Bulletin of the *Transilvania* University of Brasov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 16(65) Special Issue 2023
https://doi.org/10.31926/but.fwiafe.2023.16.65.3.4

COMPARATIVE RESEARCH REGARDING THE HYDROPHILIC EXTRACTIVES CONTENT IN TURKEY OAK AND SESSILE OAK WOOD AND THEIR RELATED STAINING SUSCEPTIBILITY BY REACTION WITH IRON

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Abstract: *The paper presents the experimental results concerning the amount of extractable substances and the color changes determined by the reaction of the tannins contained in wood with iron. Tests were performed comparatively with sapwood and heartwood samples of two Quercus species, namely Turkey oak (Quercus cerris L.) and sessile oak (Quercus petraea (Matt.) Liebl.). The findings show that Turkey oak sapwood has an unexpectedly high amount of extractable substances, higher than the heartwood of the same species. This result was obtained with three extraction methods (cold water, hot water, and NaOH 1% solution). The FTIR investigation of the extracts impregnated on quantitative filter paper seems to confirm these results. Visual assessment and color measurements after applying* $FeCl_3$ *highlight that both oak species are prone to staining in contact with iron due to their tannin content. The values of the total colour difference between the state and after the reaction with* $FeCl_3$*, lower fer for Turkey oak compared to sessile oak, correlate with a lower extractive content of this wood species.*

Key words: *Turkey oak, sessile oak, extractives, tannin, colour change.*

1. Introduction

When characterising a wood species, the main chemical composition, as well as the secondary compounds are important, especially the extractives. Extractives represent a mixture of low- and high-molecular weight compounds that can be extracted from wood with cold/hot water and organic solvents. According to their chemical structure, extractives can be divided into three main classes: aliphatic compounds, terpenes, and phenols (polyphenols). The first two main classes

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of extractives are lipophilic compounds, which may be extracted in non-polar or low-polar solvents (*e.g.* cyclohexane, hexane, acetone, mixtures cyclohexane-ethanol, benzene-ethanol), while the phenolic compounds, which include the various types of tannins, are hydrophilic and can be extracted in polar solvents (*e.g.* water, ethanol, mixtures ethanol-water) [14]. In alkaline solutions (*e.g.* NaOH 1%), the solubility of phenolic compounds increases [13]. Also, alkaline solutions with pH of 9-10 are capable to dissolve non-structural wood compounds, such as dyes, simple sugars, resin, and fatty acids even at room temperature. Furthermore, pentosans may undergo degradation in alkaline environment and can be dissolved in alkaline conditions [5].

Each wood species is different with respect to its secondary chemical composition. Most of the extractives are located in the heartwood, being responsible for the darker colour of this tree part [10].

Even if the extractable substances are present in wood in small quantities, they can affect certain wood characteristics decisively. For instance, they may confer wood a specific taste, smell, and colour. Being situated in the intermicellar, interfibrillar, intercellular spaces and in the lumen of the cells, certain classes of extractives may influence the swelling of wood and have a positive influence on the natural durability of wood [7, 9, 17], but they also have a negative influence on some technological processes (*e.g.* gluing, finishing) [12]. During processing, they can cause more severe tool wear [3], and during drying, they may cause discolorations [11, 15, 19]. In the case of oak wood, which is generally known as being rich in different types of tannins, specific discolorations can be generated by the chemical reaction between the tannin and the ferrous metallic parts, which leads to grey-blue/black spots on the wood surface, in the contact area. Oxidative stains are also frequent during the conventional drying of oak wood [20, 21]. If the temperature rises above 30°C while the wood still contains free water, the edge zones of the board may turn dark reddish. Depending on the tannin content and concentration, the discoloration occurs more severely, the higher the temperature is. The discoloration is often uneven because of the different accessibility of the wooden tissue to atmospheric oxygen, and also because of the uneven distribution of the tannins within the wood volume.

According to Holzatlas [8], the content of extractable substances for common oak wood (*Quercus robur* L., *Quercus petraea* (Matt.) Liebl.) is 2.9-7.3% for cold water extraction, and 5.4-12.2% for hot water extraction. Another source [16] indicates 8.12% for hot water extraction, and 23.8% for NaOH extraction in the case of *Quercus robur* (L.) wood.

