# Phenolic Group Distribution as a Function of Molecular Weight in Lignins and Their Fractions

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**ABSTRACT:** An analytical approach based on phenol fluorescence labeling with the dansyl fluorophore coupled with gel permeation chromatography using UV and fluorescence detection was applied in an effort to increase the understanding of the phenolic group distribution in different lignin specimens. Selective derivatization with dansyl chloride of lignin phenolic functionalities was quantitatively achieved under mild reaction conditions and was applied to acidolytic and kraft lignins from softwood and hardwood and to kraft lignin fractions obtained by solvent fractionation. Acetylated lignins and labeled lignins were analyzed to discern the lignin aromatic skeleton input from the response of dansyl-linked phenols, successively, allowing for estimation of the phenolic group distribution as a function of molecular weight for different lignins before and after solvent fractionation.



**KEYWORDS:** *technical lignins, lignin fractionation, phenol, dansyl, fluorescence, molecular weight*
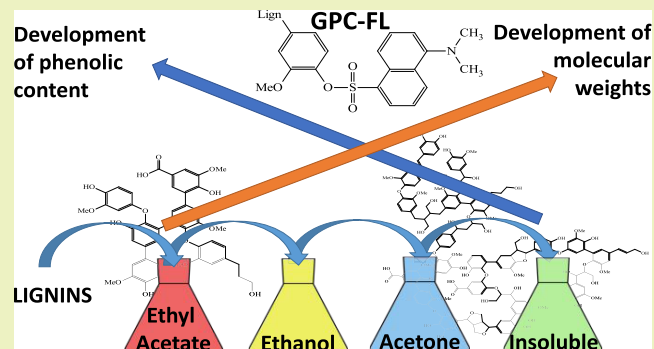
## INTRODUCTION

Lignin is a three-dimensional, heterogeneous, and irregular polyphenolic macromolecule and the second most abundant natural polymer after cellulose in land-based biomass. Along with hemicelluloses, these biopolymers constitute the so-called lignocellulosic materials. Lignin is constituted by three phenolic monomers, named *para*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units when linked to the lignin polymer through ether and carbon−carbon bonds.[1] The structure of lignin and the amount of three monomeric units depend on the botanical origin and the type of lignocellulosic biomass. Approximately 50 million tons of technical lignins are annually produced from spent pulping liquors, mainly from the sulfite and kraft processes[2] on lignocellulosic biomasses. During those processes, intermolecular linkages are broken and modified and the already complicated lignin structure is strongly affected.[3] The heterogeneity of the resulting technical lignins has been recognized as one of the major drawbacks of its valuable valorization.[4] Recently, several papers were focused on fractionation approaches to extract more homogeneous lignin preparation in terms of molecular weight and functional group distribution.[5] It was already demonstrated that sequential ultrafiltration of kraft lignin can fractionate and elucidate molar mass-dependent changes in the lignin structure and characteristics, revealing that the smaller fractions are also the richest in phenolic groups.[6−8] Selective solvent extraction,[9] the oldest and more diffuse fractionation technique,[10−12] takes advantage of the different solubilities of lignin in solvents with varying

polarities.[13] In general, the lower the solvent polarity, the lower the average molecular weight of the fraction and the largest the phenolic content.[14−16] Fractionation by successive precipitation, obtained either by a stepwise reduction of the pH value of black liquor or especially by the addition of a non-solvent to an organic lignin solution, generally results in high lignin yield and highly monodispersed fractions.[17,18]

Despite these efforts, a strong contribution to the field of analytical chemistry of lignin is still necessary to deeply understand and completely control lignin valorization. This is why, recently, a novel analytical approach based on phenol fluorescence labeling using the dansyl fluorophore, coupled with gel permeation chromatography equipped with UV and fluorescence detector, was developed in an effort to increase the understanding of phenolic group distribution in different lignin specimens.[19] The presented study focused on the possibility to obtain precise knowledge regarding the distribution of phenolic groups in different lignins as a function of their molecular weight, avoiding the need for preliminary lignin fractionation. Fractionation procedures are, as a matter of fact, expensive in terms of time, resources, and
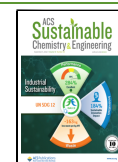
effort whereas, according to the method described herein, GPC analysis performed on a specimen of the unfractionated lignin sample might be enough to select a specific fraction of interest in downstream processing. Hence, the generally proposed workflow involving fractionation followed by analyses of the fractions might be replaced by the inverse, more holistic approach of analysis-fractionation. In particular, the proposed analytical method is based on the selective fluorescence labeling of lignin phenolic moieties with dansyl chloride. The phenols were chosen as the main representative and versatile functional group on account of their properties and ease of conversion to other reactive species. The selectivity and yield of the labeling reaction were determined by quantitative phosphorylation of phenols followed by $^{31}P$ NMR. The fluorescence-labeled lignin, along with the acetylated reference, was then analyzed by the abovementioned gel permeation chromatography (GPC) set-up. The comparison between the acetylated and dansylated GPC profiles was correctly evaluated and computed, and provided helpful information about phenols concentrations over different molecular weight ranges.

## ■ EXPERIMENTAL SECTION

**Reagents and Materials.** All solvents and reagents were purchased from Merck Life Science-Italia and used as received. Technical lignins from the kraft process (softwood kraft, SWK; hardwood kraft, HWK) were kindly provided by local factories. Reference lignin from *Eucalyptus* sp. (HW) and *Pinus* sp. (SW) was extracted by the acidolytic process following the procedure reported in the literature.[20]

**Lignin Fractionation.** Kraft lignins were fractionated following the procedure reported elsewere,[21] by successive extractions with organic solvents. Four fractions were obtained: the fraction soluble in ethyl acetate (EA), the fraction soluble in ethanol (ET), the fraction soluble in acetone (AC), and the insoluble residue (INS). The extractions were carried out on 200 g of dried softwood and hardwood kraft lignins using 1 L of the corresponding solvent. At each step, the undissolved material was retrieved by vacuum-assisted filtration and suspended for a second identical extraction. The solubilized fractions were recovered by evaporating the solvent and weighted.

**Lignin Dansylation.** Lignin samples were dansylated following the procedure reported.[19] In a typical experimental procedure, 100 mg of lignin and 5 mg of tetrabutylammonium bromide (TBAB) were dissolved in 20 mL of carbonate buffer solution (pH 10, 0.1 M) and heated to 50 °C in an oil bath. A certain amount of dansyl chloride (20% molar excess to the total phenolic content as calculated from $^{31}P$ NMR) was then solubilized in acetonitrile (10 mL) and added to the lignin solution. The mixture was reacted for 90 minutes at 50 °C under vigorous stirring. At the end of the reaction, dansylated lignin was recovered by centrifugation (4000 rpm, 10 min, 2 cycles of washing with acidic water) after regeneration in cold acidic water (HCl 0.1 M).

**Lignin Acetylation.** Starting lignin samples and dansylated lignin samples (ca. 50 mg) were acetylated using a 1:1 pyridine/acetic anhydride mixtures (2 mL, 40 °C, 24 h) and recovered by stripping with EtOH, toluene, and chloroform before being subjected to GPC analysis.

**$^{31}P$ NMR Analyses.** Quantitative $^{31}P$ NMR analyses were performed using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane as the phosphorus derivatizing agent. The $^{31}P$ NMR data reported in this paper are the average of three experiments. The maximum standard deviation was 0.02 mmol/g.

