



## SQUEAKY CLEAN CELLULOSE: COMPARING PRETREATMENT EFFECTIVENESS ON SINGLE TREE RINGS AND WOODEN LATHS

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**ABSTRACT.** Obtaining accurate radiocarbon (<sup>14</sup>C) results from wood samples requires the extraction of cellulose. In the past, this has been done using different combinations of methods including acidified bleaching, acid-base-acid, and strong bases. This often becomes a time-consuming task in most analyses, especially when single ring isotope chronologies are needed from wood samples. Using <sup>14</sup>C and Fourier-transform infrared spectroscopy (FTIR), we tested four different pretreatment methods to determine their ability to produce high quality cellulose. We then adjusted the best methods to determine a method for pretreating wood laths of multiple rings. A sequence of base-acid-base-acid-bleach + strong base (BABAB+) and BABAB produced the most accurate results when compared to the Bomb20 Northern Hemisphere Zone 1 curve with an average difference in  $\Delta^{14}\text{C}$  of 1.3‰ and 5.8‰ respectively. These methods were adjusted to pretreat an entire wood lath and a comparison of the FTIR results suggest that our adjusted BABAB+ and BABAB produced high quality cellulose comparable to that of an individually pretreated ring. The possibility to pretreat wood lath samples has the potential to more than double the number of tree rings which can be pretreated in a week. This is a significant reduction in time when creating long tree ring chronologies.

**KEYWORDS:** chemical analysis, dating, dendrochronology.

### INTRODUCTION

Dendrochronology and dendroclimatology often require multiple series of tree rings to create a chronology with adequate sample depth. As a result, hundreds of rings need to be pretreated before they are analysed. This makes for a time consuming and laborious task that can create a bottleneck in processing samples leading to reduced efficiency in the laboratory.

#### Cellulose Extraction in the Past

The average composition of pine wood species is  $\alpha$ -cellulose (40–45%), hemicellulose (20–25%), lignin (23–34%), and resins and extractives (<10%) (Rowell et al. 2005). For radiocarbon analysis,  $\alpha$ -cellulose is often the preferred compound to extract as it most accurately represents the atmospheric radiocarbon content from the growth year.

Green (1963) outlines the commonly used Jayme-Wise method whereby fine wood meal is first treated using a solvent extraction of 2:1 benzene-ethanol followed by 95% ethanol to remove resins. This is followed by treatment with acidified (glacial acetic acid) NaClO<sub>2</sub> and heated in a steam bath to 70°C. Acidified NaClO<sub>2</sub> is periodically and heated for varying times depending on wood types. A modification of this method by Leavitt and Danzer (1993) using toluene instead of benzene, has become one of the most common methods used to extract holocellulose.

Since the turn of the 21st century, there have been a few projects with the aim of processing wood samples in strips of wood (laths) rather than as individual ring samples. This is an

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important concern as the capacity to process samples is often the major limiting factor in analysis as it is both time and labor-intensive.

Loader et al. (2002) made one of the first attempts to extract holocellulose before separating out the individual annual rings by soaking cores in acidified  $\text{NaClO}_2$ . This method was unable to produce accurate  $\delta^{13}\text{C}$  results when compared to individually prepared samples. In later years, lath preparation of samples was successfully done by Li et al. (2011) where laths were treated using the standard Jayme-Wise method (Green 1963). This did however come with difficulties, as laths were fragile and often broke, thus requiring extra support. Kagawa et al. (2015) successfully created cases made from polytetrafluoroethylene (Teflon) to support wooden lath samples throughout a Jayme-Wise pretreatment to address this problem while maintaining sample quality. In the present study, we use silica webbing tape to support the lath samples.

### **FTIR and Radiocarbon as Proxies for Contaminant Removal**

Testing the effectiveness of different methods required the use of different proxies and carefully selected samples. We chose to use Fourier-transform infrared spectroscopy (FTIR) and radiocarbon to determine the extent of contaminant removal. Radiocarbon results were compared to the IntCal20 and Bomb21 calibration curves (Hua et al. 2022; Reimer et al. 2020). In conjunction, FTIR offers a visual representation of the presence or absence of wood components when compared to untreated wood.

The use of FTIR in the identification of various wood components has been used effectively in many studies ranging from the paper and pulp industry (Bouchard and Douek 1993) to similar projects investigating the preparation of wood samples for isotope analysis (e.g. Rinne et al. 2005; van der Wal 2021). FTIR has been shown as a powerful tool in understanding wood composition and testing the removal of contaminants when extracting cellulose.

