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CHEMICAL CHARACTERISATION OF EUROPEAN BEECH (*Fagus sylvatica* L.) MATURE WOOD AND FALSE HEARTWOOD

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ABSTRACT

False heartwood in European beech (*Fagus sylvatica* L.) is a significant defect reducing the usability of the wood for aesthetic reasons. The aim of this work was to compare the chemical composition of mature wood and false heartwood of beech regarding their different coloring. The content of the main wood components, extractives, polyphenolic compounds, and soluble carbohydrates was determined. In addition, FTIR (Fourier transform infrared spectroscopy) analysis of mature wood, border zones and false heartwood was also performed. Chemical analyses showed a slightly higher content of holocellulose in false heartwood compared to mature wood. The differences in cellulose and lignin content are minimal. The content of both lipophilic and hydrophilic extractives is higher in mature wood. Hydrophilic extract from mature wood contains more phenolics and soluble carbohydrates compared to false heart. According to ATR-FTIR (Attenuated total reflectance Fourier transform infrared spectroscopy) analysis it can be assumed that there is an increase in the content of polyphenolic extractives in the border zone, especially in the darkest colored zone next to the false heart.

Keywords: false heartwood; European beech; chemical composition; polyphenolic extractives; FTIR.

INTRODUCTION

European beech (*Fagus sylvatica* L.) is widely available across Europe, and it is the dominant hardwood species in the Slovak Republic. Beech wood serves primarily to produce furniture, lumber, veneer, flooring, plywood, and turned objects. Slovak consumers most often buy seating furniture, dining furniture and cabinets made of beech wood (Sedliačiková and Moresová, 2022). However, the use of beech wood is restricted by various wood defects. One of them is the presence of false heartwood, which reduces the usability of the wood for aesthetic reasons (Klement and Vilkovská, 2019).

False heartwood formation in beech is facultative, in contrast to the obligatory colored heartwood forming species. Its occurrence frequency, extent, and type are not predictable (Hofmann *et al.*, 2022). The existence of false heartwood in still-standing beeches is not visible on the surface, however, it can be predicted based on tree age, average growth rate, and the number of bark injuries (dead branches, knots, large scars) through which oxygen can enter the stem (Knoke, 2003).

For many years, research has focused on finding the causes of false heartwood. Some authors (Kúdela and Čunderlík, 2012, Klement and Vilkovská, 2019) state that false

heartwood formation is associated with the breaking down of the water transport system and decreasing vitality of the parenchyma tissues. According to Račko and Čunderlík (2010) the occurrence of ripewood zone and the oxygen penetration into structure of wood are two main causes of false heartwood formation. Oxygen penetrated into ripewood zone in the central part of trunk via wounding the trunk and breaking the branches. Since heartwood formation begins only if the oxygen concentration in the ripewood zone is large, false heartwood formation depends on largeness of branch and trunk wounds. The wounds are caused by silvicultural treatments in the forest, by wildlife or by naturally occurring events, such as wind, storms, or snow. As regards ripewood zone, its width depends on the growth condition in the forest stands and increases as the trees age (Račko and Čunderlík, 2007). In accordance with the above Suchomel and Gejdoš (2010) recommend reducing the occurrence of the false heartwood the selection for nutrients richer soils for cultivation of beech forest, shorter felling periods, less intensive cultivation, and treatment of damaged locations on the trunk surface.

A false heart is characterized by a darker color of the wood (Slabejová, 2013) whereas "healthy" false heartwood and the decay-induced discoloration are not easily distinguishable (Hörnfeldt *et al*., 2010). False heart can occur in different forms such as round, marble, spattering or rot heartwood (Trenčiansky *et al*., 2017). Dzurenda (2023) numerically documented the visual color differences between the color of beech mature wood and the color of sapwood as well as between the color of false heartwood and sapwood through the values of total color differences. The color difference between mature wood and sapwood belongs to the category of visible changes with $\Delta E^* = 3.5$. The difference between the color of the false heartwood and the color of the sapwood reached the value $\Delta E^* = 18.1$ and belongs to the category of significant color changes.

