

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Compositional Variability of Lignin in Biomass

Ana Lourenço and Helena Pereira

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.71208>

## Abstract

The objective of this chapter is to provide a concise overview of lignin composition and structure in different species and materials (wood, barks and nonwood plants). It includes a brief review on the lignin precursors and their polymerization as well as of the analytical tools used for lignin characterization from wet chemical to spectroscopic methods. Wood of gymnosperms is characterized by high lignin content (25–35%) and a HG-type of lignin with more guaiacyl (G) units and a small portion of *p*-hydroxyphenyl (H) units. Wood of angiosperms has a lignin content of 15–28%, with a GS-lignin having different proportions of syringyl (S) units. Nonwoody monocotyledon species have different lignin content (9–20%) and a HGS type of lignin, characterized by a high proportion of H units. Bark lignin content ranges from 13 to 43% and is of HGS-type with species-specific composition and different in the bark components, phloem and cork. Lignin composition and macromolecular structure are key issues to understand the properties of lignocellulosic materials and to design a lignin-based pathway within biomass biorefineries. The available information on lignin composition is still limited to a few species and plant components. This is certainly an area where more research is needed.

**Keywords:** analytical tools, biomass, lignin composition, monolignols, S/G ratio

## 1. Introduction

Lignin has been the subject of continuous and intensive research over the last century. The Web of Science shows that since 1908 more than 27,000 publications were published with the topic “lignin,” including articles, reviews, book chapters, notes and proceedings, under different subject areas, e.g., plant science, biotechnology, applied microbiology, chemistry, wood, pulp & paper, materials, energy and fuels.

Lignin is the second most abundant biopolymer in nature and accounts for almost 30% of the plants [1]. Its deposition in the cell wall is of great importance for plant development: (i) it

provides rigidity and strength to the cell wall, giving mechanical support for the plant organs; (ii) it presents hydrophobicity favoring the transport of water and solutes in the vascular system and (iii) it protects the cell against pathogens [1–5]. Lignin is linked to the other structural components of the cell wall—cellulose and hemicelluloses—by covalent linkages, forming lignin-carbohydrate complex (LCC) [6].

The first lignin studies were mainly driven by its importance for the pulp & paper industry, where the objective is to remove lignin from the wood cells to obtain a fibrous product rich in cellulose. Thus, the studies on lignin, whether related to content, composition or structure, were focused on pulpwoods [7–11]. Lignin was also studied in herbaceous plants [12–16] partly triggered by digestability and dietary conversion issues in animal feed [2]. More recently, other wood species as well as various lignocellulosic residues and wastes attracted attention within the biorefinery concept providing opportunities for production of green chemicals, bioproducts and energy, calling for the need to include lignin valorization.

The lignin content shows a large variability between species: in general, in monocotyledons, it ranges between 5 and 12%, in softwoods between 25 and 35% and in hardwoods between 15 and 30%. The structural arrangement of lignin also differs between these three groups. This chapter makes a review on the compositional variability of lignin in various species and biomass components after an introductory compilation of the macromolecular assembly and the analytical tools used in lignin research.

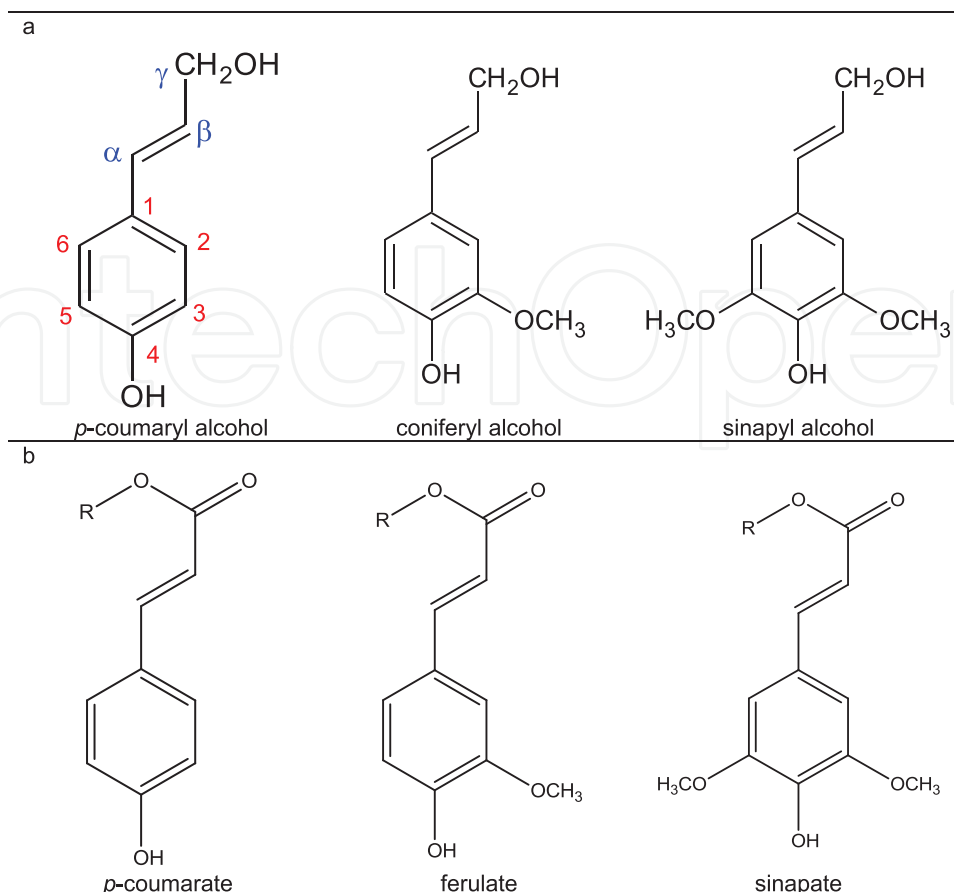
## 2. The molecular construction of lignin

### 2.1. Precursors and monomers

Lignin is a heterogeneous aromatic polymer mainly constituted by three precursors: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (**Figure 1**). These precursors are often mentioned as phenylpropane or C<sub>9</sub> units, where the hydroxyl group is linked to the C<sub>4</sub> and substitutions with one or two methoxyl groups may be present at the C<sub>3</sub> and C<sub>5</sub>. Therefore, the aromatic ring of the three alcohols is called *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) if the ring is unmethoxylated, or has one or two methoxyl groups, respectively. The side-chain carbons are designated as  $\alpha$ -,  $\beta$ - and  $\gamma$ -, with C $\alpha$  attached to the aromatic C<sub>1</sub>.

Researchers have recently recognized that lignin polymerization may involve other monomers, such as hydroxycinnamic acids and aldehydes, coniferyl and sinapyl acetates and coumarates [17–19].

The deposition of lignin and cellulose in the cell wall proceeds in three phases and starts after the deposition of pectins and the formation of the secondary wall S<sub>1</sub> layer has begun [3, 20]. The first phase starts by the lignification at cell corners and middle lamella; the second phase corresponds mainly to the deposition in the S<sub>2</sub> layer of cellulose in microfibrils and of xylan and mannan, with lignin being slowly added; in the third phase, lignin deposition proceeds extensively across the cell wall after the deposition of cellulose in the microfibrils of the S<sub>3</sub> layer.



**Figure 1.** Chemical structures of the primary lignin precursors (a) and the hydroxycinnamates (b). R = H. Adapted from Ralph [19].

The composition of lignin changes during cell development. According to Terashima and Fukushima [20], in conifers, the middle lamella and cell corners are first enriched in *p*-hydroxyphenyl lignin, followed by the deposition of guaiacyl lignin in the middle lamella and in the secondary wall, while a small amount of syringyl lignin may be deposited later in the secondary wall. Under mechanical stress, gymnosperm trees form compression wood where the cells are characterized by a higher lignin content and an increased proportion of *p*-hydroxyphenyl lignin [2]. This differentiation of monolignol incorporation in the cell wall at different stages of xylem development and cell wall regions suggests a tightly regulated pathway for the lignin unit formation [21]. Lignin deposition and composition depend on the environmental conditions and are subject to modulation at different levels during normal development and in response to different stresses [1, 2]. It is still unclear whether lignin biosynthesis is explained by a single pathway in all species and tissues and under every environment conditions [2, 4].

Lignin heterogeneity may be related to enzyme diversity and specificity regarding substrates, thereby affecting the metabolite flux into diverse branches of the biosynthetic pathway [2]. A clear example is the difference in lignin type between softwoods and hardwood: softwood lignin is mainly constituted by G units and minor amounts of H units, whereas hardwood lignin has G and S units.

## 2.2. Polymerization and molecular assembly

The theory underlying lignification was presented by Freudenberg and Neish [22] based on chemical processes involving the oxidative coupling of phenols and addition of the available phenolic substrates to the polymer [23, 24]. The oxidation produces a phenolic radical with unpaired electron density delocalized at the C1, C3, C5 and O-4 positions of the aromatic ring and at the propanolic C $\beta$ , forming resonance structures.

The lignin polymerization starts with the coupling of two monomeric radicals and continues by coupling of monomer radicals with phenoxy radicals formed on the growing polymer [25]. This concept explains some features of the lignin composition and structure, e.g., the evidences that other monomers such as coniferyl and sinapyl acetates and coumarates are also incorporated in the polymer [17–19]. Although a lignin polymerization model based on a protein-controlled radical coupling was proposed [26–29], the idea was not fully accepted by the scientific community given its flaws [30]. It was proved that lignification is malleable to plant needs and the polymer can be manipulated by changing the lignin-biosynthetic pathway genes [17, 31], and plants may incorporate other monomers into the lignin [32, 33].

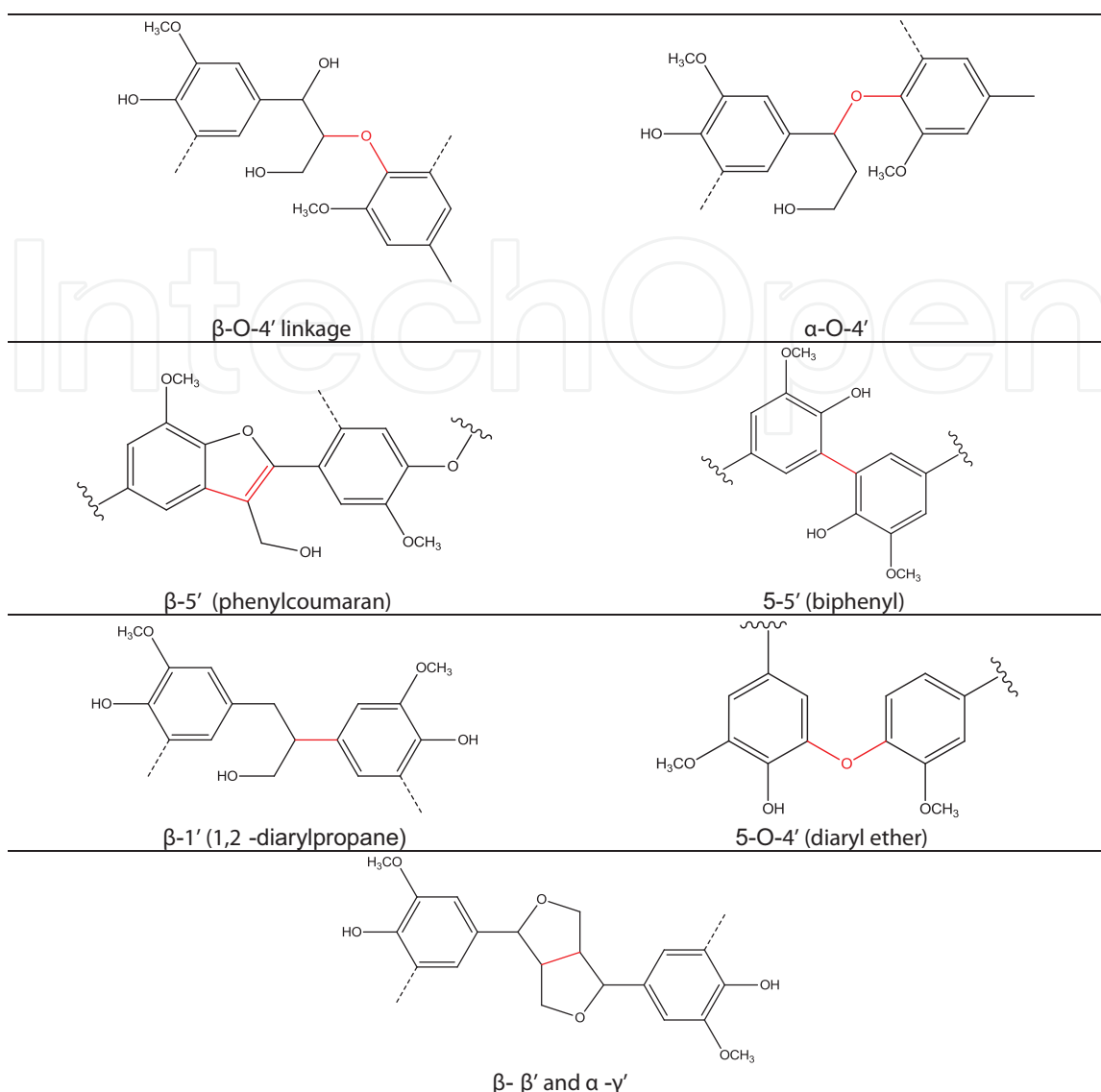
The dehydrogenation of the lignin monomers is made by peroxidases (or peroxidase-H<sub>2</sub>O<sub>2</sub> system) that are capable of removing a proton from the phenolic hydroxyl forming the resonance-stabilized free radicals, using the H<sub>2</sub>O<sub>2</sub> produced by the peroxidase enzyme as an electron-acceptor substrate [34]. Laccase is a phenoloxidase also related to lignin biosynthesis [35].

