Wood Processing Waste – Contamination with Microscopic Fungi and Contents of Selected Bioactive Compounds

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Wood processing work stations produce contaminants that affect air quality in plant production facilities. A significant portion of these contaminants consists of spores and hyphae of microscopic fungi. Their presence in respirable and settled dust directly affects the health of the employees working in those facilities. Moreover, microscopic fungi interact with the components of wood, causing its degradation. Thus, several factors affecting the quality of ambient air were analyzed in samples collected from all accessible locations where wood waste is accumulated in the plant. The samples were tested for their concentrations of ergosterol, total phenolics, and antioxidant activity as well as their contents of endogenous wood sterols such as desmosterol, cholesterol, lanosterol, stigmasterol, and beta-sitosterol. The analyses showed that wood waste, despite the varied location and exposure time, promotes the growth of microscopic fungi. Several significant correlations between the analyzed parameters became evident, which made it possible to design the sterol bioconversion mechanism for wood, taking place as a result of the growth of microscopic fungi on the wood material.

Keywords: Antioxidant activity; Ergosterol; Free phenolic acids; Microscopic fungi; Wood dust

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INTRODUCTION

Wood processing generates several waste products such as bark, chips, and dusts of varying particle size. Most of the sawdust and shavings can be processed further. Due to its small dimensions and proximity to working surfaces, some of this waste constitutes secondary air pollution source in wood processing facilities.

Microbial growth is observed in wood waste deposited in these facilities. These organisms may develop on all surfaces and under a wide range of environmental conditions. In particular, wood processing plants provide suitable conditions promoting their growth such as elevated temperature and relative humidity (Prażmo and Dutkiewicz 2000; Dutkiewicz *et al*. 2001; Krysińska-Traczyk *et al*. 2002; Rogoziński *et al*. 2014). As a result of fungi, wood material that is being stored for further processing loses both weight and quality (Marutzky and Keserue 1981a; Marutzky and Keserue 1981b). As a result of fungal infection, particle board emits substances harmful to health and loses its mechanical properties (Mirski *et al*. 2014).

Additional factors promoting the growth of microscopic fungi on wood waste include the relatively long duration of its deposition, small particle dimensions, and self-heating of the wood dust and chip mass. In wood processing plants, sources of air pollution containing spores of microscopic fungi include both wood delivered to the plants as well as primary and secondary contamination. The latter may originate from the exhaust system, in which organic waste (*e.g.*, wood dust, bark dust) is deposited for sufficiently long periods to promote the growth of fungal microflora.

At the exposure of wood to the action of microscopic fungi, wood cells undergo biodegradation, which may even lead to their complete decomposition, thus producing nutrients sustaining the growth of microscopic fungi. Earlier studies confirmed a significant role of endogenous sterols in wood during the action of microscopic fungi on wood dust and other wood waste materials. Because endogenous sterols are a major structural material of wood cell walls, they are reduced or transformed by the cells of microscopic fungi (Rogoziński *et al*. 2014).

Within this study, investigations concerning sterols, both as wood components and those of fungal origin, were extended to cover other factors including the antioxidant activity of bioactive wood components and the content of free phenolics. The potential correlation between the analyzed metabolites during the action of microscopic fungi on wood processing by-products was examined.

The aim of this study was to analyze contents of endogenous wood sterols, fungal sterols, the antioxidant activity of extracts obtained from wood dust and waste, and free phenolics to determine a dependence between the above-mentioned parameters.

EXPERIMENTAL

Description of Mechanical Technology in the Sawmill

The technological process performed at the investigated sawmill comprises production of wooden structural elements for garden architecture items. In the first stage, logs are transported by a conveyor (station 1) from the storage yard to the saw machine. Then roundwood is sawed (work station 2 – saw machine), and planks are produced. Planks are subsequently cut using a multi-rip saw (work station 3) and a cross-cut saw (work station 4) to create smaller-sized assortments. Then, the elements are impregnated and dried. Dry elements are planed (work station 5 – four-side planer) to provide adequate surface quality and then cut to size on the cross-cut saw machine. The finished components are transported to the stations where wooden garden architecture items are assembled. Thus, the area of the sawmill may be divided into three technological zones: the log sawing room, the plank machining room, and the assembly room.

In the sawmill, large amounts of bark waste are processed. Finally, the bark is sorted into size classes and automatically packaged into retail packages.

