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ABSTRACT

Lignin, the second most abundant biopolymer in earth, represents nature's largest carbon sources, has high polydispersity and uncertain reactivity, that limit efficient modification/degradation and use of products obtained. In order to decrease by mechanochemical means the average molecular mass and polydispersity of lignin fragments of the CIMV organosolv lignin named Biolignin™, we applied a sodium hydroxide-assisted planetary ball milling process. Experimental conditions were first optimized on purified Biolignin™ then to Biolignin™ without prior purification in 20ml jars before applying it successfully to a 500 ml planetary ball milling apparatus. Extensive, quantitative HSQC NMR, IR and GPC studies, overtaken supported our three most important findings: reduction of the needed quantity of sodium hydroxide of 75% compared to the literature, obtention of halve (-51%) the occurrence of β-O-4'

linkages in lignin fragment, an optimum of 14 wt% of moisture is useful in the degradation process.

Keywords: organosolv lignin degradation, mechanical milling, β-O-4-linkages cleavage

1. Introduction

Lignin, the second most abundant biopolymer in earth after cellulose, represents nature's largest carbon sources with an annual production of about 20 billion tons. In contrast to cellulose and hemicellulose, lignin is highly branched heterogenous polyphenolic material, without a recurring structural unit in the biomass. In that respect, lignin has high polydispersity and consequently uncertain reactivity, two important factors that limit efficient modification/degradation and use of products obtained. Mechanical energy and mechanochemistry, besides the fact that allowed the development of sustainable and efficient chemical transformations by promoting the formation of new bonds and non-covalent interactions, has also demonstrated its potential in the selective cleavage of specific chemical bonds.

Lignins can be obtained by various methods resulting in different characteristics. Technical lignins mainly gathered kraft, sulphite, soda-anthaquinone and organosolv lignins. Organosolv lignins are the most recent and promising ones. Organosolv processes are considered to be the most efficient and environmentally friendly way to extract lignin from biomass. It allows the obtention of lignin with a relatively lower molecular weight and more homogeneous fragments resulting a lower polydispersity compared to others technical lignins. However, even these more homogeneous lignins are still suitable for direct use to a limited extent.

The valorisation of lignins in the era of green and environmental preserved world, generated a tremendous activity in fundamental (and consequently industrial) research during the last two decades. The quest for lignin derived chemical platforms (low molecular mass compounds and essentially monomers), oriented important work on homogenous, heterogenous catalysis, use of alternative solvents, and thermochemical, oxidative, photocatalytic or biochemical depolymerization methods (Zhou *et al.* 2022 and Sun *et al.* 2018 and references therein). Usually, each method is associated with its own benefits and drawbacks; it is also important to point out that each kind of lignin is unique (purity, dispersity, molecular weight…) so each method should be adapted to the lignin studied. Oxidative depolymerization of lignin is one of the most studied aspects (Abdelaziz *et al.* 2022).

Some ten years ago, mechanochemistry entered in the field of lignin degradation. In 2013, Kleine *et al.* reported a pioneering work on the lignin depolymerization *via* mechanochemical grinding and in the presence of solid bases (Kleine *et al.* 2013). Mechanochemical oxidative processing is also reported since the last decade by many authors. As an example, the mechanochemical oxidation and cleavage of the β-O-4 in lignin systems has been reported in the presence of catalytic amounts of HO-TEMPO/KBr/Oxone, resulting in monomeric products efficiently in order to be applied in upscale batch reactions (Dabral *et al.* 2018). Very recently, a novel mechano-enzymatic strategy was developed (Xu *et al.* 2023) for lignin depolymerization leading into functional aromatic monomers considered as high value chemicals for pharmaceutical, material and energy industries.

Also, very recently, surface modification of a kraft lignin by grinding with sodium percarbonate and sodium hydroxide as an oxidant can lead to reduction in polydispersity and improved water adsorption (Fink *et al.* 2023).

The aim of our study concerns a specific organosolv lignin named Biolignin™
The objective of this work is to halve the average molecular weight lignin fragments of Biolignin™ without highly modifying its functional groups content in order to avoid any decrease of its reactivity and to enhance its ability to substitute phenol or polyols in various industrial applications.
Indeed, in some applications in which Biolignin™ was used as the substitute of petrochemical molecules (phenol, PEG) (Tachon *et al.*, 2016; Llovera *et al.*, 2016), the limiting factor of the substitution might be the molecular mass of lignin fragments. Lignin fragments are not totally linear and reactive groups such as phenolic and aliphatic OH, could be sterically shielded, affecting the global reactivity of lignin. Decreasing the length of lignin fragment might enhance the accessibility of such functional groups for further coupling reactions.

2. Materials and methods

2.1.Extraction and solvent fractionation of the organosolv lignin

The organosolv lignin studied below is named Biolignin™. Biolignin™ was extracted at pilot scale from wheat straw at the CIMV pilot plant (Compiègne, France). The CIMV organosolv process consists in a fractionation of lignocellulosic materials thanks to a mixture of acetic acid/formic acid/water (55:30:15, w/w/w) during 3.5 hours at 105°C and at atmospheric pressure. The lignin is separated from the extraction liquor by adding water to the concentrated liquor to induce its precipitation. It is finally recovered

by filtrating the above liquid suspension (Figure 1) (Benjelloun-Mlayah and Delmas 2019).

[**Figure 1]**

In order to increase the lignin content (increase the purity) and to decrease the polydispersity of lignin fragments, the Biolignin™ was sequentially fractionated with diethyl ether (99.7% stabilized with ~6 ppm of BHT, PanReac), dichloromethane (99.8% stabilized with ~20 ppm of amylene, PanReac) and methanol (99.9% UV-IR-HPLC-GPC Grade, PanReac) as illustrated in Figure 2. This purification was previously developed by the CIMV research group in order to obtain a purified Biolignin™, highly concentrated in similar lignin fragments and gathered nearly 50% (w/w) of unfractionated Biolignin™ (unpublished results).

Before fractionation, the Biolignin™ was dried at 50°C in a drying oven, until obtaining at least 96% of Dry Matter (DM). The dry Biolignin™ was then grounded in a ceramic mortar to enhance its contact surface.

[**Figure 2]**

An amount (100g) of dried and grounded Biolignin™ was introduced in a 2L-cylindrical reactor equipped of a frit n°3 (pore size: 16-40 µm). Then, 500 mL of the respective solvent was added and the mixture was mechanically stirred during 1 hour at room temperature. After removing the solvent by pumping through the frit, 500 mL of the identical fresh solvent was added and the mixture stirred during 1 hour. This procedure was repeated several times until the majority of the soluble molecules seemed to be eluted (final filtrate nearly colorless). The next solvent was then added to the remaining Biolignin™ sample and the same procedure was applied.

All the filtrates of each solvent were combined and evaporated under reduced pressure to obtain the three global fractions: F1 (6.4% ±0.5 w/w), F2 (16.2% ±1.2 w/w) and Purified Biolignin^{TM} (48.4% ±2.2 w/w). The insoluble residue (29.0% ±2.7 w/w) was composed of the remaining solid residue after methanol elution. F1 was recovered as a brown-yellow sticky residue, F2 as brown-yellow flakes and Purified Biolignin^{TM} as sparkled black flakes.

2.2. Biolignin^{TM} content analyses

2.2.1. Ash content

Ash content was determined by the combustion of 1g of accurately weighted sample (accuracy: 10^{-4}) with a known moisture content. The sample was placed in an oven at 600°C during 24h. The weight of ash remaining was calculated as a percentage of the original dry weight of sample. At least two measurements were done on each sample.

2.2.2. Elemental analysis and proteins content

Elemental analyses were performed on a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer. 2.0 mg (+/- 0.5mg) of sample were weighed. Only C, H and N elements were analyzed. The proteins content was deduced from the % of N using the nitrogen-to-protein conversion factor (5.4 for wheat straw, Marriotti et *al.* 2008). At least two measurements were done on each sample.

2.2.3. Cellulose and hemicellulose content in lignin

Residual cellulose and hemicellulose contents of lignin fractions were determined based on NREL method (Sluiter *et al.* 2008): The glucose and xylose contained in the fraction

were quantified by HPLC, after chemical hydrolysis. The hydrolysis was conducted in 2 steps: 0.2 g of dry lignin sample was powdered in 1.6 ml of H_2SO_4 72% (12 M) and stirred at room temperature overnight, followed by stirring at 30°C for 1 hour. For the second step, 22.4 g of distilled water was added and the mixture was maintained at 120°C for 1 hour.

Then, the samples were cooled at room temperature before their analysis by HPLC for sugars quantification. The following HPLC conditions were used: Eluent: H_2SO_4 0.005 M; Flow rate: 0.6 ml/min; Column Hi-Plex H column (300 x 7.7 mm), temperature 80°C; Detector Refractometer, temperature 50°C. At least two measurements were done on each sample.

2.2.4. Infrared spectroscopic analyses

Infrared analyses were conducted with an attenuated total reflectance system (ATR) on a PerkinElmer Spectrum 100 Universal ATR-FTIR instrument equipped with a diamond/ZnSe crystal single reflection. 10 mg of dried sample were placed on the crystal plate on which a constant pressure of 85 N/mm² was applied. Each spectrum was obtained after 8 scans at a resolution of 4 cm^{-1}. At least two measurements were done on each sample.

2.2.5. Gel Permeation Chromatography (GPC)

The molecular-average weights of samples were determined by Gel Permeation Chromatography (GPC). GPC analyses were carried out on a Water 1515 Isocratic HPLC Pump equipped with a Water 2414 Refractive Index Detector. Three stainless steel columns (Phenomenex, Phenogel. 300 x 7.8 mm. 5 µm) connected in series and

packed with a styrene-divinylbenzene copolymer of porosity 100, 500 and 10^3 Å, respectively, were used. The following operating conditions were employed: Eluent, THF (Panreac, UV-IR-HPLC-GPC Grade); temperature, 38°C; sample concentration, 5 mg/mL; injection volume, 20 µL; flow rate, 0.8 mL/min. Polystyrene (PS) standards used for the GPC calibration curve have a molecular weight of: 4910, 3180, 2590, 2170, 1530, 990, 770, 580 and 380 Da. The standards concentration in THF was set at 2 mg/mL.