**GPC−UV−Fluorescence Analyses.** GPC analyses were performed on an Agilent 1260 Infinity liquid chromatography system, equipped with an autosampler (Agilent 1260 Vialsampler, injection volume 25 $\mu$L) and connected in series to an Agilent 1260 DA VL detector (set at 280 nm) and an Agilent 1260 FL detector ($\lambda$

excitation: 390 nm, $\lambda$ emission: 550 nm for all samples). The composition of the GPC-column system was as follows (following the solvent flow direction): Agilent PLgel 5 $\mu$m (500 Å), Agilent PLgel 5 $\mu$m (1000 Å), and Agilent PLgel 5 $\mu$m (10,000 Å). Samples were dissolved in tetrahydrofuran (THF, accurately prepared at the concentration of 1 mg/mL starting from more concentrated stock solutions) and analyzed using THF as the eluent (Fluka 99.8%) at a flow rate of 1 mL/min. Standards of polystyrene from Polymer Laboratories were used for calibration ($3 \times 10^6$−162 g/mol). The evaluation of the number-average molecular weight ($M_n$) and the weight-average molecular weight ($M_w$) of the samples was performed. Moreover, the ratio $I = M_w/M_n$, defined as the polydispersity index was calculated. The $M_n$ and $M_w$ values reported are the average of three analyses (standard deviation $M_w$: 500 g/mol; $M_n$: 100 g/mol).

**Chromatographic Data Elaboration.** GPC chromatograms were acquired for acetylated and dansylated specimens as both UV (280 nm) and fluorescence ($\lambda_{exc}$: 390 nm, $\lambda_{em}$: 550 nm) profiles to obtain information about the distribution of phenols in the examined lignin samples against lignin skeleton response. A basic assumption was made on the behavior of the molar extinction coefficient and the fluorescence intensity upon the variation of the molecular weight: both were considered constant and independent from the species constituting acetylated and dansylated lignin samples. This means that the UV response of lignin and the fluorescence response of dansyl groups linked to aromatic rings was considered unaffected by changes in the molecular weight and, especially, by the variegated chemical structure offered by lignin.

In this view, the four chromatograms obtained after GPC analysis were exploited as follows:

(1) The UV profile of a generic acetylated lignin sample was used to determine the representative mass fraction (w/w) of a specific molecular weight range (ranges $b \rightarrow a$ are taken as follows >10,000 g/mol; 10,000−5000 g/mol; 5000−3000 g/mol; 3000−2000 g/mol; 2000−1000 g/mol; <1000 g/mol). Accordingly, the total UV absorbance calculated over a specified range $\sum_{n=a}^{b} abs(n)$ was divided by the total UV absorbance calculated over the whole molecular weight distribution $\sum_{n=200}^{\infty} abs(n)$ according to eq 1

$$g \; lignin(b \rightarrow a)/g = \frac{\sum_{n=1}^{b} abs(n)}{\sum_{n=200}^{\infty} abs(n)} \qquad (1)$$

(2) The fluorescence profile of a generic acetylated lignin sample was used to correct the fluorescence signal of the corresponding dansylated specimen as follows: the acetylated lignin chromatogram was normalized using the proper weight conversion factor (whose calculation has been described in the previous section) and then subtracted from the fluorescence chromatogram of the analogous dansylated sample to remove the auto-fluorescence contribution of the lignin skeleton. When the auto-fluorescence of nondansylated lignin samples was negligible, this step was skipped.

(3) The UV profile of a generic dansylated sample was used to estimate the change in the hydrodynamic volume of the corresponding lignin after dansylation.

(4) The elaboration of the fluorescence profile of a generic dansylated sample provided the representative phenolic molar fraction (mol/mol) referred to as a specified molecular weight range (ranges $b \rightarrow a$ are the same as those listed in point 1). The phenolic molar fractions were obtained according to the following relation (eq 2)

$$PhOH \; mol(b \rightarrow a)/mol = \frac{\sum_{n=a}^{b} fluo(n)}{\sum_{n=200}^{\infty} fluo(n)} \qquad (2)$$

where $\sum_{n=a}^{b} fluo(n)$ is the fluorescence intensity over a specified molecular weight range $b \rightarrow a$; $\sum_{n=200}^{\infty} fluo(n)$ is
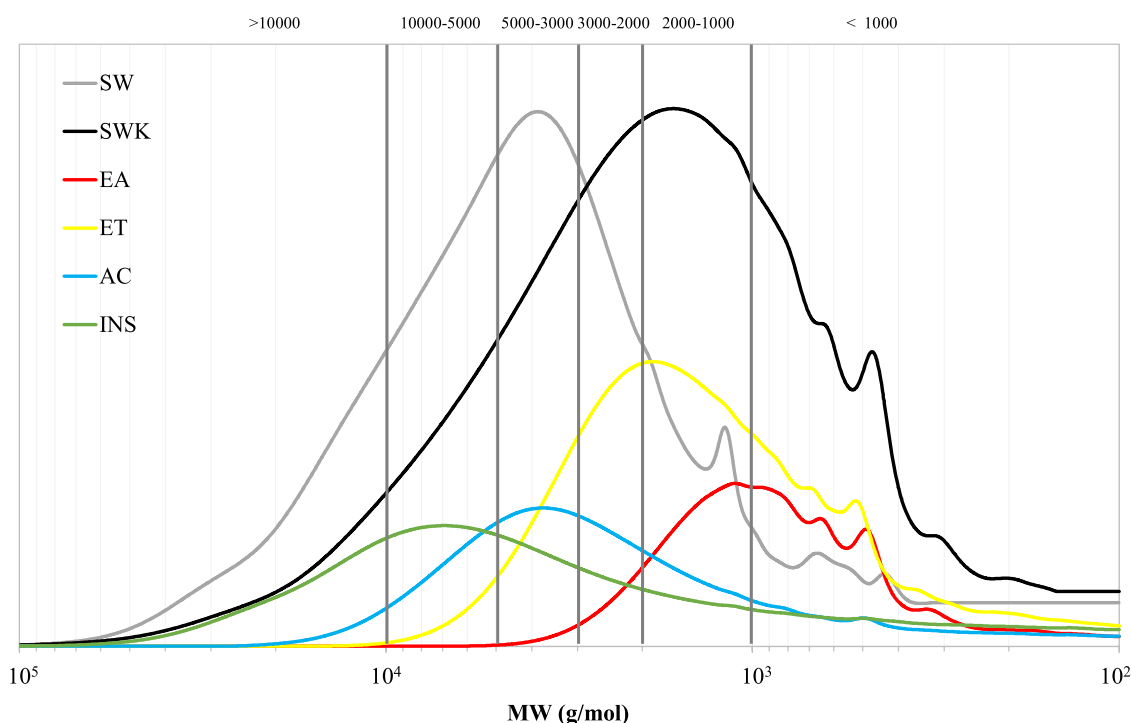
**Figure 1.** GPC−UV (280 nm) elution profiles of acidolytic SW lignin, SWK lignin, and its solvent fractions (EA, ET, AC, INS) after acetylation. The relative intensity is normalized on the basis of extraction yields.

**Table 1. Fractionation Yields, GPC−UV (280 nm) Data and Absolute Weight Fractions of Acidolytic SW Lignin, SWK Lignin, and Its Solvent Fractions (EA, ET, AC, and INS) after Acetylation (±0.01)[a]**

| | w/w | (g/mol) | | PD | (w/w) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | fractionation yield | $M_n$ | $M_w$ | | <1000 | 1000−2000 | 2000−3000 | 3000−5000 | 5000−10,000 | >10,000 |
| SW | n.a. | 5700 | 13,500 | 2.37 | 0.11 | 0.13 | 0.15 | **0.24** | **0.23** | 0.14 |
| SWK | n.a. | 3350 | 10,900 | 3.27 | **0.23** | **0.28** | 0.16 | 0.15 | 0.12 | 0.06 |
| EA | 0.17 | 1100 | 1550 | 1.38 | **0.48** | **0.41** | 0.09 | 0.02 | 0.00 | 0.00 |
| ET | 0.40 | 1900 | 2900 | 1.54 | **0.26** | **0.35** | **0.21** | 0.14 | 0.03 | 0.00 |
| AC | 0.20 | 3550 | 5750 | 1.62 | 0.12 | 0.18 | 0.19 | **0.27** | **0.21** | 0.03 |
| INS | 0.22 | 6600 | 14,600 | 2.21 | 0.06 | 0.12 | 0.11 | 0.19 | **0.29** | **0.23** |

[a]In bold are the most abundant mass fraction (>0.2 w/w).

the total fluorescence intensity calculated over the whole molecular weight range.

