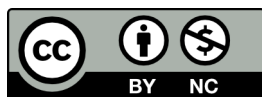


Article:

SABLÍK, Pavel; GIAGLI, Kyriaki; PASCHOVÁ, Zuzana; ORAVEC, Michal; GRYC, Vladimír; RADEMACHER, Peter. 2018. FexIKA method parameters affecting black locust heartwood extraction yield. *BioResources*. 13(2), 4224-4238. ISSN 1930-2126. Available at: <https://doi.org/10.15376/biores.13.2.4224-4238>

This article is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License, CC BY-NC
(<https://creativecommons.org/licenses/by-nc/4.0/>)

This license enables reusers to distribute, remix, adapt, and build upon the material in any medium or format for noncommercial purposes only, and only so long as attribution is given to the creator.



FexIKA Method Parameters Affecting Black Locust Heartwood Extraction Yield

Pavel Sablík, ^{a,*} Kyriaki Giagli, ^a Zuzana Paschová, ^a Michal Oravec, ^b Vladimír Gryc, ^a and Peter Rademacher ^{a,c}

The influence of certain adjustable parameters (sample property, temperature, and solvent type) on the advanced *fex*IKA extraction outputs was determined. Extracts were obtained from black locust (*Robinia pseudoacacia* L.) heartwood and analysed by high-performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS). The amount of extract yield remained similar, regardless of the wood particle size, whereas the total amount of phenolic compounds gradually decreased when the particle size increased. The highest amounts of extractives were obtained at higher temperatures, and at 170 and 200 °C, a significant influence from the temperature on the chemical composition was noticed. Namely, the phenol and robinetin yields increased, while the rest of the main phenolic compounds were degraded. Additionally, at higher temperatures (170 and 200 °C), two newly formed furfural compounds were detected.

Keywords: Extractives; Relative permittivity; Phenolic compounds; Heat stress; HPLC-HRMS; *Robinia pseudoacacia* L.

Contact information: a: Mendel University in Brno, Faculty of Forestry and Wood Technology, Department of Wood Science, Zemědělská 3, CZ-613 00 Brno, Czech Republic; b: Global Change Research Institute CAS, Bělidla 4a, CZ-603 00 Brno, Czech Republic; c: University of Sopron, EFKK Research Center for Forestry and Wood Industry, Bajcsy-Zs. u. 4, H-9400 Sopron, Hungary; * Corresponding author: pavel.sablik@ikea.com

INTRODUCTION

Various enhanced extraction methods, *i.e.*, Soxhlet, ultrasonic bath, microwave-assisted, pressurised fluid extraction, supercritical fluid extraction, and *fex*IKA, can be employed for the extraction of chemical compounds (Szyrwińska and Lulek 2001; Martens *et al.* 2002; Schwanninger and Hinterstoisser 2002). The selection of the extraction technique is considered to be a decisive step in the analytical cycle, and hence, special emphasis has to be given to the accurate choice of extraction technique and sample pre-treatment procedures (Martens *et al.* 2002). In particular, to maximize the extraction yield from wood matrices, numerous parameters, such as the influence of material pre-treatments (grinding and moisture removal), temperature, extraction time, solvent type, and chemical characteristics, should be extensively studied and specified (Guerrero *et al.* 2008).

The particle size of ground wood is directly related to the extraction quantity and yield (Ottone and Baldwin 1981). Grozdits and Chang (1984) suggested the use of particle sizes smaller than 0.23 mm for extracting polyphenols from white fir bark. A recent study showed that the highest total extraction yield from spruce wood was achieved after using the finest (< 0.1 mm) ground wood fraction (Song *et al.* 2012). Spectrocolorimetric tests revealed a trend: the smaller the particle size of ground wood is, the larger is the extraction amount. Nevertheless, similar amounts of extracts have been

obtained from particles that were 0.4 to 1 mm in size (Gião *et al.* 2009; Mustapha *et al.* 2012; Song *et al.* 2012; Li *et al.* 2013).

When wood was exposed to high temperatures (> 100 °C), major chemical changes affected its properties (Esteves and Pereira 2009; Čermák *et al.* 2015). The chemical reactions that occurred during thermal stress often led to the degradation of products, which affected the chemical compounds of the cell wall (Bekhta and Niemz 2003; Chen *et al.* 2012). Thermal-based changes are complex and far from being entirely understood. A *fexIKA* apparatus set up generally requires approximate temperatures that are near (20% to 30% above) the solvent boiling point (Schwanninger and Hinterstoisser 2002). According to the *fexIKA* manufacturer, it is theoretically feasible to reach a maximum temperature of 220 °C on the heating/cooling block and basic flask walls. Nevertheless, at this temperature, the extracted chemical compounds could be modified or degraded (Niemz *et al.* 2010). Therefore, the detection of changes in the wood constituents caused by thermal stress on a molecular level is fundamental.

A vast number of various wood chemical compounds can be obtained by using polar and/or nonpolar solvents (Sergent *et al.* 2014). Mészáros *et al.* (2007) reported that the quality of the applied solvents remarkably affected the amount and composition of the extracts. According to the extractive dissolving properties, these chemicals could be selectively removed from the wood matrix. Therefore, one of the factors that affects the extraction efficiency is the concentration and type of solvent. Different relative polarities of the extraction solvents could influence the amount of wood extractives obtained (Soon and Chiang 2012). Hence, extractive compounds can be classified based on the relative polarity of the solvent used to extract them.

Black locust wood (*Robinia pseudoacacia* L.) contains a large amount of extracts, such as tannins, flavonoids (*e.g.*, robtein, butein), flavanones, flavanonols (*e.g.*, dihydrorobinetin), flavonols (*e.g.*, robinetin), stigmaterol, choline, syringenin, starch, simple sugars, water-soluble proteins, and related metabolites (Chow *et al.* 1996; Mészáros *et al.* 2007). Black locust wood exhibits an exceptional resistance to biodegradation (Sablík *et al.* 2016). This trait is attributed to the considerably large quantity of extractive compounds identified in the ethanol extract, which includes robinetin and dihydrorobinetin. These chemical substances have allelopathic properties and the ability to inhibit biotic growth (Hashemi and Kanani 2012). Latorraca *et al.* (2011) found high concentrations of phenolic compounds and flavonoids in black locust heartwood, especially in the axial parenchyma and vessels (cell walls). The black locust heartwood extractive content is responsible for the particular colour and decay resistance of this species, although according to Stringer (1992), the total content of the dry main stem matter was found to be low (6.2% to 8.3%). Furthermore, the hot water extract amounts ranged from 5.2% to 8.6% (Hart 1968; So *et al.* 1983).

