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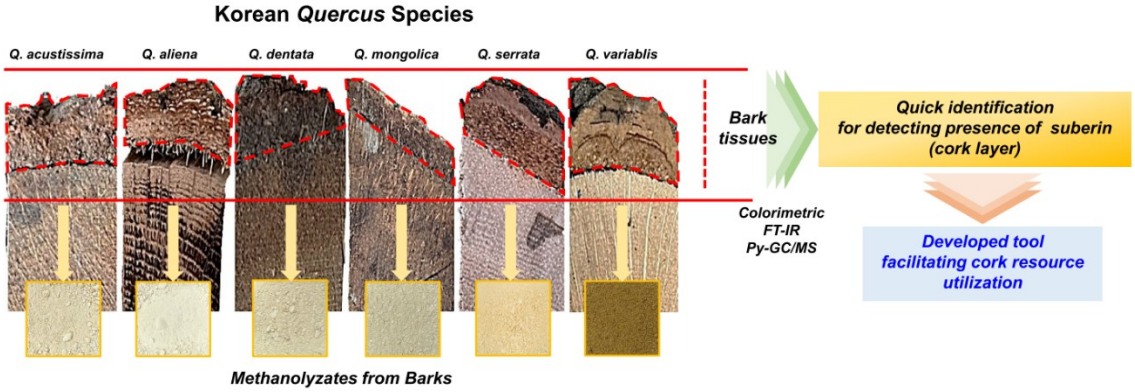
DOI: 10.15376/biores.20.4.9033-9050

GRAPHICAL ABSTRACT



Integrated Analysis of Cork Presence in Korean Oak Barks Using Visual Inspection, Colorimetry, FT-IR, and Py-GC/MS

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Six oak species are native to Korea, but a visible cork layer is present only in *Quercus variabilis*. The potential of the other Korean oak species for cork applications remains unexplored. This study used colorimetry, FT-IR, and Py-GC/MS to compare the cork composition of *Quercus acutissima*, *Q. aliena*, *Q. dentata*, *Q. mongolica*, *Q. serrata*, and *Q. variabilis*. The analysis focused on suberin and lignin, the main cork components, by identifying their pyrolysis products. Methanolysis with 3% $NaOCH_3$ revealed approximately 20% suberin content in all species except *Q. variabilis*, which showed around 40%. Py-GC/MS differentiated suberin-derived fatty acids—found exclusively in cork tissue—from other fatty acids present in the rhytidome. *Q. variabilis* exhibited 2 to 8 times higher levels of suberin-derived fatty acids and abundant lignin monomers, mainly guaiacyl (G) units. In contrast, lignin monomers were undetectable *in Q. acutissima*, *Q. aliena*, and *Q. dentata*, suggesting either very low levels or concentrations below the instrument's detection limit. Syringyl (S) monomers were also absent in *Q. mongolica* and *Q. serrata*. These findings suggest that lignin composition, along with visual cork layer assessment, can help evaluate the cork potential of Korean oak species and identify viable substitutes for commercial cork.

DOI: 10.15376/biores.20.4.9033-9050

Keywords: Korean oak species; Cork composition; Suberin; Pyrolysis-gas chromatography/mass spectrometry; Lignin

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INTRODUCTION

In the Republic of Korea, forests account for 42% of the total land area and predominantly comprise pine (*Pinus*, 21.9%) and oak (*Quercus*, 24.2%) tree species, with oak trees being most abundant in Gangwon-do Province (Korea Forest Service 2022). A recent Republic of Korea forestry policy to expand the utilization of broadleaf species has led to increased research interest in Korean oak species, which have traditionally been used as fuel for firewood and charcoal but are more recently being exploited for building materials and the production of biochemicals such as bioethanol (Yang *et al.* 2017; Jeong *et al.* 2019; Lee *et al.* 2020; Song and Kim 2020; Kim *et al.* 2020). The bark of oak trees is typically discarded after use or burned to make charcoal; however, this unused bark contains a unique outer bark tissue called cork, a scientifically and economically valuable resource worldwide (Cardoso *et al.* 2017). Unlike wood, in which cellulose is the main

component (approximately 50%), cork tissue predominantly comprises suberin polymers (>40%) (Graça and Santos 2006, 2007). Suberin is a polyester polymer located in the plant cell wall that comprises aromatics, including a lignin-like polymer, and long-chain fatty acids, such as α,ω-diacids, and ω-hydroxyacids, protecting against water loss, pathogens, and heat. Morphologically, cork has a polygonal porous, cellular structure that boasts impermeability, insulation, shock absorption, and microbial resistance (Pereira *et al.* 1987).

Most research on cork products has been done in Europe, where the native *Quercus suber* L. is the dominant oak, particularly in the Mediterranean region, where it plays a major ecological and economic role. Cork is commonly used for wine bottle stoppers and other household items, but it has more recently been applied to construction in road paving, cork foam, and cork board (Alexandre *et al.* 2020; Correia *et al.* 2022). As cork can be continuously harvested every 9 to 10 years if the cambium layer remains undamaged in Portugal, it is expected to become increasingly popular as a renewable resource that can be extracted without damage to forests (Paulo and Tomé 2010; Oliveira and Costa 2012; Sierra-Pérez *et al.* 2015). *Quercus variabilis*, which is widely distributed in Asia, including Korea, China, and Japan, exhibits thick cork tissues (Ferreira *et al.* 2016; De Carvalho and Caramujo 2018). Although some native Korean cork is used as a raw material for road paving and building interiors, most cork is imported from China and European countries such as Portugal and Spain (APCOR 2021). Previous research on Korean oak as a cork-producing tree species has focused on using xylem, with little research into the composition of cork or suberin. Cork produced in Europe predominantly consists of second-harvest cork, which exhibits very different characteristics from virgin (first-harvest) cork (Leite and Pereira 2017; Paulo and Santos 2023; González Adrados *et al.* 2024). Several recent studies have analyzed the virgin cork tissue of Korean *Q. variabilis* (Prasetia *et al.* 2022; Kim *et al.* 2024), particularly comparing *Q. variabilis* cork to that of *Q. suber.*