The samples were injected without prior derivatization. To limit the presence of insoluble particles in the injected samples, they were previously dissolved in a mixture of 1,4-Dioxane/Methanol (1:1, v/v), (Panreac, UV-IR-HPLC Grade) to reach a concentration of 40 mg/mL. When necessary (samples degraded by NaOH), the samples were neutralized by addition of hydrochloric acid in an amount equivalent to the sodium hydroxide. The solution was then diluted with THF (HPLC Grade) to obtain a final concentration of 10 mg/mL. The solution was then filtrated through a 0.45 µm membrane before analysis. At least two measurements were done on each sample. The molecular weight of lignin was quantified relative to polystyrene standards and was therefore not absolute. M_n represent the number average Molecular weight, M_w, the weight average molecular weight and I the polydispersity index of the polymer ($I=M_w/M_n$).

2.2.6. Quantitative ^{31}P NMR

Hydroxyl groups and aromatic carboxylic acids of lignin samples were quantified by ^{31}P-NMR. Each sample was phosphitylated with 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (95%, Sigma Aldrich, St. Louis, MO, USA). For these experiments,

endo-N-hydroxy-5-norbornene-2,3-dicarboximide (97%, Sigma Aldrich, St. Louis, MO, USA) was selected as the internal standard. The whole experimental procedure is described by Cachet and Benjelloun-Mlayah (2021). At least two measurements were done on each sample.

2.2.7. ^{1}H-^{13}C 2D-NMR experiments

NMR experiments were performed at 25°C on a Bruker Advance 500 MHz spectrometer equipped with a 5 mm gradient cryoprobe with inverse geometry (5 mm CPTCI 1H-31P/13C Z-GRD Z44913/0001). 125 mg of each sample (without prior derivatization) were dissolved in 750 µL of DMSO-d_6. Chemical shifts (δ in ppm) are referenced to the carbon (δ_C 39.53 ppm) and the residual proton (δ_H 2.50 ppm) signals of DMSO-d_6 (99.8%D, Eurisotop, Saint Aubin, France)

The two-dimensional heteronuclear single quantum coherence (HSQC) - NMR experiments were conducted using Bruker's "hsqcetgp" pulse with spectral widths of 6002 Hz (from 11.5 to -0.50 ppm) and 22638 Hz (from 175 to -5 ppm) for the ^{1}H- and ^{13}C- dimension, respectively. The number of collection complex points was 1024 for the ^{1}H- dimension with a recycle delay of 2 s. The number of transients was 16, and 1024 times increments were always recorded in the ^{13}C- dimension. The $^{1}J_{CH}$ used was 145 Hz. Processing used typical squared sine-bell apodization for the ^{1}H- and ^{13}C- dimension. Prior to Fourier transformation, the data matrixes were zero-filled up to 1024 points in the ^{13}C- dimension.

The semi-quantitative analysis of the volume integrals (uncorrected) of the HSQC correlation peaks was performed using MestRec 4.9.9.3 processing software. This

quantification was conducted following the procedure described by Wen *et al.* (2013) and by Sette *et al.* (2011).

2.3. Mechanochemical reactions

Mechanochemical reactions were mainly inspired by the work of Brittain *et al*. (2018). Ball-milling of lignin was performed in two ZrO_2 jars (20 mL) in parallel, at room temperature using a planetary Fritsch Pulverisette 7 Premium Line ball mill. The jars were equipped with five milling balls each (10 mm diameter, ZrO_2). The ball-milling was carried out at 800 rpm for 15 to 120 min. To avoid overheating at long milling times, the mill was stopped for 5 min after every 30 min of milling. The milling jars were filled with crude or purified Biolignin™ and sodium hydroxide powder (97%, Sigma Aldrich, St Louis, MO, USA). The quantity of solid products in each jar was set at 700 to 1000 mg according to the experiment. Preliminary experiments confirmed that no positive or negative effects were observed varying the quantity of solid products from 700 mg to 1000 mg. The ratio of Biolignin™/NaOH was adjusted from 1:1 (w/w) to 4:1 (w/w) according to the experiment. The samples were left to sit for 5 min–24 h before being prepared for molecular mass analysis in order to study the quenching effect of the reaction. Each jar was analyzed separately by GPC in order to evaluate the repeatability of the experiment. If the two results of a same experiment were too different (*i.e.* >10%), the experiment was repeated twice. The values reported on this study are the average of the values obtained in experiments under the same conditions. Before analyses, the grinded samples were neutralized by solubilization in water (50 mg/mL) and slowly adding dropwise concentrated HCl (37%, Pharma Grade, PanReac) in an amount equivalent to the sodium hydroxide used for the reaction. The pH value

was checked with pH test stripes (pH 1-14, ChemSolute) and adjusted if needed in order to precipitate lignin. The medium was centrifuged (4000 RPM, 2x 15 min). The solid lignin was then separated to the aqueous phase and washed 3 times with UHQ H_2O by filtration on a frit n°3 (pore size: 16-40 µm). The final solid was dried at 50°C in a drying oven during 24 hours.

Some experiments were assisted by a small amount of liquid in order to enhance the reactivity of the depolymerization. The amount of liquid was set in order to respect the conditions of Liquid-assisted grinding (LAG) empirically defined by the parameter η [η=V (liquid; µL) / m (solid reagents; mg)]. LAG conditions are operating when values of η range 0–2 µL/mg, the reaction appears independent of reactant solubility (Ying *et al.* 2021).

For ion scavenging tests, methanol (0.27µL/mg of Biolignin™, η 0.22) was added to the lignin/sodium hydroxide mixture in the milling jar. To investigate the influence of the moisture content, water was added to the milling jar to increase the total moisture content in the sample from its original value of 4 wt% to the desired moisture content.

A control experiment was made milling *ca.* 700 mg of purified Biolignin™ without sodium hydroxide powder (97%, Sigma Aldrich, St Louis, MO, USA) in a 20 mL ZrO_2 jar with five milling balls (10 mm diameter, ZrO_2) during 120 min, with a 5 min stop every 30 minutes to cool down the system.

To test the scale up of the reaction, ball-milling of lignin was performed in a Tungsten Carbide (WC) jar (500 mL) using a Retcsh PM 100 ball mill at room temperature. The jar was equipped with 10 or 20 milling balls according to the experiment (20 mm diameter, WC). The ball-milling was carried out at 650 rpm for 60 minutes. To avoid overheating at long milling times, the mill was stopped for 10 min after every 15 min of

milling. The milling jar was filled with Biolignin^{TM} and sodium hydroxide powder. The quantity of products was set at 21.0g ± 0.5g. The ratio of Biolignin^{TM}/Sodium hydroxide was set at an equimolar ratio between C9-Unit (162g/ mol) and NaOH. The influence of the moisture content was made by adding water to the milling jar to increase the total moisture content in the sample from its original value of 4 wt% to the desired moisture content.

3. Results and discussion

3.1. Extraction and fractionation of lignin from wheat straw

The mechanochemistry is a method of choice to obtain valuable products in a green way. Thus, the more environmentally friendly production of organosolv lignins among technical lignins, leads us to select this kind of lignin. Moreover, organosolv lignins are composed of relatively homogeneous lignin fragments. Indeed, less energy will be required to obtain more homogeneous fragments with a low average molecular weight than if another technical lignin is used.

To obtain the organosolv lignin studied below, the wheat straw was treated at atmospheric pressure with a mixture of organic acids (acetic acid and formic acid) and water at 105°C during 3.5 hours (Benjelloun-Mlayah and Delmas 2019). These conditions allowed the dissolution of lignin fragments and hemicelluloses in the acidic media. After filtration to remove the solid part (cellulose fraction), the lignin was precipitated and separated from the extraction liquor. The precipitated fraction mainly composed of lignin, is named Biolignin^{TM} and represents 27% (w/w, Dry Matter) of the initial wheat straw.

The Biolignin^{TM} was not entirely composed of lignin fragments. Some constituents of wheat straw, like ashes, residual carbohydrates and proteins, were also precipitated with

lignin fragments during its separation from the extraction liquor. Table 1 summarized and quantified these constituents (see experimental sections 2.2.1 to 2.2.3).

[**Table 1]**

As, showed in Table 2, lignin fragments of Biolignin^{TM} had restricted distribution ($M_w/M_n < 2$). However, this distribution and the impurities contained in the Biolignin^{TM} fraction (Table 1) could have a negative influence for depolymerization/degradation of lignin fragment by mechanochemistry. In literature, most of the time, the depolymerization of lignin is optimized using synthetic lignin models (dimers or trimers) before testing on natural lignin (Dabral et *al.* 2018, Sun et *al.* 2020, Xu et *al.* 2023). For the study below, a direct use of lignin fragments was chosen, driving the experiments by previous works done on lignin models (Brittain *et al.* 2018, Dabral *et al.* 2018). However, to overcome the potential negative effects of impurities and lignin fragments distribution, we proceeded to the fractionation of Biolignin^{TM} in order to obtain a purified fraction with a narrower distribution. This purified fraction is considered as a lignin model of the selected crude organosolv lignin.

[**Table 2**]

3.2. Purification of Biolignin^{TM}

The aim of the fractionation was to isolate a homogeneous lignin fraction with a narrower molecular mass than Biolignin^{TM} which can represent the pattern of Biolignin^{TM} (*i.e.* same types of intra-linkages and reactive groups). Three fractions were isolated from the initial Biolignin^{TM} by sequential extraction with diethyl ether (F1), dichloromethane (F2), methanol (Figure 2). The solid residue obtained after the last solvent extraction (*i.e.* Methanol) represented 29.0 (± 2.7)% w/w of the initial Biolignin^{TM} sample and was highly insoluble in a large panel of organic solvents.

Methanol fraction represented the main part of the unfractionated materiel with a yield of 48.4 (± 2.2)% (w/w) while fractions F1 and F2 were respectively obtained with the yields of 6.4 (± 0.5)% and 16.2 (± 1.2)% w/w (Table 3). The four fractions were analyzed by Gel Permeation Chromatography (GPC) in order to determine their molecular weight. The results of GPC analysis (Table 3) showed that fractionation yielded fractions of increasing molecular weight. With a M_n and M_w of, respectively, 611 and 784 g/mol, 747 and 1116 g/mol, 989 and 1579 g/mol, the distributions of the three first fractions were lower than the one of the initial Biolignin™ material (M_n 999 g/mol and M_w 1743 g/mol). Fractions F1 and F2 gathered molecules with the lowest average molecular weight but did not yield a large part of the parent Biolignin™ (F1+F2 < 25% (w/w)). Due to their low yield, these two fractions cannot be considered as a pattern of Biolignin™.