Quercus cerris L. (Turkey oak) wood has been studied less. A total extractable content of 6.7% for the heartwood of this species was reported by Bajroktari [1]. Another source [18] applied warm water extraction and obtained an average of 7.03 ± 1.83% (with sawdust samples made of 50% sapwood and 50% heartwood). The value is significantly lower than in the case of *Quercus robur* (L.), where Carmona [2] reported a percentage of 14.8-15.7% for the total extractable substances (where the extractives in ethanol and water represent about 93% of the total extractives).

2. Objective

The main objective of this research was to determine the similarities and differences between Turkey oak wood (*Quercus cerris* L.) and sessile oak wood (*Quercus petraea* Matt. Lieb.l) with regard to the amount of extractable substances, soluble in cold water, hot water, and NaOH, as well as regarding the colour changes by the reaction of the tannins with iron.

3. Materials and Methods

3.1. Location

The wooden samples used in this experimental research were obtained from discs cut at 1/3 of the tree height from two freshly harvested trees originating from the same region, namely the Southern Sub-Carpathians, Valcea county, Romania (45°N 24°E). The plot characteristics are presented in Table 1.

Plot characterisation Table 1

Geographic region	Southern Sub-Carpathians
Altitude	410 m
Climate	Continental moderate climate, specific to submontane plateau area Average annual temperature: 10.5°C Average annual rainfall: 578.6 mm Average wind speed < 1m/s, max. 2.1m/s
Type of soil	Luvosoil with a defective air-hydric regime; the water from the rainfall crosses the upper layers easily, but the clay layer laboriously. The humus content is approx. 2%, pH≤5.
Type of forest	Mixed hardwood forest (pedunculate oak, sessile oak, Turkey oak, beech)

The investigation of the secondary chemical compounds comprised the following tests:

- Determination of the amount of substances soluble in cold water, hot water, and NaOH;
- FTIR analysis of the extracts;
- Colour assessment of the solid wood samples, before and after applying $FeCl_3$ solution, in order to highlight the presence of tannins and the related staining susceptibility of the wooden material.

3.2. Determination of Extractable Substances

Sawdust probes (Figure 1) were taken from the sapwood and heartwood of the two species. These were screened, and the fraction which passed through the 0.5 mm sieve meshes, but did not pass through the 0.25 mm ones, was used within the tests.

The moisture content of the screened sawdust samples was determined by the oven-dry method. Four replicates, weighing 2 ± 0.0001 g each, were used for each wood assortment.

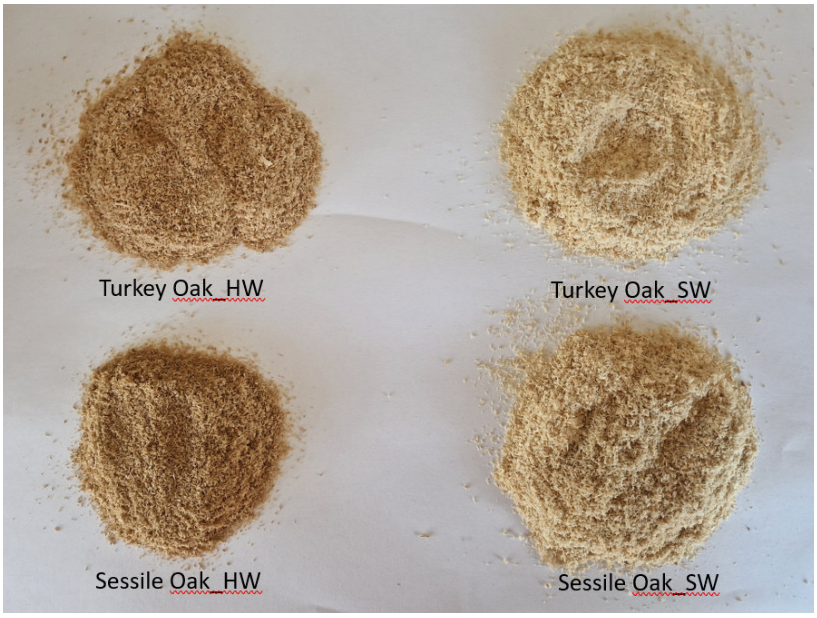


Fig. 1. *Sawdust samples from Turkey oak sapwood/heartwood, and sessile oak sapwood/heartwood, used for the determination of extractable substances*

