



Article Structural and Thermal Characterization of Milled Wood Lignin from Bamboo (*Phyllostachys pubescens*) Grown in Korea

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Abstract: The structural and thermal characterization of milled wood lignin (MWL) prepared from bamboo (*Phyllostachys pubescens*) grown in Korea was investigated, and the results were compared with bamboo MWLs from other studies. The C₉ formula of the bamboo MWL was C₉H_{7.76}O_{3.23}N_{0.02} (OCH₃)_{1.41}. The Mw and Mn of MWL were 13,000 and 4400 Da, respectively, which resulted in a polydispersity index (PDI) of 3.0. The PDI of the prepared MWL was higher than other bamboo MWLs (1.3–2.2), suggesting a broader molecular weight distribution. The structural features of MWL were elucidated using FT-IR spectroscopy and NMR techniques (¹H, ¹³C, HSQC, ³¹P NMR), which indicate that MWL is of the HGS-type lignin. The major lignin linkages (β-O-4, β-β, β-5) were not different from other bamboo MWLs. The syringyl/guaiacyl ratio, determined from ¹H NMR, was calculated as 0.89. ³¹P NMR revealed variations in hydroxyl content, with a higher aliphatic hydroxyl content in MWL compared to other bamboo MWLs. Thermal properties were investigated through TGA, DSC, and pyrolysis-GC/MS spectrometry (Py-GC/MS). The DTG_{max} of MWL under inert conditions was 287 °C, and the T_g of MWL was 159 °C. Py-GC/MS at 675 °C revealed a syringyl, guaiacyl, *p*-hydroxyphenyl composition of 17:37:47.

Keywords: bamboo; Phyllostachys pubescens; milled wood lignin; structural analysis; thermal analysis

1. Introduction

According to the Food and Agriculture Organization (FAO), the annual rate of deforestation was estimated at 10 million ha between 2015 and 2020. Over the past three decades, an estimated 420 million ha of forest have been lost since 1990 [1]. This rampant forest destruction contributes to approximately 20% of global carbon emissions [2]. Moreover, the excessive use of wood has faced strong criticism due to the continuous depletion of forest resources worldwide [3]. As the world's wood resources decline, there is a growing need for lignocellulosic biomass to replace them. Among these lignocellulosic biomass, bamboo is an emerging biomass that can replace wood because of its short production cycle and high amount of biomass per unit area. In addition, the remarkable regeneration capability and minimal maintenance requirements make bamboo an environmentally friendly alternative [3–5]. The Food and Agriculture Organization (FAO) reports that bamboo is widely grown in tropical and subtropical climatic zones, including in East, Southeast, and South Asia [6]. In historical China, bamboo found diverse applications, such as arrowheads, baskets, writing scrolls, pens, paper, boats, shoes, and construction materials [7]. In modern-day China, bamboo utilization spans various applications, including bamboo shoots, ceiling/flooring interiors, scaffolding, timber, furniture, and crafts [3,8]. According to China's 9th National Forest Inventory, China's bamboo forest area gradually increased from 2014 to 2018, with a total bamboo forest area of 6.73 million ha [6]. In this light, China's research on bamboo remained active over these years. However, in Korea, bamboo consumption is declining, leading to the desolation of domestic bamboo forests [9]; hence, there has not been much research on bamboo in Korea.



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Bamboo can be processed into a variety of products, including particleboards, plywood, laminated bamboo, bamboo composites, and bamboo fiber [10–12]. Truly, the utilization of bamboo is limited due to its hollow structure, and certain bamboo stems may not be affected by certain types of chemicals due to their waxy skin [13]. These impermeable properties may limit chemical treatment to improve mechanical properties for potential applications [3]. In addition to these problems, numerous obstacles still impede the efficient utilization of bamboo resources. Therefore, overcoming these hurdles requires further research efforts.

On the other hand, bamboo can be easily delignified compared to other woody biomass [14,15]. In other words, it offers the advantage of producing pulps, as well as lignin, more readily compared to other conventional methods. Additionally, bamboo lignin contains a substantial amount of *p*-hydroxyphenyl units, a feature nearly absent in softwood and hardwood lignins [16]. The high reactivity of this unit, with vacant positions at 3 and 5, is expected to significantly contribute to future lignin utilization.

In this study, the structural and thermal characteristics of lignin were investigated as a fundamental study for the future utilization of bamboo grown in Korea. In addition, the results obtained were compared with previously reported bamboo lignin.

2. Results and Discussion

2.1. Chemical Composition of Bamboo

Analysis of ash, extracts, carbohydrates, and lignin content was performed to determine the chemical composition of bamboo, and the results are shown in Table 1. The alkaline extract yield was approximately 30%, surpassing that of cold-water, hot-water, and organic solvent extracts. Li et al. [17] investigated changes in the maturation of bamboo (*Phyllostachys pubescens*). The ash, Klason lignin, holocellulose, and α -cellulose content were close to the reported values of 3-year-old bamboo culms.

Ash (%)	1.24 ± 0.01
Extracts (%)	
Cold water	5.77 ± 0.02
Hot water	9.85 ± 0.04
1% NaOH	29.69 ± 0.10
Alcohol-benzene	4.77 ± 0.07
Carbohydrate (%)	
Holocellulose	69.25 ± 0.44
α-Cellulose	44.31 ± 0.16
Hemicellulose *	24.94
Lignin (%)	
Klason	27.20 ± 0.09
Acid-soluble	0.51 ± 0.01
Total	27.71

Table 1. Chemical composition of bamboo.

* Hemicellulose (%) = holocellulose— α -cellulose.

