

EXTRACTION OF PHENOLIC AND LIPOPHILIC COMPOUNDS FROM SPRUCE (*PICEA ABIES*) BARK USING ACCELERATED SOLVENT EXTRACTION BY ETHANOL

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ABSTRACT

The extractive substances from spruce bark (*Picea abies*) were studied by accelerated solvent extraction (ASE). ASE was carried out by ethanol and the temperature was 80, 120 and 160°C. Structural characterizations of the extractives were carried out by using FTIR and GC/MS analysis. It was found that with increasing temperature increased extractivity of substances in all evaluated samples. The FTIR analysis was used to identify the extracted substances. By GC/MS the main groups identified included fatty acids, sterols, waxes, sterols and steroids.

KEYWORDS: Accelerated solvent extraction, spruce bark, GC-MS.

INTRODUCTION

Biomass plays an important role in future energy infrastructure to produce electricity and heat, but also for the production of chemicals and fuels. Last but not least, it is a unique source of basic building raw materials which are used for production of various composite materials (paper, particle board, biocomposites, bioplastics and other) (Boerrigter and Rauch 2006). Due to increasing prices of raw materials, fuel and energy, it is necessary to turn our attention to finding new methods and technology involved to these individual substances. Biomass can be converted into a used diversified materials, biofuels and biochemicals especially by modernization of the existing biorefinery technologies and developing new procedures. Modern processes include fractionation of biomass processing, pressing or extraction, liquefaction, combustion, pyrolysis, hydrolysis, calcination, gasification, anaerobic digestion and fermentation (Ajuong and Breese 1998; Boeriu et al. 2004). Sources of biomass are wood, wood and vegetable waste, agricultural

and industrial residues, waste paper and municipal solid waste, bio-solids, waste from food processing, animal waste, aquatic plants, algae, and the like (Demirbas 2009a, b). In terms of the potential use of renewable raw material is therefore necessary to draw attention to the use of industrial tree species such as pine and spruce and the processing of waste (bark). A simple and clean fractionation of the main components of biomass represents a very important step in the "clean", renewable carbon economy. Fractionation on raw materials is an essential operation for almost all processes acquiring other products. If we can easily separate the different components, we gain a significant source of raw material (Ignat et al. 2013; Košíková et al. 2006; Spiridon et al. 1995). These can be further used as a starting material for new composites, but also value-added chemicals as well as pharmaceutical goods.

Spruce is widely used in the manufacture of pulp and paper. Pulp and paper industry produces annually a considerable amount of biomass waste. This waste (e.g. leaves, branches from mining) and bark removed from logs for pulp production is most often treated as an energy source (Domingues et al. 2009). The most suitable process is the isolation of substances with added value which are represented in this collection. Extractive differs in chemical structure, but also the physico-chemical and colloid - chemical properties. The amount and nature of the isolated compounds greatly depends on the isolation, for the isolation is possible to use different methods: Extraction in a Soxhlet apparatus (Garcia - Ayuso et al. 1998), Soxtec extraction, accelerated solvent extraction - ASE, ultrasound-assisted extraction (Junior et al. 2006), supercritical fluid extraction - SFE (Turner et al. 2002), pressurized liquid extraction - PLE (Giergielewicz-Mozajska et al. 2001) and microwave-assisted extraction - MAE (Lopez-Avila and Wilson 2000; Halko and Dutta 2007). In this study, therefore, extractive compounds from spruce bark treated in accelerated solvent extraction by ethanol were subsequently characterized by GC-MS analyses. In addition, infrared spectroscopic analysis was also conducted.

MATERIAL AND METHODS

Sampling methods

The dried samples (Spruce bark) were milled using a knife mill. For isolation of extractive compounds, the milled samples were extracted with ethanol in a Soxhlet apparatus for 8 h. And other extractions were used by accelerated solvent extraction by ethanol. Extractions were performed with the model 200 Accelerated Solvent Extractor, Dionex ASE 350. The extraction pressure (1500 psi) was imposed by the ASE 350 apparatus. Samples, typically 6 g (milled samples), were placed into stainless steel extraction chambers. Parameter of static time (time for reaching final temperature) was 5 min for 80°C, 6 min for 120°C and 8 min for 160°C. After static time sample was flushed with 50 % volume of used extraction ethanol in stainless steel cell and extract was collected in vial. Samples were sequentially extracted three times. Two replicate samples were used for each sample. All extracts were evaporated to dryness.