Each work station where mechanical processing of wood is performed is connected to an exhaust system that removes chips and dust generated during wood machining. In the analyzed sawmill, there are three exhaust systems ending with cyclones.

Dust Sampling

Three groups of wood dust deposited on surfaces in the sawmill were sampled (Table 1). The sampled areas included:

- Surfaces of work stations performing wood machining operations including a log conveyor and a bark packaging and sorting station (A),
- Areas surrounding the cyclones (B),
- Surfaces in the three technological zones (C).

Wood dust samples for analyses, each comprising approximately 5 g of settled dust, were collected using sterile spatulas as described by Rogoziński *et al*. (2014). Samples from group A surfaces were collected after working shifts in three replications. The dust collected had settled on these surfaces during one shift. Samples from surfaces B and C were also collected three times, but the dust was deposited there for a longer period than one working shift.

Table 1. Working Stands from Which Samples Were Collected

No. of sampled surface	Description of sampled surface	The group of sampled area
1	chain conveyor for logs to frame sawing machine, fresh waste (wood and bark)	A
2	frame sawing machine, fresh chips	A
3	multi-saw machine, fresh chips	A
4	heavy cross sawing machine, fresh chips	A
5	four-side planers, fresh chips	A
6	small cross sawing machine at assembly department , fresh chips	A
7	cyclone, end of pipeline from frame sawing machine, fresh chips (wood and bark)	B
8	cyclone, end of pipeline from multi-saw machines, fresh chips	B
9	cyclone, end of pipeline from planers, thicknessers, four-side planers; fresh chips	B
10	cyclone, end of pipeline from planers, thicknessers, four-side planers; musty chips	B
11	belt conveyor for lumber, musty chips (wood and bark)	C
12	woodworking room, planers, thicknessers; musty chips	C
13	assembly position, mouldy chips from floor	C
14	line of bark packing, fresh waste from floor	C

Sterols

Sterols were determined following basic hydrolysis by microwave-assisted extraction. Prior to analysis samples were dissolved in 1 mL of methanol and filtered through 13 mm syringe filters with 0.22 µm pore diameter (Fluoropore Membrane Filters). Contents of sterols were analysed using an Aquity H class UPLC system equipped with an Waters Acquity PDA detector (Milford, MA, USA). Chromatographic separation was performed on a Acquity UPLC® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 µm) (Waters). The elution was carried out isocratically using following mobile phase composition: A, acetonitrile 10%; B, methanol 85%; C, water 5%, flow 0.5 mL/min. Measurements of sterols concentrations were performed using an external standard at wavelengths λ = 210 nm (desmosterol, cholesterol, lanosterol, stigmasterol, β-sitosterol). Compounds were identified based on a comparison of retention times of the examined peak

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 0.1 mg/kg (Stuper-Szablewska *et al.* 2017).

Total Polyphenolic Contents

Samples of 50 g were collected for analyses of polyphenols. Samples were ground using a laboratory mill (WŻ-1). Phenolic compounds were extracted with 80% MeOH were extracted with ultrasonic bath. Samples were filtered on a paper filter using a water pump. Next, the combined extracts were evaporated until dry in an evaporator. The phenolic compounds were transferred quantitatively to a vial using 4 mL of MeOH and dried in a stream of nitrogen. The total content of polyphenols was assayed according to Heimler *et al.* (1995). Folin-Ciocalteu reagent was added to the extracts, the mixture was supplemented with 1.25 mL of 7% aqueous Na_2CO_3 solution and 1 mL of deionized water (Singleton and Rossi 1965). After 90 min incubation in the dark, the absorbance was read at a wavelength of 760 nm in relation to water (Helios spectrophotometer, Thermo Electron Corp.). The results were expressed in mg gallic acid per 100 g of dry mass sample.

$ABTS^{\cdot+}$ Method (antioxidant activity determination)

For $ABTS^{\cdot+}$ generation from ABTS salt, 3 mM of $K_2S_2O_8$ were reacted with 8 mM ABTS salt in distilled. They were incubated in deionized water for 16 h at room temperature in the dark. The $ABTS^{\cdot+}$ solution was then diluted with pH 7.4 with PBS (phosphate buffer) to obtain an initial absorbance of 1.5 at 730 nm. Reactions were complete within 30 min. Samples and standards (100 μm) were reacted with the $ABTS^{\cdot+}$ solution (2900 μm) for 30 min. Trolox was used as a standard (Re *et al*. 1999).