According to the work of Lange and coworkers on wheat straw Biolignin™ (Lange et *al.* 2016), this lignin has an average molecular weight of a C9-unit of 162 g/mol. Even if the average number molecular weight (M_n) of purified Biolignin™ and Biolignin™ are almost the same (*ca.* 6 C9-units), the average weight molecular weight (M_w) decreases of one C9-unit in purified Biolignin™ (9.7 u *vs.* 10.8 u, respectively, Table 3). This offers us the opportunity to explore first the degradation conditions on the purified Biolignin™ that we consider as "lignin model" more homogeneous than its parent one.

[Table 3]

Thus, in regards to its GPC profile and its yield from Biolignin™, the third fraction, named Purified Biolignin™ seemed to be a good candidate as a Biolignin™ model to optimize the degradation of lignin fragments by mechanochemistry. The chemical characterization of this fraction is described below.

3.3. Composition and ATR-FT-IR of purified Biolignin™ fraction

Biolignin™ and purified Biolignin™ are not entirely composed of lignin fragments. However, after fractionation, purified Biolignin™ contains 31% less impurities than Biolignin™ (8.94% *vs.* 12.92%, Table 4). This fractionation can thus be considered as an efficient purification.

[Table 4]

In order to verify that purified Biolignin™ could be used to represent the behavior of Biolignin™ during mechanochemical degradation, a comparison of the ATR-FT-IR profile of purified and parent Biolignin™ was done. As it is indicated on Table 5, the global shape of these two spectra were similar and perfectly fit with the usual IR profile of lignins containing Guayacyl/Syringyl/ *p*-Hydroxyphenyl units (GSH-lignins) (Bykov 2008, Cronin et *al.* 2017). Then, the purification of Biolignin™ did not seem to drastically modify the chemical structure of lignin fragments.

It could be noted that F1, F2 and the insoluble residue did not show an ATR-FT-IR profile that totally fit with lignin profile. The only spectrum with some characteristic lignin IR bands was the one of insoluble residue, however its global shape did not fit with the Biolignin™ spectrum. See **Table S1** for more details.

[Table 5]

3.4. NMR analysis of Biolignin™ and purified Biolignin™

Table 6 highlights the amount of hydroxyl groups and carboxylic acids quantified by ^{31}P-NMR. The following signal ranges were integrated: 150.2-144.6, 143.7-140.2, 140.2-138.6, 138.6-136.9 and 135.6-133.7. They were attributed to, respectively,

aliphatic hydroxyls, phenolic hydroxyls of S-units and/or condensed G-units, G-units, H-units and carboxylic acids (Argyropoulos et *al.* 2021).

The ^{31}P-NMR quantification supported the previous hypothesis that lignin fragments of Biolignin^{TM} and Purified Biolignin^{TM} are similar. However, a slight decrease of free phenolic hydroxyls of S-, condensed G- and G-units could be pointed on purified Biolignin^{TM} compared to Biolignin^{TM}. This loss of -0.25mmol/g will be considered in the following as too weak to influence the behavior of lignin fragments during their mechanochemical cleavage. Thus, the ^{31}P NMR analysis validates the use of purified Biolignin^{TM} as a model of Biolignin^{TM} for the further the mechanochemical reactions.

[Table 6]

Several types of linkages exist in lignins. Their type and ratio are dependent on the plant source (Strassberger *et al.*, 2014). The most common linkages found in wheat straw Biolignin^{TM} are shown in Figure 3.

With nearly 90% of the total identified linkages, the 3 major linkages in Biolignin^{TM} and purified Biolignin^{TM} are β-O-4' (**1**), β-5' (**2**) and β-β' (**3**) (Table 7). 1,2-Diarylpropane (**4**), monotetrahydrofuran (**5**) and α,β-diarylether (**6**) represented only 7.4% and 10.9% of the total identified substructures in, respectively, Biolignin^{TM} and Purified Biolignin^{TM}.

With 69.7% and 73.6% of the identified substructures of, respectively, Biolignin^{TM} and Purified Biolignin^{TM} (Table 7), β-O-4' ether unit is the predominant linkage (Figure 3, Substructure **1**). This linkage is the most common one in lignin (40-70 wt%) and is among the most readily cleaved (Cui *et al.*, 2021). The energy necessary to cleave this linkage varies between 68.2 and 71.8 kcal/mol, depending on substitution pattern, which is weak compared to other lignin linkages (Sun *et al.*, 2018). The large presence and the

reactivity of β-O-4' lignin units have made them a target for many depolymerization/ degradation strategies.

In view of this, we mainly focused on the cleavage of this linkage under mechano-chemical conditions. In this work, we did not optimize the experiments on the production of phenolic monomers, as it is largely described in the literature (Sun *et al.*, 2018; Liu *et al.*, 2020). The aim of the study below is to decrease substantially the average molecular weight of Biolignin^{TM} in order to enhance its ability to substitute phenol or polyols in various industrial applications. Halving the average molecular weight might be an acceptable goal to enhance the accessibility of functional groups for further coupling reactions.

The main goal of our study is to decrease the average molecular mass and the polydispersity of lignin fragments of an organosolv lignin source without highly modifying its functional groups content in order to avoid any decrease of its reactivity. This study is not trying to obtain platform molecules (vanillin etc..) from lignin but to partially depolymerize lignin fragments in order to optimize its use in applications (polyurethanes, phenolic resins, epoxy resins etc..) without any functional modification.

[Table 7]

[Figure 3]

3.5. Mechanochemical cleavage of purified Biolignin^{TM} with sodium hydroxide

The double objective of this mechanochemical cleavage is to decrease the average relative molecular mass and the polydispersity of lignin fragments but also to optimize a protocol using cheap and widely available reagents. The literature highlighted promising results using sodium hydroxide to depolymerize an organosolv lignin (Kleine *et al.* 2013; Brittain *et al.* 2018). In contrast to Brittain and co-workers who tried to

optimize their experimental conditions in view of maximizing the production of monomers, this work focused on reaching a final homogeneous fraction, with roughly half of the original average molecular mass minimizing the condensation reaction as it could occur during lignin depolymerization (Li *et al.*, 2015). To our knowledge, only Fink *et al.* had the same approach using Kraft lignin with sodium percarbonate (Fink *et al.* 2023). In contrast to our study, Fink and co-workers worked on a Kraft lignin, which is highly branched, very heterogeneous and has higher average molar mass than organosolv lignins (~1500-25000 g/mol *vs.* 500-5000 g/mol for organosolv lignins) and consequently significantly less reactive than organosolv lignins. The author tried to cleave, to homogenize lignin fragments from Kraft lignin but also to increase some functional groups (mainly carbonyl groups) in order to improve its reactivity. In our case, Biolignin^{TM} is already very reactive. The incorporation of new functional groups is unnecessary. The main point we focused on is the limitation of condensation reaction often observed during the depolymerization of lignin (Li *et al.*, 2015).

In order to optimize the conditions of the reaction, purified Biolignin^{TM} was chosen as an initial substrate. The experiments are described in Table 8.

[Table 8]

3.5.1 Optimization of the milling time and the quantity of sodium hydroxide

In their study, Brittain *et al.* (2014) used lignin and NaOH in an equimass ratio. In order to evaluate the performance of the degradation on purified Biolignin^{TM}, our four first experiments were also done with this ratio (Table 8, experiments D1 to D4) allowing a decrease of the average molecular mass of *ca.* 30% in only 15 min-milling and reach its lower average molecular mass after 60 min-milling (*ca.* -50%, See supporting information Fig. S1). However, the quantity of NaOH seem to be overdosed according

to the possible reaction mechanism between lignin and NaOH (Sun *et al.* 2020). Sodium hydroxide reacts with $C_{\beta}H$ of arylglycerol-β-arylether substructure (Figure 3, Substructure 1) by breaking β-O-4' linkage, the major linkage of purified Biolignin^{TM}. It seems to be more consistent if a mole of NaOH is added for a mole of arylglycerol-β-arylether substructure (equimolar ratio). For convenience, we worked on the average C9-unit of Biolignin^{TM} as the reference unit instead of the arylglycerol-β-arylether substructure. Lange *et al*. determined the average C9-unit of wheat straw Biolignin^{TM} at 162g/mol (Lange *et al.*, 2016). Thus, the experiments D5 to D10 (Table 8) were conducted with a molar ratio C9-unit/NaOH of 1:1 (D5, D7, D9) and 1:2 (D6, D8, D10). It is interesting to note that an equimass ratio purified Biolignin^{TM}/ NaOH is equivalent to a 1:4 molar ratio C9-unit/NaOH. Decreasing NaOH quantity resulted in a final fraction with higher average molecular mass. Indeed, instead of a decrease of *ca.* 50% after 60 min-milling, the average molar mass decreased of 28% and 36% for, respectively, 1:1 ratio C9-unit/NaOH (D7) and 1:2 ratio C9-unit/NaOH (D8). However, the study of FT-IR spectra gave some precious information (the normalized absorbances of measured FT-IR spectra can be found on Table S2). Sodium hydroxide cannot cause a ring cleavage, then, spectra were normalized against the vibration of the aromatic framework at 1510 cm^{-1} which is supposed to not vary. A simultaneous decrease of intensity at 1210 cm^{-1} (vibration of C-O-C) and 1140 cm^{-1} (C-O stretch, Faix, O. 1991) indicates a cleavage of the ether bonds (Fink et *al.* 2023). Figure 4 represents the normalized FTIR absorption values of these two vibration bands exposed to different quantity of sodium hydroxide and time of milling. It clearly appeared that, after only 30 min-milling, the equimass ratio purified Biolignin^{TM}/ NaOH led to a significant increase of intensity at 1210 cm^{-1} and 1140 cm^{-1} while the (1:1) or (1:2) C9-unit/ NaOH molar

ratio show a decrease of these bands. Thus, it seemed that the milder conditions allowed the cleavage of ether bonds without any recombination of fragments while the use of an equimass quantity of NaOH leads to a cleavage of C-O-C bond quickly following by a rearrangement of new fragments, increasing the intensity at 1210 and 1140 cm^{-1}.

