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THE EFFECTS OF NATURAL DEGRADATION ON THE CHEMICAL COMPOSITION OF PEDUNCULATE OAK STUMP (*QUERCUS ROBUR* L.)

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ABSTRACT

After trees are cut down, the stumps usually remain in the forest. Recently, there has been a growing interest in utilising stump biomass as an energy resource or alternative raw material to produce various chemicals. However, stumps left in the forest are exposed to various biotic (microorganisms, insects) and abiotic factors (UV radiation, precipitation, low/high temperatures, oxygen from the air, and atmospheric pollutants), causing their degradation over time.

This paper investigates the changes in the chemical composition of the xylem of pedunculate oak stumps (*Quercus robur* L.) after two years of natural degradation. In that aspect, the samples of the freshly cut stump were compared to the samples obtained from the stump that was exposed in the forest environment for two years. During this period, the cellulose and ash content decreased by 3.7% and 30%, respectively, while the lignin content increased by 5%. It was also found that after two years, the content of wood extractives in the oak stump increased by 15%. To detect natural products of stump wood extracts, the High-Performance Thin-Layer Chromatography (HPTLC) technique and post-chromatographic derivatisation with ASA (*p*-anisaldehyde/sulphuric acid) reagent were used. ASA is a widely used reagent for the detection of terpenoids, steroids, and carbohydrates through colour differentiation. The developed colours are indicative of the chemical nature of the compounds. Compounds visualised under white light after derivatisation and heating show that extracts from the stump wood, following two years of natural degradation, have more terpenoids, producing blue, purple, or brown zones. A decrease in the intensity of the grey and green spots under white light can also be observed, indicating that the chemical profile of the wood extract is less rich in steroids and allylic alcohols. Terpenoids are not only important for the tree's defence mechanisms but also for their role in the production of aromatic compounds that contribute to the wood's uses in industries like winemaking.

Keywords: *Quercus robur* L., natural degradation, stump, chemical composition, HPTLC.

1. INTRODUCTION

After tree felling, stumps usually remain in the forest (Walmsley and Godbold, 2010) and represent a potential source of carbon (C) and nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) (Wang et al., 2022; Palviainen et al., 2015; Sucre and Fox, 2009; Persson, 2013; Lasota, 2018). The degradation of stumps contributes to nutrient cycling within forest ecosystems, enriching forest soils with organic minerals, nitrogen (N), and

carbon (C), thereby supplying nutrients essential for the growth of trees in future forest generations (Deng et al., 2018; Sucre and Fox, 2009; Persson, 2013).

The removal of stumps can have negative consequences for soil structure and fertility, increase erosion, and reduce organic carbon stocks (Moffat et al., 2011; Sucre and Fox, 2009; Persson, 2013; Olsson et al., 1996). Moreover, stumps serve as habitats for numerous forest organisms, so their removal may threaten the survival of these populations and negatively impact forest biodiversity (Moffat et al., 2011; Walmsley and Godbold, 2010; Persson, 2013). On the other hand, decaying stumps increase the risk of pests and diseases (Walmsley and Godbold, 2010; Moffat et al., 2011; Persson, 2013), which is why in some countries such as the United Kingdom, the northwestern United States, and Canada, commercial stump removal is practiced as a method of rot prevention (Cleary et al., 2013; Persson, 2013), while in Finland and Sweden, stumps are utilized for bioenergy production (Hakkila, 2004; Walmsley and Godbold, 2010; Persson, 2013).

