Methylated Fatty Acids from Heartwood and Bark of *Pinus sylvestris*, *Abies alba*, *Picea abies*, and *Larix decidua*: Effect of Strong Acid Treatment

Mohamed Z. M. Salem,[a,*] Ramadan A. Nasser,[a,b] Aleš Zeidler,[c] Hosam O. Elansary,[d,e] Ibrahim M. Aref,[b] Martin Böhm,[f] Hayssam M. Ali,[g,h] and Abdalla I. Ahmed [b]

Methylated fatty acid (FA) compounds in the heartwood and bark of some softwood species, specifically *Pinus sylvestris*, *Abies alba*, *Picea abies*, and *Larix decidua*, grown in the Czech Republic were evaluated. Strong H_2SO_4 was used for methylation of the lipids. The highest content of lipid was found in *P. abies* bark (40.132 mg/g o.d. sample), and the lowest content was in *A. alba* wood (11.027 mg/g o.d. sample). The highest concentration of FAs was observed in *L. decidua* bark. The highest percentages of FAs in wood of *P. sylvestris* were arachidic acid and oleic acid. In bark, the highest percentages of FAs were stearic acid, palmitic acid, and oleic acid. The FAs with the highest concentrations in *A. alba* wood were arachidic acid, palmitic acid, pentadecanoic acid, and margarinic, and those in bark were behenic acid, lignoceric acid, and arachidic acid. *P. abies* wood FAs showed arachidic acid, palmitic acid, and margarinic acid, and the bark contained lignoceric acid and arachidic acid. The FAs of *L. decidua* wood were arachidic acid, palmitic acid, and stearic acid, and in bark they were pentacosylic acid, docosahexaenoic acid (DHA), lignoceric acid, arachidic acid, and behenic acid. The lack of typically dominant unsaturated fatty acids (*e.g.* 18:1, 18:2), compared to literature values were attributed to the application of strong acid for the hydrolysis.

Keywords: Chemical composition; Fatty acids; Strong acid; Wood; Bark; Pinus sylvestris; Abies alba; Picea abies; Larix decidua

*Contact information: a: Forestry and Wood Technology Department, Faculty of Agriculture (EL–Shatby), Alexandria University, Egypt; b: Range and Forestry Applied Research Unit, Plant Production Dept., Food and Agricultural Sciences Collage, King Saud University, Saudi Arabia; c: Department of Wood Processing, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic; d: Department of Floriculture, Ornamental Horticulture and Garden Design, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt; e: Biodiversity Institute of Ontario, University of Guelph, Guelph, ON, Canada; f: Department of Wood Products and Wood Constructions, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic; g: Timber Trees Research Department, Sabahia Horticulture Research Station, Horticulture Research Institute, Agriculture Research Center; Alexandria, Egypt; h: Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia;*Corresponding author: zidan_forest@yahoo.com*

INTRODUCTION

Biomass, mainly wood, is a great source for phytochemicals, which are useful in many areas of industries. The chemical composition of various softwood species has been the subject of numerous studies focused on North American and Central European species (Kubeczka and Schultze 1987; Sjödin *et al.* 1993; Hennig *et al.* 1994). Softwood species including pines (*Pinus*), spruces (*Picea*), larches (*Larix*), and *Pseudotsuga* are

prolific resin producers and have well-established systems of horizontal and vertical ducts filled with resin (Parham and Gray 1984; Simoneit *et al.* 2000). The Pinaceae family contains 12 genera: *Abies*, *Cathaya*, *Cedrus*, *Keteleeria*, *Larix*, *Nothotsuga*, *Picea*, *Pinus*, *Pseudolarix*, *Pseudotsuga*, *Tsuga*, and *Hesperopeuce;* where *Pinus* is the largest, *Abies* ranked second, and *Picea* third (Page 1990; Wolff *et al.* 2001, 2002).

Fatty acids (FAs) grouped into saturated and unsaturated forms have been studied in many published works. The FAs and sterols, in contrast, are nutrients for attacking fungi; therefore, they reduce the durability of the wood (Fries *et al.* 2000). However, in the study of Salem *et al.* (2014a,b), some antifungal properties were found for the FAs. Additionally, the unsaturated FAs with additional reactions can form saturated FAs, which should decrease the relative amount of unsaturated FAs (Sjöström 1981).

