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Partial structural characterization and antioxidant activity of a phenolic–xylan from *Castanea sativa* hardwood

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ABSTRACT

4-*O*-Methylglucuronoxylans (MGX) were isolated from chestnut wood sawdust using two different procedures: chlorite delignification followed by the classical alkaline extraction step, and an unusual green chemistry process of delignification using phthalocyanine/H_2O_2 followed by a simple extraction with hot water. Antioxidant properties of both MGX were evaluated against the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) by electronic spin resonance (ESR). IC_{50} of water-extracted MGX was found to be less than 225 $\mu g\,mL^{-1}$, in contrast with alkali-extracted MGX for which no radical scavenging was observed. Characterization of extracts by colorimetric assay, GC, LC–MS and NMR spectroscopy provided some clues to understanding structure–function relationships of MGX in connection with their antioxidant activity.

1. Introduction

Every year the wood industry is responsible for the production of millions of tons of waste. By-products such as sawdust, bark or chips are rich in polysaccharides, especially cellulose and hemicelluloses. Although hemicelluloses received great interest in recent years because of their biological properties (antitumoral, prebiotic, antimicrobial or antioxidant activities) [1], extraction of these molecules from woody waste requires the use of toxic chemicals that can hamper their valorization at industrial scale.

Among these interesting biological activities, the antioxidant properties of hemicelluloses were particularly studied for their industrial application potential. Antioxidant products are widely used in food and cosmetic industries to delay oxidation. Indeed, oxidation of lipids in food is responsible for the formation of bad flavors and undesirable chemical compounds which may be detrimental to the health. Following the consumers' increasing preference for fresh and natural products, the search for natural antioxidants as a substitute for food additives is growing. Numerous plants and their respective wastes have been screened for this purpose. If evaluations of antioxidant properties of residues from oil, juice, peels, seeds, or marc have already been explored, only a few papers have reported the extraction of antioxidant polysaccharides from wood by-products [2]. In Limousin region in France, forests cover one-third of the territory with roughly 572,000 ha, 75% of which are hardwood and one of the principal species is the chestnut (*Castanea sativa* Mill.). Intensive use generates large volumes of waste and our laboratory currently devotes a significant part of these research activities finding applications with high added value for their molecular constituents. So, in connection with our research program on fractioning the components of forest biomass and the search for new ways of using forestry industry sub-products, especially their polysaccharide fraction, the aim of this work was to study the antioxidant properties of hemicelluloses isolated from hardwood sawdust and for this purpose, we gave special focus to chestnut wood. Moreover, the current interest for green chemistry brought us to develop a biomimetic alternative delignification process of sawdust. A small number of microorganisms, such as white-rot fungi, are known to degrade lignin by the means of enzymes such as laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP) whose combined activities allow the selective oxidative delignification of woody tissues. Because of their extraction and purification are not readily done and their use is non-easy and based on our know-how in the synthesis of tetrapyrrolic macrocycles for oxidation reaction applications, we developed recently a greener delignification of chestnut sawdust

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using tetrasulfophthalocyanine as biomimetic catalyst that allows in a second step non-destructively hot-water extraction of xylans.

Predominant in hardwood hemicellulose, especially in chestnut wood, xylans are known for their biological properties, particularly their antitumoral activity [3]. To our knowledge, the literature is scarce regarding the antioxidant properties of native or modified xylans [4].

Thus, this paper reports the antioxidant properties of 4-*O*-methylglucuronoxylans isolated from chestnut wood sawdust using two different extraction procedures: a classical one using toxic and noxious products and another one using the green chemistry process developed in our laboratory. The two final products were assayed for their antioxidant properties and the relationship between structure and activity was investigated.

2. Materials and methods

2.1. Materials

C. sativa sawdusts were obtained from a local sawmill. Hydrogen peroxide (35%) was purchased from Acros. DPPH was supplied from Sigma (St. Louis, MO).

2.2. General methods

All extracts (see below) were evaporated at temperature <40 °C under reduced pressure. The centrifugation conditions were 10,000 rpm for 15 min at 25 °C. Iron tetrasulfophthalocyanine (FePcS) was synthesized according to Klein-Koerkamp [5] and all structural characteristics were in accordance with our previous paper [6].

2.3. Isolation of (4-O-methyl-α-D-glucurono)-D-xylans (MGXs)

Sawdust was dried 48 h at 60 °C in a ventilated oven, then ground and sieved (500 μm). Ground sawdust was then extracted in a Soxhlet extractor with 80% EtOH (solid/liquid ratio: 1/20) at 80 °C, freeze-dried and stored until purification. The dewaxed sawdust was depectinized using a 1% ammonium oxalate solution (2 h at 80 °C, 50 mL g^{-1} of dewaxed sawdust). Then, two protocols were used for glucuronoxylan extraction.