After formation of the phenoxy radicals, the reaction is no longer controlled by enzymes but is a random radical polymerization process at the reactive sites [34]. The most reactive positions are the phenoxy oxygen and the C $\beta$  that readily couple into aryl-ether linkages; the  $\beta$ -O-4' linkage is predominant in lignin, e.g., almost 50% of all intermonomeric linkages in softwoods and 60% in hardwoods [36]. Overall, the coupling of the lignin monomers may be by ether bonds ( $\beta$ -O-4', 4-O-5', 1-O-4') and by carbon-carbon bonds (5-5',  $\beta$ -5',  $\beta$ - $\beta'$ ,  $\beta$ -1') is often called condensed bonds [25]. Some of these linkages are shown in **Figure 2**.

## 2.3. Analytical tools and lignin compositional indicators

Lignin quantification is usually made through wet chemistry by acid hydrolysis with sulfuric acid, using standard methods, e.g., TAPPI T222 om-11 and UM 205 om-83, respectively, for Klason lignin (obtained as a solid residue) and acid-soluble lignin (measured at 205 mm in the solution) that together make up the total lignin in the sample [37]. The procedure was optimized for wood and may lead to overestimation if the raw material is rich in ash and proteins or contains carbohydrate degradation products such as furfural and hydroxymethyl furfural. In spite of these shortcomings, most of the available data on lignin content of lignocellulosic materials refer to Klason lignin determinations and therefore establish a comparative reference. More recently, lignin content has been calculated from analytical pyrolysis or estimated using FTIR and NIR spectra modeling, as described subsequently.

As regards the study of lignin composition, the ideal would be to have an isolated pure lignin, e.g., recovered after removal of extractives, cellulose and hemicelluloses, without chemical



**Figure 2.** Linkages present in the lignin polymer. Adapted from Dimmel [25].

modification of the original lignin. This proved unfeasible although some procedures approach the requirements [38]. Lignin is frequently isolated by the classical Björkman method [39] and called milled wood lignin (MWL) if wood is the starting material. The lignin is obtained by milling the extracted sample in a planetary ball mill, followed by extraction with dioxane; after evaporation, the lignin is dissolved in acetic acid, precipitated into water, dried and dissolved in dichloroethane:ethanol solution and precipitated into ethyl ether [39]. MWL is still contaminated with carbohydrates [39] and represents only a part of the total cell wall lignin [40] whose structural features are correlated with the yield and, to a less degree, milling time [41]. Other procedures involving enzymes, e.g., cellulolytic enzymes, to remove the carbohydrates are used to increase the lignin yield [42].

Much of the present understanding of the composition and structure of lignin is based upon interpretation and extrapolation of data obtained from chemical degradative methods that



include thioacidolysis, nitrobenzene oxidation, derivatization followed by reductive cleavage and analytical pyrolysis. Nondestructive methods, such as Fourier transform infrared (FTIR), near-infrared spectroscopy (NIRS) and nuclear magnetic resonance (NMR), are also used for lignin characterization.

### 2.3.1. Thioacidolysis

Thioacidolysis involves the solvolysis of the extractive-free material in dioxane/ethanediol (9/1) containing boron trifluoride etherate [43, 44]. The reaction depolymerizes part of the lignin and the released monomers can be analyzed, e.g., by GC-MS allowing to estimate the amount and composition of uncondensed alkyl ether structures [43]. Most studies were focused on wood but thioacidolysis was also applied to other materials, e.g., cork [45]. Calculation of the S/G ratio is usually made.

### 2.3.2. Nitrobenzene oxidation (NO)

Alkaline nitrobenzene oxidation leads to formation of aromatic aldehydes (*p*-hydroxybenzaldehyde, vanillin, syringaldehyde) as the major compounds, with benzoic acids (*p*-hydroxybenzoic, vanillic and syringic acids) and dehydrodivanillin or acetoguaiacone in minor amounts [46]. The yield of the compounds is related to the lignin structure in the raw material: vanillin is the major product from softwoods, syringaldehyde and vanillin are produced in great amounts from hardwoods and *p*-hydroxybenzaldehyde is produced from grasses [46, 47]. Nitrobenzene oxidation provides a satisfactory yield of *p*-hydroxybenzaldehyde, vanillin and syringaldehyde from the lignin H, G and S units [48]. The calculation of the S/G ratio is based on the obtained syringaldehyde and vanillin [49].

### 2.3.3. Derivatization followed by reductive cleavage (DFRC)

DFRC provides information on the occurrence of acylated  $\gamma$ -OH units [50–53]. The derivatization is made with acetyl bromide in acetic acid (called DFRC) or propionyl bromide in propionic acid (DFRC modified) at 50°C. The products are dissolved in dioxane/propionic acid/water (5:4:1, v/v/v), with the addition of zinc for the reductive cleavage, and the final derivatization step made with acetic anhydride or propionic anhydride depending of the method chosen (DFRC or DFRC modified) [53]. The lignin degradation compounds are collected after evaporation and analyzed by GC/MS.

### 2.3.4. Analytical pyrolysis

Pyrolysis transforms a nonvolatile compound into a volatile degradation mixture by heat in the absence of oxygen and by the breaking of chemical bonds using thermal energy [54, 55]. The ground biomass sample (particle sizes from mm to  $\mu$ m) is heated at temperatures from 400 to 1000°C, in the absence of oxygen, for 300 s to less than 0.5 s, producing charcoal (solid), bio-oil (liquid) and fuel gas products [56, 57]. Analytical pyrolysis was perfected for the pyrolysis of small samples for analytical purposes: a mixture of volatile compounds derived from the three macromolecular constituents of biomass (cellulose, hemicelluloses and lignin) is obtained and separated through a capillary column, identified by mass spectrometry (MS) and quantified by

a flame ionization detector (FID). The MS identification of pyrolysis products was mostly made by Faix et al. [58–61] and by Ralph and Hatfield [62]. Nowadays, quantification is also made by Py-GC/MS [63]. Pyrolysis is particularly interesting to characterize the lignin monomeric composition into the phenolic S, G and H precursor monomers. The ratio of monomers is given in the form of H:G:S or, more frequently, as the S/G ratio.

### 2.3.5. Fourier transform infrared (FTIR)

FTIR spectroscopy is a rapid technique for lignin characterization that permits determination of the lignin monomeric composition, of methoxy and carbonyl groups, and the calculation of the ratio of phenolic to aliphatic groups [64]. It is based on the interaction of infrared radiation (4000–500 nm wavelength) with the sample and the fact that each molecule absorbs energy characteristic from its specific intramolecular bonds. Some of the bands related to lignin are found at 1600 and 1500  $\text{cm}^{-1}$  due to the aromatic skeleton vibration of the benzene ring, at 1300 and 1200  $\text{cm}^{-1}$  related to syringyl and guaiacyl lignin units and the bands at 1716 and 1711  $\text{cm}^{-1}$  are attributed to phenol esterification and the alcohol of the propanoic chain ( $\text{C}\alpha$  and  $\text{C}\gamma$ ) [65, 66]. The spectral acquisition data can be made in transmission mode (TR), attenuated total reflectance (ATR), diffuse reflectance (DR) and photoacoustic (PA). Most lignin studies have used FTIR-TR but FTIR-DR is suitable to study the wood surface oxidation and weathering [64]. Watkins et al. [66] used FTIR-ATR to characterize organosolv lignins. FTIR spectral data were also used to model lignin content in wood of, e.g., *Picea sitchensis* [67], *Picea abies* [68] and *Eucalyptus globulus* [69].

### 2.3.6. Near-infrared spectroscopy (NIRS)

NIRS is a fast nondestructive technique that requires minimal or no sample preparation. The NIR region spans the wavelength range 780–2500 nm (12,821–4000  $\text{cm}^{-1}$ ), in which absorption bands correspond mainly to overtones and combinations of fundamental molecular vibrations, especially stretching and bending [70, 71]. The NIR spectrum is a superposition of scatter and light absorbance signals [72] and consequently contains information specific to the molecular vibrational aspects and their physical environments. NIRS methods require multivariate calibration algorithms (PCA, SIMCA, PCR or PLSR) usually referred to as chemometric methods to model spectral response to chemical or physical properties of a calibration sample set [71]. Spectra pre-processing is used to eliminate or minimize variability not related to the investigated property [72] and include normalization, derivatives (usually first or second), multiplicative scatter correction (MSC), standard normal variate (SNV), de-trending (DT) or a combination thereof [70]. NIRS has been successfully applied to evaluate different features of lignocellulosic materials [73, 74]. For instance, it was used to estimate Klason lignin content in *E. camaldulensis* and *L. leucocephala* wood [75], and lignin composition in maritime pine wood [76], and in *E. camaldulensis* and *E. urophylla* wood [44] by screening the H/G and S/G ratios, respectively.

### 2.3.7. Two-dimensional nuclear magnetic resonance (2D NMR)

Nuclear magnetic resonance spectrometry (NMR) is based on the measurement of absorption of electromagnetic radiation in the radiofrequency region of 4–900 MHz [77]. The NMR parameters, chemical shifts ( $\delta$ ), coupling constants ( $J$ ), relaxation times and signal intensities, are related to the



electronic structure and chemical environments of nuclei involved in the resonance phenomenon [78]. NMR spectroscopy can be proton ( $^1\text{H}$ ) NMR or carbon ( $^{13}\text{C}$ ), and a two-dimensional (2D)  $^{13}\text{C}$ - $^1\text{H}$  correlation spectrum has also been used. The acquisition can be made by: i) homonuclear correlation spectroscopy: COZY, TOCSY meaning that the protons correlate with the other protons; and ii) heteronuclear correlation ( $^{13}\text{C}$ - $^1\text{H}$ ): HMQC or HSQC where each carbon correlates with its attached proton; HMQC-TOCSY or HSQC-TOCSY where a carbon correlates with the proton attached to him and to other protons in the same coupling network [79]. The 2D HSQC NMR spectra are analyzed in the aromatic and the aliphatic regions. The aromatic region contains the correlations of C2-H2 at  $\delta_{\text{C}}/\delta_{\text{H}}$  100–150/6.0–9.0 and includes the hydroxycinnamates and cinnamyl alcohol end-groups [49]. The H:G:S relation is quantified by volume integration of the contours. Also, the presence of acylated structures in lignin is identified in this region (especially in C $\gamma$ ). The aliphatic-oxygenated region (around  $\delta_{\text{C}}/\delta_{\text{H}}$  50–90/2.5–6.0) provides information on the inter-unit bonds in lignin. Signals from the  $\beta$ -O-4' alkyl-aryl ethers, phenylcoumarans, resinols and dibenzodioxocins are a few examples of signals that are visualized in this region. The presence of polysaccharides signals is also detected here [49]. Overall, NMR is a powerful tool for structural investigation, since it allows to accurately assess the chemical structures, functionalities and nature of chemical bonds in the lignin macromolecule [78, 79], including an accurate measurement of the S/G ratio.

### 3. Lignin composition in biomass

Lignin differs naturally in content and composition between biomass materials at various levels, e.g., between species, within species and between components (such as wood and bark), and is influenced by plant growth stage and environmental stress [2, 15, 62, 65, 80].

Most studies on lignin composition were focused on the economic important pulpwoods, such as the pine and spruce softwoods, and the eucalyptus and birch hardwoods. The S/G ratio has been the mostly evaluated lignin composition parameter due to its importance in the pulping reactions; it is considered a pulpwood quality trait and a selection parameter in breeding programmes. Lignin composition has also been investigated in herbaceous plants in relation with their use in animal nutrition. More recently, and with the growing interest of biomass as a feedstock for biorefineries, lignin has increasingly been investigated in different species and components, namely in barks.

**Table 1** makes a synthesis of the available information on lignin composition in wood of gymnosperms and angiosperms, in biomass from monocotyledons and barks. **Figure 3** compares the monomeric composition of lignin (S/G) in wood and bark for the species for which this determination was made.