The positions of the relative peak intensities indicate the presence of different functional groups. Wood spectra generally show absorbance around  $3300\text{ cm}^{-1}$  indicating O-H bonds and a slightly smaller peak around  $2900\text{ cm}^{-1}$  indicating the presence of C-H bonds. The region between  $2500\text{ cm}^{-1}$  and  $2000\text{ cm}^{-1}$  generally shows no absorption, as this region indicates the presence of triple bonds (of which are not abundant in most wood components). For this study, wavenumbers between  $1800\text{ cm}^{-1}$  and  $1400\text{ cm}^{-1}$  are of interest, as this region records the absorbance of double bonds (C=O and C=C). These double bonds are present in lignin, resin, and other minor extractives.

In this study, we test the ability of different methods to produce “clean” cellulose in the shortest time by using  $^{14}\text{C}$  measurements and Fourier-Transform Infrared Spectroscopy (FTIR) analysis as a proxy for pretreatment efficiency. To do this, we compare four different pretreatment methods on individual pine tree rings (*Pinus sylvestris* L.) from central Norway from the 1930s to determine the extent of contaminant removal. We then use the best performing methods on thin wood laths containing multiple rings and analyze using FTIR to understand the potential of lath pretreatment.

### **MATERIALS**

To test the different methods, samples were taken from a pine tree which started its growth in AD 1899 around Vassfjellet (close to the city of Trondheim in central Norway). This tree was

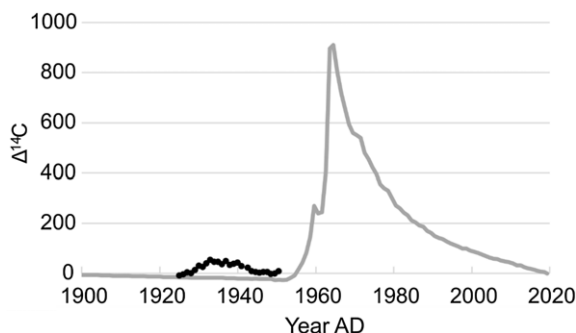


Figure 1 Intcal20 (1850–1941; Reimer et al. 2020) and Bomb calibration curve NH1 (1942–2019; Hua et al. 2022) plotted in gray against the  $\Delta^{14}\text{C}$  results of untreated tree rings from 1925 to 1950 (black).

felled in AD 2018 and these ages were confirmed using dendrochronological cross matching against the central Norway master curve by Thun (2002). At the time of felling, the heartwood-sapwood boundary was around the AD 1974 ring. The tree is ca. 45 cm in diameter with an average ring width of 1.7 mm. A 5cm thick disc was cut from the trunk and was used for sample material in these experiments.

### False Bomb Peak

The transition of wood from sapwood to heartwood has been shown to incorporate  $^{14}\text{C}$  from the atmosphere at the time of transition (Cain and Suess 1976; Olsson 1980; Stuiver and Quay 1981). This becomes important for samples where the conversion from sapwood to heartwood happened during the bomb spike period as these rings would incorporate a higher  $^{14}\text{C}$  concentration than that of the original ring cellulose. This effect makes for  $^{14}\text{C}$ -enriched tree rings which are good candidates for testing the effectiveness of pretreatment methods. Figure 1 shows the radiocarbon results of 25 tree ring samples (1925–1950) of untreated wood plotted against the NH1 atmospheric  $^{14}\text{C}$  bomb curve (Hua et al. 2022) over 1942–2019 and IntCal20 (Reimer et al. 2020) during the period 1850–1941. Our untreated wood samples before the 1930s contain between 3‰ and 15‰ more  $^{14}\text{C}$  than the atmosphere during their growing period. This increases in the 1930s with 1933 having the biggest difference between untreated wood and the curve (70‰ difference). This increased  $\Delta^{14}\text{C}$  marks the “false bomb spike” for this tree. It is for this reason that we use tree ring samples for 1933–1937 to test pretreatment methods.

### METHODS

For the pretreatment of individual rings, the earlywood (EW), latewood (LW), and whole ring (WR) samples were cut for each year (1933–1937). The disc was cut into smaller pieces using an electric band saw. Thin shavings were cut using handheld scalpels from these pieces for each year, taking care to keep EW and LW portions separate for pretreatment. These samples were then pretreated using the methods outlined in Table 1 and described below. This resulted in a total of 75 samples including 15 samples that would not be pretreated. Sample weights ranged between 4 mg and 6 mg for 17.5% NaOH and Solvent extraction, and between 10mg and 70mg for BABAB and BABAB+ treatments.

