

## Contribution to Understanding the Occurrence of Extractives in Red Heart of Beech

Viljem Vek,<sup>a</sup> Primož Oven,<sup>a,\*</sup> Ida Poljanšek,<sup>a</sup> and Thomas Ters<sup>b</sup>

Common beech (*Fagus sylvatica*) is one of the deciduous tree species characterized by the formation of a discolored red heart in the central part of the stem. The aim of this work was to review data in existing literature and to present original results on the extractives present in sapwood and the red heart of beech. Samples of sapwood and red heart were taken from freshly felled beech trees and extracted with a speed extractor. The content of lipophilic and hydrophilic extractives was determined gravimetrically and further evaluated by gas chromatography. The beech wood contained, on average, 1.04% lipophilic and 3.71% hydrophilic extractives. Even though the gravimetrically determined content of lipophilic extractives was comparable in the sapwood and the red heart, saturated fatty acids, fatty alcohols, and free sterols were dominant in the red heart. Sapwood contained a larger amount of total hydrophilic extractives. Mono- and oligosaccharides, sugar acids and alcohols, carboxylic acids, simple phenols, and flavonoids were identified as the prevailing hydrophilic solubles in sapwood, whereas the concentration of sugar alcohols was higher in the red heart. The composition and character of the extractives in the wood of red-hearted beech should be considered the relevant technological factor.

*Keywords:* *Fagus sylvatica*; Discoloration; Sapwood; Red heart; Extraction; GC/MS; Extractives; Variability

*Contact information:* a: University of Ljubljana, Biotechnical Faculty, Department of Wood Science and Technology, Jamnikarjeva 101, 1000 Ljubljana, Slovenia; b: Vienna University of Technology, Institute of Chemical Engineering, Karlsplatz 13, 1040 Vienna, Austria;

\* Corresponding author: primo.z.oven@bf.uni-lj.si

### INTRODUCTION

Common beech (*Fagus sylvatica* L.) accounts for approximately one third of the wood stock in Slovenian forests (Slovenian Forest Service 2011). It is an economically important tree species widely used by the wood industry (Wernsdörfer *et al.* 2005; Mali *et al.* 2009). Beech is characterized by the formation of discolored wood in the central part of the stem, often called the “red heart” (Torelli 1984). Discoloration in wood generally relates to physiological, biochemical, and chemical reactions that take place in living trees (Bauch 1984) during the thermal treatment of wood (*e.g.*, drying and steaming) or during thermal modification (Koch *et al.* 2003; Jamalirad *et al.* 2011; 2012). The formation of a red heart in the wood of a living beech tree is ascribed to physiological reactions of living parenchyma (formation of tyloses and accumulation of accessory compounds) triggered by oxygen penetration into tissues and to enzymatically-activated biochemical changes (Bauch 1984; Torelli 1984).

Accessory compounds, or extractives, play an important role in the formation of discoloration. These are low-molecular weight, non-structural components of wood located in the lumen of cells and extraneous to the lignocellulosic cell wall. From a physiological point of view, the extractable compounds of plant tissues are known to be primary and secondary metabolites (Rowe and Conner 1979; Fengel and Wegener 1984; Kai 1991; Holmbom 1999).

Depending on the solvents in which they are soluble, extractives can be divided into lipophilic and hydrophilic categories (Willför *et al.* 2006; Jansson and Nilvebrant 2009). The xylem of tree species in temperate climatic zones contain relatively small amounts of extractives, up to 5 to 10% (Umezawa 2000), but concentrations can be much larger in certain parts of a tree (*e.g.*, in branch bases, bark, and roots) (Fang *et al.* 2013; Latva-Mäenpää *et al.* 2014). Larger amounts of extractives have been found in some tropical and subtropical woods (Fengel and Wegener 1984; Holmbom 1999).

Beech wood is characterized by a relatively low amount of extractives (Rowe and Conner 1979; Kubel and Weissmann 1988). Wagenführ (1996) reported an extractive content of beech wood between 3 and 5% and a fraction of inorganic compounds between 0.3 and 1.2%.

In beech wood sawdust, Bodirlau *et al.* (2008) found 0.93% benzene-alcohol solubles, 2% hot water solubles, and 13.15% of the sawdust was soluble in 1% NaOH. In the case of fresh beech wood chips, Sixta *et al.* (2004) found that 0.2% could be extracted with dichloromethane, 1.0% with acetone, that 1.7% of the extractives were soluble in ethanol, and 2.7% were water-soluble. According to Košíkova *et al.* (2008), the extractives content of healthy sapwood, around 1.78% acetone-soluble extractives, differed significantly from that of decayed wood, which contained only 0.98%. Kubel and Weissmann (1988) reported that the contents of extractives soluble in petrol ether and diethyl ether were relatively low, only 0.2% and 0.1%, respectively. More compounds are extractable by more polar solvent mixtures, such as acetone/water (9:1, v/v) (1.6%) or ethanol/water (8:2, v/v) (1.2%). The amount of water-solubles was reported to be 0.3% (Kubel and Weissmann 1988).

When reviewing literature regarding the occurrence and composition of low-molecular weight extractives in beech tissue, it became clear that existing data provide only limited information. A review of the identified lipophilic and hydrophilic extractives in various parts of a beech tree (*e.g.*, wood (treated and untreated), bark, and leaves), is presented in Tables 1 and 2. In addition, detailed data regarding the composition of extractives in different categories of wound-associated tissues of beech has recently been presented by Vek *et al.* (2014). The cited report contributes to the understanding of physiological response of wood to wounding, but did not reveal the extractive composition of the practically inevitable red-hearted stem wood of beech.

