Janja Zule[1], Katarina Čufar[2], Vesna Tišler[2]

Lipophilic Extractives in Heartwood of European Larch (*Larix decidua* Mill.)

Lipofilni ekstraktivi srži europskog ariša (*Larix decidua* Mill.)

Original scientific paper • Izvorni znanstveni rad
Received – prispjelo: 12. 12. 2014.
Accepted – prihvaćeno: 6. 11. 2015.
*UDK: 674.032.14; 630*811.52; 630*813.23*
doi:10.5552/drind.2015.1442

ABSTRACT • *The heartwood of two European larch trees was examined for the content of lipophilic extractives. Hexane was used as a solvent for extractions, while gas chromatography with flame ionisation detection and gas chromatography coupled to mass spectrometry were applied for analyses. Different lipophilic groups of compounds, such as fatty acids, resin acids, diterpenoids, sterols, steryl esters and triglycerides were identified and quantitatively evaluated as well as individual low molecular mass components. Distribution of heartwood lipophilics in relation to the trunk heights was also determined and their most likely biological function in wood tissues discussed. The content of hexane extract increased with stem height. Various fatty and resin acids as well as diterpenoid alcohols and sterols were characterized. The predominating lipophilic compounds identified were isopimaric acid and diterpenoid alcohol larixyl acetate. Their average concentrations in examined samples was between 2.0 and 2.5 mg/g. Higher molecular mass lipophilics, e.g. steryl esters and triglycerides, were also present with concentrations between 0.5 and 2.2 mg/g.*

Key words: *European larch, heartwood, lipophilic extractives, chromatographic techniques*

SAŽETAK • *U radu je prikazan rezultat istraživanja lipofilnih ekstraktivnih tvari u srži dvaju stabala europskog ariša. Kao otapalo za ekstrakciju primijenjen je heksan, a plinska kromatografija s plameno-ionizacijskim detektorom i plinska kromatografija povezana s masenom spektrometrijom primijenjene su za kemijske analize. Različite lipofilne grupe spojeva, kao masne kiseline, smolne kiseline, diterpenoidi, steroli, sterolni esteri i trigliceridi, identificirane su u uzorcima i kvantitativno su procijenjene. Također su identificirani i procijenjeni pojedini spojevi niskih molekularnih masa. Određena je i raspodjela lipofilnih tvari u srži s obzirom na visinu stabala i objašnjena njihova najvjerojatnija biološka uloga u drvnim tkivima. Sadržaj ekstrakta heksana povećava se s visinom stabla. Različite masne i smolne kiseline, kao i diterpenoidni alkoholi i steroli, također su zastupljeni u srži europskog ariša. Dominantni identificirani lipofilni spojevi jesu isopimarična kiselina i diterpenoidni alkohol. Njihove prosječne koncentracije u ispitivanim uzorcima kreću se između 2,0 i 2,5 mg/g. Lipofilni spojevi veće molekularne mase, npr. sterolni esteri i trigliceridi, također su otkriveni u koncentracijama između 0,5 i 2,2 mg/g.*

Ključne riječi: *europski ariš, srž, lipofilni ekstraktivi, kromatografske tehnike*

[1] Author is researcher at Pulp and Paper Institute, Ljubljana, Slovenia. [2]Authors are professors at University of Ljubljana, Biotechnical Faculty, Ljubjana, Slovenia.
[1] Autorica je znanstvenica Instituta za celulozu i papir, Ljubljana, Slovenija. [2]Autorice su profesorice Sveučilišta u Ljubljani, Biotehnički fakultet, Ljubljana, Slovenija.

1 INTRODUCTION
1. UVOD

Different nonstructural wood components with relatively low molecular masses, which are well soluble in nonpolar organic solvents are classified as lipophilic extractives. Their content is much smaller than that of the main building elements, e. g. polysaccharides and lignin and averagely amounts up to only a few percents of dry plant material. Types and concentrations of wood lipophilics depend on tree species, tissue types, geographic position of growing site, climatic conditions, felling season, age and tree health. A function of lipophilic extractives in living trees is not yet fully understood, however it is well known that some compounds, such as sterols and steryl esters are components of cell membranes and take an active part in different biochemical processes, while others, e.g. triglycerides and diglycerides represent food reserve. The most important function of extractives is most probably mechanical and chemical protection of wood tissues against microbial, fungal and insect attack. Resin and wax components form typical mechanical protective layer or hydrophobic barrier while more polar phenols (lignans, flavonoids, stilbenes) exhibit biocidal properties and are as such very toxic for numerous organisms (Hawley *et al*., 1924; Scheffer and Cowling, 1966; Fengel and Wegener, 1984; Schultz *et al*., 1990, 1995).

The genus *Larix* is represented by about ten species that are mainly distributed across the cooler regions of the northern hemisphere. European larch (*Larix decidua* Mill.) is the most frequent representative of this genus in Europe. It is growing on its natural habitat or on reforested areas. It is appreciated for its timber, with good mechanical properties, attractive colour and high natural durability of its heartwood (Gierlinger *et al*., 2004). The heartwood of European larch is usually composed of about 39 % of cellulose, 30 % of hemicellulose (mannan and xylan), 28 % of lignin with the remaining portion being ash and extractives (Fengel and Wegener, 1984). Chemical composition of larch wood is variable, which is e.g. reflected in variable durability of larch heartwood, reported to range from nondurable to moderately durable (class 5 to 3, European Standard EN 350-1) (Gierlinger *et al*., 2003).