Conifers are known for the presence of unusual FAs with the first site of unsaturation at the fifth carbon atom (Δ5-UPIFAs) and also for *cis* (Z) configuration, such as *cis*-5,9-octadecadienoic (taxoleic), *cis*-5,9,12-octadecatrienoic (pinolenic), *cis*-5,11-octadecadienoic (ephedrenic), *cis*-5,11-eicosadienoic (keteleeronic), *cis*-5,11,14-eicosatrienoic (sciadonic), *cis*-5,9,12,15-eicosatetraenoic (coniferonic), and *cis*-5,11,14,17-eicosatetraenoic (juniperonic) acids (Takagi and Itabashi 1982; Piispanen and Saranpää 2002; Wolff *et al.* 2002; Lísa *et al.* 2007).

It has been reported previously that fatty acids are partially deposited in the cell lumen and pit membranes, where they decrease the permeability of wood during heartwood formation (Saranpää 1990). Moreover, secondary substances synthesized during heartwood formation must originate primarily from stored carbohydrates (Fischer and Höll 1992). The FAs (oleic, linoleic, α-linolenic, Δ5-taxoleic, pinolenic, and coniferonic acid) are valuable components in the family Pinaceae (Bağci and Karaağaçli 2004). FAs such as *cis*-5,9-octadecadienoic (taxoleic), *cis*-5,9,12-octadecatrienoic (pinolenic), *cis*-5,11-eicosadienoic (keteleeronic), and *cis*-5,11,14-eicosatrienoic acids (sciadonic) can be found in the coniferous trees (Lísa *et al.* 2007).

The free FA concentrations were negligible in the outer sapwood of Scots pine (*Pinus sylvestris* L.), but ranged between 5 and 18 mg/g (dm) in the heartwood (Piispanen and Saranpää 2002). The dominant FAs in all fractions analyzed by gas-liquid chromatography (GLC) sapwood were 16:0, 18:0, 18:1, and 18:2. 18:1 and 18:2 formed about 70% of the total triacylglycerol fatty acids (Saranpää and Nyberg 1987).

Most preceding studies have focused on specific components in the bark of Norway spruce (*Picea abies* (L.) H. Karst.), such as lipophilic extractives (Norin and Winell 1972; Ånäs *et al.* 1983). Larix species are widely distributed across Europe, North America, and Asia. The European Larch (*Larix decidua* Mill.) can grow up to 35 m high and is native to the higher regions of the Alps, Sudetes, and Carpathian mountains. Silver fir (*Abies alba* (Mill.)) is the most common tree species in Central Europe; therefore, it is important in wood production and is also very economically and environmentally significant (Ficko *et al.* 2011).

Most studies on the FA concentrations of softwood species have focused on the presence of FAs in seeds and leaves. Also, more details about the distribution and identification of FA composition of Pinaceae as taxonomic markers were reviewed by Wolff *et al.* (2001). As part of an extensive phytochemical analysis of some softwood species growing in the Czech Republic, the present work reports the authors' first investigation toward the chemical constituents of the methylated fatty acids obtained from the heartwood and bark of *Pinus sylvestris*, *Abies alba*, *Picea abies*, and *Larix decidua* as affected by strong acidic hydrolysis.

EXPERIMENTAL

Materials

Preparation of heartwood and bark samples

Freshly cut wood discs containing bark samples of Scots pine (*Pinus sylvestris* L.), European silver fir (*Abies alba* Mill.), Norway spruce (*Picea abies* (L.) H. Karst.), and European Larch (*Larix decidua* Mill. subsp. *decidua*), used in the present study were obtained at the end of 2014, by the Department of Wood Processing, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences. The ages of the trees were 33, 34, 28, and 41 years for *P. sylvestris*, *A. alba*, *P. abies*, and *L. decidua*, respectively. Then, the discs were air-dried under shade at room temperature at the Laboratory of Wood Technology (Forestry and Wood Technology Department, Faculty of Agriculture (EL–Shatby, Alexandria University, Egypt). Heartwood samples were prepared with a particle size of 0.4 to 0.6 mm. Bark samples had a particle size of 0.1 to 0.2 mm.