To determine the content of extractable substances in cold water, each sample was placed in a Berzelius glass together with 300 cm^3 of cold distilled water. The samples were left for 48 h at room temperature (20°C), being stirred periodically with a glass rod. Then, they were filtered by using 1G2 crucibles, placed in the oven at 103 ± 2°C for 6 h, and weighed every two hours until their mass became constant.

To determine the content of extractable substances in hot water, each sample was introduced into a ground flask together with 100 cm^3 of hot distilled water. The flasks with the samples were placed in a water bath at 97-100°C and kept there for 3 h. After boiling, the content of each flask was passed through a filtering crucible 1G2. After filtering, the crucibles with the resulting filtered material were placed in the oven at 103 ± 2°C until they reached constant mass.

A similar extraction method was applied to the extractives in NaOH solution, but 100 cm^3 of sodium hydroxide solution 1% was used instead of hot distilled water.

After each extraction (cold water/hot water/NaOH), the contents of soluble substances was calculated based on the measured masses and moistures, according to Equation (1):

$$\text{ExtractableSubstances}\left[\%\right] = \frac{m_1 \cdot \left(100 - U\right) - 100 \cdot m_2}{m_1 \cdot \left(100 - U\right)} \cdot 100 \qquad (1)$$

where:

m_1 is the sawdust mass before the extraction [g];
m_2 – the sawdust mass after the extraction [g];
U – the initial moisture content of the sawdust sample [%].

3.3. FTIR Analysis

Quantitative filter paper discs were impregnated by repeated immersion (5 folds with intermediate air-drying) with the solutions obtained through the three extraction methods for the Turkey oak wood samples. Using Fourier Transformed Infrared spectroscopy (FTIR), the air-dried paper discs were employed in an attempt to investigate any detectable common and differentiating chemical features among the three types of extracts and between the extractives resulting from Turkey oak sapwood compared to Turkey oak heartwood. The investigation was carried out with an Alpha-Bruker FTIR spectrometer (Germany) equipped with an ATR (Attenuated Total Reflectance) module. The spectra were recorded in the range 4,000 to 600 cm^{-1}, at a resolution of 4 cm^{-1} and 24 scans/spectrum on three seldom areas of the paper discs (two paper discs for each extract type), resulting in six spectra for each type of extract. The recorded spectra were further processed for baseline correction and smoothing, and the average spectra were computed for each category of sample using the OPUS software, version 7.2, Bruker. The average spectra were further normalized (Min-Max normalization) and compared.

3.4. Determination of Colour Reactions with Iron

To highlight the presence of tannins, three solid wood samples of each wood species with dimensions of 180 x 20 x 18 mm were analysed on the radial, tangential, and longitudinal direction, respectively, containing both the sapwood and heartwood zone. The samples were air-dried and conditioned in laboratory normal conditions. Then, by means of an AvaSpec-USB2 spectrophotometer, the colour coordinates in the CIELab system: L^* (lightness), a^* (red-green chromatic component), b^* (yellow-blue chromatic component), were determined in the initial state. Colour measurements were performed on several precise positions (actually circular areas with a diameter of 8 mm) on each sample, starting from the outer sapwood area towards the inner heartwood area. The measuring positions were situated at 20 mm intervals. Due to the difference in sapwood width in the two wood species, two measurements on sapwood and five measurements on heartwood were made for Turkey oak, whilst in the case of sessile oak one and seven measurements were taken, respectively. Then, a layer of $FeCl_3$ solution (C=5%) was applied by brushing on each sample. After 24 h the colour was measured in the same areas again. The colour changes (ΔL^*, Δa^*, Δb^*) and the global colour difference (ΔE^*) due to the reaction of iron with the tannins in the wood were assessed by calculation. The global colour difference (ΔE) was calculated, according to Equation (2):

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad (2)$$

where: ΔL^*, Δa^*, Δb^* are the modifications of each colour coordinate after the application of the $FeCl_3$ solution (state 2), compared to the initial state (state 1): $\Delta L^*=L_1^* - L_2^*$, $\Delta a^*=a_1^* - a_2^*$, $\Delta b^*=b_1^* - b_2^*$.

