Batch processing of tree ring samples for radiocarbon analysis

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Abstract

We here present the method of pretreatment of a batch of tree rings used for measuring radiocarbon with high precision at the Aarhus AMS Centre (AARAMS) at Aarhus University, Denmark. The aim was to develop an efficient and high-throughput method to be able to pretreat c. 50 samples at a time. Numerous pretreatment methods exist, and we have tested two methods for extracting α-cellulose from wood to find the most optimal for our use. One method used acetic acid and the other used HCl acid for the delignification, the former also used a final bleaching step after treatment with NaOH, whereas the latter included a step with HCI acid after NaOH treatment. This testing has been done on different background ^{14}C samples, in order to assess the contamination transferred to the samples during pretreatment. Furthermore, the extracted wood fractions have been analyzed using Fourier Transform Infrared (FTIR) spectroscopy to assess the molecular composition of the extracts, showing a successful extraction of α-cellulose. Samples were pretreated at AARAMS, and the graphitization and radiocarbon analysis of these samples were done at both AARAMS and the radiocarbon dating laboratory at Lund University to compare the graphitization and AMS machine performance. No significant offset was found between these two set of measurements. The results of these tests led to the development of a method to be used henceforth at AARAMS for radiocarbon analysis of tree rings.

Introduction

Analyzing the radiocarbon fraction in tree rings is fundamental to the radiocarbon method in order to calibrate ^{14}C years into calendar years. Substantial work has been conducted during the past c. 40 years to construct a calibration curve covering the last 50,000 years, of which the last 13,900 years are covered by tree rings (Reimer et al. 2013). The vast majority of data going into the calibration curve have decadal time resolution. However, with the discovery of fast changes in the atmospheric $^{14}C/^{12}C$, likely due to solar proton events, the so-called Miyake events (Miyake et al. 2012; Miyake, Masuda, and Nakamura 2013), there is an increasing interest in measuring ^{14}C at annual resolution. This requires a large number of tree-ring samples to be pretreated. Furthermore, to measure radiocarbon with annual resolution, it is necessary to ensure that the carbon originates from only one year and that this carbon has not been exchanged since formation. The carbon originating from cellulose has this property, whereas carbon in the lignin and hemicellulose components of wood can be exchanged with the atmosphere or cross over ring boundaries after formation (Wilson and Grinsted 1977; Leavitt and Danzer 1993; Gaudinski et al. 2005, Kagawa et al. 2006). Moreover, earlywood in ring-porous trees is less reliable for tracking annual changes in ^{14}C, due to a part of it being made of reserves from the previous year (Fogtmann-Schulz et al. 2017; Kudsk et al. 2018; McDonald et al. 2019). Therefore, latewood cellulose is the preferred component for ^{14}C measurements (Loader, Switsur, and Field 1995; Loader et al. 1997; Santos et al. 2001; Nemec et al. 2010). Cellulose has the molecular formula $(C_6H_{10}O_5)_n$, and is a long-chained, carbon-based polymer. Hemicelluloses are various polysaccharides, and cellulose and hemicelluloses are collectively referred to as holocellulose. α-Cellulose is defined as the part of holocellulose that is insoluble in 17.5 % sodium hydroxide (NaOH) at room temperature and is the material most similar to pure cellulose that can be extracted from wood samples. It consists mainly of cellulose with only a minor fraction of some resistant hemicelluloses (Green 1963). Accordingly, we produce α-cellulose for our measurements, using a method build upon the Jayme-Wise method presented in Green (1963). The two main steps are: Removal of lignins by oxidative delignification, and removal of hemicelluloses by alkaline extraction.

Some different approaches have been used for α-cellulose extraction, but methods following the overall scheme of the Jayme-Wise method have been found to give the purest α-cellulose and the most reliable results for radiocarbon analysis in terms of contamination (Gaudinski et al. 2005; Anchukaitis et al. 2008). For our measurements, we began using a revised version of the method of Loader et al. (1997) (henceforth referred to as the Loader method). This method was developed for measuring stable carbon isotopes.

Lignins are removed by bleaching using a chlorite, ClO_2, solution (Green 1963), where an acidified solution of sodium chlorite ($NaClO_2$) provides the chlorite oxidizer. Preferentially, acetic acid, CH_3COOH, (Loader method) is used, because acetic acid acts as a buffer keeping pH constant. This has proven to work well for stable isotope analysis for climate research using tree rings (e.g. Mullane, Waterhouse, and Switsur (1988); Loader et al. (1997)) but may, however, impose a problem for radiocarbon analysis as the carbon in the acetic acid might influence ^{14}C activity in the produced cellulose. Therefore, it has been suggested to replace the acetic acid with hydrochloric acid, HCl (Southon and Magana 2010). The acetic acid has a higher pH-value than hydrochloric acid, but Southon and Magana (2010) report of no loss of bleaching efficiency with hydrochloric acid. Due to the contamination concerns, we have also made radiocarbon measurements using the method of Southon and Magana (2010) (henceforth referred to as the Southon method), with some minor changes based on our experience with the Loader method.