Methods

Lipid extraction

Ten grams from each source of heartwood and bark were weighed out into a conical flask containing 10 mL of concentrated HCl and then boiled in a water bath until the entire sample had been dissolved. Then, 30 mL of diethyl ether was added to the solution to extract the fats through shaking. The extract was collected into a weighed flask after allowing the layers to separate. The extraction was repeated three more times and the solvent was distilled off. Then the fats were dried at 100 °C, cooled, and weighed using a balance (Kirk and Sawyer 1991). The lipid weighted based on one gram oven dry weight of the sample (mg lipid/gram o.d. material).

Methylation of lipid

Methylation of the lipids was carried out by weighing a sample of 50 mg of lipid from each source in a tube. The following chemicals were then added to each tube; 50 mL of the mixture from 1 mL concentrated sulfuric acid, 100 mL of methanol, and 2 mL of benzene. After the tube was sealed completely, the samples were placed in a water bath at 90 °C for 90 min. The tube was then cooled and 8 mL of water with 5 mL of petroleum ether was added. Then, the tube was thoroughly shaken and the ethereal layer was separated out and evaporated (Agoramoorthy *et al.* 2007). After methylation of the fatty acids (FAs), they were subjected to gas chromatography (GC) analysis.

Table 1. GC Condition for Analysis of Methylated Fatty Acids*

Device model	HP (Hewlett Packard) 6890 GC.		
Column	HP-5 (5% diphenyl, 95% dimethyl polysiloxane), 30 m, 0.32 mm. ID, 0.25 µm film thickness.		
Carrier gas/gas flow	Nitrogen/1 mL/min.		
Detector/temperature	FID (Flame Ionization Detector)/250 °C.		
Injector temperature, Injection volume	220 °C, 2 µL in a splitless mode.		
Oven program	Initial Temp. 150 °C for 2 min.		
Ramps	Rate °C/min	Final Temp. °C	Hold time
1	10	200	-
2	5	250	9 min

* Conditions used previously by Salem *et al.* (2014)

The compositions of the methylated FAs were identified by GC analysis using a HP (Hewlett Packard 6890 GC, USA) GC analyzer as shown in Table 1. This was done by matching their retention times with standard FAs (C_4-C_{25}) chromatographed under the same conditions (Mohamed and Awatif 1998).

Statistical Analysis

Analysis of variance (ANOVA) procedure (SAS version 8.2, 2001, USA) in a completely randomized design (CRD) was used to study the statistical differences among the studied parts of the tree species for the lipids and FAs concentrations. Fisher's least significant difference (LSD) at a 5% level of probability was used to measure the differences among the mean values.

RESULTS AND DISCUSSION

Lipid and Fatty Acids Concentrations

Table 2 presents the concentration of lipids and fatty acids (FAs) found in the heartwood and bark of *P. sylvestris, A. alba, P. abies*, and *L. decidua*. Among the studied species, the highest content of lipid was found in the *P. abies* bark (40.132 mg/g o.d. sample), followed by *P. sylvestris* heartwood (31.649 mg/g o.d. sample), and the lowest content was present in *L. decidua* heartwood (11.548 mg/g o.d. sample) as well as in *A. alba* heartwood (11.027 mg/g o.d. sample). On the other hand, the highest concentration of FAs was observed in *L. decidua* bark (175.03 mg/g of lipid) followed by *A. alba* bark (155.695 mg/g of lipid). The lowest concentration observed in heartwood and bark of *P. sylvestris* was 7.153 and 6.968 mg/g of lipid, respectively. In other studies, the concentrations of fats were 10 times higher in Scots pine than in Norway spruce, grown in Finland, and the sapwood contained more lipophilic extractives than heartwood (Ekman 1979).