Although the presence of cork in *Q. variabilis* is already known in the Asian region, numerous other oak species are also extensively distributed throughout the area (Fujiwara and Harada 2015; Gao *et al.* 2017; Wang *et al.* 2022). Therefore, a method for quickly confirming the presence of cork layers and assessing their potential for application must be developed, particularly for use in sustainable materials, insulation, and bio-based composites. The primary objective of this study was not to establish a taxonomic classification of oak species but to evaluate their cork tissues in terms of chemical composition—particularly suberin and lignin content to identify species with potential for cork utilization. A stepwise chemical analysis was performed using colorimetric, Fourier transform infrared (FT-IR), and pyrolysis gas chromatography – mass spectrometry (Py-GC/MS) methods to characterize the cork components of six Korean oak species. To address the need for identifying oak species with cork-producing potential, a systematic chemical analysis framework was developed targeting six Korean oak species (*Q. acutissima*, *Q. aliena*, *Q. dentata*, *Q. mongolica*, *Q. serrata*, and *Q. variabilis*). The analytical approach consisted of sequential extraction steps, including solvent extraction and methanolysis for suberin monomer isolation, followed by pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS) and spectroscopic analyses. This strategy enabled the comparative evaluation of cork-related chemical components particularly suberin and lignin across species. The findings were aimed to provide a scientific basis for assessing the potential of Korean oak bark as a sustainable alternative resource for cork-based applications. This study also represents the first integrated chemical approach applied to native Korean oak species, offering a valuable framework for expanding cork-related research beyond European species.

EXPERIMENTAL

Materials

Three trees of each of the six Korean oak species (*Q. acutissima*, *Q. aliena*, *Q. dentata*, *Q. mongolica*, *Q. serrata*, and *Q. variabilis*) were harvested from the academic forest of Kangwon National University, Chuncheon-si, Gangwon-do, South Korea (37° 47′ 2.8932″ N, 127° 49′ 13.368″ E). The selected trees exhibited a range of diameters at breast height (DBH, measured at approximately 1.3 m above ground level), from 204 mm in *Q. aliena* to 267 mm in *Q. variabilis*. Cambial ages, determined by growth ring analysis, ranged from 48 years in *Q. acutissima* and *Q. aliena* to 72 years in *Q. serrata* (Savero *et al.* 2024; Prasetia *et al.* 2024). The detailed information on the six Korean oak wood species is summarized in Table 1. To observe the anatomical features of bark tissues, tree cross-sections including the outer bark, phloem, and adjacent xylem were obtained and shown in Fig. 1.

For chemical analysis, only the bark (excluding xylem) was used. Bark samples were ground into powder (40 to 80 mesh, 0.425 to 0.180 mm) for subsequent analysis.

Table 1. Key information on the Six Korean *Quercus* Wood Species

Trade Name	Scientific name	Diameter (mm)	Age (years)
Jolcham Oak	*Quercus serrata* Murray	266.6 (34.8)	72 (19.7)
Mongolian Oak	*Quercus mongolica* Fisch. ex Ledeb	230.8 (18.6)	64 (1.0)
Sawtooth Oak	*Quercus acutissima* Carruth.	217.3 (45.9)	48 (0.0)
Oriental White Oak	*Quercus aliena* Blume	204.2 (42.6)	48 (3.2)
Korean Oak	*Quercus dentata* Thunb.	221.6 (13.1)	73 (8.3)
Oriental Cork Oak	*Quercus variabilis* Blume	248.7 (38.8)	63 (1.5)

Note: The diameter and age indicate the average values of the three trees, with the numbers in parentheses denoting the standard deviations.

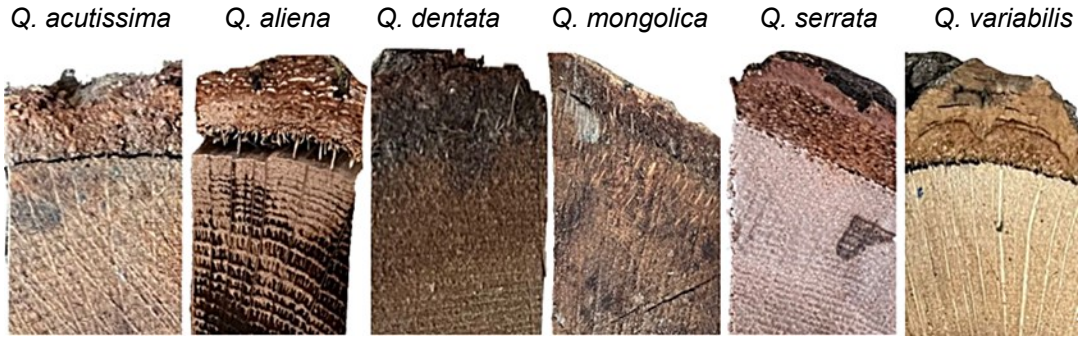

Fig. 1. Photographs of the bark sampled from six *Quercus* species used in this study. The location was the Research Forest of Kangwon National University, Chuncheon, South Korea, (37° 47′2.8932″ N, 127° 49′ 13.368″ E).

Material Preparation

Outer bark samples were manually collected from each tree's outer stem region at 1.3 m above ground level, ensuring consistency in sampling location across all specimens. Care was taken to avoid regions with visible defects, such as wounds or epicormic shoots.

The collected outer bark samples were initially air-dried under ambient laboratory conditions and ground into a fine powder using a mechanical grinder. The resulting powder

was passed through stainless steel sieves to obtain particle sizes between 40 and 80 mesh (corresponding to 0.425 to 0.180 mm), which were used for all subsequent chemical and morphological analyses.

The moisture content of the outer bark samples was determined before analysis using the oven-dry method and was found to range between 1.84% and 6.40%. All chemical composition data were calculated on an oven-dry weight basis.

Chemical Composition Analysis

Sequential solvent extraction was performed according to NREL methods to remove extractives from the outer bark, which includes fatty acids in the wood tissue (Sluiter *et al.* 2005). After weighing 2.5 g of wood powder from each oak bark sample, sequential extraction was carried out using dichloromethane, ethanol, and distilled water at 60, 80, and 100 °C, respectively, for 8 h each. The extraction durations were adjusted following the methodology described by Leite *et al*. (2020) to align with established experimental conditions. Water extraction was not effectively conducted at temperatures below 100 °C, so this temperature was selected for the extraction process. Outer bark powder samples with the extractives removed were labeled as extract-free (EF) material.