#### 3.1. Wood of gymnosperms

Softwoods are gymnosperms (mostly conifers), generally needle-leaved evergreen trees, e.g., pines (*Pinus* spp.) and spruces (*Picea* spp.). The lignin content in softwoods varies between 25 and 35% and consists almost exclusively of guaiacyl units with a low proportion of *p*-hydroxyphenyl

	Total lignin (% o.d.)	H:G:S	S/G or H/G*	Linkages (%) β-O-4', phenylcoumarans, resinols	Acylation (%)	Ref.
<b>Gymnosperms</b>						
<i>Pice abies</i>						
wood	25-29%	-	-	69%, 18%, 10%	no	[37, 68, 80, 93, 84]
<i>Pinus pinaster</i>						
wood	23-30%	-	0.041	-	-	[91-94]
bark	33%	20:80:0	0.25*	-	-	[167]
<i>Pinus taeda</i>						
wood	28%	-	0.01*	-	-	[95]
bark	33-44%	-	0.59*	-	-	[95, 166]
<i>Pinus sylvestris</i>						
wood	19-25%	-	0.048*	-	-	[96-98]
<b>Angiosperms Dicotyledons</b>						
<i>E. globulus</i>						
wood	15-28%	-	1.5-2.9	76%, 2%, 17%	no	[105-113]
younger trees	-	1:4:6	1.3	68%, 5%, 18%	-	[108]
older trees	-	1:10:39	3.8	69%, 1%, 19%	-	[108]
bark	-	1:29:23	0.80	-	-	[179]
<i>Betula pendula</i>						
wood	22-25%	-	3.0-7.0	-	-	[116, 117]
bark (cork)	14%	1:43:6	0.1			[176, 177]
<i>Fagus sylvatica</i>						
wood	20%	1:35:26	0.75	-	-	[118, 119]
<i>Acacia mangium</i>						
wood	28%	1:1:0.1	-	40%, 43%,	-	[122]
<i>Quercus suber</i>						
wood	24%	1:44:55	1.2	77%, 9%, 8%	-	[125]
phloem	38%	1:58:41	0.7	71%, 13%, 7%	-	[125]
bark (cork)	27%	1:43:7	0.1	68%, 20%, 4%	48%, at G-units	[125, 170, 173]
<i>Tectona grandis</i>						
sapwood	32-43%					
	32%	1:34:24;	0.7	-	-	[126]

	Total lignin (% o.d.)	H:G:S	S/G or H/G*	Linkages (%) β-O-4', phenylcoumarans, resinols	Acylation (%)	Ref.
heartwood	32%	1:29:23	0.8	-	-	[126]
bark	22%	1:11:9	0.8	-	-	[126, 178]
<i>Cynara cardunculus</i>						
stalks	16-19%	0:58:42	0.7	70%, 14%, 7%	12%, acetates (S-units)	[131, 134–136]
depithed stalks	23-26%	1:6:8	1.3	-	-	[135]
pith	19-25%	1:4:9	2.1	-	-	[135]
<b>Monocotyledons</b>						
<i>Arundo donax</i>						
stalks	20%	1:61:38	0.62	79%, 8%, 10%	43%	[139, 142]
internode	16-22%	1:2:0.5	1.2	49%, -, -	-	[140, 143]
node	15-20%	1:2:0.6	1.1	-	-	[140, 143]
<i>Miscanthus x giganteus</i>						
stalks	13%	1:13:11	0.7	93%, -, -	46% acetate or p-coumarate	[144–146]
<i>Triticum</i>						
stalks	5-16%	1:15:5		75%, 11%, -	10%, acetates in G-units (12%), in S (1%)	[14, 15, 149, 154]
<i>Musa acuminata</i>						
rachis	11%	1:0.7:1	1.4	0.32/C <sub>6'</sub> -, -	-	[159, 160]
leaf sheaths	13%	1:2:0.5	0.25	-	-	[159, 160]
floral stalks	11%	1:1.6:1	0.63	0.12/C <sub>6'</sub> -, -	-	[159, 160]
petioles/midrib	18%	1:1.9:2.1	1.1	-	-	[159, 160]
leaf blades	24%	1:9.3:6.3	0.60	-	-	[159, 160]

**Table 1.** Lignin composition in wood of gymnosperms, angiosperms, in biomass from monocotyledons and barks.

units (HG-lignin) with a methoxyl content of 15–16% [81]. In the compression wood of softwoods, the lignin is enriched in condensed structures such as H units [4] and G units [82] depending on the species.

### 3.1.1. *Picea*

*Picea abies* (Norway spruce) is a fast-growing conifer that is mostly planted in the North of Europe for timber and pulp production. Spruce lignin ranges from 25.3 to 28.6% [37, 80] and was estimated as 28.5% using FTIR modeling [68]. It is composed predominantly of G units with minor amounts of H units [83, 84]. Wood and MWL were analyzed by 2D NMR: the main linkage was the  $\beta$ -O-4' aryl ether bond (69 and 65% of the total intermonomeric bonds), followed by phenylcoumarans (18%), resinols (10 and 11%), dibenzodioxocins (3 and 5%), with no acylation [84]. Similar results were presented by Capanema et al. [85] who compared different structural models for spruce MWL. Lawoko et al. [86] showed the presence of covalent linkages between spruce lignin and carbohydrates. The potential of spruce lignin genetic modification was evaluated by Wadenbäck [87] who could reduce the lignin content and *p*-hydroxyphenyl fraction by 5 and 23%, respectively. The developmental lignification in Norway spruce was studied by Koutaniemi et al. [88, 89] who were able to identify a group of potential genes for the monolignol polymerization. Recently, the spectra of spruce MWL, black liquor and lignin models were studied by  $^{13}\text{C}$ -NMR and it was found that the signals of some carbons can shift if using different solvents and ionization processes [90].

### 3.1.2. *Pinus*

Maritime pine (*Pinus pinaster*) is a fast-growing pine adapted to Mediterranean climates and used for construction and pulping. A total lignin content of 23% was reported by Esteves et al. [91] and a Klason lignin content between 25.8 and 35.3% by Alves et al. [92], whereas lignin content determined by Py-GC/FID was between 23.0 and 29.6% [92]. Pine lignin is a HG-lignin, with H/G ratio values from 0.041 to 0.113 [76, 93]. The prediction of lignin content and composition by NIRS was possible [76, 93]. Baptista et al. [94] using permanganate analysis showed that noncondensed structures predominate in MWL comparatively to lignins isolated from black liquors: the ratio condensed/noncondensed structures was 0.17 in MWL and increased to 1.06 in one of the isolated lignins. The NMR analysis revealed that the delignification induced the formation of new condensed structures ( $\alpha$ -5,  $\beta$ -6 or 5-6) and arabinoglucoronoxylans were the hemicelluloses preferentially linked to the lignin.

Loblolly pine (*P. taeda*) has a total lignin content of 28%, and a H/G ratio of 0.01, i.e., guaiacyl units largely predominate over *p*-hydroxyphenyl units [95].

Lignin content of *Pinus sylvestris* ranges between 18.8 and 24.5% [96, 97]. The H/G ratio ranges from 0.042 to 0.048 (NIR) in trees with a Klason lignin content ranging from 26.9 and 27.8% [98]. The lignin deposition in early and latewood xylem cells was studied by Antanova et al. [99]: the lignification of earlywood cells occurs faster, decreasing the intensity towards the end of latewood cell differentiation.

### 3.1.3. *Pseudotsuga menziesii*

Douglas-fir wood is used for timber and pulping, mainly in North America and also in Europe. The lignin content can range from 19.7 to 32.8% [100, 101]. The MWL was characterized as a HG-type, presenting an total amount of  $\beta$ -O-4' aryl ether bonds of approximately 1700  $\mu\text{mol/g}$  and

a total amount of phenolic hydroxyl groups of 1500  $\mu\text{mol/g}$ , with 40% of condensed structures, and the average molecular weight was 7400 g/mol [42].

### 3.2. Wood of angiosperms

Hardwoods belong to angiosperms, typically broadleaf deciduous trees. Lignin in hardwoods is constituted mainly by guaiacyl and syringyl units (GS-lignin), with a methoxyl content of 21%, and the  $\beta\text{-O-4'}$  as the most common linkage, with a proportion of 71% or higher of the intermonomeric linkages [81, 102, 103].

#### 3.2.1. *Eucalyptus*

*Eucalyptus globulus* is a fast-growing hardwood species that is highly appreciated for pulping [104]. The lignin content determined by wet chemical analysis ranges from 15 to 28% [105–107]. Using the acetyl bromide method, Rodrigues et al. [69] obtained a lignin content of 23–34% (in extractive-free base), and a similar range by FTIR modeling. The monomeric composition analysis shows that it is a GS-lignin with minor amounts of H units. The monomeric composition varies with tree age (1-month, 18-months, 9 years) with H units decreasing and S units increasing: the H:G:S was 1:4:6 (youngest trees) and 1:10:39 (oldest trees) [108]. Sapwood and heartwood present a H:G:S relation of, respectively, 1:8:29 and 1:11:39 [109]. The S/G ratio ranged from 1.5 to 6.4 determined by analytical pyrolysis [109–112] and 2.8 by 2D NMR [84]. Eucalypt MWL was characterized by a predominance of  $\beta\text{-O-4'}$  aryl ether bond (76%), followed by resinol structures (17%) and in minor amounts of phenylcoumarans (2%), spirodienones (2%) and cinnamyl alcohol end groups (3%) [84]. The presence of phenylcoumarans and cinnamyl end groups decreased with age [108]. Eucalypt lignin presents no acylation [84]. Evtuguin et al. [113] obtained similar results with eucalypt lignin isolated by a modified mild acidolysis: high abundance of  $\beta\text{-O-4}$  (0.56/C6) structures, units linked by  $\alpha\text{-O-4}$  bonds (0.23/C6), low presence of phenylcoumaran structures (0.03/C6) and slightly higher amounts of  $\beta\text{-}\beta$  substructures (0.13/C6).

MWL from *E. nitens* and *E. grandis* revealed similar characteristics of those of *E. globulus* lignin: predominance of syringyl units,  $\beta\text{-O-4'}$  aryl ether bonds (83 and 77%, respectively), resinols (9 and 10%) and phenylcoumarans (5 and 8%) [63].

Lignin content differs between eucalyptus species: Neiva et al. [114] reported values of 21.6% (*E. maculata*), 24.0% (*E. camaldulensis*), 24.8% (*E. globulus*), 26.0% (*E. ovata*), 26.6% (*E. sideroxylon* and *E. saligna*), 26.8% (*E. rudis* and *E. viminalis*), 27.1% (*E. botryoides*), 27.8% (*E. grandis*), 29.9% (*E. propinqua*) and 30.8% (*E. resinifera*). The lignin content in *E. urophylla* was 29.9% and presents a S/G of 2.4 [115].

#### 3.2.2. *Betula pendula*

*B. pendula* (silver birch) is a deciduous tree used for timber and pulping. Total lignin content ranges from 22 to 25% [116, 117], and its monomeric composition showed that it is a GS-lignin as determined by thioacidolysis and by oxidation with copper with a S/G ratio ranging from 3 to 7 depending on the tissue, e.g., in lignified xylem, the ratio reached 7 [116].



### 3.2.3. *Fagus sylvatica*

The European beech (*F. sylvatica*) wood is one of the most important and wide spread trees in Europe with a wide range of uses from furniture, musical instruments and pulp. The lignin content in sapwood and heartwood was, respectively, 22.9 and 24.9% [118]. The monomeric composition showed that beech lignin is a HGS-type with a H:G:S of 1:35:26, corresponding to a S/G of 0.75 [119].

### 3.2.4. *Acacia*

Acacia species are fast-growing trees used for timber and pulp production. Total lignin in *A. melanoxylon* ranged from 21.0 to 28.2% [120, 121], whereas in *A. mangium* the value was 28% [122]. Overall, the lignin is of the HGS-type with proportions differing between species. Lignin from *A. mangium* presented a H:G:S relation of 1:16:16 (by NMR) and 1:21:12 (by permanganate oxidation), showing a very low content of H units, with a corresponding S/G of 0.98 and 0.56 [122]. The lignin presented a high degree of condensation and low content of  $\beta$ -O-4' structures that are associated to the low reactivity during pulping [122]. *A. mearnsii*, *A. mangium*, *A. auriculiformis* and hybrids were studied by Nawawi et al. [123] to relate lignin chemical characteristics (content and proportion of aromatic ring types) with pulpability; woods with higher syringyl ratios were easier to delignify due to the higher reactivity of the  $\beta$ -O-4' structures.

### 3.2.5. *Quercus*

*Quercus suber* (cork oak), native to southwestern Europe and northwestern Africa, has a great economic importance as a producer of cork, the raw material used for the world-known wine cork stoppers [124]. The cork oak wood has a lignin content of 23.6%, and its MWL is characterized by a H:G:S molar ratio of 1:44:55, a S/G ratio of 1.2 (from NMR data), predominance of alkyl-aryl ethers ( $\beta$ -O-4') (77%), lower amounts of condensed linkages and is scarcely acetylated, mainly over S units [125].

### 3.2.6. *Tectona*

*Tectona grandis* (teak), a tropical hardwood that grows naturally in Southeast Asia and has been planted in other countries, is one of the commercially most valuable timber species. Lignin content ranges from 32 to 43% [126–128]. Teak lignin composition was studied in sapwood and heartwood by Lourenço et al. [126]: teak has a GS-lignin, with monomeric composition determined by Py-GC/MS(FID) of H:G:S 1:34:24 (sapwood) and 1:29:23 (heartwood) and S/G ratio of 0.71 and 0.81, respectively.

### 3.2.7. *Cynara cardunculus*

*Cynara cardunculus* (cardoon) is an example of an angiosperm plant that is nonwoody (i.e., it forms only primary xylem) and has high productivities in Mediterranean countries [129, 130]. Cardoon is traditionally used for cheese making, fodder and human food [129] and has been researched as a multipurpose resource, e.g., for pulping and energy [131–133]. Lignin content in the stalks varies from 16.4 to 19.2% [131, 134, 135]. The lignin monomeric composition of



cardoon stalks separated in depithed and pith regions shows a H:G:S relation of 1:6:8 and 1:4:9, respectively, determined by Py-GC/MS (FID), corresponding to a S/G ratio of 1.3 and 2.2, respectively [136]. Whole stalks lignin was studied after isolation by the Björkman method and characterized by Py-GC/MS, 2D NMR and DFRC' [136]. The 2D NMR analysis showed that the isolated lignin has a H:G:S relation of 0:58:42, i.e., a S/G ratio of 0.7 and a predominance of  $\beta$ -O-4' alkyl-aryl ether structures (70% of all inter-unit linkages), followed by a considerable amount of condensed structures: phenylcoumarans ( $\beta$ -5', 14%), resinols ( $\beta$ - $\beta$ ', 7%), spirodienones ( $\beta$ -1', 5%) and dibenzodioxocins (5-5', 4%). The lignin was partially acylated (12%) at the carbon  $\gamma$ -OH by acetate groups that were preferentially attached over S units (32% of S units and only 1% of G units). Lourenço et al. [136] showed that sinapyl acetate acts as a real monolignol involved in the lignification of cardoon stalks.

### 3.3. Biomass of monocotyledons

Monocotyledons are angiosperm flowering plants with seeds typically containing only one cotyledon that include the families *Poacea* (grasses, bamboos and sugar cane), *Arecaceae* (palms) and *Musaceae* (bananas) with several species having a great economic importance. Lignin in monocotyledon species is structurally different from softwood and hardwood lignin: it is a HGS-lignin with a higher proportion of *p*-hydroxyphenyl units, a significant amount of hydroxycinnamate esters and some acylation by *p*-coumarates in the S units [4]. The structure and biogenesis of the cell walls of grasses were reviewed by Carpita [137], who concluded that in spite of the differences between grasses and other plants (e.g., in wall composition), the grass cells respond similarly to environmental signals and growth regulators. A review of herbaceous lignin was also published by Buranov and Mazza [15] describing the lignin in different straws (wheat, rice, flax and corn).