Table 2. Concentration of Lipid and Fatty Acids in Heartwood and Bark of the Studied Softwood Species

Tree species	Tree Part	lipid (mg/g o.d. sample)	FA con. (mg/g lipid)
Pinus sylvestris	Heartwood	31.649b*	7.153e*
	bark	14.115d	6.968e
Abies alba	Heartwood	11.027e	17.478d
	bark	25.733c	155.695b
Picea abies	Heartwood	12.810e	18.661d
	bark	40.132a	80.624c
Larix decidua	Heartwood	11.548e	19.586d
	bark	27.769c	175.030a

* Means with the same letters within the same column are not significantly different according to $LSD_{0.05}$

Also, large differences in the FA compositions of different lipid classes in *P. sylvestris* and *P. abies* woods have been demonstrated previously (Assarsson and Åkerlund 1967). Other works reported that the concentrations of neutral lipids and free FAs in the wood of Norway spruce were lower than the concentrations reported for Scots pine (Fischer and Höll 1992; Saranpää and Piispanen 1994).

The variation in the concentration between previous and present works could be related to reasons including that the amount of live cells in the xylem of fertilized trees was higher than in non-treated control trees, a high growth rate, tree vigor, nitrogen concentration in the stem wood, the carbon/nutrient balance hypothesis, and geographic and environmental locations (Kramer and Kozlowski 1979; Bryant *et al.* 1983; Stockfors and Linder 1998).

Methylated Fatty Acids in Heartwood and Bark of *P. sylvestris*, *A. alba*, *P. abies*, and *L. decidua*

Table 3 summarizes the methylated FAs and their concentration reported in the heartwood and bark of *P. sylvestris, A. alba, P. abies,* and *L. decidua*.

Fatty Acids in *Pinus sylvestris* Heartwood and Bark

The highest percentages of FAs in the heartwood of *P. sylvestris* were arachidic acid (55.100%), oleic acid (10.51%), tricosylic acid (9.862%), palmitic acid (8.816%), stearic acid (6.784%), linoleic acid (4.014%), and pentadecanoic acid (4.125%). The bark contained the following FAs; stearic acid (54.112%), palmitic acid (16.292%), oleic acid (14:500%), and pentadecanoic acid (7.973%).

According to the study of Saranpää and Nyberg (1987), the resin acids and FAs occur at low concentrations in the sapwood of *P. sylvestris*. In previous published works, the most abundant FAs of triacylglycerols were found to be oleic (18:1), linoleic (18:2ω6, 18:2Δ5,9), linolenic (pinolenic, 18:3Δ5,9,12 and 18:3ω3), and eicosatrienoic acid (20:3Δ5,11,14 and 20:3ω6), also, the concentration of linoleic acid comprised 39% to 46% of the triacylglycerol fatty acids (Piispanen and Saranpää 2002) of *P. sylvestris* grown in Northern and Southern Finland. It was reported that the free FAs in heartwood are formed by the hydrolysis of sapwood triacylglycerols of Scots pine (Saranpää and Nyberg 1987). In another study, oleic and linoleic acid together comprised about 70% of the total triacylglycerol FAs, with linoleic acid making the major contribution of 39% to 46% (Saranpää and Nyberg 1987).

Fischer and Höll (1992) reported that oleic acid is the principal FA of triacylglycerols in stems of Scots pine trees grown in southern Germany. Sapwood fractions of Scots pine analyzed by GC observed the most important FAs as being 16:0 (palmitic acid), 18:0 (stearic acid), 18:1 (oleic acid), 18:2 (linoleic acid, the dominant fatty acid in all fractions), 18:3, and 20:3 (Saranpää and Nyberg 1987). In contrast, noticeable amounts of free FAs were present only in the heartwood.

Fatty acids in *Abies alba* Heartwood and Bark

The heartwood of *A. alba* was found to contain the following FAs: arachidic acid (37.417%), palmitic acid (22.019%), pentadecanoic acid (15.139%), margarinic acid (12.234%), stearic acid (5.831%), myristic acid (5.104%), and erucic acid (cis-13) (2.137%). The bark was found to contain behenic acid (41.777%), lignoceric acid (21.086%), arachidic acid (18.016%), tricosylic acid (4.522%), stearic acid (3.488%), heptadecenoic acid (2.548%), undecanoic acid (2.204%), and cis-10-pentadecenoic acid (2.066%).