[Figure 4]

Seeking to avoid any recombination of cleaved lignin fragments, the equimass ratio reaction was ruled out. According to GPC and FTIR analyses, the improvement of C-O-C cleavage between reactions with (1:2) and (1:1) C9-Units/NaOH was not sufficiently significant to favor the use of a (1:2) C9-Units/NaOH ratio.

Then, in the following, the mechanochemical reaction between lignin and sodium hydroxide will be equimolar (1:1 C9-Unit/NaOH), thus, leading to a drastic reduction of the quantity of NaOH (*i.e.* -75%) compared to the literature (Kleine et *al.* 2013, Brittain et *al.* 2018).

The three samples D5, D7 and D9, obtained after the (1:1) C9-unit/NaOH mechanochemical reaction were analyzed by HSQC NMR spectroscopy and compared with unmilled lignin sample. Figure 5 represents the evolution of the three main linkages (β-O-4', β-5' and β-β') in which the aromatic unit (C9-unit) is defined as the internal standard (IS) of the sample. Indeed, aromatics are not supposed to vary during the discussed mechanochemical degradation because of their resistance to cleavage with NaOH. This method used a cluster of signals that are representative to all C9-units (*i.e.* IS) as following:

I_{C9} units = $0.5IS_{2,6} + IG_2 + 0.5IH_{2,6}$.

476 Where $IS_{2,6}$ is the integration of $S_{2,6}$, IG_2 is the integral value of G_2 and $IH_{2,6}$ is the
477 integral value of $H_{2,6}$. This formula was determined by Wen and co-workers for grass
478 lignin (Wen *et al.* 2012, Wen *et al.* 2013)
479 I_{C9} represents the integral value of the aromatic ring. According to the internal standard
480 (I_{C9}), the amount of I_X% could be obtained by the following formula:
481 $I_X\% = I_X/I_{C9} \times 100\%$
482 Where I_X is the integral value of the α-position of the substructures 1 (β-O-4), 2 (β-5),
483 and 3 (β-β).
484 The inter-unit bonding spin system shows three different signals due to CH and CH_2
485 groups in the positions α, β and γ of the side chain (Figure 3). T_2 relaxations of each
486 signal is dependent on carbon substitution. Then, only the CH signals were selected for
487 quantitative analyses. Among the CH signals for each spin system, we focused on the
488 better-resolved resonances that did not overlap with signals. Hence, for quantification,
489 the following signals were used: 1-α, 2-α and 3-β (Figure 6). These selected signals are
490 in accordance with those selected in literature (Sette et *al.* 2011).
491 **[Figure 5]**
492 **[Figure 6]**
493 Before milling, purified Biolignin™ contained, respectively, 20.5%, 4.3% and 1.6% of
494 Arylglycerol-β-arylether (β-O-4), Phenylcoumaran (β-5) and Resinol (β-β). After 2h of
495 milling purified Biolignin™ without any presence of sodium hydroxide (C1
496 experiment), this ratio reached 19.8%, 4.5% and 1.7% confirming that the mechanical
497 energy given during the milling is insufficient to cleave lignin linkages.
498 Focusing on β-O-4' linkage, it clearly appeared that the main C-O-C cleavage occurred
499 during the first 60 min of milling. Reaching 5.9% after 60 min of milling, more than

71% of the initial β-O-4' are cleaved. Then, with a slower degradation rate, more than 86% of the initial β-O-4'are cleaved after 120 min of milling. Considering the energy cost of milling (Sealy *et al*. 2016) and setting the milling duration at 60 min seemed more beneficial from the perspective of the process scale-up to industrial scale.

We expected that resinol (Substructure 3, β-β' linkage) was not impacted by the degradation, because of the resistance of C-C bonds to cleavage with basic catalysts (Zakzeski et *al.* 2010, Kleine et *al.* 2013). However, the HSQC quantification seemed to indicate that a cleavage of this linkage occurred. From 2.0% before milling, this linkage reached 0.2% after 60 min of milling. After 120 min of milling, an odd phenomenon appeared: the quantity of β-β' linkage increased until reaching 0.8%. It may indicate that some slight rearrangements occurred after 60 min of milling.

Phenylcoumaran (Substructure 2, β-5' linkage) was also affected by the mechanochemical degradation. From 4.3% before milling, this linkage slowly decreased, reaching 1.9%, then 1.4% after, respectively 60 min and 120 min of milling.

The above discussed cleavages were visually observed on Figure 6 where the intensity of correlation spots of 1-α, 2-α and 3-β progressively decreased with the milling time.

Hence, in view of the above results, the best compromise between the efficiency of the cleavage of purified Biolignin™ and the quantity of sodium hydroxide/ time of milling was the use of an equimolar ratio C9-unit/NaOH during 60 min of milling.

3.5.2 Influence of moisture content

Cleavage of lignin is mainly a hydrolysis reaction under strong basic conditions (Klapiszewski et *al.* 2018). A molecule of water is required for each C-O-C bond cleavage. In the present study, the moisture content of Purified Biolignin™ was

524 increased from 4 wt% (D7, initial moisture of Purified Biolignin^{TM}) to 24% (D14)
525 (Table 8) by adding water with purified Biolignin^{TM} and sodium hydroxide in the two
526 jars before grinding. Mechanochemistry has the advantage of being a solvent-free
527 technique. However, adding a small amount of liquid could enhance the reactivity. In
528 order to be considered as a solvent-free reaction, it has to respect the Liquid-Assisted
529 Grinding (LAG) zone (*i.e.* $0<\eta<2$ μL/mg) (Ying *et al.* 2021). Beyond $\eta = 2$ μL/mg, the
530 added solvent is too high to consider the reaction as solvent-free. The solvent could
531 slurrying the medium and affect the efficiency of the grinding. The η value was
532 calculated for each experiment in order to always respect the LAG condition. The
533 calculated η value is indicated on Table 8.
534 The average relative molecular mass of the grinded samples with an increase of
535 moisture (D7 to D14) is illustrated on Table 9.
536 Surprisingly, the average molecular mass of milled sample constantly decreases until
537 D13, the experiment with a moisture content of 18 wt%. At this point, the
538 mechanochemical reaction allowed an average molecular mass reduction of *ca.* 40%, in
539 other words it allowed the obtention of a fraction with lignin fragments with *ca.* 4 C9-
540 units. Keeping on mind the industrialization of the process, this is a particularly
541 interesting result because it could suggest the use of a less dry lignin, thus implying
542 much less energy cost compared to the actual one for lignin drying,
543 When the sample contains 24 wt% of water (Experiment D14), the average molecular
544 mass increases, reaching the same weight, or even worse, than D7, the sample with only
545 4 wt% water. However, this phenomenon was already observed by Brittain and co-
546 workers (Brittain et *al.* 2018). For higher percentage of water (*i.e.* ≥ 24 wt% in the

present study), the mixture grinded is no longer a powder but rather a slurry affecting the efficiency of the mechanochemical conditions as it was on lignin/NaOH powder.

[Table 9]

3.5.3 Influence of methanol as a scavenger for reactive intermediates

In the literature, the biggest challenge of lignin depolymerization is to avoid repolymerization/ condensation reactions. Lignin hydrolysis with a basic catalyst generates unstable intermediates. These reactive intermediates are easily recombined in new oligomers with C-C linkages, unbreakable in the presence of basic catalysts (Li et *al.* 2015).

Brittain and co-workers (Brittain et *al.* 2018) had promising results using methanol as a scavenger to prevent any repolymerization reaction during the milling of lignin and sodium hydroxide. In their study, they added 0.40 mL of methanol for 1.50 g of lignin and 1.50 g of sodium hydroxide, so 0.27µL/mg of lignin. They observed a quicker and a more efficient depolymerization, with a final average relative molecular mass 40% smaller compared to the samples milled with sodium hydroxide in absence of methanol. In the following, methanol was added before the reaction between Purified Biolignin^{TM} and sodium hydroxide using (1:1) C9-Unit/NaOH ratio (η = 0.27µL/mg of lignin). Two milling time were tested, 30 min (D15) and 60 min (D16), and compared with the samples milled without methanol (*i.e.* D5, D7 and D13, Table 8). The average molecular mass of the compared samples is shown in Table 10).

[Table 10]

Two samples, D5 and D15, were milled during 30 minutes without and in the presence of methanol. The results obtained indicated that methanol increases obviously the rate of depolymerization. With only 30 min of milling, the presence of methanol to the

system allowed the recovery of lignin fragments 29% smaller than in its the absence (in regards to M_n value). However, contrary to Brittain et *al.* (2018) findings, milling for a longer time in presence of methanol (*i.e.* D16, 60 min of milling) did not improve the efficiency of the depolymerization. The obtained fragments had nearly the same average relative molecular mass than those obtained after 30 min of milling. A slight increase of FTIR bands 1210 cm^{-1} and 1140 cm^{-1} between D15 and D16 indicated that D16 contains more C-O-C linkages than D15 (See Supporting information, Table S2). According to Aboagye and co-workers, this could be the result of additional methoxy groups (Aboagye et *al.* 2023). Concerning our experiment, the band on D16 FTIR spectrum is 7% and 12% higher than D15 FTIR and Purified Biolignin™ spectrum.

It is interesting to note that D13 experiment (60 min milling with 18 wt% H_2O, without MeOH) gave same, or slightly smaller lignin fragments than those obtained by D15 experiment (30 minutes milling with a dry lignin and the presence of MeOH). In view of these results and considering an environmentally friendly process, it seems more advantageous to optimize the process without the presence of methanol, even if the needed milling time is twice longer.