#### 3.3.1. *Arundo donax*

*Arundo donax* (giant reed) is a perennial plant that grows in Mediterranean climates with high biomass production and potential for pulp, chemicals and energy [138]. The lignin content in the whole stalk is 21.1% [139] with 16–22% in the internodes and 15–20% in nodes [140]. The lignin is of HGS-type mainly constituted by guaiacyl and syringyl units with some *p*-hydroxyphenyl units [141]. The lignin in the whole stalk has a S/G ratio of 0.62 and a high content of  $\beta$ -O-4' alkyl-aryl ether structures (79%) and less of other structures, such as resinol (9%), phenylcoumaran structures (8%) and spirodienone (3%), and is highly acylated in the C $\gamma$  (43%) [142]. In internodes and nodes, the H:G:S relation was 1:2:0.5 and 1:2:0.6, respectively, corresponding to S/G ratios of 0.25 and 0.3 [140]. Higher values of S/G ratio of 1.23 (internodes) and 1.13 (nodes) were also reported [143]. Internode lignin presented in relation to node lignin more  $\beta$ -O-4' linkages (0.49 *vs.* 0.32 per aromatic unit), suggesting that internode lignin is of a less condensed nature [143].

#### 3.3.2. *Miscanthus*

*Miscanthus sinensis*, *M. sacchariflorus*, *M. tinctorius* and the hybrid *Miscanthus x giganteus* (*M. sinensis*  $\times$  *M. sacchariflorus*) show potential as energy crops [144]. Lignin content varies

from 9.2% in *M. sinensis* to 12.6% in *M. x giganteus* [144]. *M. x giganteus* lignin (MWL) is of HGS-type with a relation of 1:13:11 [145], a S/G ratio of 0.7 and is highly acylated at the C $\gamma$  side chain (46%) with acetate or *p*-coumarate groups [146] and has a predominance of  $\beta$ -O-4' linkages (93% of all linkages, Brosse et al. [144]). Lignins isolated by mild formosolv (AL), alkaline organosolv (BL) and cellulolytic enzymes (CL) showed differences: CL lignin contained more carbohydrates (12.8%), more  $\beta$ -O-4' linkages (82%) and the lowest S/G ratio of 0.7 [147]. Organosolv lignins isolated using different ethanol concentrations (65–95%) showed less carbohydrate content (3.6–1.1%) and molecular weight (2.72–2.25 Ka) with the increase of ethanol concentration, but an increase of *p*-coumaric and ferulic acids while no effect was found in the S/G ratio (0.63) [148].

### 3.3.3. *Triticum*

Wheat is extensively cultivated for seed production, leaving the straw as a widely available residue with great potential for bioenergy, including bioethanol [15, 149]. The lignin content in wheat straw ranges from 5 to 16% [15, 149]. It is a HGS-type of lignin with a proportion of 1:11:5 [14] and 1:10:9 [15], corresponding to S/G ratios of 0.45 and 0.9, respectively. Milled straw lignin presents *p*-coumarates and ferulates, and the flavone triclin was also found to be incorporated into the polymer [14]. The *p*-coumaric acid is ester linked to lignin [150], whereas ferulic acid is linked by ether bonds (52–68%) and ester bonds (32–48%) forming ester-ether bridges in the lignin fragments [151, 152]. Ferulic acid is also ester linked to polysaccharides [15, 153]. The main structures in wheat straw lignin are  $\beta$ -O-4'-ethers (~75%), followed by phenylcoumarans (~11%) among others such as pinorelinol [14, 154]. The lignin is partially acylated (around 10%) at the C $\gamma$ , predominantly with acetates in the guaiacyl units (12%) and in minor amounts in syringyl units (1%) [14].

### 3.3.4. *Bamboo*

*Phyllostachys pubescens* is one of the bamboo species that is used for different purposes, as a material, e.g., flooring, furniture and mats, and as a fiber source. Its lignin content can vary from 14.6 to 18.3% (2-month-old bamboo) and 25.4 to 27.1% (3-year-old bamboo) [155]. Bamboo culm is divided into green, yellow, timber and pith. Milled wood lignins were isolated from green (MWLg) and yellow bamboo (MWLy) and the lignin found to be of a HGS-type with values of 1:9:7 (MWLg) and 1:9:10 (MWLy) and a S/G of, respectively, 0.74 and 1.16 [156]. The main substructures in MWLg and MWLy were  $\beta$ -O-4 alkyl-aryl ether (38.2 and 39.8%, respectively), resinol (6.9 and 6.3%), phenylcoumaran (3.8 and 2.9%), spirodienone (1.7 and 2.1%) and  $\alpha,\beta$ -diaryl ether (0.4 and 0.3%). The lignin of green bamboo presented lower acylation degree (17.2 vs. 21.1%), and in both lignins, triclin was detected [156]. Wen et al. [157] studied MWL of *P. pubescens* of control and torrefied samples. Torrefication promoted depolymerization, demethoxylation, bond cleavage and condensation reactions. The torrefied samples at 275 and 275°C were enriched in lignin content but dramatically different from the original lignin with almost no  $\beta$ -O-4 linkages, resinol and phenylcoumaran that were not detected by 2D NMR. Also, the presence of H units increased; in the starting material, the H:G:S relation was 1:15:34 (S/G of 2.26) and in the torrefied sample at 300°C, the relation was 1:0.3:0.8 (S/G of 2.37). Shao et al. [158] treated bamboo by steam explosion and its MWL

presented a reduction of the  $\beta$ -aryl ether linkages and a reduction of ester bonds between lignin and *p*-coumaric acid.

### 3.3.5. Banana

*Musa acuminata* Colla var. cavendish (Dwarf Cavendish) is one of the banana varieties under commercial production [159]. The banana plant has a HGS-type of lignin but its content and composition differ in the different morphological parts [160]. The lignin content varied from 10.5 to 24.3%: in rachis, 10.5%; floral stalks, 10.7%; leaf sheaths, 13.3%; petioles/midrib, 18.0% and leaf blades, 24.3%. By nitrobenzene oxidation, the lignin composition determined as follows: the G units predominated in leaf blades (with a H:G:S of 1:9.3:6.3), in floral stalk (1:1.6:1) and leaf sheaths (1:2:0.5), and the S/G ratios were 0.68, 0.63 and 0.25, respectively. The S units slightly predominated in petioles/midrib (1:1.9:2.1) and rachis (1:0.7:1.0) with S/G ratio values of 1.10 and 1.42. Oliveira et al. [159] further studied the lignin composition in floral stalks (LFS) and raquis (LR) after lignin isolation by dioxane method. LR presented significantly higher amount of  $\beta$ -O-4' structures (0.32/C6 vs. 0.12/C6) and higher molecular weight (5400 Da vs. 3750 Da) comparatively to LFS. LFS had more condensed structures (~72%) such as  $\beta$ -5' and 5-5' types, whereas in LR the more abundant were the 4-O-5'-diaryl ether structures. Both lignin presented H units linked to coumarates by ester bonds, and both were structurally associated with suberin-like components.

## 3.4. Barks

Barks are complex and heterogeneous components of plants that include phloem and periderm and eventually rhytidome (periderms interspersed by phloem), as schematically represented in Sen et al. [161]. Phloem is produced by the cambium and the periderm by the phellogen [162]. Barks are a largely available residue from the timber and pulp industries mostly used for energy but increasingly considered as potential feedstocks for biorefineries given their chemical and structural diversity [163, 164]. Cork is one component of bark periderms that may attain considerable proportions in some species [165]. The cork from *Quercus suber* (cork oak) is the basis of an important industrial chain and therefore has been extensively studied (as reviewed by Pereira [124]). But overall the literature on barks is limited and little information is available on lignin composition and structural features except for a few cases.

### 3.4.1. Pinus barks

In *Pinus taeda*, bark lignin ranged from 32.9 to 43.5% [95, 166]. It is mainly constituted by guaiacyl and *p*-hydroxyphenyl units, with reported H/G ratio values of 0.59 (the value for wood lignin is 0.01) [95] and 0.28 [166]. Compared to wood lignin, bark lignin contains more condensed structures (5-5' or  $\beta$ -5'), less dibenzodioxocin and  $\beta$ -O-4' structures and fewer methoxyl groups [95].

*P. pinaster* bark has a Klason lignin content of 33.2% (% o.d. initial bark) determined after extraction of the polyphenolic tannin compounds; it is a HG-lignin composed of *p*-hydroxyphenyl and guaiacyl units with a H/G ratio of 0.25 [167].

### 3.4.2. *Quercus* barks

*Quercus suber* cork has a total lignin content ranging from 17.1 to 36.4% [168, 169]. The lignin monomeric composition of cork was assessed after isolation by the Björkman method by Marques [170–173] and Lourenço et al. [125]. The S/G ratio of cork was 0.029 determined by Py-GC/FID and 0.1 by NMR, revealing a strong predominance of guaiacyl units that was also confirmed by FTIR analysis [170]. Cork lignin in young trees was characterized by 2D NMR as a HGS-lignin constituted mainly by guaiacyl units, with a H:G:S of 1:43:7 and a S/G ratio of 0.16. The alkyl-aryl ethers ( $\beta$ -O-4') were the predominant structures (68–77%), but it also contained condensed structures of phenylcoumarans ( $\beta$ -5', 18–20%) and dibenzodioxocins (5-5', 1–5%) [125, 173]. The cork lignin is partially acylated at the C $\gamma$  of the side chain (48–50%), mainly over G units [125, 173]. Ferulic acid was present in cork lignin (6%) and considered to participate in the crosslinking between lignin and suberin [173]. Lopes et al. [174] isolated the cork lignin by an organosolv protocol and characterized it by permanganate oxidation. The lignin presented a H:G:S relation of 1:47:3 with a S/G ratio of 0.05.

Lourenço et al. [125] also studied the lignin content and composition in the bark phloem after isolation by Björkman method. The lignin content was 38.4% (% o.d material), with a H:G:S ratio of 1:58:41 and a S/G ratio of 0.7 (from NMR). The lignin was characterized mainly by  $\beta$ -O-4' alkyl-aryl ethers (71%), with low amounts of condensed linkages, and was scarcely acetylated, mainly over S units.

Cork from *Quercus cerris* bark has a lignin content of 28.1% [175], and the monomeric composition is mainly of G units, with a low proportion of S and H units, respectively, 93.7, 2.7 and 3.6%, that correspond to a S/G ratio of 0.03 [176].

### 3.4.3. *Betula pendula* barks

Cork from *Betula pendula* presents a lignin content of 14.3% [177], characterized predominantly by guaiacyl units (85.7%) with a minor proportion of syringyl units (11.9%) and *p*-hydroxyphenyl (2.4%), which correspond to a S/G of 0.14, determined by Py-GC-MS/FID [176].

### 3.4.4. *Tectona grandis* barks

Teak bark has a lignin content of 22.4% determined by wet chemistry [178]. It has a GS-lignin with a composition in H:G:S of 1:11:9, and a S/G ratio of 0.8 [126].

### 3.4.5. *Eucalyptus globulus* barks

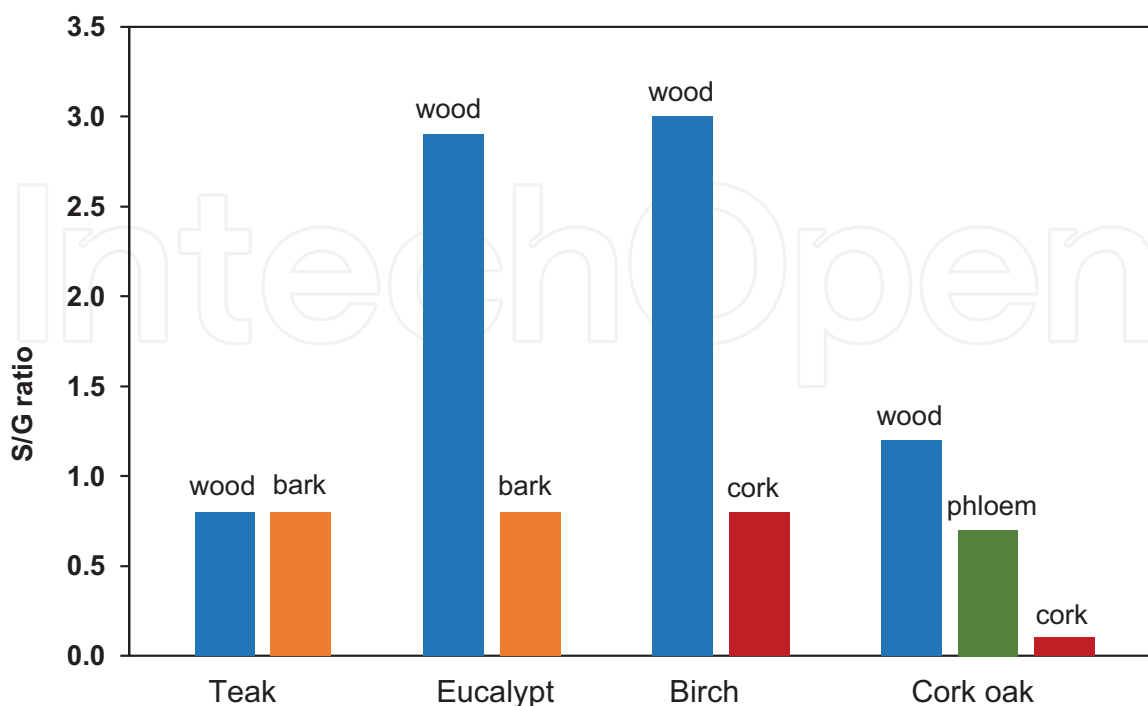
Lignin from eucalypt bark was isolated by mild acidolysis and characterized by nitrobenzene oxidation (NO) and NMR ( $^{13}\text{C}$  and  $^{31}\text{P}$  NMR) [179]. Bark lignin was of HGS-type, with a H:G:S relation of 1:6:18 ( $^{13}\text{C}$  NMR). The S/G ratio values differed between techniques, with the higher values attained by the nitrobenzene oxidation since it only quantifies the noncondensed structures: 1.5 (NMR), 1.5 ( $^{31}\text{P}$  NM), 3.17 ( $^{13}\text{C}$  NMR) and 5.9 (NO).