Table 3. Concentration of the Identified Methyl Ester of Fatty Acids

FA		Relative percentage (%)							
		Pinus sylvestris		***Abies alba***		***Picea abies***		***Larix decidua***	
No. of Carbon atom	Common name	Heart-wood	Bark	Heart-wood	Bark	Heart-wood	Bark	Heart-wood	Bark
C10:0	Capric acid	0.690	-	-	0.028	-	0.006	-	-
C11:0	Undecanoic acid	-	-	-	2.204	-	0.559	-	-
C12:0	Lauric acid	-	-	-	-	-	0.224	-	-
C14:1	Tetradecenoic acid	-	-	-	-	-	-	-	0.559
C14:0	Myristic acid	-	-	5.104	-	-	2.302	-	0.554
C15:1	cis-10-Pentadecenoic acid	-	-	-	2.066	-	-	-	-
C15:0	Pentadecanoic acid	4.125	7.973	15.139	-	6.147	2.424	5.268	1.168
C16:1ω7	9-hexadecenoic acid	-	-	-	1.395	-	-	-	-
C16:0	Palmitic acid	8.816	16.292	22.019	-	11.197	2.629	9.340	2.441
C17:1	Heptadecenoic acid	-	-	-	2.548	-	-	-	-
C17:0	Margarinic acid	-	-	12.234	-	8.135	0.480	6.061	1.797
C18:1	Oleic acid	10.51	14:500	-	-	-	-	-	-
C18:2ω6	linoleic acid	4.014	10.851	-	-	-	0.323	-	-
C18:0	Stearic acid	6.784	54.112	5.831	3.488	4.693	2.742	6.974	2.414
C20:5ω3	Cis-5,8,11,14,17-Eicosapentaenoic acid (EPA)	-	-	-	-	-	3.633	-	6.753
C20:0	Arachidic acid	55.100	-	37.417	18.016	58.319	24.809	66.744	13.347
C22:1	erucic acid (*cis*-13)	-	-	2.137	-	-	1.460	-	-
C22:6ω3	Docosahexaenoic acid (DHA)	-	-	-	-	-	-	-	21.943
C22:2	Cis-13,16-docosadienoic acid	-	-	-	-	-	3.051	-	0.603
C22:0	Behenic acid	-	0.272	-	41.777	-	3.001	-	11.169
C23:0	Tricosylic acid	9.862	-	-	4.522	-	-	-	-
C24:0	Lignoceric acid	-	-	-	21.086	-	29.513	-	14.413
C25:0	Pentacosylic acid	-	-	-	-	-	17.586	-	22.759

Fatty Acids in *Picea abies* Heartwood and Bark

GC analysis of methylated FAs from heartwood of *P. abies* reported the following main FAs compounds: arachidic acid (58.319%), palmitic acid (11.197%), margarinic acid (8.135%), pentadecanoic acid (6.147%), and stearic acid (4.693%). In bark, the following FAs were found: lignoceric acid (29.513%), arachidic acid (24.809%), cis-13,16-docosadienoic acid (3.051%), pentacosylic acid (17.586%), cis-5,8,11,14,17-eicosapentaenoic acid (EPA) (3.633%), behenic acid (3.001%), stearic acid (2.742%), palmitic acid (2.629%), pentadecanoic acid (2.424%), and myristic acid (2.302%). FAs ranged from 1.5 to 0.27 mg/g of dry bark (Krogell *et al.* 2012) and from 0.009 to 0.011% DW of wood (Anttonen *et al.* 2002).

The free FAs have been reported as 0.6 to 1.3 mg/g of o.d. wood (Bertaud and Holmbom 2004). The spruce wood oleoresin also contains *n*-alkanes in homologous series ranging from C11 to C33, with C22 to C27 as the main constituents (Assarsson and Åkerlund 1967). In *P. abies,* pinolenic (Ekman 1980), taxoleic, coniferonic, sciadonic and juniperonic acids are found in many species of conifers (Jamieson and Reid 1972). Also, polyunsaturated acids (19:1, 5,9-19:2, 9,12-19:2, and 5,9,12-19:3 acids) have been detected in *P. abies* wood extracts (Ekman 1980).

Fatty Acids in *Larix decidua* Heartwood and Bark

The FAs components present in the heartwood of *L. decidua* were arachidic acid (66.744%), palmitic acid (9.340%), stearic acid (6.974%), margarinic acid (6.061%), and pentadecanoic acid (5.268%). The major FAs in bark were pentacosylic acid (22.759%), docosahexaenoic acid (DHA) (21.943%), lignoceric acid (14.413%), arachidic acid (13.347%), behenic acid (11.169%), cis-5,8,11,14,17-eicosapentaenoic acid (EPA) (6.753%), palmitic acid (2.441%), and stearic acid (2.414%).