The natural durability and mechanical properties of false heartwood do not differ much from those of mature beech wood (Koch *et al.*, 2003). However, in the case of false heartwood, the presence of mechanical barriers (tyloses) that arise as a result of oxygen penetration affects not only the length of the drying process (Koch *et al.*, 2003) but contributes also to issues with impregnation (Hörnfeldt *et al*., 2010).

As the differences in several properties of wood are dependent on different chemical composition, the aim of this article is to compare the chemical composition of mature wood and false heartwood of European beech. The focus is put on the content of the main wood components, extractives, polyphenolic compounds, and soluble carbohydrates. To find out the differences in chemical composition not only between mature wood and false heart, but also at their boundary, the individual zones of beech wood were subjected to ATR-FTIR (Attenuated total reflectance Fourier transform infrared spectroscopy) analysis.

MATERIALS AND METHODS

Materials

European beech (*Fagus sylvatica* L.) wood harvested from the Štiavnické vrchy locality (Slovakia), was studied. For research, 25 lumber-logs with healthy round false heartwood were selected. Blanks with dimensions of 32 × 50 × 800 mm were produced by spreading the central lumber with a thickness of h = 50 mm and transverse handling. Three pieces of blanks were randomly selected from each central lumber.

Thin sections with distinct mature wood, border and false heartwood zones were prepared for ATR-FTIR (Attenuated total reflectance Fourier transform infrared spectroscopy) analysis (Fig. 1). Mature wood and false heartwood were separated for

chemical analyses. The samples were disintegrated into sawdust, and the 0.5-1 mm fraction was used for analysis.

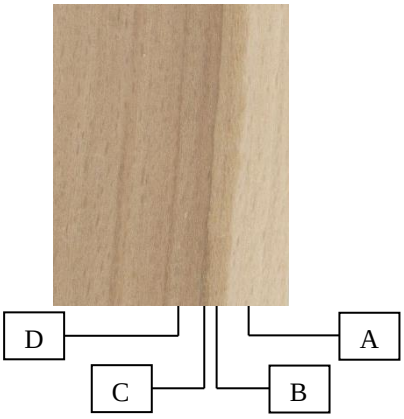


Fig. 1 Zones of beech wood analysed by ATR-FTIR spectroscopy (A – mature wood, B – border zone next to the mature wood, C – border zone next to the false heart, D –false heart)

Chemical analyses

In both samples, mature wood and false heartwood, the contents of extractives and main wood components were determined. All measurements were performed on three replicates per sample. The results were expressed in percentage of dry mass. Moreover, extraction with a methanol-water mixture was also performed to determine the total phenolic content and total soluble carbohydrates. Chemical analyses were performed according to the following procedures:

Ethanol-toluene solubility of wood according to ASTM D 1107-96 (2013)

2 g of sawdust were extracted with ethanol-toluene (2:1, v/v) for 7 h in a Soxhlet apparatus. The resulting extract was distilled off in a vacuum evaporator and dried in an oven at t = 103 (± 2) °C to constant weight.

Hot-water solubility of wood according to ASTM D 1110-84 (1995)

2 g of sawdust were heated in a conical flask with 100 ml of distilled water on a boiling water bath for 3 h. After filtering and washing (200 ml of boiling distilled water), the sample was dried in an oven at t = 103 (± 2) °C to constant weight.

Polysaccharide fraction (holocellulose) according to Wise method (Kačík and Solár, 2000)

Extracted sawdust (5 g) was treated with $NaClO_2$ (1.5 g) in the presence of acetic acid (10 drops) for 1 h at t = 80–90 °C. This procedure was repeated 3-fold. After washing (water, acetone), cooling and filtering, the sample was dried in an oven at t = 103 (± 2) °C to constant weight.

Cellulose according to Kürschner-Hoffer method (Kačík and Solár, 2000)

Extracted sawdust (1 g) was boiled with a mixture of concentrated HNO_3 and 95% ethylalcohol (1:4) in a flask under reflux for 1 h. This procedure was repeated 3-fold. After filtering and washing (ethanol + HNO_3, hot water), the sample was dried in an oven at t = 103 (± 2) °C to constant weight.