The aim of this study was therefore to qualitatively and quantitatively evaluate the composition of low-molecular weight extractives in the sapwood and red heart of common beech (*Fagus sylvatica* L.) by means of gas chromatography coupled with mass spectrometry.

**Table 1.** Occurrence of Lipophilic Extractives in the Wood and Other Parts of a Beech Tree

Compound	Type of beech tree tissue	Reference
Lauric (C12:0) acid	Sapwood dust	Kubel and Weissmann (1988)
Myristic (C14:0) acid	Sapwood dust	Kubel and Weissmann (1988)
Palmitic (C16:0) acid	<sup>1</sup> Sapwood dust, <sup>2</sup> wood chips	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Zule and Može (2003)
Stearic (C18:0) acid	<sup>1</sup> Sapwood dust, <sup>2</sup> wood chips	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Zule and Može (2003)
Arachidic (C20:0) acid	Sapwood dust	Kubel and Weissmann (1988)
Behenic (C22:0) acid	Sapwood dust	Kubel and Weissmann (1988)
Lignoceric (C24:0) acid	Sapwood dust	Kubel and Weissmann (1988)
Palmitoleic (C16:1) acid	Sapwood dust	Kubel and Weissmann (1988)
Oleic (C18:1) acid	<sup>1</sup> Sapwood dust, <sup>2</sup> wood chips	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Zule and Može (2003)
Elaidic (C18:1) acid	Sapwood dust	Kubel and Weissmann (1988)
Linoleic (C18:2) acid	<sup>1</sup> Sapwood dust, <sup>2</sup> wood chips	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Zule and Može (2003)
Linolenic (18:3) acid	Sapwood dust	Kubel and Weissmann (1988)
Hydroxyoctadecadiene acid	Sapwood dust	Kubel and Weissmann (1988)
Dehydroabietic acid	Sapwood dust	Kubel and Weissmann (1988)
Squalene	Dried wood	Pišova and Souček (1973)
Cycloartenyl acetat	Dried wood	Pišova and Souček (1973)
$\beta$ -amyrin acetate	Dried wood	Pišova and Souček (1973)
Acetyl methyl betulinate	Dried wood	Pišova and Souček (1973)
$\beta$ -carotene	Sapwood	Masson <i>et al.</i> (1997)
Lutein	Sapwood	Masson <i>et al.</i> (1997)
$\beta$ -sitosterol	<sup>1</sup> Sapwood dust, <sup>2</sup> wood chips	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Zule and Može (2003)
$\beta$ -Sitostanol (dihydrositosterol)	<sup>1</sup> Dried wood, <sup>2</sup> sapwood dust	<sup>1</sup> Pišova and Souček (1973), <sup>2</sup> Kubel and Weissmann (1988)
Campesterol	Sapwood dust	Kubel and Weissmann (1988)

**Table 2.** Occurrence of Hydrophilic Extractives in the Wood and Other Parts of a Beech Tree

Compound	Type of beech tree tissue	Reference
Glucose	<sup>1</sup> Sapwood, <sup>2</sup> sapwood dust, <sup>3</sup> wood condensate	<sup>1</sup> Dietrichs (1964b), <sup>2</sup> Kubel and Weissmann (1988), <sup>3</sup> Irmouli <i>et al.</i> (2002)
Galactose	<sup>1</sup> Sapwood dust, <sup>2</sup> wood condensate	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Irmouli <i>et al.</i> (2002)
Arabinose	<sup>1</sup> Sapwood dust, <sup>2</sup> wood condensate	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Irmouli <i>et al.</i> (2002)
Fructose	<sup>1</sup> Sapwood, <sup>2</sup> sapwood dust, <sup>3</sup> wood condensate	<sup>1</sup> Dietrichs (1964b), <sup>2</sup> Kubel and Weissmann (1988), <sup>3</sup> Irmouli <i>et al.</i> (2002)
Xylose	<sup>1</sup> Sapwood dust, <sup>2</sup> wood condensate	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Irmouli <i>et al.</i> (2002)