Micro FT-IR

Substances extracted from each sample dissolved in 5 ml of methanol, individually. On body-plate were loaded 5 layers onto each such solution (1 drop 5 µL). Between the depositions of each layer, the solution is allowed to dry on a body-plate. Measurements of sample spectra were performed using an FT-IR microscope (UMA 600 series IR microscope interfaced with a FTS Excalibur 30 MX FTIR spectrometer). The microscope was equipped with a motorized x5y stage, a 4x and 16x objective, a broadband mercury cadmium telluride detector, and slide-

on attenuated total reflection (ATR) germanium objective (Varian 600 UMA, Palo Alto, CA). Spectra were collected from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} co-adding 100 scans to improve the signal-to-noise ratio. The spectrometer was controlled using Win-IR Pro control software. Spectra were displayed in terms of absorbance obtained by rationing the single beam spectrum against that of the air background. All recorded spectra were mathematically converted to Kubelka – Munk intensity. Two replicate spectra were collected for each sample.

Derivatization

From the published derivatization techniques is shown as the preferred alkylation (methylation) with DMF-DMA (N, N-dimethylformamide dimethyl acetal). As solvent for derivatization was used pyridine. For derivatization of 50 mg dried sample was used a mixture (1:1) 0.5 ml pyridine and 0.5 mL of DMF-DMA. Derivatization was carried out at 75°C and the time was 15 min.

GC MS analysis

The GC/MS analysis was performed on a gas chromatograph (Agilent 7890 GC) coupled with mass detector (Agilent 5975C) which ran electron ionization equipped with a capillary column (HP-5MS, 30 m \times 250 μm i.d., 0.25 μm film thickness; Agilent). Helium was used as carrier gas at a rate of 2 mL/min. Chromatograph oven temperature program was 120°C held for 2 min, then the heating of 10°C.min⁻¹ to 300°C. The final temperature was held for 10 min. Recording and evaluation of data was performed by using ChemStation software E 02/01/1177 and identification of compounds using electronic libraries NIST and Wiley.

RESULTS AND DISCUSSION

The usage of ASE is one of important method for the obtaining lipophilic compounds from lignocellulosic materials and forest waste.

Tab. 1: Yield of extraction compounds with bark of *Picea abies* obtaining by ASE and Soxhlet extraction.

Methods	ASE			Soxhlet ex.
Temperature (°C)	80	120	160	~78
Total time (min.)	35	36	38	540
Yield (%)	7.5±0.2	10.5±0.3	13.8±0.2	10.2±0.2

Extractives obtained by temperature 80°C from spruce bark was 7.5±0.2 %, what is 1.4 times less than by temperature 120°C (10.5±0.3 %). Yield extracted by temperature 120°C is 1.3 times lower than by 160°C, with extraction efficiency 13.8±0.2 %. Besides ASE was spruce bark also extracted with Soxhlet apparatus (Tab. 1). Yield by this method was 10.2±0.2 %, what is similar with ASE by temperature 120°C (10.5±0.3 %). Significant difference between these two methods was in time of extraction, by ASE it was 36 min (15 times lower consumption of time) and by Soxhlet 540 min. From this information, it is clearly possible to conclude that ASE is due to elevated temperature and pressure, more efficient and faster than Soxhlet extraction method.

ATR/FTIR or DRIFT (Hoang et al. 2005; Boeriu et al. 2004; Rodrigues et al. 2001) spectra is a useful technique for studying wood/pulp decay chemistry as well as an analytical technique for estimation of the chemical composition and functional properties of lignin and polysaccharide/pulp (Jablonský and Kaziková 2011) and extractive compounds (Ajuong and

Breese 1998). Extracts of the samples were subjected to infrared spectroscopic analysis for the identification of different types of compounds that might be found in the extracts according to previous publications (Ajuong and Breese 1998; Che-Man and Setiowaty 1999; Rodrigues et al. 2001).