The two different methods tested in this study also differ in number and length of bleaching steps as elaborated in the following sections. The length of the delignification is investigated by comparing infrared spectra of samples being delignified for variable amounts of time.

Moreover, the Southon method finishes with an acid wash after the alkaline extraction. This is because a strong alkaline solution can absorb carbon dioxide from ambient air forming sodium carbonate, which is a potential contamination source for radiocarbon analysis. A subsequent acid wash will dissolve such sodium carbonate. Loader et al. (1997) also includes this step, but it was not included in the revised version followed in this study. By comparing radiocarbon ages of background samples pretreated with and without this acid wash, such contamination is investigated.

Here we will report on a method that allows up to 48 samples to be pretreated per batch. Furthermore, we will report on modifications to the method that we have made in order to make sample pretreatment more efficient.

Samples

An oak (*Quercus* sp.) wood sequence was acquired from the Danish Oak Chronology by the Danish National Museum. This sequence was dendrochronologically dated (Eriksen 1996), and is referred to as Sch. Latewood (LW) from a single thick ring from Sch, dated to AD 1420 (AAR-26314) was divided in 34 different samples and subjected to different steps of the pretreatment (the Loader method as described below) in order to test the purity of the extracted samples. These samples were not radiocarbon analyzed.

Twenty annual LW samples from the years 1439-1458 AD from Sch (AAR-22701-22720) were pretreated using the full Loader method. These samples were used to assess the quality of the graphitization and AMS measurements, since the sample material was divided in two after the pretreatment. One part was radiocarbon analyzed at Aarhus AMS Centre (AARAMS); the other part was radiocarbon analyzed at the radiocarbon dating laboratory at Lund University.

In order to assess the background contamination at AARAMS, two different backgrounds went through the pretreatment methods described below. One was a piece of oak stratigraphically dated to the Eemian (Adolphi et al. 2013), and the second was a piece of kauri from Marine Isotope Stage 7 (MIS7) provided by P. Reimer at Queen's University Belfast. Two Eemian oak samples and four MIS7 Kauri samples were cut from the pieces and pretreated following the AARAMS method, based on the Southon method, and the rest of the samples were pretreated using the Loader method.

We also measured a charcoal background that is routinely used in the AARAMS laboratory for reference and comparison. This background was not pretreated as a part of this study. It was pretreated in a similar way as the wood samples but following an acid-base-acid (ABA) pretreatment procedure instead (Brock et al. 2010).

Methods

The wood samples were cut into pieces with approximate dimensions of 15 mm by 5 mm spanning the available annual rings. This size gave enough wood to ensure that enough α-cellulose for two AMS samples (2.13-2.54 mg for each sample) was available for narrow rings. No more than 30.0 mg of wood from each sample was used, since more material would require a larger volume of reagents in the pretreatment. The pieces were cut under a microscope with a scalpel into annual samples, with earlywood (EW) and latewood (LW) being separated. To avoid effects of photosynthates produced the previous year being incorporated into the ring being analyzed, only the LW samples were used for 14C dating. The pretreatment from bulk wood LW samples to cellulose was modified from Loader et al. (1997) and Southon and Magana (2010) and summarized in Table 1.

Table 1: The main steps of pretreatment of the revised Loader method (Loader et al. 1997) and Southon method (Southon and Magana 2010) are shown in the table as well as the resulting procedure used in the AARAMS lab. Between each part of the pretreatment, the samples are rinsed with deionized or Milli-Q water to neutral pH.*

Procedure	Loader method	Southon method	AARAMS method
Delignification	$NaClO_2$ and CH_3COOH 80 °C 6 additions 6 hours in total	$NaClO_2$ and HCl 70 °C New addition if necessary 4-6 hours in total	$NaClO_2$ and HCl 70 °C 2 additions 6 hours in total
Alkaline extraction	10 % NaOH 80 °C, 45 min. 17 % NaOH Room temp., 45 min.	20 % NaOH Room temp., 1 hour	17 % NaOH Room temp., 1 hour
Final steps	$NaClO_2$ and CH_3COOH 80 °C 2 additions 2 hours in total	1 M HCl 70 °C, 30 min.	1 M HCl 70 °C, 30 min.

*Samples prepared in conventional water bath, with additional final bleaching stage and ultrasonic homogenisation after preparation to α-cellulose.

The experimental setup is shown in Figure 1a. Each annual LW sample was placed in a special glass vial made by the local glassblower (Figure 1b), modified from McCarroll and Loader (2006). This vial consists of a vessel 5 cm in height, 2 cm in diameter with a glass filter of porosity 2 in the bottom, i.e. a pore size of 40-100 μm. Under the filter, the vessel is connected to a narrow tube, 6 mm in diameter. 48 glass vials were placed in a water bath. The vials were connected by tubes to a diagraph vacuum pump. Pumping of waste chemicals were controlled by valves (see Figure 1a).

After all steps of the pretreatment (Table 1), the samples were transferred to 2.0 ml Eppendorf tubes. If the sample consisted of inhomogeneous material such as a mix of EW and LW or a mix of several years, it was homogenized using a Hielscher Ultrasonic sonotrode (UP200st) to ensure the sample was completely homogeneous (Laumer et al. 2009). In this study, this was only done to the background samples, since the Sch samples consisted of wood from only one annual LW ring. Next, the samples were freeze dried, leaving dry α-cellulose, and subsequently weighed to determine the α-cellulose yield.

