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Characterization of condensed tannins and carbohydrates in hot water bark extracts of European softwood species

Sauro Bianchi [a,*], Ivana Kroslakova [b], Ron Janzon [c], Ingo Mayer [a], Bodo Saake [c], Frédéric Pichelin [a]

[a] Bern University of Applied Sciences, Architecture Wood and Civil Engineering, Solothurnstrasse 102, 2502 Biel, Switzerland
[b] Zurich University of Applied Sciences, Institute of Chemistry and Biological Chemistry, Einsiedlerstrasse 31, 8820 Wädenswil, Switzerland
[c] University of Hamburg, Department of Chemical Wood Technology, Leuschnerstraße 91b, 21031 Hamburg, Germany

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A B S T R A C T

Condensed tannins extracted from European softwood bark are recognized as alternatives to synthetic phenolics. The extraction is generally performed in hot water, leading to simultaneous extraction of other bark constituents such as carbohydrates, phenolic monomers and salts. Characterization of the extract's composition and identification of the extracted tannins' molecular structure are needed to better identify potential applications.

Bark from Silver fir (*Abies alba* [Mill.]), European larch (*Larix decidua* [Mill.]), Norway spruce (*Picea abies* [Karst.]), Douglas fir (*Pseudotsuga menziesii* [Mirb.]) and Scots pine (*Pinus sylvestris* [L.]) were extracted in water at 60 °C. The amounts of phenolic monomers, condensed tannins, carbohydrates, and inorganic compounds in the extract were determined. The molecular structures of condensed tannins and carbohydrates were also investigated (HPLC-UV combined with thiolysis, MALDI-TOF mass spectrometry, anion exchange chromatography).

Distinct extract compositions and tannin structures were found in each of the analysed species. Procyanidins were the most ubiquitous tannins. The presence of phenolic glucosides in the tannin oligomers was suggested. Polysaccharides such as arabinans, arabinogalactans and glucans represented an important fraction of all extracts. Compared to traditionally used species (Mimosa and Quebracho) higher viscosities as well as faster chemical reactivities are expected in the analysed species. The most promising species for a bark tannin extraction was found to be larch, while the least encouraging results were detected in pine. A better knowledge of the interaction between the various extracted compounds is deemed an important matter for investigation in the context of industrial applications of such extracts.

1. Introduction

Condensed tannins, or proanthocyanidins, are natural polyphenolic oligomers made of flavan-3-ol units. They are recognized as suitable natural substitutes in the formulation of wood adhesives (Yazaki and Collins, 1994; Roffael et al. 2000; Pichelin et al., 2006; Pizzi, 2008), foamed resins (Lacoste et al., 2013) and heavy metal removal systems (Palma et al., 2003). Industrially used tannins are mostly extracted from the bark of Black wattle (*Acacia mearnsii* [De Wild.]) and the heartwood of Quebracho (*Schinopsis lorentzii* [Engl.]). The bark of softwood species has also been reported as a valuable source of condensed tannins (Porter, 1989; Foo and Karchesy, 1989; Matthews et al., 1997a; Karonen et al., 2004; Krogell et al., 2012). In Switzerland, 425,000 m^3 of bark was produced in 2013, the majority of which was burned for energy production (BAFU, 2014). Thus, softwood bark represents an important source of condensed tannins in Switzerland. In particular, Silver fir (*Abies alba* [Mill.]), Norway spruce (*Picea abies* [Karst.]), Scots pine (*Pinus sylvestris* [L.]), European larch (*Larix decidua* [Mill.]) and Douglas fir (*Pseudotsuga menziesii* [Mirb.]) are species of special interest, representing more than 95% of the total Swiss softwood growing stock (BAFU, 2014). These species are also among the most common softwoods in Central and Northern Europe, where round softwood production in 2013 was close to 250 million m^3. As bark corresponds to 10% of the log volume, 25 millions m^3 of it is thus theoretically available in Europe.

Properties of condensed tannins are significantly influenced by the molecular structure of their flavan-3-ol units (Fig. 1) and by their degree of polymerization.

Oligomers made of catechin or gallocatechin units showed up to tenfold shorter gelling times in the condensation reaction with

* Corresponding author.
E-mail address: sauro.bianchi@bfh.ch (S. Bianchi).

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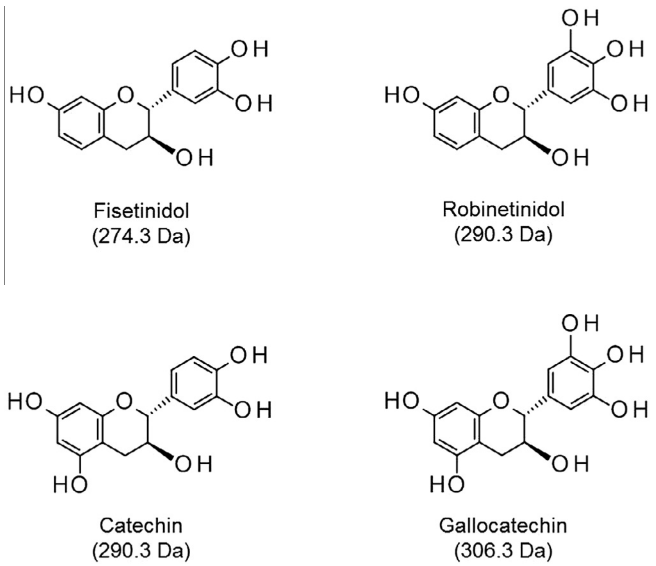

Fig. 1. Molecular structures of the most common flavan-3-ols units in the condensed tannins.

formaldehyde than oligomers made of fisetinidol or robinetinidol units (Pizzi and Stephanou, 1994; Garnier et al., 2002). Differences in reaction rates have been identified even between the stereoisomers catechin and epicatechin (Takagaki et al., 2000). Higher viscosities and shorter gelling times have also been correlated with higher degrees of polymerization (Garnier et al., 2001). Likewise, an enhancement of the heavy metal chelating ability has been reported with increasing degrees of hydroxylation and polymerization (Yoneda and Nakatsubo, 1998).