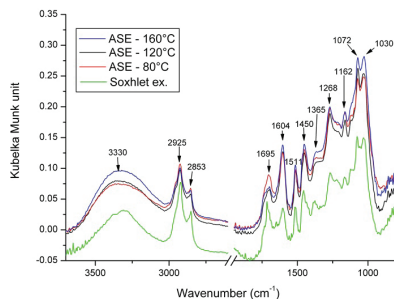


Fig. 1: Infrared spectra of bark extracts from *Picea abies* (ASE 80°C, 120°C, 160°C and Soxhlet ex.) in the range from 3800 to 800 cm^{-1} .

Peaks in the ~ 3330 , 2925, 2853, 1714, 1695, 1604 1511, 1450, 1365, 1268, 1162 and 1030 cm^{-1} regions were evident in the IR profile of the extracts of bark; the peaks at ~ 3300 , 2925, ~ 2853 , 1072 and 1030 cm^{-1} , were generally the highest (Fig. 1). A broad band at $\sim 3300 \text{ cm}^{-1}$ is assigned to the hydroxyl group stretching vibration. These strong intermolecular bonded hydroxyl stretch band is assigned to stretching vibration in sterols, mono- and diglycerides or co-extracted polysaccharides (Sun and Sun 2001; Ajuong and Breese 1998; Che-Man and Setiowaty 1999). The presence of bands in the ~ 2925 and 2853 cm^{-1} regions indicates the methylene and methyl stretching frequencies, respectively. The affected regions $\sim 1268 - 950 \text{ cm}^{-1}$ in the spectrum of extracts include those bands that are chiefly allocated to co-extracted polysaccharides. In the “finger print region” of the extract spectrum an aryl alkyl ether symmetrical stretching band is recorded at wavenumber $\sim 1072 \text{ cm}^{-1}$ and the strong, sharp absorptions appearing at $\sim 1030 \text{ cm}^{-1}$ are caused by C-O single bonded stretching in the acetates of primary alcohols. The strip recorded at 1162 cm^{-1} emanates probably from carbon to oxygen single bonded stretching in saturated esters.

There are several papers in which the authors deal with obtaining compounds from spruce bark. In work Ház et al. was used supercritical CO₂ extraction with ethanol (Ház et al. 2013). Resin acids, fatty acids, terpenes, terpenoid alcohols and waxes have been identified. Paper of Co et al. (2011) was aimed for obtaining antioxidants from spruce bark extracts. Here were identified compounds such as stilbene glucosides, astringin (as stilbenoid), piceid and isorhapontin. Kylliäinen and Holmbom (2004) studied aqueous extracts of the bark of *Picea abies*. In this work saccharides (mono-, di-, and polysaccharides such as e.g. glucose, galactose, galacturonic acid), stilbenes, stilbene glycosides, tannins and resin acids were determined. Ajuong and Berkinshaw (2004) confirmed that the ethanol extract of spruce chips contains condensed tannins, flavonoids, and phenolic compounds. In Tab. 2 can be seen semi-quantitative comparison of data which represented substances in samples. It was confirmed wider representation of substances at higher temperatures compared to most likely dropped temperatures. This parameter is therefore selective in the case of the acquisition of certain species / groups of substances, which represents valuable information for targeted extraction of substances that are released at lower temperatures or in the case of obtaining the widest composition by ASE at temperature 160°C. Composition of lipophilic extracts was confirmed in other experiments by different types of extraction (Patinha et

al. 2013; Patel et al. 2011; Feng et al. 2013). The analysis of the compounds extracted from spruce bark points out wide group of substances such as fatty acids, aliphatic hydrocarbons, mono-, di-, tri-, sesqui-, terpenes, sterols and steroids (Tab. 2). The most significant representation in terms of quantity is methyl dehydroabietate, and other derivatives of abietic acid, terpenes, fatty acids and waxes which also support the work (Holser and Akin 2008).

Tab. 2: Composition of extractive compounds (%) from *Picea abies* bark.

