



Condensed tannins from pine bark: A novel wood surface modifier assisted by laccase



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ARTICLE INFO

Article history:

Received 19 December 2016

Received in revised form 2 March 2017

Accepted 25 March 2017

Available online 13 April 2017

Keywords:

Laccase

Condensed tannins

Hydroxypropylation

Grafting

Pinus radiata

ABSTRACT

Condensed tannins (CTs) from *Pinus radiata* bark were chemically modified with propylene oxide in order to improve their hydrophobicity for beech wood (*Fagus sylvatica* L.) surface modification. CTs and hydroxypropylated tannins (HPTs) were used for laccase catalyzed wood surface grafting at different pH (3, 7, and 10) for the first time. The impact of the modification on the wood surfaces was evaluated by FT-IR mapping, Confocal Fluorescence Emission Microscopy (CFEM) and Water Uptake Behavior (WUB). The effect of the enzyme was evaluated by measuring the total phenolic content (TPC) of the washing solution. The leaching of CTs and HPTs was decreased due to the enzyme activity. HPTs showed up to 30% less leaching after the surface coating in comparison to CTs. CFEM results suggested that the best grafting conditions occurred at pH 10, for both polyphenols used. CT- and HPT-treated samples increased their surface hydrophobicity, which resulted in a lower water uptake, during the first 12 h. The results highlighted that the enzymatic grafting of CTs and HPTs on beech wood surfaces can be a promising strategy for the functionalization of lignocellulosic materials in order to provide new properties for a wide range of applications.

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1. Introduction

Condensed tannins (CTs) are one of the most abundant secondary metabolites in nature. These high-molecular-weight (M_w) polyphenols can be found mainly in the bark of conifers and legumes. Due to the biological properties of CTs, several polyphenols are involved in the defensive mechanisms of plants against pathogen agents like fungi and bacteria, as well as in UV-light protection (Shashank and Abhay, 2013; Tyagi et al., 2015). CTs from mimosa (*Acacia mearnsii*), quebracho (*Schinopsis balansae* and *Schinopsi lorentzii*), and pine (*Pinus pinaster*) are already commercially available. However, applications are mainly focused on the adhesives industry. Conventional uses for CTs include several applications such as food preservation, leather tanning and more specifically as nutraceutical chemicals (Pizzi, 2008; De Bruyne

et al., 1999; Cobb, 2014; Bianchi et al., 2015; Lacoste et al., 2013; Matamala et al., 2000).

In order to diversify physicochemical properties, the derivatization of CTs could increase their range of potential use (García et al., 2016a, 2016b). For instance, hydroxypropylation is a recognized pathway to tailor the reactivity and increase the hydrophobicity of polyphenols (García et al., 2015a, 2015b). Such derivatization reaction changes the solubility pattern of CTs, as well as related physicochemical properties to a great extent.

On the other hand, the last decade has seen an increasing interest in wood protection using natural preservatives (Singh and Singh, 2012). One of the main drawbacks in the use of natural wood protectors is their leaching under exposure to atmospheric conditions. Laccase, which is an enzyme produced mainly by lignin degrading fungi, is an effective catalyst with a high potential to prevent the leaching of phenolic compounds grafted on wood. The enzyme has the ability to oxidize phenolic compounds, thereby allowing the covalent bonding of the phenolic extractives to wood surfaces (Fernández-Fernández et al., 2014).

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By improving the physical and biological properties of the phenolic compounds due to chemical derivatization and via an irreversible coupling, it can be possible to modify the wood's properties in an environmentally friendly way (Rättö et al., 2004; Widsten et al., 2010). In comparison to conventional treatments, the grafted phenolic compounds may improve wood preservation, as CTs from the outer bark layers chemically protect trees from environmentally and biotically induced degradation.

Therefore, this study deals with the combined effect of pilot plant extracted CTs or synthesized hydroxypropylated tannins (HPTs) together with the phenoloxidase laccase on the surface properties of beech wood (*Fagus sylvatica* L.). The hydroxypropylation of CTs was utilized in order to tailor reactivity and to improve hydrophobicity on the wood surface (García et al., 2013). The impact of the treatments on the wood's features was assessed by several physicochemical and molecular methods.

2. Materials and methods

2.1. Materials

CTs from pine bark (*P. radiata* (D. Don)) were supplied by the UDT (Unidad de Desarrollo Tecnológico), which belongs to the University of Concepción, Biobío, Chile. CTs were extracted under pilot-scale conditions at 120 °C for 120 min in a batch reactor using a methanol:water solution (1:6 w/v). In a second step, the hydrophobic CTs extract was precipitated by vacuum evaporation (5 mbar). This hydrophobic phenolic fraction comprised low M_w compounds such as stilbenes (Resveratrol), flavonoids (Taxifolin), and terpenoids (Dehydroabietic acid), and catechin oligomers (degree of polymerization (DP): 1–4). This hydrophobic fraction was washed with distilled water and oven-dried.

Beech wood was imported from the USA by Maderas Tarapacá (Santiago, Chile).