3.6. Mechanochemical cleavage of Biolignin™ with sodium hydroxide

3.6.1. Reaction on P7 Premium Line ball mill (20 mL ZrO_2 jars with 5 ZrO_2 balls)

The promising results obtained using purified Biolignin™ encouraged us to directly test the mechanochemical cleavage on Biolignin™, without any prior purification (C2, D17, D18, 19, Table 11). The experiments were tested on Biolignin™ using the same planetary mill as above (Fritsch Pulverisette 7 Premium Line ball mill). For C2

595 experiment, Biolignin^{TM} was milled without sodium hydroxide. The average relative
596 molecular mass of the recovered sample confirms that grinding Biolignin^{TM} without the
597 presence of an appropriate reagent did not allow a significant depolymerization.
598 Concerning the experiment D19, using a Biolignin^{TM} of 18 wt% of H_2O, the reaction
599 mixture was not recoverable. After milling, a very sticky slurry surrounded the balls.
600 The scale up of such process seemed to be too hazardous to be considered. Most
601 gratifyingly, the mechanochemical cleavage of Biolignin^{TM}, without its prior
602 purification, afforded lignin fragments in final samples *ca.* 40% smaller than in the
603 initial Biolignin^{TM} (Table 11), indicating the presence of oligomers with less than 4 C9-
604 units in average.
605 Comparison of 4%wt and 14%wt water (D17, D18 experiments respectively) showed
606 that M_n values are similar while M_w showed 10% decrease for experiment D18 with
607 14%wt water (Table 11). Using a wetter lignin did not significantly affect the M_n value
608 of the milled sample but decrease the M_w, resulting the recovery of a more
609 homogeneous fraction.
610 Most importantly, our findings indicate the same decrease as the optimized reaction
611 with purified Biolignin^{TM} (D13, Purified Biolignin^{TM}/ NaOH 1:1 C9-unit/NaOH + 18
612 wt% H_2O, 60 min milling, Table 9) was observed.
613 [**Table 11]**
614 Before milling, Biolignin^{TM} contained, respectively, 16.8/100 C9, 4.9/100 C9 and
615 1.3/100 C9 of Arylglycerol-β-arylether (β-O-4'), Phenylcoumaran (β-5') and Resinol
616 (β-β'). After 60 min of milling and in the presence of 4% (w/w) and 14% (w/w) of H_2O,
617 the occurrence of β-O-4' decreased of 38% and more than 44%, respectively (Figure 7).
618 These decreases were weaker than those observed on Purified Biolignin^{TM}, however,

619 Biolignin^{TM} contains more hemicelluloses than purified Biolignin^{TM}. A part of NaOH
620 was probably used to cleave C-O-C linkages between lignin fragments and
621 hemicellulose. The ^{31}P-NMR spectrum gave also some precious information.
622 The 4-O-5' linkage of condensed G-units significantly decreased (Table 12) while the
623 other moieties seemed almost constant before and after the mechanochemical
624 degradation. Surprisingly, the ^{31}P-NMR spectrum seemed to indicate NaOH cleavage of
625 C-O-C of 4-O-5' linkage. This cleavage was not distinguished by HSQC analysis
626 because no HSQC correlations are observed for carbons in positions 4, 4', 5 and 5' of
627 the aromatics. The combination of both NMR techniques was thus essential for a better
628 understanding of all the different cleavages produced during the reaction.
629 The ^{31}P-NMR spectrum also confirmed that reactive groups of Biolignin^{TM} (mostly
630 phenolic and aliphatic OH) are almost unchanged after degradation (Table 12). This
631 point was primordial to validate the degradation without affecting the reactivity of the
632 sample.
633 The C-O-C cleavage of β-O-4' linkage seemed to be improved when Biolignin^{TM}
634 moisture is increased ((*i.e.* 14% H_2O (w/w) *vs.* 4% H_2O (w/w)). This is an interesting
635 result that encourages us to reduce the Biolignin^{TM} drying rate.
636 [**Figure 7**]
637 In view of the above results, using a mechanical reaction with sodium hydroxide,
638 impurities of Biolignin^{TM} do not impact on the efficient cleavage of lignin fragments.
639 [**Table 12**]
640 *3.6.2. Scale up and Optimization of the reaction between Biolignin^{TM} and NaOH on*
641 *Retcsh PM 100 ball mill*
642

643 The positive results described above were enough encouraging to scale up the reaction
644 to the planetary 500 mL mill available in the CIMV lab (Retcsh PM 100 ball mill
645 equipped with a tungsten carbide (WC) jar and 20 mm diameter WC balls). The
646 maximum RPM of this mill is 650, 150 RPM less than the previous planetary mill used.
647 The density of WC (14.80 g/cm^3) is higher than ZrO_2 (6.05 g/cm^3), thus providing in
648 principle better results due to the capability of imparting strong impact forces to the
649 particles (Sitotaw et *al.* 2023). Then, the large scale mechanochemical cleavage of
650 Biolignin^{TM} was tested adapting some parameters to the available materials. Instead of
651 650 mg, the initial Biolignin^{TM} added to the system was 17.000g, a 26-fold greater
652 quantity than in prior experiments (Table 13).
653 **[Table 13]**
654 At the beginning, 10 WC balls was used at 650 RPM during 60 minutes (D20). The
655 milled sample recovered after this experiment has lignin fragments 41-45% smaller than
656 those of the initial Biolignin^{TM} (Table 13). This was the smallest lignin fragments
657 recovered from all our experiments described above.
658 In order to improve the reaction, 20 WC balls (Experiment D21) were used instead of
659 the initial 10 WC balls (Experiment D20). Increasing the number of ball mills gave
660 positive results. The recovery fraction contained an average of trimers of lignin (51-
661 55% smaller than the initial Biolignin^{TM}). A lignin fraction containing trimers seems to
662 be very promising to improve its use on applications avoiding issues of sterically
663 shielded reactive groups.
664 [**Figure 8**]
665 After 60 min of milling with 10 WC-balls and 20 WC-balls in the WC-500mL jar, the
666 occurrence of β-O-4' linkages decreased of 35.4% and more than 51.0%, respectively

(Figure 8). Hence, doubling the number of milling balls allowed a significant decrease of occurrence of β-O-4' linkages.

So, to summarize this part, the final protocol allowed to halve the average molecular mass of lignin fragments and to halve the occurrence of β-O-4' linkages of Biolignin™ without any prior purification.

4. Conclusion

Our study aimed to decrease by mechanochemical means the average molecular mass and the polydispersity of lignin fragments of the organosolv lignin named Biolignin™, without highly impacting its functional groups content. This is the first mechano-chemical study on this kind of lignin. The reaction was firstly optimized on "purified Biolignin™", containing more uniform lignin fragments and less impurities than Biolignin™. Comparative studies between Biolignin™ and purified Biolignin™ by their ATR-FT-IR profile and by ^{31}P-NMR quantification demonstrated that the chemical structure of their lignin fragments is similar, thus validating the use of purified Biolignin™ as a model of Biolignin™.

For both studies on purified Biolignin™ and Biolignin™, quantitative HSQC NMR, IR and GPC studies, supported our findings.

An important point highlighted by our study is the efficiency of the reaction when using an equimolar quantity (and not equimass) of sodium hydroxide related to C-9 unit of Biolignin™ needed to break interunits lignin bonds. This corresponds to a 75% reduction of sodium hydroxide quantity compared to the literature. Under these conditions and after 60 min-grinding in the planetary ball milling apparatus, more than 71% of the initial β-O-4' bonds are cleaved.

691 The effect of Liquid Assisted Grinding (LAG), using water or methanol, showed a
692 slightly better result with water. The mechanochemical reaction with a 18 wt% moisture
693 content allowed an average molecular mass reduction of *ca.* 40%, indicating the
694 obtention of lignin fragments with *ca.* 4 C9-units. Analogous results were observed on
695 Purified Biolignin™ and on Biolignin™.
696 Mechanochemical results with Biolignin™ under LAG conditions are particularly
697 important. Obtention of Biolignin™ at 18 wt% moisture content implies much less
698 energy input needed. This, in conjuction with the use of an equimolar ratio of sodium
699 hydroxide could be the base of an economically feasible industrialized process for the
700 mechanochemical cleavage Biolignin™.
701 Finally, for the first time the mechanochemical cleavage was conducted in a planetary
702 500mL mill. For a total of 14 wt% moisture on Biolignin™ and an equimolar amount of
703 sodium hydroxide, a 60 min grinding afforded halve (-51%) the average molecular mass
704 of lignin fragments and decreased for more than 44% the occurrence of β-O-4' linkages
705 in lignin fragment, suggesting the feasibility of scaling-up.

706 The significant improvements (sodium hydroxide reduction and moisture precise
707 content) can pave the way for using less energetically demanding process for obtaining
708 Biolignin™ and also envision an industrial scale up for lignin cleavage.

709 In addition, we envision to explore in the near future the combined opportunities that
710 might be offered by our results and the potential oxidative cleavage of Biolignin™.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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Mechanochemical cleavage of lignin in presence of sodium hydroxide to produce a homogeneous lignin fraction optimized for a direct use in applications

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Figure captions

Fig. 1. CIMV organosolv biorefinery process

Fig. 2. Scheme of fractionation of the Biolignin™

Fig. 3. 6 main lignin moieties present in Biolignin™ and Purified Biolignin™

Fig. 4. Normalized FTIR absorption values of Alkyl-aryl ether asymmetric C-O vibration (1210 nm) and C-O stretching (1140 nm) exposed to different quantity of sodium hydroxide

Fig. 5. Evolution of the 3 main linkages of lignin samples (β-O-4', β-5' and β-β') determined by quantitative HSQC (/100 C9 unit)

Fig. 6. HSQC NMR Spectra of Purified Biolignin™ before milling (a); after 120 min milling without the presence of NaOH (C1, b), and with NaOH after 30 min milling (D5, c), 60 min milling (D7, d), 120 min milling (D9, e)

Fig. 7. Occurrence of β-O-4', β-5' and β-β' (/100 C9 unit) in Biolignin™, D17 and D18 as determined by quantitative NMR HSQC

Fig. 8. Occurrence of β-O-4', β-5' and β-β' (/100 C9 unit) in Biolignin™, after milling with 10 balls and 20 balls as determined by quantitative NMR HSQC