Several authors have so far reported on the content of lipophilic extractives in different larch species. Nair and von Rudloff (1959, 1960) studied and compared chemical composition of heartwood extractives of Tamarack (*Larix laricina*) and Alpine larch (*Larix lyalii*). Norin and coauthors (1965) determined the structures and configurations of the diterpenoids larixol, larixyl acetate and 13-epimanool, which were isolated from the oleoresin of European larch. Larixol was reported to be one of the main neutral constituents of Siberian larch (*Larix sibirica*) as well. Mills (1973) analysed oleoresin of most larch species growing in England. He found out that European larch contained large quantities of larixyl acetate together with lesser amounts of the free diol larixol and epimanool. The neutral fraction was composed of aldehydes including dehydroabietal and isopimaral. Siberian larch contained epimanool and epitorulosol, as well as hydrocarbons such as monocyclic thunbergene and biformene and compounds abietadiene, abietal and abietol. All investigated larches also contained resin acids of abietic and pimaric type. The most striking feature was that two species, namely European and Japanese larch (*Larix gmelinii*) contained large amounts of larixol and larixyl acetate, which were not detectable in the others. Western larch and Japanese larch (*Larix kaempferi*) had high amounts of thunbergol, while the latter was completely absent from Tamarack and Alpine larch. Giwa and Swan (1975) studied extractives of Western larch, isolated from the heartwood by the help of solvents of different polarity. The lipophilic portion was composed of sandaracopimaric, isopimaric, abietic and dehydroabietic acid, β-sitosterol, larixol and larixyl acetate. Viitanen and coauthors (1997) examined durability of European, Siberian and Japanese larch against brown-rot fungi and found out that it was related to the extractives content. They analysed diethylether extracts and proved that some resin acids inhibited the growth of certain fungi, in spite of the fact that their content was as small as about 0.1 %. Staccioli and colleagues (1997) compared heartwood extractives of living and subfossil European larch and established different age dependent, structural changes. Krutov and co-workers (1988) examined extractives of Siberian larch in which they identified numerous neutral diterpenoid components. Babkin and coauthors (2001) also analysed resinous substances in Siberian and Japanese larch. The latter were a complex mixture of neutral diterpenoids and resin acids. Ohtsu and co-workers (1998a, 1998b, 1998c) identified some interesting diterpenes in cones of Japanese larch as well as tetracyclic triterpenes in needles and bark of the same tree species, while Xue (2004) reported about identification of two new diterpenoids of labdane type in Chinese larch (*Larix chinensis*). Most authors analysed heartwood samples at the representative height of 1.2 to 1.5 m, while there is practically no data on the content of extractives at different positions along the trunk.

The purpose of the present research was to make a detailed chemical characterization of lipophilic extractives in the heartwood of European larch, and to determine their variability in longitudinal direction within a tree and between two trees.

2 MATERIAL AND METHODS
2. MATERIJAL I METODE

2.1 Samples
2.1. Uzorci

Two larch trees (*Larix decidua* Mill.) were felled at the end of June, 2009 in the Alpine region of Slovenia at the altitude of 1000 m. They were growing in a mixed forest among beech and spruce trees. Both were about 180 years old, about 30 m high and with a diameter at breast height (DBH) above 50 cm. The trees were healthy and without visible damage.

About 5 cm thick discs were cut from the trunks at the base (50 cm above ground) as well as at the

heights of 8, 18 and 28 m. Discs were debarked and representative sections of heartwood were cut into smaller pieces, which were subsequently frozen at -24 °C till analysis.

2.2 Drying and grinding
2.2. Sušenje i mljevenje

Frozen samples were first conditioned at room temperature, after which they were cut into short splinters, freeze-dried for 24 hours (Martin Christ Gefriertrocknungsanlagen GmbH) and ground by means of a Wiley laboratory mill (100 mesh) to wood meal. The latter was freeze-dryed again for 24 hours in order to remove volatile compounds.

2.3 Extraction
2.3. Ekstrakcija

Extractions were conducted by means of an accelerated solvent extraction system (ASE) using the instrument Dionex ASE 200. About 5 g of each freeze-dried powdered sample was weighed into a metal extraction cell and extracted with hexane (V-50 ml) to isolate lipophilic fraction. The temperature of the extraction was 90 ºC, pressure 13.8 MPa and extraction time 10 minutes (2 static cycles with static time of 5 minutes). The extraction was performed under the stream of nitrogen.

2.4 Derivatization of extractives
2.4. Derivatizacija ekstraktiva

All hexane extracts were derivatized prior to chromatographic analyses (GC-FID, GC-MS), by which components with hydroxyl groups, such as alcohols, sterols and acids were converted to the corresponding trimethylsilyl (TMS) derivatives, namely ethers and esters. 2 ml of internal standard solution were added to each extract, containing about 0.5 mg of extractive compounds. Internal standards were heneicosanoic acid (S1), betulinol (S2), cholesteryl heptadecanoate (S3) and 1.3-dipalmitoyl-2-oleyl glycerol (S4) all having concentration of 0.02 mg/ml. The mixture of a sample and standards was dried under the stream of N_2 and in vacuum desiccator at 40 ºC, after which sylilation reagents were added: 80µl BSTFA (bis-trimethylsilyl-trifluoroacetamide) and 20 µl TMCS (trimethylchlorosilane) in 20 µl of pyridine. The reaction mixture was heated for 1 hour at 70 ºC, then it was cooled and injected into gas chromatograph (Willfor, 2007).

2.5 Chromatographic analyses of extractives
2.5. Kromatografska analiza ekstraktiva

2.5.1 GC-FID analysis on short capillary column
2.5.1. GC-FID analiza na kratkoj kapilarnoj koloni

The main extractive groups in hexane extracts were determined by means of gas chromatography with flame ionization detector (GC-FID) on the Perkin Elmer Clarus 500 instrument. The separation was performed on a short capillary column HP-1 (6 m x 0.53 mm x 0.15 µm) using the following experimental conditions – oven temperature program: 100 ºC (1.5 min), 12 ºC/min, 340 ºC (5 min); carrier gas H_2 (7 ml/min, programed flow); PSS injector – temperature program of heating: 80 ºC (0,1 min), 50 ºC/min, 110 ºC, 15 ºC/min, 330 ºC (7 min); FID detector: 340 ºC; injection volume 0.5 µl (on column). Heneicosanoic acid (S1) was used for quantification of free fatty and resin acids as well as diterpenoids, while betulinol (S2) was applied for determination of sterols. Steryl esters were quantified against cholesteryl heptadecanoate (S3) and triglycerides against 1.3-dipalmitoyl-2-oleyl glycerol (S4). No correction factor was applied for the calculation of lipophilics. The concentrations of individual extractive groups were expressed as mg per gram of dry sample weight where the quantification limit was about 0.01 mg/g (Willfor, 2007).