All Δ5-UPIFAs (5,9-18:2 and 5,9,12-18:3 acids) have been characterized in the leaves of many *Picea* and *Larix* species (Jamieson and Reid 1972) and also in *P. abies* wood (Holmbom and Ekman 1978; Ekman 1980). It was reported that in most of the softwood species, the C18 Δ5-olefinic acids (5,9-18:2 and 5,9,12-18:3 acids) are present in considerably higher amounts than the C20 Δ5-olefinic acids (5,11-20:2 and 5,11,14-20:3 acids) (Wolff *et al.* 2001). Arachidonic acid was found in the cambium zone of *L. sibirica* (Groenewald and van der Westhuizen 1997), although thorough studies of *P. abies* wood extracts did not mention such an occurrence (Holmbom and Ekman 1978; Ekman 1980).

CONCLUSIONS

1. The highest percentages of FAs in the heartwood of *P. sylvestris* were arachidic acid, oleic acid, tricosylic acid, and palmitic acid. In bark, stearic acid, palmitic acid, and oleic acid were the most abundant.
2. The FAs most prominent in the heartwood of *A. alba* were arachidic acid, palmitic acid, pentadecanoic acid, and margarinic acid. In the bark of *A. alba*, the most prominent FAs presented were behenic acid, lignoceric acid, and arachidic acid.
3. The heartwood of *P. abies* exhibited the following main FA compounds: arachidic acid, palmitic acid, and margarinic acid. In the bark of *P. abies*, the main FA compounds seen were lignoceric acid and arachidic acid.
4. FAs in the heartwood of *L. decidua* were arachidic acid, palmitic acid, stearic acid, and margarinic acid. FAs in the bark of *L. decidua* were pentacosylic acid, docosahexaenoic acid (DHA), lignoceric acid, arachidic acid, behenic acid, and cis-5,8,11,14,17-eicosapentaenoic acid (EPA).
5. The present results indicated changes in the heartwood and bark FAs from *P. sylvestris*, *A. alba*, *P. abies*, and *L. decidua*. Some of these changes may influence the suitability of such wood for different end-use purposes.

6. The lack of typically dominant unsaturated fatty acids could be related to the use of strong acids in the present study.

ACKNOWLEDGMENTS

The authors would like to extend their sincerest appreciation to the Deanship of Scientific Research at King Saud University for the funding of this research through the Research Group Project No. RGP-VPP-226.

REFERENCES CITED

Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V., and Hsu, M. J. (2007). "Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India," *Braz. J. Microbiol.* 38(4), 739-742. DOI: 10.1590/S1517-83822007000400028

Ånäs, E., Ekman, R., and Holmbom, B. (1983). "Composition of nonpolar extractives in bark of Norway spruce and Scots pine," *J. Wood Chem. Technol.* 3(2), 119-130. DOI: 10.1080/02773818308085155

Anttonen, S., Manninen, A. M., Saranpää, P., Kainulainen, P., Linder, S., Vapaavuori, E. (2002). "Effects of long-term nutrient optimisation on stem wood chemistry in *Picea abies*," *Trees* 16(6), 386-394. DOI: 10.1007/s00468-002-0181-7

Assarsson, A., and Åkerlund, G. (1967). "Studies in wood resin, especially the change in chemical composition during seasoning of the wood. V. Changes in composition of non-volatile extractives during water seasoning of unbarked spruce, pine, birch, and aspen logs," *Svensk Papperstidn.* 70(6), 205-212.

Bağci, E., and Karaağaçli, Y. (2004). "Fatty acid and tocochromanol patterns of Turkish pines," *Acta Biol. Cracov. Bot.* 46, 95-100.

Bertaud, F., and Holmbom, B. (2004). "Chemical composition of earlywood and latewood in Norway spruce heartwood, sapwood and transition zone wood," *Wood Sci. Technol.* 38(4), 245-256. DOI: 10.1007/s00226-004-0241-9

Bryant, J. P., Chapin, F. S., and Klein, D. R. (1983). "Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory," *Oikos* 40(3), 357-368. DOI: 10.2307/3544308

Ekman, R. (1979). "Analysis of the nonvolatile extractives in Norway spruce sapwood and heartwood," *Acta Acad. Abo. (Ser. B)* 39(4), 1-20.