Table 1 Outline of the four pretreatment methods tested: 17.5% NaOH (Rowell et al. 2005), Solvent extraction (Bruhn et al. 2001), BABAB (Němec et al. 2010), and BABAB+. The amounts and times indicated are those used for individual samples. These were increased for the pretreatment of laths.

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8	Step 9
NaOH	Sample was submerged in 17.5% NaOH solution for 1–1.5 hours at room temperature.	3x H <sub>2</sub> O rinse (pH=10).	5 mL 0.4% HCl for 30 minutes. Rinsed in H <sub>2</sub> O and dried (65 °C).						
Solvent extraction	3× 15 minute boil in Tetrahydrofuran.	3× 15 minute boil in chloroform.	3× 15 minute boil in petroleum ether.	3× 15 minute boil in acetone.	3× 15 minute boil in methanol.	3× 15 minute boil in distilled water.	Left to dry in the drying oven (65°C).		
BABAB	5 mL of 4% NaOH overnight at 75°C followed by H <sub>2</sub> O rinse until pH=10.	5 mL of 4% HCl for 1 hour followed by H <sub>2</sub> O rinse until pH=3.	5 mL of 4% NaOH for 1.5 hours followed by H <sub>2</sub> O rinse until pH=10.	5 mL of 4% HCl for 1 hour followed by H <sub>2</sub> O rinse until pH=4.	5 mL of 10% NaClO <sub>2</sub> , 5 mL H <sub>2</sub> O, 2 drops of 4% HCl (pH=2) for 2 hours.	Ultrasonic bath at room temperature for 15 minutes.	Rinsed with H <sub>2</sub> O until pH=4.	Bleach step repeated if necessary otherwise fraction is dried (65°C).	
BABAB+	5 mL of 4% NaOH overnight at 75°C followed by H <sub>2</sub> O rinse until pH=10.	5 mL of 4% HCl for 1 hour followed by H <sub>2</sub> O rinse until pH=3.	5 mL of 4% NaOH for 1.5 hours followed by H <sub>2</sub> O rinse until pH=10.	5 mL of 4% HCl for 1 hour followed by H <sub>2</sub> O rinse until pH=4.	5 mL of 10% NaClO <sub>2</sub> , 5 mL H <sub>2</sub> O, 2 drops of 4% HCl (pH=2) for 2 hours.	Ultrasonic bath at room temperature for 15 minutes.	Rinsed with H <sub>2</sub> O until pH = 4 Bleach step repeated if necessary.	5 mL of 17.5% NaOH for 1 hour followed by H <sub>2</sub> O rinse until pH=10.	5 mL 0.4% HCl for 30 minutes. Rinsed once in H <sub>2</sub> O and dried (65°C).

### 17.5% NaOH

The use of a strong base is important for the isolation of  $\alpha$ -cellulose from holocellulose (Rowell et al. 2005) as it is insoluble in 17.5% NaOH unlike hemicellulose. We therefore experiment with treating the wholewood with only 17.5% NaOH to test the ability of a strong base in creating a crude fraction with properties similar to cellulose.

### Solvent Extraction

Solvent extractions are often the starting point of many pretreatments of wood tissue (Leavitt and Danzer 1993; Loader et al. 1997). A common mix of solvents has often included the use of toluene and ethanol to remove resinous material and other extractives. A solvent extraction alone is not adequate as resins and extractives do not make up more than 10% of pine wood samples. However, in the present case, as we are aware that the extractives are enriched in radiocarbon, this method should prove fairly effective in improving the results. The eluotropic sequence used in this project is taken from Bruhn et al. (2001) and includes the use of six different solvents in the following order: tetrahydrofuran, chloroform, petroleum ether, acetone, methanol, and water. This choice of solvents, from non-polar to polar, ensures that each solvent washes out the previous one.