2.5.2 GC-FID analysis on long capillary column
2.5.2. GC-FID analiza na dugoj kapilarnoj koloni

The determination of individual fatty and resin acids, diterpenoids and sterols was accomplished by means of GC-FID on the Perkin Elmer AutoSystem XL instrument. HP-1 (25 m x 0.20 mm x 0.11 µm) capillary column was used for separation of compounds under the following experimental conditions: temperature program of column heating: 120 ºC, 6 ºC/min, 300 ºC (10 min); carrier gas H_2 (0,8 ml/min); split injector (1:20) – 160 ºC, 8 ºC/min, 260 ºC (15 min); FID detector: 320 ºC; injection volume 1 µl. Heneicosanoic acid (S1) was used as internal standard for quantification of individual acids and diterpenoids, while betulinol (S2) was used as standard in determination of sterols. All results are expressed as mg per gram of dry sample weight where the limit of quantification was about 0.01 mg/g (Willfor, 2007).

2.5.3 Identification of extractive compounds by GC-MS
2.5.3. Identifikacija ekstraktivnih spojeva uz pomoć GC-MS-a

Characteristic components of the selected hexane extracts were identified by means of gas chromatography coupled to mass spectroscopy (GC-MS). The analyses were performed on the HP 6890-5973 GC-MSD instrument. The separation was carried out on the HP-1 (30 m x 0.25 mm x 0.25 µm) capillary column under the following experimental conditions: temperature program of column heating 80 ºC, 8 ºC/min, 290 ºC; carrier gas He (0.9 ml/min); split injector (1:15) – 260 ºC; MS-EI detector (source temp. 280 ºC, 70 eV, quadrupole temp. 180 ºC). Mass range (m/z) was between 10 and 1050. For positive identification of individual compounds the mass spectra of their chromatographic peaks were compared with spectra of pure compounds from the Wiley Registry NIST 2008 Mass Spectral Library. On both GC-FID and GC-MS chromatograms there was practically the same sequence of chromatographic peaks of individual compounds as similar long capillary columns were used, by which reliability of identification was ensured and quantitative work facilitated (Willfor, 2007).

2.6 Gravimetric determination of the content of lipophilic extractives
2.6 Gravimetrijsko određivanje sadržaja lipofilnih ekstraktiva

10 ml aliquots of selected hexane extracts were dried under the stream of N_2 and in vacuum desiccator at 40 ºC until a constant weight was reached. The gravimetric amount of extractives was calculated as mg per gram of dry sample weight.

All quantitative determinations of lipophilic extractives in the samples of heartwood were performed in two parallels. The present results are average values of individual determinations.

3 RESULTS AND DISCUSSION
3. REZULTATI I RASPRAVA

3.1 Gravimetric determination of hexane extracts
3.1. Gravimetrijsko određivanje heksanskih ekstrakata

Hexane, as one of the most nonpolar solvents, was selected for extraction and separation of lipophilic fraction from more polar components, e.g. polyphenols, which are not soluble in it.

The content of hexane extract in the heartwood samples increased with stem height. The measured values were between 8 and 16 mg/g, calculated on dry mass of wood. There was no significant difference be-

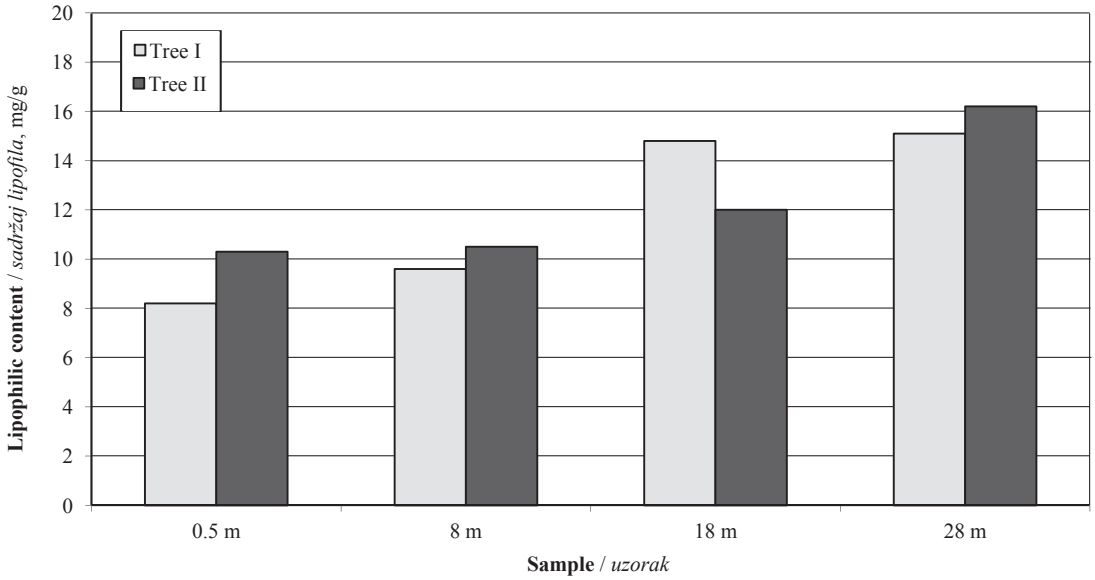

Figure 1 Hexane extract content in heartwood at different heights of two larch trees
Slika 1. Sadržaj heksanskog ekstrakta srži stabla 1 i 2 na različitim visinama