Mannose	<sup>1</sup> Sapwood dust, <sup>2</sup> wood condensate	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Irmouli <i>et al.</i> (2002)
Rhamnose	Sapwood dust	Kubel and Weissmann (1988)
Saccharose	<sup>1</sup> Sapwood, <sup>2</sup> wood condensate	<sup>1</sup> Dietrichs (1964b), <sup>2</sup> Irmouli <i>et al.</i> (2002)
Raffinose	Wood condensate	Irmouli <i>et al.</i> (2002)
Stachyose	Wood condensate	Irmouli <i>et al.</i> (2002)
Starch	Sapwood	Dietrichs (1964a); Dietrichs (1964b)
Glycerol	Wood chips	Zule and Može (2003)
Xylitol	Wood chips	Zule and Može (2003)
Kaempferol	Leaves	Pirvu <i>et al.</i> (2010)
Apigenin,	Leaves	Pirvu <i>et al.</i> (2010)
Quercetin,	Leaves	Pirvu <i>et al.</i> (2010)
Caffeic acid derivates	Leaves	Pirvu <i>et al.</i> (2010)
Epicatechin	<sup>1</sup> Leaves, <sup>2</sup> mycorrhizal roots, <sup>3</sup> sapwood and discoloured wood,	<sup>1</sup> Feucht <i>et al.</i> (1994); Feucht <i>et al.</i> (1997), <sup>2</sup> Beyeler and Heyser (1997), <sup>3</sup> Hofmann <i>et al.</i> (2004; 2008)
Catechin	<sup>1</sup> Sapwood dust, <sup>2</sup> leaves, <sup>3</sup> mycorrhizal roots, <sup>4</sup> bark, <sup>5</sup> sapwood and reaction zone, (discoloured wood), <sup>6</sup> wood chips, <sup>7</sup> steamed and kiln-dried wood, <sup>8</sup> sapwood and discoloured wood, <sup>9</sup> knot, <sup>10</sup> dried wood, <sup>11</sup> wood chips, <sup>12</sup> leaves	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Feucht <i>et al.</i> (1994); Feucht <i>et al.</i> (1997), <sup>3</sup> Beyeler and Heyser (1997), <sup>4</sup> Dubeler <i>et al.</i> (1997), <sup>5</sup> Baum and Schwarze (2002), <sup>6</sup> Zule and Može (2003), <sup>7</sup> Koch <i>et al.</i> (2003), <sup>8</sup> Hofmann <i>et al.</i> (2004; 2008), <sup>9</sup> Pietarinen <i>et al.</i> (2006), <sup>10</sup> Mounguengui <i>et al.</i> (2007), <sup>11</sup> Lekounougou <i>et al.</i> (2008), <sup>12</sup> Pirvu <i>et al.</i> (2010)
Catechin glycoside	<sup>1</sup> Sapwood dust, <sup>2</sup> steamed and kiln-dried wood	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Koch <i>et al.</i> (2003)
Taxifolin glycoside	<sup>1</sup> Sapwood dust, <sup>2</sup> steamed and kiln-dried wood	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Koch <i>et al.</i> (2003)
Taxifolin-O-pentoside	Wood dust	Mammela (2001)
Taxifolin-O-hexoside	Wood dust	Mammela (2001)
Quercetin-O-pentoside	Wood dust	Mammela (2001)
Quercetin-O-hexoside	Wood dust	Mammela (2001)
Tannins, proanthocyanidins	<sup>1</sup> Sapwood dust, <sup>2</sup> leaves, <sup>3</sup> mycorrhizal roots, <sup>4</sup> sapwood and reaction zone (discoloured wood), <sup>5</sup> leaves	<sup>1</sup> Kubel and Weissmann (1988), <sup>2</sup> Feucht <i>et al.</i> (1994); Feucht <i>et al.</i> (1997), <sup>3</sup> Beyeler and Heyser (1997), <sup>4</sup> Baum and Schwarze (2002), <sup>5</sup> Behrens <i>et al.</i> (2003)
Kojic acid	Wood shavings/turnings	Challinor (1996)
3,4-Dimethoxybenzaldehyde	Wood shavings/turnings	Challinor (1996)
3,4-Dimethoxybenzoic acid methyl ester	Wood shavings/turnings	Challinor (1996)

3,4,5-Trimethoxybenzaldehyde	Wood shavings/turnings	Challinor (1996)
3,4,5-Trimethoxybenzoic acid methyl ester.	Wood shavings/turnings	Challinor (1996)
Sinapyl alcohol	Steamed and kiln-dried wood	Koch <i>et al.</i> (2003)
Coniferyl alcohol	Steamed and kiln-dried wood	Koch <i>et al.</i> (2003)
2,6-Dimethoxybenzoquinone	<sup>1</sup> Steamed and kiln-dried wood, <sup>2</sup> dried wood	<sup>1</sup> Koch <i>et al.</i> (2003), <sup>2</sup> Mounguengui <i>et al.</i> (2007)
Protocatechuic acid	Dried wood	Mounguengui <i>et al.</i> (2007)
Vanillic acid	<sup>1</sup> Wood condensate, <sup>2</sup> dried wood	<sup>1</sup> Irmouli <i>et al.</i> (2002), <sup>2</sup> Mounguengui <i>et al.</i> (2007)
Vanillin	<sup>1</sup> Wood condensate, <sup>2</sup> dried wood	<sup>1</sup> Irmouli <i>et al.</i> (2002), <sup>2</sup> Mounguengui <i>et al.</i> (2007)
Syringic acid	<sup>1</sup> Wood condensate, <sup>2</sup> dried wood	<sup>1</sup> Irmouli <i>et al.</i> (2002), <sup>2</sup> Mounguengui <i>et al.</i> (2007)
Coniferaldehyde	Dried wood	Mounguengui <i>et al.</i> (2007)
Synapic acid	Dried wood	Mounguengui <i>et al.</i> (2007)
p-Hydroxybenzoic acid	Wood condensate	Irmouli <i>et al.</i> (2002)
Syringaldehyde	Wood condensate	Irmouli <i>et al.</i> (2002)
cis-Coniferin	Bark	Dubeler <i>et al.</i> (1997)
cis-Syringin	Bark	Dubeler <i>et al.</i> (1997)
cis-Isoconiferin	Bark	Dubeler <i>et al.</i> (1997)
(2R,3R)-(+)-Glucodistylin (Taxifolin-3-glucopyranoside)	Bark	Dubeler <i>et al.</i> (1997)
(2S,3S)-(-)-Glucodistylin (Taxifolin-3-glucopyranoside)	Bark	Dubeler <i>et al.</i> (1997)
2R,3R-Taxifolin-3-D- $\beta$ -xylopyranosid	Bark	Dubeler <i>et al.</i> (1997)
Ascorbic acid	Leaves	Kunert and Ederer (1985)
$\alpha$ -Tocopherol	Leaves	Kunert and Ederer (1985)

## EXPERIMENTAL

### Materials

Six adult beech trees with red heart were felled in the forest area of Kočevski Rog, Slovenia. Selected trees were from 113 to 221 years old, had diameters (at 1.3 m above the ground) ranging from 43 cm to 74 cm and were 24 to 32 m high. Stem discs were sawn from each harvested tree and samples of functional sapwood (S) and red heart (RH) were taken from each disc.

