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Infrared spectroscopy and multivariate analysis to appraise α-cellulose extracted from wood for stable carbon isotope measurements

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ABSTRACT

Wood is a heterogeneous material mainly constituted of cellulose, hemicelluloses, lignin, and extractives which all have different isotopic signatures. For applications where $\delta^{13}C$ measurement of α-cellulose is required, it is important that residues of the other constituents remain below an acceptable level. The laboratory method most widely used for the extraction of α-cellulose consists of several successive treatments: organic solvents and boiled water, acidified sodium chlorite ($NaClO_2$) application, and finally a NaOH treatment. Different variants of this method were tested systematically to optimize the extraction of α-cellulose from wood samples of four tree species: oak, beech, poplar, and pine. Mid-infrared spectroscopy in Attenuated Total Reflection mode (IR-ATR) combined with a curve resolution method (Bayesian Positive Sources Separation statistical analysis) was used to monitor the residues of other wood constituents in extracted α-cellulose. IR-ATR spectroscopy was shown to be sensitive enough to detect residual compounds in α-cellulose extracts below a concentration which does not present a measurable bias for $\delta^{13}C$ measurements. For all tree species, a residual concentration of lignin below the bias threshold for $\delta^{13}C$ measurements was reached with fewer additions of acidic $NaClO_2$ than usually reported. Further the NaOH treatment step was not necessary to remove the hemicelluloses from oak and beech. Infrared spectroscopy combined with a curve resolution method is appropriate to improve α-cellulose extraction species-specifically for reliable stable carbon isotope $\delta^{13}C$ measurements. It allows to check the extracted α-cellulose and to reduce the consumption of chemicals, the extraction time and the loss of α-cellulose.

1. Introduction

Trunk wood is a spatial and temporal integrator of the carbon assimilated by a tree and thus of its organic signature ($\delta^{13}C$, the measure of the carbon stable isotope ratio $^{13}C/^{12}C$) (Farquhar et al., 1982). Stable isotopes of carbon appear to provide a strong proxy indicator for reconstructing precisely dated annual climatic history not only from living but also from subfossil trees (Stuiver and Braziunas, 1987; Loader et al., 1995; Robertson et al., 2008). The major constituents of wood; i.e. cellulose, lignin, hemicelluloses and extractives, have different $\delta^{13}C$ values due to the different biological pathway used for their synthesis (Wilson and Grinsted, 1977; Benner et al., 1987; Dungait et al., 2008). A difference in isotopic composition between wood components will result in an apparent variability of whole wood $\delta^{13}C$ if there is a variability of the relative quantity of the components among samples. All three components of wood can show relatively large variations, within as well as between species (Chen et al., 2010). Consequently it was suggested that α-cellulose from wood is more appropriate to measure $\delta^{13}C$ rather than the bulk wood due to its molecular homogeneity (Au and Tardif, 2009). Most dendro-isotopical studies have concentrated on the analysis of α-cellulose as the dominant and most easily isolated component of the wood (McCarroll and Loader, 2004). The benefice and the necessity to have knowledge of the cellulose purity or at least isotopic fidelity for the current and future stable isotope studies for dendroclimatology community was already shown (Brookman and Whittaker, 2012). In fact these studies are recognized as a mean of high resolution terrestrial paleoclimate reconstruction which even over multicentennial scales are based only on small amplitude changes in the isotopic record (<6‰) (Kress et al., 2010). These recommendations also concern carbon isotope ^{13}C studies on fossil wood or decayed

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wood pieces unlikely to be in their original condition which generate changes in wood component proportions and in their isotopic values (Schleser et al., 1999).

The ideal α-cellulose extraction process for carbon isotopic analyses aims at removing the extractives, lignin, pectin and hemicelluloses from a wood sample; and at avoiding the degradation of the cellulose polymer. The most widely used methods are variants of the method described by Jayme (Jayme, 1942) and Wise (Wise, 1945) which uses solvents and boiled water to remove extractives (i.e. oils, resins, inorganic salts, starch, low molecular polysaccharides…), then acidified sodium chlorite to digest the lignin, and finally alkaline solutions to remove the hemicelluloses and to produce the "analytical α-cellulose" defined as a "highly refined anhydroglucose alkali-resistant cellulose". During the delignification process, $NaClO_2$ is consumed and should be added in sufficient quantity. Since Green (Green, 1963), the most frequent concentration of the $NaClO_2$ solution used was close to 2%, which was applied for 1 h at 70 °C in successive additions. However, some authors have changed the concentration and the duration of this $NaClO_2$ application (Leavitt and Danzer, 1993; Gaudinski et al., 2005). The conditions of using the alkaline solution also vary widely in concentration, temperature, and time among publications (Wise, 1945; Rapson and Morbey, 1959; Loader et al., 1997; Rinne et al., 2005). No clear justifications were proposed about the choice of those experimental conditions, and only few authors have tested their modifications of the original extraction protocol and their applications on tree species (Leavitt and Danzer, 1993; Gaudinski et al., 2005; Rinne et al., 2005). To study the effects of variants of the original methods on the purity of the extracted cellulose as well as to control the quality of extractions for isotopic analyses, a non-invasive method to monitor the presence of residual wood components in the cellulose is needed.

