

# **Infrared spectroscopy and multivariate analysis to appraise $\alpha$ -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  $\delta^{13}\text{C}$  measurement of  $\alpha$ -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  $\alpha$ -cellulose consists of several successive treatments: organic solvents and boiled water, acidified sodium chlorite ( $\text{NaClO}_2$ ) application, and finally a  $\text{NaOH}$  treatment. Different variants of this method were tested systematically to optimize the extraction of  $\alpha$ -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  $\alpha$ -cellulose. IR-ATR spectroscopy was shown to be sensitive enough to assess the significant occurrence of residual compounds in  $\alpha$ -celluloses extracted from different species. For all tree species, sufficient removal of lignin for isotopic analyses was reached with fewer additions of acidic  $\text{NaClO}_2$  than usually reported; further the  $\text{NaOH}$  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  $\alpha$ -cellulose extraction species-specifically for reliable stable carbon isotope  $\delta^{13}\text{C}$  measurements. It allows to check the purity of the  $\alpha$ -cellulose extract and to reduce the consumption of chemicals, the extraction time and the loss of  $\alpha$ -cellulose.

## Keywords

Wood,  $^{13}\text{C}$  composition,  $\alpha$ -cellulose extraction, mid-infrared spectroscopy, multivariate statistical analysis

## 1. Introduction

Trunk wood is a spatial and temporal integrator of the carbon assimilated by a tree and thus of its organic signature ( $\delta^{13}\text{C}$ , the measure of the carbon stable isotope ratio  $^{13}\text{C}/^{12}\text{C}$ ) (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). The major constituents of wood; *i.e.* cellulose, lignin, hemicelluloses and extractives, have different  $\delta^{13}\text{C}$  values due to the different biological pathway used for their synthesis (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  $\delta^{13}\text{C}$  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  $\alpha$ -cellulose from wood is more appropriate to measure  $\delta^{13}\text{C}$  rather than the bulk wood due to its molecular homogeneity (Leavitt and Danzer, 1993). Most dendro-isotopical studies have concentrated on the analysis of  $\alpha$ -cellulose as the dominant and most easily isolated component of the wood (McCarroll and Loader, 2004). The beneficence 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 concerns carbon isotopes studies on fossil wood or decayed 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  $\alpha$ -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  $\alpha$ -cellulose” defined as a “highly refined

anhydroglucose alkali-resistant cellulose". During the delignification process, NaClO<sub>2</sub> is consumed and should be added in sufficient quantity. The most frequent concentration of the NaClO<sub>2</sub> solution used was close to 2%, which was applied for one hour at 70°C in successive additions. However, some authors have changed the concentration and the duration of this NaClO<sub>2</sub> 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; 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; Rinne et al., 2005; Gaudinski 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; Mohebbi, 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  $\alpha$ -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  $\alpha$ -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., 2006) 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  $\alpha$ -cellulose for unbiased  $\delta^{13}\text{C}$  isotope measurements. For this purpose, we have systematically tested several experimental conditions for  $\alpha$ -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  $\delta^{13}\text{C}$  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 hemicelluloses residues in cellulosic samples for the range of extraction conditions, and their impact on the purity, yield and isotopic  $\delta^{13}\text{C}$  values of the extracted  $\alpha$ -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  $\alpha$ -cellulose from wood pulp of broadleaf trees were purchased from Fluka (batch 22182), Whatman, and Sigma (batch C6429), respectively. Organosolv extracted lignin from a mixture of trees (50% maple, 35% birch, 15% poplar), and Kraft process extracted lignin from fir were purchased from Aldrich (Batches 371017 and 471003, respectively). Klason process extracted lignin from *Quercus petraea* was provided by T. Eglin (University of Orsay, France). Galactomannan ( $\beta$ 1-4) from *Ceratonia siliqua* and poly( $\beta$ -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<sub>2</sub>, H<sub>2</sub>O} (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 (*Quercus petraea*, oak), and two diffuse porous species (*Fagus sylvatica*, beech and *Populus deltaoides 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, France), and then milled for 1 min. at 30 Hz in a ball-mill (Retsch MM301, Germany), 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 lignin (Aldrich) or D-galactomannan (Sigma) in the cellulose (Fluka) matrix (Table SM1 in Supplementary Material or SM). These powders were milled separately for 1 min, at 30 Hz in a stainless steel ball-mill (Retsch MM301, Germany) with one stainless bead. Then the synthetic mixtures of the commercial compounds were homogenised by vortex-mixing before the  $\delta^{13}\text{C}$  measurements and the recording of the infrared spectra.

### 2.4. *Cellulose extraction procedure*

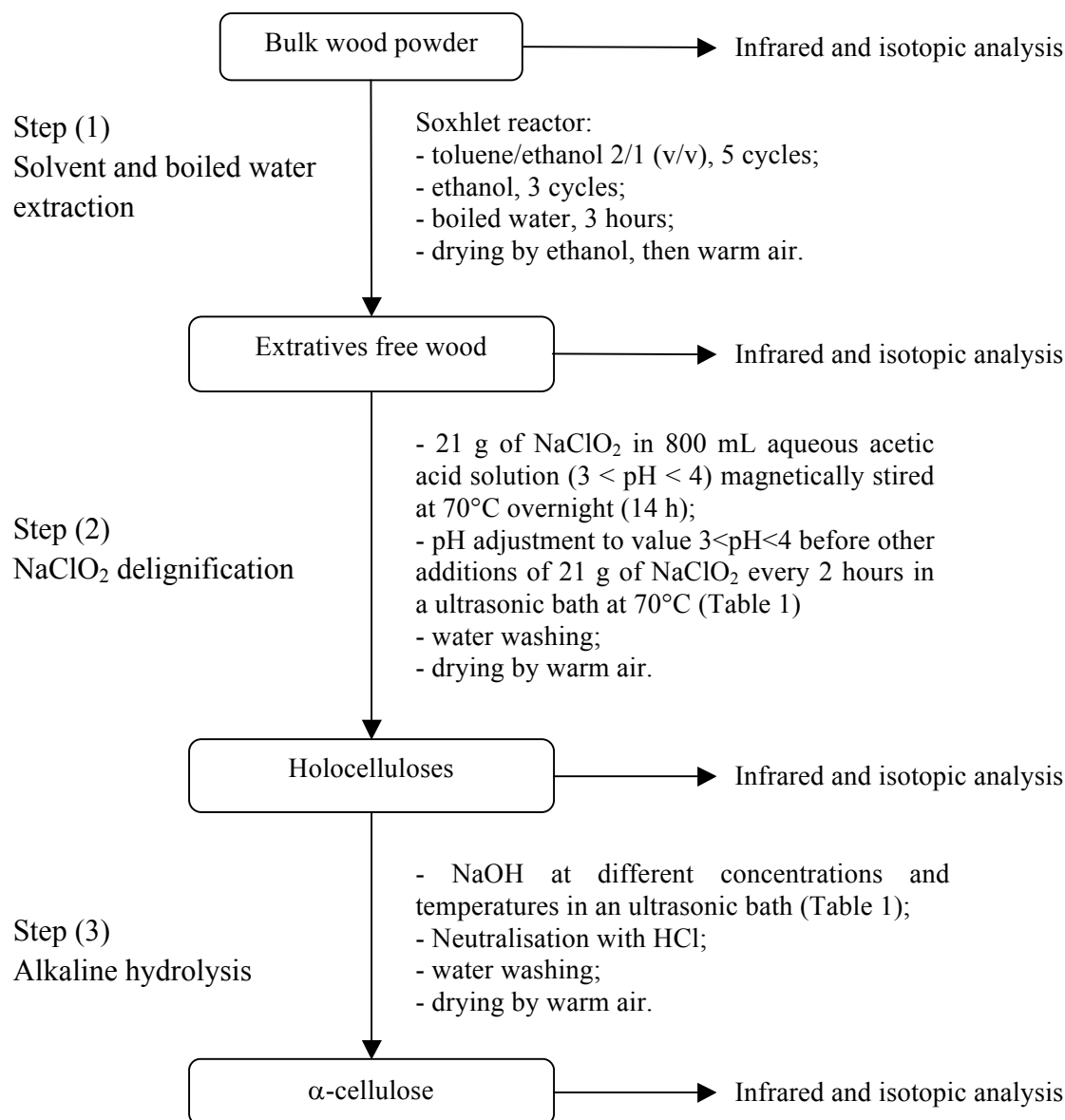
The method adopted for the extraction of the cellulosic materials from the wood is a variant of the acidified sodium chlorite extraction method as originally described by Jayme (Jayme, 1942) and Wise (Wise, 1945), and consists in three steps resulting in three types of material: extractives free wood, then holocellulose, and finally analytical, alkali resistant,  $\alpha$ -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  $\alpha$ -cellulose for each wood tested were analysed by mass spectrometry and mid-infrared spectroscopy (details are given below).