In the context of increasing demand for renewable energy sources and the reduction of fossil fuel use, interest in utilising stumps as a raw material for bioenergy and chemical production is growing (Bjorheden, 2006; Walmsley and Godbold, 2010). Stumps make up the largest portion of coarse woody biomass remaining after tree harvesting and may account for up to 20% of additional biomass (Richardson et al., 2002; Hakkila and Aarniala, 2004; Walmsley and Godbold, 2010). In some European countries, particularly in Scandinavia, stumps and coarse roots are commercially removed for the production of fibre and bioenergy (Moffat et al., 2011; Persson, 2013; Walmsley and Godbold, 2010; Hakkila, 2004; Walmsley and Godbold, 2010). As early as the 17th century, and especially during the 19th century in Sweden and Finland, stumps were used for the production of tar and other wood-derived products (Karlsson, 2007; Walmsley and Godbold, 2010; Persson, 2013). Partially decomposed stumps found in forests are also a potential resource for the production of various wood-based products (Rahmati et al., 2019).

Stump decomposition is a complex and long-term process influenced by numerous biotic and abiotic factors, including climatic conditions, soil properties (Moffat et al., 2011), microbial activity, wood species, and the physico-chemical properties of the stump itself (van Geffen et al., 2010; Moffat et al., 2011; Deng, 2018; Zhu et al., 2017; Lasota, 2018; Erdenebileg et al., 2020). White-rot and brown-rot fungi play a key role in the degradation of cellulose, hemicellulose, and lignin, leading to progressive mass loss and changes in the density of woody biomass (Rahmati et al., 2019). The decomposition rate is also significantly affected by the C:N ratio, extractive content, and tissue type (heartwood, sapwood, bark) (Moffat et al., 2011; Lasota, 2018; Kanbayashi et al., 2021). Generally, nutrient-rich tissues such as sapwood decompose faster than heartwood, which is rich in extractives with antifungal properties (Moffat et al., 2011). Over time, the proportion of carbohydrates decreases while the lignin-to-nitrogen ratio increases, which slows down the decomposition rate (Moffat et al., 2011).

Pedunculate oak (*Quercus robur* L.) is one of the most abundant and ecologically important hardwood species in Serbia, especially in the floodplain forests of Vojvodina along major rivers such as the Sava and Danube, as well as in regions like Mačva and Pomoravlje (Banković, 2009). This species is valued for its natural durability and high concentration of polyphenolic extractives, which contribute to its resistance to biological degradation and define its potential use in various industries.

Despite its wide distribution and economic importance, changes in the chemical composition occurring during the natural degradation of pedunculate oak stumps have not been sufficiently studied. Understanding these variations can help improve resource usage, especially in light of the growing demand for the sustainable use of woody biomass. This study aims to examine changes in the chemical composition of the xylem of pedunculate oak (*Quercus robur* L.) stumps after two years of natural degradation in forest conditions and to evaluate their potential for industrial or energy purposes.

2. MATERIAL AND METHODS

Samples for chemical composition analysis were taken from pedunculate oak stumps (*Quercus robur* L.) from the same site within the “Morović” forest management unit (Public Enterprise “Vojvodinašume”, Serbia). Two categories of samples were examined: stumps from recently felled trees (Qr) and stumps in the degradation phase, originating from trees cut two years prior (DQr). The

stumps originated from trees of approximately the same age (around 110 years), eliminating variability related to tree age.

From each stump, discs approximately 3 cm thick were cut and air-dried for three months. After removing the bark, the remaining xylem was ground and sieved. For analysis, the particle fraction of 0.5–1 mm was used, following TAPPI standard T 257 cm-02.

The moisture content of the stump samples was determined gravimetrically by drying to constant mass at $105 \pm 2^\circ\text{C}$ (TAPPI T 264 cm-97) and was $7.77 \pm 0.11\%$ for the Qr sample and $7.60 \pm 0.10\%$ for the DQr sample.

The chemical characterisation of the stumps (Qr and DQr) included the determination of cellulose content using the Kürschner-Hoffer method (Browning, 1967); extractives soluble in toluene:ethanol (2:1, v/v) after 8 hours of extraction in a Soxhlet apparatus (ASTM D1105-21); extractives soluble in hot water after 3 hours of extraction (TAPPI T 207 cm-99; ASTM D1110-21); ash content at 900°C (TAPPI T 413 om-22); and lignin content, determined as the sum of acid-insoluble lignin (TAPPI T 222 om-11) and acid-soluble lignin measured at a wavelength of 205 nm using a UV spectrophotometer Vision-600 (TAPPI T UM 250). All analyses were performed in triplicate, and the results are expressed as mean values with standard deviations. The hemicellulose content was calculated as the difference between the total wood mass (100%) and the sum of the other wood components.