Methanolysis was performed to extract suberin, the primary component of cork. The EF sample (1.5 g) was reacted in 100 mL (w/v) of 3% $NaOCH_3$ methanol solution for 3 h, filtered, and then reacted again in 100 mL of methanol for 30 min. The methanolysis-derived extract containing suberin monomers was subjected to a neutralization step. The extract was slowly added to a 1% acetic acid solution under gentle stirring at room temperature and maintained for 1 h. After neutralization, the mixture was thoroughly washed with distilled water until the pH reached 6.5 to 7. The neutralized suberin extract was then dried at 60 °C for 24 h and stored in a desiccator until further use. The resulting methanolysis product is hereafter referred to as the methanolysis-derived extract (suberin monomers, MZ) (García-Vallejo *et al.* 1997; Conde *et al.* 1999; Costa 2019).

The remaining powders removed with extractives and suberin monomers were labeled as desuberinised material; this again emphasizes that the samples consisted of extracts and suberin-removed material from the outer bark powder, leaving only polysaccharides and lignin.

Visual Observation

Following solvent extraction and methanolysis stages, the color of each sample (EF, desuberinised, and suberin monomers) was measured to compare the color differences after removing different components from the outer bark. The color difference values were measured and recorded using a color difference meter (CR-10 Plus, KONICA MINOTA, Japan) according to CIELAB color space employs three values, L^*, a^*, and b^*. L^* indicates brightness from 100 (white) to 0 (black), whereas a^* and b^* represent chromaticity, where $+a^*$, $-a^*$, $+b^*$, and $-b^*$ indicate redder, greener, yellower, and bluer colors, respectively. The L^*, a^*, and b^* values for each sample were determined according to previous research (Moya *et al.* 2012; Costa *et al.* 2019; Hirata *et al.* 2020; Wu *et al.* 2021; Liu *et al.* 2024).

ATR Fourier Transform Infrared Spectroscopy Analysis

ATR-FTIR analysis was conducted using a Nicolet™ iS5 spectrometer (Thermo Fisher Scientific, USA) equipped with a diamond ATR crystal to identify the functional groups in extractive-free (EF) material and suberin monomers. FT-IR spectra were collected by scanning 64 times in the 4.000 to 500 cm^{-1} range. FT-IR spectra in the ranges

of 3.000 to 2.942 cm^{-1}, 2.940 to 2.850 cm^{-1}, 1.738 to 1.730 cm^{-1}, 1.600 to 1.500 cm^{-1}, and 1.000 to 700 cm^{-1} represent fatty acids and aromatic lignin-like components, *i.e.*, potential components of the suberin monomers.

PY-GC/MS Analysis

Py-GC/MS was performed to analyze the aromatic and fatty acid substances comprising the suberin monomers of the outer bark. Approximately 0.7 mg of each sample was placed in a quartz tube, to which 3.9 mg/mL of fluoranthene in methanol was added as an internal standard for quantitative analysis. The coil type used was a CDS Pyroprobe 5000 (CDS Analytical Inc., USA). The samples were heated to 600 °C at a rate of 10 °C/ms for 20 s before maintaining an internal temperature of 250 ℃ during pyrolysis. After pyrolysis, the substances were transferred to a GC/MS instrument (Agilent 7890A/Agilent 5975C, USA) with a flame ionization detector and DB-5 capillary column (30 m × 0.25 mm × 0.25 μm). Qualitative analysis of pyrolysis products was conducted based on the NIST 20 Mass Spectral Library (National Institute of Standards and Technology).

Statistical Analysis

Statistical analyses were conducted to evaluate significant differences among experimental groups following solvent extraction and methanolysis. One-way analysis of variance (ANOVA) was performed at a significance level of $p < 0.05$ to compare extractive content, suberin monomers, and residual components among oak species. When significant differences were detected, Tukey's post hoc test was applied for multiple comparisons (San-Emeterio *et al.* 2023; Rani and Whitcomb 2025). For pyrolysis gas chromatography/ mass spectrometry (Py-GC/MS) data, the Kruskal-Wallis H test was employed to assess significant differences in chemical composition among oak species. If significant differences ($p < 0.05$) were observed, then hierarchical clustering using Ward's method was applied to classify species based on chemical composition profiles, with a 1.2 distance threshold for optimal separation. Data were expressed as median ± interquartile range (IQR), and groups with statistically significant differences were assigned distinct superscript letters (a, b, c, d) (He *et al.* 2024; VanderRoest *et al.* 2024).

All statistical analyses were conducted using IBM SPSS Statistics (Version 29.0, IBM Corp., Armonk, NY, USA). Before applying parametric tests, data normality was assessed using the Shapiro-Wilk test. If data did not meet normality assumptions, appropriate nonparametric tests were conducted.

RESULTS AND DISCUSSION

Chemical Composition

The percentage of extractives and suberin monomers of the outer bark samples of six Korean oak species are shown in Table 2. The suberin monomers contain suberin-derived fatty acid chains, the main component of cork, and non-suberin fatty acids. For all oak species except *Q. variabilis*, the suberin monomers accounted for approximately 20%, whereas in *Q. variabilis*, it accounted for 40.5%. Previous studies have reported high suberin contents of 42.3% in the cork layer of *Q. suber* (Pereira 2013; Fu *et al.* 2020). Although it showed a significant suberin monomers content of around 40%, a detailed compound structure analysis is necessary to clarify whether these suberin monomers are derived from cork suberin or aliphatic substances within the outer bark. This uncertainty

arises because the outer bark samples used in this study included both cork and non-cork tissues, as physical separation of the cork layer was not feasible. Therefore, some aliphatic compounds detected during analysis may originate from non-cork parts of the rhytidome. This limitation has been noted and is considered in the interpretation of suberin content.

Table 2. Proportion (%) of Extractives and Suberin Monomers Obtained from the Outer Bark of Six *Quercus* Species

Species	*Q. acutissima*	*Q. aliena*	*Q. dentata*	*Q. mongolica*	*Q. serrata*	*Q. variabilis*
Extractives	11.68[a] (±0.39)	17.83[a] (±0.16)	15.50[a] (±1.08)	23.26[a] (±0.73)	16.50[a] (±0.39)	12.63[a] (±0.55)
Suberin monomers	19.6[b] (±0.33)	17.46[b] (±0.38)	18.91[b] (±0.17)	18.69[b] (±0.45)	18.11[b] (±0.23)	40.52[a] (± 1.27)

Note: Values are expressed as mean ± standard deviation. Superscript letters (a, b) within the same row indicate statistically significant differences among the six *Quercus* species ($p < 0.05$), based on one-way ANOVA followed by post-hoc multiple comparisons.