Infrared spectroscopy (ATR-FTIR, Attenuated Total Reflectance Fourier Transform InfraRed) was conducted using an instrument from PerkinElmer to characterize the quality of the cellulose pretreatment at different stages during the pretreatment process.
Infrared radiation with wavenumber from 4000 to 400 cm^{-1} was passed through the sample and the amount of reflected radiation was measured. Different molecular bonds absorb radiation at different wavelengths, so the absorption spectrum will depend on the molecular constituents of the sample.

Before the first spectrum of a sample was taken, a background spectrum without sample was taken, which was then subtracted from the following spectra. A sample was then placed in the instrument, and 4 scans were averaged into one spectrum using the software Spectrum™ 10 (PerkinElmer). Between each sample, the sample plate was wiped with lens paper dampened with ethanol.

The spectra were normalized such that the absorbance values were between zero and one. Zero means no light is absorbed; one means maximum light is absorbed. Defining the peak absorbance (found in all spectra between approximately 1140 and 920 cm^{-1}) as y_1 and the absorbance in a flat area at 1877 cm^{-1} with no significant absorption as y_0, the normalized absorbance values (y_n) are calculated as:

$$y_n = \frac{y_i - y_0}{y_1 - y_0} \, ,$$

where the non-normalized absorbance values are denoted y_i.

For AMS analysis the α-cellulose samples were converted into CO_2 using torch sealed tubes containing pre-baked CuO. The CO_2 samples were then graphitized using an iron catalyst and H_2 atmosphere, and subsequently analyzed on the 1 MV AMS system from High Voltage Engineering Europe at AARAMS (Olsen et al. 2017). A small subset of samples was analyzed at the radiocarbon dating laboratory at Lund University in Sweden (Skog, Rundgren, and Sköld 2010; Adolphi et al. 2013).

Radiocarbon ages are reported as conventional ^{14}C dates in ^{14}C yr BP based on the measured $^{14}C/^{12}C$ ratio corrected for the natural isotopic fractionation using online $^{13}C/^{12}C$ by normalizing the result to the standard $\delta^{13}C$ value of -25 ‰ VPDB (Stuiver and Polach 1977). $F^{14}C$ values are calculated as described in Reimer, Brown, and Reimer (2004).

Results and discussion

In order to pretreat a large batch of samples efficiently we have modified the pretreatment method by testing the different steps of the Loader method and comparing with our revised version of the Southon method (called the AARAMS method). Our overall aim is to obtain the purest possible cellulose sample and to demonstrate a low background contamination.

Pretreatment and infrared spectroscopy

Placement of individual samples in separate vials (Figure 1b) makes it possible to have the chemicals reacting with the sample and afterwards filtering the solution out through the tube, leaving the sample material behind. This reduces the loss of material, since the sample material is kept in the same container throughout the whole procedure.

In the Loader method, the bleaching solution is changed after 1 hour. Six additions are made in total before the alkaline (NaOH) extraction, and another two additions are made after the alkaline extraction (Table 1). Infrared spectra from samples that had been bleached for 1, 2, ..., 6 hours (Figure 2a) were inspected and visually compared to investigate the significance of the length of the delignification. The biggest changes were seen in the first two hours of bleaching, and after 3 hours of bleaching, no significant changes were observed. This means that the 8 hours of bleaching in the Loader method is unnecessary for our samples, and 3 or 4 hours are enough to remove most lignin in these oak samples. The same conclusion was reached by Loader et al. (1997). The method has since been altered to include 8 hours of bleaching, and initially we followed this revised method in order to ensure complete delignification of our samples. Absorption spectra from samples that were bleached for 2 hours before alkaline extraction and then bleached for another two hours after alkaline extraction were compared with spectra of samples that were bleached for 4 hours before alkaline extraction. The absorption spectra showed no observable difference between these two approaches.

In the Southon method, the bleaching solution is changed if needed, by monitoring the color of the solution. The solution has a yellow color due to the dissolution of chlorite. But when all chlorite ions have reacted, the solution loses its color, and no more bleaching takes place. When this happens, the solution is changed to a freshly mixed acidified sodium chlorite solution. The samples are bleached 4 to 6 hours in total. The delignification is terminated when the color of a sample is observed to be white. No bleaching takes place after the alkaline extraction. In our pretreatment, we found the yellow color to weaken and disappear from the solution of many samples after 3 hours, thus for simplicity we decided to change the bleaching solution of all samples to a freshly mixed acidified sodium chlorite solution after 3 hours. Determination of sufficiently white color of the samples themselves proved difficult due to the color of the solution in which they were submerged, so for consistency, we bleached all samples for a total of 6 hours.