All the samples obtained were ground successively with Retsch mills SM 2000 and ZM 200 (Germany), producing particles that pass through a 35-mesh screen (500  $\mu$ m sieve). The wood meals were then freeze-dried in a Christ Alpha 1-4LD lyophilisator (Germany) at -54 °C and 0.054 mbar.

## Methods

### Extraction

Half a gram of each wood sample was extracted using a Büchi E 916 speed extractor (Switzerland). Lipophilic compounds were extracted at 90 °C and 110 bars with cyclohexane, while hydrophilic extractives were extracted at 100 °C and 110 bars with a methanol/water mixture (95:5, v/v). Two 15-min static cycles were applied for each solvent (Vek 2011). The acquired extracts were cooled and stored in the dark until further analysis. The content of all lipophilic and hydrophilic extractives was determined gravimetrically. The results were expressed in milligrams of soluble matter per gram of dry wood (mg/g).

### Gas chromatography

Before chromatographic analysis, all extracts were silylated with 80 µL of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA), 20 µL of trimethylchlorosilane (TMCS), and 20 µL of pyridine, as proposed by Willför *et al.* (2003). Heneicosanoic acid and betulinol were used as internal standards (marked ISTD on the chromatograms). The separation of beech wood extractives was performed on an Agilent 7890A GC-FID chromatograph (USA) equipped with a DB-5 column (30 m x 250 µm x 0.25 µm). Extractives were identified by GC-MS (Agilent 5975C MSD) analysis of the silylated extracts using a HP-5MS column (30 m x 250 µm x 0.25 µm). In both cases, the temperature program of the column oven was 150 °C (1 min), 4 °C/min to 220 °C, and 20 °C/min to 320 °C (6.5 min) at a helium flow rate of 1.4 mL/min, a split ratio of 10:1, and 260 °C injector and 330 °C FID temperatures. The recorded chromatograms and mass spectra were processed with Agilent ChemStation B.04.02 and MSD Productivity Chemstation E.02.00 software. The final results were calculated on a freeze-dried wood basis and expressed in milligrams of identified compound per gram of dry wood (mg/g).

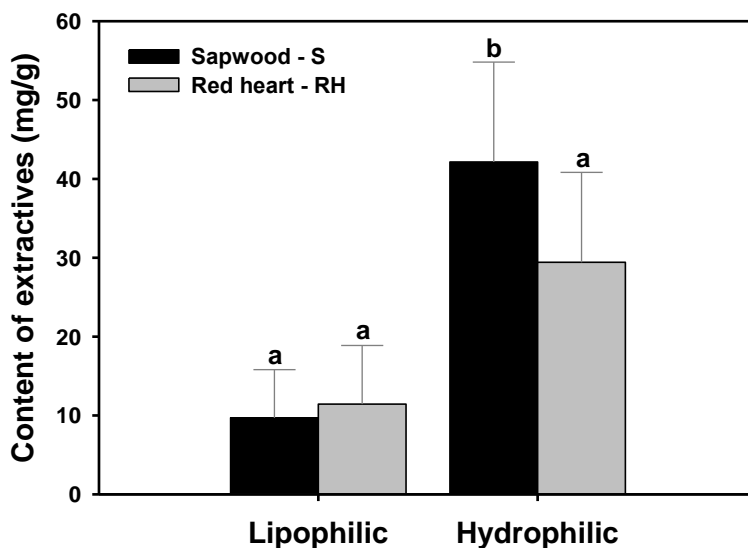
### Statistics

Basic statistical analysis of the extractive contents was performed using Statgraphics software (USA). Values of the measurements were first checked for normal distribution. Significant differences were then investigated by ANOVA at the 0.95 confidence level. The contents of lipophilic and hydrophilic extractives were further compared by means of the multiple range test (Fisher's least significant difference (LSD) procedure).

## RESULTS AND DISCUSSION

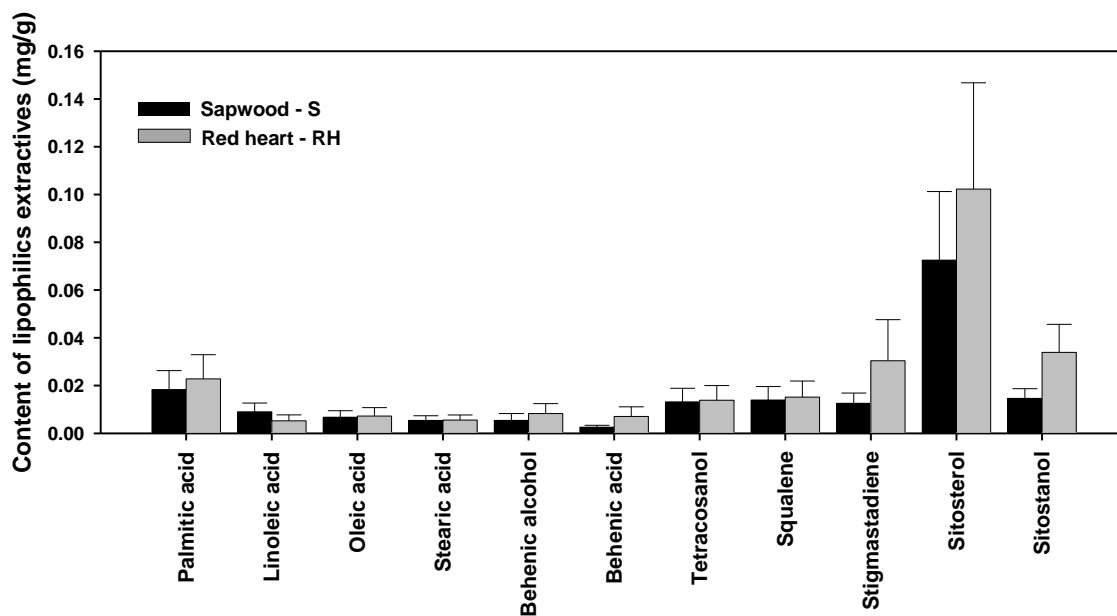
The contents of compounds soluble in cyclohexane and methanol were determined gravimetrically and are presented in Fig. 1. Beech wood (sapwood and red heart) contained, on average, 10.35 mg/g lipophilic extractives, while the average content of hydrophilic extractives was 37.05 mg/g. The content of compounds soluble in methanol was significantly larger than the total lipophilic extractives (ANOVA,  $F = 91.75$ ,  $p < 0.00001$ ). The amount of hydrophilic extractives was larger in sapwood (S) than in red heart (RH), whereas differences in lipophilic extractives between tissues were not significant (Fig. 1).