### 3.5. Variability of lignin composition at tissue and cell levels

Few studies have compared lignin composition in different biomass components of the same plant (**Figure 3**). For *Quercus suber*, lignin was characterized in wood, phloem and cork, revealing a distribution of H:G:S quite distinct. Wood and phloem were enriched in S-lignin with H:G:S values of 1:45:55 and 1:58:41, respectively, whereas cork was considerably enriched in G units and with minor proportions of H and S units (2:85:13) [125]. Similar differences were found in the proportions of interunit linkages: wood and phloem presented more alkyl-aryl ethers ( $\beta$ -O-4') representing 77 and 71% in accordance to the predominance of S units in these tissues, whereas lower value was reported in cork (68%). Therefore, the main condensed structures were found in cork: 20% phenylcoumarans and 5% dibenzodioxocins.

Variation of lignin composition also occurs at cellular level. Lignin formation and composition are cell specific, e.g., lignin differs between tracheary elements, vessels and sclerenchyma cells, and presents a distinctive feature at subcellular localization [2, 180]. During the early phases of xylem lignification, the H units are incorporated in the cell and G units are present in the middle lamella and cell corners, whereas in the next phase, the lignification of the cell primary wall and outer layers of secondary wall is mainly by G units [181–183]. In *Arabidopsis*, xylem vessels have a predominance of H units and cell corners and middle lamella have a G-lignin, while the fibers are rich in S units [184]. In white birch, the vessels secondary wall has a G:S relation of 88:12, the fibers 12:88 and the ray parenchyma 49:51 [185]. In *Acer* species, Watanabe and Fukazawa [186] observed several patterns: in some, vessels and fibers were richer in S units, whereas in others, vessels and fibers were richer in G units; but it was also observed that vessels were richer in G units and fibers richer in S units. Saito et al. [187] confirmed this last observation in *A. micranthum*, as well as Wu et al. [188] when they analyzed 25 Chinese hardwoods species.



**Figure 3.** Variation of S/G in wood and barks of *Tectona grandis* (teak) and *Eucalyptus globulus* (eucalypt), wood and cork of *Betula pendula* (birch) and wood, phloem and cork of *Quercus suber* (cork oak).

## 4. Concluding remarks

This chapter provides an overview on lignin content and composition, showing its complexity as a polymer and variability between and within plant species. The lignin polymer is built with three monomers (*p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol), which are known since the early lignin research studies, to which other monomers were more recently added (hydroxycinnamic acids and aldehydes, coniferyl and sinapyl acetates). However, the biosynthetic pathway of monomer formation and lignin construction are still open research areas seeking to explain the variability that is found as well as the flexibility in lignin construction.

Lignin diversity is well demonstrated when analyzing the available data on the lignin monomeric composition of various species and biomass components. The usual classification of lignin in three types—HG-lignin, GS-lignin and HGS-lignin—broadly assigned to softwoods, hardwoods and monocots, respectively, is a crude generalization and do not encompass the diversity found within each group. It is true that the knowledge on lignin composition and structure is still restricted to a limited number of species and plant components. This is clearly an area in which more research is needed. The various analytical tools that have been developed, including wet chemistry, spectroscopic, magnetic and pyrolytic methodologies allow a better insight into lignin structure and the possibility of making a much more extensive coverage of biomass materials.

Lignin plays an important role in plant cell walls providing support and protection, and it is the second most abundant polymer in nature after cellulose. Increased knowledge on lignin will therefore contribute to our understanding of plant physiology and adaptation, as well as support a lignin platform within future biorefineries providing combined valorization routes for chemicals, materials and energy.

## Acknowledgements

The support for this work was provided by Fundação para a Ciência e a Tecnologia (FCT) through funding of the Forest Research Center (UID/AGR/00239/2013). Ana Lourenço acknowledges support from FCT through a postdoctoral grant (SFRH/BPD/95385/2013). A word of appreciation to Vanda Oliveira, Duarte M. Neiva and Jorge Gominho for their help.

## Author details

Ana Lourenço\* and Helena Pereira

\*Address all correspondence to: [analourenco@isa.ulisboa.pt](mailto:analourenco@isa.ulisboa.pt)

Forest Research Center, School of Agriculture, University of Lisbon, Lisboa, Portugal



## References

- [1] Boudet A. Lignins and lignification: Selected issues. *Plant Physiology and Biochemistry*. 2000;**38**:81-96. DOI: 10.1016/S0981-9428(00)00166-2
- [2] Campbell MM, Sederoff RR. Variation in lignin content and composition. Mechanism of control and implications for the genetic improvements of plants. *Plant Physiology*. 1996; **110**:3-13
- [3] Donaldson LA. Lignification and lignin topochemistry – An ultrastructural view. *Phytochemistry*. 2001;**57**:859-873. DOI: 10.1016/S0031-9422(01)00049-8
- [4] Boerjan W, Ralph J, Baucher M. Lignin biosynthesis. *Annual Review of Plant Biology*. 2003;**54**:519-546. DOI: 10.1146/annurev.arplant.54.031902.134938
- [5] Vanholme R, Morreel K, Ralph J, Boerjan W. Lignin engineering. *Current Opinion in Plant Biology*. 2008;**11**:278-285. DOI: 10.1016/j.pbi.2008.03.005
- [6] Henriksson G. Lignin. In: Ek M, Gellerstedt G, Henriksson G, editors. *Pulp and Paper Chemistry Technology. Wood Chemistry Wood Biotechnology*. Vol. 1. Berlin: Walter de Gruyter GmbH Co; 2009. p. 121-145
- [7] Rydholm SA. *Pulping Process*. Hertfordshire: Interscience Publishers, John Wiley Sons; 1965
- [8] Biermann CJ. *Essentials of Pulping and Papermaking*. San Diego: Academic Press; 1983
- [9] Biermann CJ. *Handbook of Pulping and Papermaking*. 2nd ed. San Diego: Academic Press Limited; 1996
- [10] Gellersted G, Lindfors E. Structural changes in lignin during kraft pulping. *Holzforschung*. 1984;**38**:151-158. DOI: 10.1515/hfsg.1984.38.3.151
- [11] Gierer J. Chemistry of delignification. Part 1: General concept and reactions during pulping. *Wood Science and Technology*. 1985;**19**:289-312. DOI: 10.1007/BF00350807
- [12] del Río JC, Gutiérrez A, Rodríguez IM, Ibarra D, Martínez AT. Composition of non-woody plant lignins and cinnamic acids by Py-GC/MS, Py/TMAH and FT-IR. *Journal of Analytical and Applied Pyrolysis* 2007;**79**:39-46. doi:10.1016/j.jaap.2006.09.003
- [13] del Río JC, Prinsen P, Rencoret J, Nieto L, Jiménez-Barbero J, Ralph J, Martínez AT, Gutiérrez A Structural characterization of the lignin in the cortex and pith of elephant grass (*Pennisetum purpureum*) stems. *Journal of Agricultural and Food Chemistry* 2012;**60**:3619-3634. DOI: 10.1021/jf300099g
- [14] del Río JC, Rencoret J, Prinsen P, Martínez AT, Ralph J, Gutiérrez A Structural characterization of wheat straw lignin as revealed by analytical pyrolysis, 2D-NMR, and reductive cleavage methods. *Journal of Agricultural and Food Chemistry* 2012;**60**:5922-5935. DOI: 10.1021/jf301002n

- [15] Buranov AU, Mazza G. Review: Lignin in straw of herbaceous crops. *Industrial Crops and Products*. 2008;**28**:237-259. DOI: 10.1016/j.indcrop.2008.03.008
- [16] Marques G, Rencoret J, Gutiérrez A, del Río JC Evaluation of the chemical composition of different non-woody plant fibers used for pulp and paper manufacturing. *The Open Agriculture Journal*. 2010;**3**:93-101. DOI: 10.2174/1874331501004010093
- [17] Ralph J, Lundquist K, Brunow G, Lu F, Kim H, Schatz PF, Marita JM, Hatfield RD, Ralph SA, Christensen JH, Boerjan W. Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids. *Phytochemistry Reviews*. 2004;**3**:29-60
- [18] Grabber JH, Schatz PF, Kim H, Lu F, Ralph J. Identifying new lignin bioengineering targets: 1. Monolignol-substitute impacts on lignin formation and cell wall fermentability. *BMC Plant Biology*. 2010;**10**:114-127. DOI: 10.1186/1471-2229-10-114
- [19] Ralph J. Hydroxycinnamates in lignification. *Phytochemistry Reviews*. 2010;**9**(1):65-83. DOI: 10.1007/s11101-009-9141-9
- [20] Terashima N, Fukushima K. Heterogeneity in formation of lignin – XI: An autoradiographic study of the heterogeneous formation and structure of pine lignin. *Wood Science and Technology*. 1988;**22**:259-270
- [21] Dixon RA, Chen F, Guo D, Parvathi K. The biosynthesis of monolignols: A “metabolic grid”, or independent pathways to guaiacyl and syringyl units? *Phytochem*. 2001;**57**:1069-1084. DOI: 10.1016/S0031-9422(01)00092-9
- [22] Freudenberg K, Neish AC. *Constitution and Biosynthesis of Lignin*. Berlin, Germany: Springer-Verlag; 1968. p. 132. ISBN 978-3-540-04274-7
- [23] Sarkanen KV, Ludwig CH, editors. *Lignins, occurrence, formation, structure and reactions*. California: Wiley-Interscience; 1971. p. 916. ISBN 0471754226
- [24] Brunow G, Lundquist K, Gellerstedt G. Lignin. In: Sjöström E, Alén R, editors. *Analytical Methods in Wood Chemistry, Pulping and Papermaking*. Berlin, Germany: Springer-Verlag; 1999. p. 77-124
- [25] Dimmel D. Overview. In: Heitner C, Dimmel DR, Schidt JA editors. *Lignin and lignans. Advances in Chemistry*. Taylor and Francis Group. New York, USA: CRC Press; 2010. p. 1-10
- [26] Lewis NG, Davin LB. The biochemical control of monolignol coupling and structure during lignan and lignin biosynthesis. In: Lewis NG, Sarkanen S, editors. *Lignin and Lignan Biosynthesis, Vol 697*. American Chemistry Society Symposium Series. Washington, USA. 1998. p. 334-361
- [27] Gang DR, Costa MA, Fujita M, Dinkova-Kostova AT, Wang HB, Burlat V, Martin W, Sarkanen S, Davin LB, Lewis NG. Regiochemical control of monolignol radical coupling: A new paradigm for lignin and lignin biosynthesis. *Chemistry and Biology*. 1999;**6**:143-151. DOI: 10.1016/S1074-5521(99)89006-1

- [28] Davin LB, Lewis NG. Dirigent proteins and dirigent sites explain the mystery of specificity of radical precursor coupling in lignin and lignin biosynthesis. *Plant Physiology*. 2000;**123**:453-461. DOI: 10.1104/pp.123.2.453
- [29] Davin LB, Lewis NG. Lignin primary structures and dirigent sites. *Current Opinion in Biotechnology*. 2005;**16**:407-415. DOI: 10.1016/j.copbio.2005.06.011
- [30] Ralph J, Brunow G, Harris PJ, Dixon RA, Schatz PF, Boerjan W. Cap 2. Lignification: are lignins biosynthesized via simple combinatorial chemistry or via proteinaceous control and template replication? In: Daayf F, Lattanzio V, editors. *Recent Advances in Polyphenol Research*. Oxford, UK: Wiley-Blackwell; 2008. p. 36-99
- [31] Sederoff RR, MacKay JJ, Ralph J, Hatfield RD. Unexpected variation in lignin. *Current Opinion in Plant Biology*. 1999;**2**:145-152
- [32] Ralph J, MacKay JJ, Hatfield RD, O'Malley DM, Whetten RW, Sederoff RR. Abnormal lignin in a loblolly pine mutant. *Science*. 1997;**277**:235-239. DOI: 10.1126/science.277.5323.235
- [33] Ralph J, Hatfield RD, Piquemal J, Yahiaoui N, Pean M, Lapierre C, Boudet AM. NMR characterization of altered lignins extracted from tobacco plants down-regulated for lignification enzymes cinnamyl alcohol dehydrogenase and cinnamoyl-CoA reductase. *Proceedings of the National Academy of Sciences*. 1998;**95**:12803-12808. DOI: 10.1073/pnas.95.22.12803
- [34] Argyropoulos DS, Menachem SB. Lignin. In: Scheper T, editor. *Advances in Biochemical Engineering/Biotechnology*. Berlin, Germany: Springer-Verlag; 1997;**57**:127-158
- [35] O'Malley DM, Whetten R, Bao W, Chen C, Sederoff R. The role of laccase in lignification. *The Plant Journal*. 1993;**4**(5):751-757. DOI: 10.1046/j.1365-313X.1993.04050751.x.
- [36] Adler E. Lignin chemistry – Past, present and future. *Wood Science and Technology*. 1977;**11**:169-218
- [37] Dence CW. The determination of lignin. In: Lin SY, Dence CW, editors. *Methods in Lignin Chemistry*. Berlin: Springer-Verlag; 1992. p. 33-62
- [38] Dence CW, Lin SY. Introduction. In: Lin SY, Dence CW, editors. *Methods in Lignin Chemistry*. Berlin: Springer-Verlag; 1992. p. 3-21
- [39] Björkman A. Studies on finely divided wood. Part I. Extraction of lignin with neutral solvents. *Sven Papperstidn*. 1956;**13**:477-485
- [40] Fujimoto A, Matsumoto Y, Chang H, Meshitsuka G. Quantitative evaluation of milling effects on lignin structure during the isolation process of milled wood lignin. *Journal of Wood Science*. 2005;**51**:89-91. DOI: 10.1007/s10086-004-0682-7
- [41] Capanema E, Balakshin M, Katahira R, Chang H, Jameel H. How well do MWL and CEL preparations represent the whole hardwood lignin? *Journal of Wood Chemistry and Technology*. 2015;**35**:17-26. DOI: 10.1080/02773813.2014.892993