A one-way analysis of variance (ANOVA) with a 95% confidence level was used to determine the significance of differences between fresh and degraded stump samples.

A CAMAG Linomat 5 (Muttentz, Switzerland) was used to apply 20 μL of extracts in 6 mm bands onto 20 cm \times 10 cm HPTLC glass silica gel plates. The bands were positioned 8 mm from the plate's lower edge, with a minimum of 13 mm separating them on either side. Using the mobile phase ethyl acetate: *n*-hexane: formic acid: water (11:2:1:0.5 v/v/v/v) in a saturated Twin Trough Chamber (20 cm \times 10 cm), chromatographic development was performed, and the mobile phase was allowed to migrate a distance of 70 mm. After being taken in TIFF format, the pictures of the acquired HPTLC chromatograms were processed with VideoScan (version 1.02, CAMAG). To identify natural oxidisable molecules, a *p*-anisaldehyde/sulphuric acid (ASA) reagent was used for derivatisation. The zones were then visible after 10 minutes of heating on a plate at 110°C . The ASA reagent was freshly prepared by mixing 10 mL of glacial acetic acid with 85 mL of methanol. To this chilled solution, 5 mL of concentrated sulphuric acid and 0.5 mL of *p*-anisaldehyde were added (Martelanc et al., 2016). All chemicals and reagents for this investigation were purchased from commercial suppliers. Formic acid, sulphuric acid, acetic acid (glacial, 100%), methanol, and HPTLC silica gel 60 (Art. 105461) glass plates were purchased from Merck KGaA (Darmstadt, Germany). Ethyl acetate was supplied by Centrohem (Stara Pazova, Serbia). *n*-Hexane, *p*-anisaldehyde, and standards of rosmarinic acid (96%), β -sitosterol ($\geq 95\%$), and oleanolic acid ($\geq 97\%$) were bought from Sigma Aldrich Chemie GmbH (Steinheim, Germany).

3. RESULTS AND DISCUSSION

The chemical composition of Qr and DQr pedunculate oak (*Quercus robur* L.) stumps is presented in Figures 1 and 2. The examined parameters indicate significant alterations in the chemical composition of oak stumps over two years of natural decay.

As shown in Figure 1, the contents of cellulose and hemicellulose in degraded stump samples (41.44% and 13.86%, respectively) are significantly lower compared to those in fresh stumps (42.99% and 15.26%, respectively). In contrast, the lignin content in DQr samples (29.93%) is significantly higher compared to Qr samples (28.51%). Rahmati et al. (2019) also reported a greater degree of cellulose and hemicellulose degradation compared to lignin in stumps of Oriental beech (*Fagus orientalis* Lipsky) over a felling period of 2 to 25 years.