Acid-insoluble lignin according to ASTM D 1106-96

Extracted sawdust (1 g) was treated with 15 ml of 72 % H_2SO_4, intense stirred for 1 min at t = 12–15 °C, and then allowed to stand for 2 h at a room temperature (20 °C). Consequently, it was quantitatively transferred to a boiling flask, diluted to 3 % concentration with 560 ml of distilled water and boiled under reflux for 4 h. The brown lignin precipitate settled, filtered through a weighed glass filter, and thoroughly washed with hot water (500 ml) followed by drying in an oven at t = 103 (± 2) °C to constant weight.

Extraction with methanol-water

2 g of sawdust were extracted with methanol-water (1:1) using an automated solvent extractor Dionex ASE 350 (Thermo Fisher Scientific, USA). The extraction conditions were as follows: 50 °C extraction temperature; 3 extraction cycles; 5 minutes static time per cycle; 50% of the cell volume as flush volume; 90 s of purging with nitrogen. Both samples (mature wood and false heart) were extracted in three replicates. The gained extract volume was adjusted with the same solvent to get 25 ml of extract. An aliquot of 2 ml served for further analysis, while the remaining 23 ml were left for extractive content determination. Finally, the mixture containing solids (after partial solvent evaporation) was dried in an oven t = 103 (± 2) °C to constant weight. The extractive content was determined gravimetrically and expressed in percentage of dry mass.

Total phenolic content (TPC)

An aqueous solution of gallic acid served as a standard for calibration of the total phenolic content. Calibration solutions of 5 ml volume covered the concentration range of gallic acid between 2 – 30 μg/ml. The solution was vortexed, 0.2 ml of Folin-Ciocalteu reagent was added to each tube containing diluted standard solution, vortexed again, and 3 min later 1 ml of 20 wt.% solution of sodium carbonate was pipetted to each tube (Čermák *et al*., 2019). The mixture was vortexed and incubated for 30 min. The absorbance of solutions was then measured at the wavelength of 700 nm against demineralized water using UV-VIS spectrophotometer (Shanghai Metash Instruments, China).

The phenolic compounds present in the methanol-water extract were exposed to Folin-Ciocalteu reagent and proceeded as done in the case of gallic acid for calibration. Briefly, extract aliquot of 0.1 ml was diluted with demineralized water to get final volume of 5 ml. Each methanol-water extract was analysed in two parallel measurements. Six values for mature wood and six values for false heartwood were obtained. The amount of phenolic compounds present in extracts was expressed in milligrams of gallic acid equivalents per gram dried weight (mg GAE/g dw).

Total soluble carbohydrates (TSC)

The total carbohydrate content was expressed using phenol-sulfuric acid assay (Dubois *et al*., 1956) with an adaption for wood according to the protocol of Čermák *et al*. (2019). The calibration was performed with an aqueous solution of D-(+)-Glucose within the concentration range 12.5 – 125 μg/ml and the response was plotted as a linear curve (R^2=0.9988). Each tube contained 2 ml of diluted standard solution. 1 ml of fresh 5 wt.% phenol solution was added to each tube, followed by addition of 5 ml of concentrated sulfuric acid. After cautious mixing, the tubes were left for: 10 min cooling down (at room temperature); 20 min in water bath (at 30 °C); and finally, 30 min for color stabilization (at room temperature). Afterwards, absorbance of the samples was recorded at 490 nm against demineralized water. The aliquot volume of 0.2 ml sample of methanol-water extract was diluted with demineralized water to final volume of 2 ml and further steps were done under the same conditions as with calibration solutions of D-(+)-Glucose. Each methanol-water

extract was analysed in two parallel measurements. Six values for mature wood and six values for false heartwood were obtained. The final concentration of soluble carbohydrates present in the extract was expressed in milligrams of glucose equivalents per gram dried weight (mg GluE/g dw).

UV-VIS spectrum scan

The spectra of extracts from mature wood and false heartwood were scanned using UV-VIS spectrophotometer Agilent Cary 60 (USA). The measured spectrum was in the range 200–500 nm and the scan rate was set at 150 nm/min. Prior to the measurement of extracts, response within the range was baseline corrected by measurement of extraction solution (i.e. methanol-water 1:1).