The advanced *fexIKA* method has been established as a successful technique for extracting accessory compounds from wood matrices, and it has been found to be less solvent and time consuming than Soxhlet extraction (Szyrwińska and Lulek 2001; Schwanninger and Hinterstoisser 2002). Nevertheless, further research into the influence of the extraction process variables, such as material size, temperature, and solvent type, on the extraction yield is required for optimizing the outputs of *fexIKA*. In this study, the authors performed qualitative and quantitative analyses on black locust heartwood extracts. The authors aimed to optimise the *fexIKA* extraction procedure by assessing the influence of three adjustable parameters, the wood particle size, applied temperature (thermal stress) and solvent type, on the quantity and quality of the extract yield.

EXPERIMENTAL

Materials and Methods

Sample preparation

Air-dried heartwood samples were obtained from a 70-year-old log of black locust, growing in South Moravia, Czech Republic (290 m a.s.l.). Mean annual precipitation is 510 mm (base on Czech hydrometeorological institute figures available at <http://portal.chmi.cz/>).

The samples were 0, 1.3, 4, and 6 m in height and were manually chipped and ground with a Retsch SM300 cutting mill (Haan, Germany). Four gradient analytical sieves were used for passing the samples through 4.0-, 2.0-, 1.0-, and 0.5-mm screens. Subsequently, an IKA A10 Yellow Line analytical grinder (Staufen, Germany) was used for preparing the wood powder (0.5 μm) obtained from all of the samples. Both types of mills were chosen to avoid sample overheating during preparation, as well as to prevent any contamination of the sampling material with metal particles that may have detached from the equipment during processing. Additionally, to simulate industrial conditions, veneer-sized samples (1.0 x 15.0 x 30.0 mm tangential/longitudinal/radial respectively) were included in the research. Although TAPPI T204CM-07 (2007) suggests 2.0 ± 0.1 g as the minimum required amount of material for one single extraction, the authors prepared 10.0 ± 0.1 g in order to increase the extract yield quantity, as well as for measurement precision.

Three different sample sets (A, B, C) were prepared to examine the three adjustable parameters of the extraction: (1) Sample set A for the particle size parameter, (2) sample set B for the heat treatment, and (3) sample set C for the solvent type parameter. Tables 1 and 2 describe the basic parameters applied for the *fex*IKA extraction as well as the individual parameters for each sample set.

The solvents were chosen and ranked based on the polarity. The polarity of solvents and solvent mixtures is described by the physical property called relative permittivity (ϵ_r), which is a relative measure of the chemical polarity. Water and methanol were used for the phenolic compounds extraction and are polar solvents. Additionally, middle-polar solvents, ethanol and acetone, and two nonpolar solvents, benzene and cyclohexane, were tested and compared with the polar solvents. Distilled water-methanol mixtures (dw:m) that had ratios of 1:1 v/v and 1:4 v/v were also used to reduce the toxicity of the final mixtures in order to decrease the extraction process cost and obtain a wider range of compounds.

The wood samples were subjected to heat treatment at temperatures as described in Table 1 for 24 h. The treatment was carried out in electrical laboratory oven VENTICELL 55 (Munich, Germany).

Reagents and standards

The organic solvents used for the quantitative analysis, *i.e.* cyclohexane, benzene, acetone, methanol, and ethanol, were purchased from PENTA s.r.o. (Prague, Czech Republic). All of the tested solvents were of analytical grade.

To perform a qualitative analysis of the compounds, the authors used acetonitrile hypergrade for LC-MS LiChrosolv^R, which was supplied by Merck KGaA (Darmstadt, Germany). The acetic acid was obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). A Purelab Classic (ELGA LabWater, High Wycombe, UK) was used to generate high purity water for the preparation of the aqueous mobile phase. All of the

standards for the phenolic compounds and sugars were purchased from Sigma-Aldrich (Prague, Czech Republic).

Table 1. Basic Parameters Applied for the *fex*lKA Extraction and Parameters used for Preparing the sample sets A and B. Dw:m; Distilled Water-Methanol

Mixture of heights [m]	0.0; 1.3; 4.0; 6.0						Basic parameters of extraction
Samples per set	40						
Sample weight [g]	10.0 ± 0.1						
Used solvent	dw:m (1:1)						
Solvent volume [mL]	100.0 ± 1.0 %						
Relative permittivity	42.4						
Temperature of the extraction [°C]	<110						
Time/cycle of the extraction [min]	30						
Sample set	A1	A2	A3	A4	A5	A6	Parameters for the particle size sample set
Particle size [mm]	≤0.5 [µm]	≤0.5	≤1.0	≤2.0	≤4.0	1.0*15.0*30.0	
Heat treatment [°C]	none						
Sample set	B1	B2	B3	B4	B5	B6	Parameters for the heat treatment sample set
Particle size [mm]	≤ 0.5						
Heat treatment [°C]	none	110	120	140	170	200	

Table 2. Basic Parameters Applied for the *fex*lKA Extraction and Parameters used for Preparing the Sample Set C. Dw:m; Distilled Water-Methanol

Mixture of heights [m]	0.0; 1.3; 4.0; 6.0								Basic parameters of extraction
Samples per set	40								
Sample weight [g]	10.0 ± 0.1								
Solvent volume [mL]	100.0 ± 1.0								
Sample set	C1	C2	C3	C4	C5	C6	C7	C8	Parameters for the solvent sample set
Particle size [mm]	≤ 0.5								
Used solvent	Cyclohexane	Benzene	Acetone	Ethanol	Methanol	dw	dw:m (1:1)	dw:m (1:4)	
Relative permittivity	2.0	2.2	21.0	25.0	33.0	80.0	42.4	56.5	
Temperature of the extraction [°C]	<95	<92	<75	<95	<85	<130	<110	<110	
Time/cycle of the extraction [min]	10	22	32	22	35	30	30	25	
Heat treatment [°C]	none								

Extraction

The ground black locust heartwood samples were extracted using a *fexIKA* vario control Soxhlet apparatus (Staufen, Germany). The samples were placed directly on the set of filters (base filter PTFE and three protective cellulose filters) of the apparatus. This configuration immediately achieved the maximum material-solvent interaction when the extraction process began. The extraction process ran for three individual phases (Fig. 1), which corresponded to one cycle. Four extraction cycles were performed in total for each sample.