(5) The obtained data are meant to be used in the expression

$$\text{PhOH}\frac{\text{mmol}}{g}(b \rightarrow a) = \frac{\text{mmol PhOH } (b \rightarrow a)}{g \text{ lignin } (b \rightarrow a)} \times [\text{phOH}]$$

(3)

a molecular weight range-dependent version of the equation usually exploited for the average assessment of lignin phenolic moieties quantification. [PhOH] was the total phenol content retrieved by ^{31}P NMR. The phenol content, calculated from GPC-FL data reported in this paper, is the average of three experiments. The maximum standard deviation was 0.1 mmol/g.

■ **RESULTS AND DISCUSSION**

**GPC−UV and ^{31}P NMR Characterization of Lignin Fractions.** Acidolytic and kraft lignin samples from SW and HW, and kraft lignin fractions obtained by solvent fractionation were initially structurally characterized using standard GPC analyses after acetylation and reliable quantitative ^{31}P NMR spectroscopy after phosphitylation.[9] Figure 1 shows the

elution traces obtained for the GPC analyses of the softwood lignins, and Table 1 lists, among others, the numerical results.

For the solvent fractionation samples, *i.e.*, EA, ET, AC, and INS, the gravimetric extraction yields were measured. Ethyl acetate was able to solubilize 17% of the starting SWK, then ethanol extracted 40% and acetone 20%, while 22% of SWK remained as an insoluble residue. Each acetylated specimen was analyzed at λ = 280 nm *via* GPC−UV. For comparison purposes also acidolytic lignin of the same botanical origin was analyzed. As already reported, the SWK solvent fractions were characterized by lower polydispersity than unfractionated SWK,[9] while the molecular weights increased ($M_n$ and $M_w$) following the order EA, ET, AC and INS. Acidolytic lignin SW was characterized by a higher molecular weight with respect to SWK lignin.

For each sample, the absolute weight fractions of a specific molecular weight range (>10,000, 10,000−5000, 5000−3000, 3000−2000, 2000−1000, and <1000 g/mol) were calculated from the GPC profile according to eq 1. In bold are highlighted the more representative fractions for each specimen (>0.2 w/w). The data are also reported graphically in
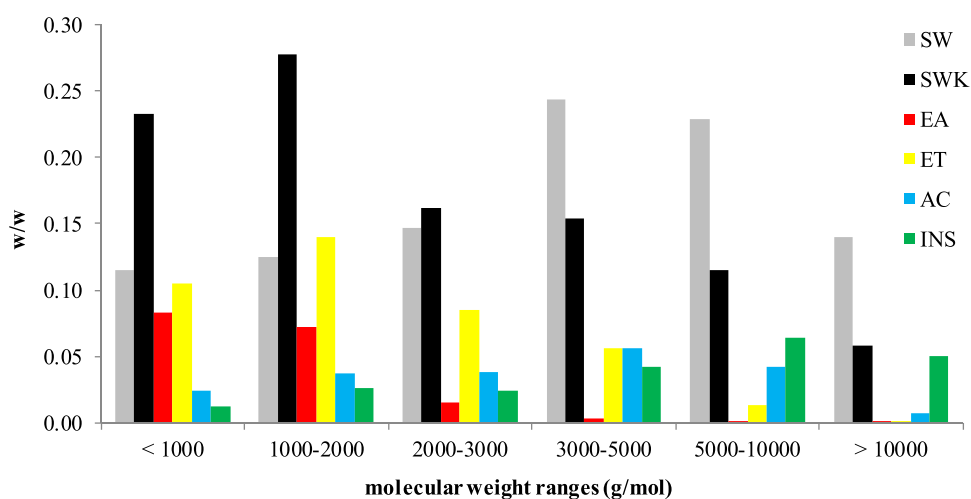
**Figure 2.** Relative weight fractions (w/w) for acidolytic SW lignin, SWK lignin, and its solvent fractions (EA, ET, AC, and INS) of the specific molecular weight ranges (>10,000, 10,000-5000, 5000−3000, 3000−2000, 2000−1000, and <1000 g/mol).
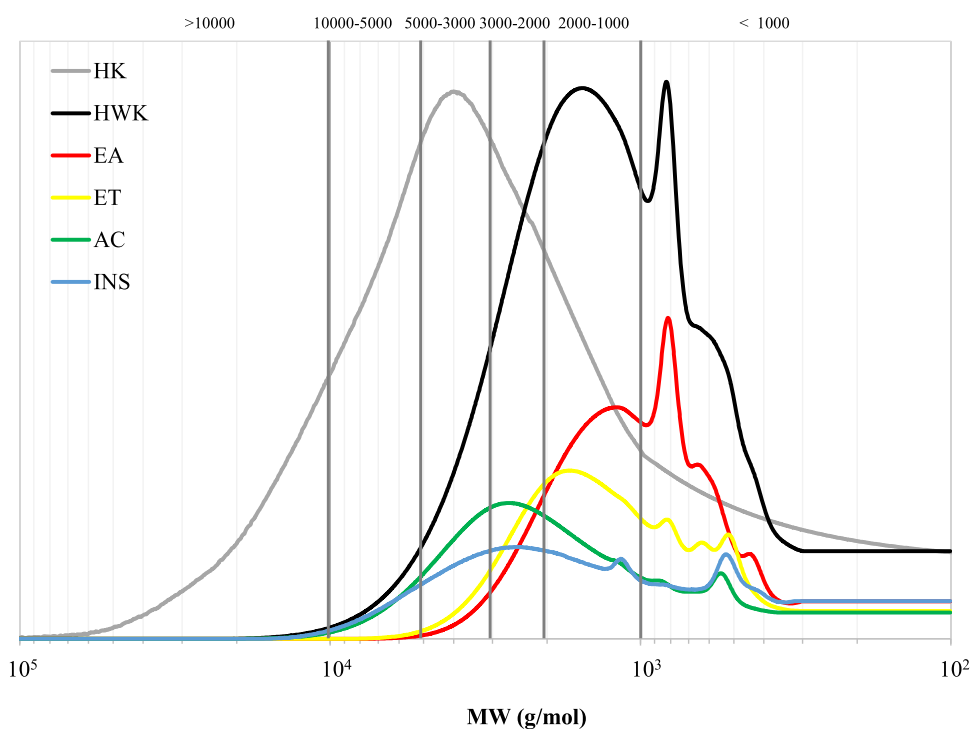


**Figure 3.** GPC−UV (280 nm) elution profiles of acidolytic HW lignin, HWK lignin, and its solvent fractions (EA, ET, AC, and INS) after acetylation. The relative intensity is normalized on the basis of extraction yields.