**Table 1** Chemical treatments for the cellulose extraction for oak, beech, poplar and pine wood samples. Refer to Fig. 1 for more details about solvent extraction, delignification and alkaline hydrolysis.

Treatment	Step (1)	Step (2)	Step (3)		
	Solvent and water boiled extraction	Delignification	NaOH Concentration (%) (w/v)	Temperature (°C)	
	(same process on all the treatments)	Number of NaClO <sub>2</sub> additions / total time of NaClO <sub>2</sub> treatment (hours)			
Soft treatments	0	yes	0 / 0	-	
	0A	yes	0 / 0	39 <sup>b</sup>	
	2	yes	2 / 16	-	
	2A	yes	2 / 16	39 <sup>b</sup>	
	5	yes	5 / 22	-	
	5A	yes	5 / 22	0.4	39 <sup>b</sup>
Hard treatments	5B <sup>a</sup>	yes	5 / 22	10	39 <sup>b</sup>
	5C <sup>a</sup>	yes	5 / 22	10	80
	5D <sup>a</sup>	yes	5 / 22	17	39 <sup>b</sup>
	5E <sup>a</sup>	yes	5 / 22	10 & 17	80 & 39 <sup>b</sup>

<sup>a</sup> Not performed on beech

<sup>b</sup> Measured temperature without freezing the NaOH solution



**Fig. 1.** Flow scheme showing the chemical protocol of  $\alpha$ -cellulose extraction from wood for infrared spectroscopy and carbon isotope ratio analysis. Refer to Table 1 for more details about solvent extraction, delignification and alkaline hydrolysis.

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  $\mu$ m (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 dried in an oven at 60°C for 36 h, and then immediately weighted (balance Sartorius analytic, delta range 212 g x 0.1mg). The proportions of extractives (Pe), holocellulose (Pho) and  $\alpha$ -cellulose (Pa) were calculated using the oven-dried mass of extractives, holocellulose and  $\alpha$ -cellulose relative to the original mass of the oven-dried wood powder.

Treatments 0 to 5A (Table 1) that do not use NaOH or low concentration of NaOH are hereafter called “soft treatments”. Treatments 5B to 5E are called “hard treatments”. The commercial cellulose sample from spruce wood (Fluka) was cleaned with the  $\alpha$ -cellulose extraction process using treatment 5B (Table 1) and dried before being used as a cellulose standard.

## 2.5. Carbon isotope analysis

For the  $\delta^{13}\text{C}$  and the %C measurements, 1 mg of material was weighed into 4 mm  $\times$  6 mm tin capsules (Elementary microanalysis, Okehampton, UK). The isotopic analyses were performed with an Elementar Analyser (Carlo Erba, Milano) coupled to a Finnigan continuous flow isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany) at the stable isotope facility at Functional Ecology PTEF (OC 081) at INRA Nancy, France. The carbon isotope composition was expressed according to the conventional  $\delta$  (‰) notation:  $\delta^{13}\text{C} = [(R_s - R_{\text{PDB}})/R_{\text{PDB}}] \times 1000$  where  $R_s$  and  $R_{\text{PDB}}$  are the molar abundance ratios of the carbon isotopes  $^{13}\text{C}/^{12}\text{C}$  in the sample and the Vienna Pee Dee Belemnite (VPDB) standard, respectively. The mean standard deviation of the used standard (saccharose) for  $\delta^{13}\text{C}$  measurements was 0.11‰.

## 2.6. Statistical analysis of the carbon isotope data

The isotopic composition of artificial mixtures against the pure cellulose standard (spruce wood  $\alpha$ -cellulose from Fluka), and the effect of residual constituents on the isotopic composition of extracted  $\alpha$ -cellulose were evaluated using ANOVA with the following model:  $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ , where  $Y_{ij}$  is the response variable;  $\mu$  the intercept;  $\alpha_i$  the treatment effect and  $\varepsilon_{ij}$  the residue. A Dunnett contrast test was used to evaluate modalities of the artificial mixtures *versus* the pure

spruce wood  $\alpha$ -cellulose standard as a control. We used multiple pairwise comparisons of means with the Tukey contrast test to evaluate the effect of residual constituents on the isotopic composition of extracted  $\alpha$ -cellulose. All the statistics were fitted with the R software (R-Development-core-Team, 2008). Multiple comparison tests were performed on parameters of interest with the multcomp R-package. Normality and homoscedasticity were tested graphically with residual *versus* predicted and normal quantile to quantile plots. When necessary, variables were normalized for the ANOVA analysis by the arcsine of the square root or the logarithm transformations. The tests were taken as significant when  $p < 0.05$ . Means are expressed with their standard deviation (SD).

## 2.7. Mass balance equation

The mass balance equation used to estimate the  $\delta^{13}\text{C}$  values for the compounds in woods is given as:

$$\delta^{13}\text{C}_{\text{extractives}} = \frac{\delta^{13}\text{C}_{\text{wood}} - \delta^{13}\text{C}_{\text{extractives free}} \times Pe}{1 - Pe}$$

$$\delta^{13}\text{C}_{\text{lignin}} = \frac{\delta^{13}\text{C}_{\text{extractives free}} - \delta^{13}\text{C}_{\text{holocellulose}} \times \frac{Pho}{Pe}}{1 - \frac{Pho}{Pe}}$$

$$\delta^{13}\text{C}_{\text{hemicelluloses}} = \frac{\delta^{13}\text{C}_{\text{holocellulose}} - \delta^{13}\text{C}_{\alpha\text{-cellulose}} \times \frac{Pa}{Pho}}{1 - \frac{Pa}{Pho}}$$

Where  $Pe$ ,  $Pho$  and  $Pa$  are the proportions of extractives, holo- and  $\alpha$ -cellulose in the wood samples, respectively. The precision of these isotopic estimations were computed using the error propagation calculation. The standard deviations of  $\delta^{13}\text{C}$  and proportions were estimated from three replicated extractions.

## 2.8. Infrared spectroscopy

Fourier Transform Infrared (IR) spectra in Attenuated Total Reflexion mode (ATR) were recorded between 4000 and 700  $\text{cm}^{-1}$  on a Bruker Vector 22 spectrometer equipped with a KBr

beam splitter and a deuterated triglycine sulphate (DTGS) thermal detector. The resolution of the single beam spectra was  $4\text{ cm}^{-1}$ . A nine-reflection diamond ATR accessory (DurasamplIR™, SensIR Technologies) was used for acquiring the spectra. The incidence angle was  $45^\circ$  and the refraction index of the crystal was 2.4. The number of bidirectional double-sided interferogram scans was 100, which corresponds to a one-minute accumulation. All the interferograms were Fourier processed using the Mertz phase correction mode and a Blackman-Harris three-term apodization function. No ATR correction was performed. IR-ATR spectra are shown with an absorbance scale corresponding to  $\log(R_{\text{reference}}/R_{\text{sample}})$ , where R is the internal reflectance of the device. Recording of the spectra, data storage and data processing were performed using the Bruker OPUS 3.1 software. The measurements were performed at  $21 \pm 1^\circ\text{C}$  in an air-conditioned room. About 40 mg on beforehand dried powder were milled as described above and laid on the ATR crystal at ambient humidity. Water vapour subtraction was performed when necessary.