Infrared spectroscopy (IR) is a valuable tool for the analysis of the biochemical composition of wood (Pandey and Pitman, 2003; Mohebby, 2005). It has been applied to monitor the extraction of cellulose from *Pinus sylvestris* (Rinne et al., 2005) or to verify the purity of extracted α-cellulose from woods of different tree species (Brendel et al., 2000; Anchukaitis et al., 2008; Brookman and Whittaker, 2012). Chemometric (statistical) analyses on series of infrared spectra have been used to assess the physical properties and the chemical composition of woods (Chen et al., 2010; González-Peña and Hale, 2011). However, to the best of our knowledge, the analysis of the infrared spectra for the estimation of residual compounds in the α-cellulose obtained from processes of extraction from different tree species has not been carried out. The Bayesian Positive Source Separation (or BPSS) curve resolution method (Moussaoui et al., 2006a,b) can be used to estimate the spectra of a mixture of unknown components (here cellulose and residuals) as well as the relative concentrations of these components, from the vibrational spectra recorded.

In the present work, we aimed at obtaining a satisfactory quality of the chemically extracted α-cellulose for unbiased $\delta^{13}C$ isotope measurements. For this purpose, we have systematically tested several experimental conditions for α-cellulose extraction. Infrared spectroscopy associated with the BPSS chemometric method was used to monitor residual compounds in cellulose which was extracted from wood using a range of conditions. For this, (i) we needed and provided spectral information on commercially available cellulose, hemicelluloses and lignin compounds to judge their value as standards representative of the components of the bulk wood; (ii) we compared the detection limits of infrared spectroscopy and $\delta^{13}C$ on synthetic mixtures of commercial lignin and hemicellulose compounds in a commercial cellulose matrix; and (iii) we evaluated from the infrared spectra, the occurrence of extractives, lignin and hemicellulose residues in cellulosic samples for the range of extraction conditions, and their impact on the purity, yield and isotopic $\delta^{13}C$ values of the extracted α-cellulose. This was done for wood of different tree species, chosen to represent a range of wood types (gymnosperm, angiosperm ring-porous and angiosperm diffuse-porous woods).

2. Materials and methods

2.1. Chemicals

Celluloses from spruce wood, from cotton fibres, and α-cellulose from wood pulp of broadleaf trees were purchased from Fluka (batch 22182), Whatman, and Sigma (batch C6429), respectively. Organosolv lignin from a mixture of trees (50% maple, 35% birch, 15% poplar), and Kraft lignin from fir were purchased from Aldrich (Batches 371017 and 471003, respectively). Klason lignin from *Quercus petraea* was provided by T. Eglin (University of Orsay, France). Galactomannan (β1-4) from *Ceratonia siliqua* and poly(β−D-xylopyranose1-4) from *Fagus sylvatica* were purchased from Sigma (batches 48230 and x4252, respectively). NaOH (97%, Normapur) was purchased from Carlo Erba. Toluene (99.5%), {$NaClO_2$, H_2O} (96%), ethanol (95% and 99.8%), and glacial acetic acid (99%) were purchased from VWR.

2.2. Plant material

Four different woods from tree species with different structures of the vascular system were used for the experiments to maximize the differences in biochemical composition: three broadleaf (angiosperm) species consisting in one ring porous (*Q. petraea*, oak), and two diffuse porous species (*F. sylvatica*, beech and *Populus deltaoïdes x nigra I214*, poplar); and one conifer (gymnosperm) species (*Pinus pinaster*, pine). All the woods were from adult trees and several rings of a cross section were pulverized using a ring mill (Sodemi CB2200, France), and then 5 g were milled for 1 min. at 30 Hz in a ball-mill (Retsch MM301, Germany) using 10 mL grinding jars with one stainless steel ball (diameter of 6 mm), to yield a representative, homogeneous sample of sufficient quantity for all experiments.

2.3. Synthetic mixtures

To determine the detection limits of lignin and hemicelluloses (galactomannan) in a cellulose matrix, several synthetic mixtures were made with 2, 4, 6 or 10 wt.% of Kraft lignin (Aldrich) or D-galactomannan (Sigma) in the cellulose (Fluka) matrix (Table SM1 in Supplementary Material or SM). These powders were milled separately in the same conditions as reported for plant material. Then the synthetic mixtures of the commercial compounds were homogenised by vortex-mixing before the $\delta^{13}C$ measurements and the recording of the infrared spectra.

2.4. Cellulose extraction procedure

The method adopted for the extraction of the cellulosic materials from the wood is a variant of the acidified sodium chlorite extraction method as originally described by Jayme (1942) and Wise (1945), and consists of three steps resulting in three types of material: extractives free wood, then holocellulose, and finally analytical, alkali resistant, α-cellulose. The chemical protocol and the name of each chemical treatment for all the variants of the extraction method are presented in Table 1 and the corresponding flow scheme is shown in Fig. 1. Bulk wood, extractives free wood, holocelluloses and α-cellulose for each wood tested were analysed by mass spectrometry and mid-infrared spectroscopy (details are given below).

The powdered woods were dried at 60 °C for 24 h in the oven. Then, for each wood sample, aliquots of 0.15 g were put into individual pouches made of PTFE pre-cut filter membranes with a pore size of 1 μm (Alltech, France). The filter membrane was shaped into a pouch which was closed with a Teflon ribbon ("sample pouches"). Three replicated extractions have been performed on the same sample material. The "sample pouches" destined for spectrometric and isotopic analyses at each step of the extraction process were collected and the respective content was transferred from the pouches into glass sampling tubes and