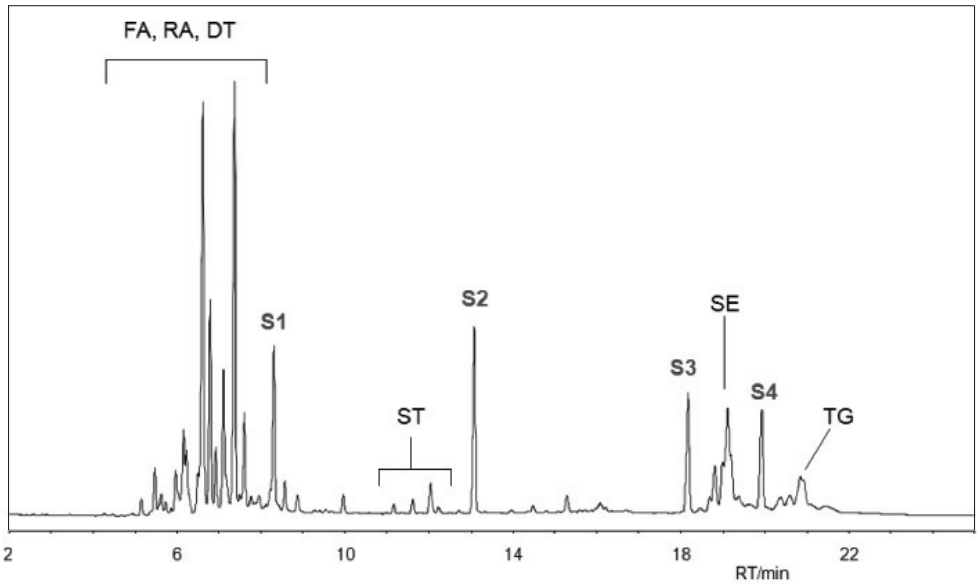

Figure 2 GC-FID (short column) chromatogram of heartwood hexane extract (tree 1 – 0.5 m stem height) (FA – fatty acids, RA – resin acids, DT – diterpenoids, ST – sterols, SE – steryl esters, TG – triglycerides, S1-S4 – internal standards)
Slika 2. GC-FID kromatogram (kratka kolona) heksanskog ekstrakta srži (stablo 1 – 0,5 m visina stabla) (FA – masne kiseline, RA – smolne kiseline, DT – diterpenoidi, ST – steroli, SE – sterolni esteri, TG – trigliceridi, S1-S4 – interni standardi)

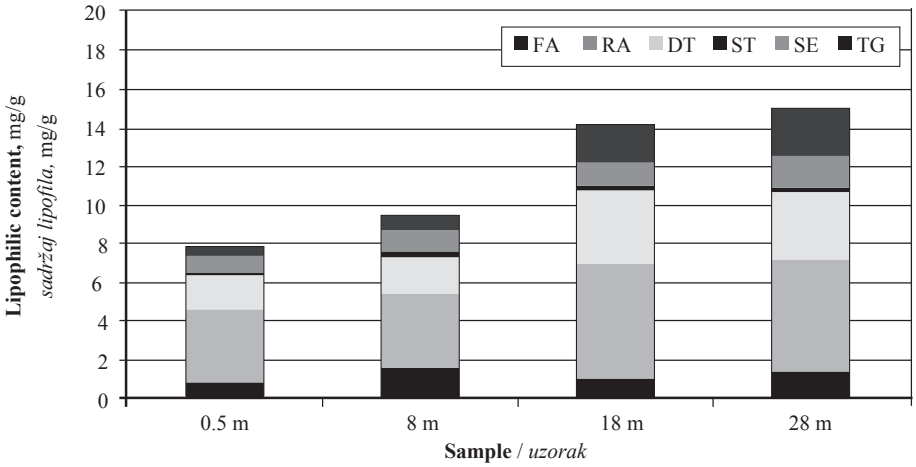

Figure 3 Content and composition of lipophilic fraction of heartwood of tree 1 at different heights (FA – fatty acids, RA – resin acids, DT – diterpenoids, ST – sterols, SE – steryl esters, TG – triglycerides)
Slika 3. Sadržaj i sastav lipofilne frakcije srži stabla 1 na različitim visinama (FA – masne kiseline, RA – smolne kiseline, DT – diterpenoidi, ST – steroli, SE – sterolni esteri, TG – trigliceridi)

tween the two trees. The amount of hexane extract vs. stem height is presented in Figure 1.

The average concentration of hexane extract in the heartwood of tree 1 was calculated to be 11.6 ± 3.5 mg/g and in the heartwood of tree 2 it was 12.0 ± 2.8 mg/g, respectively.

3.2 Determination of typical compound groups (GC-FID)
3.2. Određivanje tipičnih grupa spojeva (GC-FID)

The composition of lipophilic groups in hexane extracts was determined by GC-FID using short capillary column. A typical short column chromatogram of a heartwood hexane extract is shown in Figure 2.

The analysis on a short capillary column (5 m) with relatively high carrier gas flows and use of temperature programmable injection, supported by a relevant computer program for calculation of complex peak areas, enables separation and detection of a series of compounds with very different volatilities and chemical properties on a single chromatographic column. The most significant property of these short column chromatograms is somewhat worse separation of peaks, meaning that compounds are separated as characteristic groups (broad, overlapping peaks) and not as sharp peaks, representing individual compounds. In this way, it is possible to qualitatively and quantitatively determine the composition of various lipophilic groups in wood extracts in a single chromatographic analysis (Sjöstrom and Alen, 1999).

The content and composition of lipophilic fraction in the heartwood of trees I and II related to the stem height is presented in Figures 3 and 4.