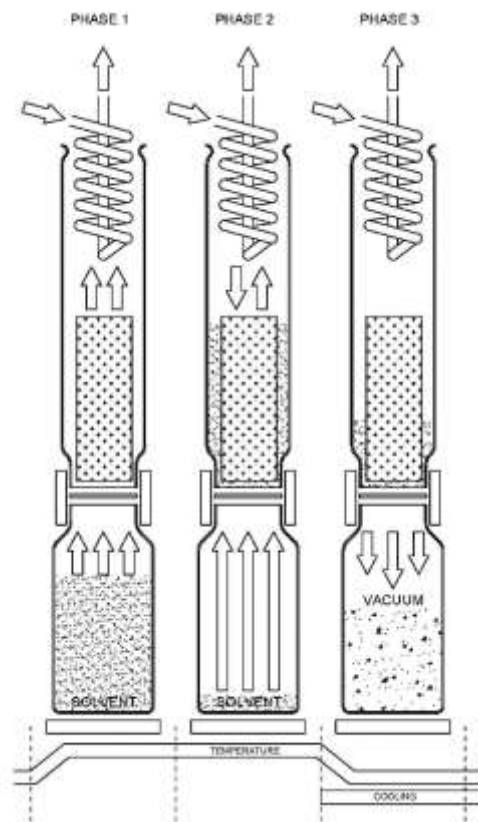


Fig. 1. FexIKA extraction process phases

Phase 1: Heating of the solvent to the boiling point and evaporation. The solvent vapors penetrate through the set of filters into the material. At the beginning of the extraction process, the solvent vapors condense inside the extraction material as well as on the walls of the extraction tube.

Phase 2: The solvent vapors condense on the cooling element at the top of the extraction tube and falls in drops on the sample. The condensed solvent is heated by the vapors in the extraction tube amplifying the intensity of the extraction in the boiling solvent. This technique ensures the effectiveness of the extraction kinetics during the whole process.

Phase 3: The solvent in the basic vessel completely evaporates, condensing into the extraction tube. The induced cooling of the heating block (switching off the solenoid valve) interacts with the condensing of the solvent, causing suction in the basic vessel.

The difference between the atmospheric and the tube's inner pressure conveys the extracts into the basic vessel through the filter.

Evaporation

The extraction process was followed by the evaporation of the samples in a rotary evaporator (INGOS RVO 400, Prague, Czech Republic). The heating temperature for evaporation was set according to the solvent type. The rotation of the flask was set to 15 RPM and the vacuum pressure was 100 mbar. Finally, the samples were dried at 60 °C until a constant weight was achieved and then conditioned in a desiccator before being weighed (Craig *et al.* 1950).

High performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS)

A Dionex Ultimate 3000 (ThermoFisher Scientific, USA/Dionex RSLC, Dionex, Waltham, MA, USA) and a Hypersil Gold column (150 mm x 2.1 mm x 3 µm, ThermoFisher Scientific) were used for high-performance liquid chromatography (HPLC). The flow rate of the mobile phases was 0.3 mL/min, and the column temperature was set to 30 °C.

The HPLC mobile phase consisted of (A) acetonitrile and (B) water containing 0.1% acetic acid. Both the A and B mobile phases were filtered and degassed for 10 min in an ultrasonic bath before use. Gradient elution chromatography was performed starting with 10% of A and 90% of B for 5 min. Over the time interval of 5 to 20 min, the A percent concentration was linearly increased to 90%, while the B percent concentration respectively decreased to 10%. This composition was maintained for 5 min (A: 90% and B: 10%), before the system was again equilibrated to the initial conditions (A: 90% → 10% and B: 10% → 90%). The mobile phase lasted for 25 min in total. The injection volume of the samples was 5 µL. The wavelengths 254, 272, 274, and 331 nm were monitored using a DAD detector (ThermoFischer, Waltham, MA, USA).

Additionally, mass spectrometry (MS) and sequential mass spectrometry (MSⁿ) were performed using a LTQ Orbitrap XL- high resolution mass spectrometer (ThermoFisher Scientific) equipped with a HESI II (heated electrospray ionization) source. High-resolution mass spectrometry (HRMS) was performed in the Full Scan mode with a resolution of 60,000. Full scan spectra were acquired over the mass range of 50 to 1000 m/z in the positive mode and from 65 to 1000 m/z in the negative mode. The resolution and sensitivity of the Orbitrap were controlled with the injection of a mixed standard after analyzing all 25 samples, and the resolution was also confirmed by the lock masses (phthalates). Blanks of pure mobile phases were also analysed during sequencing. The compounds were analysed in the mass library, which was created from the measurement of standards in the MS and MSⁿ modes on the Orbitrap. All peaks of interest were confirmed by isotope ratios, fragments and dimers. All of the samples were injected twice, once under a positive injection mode and then under a negative injection mode. Furthermore, the HPLC method as performed, provided also data for the saccharides groups, *i.e.*, the sum of pentoses, hexoses and disaccharides. In this study, the groups were named after the most representative saccharide. Namely, xyloses represent the pentoses group (xyloses equivalent), glucoses represent the hexoses group (glucoses equivalent) and saccharose for the disaccharides (saccharose equivalent).

RESULTS AND DISCUSSION

Influence of Particle Sizes on the Extraction Output

The amount of extraction yield (dw:m, 1:1, v/v) from the dry wood ranged from 7.3% (4.0 mm) to 8.1% (0.5 mm). The results were found to be significantly different ($F=4.04$; $p=0.003474$). The heartwood powder results were also within this range (7.5%), but were lower than the extraction yield of the 0.5 and 1.0 mm samples. Namely, the 0.5 mm sample provided the highest extraction yield. The total amount of extract content obtained from the veneer-sized samples was 7.3% after two subsequent extractions, and found to be significantly different from the 0.5 mm and 1.0 mm samples ($p=0.004094$ and $p=0.044753$, respectively) (Fig. 2).