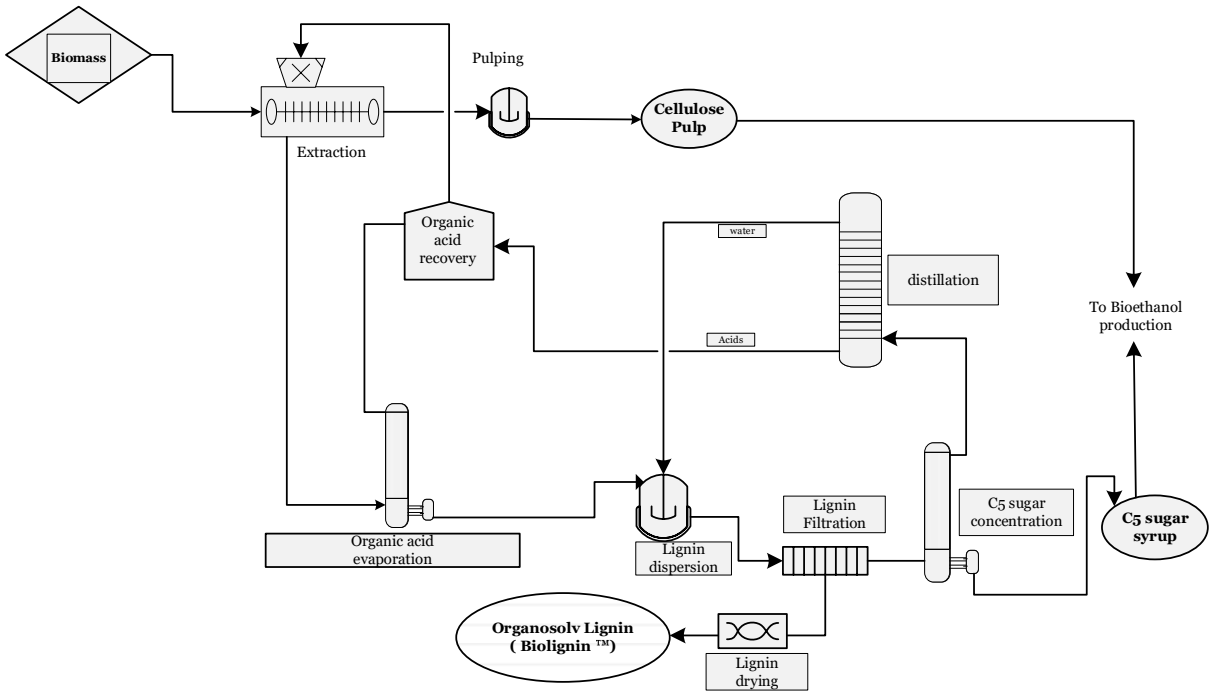


Figure 1 – CIMV organosolv biorefinery process

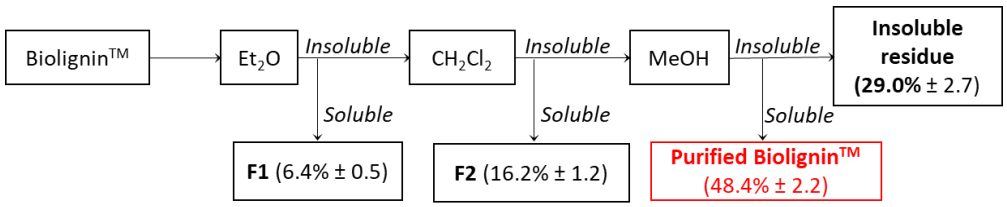


Figure 2 – Scheme of fractionation of the Biolignin™

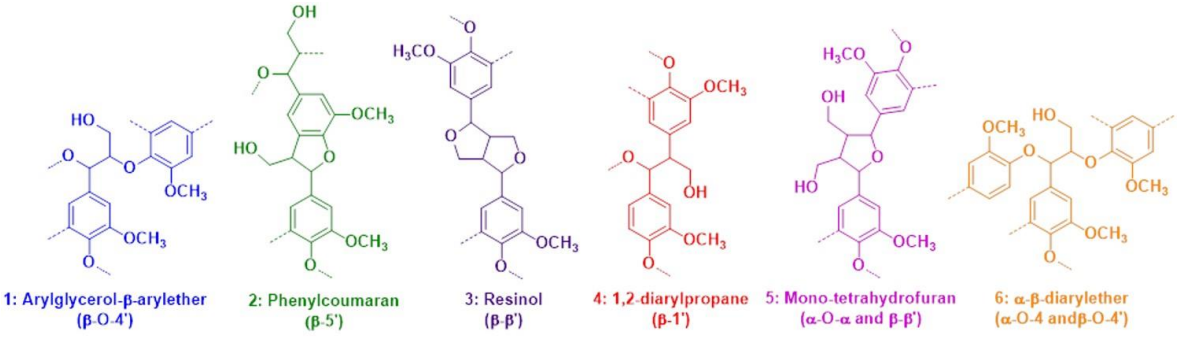


Figure 3 – 6 Main lignin moieties present in Biolignin™ and Purified Biolignin™

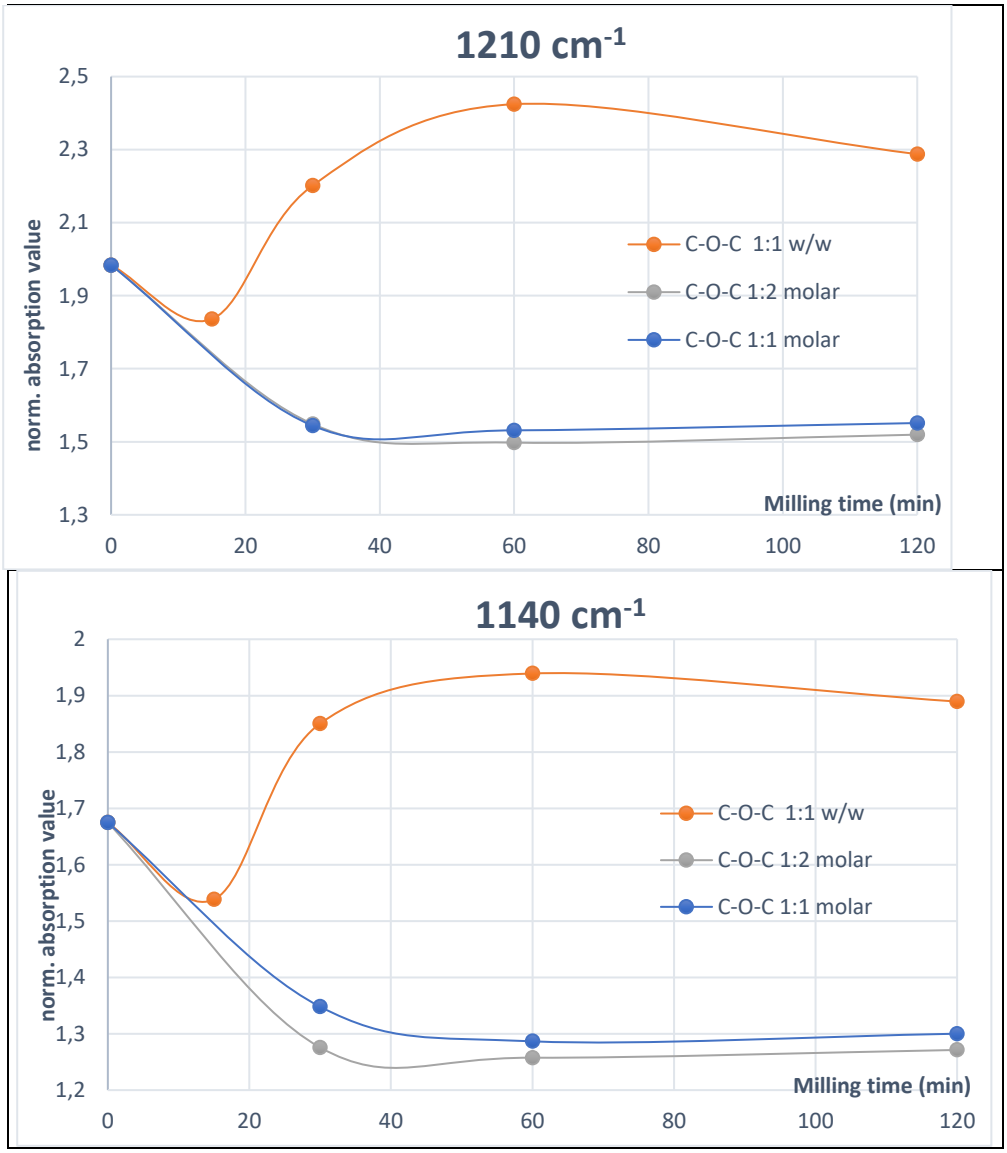


Figure 4 – Normalized FTIR absorption values of Alkyl-aryl ether asymmetric C-O vibration (1210 nm) and C-O stretching (1140 nm) exposed to different quantity of sodium hydroxide

	Milling Time (min)	$I_{\beta\text{-}O4'}$ (/100C9)	$I_{\beta\text{-}5'}$ (/100C9)	$I_{\beta\text{-}\beta'}$ (/100C9)
Purified Biolignin™	0	20.5	4.3	1.6
D5	30	6.4	2.5	0.7
D7	60	5.9	1.9	0.2
D9	120	2.8	1.4	0.8