ATR-FTIR spectroscopy

ATR-FTIR analysis was carried out using a Nicolet iS10 FTIR spectrometer equipped with Smart iTR attenuated total reflectance (ATR) sampling accessory with diamond crystal (Thermo Fisher Scientific, Madison WI, USA). The spectra were measured in the wavenumber range from 4000 cm^{-1} to 650 cm^{-1}, whereas the resolution was set at 4 cm^{-1}. The number of scans were 32 scans for each analysis. Eight analyses were performed per each zone of wood. The spectra were normalised to the band maximum at around 1370 cm^{-1}. OMNIC 8.0 software (Thermo Fisher Scientific, Madison WI, USA) was used for evaluation.

RESULTS AND DISCUSSION

The relative content of chemical components of both, mature wood, and false heartwood of beech, are shown in Tab.1.

Tab. 1 Chemical characteristics of mature wood and false heartwood of beech (TEE – toluene-ethanol extractives, HWE – hot water extractives).

	TEE (%)	HWE (%)	Hollocellulose (%)	Cellulose (%)	Lignin (%)
Mature wood	2.50 (0.10)	1.80 (0.04)	75.31 (0.04)	40.57 (0.09)	20.39 (0.08)
False heartwood	1.28 (0.13)	1.46 (0.04)	76.27 (0.17)	40.00 (0.18)	20.98 (0.14)

* standard deviation in parentheses

The content of polysaccharides, the major component of wood, is almost 1 % higher in false heartwood, than in mature wood (76.27 %. resp. 75.31 %). It is a sign of a higher proportion of hemicelluloses, because cellulose shows a comparable proportion in both samples. The difference in lignin content is also minimal. Nečesaný (1958) reports a similar distribution of the main wood components in sapwood and false heartwood of beech, but with somewhat different values for the individual components between the two zones of the tree trunk. Both groups of extractives, TEE and HWE, have a higher proportion in mature wood than in false heartwood. Lipophilic substances, fatty acids, sterols, sterol esters and triglycerides are released into the toluene-ethanol mixture. Hot water removes phenolic compounds, sugars, starches and coloring matters (Vek *et al*., 2016). The total content of extractives is 4.30 % in mature wood, and 2.74 % in false heartwood. In spite of their lower proportion, they play important functions and affect many properties of wood, such as color, resistance to biotic damage or heating value. Vek *et al.* (2014) qualitatively and

quantitatively evaluated the composition of low-molecular weight extractives in the sapwood and false heartwood of European beech. They found a comparable content of lipophilic extractives in sapwood and false heartwood, while the total content of hydrophilic extractives was higher in sapwood. False heartwood contained significantly larger amounts of saturated fatty acids, fatty alcohols, and triterpenoids than sapwood, however, these compounds do not contribute to wood color. Sapwood contained larger concentrations of mono- and oligosaccharides, sugar acids, carboxylic acids, simple phenols, and flavonoids than false heartwood. The content of β-sitosterol, the characteristic compounds of the non-polar extracts, was higher in false heart. Catechin, the characteristic compounds of the polar extracts, prevailed in the sapwood.

Extraction with a methanol-water mixture was also performed to determine the total phenolic content and total soluble carbohydrates. Hydrophilic extractives are released into this extraction solution, similarly to hot water. In this case, the content of extractives was also higher in mature wood than in the false heartwood, namely 1.7 times (Tab. 2). Similar extractive content in beech sapwood (2.41%) was also detected by Sablík *et al*. (2016), who used the same solvent.

Tab. 2 Content of the methanol/water extractives (MWE) in mature wood and false heartwood of beech, and the total phenolic content (TPC) and total soluble carbohydrates (TSC) in these extracts

	MWE (%)	TPC (mg GAE/g dw)	TSC (mg GluE/g dw)
Mature wood	2.19 (0.02)	1.63 (0.03)	5.64 (0.12)
False heartwood	1.32 (0.03)	0.62 (0.02)	1.16 (0.03)