Qualitative analysis showed that the extractable lipophilic fraction of the wood of red hearted beech consisted of various saturated (palmitic C16:0, stearic C18:0, and behenic C22:0 acid) and unsaturated (linoleic C18:2 and oleic C18:1 acid) fatty acids. Palmitic acid was the most abundant fatty acid (Fig. 2 and Fig. 3).



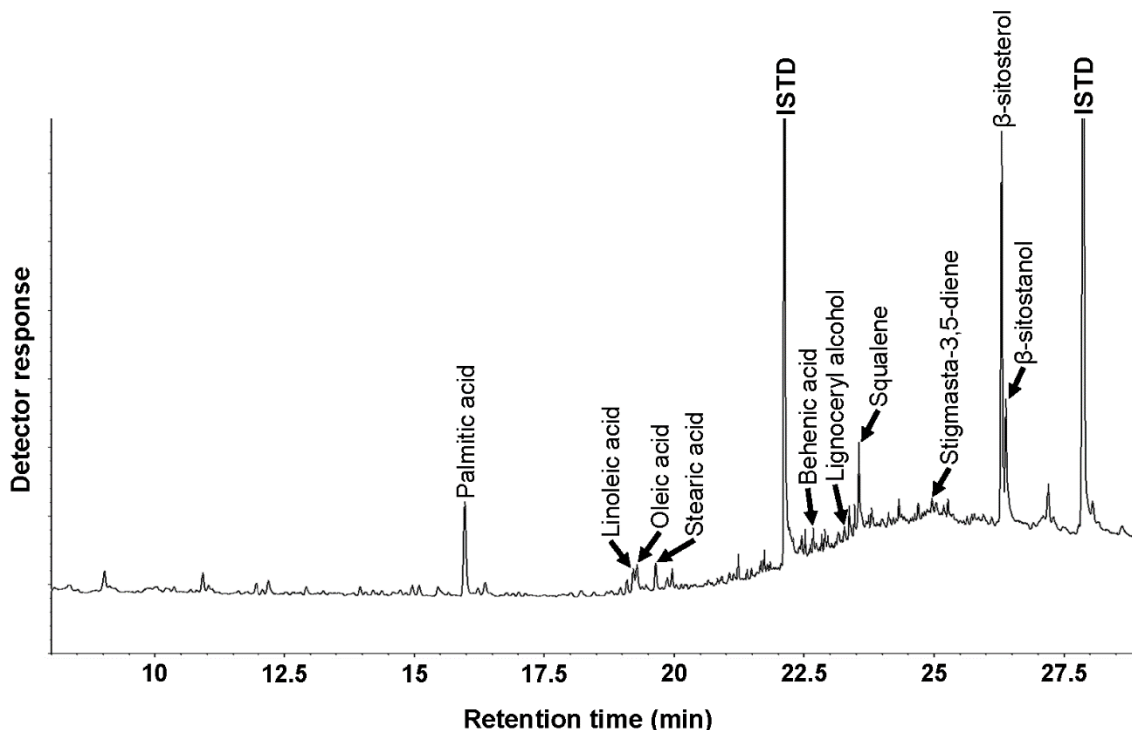
**Fig. 1.** Content of total lipophilic and hydrophilic extractives in the sapwood and red heart of beech. <sup>a, b</sup> Different letters at the top of the error bars within each set indicate statistically significant differences (Fisher's least significant difference (LSD) test at a 95% confidence level)

As reported by Perra *et al.* (1993), the mentioned acids also represent a part of the aliphatic fraction in suberin, which reportedly occurs in the red heart of beech (Pearce 1996; Torelli and Oven 1996; Oven *et al.* 1999; Schwarze and Baum 2000). Fatty alcohols (behenyl C22:0 and lingoceryl C24:0 alcohol) and triterpenoids (squalene, stigmastadiene,  $\beta$ -sitosterol, and  $\beta$ -sitostanol) were also identified in the lipophilic beech wood extracts (Fig. 2 and 3).