### BABAB

This is a holocellulose extraction method from Němec et al. (2010), which combines two common concepts for holocellulose extraction: the acid/base/acid (ABA) pretreatment sequence and an acidified bleach treatment. The ABA sequence is commonly used in pretreatments to remove carbohydrates and, in the case of wood, leaves behind cellulose and lignin (Gaudinski et al. 2005). Němec et al. (2010) modified this and preceded this step with a long overnight soak in 4% NaOH, which disrupts the wood structure allowing for a more effective use of the ABA steps. This is then followed by an acidic bleaching step to remove the lignin. The remaining fraction from this method is holocellulose. From start to finish, samples pretreated using the BABAB method require one overnight soak and 8 hours of additional treatment, not including sample cutting and drying times.

### BABAB+

This method uses the BABAB holocellulose extraction (described above) followed by a strong base treatment (17.5% NaOH) for 3.5 hours to remove hemicellulose. Degraded samples may require less treatment time. A short 0.4% HCl step to remove atmospheric carbon that dissolved in the alkali solution ends the preparation. Using this method, we expect to extract a pure  $\alpha$ -cellulose of sufficiently high quality for precise radiocarbon measurements as opposed to the holocellulose extracted using the BABAB method.

### Lath Pretreatment

The lath pretreatment began with the use of an electric band saw to cut 1 mm to 3 mm thick laths from a wood disc (approximately 3 cm thick). Two laths containing 102 rings each were pretreated using BABAB and BABAB+ methods in long sample boats (see Supplementary material, Figure 1). The sample boats are made of borosilicate glass and are 300 mm long, 58 mm wide, and 28 mm deep.

To compensate for the thickness of material being pretreated, the amount of solution and the submerging times were increased to full-day submersion. The laths were pretreated in 100 mL of the solutions for at least double the time outlined in Table 1. It became particularly important to perform the first NaOH step twice, adding fresh solution the second time, as well as performing multiple bleach steps until the whole lath was white. To provide extra support to the sample during the bleaching steps, 30 cm strips of silica webbing tape (2.5 cm wide) were placed beneath the lath to help move the sample in one piece.

While the BABAB treatment could be completed with the lath intact, the strong base step at the end of BABAB+ proved to be too aggressive. The laths had to be cut prior to this last step, which was done on individual rings, thus not completed as an in-tact lath.

### FTIR and Radiocarbon Analysis

All samples were left in a drying oven at 65°C until completely dry after pretreatment. Samples were then combusted in an elemental analyzer and graphitized in an automatic system using a H<sub>2</sub>-Fe reaction (Seiler et al. 2019). The graphite was measured at the Trondheim 1MV AMS system following standard procedures (Nadeau et al. 2015).

FTIR measurements were done using a PerkinElmer Spectrum 400 FT-IR/FT-NIR Spectrometer. Absorption was measured between 4000 cm<sup>-1</sup> and 550 cm<sup>-1</sup> in 32 scans per sample. Background measurements were also taken in between each sample. The results were normalized using the Spectragryph (version 1.2.16.1) to allow for easier comparison.

## RESULTS AND DISCUSSION

### Individual Ring Pretreatments

#### FTIR

FTIR analysis of the pretreated fractions and untreated wood show a progression of “contaminant” removal between wavenumbers 1400 and 1800 cm<sup>-1</sup>. This region mostly represents the bending and stretching of double bonds (C=O and C=C) neither of which should be present in α-cellulose. Untreated wood has the highest absorbance in this region due to the presence of lignin and wood extractives. Five peaks were identified in this region (labeled 1–5 in Figure 2). Figure 2 shows the FTIR spectra for the 1935 EW pretreated samples. There was no significant visible difference in the spectra between the years and between earlywood, latewood, and whole wood samples.

Peak 1 (1734 cm<sup>-1</sup>) typically represents C=O bonds found in esters, ketones, aldehydes, and acids. This peak can be detected in untreated wood as well as the NaOH and solvent extraction samples. This peak has been attributed to the presence of lignin in wood (Pandey and Pitman 2003; Rinne et al. 2005). We do not expect a solvent extraction to remove lignin, but this peak is reduced in samples treated with solvent extraction. It is therefore possible that absorbance in this peak may also be caused by resin or other extractives.

Peak 2 (1698 cm<sup>-1</sup>), associated with resins and extractives, is only observed in the untreated wood and the NaOH pretreatment sample although absorption is somewhat reduced. Solvent extraction, BABAB and BABAB+ pretreated samples do not show absorbance of this band. This is to be expected as resins and extractives are secondary components that are relatively easy to remove.