2.2. Chemical and Structural Characterization of Bamboo MWL

2.2.1. Elemental Composition of MWL

The C, H, O, N, S, and OCH₃ contents of MWL are shown in Table 2. The methoxyl content was estimated from the integration ratio of protons originating from aromatic rings and methoxyl groups in the ¹H NMR, following the method outlined by Abreu [18]. The C, H, and O contents of the MWL prepared in this study were similar to MWL-A from *Phyllostachys acuta*. On the other hand, the C content of MWL was lower than that of MWL-X from an unknown bamboo species. The methoxyl content of MWL was found to be higher than the other two bamboo MWLs. This result may be due to the presence of fewer *p*-hydroxyphenyl (H) units, which have no methoxy groups, in the two bamboo lignins, as shown in Table 2 for comparison. In Table 3, the C₉ molecular formula and

formula weight of MWL and other bamboo MWLs are listed. The formula weight of MWL was approximately 214 Da, higher than that of the other two bamboo MWLs.

	Elemental Analysis (%)						Poforonco
-	С	Н	0	Ν	S	OCH ₃	- Kelelelice
MWL	58.39	5.66	34.66	0.12	-	20.47	This study
MWL-A MWL-X	58.78 63.10	5.96 5.67	34.97 31.23	0.28	- -	19.48 17.74	[19] [20]

Table 2. Elemental analyses and methoxyl contents of MWLs.

MWL: *P. pubescens*, A: *P. acuta*, X: unknown bamboo species. Methoxyl content was calculated by the integrations of the aromatic and methoxyl signals in ¹H NMR spectrum [18].

Table 3. C9 f	ormula	and formul	a weight	of MWLs.

	C ₉ Formula	Formular Weight (Da)	Reference
MWL	$C_9H_{7.76}O_{3.23}N_{0.02}(OCH_3)_{1.41}$	214.17	This study
MWL-A	C ₉ H _{7.67} O _{2.72} (OCH ₃) _{1.52}	206.38	[19]
MWL-X	C9H7.53O2.65(OCH3)1.10	191.59	[20]

MWL: P. pubescens, A: P. acuta, X: unknown bamboo species.

2.2.2. Molecular Weight (MW) Distribution, Average MW, and Polydispersity

The weight-average (\overline{Mw}) and number-average (\overline{Mn}) MW, along with the polydispersity index (PDI), of acetylated MWL (Ac-MWL), were determined using gel permeation chromatography (GPC). The MW distribution of Ac-MWL is illustrated in Figure 1, and the corresponding \overline{Mw} , \overline{Mn} , and PDI values are presented in Table 4. For comparison, data on previously reported bamboo MWLs are provided in Table 4. The \overline{Mw} and \overline{Mn} of Ac-MWL were approximately 13,000 Da and 4400 Da, respectively. The \overline{Mw} of Ac-MWL prepared in this study was higher than that of all other bamboo MWLs. In addition, the PDI of Ac-MWL was also higher than that of all other bamboo MWLs, indicating a broader distribution of MW. The Mw, Mn, and PDI differed even from the same bamboo species. This result is likely attributed to the different climatic zones wherein the bamboo was grown, since the Korean-grown bamboo is from temperate regions and the Chinese-grown bamboo is from subtropical regions. The Korean-grown bamboo was believed to have thicker and more rigid culms to withstand colder temperatures and harsher climatic conditions. This robustness was thought to contribute to the greater strength and density of Korean-grown bamboo, potentially resulting in a higher Mw.

Table 4. Average MW and PDI of Ac-MWLs.

	$\overline{\mathbf{M}}\mathbf{w}$ (Da)	Mn (Da)	PDI ($\overline{M}w/\overline{M}n$)	References
Ac-MWL	13,279	4436	3.0	This study
Ac-MWL-A	12,090	5410	2.2	[19]
Ac-MWL-S	6080	3230	1.9	[21]
Ac-MWL-P	6050	3400	1.8	[21]
Ac-MWL-B	7692	4406	1.8	[22]
Ac-MWL-N	9420	7458	1.3	[23]

Ac-MWL: P. pubescens, A: P. acuta, S, P: stem, pith of P. pubescens, B: Dendrocalamus brandisii, N: Neosinocalamus affinis.



Figure 1. MW distribution of Ac-MWL.

2.2.3. FT-IR Spectroscopy

FT-IR spectroscopy was conducted for the determination of functional groups in MWL. In Figure 2, the FT-IR spectrum of MWL is presented. The bands have been assigned according to the work of Faix [24], and the assignments are detailed in Table 5. The observed spectral features of MWL aligned with those of HGS-type lignins found in other bamboo MWLs [19,21,24]. Notably, the band at 834 cm⁻¹, corresponding to C–H out-of-plane vibrations in H units, along with a characteristic shoulder at 1160 cm⁻¹—typical for HGS type—was observed. In addition, the C=O stretching related to unconjugated ketone, carbonyl, and ester groups was assigned at 1718 cm⁻¹, aromatic skeleton vibrations at 1594, 1503, and 1419 cm⁻¹, syringyl (S)-related bands at 1130 and 1123 cm⁻¹, and guaiacyl (G)-related bands at 1330, 1266, 1222, and 1033 cm⁻¹.

Table 5. Assignment of FT-IR spectrum of MWL.

Band (cm ⁻¹)	Assignments
3441	O–H stretching
2843-2937	C-H stretching in methyl, methylene groups
1718	C=O stretching in unconjugated ketone, carbonyl, and ester groups
1664	C=O stretching in conjugated <i>p</i> -substituted aryl ketone
1594	Aromatic skeleton vibration plus C=O stretching; S > G: G _{condensed} > G _{etherified}
1503	Aromatic skeleton vibration $(G > S)$
1462	C–H deformations (asymm in –CH ₃ and –CH ₂ –)
1419	Aromatic skeleton vibration combined with C-H in plane deformations
1365	Aliphatic C–H stretching in CH_3 and phenolic OH
1330	Condensed S and G ring (G ring bound via position 5)
1266	G ring plus C=O stretching (G-methoxyl C–O)
1222	$C-O + C-O + C=O$ stretching ($G_{condensed} > G_{etherified}$)
1160	Typical for HGS lignins; C=O in ester groups (conj.)
1123	Aromatic C–H in-plane deformation (S)
1089	C–O deformation in sec-alcohols and aliphatic ethers
1022	Aromatic C–H in-plane deformation (G > S) + C–O deformation in primary alcohols + C–H
1055	stretching (unconjugated)
921	C-H out of plane (aromatic ring)
834	C–H out of plane in positions (2 and 6 of S + in all positions of H units)



Figure 2. FT-IR (ATR) spectrum of MWL.