3.5. Statistical Data Analysis

All the experimentally obtained values were statistically analysed in order to establish if there is a significant difference between the four considered wood assortments: *Quercus cerris* (L.) sapwood, *Quercus cerris* (L.) oak heartwood, *Quercus petraea* (Matt. Liebl.) sapwood, and *Quercus petraea* (Matt. Liebl.) heartwood.

The statistical analysis was performed by means of Data Analysis ToolPack, Microsoft Excel. One Way Analysis of Variance was run to figure out if there is a significant difference among the four analysed groups. In addition, the t-test for two independent groups was applied as Post Hoc test together with Bonferroni correction to reveal which pairs of mean differences are statistically significant. The Bonferroni correction requires to divide the adopted significant alpha level, namely 5% in this study, by the number of analysed groups, namely four. Therefore, the significant alpha level used in the Post Hoc analysis was equal to 0.0125. The means that do not share the same letter are significantly different from each other at this significance level

4. Results

4.1. Contents of Extractable Substances

According to previous research [4], sessile oak heartwood contains the highest amount of extractable substances among the four analysed wood types. It is surprisingly followed by Turkey oak sapwood, which registered similar values with sessile oak sapwood after the water extractions, but a significantly higher value after the NaOH extraction. The lowest amount of secondary compounds was recorded for the Turkey oak heartwood. The hierarchy of the four wood types according to the amount of extractable substances was the same for all three extraction methods applied (Figure 2).

There is a significant difference between the content of extractable substances in Turkey oak sapwood and heartwood, but only in the case of the cold/hot water extraction. When using NaOH, the differences between the two types of wood are much lower and statistically not significant [4].

4.2. FTIR Analysis of the Extracts

The FTIR spectra of Turkey oak sapwood (C-A) and heartwood (C-D) extracts impregnated on paper discs are shown in Figure 3. They are almost similar to those of the reference (unimpregnated) support paper (Href) due to the low concentration of the obtained extracts. From this point of view, the FTIR analysis, with a detection limit of about 5%, is not highly relevant, but it still allowed a comparison between the types of extracts, and between the sapwood and heartwood extracts, respectively.

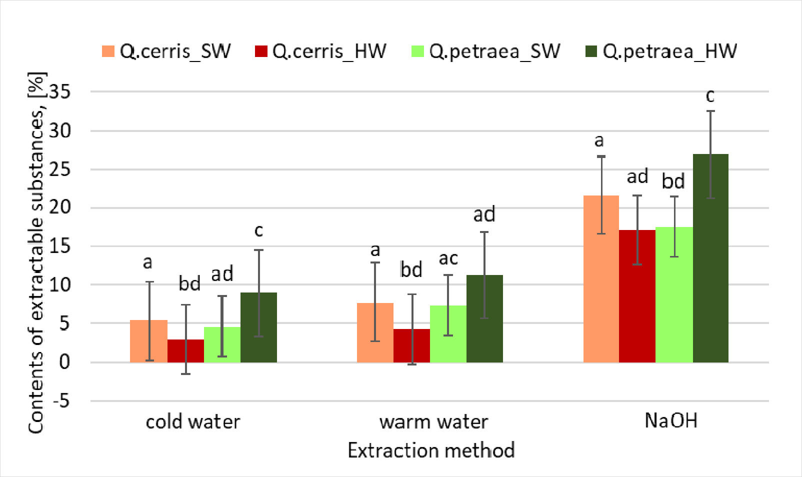


Fig. 2. *Contents of extractable substances from Turkey oak and sessile oak heartwood (HW) and sapwood (SW), depending on the extraction method (the different letters on the bars indicate a statistically significant difference between the compared values)*