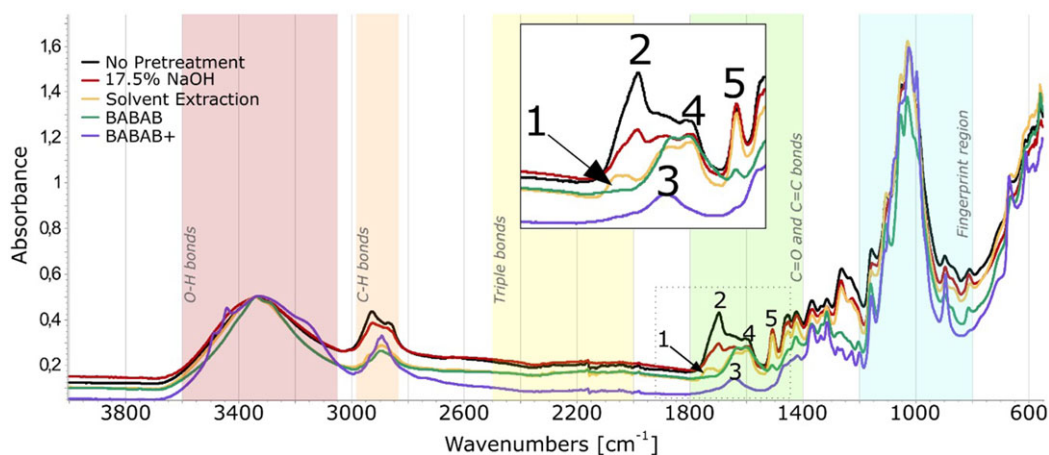


Figure 2 FTIR spectra of the different pretreatment methods: No pretreatment (black), 17.5% NaOH (red), solvent extraction (yellow), BABAB (green), and BABAB+ (purple). (Please see online version for color figures.)

Peak 3 ( $1641\text{ cm}^{-1}$ ) is seen in all fractions. While we did not expect to see this in the BABAB+ pretreated wood fraction (as it suggests the presence of double bonds), this is not uncommon in  $\alpha$ -cellulose as it is associated with the extra moisture (Rinne et al. 2005; Hajdas et al. 2017). Rinne et al. (2005) also tested if the water used during pretreatment could be detected in the  $\delta^{18}\text{O}$  analysis of the cellulose and found that it did not make a significant difference.

Peak 4 ( $1594\text{ cm}^{-1}$ ) was observed in all samples other than BABAB+ pretreatment. This peak highlights the efficiency of the extra 17.5% NaOH after a BABAB procedure and, ultimately, the removal of hemicellulose from the wood. Similar to peak 2, this justifies the need to remove lignin, resins, and extractives for complete cellulose extraction as there was very little reduction in absorption from the sample treated only with 17.5% NaOH.

Peak 5 ( $1509\text{ cm}^{-1}$ ) is only completely removed by BABAB+ pretreatment; however, a BABAB pretreatment removes a large portion of the peak. This peak has been attributed to the presence of lignin (Pandey and Pitman 2003), thus suggesting that, in this case, the BABAB pretreatment has not been completely successful in removing lignin. Nĕmec et al. (2010) suggests repeated bleaching steps may be required, this may be necessary for well-preserved woods.

The FTIR spectra obtained from BABAB+ pretreatment is visually similar to spectra obtained from cellulose extracted from a modified Jayme-Wise on *Pinus* wood Kagawa et al. (2015). This suggests that the BABAB+ pretreatment outlined here is capable of producing high quality  $\alpha$ -cellulose. This is followed by a BABAB pretreatment that is still effective in removing contaminant components but only produces holocellulose.

### Lath Pretreatment

The spectra shown in Figure 2 suggest that to obtain “clean” enough samples we would need to perform BABAB or BABAB+ pretreatments. However, the capacity to perform these pretreatments is limited by time and sample pretreatment capacity. Making precise cuts to separate rings (in some instances making sub-annual cuts) for a whole tree sequence before