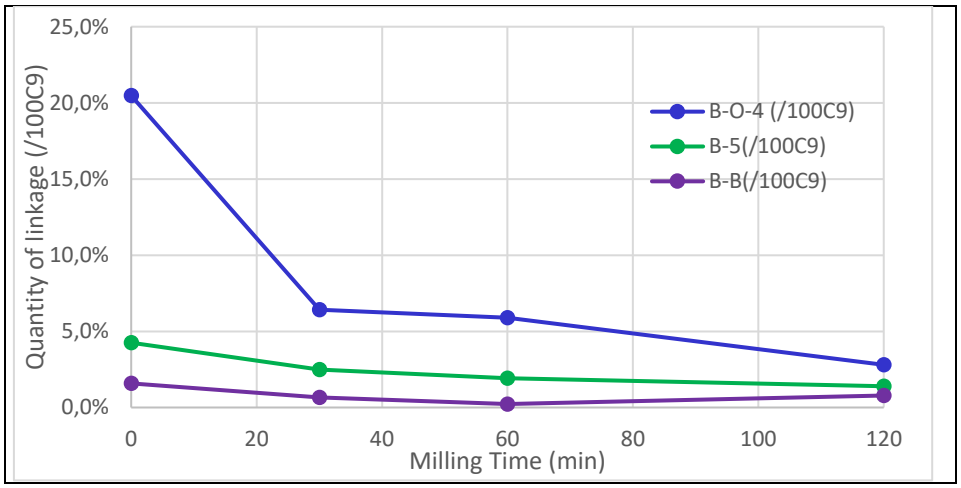
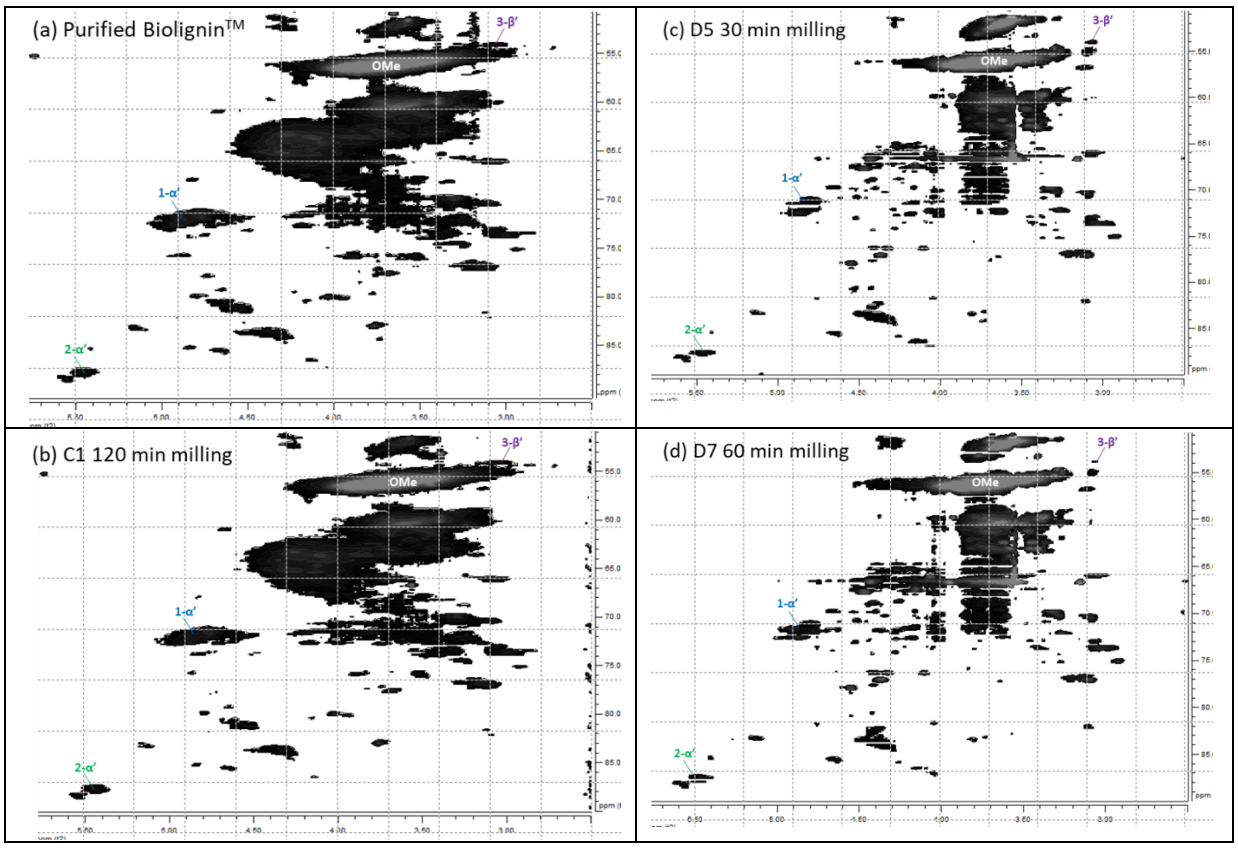


Figure 5 – Evolution of the 3 main linkages of lignin samples (β-O-4', β-5' and β-β') determined by quantitative HSQC (/100 C9 unit)



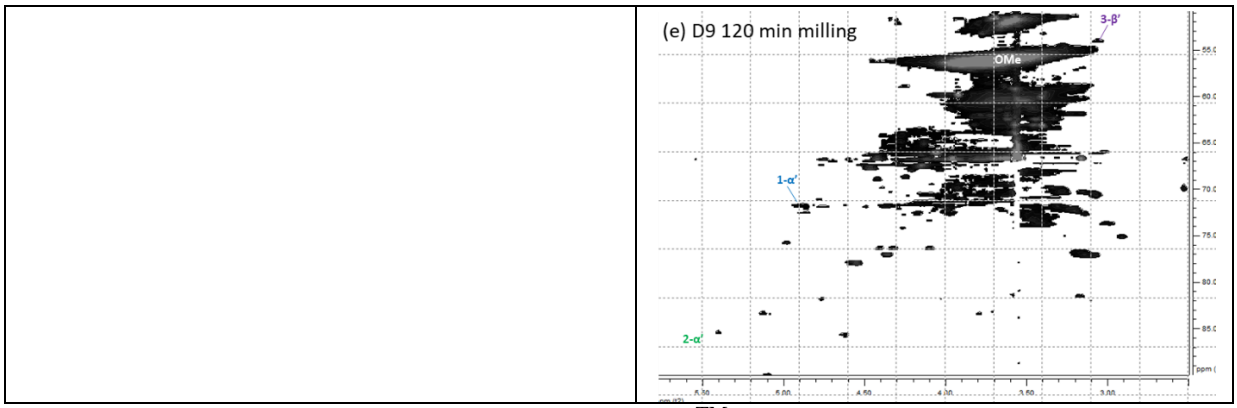


Figure 6 – HSQC NMR Spectra of Purified Biolignin^{TM} before milling (**a**); after 120 min milling without the presence of NaOH (C1, **b**), and with NaOH after 30 min milling (D5, **c**), 60 min milling (D7, **d**), 120 min milling (D9, **e**)

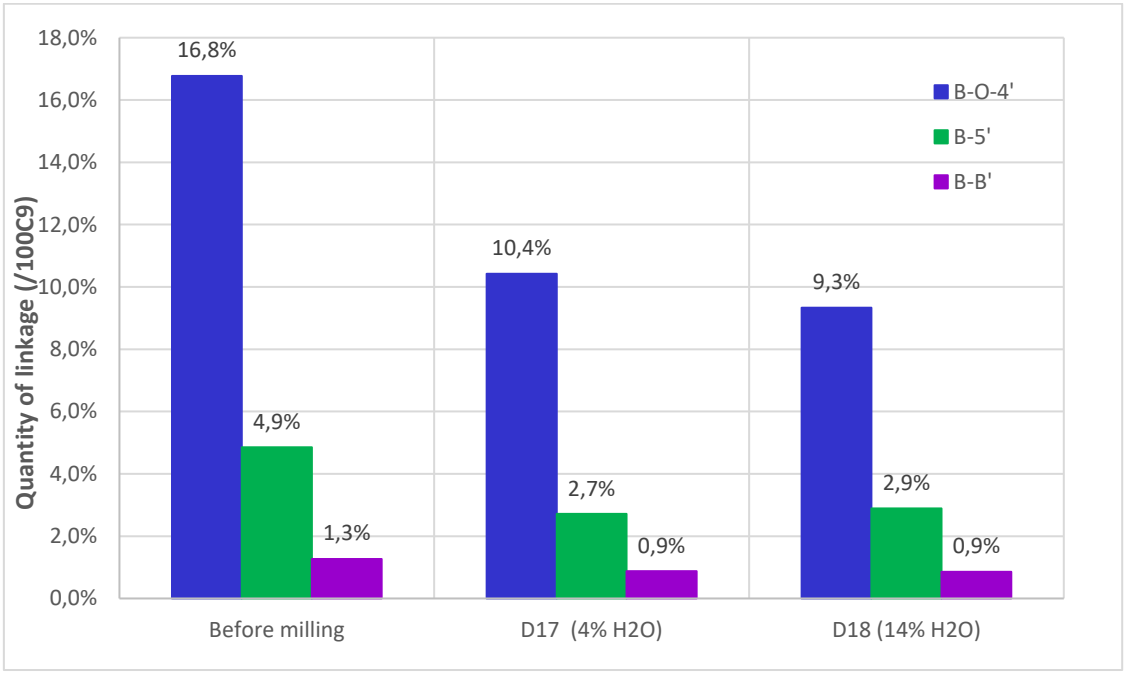


Figure 7 - Occurrence of β-O-4', β-5' and β-β' (/100 C9 unit) in Biolignin^{TM}, D17 and D18 as determined by quantitative NMR HSQC

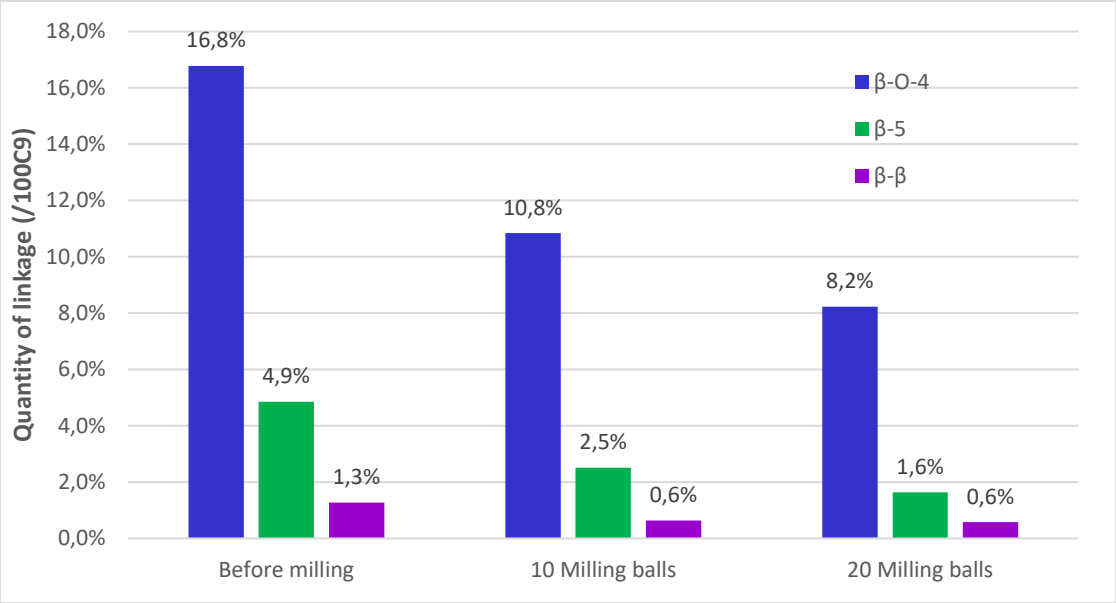


Figure 8 - Occurrence of β-O-4', β-5' and β-β' (/100 C9 unit) in Biolignin^{TM}, after milling with 10 balls and 20 balls as determined by quantitative NMR HSQC