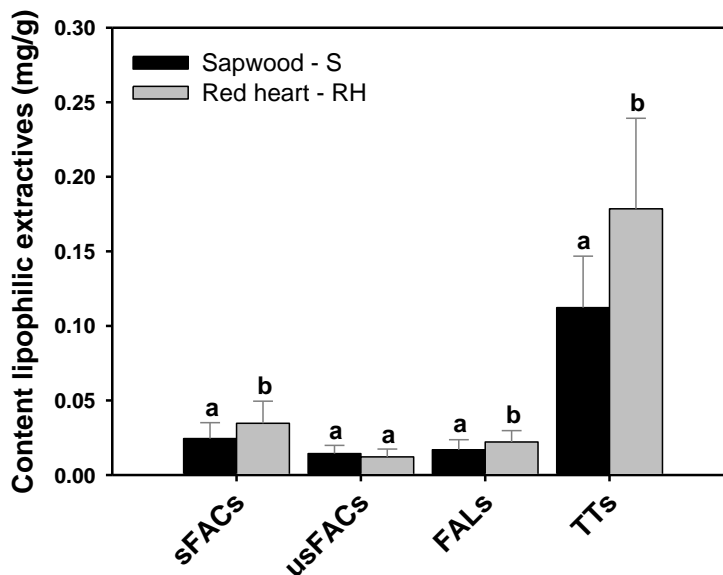


**Fig. 2.** Contents of identified lipophilic extractives in the cyclohexane extracts of sapwood and red heart of beech

Triterpenoids were the most abundant group of extractives in the cyclohexane extracts of beech, among which free sterols were the dominant compounds (Figs. 5 and 6).  $\beta$ -sitosterol was a distinctive compound as shown by the most intense peak on the chromatograms of the cyclohexane extracts (Fig. 3).



**Fig. 3.** GC-FID chromatogram of the cyclohexane extract of red heart of beech (*Fagus sylvatica* L.). ISTD - internal standard

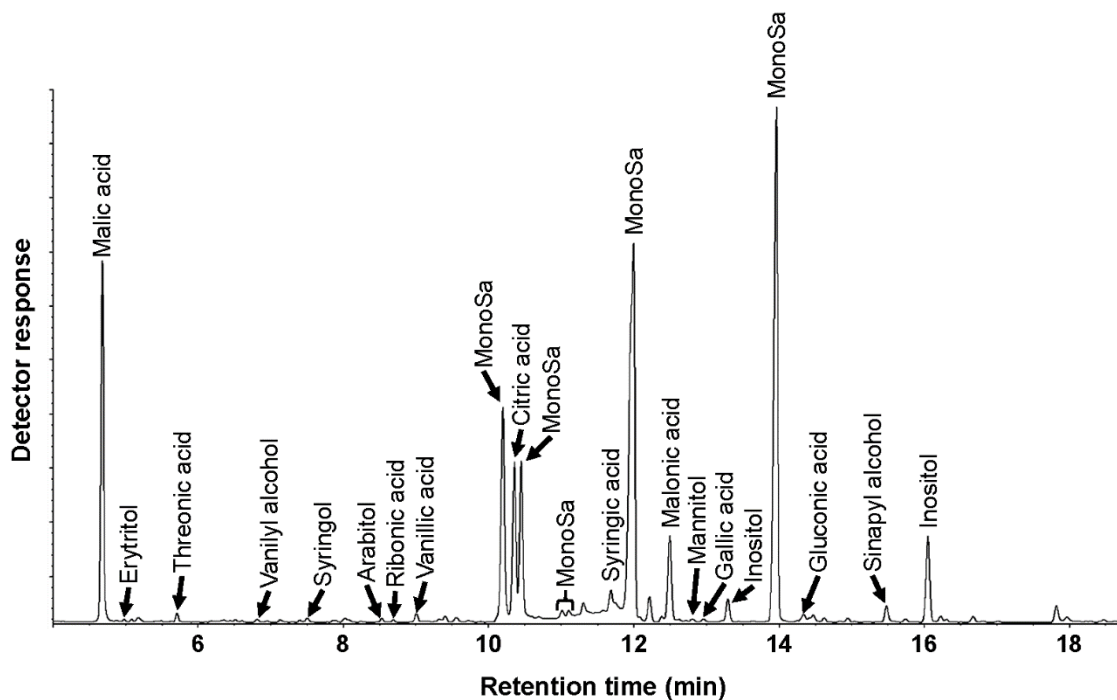


**Fig. 4.** Differences in the content of individual groups of lipophilic extractives between sapwood and red heart of beech. <sup>a, b</sup> Different letters at the top of the error bars within each set indicate statistically significant differences (Fisher's least significant difference (LSD) test at a 95 % confidence level). sFACs - saturated fatty acids, usFACs - unsaturated fatty acids, FALs - fatty alcohols, TTs - triterpenoids



As presented in Fig. 2, stigmasta-3,5-diene is also a characteristic compound of the nonpolar fraction of the extractives in beech wood. It belongs to the group of sterenes known as the dehydration compounds of sterols (Gallina Toschi *et al.* 1996; Amelio *et al.* 1998). Acyclic triterpene squalene was also associated with a relatively strong peak (Fig. 3). The contents of individual lipophilic compounds were different in the sapwood and red heart but the red heart contained significantly greater amounts of saturated fatty acids, fatty alcohols, and triterpenoids than sapwood (Fig. 4).