## 2.9. *Multivariate curve resolution with the Bayesian Positive Source Separation method*

The resolution of the pure component spectra from the “mixture” infrared spectra without any *a priori* information was performed with a statistical method of spectral mixture analysis called BPSS, for Bayesian Positive Source Separation (Moussaoui et al., 2006). Singular value decomposition (SVD) was used to estimate the rank of the data sets. The usual approximation is that the number of large singular values, significantly different in magnitude from the noise related singular values, indicates the rank of the analysed matrix. In principle, a successful separation can be achieved by using the appropriate number of components. An advantage of BPSS is that the number of components can be introduced into the algorithm and several trials can be conducted within minutes until the error matrix does not carry recognizable spectral features. Thus, the number of components may be chosen from SVD, but it is ultimately the algorithm that shows whether the number of requested components is correct. The lack of fit is used to assess how the estimated pure component spectra and mixing coefficients can reproduce the observed data. By assuming a known number of components, the mixture analysis aim is to estimate the pure component spectra and the mixing coefficient profiles from the mixture spectra. Without any calibration reference, it is possible to obtain relative concentrations by using BPSS and a mass-balance constraint. It must be emphasized that these concentrations are relative

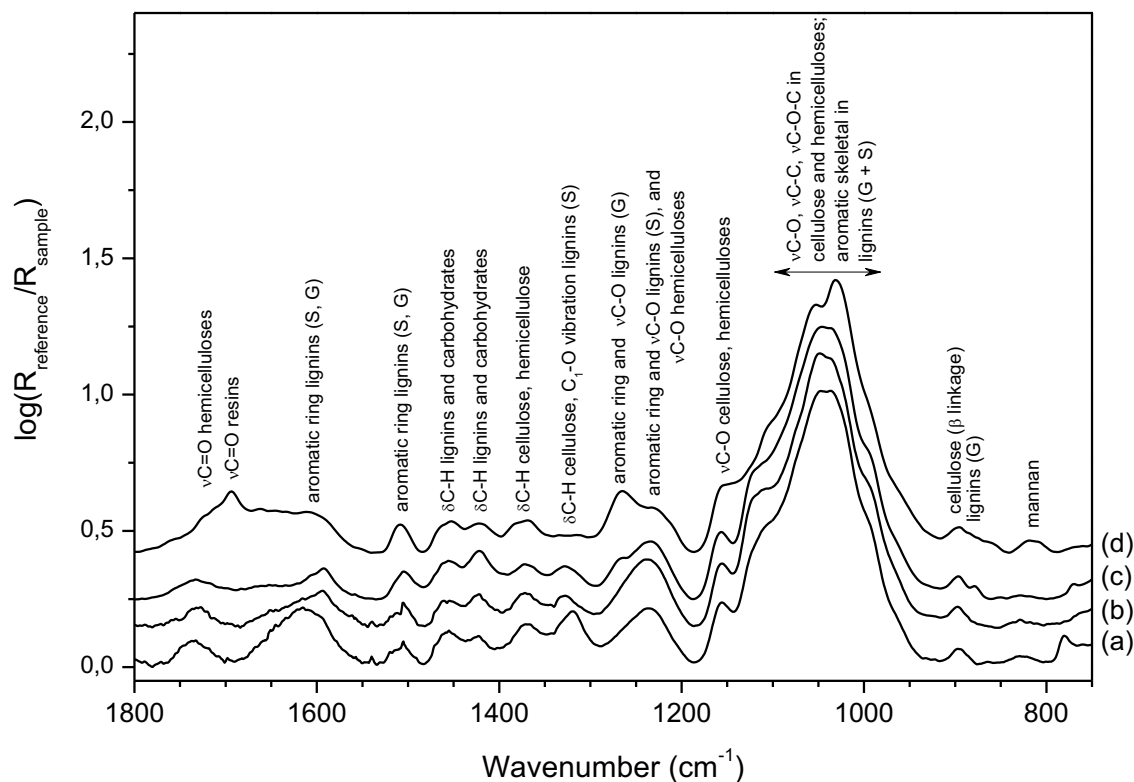
concentrations since they are weighted by the intensity of the normalized pure component spectra and not by a separate calibration.

The analyses were performed in spectral region 1800-730  $\text{cm}^{-1}$  on 5-points (1800, 1542, 1186, 850 and 800  $\text{cm}^{-1}$ ) baseline corrected spectra normalized with respect to the peak maximum of each spectrum in the 1000-1100  $\text{cm}^{-1}$  region. IR-ATR spectra were recorded on each sample from each chemical treatment. Thirty spectra were analysed for oak, poplar and pine woods each and 18 spectra for beech wood. Two or three principal components were estimated (see the Results and discussion section). In all cases, the residual variance was at 3%. The BPSS results are presented in two forms: (1) normalized estimated pure component spectra of each component, and (2) estimated relative concentration of each component against the hardness of the chemical treatment. The BPSS results are shown for each treatment; therefore the results are the mean of the triplicate samples. Matlab 7.1 (The Matworks, MA, USA) and the BPSS code were used for all the calculations. The BPSS code is available upon request.

### 3. Results and discussion

#### 3.1. Bulk woods and their components as a function of the tree species

As a first estimation of the biochemical differences among the different chosen tree species, the IR-ATR spectral features of the bulk wood powders were compared (Fig. 2). Main assignments of the IR-ATR spectra are given in accordance with the literature (Malikova et al., 1997; Pandey and Pitman, 2003; Rinne et al., 2005; Mohebbi, 2005; González-Peña and Hale, 2011). The spectral features are dominated by the absorptions in the 1200-850  $\text{cm}^{-1}$  region, and they are mainly assigned to various vibration modes of cellulose, hemicellulose and lignin (Fig. 2). In the spectrum of the pine wood, the specific band at 1694  $\text{cm}^{-1}$  is characteristic of resins occurring only in conifer woods (Rinne et al., 2005). The characteristic bands of lignin in regions free of neutral polysaccharides signatures show different relative band intensities among the different tree species (1607-1592  $\text{cm}^{-1}$ , 1514-1505  $\text{cm}^{-1}$ , 1300-1200  $\text{cm}^{-1}$ , assigned to the aromatic rings vibration associated with stretching C=O, to aromatic ring vibrations, and to C-O stretchings of syringyl (S) and guaiacyl (G) moieties, respectively).



**Fig. 2.** IR-ATR spectra of (a) oak (b) beech (c) poplar, and (d) pine wood powders. Principal assignments are indicated. The spectra are normalized with respect to the peak maximum of each spectrum in the 1100-1000  $\text{cm}^{-1}$  region. For clarity, spectra were shifted vertically. Key:  $\nu$ , stretching vibration;  $\delta$ , bending vibration, S, syringyl structure; G, guaiacyl structure.

The oak wood spectrum shows the highest content of aromatic compounds followed by the pine wood (see the bands near 1600  $\text{cm}^{-1}$ ). The oak and the beech woods show almost only S type lignin whereas S moieties are preponderant with respect to G moieties in poplar wood (see the band ratios near 1220 and 1270  $\text{cm}^{-1}$ , and near 1600 and 1510  $\text{cm}^{-1}$  (Pandey and Pitman, 2003; Mohebbi, 2005)). On the contrary, G type lignin dominated in the spectrum of pine wood. The band near 1230  $\text{cm}^{-1}$  is mainly assigned to S type lignin but this band also results partially from the C–O stretching in xylan (acetyl ester), which is, however in low content in the pine wood (Pandey and Pitman, 2003; Mohebbi, 2005). The band at 1735-1731  $\text{cm}^{-1}$  is assigned to unconjugated C=O stretching in hemicelluloses (xylans), and it is the most intense in the spectrum of the oak wood, reflecting the high content of xylan in hardwoods (Pandey and Pitman, 2003). This band does not occur in the pine wood spectrum, where another band assignable to other hemicelluloses is present near 1725  $\text{cm}^{-1}$ . The spectrum of this latter wood also showed the

occurrence of mannans through the band at  $809\text{ cm}^{-1}$  (Malikova et al., 1997). This band was not present in the wood spectra of the other species, showing the diversity of hemicellulosic compounds in the woods studied. Overall, the presence of different compounds, as well as their specificity (S versus G type lignin, xylan versus mannan type hemicelluloses), varied strongly among tree species indicating that a common cellulose extraction procedure might not result in similar results for all species.

### 3.2. Carbon isotopic composition and yield measurements at each step of the cellulose extraction

The  $\delta^{13}\text{C}$  values of bulk wood material differed among species (Table 2), probably as well due to differences in the chemical composition of the wood as to differences in physiological processes and their responses to environmental conditions prevailing during the assimilation of  $\text{CO}_2$  (Ehleringer, 1993).

The solvent and boiled water extraction step was maintained for all the woods because the removal of linking sites with the removal of the extractives would give a higher accessibility to lignin in reactive oxidizing with  $\text{NaClO}_2$  (Bag et al., 2011). Treatment 0 (Table 1) reduced the mass of the original sample by 6 % for pine and poplar, by 12% for beech, and up to 15% for oak (Fig. 3(I)). Among the four tree species, only the  $\delta^{13}\text{C}$  value of the pine increased significantly (less negative by 0.3‰, Fig. 3(II)). Harlow et al. (Harlow et al., 2006) showed on 44 tree species a similar high  $\delta^{13}\text{C}$  offset between bulk wood and extractives free wood. However, such a  $\delta^{13}\text{C}$  offset is not always detected for conifers (Taylor et al., 2008), suggesting that the extractives compounds normally removed with this chemical treatment change either in concentration or in isotopic composition.