Mechanochemical cleavage of lignin in presence of sodium hydroxide to produce a homogeneous lignin fraction optimized for a direct use in applications

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Table captions

Table 1 – Identification and quantification of the impurities within the Biolignin™ fraction

Ashes content (%, Dry Matter)		1.02
Carbohydrates content	**Glucose (%, DM)**	2.31
	Xylose (%, DM)	1.83
Protein content (%, DM)		7.80

Table 2 – Molecular profile of Biolignin™ determined by GPC

Mn (g/mol)	999
Mw (g/mol)	1743
P (M_w/M_n)	1.8

Table 3 – Molecular weight distribution in Biolignin™ and fractions

	Solvent	**Yield (%)**	**M_n (g/mol)**	**M_w (g/mol)**	**P (M_w/M_n)**
F1	Et_2O	6.4 (±0.5)	611 (3.8 u*)	784 (4.8 u)	1.3
F2	CH_2Cl_2	16.2 (±1.2)	747 (4.6 u)	1116 (6.9 u)	1.5
Purified Biolignin™	MeOH	48.4 (±2.2)	983 (6.1 u)	1579 (9.7 u)	1.6
Insoluble residue	-	*29.0 (±2.7)*	*Highly insoluble but > 1700 g/mol*		
Biolignin™	-	-	999 (6.2 u)	1743 (10.8 u)	1.8

* u = C9-unit of wheat straw Biolignin™ (Lange et *al.* 2016)

Table 4 - Identification and quantification of the impurities within the Biolignin™ fractions

	Purified Biolignin™	**Biolignin™**
Ashes content (%, Dry Matter)	0.44	1.02
Hemicelluloses (%, DM)	2.3	4.1
Protein content (%, DM)	6.2	7.8

Total (%, DM)	8.94	12.92

Table 5 - ATR-FT-IR of purified Biolignin™ and Biolignin™

Purified Biolignin™	Biolignin™	
3368	3377	O-H Stretch
2925	2936	C-H Stretch in CH_3/or and CH_2 groups
2850	2855	C-H Stretch in O-CH_3 groups
1713	1714	C=O Stretch in unconjugated ketones, carboxyls and ester groups
1653	1654	Ring conjugated C=C Stretch of **Coniferyl/ Synapyl alcohol**
1599	1598	Aryl ring Stretching, symmetric
1509	1508	Aryl ring Stretching, asymmetric
1458	1456	C-H deformation asymmetric in CH_3 and CH_2
1422	1425	Aromatic skeletal vibration combined with C-H in-plane deformation
1372	1363	C-H deformation asymmetric of O-CH_3 groups
1330	1330(sh)	**Syringyl ring** breathing with C-O stretch
1227	1222	C=C stretching of **Guaiacyl ring**, Phenolic OH, alkyl-aryl ether asymmetric C-O-C
1159	1160	Aromatic C-H in plane deformation, typical of **Guaiacyl units**
1140	1140	Aromatic C-H in-plane deformation; typical of **Guaiacyl units**; whereby G Condensed > etherified (typical for S units); C-O Stretching
1118	1118	Aromatic C-H in plane deformation, typical of **Syringyl units,** secondary alcohols
1029	1029	Aromatic C-H in plane deformation of **Guaiacyl units**, C-O deformation in primary alcohols
869	869	C-H deformation out of plane, Aromatic ring
839	835	C-H deformation out of plane in position 2 and 6 of **Syringyl and in all *p*-Hydroxyphenyl units**

* sh: Shoulder

Table 6 – ^{31}P NMR quantification of Biolignin™ and purified Biolignin™ using endo-N-hydroxy-5-norbornene-2,3-dicarboximide as an internal standard

Quantification (mmol/g)	Biolignin™	Purified Biolignin™
-COOH	0.76	0.65
H-units phenolic -OH	0.39	0.39
G-units phenolic -OH	0.83	0.74
S-units and condensed G-units phenolic -OH	1.09	0.93
Aliphatic - OH	2.05	2.00
Total -OH (mmol/g)	**4.36**	**4.06**
Total Alk-OH (mmol/g)	2.05	2.00
Total Ar-OH (mmol/g)	2.31	2.06

Table 7 – 6 main lignin moieties of Biolignin™ and Purified Biolignin™ quantified by HSQC

Name	Ref. signal	Biolignin™	Purified Biolignin™
Arylglycerol-β-arylether (β-O-4') substructure (1)	**$C_\alpha H$ (ref)**	**69.7%**	**73,6%**
Phenylcoumaran (β-5') substructure (2)	**$C_\alpha H$**	**15.4%**	**10.7%**
Resinol (β-β') substructure (3)	**$C_\beta H$**	**7.6%**	**5.1%**
1,2-diarylpropane (β-1') substructure (4)	**$C_\alpha H$**	**0.4%**	**1.1%**
Monotetrahydrofuran (α-O-α' and β-β') substructure (5)	**$C_\alpha H$**	**3.8%**	**3.1%**

α.β-diarylether (α-O-4' and β-O-4") substructure (6)	**$C_\alpha H$**	**3.2%**	**6.7%**

Table 8 – Mechanochemical experiments tested on Purified Biolignin™ and their conditions

		Purified Biolignin™ (mg)	NaOH (mg)	H_2O (µL)	MeOH (µL)	Reaction time (min)	wt% H_2O	η*
	C1	700	0	0	0	120	4%	0.03
Optimization of reaction time	**D1**	350	350	0	0	15	4%	0.03
	D2	350	350	0	0	30	4%	0.03
	D3	350	350	0	0	60	4%	0.03
	D4	350	350	0	0	120	4%	0.03
Optimization of quantity of NaOH	**D5**	650	160.5	0	0	30	4%	0.03
	D6	650	321	0	0	30	4%	0.03
	D7	650	160.5	0	0	60	4%	0.03
	D8	650	321	0	0	60	4%	0.03
	D9	650	160.5	0	0	120	4%	0.03
	D10	650	321	0	0	120	4%	0.03
H_2O effect	**D11**	650	160.5	41.6	-	60	10%	0.08
	D12	650	160.5	72.1	-	60	14%	0.11
	D13	650	160.5	105.9	-	60	18%	0.14
	D14	650	160.5	141.2	-	60	24%	0.22
MeOH effect	**D15**	650	160.5	-	175.5	30	4%	0.22
	D16	650	160.5	-	175.5	60	4%	0.22

*η: empiric parameter which determine if the reaction respect the Liquid-assisted Grinding (LAG) condition (*i.e.* 0<η<2 µL/mg). η=V (liquid; µL)/m (solid reagents; mg) (Ying et *al.* 2021)

Table 9 – Average relative molecular mass of milled samples as a function of its moisture content, as determined by GPC

	wt% H_2O	M_n (g/mol)	M_w (g/mol)
Purified Biolignin™	4%	983 (6.1 u)	1579 (9.7 u)
D7	4%	797 (4.9 u)	1143 (7.1 u)
D11	10%	720 (4.4 u)	1038 (6.4 u)
D12	14%	688 (4.2 u)	1028 (6.3 u)
D13	18%	617 (3.8 u)	911 (5.6 u)
D14	24%	855 (5.3 u)	1167 (7.2 u)

* u = C9-unit of wheat straw Biolignin™ (Lange et *al.* 2016)

Table 10 - Average relative molecular mass determined by GPC of milled samples with or without the presence of methanol as a scavenger

	wt% H_2O	*MeOH*	*Milling time (min)*	M_n (g/mol)	M_w (g/mol)
Purified Biolignin™	4%	-	-	983 (6.1 u)	1579 (9.7 u)

D5	4%	-	30	901 (5.5 u)	1201 (7.4 u)
D7	4%	-	60	797 (4.9 u)	1143 (7.1 u)
D13	18%	-	60	617 (3.8 u)	911 (5.6 u)
D15	4%	Yes	30	638 (3.9 u)	988 (6.1 u)
D16	4%	Yes	60	645 (4.0 u)	1010 (6.2 u)

* u = C9-unit of wheat straw Biolignin™ (Lange et al. 2016)

Table 11 – Average relative molecular mass of milled Biolignin™ samples as a function of its moisture content, as determined by GPC

	Biolignin™ (mg)	**NaOH (mg)**	**H_2O (µL)**	**Reaction time (min)**	**wt% H_2O**	**η**	**M_n (g/mol)**	**M_w (g/mol)**
Biolignin™	**-**	**-**	**-**	**-**	**4%**		999 (6.2 u)	1743 (10.8 u)
C2	700	0	0	60	4%	0.03	978 (6.0 u)	1662 (10.3 u)
D17	650	160.5	0	60	4%	0.03	607 (3.8 u)	1015 (6.3 u)
D18	650	160.5	72.2	60	14%	0.11	591 (3.7 u)	903 (5.6 u)
D19	650	160.5	105.8	60	18%	0.14	-	-

* u = C9-unit of wheat straw Biolignin™ (Lange et *al.* 2016)

Table 12 – ^{31}P NMR quantification of COOH and OH groups and Biolignin™, D17 and D18

Quantification (mmol/g of sample)	**Biolignin™**	**D17**	**D18**
-COOH	0.76	1.03	1.04
Phenolic - OH of H-unit	0.39	0.32	0.31
Phenolic - OH of G-unit	0.83	0.76	0.79
Phenolic - OH of S-unit	0.52	0.48	0.51
5-5' condensed unit	0.25	0.22	0.24
4-O-5' condensed unit	**0.46**	**0.08**	**0.07**
Aliphatic - OH	2.05	1.95	1.87

Table 13 - Mechanochemical experiments on Biolignin™ tested, their conditions and the average molecular mass of the final sample as determined by GPC

	Biolignin™ (g)	**NaOH (g)**	**Number of balls**	**Reaction time (min)**	**wt% H_2O**	**η**	**M_n (g/mol)**	**M_w (g/mol)**
D20	17.000	4.197	10	60	4%	0.03	592 (3.6 u)	939 (5.8)
D21	17.000	4.197	20	60	4%	0.03	484 (3.0 u)	772 (4.8 u)