Industrial Crops & Products 108 (2017) 327–335

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Cellular level distributions of Scots pine heartwood and knot heartwood extractives revealed by Raman spectroscopy imaging

Tiina Belt[a,1,*], Tobias Keplinger[b,c,1], Tuomas Hänninen[a], Lauri Rautkari[a]

[a] Aalto University, School of Chemical Engineering, Department of Bioproducts and Biosystems, P.O. Box 16300, 00076 Aalto, Finland
[b] Wood Materials Science, Institute for Building Materials, ETH Zurich, Stefano-Franscini-Platz 3, 8093 Zurich, Switzerland
[c] Applied Wood Materials, EMPA Swiss Federal Laboratories for Materials Science and Technology, Ueberlandstrasse 129, 8600 Dübendorf, Switzerland

ARTICLE INFO

Keywords:
Extractives
Heartwood
Imaging
Pinosylvin
Raman spectroscopy
Scots pine

ABSTRACT

Wood extractives are biologically active secondary metabolites that help protect wood and wood products from decay and other forms of biological attack. Despite the influence of distribution on their ability to protect wood, very few studies have investigated the distributions of extractives on a cellular level. In this paper, the distributions of extractives were studied in Scots pine (*Pinus sylvestris* L.) heartwood (HW) and knot heartwood by confocal Raman spectroscopy imaging. Pinosylvins, the antifungal phenolic extractives of pine, were found to be present in the cell walls, middle lamella, and lumina of tracheids. Their distribution suggested the existence of two different mechanisms of deposition and revealed similarities to the distribution of lignin. The potential binding of pinosylvins to lignin and their relatively low concentration in HW cell walls could explain why Scots pine HW is, on average, only moderately resistant to decay. Resin acids, the most abundant group of extractives in pine, were detected only within the lumina of tracheids and ray cells, where they may contribute to the reduced permeability of HW. The extractives distributions presented here help us understand the properties of HW and provide a deeper understanding of the origins of natural durability, which is of value in the current efforts to develop more environmentally friendly means of wood protection.

1. Introduction

Wood is a complex natural composite consisting primarily of cellulose, hemicelluloses, and lignin. In addition to these structural polymers, wood also contains secondary metabolites called extractives, which occur primarily in the heartwood (HW) and knot heartwood (KHW) of trees. Although they are often low in abundance compared to the structural components, the extractives have a significant effect on the properties of wood, most notably its resistance to decay and other forms of biological attack (Hillis, 1987; Taylor et al., 2002). Due to the economic and biological significance of decay resistance, the chemical composition, properties, and formation of extractives have been extensively studied (Hillis, 1987; Taylor et al., 2002; Kampe and Magel, 2013).

In Scots pine (*Pinus sylvestris* L.), a commercially important species in northern Europe, the HW and KHW extractives consist mainly of the phenolic pinosylvins and the hydrophobic resin acids and fatty acids (Piispanen and Saranpää, 2002; Willför et al., 2003; Ekeberg et al., 2006; Hovelstad et al., 2006; Fang et al., 2013). The knots of Scots pine are often particularly rich in extractives and contain lignans in addition to the HW extractives (Willför et al., 2003, 2004; Hovelstad et al., 2006; Fang et al., 2013). Pinosylvins and resin acids have both been linked to the decay resistance of pine HW (Harju et al., 2002; Venäläinen et al., 2004; Leinonen et al., 2008), and attempts have even been made to utilize these compounds as environmentally friendly wood protection agents (Celimene et al., 1999; Lu et al., 2016).

Despite extensive characterization of composition and properties, very little is known about the deposition pathways and cellular level distributions of Scots pine extractives. Knowledge of the incorporation and formation of extractives is necessary to gain a full understanding of HW formation, but the distribution of extractives also has a significant effect on their ability to modify wood properties (Taylor et al., 2002). The distribution of pinosylvins is of particular interest, due to the disparity that exists between the high antifungal activity of pinosylvins and the moderate decay resistance of the HW of various pine species (Hart and Shrimpton, 1979). The decay resistance and pinosylvins content Scots pine HW are highly variable (Bergström et al., 1999; Fries et al., 2000; Harju and Venäläinen, 2002; Venäläinen et al., 2004), and the average resistance of its HW is typically classified as only moderate or slight (Jebrane et al., 2014; Plaschkies et al., 2014). It has been

* Corresponding author.
E-mail address: tiina.belt@aalto.fi (T. Belt).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.indcrop.2017.06.056
Received 28 April 2017; Received in revised form 22 June 2017; Accepted 26 June 2017

suggested that the inability of pinosylvins to confer significant decay resistance to wood may be due to their distribution or binding to lignin (Hart and Shrimpton, 1979).

In other wood species, a small number of studies have been performed to investigate the distribution of HW extractives (Kuo and Arganbright, 1980; Streit and Fengel, 1994; Nagasaki et al., 2002; Zhang et al., 2004; Imai et al., 2005). However, most of these studies have used non-specific staining rather than methods that allow for the visualization of defined chemical components. One of the most promising methods for distribution mapping of specific chemical components is confocal Raman spectroscopy imaging. Raman imaging yields spatially resolved chemical information without staining or labeling and has previously been used to investigate the distributions of wood cell wall polymers (Agarwal, 2006; Gierlinger and Schwanninger, 2006; Hänninen et al., 2011; Ji et al., 2013; Gierlinger, 2014; Zhou et al., 2014) and extraneous compounds inserted into the cell walls (Ermeydan et al., 2012; Keplinger et al., 2015; Merk et al., 2015).