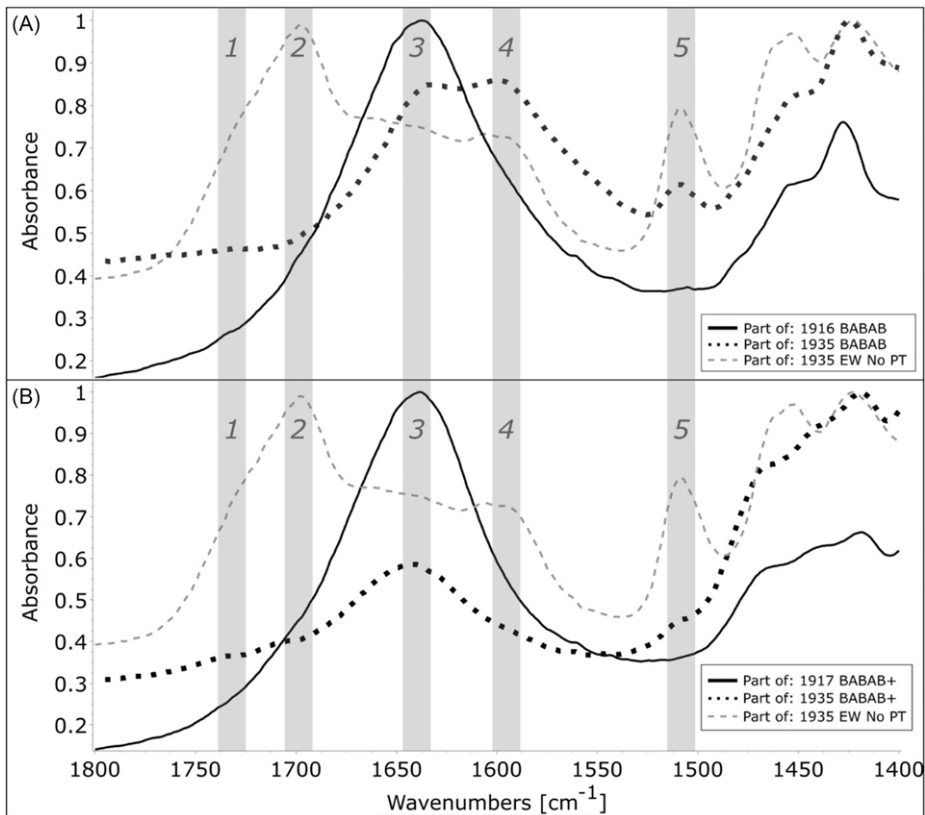


Figure 3 A. FTIR spectra of a BABAB lath pretreated ring, an individually pretreated ring and untreated wood sample. B. FTIR spectra of a BABAB+ lath pretreated ring, an individually pretreated ring, and an untreated wood sample.

pretreatment is also time consuming (compared to a few minutes to cut a lath from a disc with a bandsaw or cutting a core in half with a handheld electric mini-saw). With BABAB and BABAB+ being the most effective but time-consuming pretreatments within this project, we decided to alter and adjust these methods to test if they can be used for pretreating a wood lath sample.

Figure 3 shows the comparison of BABAB and BABAB+ when done on individual rings, rings from the lath, and untreated wood. These spectra show that, with time adjustments, a lath pretreatment can produce a good quality cellulose comparable to that of a single ring pretreatment. When comparing the BABAB+ method (Figure 3B), the lath pretreatment shows little absorbance around the untreated peak positions 1, 2, 4, and 5. The amplitude of peak 3 is likely caused by excess water in the sample as they might not have been dried sufficiently. This result gives us confidence in scaling the method for laths. The comparison of the BABAB pretreated samples (Figure 3A) suggest that using the time adjusted BABAB method for a lath results in improved quality of cellulose. Peaks 4 and 5 almost disappear in the lath sample unlike the in the individual ring test. For the individual ring pretreatments, only BABAB+ was able to remove these two peaks. This suggests that extended submersion times



can compensate for not using the ultrasonic bath or the addition of a strong base to remove hemicellulose.

### Ring vs. Lath

The sample boats are relatively compact and can accommodate 25-cm-long laths (usually more than 100 rings). Using two boats, a BABAB pretreatment can produce over 200 samples in five days. Pretreatment of an entire lath comes with its own difficulties and limitations. A major one being the structural integrity of the sample throughout the pretreatment. We found it increasingly difficult to keep the samples intact with each bleaching step. Taking pictures of the lath and easily distinguishable sequences before pretreatment and throughout the process made it easier to determine the ring year. This is important to note when using this method as it requires an attention to detail in order not to lose or add a ring. The very nature of the bleach steps introduces a second problem: the difficulty to distinguish rings. However, with good lighting and photographs of easily identifiable sequences, the rings can still be identified. Making sub-annual cuts was easier at this stage compared to cutting untreated wood. Treating a lath also poses a limit to the type of samples that can be done. In this case, samples cut from a thick slab of wood were available, but it is common in dendro- studies to only have access to cores obtained with a boring device. Cutting these cores into 2mm thick laths may result in narrow rings yielding samples that are too small for analysis. We can also appreciate that the samples chosen in these experiments were selected because their wider rings permit easier identification of ring boundaries. It is often the case that wood samples in climate studies have narrow rings thus making lath pretreatment more difficult.