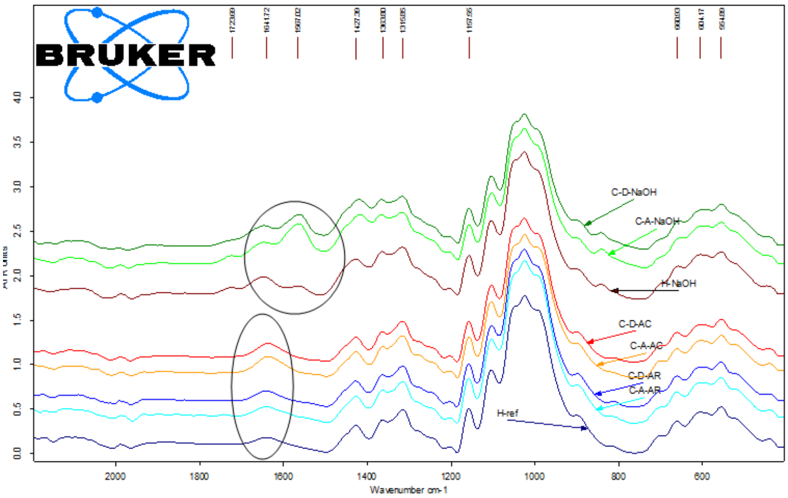


Fig. 3. *FTIR analysis of extracts from Turkey oak sapwood and heartwood*

Thus, the similar absorption bands show that there are no qualitative differences between the extracts from sapwood and heartwood detectable by FTIR, regardless of the extractive process (cold water, hot water, NaOH).

For the cold and hot water extracts, a small intensification of the absorption band at 1,640 cm^{-1} could be observed compared to the reference paper. Since this absorption is attributable both to the water absorbed in the support paper and to some compounds with conjugated carbonyl groups, aromatic ketones, –OH groups in p-substituted aryl-ketones, this intensification of the 1640 cm^{-1} absorption can be due to: some components with an aromatic ketone structure and/or conjugated carbonyl groups, –OH groups in p-substituted aryl-ketones, possibly present in extractable organic compounds or to a higher content of absorbed water in the support paper.

However, the intensification of this absorption is more pronounced for the paper impregnated with hot water extracts compared to cold water extracts, which correlates with a more pronounced hot solubilisation of water-soluble compounds at higher temperature. In this context we can consider that the intensification of this band could be associated with a series of hydrophilic extractable organic compounds containing conjugated carbonyl groups.

Also, for the samples impregnated with hot water extracts, the intensity of the absorption band at 1640 cm^{-1} is higher for the samples taken from sapwood compared to those from heartwood, which could suggest a higher content of extractables soluble in hot water, in sapwood than in heartwood. This result correlates well with the results displayed in Figure 2. Nevertheless, it is noteworthy that absorption differences in the support paper and the different distribution of retained extractables (middle/edge) may influence these results, so a firm quantitative conclusion cannot be formulated.

For the NaOH 1% solution, two additional absorptions are observed in the spectrum of the control paper impregnated with NaOH (H-NaOH) at 1,724 cm^{-1} and 1,567 cm^{-1}. For the paper discs impregnated with the NaOH 1% extracts obtained from Turkey oak sapwood and heartwood, a significant intensification of the absorption at 1567 cm^{-1} is clearly observed. The absorption for the C=C double bonds in the aromatic ring (but not only) is situated in this region of the IR spectrum (1,500-1,600 cm^{-1}), so the intensification of this band could be associated with extractable phenolic compounds (low molecular phenols) solubilised in a basic medium, possibly even from hydrolysable tannins (esters of gallic, digallic, and ellagic acid with monosaccharides), saponified in a basic medium. The intensification of the absorption at 1567 cm^{-1} appears to be higher for the sapwood extracts compared to the heartwood extracts, which correlates with the numeric data displayed in Figure 2.