Visual Observations

Compounds in wood with phenolic and double-bond structures determine wood color (Marques *et al.* 2006); lignin and phenolic extracts are responsible for brown or yellowish colors in wood (Hu *et al.* 2020). Considering the polymeric structure of suberin, it was predicted that the color of suberin monomers would be determined by the linked lignin-like monomeric structure instead of the general fatty acids. The visual analysis results are shown in Fig. 2.

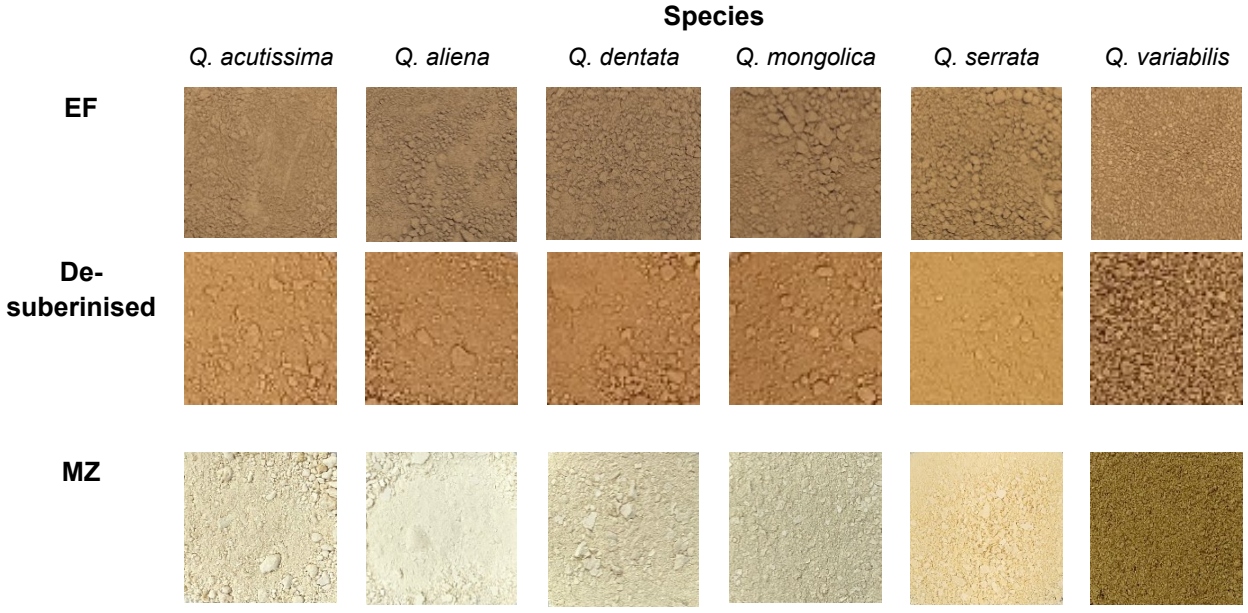

Fig. 2. Qualitative color results of extract-free (EF), de-suberinised material, and methanolyzates (Desuberinised) of six Korean *Quercus* species (EF = methanolyzates + lignin + carbohydrate; Desuberinised = lignin + carbohydrate; MZ = potential suberin-containing extract)

Table 3. Quantitative Color Measurements of EF, Desuberinised, and Suberin Monomers Samples from Six Korean *Quercus* Species

	Q. acutissima			*Q. mongolica*			*Q. aliena*		
	*L**	*a**	*b**	*L**	*a**	*b**	*L**	*a**	*b**
EF	40.8	13.7	20.1	29.5	17.8	24.2	31.6	15.5	21.0
Desuberinised	25.0	16.9	23.6	23.9	17.0	23.1	21.7	15.6	20.7
Suberin monomers	53.3	14.2	22.0	58.1	13.7	21.8	58.2	12.7	20.7
	Q. serrata			*Q. dentata*			*Q. variabilis*		
	*L**	*a**	*b**	*L**	*a**	*b**	*L**	*a**	*b**
EF	42.3	13.6	23.5	30.2	16.0	20.6	30.5	16.6	22.3
Desuberinised	26.3	16.7	25.8	25.9	12.8	13.0	23.0	15.0	20.3
Suberin monomers	57.0	15.3	30.8	59.3	13.3	20.0	38.0	11.5	10.9

After the removal of the primary extract, the EF samples contained ash, suberin, lignin, and polysaccharides. The EF showed a brown color, with no significant differences between species. In contrast, the desuberinised bark obtained after methanolysis of the EF is a sample with all types of fatty acids and suberin removed, indicating the suberin monomers had been eliminated from the de-suberinised bark, leaving only lignin and polysaccharides. Finally, the suberin monomers showed a whiter color and high *L** values for all species except *Q. variabilis* and *Q. serrata* (Table 2). The suberin monomers of *Q. serrata* showed higher *b** values than those of other oak species, indicating a more yellow color. Phenolic compounds and those with double bonds contribute to yellow and brown coloration. These results suggest that differences in the concentration of suberin and its associated components influence the coloration of the samples. For example, Miranda *et al*. (2024) observed a lightening of the cork surface color through an outdoor weathering test. Furthermore, FT-IR and Py-GC/MS analyses confirmed the degradation of long chain fatty acids (Miranda *et al.* 2024). Of course, it would be somewhat premature to draw a definite conclusion about the presence of cork suberin in the outer bark based solely on the color data. A structural analysis of the suberin monomers is necessary where suberin is expected to be present. In addition, the color characteristics of the extracted fractions were evaluated using the CIELAB system (Table 3). The EF samples generally showed darker tones (L* ≈ 30–42), whereas the MZ fractions exhibited markedly higher lightness (L* ≈ 53–59). A striking exception was *Q. variabilis*, whose MZ fraction displayed an unusually low L* value (38.0), remaining as dark as the EF samples. This atypical behavior suggests that the suberin-related components of *Q. variabilis* differ substantially from those of the other oak species, resulting in reduced lightening after depolymerization.