RESULTS AND DISCUSSION

Studies conducted to date on the metabolic reactions taking place in wood cells under the influence of the growth of microscopic fungi have been concerned primarily with forest ecosystems (Jörgensen 1961; Rayner and Boddy 1988; Dix and Webster 1995; Küffer et al. 2008; Chong *et al.* 2009). Within this study, the current knowledge on the metabolomics of mycobiota in relation to wood was used to examine the environment of wood processing plants. This problem was investigated in view of the confirmed serious health hazard connected with the presence of mycelial hyphae and spores of microscopic fungi both in respirable dust and in wood waste (Dykewicz *et al*. 1988; Halpin *et al*. 1994; Alwis *et al*. 1999; Dutkiewicz *et al*. 2001; Krysińska-Traczyk *et al*. 2002; Oppliger *et al*. 2005; Rogoziński *et al*. 2014). Presented analyses were conducted on waste generated during various stages of wood processing. Samples were collected from all accessible locations where wood waste is accumulated in the plant. Fine dust and waste originating from 14 work stations were tested in terms of their contamination with microscopic fungi (Fig. 1a). For this purpose the concentration was recorded for ergosterol (ERG) as a chemical marker for the quantity of mycobiota in the analysed material (Perkowski *et al*. 2008; Szwajkowska-Michałek *et al*. 2010; Rogoziński *et al*. 2014; Stuper-Szablewska and Perkowski 2014). The level of this sterol, characteristic of microscopic fungi, varied greatly in all analyzed samples and ranged from 1.16 mg/kg recorded in green wood to 57.41 mg/kg recorded in samples deposited over extended periods of time and containing large amounts of bark. The varied levels of contamination with mycobiota showed significant dependencies between ERG concentration and the type of analyzed samples. A directly

proportional dependence was found between the level of contamination with mycobiota and the deposition time of wood dust and waste. Another dependence was determined concerning the composition of analyzed samples. A higher ERG concentration was recorded in samples containing bark, whereas samples containing internal wood components were characterized by lower ERG concentrations. It was shown during growth of microscopic fungi on wood surfaces in dust or waste that while the material is considered to be inanimate, it still contains active substances protecting the wood against microbiological degradation over a specific period of time. Phenolic compounds are these agents and they are characteristic of all tree species growing in the temperate zone (Rayner and Boddy 1988; Charlwood and Rhodes 1990; Davin *et al*. 1992; Wallace and Fry 1994; Evensen *et al*. 2000; Hiltunen *et al*. 2006; Zarzyński 2009; Szwajkowska-Michałek *et al*. 2010; Szwajkowska−Michałek *et al.* 2019).

The dusts and waste materials tested in this study varied in terms of their deposition time and for this reason the total phenolic concentration was recorded, based on the determination of free phenolic acids (FPA) (Fig. 1b). The results showed that except for sample 14, no significant differences were found between individual samples in terms of their FPA contents. The correlation coefficient between FPA concentration and ERG was high, amounting to 0.4360 (Table 2), but it was non-significant at the significance level of 0.05 for the investigated population of samples. An almost 3-fold greater FPA content was found in sample 14 which contained mainly cyclone waste as a result of its composition of both green wood fragments and a high share of bark. Bark is the first barrier protecting the tree interior against external factors including fungal infection. Therefore, the bark accumulates most of the substances participating in immune and defense mechanisms (Stanley 1969; Wargo 1981; Zucker 1983; Shaw 1985; Wargo 1988; King and Young 1999; Sirmah 2009).

Table 2. Correlation Matrix for Endogenous Wood Sterols, Ergosterol (ERG), Free Phenolic Acids (FPA), and Antioxidant Activity (ABTS)

	ERG	ABTS	FPA	desmosterol	cholesterol	lanosterol	sigmasterol	β-sitosterol
ERG	1							
ABTS	0.5612*	1						
FPA	0.4360	0,8824	1					
desmosterol	0.3749	0.6589*	0.6553*	1				
cholesterol	-0.0290	0.4837	0.6676*	0.4310	1			
lanosterol	0.1659	0.5600*	0.3515	0.3599	0.6311*	1		
sigmasterol	0.2146	0.5857*	0,4730	0,7891*	0.3176	0,4201	1	
β-sitosterol	-0.0009	-0.4662	-0.3118	-0.6159*	-0.2049	-0.3688	0,8681**	1