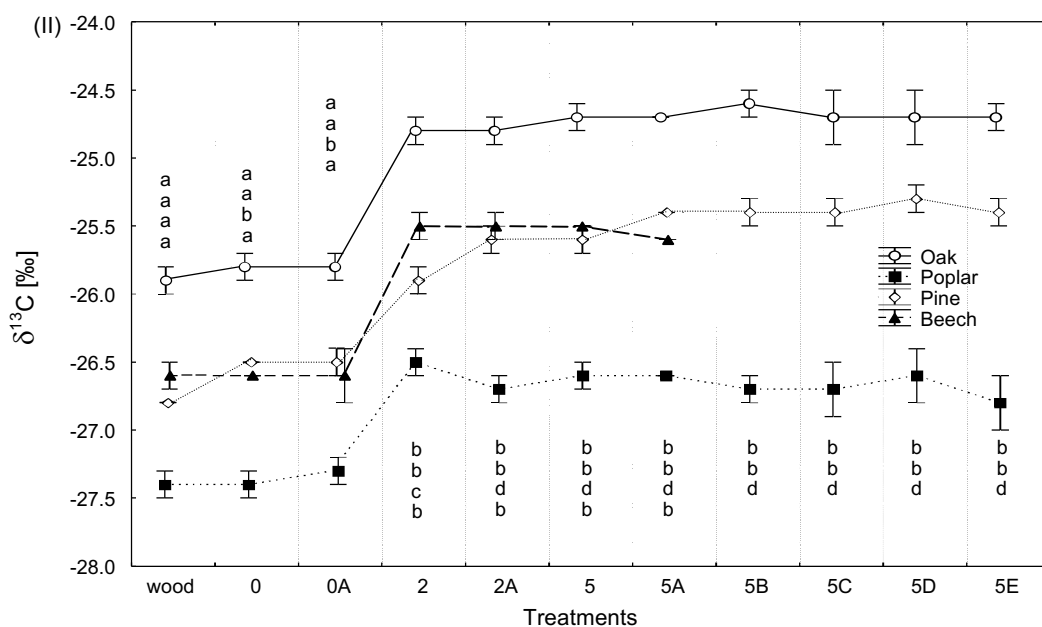
For all the tree species, the delignification with treatment 2 led to a significant loss of material. About 40% for oak, beech and poplar, and  $\approx 20\%$  for pine extracted dry material was recovered with respect to treatment 0. Relative to bulk wood powder, the acquired holocellulose extract was  $\approx 45\%$  for oak and beech,  $\approx 55\%$  for poplar, and  $\approx 75\%$  for pine (Fig. 3(I)). These values were close to those obtained after 4 or 5  $\text{NaClO}_2$  treatments in other published extractions (Harlow et al., 2006).

**Table 2** Measured  $\delta^{13}\text{C}$  values for the bulk wood, holocellulose (from treatment 2), and  $\alpha$ -cellulose (from treatment 5B) for oak, beech, poplar and pine; and the estimated  $\delta^{13}\text{C}$  values for their extractives, lignin and hemicelluloses using the data of Fig. 3 and isotopic mass balance calculations. The standard deviations ( $\pm\text{SD}$ ) for wood, holo- and  $\alpha$ -cellulose are estimated from 3 replicated extractions of the same wood sample, whereas the SD of extractives, lignin and hemicelluloses are estimated using error propagation calculations.

Tree species	Measured $\delta^{13}\text{C}$ (‰)			Estimated $\delta^{13}\text{C}$ (‰)		
	Bulk wood	Holocellulose	$\alpha$ -cellulose	Extractives	Lignin	Hemicelluloses
<i>Quercus petraea</i>	-25.9 $\pm$ 0.1	-24.7 $\pm$ 0.1	-24.6 $\pm$ 0.1	-26.5 $\pm$ 0.7	-26.9 $\pm$ 0.1	<sup>a</sup>
<i>Fagus sylvatica</i>	-26.6 $\pm$ 0.1	-25.5 $\pm$ 0.1	-	-26.7 $\pm$ 0.7	-27.7 $\pm$ 0.2	-
<i>Populus deltaoides</i>	-27.4 $\pm$ 0.1	-26.6 $\pm$ 0.1	-26.7 $\pm$ 0.1	-28.5 $\pm$ 1.4	-28.6 $\pm$ 0.2	-25.1 $\pm$ 1.0
<i>Pinus pinaster</i>	-26.8 $\pm$ 0.0	-25.6 $\pm$ 0.1	-25.4 $\pm$ 0.1	-30.9 $\pm$ 0.7	-29.0 $\pm$ 0.3	-26.7 $\pm$ 0.4

<sup>a</sup> Not estimated because hemicelluloses were already extracted with treatment 2 (see Fig. 5).

Similarly, the carbon isotopic composition of the holocellulosic extracts with treatment 2 showed a significant increase in  $\delta^{13}\text{C}$  values for all the tree species compared to treatment 0 (about +1‰ for oak, beech and poplar, and +0.6 ‰ for pine, Fig. 3(II)), which is similar to values found in the literature for oak (Loader et al., 2003) and for a broad set of species (Harlow et al., 2006). Using mass balance calculations and error propagation, the isotopic shift between  $\alpha$ -cellulose and lignin could be estimated at -3.6‰ $\pm$ 0.3 for pine, -2.3‰ $\pm$ 0.1 for oak and -1.9‰ $\pm$ 0.2 for poplar (Table 2), suggesting significant differences especially between conifer and broadleaf species as it has been observed before (Benner et al., 1987). This difference in isotopic composition might be due to differences in lignin monomer compositions, as differences in the isotopic composition among lignin monomers have been observed before (Goñi and Eglinton, 1996).



**Fig. 3.** (I) Percentage of remaining dry material after each extraction step (%), and (II)  $\delta^{13}\text{C}$  values (‰) for each extracted dry material from wood (see Table 1 and Fig.1) for oak, poplar, pine and beech. Each value represents a mean of 3 replicates. Letters indicate significant differences ( $P < 0.01$ ) within species among treatments; the letters are given for Oak, Poplar, Pine and Beech, respectively. The lines have been added to improve the reading.



The delignification with treatment 5 did not lead to changes in the percentage of the extracted dry material with respect to treatment 2, except for pine for which a significant decrease of 10% was observed (acquired holocellulose extract of 64%), suggesting a further removal of a component with a negative isotopic composition, such as lignin (Table 2). This indicates that the delignification process might be different between gymnosperm and angiosperm tree species.

A NaOH treatment of 0.4% without preliminary de-lignification (treatment 0A) had no significant effect on the extractives content and its  $\delta^{13}\text{C}$  value independently of the tree species. The same NaOH treatment with preliminary de-lignification (treatment 2A) had no effect on the proportion of holocellulose extract for angiosperm species, but decreased the proportion of holocellulose extract of pine by 10% with an increase of the  $\delta^{13}\text{C}$  value of +0.3‰ with respect to treatment 2. This suggests that the hemicelluloses subtraction might be different between gymnosperm and angiosperm tree species. For pine, a concurrent increase in  $\delta^{13}\text{C}$  with increasing NaOH treatments, suggested a further removal of a component with a negative isotopic composition. Using mass balance calculations and error propagation, the isotopic shift between  $\alpha$ -cellulose and hemicelluloses could be estimated at  $-1.3\text{‰}\pm 0.4$  for pine (Table 2). This would be in agreement with the more  $^{13}\text{C}$  depleted structural sugar carbohydrates relative to bulk wood for *Juniperus virginiana* (Teece and Fogel, 2007). The same NaOH treatment with stronger preliminary de-lignification (treatment 5A) had no further effect on the amount of holocellulose extracts or  $\delta^{13}\text{C}$  values compared to either 2A (softer delignification) or 5 (no NaOH treatment) for all the tree species. However, a stronger NaOH treatment after treatment 5 (treatment 5B) diminished by 18% and 11% the  $\alpha$ -cellulose extract for the samples of pine and poplar, respectively, with respect to treatment 5, but without changing the isotopic composition for oak, pine, and poplar. Also for these last tree species, even strong NaOH treatments, *i.e.* treatments 5C and 5D, did not lead to additional  $\delta^{13}\text{C}$  shifts or to differences in the amount of  $\alpha$ -cellulose extracted with respect to treatment 5B. These results would suggest that either (i) most of the hemicelluloses were already removed before NaOH treatment for oak; (ii) the pine's and poplar's hemicelluloses removed by NaOH at a concentration of 10% at 39°C would have  $\delta^{13}\text{C}$  values close to the one of the extracted  $\alpha$ -cellulose. Overall, for the here tested angiosperm species, the NaOH treatment does not seem to be necessary to produce cellulose with a  $\delta^{13}\text{C}$  value not influenced by residual hemicelluloses. However, if the carbon isotopic value of hemicelluloses

changes, it is necessary to add a NaOH treatment to remove them. For pine, one treatment of 10 % at 39°C would be sufficient to reach a similar robust estimate of the celluloses  $\delta^{13}\text{C}$  values.

