Chapter 3

Compositional Variability of Lignin in Biomass

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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 C9 units, where the hydroxyl group is linked to the C4 and substitutions with one or two methoxyl groups may be present at the C3 and C5. 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 α-, β- and γ-, with Cα attached to the aromatic C1.

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 S1 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 S2 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 S3 layer.

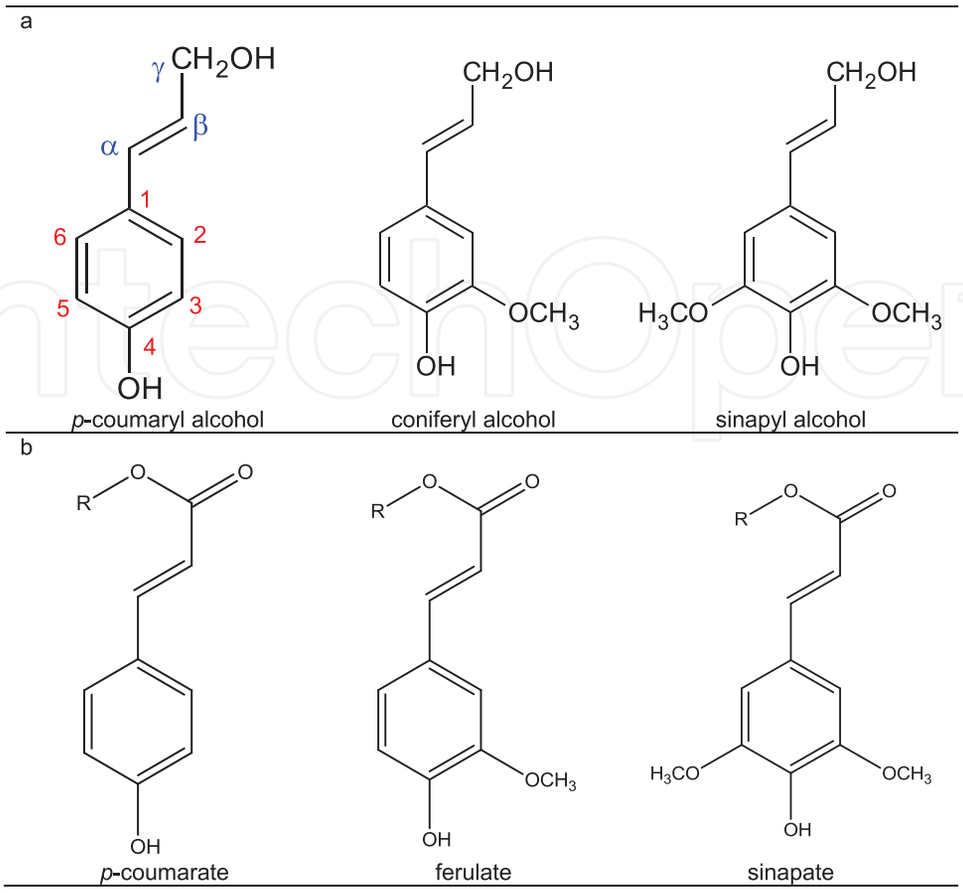

Figure 1. Chemical structures of the primary lignin precursors (a) and the hydroxycinnamates (b). R = H. Adapted from Ralph [19].

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 Cβ, 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_2O_2 system) that are capable of removing a proton from the phenolic hydroxyl forming the resonance-stabilized free radicals, using the H_2O_2 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 Cβ that readily couple into aryl-ether linkages; the β-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 (β-O-4′, 4-O-5′, 1-O-4′) and by carbon-carbon bonds (5–5′, β-5′, β-β′, β-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