Functional Group Changes Identified by ATR-FTIR

Figure 3 shows the FT-IR spectra of EF and suberin monomers from all six oak species. None of the EF showed substantial differences in FT-IR spectra, except for that of *Q. variabilis*, where an isolated peak was observed at 2800 to 2900 cm^{-1}. Conversely, the peak at 2800 to 2900 cm^{-1} was observed in the suberin monomers of all species, but the relative peak size intensity was significantly larger in *Q. variabilis*. Moreover, the suberin monomers of *Q. variabilis*, which is assumed to be the most favorable substitute for *Q. suber* among Korean oak species, showed clear C-H stretching vibrations at 2918 cm^{-1} and 2848 cm^{-1}, which are characteristic peaks of aliphatic chain structures (Lopes *et al.* 2020; Şen and Pereira 2022).

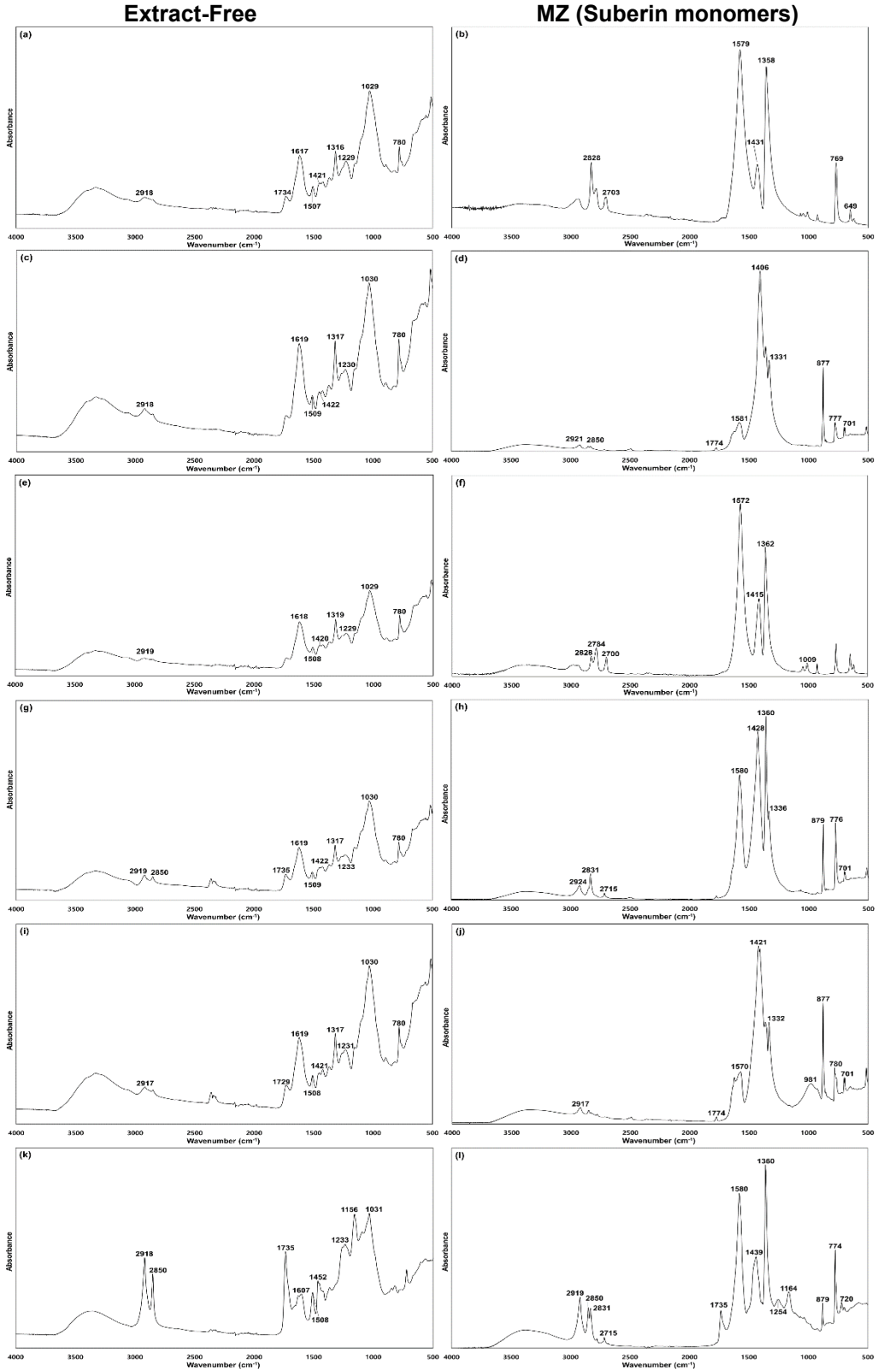

Fig. 3. FT-IR spectra of EF and De-suberinised isolated from six Korean *Quercus* species: a) and b) *Q. acutissima;* c) and d) *Q. aliena*, e) and f) *Q. dentata*, g) and h) *Q. mongolica*, i) and j) *Q. serrata*, and k) and l) *Q. variabilis*

Similarly, Sen and Pereira (2022) observed symmetric and asymmetric methylene (CH_2) stretching peaks in the corresponding areas in *Q. cerris* cork. They suggested that peaks at 2850 and 2920 cm^{-1} are characteristic of suberin (Marques and Pereira 2014). This implies that the fatty-acid chain structure remains in EF even after the initial removal of fatty acids and is related to aliphatic substances in the suberin structure. Prasetia (2024) compared the FT-IR spectra of suberin from the virgin cork of *Q. variabilis* to that of *Q. suber* and identified suberin-derived peaks at 2919, 2847, 1735, and 1162 cm^{-1}, similar to the peak positions observed in this study (Lopes *et al.* 2000).