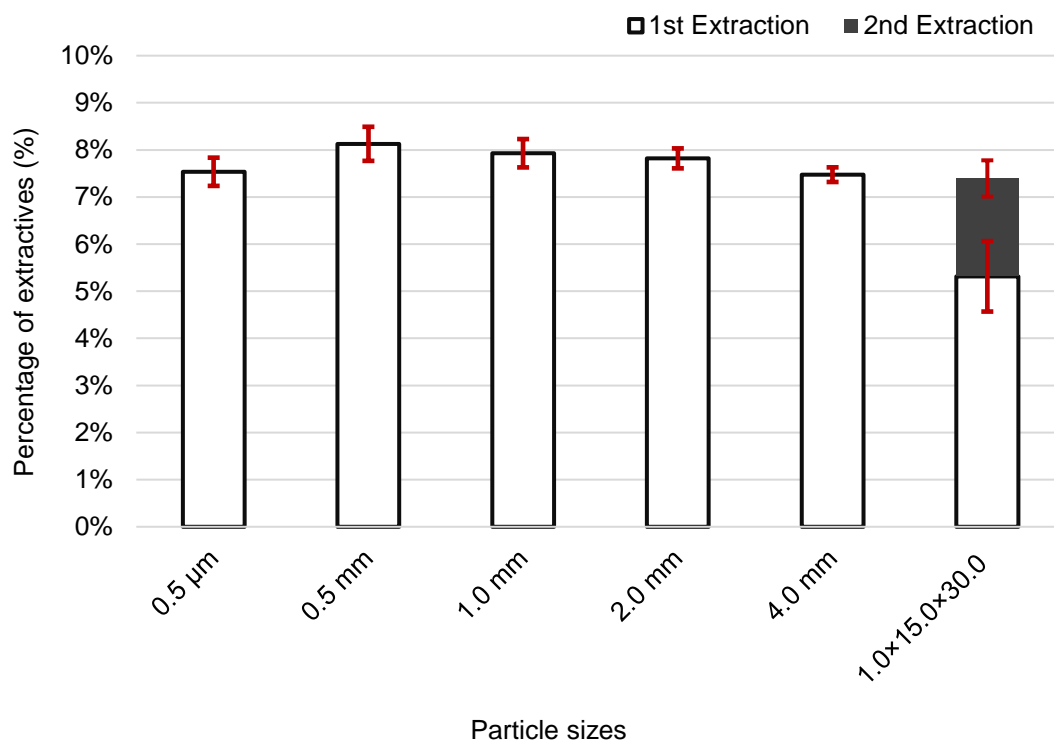


Fig. 2. Amounts of extract from black locust heartwood (dry wood) after using various wood particle sizes; Water-methanol mixture (1:1, v/v) was used as the extraction solvent (error bars stand for standard deviation).

The extraction yield amount compared well with the results reported in the literature (Hart 1968; So *et al.* 1980; Stringer 1992), although the authors had to compare used methodology findings with other extraction methods, due to the fact that *fexIKA* method is unfamiliar and not frequently used.

The HPLC-HRMS results (Table 3) showed that the amount of the most important phenolic compounds extracted from the black locust heartwood gradually decreased as the particle size increased from wood powder to 4.0 mm. The basic phenol amount decreased from 83 to 38 µg/mL for dihydrorobinetin (from 161 to 135 µg/mL). Although robinetinidin presented constant values for the powder to 2.0 mm particle sizes, the bigger particle sizes adversely affected the compound output (from 1780 to 1570 µg/mL). In line with the results above, the total yield of the chemical compounds followed a

decreasing trend, which decreased from 2100 µg/mL for the wood powder to 1770 µg/mL for the largest particle size (4.0 mm).

Table 3. HPLC-HRMS Analysis Results of the Extractives Obtained from the Different Wood Particle Sizes

Extractives (µg/mL)	Powder	Sieve 0.5	Sieve 1.0	Sieve 2.0	Sieve 4.0
Robinetinidin	1780	1800	1770	1760	1570
Dihydrorobinetin	161	153	153	154	135
Phenol	82.6	37.8	50.0	43.9	38.3
Robinetin	46.3	45.6	16.9	8.82	4.93
Fisetin	10.6	10.7	10.8	11.0	9.78
Catechin	6.64	5.99	5.50	5.97	5.23
Gallic acid	5.44	2.41	3.02	2.50	2.93
Epicatechin	0.748	0.198	0.326	0.184	0.257
Pyrocatechol	0.300	0.120	0.200	0.100	0.136
Syringic acid	0.168	0.071	0.074	0.063	0.069
Syringaldehyde	0.036	0.015	0.015	0.013	0.018
Sum of phenol. comp.	2090	2020	2010	1990	1770
Xyloses equivalent	1.55	1.39	1.45	1.82	1.40
Glucoses equivalent	0.548	0.505	0.507	0.651	0.589
Saccharoses equivalent	0.179	0.123	0.115	0.154	0.261
Sum of saccharides	2.28	2.02	2.07	2.63	2.27

Original concentration was 10.0 ± 0.1 g of sample extracted with 100 mL of water-methanol mixture (1:1, v/v)

A similar extraction yield amount was found regardless of the particle size, *i.e.*, 7.4% (wood powder) to 7.3% (4.0 mm). Nevertheless, the fact that the HPLC-HRMS qualitative analysis revealed that the total amount of phenolic compounds extracted by the *fexIKA* method gradually decreased as the particle size increased from wood powder to 4.0 mm was in line with the prevailing notion that the relationship between the particle size and extraction yield is inversely proportional (Ottone and Baldwin 1981; Song *et al.* 2012). The extracts of black locust heartwood are mostly polyphenols, primarily flavonoids that occur principally as free aglycones (Roux and Paulus 1962; Kubota and Hase 1966; Sanz *et al.* 2011; Sergent *et al.* 2014). It was found that the basic phenol amount decreased from 83 (wood powder) to 38 µg/mL (4.0 mm). According to previous studies, two flavonoids detected in the black locust heartwood, dihydrorobinetin and robinetin, were the main extractive compounds with anti-fungal properties (Freudenberg and Hartmann 1953). In this study, the dihydrorobinetin yield was not strongly influenced by the different particle sizes (from 161 to 135 µg/mL), whereas robinetinidin was affected when the particles were larger than 2.0 mm. The high content of these two phenolic compounds detected in the black locust mature heartwood extract is considered to be the key factor for the high decay resistance of black locust wood (Magel *et al.* 1994; Dünisch *et al.* 2010; Sergent *et al.* 2014).