2.2.4. ¹H NMR Analysis

The ¹H NMR spectrum of Ac-MWL is presented in Figure 3. The aromatic region (7.20–6.25 ppm) revealed the presence of G and S phenylpropane (C_9) units. The aliphatic content was higher than the aromatic content.



Figure 3. ¹H NMR spectrum of Ac-MWL.

Signal assignments in the ¹H NMR spectrum of Ac-MWL are listed in Table 6, based on literature data [25,26]. To estimate the distribution of protons per C₉ structural unit in Ac-MWL, integration ratios and their C₉ molecular formulas were used [27]. The methoxyl content in the C₉ molecular formula for MWL was 1.41, multiplied by 3 to yield 4.23—the total number of protons in the methoxyl groups. Integration values for other structural components were normalized to the methoxyl protons in a single C₉ unit. However, certain quantitative conclusions cannot be drawn due to overlapping signals, carbohydrate inclusions, and uncertainties in range assignments.

Ppm	Main Assignments	Ac-MWL
7.20-6.80 *	Aromatic proton in G units	1.05
6.80-6.25	Aromatic proton in S units	0.93
6.25-5.75	H_{α} of β -O-4 and β -1 structures	0.47
5.75-5.24	H_{α} of β -5 structures	0.24
5.20-4.90	H of xylan residues	0.29
4.90-4.30	H_{α} and H_{β} of β -O-4 structures	1.47
4.30-4.00	H_{α} of β - β structures, H of xylan residues	0.88
4.00-3.48	H of methoxyl groups	4.23
2.50-2.22	H of aromatic acetates	0.76
2.22-1.60	H of aliphatic acetates	4.38

Table 6. ¹H NMR assignments and distribution of protons per C₉ structural unit of Ac-MWL.

* From reference, it was 7.25–6.80, but CDCl₃ solvent peak was detected at 7.24, thus, the chemical shift was adjusted.

The arylglycerol β -O-4 aryl ether linkage (6.25–5.75 ppm, 4.90–4.30 ppm) is the main intermonomeric linkage found in native lignin [25]. The H_{α} and H_{β} contents from Ac- β -O-4 structures were highest among the linkages. The aromatic protons per C₉ unit for Ac-MWL were determined to be 0.93 for S units and 1.05 for G units. The S/G molar ratio of MWL based on ¹H NMR was determined to be 0.89.

To ascertain the number of aliphatic and phenolic hydroxyl groups per C₉ unit, corresponding acetyl group signals (2.50–1.60 ppm) were examined. The Oac/OCH₃ mole ratio was calculated as (0.76 + 4.38)/4.23, resulting in 1.22. Consequently, the total Oac/C₉ ratio was determined as $(1.41 \text{ OCH}_3) \times (1.22 \text{ Oac}/1 \text{ OCH}_3)$, yielding 1.72. The number of aliphatic Oac/OCH₃ was calculated as $(1.41 \text{ OCH}_3/C_9) \times (4.38 \text{ Oac}/4.23 \text{ OCH}_3)$, resulting in 1.46, while the number of phenolic Oac/OCH₃ was calculated as $(1.41) \times (0.76/4.23)$, resulting in 0.25. Therefore, the estimated number of aliphatic and phenolic hydroxyl groups per 100 C₉ units of MWL was 146 and 25, respectively.

2.2.5. ¹³C NMR Analysis

The structural features and linkages within MWL were further elucidated through ¹³C NMR analysis. In Figure 4, the ¹³C NMR spectrum of MWL is presented, and Table 7 provides the chemical shifts along with their assignments based on previously reported works [16,21,28,29]. The NMR spectrum was divided into four regions: C=O, aromatic, side chain, and aliphatic.



Figure 4. ¹³C NMR spectrum of MWL.

Signal No.	ppm	Assignments
1	170.1	A set al C. O in all shalls (where all
2	169.5	Acetyl C=O in alcohols/phenois
3	166.3	C-9 in <i>p</i> -CE
4	162.0	C-4 in H
5	159.9	C-4 in <i>p</i> -CE
6	152.2	C-3/C-5 in etherified S
7	149.2	C-4 in etherified G, C-3 in etherified G with α -CO
8	147.1	C-3 in G, C-3/C-5 in nonetherified S, C-3 in 5-5 biphenyl
9	145.4	C-4 in nonetherified G, C_{α} in <i>p</i> -CE
10	138.0	C-4 in etherified S
11	134.9	C-1 in etherified S, C-4 in nonetherified S
12	134.4	C-1 in etherified G
13	133.3	C-1 in nonetherified G
14	132.3	C-1 in nonetherified S
15	130.2	C-2/C-6 in <i>p</i> -CE
16	127.9	C-2/C-6 in H
17	125.0	C-1 in <i>p</i> -CE
18	119.1	C-6 in G
19	115.8	C-3/C-5 in <i>p</i> -CE
20	115.2	C-5 in G, C-3/C-5 in H, C-8 in <i>p</i> -CE
21	111.2	C-2 in G
22	106.5	C-2/C-6 in S with α -CO
23	104.2	C-2/C-6 in S, C-4 in β - β resinol
24	103.4	C-2/C-6 in S
25, 26	101.8, 99.5	Residual carbohydrates
27	86.9	C-α in β-5 phenylcoumaran
28	86.1	C-β in β-O-4
29	85.0	$C-\alpha$ in $\beta-\beta$ resinol
30	84.5-81.3	C-β in β-O-4
31	75.6	Residual carbohydrates
32	75.3	C- α in β -1, residual carbohydrates
33	73.4	C- α in β -O-4, residual carbohydrates
34	72.2	$C-\alpha$ in β -O-4
35	71.7	$C-\gamma$ in $\beta-\beta$ resinol
36	68.2	NA *
37	64.9	C- γ in β -5 phenylcoumaran
38	62.7	C- γ in β -5 phenylcoumaran, β -O-4 with α -CO
39	60.1	C- γ in β -O-4
40	55.8	OCH ₃ in S and G
41	29.0	CH_2 in aliphatic side chain
42	20.9	CH_3 in acetyl

Table 7. ¹³C NMR assignment of MWL.