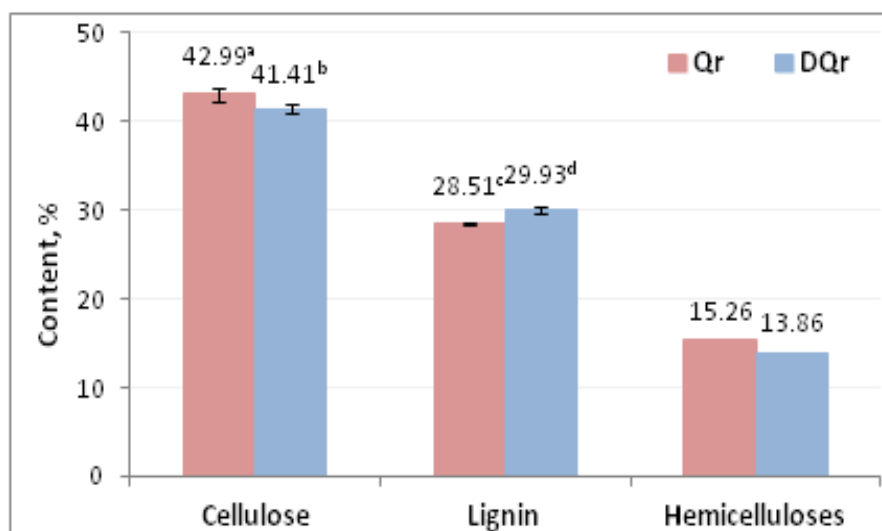


Figure 1. Content of structural components from a freshly felled tree (Qr) and from a tree felled two years ago (DQr) pedunculate oak stumps (% dry mass). Different letters (^{a, b, c, d}) indicate statistically significant differences ($p < 0.05$).

The crystalline structure of cellulose contributes to its resistance to enzymatic degradation (Teeri and Henriksson, 2009). Hemicellulose does not form crystalline structures like cellulose, making it more accessible and sensitive to enzymatic hydrolysis and microbial degradation (Saha, 2003). Additionally, hemicellulose provides a physical barrier around cellulose microfibrils, thereby protecting them from the action of hydrolytic enzymes of microorganisms (Rahmati et al., 2019). As a result, hemicellulose is more exposed to microbial attack, especially in the early stages of degradation. This explains the higher degradation rate of hemicellulose, whose content in DQr samples is approximately 9.4% lower compared to Qr, while cellulose content is about 3.7% lower.

Polysaccharides are embedded in a lignin matrix that protects them from microbial attack (Nilsson, 2009). During natural wood degradation, polysaccharides degrade faster than lignin, which is the most resistant component of the cell wall to biological degradation (Rahmati et al., 2019). Lignin's resistance to enzymatic degradation is due to its complex, highly branched heteropolymeric structure, as well as its pronounced aromatic and hydrophobic nature (Kai et al., 2018; Sánchez and Alméciga-Díaz, 2011; Rahmati et al., 2019). Consequently, the total lignin content in partially degraded stumps is approximately 5% higher than in freshly cut stumps. According to Moffat et al. (2011), increased lignin content after partial degradation slows down the rate of further decay.

The observed differences in the content of structural cell wall components between fresh and degraded stump samples indicate more intensive degradation of polysaccharides during natural stump decomposition, which is consistent with known patterns of lignocellulosic biomass decay (Rahmati et al., 2019). Microorganisms involved in lignin degradation include specific fungi and bacteria commonly found in soil and decaying vegetation (Kai et al., 2018; Sánchez et al., 2011).

Cell wall polysaccharides are mostly selectively degraded by brown- and soft-rot fungi, and to a lesser extent by white-rot fungi (Tomak, 2014; Rahmati et al., 2019; Nilsson, 2009), as well as by certain aerobic and anaerobic bacteria (Teeri and Henriksson, 2009). In the early stages of degradation, brown-rot fungi selectively degrade hemicellulose, which is more sensitive to microbial breakdown, and subsequently degrade cellulose, leaving lignin as a slightly modified residue (Nilsson, 2009; Tomak, 2014; Rahmati et al., 2019; Kai et al., 2018).

Brown-rot fungi are responsible for the lower polysaccharide content and higher lignin content in decaying oak stumps because polysaccharides degrade more quickly than lignin. Brown-rot fungi lead to significant decomposition and mass loss, resulting in reduced density and diminished mechanical properties of the wood (Tomak, 2014; Rahmati et al., 2019).

Figure 2 shows the content of non-structural components (extractives and mineral substances) in freshly cut oak stumps and oak stumps after two years of natural forest degradation.