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

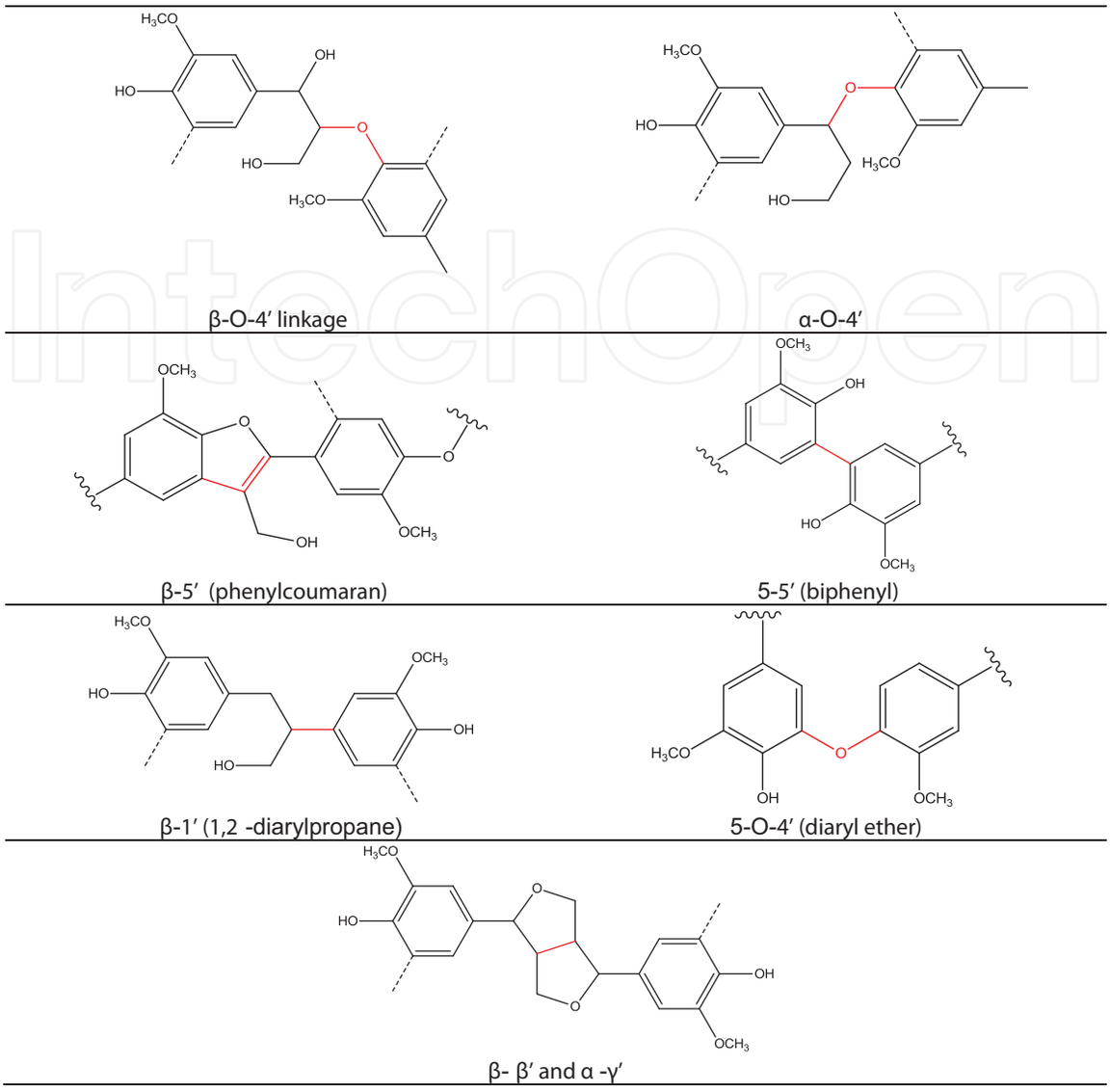

Figure 2. Linkages present in the lignin polymer. Adapted from Dimmel [25].

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 γ-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 μm) 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 cm^{-1} due to the aromatic skeleton vibration of the benzene ring, at 1300 and 1200 cm^{-1} related to syringyl and guaiacyl lignin units and the bands at 1716 and 1711 cm^{-1} are attributed to phenol esterification and the alcohol of the propanoic chain (Cα and Cγ) [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 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 (δ), 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}H) NMR or carbon (^{13}C), and a two-dimensional (2D) ^{13}C-^{1}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}C-^{1}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 δ_C/δ_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 Cγ). The aliphatic-oxygenated region (around δ_C/δ_H 50–90/2.5–6.0) provides information on the inter-unit bonds in lignin. Signals from the β-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.
Gymnosperms						
Pice abies						
wood	25-29%	-	-	69%, 18%, 10%	no	[37, 68, 80, 93, 84]
Pinus pinaster						
wood	23-30%	-	0.041	-	-	[91–94]
bark	33%	20:80:0	0.25*	-	-	[167]
Pinus taeda						
wood	28%	-	0.01*	-	-	[95]
bark	33-44%	-	0.59*	-	-	[95, 166]
Pinus sylvestris						
wood	19-25%	-	0.048*	-	-	[96–98]
Angiosperms Dicotyledons						
E. globulus						
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]
Betula pendula						
wood	22-25%	-	3.0-7.0	-	-	[116, 117]
bark (cork)	14%	1:43:6	0.1			[176, 177]
Fagus sylvatica						
wood	20%	1:35:26	0.75	-	-	[118, 119]
Acacia mangium						
wood	28%	1:1:0.1	-	40%, 43%,	-	[122]
Quercus suber						
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]
Tectona grandis	32-43%					
sapwood	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]
Cynara cardunculus						
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]
Monocotyledons						
Arundo donax						
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]
Miscanthus **x** *giganteus*						
stalks	13%	1:13:11	0.7	93%, -, -	46% acetate or *p*-coumarate	[144–146]
Triticum						
stalks	5-16%	1:15:5		75%, 11%, -	10%, acetates in G-units (12%), in S (1%)	[14, 15, 149, 154]
Musa acuminata						
rachis	11%	1:0.7:1	1.4	0.32/C_6, -, -	-	[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_6, -, -	-	[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 β-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}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 (α-5, β-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 β-O-4′ aryl ether bonds of approximately 1700 μmol/g and

a total amount of phenolic hydroxyl groups of 1500 μmol/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 β-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 β-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 β-O-4 (0.56/C6) structures, units linked by α-O-4 bonds (0.23/C6), low presence of phenylcoumaran structures (0.03/C6) and slightly higher amounts of β-β substructures (0.13/C6).