Chromatographically determined total concentration of lipophilics (the sum of individual lipophilic group concentrations) increased with the height in both trees just the same as gravimetrically determined content of hexane extract. Concentrations of free fatty acids ranged between 0.9 and 1.8 mg/g, while the con-

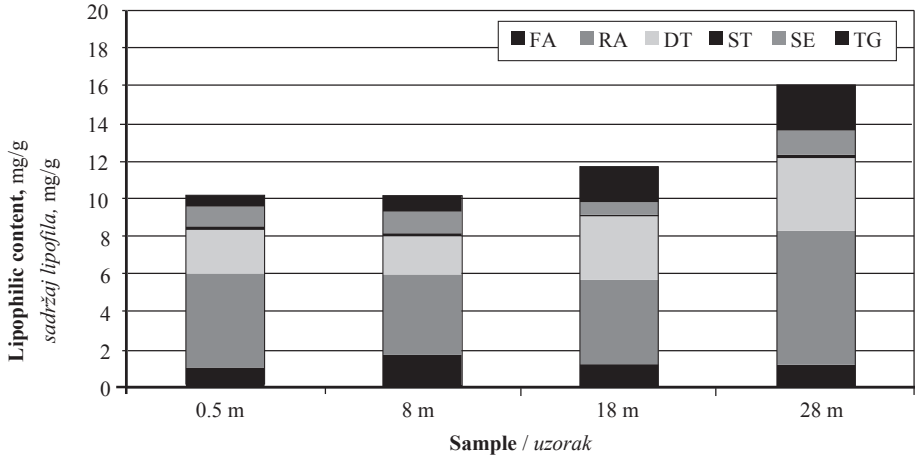

Figure 4 Content and composition of lipophilic fraction of heartwood of tree 2 at different heights (FA – fatty acids, RA – resin acids, DT – diterpenoids, ST – sterols, SE – steryl esters, TG – triglycerides)
Slika 4. Sadržaj i sastav lipofilne frakcije srži stabla 2 na različitim visinama (FA – masne kiseline, RA – smolne kiseline, DT – diterpenoidi, ST – steroli, SE – sterolni esteri, TG – trigliceridi)

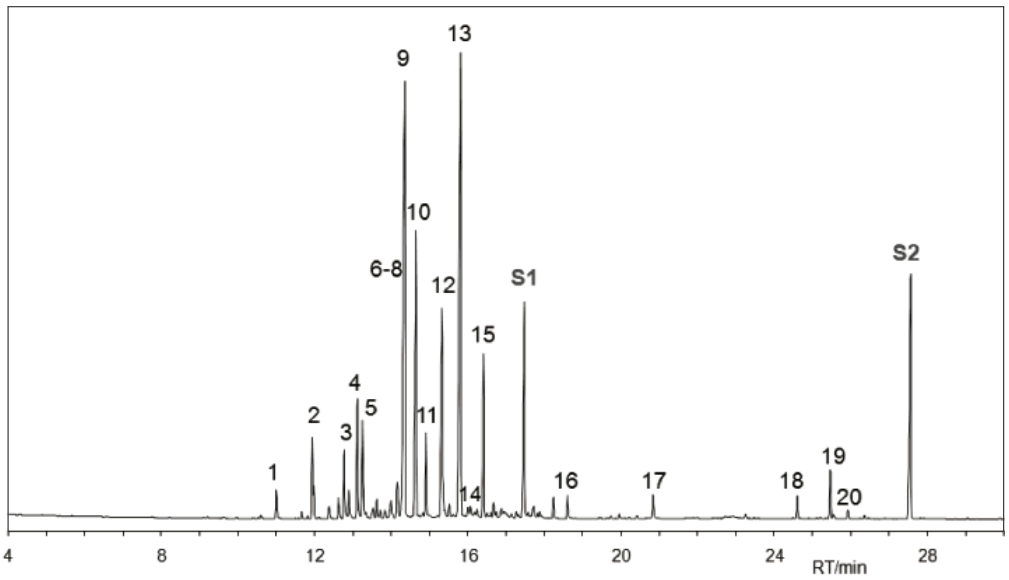

Figure 5 GC-FID (long column) chromatogram of heartwood hexane extract (tree 1 – 0.5 m height) (1 – palmitic, 2 – epimanool, 3 – pinolenic, 4 – linoleic, 5 – oleic, 6 – stearic, 7 – pimaric, 8 – sandaracopimaric, 9 – isopimaric, 10 – palustric, 11 – dehydroabietic, 12 – abietic, 13 – larixyl acetate, 14 – arachidic, 15 – neoabietic, 16 – behenic,17 – lignoceric, 18 – campesterol, 19 – β-sitosterol, 20 – cycloartenol, S1 and S2 – internal standards)
Slika 5. GC-FID kromatogram (duga kolona) heksanskog ekstrakta srži (stablo 1 – 0,5 m visina stabla) (1 – palmitinska, 2 – epimanol, 3 – pinolenska, 4 – linolna, 5 – oleinska, 6 – stearinska, 7 – pimarna, 8 – sandarakopimarna, 9 – isopimarna, 10 – palustrinska, 11 – dehidroabietinska, 12 – abietinska, 13 – lariksil acetat, 14 – arašidna, 15 – neoabietinska, 16 – behenska, 17 – lignocerinska, 18 – kampesterol, 19 – β-sitosterol, 20 – cikloartenol, S1 i S2 – interni standardi)

centrations of resin acids and other diterpenoids were much higher, which was between 5.6 and 11 mg/g. Concentrations of free sterols in no case exceeded 0.3 mg/g, while the contents of higher lipophilics, e.g. steryl esters and triglycerides, were comparable and in the range between 0.5 and 2.2 mg/g. High increase of triglycerides was the most distinctive in the heartwood of both trees at 28 m, which was the top position. The measured quantities were 5 times bigger than at the base of the tree at 0.5 m. The most important fact is that the concentrations of all lipophilic groups exhibited increasing trend in respect to the stem height.