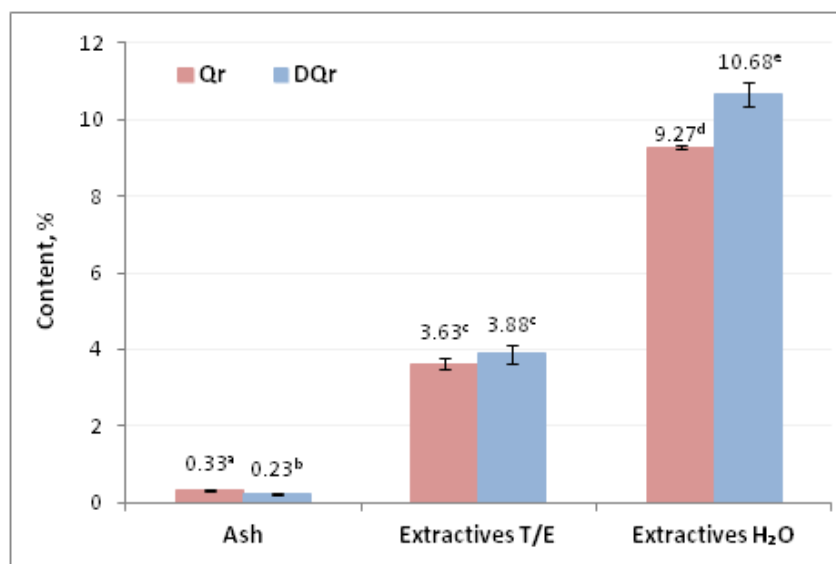


Figure 2. Content of non-structural components from a freshly felled tree (Qr) and from a tree felled two years ago (DQr) pedunculate oak stumps (% dry mass). Different letters (^{a, b, c, d, e}) indicate statistically significant differences ($p < 0.05$).

Regarding non-structural wood components, *Quercus robur* L. stumps after two years of degradation exhibited higher extractive content and lower ash content compared to fresh stumps (Figure 2). Ash content was used to represent the mineral concentration during full combustion at 900°C (TAPPI T 413 om-22). The overall mineral content in wood is generally low, amounting to 0.33% in fresh *Q. robur* L. stumps. During natural stump decomposition, the content of mineral elements decreases further (Xie et al., 2024), resulting in approximately 30% lower ash content in degraded oak stumps compared to fresh ones.

This significantly reduced ash content in degraded samples is the result of a combination of leaching and biological uptake of mineral substances due to atmospheric influences and microbial activity. During the decomposition of stumps, rainwater and groundwater over time wash inorganic ions (such as K⁺, Mg²⁺, Ca²⁺, and others like phosphate) from the wood and transport them into deeper soil layers. It is one of the main reasons for the reduction of inorganic content and is particularly prominent in the early stages of decomposition (Lasota, 2018; Xie et al., 2024).

In addition, during wood decomposition, minerals are transformed from free inorganic forms into forms that are no longer free inorganic compounds (Lasota, 2018; Filipiak, 2018; Khanina et al., 2024). As the organic matter in wood breaks down into simpler molecules, part of the inorganic content becomes bound into newly formed organic complexes (e.g., humus), which increases soil fertility but reduces the availability of free inorganic ions (Filipiak, 2018; Khanina et al., 2024). Furthermore, surrounding plants and wood-degrading microorganisms, such as fungi and bacteria, absorb and assimilate some of the inorganic ions for their metabolism, thus contributing to the further reduction of free inorganic substances in the wood (Lasota, 2018; Filipiak, 2018; Khanina et al., 2024).

The accumulation of low-molecular-weight degradation products, such as sugars, phenolics, and organic acids, during the degradation process can be seen by the observed increase in extractives soluble in toluene, ethanol, and hot water in decayed oak stumps by around 6.9% and 15%, respectively. Onuchin et al. (2018) reported that during wood decay, the content of starch and pentosans generally decreases, while the content of extractives soluble in water and benzene increases, which is consistent with the results of these studies.