Based on the peak profiles in the 1000 to 1800 cm^{-1} region of the suberin monomer spectra, the six *Quercus* species were classified into two groups: Group A (*Q. acutissima*, *Q. mongolica*, and *Q. variabilis*) and Group B (*Q. aliena*, *Q. dentata*, and *Q. serrata*). A distinct peak at 1735 cm^{-1} corresponds to C=O stretching vibrations, while a peak at 1580 cm^{-1} is attributed to the C–C stretching of aromatic rings. Aromatic C–H stretching vibrations were also observed at 1360 cm^{-1}. A peak at 1164 cm^{-1}, corresponding to the C–O–C stretching vibration of aliphatic ester bonds, was also clearly identified in *Q. variabilis*, in association with the C=O stretching band at 1735 cm^{-1}(Rocha *et al.* 2001; Huang *et al.* 2012). In a previous FT-IR analysis of suberin-derived fatty acids obtained from birch bark, Rizhikovs *et al.* (2022) reported the appearance of a C=O peak of typical esters at 1732 cm^{-1} in suberin, as well as peaks at 1626 cm^{-1}, corresponding to the C=C stretch from conjugated carbonyl groups, which are typically aromatic. The peaks at 1463 cm^{-1} and 1375 cm^{-1} indicate asymmetric and symmetric C–H deformations of the aliphatic regions, generally characteristic of suberin in various plant species (Rizhikovs *et al.* 2022).

These peaks were also observed in suberin monomers of *Q. variabilis*, suggesting structural features common to suberin across different species. The suberin monomers of *Q. variabilis* thus exhibited more pronounced FT-IR peaks related to aliphatic and aromatic derivatives than those of the other oak species. To investigate these compounds in more detail, a Py-GC/MS analysis was conducted.

Py-GC/MS of Suberin Monomers

Analytical pyrolysis provides a rapid means to quantify carbohydrates and lignin. This method was applied to suberin monomers derived from cork bark to investigate their thermal decomposition behavior and structural features.

Qualitative analysis

The Py-GC/MS results of suberin monomers are shown in Figs. 4 and 5. Aliphatic substances were detected with high sensitivity in *Q. variabilis*, and the carbon chain lengths of alkene and alkadiene ranged from 8 to 20.

The main detected substances in *Q. variabilis* included 1-Nonene (C9:1), 1-Decene (C10:1), and 1,4-Octadiene (C8:2). *Q. serrata* showed similar aliphatic substances to *Q. variabilis*; however, long-chain fatty acids, a major suberin component, were undetected. This result supports the previous study's findings (Sen *et al.* 2022), which reported that fatty-acid substances were not easily detected during pyrolysis at 550 to 900 °C. Although the term "suberin monomers" is used throughout this study, it should be noted that the compounds detected through pyrolysis may also include oligomeric fragments or partially depolymerized structures. These are generated due to the thermal degradation processes involved in Py-GC/MS. Therefore, the term is used in a functional sense to describe analyzable suberin-derived compounds, rather than strictly defined single-unit monomers. Regarding non-phenolic aromatic substances, toluene, 2,3-xylenol, and indane were

commonly found in *Q. variabilis* and *Q. serrata*. However, phenolic aromatic substances were predominantly found in *Q. variabilis*, with relatively little observed in *Q. serrata*.