* NA: not assigned.

The signals at positions 1 and 2 correspond to carbonyl groups in MWL. Signals at positions 25, 26, 31, 32, and 33 were attributed to residual carbohydrates, which likely originated from impurities such as traces of hemicelluloses associated with some MWL substructures. The intense signal at position 40 is indicative of OCH₃ in both S and G units.

In the aromatic region (166 to 103 ppm) of the spectrum, several peaks were assigned to S, G, and H units. For S units: C-3/C-5 etherified (6), C-5 nonetherified (8), C-4 etherified (10), C-1 etherified and C-4 nonetherified (11), C-1 nonetherified (14), C-2/C-6 with α -carbonyl (22), C-2/C-6 (23, 24). For G units: C-4 etherified and C-3 etherified G with α -carbonyl (7), C-3 (8), C-4 nonetherified (9), C-1 etherified (12), C-1 nonetherified (13), C-6 (18), C-5 (20), C-2 (21). For H units: C-4 (4), C-2/C-6 (16), and C-3/C-5 (20). Additionally, six peaks were assigned to C-9 (3), C-4 (5), C-2/C-6 (15), C-1 (17), C-3/C-5 (19), and C-8 (20) in *p*-coumaric ester (*p*-CE). The NMR analysis indicated that a considerable amount of *p*-coumaric acid (*p*-CA) is etherified at the γ -position [21,30–33]. The ¹³C NMR results confirmed that MWL is an HGS-type lignin, consistent with the FT-IR results.

The etherified S/nonetherified S unit ratio was estimated based on the peak height ratio at 152.2/147.1 ppm, while the etherified G/nonetherified G ratio was derived from resonance ratios at 149.4/145.5 ppm [34]. The values of etherified S/nonetherified S (4.4) and etherified G/nonetherified G (1.5) suggested a greater involvement of S units in

In the side chain region (87 to 57 ppm) of the spectrum, several peaks were assigned to β -O-4, β - β resinol, β -1, and β -5. For β -O-4: C- α (**33**, **34**), C- β (**28**, **30**), C- γ (**39**), C- γ in β -O-4 with α -carbonyl (**38**); for β - β : C- α (**29**), C- γ (**35**), C-4 (**23**); for β -1: C- α (**32**); and for β -5: C- α (**27**), C- γ (**37**, **38**). No notable differences were observed in the ¹³C NMR results of MWL compared to other bamboo MWLs.

Since MWL is a macromolecule, some overlapping signals were observed in the ¹³C NMR spectrum. Therefore, 2D HSQC NMR analysis was performed to enhance spectral resolutions in intercoupling bonds and linkages within lignin substructures.

2.2.6. 2D HSQC NMR Analysis

Figure 5 depicts the side chain (120–70/5.0–3.0 ppm) and aromatic (160–100/8.0–6.0 ppm) regions of MWL in the HSQC spectrum. The aliphatic region was excluded from the discussion, as notable information was not provided. Cross-signals and their assignments, derived from previously reported works by Wen et al. [21,35,36], are shown in Table 8. The substructures present in MWL, along with their corresponding notations, are presented in Figure 6.



Figure 5. (a) Side chain and (b) aromatic region of the HSQC spectra of MWL.

Fable 8. Assignments of ¹³	C/ ¹ H correlation si	gnals in the HMQC s	pectra of MWL
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	Notation	$\delta_{\rm C}/\delta_{\rm H}$	Main Assignments
	C _β	51.5/3.70	C_{β} – H_{β} in β -5 phenylcoumaran (E)
	B _β	54.1/3.04	C_{β} – H_{β} in β - β resinol (B)
	Ome	56.6/3.71	C–H in methoxyls
	A_{γ}	60.2/3.38-3.89	C_{γ} -H _{γ} in β-O-4 (A)
	C_{γ}	62.8/3.73	C_{γ} – H_{γ} in β -5 phenylcoumaran (E)
	$\dot{F_{\gamma}}$	63.3/4.11	C_{γ} – H_{γ} in <i>p</i> -hydroxycinnamyl alcohol end-group (F)
	B_{γ}	71.8/4.16, 72.1/3.80	C_{γ} - H_{γ} in β - β resinol (B)
	A _α	72.8/4.85	C_{α} – H_{α} in β -O-4 (A)

Notation	$\delta_{\rm C}/\delta_{\rm H}$	Main Assignments
A _{β(G/H)}	82.4/4.34	C_{β} – H_{β} in β -O-4 (A) linked to a G/H units
B_{α}	85.8/4.64	C_{α} -H _{α} in β - β resinol (B)
A _{B(S)}	86.6/4.10	C_{β} – H_{β} in β -O-4 (A) linked to a S units
S _{2.6}	104.8/6.69	$C_{2,6}$ -H _{2,6} in syringyl units (S)
S'' _{2.6}	107.0/7.30	$C_{2.6}$ -H _{2.6} in oxidized (C_{α} OOH) syringyl units (S'')
S'2,6	107.2/7.19	$C_{2,6}$ -H _{2,6} in oxidized (C_{α} =O) syringyl units (S)
G ₂	111.9/6.97	C_2 – H_2 in guaiacyl units (G)
H _{3.5}	113.8/6.67	$C_{3,5}$ - $H_{3,5}$ in H units (H)
G ₅	115.4/6.68	C_5 – H_5 in guaiacyl units (G)
p-CE _{3,5}	116.1/6.78	$C_{3,5}$ – $H_{3,5}$ in <i>p</i> -coumarate (PCE)
$p-CE_8$	116.2/6.26	C_8 – H_8 in <i>p</i> -coumarate (PCE)
G ₆	119.8/6.80	C_6 – H_6 in guaiacyl units (G)
H _{2,6}	128.3/7.17	$C_{2,6}$ -H _{2,6} in H units (H)
p-CE _{2,6}	130.6/7.48	$C_{2,6}$ – $H_{2,6}$ in <i>p</i> -coumarate (PCE)
X2	73.1/3.05	C_2 – H_2 in β -D-xylopyranoside
X ₃	74.6/3.26	C_3 – H_3 in β -D-xylopyranoside
X_4	76.0/3.50	C_4 – H_4 in β -D-xylopyranoside
X ₂₂	74.0/4.49	C_2 – H_2 in 2-O-acetyl- β -D-xylopyranoside
X ₃₃	75.4/4.78	C_3 – H_3 in 3-O-acetyl- β -D-xylopyranoside