Within this study, antioxidant activity was determined based on the reaction with the ABTS cation radical (Fig. 1c). Antioxidant activity was highly correlated with FPA content (Table 2), and it was highest for samples containing green wood fragments and bark. Moreover, a correlation was found between ERG concentration and antioxidant activity. This dependence confirms the above-mentioned theory on the action of defense mechanisms in wood after the cessation of vital functions in trees. Non-enzymatic defense

mechanisms consisting in overproduction of specific bioactive compounds cause a significant increase in antioxidant activity. This mechanism is activated during the growth of microscopic fungi on the surface of plant material.

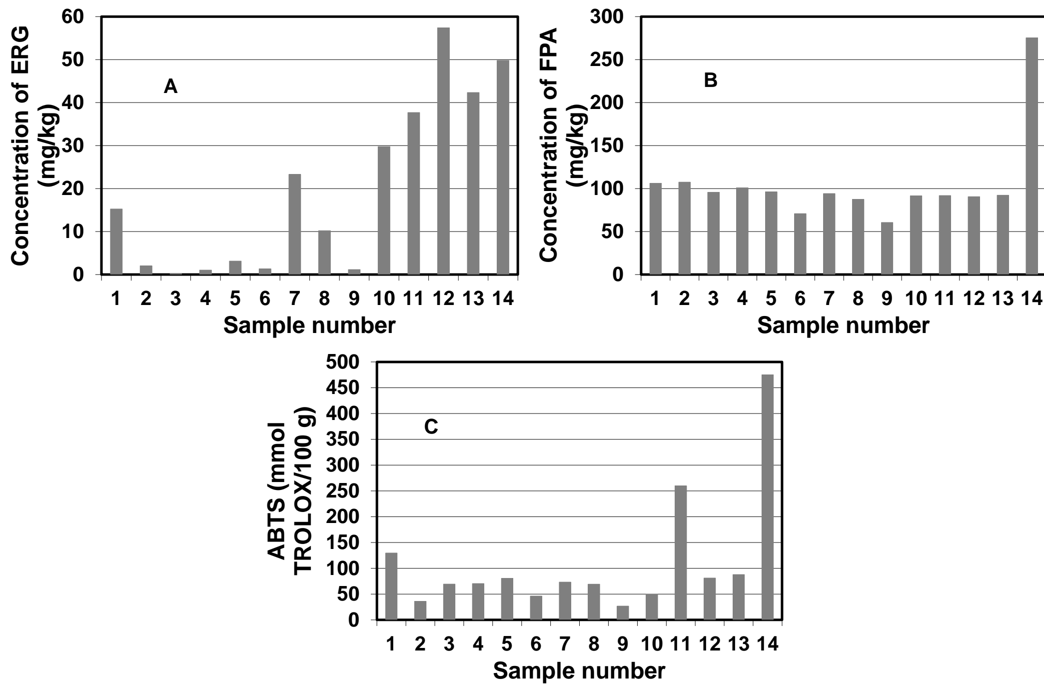

Fig. 1. Concentrations of ergosterol (ERG) mg/kg (a) and free phenolic acids (FPA) mg/kg (b), antioxidant activity by ABTS (c) in wood dust samples.