The next step is alkaline extraction with sodium hydroxide to remove hemicelluloses. The Loader method uses two additions of NaOH, first a 10 % added to the samples at 80° C, next a 17 % solution added at room temperature. These two solutions remove different hemicelluloses. Spectra of samples that had been subjected to either one or two NaOH solutions were visually compared. The infrared spectra of our samples showed no difference, meaning one step with a 17 % NaOH solution is sufficient to remove the hemicelluloses from these samples. The Southon method uses only one addition of NaOH; a 20 % solution added at room temperature. In our pretreatment following the Southon method (called the AARAMS method), we chose to use a 17 % sodium hydroxide solution instead due to the definition of α-cellulose (Green 1963).

Overall, the result of the pretreatment is investigated by visually comparing spectra of samples that have not undergone any pretreatment (wholewood), samples that have only been delignified (holocellulose) following the Loader method, and samples that went through the whole pretreatment following the Loader method (α-cellulose). These spectra are also compared to an infrared spectrum of an acquired microcrystalline cellulose powder produced by Acros Organics BVBA. These four spectra are shown in Figure 2b.

Some differences between the spectra are clearly visible. In Figure 2b most of the peaks are marked with a letter. Peak B (at 640 cm^{-1}) is from water linked to cellulose (Fan, Dai, and Huang 2012) and is present in all spectra. Peaks C ((at 1592 cm^{-1}) and D (at 1502 cm^{-1}) are from lignin (Stewart et al. 1995) and they are removed after the delignification, even though a small hint of a peak remains afterwards in all spectra at peak D. Peaks A (at 1729 cm^{-1}) and I (at 1230 cm^{-1}) are due to hemicelluloses (Pandey and Pitman 2003; Fan, Dai, and Huang 2012). Peak A disappears after the NaOH wash, but there is still absorbance in the area around peak I, but the peak at 1230 cm^{-1} is no longer there. This is because there are many other peaks in this area from cellulose, which will then be more pronounced after removal of the hemicelluloses (Fang, Sun, and Tomkinson 2000), which is also seen by the fact that the spectra of the α-cellulose and microcellulose are similar in this area. The peaks E (at 1457 cm^{-1}) and F (at 1421 cm^{-1}) are more pronounced in the wholewood, but there is still some absorbance present in the other spectra. Fang, Sun, and Tomkinson (2000), Pandey and Pitman (2003), and Fan, Dai, and Huang (2012) all discuss different peaks from both cellulose, hemicelluloses, and lignin in this area, and it is difficult to tell the cause of this absorbance. The lignin absorbance probably causes the drop around peaks E and F from the wholewood spectrum to the holocellulose spectrum. The rest of the peaks (G at 1368 cm^{-1}, H at 1323 cm^{-1}, J at 1157 cm^{-1}, K at 1030 cm^{-1}, and L at 895 cm^{-1}) are present in all the spectra, and they are reported to be from cellulose as would be expected, when they are not removed in the process of pretreatment (Stewart et al. 1995; Fang, Sun, and Tomkinson 2000; Pandey and Pitman 2003; Fan, Dai, and Huang 2012). However, Pandey and Pitman (2003) report the peaks G and J to be both from cellulose and hemicelluloses.

Overall, the spectra of the extracted α-cellulose and the microcellulose are similar, meaning a successful extraction was achieved.

Radiocarbon analysis

In this study, we used three different backgrounds: Charcoal, MIS7 Kauri, and Eemian oak. The measured F^{14}C values for the charcoal samples is shown in Table 2, and the measured F^{14}C values for the MIS7 Kauri and Eemian oak samples are shown in Table 3 and Figure 3. Five Eemian oak samples and one MIS7 Kauri sample were measured in Lund. The charcoal background was used to assess the graphitization and AMS measurements of the samples, by comparing the radiocarbon ages of this background obtained in this study by radiocarbon ages obtained routinely in AARAMS of this background. The mean of this background was found in this study to be 48100 ± 3500 BP (F^{14}C: 0.0025 ± 0.0011), which agrees well with the mean based on all measurements in AARAMS of this background of 48800 ± 3500 BP (F^{14}C: 0.0023 ± 0.0010) (Figure 3).

Table 2: The charcoal backgrounds used in the project with 1 standard deviation in parantheses.

Sample ID, type, pretreatment	F^{14}C
AAR-1351, charcoal, ABA	
	0.0042(3)
	0.0038(3)
	0.0020(1)
	0.0017(1)
	0.0013(1)
	0.0017(1)
	0.0022(1)
	0.0016(1)
	0.0029(2)
	0.0024(1)
	0.0041(1)
Mean	**0.0025(11)**

Table 3: The MIS7 Kauri and Eemian oak backgrounds used in the project with 1 standard deviation in parentheses.