Table 8. Cont.



Figure 6. Substructures present in MWL from 2D HSQC NMR.

In the side chain region (Figure 5a), β -O-4 (A), β - β resinol (B), and β -5 phenylcoumarans (C) moieties were detected. For β -O-4 moieties, C_{α} -H_{α} (A_{α}) at 72.8/4.85 and C_{γ} -H_{γ} (A_{γ}) at 60.2/3.38–3.89 were observed. C_{β} -H_{β} in β -O-4 substructures linked to a G/H unit (A_{β (G/H)}) and S unit (A_{β (S)}) were at 84.6/4.26 and 86.6/4.10 ppm, respectively. The signals at 85.8/4.64 and 54.1/3.04 were assigned to C_{α} -H_{α} (B_{α}) and C_{β} -H_{β} (B_{β}), respectively, whereas 71.8/4.16 and 72.1/3.80 were assigned to C_{γ} -H_{γ} (B_{γ}) in β - β moieties. For β -5 moieties, C_{β} -H_{β} (C_{β}) at 51.5/3.70 and C_{γ} -H_{γ} (C_{γ}) at 62.8/3.73 were observed. The signals of methoxyl and the β -O-4 moiety were prominent in this region. Additionally, the signal at 63.3/4.11 ppm was assigned to C_{γ} -H_{γ} in *p*-hydroxycinnamyl alcohol end-groups (F_{γ}). Carbohydrate-associated signals were also found, including signals from β -D-xylopyranoside moieties (X₂, X₃, X₄) and 2-O- and 3-O-acetyl- β -D-xylopyranoside moieties (X₂₂, X₃₃), as reported by Kim and Ralph [37] and Wen et al. [35,36].

In the aromatic region (Figure 5b), signals from S, G, and H moieties were highly visible. S moieties ($S_{2,6}$, $S'_{2,6'}$, and $S''_{2,6}$) were in the range of 104.8–107.2/7.30–6.69 ppm. G moieties were situated at 111.9/6.97, 115.4/6.68, and 129.8/6.80 ppm for C₂–H₂, C₅–H₅, and C₆–H₆, respectively. H moieties were observed at 113.8/6.67 ppm for C_{3,5}–H_{3,5} and 128.3/7.17 ppm for C_{2,6}–H_{2,6}. Additionally, three cross signals were assigned to C_{3,5}–H_{3,5} (116.1/6.78

ppm), C₈–H₈ (116.2/6.26 ppm), and C_{2,6}–H_{2,6} (130.6/7.48 ppm) in *p*-CE. The HSQC NMR results confirmed MWL as an HGS-type lignin, consistent with the FT-IR and ¹³C NMR results. Signals related to spirodienone, ferulate, and cinnamaldehyde end-groups were observed in bamboo MWL from *P. pubescens* grown in China [21], but not in bamboo MWL grown in Korea.

2.2.7. ³¹P NMR Analysis

The hydroxyl and carboxyl content of MWL were determined using the ³¹P NMR method based on Argyropoulos et al. [38], which allows the quantification of different types of hydroxyl groups, including aliphatic and phenolic hydroxyl groups, as well as G, S, H, and C₅ condensed phenolic hydroxyl groups. In Figure 7a, the full ³¹P NMR spectrum of phosphitylated MWL is presented, and Figure 7b shows the enlarged hydroxyl group region of interest (150–134 ppm). A sharp peak at 174 ppm was attributed to the excess amount of unreacted TMDP, indicating the complete derivatization of all hydroxyl groups in MWL.



Figure 7. (a) ³¹P NMR spectrum and (b) enlarged spectrum of MWL.

The aliphatic and phenolic (C₅-substituted + S, G, and H) hydroxyl, and carboxyl contents of MWL, calculated from the ³¹P NMR result, are listed in Table 9. The hydroxyl content was compared to other bamboo MWLs based on published data [23,39]. For MWL, the total hydroxyl content was found to be 8.19 mmol/g MWL. The hydroxyl group of the H units was similar in all bamboo MWLs. The aliphatic and total hydroxyl contents in MWL were notably higher, while the C₅-substituted+S content was lower compared to MWL-Y and MWL-N. The carboxyl content of MWL was determined to be 0.23 mmol/g MWL, close to MWL-N and slightly lower than MWL-Y.

Table 9. Hydroxyl and carboxyl contents of MWLs.

	Amount (mmol/g MWL)							Defense
	Ali OH	Ph OH	C ₅ -sub OH + S OH	G OH	Н ОН	Total OH *	СООН	- Kererences
MWL	6.74	1.45	0.18	0.51	0.76	8.19	0.23	This study
MWL-N MWL-Y	4.52 3.71	1.50 1.93	0.28 0.59	0.48 0.58	0.74 0.76	6.02 5.64	0.24 0.30	[23] [39]

MWL: *P. pubescens*, N: *Neosinocalamus affinis*, Y: unknown bamboo species. Ali: aliphatic, Ph: phenolic, C_5 -sub: C_5 -substituted; * Total OH = aliphatic OH + aromatic OH.