This assumption correlates with some literature data. Thus, Seikel et al. [18] identified several low molecular phenolic compounds in the extract of *Quercus alba* (L.), such as sinapadehyde, coniferylaldehyde, syringaldehyde, vanillin, p-hydroxy-benzaldehyde propioguaiacone. Also, though hydrolysable tannins occur less frequently in wood compared to the condensed tannins, they seem to be characteristic for the oak species. The study of the extractives of several oak species (*Q. petraea* (Matt. Liebl.), *Q. suber* (L.), *Q. ilex* (L.)) compared to some tropical woods revealed the presence of hydrolysable tannins only in the oaks [6]. Gallotannins were detected in the sapwood and heartwood of *Q. alba* (L.) and *Q. rubra* (L.) [18].

At the same time, a decrease in absorptions at 1,363, 1,315 cm^{-1} and a relative intensification of absorption at 1,427 cm^{-1} was observed for the paper discs impregnated with the NaOH extracts.

4.3. Color Reactions with Iron

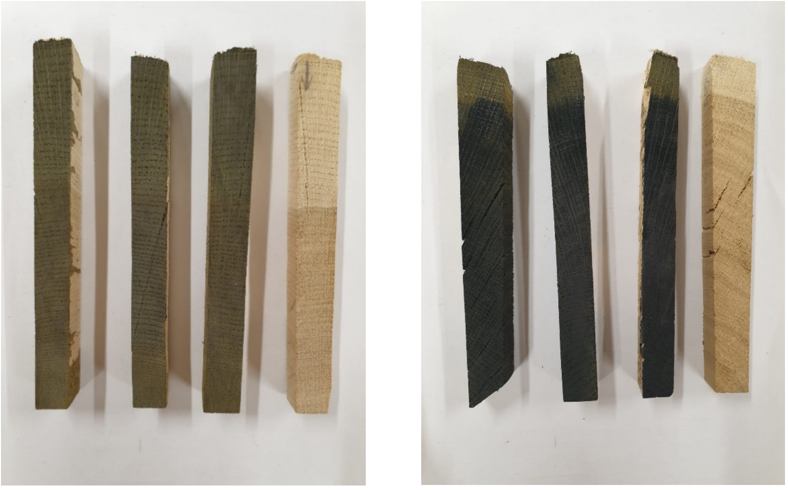
Table 2 presents the results concerning the colour of the four types of wood before and after the application of $FeCl_3$

solution. As it can also be observed in Figure 4, in the initial state, the heartwood is darker than the sapwood for both species (lower L*). According to the L*-coordinate (lightness) measured on the two species in the initial state, the Turkey oak heartwood is darker than the sessile oak heartwood, but the Turkey oak sapwood is lighter than the sessile oak sapwood.

Table 2

Colour assessment before and after applying $FeCl_3$ on Quercus cerris (L.) and Quercus petraea (Matt. Liebl.) heartwood and sapwood samples

Wood	Initial state			After $FeCl_3$		
	L*	a*	b*	L*	a*	b*
Turkey oak SW	73.67 (0.90)	4.05 (0.16)	15.77 (1.26)	40.93 (2.86)	-1.16 (1.91)	11.41 (2.21)
Turkey oak HW	61.55 (0.99)	6.34 (0.28)	16.07 (0.31)	34.97 (0.58)	-1.23 (0.49)	6.51 (0.7)
Sessile oak SW	76.5 (3.15)	3.05 (0.58)	16.48 (1.32)	37.08 (6.31)	-1.52 (1.37)	5.95 (5.61)
Sessile oak HW	60.35 (2.80)	5.94 (0.75)	18.72 (1.52)	27.94 (0.32)	-0.08 (0.34)	-2.24 (0.74)



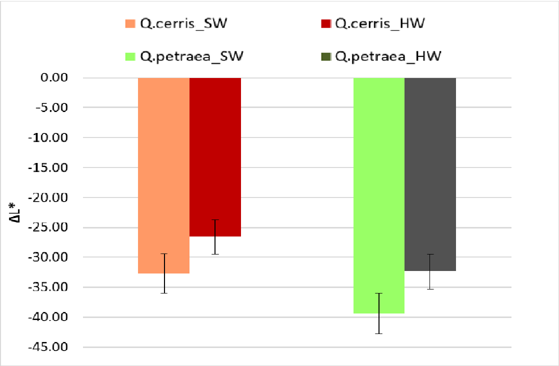
a. b.