- [42] Guerra A, Filpponen I, Lucia LA, Argyropoulos DS. Comparative evaluation of three lignin isolation protocols for various wood species. *Journal of Agricultural and Food Chemistry*. 2006;**54**:9696-9705. DOI: 10.1021/jf062433c
- [43] Roland C, Monties B, Lapierre C. Thioacidolysis. In: Lin S Y, Dence CW editors. *Methods in Lignin Chemistry*, Springer-Verlag, Berlin; 1992. p. 334-349
- [44] Ramadevi P, Hedge DV, Varghese M, Kamalakannan R, Ganapathy SP, Gurumurth DS. Evaluation of lignin syringyl/guaiacyl ratio in *Eucalyptus camaldulensis* across three diverse sites based on near infrared spectroscopic calibration modelling with five eucalyptus species and its impact on kraft pulp yield. *Journal of Near Infrared Spectroscopy*. 2016;**24**:529-536
- [45] Marques AV, Pereira H, Meier D, Faix O. Structural characterization of cork lignin by thioacidolysis and permanganate oxidation. *Holzforschung*. 1999;**53**:167-174. DOI: 10.1515/HF.1999.028
- [46] Chen CL. Nitrobenzene and cupric oxide oxidations. In: Lin SY, Dence CW, editors. *Methods in Lignin Chemistry*. Springer-Verlag; 1992. pp. 301-321. ISBN 3-540-50295-5
- [47] Min D, Xiang Z, Liu J, Jamaeel H, Chiang V, Jin Y, Chang H. Improved protocol for alkaline nitrobenzene oxidation of woody and non-woody biomass. *Journal of Wood Chemistry and Technology*. 2015;**35**:52-61. DOI: 10.1080/02773813.2014.902965
- [48] Lapierre C. Determinating lignin structure by chemical degradations. In: Heitner C, Dimmel DR, Schmidt JA, editors. *Lignin and Lignans*. USA: CRC Press; 2010. pp. 11-48
- [49] Mansfield SD, Kim H, Lu F, Ralph J. Whole plant cell wall characterization using solution-state 2D NMR. *Nature Protocols*. 2012;**7**(9):1579-1589. DOI: 10.1038/nprot.2012.064
- [50] Lu F, Ralph J. The DFRC method for lignin analysis. Part 1. A new method for  $\beta$ -aryl ether cleavage: lignin model studies. *Journal of Agricultural and Food Chemistry* 1997;**45**:4655-4660. DOI: S0021-561(97)00539-6
- [51] Lu F, Ralph J. Derivatization followed by reductive cleavage (DFRC method), a new method for lignin analysis: Protocol for analysis of DFRC monomers. *Journal of Agricultural and Food Chemistry* 1997;**45**:2590-2592. DOI: S0021-8561(97)00258-6
- [52] Lu F, Ralph J. Efficient ether cleavage in lignins: the derivatization followed by reductive cleavage procedure as a basis for new analytical methods. In: Lewis NG, Sarkanen S. editors. *Lignin and Lignan Biosynthesis*. New Orleans, USA: American Chemical Society; 1998. p. 294-322
- [53] Ralph J, Lu F. The DFRC method for lignin analysis. 6. A simple modification for identifying natural acetates in lignin. *Journal of Agricultural and Food Chemistry*. 1998;**46**:4616-4619. DOI: 10.1021/jf980680d
- [54] Meier D, Faix O. Pyrolysis-gas-chromatography-mass spectroscopy. In: Lin SY, Dence CW, editors. *Methods in Lignin Chemistry*. New York: Springer Series in Wood Science; 1992. p. 177-199

- [55] Wampler TP. Analytical pyrolysis: An overview. In: Wampler TP, editor. Applied Pyrolysis Handbook. 2nd ed. New York: Taylor Francis Group; 2007
- [56] Bahng M, Mukarakate C, Robichaud DJ, Nimlos MR. Current technologies for analysis of biomass thermochemical processing: A review. *Analytica Chimica Acta*. 2009;**651**:117-138. DOI: 10.1016/j.aca.2009.08.016
- [57] Demirbas A, Arin G. An overview of biomass pyrolysis. *Energy Sources*. 2002;**24**:471-482. DOI: 10.1080/00908310252889979
- [58] Faix O, Meier D, Fortman I. Thermal degradation products of wood. A collection of electron-impact (EI) mass spectra of monomeric lignin derived products. *Holz als Roh-und Werkstoff*. 1990;**48**:351-354
- [59] Faix O, Meier D, Fortman I. Thermal degradation products of wood. Gas chromatographic separation and mass spectrometric characterization of monomeric lignin derived products. *Holz als Roh-und Werkstoff*. 1990;**48**:281-285
- [60] Faix O, Fortman I, Bremer J, Meier D. Thermal degradation products of wood. Gas chromatographic separation and mass spectrometric characterization of polysaccharide derived products. *Holz als Roh-und Werkstoff*. 1991;**49**:213-219
- [61] Faix O, Fortman I, Bremer J, Meier D. Thermal degradation products of wood. A collection of electron-impact (EI) mass spectra of polysaccharide derived products. *Holz als Roh-und Werkstoff*. 1991;**49**:299-304
- [62] Ralph J, Hatfield RD. Pyrolysis-GC-MS characterization of forage materials. *Journal of Agricultural and Food Chemistry*. 1991;**39**:1426-1437. DOI: 10.1021/jf00008a014
- [63] Rencoret J, Marques G, Gutierrez A, Ibarra D, Li J, Gellersted G, Santos I, Jimenez-Barbero J, Martinez A, del Rio JC. Structural characterization of milled wood lignins from different eucalypt species. *Holzforschung* 2008;**62**: 514-526. DOI: 10.1515/HF.2008.096
- [64] Faix O. Fourier transform infrared spectroscopy. In: Lin SY, Dence CW, editors. *Methods in Lignin chemistry*. Berlin, Germany: Springer-Verlag; 1992. p. 83-109. ISBN 3-540-50295-5
- [65] Faix O. Classification of lignins from different botanical origins by FTIR. *Holzforschung*. 1991;**45**:21-27
- [66] Watkins D, Nuruddin M, Hosur M, Tcherbi-Narteh A, Jeelani S. Extraction and characterization of lignin from different biomass resources. *Journal of Materials Research and Technology*. 2015;**4**(1):26-32. DOI: 10.1016/j.jmrt.2014.10.009
- [67] Costa-e-Silva J, Nielsen BH, Rodrigues JC, Pereira H, Wellendorf U. Rapid determination of the lignin content in Sitka spruce (*Picea sitchensis* (bong.) Carr.) wood by Fourier transform infrared spectrometry. *Holzforschung*. 1999;**53**(6):597-602
- [68] Hannrup B, Cahalan C, Chantre G, Grabner M, Karlsson B, Le Bayon I, Jones GL, Muller U, Pereira H, Rodrigues JC, Rosner S, Rozenberg P, Wilhelmsson L, Wimmer R. Genetic parameters of wood properties in *Picea abies*. *Scandinavian Journal of Forest Research*. 2004;**19**(1):14-29. DOI: 10.1080/02827580310019536



- [69] Rodrigues JC, Faix O, Pereira H. Determination of lignin content of *Eucalyptus globulus* wood using FTIR spectroscopy. *Holzforschung*. 1998;**52**(1):46-50. DOI: 10.1515/hfsg.1998.52.1.46
- [70] Blanco M, Villarroya I. NIR spectroscopy: A rapid-response analytical tool. *Trends in Analytical Chemistry*. 2002;**21**(4):240-250
- [71] Workman J Jr., Weyer L. Practical Guide to Interpretive Near-Infrared Spectroscopy. Taylor & Francis Group. New York, USA: CRC Press; 2008. p. 344
- [72] Sandak J, Sandak A, Meder R. Assessing trees, wood and derived products with near infrared spectroscopy: Hints and tips. *Journal of Near Infrared Spectroscopy*. 2016;**24**:485-505
- [73] Schwanninger M, Rodrigues JC, Fackler K. A review of band assignments in near infrared spectra of wood and wood components. *Journal of Near Infrared Spectroscopy*. 2011; **19**:287-308. DOI: <https://doi.org/10.1255/jnirs.955>
- [74] Tsuchikawa S, Schwanninger M. A review of recent near-infrared research for wood and paper. *Applied Spectroscopy Reviews*. 2013;**48**:560-587. DOI: 10.1080/05704920601036707
- [75] Ramadevi P, Meder R, Varghese M. Rapid estimation of kraft pulp yield and lignin in *Eucalyptus camaldulensis* and *Leucaena leucocephala* by diffuse reflectance near-infrared spectroscopy (NIRS). *Southern Forests: A Journal of Forest Science*. 2010;**72**(2):107-111. DOI: 10.2989/20702620.2010.507462
- [76] Alves A, Schwanninger M, Pereira H, Rodrigues J. Calibration of NIR to assess lignin composition (H/G ratio) in maritime pine wood using analytical pyrolysis as the reference method. *Holzforschung*. 2006;**60**:29-31. DOI: 10.1515/HF.2006.006
- [77] Skoog DA, Holler FJ, Crouch SR. Principles of instrumentation analysis. Belmont, USA: Harris D publisher; 2007. ISBN-10:0-495-01201-7
- [78] Robert, D. Characterization in solution: Spectroscopic methods. 5.4. Carbon-13 nuclear magnetic resonance spectrometry. In: Lin SY, Dence CW, editors. *Methods in Lignin Chemistry*, Berlin: Springer-Verlag; 1992. p. 250-273
- [79] Ralph J, Landucci LL. NMR of lignins. Heitner C, Dimmel DR, Schmidt JA, editors. *Lignin and lignans: advances in chemistry*. New York, USA: CRC Taylor & Francis Group; 2010. ISBN 978-1-57444-486-5
- [80] Cristiernin M. Structure of lignins in developing xylem of Norway spruce. *Plant Physiology and Biochemistry*. 2006;**44**:693-699. DOI: 10.1016/j.plaphy.2006.10.015
- [81] Rowell RM, Pettersen R, Han JS, Rowell JS, Tshabalala MA. Cell wall chemistry. Part 1. Structure and chemistry. In: Rowell RM, editors. *Handbook of Chemistry and Wood Composites*. Florida: Taylor Francis; 2005
- [82] Kutsuki H, Higuchi T. Activities of some enzymes of lignin formation in reaction wood of *Thuja orientalis*, *Metasequoia glyptostroboides* and *Robinia pseudoacacia*. *Planta*. 1981; **152**:365-368



- [83] Lundquist K. <sup>1</sup>H NMR spectral studies of lignins. *Nordic Pulp & Paper Research Journal*. 1992;**1**:4-16
- [84] Rencoret J, Marques G, Gutiérrez A, Nieto L, Santos JI, Jiménez-Barbero J, Martínez AT, del Río JC. 2-HSQC-NMR analysis of lignin in wood (*Eucalyptus globulus* and *Picea abies*) and non-woody (*Agave sisalana*) ball-milled plant materials at the gel state. *Holzforchung*. 2009;**63**:691-698. DOI: 10.1515/HF.2009.070
- [85] Capanema EA, Balakhian MY, Kadla JF. A comprehensive approach for quantitative lignin characterization by NMR. *Journal of Agricultural and Food Chemistry*. 2004;**52**:1850-1860. DOI: 10.1021/jf035282b
- [86] Lawoko M, Henriksson G, Gellerstedt G. Characterisation of lignin-carbohydrate complexes (LCCs) of spruce wood (*Picea abies* L.) isolated with two methods. *Holzforschung*. 2006;**60**(2):151-161. DOI: 10.1515/HF.2006.025
- [87] Wadenbäck J. 2006. Lignin studies of transgenic Norway spruce. [Doctoral thesis] Uppsala. ISBN 91-576-7113-3 (pdf), Available from: <https://pub.epsilon.slu.se/1237/>
- [88] Koutaniemi S. Lignin biosynthesis in Norway spruce: From model system to the tree. Academic dissertation; 2007. ISBN 9789521042928 (pdf), Available from: <http://ethesis.helsinki.fi>
- [89] Koutaniemi S, Warinowski T, Kärkönen A, Alatalo E, Fossdal CG, Saranpää P, Laakso T, Fagerstedt KV, Simola LK, Paulin L, Rudd S, Teeri TH. Expression profiling of the lignin biosynthetic pathway in Norway spruce using EST sequencing and real-time RT-PCR. *Plant Molecular Biology*. 2007;**65**:311-328. DOI: 10.1007/s11103-007-9220-5
- [90] Fedulina TG, Kiryushina MF, Shevchenko SM, Pranovich AV. Effects of solvation and ionization on <sup>13</sup>C NMR spectra of lignin model compounds, spruce milled wood lignin and kraft lignin. *Journal of Wood Chemistry and Technology*. 2017;**37**(4):241-250. DOI: 10.1080/02773813.2016.1272126
- [91] Esteves B, Gominho J, Rodrigues JC, Miranda I, Pereira H. Pulping yield and delignification kinetics of heartwood and sapwood of maritime pine. *Wood Chemistry and Technology*. 2005;**25**(4):217-230. DOI: 10.1080/02773810500366656
- [92] Alves A, Schwanninger M, Pereira H, Rodrigues J. Analytical pyrolysis as a direct method to determine the lignin content in wood. Part 1: Comparison of pyrolysis lignin with Klason lignin. *Journal of Analytical and Applied Pyrolysis*. 2006;**76**:209-213. DOI: 10.1016/j.jaap.2005.11.004
- [93] Alves A, Rodrigues J, Wimmer R, Schwanninger M. Analytical pyrolysis as a direct method to determine the lignin content in wood. Part 2: Evaluation of the common model and the influence of compression wood. *Journal of Analytical and Applied Pyrolysis*. 2008;**81**:167-172. DOI: 10.1016/j.jaap.2005.11.004
- [94] Baptista C, Robert D, Duarte AP. Relationship between lignin structure and delignification degree in *Pinus pinaster* kraft pulps. *Bioresource Technology*. 2008;**99**:2349-2356. DOI: 10.1016/j.biortech.2007.05.012

