractuated sample indicating that acetone may induce lignin aggregation [23]. Hence,

| Table 2 | Carbon, hydrogen, nitrogen and sulphur composition of the lignin fractions. |
|---------|---------------------------------|
| Samples | Carbon (%) | Hydrogen (%) | Nitrogen (%) | Sulphur (%) |
| OSL-INS | 66.08 | 7.66 | 0.93 | 0.00 |
| SSL-INS | 27.07 | 3.39 | 1.81 | 3.26 |
| SKL-INS | 58.28 | 5.79 | 0.40 | 1.07 |
| OSL-PREC | 69.26 | 5.46 | 1.13 | 0.07 |
| SSL-PREC | 63.05 | 5.47 | 1.65 | 0.07 |
| SKL-PREC | 66.88 | 5.51 | 0.15 | 1.45 |
| OSL-SOL | 63.88 | 5.79 | 0.95 | 0.03 |
| SSL-SOL | 54.46 | 4.87 | 1.74 | 2.32 |
| SKL-SOL | 63.18 | 5.39 | 0.15 | 2.12 |

| Table 3 | Molecular weight of lignins from the aqueous acetone fractionation. |
|---------|---------------------------------|
| Samples | Mn (g/mol) | Mw (g/mol) | PD |
| OSL | 1490 | 2520 | 1.7 |
| SSL | 1970 | 4170 | 2.1 |
| SKL | 2080 | 4130 | 2.0 |
| OSL-INS | 1660 | 3850 | 2.3 |
| SSL-INS | 2340 | 36120 | 15.5 |
| SKL-INS | 2590 | 11230 | 4.3 |
| OSL-PREC | 1630 | 2820 | 1.7 |
| SSL-PREC | 2810 | 6240 | 2.2 |
| SKL-PREC | 2530 | 5600 | 2.2 |
| OSL-SOL | 1080 | 1590 | 1.5 |
| SSL-SOL | 1500 | 2580 | 1.7 |
| SKL-SOL | 1390 | 2260 | 1.6 |

* Number average.  
b Molecular weight average.  
c Polydispersity = Mw/Mn.
these high values for the molar mass and polydispersity could be explained by this phenomenon. It clears that 0.1 M sodium hydroxide solution at room temperature (experimental conditions) did not dis-aggregate lignin molecules in these two samples. Interestingly, no aggregation of organosolv (OSL) lignin was observed, which agrees with the results in literature for aqueous acetone fractionation of organosolv lignin [44].

The PREC fractions possessed much more uniform lignin molar mass distribution than the INS fractions. The molar mass and polydispersity of soda straw lignin and softwood kraft lignin were very similar, whereas the PREC fraction of organosolv lignin possessed clearly lower molar mass and polydispersity (Table 3 and Fig. 6).

The SOL fractions possessed remarkably lower molar mass and polydispersity than the PREC fraction. This result agrees with literature [32,33]. The SOL fraction of organosolv lignin possessed lower molar mass and polydispersity than the SOL fractions of soda straw or softwood kraft lignin.

3.7. Functional groups

The amount of hydroxyl groups, consisting of aliphatic hydroxyl groups, phenolic hydroxyl groups (syringyl and condensed units, guaiacyl units and p-hydroxyphenyl units) and carboxyl groups was determined by $^{31}$P NMR after derivatising lignin samples with
phosphorus-containing reagent. The $^{31}$P NMR spectra in Fig. 7A illustrate clearly the distinct differences of straw and softwood lignins. Straw lignin possesses notable amount of syringyl units as well as $p$-hydroxyphenyl units. On the other hand, softwood lignin contains only very few para-hydroxyphenyl moieties, it is practically free from syringyl units and therefore the bands between 140 and 145 ppm originate from C5-condensed aromatic structures. The insoluble fraction of the SSL did not dissolve in the solvent used for this measurement because of its poor solubility and could thus not be measured.

Fractons with the highest molar mass (INS fractions) for the three lignin samples showed the minimum content of total phenolic hydroxyl groups. On the contrary, the maximum content of total phenolic hydroxyl groups was achieved in the most water-soluble fractions, which also showed lowest molar mass (Tables 3 and 4).

$\beta$-ether units are the major substructures in lignins [45] and when a lignin samples is depolymerised by the cleavage of $\beta$-aryl ether linkage, the molar mass is decreased and new phenolic hydroxyl groups are formed [23]. This correlation between the molar mass and the number of phenolic hydroxyl groups in lignin fractions is also presented in Fig. 8A and it has been previously reported in other studies [16,27].

No clear trend is observed in the content of aliphatic hydroxyl groups according to the molar mass. However, in other fractionation processes have been found that low molar mass lignin fractions possess less content of aliphatic hydroxyl groups [16,27]. This is consistent with the low content of aliphatic hydroxyl groups presented in soluble fraction (SOL) of wheat straw organosolv lignin (Table 4). All this agrees with the low content of residual carbohydrates in this fraction (0.8%) compared to the same SOL fraction for the other two samples (Fig. 4).

On the other hand, the content of carboxylic acid structures was higher in the fractions with the lowest molar mass (SOL fractions) for the three studied lignin samples. This result is consistent with the high solubility of this fraction in solutions with high water content. A similar phenomenon has been previously reported for different solvent fractionation processes [16,23]. This correlation between the molar mass and the number of carboxylic acid groups in lignin fractions is also presented in Fig. 8B.

