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Isolation of suberin from birch outer bark and cork using ionic liquids: A new source of macromonomers

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ABSTRACT

Cholinium hexanoate, a biocompatible and biodegradable ionic liquid, was recently demonstrated to efficiently and selectively extract suberin domains from cork, combining high extraction efficiency with isolation of a partial depolymerised material. In the present paper, we report a comparative study of the characterisation of suberin extracted from birch outer bark and from cork using cholinium hexanoate. It became apparent that both extracted suberin samples showed still a cross-linked nature, *i.e.* likely to be closely related to *in situ* suberin. Suberin samples were mainly constituted by oligomeric or polymeric structures in turn essentially composed by long chain hydroxyacids monomers. Their high thermal stability together with the oligomeric/polymeric nature, open new perspectives for suberin use as macromonomers in the development of bio-based polymeric materials. This also contributes for the valorisation of suberin rich agro-forest residues.

1. Introduction

Knowledge on natural polymers, such as starch, cotton, proteins and wool, is ancient and originated in the 19th century (Sperling, 2006; Gandini, 2011). During the last decade, we have been witnessing a renewed and exponential increase of interest in the production of chemicals, materials, fuels and energy obtained from renewable resources. This is especially true in the so-called biorefinery concept (Kamm et al., 2000; Mosier et al., 2005), which involves, in many cases, application of valuable components from by-products of agro-forest industries, such as suberin from cork residues.

Suberin, a complex aromatic–aliphatic cross-linked biopolyester, is widespread in the plant Kingdom but it is particularly abundant in *Quercus suber* L. cork (30–50 wt%) and *Betuta pendula* outer bark (40–50 wt%) (Pereira, 1988; Lopes et al., 2000a,b, 2001; Gandini et al., 2006; Pinto et al., 2009). This hydrophobic biopolyester plays a key role as a protective barrier between the plant and the environment (Pollard et al., 2008). Suberin constitutes a major natural source of valuable compounds such as ω-hydroxyacids, α,ω-dicarboxylic acids and corresponding mid-chain epoxy or dihydroxy derivatives (Gandini et al., 2006; Pinto et al., 2009). These compounds have attracted considerable attention as building blocks for polymer synthesis (Gandini et al., 2006; Olsson et al., 2007; Sousa et al., 2008, 2011).

Wastes derived from birch kraft pulp mills and cork industries are produced in large amounts, corresponding to ~3.4 wt% (Ekman, 1983; Paper and wood insights, 2006) and ~23 wt% (Gil, 1988) of the total production, respectively. Up to present, their exploitation is often limited to burning in biomass boilers to produce energy. However, substantial valorisation can be attained if valuable components are extracted prior to burning.

Suberin can be isolated from cork and birch outer bark residues by a set of well defined depolymerisation methodologies. They normally require harsh chemical processes of ester bond cleavage through alkaline methanolysis with sodium methoxide, or by aqueous alkaline hydrolysis (Ekman and Eckerman, 1985; Gandini et al., 2006). Suberin partial depolymerisation can also be achieved using more gentle (though less efficient) extraction processes, *e.g.* calcium oxide methanolysis (Graça and Pereira, 1997, 1999, 2000a,c).

Advances in suberin extraction under milder and environmentally benign conditions will certainly foster its wider application. Recently it has been demonstrated that extraction of suberin from cork can also be attained using cholinium hexanoate as solvent (Garcia et al., 2010; Ferreira et al., 2012). This biocompatible and

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biodegradable ionic liquid (Petkovic et al., 2010), was able to promote a specific and efficient extraction of the suberin domains from cork. The isolated suberin will certainly display distinct properties from those obtained by conventional depolymerisation methods. This observation, together with the environmental sustainability of the ionic liquid extraction process, opens perspectives for new applications, directly or after chemical modification.

The successful extraction of suberin from cork with cholinium hexanoate (Garcia et al., 2010; Ferreira et al., 2012) prompted us to isolate also suberin from birch outer bark. Our aim is to carry out a comparative study focussing on the chemical composition and the thermal behaviour of suberin samples isolated from cork and birch outer bark. The data makes apparent the high versatility of the ionic liquid mild extraction process for the isolation of oligomeric/polymeric suberin fractions displaying a thermal behaviour comparable to that of the starting materials.

2. Materials and methods

2.1. Cork and birch outer bark samples

Granulated cork was obtained from the cork producers Amorim & Irmãos SA (St^{a} Maria de Lamas, Portugal). *Betula pendula* outer bark samples were collected from the debarking line at a birch kraft pulp mill in Finland. The industrial birch outer bark was ground in a laboratory mill to pass a 6-mm screen, followed by separation in water into floating outer bark and sedimented inner bark.

Cork and birch outer bark samples were ground to a powder (<1 mm) using a centrifuge mill (Retsch) and the soluble extractives removed by Soxhlet extraction with solvents of increasing polarity (dichloromethane, ethanol and water) as previously described by Gil et al. (1997). The extractive-free powders, hereafter defined solely as cork and birch outer bark (starting materials), were further washed in an excess of deionised water, and then dried prior to use.