The purity of the extracted tannins represents yet another critical point. Bark extraction is typically performed with hot water. This method also yields the extraction of smaller phenolic compounds, carbohydrates and inorganic salts. Condensed tannins are therefore only a component of bark extracts, which chemical and physical characteristics could be significantly affected by their actual composition (Pizzi, 2008). The presence of polysaccharides is of particular concern, as the latter are related to an increase in the viscosity of resin formulations (Weissman, 1985; Garnier et al., 2001).

A detailed characterization of bark extracts is therefore important to better understand their properties and limitations.

Condensed tannins extracted from softwood bark have generally been associated with procyanidins (PC) and prodelphinidins (PD), oligomers composed mainly of catechin and gallocatechin units, respectively (Porter, 1989; Foo and Karchesy, 1989; Matthews et al., 1997a; Karonen et al., 2004; Navarrete et al., 2010). Nevertheless, the occurrence of building blocks other than simple flavan-3-ols, such as flavan-3-ol esters, glucosides, stilbenes and lignans has been suggested (Zhang and Gellerstedt, 2008; Ucar et al., 2013; Bianchi et al., 2014).

Bark polysaccharides, apart from cellulose, have been described as galactoglucomannans and arabinomethylglucuronoxylans (Timell, 1961; Dietrichs et al., 1978), but the presence of glucans (callose), arabinans and pectins has also been reported (Timell, 1964; Fu et al., 1972; Fu and Timell, 1972; Weissman, 1981, 1985; Krogell et al., 2012; Le Normand et al., 2014).

With the exception of spruce (Kemppainen et al., 2014), softwood bark extracts have so far been characterized with a focus on selected components (e.g. condensed tannins, distinct phenolic monomers, polysaccharides) using different and often not comparable sample preparation or analysis methods. A systematic study on diverse softwood species covering both the analysis of the condensed tannins structure and the characterization of the co-extracts, is still lacking.

In this study the composition of hot water extracts from five European softwood barks (Silver fir, European larch, Norway spruce, Douglas fir and Scots pine) were investigated. The presence of total phenolics; tannins; inorganic compounds; free and bound carbohydrates in the extracts were assessed. The molecular structure of extracted tannins and carbohydrates was analysed by liquid chromatography and mass spectrometry.

2. Results and discussion

2.1. Extraction yield and extract composition

The total extraction yield of softwood barks ranged from 26.9 to 120.2 g/kg of dry bark, corresponding to pine and Silver fir, respectively (Table 1). Similar yields have been reported for hot water extractions of European softwood bark (Weissman, 1981, 1985; Bertaud et al., 2012; Kemppainen et al., 2014). In these studies, consistent differences in yield were observed not only among different bark species, but also within the same species. Dissimilarity in the extraction temperature, time, water to bark ratio, size of the bark particles and bark storage conditions before extraction are factors that may account for observed variations.

The measured extraction yields were considerably lower than those of the most common commercial tannin species (e.g. Mimosa, Quebracho), which range between 150 and 330 g/kg dry material (Sealy-Fisher and Pizzi, 1992; Hoong et al., 2011).

All bark extracts showed the presence of phenolic compounds, carbohydrates, and inorganic compounds (Table 1). Total extracted phenolics, assessed by Folin–Ciocalteau assay, represented between 13.0% and 46.7% of the extract, corresponding to total phenolic yields between 3.5 and 42.8 g of epicatechin equivalent (gECE) per kg of dry bark (Table 1). Pine showed the lowest yield while larch showed the highest. Other studies on European softwood bark extracts have reported values in the same range (Jerez et al., 2009; Yesil-Celiktas et al., 2009; Bertaud et al., 2012). Previously performed Folin–Ciocalteau assays on commercial Mimosa and Quebracho extracts showed total phenolic concentrations equal to 40.7% and 42.1%, respectively. With the exception of larch, therefore, all the investigated softwood species showed a lower concentration of phenolics than commercial extracts.

More detailed information on the phenolic extracts was obtained after separation by solid phase extraction (SPE) of the crude extract in the following three fractions:

- F0 = carbohydrates and phenolic acids;
- F1 = phenolic monomers;
- F2 = phenolic oligomers (e.g. condensed tannins).

The Folin–Ciocalteau assay on the single fractions showed that most of the phenolic compounds were re-collected in F1 and F2 (Table 1), indicating the presence of both phenolic monomers and tannins in all species. The phenolic acids (F0) contributed only minimally to the total extracted phenolics across all species. A high ratio of condensed tannins was indicated for spruce and pine, which showed higher phenolic concentrations in F2 than in F1. Conversely, a prevalence of phenolic monomers appears in Douglas fir extracts. The impact of phenolic monomers on the chemical and physical characteristics of the extracts hasn't yet been thoroughly investigated. Though they are generally regarded as simple extract constituents that merely dilute the tannin concentration in the extracts (Pizzi, 2008), they may also play a role in condensation reactions.

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