**Table 2. Fractionation Yields, GPC−UV (280 nm) Data, and Absolute Weight Fractions of Acidolytic HW Lignin, HWK Lignin, and Its Solvent Fractions (EA, ET, AC, and INS) after Acetylation ($\pm0.01$)[a]**

|  | w/w | (g/mol) | | PD | (w/w) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
|  | fractionation yield | $M_n$ | $M_w$ | | <1000 | 1000−2000 | 2000−3000 | 3000−5000 | 5000−10,000 | >10,000 |
| HW | n.a. | 4700 | 11930 | 2.54 | 0.08 | 0.17 | 0.17 | **0.27** | **0.22** | 0.10 |
| HWK | n.a. | 1685 | 2850 | 1.69 | **0.30** | **0.38** | 0.18 | 0.10 | 0.03 | 0.00 |
| EA | 0.32 | 1215 | 1700 | 1.40 | **0.42** | **0.44** | 0.12 | 0.03 | 0.00 | 0.00 |
| ET | 0.25 | 1475 | 2090 | 1.42 | **0.30** | **0.43** | 0.20 | 0.07 | 0.00 | 0.00 |
| AC | 0.23 | 2365 | 3770 | 1.59 | **0.22** | **0.27** | **0.22** | 0.22 | 0.08 | 0.00 |
| INS | 0.20 | 2470 | 4000 | 1.62 | **0.21** | **0.27** | **0.20** | 0.21 | 0.10 | 0.01 |

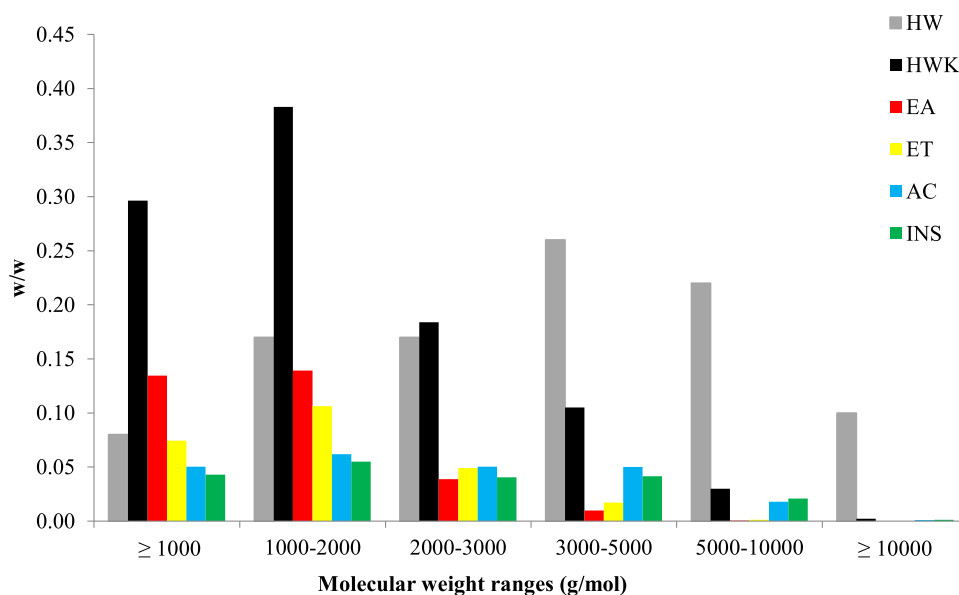[a]In bold are the most abundant fraction (>0.2 w/w).

**Figure 4.** Relative weight fractions (w/w) for acidolytic HW lignin, HWK lignin, and its solvent fractions (EA, ET, AC, and INS) of specific molecular weight ranges (>10,000, 10,000−5000, 5000−3000, 3000−2000, 2000−1000, and <1000 g/mol).

**Table 3. $^{31}$P NMR Data Obtained for Acidolytic SW, SWK Lignin, and Its Solvent Fractions Derived Thereof (Confidence Interval ±0.03 mmol/g, 0.9, 3)**

|  | aliphatic OH | condensed aromatic OH | G-type aromatic OH | H-type aromatic OH | total aromatic OH | acidic COOH |
|---|---|---|---|---|---|---|
|  |  |  | [mmol/g] |  |  |  |
| SW | 3.43 | 0.33 | 0.76 | 0.10 | 1.18 | 0.13 |
| SWK | 1.91 | 1.84 | 2.13 | 0.24 | 4.21 | 0.42 |
| EA | 0.94 | 1.80 | 2.99 | 0.23 | 5.02 | 0.56 |
| ET | 1.75 | 1.91 | 2.33 | 0.25 | 4.49 | 0.36 |
| AC | 1.91 | 1.97 | 1.80 | 0.26 | 4.03 | 0.38 |
| INS | 2.82 | 1.51 | 1.46 | 0.17 | 3.14 | 0.36 |

**Scheme 1. Main Phenol Groups Quantified by $^{31}$P NMR**



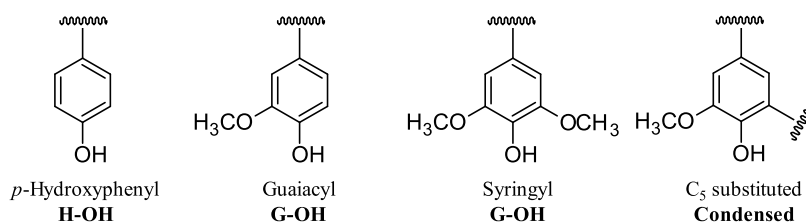| *p*-Hydroxyphenyl | Guaiacyl | Syringyl | C$_5$ substituted |
|---|---|---|---|
| **H-OH** | **G-OH** | **G-OH** | **Condensed** |

Figure 2. For the EA, ET, AC, and INS samples, the relative weight fractions were calculated from the absolute ones (Table 1), multiplying those values stemming from the EA, ET, AC, and INS samples by the corresponding fractionation yields. In order to assess the validity of the solvent fractionation, the relative weight fraction for the EA, ET, AC, and INS samples of a specific molecular weight range have been summed up ("calculated w/w") and compared with the original weight fractions of the SWK sample ("experimental w/w"). In Figure S1, the correlation between the experimental w/w and calculated w/w values is reported. Considering the good correlation found and the data reported in the literature,[9] the GPC data were considered consistent.

The same procedure has been applied to acidolytic hardwood lignins and hardwood kraft lignin, before and after solvent fractionation. Figure 3 shows the elution traces obtained for the GPC analyses of the hardwood lignins, and Table 2 lists, among others, the numerical results.

Concerning the HW lignin data, similar considerations as those applied for SW lignins regarding the efficiency of solvent fractionation at reducing polydispersity were taken. For the EA, ET, AC, and INS samples, the relative weight fractions were calculated from the absolute ones (Table 2), multiplying those values stemming from the EA, ET, AC, and INS samples by the corresponding fractionation yields and reported in Figure 4. The extraction procedure has also been validated (Figure S2).

Characterization results of softwood lignins and solvent fractionation samples by $^{31}$P NMR are reported in Table 3 along with a schematic representation of the main phenol groups, reported in Scheme 1.