2.3. Thermal Characterization of Bamboo MWL2.3.1. TGA

TGA is widely employed for examining the thermal behavior and thermal and thermooxidative stability of lignin. Figure 8a,b show the thermogravimetric (TG) and derivative TG (DTG) curves of MWL, respectively, under oxidative and inert conditions. In the TG and DTG curves, two crucial temperatures are observed. The onset temperature marks the point at which decomposition begins, indicating the initiation of gas release during thermal oxidation or pyrolysis experiments. The other temperature, DTG_{max}, represents the point at which maximum thermal degradation occurs and is considered a parameter for determining the thermal stability of lignins [40].



Figure 8. (a) TG and (b) DTG curves of MWL under oxidative and inert conditions.

The decomposition process of the lignin sample can be divided into several stages. In the initial stage before 120 °C, the weight loss is attributed to the evaporation of moisture remaining in the lignin samples [41] and low MW volatiles. Under oxidative conditions at 120 °C, a weight loss of 1.9% in MWL was noted (Table 10). The onset temperature was 234 °C and DTG_{max} was 544 °C. The weight losses at 400 °C and 500 °C were approximately 43% and 65%, respectively. There were no weight changes beyond 590 °C, and the ash content obtained at 800 °C was 0.3%.

	Condition	Composition (%)			Temperature (°C)		Defense	
	Contaction	120 °C (Volatiles)	400 °C	500 °C	800 °C (Ash/Residue)	Onset	DTG _{max}	Kelerence
MWL	Oxidative	1.9	56.7	34.9	0.3	234	544	This study
MWL		1.8	89.4	63.9	28.7	235	287	
MWL-N	Inert	-	-	-	32.9	-	367	[23]
MWL-Y	· · ·	-	-	-	21.0	-	360	[39]

Table 10. Composition, onset temperature, and DTG_{max} of MWL (oxidative, inert).

MWL: P. pubescens, N: Neosinocalamus affinis, Y: unknown bamboo species.

Under inert conditions at 120 °C, a weight loss of 1.8% in MWL was observed (Table 10). The onset temperature of MWL was found to be 235 °C, nearly the same as that of oxidative condition (234 °C). In the 200–400 °C region of the DTG curve, two major bands were observed at 287 °C and 370 °C (Figure 8b). The weight loss in this region is attributed to the cleavage of interunit linkages in lignin, releasing monomeric phenols into the vapor phase [42]. Specifically, the peak at 287 °C signifies the degradation of aliphatic side chains, particularly the scission of β -O-4 ether linkages, while the peak at 370 °C indicates the degradation of methoxyl groups [43], based on information regarding gases released during pyrolysis [44]. Between 400 and 600 °C, the weight loss is primarily attributed to the decomposition or condensation of the aromatic ring [42,45]. The weight losses at 400 °C and 500 °C were approximately 11% and 36%, respectively. MWL underwent continuous carbonization at temperatures ranging from 600 to 800 °C, with a residual content of 28.7% at 800 °C.

In Table 10, the DTG_{max} and residual content of other bamboo MWLs are presented in comparison to MWL. The DTG_{max} of MWL was lower (287 °C) than that of other bamboo MWLs (360 and 367 °C). This difference may be attributed to higher S unit content. The residue content of MWL was higher than MWL-Y and lower than MWL-N.

Furthermore, under inert conditions, the DTG_{max} (287 °C) was lower than that obtained under oxidative conditions (544 °C). This can be attributed to the difficulty involved in degrading oxidized condensed aromatic moieties [43].

2.3.2. DSC

The thermal behavior of MWL, expressed as heat flow with respect to temperature, was determined over the range of 20–240 °C. In Figure 9, the 1st heating, cooling, and 2nd heating cycles of MWL are illustrated. During the 1st heating cycle, the thermal history of MWL, i.e., moisture absorbed during storage conditions, residual solvents, and drying methods, was removed. Upon reaching 240 °C, a temperature exceeding its melting transition, MWL was allowed to cool at 20 °C. An exothermic peak observed during the cooling cycle indicated the solidification of the lignin melt. The 2nd heating cycle reveals the true thermal behavior of the sample and is known to provide a reliable estimate of the glass transition temperature (T_g). T_g is an important transition temperature, at which amorphous polymers shift from a glassy to a rubbery state. In the 2nd heating cycle, the T_g of MWL was determined to be 159 °C.



Figure 9. DSC profile of MWL.

2.3.3. Pyrolysis GC/MS (Py-GC/MS)

Py-GC/MS was conducted at 675 °C to analyze the composition of MWL. The choice of pyrolysis temperature was based on TGA, where 675 °C was selected due to its minimal observed weight change. Compound identification was achieved by comparing mass spectra with published data from NIST and Wiley libraries [46,47], along with bamboo Py-GC/MS data [20,22,48]. The pyrogram of MWL is shown in Figure 10, and the resulting pyrolysis products with their relative compositions are listed in Table 11. Sixteen mono-lignol compounds were identified during pyrolysis products of MWL were categorized into three groups: H lignin derivatives (peaks 1, 2, 4, 6), G lignin derivatives (peaks 3, 5, 8, 10, 11, 12, 13), and S lignin derivatives (peaks 7, 9, 14, 15, 16). The major pyrolysis products released were 4-vinylphenol (6), 4-vinylguaiacol (8), guaiacol (3), syringol (9), and 4-methylphenol (2). These five major pyrolysis products constituted 68% of the total relative composition of MWL. Among them, 4-vinylphenol was the most abundant, accounting for approximately 30% of the total relative composition, consistent with previously reported

findings from Saiz-Jimenez and De Leeuw [48] and Li et al. [20]. The S:G:H composition of MWL was determined to be 16:37:47. The calculated S/G ratio for MWL was 0.43, aligning with a previously published result (0.4) from Bai et al. [22]. The S/G ratio derived from Py-GC/MS differed from the S/G ratios obtained through ¹H NMR (0.89). However, both methods concurred that the S content was lower than the G content.



Figure 10. Pyrogram of MWL at 675 °C.