* standard deviation in parentheses

As for phenolics, in our experiment, their content was 2.6 times higher in mature wood extract than in false heart (Tab. 2). Lower values of total phenolic content in false heartwood than in sapwood were found also by other researchers (Vek *et al*., 2013, Vek *et al*., 2015, Albert *et al*., 2002). The content of the total soluble carbohydrates in wood is closely related to the content of phenolic substances. In mature wood, 4.9 times higher concentration of total soluble carbohydrates than in red-coloured heartwood was observed. This result is in accordance with observation by Visi-Rajczi *et al.* (2003). They found glucose, fructose, and sucrose such main components of soluble carbohydrates in beech. In beech with false heartwood their amount rises before the colour boundary and decreases behind it sharply, while it is negligible in the false heartwood. In beech without false heartwood, a continuous and monotonic decrease towards the pith was observed, but high concentrations of carbohydrates were still found near the pith. According to Albert *et al.* (2002) the beech tree accumulates some amounts of sucrose in the tissues in front of the border and presumably synthetizes them to *in situ* phenols.

The above-mentioned trend (higher contents of phenolics and soluble carbohydrates in mature wood) was confirmed by the UV-VIS spectrum of methanol/water extracts (Fig. 2). Absorption maxima in both extracts were at around 230 and 280 nm, while the second maximum left a shoulder at around 300–350 nm. The mature wood extract had broader absorption peaks at these wavelengths and obviously higher absorbance at around 280 nm indicating a wider variety of present substances absorbing in this region. No absorption peaks in the visible region of light were detected. A wide variety of flavonoids and phenolic acid derivatives present in mature wood (Hofmann *et al*., 2022) might explain the higher absorbances of mature wood extract. Such absorption peaks between 230 and 290 nm are typical chromophores in flavonoids (Wei *et al.*, 2022). The absence of further peaks (around

350 nm or 510 nm) suggests occurrence of flavan-3-ols (e.g. catechins) (Andersen, Markham, 2006) in both extracts as the most likely flavonoid type.