MWL from *E. nitens* and *E. grandis* revealed similar characteristics of those of *E. globulus* lignin: predominance of syringyl units, β-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 β-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 delignifiy due to the higher reactivity of the β-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 (β-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*

C*ynara 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 β-O-4′ alkyl-aryl ether structures (70% of all inter-unit linkages), followed by a considerable amount of condensed structures: phenylcoumarans (β-5′, 14%), resinols (β-β′, 7%), spirodienones (β-1′, 5%) and dibenzodioxocins (5-5′, 4%). The lignin was partially acylated (12%) at the carbon γ-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 β-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 Cγ (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 β-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 x 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 Cγ side chain (46%) with acetate or p-coumarate groups [146] and has a predominance of β-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 β-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 tricin 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 β-O-4′-ethers (~75%), followed by phenylcoumarans (~11%) among others such as pinoresinol [14, 154]. The lignin is partially acylated (around 10%) at the Cγ, 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 β-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 α,β-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, tricin 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 β-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 β-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 β-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 β-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 β-5′), less dibenzodioxocin and β-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 (β-O-4′) were the predominant structures (68–77%), but it also contained condensed structures of phenylcoumarans (β-5′, 18–20%) and dibenzodioxocins (5-5′, 1–5%) [125, 173]. The cork lignin is partially acylated at the Cγ 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 β-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}C and ^{31}P NMR) [179]. Bark lignin was of HGS-type, with a H:G:S relation of 1:6:18 (^{13}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}P NM), 3.17 (^{13}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 (β-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.

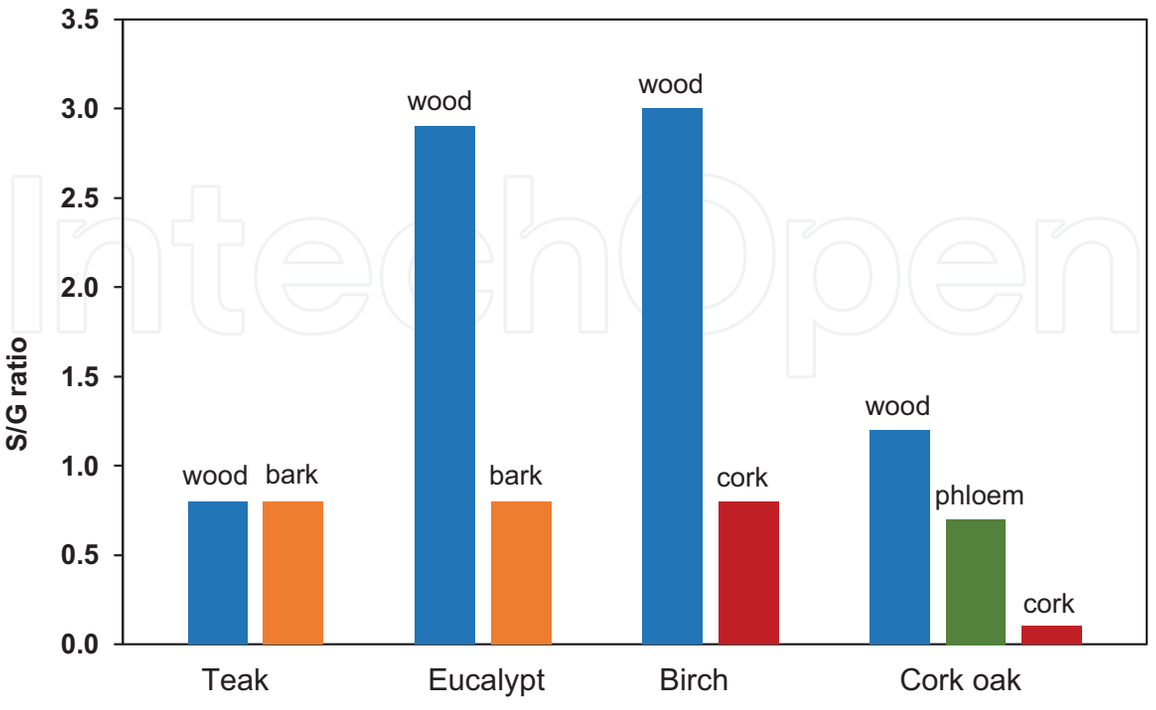

Figure 3. Variation of S/G in wood and barks of *Tectona grandis* (teak) and *Eucalyptus globulus* (eucalypt), wood and cork of *Betula pendula* (birch) and wood, phloem and cork of *Quercus suber* (cork oak).

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.

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