Fig. 4. *Colour modification of Turkey oak (a.) and sessile oak (b.) wood samples after applying $FeCl_3$*

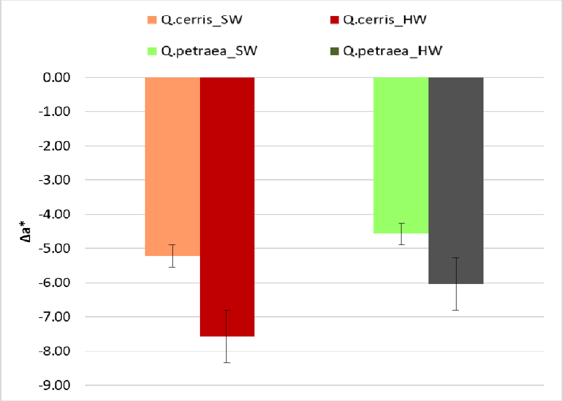
After applying $FeCl_3$, the *L** – coordinate decreases severely. The *a** – coordinate gathers negative values for all four wood types, meaning that the colour turns green rather than red. The *b** – coordinate increases for three of the four wood types (both sapwoods and Turkey oak heartwood), meaning that the yellow shade becomes more intense, while with sessile oak heartwood the *b** – coordinate becomes negative, which means that the colour of this wood comes closer to blue than to yellow.

Figure 5 presents the values of the colour change for each coordinate, as well as the total colour change (ΔE^*).

The negative values of ΔL^* show that all wood types became darker after the application of $FeCl_3$. The strongest darkening was recorded for the sessile oak sapwood (ΔL^* =-39.42 ± 3.56), followed by the Turkey oak sapwood (-32.74 ± 2.14), and very close to this, the sessile oak sapwood (ΔL^* =-32.42 ± 2.87). The weakest darkening was recorded for the Turkey oak heartwood (ΔL^* =-26.58 ± 0.68).



a.



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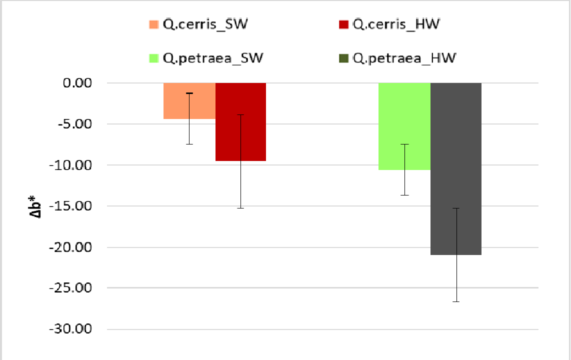
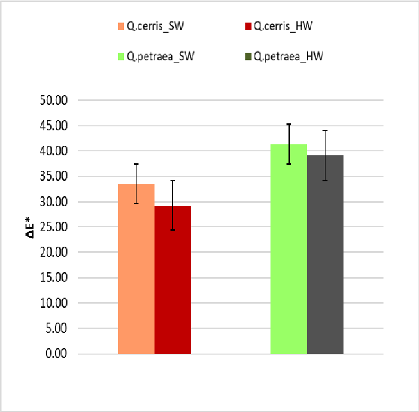


c.



d.

Fig. 5. *Colour modification of Turkey oak and sessile oak heartwood and sapwood samples after applying $FeCl_3$: a. change of luminosity (ΔL*); b. change of red-green coordinate (Δa*); c. change of yellow-blue coordinate (Δb*); d. total color change (ΔE*)*

The negative values of *Δa** show that all wood types turned green (on the red – green axis) after the application of $FeCl_3$. The tendency (demonstrated by higher *Δa** values) is stronger for the sessile oak heartwood, as well as for the Turkey oak sapwood and heartwood.

The negative values of *Δb** show that all wood types turned blue (on the yellow – blue axis) after the application of $FeCl_3$. The highest decrease of the *b** coordinate (Δb* =-20.96 ± 0.89) was obtained for the sessile oak heartwood, and the lowest (Δb* =-4.36 ± 1.88) was obtained for the Turkey oak sapwood.