- [95] Huang F, Singh PM, Ragauskas AJ. Characterization of milled wood lignin (MWL) in loblolly pine stem wood, residue and bark. *Journal of Agricultural and Food Chemistry*. 2011;**59**:12910-12916. DOI: 10.1021/jf202701b
- [96] Toivanen T, Alén R. Variations in the chemical composition within pine (*Pinus sylvestris*) trunks determined by diffuse reflectance infrared spectroscopy and chemometrics. *Cellulose*. 2006;**13**:53-61. DOI: 10.1007/s10570-005-9016-1
- [97] Zaman A, Alén R, Kotilainen R. Thermal behavior of scots pine (*Pinus sylvestris*) and silver birch (*Betula pendula*) at 200-230°C. *Wood and Fiber Science*. 2000;**32**(2):138-143
- [98] Fernandes C, Gaspar MJ, Pires J, Alves A, Simões R, Rodrigues JC, Silva ME, Carvalho A, Brito JE, Lousada JL. Physical, chemical and mechanical properties of *Pinus sylvestris* wood at five sites in Portugal. *iForest*. 2017;**10**:669-679. DOI: 10.3832/for2254-010
- [99] Antanova GF, Varksina TN, Zheleznichenko TV, Stasova VV. Lignin deposition during earlywood and latewood formation in scots pine stems. *Wood Science and Technology*. 2014;**48**:919-936. DOI: 10.1007/s00226-014-0650-3
- [100] Socha AM, Plummer SP, Stavila V, Simmons BA, Singh S. Comparison of sugar content for ionic liquid pretreated Douglas-fir woodchips and forest residues. *Biotechnology for Biofuels*. 2013;**6**:61-71. DOI: 10.1186/1754-6834-6-61
- [101] Alvarez-Vasco C, Ma R, Quintero M, Gio M, Geleynse S, Ramasamy KK, Wolcott M, Zhang X. Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): A source of lignin for valorization. *Green Chemistry*. 2016;**18**:5133-5141. DOI: 10.1039/c6gc01007e
- [102] Cristiernin M, Ohlsson AB, Berglund T, Henriksson G. Lignin isolated from primary walls of hybrid aspen cell cultures indicates significant differences in lignin structure between primary and secondary cell wall. *Plant Physiology and Biochemistry*. 2005;**43**: 777-785. DOI: 10.1016/j.plaphy.2005.07.007
- [103] Abreu HS, Latorraca JVF, Pereira RPW, Monteiro MBO, Abreu FA, Amparado KF. A supramolecular proposal of lignin structure and its relation with the wood properties. *Anais da Academia Brasileira de Ciências*. 2009;**81**(1):137-142
- [104] Pereira H, Miranda I, Gominho J, Tavares F, Quilhó T, Graça J, Rodrigues J, Shatalov A, Knapic S. *Qualidade Tecnológica Do Eucalipto Eucalyptus globulus*. Lisboa: Centro de Estudos Florestais ed., Instituto Superior de Agronomia, Universidade Técnica de Lisboa; 2010. 377 p
- [105] Kojima Y, Isaji S, Yoon S, Ona T. Selection criteria of *Eucalyptus globulus* Labill. For production of chemithermomechanical pulps (CTMP). *Holzforschung*. 2008;**62**:71-76. DOI: 10.1515/HF.2008.010
- [106] Miranda I, Pereira H. The variation of chemical composition and pulping yield with age and growth factors in young *Eucalyptus globulus*. *Wood and Fiber Science*. 2002; **34**(1):140-145

- [107] Pereira H. Variability in the chemical composition of plantation eucalypts (*Eucalyptus globulus* Labill.). Wood and Fiber Science. 1988;**20**(1):82-90
- [108] Rencoret J, Gutiérrez A, Neto L, Jiménez-Barbero J, Faulds CB, Kim H, Ralph J, Martínez AT, del Río JC. Lignin composition and structure in young versus adult *Eucalyptus globulus* plants. Plant Physiology 2011;**155**:667-682
- [109] Lourenço A, Gominho J, Marques AV, Pereira H. Variation of lignin monomeric composition during kraft pulping of *Eucalyptus globulus* heartwood and sapwood. Journal of Wood Chemistry and Technology. 2013;**33**:1-18. DOI: 10.1080/02773813.2012.703284
- [110] Rodrigues J, Meier D, Faix O, Pereira H. Determination of tree to tree variation in syringyl/guaiacyl ratio of *Eucalyptus globulus* wood lignin by analytical pyrolysis. Journal of Analytical and Applied Pyrolysis. 1999b;**48**:121-128. DOI: 10.1016/S0165-2370(98)00134-X
- [111] del Río JC, Gutiérrez A, Hernando M, Landín P, Romero J, Martínez AT. Determining the influence of eucalypt lignin composition in paper pulp yield using Py-GC/MS. Journal of Analytical and Applied Pyrolysis 2005;**74**:110-115. DOI: 10.1016/j.jaap.2004.10.010
- [112] Alves A, Simoes R, Stackpole DJ, Villancourt RE, Potts BM, Schwanninger M, Rodrigues J. Determination of syringyl/guaiacyl ratio of *Eucalyptus globulus* wood lignin by near infrared based partial least squares regression models using analytical pyrolysis as the reference method. Journal of Near Infrared Spectroscopy. 2011;**19**(5):343. DOI: 10.1255/jnirs.946
- [113] Evtuguin DV, Neto CP, Silva AMS, Domingues PM, Amado FML, Robert D, Faix O. Comprehensive study on the chemical structure of dioxane lignin from plantation *Eucalyptus globulus* wood. Journal of Agricultural and Food Chemistry. 2001;**49**:4252-4261. DOI: 10.1021/jf010315d
- [114] Neiva DM, Araújo S, Lourenço A, Gominho J, Fernandes L, Simões R, Pereira H. Chemical composition and kraft pulping potential of 12 *Eucalypt* species. Industrial Crops and Products. 2015;**66**:89-95. DOI: 10.1016/j.indcrop.2014.12.016
- [115] Hein PRG, Lima JT, Chaix G. Effects of sample preparation on NIR spectroscopic estimation of chemical properties of *E. urophylla* S.T. Blake wood. Holzforschung. 2010;**64**:45-54. DOI: 10.1515/HF.2010.011
- [116] Fagerstedt KV, Saranpää Tapanila T, Immanen J, Serra JAA, Nieminen K. Determining the composition of lignins in different tissues of silver birch. Plants 2015;**4**:183-195. DOI: 10.3390/plants4020183
- [117] Chen C, Alén R, Lehto J, Pakkanen H. Combustion properties of birch (*Betula pendula*) black liquors from sulfur-free pulping. Journal of Wood Chemistry and Technology. 2016;**36**(6):401-411. DOI: 10.1080/02773813.2016.1203945
- [118] Szczepkowski A, Nicewicz D, Koczon P. The relationship between tree health and chemical composition of beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) wood and polish provenances. Acta Scientiarum Polonorum Silvarum Colendarum Ratio et Industria Lignaria. 2007;**6**(3):77-88

- [119] Choi JW, faix O, Meier D. Characterization of residual lignins from chemical pulps of spruce (*Picea abies* L.) and beech (*Fagus sylvatica* L.) by analytical pyrolysis-gas chromatography/mass spectrometry. *Holzforschung*. 2001;**55**:185-192. DOI: 10.1515/HF.2001.031
- [120] Lourenço A, Baptista I, Gominho J, Pereira H. The influence of heartwood in the pulping properties of *Acacia melanoxylon* wood. *Journal of Wood Science*. 2008;**54**:464-469. DOI: 10.1007/s10086-008-0972-6
- [121] Santos AJA, Anjos O, Pereira H. Prediction of blackwood kraft pulps yields with wood NIR-PLSR models. *Wood Science and Technology*. 2016;**50**(6):1307-1322. DOI: 10.1007/s00226-016-0837-x
- [122] Pinto PC, Evtuguin DV, Neto CP. Chemical composition and structural features of the macromolecular components of plantation *Acaia mangium* wood. *Journal of Agricultural and Food Chemistry*. 2005;**53**:7856-7862. DOI: 10.1021/jf058081b
- [123] Nawawi DS, Syafii W, Tomoda I, Uchida Y, Akiyama T, Yokoyama T, Matsumoto Y. Characteristics and reactivity of lignin in *Acacia* and *Eucalyptus* woods. *Journal of Wood Chemistry and Technology*. 2017;**37**(4):273-282. DOI: 10.1080/02773813.2017.1291684
- [124] Pereira H. *Cork: Biology, Production and Uses*. 1st ed. Elsevier; 2007. 336 p. ISBN-13: 978-0-444-5296-1
- [125] Lourenço A, Rencoret J, Chematova C, Gominho J, Gutiérrez A, del Río JC, Pereira H. Lignin composition and structure differs between xylem, phloem and phellem in *Quercus suber* L. *Frontiers in Plant Science* 2016;**7**:1612. DOI: 10.3389/fpls.2016.01612
- [126] Lourenço A, Neiva D, Gominho J, Marques AV, Pereira H. Characterization of lignin in heartwood, sapwood and bark from *Tectona grandis* using Py-GC-MS/FID. *Wood Science and Technology*. 2015;**49**(1):159-175. DOI: 10.1007/s00226-014-0684-6
- [127] Miranda I, Sousa V, Pereira H. Wood properties of teak (*Tectona grandis*) from a mature unmanaged stand in East Timor. *Journal of Wood Science*. 2011;**57**:171-178. DOI: 10.1007/s10086-010-1164-8
- [128] Windeisen E, Klassen A, Wegener G. On the chemical characterization of plantation teakwood from Panama. *Holz als Roh- und Werkstoff*. 2003;**61**:416-418. DOI: 10.1007/s00107-003-0425-2
- [129] Fernández J, Curt MD, Aguado PL. Industrial applications of *Cynara cardunculus* L. for energy and other uses. *Industrial Crops and Products*. 2006;**24**:222-229. DOI: 10.1016/j.indcrop.2006.06.010
- [130] Gominho J, Lourenço A, Curt MD, Fernández J, Pereira H. *Cynara cardunculus* in large scale cultivation. A case study in Portugal. *Chemical Engineering Transactions*. 2014;**37**: 529-534
- [131] Gominho J, Fernandez J, Pereira H. *Cynara cardunculus* L. – A new fibre crop for pulp and paper production. *Industrial Crops and Products*. 2001;**13**:1-10. DOI: 10.1016/S0926-6690(00)00044-3



- [132] Sengo I, Gominho J, d'Orey L, Martins M, d'Almeida-Duarte E, Pereira H, Ferreira-Dias S. Response surface modeling and optimization of biodiesel production from *Cynara cardunculus* oil. *European Journal of Lipid Science and Technology*. 2010;**112**:310-320. DOI: 10.1002/ejlt.200900135
- [133] Abelha P, Franco C, Pinto F, Lopes H, Gulyurtlu I, Gominho J, Lourenço A, Pereira H. Thermal conversion of *Cynara cardunculus* L. and mixtures with *Eucalyptus globulus* by fluidized bed combustion and gasification. *Energy & Fuels*. 2013;**27**(11):6725-6737. DOI: 10.1021/ef401246p
- [134] Ballesteros M, Negro MJ, Manzanares P, Ballesteros I, Sáez F, Oliva JM. Fractionation of *Cynara cardunculus* (cardoon) biomass by dilute-acid pretreatment. *Applied Biochemistry and Biotechnology*. 2007;**136-140**:239-252. DOI: 10.1007/s12010-007-9055-1
- [135] Lourenço A, Neiva DM, Gominho J, Curt MD, Fernández J, Marques AV, Pereira H. Biomass production of four *Cynara cardunculus* clones and lignin composition analysis. *Biomass and Bioenergy*. 2015;**76**:86-95. DOI: 10.1016/j.biombioe.2015.03.009
- [136] Lourenço A, Rencoret J, Chemetova C, Gominho J, Gutiérrez A, Pereira H, del Río JC. Isolation and structural characterization of lignin from cardoon (*Cynara cardunculus* L.) stalks. *Bioenergy Research*. 2015;**8**(4):1946-1955. DOI: 10.1007/s12155-015-9647-5
- [137] Carpita NC. Structure and biogenesis of cell walls of grasses. *Annual Review of Plant Physiology and Plant Molecular Biology*. 1996;**47**:445-476. DOI: 10.1146/annurev.arplant.47.1.445
- [138] Borin M, Barbera AC, Milani M, Molari G, Zimbone SM, Toscano A. Biomass production and N balance of gigante reed (*Arundo donax* L.) under high water and N input in Mediterranean environments. *European Journal of Agronomy*. 2013;**51**:117-119. DOI: 10.1016/j.eja.2013.07.005
- [139] Shatalov AA, Pereira H. Kinetics of organosolv delignification of fibre crop *Arundo donax* L. *Industrial Crops and Products*. 2005;**21**:203-210. DOI: 10.1016/j.indcrop.2004.04.010
- [140] Neto CP, seca A, Nunes AM, Coimbra MA, Domingues F, Evtuguin D, Silvestre A, Cavaleiro JAS. Variations in chemical composition and structure of macromolecular components in different morphological regions and maturity stages of *Arundo donax*. *Industrial Crops and Products*. 1997;**6**(1):51-58. DOI: 10.1016/S0926-6690(96)00205-1
- [141] Faix O, Meier D, Beinhoff O. Analysis of lignocelluloses and lignins from *Arundo donax* L. and *Miscanthus sinensis* Anderss, and hydroliquefaction of *Miscanthus*. *Biomass*. 1989;**18**:109-126. DOI: 10.1016/0144-4565(89)90088-7
- [142] You T, Mao J, Yuan T, Wen J, Xu F. Structural elucidation of the lignins from stems and foliage of *Arundo donax* Linn. *Journal of Agricultural and Food Chemistry*. 2013;**61**:5361-5370. DOI: 10.1021/jf401277v
- [143] Seca AML, Cavaleiro JAS, Domingues FMJ, Silvestre AJD, Evtuguin D, Neto CP. Structural characterisation of the lignin from the nodes and internodes of *Arundo donax* reed. *Journal of Agricultural and Food Chemistry*. 2000;**48**:817-824. DOI: 10.1021/jf9910988