There was not a significant variance in the number of extractives soluble in toluene:ethanol between Qr and DQr samples, suggesting that components of this fraction degrade relatively slowly. However, after two years of natural degradation, the content of water-soluble extractives in oak stumps increased by approximately 15%.

Species of the genus *Quercus* are known for their high polyphenol content (Fernandez de Simon et al., 1996), including volatile phenols, phenolic acids, and ellagitannins (Zhang et al., 2015). These polyphenols are water-soluble and play a key role in enhancing the natural durability of wood and preventing its degradation by fungi (Valette et al., 2017; Rahmati et al., 2019; Chang et al., 2010). In addition to the heartwood and bark, stumps are particularly rich in extractives (Stefanescu et al., 2022).

Polyphenolic compounds are characterised by high antioxidant, antibacterial, and antifungal activity, which makes them toxic to microorganisms (Agarwal, 2021; Rahmati et al., 2019; N'Guessan et al., 2023), contributing to the wood's resistance to biodegradation (Taylor et al., 2002; Valette et al., 2017; Rahmati et al., 2019). Their biological activity against wood-degrading microorganisms is one of the main reasons for the low degradation rate of these components (Rahmati et al., 2019) and may explain the increased content of water-soluble substances after two years of oak stump degradation (N'Guessan et al., 2023).

The high-performance thin-layer chromatography method and post-chromatographic derivatisation with ASA reagent were employed to identify natural products of stump wood extracts (Jork et al., 1994). Distinct chromatographic profiles of the extracts were obtained using a mobile phase composed of ethyl acetate, *n*-hexane, formic acid, and water (11:2:1:0.5, v/v/v/v), optimised to achieve maximum separation of phenolic compounds, terpenoids, and steroids in water extracts from fresh and biodegraded stump wood. The separated compounds were visualised by characteristic colours following derivatisation with ASA reagent under white light, which facilitates the detection of diverse organic compounds (Wagner et al., 1984; Agatonovic-Kustrin et al., 2021). Standards of rosmarinic acid, β -sitosterol, and oleanolic acid were used for compound identification and colour comparison (Figure 4). Rosmarinic acid, a phenolic compound, showed a purple to brownish-purple zone after derivatisation with ASA reagent. β -Sitosterol, a phytosterol, developed a dark violet to purple colouration with greyish hues, reflecting its structural similarity to steroids. Oleanolic acid, representing triterpenoids, showed purple to violet zones, consistent with the typical responses of this class under the reagent.

HPTLC analysis revealed distinct differences in the chemical profiles of the xylem extracts from fresh and naturally degraded *Quercus robur* L. stumps. The DQr extract exhibited a visibly richer chromatographic profile compared to the Qr extract, particularly under post-derivatisation visualisation. A prominent purple zone at $R_F \approx 0.73$ -0.75 was observed in the DQr extract, less intense in the Qr. This zone corresponds in both colour and R_F value to the oleanolic acid standard, suggesting a higher content of triterpenoid compounds in the degraded sample. Conversely, a zone at $R_F \approx 0.70$ -0.72, corresponding to the β -sitosterol standard, indicates a reduction in phytosterol content after two years of natural degradation in forest conditions (Figure 4).

In the R_F range of approximately 0.40 to 0.60, several purple-coloured zones were observed in the DQr extract following derivatisation with ASA reagent. These zones were either absent or barely visible in the Qr extract, suggesting a degradation-induced accumulation or transformation of specific compounds over time. Based on their R_F values, these zones likely correspond to triterpenoid derivatives or sesquiterpenes, or complex phenolic derivatives such as glycosides, which are known to develop purple to violet colouration under this reagent. The appearance of these compounds exclusively or more prominently in the degraded wood extract may indicate biochemical changes in the xylem matrix during the natural degradation process, possibly due to enzymatic or microbial activity enhancing the release or formation of such semi-polar terpenoid structures.

