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THE CONTENT OF BIOACTIVE COMPOUNDS IN CHIPS FROM THE WOOD PROCESSING LINE

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Abstract

Industrial wood waste is the effect of mechanical treatment of wood and wood products. This group of wastes includes bark, sawdust, wood chips, sawmill dust, dust and chips Analyses were conducted on four sterols: desmosterol, campesterol, stigmasterol, β-sitosterol and organic acids (malonic, citric, quinic, succinic, lactic, maleic, fumaric, oxalic) using UPLC in bark of three species of deciduous trees (oak, beech, hornbeam) and two conifers (spruce, pine). In the bark of deciduous trees these sterols were not found. Deciduous trees are poorer in organic acids than in coniferous ones. The spruce contained all the acids determined in the experiment The bark of the oak contained mainly citric acid and small amounts of fumaric and oxalic acid

Key words: *bark, sterols, organic acids, UPLC analysis, woodworking*

INTRODUCTION

Industrial wood waste is the effect of mechanical treatment of wood and wood products. This group of wastes includes bark, sawdust, wood chips, sawmill dust, dust and chips.

The amount of waste generated during machining, ranging from raw material abrasion to finished product is very high and in many cases exceeds the mass of finished product elements. That is why some of the wood waste from the wood industry companies is used for the production of board materials, and the rest is used in agriculture and horticulture and as fuel for energy purposes. Full use of this industrial raw material allows manufacturing plants to produce products with high technical advantages, used in many areas of everyday life [1]. Waste management is an important element of the wood industry. Ensuring their safety elimination or deactivation of chemical and microbiological factors is a necessary condition that must be met in order for the waste material to be used. One of the most commonly used methods is drying wood in order to obtaining semi-products and waste.

It consists in reducing the humidity to the level of hygroscopic equilibrium with ambient conditions corresponding to the environment in which the products made from this raw material will be used. An additional purpose of drying is to get rid of, among others, fungi and their spores, which can settle dead wood, leading to its degradation. The effect of high temperature also causes the deactivation of biologically active compounds of wood among them enzymes and the breakdown of high molecular weight antioxidant compounds. Literature studies show that the main residues after the wood drying process are low molecular weight compounds, such as organic acids. It also leads to complete disintegration of eg: endogenous sterols [2, 3, 4]. In the light of the few studies on this subject, it was

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decided to carry out analyzes of endogenous wood sterols and the content of organic acids after the drying process in waste from the wood processing line.

MATERIAL AND METHODS

Tested material

The bark of three species of deciduous trees (oak, beech, hornbeam) and two conifers (spruce, pine) was tested in three replications. After drying, the bark was milled using a cutting mill Pulverisette 19 (Fritsch, Germany) and analyzed for the content of sterols (campesterol, desmosterol, stigmasterol, beta-sitosterol) and organic acids.

Analysis of the content of sterols

Methanol and acetonitrile were HPLC grade (Sigma-Aldrich, St. Louis, MO, USA); hydrochloric acid and natrum hydroxide were purchased from POCH S.A (Gliwice, Poland); deionized water was prepared using a Millipore Milli-Q system (Millipore, MA, USA). The standards of campesterol, desmosterol, stigmasterol, β-sitosterol were purchased from Sigma-Aldrich (St. Louis, MO, USA.).

Sterols were determined following microwave assisted basic hydrolysis. Samples of 100 mg material were placed into 17 ml culture tubes, suspended in 1 ml of methanol, treated with 0.1 ml of 2 M aqueous NaOH and sealed tightly. Then the culture tubes were placed within 250 ml plastic bottles, sealed tightly and placed inside a microwave oven (Whirpool model AVM 401/WH) operating at 2450 MHz and 900 W maximum output. Samples were irradiated (370 W) for 20 s, after c. 5 min irradiated again for an additional 20 s and extracted with pentane (HPLC grade, Sigma-Aldrich, Steinheim, Germany) (3 × 4 ml) within the culture tubes. The combined pentane extracts were evaporated to dryness in a gentle stream of a high purity nitrogen using a RapidVap Evaporator (Labconco, Kansas, MO, USA). The extracts were stored at -25ºC until analysis. Prior to analyses the samples were dissolved in 1 ml of methanol, filtered through 13mm syringe filters with a 0.22 μm pore diameter (Fluoropore Membrane Filters).

Contents of sterols were analysed using an Aquity H class UPLC system equipped with a Waters Acquity PDA detector (Waters, USA). Chromatographic separation was performed on an Acquity UPLC® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 μm) (Waters, Ireland). The elution was carried out isocratically using the following mobile phase composition: A, acetonitrile 10%; B, methanol 85%; C, water 5%; at a flow rate of 0.5 mL/min.

Measurements of sterol concentrations were performed using an external standard at wavelengths λ = 210 (desmosterol, stigmasterol, β-sitosterol, campesterol). Compounds were identified based on a comparison of retention times of the examined peaks with that of the standard and by adding a specific amount of the standard to the tested sample and repeated analyses. The limit of detection was 1 mg/kg.