2.2. Chemicals

Cholinium hexanoate was synthesised by dropwise addition of hexanoic acid to aqueous cholinium hydrogen carbonate (Sigma ~80% in water) in equimolar quantities, as described by Petkovic et al. (2010). The ionic liquid was dried prior to use by stir-heating *in vacuo* (40–50 °C, *ca.* 0.01 mbar). Dimethyl sulfoxide (DMSO) of analytical grade was purchased from Sigma.

2.3. Suberin extraction

The extraction process followed an optimal methodology previously described by Ferreira et al. (2012). Briefly, the cholinium hexanoate (T_m = 60.57 °C) was mixed with cork or birch outer bark (ionic liquid:powder ≈ 9:1 wt/wt) and kept at 100 °C during 4 h, with stirring. At the end of the extraction process the mixture was filtrated (DMSO was added, 5–10 times the reaction volume, in order to assist the filtration step) and the insoluble residue washed thoroughly with an excess of water. The filtrate was kept at 4 °C for a period of 1 h, leading to the precipitation of the extracted suberin, which was then isolated by centrifugation, washed twice with an excess of water, and dried.

2.4. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra were collected on a Bruker IFS66/S FTIR spectrometer (Bruker Daltonics, MA, USA) using a single reflection ATR cell (DuraDisk, equipped with a diamond crystal). Data were recorded at room temperature, in the range of 4000–600 cm^{-1}, by accumulating 128 scans with a resolution of 4 cm^{-1}. Five replica spectra were collected for each sample in order to evaluate reproducibility (OPUS v5.0).

2.5. ^{13}C cross polarization/magic angle spinning nuclear magnetic resonance spectroscopy (^{13}C CP/MAS NMR)

^{13}C CP/MAS NMR spectra were recorded at 9.4 T on a Brüker 400 spectrometer using 9 kHz spinning rate and MAS with proton 90° pulses of 4 μs. Chemical shifts are given in ppm from glycine. The NMR spectra were processed and analysed with MestreNova v. 6.0 (MestreLab Research S.L.).

2.6. Gas chromatography–mass spectrometry (GC–MS)

A Trace GC 2000 Series gas chromatograph equipped with a Thermo Scientific DSQ II mass spectrometer was used. The GC–MS was first calibrated with pure reference compounds (representative of the major classes of compounds present in suberin) relative to *n*-hexadecane (internal standard). Compounds identification was based on the equipment spectral library (Wiley–Nist) and on previously published data, focussing their EI-MS fragmentation patterns and/or retention times (Ekman and Eckerman, 1985; Cordeiro et al., 1998a; Lopes et al., 2000a; Pinto et al., 2009). Replicates were done to guarantee low variability and each analysis repeated twice. Each sample was analysed by two complementary methods:

- Method 1, suberin samples were converted to the corresponding trimethylsilyl (TMS) derivatives and analysed quantitatively by GC–MS, allowing the identification of monomeric structures present in the mixture. In brief, suberin samples (*ca.* 15 mg) were reacted with 250 μL of pyridine, 250 μL of *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide and 50 μL of trimethylchlorosilane during 30 min at 70 °C (Ekman, 1983).
- Method 2, in order to analyse the composition of the oligomeric/polymeric fraction of suberin, samples were, prior to the silylation, submitted to an alkaline hydrolysis to release hydrolysable monomeric constituents. Briefly, suberin samples were treated with a solution of 0.5 M NaOH in methanol/water (1:1, v/v), at 95 °C, during 4 h (Sousa et al., 2006). The mixture was cooled to room temperature, acidified to pH 3–3.5 with 1 M HCl, extracted three times with dichloromethane, and the combined organic extracts were dried in a rotary evaporator. Finally, samples were trimethylsilylated as mentioned above, prior to GC–MS analysis.

2.7. Thermogravimetric analysis (TGA)

TGA data were obtained using a TGA-Q50 TA Instruments. All samples were run in crimped aluminium pans with pinhole under a nitrogen atmosphere (100 cm^3 min^{-1}). Samples were heated up to 600 °C, at a heating rate of 10 °C min^{-1}. Universal Analysis, version 4.4A software was used to determine: degradation temperature ($T_{x\%,\,deg}$), onset temperature (T_{onset}), maximum decomposition temperature ($T_{d,max}$), weight of water adsorbed by the sample in equilibrium with atmosphere (wt_{H_2O}), weight of the solid residue remaining at 600 °C ($wt_{600\,°C}$) and derivative thermograms (DTGA). $T_{x\%,\,deg}$ and T_{onset} were respectively defined as the temperature of a specific weight loss and as the intersection of the baseline weight with the tangent of the weight *vs.* temperature curve as decomposition occurs. $T_{d,max}$ and wt_{H_2O} were respectively defined as the derivative curve (dwt/dT) maximum and the weight loss occurring since the beginning of the experiment until 100 °C.