Ekman, R. (1980). "New polyenoic fatty acids in Norway spruce wood," *Phytochemistry* 19(1), 147-148. DOI: 10.1016/0031-9422(80)85035-7

Ficko, A., Poljanec, A., and Boncina, A. (2011). "Do changes in spatial distribution, structure and abundance of silver fir (*Abies alba* Mill.) indicate its decline?," *For. Ecol. Manage.* 261(4), 844-854. DOI: 10.1016/j.foreco.2010.12.014

Fischer, C., and Höll, W. (1992). "Food reserves in Scots pine (*Pinus sylvestris* L.). II. Seasonal changes and radial distribution of carbohydrate and fat reserves in pine wood," *Trees* 6(3), 147-155. DOI: 10.1007/BF00202430

Fries, A., Ericsson, T., and Gref, R. (2000). "High heritability of wood extractives in *Pinus sylvestris* progeny tests," *Can. J. For. Res.* 30(11), 1707-1713. DOI: 10.1139/x00-100

Groenewald, E. G., and van der Westhuizen, J. (1997). "Prostaglandins and related substances in plants," *Bot. Rev.* 63(3), 199-220. DOI: 10.1007/BF02857948

Hennig, P., Steinborn, A., and Engewald, W. (1994). "Investigation of the composition of *Pinus peuce* needle oil by GC-MS and GC-GC-MS," *Chromatographia* 38(11-12), 689-693. DOI: 10.1007/BF02269622

Holmbom, B., and Ekman, R. (1978). "Tall oil precursors of Scots pine and common spruce and their change during sulphate pulping," *Acta Acad. Abo. (Ser. B)* 38(3), 1-11.

Jamieson, G. R., and Reid, E. H. (1972). "The leaf lipids of some conifer species," *Phytochemistry* 11(1), 269-275. DOI: 10.1016/S0031-9422(00)90002-5

Lísa, M., Holčapek, M., Řezanka, T., Kabátová, N. (2007). "High-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry and gas chromatography–flame ionization detection characterization of Δ5-polyenoic fatty acids in triacylglycerols from conifer seed oils," *J. Chromatogr. A*, 1146 (1) 67-77. DOI: 10.1016/j.chroma.2007.01.122

Kramer, P.J., and Kozlowski, T.T. (1979). *Physiology of Woody Plants*, Academic Press, New York.

Kirk, R. S., and Sawyer, R. (1991). *Pearson's Chemical Analysis of Foods*, 9th Ed., Longman Scientific and Technical, Harlow, Essex, UK.

Krogell, J., Holmbom, B., Pranovich, A., Hemming, J., and Willför, S. (2012). "Extraction and chemical characterization of Norway spruce inner and outer bark," *Nord. Pulp Pap. Res. J.* 27(1), 6-17. DOI: 10.3183/NPPRJ-2012-27-01-p006-017

Kubeczka, K. H., and Schultze, W. (1987). "Biology and chemistry of conifer oils," *Flav. Fragr. J.* 2(4), 137-148. DOI: 10.1002/ffj.2730020402

Mohamed, H. M. A., and Awatif, I. I. (1998). "The use of sesame oil unsaponifiable matter as a natural antioxidant," *Food Chem.* 62(3), 269-276. DOI: 10.1016/S0308-8146(97)00193-3

Norin, T., and Winell, B. (1972). "Extractives from the bark of Common spruce, *Picea abies* L. Karst," *Acta Chem. Scand.* 26(6), 2289-2296. DOI: 10.3891/acta.chem.scand.26-2289

Page, C. N. (1990). "Gymnosperms: *Coniferophytina* (Conifers and Ginkgoids)," in: *The Families and Genera of Vascular Plants*, K. Kubitski (ed.), Vol. 1, *Pteridophytes and Gymnosperms*, K. U. Kramer and P. S. Green (eds.), pp. 319-329, Springer-Verlag, Berlin.