### <sup>14</sup>C Measurements

Radiocarbon measurements for the pretreated fractions from individual rings are shown in Table 2. The difference between these results and the IntCal20 curve from 1933 to 1937 ( $\Delta^{14}\text{C}$ ) is shown in Figure 4. Due to natural variations and regional variation, we expect some deviation from the exact values of the IntCal20 curve but still use the curve as a guide for expected values. The untreated wood samples show the highest difference in  $\Delta^{14}\text{C}$  with low accuracy and repeatability. This is reflected in the FTIR results, showing high absorption between  $1800\text{ cm}^{-1}$  and  $1400\text{ cm}^{-1}$ . The samples soaked in 17.5% NaOH have a much smaller difference ( $\sim 30\%$  less) in  $\Delta^{14}\text{C}$  compared to untreated wood. This suggests the partial removal of contaminant constituents in the wood; however, these samples are still far from the Intcal20 values. The FTIR results for the NaOH treatment show absorption around Peak 2, which is mostly caused by resin. The large difference between the samples and the Intcal20 values support that Peak 2 records the presence of resins. Solvent extraction pretreatment produced the next best results with more consistency. This is consistent with the FTIR results that show samples treated by solvent extraction exhibit a spectrum closest to those of BABAB and BABAB+. The 1937 and 1934 rings even showed solvent extracted samples performing better than BABAB treated samples. While this was not a consistent trend, it does show the effectiveness of this Soxhlet sequence for these samples. It is important to note that the effectiveness of this method may be biased to this study as it is to our understanding that the resin and extractives which are later incorporated into the wood contain bomb radiocarbon. However, resin and extractives make up a small component of the whole wood. This is clear in the FTIR results (Figure 2) where solvent extraction reduced peaks relating to resins but not lignin.

Table 2 Radiocarbon results of untreated wood samples and the four different pretreatment methods for the years 1933–1937.

$\Delta^{14}\text{C}$ Results (‰)										
	No PT	$\Delta^{14}\text{C}$ (‰)	NaOH	$\Delta^{14}\text{C}$ (‰)	Soxhlet	$\Delta^{14}\text{C}$ (‰)	BABAB	$\Delta^{14}\text{C}$ (‰)	BABAB+	$\Delta^{14}\text{C}$ (‰)
1933	EW	59.5 ± 1.4	EW	16.2 ± 1.3	EW	-1.1 ± 1.4	EW	-15.3 ± 1.4	EW	-14.2 ± 2.2
	LW	35.2 ± 1.8	LW	22.9 ± 1.4	LW	-2.9 ± 1.4	LW	-13.8 ± 1.9	LW	-14.0 ± 1.4
	WR	53.8 ± 1.6	WR	17.7 ± 1.5	WR	0.9 ± 1.8	WR	-6.6 ± 1.9	WR	-13.6 ± 1.8
1934	EW	37.1 ± 1.6	EW	5.3 ± 1.6	EW	0.5 ± 1.8	EW	-10.9 ± 1.9	EW	-14.2 ± 1.4
	LW	40.3 ± 1.3	LW	2.7 ± 1.5	LW	-7.6 ± 1.6	LW	-13.4 ± 1.2	LW	-19.0 ± 1.6
	WR	45.1 ± 1.9	WR	4.0 ± 1.4	WR	-0.4 ± 1.5	WR	-6.7 ± 2.2	WR	-16.5 ± 3.0
1935	EW	56.7 ± 1.6	EW	22.1 ± 1.6	EW	0.7 ± 1.4	EW	-11.6 ± 1.4	EW	-16.2 ± 1.5
	LW	40.1 ± 1.6	LW	28.7 ± 2.9	LW	-2.8 ± 1.3	LW	-13.7 ± 1.4	LW	-14.5 ± 1.6
	WR	38.5 ± 2.1	WR	4.2 ± 1.6	WR	-3.9 ± 1.4	WR	-15.1 ± 1.5	WR	-16.6 ± 1.5
1936	EW	43.5 ± 2.1	EW	14.2 ± 1.3	EW	-2.6 ± 1.3	EW	-11.3 ± 1.4	EW	-18.2 ± 2.3
	LW	39.7 ± 1.3	LW	9.4 ± 1.3	LW	-1.1 ± 1.3	LW	-13.5 ± 1.5	LW	-18.6 ± 1.6
	WR	48.6 ± 1.3	WR	22.6 ± 1.5	WR	-6.9 ± 1.4	WR	-12.5 ± 1.7	WR	-18.3 ± 1.5
1937	EW	33.0 ± 2.2	EW	3.1 ± 1.5	EW	-5.0 ± 1.4	EW	-18.5 ± 1.9	EW	-18.4 ± 1.5
	LW	41.3 ± 1.8	LW	17.2 ± 2.5	LW	-2.4 ± 1.3	LW	-19.4 ± 2.1	LW	-22.7 ± 1.4
	WR	49.3 ± 2.0	WR	29.8 ± 2.0	WR	-9.6 ± 1.9	WR	-19.8 ± 2.0	WR	-20.0 ± 1.7