In the Qr extract, a prominent yellow-to-ochre-green zone was detected at $R_F \approx 0.02$ -0.03 following derivatisation. This low-mobility band likely corresponds to highly polar compounds, such as phenolic glycosides or hydrolysable tannins. The absence of this zone in the DQr extract may suggest degradation or leaching of these compounds during long-term exposure to natural environmental conditions.

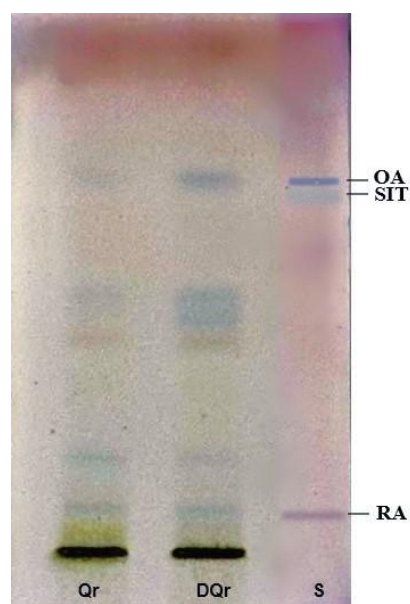


Figure 3. HPTLC profiles of xylem extracts from fresh (Qr) and naturally degraded (DQr) *Quercus robur* L. stumps and standards (S) of rosmarinic acid (RA), β -sitosterol (SIT), and oleanolic acid (OA) after derivatisation with ASA reagent, white light.

Based on the obtained chemical composition results, it can be concluded that degraded *Quercus robur* L. stumps still represent a significant source of valuable components, particularly lignin and water-soluble extractives, and have the potential to contribute to sustainable forest resource management and the utilisation of residual biomass.

The increased lignin content indicates their suitability as a raw material for the production of bio-based chemicals, such as phenolic derivatives, biopolymers, or components for the industrial production of adhesives and resins.

Given the substantial amount of residual carbon (in the form of cellulose and especially lignin), degraded stumps, following appropriate treatment, may also be used as biofuel (solid biofuel or pellets). It is supported by the increased lignin content, which has a higher heating value (26.7 MJ/kg) (Runge, Wipperfurth and Zhang, 2013) than cellulose and hemicellulose (17.3 MJ/kg and 16.2 MJ/kg, respectively (Döring, 2013)). Additionally, the lower content of non-combustible inorganic substances, which negatively affect the heating value of wood (Döring, 2013; Runge, Wipperfurth and Zhang, 2013), further supports the increased energy potential of decomposing stumps. Naturally, further research is needed to confirm and quantify this hypothesis and to optimise stump processing methods for bioenergy or biochemical applications.

The increased content of water-soluble extractives, such as tannins and other polyphenols, along with the HPTLC results indicating elevated levels of terpenoids, points to the presence of bioactive compounds in decomposed oak stumps. This opens up the possibility of utilising decomposed stumps as a source for the extraction of bioactive compounds, with potential applications in phytopharmacy, cosmetics, or wood protection. Accordingly, a comprehensive qualitative and quantitative analysis, including total phenolic content (TPC) and a phenolic profile of the extractives, would be necessary to evaluate their industrial potential.

4. CONCLUSION

The results of this study have shown that significant changes in the chemical composition of *Quercus robur* L. stumps occur over two years of natural degradation. A decrease in the content of cellulose, hemicellulose, and mineral matter was observed, while the content of lignin and water-soluble extractives increased. These changes reflect the selective degradation of wood components under the influence of microorganisms and abiotic factors.

Despite the degradation, the results of the chemical analysis indicate that decomposed stumps still contain considerable amounts of lignocellulosic material and bioactive compounds, making them suitable for further utilisation in various industrial sectors. Their valorisation could contribute to reducing forestry waste, increasing the efficiency of biomass utilisation, and supporting the development of sustainable technologies based on renewable resources.

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