In this study, Raman spectroscopy imaging was applied to the study of native wood extractives. Extractives distributions were investigated in Scots pine HW and KHW, with a focus on pinosylvins. The study aimed to provide additional insight into HW formation and increase our understanding of the properties and behavior of HW, particularly in terms of natural durability. A deeper understanding of the origins of natural durability will help in the current efforts to develop more environmentally friendly means of wood protection. New insights into the distributions of different types of extractives also provide valuable information on the inherent transportation pathways within wood, which is of great interest in the field of wood modification and functionalization.

2. Materials and methods

2.1. Chemicals

Pinosylvin, pinosylvin monomethyl ether, abietic acid, isopimaric acid, linoleic acid, and oleic acid were purchased from Sigma Aldrich and were used as received.

2.2. Wood material

Two green Scots pine logs were obtained from a sawmill in southern Finland and stored frozen until use. The sapwood and heartwood samples were both prepared from one log, which was mature (70 annual rings) and free of defects. A disc approx. 50 mm thick was sawn from the log, and a strip approx. 80 mm wide was sawn through the center of the disc. Sapwood (SW), outer heartwood (OWH), and middle heartwood (MHW) sections, each containing approx. 5 annual rings, were prepared from the strip as shown in Fig. 1a. HW was visually identified by its lighter color due to its lower moisture content. Each section was cut in half across the grain: one half was cut with a razor blade into sticks with a 5 × 5 mm cross-section, while the other half was cut into small pieces and ground in a Wiley mill.

Knot samples were prepared from the other log (27 annual rings) which was rich in knots. A thick disc containing a whorl of branches was sawn from the log, and the disc was split into four sections. Live knots were removed from each section (Fig. 1b,c) and trimmed until only knot heartwood (KHW) remained (Fig. 1d). One half of the knot material was cut into sticks as described above, while the other half was again ground in a Wiley mill.

After preparation, the wood sticks and powders were air-dried. A small portion of each air-dried powder was also dried at 105 °C to determine the residual moisture content of the powders. Each powder was then Soxhlet extracted with acetone (6 h), and the composition of each extract was analyzed by GC–MS. Some of the sticks were also extracted with acetone, after which the extracted and unextracted sticks were used for Raman spectroscopy imaging.

2.3. GC–MS analysis

A small aliquot of each extract and the internal standard (heneicosanoic acid) was added to a vial and the solvent evaporated under vacuum. The extracts were redissolved in 700 µL of pyridine and trimethylsilylated at 70 °C for 20 min after addition of 300 µL N,O-bis (trimethylsilyl)trifluoroacetamide with 5% chlorotrimethylsilane. The compounds present in the extracts were identified and quantified using a Thermo Scientific ISQ series single quadrupole mass spectrometer, coupled with a Trace 1300 gas chromatograph. The column used was TR-5MS (30 m x 0.25 mm i.d., 0.25 µm film thickness), and the oven temperature program was set to 2 min at 100 °C, 15 °C/min to 280 °C, and 15 min at 280 °C. Helium was used as the carrier gas (1 mL/min), and the mass spectra were recorded in the 50–700 (m/z) range at an ionization energy of 70 eV. Each extract was analyzed in duplicate.

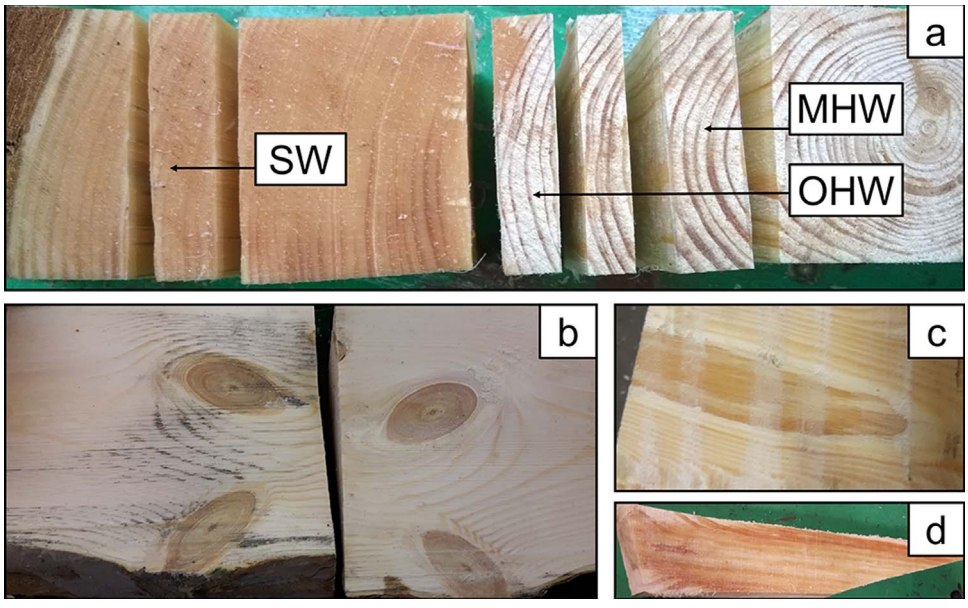

Fig. 1. Sampling of sapwood (SW), outer heartwood (OHW), middle heartwood (MHW) and knot heartwood (KHW). The position of SW, OHW, and MHW in the cross section of the disc (a), and the processing of knot samples, showing knots in the disc sections (b), a knot removed from one section (c), and a final trimmed KHW sample (d).