The hydrophilic fraction was much richer in compounds than the cyclohexane extracts. In addition to sugar alcohols (erythritol, syringol, and arabitol), sugar acids (ribonic, threonic, gluconic, and glucuronic acid), carboxylic acids (malic, malonic, and citric acid), various simple phenols (vanilyl alcohol, syringol, vanillic acid, syringic acid, gallic acid, and sinapyl alcohol) and flavonoids (catechin, taxifolin, and an unidentified flavanol-type compound). The methanolic extracts contained a large number of mono- and oligosaccharides (Fig. 5 and Fig. 7). Among them, trehalose, saccharose, and raffinose were identified (Fig. 6). Monosaccharides showing fairly similar mass spectra were not identified in detail. However, typical wood sugars such as glucose, galactose, arabinose, fructose, xylose, mannose, and rhamnose were identified as beech wood monosaccharides by Kubel and Weissmann (1988). Xylitol and glycerol (*i.e.*, sugar alcohols) have also been found in the extract of beech wood chips (Zule and Može 2003).



**Fig. 5.** GC-FID chromatogram of the methanol extract of beech sapwood (*Fagus sylvatica* L.), showing various monosaccharides (MonoSa), sugar alcohols, and acids

The GC/MS analysis revealed the presence of vanillin, vanillic and syringic acid, inositols, and dilignols in the methanolic extracts of beech wood (Fig. 5 and 6). Catechin was the most abundant phenolic compound found in the hydrophilic beech wood extract (Fig. 6 and 7). Flavonoid glycosides were not identified in this investigation, probably due to the solvents used and the analytical techniques applied (Willför *et al.* 2006). Our results are in agreement with the literature data summarized in Table 2. As shown in Fig. 7, the

highest proportion of identified hydrophilic extractives in the wood of beech, both in sapwood and red heart, consisted of mono- and oligosaccharides.

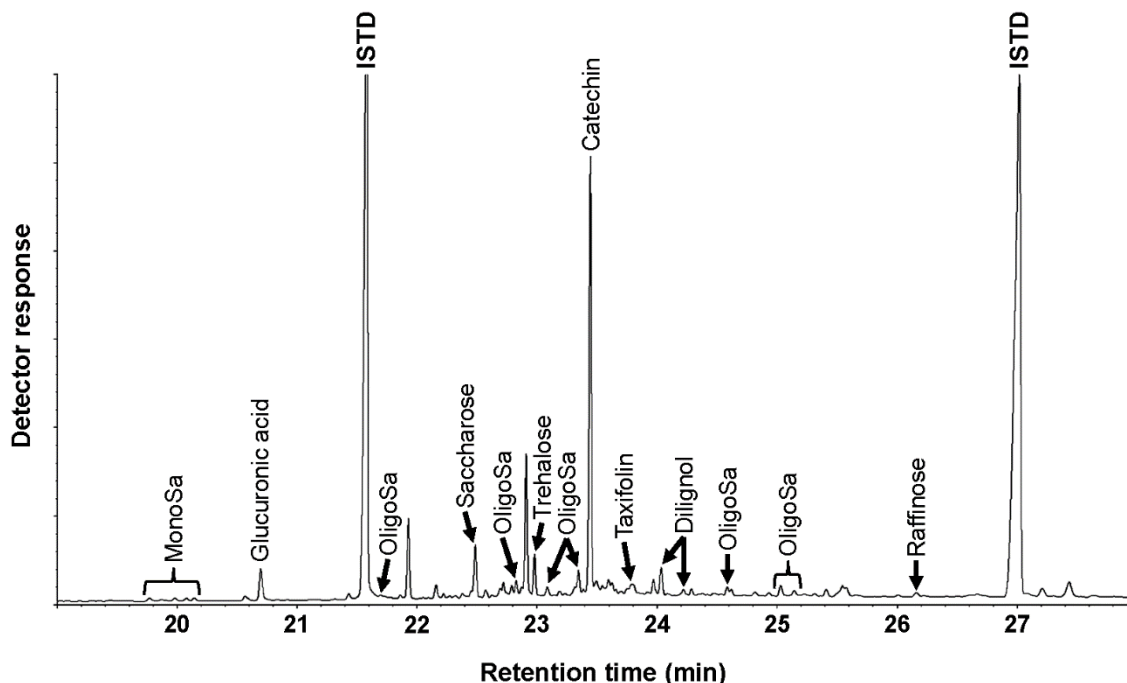


Fig. 6. GC-FID chromatogram of the methanol extract of beech sapwood (*Fagus sylvatica* L.), showing various oligosaccharides (OligoSa), dilignols, and phenolic compounds. ISTD - internal standard