3.3 Identification of individual compounds (GC-MS)
3.3. Identifikacija pojedinačnih spojeva (GC-MS)

For accurate and unambiguous identification of individual low molecular mass components, as for example free fatty acids, diterpenoids, sterols and fatty alcohols in hexane extracts, a selected sample of heartwood at 0.5 m from tree I was analysed by GC-MS technique. Among fatty acids unsaturated oleic, linolenic and pinolenic predominated, while isopimaric acid and larixyl acetate were the most abundant components among resin acids and diterpenoids. β-sitosterol proved to be the most abundant sterol in all heartwood samples.

3.4 Determination of individual compounds (GC-FID)
3.4. Određivanje pojedinih spojeva (GC-FID)

All samples were later chromatographed on a similar long capillary column using similar experimental conditions as in the case of the GC-MS analysis in order to analyse qualitative and quantitative composition of free fatty acids, resin acids, diterpenoid compounds, free sterols and fatty alcohols. A typical long capillary column chromatogram of a heartwood hexane extract is presented in Figure 5. Steryl esters, waxes and triglycerides were not eluted at the applied conditions as they were not volatile enough to be determined directly in this way.

The examined samples had practically the same chemical composition, however they differed accord-

Table 1 Composition of free fatty acids in heartwood of tree 1 at different heights
Tablica 1. Sastav jednostavnih masnih kiselina srži stabla 1 na različitim visinama

Sample *Uzorak*	**16:0** mg/g	**18:0** mg/g	**18:1** mg/g	**18:2** mg/g	**18:3** mg/g	**20:0** mg/g	**22:0** mg/g	**24:0** mg/g	**Total** *Ukupno* mg/g
0.5 m	0.06	0.02	0.26	0.29	0.16	0.02	0.04	0.01	0.86
8 m	0.14	0.03	0.48	0.57	0.29	0.03	0.07	0.02	1.6
18 m	0.09	0.03	0.31	0.31	0.14	0.07	0.11	0.02	1.1
28 m	0.12	0.04	0.54	0.37	0.16	0.08	0.11	0.01	1.4
Average *Prosječ.*	0.10	0.03	0.40	0.38	0.19	0.05	0.08	0.01	1.2

16:0 – palmitic, 18:0 – stearic, 18:1 – oleic, 18:2 – linoleic, 18:3 – pinolenic, 20:0 – arachidic, 22:0 – behenic, 24:0 – lignoceric / 16:0 – palmitinska, 18:0 – stearinska, 18:1 – oleinska, 18:2 – linolna, 18:3 – pinolenska, 20:0 – arašidna, 22:0 – behenska, 24:0 – lignocerinska

Table 2 Composition of free fatty acids in heartwood of tree 2 at different heights
Tablica 2. Sastav jednostavnih masnih kiselina srži stabla 2 na različitim visinama

Sample *Uzorak*	**16:0** mg/g	**18:0** mg/g	**18:1** mg/g	18:2 mg/g	**18:3** mg/g	**20:0** mg/g	**22:0** mg/g	**24:0** mg/g	**Total** *Ukupno* mg/g
0.5 m	0.07	0.02	0.33	0.35	0.19	0.03	0.06	0.01	1.1
8 m	0.10	0.02	0.48	0.68	0.38	0.03	0.05	0.01	1.8
18 m	0.09	0.03	0.32	0.34	0.19	0.08	0.15	0.02	1.2
28 m	0.06	0.02	0.53	0.29	0.17	0.08	0.10	0.01	1.2
Average *Prosječ.*	0.08	0.02	0.42	0.42	0.23	0.05	0.09	0.01	1.3

16:0 – palmitic, 18:0 – stearic, 18:1 – oleic, 18:2 – linoleic, 18:3 – pinolenic, 20:0 – arachidic, 22:0 – behenic, 24:0 – lignoceric / 16:0 – palmitinska, 18:0 – stearinska, 18:1 – oleinska, 18:2 – linolna, 18:3 – pinolenska, 20:0 – arašidna, 22:0 – behenska, 24:0 – lignocerinska

Table 3 Composition of resin acids and diterpenoids in heartwood of tree 1 at different heights
Tablica 3. Sastav smolnih kiselina i diterpenoida srži stabla 1 na različitim visinama

Sample *Uzorak*	**PI** mg/g	**SA** mg/g	**IZ** mg/g	**PA** mg/g	**DeAB** mg/g	**AB** mg/g	**NeAB** mg/g	EPI mg/g	**LAc** mg/g	**Total** *Ukupno* mg/g
0.5 m	0.05	0.09	1.3	0.65	0.15	0.52	0.34	0.14	1.3	4.6
8 m	0.06	0.14	1.8	0.62	0.30	0.65	0.23	0.47	1.4	5.7
18 m	0.12	0.18	3.0	0.95	0.56	0.79	0.27	1.2	2.6	9.7
28 m	0.14	0.15	3.0	0.87	0.63	0.76	0.26	0.70	2.8	9.3
Average *Prosječ.*	0.1	0.1	2.3	0.8	0.4	0.7	0.3	0.6	2.0	7.3

PI – pimaric, SA – sandaracopimaric, IZ – isopimaric, PA – palustric, DeAB – dehydroabietic, AB – abietic, NeAB – neoabietic, EPI – epimanool, LAc – larixyl acetate
PI – pimarna, SA – sandarakopimarna, IZ – izopimarna, PA – palustrinska, DeAB – dehidroabietinska, AB – abietinska, NeAB – neoabietinska, EPI – epimanol, LAc – lariksil acetat

Table 4 Composition of resin acids and diterpenoids in heartwood of tree 2 at different heights
Tablica 4. Sastav smolnih kiselina i diterpenoida u srži stabla 2 na različitim visinama