Influence of Thermal Stress on the Extraction Results

The quantitative analysis showed that the average extraction yield gradually increased with the temperature (Fig. 3). The results were found to be significantly different ($F=963.11$; $p=0.0000$). The highest *fexIKA* extractive amounts were obtained after applying higher temperatures (13.1% and 12.9% of compounds for 170 and 200 °C respectively) and proved by ANOVA to be similar to each other.

The qualitative analysis of the samples treated at 170 and 200 °C showed a remarkable influence from the temperature on the chemical composition. During heating at low temperatures (20 to 150 °C), the wood dried up, initially from the loss of free water, and then from losing bound water (Esteves and Pereira 2009). Heating wood at high temperatures (160 to 260 °C) resulted in degradation that caused chemical changes to the wood structure (Esteves and Pereira 2009; Čermák *et al.* 2015).

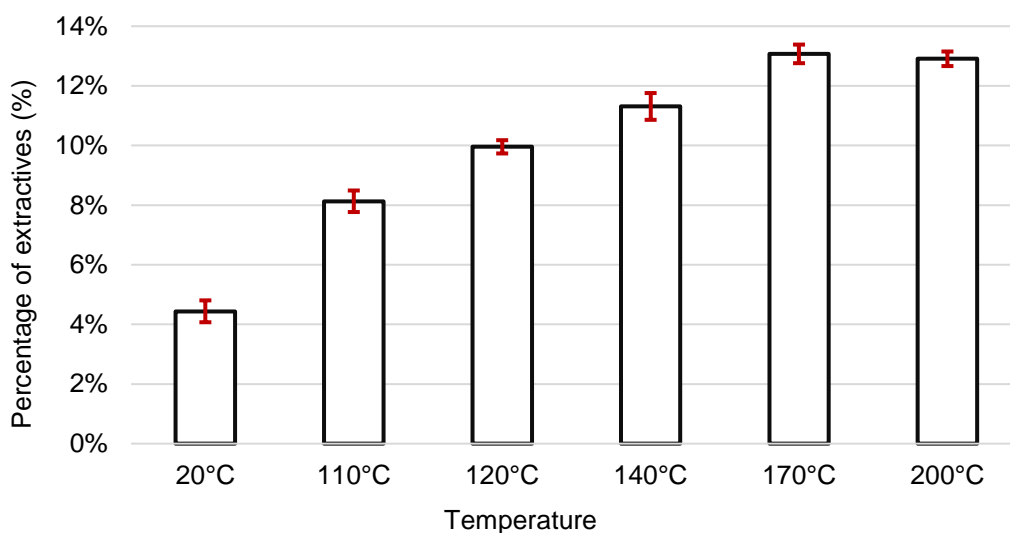


Fig. 3. The percentage of extractives from the dry black locust heartwood, after heat treatment (error bars stand for standard deviation).

The HPLC-HRMS analysis showed a high variation in the chemical composition of the extracts treated at temperatures from 20 to 200 °C. Robinetin had a concentration of 40 µg/mL at 20 °C, which then continuously increased for the 110 (4.6 µg/mL) to 200 °C (27 µg/mL) heat treatments. Similarly, the phenol concentration linearly increased from 38 to 139 µg/mL as the heat treatment temperature increased from 110 to 200 °C. The two remaining phenolic compounds decreased in concentration as the temperature increased. Dihydrorobinetin was detected at concentrations of 5.5 to 161 µg/mL from 200 to 20 °C, respectively. Robinetinidin showed similar results when the temperature increased from 20 to 140 °C, and then the concentration drastically decreased at 170 and 200 °C. When the temperature exceeded 140 °C, two new compounds were detected. Furfural was found when the wood was treated at 170 (50 µg/mL) and 200 °C (350 µg/mL). Additionally, at 200 °C, a second chemical compound, 5-methylfurfural, was detected (0.01 µg/mL).

The optimal output occurred for the 120 °C heat treatment (2340 µg/mL), when compared with the results obtained from the 110 (2020 µg/mL) and 140 °C (2240 µg/mL) heat treatments. The qualitative analysis of the extracts obtained from the samples treated at 170 and 200 °C showed a major influence from the temperature on the chemical

composition (Table 4). A gradual increase of the saccharides was noticed along with the increase of the temperature. Apparently, higher temperatures degraded the cellulose and hemicelluloses to simpler saccharides.

At higher temperatures (170 and 200 °C), two new furfural compounds were detected. Esteves and Pereira (2009) reported that hemicelluloses were the most affected compounds during heat treatment. The thermal degradation started with deacetylation, and the released acetic acid acted as a depolymerization agent, which increased the polysaccharide decomposition. Acid-catalyzed degradation led to the formation of formaldehyde, furfural, and other aldehydes, and hemicelluloses were concurrently dehydrated by the decreased amount of hydroxyl groups. The wood extractives were degraded or destroyed when new extractable compounds emerged because of the wood degradation.

Table 4. HPLC-HRMS Analysis Results of the Extractives Obtained from the Heat-Treated Samples of Black Locust Heartwood

Extractives (µg/mL)	20 °C	110 °C	120 °C	140 °C	170 °C	200 °C
Robinetinidin	1870	1800	2080	1880	950	208
Dihydrorobinetin	161	153	174	162	88.4	5.55
Phenol	50.2	37.8	64.3	174	183	139
Robinetin	39.7	4.56	4.15	5.74	4.26	27.2
Fisetin	9.28	10.7	1.25	6.67	11.6	11.4
Catechin	6.41	5.99	1.12	3.02	0.432	0.379
Gallic acid	1.93	2.41	4.37	5.41	3.33	2.39
Epicatechin	0.166	0.198	0.126	0.843	0.513	0.583
Pyrocatechol	0.124	0.120	1.10	0.260	0.440	0.260
Syringic acid	0.086	0.071	0.130	0.116	0.597	5.75
Syringaldehyde	0.010	0.015	0.070	0.180	0.658	1.42
Sum of phenol. comp.	2140	2010	2330	2240	1240	402
Xyloses equivalent	1.40	1.39	1.73	1.48	3.00	5.18
Glucoses equivalent	0.531	0.505	0.593	0.412	0.289	0.164
Saccharoses equivalent	0.624	0.601	0.748	0.447	0.246	0.037
Sum of saccharides	3.45	3.06	3.85	3.33	5.44	6.37
Furfural	nd	nd	nd	nd	50.0	350
5-methylfurfural	nd	nd	nd	nd	0.002	0.007

Original concentration was 10.0 ± 0.1 g of sample extracted with 100 mL of Dw:m mixture (1:1, v/v); nd = Not Detected

Influence of Solvent Types on the Extraction Output

The analysis of the effect of the various solvent types on the extraction showed that the two low ϵ_r indexed solvents (cyclohexane and benzene) resulted in low extraction yields (on average 0.4% and 4.9%, respectively). Methanol dissolved 7.5% of the soluble compounds (Fig. 4). The hot water extraction demonstrated a lower extract yield (7.2%) than ethanol, which managed to dissolve 8.1% of the extracted soluble compounds. The ANOVA confirmed these results ($F=365.05$; $p=0.0000$), and revealed no direct relationship between the extract yield amounts and ϵ_r .