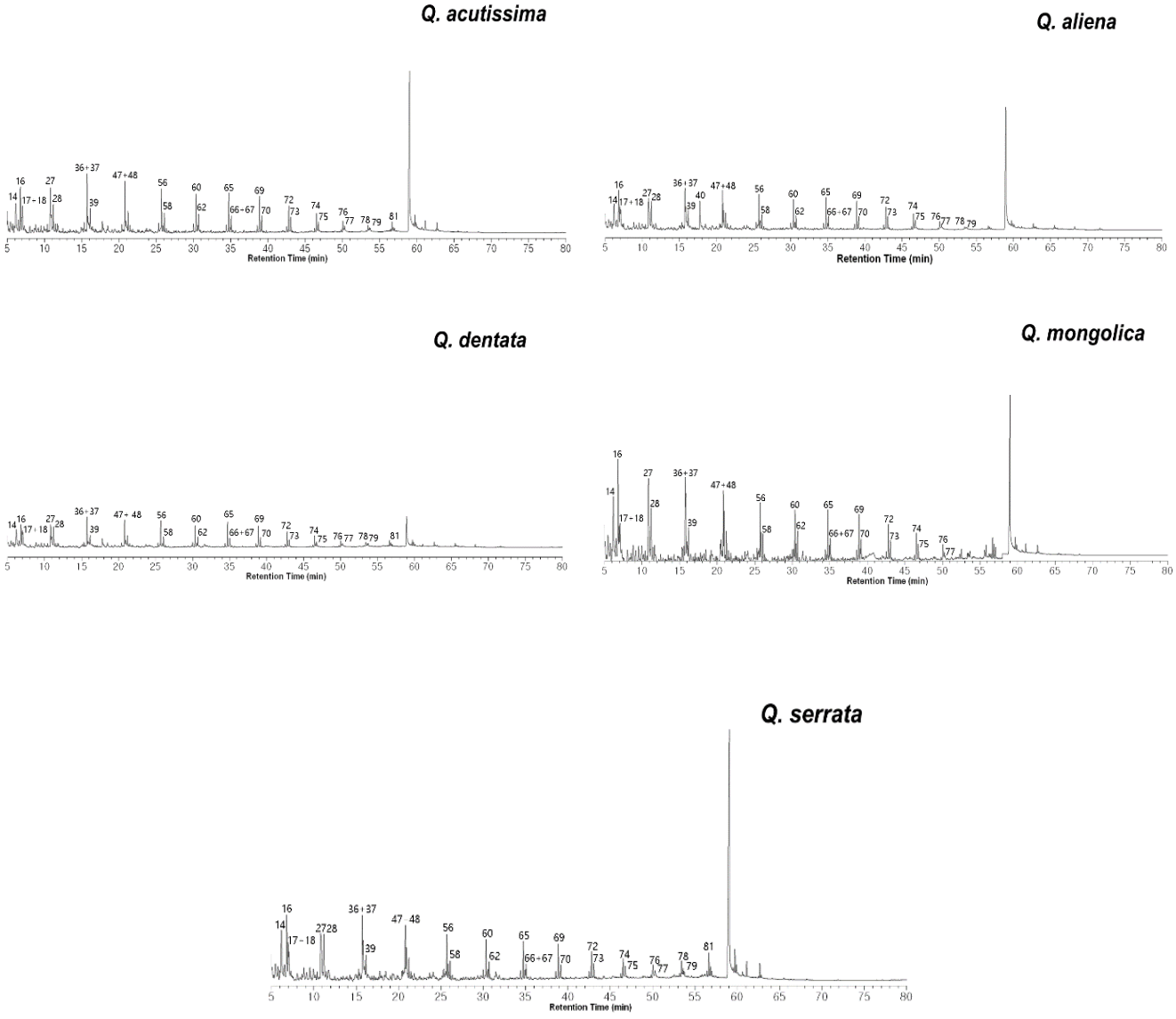

Fig. 4. Py-GC/MS pyrograms of MZ in *Q. acutissima; Q. aliena; Q. dentata; Q. mongolica;* and *Q. serrata*. 14: Toluene, 16: 2-Octene, 17: Cyclopentanone, 18: Octane, 27: 1-None, 28: Benzene, ethyenyl-, 36: 1-Decene, 37: 2-Octanone, 39: Decane, 40: 1-Hexanol-2-ethyl, 47: Cyclopropane, 1-methyl-2-pentyl-, 48: Undecane, 56: 1-Dodecene, 58: Dodecane, 60: 1-Tridecene, 62: Tridecane, 65: (3Z)-3-Tetradecene, 66: 2-Dodecanone, 67: Tetradecane, 69: 1-Pentadecne, 70: Pentadecane, 72: (cis)-2-nonadecene, 73: Hexadecane, 74: (cis)-2-nonadecene, 75: Dodecane, 2,6,11-trimethyl-, 76: 1-Octadecane, 77: Octadecane, 78: Z-5-Nonadecene, 79: Nonadecene, 81: (3E)-3-Icosene, 86: 1-Nonadecene

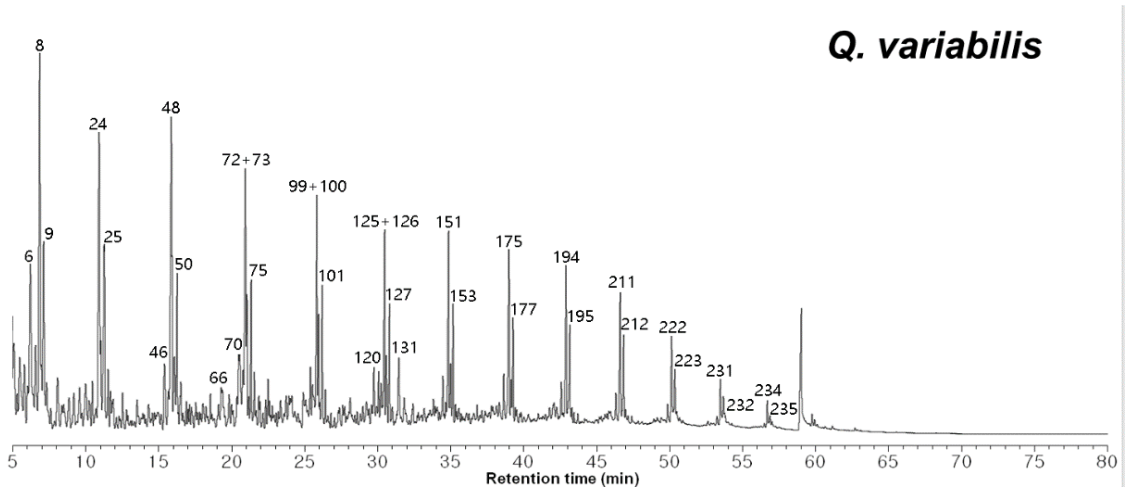

Fig. 5. Py-GC/MS pyrograms of MZ in *Q. variabilis*. 6: Toluene, 8: 2-Octene, 9: Octane, 24: Heptanone, 25: Nonane, 46: 1,9-Decadiene, 48: 1-Decene, 50: Decane, 66: ρ-Cresol, 70: Phenol, 3-methyl-, 72: 1-Undecene, 73: 2-Nonanone, 75: Undecane, 99: 1-Dodecene, 100: 2-Decanone, 101: Dodecane, 125: 1-Tridecene, 126: 2-Undecanone, 127: Tridecane, 131: 2-Methoxy-4-vinylphenol, 151: 2-Tetradecene, 153: Tetradecane, 175: 1-Pentadecene, 177: Pentadecane, 194: 1-Hexadecene, 195: Hexadecane, 211: 1-Heptadecene, 212: Heptadecane, 222: 1-Octadecene, 223: Octadecane, 231: 1-Nonadecene, 232: Nonadecene, 234: 1-Nonadecene, 235: Cyclohexadecane, 240: n-Npnadecanol-1, 241: Docosane

Quantitative analysis

The results of quantitative analysis using internal standard substances are summarized in Table 4. *Q. variabilis* had approximately twice as many fatty-acid-derived substances as *Q. dentata*, which had the second-highest aliphatics.

Table 4. Py/GC Results for the Pyrolyzed Suberin Monomers of Six *Quercus* Species (mg/g)

Products	*Q. acutissima*[a]	*Q. aliena*[a]	*Q. dentata*[c]	*Q. mongolica*[b]	*Q. serrata*[a]	*Q. variabilis*[d]
Aliphatics	**18.46**	**17.87**	**48.74**	**5.54**	**10.11**	**98.62**
Alkanes	5.54	4.04	10.21	0.88	2.74	22.96
Other alkanes	1.03	2.09	9.39	0.89	1.85	5.16
α-alkenes	8.60	10.53	27.42	2.41	2.74	49.19
Other alkenes	0.82	1.07	1.72	0.44	1.00	7.56
Alkadienes	1.95	0.14	n.a.	0.40	1.66	9.18
Other alkadienes	0.52	n.a.	n.a.	0.52	1.24	4.57
Aromatics	**3.33**	**1.55**	**8.58**	**1.89**	**4.85**	**46.78**
Phenolic	n.a.	n.a.	n.a.	0.62	0.59	17.05
Non-phenolic	3.33	1.55	8.58	1.27	4.26	29.73
Others	**3.37**	**6.78**	**5.92**	**2.40**	**3.90**	**25.05**

Note: Values are expressed as median ± interquartile range (IQR). Different superscript letters (a–d) within the same row indicate statistically significant differences among *Quercus* species ($p < 0.05$), based on the Kruskal–Wallis test followed by hierarchical clustering. "n.a." indicates values not detected or excluded from statistical analysis due to being below the quantification limit.