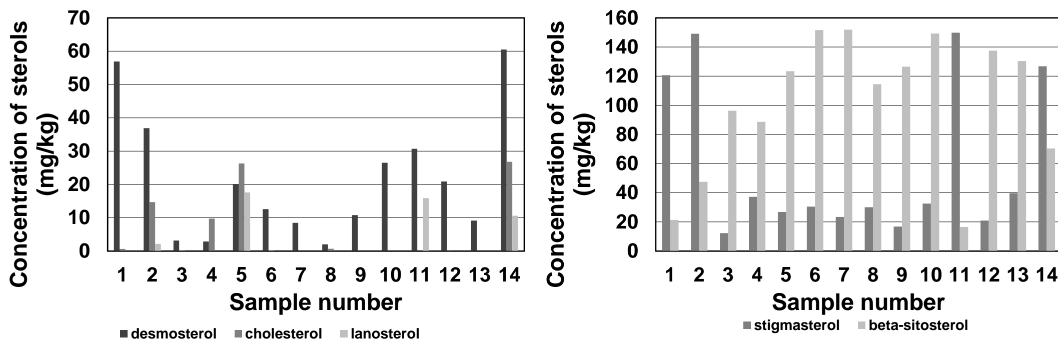

Fig. 2. Concentrations of endogenous wood sterols in wood dust

The last stage in the study comprised analyses of endogenous wood sterols. The contents of these compounds varied in all samples (Fig. 2). A significant, inversely proportional dependence was observed between the concentrations of beta-sitosterol and stigmasterol (Table 2). As suggested in previous studies microscopic fungi utilize endogenous sterols of wood during their growth on wood surfaces (Stuper-Szablewska *et al*. 2017). However, this study shows that the fungi may use the endogenous sterols in wood to biosynthesize sterols of fungal origin such as cholesterol or lanosterol. This is indicated by significant correlations between these sterols. Inversely proportional dependencies were found between endogenous wood sterols and sterols produced by microscopic fungi (Table 2). Based on this analysis and on current knowledge on the subject concerning other plants, a mechanism of sterol bioconversion was proposed for wood (Fig. 3) (Griebel and Zeier 2010; Miras-Moreño *et al*. 2016).

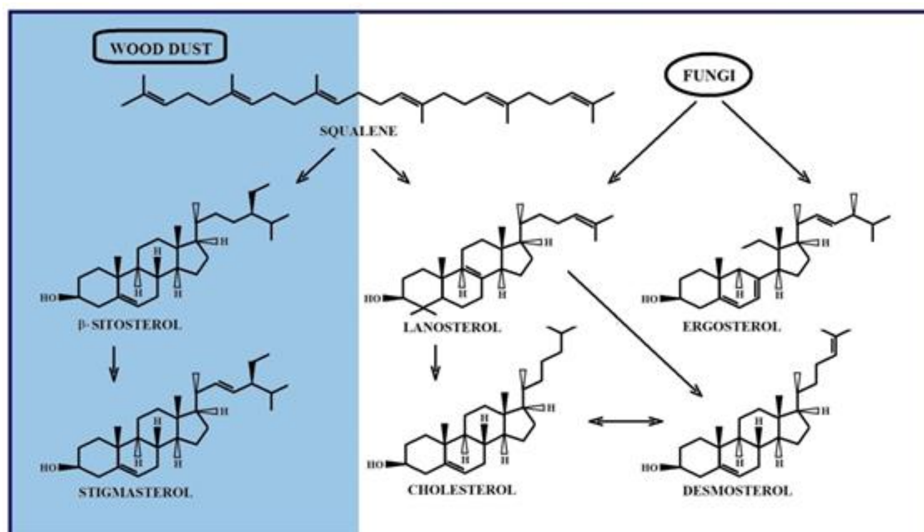

Fig. 3. A hypothetical mechanism for bioconversion of endogenous wood sterols during infection

The presence of phenolic compounds in tree bark is connected with their role in the defense mechanism in the case of attack by pathogenic fungi such as the *Armillaria* species. Harju *et al*. (2003) observed an increase in the concentration of phenolic compounds in the wood of Scots pine specimens resistant to attack by *Armillaria* fungi. According to Zarzyński (2009), the total amount of organic substances in the wood of an individual tree species is positively correlated with its density. Species characterized by "heavy" wood such as *Tabebuia* spp., *Hymnaea* spp., or *Millettia laurentii* contain markedly higher amounts of these compounds in comparison to species with light wood, such as *Triplochiton scleroxylon*, *Pinus sylvestris,* or *Aucoumea klaineana*. When compared to European species, wood of exotic species is more abundant in phenolic compounds (Zarzyński 2009).

CONCLUSIONS

1. This study showed that wood wastes, despite the differences in the place of their deposition and exposure time, are favorable materials for promoting the growth of microscopic fungi, which may pose a health hazard for workers in wood conversion and processing plants.
2. An important fact previously neglected in literature on the subject shows that wood wastes maintain their antimicrobial activity for an extended period after the cessation of vital functions in the wood. However, it is evident that green wood waste containing bark exhibits a greater antioxidant activity and contains higher levels of bioactive compounds such as polyphenols than wood wastes deposited over longer periods.
3. No studies have been conducted to date to identify correlations between extremely different metabolites, for example, those found in fungi and wood. These research results need to be verified in a model experiment in order to confirm the presented observations and conclusions.

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