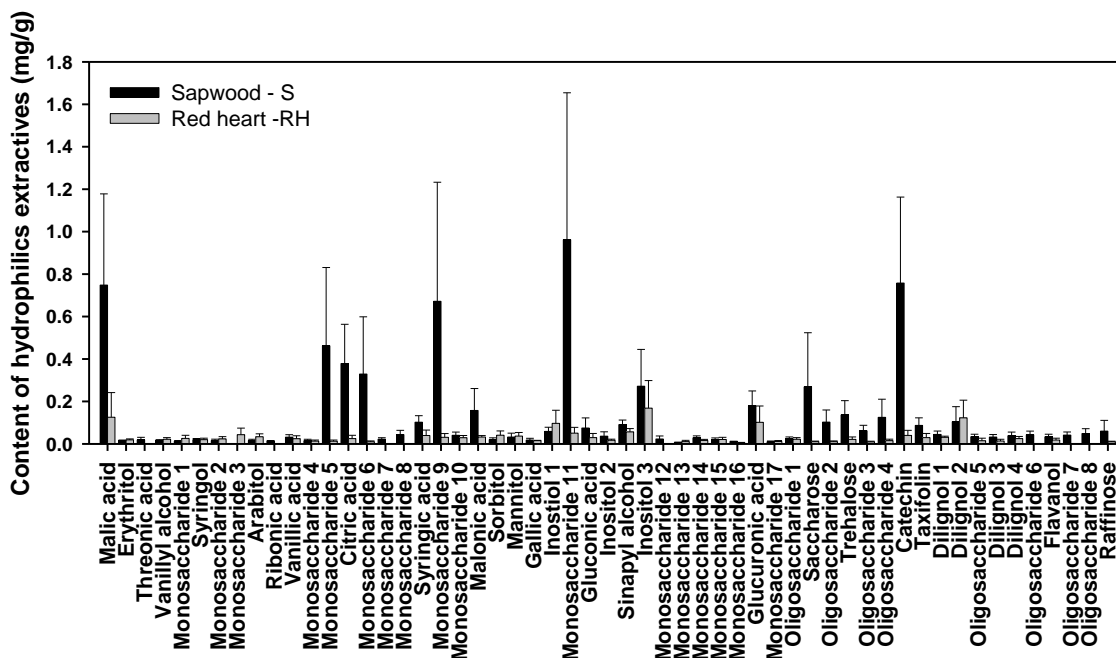
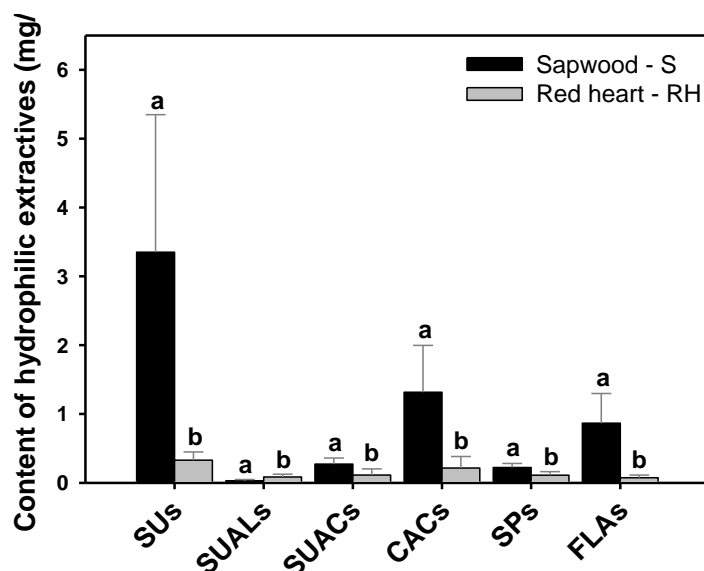


Fig. 7. Contents of identified hydrophilic extractives in the methanol extracts of sapwood and red heart of beech

Methanol extracts were also characterized by relatively large carboxylic acid content. Compared to sugar alcohols, sugar acids, and simple phenols, the flavonoid content was significantly larger in sapwood than in red heart (Fig. 8). The samples of sapwood contained larger concentrations of mono- and oligosaccharides, sugar acids, carboxylic acids, simple phenols, and flavonoids, while the methanolic red heart extracts yielded more sugar alcohols (Fig. 8). The lower contents of phenolic extractives in red heart can be explained by the participation of these compounds in the formation of chromophores, which contribute to the characteristic color of the red heart of beech wood (Hofmann *et al.* 2004).

Catechin is regarded as the major precursor for the development of chromophoric compounds. Furthermore, the discoloration mechanism in beech wood can be understood as the condensation of catechin monomers to polymeric forms. It has been suggested that during the thermal treatment of beech wood, the condensation of catechin similarly contributes to a brown staining pigment, whereas other extractives (*e.g.*, 2,6-dimethoxybenzoquinone and taxifolin) contribute to the final color of discolored tissues (Koch *et al.* 2003).



**Fig. 8.** Differences in the content of individual groups of hydrophilic extractives between sapwood and red heart of beech. <sup>a, b</sup> Different letters at the top of the error bars within each set indicate statistically significant differences (Fisher's least significant difference (LSD) test at a 95 % confidence level). SUS - soluble sugars, SUALs - sugar alcohols, SUACs - sugar acids, CACs - carboxylic acids, SPs - simple phenols, FLAs - flavonoids

Knowledge of the qualitative and quantitative composition of the extractives in different parts of beech wood is very important for optimizing thermal treatment and technical drying processes. The color irregularity of beech wood elements could potentially be mitigated. A better understanding of the chemical character of non-structural components in red heart of beech would have a favorable impact on the adhesion properties, surface modification of wood, and properties of beech wood-based composites. Finally, some extractives of beech wood with proven bioactive properties are considered to be relevant antioxidants (Malterud *et al.* 1985; Dubeler *et al.* 1997; Baum and Schwarze 2002; Välimaa *et al.* 2007; Liu *et al.* 2008) and could represent attractive compounds for commercial extraction.

## CONCLUSIONS

1. The extracts of sapwood and red heart of beech contained saturated and unsaturated fatty acids, fatty alcohols, various triterpenoids, numerous mono- and oligosaccharides, sugar alcohols and acids, di- and tricarboxylic acids, various simple phenols, and flavonoids.
2.  $\beta$ -sitosterol and catechin were the characteristic compounds of the non-polar and polar extracts, respectively. The content of  $\beta$ -sitosterol was higher in red heart, while catechin prevailed in sapwood of red hearted beech trees.
3. Red heart contained significantly larger amounts of saturated fatty acids, fatty alcohols, and triterpenoids than sapwood.
4. Sapwood contained larger concentrations of mono- and oligosaccharides, sugar acids, carboxylic acids, simple phenols, and flavonoids than red heart.
5. The high variability in the extractives content in the wood of beech can be attributed to the physiological processes involved in the transformation of sapwood to red heart.

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