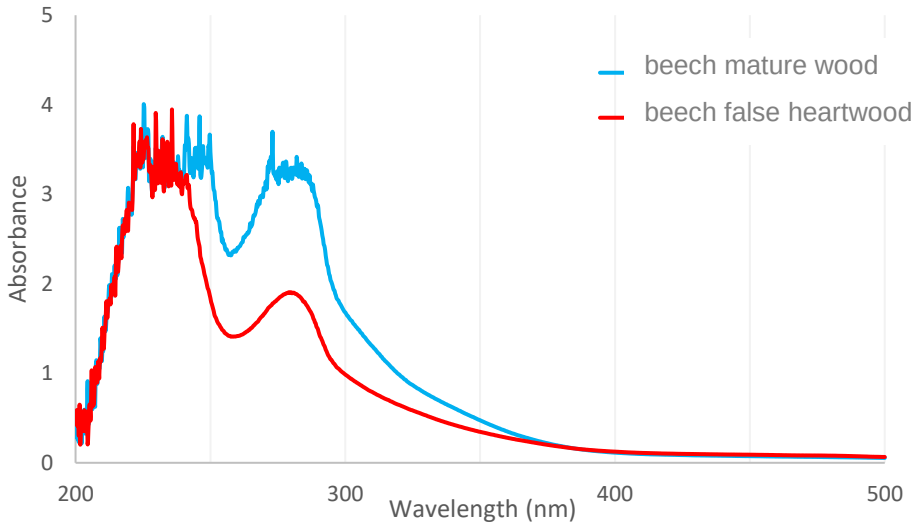


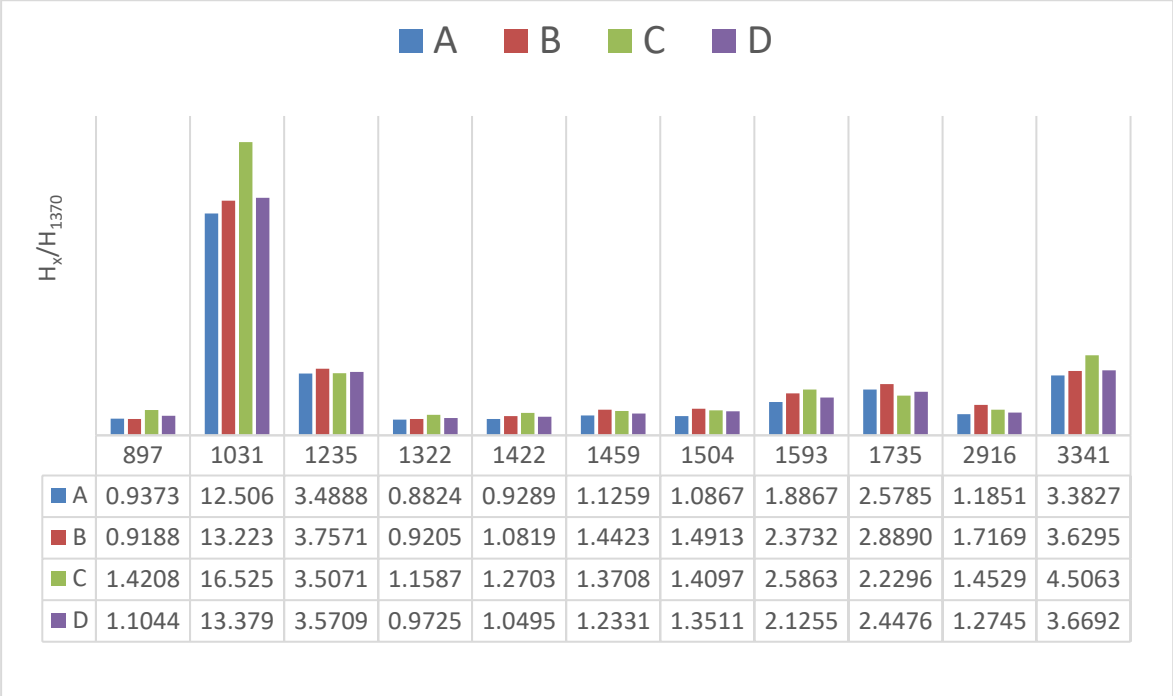
Fig. 2 UV-VIS absorption spectrum of extract from beech mature wood (blue line) and beech false heartwood (red line) in the wavelength range 200–500 nm after baseline correction

However, the exact composition or predominantly present flavonoid group responsible for this spectrum would need to be deeper investigated, since UV region is not very compound specific as many compounds absorb in this wavelength region. Moreover, extractability into the solvent also plays an important role and can have an impact on the quantitative and qualitative analyses. Polyphenols of higher molecular weight or single polyphenols chemically bonded to cell wall polymers can not easily be extracted and are so-called non-extractable polyphenols (NEPP) (Pérez-Jiménez *et al.*, 2014).

According to literature (Albert *et al.*, 2002) in the formation of false heartwood in beech trees intensive metabolical processes occur at the colour boundary. Knowledge of the radial distribution of the chemical components of wood can contribute to clarifying the formation of false heart in beech wood. In Fig. 3 relative intensities of characteristic absorption bands of beech wood components are shown. The interpretation of FTIR spectra was performed based on the literature (Bhagia *et al.*, 2022, Hon, Shiraishi, 2000, Németh, *et al.* 2016, Stark *et al.* 2015).

Stretching vibrations of hydroxyl -OH and of intra- and intermolecular hydrogen bonds are located at the wavenumber 3341 cm^{-1}. Hydroxyl groups are found in all components of wood – polysaccharides, lignin and in some groups of extractives, especially in phenolic substances. The related intensity of this peak is the highest in zone C, which is the border zone next to the false heart, and the lowest in mature wood. The overlapping bands with maximum of intensity about 2916 cm^{-1} is associated with C-H symmetric and asymmetric stretching vibrations in methyl and methylene groups. The maximum of absorption band, characteristic of the C=O group in ketones, aldehydes, carboxyl acids, and esters, lies at 1735 cm^{-1}. The relative peak intensity decreases in order of zones B > A > D > C. Generally, higher intensity of this band in FTIR spectra may be associated with a higher proportion of hemicelluloses which are rich in acetyl groups in xylan. However, chemical analyses determined a lower proportion of hemicelluloses and higher proportion of extractives in the mature wood compared to false heart. For this reason, it can be assumed that some groups of extractives contribute to the higher intensity of this peak in

zone A compared to zone D. In addition, carbonyl groups are also formed during oxidation reactions of wood components (Pandey, 2005, Liu *et al*., 2016, Feist, Hon,1984).