In order to assess the validity of solvent fractionation, absolute quantifications in mmol/g for EA, ET, AC, and INS were multiplied by the corresponding fractionation yield and then summed ("calculated mmol/g") for each functional group. In Figure S3, the calculated values were correlated with the original functional group distribution of the SWK sample,

**Table 4. ^{31}P NMR Data Obtained for Acidolytic HW, HWK Lignin, and Its Solvent Fractions (EA, ET, AC, and INS) Derived Thereof (Confidence Interval ±0.03 mmol/g, 0.9, 3)**

| | aliphatic OH | S-type and condensed aromatic OH | G-type aromatic OH | H-type aromatic OH | total aromatic OH | acidic COOH |
|---|---|---|---|---|---|---|
| | | | [mmol/g] | | | |
| HW | 3.62 | 0.95 | 0.65 | 0.07 | 1.60 | 0.24 |
| HWK | 1.02 | 2.12 | 0.71 | 0.10 | 2.93 | 0.38 |
| EA | 0.64 | 2.46 | 0.75 | 0.08 | 3.29 | 0.70 |
| ET | 0.96 | 2.33 | 0.78 | 0.13 | 3.24 | 0.29 |
| AC | 1.21 | 2.24 | 0.82 | 0.13 | 3.19 | 0.31 |
| INS | 1.30 | 1.59 | 0.57 | 0.07 | 2.24 | 0.28 |
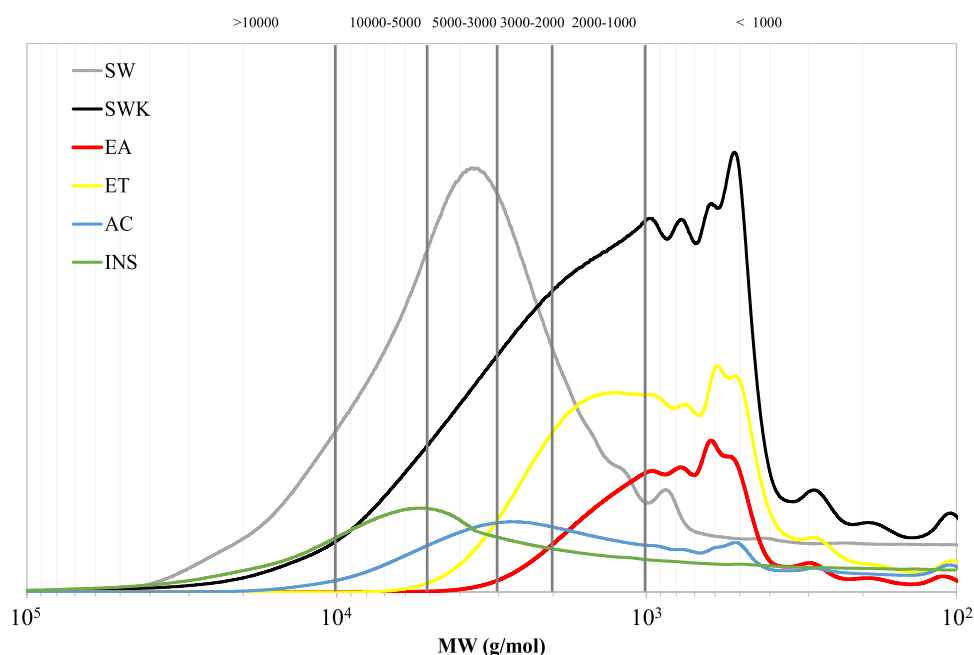


**Figure 5.** GPC-FL ($\lambda_{exc}$: 390 nm, $\lambda_{em}$: 550 nm) profiles of acidolytic SW lignin, SWK lignin and its solvent fractions (EA, ET, AC, and INS) after dansylation/acetylation. The relative intensity is calculated based on phenol fractionation yields.

**Table 5. GPC-FL Data and Phenol Weight Fractions for Acidolytic SW Lignin, SWK Lignin and Its Solvent Fractions (EA, ET, AC, and INS). Values in Bold Are the Most Abundant Fraction (>0.2 w/w)**

| | mol/mol | (g/mol) | | PD | mol/mol | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | aromatic OH fractionation yield | $M_n$ | $M_w$ | | <1000 | 1000−2000 | 2000−3000 | 3000−5000 | 5000−10,000 | >10,000 |
| SW | n.a. | 4800 | 10300 | 2.14 | 0.14 | 0.13 | 0.15 | **0.27** | **0.21** | 0.10 |
| SWK | n.a. | 2850 | 8450 | 6.20 | **0.36** | **0.25** | 0.13 | 0.12 | 0.09 | 0.04 |
| EA | 0.21 | 980 | 1450 | 1.48 | **0.59** | **0.33** | 0.07 | 0.01 | 0.00 | 0.00 |
| ET | 0.43 | 1600 | 2900 | 1.85 | **0.40** | **0.30** | **0.16** | 0.11 | 0.03 | 0.00 |
| AC | 0.20 | 2500 | 6000 | 2.37 | 0.19 | 0.22 | 0.19 | **0.22** | **0.15** | 0.04 |
| INS | 0.16 | 7400 | 17200 | 3.61 | 0.12 | 0.16 | 0.13 | 0.20 | **0.25** | **0.14** |

*i.e.*, the "experimental mmol/g". Considering the good correlation found and the data reported in the literature,[9] the ^{31}P NMR data were considered reliable. Characterization results of hardwood lignins and solvent fractionation samples by ^{31}P NMR are reported in Table 4, while the correlation is shown in Figure S4.

All GPC and ^{31}P NMR-derived data carefully validated were used for the distribution of phenols as a function of the molecular weight as describe below.

**GPC Fluorescence Data.** All the lignin samples were dansylated, acetylated, and analyzed by fluorescence-based GPC ($\lambda_{exc}$: 390 nm, $\lambda_{em}$: 550 nm). The numerical values for the elution profiles of SW lignins shown in Figure 5 are reported as numerical outputs in Table 5.

Initially, for the solvent fractionation samples, *i.e.* EA, ET, AC, and INS, the phenol fractionation yield in mol/mol was calculated by multiplying the fractionation yield in (w/w), as reported in Table 1, by the corresponding total phenol content in mmol/g reported in Table 3, and dividing the result by the phenol content of SWK, *i.e.* 4.21 mmol/g.

GPC-stemming results were calculated on the basis of chromatograms; due to the selectivity of the FL detector for the dansyl unit attached to the phenols, data correspond to the phenol molecular weight distributions. The auto-fluorescence phenomenon in lignin, known to be promoted by the occurrence of aromatic structures such as phenylcoumarones and stilbenoids[22] is taken into account and corrected. The first important observation that is obtained by this analytical
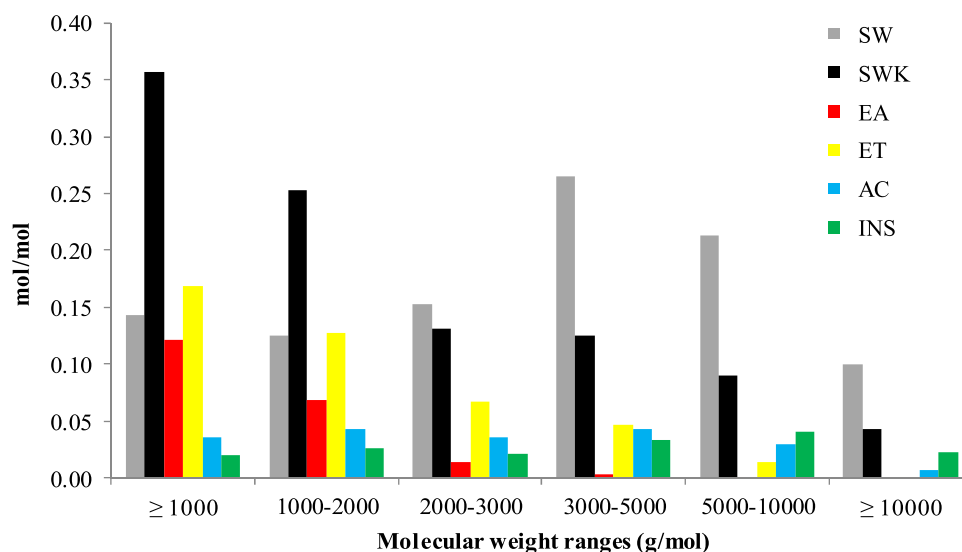
**Figure 6.** Relative molar fraction of phenols in mol/mol for acidolytic SW lignin, SWK lignin and its solvent fractions (EA, ET, AC, and INS) with specific molecular weight ranges, *i.e.*, >10,000, 10,000−5000, 5000−3000, 3000−2000, 2000−1000 and <1000 g/mol.
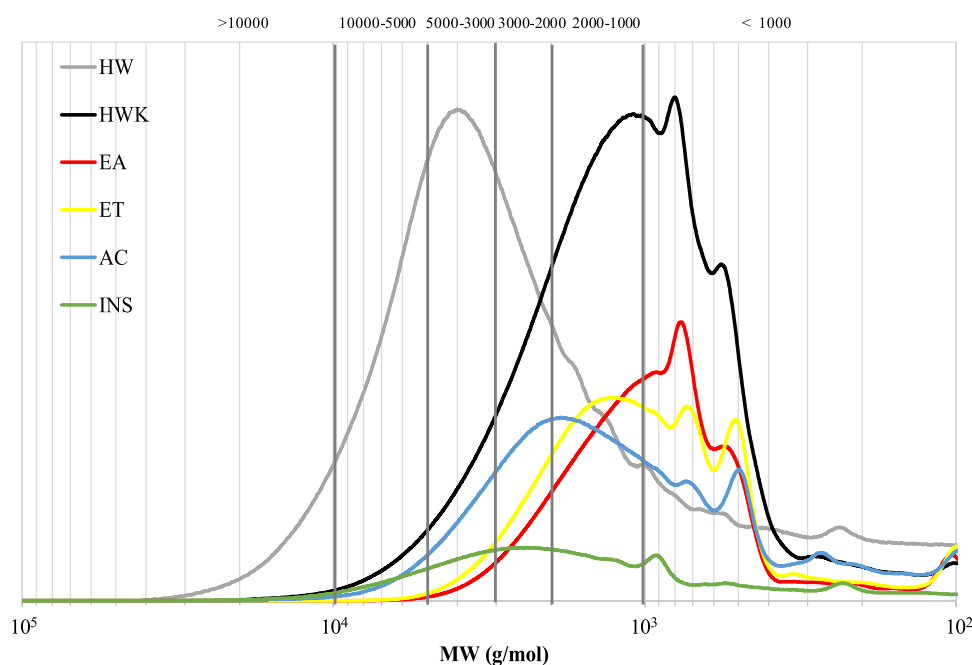