As for aromatics, approximately 17 times more phenolic substances were detected in *Q. variabilis* than in *Q. serrata* and *Q. mongolica*, with the remaining three species characterized by an absence of phenolic substances. As a cell wall component in cork, suberin represents a complex of fatty acids and lignin-like monomers (Sen *et al.* 2016).

According to the results, the suberin monomers of the five species except for *Q. variabilis* predominantly comprised fatty acids in normal bark, whereas fatty-acid and lignin-like substances derived from suberin were only identified in *Q. variabilis*. A Kruskal-Wallis test revealed significant differences among the six oak species ($p < 0.05$). Further hierarchical clustering analysis classified them into four groups (a, b, c, d) based on their chemical composition (see Table 4 for details).

Lignin Moieties in Suberin Monomers

According to Py-GC/MS, phenolic (lignin-like) aromatics were only detected *in Q. variabilis*, *Q. serrata*, and *Q. mongolica*. Therefore, the three structural units of lignin, namely guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H), were analyzed in the suberin monomers of these species to compare the lignin-like structures linked to fatty-acid chains (Table 5).

Table 5. Ratio of Lignin Moieties in Suberin Monomers

Type of lignin	*Q. mongolica*	*Q. serrata*	*Q. variabilis*
H	2.26	0.23	5.57
G	0.26	0.49	14.98
S	n.d	n.d	1.64
S/G	n.d	n.d	0.11
H:G:S	1.0:0.1:0	1.0:2.1:0	1.0:2.7:0.3
n.d.: not detected. The compound was not detected under the experimental conditions.			

In all three species, syringyl (S) units were not detected, which may indicate that their concentrations were below the detection limit of the method used. *Q. variabilis* exhibited notably higher levels of G, S, and H compared to the other two species, with an S/G ratio of 0.11 and an H:G:S ratio of 1.0:2.7:0.3, respectively. These results are consistent with those reported for cork in representative species like *Q. suber* and *Q. cerris* (Marques and Pereira 2013). Oak bark generally contains more G-unit lignin derivatives compared to the xylem of other common broadleaf trees (Grzybek *et al.* 2021).

In comparison, *Q. mongolica* showed a relatively higher proportion of H- and G-units, whereas *Q. serrata* and *Q. variabilis* exhibited similar trends, with dominant G-units and no detectable S-units. In summary, although lignin structural units were identified in the three species, the absence of syringyl-type lignin was common across all suberin monomer profiles. Most research on cork lignin composition has focused on *Q. suber*, with studies on isolated lignin (milled cork lignin) indicating a nearly complete composition of G-unit, accounting for 95.4% of the three lignin precursors (S/G ratio of 0.03) or 90.9% (S/G ratio of 0.10) (Lourenço *et al.* 2016). Conversely, analytical pyrolysis conducted directly on extractive-free cork has shown that the proportion of G-units is around 65.6% of the total lignin precursors (S/G ratio of 0.12). The lignin composition for extractive-free corks in other species, such as Betula pendula and *Q. cerris*, also varies. For example, *Q. cerris* cork contains 95.2% G-unit (S/G ratio of 0.03), while B. pendula cork has 85.8% (S/G ratio of 0.14). The structures within the suberin extracted through methanolysis differed depending on the species. In previous extraction processes, some species showed

no phenolic compounds in the methanolyzates, while others contained phenolic compounds but with varying lignin monomer content and composition ratios. These findings are significant in overcoming the challenges of identifying cork solely by visual observation. By performing Py/GC analysis on small samples of oak bark in the field, it would be possible to identify the species and verify the presence of suberin in the cork.

Limitations and Needs for Further Research

While this study focused on the chemical composition of cork-containing outer bark samples, it did not directly assess the macroscopic harvestability or anatomical continuity of cork layers from each species. Future work will aim to incorporate these aspects, including structural evaluations of cork slab formation, in order to better align chemical characteristics with practical utilization potential especially for *Q. variabilis*, which has been identified as a promising candidate.

CONCLUSIONS

1. This study introduced a stepwise chemical analysis method to classify six Korean oak species. The methanolysis and pyrolysis gas chromatography / mass spectrometry (Py-GC/MS) methods distinguished the chemical compositions of suberin and lignin-like substances, providing a scientific basis for species identification with potential cork applications.
2. The results revealed that *Quercus variabilis* exhibited a significantly higher suberin-derived fatty acid content (40%) than the other species (20%). Additionally, it contained abundant lignin monomers, predominantly guaiacyl (G) units, whereas *Q. mongolica* and *Q. serrata* lacked syringyl (S) units. The presence of phenolic aromatic compounds in *Q. variabilis*, *Q. serrata*, and *Q. mongolica* further differentiated these species.
3. These findings suggest that lignin composition and cork layer development serve as effective markers for distinguishing oak species. Furthermore, this study highlights the limitations of traditional visual identification methods, emphasizing the necessity of integrating Py-GC/MS analysis for cork verification.
4. As a sustainable and economically valuable resource, cork from *Q. variabilis* presents a viable option for domestic production in Korea. Utilizing this species could reduce reliance on imported cork while promoting sustainable forest management and economic development. Future research should refine the chemical characterization of Korean oak cork and explore its industrial applications to enhance its utilization across various sectors.

ACKNOWLEDGEMENTS

This research was funded by the R&D Program of the Korea Forest Service (Korea Forestry Promotion Institute), grant number 2021350C10-2323-AC03.

Author Contributions

Conceptualization: B.K., K.-C.P., S.-Y.P.; Resources: N.-H.K.; Methodology: B.K., K.-C.P., P.D., J.-H.K., X.J., J.-W.C., S.-Y.P.; Investigation: B.K., K.-C.P., P.D., J.-H.K., X.J., S.-Y.P.; Data curation: B.K., K.-C.P., S.-Y.P.; Writing—original draft: B.K.; Writing—review and editing: S.-Y.P.; Visualization: B.K., S.-Y.P.; Funding acquisition: N.-H.K.; Supervision: S.-Y.P. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data are contained within the article.

Conflicts of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Article submitted: June 19, 2025; Peer review completed: August 1, 2025; Revised version received: August 6, 2025; Accepted: August 14, 2025; Published: August 25, 2025.
DOI: 10.15376/biores.20.4.9033-9050