Pretreatment	Loader method	AARAMS method
Sample ID, type	F^{14}C	F^{14}C
AAR-21745, MIS7 Kauri, Measured at AARAMS	0.0044(3)	0.0036(2)
	0.0028(1)	0.0037(2)
	0.0020(1)	0.0022(1)
	0.0038(1)	0.0020(1)
	0.0042(1)	
	0.0040(1)	
	0.0051(2)	
	0.0036(2)	
	0.0033(3)	
	0.0040(1)	
	0.0030(2)	
	0.0032(1)	
	0.0038(1)	
	0.0071(2)	
	0.0031(1)	
	0.0037(1)	
Mean	**0.0038(11)**	**0.0029(9)**
AAR-21745, MIS7 Kauri, Measured in Lund	0.0041(1)	
AAR-25431, Eemian oak, Measured at AARAMS	0.0043(2)	0.0028(1)
	0.0051(2)	0.0024(1)
	0.0073(3)	
	0.0045(2)	
Mean	**0.0053(14)**	**0.0026(3)**
AAR-25431, Eemian oak, Measured in Lund	0.0053(2)	
	0.0060(2)	
	0.0060(2)	
	0.0051(2)	
	0.0061(2)	
Mean	**0.0057(4)**	

The F^{14}C values of the backgrounds all lie in the range 0.0013–0.0073, which corresponds to ^{14}C ages between 53400 and 39500 BP. Seventy-nine percent of the samples have F^{14}C values below 0.0050, corresponding to a ^{14}C age of 42600 BP. The background F^{14}C values are comparable to the values of the charcoal background routinely measured in AARAMS of 0.0023 ± 0.0010 during the period from 2016 – 2020 (Figure 3). Note that the charcoal F^{14}C values presented in Table 2 are a subset of these, which were processed along with the extracted cellulose backgrounds presented in Table 3.

The Eemian oak samples pretreated using the Loader method are in average 5700 ± 2300 ^{14}C yr younger than the samples pretreated following the AARAMS method (Table 3, Figure 3). This may indicate a higher modern contamination when using the Loader method, in accordance with a possible contamination originating from the acetic acid step. However, this result is only based on a total of 6 samples, hence may not include the full scatter of background F^{14}C values (Figure 3). The means of the MIS7 Kauri samples from the two batches of different pretreatments are not significantly different (Table 3), though, indicating that the contamination originating from the pretreatment chemicals is likely small.

The background samples dated in Lund show good agreement with the samples measured at AARAMS (Table 3). Eemian oak samples dated in Lund are normally pretreated following the BABAB procedure (Nemec et al. 2010), and the values obtained here are older than the samples that we pretreated, namely F^{14}C: 0.0034 ± 0.0007 (Adolphi et al. 2013). This difference warrants further testing, but a reason could be that the sample amount per batch is larger in the Lund radiocarbon laboratory, which could reduce the influence of modern contamination.

Table 4: Samples from the Sch wood measured both at AARAMS and at the radiocarbon dating laboratory at Lund University. All samples are pretreated following the Loader method. Subsequently, the α-cellulose is divided in two, one sample is measured at AARAMS and the other sample is measured in Lund. The steps following the pretreatment are carried out at the different labs. The difference between the two values measured for two samples from the same year is also shown as well as the difference in terms of the standard deviation. The two samples from the year 1446 AD have the biggest deviation (3.5 σ) and could be analytical outliers. Therefore, the mean and weighted mean are calculated both with and without these samples.

Sample ID AAR-	Year (AD)	AARAMS ^{14}C Age (BP)	Lund ^{14}C Age (BP)	Diff. ^{14}C years	Dev σ	Diff. ^{14}C years	Dev σ
22720	1439	514 ± 31*	506 ± 25	8 ± 40	0.2	8 ± 40	0.2
22719	1440	441 ± 21	491 ± 25	-50 ± 33	-1.5	-50 ± 33	-1.5
22718	1441	483 ± 20	473 ± 25	10 ± 32	0.3	10 ± 32	0.3
22717	1442	476 ± 20	476 ± 25	0 ± 32	0.0	0 ± 32	0.0
22716	1443	458 ± 16	458 ± 25	0 ± 30	0.0	0 ± 30	0.0
22715	1444	435 ± 18	454 ± 25	-19 ± 31	-0.6	-19 ± 31	-0.6
22714	1445	454 ± 30	452 ± 25	2 ± 39	0.1	2 ± 39	0.1
22713	1446	519 ± 22	404 ± 25	115 ± 33	3.5		
22712	1447	473 ± 27	471 ± 25	2 ± 37	0.1	2 ± 37	0.1
22711	1448	434 ± 27	422 ± 25	12 ± 37	0.3	12 ± 37	0.3
22710	1449	421 ± 16	464 ± 25	-43 ± 30	-1.4	-43 ± 30	-1.4
22709	1450	431 ± 19	460 ± 25	-29 ± 31	-0.9	-29 ± 31	-0.9
22708	1451	415 ± 31	406 ± 25	9 ± 40	0.2	9 ± 40	0.2
22707	1452	473 ± 28	445 ± 25	28 ± 38	0.7	28 ± 38	0.7
22706	1453	407 ± 20	405 ± 25	2 ± 32	0.1	2 ± 32	0.1
22705	1454	420 ± 18	428 ± 25	-8 ± 31	-0.3	-8 ± 31	-0.3
22704	1455	417 ± 19	438 ± 25	-21 ± 31	-0.7	-21 ± 31	-0.7
22703	1456	397 ± 24	428 ± 25	-31 ± 35	-0.9	-31 ± 35	-0.9
22702	1457	415 ± 19	443 ± 25	-28 ± 31	-0.9	-28 ± 31	-0.9
22701	1458	486 ± 26*	393 ± 25	93 ± 36	2.6	93 ± 36	2.6
		Mean		3 ± 39	0.0 ± 1.2	-3 ± 30	-0.1 ±0.9
		Weighted mean		0 ± 8		-6 ± 8	
		Reduced χ^2		1.4 ≤ 1.5		0.8 ≤ 1.5	

*Determined as age outlier in Fogtmann-Schulz et al. (2019).