Analysis of the content of organic acids

The analysis of LMWOAs was performed using a Waters Acquity H-class UPLC system. Separation was achieved on an Acquity UPLC BEH C18 column (150 mm × 2.1 mm, 1.7 μm, Waters) thermostated at 35°C. The gradient elution was performed with water and acetonitrile (both containing 0.1% formic acid, pH=2) at a flow rate of 0.4 mL/min. Detection was carried out in a Waters Photodiode Array Detector (Watres Corporation, Milford, MA, USA) at λ = 280 nm as the preferred wavelength for acetic, citric, fumaric,

lactic, malic, maleic, malonic, oxalic, quinic and succinic acids. Compounds were identified by comparing of retention time of the analyzed peaks with the retention time of standards or by adding a specific amount of the standard to the analyzed samples and a repeated analysis. The LMWOAs found were quantified by comparing of the area of their peaks recorded at 280 nm with calibration curves obtained from commercial standards of each compound obtained from Sigma (St. Louis, MO, USA). The results were expressed in milligrams per gram of dry weight (mg/g).

RESULTS AND DISCUSSION

As part of this work, the concentration of the four most important sterols (desmosterol, stigmasterol, β-sitosterol, campesterol) wood bark was tested. In the bark of deciduous trees after the drying process, these sterols were not found (Table 1) with the exception of beech bark and hornbeam, where only campesterol was identified. However, they were found in bark samples from conifers. In the bark of pine and spruce, capesterol occurred at the highest concentration compared to other sterols. Beta-sitosterol occurred in the lowest concentration in the bark of conifers (at the level of 1.6 to 1.78 mg/kg) (Table 1).

Table 1. Average concentration of endogenous wood sterols (mg / kg) in the analyzed bark of deciduous and coniferous trees

Trees species	Sterols (mg/kg)			
	Campesterol	Desmosterol	Stigmasterol	Beta-sitosterol
pine	270,4	2,5	1,9	1,78
spruce	247,9	17,2	5,6	1,6
hornbeam	222,17	< LOD	< LOD	< LOD
beech	18,63	< LOD	< LOD	< LOD
oak	< LOD	< LOD	< LOD	< LOD

The concentration of endogenous wood sterols is related to the level of mycobiota pollution as observed in the tested samples. The role of sterols in plant tissues during infection with pathogens has not been sufficiently explained. Bark is dead tissue, therefore the role of sterols can not be discussed in the context of defense mechanisms in plants. During the decomposition of wood, microscopic fungi degrade reserve substances. Substances released during degradation include phytosterols contained in cell membranes and cell walls. The susceptibility of plant organisms to microbial infections is related to the stability and impermeability of cell membranes, and sterols are indispensable components of cell walls and cell membranes [5]. The sterols contained in dead cells released from plant tissues as a result of fungal action are identifiable. The higher the fungus contamination, the higher the content of free endogenous sterols due to the amount of decomposed wood cells [6].
An important component of wood with a biogenic effect are organic acids. According to Siegelman (1964) [7], these acids play an important role in the biosynthesis of phenolic compounds in trees, they are also the products of their decomposition.

Organic acids are involved in the regulation of a wide range of basic cellular processes, e.g. biochemical and physiological processes. They act as signal relays [8], as well as a modulator of transport through biological membranes [9]. It has been shown that the metabolism of organic acids in the cytosol is involved in abiotic reactions stressful [10]. Organic acids are also involved in the chemical modification of proteins, for example in acetylation or succinylation [11, 12].

Table 2. The content of organic acids in the bark of conifers and deciduous trees (mg/g)

Trees species	Malonic acid	Citric acid	Quinic acid	Succinic acid	Lactic acid	Maleic acid	Fumaric acid	Oxalic acid
pine	0,12	400,6	41,84	12,95	< LOD	< LOD	7,8	3,35
spruce	0,12	349,93	297,5	42,17	37,64	49,92	7,97	11,89
hornbeam	0,04	432,36	< LOD	< LOD	< LOD	< LOD	6,16	2,8
beech	< LD	137,18	30,9	45,21	< LOD	< LOD	6,19	3,8
oak	< LD	356,8	< LOD	< LOD	< LOD	< LOD	5,33	0,6