Tabl	1. Pyrolysis products and relative composition at 675 $^\circ$	С.

No.	Compound	Туре	Formula	RRT ^a	MW	<i>m/z</i> ^b	Relative Composition (%)
1	2-Methylphenol	Н	C ₇ H ₈ O	0.91	108	108, 77	3.5
2	4-Methylphenol	Н	C ₇ H ₈ O	0.96	107	107 , 77	7.0
3	Guaiacol (G)	G	C7H ₈ O ₂	1.00	124	124, 109, 81	10.3
4	4-Ethylphenol	Н	$C_8H_{10}O$	1.17	122	122, 107	4.5
5	4-Methylguaiacol	G	$C_8H_{10}O_2$	1.24	138	138, 123, 95	3.4
6	4-Vinylphenol	H/PCA	C ₈ H ₈ O	1.29	120	120 , 91	31.7
7	3-Methoxycatechol	S	$C_7H_8O_3$	1.39	140	140, 125, 97	1.4
8	4-Vinylguaiacol	G/FA	$C_9H_{10}O_2$	1.50	150	150 , 135	11.3
9	Syringol (S)	S	$C_8H_{10}O_3$	1.57	154	154, 139, 93	7.3
10	Vanillin	G	$C_8H_8O_3$	1.66	152	152, 151	5.2
11	(E)-Isoeugenol	G	C ₁₀ H ₁₂ O ₂	1.76	164	164 , 149	2.9
12	4-Propylguaiacol	G	$C_{10}H_{14}O_2$	1.77	166	166, 137	1.5
13	Acetylguaiacol	G	$C_9H_{12}O_2$	1.82	166	166, 151	2.6
14	4-Allylsyringol	S	$C_{11}H_{14}O_3$	2.02	194	194 , 91	1.2
15	Syringaldehyde	S	$C_9H_{10}O_4$	2.11	182	182 , 181	4.1
16	Acetosyringone	S	$C_{10}H_{12}O_4$	2.23	196	196 , 181	2.0

PCA: *p*-coumaric acid, and FA: ferulate; ^a RRT: relative retention time, guaiacol as the reference; ^b m/z values in bold: base peak; only m/z values > 30% of the base peak are included.

3. Materials and Methods

3.1. Materials

The bamboo powder, prepared from a 2–3-year-old bamboo (*P. pubescens*) culm, was supplied by Songjuk Industry, located in Hamyang, Gyeongnam, Republic of Korea. The bamboo powder was air dried at room temperature for a week, 40 mesh passed powders were used.

The reagents, sodium hydroxide (NaOH, 93%), sodium chlorite (NaClO₂, 78%), ethanol (HPLC grade), benzene (EP), acetic anhydride (EP), and tetrahydrofuran (THF, 99.9%) were purchased from Duksan Pure Chemical (Seoul, Republic of Korea). Anhydrous ethyl ether (EP) and acetic acid (EP) were purchased from Samchun Chemical (Seoul, Republic of Korea). 1,2-dichloroethane (GR) and sulfuric acid (H₂SO₄, 93%), were purchased from Duksan Pharmaceutical (Sangju-si, Republic of Korea) and Daejung Chemicals & Metals (Siheung, Republic of Korea), respectively. 1,4-Dioxane (HPLC grade) was purchased

from Wako Chemical (Tokyo, Japan), anhydrous pyridine (GR) from Kanto Chemical (Tokyo, Japan), toluene (HPLC grade) from Fisher-Scientific Korea (Seoul, Republic of Korea), chloroform-*d* (CDCl₃) and dimethyl sulfoxide-*d*₆ (DMSO-*d*₆) from Eurisotop (Saint-aubin-des-bois, France), *N*-hydroxy-5-norbornene-2,3-dicarboximide (NHND, 97%) from AlfaAesar (Heysham, UK), chromium (III) acetylacetonate (97%) from AlfaAesar (Ward Hill, MA, USA), and 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP, 95%) from Sigma-Aldrich (St. Louis, MO, USA). All reagents and solvents were used without further purification. Molecular sieves (pore diameter 4Å, 1.6 mm pellet) were purchased from Yakuri Pure Chemicals (Tokyo, Japan).

3.2. Chemical Composition of Bamboo

The ash, extracts, and lignin were measured following TAPPI test methods [49–54]. Acid-soluble lignin was determined in accordance with the TAPPI standard method UM 250 [54], using an absorption coefficient of 110 L/g·cm. Holocellulose content was determined using the Wise method [55], and α -cellulose was determined using the TAPPI test method T203 om-83 [56]. Hemicellulose content was calculated by subtracting the α -cellulose value from the holocellulose value.

3.3. Preparation of MWL

The thoroughly dried, extractive-free bamboo powder was used for the preparation of MWL. Six grams of bamboo powder were placed in a 500-mL stainless-steel jar and filled with toluene. The jar, containing the sample, was then mounted on a vibratory ball mill and treated for 100 h. After milling, the MWL was isolated and purified according to the Björkman method [57].

3.4. Elemental Analysis

The MWL was vacuum dried under P_2O_5 at ambient temperature for 24 h prior to elemental analysis. C, H, O, N, and S analyses were performed using an Elemental Analyzer (IT/Flash 2000, Thermo Fisher Scientific, Waltham, MA, USA) at the Center for University-wide Research Facility, Jeonbuk National University (CURF, JBNU).

3.5. Acetylation of MWL

For the acetylation, 50 mg of MWL was dissolved in 1 mL of pyridine and 1 mL of acetic anhydride. The reactions of quenching, filtering, washing, and drying were carried out in the same manner as described by Mun et al. [58]. The acetylated MWL was designated as Ac-MWL.

3.6. Determination of MW

The average MW of MWL was determined by gel permeation chromatography (GPC). One milligram of Ac-MWL was dissolved in 1 mL of THF in a 10-mL conical beaker. The beaker was sonicated for 5 s and then filtered through a 0.45 μ m PTFE syringe filter (Chemco Scientific, Seoul, Republic of Korea). The filtrate was transferred into a 2-mL vial and diluted 2 times with THF. The GPC (Waters, Milford, MA, USA) was conducted at CURF under the conditions shown in Table 12.