**Figure 7.** GPC-FL ($\lambda_{exc}$: 390 nm, $\lambda_{em}$: 550 nm) profiles of acidolytic HW lignin, HWK lignin, and its solvent fractions (EA, ET, AC, and INS) after dansylation/acetylation. The relative intensity is calculated based on phenol fractionation yields.

approach is that the kraft lignin samples from solvent fractionation are all characterized by a much lower polydispersity with respect to the unfractionated SWK in terms of phenol distribution (PD column, Table 5). Traditional approaches, based on GPC and $^{31}$P NMR analyses, are able to detect an increased homogeneity of lignin fractions in terms of molecular weight (PD column, Table 1) and an average phenol content inversely correlated with the molecular weight (Table 3). By GPC-FL and dansylation, for the first time, it was experimentally proved that solvent fractionation greatly reduces polydispersity and heterogeneity in the distribution of phenols. In particular, the SWK lignin seemed to be composed of a plethora of different macromolecules heterogeneously distributed in molecular weights (PD 3.67) and highly heterogeneous in the number of phenols: this

description is qualitatively represented by the great difference in the shape of the GPC profiles detected by UV and FL. SWK PD started from a value of 6.2, while, after fractionation, the phenol polydispersity decreased down to 1.48 for EA, 1.85 for ET, 2.37 for AC, and 3.61 for the fraction insoluble in organic solvents. Also, the comparison with acidolytic SW lignin is interesting, which, even if characterized by higher molecular weights, appeared more homogeneous (molecular weight PD 2.37, phenol PD 2.14). Again, this analytical approach highlights interesting insights into the great softwood lignin structural rearrangement that occurred during the kraft process. For each sample, the absolute molar fractions of phenols for a specific molecular weight range, *i.e.*,>10,000, 10,000−5000, 5000−3000, 3000−2000, 2000−1000, and <1000 g/mol, were calculated from the GPC profiles according

**Table 6. GPC-FL Data and Phenol Weight Fraction for HW Lignin, HWK Lignin, and Its Solvent Fractions (EA, ET, AC, and INS)[a]**

| | mol/mol | (g/mol) | | PD | mol/mol | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | aromatic OH fractionation yield | $M_n$ | $M_w$ | | <1000 | 1000−20,00 | 2000−3000 | 3000−5000 | 5000−10,000 | >10,000 |
| HW | n.a. | 3650 | 7670 | 2.10 | 0.08 | 0.17 | 0.17 | **0.30** | **0.22** | 0.06 |
| HWK | n.a. | 1800 | 3000 | 1.67 | **0.33** | **0.39** | 0.17 | 0.08 | 0.02 | 0.00 |
| EA | 0.36 | 1110 | 1460 | 1.32 | **0.44** | **0.42** | 0.12 | 0.01 | 0.00 | 0.00 |
| ET | 0.27 | 1370 | 1960 | 1.43 | **0.33** | **0.43** | 0.17 | 0.06 | 0.00 | 0.00 |
| AC | 0.25 | 2390 | 3250 | 1.36 | **0.25** | **0.33** | **0.21** | **0.15** | 0.05 | 0.00 |
| INS | 0.15 | 2600 | 4950 | 1.90 | **0.21** | **0.31** | **0.21** | **0.18** | 0.09 | 0.00 |

[a]In bold are the most abundant fraction (>0.2 w/w) as reported in Table 2.
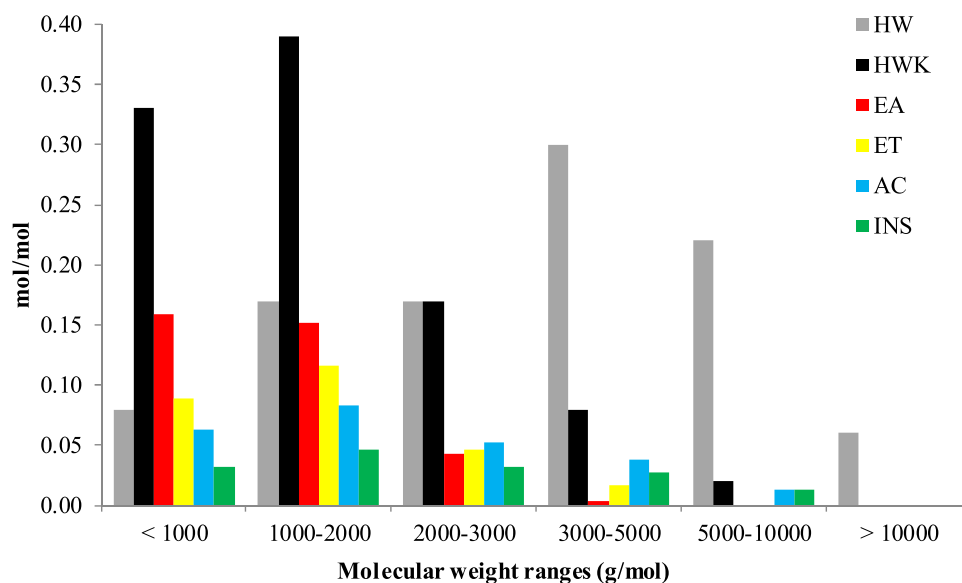


**Figure 8.** Relative molar fraction of phenols in mol/mol for acidolytic HW lignin, HWK lignin, and its solvent fractions (EA, ET, AC, and INS) of specific molecular weight ranges, *i.e.*, >10,000, 10,000−5000, 5000−3000, 3000−2000, 2000−1000, and <1000 g/mol.