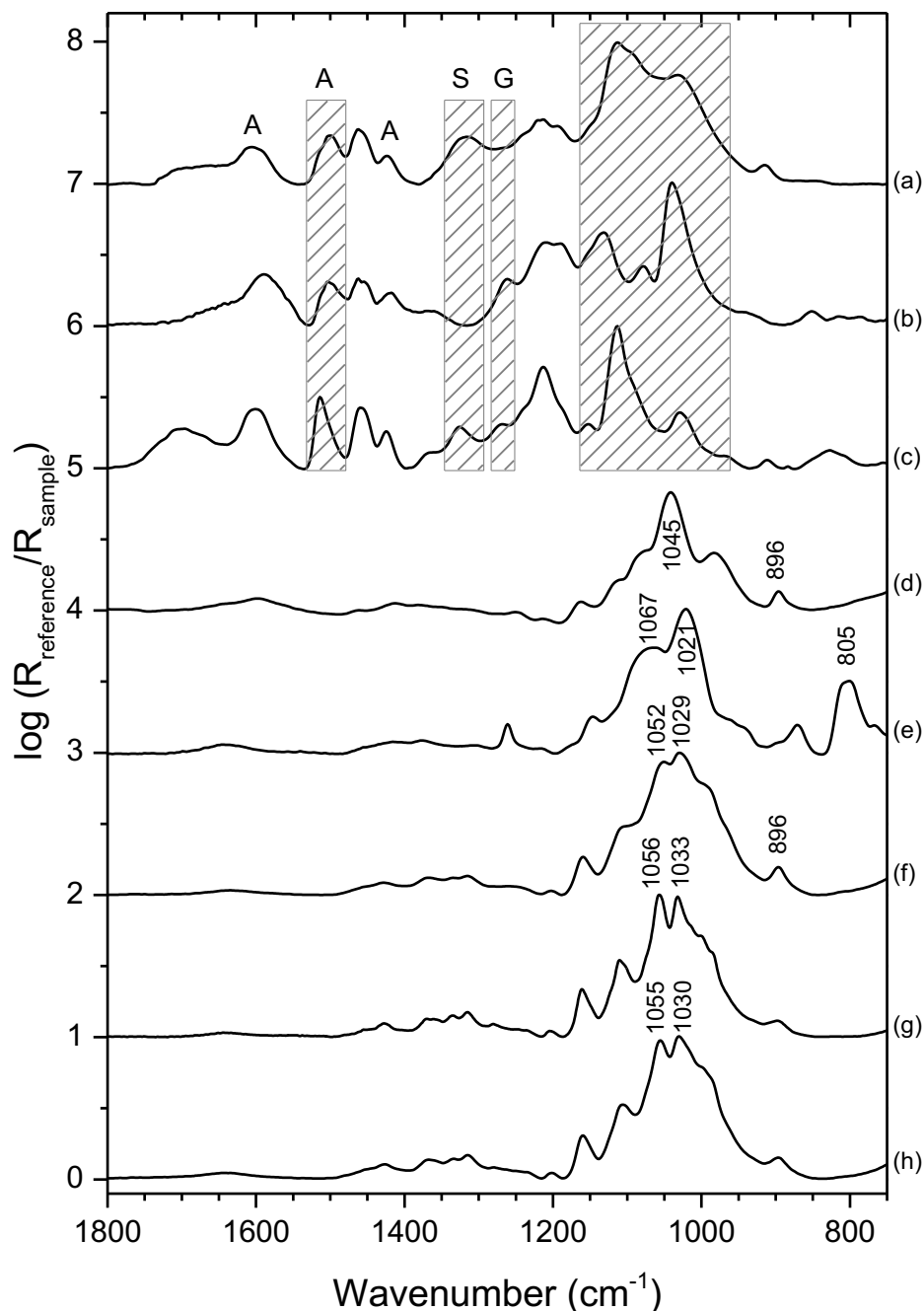
To sum up, the study of  $\delta^{13}\text{C}$  and the yield of material recovered from the different extraction steps showed that every treatment that caused an isotopic shift also showed a decrease of the mass of the extracted dry material, whereas some treatments would cause a loss of extracted material without having an impact on the  $\delta^{13}\text{C}$  value. The loss of chemical compounds is not systematically associated to a modification of  $\delta^{13}\text{C}$  values. To determine if residual compounds remain in the extracted  $\alpha$ -cellulose despite invariable  $\delta^{13}\text{C}$  values, an infrared study has been performed to have information at the molecular level for each material extract obtained for all the tree species.

### 3.3. *On the use of reference compounds for the wood constituents in a cellulose matrix*

In the idea of measuring the residual compounds in an extracted  $\alpha$ -cellulose, one way to proceed is the building of synthetic mixtures of references (lignin, hemicelluloses) in a cellulose matrix. This is usually done for well-defined chemical compounds to build a calibration curve. Several commercial available lignin, hemicelluloses, and cellulose samples were used as reference compounds.

The IR-ATR spectra of these reference compounds were different between but also within the types of compounds (Fig. 4). It was obvious for lignin compounds, for which the infrared spectra exhibit very different features. This is expressed by the spectral features near 1510-1504  $\text{cm}^{-1}$  (assigned to C=C stretchings of the aromatic rings), 1330  $\text{cm}^{-1}$  (assigned to the syringyl structure), 1260  $\text{cm}^{-1}$  (assigned to the guaiacyl structure), and in region 1160-960  $\text{cm}^{-1}$  (Fig. 4a-c), which are specific for lignin monomers (Faix, 1991). For example, it can be clearly observed on the spectra that the guaiacyl structure is preponderant in the lignin from the softwood (Fig. 4b), and that both the guaiacyl and syringyl structures occur in the lignin from hardwoods (Fig. 4c).

The spectra of the polysaccharides are dominated by absorptions in region 1200-900  $\text{cm}^{-1}$ , which are mainly assigned to C-O, C-C, and C-O-C stretchings (Marchessault, 1962; Kacuráková et al., 1999). Each spectrum is specific for each polysaccharide, and each particular polysaccharide has a specific band maximum in this region of the spectrum (Fig. 4d-h).



**Fig. 4.** IR-ATR spectra of celluloses, lignin compounds, and hemicelluloses in powder form from different commercial origins: (a) Klason process lignin from oak, (b) Kraft process extracted lignin from spruce, (c) organosolv extracted lignin from a mix of hardwood trees, (d) xylan (poly $\beta$ -1-4-D-xylopyranose) from beech, (e) galactomannan from *Ceratonia Siliqua*, (f)  $\alpha$ -cellulose from broadleaf, (g) cellulose from cotton fibres, and (h) cellulose from spruce wood. For clarity, spectra were shifted vertically. Key: A, aromatic ring; S, syringyl structure; G, guaiacyl structure.

In the anomeric region, the band at  $896\text{ cm}^{-1}$  is specific for  $\beta(1-4)$  glycosidic linkages in cellulose and xylan (Fig. 4d, f-h); and the band at  $805\text{ cm}^{-1}$  is specific for  $\beta$ -glycosidic linkages in mannans (Malikova et al., 1997) (Fig. 4e). For cellulose or  $\alpha$ -cellulose, depending on its origin, the features of the spectra between  $1200$  and  $900\text{ cm}^{-1}$  are also quite different (Fig. 4f-h). The observed variations reflected structural differences (hydrogen bonding, occurrence of minor impurities..., Fig. 4f-g), probably in relation with the initial wood, and the extraction method performed. For example, it has been shown that alkaline treatments with high concentrations of sodium hydroxide often used in  $\alpha$ -cellulose extraction lead to changes in the infrared spectra of cellulose (Oh et al., 2005). The bands in the spectrum of the cotton cellulose were narrower indicating a higher structural organisation. This is probably due to the high cellulose content of cotton, which would facilitate its extraction and would therefore preserve more easily the cellulose structure, compared to commercial cellulose extracted from wood.