Sample *Uzorak*	**PI** mg/g	**SA** mg/g	**IZ** mg/g	**PA** mg/g	**DeAB** mg/g	**AB** mg/g	**NeAB** mg/g	**EPI** mg/g	**LAc** mg/g	**Total** *Ukupno* mg/g
0.5 m	0.08	0.15	2.1	0.95	0.26	0.83	0.54	0.22	2.1	7.3
8 m	0.06	0.09	1.8	0.93	0.21	0.72	0.43	0.45	2.0	6.7
18 m	0.09	0.13	2.1	0.51	0.96	0.60	0.15	0.70	2.7	7.9
28 m	0.11	0.19	3.8	0.99	0.60	1.1	0.29	0.93	2.9	11
Average *Prosječ.*	0.1	0.1	2.5	0.8	0.5	0.8	0.4	0.6	2.4	8.2

PI – pimaric, SA – sandaracopimaric, IZ – isopimaric, PA – palustric, DeAB – dehydroabietic, AB – abietic, NeAB – neoabietic, EPI – epimanool, LAc – larixyl acetate
PI – pimarna, SA – sandarakopimarna, IZ – izopimarna, PA – palustrinska, DeAB – dehidroabietinska, AB – abietinska, NeAB – neoabietinska, EPI – epimanol, LAc – lariksil acetat

Table 5. Composition of free sterols in heartwood of tree 1 at different heights
Tablica 5. Sastav jednostavnih sterola srži stabla 1 na različitim visinama

Sample *Uzorak*	**Camp-esterol** mg/g	**β-sitosterol** mg/g	**Cycloartenol** mg/g	**Total** *Ukupno* mg/g
0.5 m	0.04	0.08	0.02	0.14
8 m	0.09	0.18	0.03	0.30
18 m	0.07	0.15	0.02	0.24
28 m	0.06	0.14	0.02	0.22
Average *Prosječ.*	0.07	0.14	0.02	0.23

Table 6. Composition of free sterols in heartwood of tree 2 at different heights
Tablica 6. Sastav jednostavnih sterola srži stabla 2 na različitim visinama

Sample *Uzorak*	**Camp-esterol** mg/g	**β-sitosterol** mg/g	**Cycloartenol** mg/g	**Total** *Ukupno* mg/g
0.5 m	0.05	0.11	0.02	0.18
8 m	0.05	0.10	0.02	0.17
18 m	0.02	0.05	0.01	0.08
28 m	0.06	0.11	0.02	0.18
Average *Prosječ.*	0.04	0.09	0.02	0.15

ing to the concentrations of individual low molecular lipophilic components. Qualitative and quantitative composition of free fatty acids, resin acids, diterpenoids and sterols is presented in Tables 1 to 6.

Unsaturated fatty acids C18 with one or more double bonds prevailed. The group was represented by oleic (C18:1), linoleic (C18:2) and pinolenic (C18:3) acids, having concentrations in the range between 0.14 and 0.68 mg/g, while the contents of saturated higher fatty acids (C16:0 – C24:0) were much lower and ranged between 0.01 and 0.12 mg/g.

The samples also contained resin acids typical for conifers, among which isopimaric acids predominated with concentrations between 1.3 and 3.8 mg/g. The concentrations increased with the height of both stems. Palustric, dehydroabietic and abietic acids were also abundant with concentrations between 0.15 and 1.1 mg/g. Other less important resin acids, in terms of quantity, such as pimaric, sandaracopimaric and neoabietic, were also detected. In addition, specific diterpenoids, typical of larch wood, which formed quite a significant portion of lipophilic fraction were also observed. The identified diterpenoid alcohols or labdans were epimanool, larixol and larixyl acetate. The concentrations of the latter were the highest and ranged between 1.3 and 2.9 mg/g. larixol eluted together with dehydroabietic acid, so it was impossible to determine its exact concentrations.

In addition to the main lipophilic groups, low amounts were also detected of several diterpenoids and fatty alcohols, which did not belong to any of the presented extractive groups. Their total amount in no case exceeded 5 % of the dry mass of the hydrophobic extractive fraction.

β-sitosterol was the main free sterol, however campesterol and cycloartenol were also detected. Total sterol concentrations ranged between 0.08 and 0.30 mg/g and no increase was observed related to the stem height.

Qualitative lipophilic composition of all examined samples in both larch trees was essentially the same with isopimaric acid and larixyl acetate as the most abundant compounds. On the other hand, the amounts of the total hexane extract as well as the concentrations of most components increased with trunk heights. This increase was most pronounced at the top of both trees at the height of 28 m, where the growth was very intensive and the observed tissues much younger and vulnerable. Relatively high amounts of triglycerides represented substantial food reserve, while resin acids and diterpenoids are known to provide physical and chemical protection against deterioration.

4 CONCLUSIONS
4. ZAKLJUČAK

A detailed chemical characterisation of the lipophilic fraction of larch heartwood was performed by means of modern chromatographic techniques, such as GC-FID and GC-MS. It was established that heartwood contained some characteristic compounds (epimanool, larixyl acetate) that could not be found in other conifer genera as for example pine (*Pinus*), spruce (*Picea*) and fir (*Abies*). All heartwood samples from different height positions of two healthy larch trees exhibited the same qualitative composition of the lipophilic fraction, however the overall concentrations of the lipophilics increased with the height of the trunk. The same held for the most important extractive groups, e.g. triglycerides, resin acids and diterpenoids, which were found to be especially abundant in the younger tissues at the top of the trees. It could be assumed that lipophilic compounds are distributed in the heartwood within a living tree in such a way as to ensure storage of food and protection against deterioration. Our study may contribute to better understanding of the chemistry and its variation within living trees.

Acknowledgement – Zahvala

Analyses were performed at the Åbo Akademi University, Turku, Finland. Authors are gratefully thankful to prof. Bjarne Holmbom and the members of his staff – Jarl Hemming, Andrey Pranovich and Markku Reunanen for substantial help and cooperation.