Cyclohexane showed, as was expected, the lowest amounts of soluble compounds (Table 5). Out of a total number of 14 different compounds, only dihydrorobinetin, fisetin, and robinetin were detected in the extract and in low amounts ($< 0.3 \mu\text{g/mL}$). Similar results were also observed for benzene. Phenol was the only compound that presented a concentration higher than $1 \mu\text{g/mL}$. In line with the literature, different solvents with various relative permittivity indices had a substantial influence on the solubility of the chemical substances (Mészáros *et al.* 2007; Sergent *et al.* 2014). Nevertheless, the relative permittivity alone was found to be an inefficient factor for selecting competent solvents for extraction.

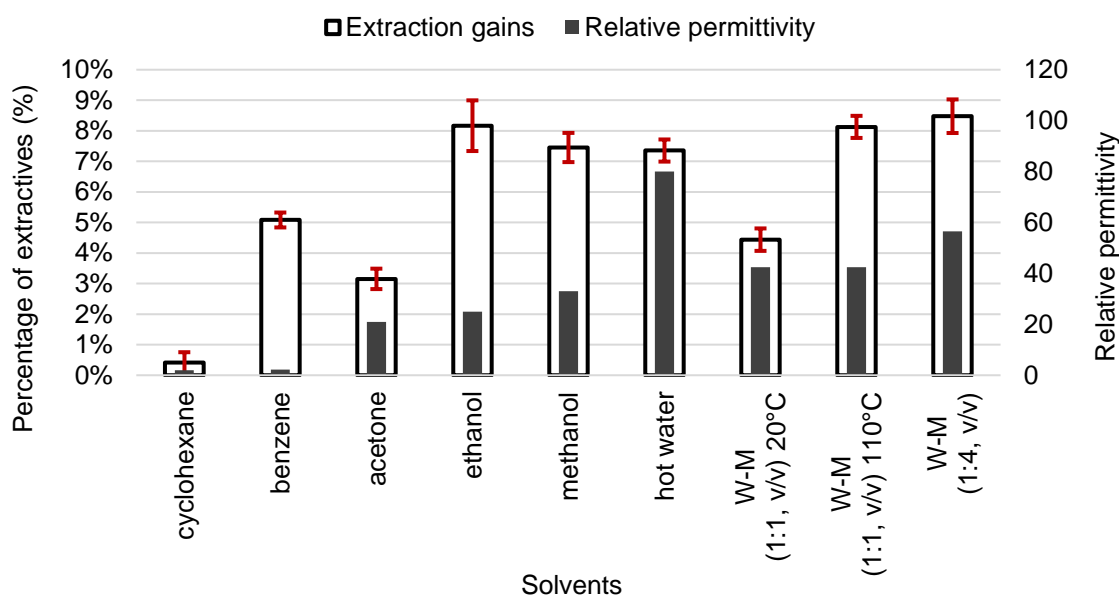


Fig. 4. Amounts of extract from black locust heartwood (dry wood) using solvents with different relative permittivity; particle size = 0.5 mm; Dw:m; Distilled Water-Methanol (error bars stand for standard deviation).

Acetone extracted the whole range of identified chemical compounds with higher amounts of dihydrorobinetin, phenol, robinetin, and robinetinidin. According to the HPLC-HRMS analysis, differences were detected between the water, methanol, and ethanol solvents. Referring to the phenolic compounds, phenol ($\text{C}_6\text{H}_5\text{OH}$) was detected in the ethanol ($44 \mu\text{g/mL}$) and water ($126 \mu\text{g/mL}$) extracts, but not in the methanol extract. Robinetinidin was detected in decreasing amounts in the water ($1930 \mu\text{g/mL}$), ethanol ($1820 \mu\text{g/mL}$), and methanol ($1590 \mu\text{g/mL}$) extracts.

Conversely, dihydrorobinetin was detected in higher amounts in the methanol ($245 \mu\text{g/mL}$) extract, followed by the ethanol ($169 \mu\text{g/mL}$) and water ($163 \mu\text{g/mL}$) extracts. Finally, ethanol was the only solvent active enough to dissolve a higher amount of robinetin ($40 \mu\text{g/mL}$). The total sum of soluble compounds clearly showed a higher output for dw:m 1:4 v/v ($2600 \mu\text{g/mL}$) than for dw:m 1:1 v/v ($2020 \mu\text{g/mL}$). The main differences were found when investigating phenol (the results doubled when using the dw:m 1:4, v/v mixture), as well as fisetin. A smaller difference was observed for robinetinidin, whereas a large difference of approximately 27% for the dw:m 1:4 v/v mixture was seen.

Water and methanol were able to dissolve hydrophobic organic compounds (olefins, aromatic hydrocarbons, or phenols), whereas the solvents with lower ϵ_r values, such as cyclohexane and benzene, managed to extract mostly lipoids, fatty acids, *etc.* The qualitative analysis of the extractives showed that acetone was a more efficient solvent than ethanol in the case of the phenolic compounds, whereas Mészáros *et al.* (2007) stated the opposite conclusion after using the Soxhlet extraction method. Eventually, the selection of the solvent type according to the relative permittivity can be an eligible parameter for the *fexIKA* extraction method in the frame of a stepwise extraction performed with solvents that have increasing ϵ_r values.