This shows the difficulty to produce standard spectra that can be used in a calibration step to determine the purity of the extracted  $\alpha$ -cellulose by using multivariate regression methods (González-Peña and Hale, 2011). However, to compare the detection limits of carbon isotope mass and infrared spectroscopies, we have performed some mixtures of these reference compounds in a cellulose matrix.

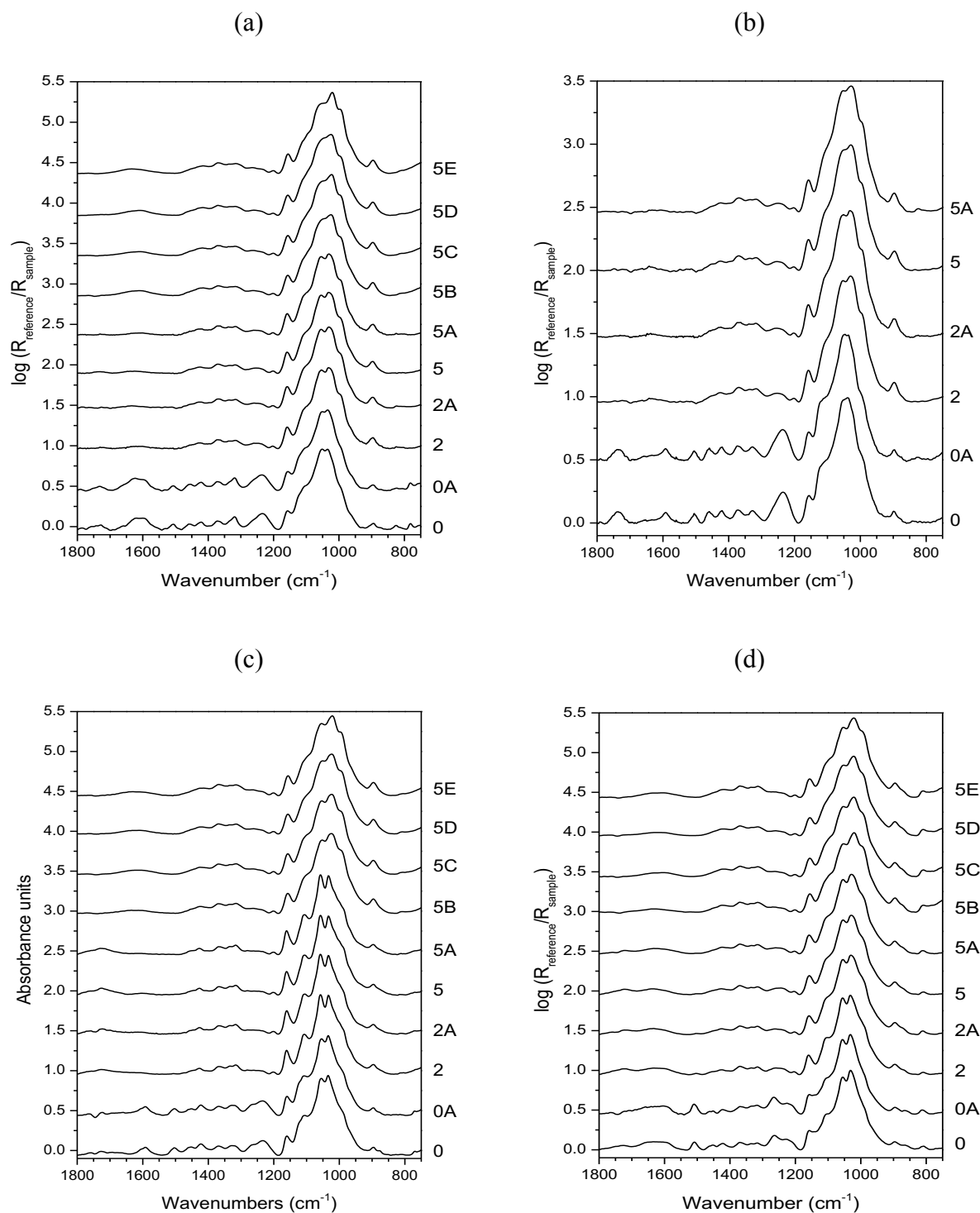
### 3.4. *Detection limits of lignin and hemicelluloses in a cellulose matrix*

The detection limit of IR-ATR should be at least as good as the sensibility of the  $\delta^{13}\text{C}$  method. Lignin was clearly detected on the IR-ATR spectra for mixtures at 4% lignin by the bands at  $1591$  and  $1509\text{ cm}^{-1}$  (Fig. SM1 in SM). Moreover, the addition of lignin to the cellulose matrix modified the typical band profiles of the sugars between  $950$  and  $1100\text{ cm}^{-1}$  on all the spectra, with respect to the spectrum of pure cellulose. This was also detected for mixtures with content of below 4% lignin, and it is probably due to interactions between both compounds. A significant shift of  $\delta^{13}\text{C}$  values was only detected for mixtures of and above 6% lignin content (Table SM1 in SM). A minimum of 4% of residual lignin content measured by chemical assays for spruce, pine and poplar woods after  $\text{NaClO}_2$  treatments was reviewed by Wise et al. (Wise et al., 1946). The detection limit of IR-ATR is therefore sufficient to detect meaningful residual

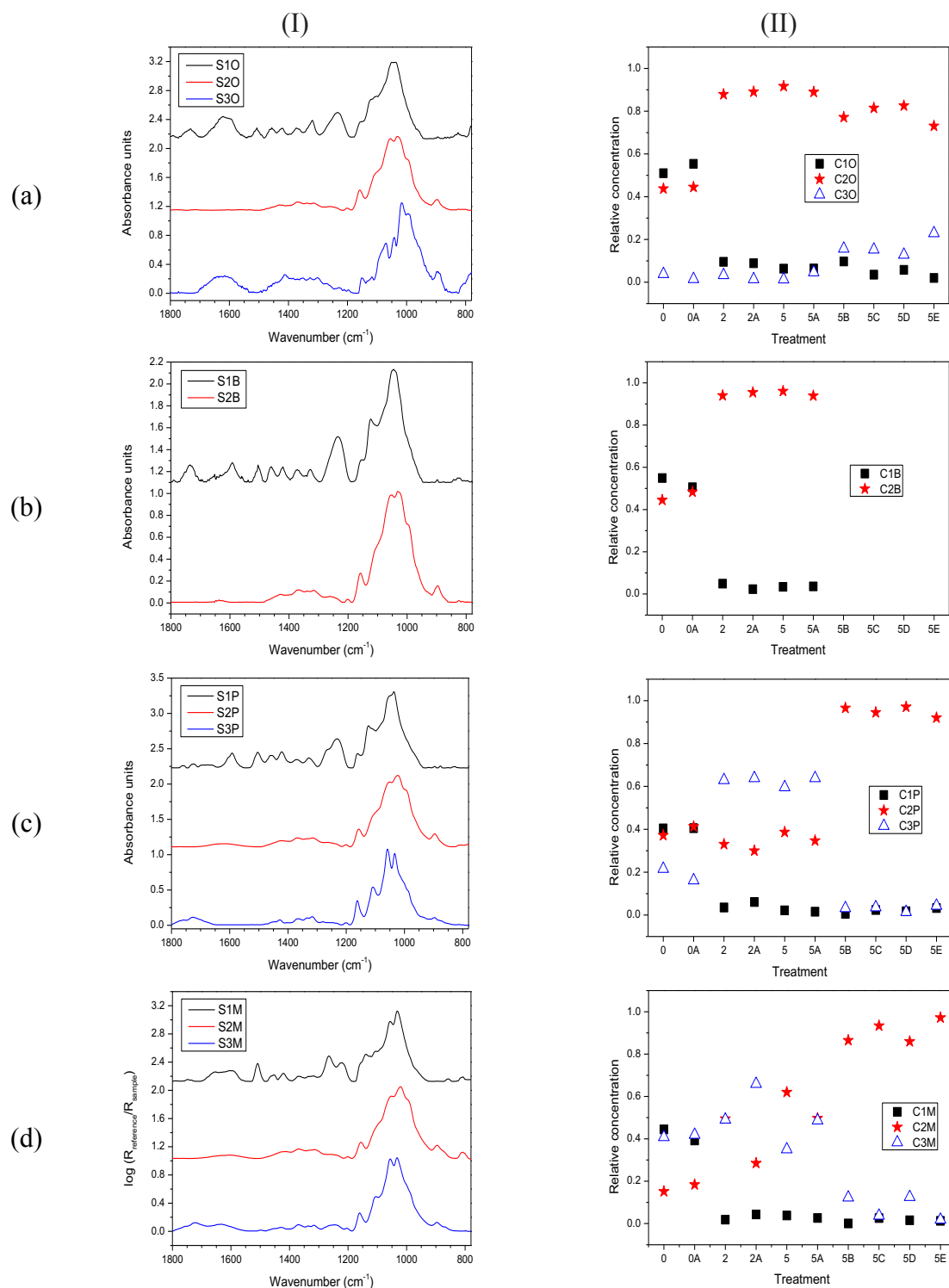
lignin in our  $\alpha$ -cellulose samples. D-galactomannan was detected on the IR-ATR spectra in the cellulose matrix above 4% by the mannan specific band at  $811\text{ cm}^{-1}$  in the cellulose matrix (Fig. SM1 in SM), whereas D-galactomannan were only detected for mixtures of and above 10% galactomannan content (Table SM1 in SM) on carbon isotope measurements. The sensibility of the  $\delta^{13}\text{C}$  analysis to some contamination is dependent on the isotopic difference between two mixed molecules: the larger the difference, the more sensible is the resulting  $\delta^{13}\text{C}$  to the contamination. Here, the isotopic difference between the commercially available lignin and cellulose (depleted by 5.3‰) or galactomannan and cellulose (enriched by 1.6‰, Table SM1 in SM) was in the same order of magnitude as what would be expected from a natural wood sample for lignin (Benner et al., 1987) and hemicelluloses (Au and Tardif, 2009). However, the relatively small difference for the D-Galactomannan could result in a lower sensitivity with respect to a natural sample, where the isotopic difference might be slightly higher. Nevertheless, the analysis of the synthetic mixtures clearly shows that the sensibility of IR-ATR is higher than the sensibility of  $\delta^{13}\text{C}$  measurements for these molecules and can therefore be used to test the  $\alpha$ -cellulose purity for carbon isotopic analysis.