Additionally, in the $^{31}$P NMR spectrum of the unfractionated OSL appears a narrow band which is only present in this sample (Fig. 7A). This band corresponds to the shift of phosphorylated alcohol groups in ethanol (146.3 ppm) [46] and it is due to the residual reagent used in the organosolv pulping process. Although this lignin sample was dried at 60°C in vacuum oven for 48 h, ethanol was not removed, which suggest that strong physical interactions have been formed as has also been discussed earlier [23]. Interestingly, this residual solvent (ethanol) was removed with the aqueous acetone fractionation process. This band related to the shifts of phosphorylated groups in ethanol, disappeared in the spectrum of PREC (Fig. 7B) and SOL (Fig. S1, supplementary material) fractions of the same samples and only remained in the INS fraction as shown in Fig. S2 (Supplementary material).

Besides, the $^{31}$P NMR spectra of wheat straw lignin fractions confirmed the presence of tricin-type flavonoids in these fractions.
It is known that wheat straw lignin contains tricin [28,47]. For signal verification of this type of flavonoid (O-methylated flavone) in the $^{31}$P NMR spectrum, a novel quantitative method was recently developed [48,49]. Based on this literature, the signals arising from this molecule can be found at 136.40, 137.67 and 141.96 and these were assigned to 7-OH, 5-OH, and 4-OH hydroxyl groups, respectively. It seems that these tricin signals were more intense in the organosolv wheat straw lignin fractions than in the soda straw lignin samples. In addition, for this OSL sample, these signals were present in the $^{31}$P NMR spectrum of the un fractionated sample, the PREC and the SOL fractions. However, for SSL sample, the tricin signals are only detected in the PREC fraction. According to Li et al. [49], the signals may be derived also from other flavonoids in addition to tricin. However, tricin has earlier been shown [47,48] to be the main flavonoid in wheat straw derived lignin samples.

As previously stated, the most soluble lignin fractions (SOL) produced by this fractionation method, showed the highest content of phenolic hydroxyl group. The presence of this group increases the reactivity of lignin towards formaldehyde when this aromatic polymer is used for phenolic resin formulations [50]. Therefore, this characteristic makes these SOL fractions suitable feedstocks for that application.

Additionally, based on the literature [27,51], this fractionation protocol could successfully separate lignin fractions with high antioxidant activity. As before, the most soluble lignin fractions presented the highest amount of phenolic hydroxyl groups and it is a beneficial factor, since these groups have positive effect on free radical scavenging activity [51]. Thus, these fractions could have a great potential for the antioxidant applications.

4. Conclusions

The presented acetone-based fractionation process separated lignin into different fractions based on their solubilities in aqueous solvents. Fractions with low molar mass were dissolved in solutions with a greater proportion of water. Lignin fractions produced with this fractionation protocol showed different functional groups and molar mass. Higher amount of phenolic hydroxyl groups and carboxylic acid groups were presented in the lignin fractions that showed the lower molar mass.

PREC fraction for organosolv and Kraft lignin showed the highest yield of the performed method. The chemical composition of the three un fractionated lignin samples was different, however, after the fractionation process this chemical composition was unified for the PREC fraction of the three lignins. Also, the carbon content was very similar between them. Thus, these results provided evidence that this fractionation protocol was applicable for different types of technical lignins to produce fractions with high purity and homogeneity. Additionally, SOL fraction showed acceptable yield percentages for the three lignins. However, the source of lignin defines the structure and composition of the produced homogeneous lignin fractions. This fraction is very useful for applications where high reactivity is required, such as resins.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijbiomac.2017.08.102.

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Table 4

| Samples | Aliphatic OH (mmol/g) | Phenolic OH (mmol/g) | Cond-S PhOH (mmol/g) | G-PhOH (mmol/g) | p-PhOH (mmol/g) | COOH (mmol/g) |
|---------|-----------------------|----------------------|----------------------|----------------|----------------|---------------|
| OSL     | 1.32                  | 2.97                 | 1.42                 | 1.06           | 0.49           | 0.48          |
| SSL     | 2.33                  | 1.27                 | 0.47                 | 0.54           | 0.25           | 0.88          |
| SKL     | 1.72                  | 3.57                 | 1.56                 | 1.73           | 0.28           | 0.44          |
| OSL-INS | 1.99                  | 1.19                 | 0.61                 | 0.42           | 0.16           | 0.29          |
| SSL-INS | –                     | –                    | –                    | –              | –              | –             |
| SKL-INS | 1.75                  | 2.10                 | 1.00                 | 0.98           | 0.12           | 0.19          |
| SSL-PREC | 2.84                | 3.13                 | 1.49                 | 1.12           | 0.53           | 0.48          |
| SKL-PREC | 1.79                 | 4.38                 | 2.01                 | 2.06           | 0.31           | 0.42          |
| OSL-SOL | 1.22                  | 3.59                 | 1.65                 | 1.38           | 0.56           | 0.74          |
| SSL-SOL | 2.87                  | 2.14                 | 0.75                 | 0.95           | 0.45           | 1.54          |
| SKL-SOL | 1.70                  | 5.91                 | 2.08                 | 3.47           | 0.37           | 0.54          |