Composition of various extracts (%)				Compounds
ASE			Soxhlet ex.	
80°C	120°C	160°C		
8.03		2.53	3.81	2,2-Dimethoxyethanol
0.95	0.76	0.37	0.55	1-Methyl-1H-pyrrole-2-carbaldehyde
		0.17		2,5-Bornanedione
		0.24		(4-Isopropenyl-1-cyclohexen-1-yl)methano
			0.30	Methyl 4-(prop-1-en-2-yl)cyclohex-1-enecarboxylate
		0.19		2,3,4,5-Tetramethyl-2-cyclopenten-1-one
	1.45	1.80	1.81	Methyl hexadecanoate
		0.89	0.66	Methyl 14-methylhexadecanoate
	0.43			Methyl isoheptadecanoate
		0.22		Methyl heptadecanoate
		0.10		Heptamethyl-3-phenyl-1,4-cyclohexadiene
			0.36	Methyl dodecanoate
		0.83	0.78	Methyl 5,9,12-octadecatrienoate
	0.34			4-Methyltricyclo[5.2.1.0 _{2,6}]decane
		0.43	0.44	Methyl 5,9-octadecadienoate
1.75	1.86	2.72	2.54	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
8.18	7.86			11-Octadecenoic acid, methyl ester
	0.97	7.84		7-Octadecenoic acid, methyl ester
		1.23	7.70	Methyl (9E)-9-octadecenoate
1.29			1.30	cis-13-Octadecenoic acid, methyl ester
	0.30	0.55	0.58	Methyl n-octadecanoate (Methyl stearate)
		0.25		Ethyl oleate
		0.21	0.40	Methyl abieta-8,13-dien-18-oate
			0.35	Methyl abieta-8,11,13-trien-18-oate
4.43	3.40	4.62	4.47	Methyl (13 α)-pimara-8(14),15-dien-18-oate
		0.22		Abieta-8,11,13-trien-18-al
9.89	9.34	7.78	7.50	Methyl pimara-7,15-dien-18-oate
		1.24	1.18	Methyl 18-methylnonadecanoate
0.75	0.66			Methyl icosanoate
1.88	1.91	1.94	2.79	Methyl abieta-6,8,11,13-tetraen-18-oate
39.19	36.32	27.57	27.48	Methyl abieta-8,11,13-trien-18-oate (Methyl dehydroabietate)
0.79	0.83	0.97	0.91	Methyl abieta-7,13-dien-18-oate (Methyl abietate)
2.61	2.68	2.35	2.81	1-Amino-2-methyl-9,10-anthraquinone
1.60	1.58	2.84	2.03	Methyl docosanoate

3.06	3.28	3.06	3.13	7-Oxodehydroabietic acid, methyl ester
		0.52		Chol-7-en-12-ol, (5.beta.,12.alpha.)-
			0.76	2,2'-(Alpha-methylbenzylidene)bis(5-methylfuran)
0.70	0.91	1.75	1.53	Tetracosanoic acid, methyl ester
			0.26	trans-4-Oxo-2-pentenoic acid
		0.21		Dienestrol
		0.34		Methyl hexacosanoate
		0.60		(3 β)-24-Oxocholest-5-en-3-yl acetate
		0.53		(3 β ,22E)-Ergosta-4,6,22-trien-3-ol
1.86	2.01	2.14	2.13	Stigmastan-3,5-diene
		0.49		gamma-Sitosterol
		0.23		(3 β)-Stigmast-5-en-3-yl acetate
		0.47		Stigmasta-3,5-dien-7-one
0.92	1.42	4.53	4.49	Other
12.12	21.71	15.04	16.97	Unknown

CONCLUSIONS

We have reported the chemical composition of the lipophilic part of extractives present in *Picea abies* bark via an accelerated solvent extraction at 80, 120, 160°C and Soxhlet extraction. The knowledge of the chemical composition of extractives components of *Picea abies* bark is important for prediction and controlling pitch problems during pulping and bleaching. Extraction process and temperature have a major effect on the extraction yield and the composition of the extracts. The main identified compounds in bark were methyl dehydroabietate and other derivatives of abietic acid, terpenes, fatty acids and waxes in terms of quantity. Information about chemical composition of bark extractives will be useful for a better utilization of this interesting plant sources. The bark could represent a potential raw material for producing valuable chemicals.

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