5 REFERENCES
5. LITERATURA

1. Babkin, V. A.; Ostroukhova, L. A.; Malkov Yu, A.; Babkin, D. V.; Onuchina, N. A.; Ivanova, S. Z., 2001: Isolation of biologically active compounds from larch wood. 11th ISWPC International Symposium on Wood and Pulping Chemistry, Nice, June 11-14, pp. 119-122.
2. Fengel, D.; Wegener, G., 1989: Wood chemistry, ultrastructure, reactions. Walter de Gruyter, Berlin-New York, 812 pp.
3. Gierlinger, N.; Jacques, D.; Schwanninger, M.; Wimmer, R.; Hinterstoisser, B.; Paques, L. E., 2003: Rapid prediction of natural durability of larch heartwood using FT-NIR spectroscopy. Canadian Journal of Forest Research, 33 (9): 1727-1736 http://dx.doi.org/10.1139/x03-092.
4. Gierlinger, N.; Jacques, D.; Schwanninger, M.; Wimmer, R.; Paques, L. E., 2004: Heartwood extractives and lignin content of different larch species (*Larix* sp.) and relationships to brown-rot decay-resistance. Trees 18: 230-236 http://dx.doi.org/10.1007/s00468-003-0300-0.
5. Giwa, S. A. O.; Swan, E. P., 1975: Heartwood extractives of a western larch tree *(Larix occidentalis* Nutt.). Wood and Fiber, 7 (3): 216-221.
6. Hawley, L. F.; Fleck, L. C.; Richard, C. A., 1924: The relation between natural durability and chemical composition in wood. Ind. Eng. Chem., 16: 699-706 http://dx.doi.org/10.1021/ie50175a015.
7. Krutov, S. M.; Kovolev, V. E.; Roshchin, V. I., 1988: Wood extractives. 5th European Workshop on Lignocellulosics and Pulp, Aveiro, Aug 30 - Sept 2, pp. 245-248.
8. Mills, J. S., 1973: Diterpenes of *Larix* oleoresins. Phytochemistry, 12: 2407-2412 http://dx.doi.org/10.1016/0031-9422(73)80447-9.
9. Nair, G. V.; Von Rudloff, E., 1959: Chemical composition of the heartwood extractives of tamarack (*Larix laricina*). Canadian Journal of Chemistry, 37: 1708-1713 http://dx.doi.org/10.1139/v59-232.

10. Nair, G. V.; Von Rudloff, E., 1960: Chemical composition of the heartwood extractives of *Larix lyallii*. Canadian Journal of Chemistry, 38: 177-181 http://dx.doi.org/10.1139/v60-023.
11. Norin, T.; Oloff, G.; Willhelm, B., 1965: The structure and configurations of larixol and larixyl acetate. Tetrahedron Lett., 39: 3523-3528 http://dx.doi.org/10.1016/S0040-4039(01)89336-9.
12. Ohtsu, H.; Tanaka, R.; Matsunaga, S., 1998a: 18-nor-Abietatrienes from the cones of *Larix kaempferi.* Journal of Natural Products, 61 (3): 406-408 http://dx.doi.org/10.1021/np9704720.
13. Ohtsu, H.; Tanaka, R.; Matsunaga, S., 1998b: Abietane diterpenoids from the cones of *Larix kaempferi*. Journal of Natural Products, 61 (10): 1307-1309 http://dx.doi.org/10.1021/np980159d.
14. Ohtsu, H.; Tanaka, R.; Michida, T.; Shingu, T.; Matsunaga, S., 1998c: Tetracyclic triterpenes and other constituents from the leaves and bark of *Larix kaempferi.* Phytochemistry, 49 (6): 1761-1768 http://dx.doi.org/10.1016/S0031-9422(98)00302-1.
15. Scheffer, T. C.; Cowling, E. B., 1966: Natural resistance to microbial deterioration. Annual Review of Phytopathology, 4: 147-170 http://dx.doi.org/10.1146/annurev.py.04.090166.001051.
16. Schultz, T. P.; Hubbard, T. F.; Fisher, J. L.; Nicholas, D. D., 1990: Role of stilbenes in the natural durability of wood: fungicidal structure-activity relationships. Phytochemistry, 29: 1501-1507 http://dx.doi.org/10.1016/0031-9422(90)80109-T.
17. Schultz, T. P., Harms, W. B.; Fisher, T. H.; McMurtrey, K. D.; Minn, J.; Nicholas, D. D., 1995: Durability of angiosperm heartwood: the importance of extractives. Holzforschung, 49: 29-34 http://dx.doi.org/10.1515/hfsg.1995.49.1.29.
18. Staccioli, G.; Sturaro, A.; Parvoli, G., 1997: GC/MS investigation on the dichloromethane extract from subfossil larch. Holzforschung, 51 (2): 107-110 http://dx.doi.org/10.1515/hfsg1997.51.2.107.
19. Viitanen, H.; Paajanen, L.; Saranpää, P.; Viitaniemi, P., 1997: Durability of larch (*Larix* spp) wood against brown-rot fungi. 28th Annual Meeting of the IRG, Whistler, BC, Canada, 26-30 May, 1-8.
20. Willför, S., 2007: Modern analytical tools for pulp and paper, Turku, 5-9 Nov., COST E41 Course.
21. Xue, J. J.; Fan, C. Q.; Dong, L.; Yang, S. P.; Yue, J. M., 2004: Novel antibacterial diterpenoids from *Larix chinensis* Beissn. Chemistry & Biodiversity, 1: 1702-1707 http://dx.doi.org/10.1002/cbdv.200490128.

Corresponding address:

JANJA ZULE, Ph.D.

Pulp and Paper Institute
Bogišičeva 8
SI-1000 Ljubljana, SLOVENIA
e-mail: janja.zule@icp-lj.si