- [144] Brosse N, Dufour A, Meng X, Sun Q, Ragauskas A. *Miscanthus*: A fast-growing crop for biofuels and chemicals production. *Biofuels, Bioproducts and Biorefining*. 2012;**6**(5):580-598. DOI: 10.1002/bbb.1353
- [145] Hage RE, Brosse N, Chrusciel L, Sanchez C, Sannigrahi P, Ragauskas A. Characterization of milled lignin and ethanol organosolv lignin from *Miscanthus*. *Polymer Degradation and Stability*. 2009;**94**:1632-1638. DOI: 10.1016/j.polymdegradstab.2009.07.007
- [146] Villaverde JJ, Li J, Ek M, Ligero P, Veja A. Native lignin structure of *Miscanthus x giganteus* and its changes during acetic acid and formic acid fractionation. *Journal of Agricultural and Food Chemistry*. 2009;**57**:6262-6270. DOI: 10.1021/jf900483t
- [147] Wang K, Bauer S, Sun R. Structural transformation of *Miscanthus × giganteus* lignin fractionated under mild formosolv, basic organosolv, and cellulolytic enzyme conditions. *Journal of Agricultural and Food Chemistry*. 2012;**60**:144-152. DOI: 10.1021/jf2037399
- [148] Bauer S, Sorek H, Mitchell VD, Ibáñez AB, Wemmer DE. Characterization of *Miscanthus giganteus* lignin isolated by ethanol organosolv process under reflux condition. *Journal of Agricultural and Food Chemistry*. 2012;**60**:8203-8212. DOI: 10.1021/jf302409d
- [149] Kim S, Dale BE. Global potential bioethanol production from wasted crops and crop residues. *Biomass and Bioenergy*. 2004;**4**:361-375. DOI: 10.1016/j.biombioe.2003.08.002
- [150] Crestini C, Argyropoulos DS. Structural analysis of wheat straw lignin by quantitative <sup>31</sup>P and 2D NMR spectroscopy. The occurrence of ester bonds and α-O-4 substructures. *Journal of Agricultural and Food Chemistry*. 1997;**45**(4):1212-1219. DOI: S0021-8561(96)00568-7
- [151] Sun R, Sun X, Zhang S. Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil palm frond fiber, and fast-growing poplar wood. *Journal of Agricultural and Food Chemistry*. 2001;**49**:5122-5129. DOI: 10.1021/jf010500r
- [152] Sun RC, Sun XF, Wang SQ, Zhu W, Wang XY. Ester and ether linkages between hydroxycinnamic acids and lignins from wheat, rice, rye, and barley straws, maize stems, and fast-growing poplar wood. *Industrial Crops and Products*. 2002;**15**(3):179-188. DOI: 10.1016/S0926-6690(01)00112-1
- [153] Sun R, Lawther JM, Banks WBA. Tentative chemical structure of wheat straw lignin. *Industrial Crops and Products*. 1997;**6**:1-8. DOI: 10.1016/S0926-6690(96)00170-7
- [154] Sun X, Sun R, Fowler P, Baird MS. Extraction and characterization of original lignin and hemicelluloses from wheat straw. *Journal of Agricultural and Food Chemistry*. 2005;**53**:860-870. DOI: 10.1021/jf040456q
- [155] Wi SG, Lee D, Nguyen QA, Bae H. Evaluation of biomass quality in short-rotation bamboo (*Phyllostachys pubescens*) for bioenergy products. *Biotechnology for Biofuels*. 2017;**10**:127. DOI: 10.1186/s13068-017-0818-9
- [156] Huang C, He J, Du L, Min D, Yong Q. Structural characterization of the lignins from the green and yellow bamboo of bamboo culm (*Phyllostachys pubescens*). *Journal of Wood Chemistry and Technology*. 2016;**36**:157-172. DOI: 10.1080/02773813.2015.1104544



- [157] Wen J, Sun S, Yuan T, Xu F, Sun R. Understanding the chemical and structural transformations of lignin macromolecule during torrefaction. *Applied Energy*. 2014;**121**:1-9. DOI: 10.1016/j.apenergy.2014.02.001
- [158] Shao S, Jin Z, Wen G, Iiyama K. Thermo characteristics of steam-exploded bamboo (*Phyllostachys pubescens*) lignin. *Wood Science and Technology*. 2009;**43**:643-652. DOI: 10.1007/s00226-009-0252-7
- [159] Oliveira L, Evtuguin D, Cordeiro N, Silvestre AJD. Structural characterization of stalks lignin from banana plant. *Industrial Crops and Products*. 2009;**29**:86-95. DOI: 10.1016/j.indcrop.2008.04.012
- [160] Oliveira L, Cordeiro N, Evtuguin DV, Torres IC, Silvestre AJD. Chemical composition of different morphological parts from 'Dwarf Cavendish' banana plant and their potential as a non-wood renewable source of natural products. *Industrial Crops and Products*. 2007;**26**:163-172. DOI: 10.1016/j.indcrop.2007.03.002
- [161] Sen A, Quilhó T, Pereira H. Bark anatomy of *Quercus cerris* L. Var. Cerris from Turkey. *Turkish Journal of Botany*. 2011;**35**:45-55
- [162] Esau K. *Anatomy of Seed Plants*. 2nd ed. New York: John Wiley & Sons; 1960. p 1-4
- [163] Miranda I, Gominho J, Mirra I, Pereira H. Chemical characterisation of barks from *Picea abies* and *Pinus sylvestris* after fractioning into different particle sizes. *Industrial Crops and Products*. 2012;**36**:395-400. DOI: 10.1016/j.indcrop.2011.10.035
- [164] Miranda I, Gominho J, Mirra I, Pereira H. Fractioning and chemical characterisation of barks of *Betula pendula* and *Eucalyptus globulus*. *Industrial Crops and Products*. 2013;**41**:299-305. DOI: 10.1016/j.indcrop.2012.04.024
- [165] Leite C, Pereira H. Cork-containing barks – A review. *Frontiers in Materials*. 2017;**3**:63. DOI: 10.3389/fmats.2016.00063
- [166] Pan S, Pu Y, Foston M, Ragauskas AJ. Compositional characterization and pyrolysis of loblolly pine and Douglas-fir bark. *Bioenergy Research*. 2013;**6**:24-34. DOI: 10.1007/s12155-012-9223-1
- [167] Fradinho DM, Neto CP, Evtuguin D, Jorge FC, Irle MA, Gil MH, Jesus JP. Chemical characterisation of bark and of alkaline bark extracts from maritime pine grown in Portugal. *Industrial Crops and Products*. 2002;**16**:23-32. DOI: 10.1016/S0926-6690(02)00004-3
- [168] Pereira H. Chemical composition and variability of cork from *Quercus suber* L. *Wood Science and Technology*. 1988;**22**:211-218. DOI: 10.1007/BF00386015
- [169] Pereira H. Variability of the chemical composition of cork. *BioResources*. 2013;**8**(2): 2246-2256
- [170] Marques AV, Pereira H, Meier D, Faix O. Quantitative analysis of cork (*Quercus suber* L.) and milled cork lignin by FTIR spectroscopy, analytical pyrolysis, and total hydrolysis. *Holzforschung*. 1994;**48**:43-50. DOI: 10.1515/hfsg.1994.48.s1.43

- [171] Marques AV, Pereira H, Meier D, Faix O. Isolation and characterization of a guaiacyl lignin from saponified cork of *Quercus suber* L. *Holzforschung*. 1996;**50**:393-400. DOI: 10.1515/hfsg.1996.50.5.393
- [172] Marques AV, Pereira H, Rodrigues J, Meier D, Faix O. Isolation and comparative characterization of a Björkman lignin from the saponified cork of Douglas-fir bark. *Journal of Analytical and Applied Pyrolysis*. 2006;**77**:169-176. DOI: 10.1016/j.jaap.2006.03.003
- [173] Marques AV, Rencoret J, Gutiérrez A, del Río JC, Pereira H. Ferulates and lignin structural composition in cork. *Holzforschung* 2015;**70**(4):275-289. DOI: 10.1515/hf-2015-0014
- [174] Lopes M, Neto CP, Evtuguin D, Silvestre AJD, Gil A, Cordeiro N, Gandini A. Products of the permanganate oxidation of cork, desuberized cork, suberin and lignin from *Quercus suber* L. *Holzforschung*. 1998;**52**:146-148. DOI: 10.1515/hfsg.1998.52.2.146
- [175] Sen A, Miranda I, Santos S, Graça J, Pereira H. The chemical composition of cork and phloem in the rhytidome of *Quercus Cerris* bark. *Industrial Crops and Products*. 2010;**31**:417-422. DOI: 10.1016/j.indcrop.2010.01.002
- [176] Marques AV, Pereira H. Lignin monomeric composition of corks from the barks of *Betula pendula*, *Quercus suber* and *Quercus cerris* determined by Py-GC-MS/FID. *Journal of Analytical and Applied Pyrolysis*. 2013;**100**:88-94. DOI: 10.1016/j.jaap.2012.12.001
- [177] Ferreira JPA, Quilhó T, Pereira H. Characterization of *Betula pendula* outer bark regarding cork and phloem components at chemical and structural levels in view of biorefinery integration. *Journal of Wood Chemistry and Technology*. 2017;**37**:10-25. DOI: 10.1080/02773813.2016.1224248
- [178] Baptista I, Miranda I, Quilhó T, Gominho J, Pereira H. Characterisation and fractioning of *Tectona grandis* bark in view of its valorisation as a biorefinery raw-material. *Industrial Crops and Products*. 2013;**50**:166-175. DOI: 10.1016/j.indcrop.2013.07.004
- [179] Costa CAE, Pinto PCR, Rodrigues AR. Evaluation of chemical processing impact on *E. globulus* wood lignin and comparison with bark lignin. *Industrial Crops and Products*. 2014;**61**:479-491. DOI: 10.1016/j.indcrop.2014.07.045
- [180] Barros J, Serk H, Granlund I, Pesquet E. The cell biology of lignification in higher plants. *Annals of Botany*. 2015;**115**:1053-1074. DOI: 10.1093/aob/mcv046
- [181] Takabe K, Fujita H, Harada H, Saiki H. Lignification process of Japanese black pine (*Pinus thunbergii* Parl.) tracheids. *Mokuzai Gakkaishi*. 1981;**27**:813-820
- [182] Takabe K, Fujita M, Harada H, Saiki H. Autoradiographic investigation of lignification in the cell-walls of *Cryptomeria* (*Cryptomeria japonica* D don). *Mokuzai Gakkaishi*. 1985;**31**:613-619
- [183] Terashima N, Fukushima K. Biogenesis and structure of macromolecular lignin in cell wall of tree xylem as studied by microautoradiography. In: Lewis N, Paice M editors. *Plant Cell Wall Polymers: Biogenesis and Biodegradation*, Vol. 399, Chap 11. Washington, USA: American Chemistry Society Symposium Series; 1989. p. 160-168

- [184] Schuetz M, Smith R, Ellis B. Xylem tissue specification, patterning, and differentiation mechanisms. *Journal of Experimental Botany*. 2012;**64**(1):11-31. DOI: 10.1093/jxb/ers287
- [185] Saka S, Goring DAI. The distribution of lignin in white birch wood as determined by bromination with TEM-EDXA. *Holzforschung*. 1988;**42**:149-153. DOI: 10.1515/hfsg.1988.42.3.149
- [186] Watanabe Y, Fukazawa K. Lignin heterogeneity of the cell walls on the genus *Acer*. *Research Bulletins of the Hokkaido University Forests*. 1993;**50**:349-389
- [187] Saito K, Watanabe Y, Shirakawa M, Matsushita Y, Imai T, Koike T, Sano Y, Funada R, Fukazawa K, Fukushima K. Direct mapping of morphological distribution of syringyl and guaiacyl lignin in the xylem of maple by time-of-flight secondary ion mass spectrometry. *The Plant Journal*. 2012;**69**:542-552. DOI: 10.1111/j.1365-313X.2011.04811.x
- [188] Wu J, Fukazawa K, Ohtani J. Distribution of syringyl and guaiacyl lignins in hardwoods in relation to habitat and porosity form in wood. *Holzforschung*. 1992;**46**:181-185. DOI: 10.1515/hfsg.1992.46.3.181