**Table 7. Estimations of the Phenol Content in mmol/g for SW Lignin, SWK Lignin and Its Solvent Fractions (EA, ET, AC, and INS) of the Specific Molecular Weight Ranges >10,000, 10,000−5000, 5000−3000, 3000-2000, 2000−1000, and <1000 g/mol (±0.15 mmol/g, 0.9, 3)[a]**

| | molecular weight ranges (g/mol) | | | | | |
|---|---|---|---|---|---|---|
| | <1000 | 1000−2000 | 2000−3000 | 3000−5000 | 5000−10,000 | >10,000 |
| | Aromatic OH in mmol/g | | | | | |
| SW | 1.47 | 1.18 | 1.22 | **1.29** | **1.10** | 0.85 |
| SWK | **6.46** | **3.85** | 3.43 | 3.40 | 3.29 | 2.63 |
| EA | **6.17** | **4.01** | 3.81 | 3.87 | nd | nd |
| ET | **6.79** | **3.85** | **3.36** | 3.52 | 3.18 | nd |
| AC | 6.26 | 4.94 | 4.04 | **3.22** | **2.95** | 4.36 |
| INS | 6.78 | 4.14 | 3.78 | 3.30 | **2.66** | **1.90** |

[a]In bold are the most abundant fractions (>0.2 w/w). nd: not detected.

to eq 2. The relative molar fractions of phenols were then calculated from the absolute ones, by multiplying the latter EA, ET, AC, and INS samples by the corresponding phenol fractionation yield reported in Table 5; resulting data are reported in Figure 6.

To validate the data, the relative molar fractions of phenols (mol/mol) for the EA, ET, AC, and INS samples of a specific molecular weight range have been summed up ("calculated mol/mol") and compared with the original molar phenol fractions of the SWK sample ("experimental mol/mol"). Figure S5 reports the correlation between the experimental w/w and

calculated w/w values. The same procedure has been applied to hardwood lignins, for acidolytic and hardwood kraft lignin and its solvent fractions. The HW lignin samples were dansylated and acetylated, and were analyzed by fluorescence-based GPC ($\lambda_{exc}$: 390 nm, $\lambda_{em}$: 550 nm). The numerical values for the elution profiles of HW lignins shown in Figure 7 are given in Table 6.

When compared to SWK lignin, the solvent fractionation of HWK lignin was able to increase the homogeneity of the phenol distribution along MW, at least for EA, ET, and AC samples, while the insoluble residue was characterized by a

**Table 8. Estimations of the Phenol Content in mmol/g for HW Lignin, HWK Lignin, and Its Solvent Fractions (EA, ET, AC, and INS) of the Specific Molecular Weight Ranges >10,000, 10,000−5000, 5000−3000, 3000−2000, 2000−1000, and <1000 g/mol (Confidence Interval ±0.15 mmol/g, 0.9, 3)[a]**

| | molecular weight ranges (g/mol) | | | | | |
|---|---|---|---|---|---|---|
| | <1000 | 1000−2000 | 2000−3000 | 3000−5000 | 5000−10,000 | >10,000 |
| | Aromatic OH in mmol/g | | | | | |
| HW | 1.75 | 1.65 | 1.72 | **1.94** | **1.67** | 1.00 |
| HWK | **3.31** | **2.97** | 2.72 | 2.24 | 2.39 | nd |
| EA | **3.45** | **3.18** | 3.25 | 1.43 | nd | nd |
| ET | **3.56** | **3.25** | **2.78** | 2.81 | 3.05 | nd |
| AC | **3.66** | **3.91** | **3.07** | 2.20 | 2.06 | nd |
| INS | **2.20** | **2.55** | **2.35** | **1.95** | 1.95 | nd |

[a]In bold are the most abundant fractions (>0.2 w/w). nd: not detected.

slightly higher PD (1.90) than unfractionated lignin. Even the comparison between HW acidolytic and kraft lignin was different with respect to SW: both in terms of molecular weight (Table 2) and phenol distribution (Table 6), the analyzed samples were characterized by quite similar polydispersity. For each sample, the absolute molar fractions of phenols for a specific molecular weight range were calculated from the GPC profiles according to eq 2. The relative molar fractions of phenols were then calculated from the absolute ones, multiplying the latter EA, ET, AC, and INS samples by the corresponding phenol fractionation yields reported in Table 6; the resulting data are reported in Figure 8. In Figure S6, the correlation between the experimental w/w and calculated w/w values is reported.

Finally, all data obtained for SW and HW lignins, as validated by the mentioned correlations, are meant to be used to generate on the basis of eq 3 a molecular weight range-dependent version of the equation usually exploited for the assessment of average quantities of phenolic lignin moieties. For SW lignins, data are reported in Table 7, while those for HW lignins are listed in Table 8. In bold are highlighted the most abundant fractions (>0.2 w/w) (Tables 1 and 2) in both cases.

**Structural Interpretation of Phenol Distributions in Molecular Weight Fractions and Comparison with Known Structural Features of Kraft Lignins.** It is well recognized that solvent fractionation strategies applied to kraft lignins are able to reduce the intrinsic heterogeneity in terms of molecular weight distribution[23] and also refine some structural chemical features[24] either functional groups (such as alcohols,[25] phenols, carboxylic acids) or structural intermonomeric motifs.[26] The recently developed selective derivatization with dansyl chloride of lignin phenolic functionalities coupled with GPC-FL[19] was used in this work to focus the investigation on this important chemical group in lignin, and verify that solvent fractionation permitted not only to obtain fractions more or less rich in phenols but those fractions were also more homogeneous in terms of phenol distribution and molecular weight distribution. The latter is quite clear from the data reported in Tables 5 and 6, in which the phenol distribution alongside the molecular weight ranges is reported. All SWK lignin fractions, *i.e.*, EA, ET, AC, and INS are characterized by a much lower phenol polydispersity than that of the unfractionated SWK lignin. For HWK, such lower phenol polydispersity characterizes only the EA, AT, and AC fractions. Interestingly, this observation seems to be connected to phenol polydispersity of unfractionated HWK lignin that is already quite low with respect to SWK lignin (Tables 5 and 6) where

the phenol polydispersity is much higher. This is an indication that for this kind of lignin the refining looks mandatory in order to exploit its performances, with respect to an intrinsically more homogenous HWK lignin. Going deep into the data, also the estimations of the phenol content in different molecular weight ranges (Tables 7 and 8) could help in understanding lignin valorization. For SWK lignin it appears that the phenol content of each molecular weight range was similar along different fractions (EA, ET, AC, INS) and the yield of extraction was determining the overall chemical properties of the fractions. The same trend can be observed for HWK lignin and its fractions. Deviation from these general conclusions is evident at high molecular weights (>5000 g/mol) in SWK and mainly in the INS fraction from HWK. In the latter case, the phenol content was more similar to acidolytic lignin, with a constant value along MWs.

In this view, the SWK ethyl acetate fraction looks particularly intriguing. Our data indicated a great reduction in polydispersity in terms of both molecular weight and phenol distribution after solvent fractionation. Moreover, this sample is characterized by the presence, for around 50% in weight (Table 1), of a fraction with a molecular weight < 1000 g/mol and around 6 mmol/g of phenol content (Table 7). Those values are consistent with a lignin oligomer (dimers, trimers, and tetramers) with one phenol per aromatic ring. For HWK, such structural features do not accompany the reduction in polydispersity. As reported by Gioia et al.,[27] where solvent fractionation was used to set up relationships between the structure and material properties for lignin-based epoxy resins, SWK provided better properties than HWK lignins by comparing fractions with similar molecular weights and reactive centers (epoxy equivalent weight). The author conclusions reported that the presence of guaiacyl units (Scheme 1) leads to the formation of more condensed units in the kraft process, which reduces molecular mobility, in contrast, hardwood kraft lignin is dominated by syringyl units with lower content of condensed units but higher content of flexible methoxy units. This observation is in agreement with our data, and could be generalized for all kraft lignin valorization strategies, in which phenols play a pivotal role[28] (thermal protection,[24,29] epoxidation,[30,31] allylation,[32,33] cyclic carbonation,[34,35] and so on).