### 3.5. *Infrared and multivariate curve resolution analyses in the course of the chemical extraction of $\alpha$ -cellulose*

The BPSS procedure of curve resolution, which allowed the avoidance of any personal subjective estimation, was used to analyse the spectra in the course of the chemical treatments as a function of their extraction strength (soft and hard treatments as previously defined). Fig. 5 shows a series of spectra recorded for each chemical treatment performed on the four woods. The BPSS analysis shown in Fig. 6(I) identified three pure component spectra from the IR-ATR spectra of the chemically treated samples from oak, poplar, and pine. The BPSS analysis estimated only two pure component spectra for samples from beech, for which only the soft treatments were performed. For all the spectra examined, the percentage of non-reconstructed data was noise. Fig. 6(II) shows the concentration profiles of each component against the chemical treatments (in the order from the softest to the hardest treatments) of the samples analysed by BPSS.



**Fig. 5.** IR-ATR spectra of (a) oak, (b) beech, (c) poplar, and (d) pine extracted dry material powders after each chemical treatment. The spectra are normalized with respect to the peak maximum of each spectrum in the 1100-1000  $\text{cm}^{-1}$  region. For clarity, spectra were shifted vertically.



**Fig. 6.** (I) Estimated BPSS IR-ATR pure component spectra (S), and (II) estimated concentration (C) profiles for individual tree species in wood for (a) oak (O), (b) beech (B), (c) poplar (P), and (d) pine (M). Samples in (II) are presented with increasing hardness of the chemical treatment (see Table 1 for the nomenclature). For clarity, spectra were shifted vertically.

### 3.5.1. Assignment of the extracted pure component spectra.

Pure component spectra called S1 (Fig. 6a-d) showed principally a mixture of lignin (through the bands near 1600, 1510, 1320, and 1260  $\text{cm}^{-1}$ ) and hemicelluloses (for example through the bands above 1700  $\text{cm}^{-1}$ ). Spectra S2 have the shape of the spectra of the commercial broadleaf  $\alpha$ -cellulose or the commercial spruce wood cellulose in region 1200-850  $\text{cm}^{-1}$  (Fig. 6(I)). For pine, an additional band at 809  $\text{cm}^{-1}$  was observed in spectrum S2M, and it was assigned to mannans. For oak, spectrum S3O was not really representative of specific compounds from the wood. Moreover, the mixing coefficients had low values which lead to a low contribution in the experimental spectra. For poplar and pine, the features of spectra S3P and S3M were roughly those of hemicelluloses in the region above 1700  $\text{cm}^{-1}$ , whereas they were very close to the profile of the cotton cellulose in region 1200-950  $\text{cm}^{-1}$  (Fig. 4g and 6(I)c,d). The assignment of these pure components spectra allowed tracing their concentrations during the cellulose extraction process.

### 3.5.2. Spectra of the samples from soft treatments.

Spectra of samples from treatments 0 and 0A were weakly modified in regions 1735, 1605 and 1200-950  $\text{cm}^{-1}$  for all the wood samples with respect to those of the bulk wood spectra (Fig. 2 and 5). It can be the result of the removal of some fatty acids, aromatic substances, and carbohydrates (Ajuong and Redington, 2004). As it was also observed by Rinne et al. (Rinne et al., 2005), resins from pine wood were already removed with treatment 0, as shown by the disappearance of the band at 1694  $\text{cm}^{-1}$  (Fig. 5(d)). Moreover, a weak decrease of a band at 1043  $\text{cm}^{-1}$  may suggest the partial removal of xylans (Fig. 2 and 5). This has also been observed for *Miscanthus x giganteus* where an extraction using a mixture of alcohol and benzene or boiling water, reduced the hemicellulose content, probably because of the auto-hydrolysis of lateral chains in various hemicelluloses (Brosse et al., 2009), but the solvent extraction step did not remove lignin for any of the tested tree species.

Spectra of samples from treatment 2 were modified for the characteristic bands of lignin and hemicelluloses. For all the tree species, concentration of compound C1 (relative to spectrum S1) rapidly decreased to between 0 and 0.1 with treatment 2 (Fig. 6(II)). This strongly suggested that lignin is removed as soon as soft treatment 2 for all the woods, and that hemicelluloses were



at least partially removed. The estimation of the detection limits of IR-ATR suggested that the lignin left was below 4% in the extracted  $\alpha$ -cellulose. Using isotopic mass balance calculations, by assuming a contamination of 4% lignin and an isotopic difference between lignin and cellulose of 3‰, the  $\delta^{13}\text{C}$  of the extracted cellulose would be biased by 0.12‰. This corresponds to the measurement precision of Isotope Ratio-Mass Spectrometers for  $\delta^{13}\text{C}$  and suggests therefore that a lignin contamination below 4% can be neglected.

For oak and beech, the spectral features of hemicelluloses also disappeared with treatment 2 (Fig. 5(a, b)), suggesting a complete removal of hemicelluloses for these species. Indeed, their characteristic bands around 1740, 1600, 1500  $\text{cm}^{-1}$  and between 1300 and 1200  $\text{cm}^{-1}$  were not detectable after this chemical treatment. The spectra of the extracted compounds obtained had the general feature of the commercial broadleaf  $\alpha$ -cellulose (Fig. 4f), and all the spectral features were very close for all the other soft treatments including those with the application of NaOH solutions (treatments 2A and 5A, Fig. 5). In addition, concentrations C2O and C2B from spectra S2O and S2B, respectively, were above 0.9 for the soft treatments (2 to 5A). This confirmed that the removal of hemicellulose was effective already at treatment 2 for oak and beech. The application of sodium hydroxide did not remove additional products, and it was therefore not necessary for those woods.

For poplar and pine, the lignin is removed at treatment 2 (Fig. 5(c,d)), whereas hemicelluloses were only partly removed. For all the soft treatments and both woods, the spectra showed residual bands near 1720  $\text{cm}^{-1}$  from hemicelluloses (C=O stretching of unconjugated ketones and/or of carboxylic acids). Another band at 810  $\text{cm}^{-1}$ , more precisely assigned to mannans (Malikova et al., 1997), was also present in the spectra of pine which indicates that mannans are neither removed by the soft treatments. This is in agreement with the BPSS analysis which showed that the infrared spectra for the extracted materials from soft treatments are composed of two predominant spectra: S2P (S2M, respectively) and spectrum S3P (S3M, respectively). The concentration profile C2P, C3P and C2M, C3M, for poplar and pine, respectively, showed evolutions with two distinct steps of extraction from treatments 2 to 5E (Fig. 6(II)c,d). The first one was reached with soft treatments 2-5A, and the second one was reached with hard treatments 5B-5E. These evolutions as a function of the chemical treatment are in accordance with the yield profile of Fig. 3(I). This suggests the occurrence of hemicelluloses in

these extracts that might correspond for pine to the residual mannans detected in the infrared spectra.