	897	1031	1235	1322	1422	1459	1504	1593	1735	2916	3341
A	0.9373	12.506	3.4888	0.8824	0.9289	1.1259	1.0867	1.8867	2.5785	1.1851	3.3827
B	0.9188	13.223	3.7571	0.9205	1.0819	1.4423	1.4913	2.3732	2.8890	1.7169	3.6295
C	1.4208	16.525	3.5071	1.1587	1.2703	1.3708	1.4097	2.5863	2.2296	1.4529	4.5063
D	1.1044	13.379	3.5709	0.9725	1.0495	1.2331	1.3511	2.1255	2.4476	1.2745	3.6692

Fig. 3 Relative intensities H_x/H_{1370} of absorption bands in ATR-FTIR spectra of beech wood zones (A – mature wood, B – border zone next to the mature wood, C – border zone next to the false heart, D – false heart)

Characteristic bands for aromatic skeletal vibrations lie at wavenumbers 1593, and 1504 cm^{-1}. The higher proportion of lignin certainly contributes to the higher proportion of the aromatic skeleton in the false heart compared to the mature wood. However, the intensities of both peaks are the highest in border zones, which indicates the highest lignin content in these locations. It is remarkable, that the same distribution can also be observed in the case of hydroxyl groups at 3341 cm^{-1}. Based on these findings, it can be assumed that the content of polyphenolic extractives increases in the border zone, especially in the darkest colored zone C. This assumption is also confirmed by other authors (Hofmann *et al*. 2022, Albert *et al.*, 2002). Hofmann *et al*. 2022 found 125 various polyphenolic compounds in European beech wood extracts. The most abundant types of compounds were the flavan-3-ols, including (+)-catechin, (-)-epicatechin and their derivatives. The second-largest group was flavonol and flavonon compounds and their conjugates. In addition, gallic acid derivatives, simple phenols, phenolic acids, and aromatic aldehydes have been identified. It was found that the concentration of many compounds increased at the color boundary and decreased sharply behind it. In the false heartwood, only free aglycones could be evidenced in low amounts, however, the presence of oxidized high-molecular-weight polymeric polyphenols was not confirmed. It is questionable whether the coloring of the false heart is caused by low molecular weight compounds identified in the zone of false heartwood or high molecular weight compounds chemically bound to the structural polymers of the cell wall as non-extractable polyphenols. The problem with insufficient isolation of phenolics from wood samples is eliminated using ATR-FTIR analysis, which enables the monitoring of functional groups and fragments of chemical compounds in the original sample. However,

it is not possible to identify individual groups of extractives from the FTIR spectra of a complex material such as wood.

The band at 1422 cm^{-1} is assigned to aromatic skeletal vibration combined with CH in-plane deformation in lignin and -CH_2 bending vibration in cellulose. Its relative intensity is decreased in the order of zones C > B > D > A. The peak with a maximum at 1322 cm^{-1} is a result of two overlapped bands. The first have a maximum at 1316 cm^{-1} and is associated with CH_2 wagging vibrations in cellulose. The second band is located at 1333 cm^{-1}, and is associated with CH in-plane bending vibrations in cellulose and syringyl ring breathing with C-O stretching vibrations in lignin. Its relative intensity decreases in the order of zones C > D > B > A. The highest peak in all spectra lies at wavenumber 1031 cm^{-1} and is assigned to C−O and C−C stretching and C−OH bending in carbohydrates. Its relative intensity is the highest in the border zone next to the false heartwood.

CONCLUSION

Based on the chemical analysis of mature wood and false heartwood in European beech (*Fagus sylvatica* L.) and the ATR-FTIR analysis of individual zones of radial section of wood, the following conclusions can be stated:

- False heartwood contains slightly more holocellulose than mature wood.
- The differences in cellulose and lignin content are minimal.
- The content of both lipophilic and hydrophilic extractives is higher in mature wood.
- The contents of phenolics and soluble carbohydrates are higher in hydrophilic extractives from mature wood than from false heartwood.
- The intensities of absorption bands of both aromatic structures and hydroxyl groups increase in the border zone, especially in the darkest colored zone next to the false heart.

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