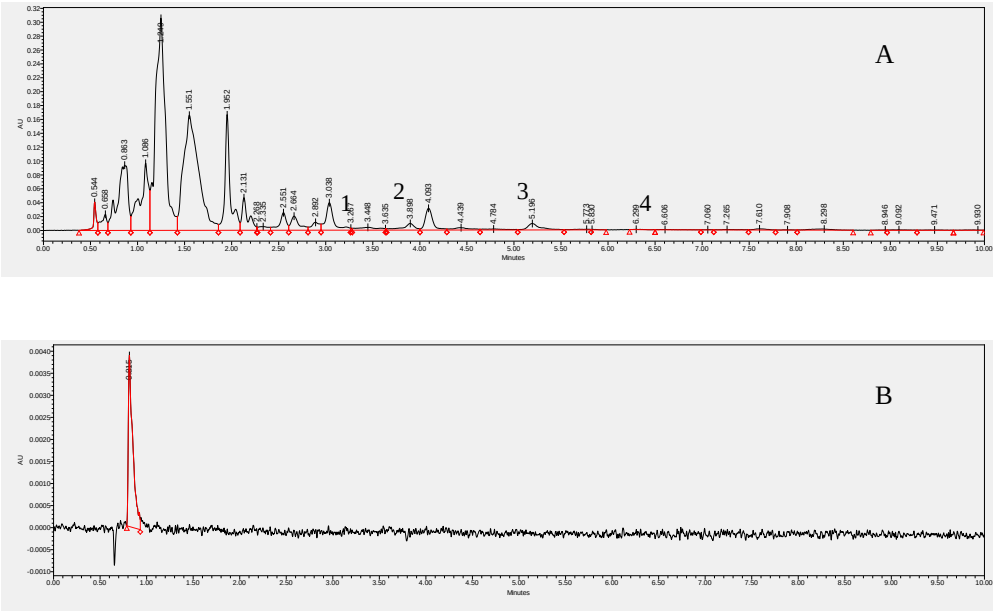

Fig. 1. An example of a spruce bark chromatogram (A) 1-campesterol, 2-desmosterol, 3-lanosterol, 4-beta-sitosterol and oak (B)

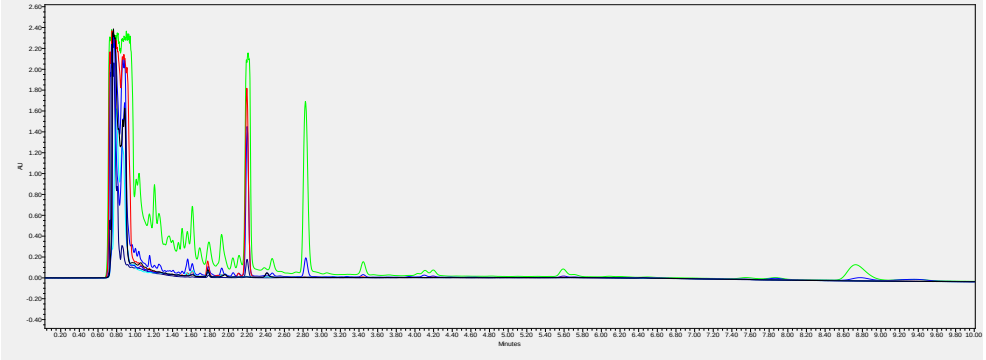

Fig. 2 Comparison of chromatograms of organic acids of all analyzed samples

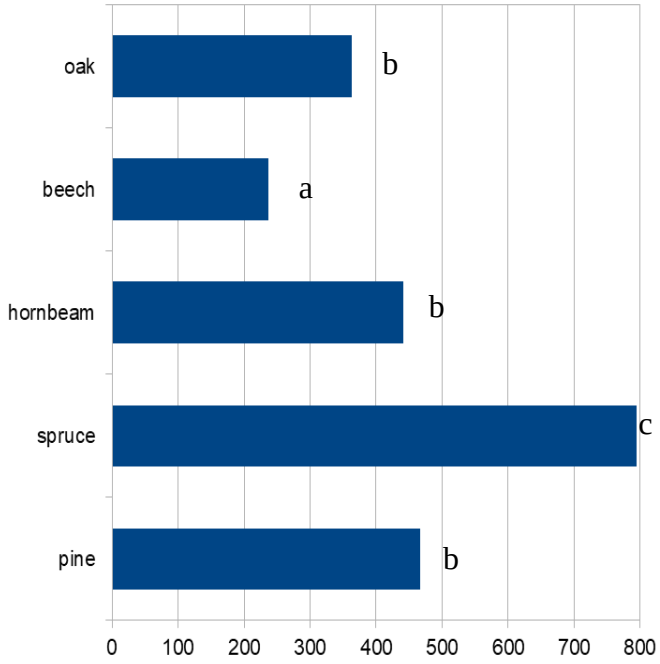

Fig. 3. Concentration of sum of organic acids [mg/g]

In the present research, the content of organic acids of oak bark, beech, hornbeam, spruce and pine was determined (Table 2, Figures 1, 2, 3). Deciduous trees are poorer in organic acids than in coniferous ones. The spruce contained all the acids determined in the experiment (malonic, lemon, quinic, amber, lactic, maleic, fumaric and oxalic), among them citric acid in the highest concentration. The bark of the oak contained mainly citric acid and small amounts of fumaric and oxalic acid (5.33 and 0.6 mg/g). The bark of hornbeam and beech proved to be slightly richer in organic acids. Beech bark, in comparison with the bark of the oak, additionally contained small amounts of malonic acid, and beech quinic and succinic acid. Previous research on this subject is limited, and knowledge about the impact of technological processes on bioactive compounds contained in it is small. Short references to organic acids in wood have been found on the basis of

literature studies. For example: Wang and Steffen [13] determined the content of organic acids in apple wood. They detected the presence of acids: amber, apple, lemon and chin. It turned out that the content of organic acids changes periodically, in spring it decreases and increases in the winter.

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