In order to independently validate the phenol distribution as delineated *via* GPC-based analyses of dansylated samples, phenol distributions can be interpreted in terms of basic structural features in connection with the information on the molecular weights of the bulk materials as delineated by standard GPC−UV protocols. The resulting basic structural
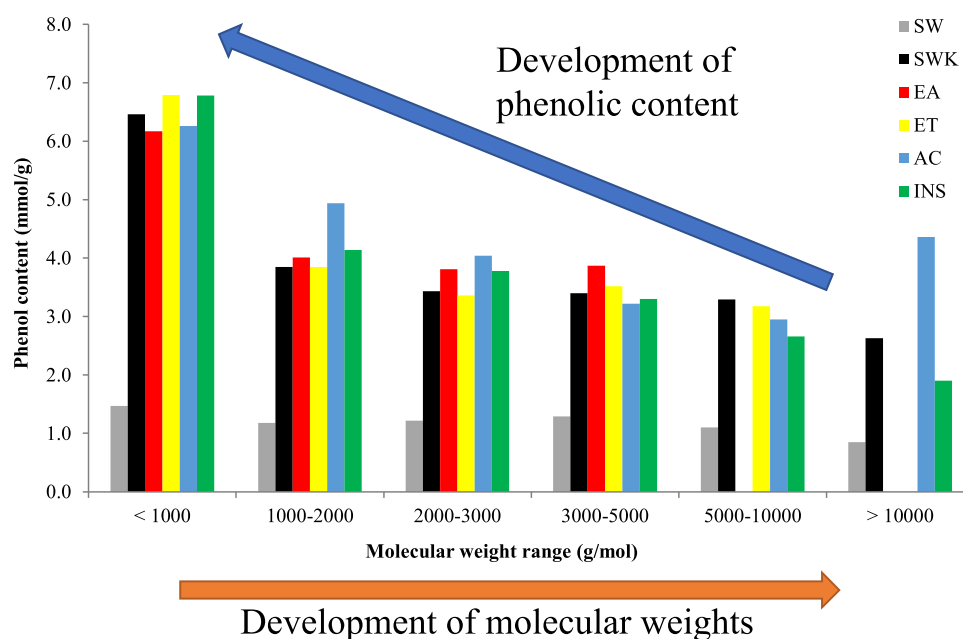
**Figure 9.** Trends emerging from the structural data as determined on the basis of GPC analysis of dansylated/acetylated SW lignins and $^{31}$P NMR spectroscopy.

aspects should, in case the dansylation approach proves universally applicable as outlined here and in principal terms before,[19] correspond to findings delineated for kraft lignins and their fractions on the basis of more sophisticated approaches such as one- and two-dimensional NMR studies. In the first analysis, comparing data obtained for SW and SWK lignins for phenolic group distribution suggests, together with bulk molecular weight analyses, that SW is considerably less degraded with respect to what is known regarding the lignin structure *in planta*: SW displays more phenolic OH-groups in the smaller molecular weight areas than in larger molecular weight regimes, but the overall distribution is quite homogeneous, *i.e.*, no sharp increase in the phenolic content in the lowest molecular weight sections, and no more pronounced depletion of these groups in higher molecular weight fractions. This corresponds to the picture of a lignin present across the range of molecular weights in the form of typical phenylpropanoid structures, exhibiting aliphatic motifs typical for softwood lignins. In contrast, SWK exhibits a significantly less homogeneous distribution of phenolic OH-groups, peaking in the small molecular weight regimes and, compared to the medium value as delineated by $^{31}$P NMR of the bulk material, depleted in higher molecular weight fractions. Together with the higher polydispersity measured for SWK lignin compared to SW, data hint at a certain degree of structural degradation with respect to what is expected for the lignin *in planta*.[26,36] Applying the same lines of interpretation to the data generated for SWK fractions, certain trends can be determined across the data presented before in Tables 2, 5, and 7, as summarized in Figure 9.

The trends correspond to what one would expect based on the literature data of SWK lignins.[37,38] All fractions exhibit "aspects" found in the bulk lignin across all MW ranges, hence the applied traditional solubility-based fractionation promotes/ augments certain structural characteristics but does not manage to fully exclude certain structural motifs in an isolated fraction. These reminiscences have been observed before.[39] Gradually changing polarity and H-bonding characteristics of

solvents lead to the expected increase in molecular weights from EA to AC, with the highest molecular weights in INS. This increase goes together with the trend of diminishing phenol content in dominating molecular weight regimes: in case INS is lower than that found for the bulk SWK material in the highest molecular weight regimes starting from 3000 g/mol (Table 7 and Figure 8). Together with data for $M_n$, $M_w$, and $I$, these findings correspond to the interpretation of softwood kraft lignins being structurally rather binary mixtures with one part being of lower molecular weights, more condensed, and richer in phenols, *i.e.*, fractions EA, ET, and AC, with the major fractions displaying a phenol content above the media, and another part being of higher molecular weight, less condensed, and poorer in phenolic, *i.e.*, the INS fraction.[26]

## ■ CONCLUSIONS

The proposed, more detailed structural analysis of lignins *via* the combination of quantitative $^{31}$P NMR analysis and comparative GPC analysis of dansylated/acetylated samples offers a means for delineating important structural aspects of isolated technical lignins, comprising their fractions, without the need for extremely expensive laboratory equipment. In this paper, the method developed by Salanti et al.[19] in 2020 was directly compared with a fractionation procedure, highlighting that lignin samples display heterogeneous molecular structures, not only in terms of molecular weights but also in terms of phenol contents, confirming the strength of this analytical approach. For the first time, it has been demonstrated that fractions obtained by simple solvent fractionation have been improved in terms of phenol distribution, and that data obtained by $^{31}$P NMR could be observed from a new perspective. The data of 4.21 for SWK and 2.93 for HWK mmol/g (Tables 3 and 4) are an average value generated by the contribution of different molecules with phenol content scattered over quite a wide range (for SWK more than HWK). After solvent fractionation, the $^{31}$P NMR data of the total phenol content could be interpreted in the same way, highlighting that those values are an average of quite less

scattered molecules, especially for the EA and ET fractions. To the best of author's knowledge, this analytical approach is the only experimental procedure that provides direct evidence of this phenomenon, with important consequences on lignin valorization. The insights generated in terms of phenolic group distributions across molecular weight regimes, interpreted on the basis of the relative importance of these regimes in the sample, allow for the estimation of chemical and physicochemical behavior of the lignin in hand on the basis of its phenol group distribution. The method proposed represents an easily accessible way that delivers a significantly deeper structural insight in terms of phenol distribution compared to the traditional UV-spectroscopy-based analysis of phenolic end-groups of the bulk material. The method presented here can thus be seen as a hands-on approach to convey crucial structural information regarding the most important functional group and its distribution in lignin. Most importantly, with respect to a combination of standard GPC analysis of acetylated lignins and quantitative $^{31}$P NMR, conventionally performed as part of the state-of-the-art analysis approach for lignins, the new method gives deeper insight. Last but not least, this approach represents an option for a "rapid" fingerprinting, which might allow for an at least rough classification of an unknown lignin sample in terms of its biorefinery origins on the basis of a simple library of characteristic lignin samples.

## ■ ASSOCIATED CONTENT

### *ⓈI* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.2c03774.

> Correlations between experimental values (weight fraction SWK and HWK, phenol content for SWK and HWK, and phenol distribution in SWK and HWK) and calculated ones (PDF)

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### Notes

The authors declare no competing financial interest.

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