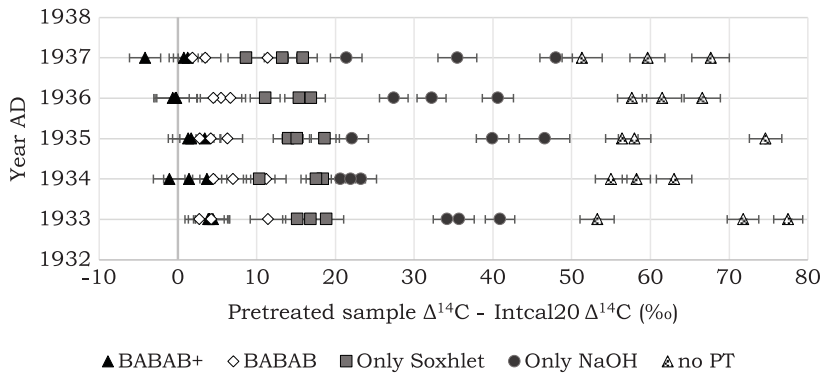


Figure 4 Difference in radiocarbon results of pretreated samples from 1933–1937 compared to the Intcal20 calibration curve.

The best performing pretreatment methods were BABAB and BABAB+. Samples pretreated with a BABAB+ method measured the closest to the Intcal20 values compared to BABAB samples. These results show the efficiency of the 17.5% NaOH step after bleaching and the small isotopic differences between holocellulose and  $\alpha$ -cellulose and ultimately the importance of isolating  $\alpha$ -cellulose for radiocarbon measurements. The results for earlywood, latewood, or whole ring samples do not show statistical differences.

## CONCLUSION

We have tested four methods of pretreatment to explore the potential of using less vigorous pretreatment methods as well as the individual capabilities of these methods to remove different wood components. The wood samples were selected to include the “false bomb peak” in which  $^{14}\text{C}$  from the bomb spike is incorporated in wood during the sapwood to heartwood transition. In addition to radiocarbon measurements, we have used FTIR to assess the purity of the samples after each pretreatment.

Based on the FTIR spectra there are clear differences in the spectra of more crude pretreatment fractions compared to the BABAB and BABAB+ extracted holo- and  $\alpha$ -cellulose. Furthermore, we can identify the peaks affected by the different solvent washes. The elutropic sequence of solvent extraction is very effective in removing secondary wood components that in this case have a much higher  $^{14}\text{C}$  content, but not enough lignin is removed by this procedure.

FTIR is a powerful proxy tool for assessing contaminant removal in cellulose. In this case, where we were aware of the source of “contamination”, we were able to avoid overestimating the effectiveness of a solvent extraction in the samples.

We also highlight that with some adjustments to the BABAB and BABAB+ methods, they can be used to pretreat long laths with multiple rings that are comparable by FTIR to individually processed rings. By increasing the time of submerging and some careful sample handling, lath pretreatment potentially doubles sample output. This is particularly important in creating tree ring chronologies, as it reduces processing time significantly.

As shown in Figure 4, BABAB+ pretreatment produced cellulose whose radiocarbon content was closest to the IntCal20 but the difference between  $\alpha$ -cellulose and holocellulose for stable isotope analyses needs to be further investigated.

### COMPETING INTEREST DECLARATION

There are no competing interests from any of the authors of this manuscript.

### SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit <https://doi.org/10.1017/RDC.2024.20>

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