Laccase enzyme from *Myceliophthora termophila* (NS51003) was kindly provided by Novozymes (Bagsværd, Denmark). The activity of the enzyme was calculated by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) oxidation assay. One unit of activity is defined as the amount of enzyme that oxidizes 1 μmol of ABTS per minute at 25 °C of temperature and pH 7 in a 0.1 M phosphate buffer).

Propylene oxide (PO) (Sigma-Aldrich, St. Louis, USA) and hydrochloric acid (Winkler Winkler Química LTDA, Santiago, Chile) were used for the chemical derivatization of CTs.

Acetic acid (Fischer Scientific, Waltham, USA), sodium acetate (JT Baker), sodium phosphate dihydrate (Riedel-de Hein, Pennsylvania, USA), sodium phosphate dibasic (Winkler Química LTDA, Santiago, Chile), potassium phosphate (Honeywell, Seelze, Germany), sodium hydroxide (Merck, Darmstadt, Germany), pyridine (Merck, Darmstadt, Germany), anhydride acetic (Winkler Química LTDA, Santiago, Chile), and tetrahydrofuran (THF) (Merck, Darmstadt, Germany) were used without further purification.

2.2. Hydroxypropylation of CTs

30 g of CTs (103.35 mmol) were dissolved in 250 mL of aqueous buffer solution (pH 14) and the pH was adjusted to 12. The addition of 15 mL of PO within 30 min and a subsequent reaction time of 24 h at room temperature (20–22 °C) under permanent stirring resulted in a PO/tannin monomeric unit ratio of 1.0–2.0. The pH was lowered to 2 by concentrated HCl (40%, v/v) in order to precipitate the CT derivatives. Hydroxypropylated CTs (HPTs) were centrifuged, washed with distilled water five times, and oven-dried (García et al., 2013).

2.3. Enzymatic treatment of beech wood

A treatment solution (TS) of polyphenols (CTs/HPTs: 5 mM) and 1.5 mL of laccase (370 U) dissolved in 60 mL of methanol/buffer (60/40%, v/v) was used to immerse three beech samples with the dimensions (20 × 20 × 5 mm, longitudinal/tangential/radial). The temperature was kept at 50 °C and the reaction was stopped after 2 h. This procedure was repeated for pH 3, 7, and 10. Afterwards, the wood samples were dried at room temperature (18–20 °C) for 24 h. Then the beech samples were washed with a methanol: distilled water solution (WS) (60:40%, v/v) at 50 °C for 30 min, and subsequently washed with distilled water three times and oven-dried for 24 h at 40 °C.

2.4. Gel permeation chromatography (GPC)

CTs and HPTs which were not grafted on the wood surface were recovered from the TS and isolated by rotary-evaporation followed by lyophilization. Polyphenols (20 g) were derivatized with anhydride acetic:pyridine (1:1, v/v) in order to reduce the interaction of the polyphenols with the chromatography column. After two days of reaction, the peracetylated compounds were washed with distilled water and oven-dried at 45 °C for 12 h. Finally, the dried derivatives were dissolved in THF (4 mL) and filtered through a plastic membrane (pore size: 0.45 μm).

Two columns (Phenogel 5u10E3A and 5u10E6A) with a filler made of polystyrene and divinylbenzene (PSDVB) were employed for the determination of the M_w . A mobile phase (THF) with a flow rate of 1 mL min^{-1} was used. The columns were kept at 30 °C during the separation and the peaks were measured at 280 nm using a UV detector.

2.5. Total phenolic content (TPC)

The washing solution was recovered and used to assess the TPC. Washing solution (0.1 mL) was mixed with 0.4 mL of distilled water and 2.5 mL of Folin-Ciocalteu solution (10% v/v). After 8 min of agitation, 2 mL of sodium carbonate (75 g L^{-1}) were added and the solution was kept at 45 °C for 15 min. Finally, the absorbance at 765 nm was measured three times. The results were expressed as relative absorbance.

2.6. Fourier transform infrared spectroscopy (FT-IR mapping)

In order to study the changes in the surface chemistry, the treated wood samples and control samples of CTs and HPTs were analyzed on a FT-IR (ATR) microscope mapping (Spotlight 400, Perkin Elmer, Waltham, USA). The pictures had an area of 200 μm^2 and a spectral resolution of 8 cm^{-1} . Each spectrum was an average of 16 scans. The size of the pixel was 6.25 μm and atmospheric and ATR correction were performed.

2.7. Confocal fluorescence emission microscopy (CFEM)

The surface of the beech wood treated with laccase and CTs/HPTs was analyzed by CFEM (Zeiss LSM780, Carl Zeiss GmbH, Jena, Germany). Control samples that were not washed after the treatment were also analyzed with the same procedure in order to understand fluorescence changes due to the treatment. The configurations used were EC Plan NeoFluar 20x NA 0.5 and EC Plan NeoFluar 40x oil NA 1.3. The fluorescence spectra were recorded using a 405 nm laser that induced fluorescence in 4.4 nm steps within the frequency range of 405–700 nm. The Z-stack pictures were recorded by using a multi-Argon laser of 488 nm that induced emission between 490 and 560 nm. The data acquisition and basic analyses were performed with Z software (Dortmund, Germany)

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