Our results suggest that hemicelluloses other than the mannan type are already removed during the delignification step. It has been shown that the development of chlorine dioxide generated with NaClO<sub>2</sub> treatments attacks a wide variety of organic compounds, including carbohydrates, pectin, and phenolic compounds (Tao et al., 2010).

### 3.5.3. Spectra of the samples from hard treatments.

As soon as treatments 5B, the band at or near 1720 cm<sup>-1</sup> disappeared in the infrared spectra for all the hard treatments for poplar and pine samples, and the spectra of the extracted compounds of poplar have the general features of the spectrum of the broadleaf  $\alpha$ -cellulose. For pine, a band at 810 cm<sup>-1</sup> (assigned to mannans) stayed in the spectra independently of the treatment performed. The spectra in the 1200-900 cm<sup>-1</sup> region of the extracted compound obtained had the general features of the commercial spruce cellulose, (Fig. 4h). The BPSS analysis for poplar and pine showed only spectrum S2P and S2M, respectively, in the spectra obtained from the hard treatments at relative concentrations C2P and C2M, respectively, above 0.9. This suggests a content of residual compounds of less than 10%. It can be shown, using isotopic mass balance calculations, that assuming a contamination of 10% hemicelluloses and an isotopic difference between hemicelluloses and  $\alpha$ -cellulose of 1.6‰ (Table SM1), the  $\delta^{13}\text{C}$  of the extracted cellulose would be biased by 0.16‰. This corresponds to the measurement precision of Isotope Ratio-Mass Spectrometers for  $\delta^{13}\text{C}$  and suggests therefore that a hemicellulose contamination below 10% can be neglected. IR-ATR is a relevant method to appraise residual of hemicellulose mannan type in  $\alpha$ -cellulose extract. The occurrence of residual mannans in pine  $\alpha$ -cellulose samples had also been observed with contaminations up to 9%, even when hardest alkaline treatments were applied (Wise et al., 1946).

For poplar and pine wood, two kinds of spectral profiles occurred in S2P (S2M), and S3P (S3M) in region 1200-850 cm<sup>-1</sup> (Fig. 6(I)c,d). These spectral profiles are very similar to the spectra of broadleaf  $\alpha$ -cellulose and of cotton cellulose for S2 and S3, respectively. Spectra of S2 and S3 types occurred in the experimental spectra for the soft treatments, whereas only one (S2) occurred in the experimental spectra for the hard treatments. Two hypotheses can be suggested for the occurrence of both types of cellulose spectra: (i) both structural celluloses coexist in the

bulk wood and the hard chemical treatments change the structure of the more organised one. (ii) There is one cellulose structure in the bulk wood; and the soft treatments did not entirely change the initial structure, whereas the hard treatments did change the structure more drastically. Despite pine being a resinous and poplar a broadleaf species (angiosperm) the impact of the extraction process on changes in the resulting  $\alpha$ -cellulose was quite similar for our tested samples.

At last for oak, the spectra of the extracted compounds were also very close to the one of the commercial hardwood cellulose except for slight changes in the relative band intensities in region  $1000\text{-}1200\text{ cm}^{-1}$ , especially in spectrum 5E with respect to spectrum 5 (Fig. 5(a)). The appearance of spectrum S3O was observed for this hardest treatment (Fig. 6(II,a), 5E), but the mixing coefficients had low values which lead to a low contribution in the experimental spectra. S3O probably originated from some degradation of the  $\alpha$ -cellulose extracted with the hardest treatments that induce small changes in the infrared spectra, and loss of extracted material (Fig. 3(I)).

To sum up, the IR-ATR study confirms for all the tree species studied the use of the same treatment to subtract resins and terpenes and the same conditions of  $\text{NaClO}_2$  treatment to remove lignin. Moreover, the partial or total subtraction of hemicelluloses already occurred. An additional treatment with  $\text{NaOH}$  can be useful to help the removal of more hemicelluloses however is sometimes not efficient to subtract all the hemicelluloses, but might deteriorate the  $\alpha$ -cellulose.

#### 4. Conclusion

Mid-infrared spectroscopy associated to a curve resolution method was relevant to test the significant occurrence of residual compounds (lignin, hemicelluloses, and extractives) in  $\alpha$ -cellulose extracted from wood for  $\delta^{13}\text{C}$  measurements. We have shown that the solvent extraction step did not remove lignin for any of the tested tree species, but we observed a partial removal of hemicellulose. The delignification was effective for all the species after only treatments with two additions of  $\text{NaClO}_2$ . Our results suggest that for oak and beech two additions of acidic  $\text{NaClO}_2$  after extractives removal were sufficient to remove lignin but also to digest the hemicelluloses. For poplar, additional  $\text{NaOH}$  treatments were necessary to remove hemicelluloses. A  $\text{NaOH}$

concentration of 10% at 39°C was necessary and sufficient to digest residual hemicelluloses for poplar. This NaOH concentration is lower than the usual NaOH concentration of 17% usually practiced in  $\alpha$ -cellulose extraction process. For pine, even after the hardest NaOH treatments (NaOH at 17% or 10% and 80°C), residual mannan compounds were still detected in  $\alpha$ -cellulose extracts. Finally, we recommend first a treatment with solvents (ethanol/toluene) followed by boiled water, then a treatment with a 2% NaClO<sub>2</sub> solution with only two successive additions instead of four or five usually performed for the removal of the lignin for the four woods studied (*Quercus petraea*, *Fagus sylvatica*, *Populus deltaoides x nigra*, *Pinus pinaster*). A treatment with NaOH of 10% at 39°C is useful for the process of  $\alpha$ -cellulose extraction of the poplar and pine to be in situation of residual hemicelluloses compounds left below the threshold which would bias the  $\delta^{13}\text{C}$  measurement. At last, NaOH treatments above 10% are not useful and, at least for oak, lead to  $\alpha$ -cellulose degradation. Although beech and poplar have both a similar wood structure, that is a diffuse porous vascular system, they differ in the  $\alpha$ -cellulose extraction process. This suggested that the  $\alpha$ -cellulose extraction protocol is rather dependent on the tree species than on the structure of the wood.

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## Supplementary Material

### Infrared spectroscopy and multivariate analysis to appraise $\alpha$ -cellulose extracted from wood for stable carbon isotope measurements

by

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**Table SM1.** Estimated  $\delta^{13}\text{C}$  values using a mass balance equation relative to commercial standards values and means ( $\pm$ SD) of measured values for  $\delta^{13}\text{C}$  of several mixed lignins (Aldrich) or D-galactomannan (Sigma) in cellulose (Fluka). Each value represents a mean of three replicates. Significant mixed values compared with Fluka cellulose samples are indicated by a  $p < 0.01$  and b  $p < 0.001$ . The correlation coefficient between estimated and measured  $\delta^{13}\text{C}$  values was 0.992 for lignin, where the isotopic difference was 5.27‰ to the used cellulose, and it was 0.856 for D-galactomannan, where the isotopic difference was 1.58‰.

Proportion in the mixture (%)	Lignin in cellulose		D-galactomannan in cellulose	
	$\delta^{13}\text{C}$ (‰) estimated	$\delta^{13}\text{C}$ (‰) measured	$\delta^{13}\text{C}$ (‰) estimated	$\delta^{13}\text{C}$ (‰) measured
0	-24.39	$-24.39 \pm 0.09$	-24.39	$-24.39 \pm 0.09$
2	-24.49	$-24.41 \pm 0.08$	-24.35	$-24.26 \pm 0.03$
4	-24.60	$-24.49 \pm 0.03$	-24.32	$-24.20 \pm 0.11$
6	-24.70	$-24.60 \pm 0.05^a$	-24.29	$-24.25 \pm 0.19$
10	-24.92	$-24.90 \pm 0.11^b$	-24.23	$-24.08 \pm 0.04^a$
100	-29.66	$-29.66 \pm 0.04$	-22.81	$-22.81 \pm 0.05$



**Fig. SM1.** IR-ATR spectra of synthetic mixtures of {cellulose from spruce wood (Fluka) + Kraft lignin from fir (Aldrich)}, and {cellulose from spruce wood (Fluka) + galactomannan from *Ceratonia Siliqua* (Sigma)}. The spectra are normalized with respect to the peak maximum of each spectrum in the 1100-1000  $\text{cm}^{-1}$  region.