Single factor ANOVA results for comparison among the analysed groups Table 3

SUMMARY						
Groups	Count	Sum	Average*	Variance	SD	
Turkey oak SW	3	100.4764684	33.49215613^{ab}	6.582179	2.565576	
Turkey oak HW	3	87.74599953	29.24866651^{b}	0.364351	0.603615	
Sessile oak SW	3	123.9707955	41.32359851^{ab}	22.37551	4.730276	
Sessile oak HW	3	117.3684525	39.12281751^{a}	3.266693	1.8074	
*the different letters on the bars indicate a statistically significant difference between the compared values						
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	269.3919895	3	89.79732982	11.02189	0.003256	4.066181
Within Groups	65.17746463	8	8.147183079			
Total	**334.5694541**	**11**				
p-value < 0.05 (adopted α); SS – sum of squares; df – degree of freedom; MS – mean square error; F – calculated value; F_{critic} – critical value.						

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Results of the t-test during Post Hoch Analysis Table 4

Sample	M	N	Variance	SD	df	t_{critic}	t	p-value	α
Turkey oak SW vs. Turkey oak HW – Not significant (p-value > α)									
Turkey oak SW	33.49	3	6.58	2.56	2	4.30	2.78	0.10	0.0125
Turkey oak HW	29.24	3	0.36	0.60					
Turkey oak SW vs. Sessile oak SW - Not significant (p-value > α)									
Turkey oak SW	33.49	3	6.58	2.56	3	3.18	2.52	0.08	0.0125
Sessile oak SW	41.32	3	22.37	4.73					
Turkey oak HW vs. Sessile oak HW - Significant (p-value < α)									
Turkey oak HW	29.24	3	0.36	0.6	2	4.30	8.97	0.0122	0.0125
Sessile oak HW	39.12	3	3.26	1.80					
Sessile oak SW vs. Sessile oak HW - Not significant (p-value > α)									
Sessile oak SW	41.31	3	22.37	4.73	3	3.18	0.75	0.50	0.0125
Sessile oak HW	39.12	3	3.26	1.80					

M-is the mean; N- sample size; SD – standard deviation; df - degree of freedom; t_{critic} – critical value; t – calculated value.

The values of the total colour change (ΔE^*) show that the sessile oak wood suffered a more intense colour darkening, both in sapwood and in heartwood, thus suggesting a higher amount of tannins than Turkey oak wood.

As in the case of the secondary chemical components, it is the sapwood of Turkey oak that contains a greater amount of tannin, considering that its colour change (ΔE) is by 14.5% higher than in the heartwood.

However, the values of ΔE^* obtained for the four types of wood are quite close, and the statistical analysis revealed a significant difference only between the total color modification of the heartwood of the two species (Tables 3 and 4), same as obtained for the amount of extractable substances.

5. Conclusions

The conducted research revealed that:

1. Turkey oak sapwood has a higher amount of extractable substances than the heartwood of this species. The FTIR analysis of the extracts impregnated on support paper sustains this result, though a firm quantitative conclusion cannot be formulated based on this type of investigation. The differences between sapwood and heartwood

were found to be statistically significant for the cold and hot water extraction, where the amount of extractable substances was 81% and 83% higher in the sapwood than in the heartwood.

2. By comparing the two *Quercus* species, the research revealed that the amount of extractable substances of the Turkey oak heartwood is significantly lower than that of the sessile oak heartwood, while the sapwood of Turkey oak wood contains fewer extractable substances than the sessile oak sapwood.
3. The above mentioned results obtained with three different extraction methods were also confirmed by the colour change measurement performed before and after applying $FeCl_3$. Thus, the colour tests clearly showed that the Turkey oak sapwood contains more tannins than the heartwood of the same species, and also that Turkey oak wood generally contains a lower amount of tannins than sessile oak wood.

The results of this research are important for the characterisation of Turkey oak wood in comparison to sessile oak wood, in order to better understand the peculiarities of its behaviour during drying, mechanical processing, gluing and finishing, and to increase its valorisation chances for furniture production.

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