Table 5. HPLC-HRMS Analysis Results of the Extractives Obtained by Using Various Solvents

Extractives ($\mu\text{g/mL}$)	Cyclohexane	Benzene	Acetone	Ethanol	Methanol	Hot water	Dw:m 1:1	Dw:m 1:4
Relative permittivity	2.0	2.2	21.0	25.0	33.0	80.0	42.4	56.5
Robinetinidin	nd	nd	2020	1820	1590	1930	1800	2290
Dihydrorobinetin	0.074	0.079	298	169	245	163	153	195
Phenol	nd	34.3	80.1	44.3	nd	126	37.8	79.9
Robinetin	0.229	0.202	16.2	40.4	8.09	0.900	4.56	1.56
Fisetin	0.125	0.064	8.84	6.99	7.57	2.54	10.7	13.1
Catechin	nd	nd	1.91	0.686	2.75	4.73	5.99	7.86
Gallic acid	nd	0.012	1.11	1.00	1.15	2.96	2.41	3.15
Epicatechin	nd	nd	0.144	0.246	0.109	2.02	0.198	0.222
Pyrocatechol	nd	nd	0.077	0.069	0.100	0.430	0.120	0.320
Syringic acid	nd	0.166	0.150	0.083	0.081	0.301	0.071	0.168
Syringaldehyde	nd	nd	0.056	0.045	nd	nd	0.015	0.021
Sum of phenol. comp.	0.428	34.8	2430	2080	1860	2230	2020	2600
Xyloses equivalent	nd	nd	3.50	2.14	1.46	1.88	1.39	2.03
Glucoses equivalent	nd	nd	0.627	0.814	0.605	0.545	0.505	0.730
Saccharoses equivalent	nd	nd	0.271	2.51	1.20	0.160	0.123	1.84
Sum of saccharides	0.00	0.00	4.40	5.46	3.72	2.59	2.02	4.60

Original concentration was 10.0 ± 0.1 g of sample extracted with 100 mL of Dw:m mixture (1:1, v/v); nd = Not Detected

Conclusively, the advanced *fexIKA* method can be suggested as a faster, less energy and solvent consuming technique for extracting accessorial compounds from wood in an optimal way. Further research on other species can confirm the findings of this study.

CONCLUSIONS

1. The advanced *fexIKA* extraction method provided the authors with results that compared well with previous studies on black locust heartwood extractives obtained by other extraction methods.
2. An inversely proportional relationship between the particle size and extraction yield was confirmed for the phenolic compounds.
3. The relative permittivity was not an adequate factor for selecting a solvent for use in *fexIKA* extraction.

4. Higher amounts of extractives were obtained by heat treating at higher temperatures.
5. During thermal treatment, the wood extractives were destroyed or degraded. They formed new extractable compounds and risked the quantity and quality accuracy of the extraction yield.

ACKNOWLEDGMENTS

The article was supported by the grant project IGA LDF_VP_2015005 “Antioxidant assay as a potential assessment of wood extractives antifungal activity” and project “The Establishment of an International Research Team for the Development of New Wood-based Materials” reg. no. CZ.1.07/2.3.00/20.0269 is co-financed by the European Social Fund and the state budget of the Czech Republic.

Special thanks go to the Global Change Research Institute CAS in Brno for the laboratory support, founded by the grant projects supported by the Ministry of Education, Youth and Sports of the Czech Republic within the National Program for Sustainability (NPU, grant No. LO1415) and National infrastructure CzeCOS ProCES (grant No. CZ.02.1.01/0.0/0.0/16_013/0001609).

REFERENCES CITED

- Bekhta, P., and Niemz, P. (2003). “Effect of high temperature on the change in color, dimensional stability and mechanical properties of spruce wood,” *Holzforschung* 57(5), 539-546. DOI: 10.1515/HF.2003.080
- Čermák, P., Rautkari, L., Horáček, P., Saake, B., Rademacher, P., and Sablík, P. (2015). “Analysis of dimensional stability of thermally modified wood affected by re-wetting cycles,” *BioResources* 10(2), 3242-3253. DOI: 10.15376/biores.10.2.3242-3253
- Chen, Y., Gao, J., Fan, Y., Tshabalala, M. A., and Stark, N. M. (2012). “Heat-induced chemical and color changes of extractive-free black locust (*Robinia pseudoacacia*) wood,” *BioResources* 7(2), 2236-2248. DOI: 10.15376/biores.7.2.2236-2248
- Chow, P., Rolfe, G. L., and Shupe, T. F. (1996). “Some chemical constituents of ten-year-old American sycamore and black locust grown in Illinois,” *Wood Fiber Sci.* 28(2), 186-193.
- Craig, L. C., Gregory, J. D., and Hausmann, W. (1950). “Versatile laboratory concentration device,” *Anal. Chem.* 22(11), 1462-1462. DOI: 10.1021/ac60047a601
- Dünisch, O., Richter, H.-G., and Koch, G. (2010). “Wood properties of juvenile and mature heartwood in *Robinia pseudoacacia* L.,” *Wood Sci. Technol.* 44(2), 301-313. DOI: 10.1007/s00226-009-0275-0
- Esteves, B. M., and Pereira, H. M. (2009). “Wood modification by heat treatment: A review,” *BioResources* 4(1), 370-404. DOI: 10.15376/biores.4.1.370-404
- Freudenberg, K., and Hartmann, L. (1953). “Constituents from *Robinia pseudoacacia*,” *Naturwissenschaften* 40(15), 413.
- Gião, M. S., Pereira, C. I., Fonseca, S. C., Pintado, M. E., and Malcata, F. X. (2009). “Effect of particle size upon the extent of extraction of antioxidant power from the plants *Agrimonia eupatoria*, *Salvia* sp. and *Satureja Montana*,” *Food Chem.* 117(3), 412-416. DOI: 10.1016/j.foodchem.2009.04.020