The first procedure is a classical protocol for MGX extraction from wood [7]. Delignification was done by an acidic sodium chlorite solution (0.47 g of sodium chlorite and 0.2 mL of glacial acetic acid per gram of sawdust) at 80 °C for 2 h. After filtration, the residue was washed with water and then air dried at 60 °C for 16 h to give the holocellulose fraction. Lastly, MGX 1 was extracted from holocellulose by refluxing during 1 h in a 4.3 M KOH solution (50 mL g^{-1} of holocellulose) with $NaBH_4$ (3 mg mL^{-1}) under continuous stirring. The hemicellulose solution was neutralized by addition of glacial acetic acid and dialyzed against water (Spectrapor MWCO 6-8000 Da). Hemicelluloses were lastly precipitated by addition of three volumes of ethanol to the aqueous solution; the pellet was recovered after centrifugation, solubilized in water and then freeze-dried and stored. The second procedure was adapted from Barbat [6]. It consists in delignification of pectin-free sawdust by a water/H_2O_2 solution (84/16 v/v, 100 mL g^{-1} of sawdust) containing iron tetrasulfophthalocyanine (FePcS) (0.056% w/v) during 100 h at room temperature followed by an aqueous extraction of MGX with water during 1 h at reflux. The filtrate was purified by ultrafiltration, then freeze-dried and stored.

2.4. Determination of the chemical composition of extracts

Total sugar content of glucuronoxylans was measured at 490 nm by the phenol–sulfuric acid method [8], using xylose as standard. Uronic acid contents were determined colorimetrically at 520 nm by the *m*-hydroxydiphenyl method [9], with glucuronic acid used to make a standard curve. Reducing sugar contents were determined by the Lever method [10] using 4-hydroxybenzoic acid. Absorbance of samples was read at 410 nm and xylose was used as reference. The degree of polymerization (DP) was established as the ratio of xylosyl monomer concentration to the reducing-end concentration. Total phenol contents were determined by spectrophotometric measurement at 760 nm after reaction with Folin–Ciocalteu reagent using gallic acid for calibration [11]. Assay of aldehyde content was performed using a method adapted from Pommerening [12] consisting in an acid–base titration after a Cannizarro reaction. For each type of determination, assays were done in triplicate.

2.5. Monosaccharide composition

Monosaccharide composition of polysaccharides was determined by gas–liquid chromatography, using a Perichrom gas chromatograph fitted with a flame-ionization detector. Monosaccharides were liberated from polysaccharides (200 μg) after methanolysis (MeOH/HCl 1 M, 24 h, 80 °C). Then the solutions (containing myo-inositol as internal standard) were evaporated under nitrogen and 1 mL of methanol was added. Samples were defatted three times with 1 mL of heptane. After a new evaporation of the solutions, trimethylsilylation was carried out with BSTFA (*N*,*O*-bis-trimethylsilyl-trifluoroacetamide)-pyridine (1:1, v/v; 200 μL) at 27 °C for 2 h. Separation of the *per*trimethyl-silylated methylglycosides was done using a CPSIL-5CB capillary column (Chrompack, 0.32 mm × 50 m), with the following temperature program 120 × –240 °C at 2 °C min^{-1}. Nitrogen was the carrier gas at 0.5 atm.

2.6. NMR spectroscopy

MGX samples were freeze-dried three times in D_2O and then dissolved into 600 μL of D_2O (99.97% purity, Euriso-top, Saclay, France). Samples were analyzed at 300 K in 5 mm o.d. BMS-005-B Shigemi tubes on Bruker DPX-400 spectrometer operating at 400.13 MHz (^1H). ^1H spectra were acquired using a 5.7 kHz spectral width with 32 K data points, 2.831 s acquisition time, and 152 scans were accumulated (^1H 90° pulse = 5.2 × μs). ^1H chemical shifts (ppm) were calibrated relative to the signals from acetone used as an internal standard, at 2.22 ppm.

2.7. Antioxidant activity

Antioxidant activity has been determined by electron spin resonance (ESR) (BrukerESP300E spectrometer). The study of the ability of the different MGXs to scavenge the stable DPPH radical was adapted from a previously described procedure [13]. Briefly, microsampling pipets were used at room temperature. ESR spectra were measured under the following conditions: 100 kHz modulation frequency, 9.78 GHz microwave frequency, 4 mW microwave power, 1.97 modulation amplitude, and 10.24 ms time constant. The tested compounds were dissolved in water at different concentrations and scattered during 10 min under ultra-sonication. The activities were evaluated after mixing 50 μL of 5×10^{-4} M 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol with 50 μL of MGX solutions. ESR spectra were recorded 3 min after mixing. Inhibition was calculated as follows: inhibition = [ref − compound]/[ref − bg], where ref and compound are the values of the double integrals for the ESR spectra of the reference (DPPH + water) and the tested solution (DPPH + MGX in water) respectively; bg represents the background signal (water). Each data is the result of the average of three independent measurements. Inhibition of 50% of the DPPH