Parham, R. A., and Gray, R. L. (1984). "Formation and structure of wood," in: *The Chemistry of Solid Wood*, R. Rowell (ed.), American Chemical Society, Washington DC, pp. 3-56.

Piispanen, R., and Saranpää, P. (2002). "Neutral lipids and phospholipids in Scots pine (*Pinus sylvestris*) sapwood and heartwood," *Tree Physiol.* 22(9), 661-666.

Salem, M. Z. M., Abdel-Megeed, A., and Ali, H. M. (2014a). "Stem wood and bark extracts of *Delonix regia* (Boj. Ex. Hook): Chemical analysis and antibacterial, antifungal, and antioxidant properties," *BioResources* 9(2), 2382-2395. DOI: 10.15376/biores.9.2.2382-2395

Salem, M. Z. M., Ali, H. M., and Mansour, M. M. (2014b). "Fatty acid methyl esters from air-dried wood, bark, and leaves of *Brachychiton diversifolius* R. Br: Antibacterial, antifungal, and antioxidant activities," *BioResources* 9(3), 3835-3845. DOI: 10.15376/biores.9.3.3835-3845

Saranpää, P. (1990). "Heartwood formation in stems of *Pinus sylvestris* L.: Lipids and carbohydrates of sapwood and heartwood and ultrastructure of ray parenchyma cells," Publ. No.14, pp. 1-22, Department of Botany, University of Helsinki, Helsinki, Finland.

Saranpää, P., and Nyberg, H. (1987). "Lipids and sterols of *Pinus sylvestris* L. sapwood and heartwood," *Trees* 1(2), 82-87. DOI: 10.1007/BF00203575

Saranpää, P., and Piispanen, R. (1994). "Variation in the amount of triacylglycerols and steryl esters in the outer sapwood of *Pinus sylvestris* L.," *Trees* 8(5), 228-231. DOI: 10.1007/BF00196626

SAS (2001). "Users guide: Statistics (release 8.02)," SAS Inst., Cary, NC.

Simoneit, B. R. T., Rogge, W. F., Lang, Q., and Jaffé, R. (2000). "Molecular characterization of smoke from campfire burning of pine wood (*Pinus elliottii*)," *Chemosphere* 2(1), 107-122. DOI: 10.1016/S1465-9972(99)00048-3

Sjödin, K., Persson M., and Norin, T. (1993). "Enantiomeric compositions of monoterpene hydrocarbons in the wood of healthy and top-cut *Pinus sylvestris*," *Phytochemistry* 32(1), 53-56. DOI: 10.1016/0031-9422(92)80105-N

Sjöström, E. (1981). *Wood Chemistry, Fundamentals and Applications*, Academic Press, New York.

Stockfors, J., and Linder, S. (1998). "Effect of nitrogen on the seasonal course of growth and maintenance respiration in stems of Norway spruce trees," *Tree Physiol.* 18(3), 155-166. DOI: 10.1093/treephys/18.3.155

Takagi, T., and Itabashi, Y. (1982). "Cis-5-oliefinic unusual fatty acids in seed lipids of Gymnospermae and their distribution in triacylglycerols," *Lipids* 17(10), 716- 723. DOI: 10.1007/BF02534657

Wolff, R. L., Lavialle, O., Pédrono, F., Pasquier, E., Deluc, L. G., Marpeau, A. M., and Aitzetmüller, K. (2001). "Fatty acid composition of Pinaceae as taxonomic markers," *Lipids* 36(5), 439-451. DOI: 10.1007/s11745-001-0741-5

Wolff, R. L., Lavialle, O., Pédrono, F., Pasquier, E., Destaillats F., Marpeau, A. M., Angers, P., and Aitzetmüller, K. (2002). "Abietoid seed fatty acid compositions—A review of the genera *Abies, Cedrus, Hesperopeuce, Keteleeria, Pseudolarix*, and *Tsuga* and preliminary inferences on the taxonomy of Pinaceae," *Lipids* 37(1), 17-26. DOI: 10.1007/s11745-002-0859-5

Article submitted: May 20, 2015; Peer review completed: September 19, 2015; Revised version received and accepted: September 21, 2015; Published: September 30, 2015.
DOI: 10.15376/biores.10.4.7715-7724