- Grozdzits, G. A., and Chang, P. C. (1984). "Abies concolor bark extractive yields as affected by process variables," *New Zeal. J. For. Sci.* 14(2), 240-248.
- Guerrero, M. S., Torres, J. S., and Nuñez, M. J. (2008). "Extraction of polyphenols from white distilled grape pomace: Optimization and modelling," *Bioresource Technol.* 99(5), 1311-1318. DOI: 10.1016/j.biortech.2007.02.009
- Hart, J. H. (1968). "Morphological and chemical differences between sapwood, discolored sapwood, and heartwood in black locust and Osage orange," *Forest Sci.* 14(3), 334-338.
- Hashemi, S. K. H., and Kanani, S. (2012). "Heartwood extractives of *Robinia pseudoacacia* wood," *J. Adv. Lab. Res. Biol.* 3(3), 156-160.
- Kubota, T., and Hase, T. (1966). "Constituents of *Robinia pseudoacacia*. I. Constituents of the heartwood of *Robinia pseudoacacia*," *J. Chem. Soc. Jpn. Pure Chem. Sect.* 87(11), 1201-1205.
- Latorraca, J. V. F., Dünisch, O., and Koch, G. (2011). "Chemical composition and natural durability of juvenile and mature heartwood of *Robinia pseudoacacia* L.," *Ann. Brazil. Acad. Sci.* 83(3), 1059-1068. DOI: 10.1590/S0001-37652011005000016
- Li, Z., Qin, M., Xu, C., and Chen, X. (2013). "Hot water extraction of hemicelluloses from aspen wood chips of different sizes," *BioResources* 8(4), 5690-5700. DOI: 10.15376/biores.8.4.5690-5700
- Magel, E., Jay-Allemand, C., and Ziegler, H. (1994). "Formation of heartwood substances in the stemwood of *Robinia pseudoacacia* L. II. Distribution of nonstructural carbohydrates and wood extractives across the trunk," *Trees* 8(4), 165-171. DOI: 10.1007/BF00196843
- Martens, D., Gfrerer, M., Wenzl, T., Zhang, A., Gawlik, B. M., Schramm, K.-W., Lankmayr, E., and Kettrup, A. (2002). "Comparison of different extraction techniques for the determination of polychlorinated organic compounds in sediment," *Anal. Bioanal. Chem.* 372(4), 562-568. DOI: 10.1007/s00216-001-1120-y
- Mészáros, E., Jakab, E., and Várhegyi, G. (2007). "TG/MS, Py-GC/MS and THM-GC/MS study of the composition and thermal behavior of extractive components of *Robinia pseudoacacia*," *J. Anal. Appl. Pyrol.* 79(1-2), 61-70. DOI: 10.1016/j.jaap.2006.12.007
- Mustapha, M. B., Adefisan, H. A., and Olawale, A. S. (2012). "Studies of tannin extract yield from *Parkia clappertoniana*'s husk," *J. Appl. Sci. Res.* 8(1), 65-68.
- Niemz, P., Hofmann, T., and Rétfalvi, T. (2010). "Investigation of chemical changes in the structure of thermally modified wood," *Maderas-Cienc. Tecnol.* 12(2), 69-78. DOI 10.4067/S0718-221X2010000200002
- Ottone, S. P., and Baldwin, R. C. (1981). "The relationship of extractive content to particle size distribution in milled yellow poplar (*Liriodendron tulipifera* L.) bark," *Wood Fiber Sci.* 13(2), 74-85.
- Roux, D. G., and Paulus, E. (1962). "Condensed tannins: 13. Inter-relationships of flavonoid components from the heartwood of *Robinia pseudoacacia*," *Biochem. J.* 82(2), 324-330.
- Sablík, P., Giagli, K., Pařil, P., Baar, J., and Rademacher, P. (2016). "Impact of extractive chemical compounds from durable wood species on fungal decay after impregnation of nondurable wood species," *Eur. J. Wood Prod.* 74(2), 231-236. DOI: 10.1007/s00107-015-0984-z
- Sanz, M., Fernández de Simón, B., Esteruelas, E., Muñoz, Á. M., Cadahía, E., Hernández, T., Estrella, I., and Pinto, E. (2011). "Effect of toasting intensity at

- cooperage on phenolic compounds in acacia (*Robinia pseudoacacia*) heartwood,” *J. Agr. Food Chem.* 59(7), 3135-3145. DOI: 10.1021/jf1042932
- Schwanninger, M., and Hinterstoisser, B. (2002). “Comparison of the classical wood extraction method using a Soxhlet apparatus with an advanced extraction method,” *Holz Roh. Werkst.* 60(5), 343-346. DOI: 10.1007/s00107-002-0312-2
- Sergent, T., Kohnen, S., Jourez, B., Beauve, C., Schneider, Y.-J., and Vincke, C. (2014). “Characterization of black locust (*Robinia pseudoacacia* L.) heartwood extractives: Identification of resveratrol and piceatannol,” *Wood Sci. Technol.* 48(5), 1005-1017. DOI: 10.1007/s00226-014-0656-x
- So, W. T., Park, S. J., Kim, J. K., Shim, K., Lee, K. Y., Hyun, J. I., Lee, H. S., Kang, S. K., and Jo, J. M. (1983). “On wood properties of imported (introduced) species grown in Korea. Wood properties of *Pinus strobus*, *Pinus sylvestris*, *Pinus banksiana*, *Picea abies* and *Robinia pseudoacacia*,” *For. Res. Inst.* 27(1), 7-31.
- Song, T., Pranovich, A., and Holmbom, B. (2012). “Hot-water extraction of ground spruce wood of different particle size,” *BioResources* 7(3), 4214-4225. DOI: 10.15376/biores.7.3.4214-4225
- Soon, L. K., and Chiang, L. K. (2012). “Influence of different extraction solvents on lipophilic extractives of *Acacia* hybrid in different wood portions,” *Asian J. Appl. Sci.* 5(2), 107-116. DOI: 10.3923/ajaps.2012.107.116
- Stringer, J. W. (1992). “Wood properties of black locust (*Robinia pseudoacacia*): Physical, mechanical, and quantitative chemical variability,” in: *Proceedings of the International Conference on Black Locust: Biology, Culture and Utilization*, East Lansing, MI, pp. 197-207.
- Szyrwińska, K., and Lulek, J. (2001). “Fexika extraction for the determination of selected polychlorinated biphenyl congeners in sediment samples,” in: *Ars Separatoria 2001: Proceedings of the XVIth International Symposium on Physico-Chemical Methods of the Mixtures Separation*, Borówno, Poland.
- TAPPI T204 CM-07 (2007). “Solvent extractives of wood and pulp,” TAPPI Technical Association of the Pulp and Paper Industry, Atlanta, GA, USA.

Article submitted: April 8, 2017; Peer review completed: July 20, 2017; Revised version received: January 8, 2018; Accepted: March 31 2018; Published: April 24, 2018.
DOI: 10.15376/biores.13.2.4224-4238