Twenty annual LW samples from the Sch oak piece were pretreated at AARAMS using the Loader method. The produced α-cellulose was subsequently divided and dated both at AARAMS and in Lund (Table 4). Overall, the agreement between AARAMS and Lund is good with weighted mean difference of 0 ± 8 ^{14}C years (reduced χ^2: 1.4 ≤ 1.5). One sample (AAR-22713) resulted in a difference of 115 ± 33 BP corresponding to 3.5 σ. Hence for

AAR-22713, AARAMS and Lund are statically different. Excluding AAR-22713 as an outlier, the weighted mean difference of the samples is -6 ± 8 ^{14}C years (reduced χ^2: $0.8 \leq 1.5$). The difference between AARAMS and Lund is 0.8 σ and thus in statistical agreement.

Conclusion

Based on a qualitative visual analysis of the placement of peaks in absorption spectra from FTIR of samples pretreated following the Loader method, the produced α-cellulose is similar to the microcrystalline cellulose, thus the pretreatment was successful in producing α-cellulose. However, some of the extra steps in the Loader method as compared to the Southon method are unnecessary, at least when pretreating oak for radiocarbon analysis. Yet, it may be necessary with other wood samples, so a careful consideration of the steps of the pretreatment is always necessary. Based on this we have developed the AARAMS method for pretreating wood samples to α-cellulose. The contamination introduced to the samples during pre-treatment and graphitization is comparable with two other background samples used in the laboratory, even though the background level slightly improved when using the AARAMS method instead of the Loader method. Based on 20 doublet samples measured both at AARAMS and in Lund the average inter-laboratory age difference calculated to -6 ± 8 ^{14}C years is insignificant.

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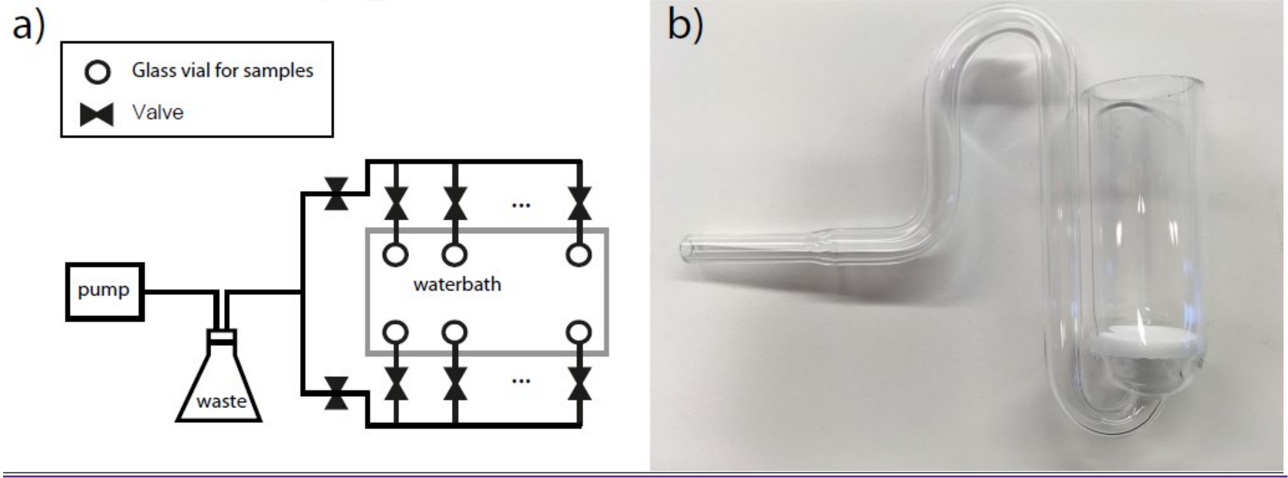
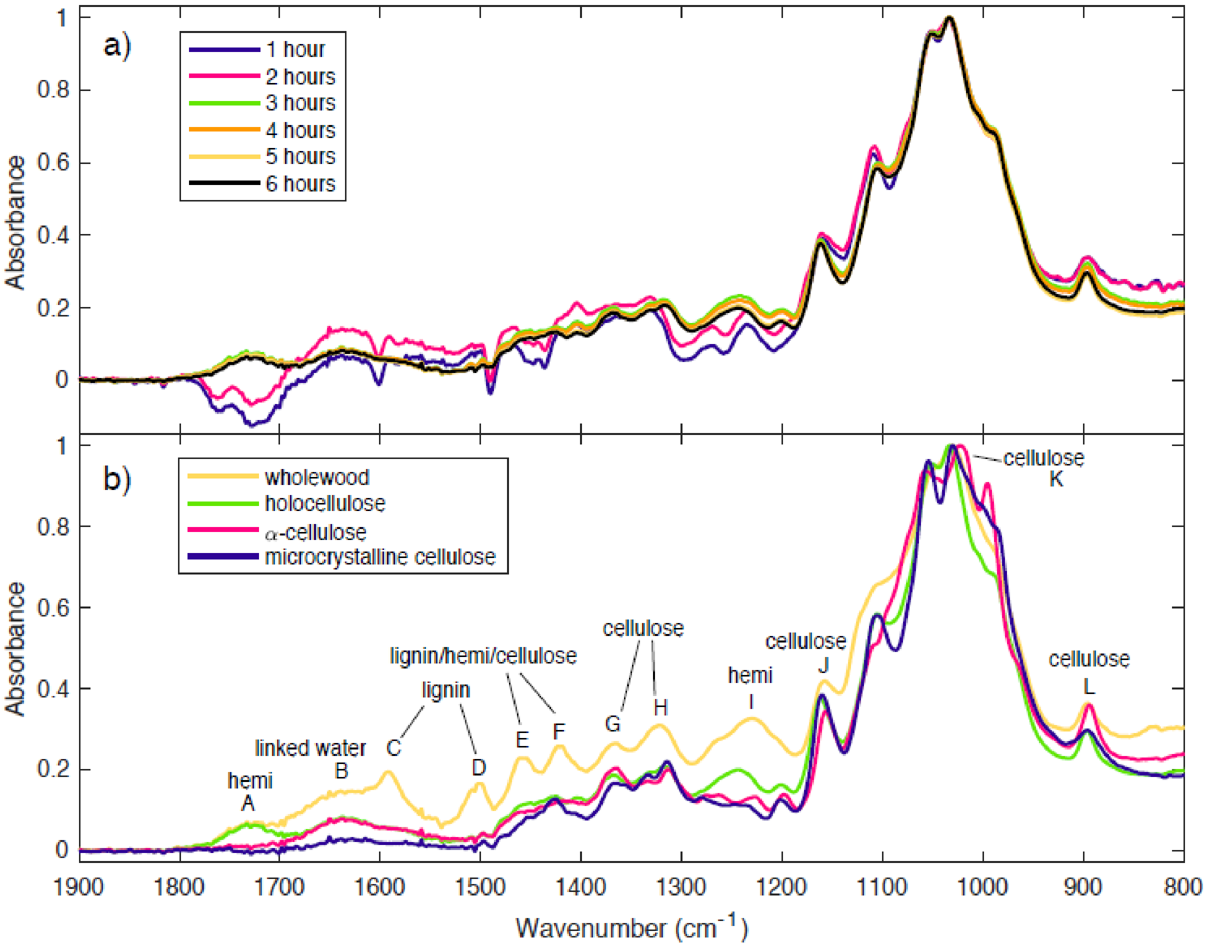