GPC Configuration	Waters (Acquity APC) System, USA			
Columns	Acquity APC 2.5 μm XT 125, Acquity APC 1.7 μm XT 200 (4.6 × 150 mm, Waters, Wexford, Ireland)			
Flow rate	0.6 mL/min			
Sample injection volume	10 µL			
Éluent	THF			
Column oven temperature	30 °C			
Detector	UV (254 nm: polystyrene standards; 280 nm: sample)			
Analysis time	10 min			
MW polystyrene standards	Red: 130,000-21,500-6540-1250 Da White: 35,500-9130-2280-266 Da			

Table 12. Analysis conditions for GPC.

3.7. FT-IR Spectroscopy

FT-IR analysis was conducted utilizing a diamond attenuated total reflectance (ATR) accessory on an FT-IR spectrophotometer (Frontier, Perkin Elmer, Shelton, CT, USA), equipped with a deuterated triglycine sulfate (DTGS) detector. The spectrum was acquired in the wavelength range of 4000–500 cm⁻¹ with a resolution of 4 cm⁻¹. The analysis was performed at the CURF, JBNU.

3.8. ¹H NMR Analysis

Ten milligrams of the Ac-MWL sample was dissolved in 0.4 mL of CDCl₃ in a 10-mL conical beaker. The beaker was sonicated for 1–2 min to dissolve the sample. The mixture was filtered through a fine glass wool suspended inside a Pasteur pipette, which was directly connected to a clean NMR tube. The conical beaker was rinsed with additional 0.3 mL of CDCl₃ and the contents were transferred as described in a previous filtration method. The measurement was conducted using the NMR spectrometer (500 MHz FT-NMR, JNM-ECZ500R, JEOL, Tokyo, Japan) at the CURF, JBNU.

3.9. ¹³C and 2D HSQC NMR Analysis

A 120 mg MWL was placed into a 5-mL vial and vacuum dried under P_2O_5 at ambient temperature for 24 h before sample preparation. The moisture-free MWL was dissolved at 0.75 mL DMSO- d_6 at 50 °C. The filtration was carried out in the same manner as for ¹H NMR samples mentioned above. The ¹³C and HSQC NMR analyses were conducted using an NMR spectrometer (600 MHz, JEOL, Tokyo, Japan) at the CURF, JBNU.

3.10. ³¹P NMR Analysis

The hydroxyl and carboxyl group content of MWL was determined through ³¹P NMR analysis following the procedure outlined by Argyropoulos et al. [38]. The sample was prepared using pyridine/CDCl₃ (1.6:1 v/v) solvent with an internal standard NHND, a relaxation agent (chromium (III) acetylacetonate), and a phosphitylating agent (TMDP). Throughout the process, maintaining a moisture-free condition was crucial. The ³¹P NMR analysis was conducted using an NMR spectrometer (600 MHz, JEOL, Tokyo, Japan) at the CURF, JBNU. The spectrum was obtained using an inverse-gated decoupling pulse sequence, a 10 s relaxation delay, and 64 scans.

3.11. TGA

A 4–8 mg MWL was placed in a standard aluminum pan and secured in a thermogravimetric analyzer (Q600 SDT, TA Instruments). The sample was heated from 20 to 800 °C at 10 °C/min under nitrogen and oxidative conditions. TGA was performed at the CURF, JBNU.

3.12. DSC

A 2–6 mg MWL was loaded in a standard aluminum pan, and the heat flow was measured by a differential scanning calorimeter (DSC Q20, TA Instruments, New Castle, DE, USA). The sample was heated from 40 to 240 $^{\circ}$ C at 10 $^{\circ}$ C/min under a nitrogen

atmosphere. The sample was cooled to 40 °C. The sample was again heated to 240 °C at the same heating rate. The glass transition temperature (T_g) was estimated from the second heating cycle. DSC was performed at the CURF, JBNU.

3.13. Pyrolysis GC/MS (Py-GC/MS)

The MWL was derivatized using trimethylsilylation, as described by Pe et al. [43]. Py-GC/MS was then conducted at the CURF under the conditions shown in Table 13.

Table 13. Analysis conditions for Py-GC/MS.

Stage	Equipment/Condition			
Pyrolysis	Equipment	Curie-point pyrolyzer (JCI-21, Japan Analytical Industry), pyrofoil (F670, JAI)		
GC/MS		GCMS-QP2010 Ultra, Shimadzu		
GC	Pyrolysis temp. Interface temp.	670 °C for 5 s 300 °C		
	Column	J & W DB-5MS (30 m \times 0.25 mm ID \times 0.25 μm , Agilent Techn.)		
CC	Carrier gas	He, 1 mL/min		
GC	Injector/Detector temp.	250 °C		
	Split ratio	1:30		
	Oven temp.	$50 \ ^{\circ}C (1 \text{ min}) \rightarrow \text{ramping } (5 \ ^{\circ}C/\text{min}) \rightarrow 320 \ ^{\circ}C (5 \text{ min})$		
MS	Ionization	Electron impact method, 70 eV		

4. Conclusions

The MWL prepared from bamboo grown in Korea was investigated through several structural and thermal characterization techniques, and the results were compared with other bamboo MWLs grown in China. The distinct difference observed in MWL was that it had a higher average molecular weight and a broader molecular weight distribution compared to other bamboo MWLs. Various spectroscopic analyses showed that the MWL was a typical grass lignin but exhibited a very high aliphatic hydroxyl content compared to other bamboo lignins. The MWL also had a considerably lower DTG_{max} (287 °C), which indicated that there were more β -O-4 ether linkages. Through this research on lignin from bamboo grown in Korea, the authors were able to obtain fundamental data on the structural and thermal characteristics of domestic bamboo, and it was confirmed that there were some structural differences from bamboo grown in temperate and subtropical regions.

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