Fig.1


a)
1 hour
2 hours
3 hours
4 hours
5 hours
6 hours
Absorbance
1
0.8
0.6
0.4
0.2
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b)
wholewood
holocellulose
α-cellulose
microcrystalline cellulose
Absorbance
1
0.8
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0.4
0.2
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hemi
A
linked water
B
C
lignin
D
lignin/hemi/cellulose
E
F
cellulose
G
H
hemi
I
cellulose
J
cellulose
K
cellulose
L
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
Wavenumber (cm^{-1})

FIG3.

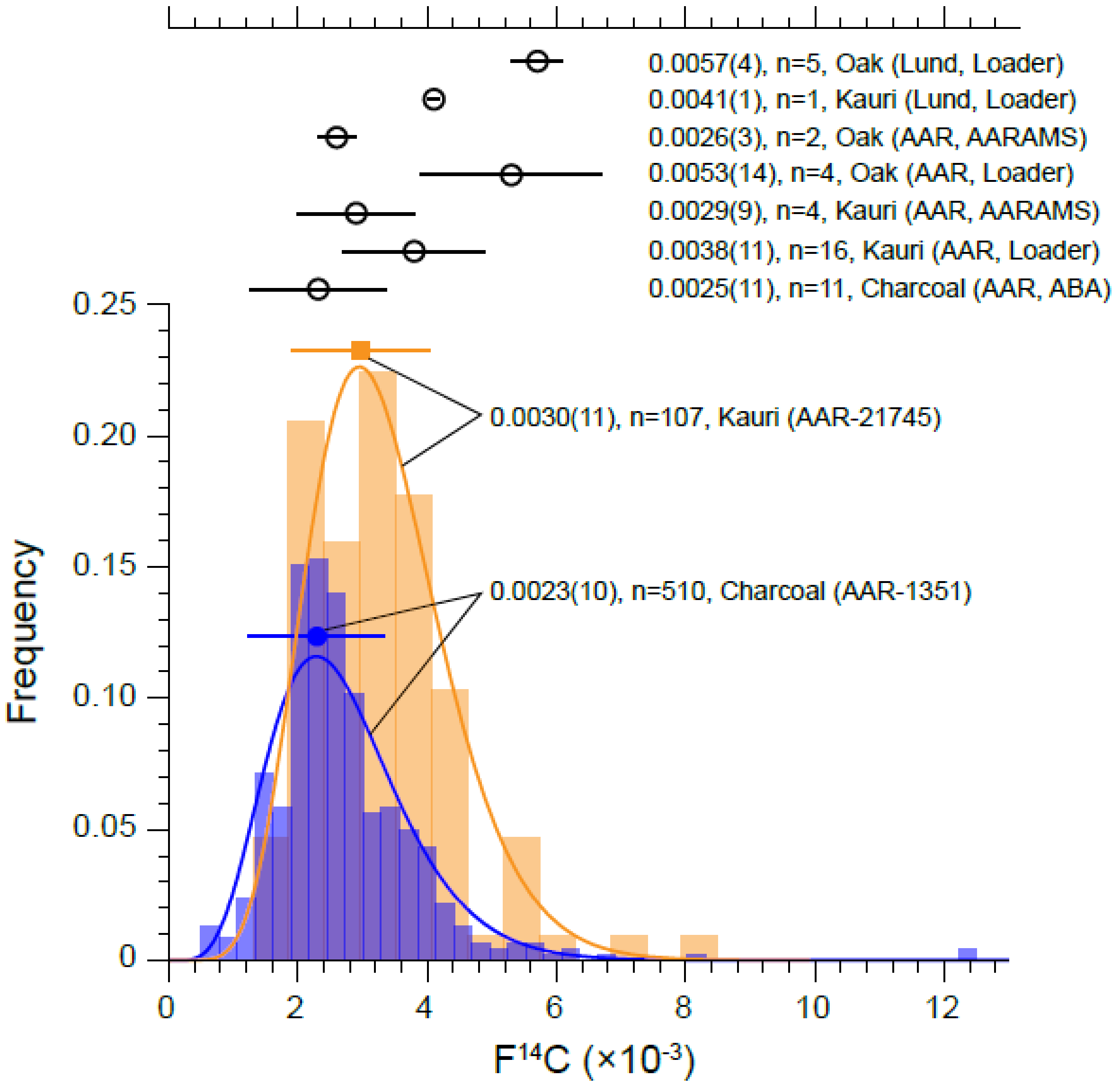
0.0057(4), n=5, Oak (Lund, Loader)
0.0041(1), n=1, Kauri (Lund, Loader)
0.0026(3), n=2, Oak (AAR, AARAMS)
0.0053(14), n=4, Oak (AAR, Loader)
0.0029(9), n=4, Kauri (AAR, AARAMS)
0.0038(11), n=16, Kauri (AAR, Loader)
0.0025(11), n=11, Charcoal (AAR, ABA)
0.0030(11), n=107, Kauri (AAR-21745)
0.0023(10), n=510, Charcoal (AAR-1351)
Frequency
0.25
0.20
0.15
0.10
0.05
0
0
2
4
6
8
10
12
F14C (×10-3)

FIGURE CAPTIONS

Figure 1: a) The experimental setup during pretreatment. 48 glass vials are placed in a water bath and connected via valves and tubes to a vacuum pump, so chemicals from individual vials can be pumped out and collected in an Erlenmeyer flask. b) A glass vial for one sample during pretreatment.

Figure 2: a) Absorbance spectra of six wholewood subsamples that have been delignified between 1 and 6 hours. b) Absorbance spectra of four samples. The peaks marked by letters are explained in the text.

Figure 3: Histogram of all Kauri (AAR-21745) and charcoal (AAR-1351) samples measured between 2016 and 2020 in AARAMS. A Gamma probability distribution is fitted to both histograms from which a mean and standard deviation are estimated for both Kauri (AAR-21745) and charcoal (AAR-1351). For comparison, the mean F^{14}C background values of Eemian oak and Kauri presented in Table 2 and 3 are also shown.

TABLE CAPTIONS

Table 1: The main steps of pretreatment of the revised* Loader method (Loader et al. 1997) and Southon method (Southon and Magana 2010) are shown in the table as well as the resulting procedure used in the AARAMS lab. Between each part of the pretreatment, the samples are rinsed with deionized or Milli-Q water to neutral pH.
(Below table:) *Samples prepared in conventional water bath, with additional final bleaching stage and ultrasonic homogenisation after preparation to α-cellulose.

Table 2: The charcoal backgrounds used in the project with 1 standard deviation in parantheses.

Table 3: The MIS7 Kauri and Eemian oak backgrounds used in the project with 1 standard deviation in parentheses.

Table 4: Samples from the Sch wood measured both at AARAMS and at the radiocarbon dating laboratory at Lund University. All samples are pretreated following the Loader method. Subsequently, the α-cellulose is divided in two, one sample is measured at AARAMS and the other sample is measured in Lund. The steps following the pretreatment are carried out at the different labs. The difference between the two values measured for two samples from the same year is also shown as well as the difference in terms of the standard deviation.
The two samples from the year 1446 AD have the biggest deviation (3.5 σ) and could be analytical outliers. Therefore, the mean and weighted mean are calculated both with and without these samples.
(Below table:) *Determined as age outlier in Fogtmann-Schulz et al. (2019).

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