MSc research project

K.S. van der Wal (s4181808)

k.s.van.der.wal@student.rug.nl

Centre for Isotope Analysis (CIO)

University of Groningen

M.W. Dee

Looking into contamination in wood samples for radiocarbon analysis using FTIR

Looking into contamination in wood samples for radiocarbon analysis using FTIR

MSc research project

K.S. van der Wal (s4181808)

k.s.van.der.wal@student.rug.nl

Centre for Isotope Analysis (CIO)

University of Groningen

M.W. Dee

Summary

This report focusses on the radiocarbon dating and analysis of wood and the problem of sample contamination that arises in the preparation process. Radiocarbon dating relies on the ratio between the heavier carbon isotope ^{14}C compared to the most abundant ^{12}C. A carbon-containing contamination may have a different $^{14}C/^{12}C$ ratio compared to the target material, therefore assigning a wrong date to an object.

In wood, all components tend to migrate throughout the structure of year rings causing contamination, except for α-cellulose. Therefore, α-cellulose is isolated from wood to act as the ultimate carbon source. This is regularly done in three steps: an optional solvent washing step to remove resin, the oxidative removal of lignin and the removal of hemicellulose to obtain the pure alpha-cellulose (Southon & Magana, 2010).

The goal of this research project entails the development of a protocol for checking the purity of the α-cellulose product derived from wood samples. Focus is laid on six wood types: resinous cypress (code MH), sequoia (SS), Kauri (KA) and three non-resinous oak samples (EO, MS and WC). All these woods are turned into both cut and milled samples.

The procedure used to purify these wood samples at the University of Groningen is as follows:

- An optional solvent wash step in small tubes using acetone (45°C, 45 min), methanol, (45°C, 45 min) and chloroform (45°C, 45 min). This step is only applied on the resinous samples.
- An acid-base-acid procedure using hydrochloric acid and sodium hydroxide to remove lignin and yield holocellulose
- An oxidation step to remove hemicellulose using sodium hypochlorite and yield the pure α-cellulose.

After each step, Fourier Transform Infrared spectroscopy (FTIR)spectra are taken to see if any structural changes occur. The final product is compared to pure α-cellulose from Sigma.

Additionally, the solvent wash is performed on pure α-cellulose to check for any solvent residuals after the solvent wash step.

Another version of the solvent wash is performed on the most resinous sample (MH) using a Soxhlet system, and acetone, methanol and chloroform for 45 min, but now at their respective boiling temperatures. A third version of the solvent wash is carried out using only acetone as a solvent and FTIR is performed on the resin to further investigate the result of the solvent wash step.

From this research, the following conclusions can be drawn:

- A clear difference in FTIR spectra peaks between resinous and non-resinous wood types can be seen
- After the whole pretreatment procedure, FTIR spectra show results that to a high extent look like pure α-cellulose. In MH, EO, MS and WC spectra a contamination side peak around 1000 cm^{-1} is visible.
- Cutting the sample is easier than milling, but gives similar results according to the FTIR spectra and is therefore preferred

- No major differences between the FTIR spectra of the wood samples before and after solvent wash are shown. The Groningen solvent wash seemed to add a peak (1000 cm^{-1}) that might be solvent. The Soxhlet procedures gave a similar result, with the resin taken out not significantly changing the spectrum. It is therefore recommended to do more research: both on the effects of a solvent wash on the purity of the α-cellulose final product and whether this step is really worthwhile.
- A set of instructions for the recording and normalization of FTIR spectra have been written. These can be found in Appendix B (page 44).

Table of Contents

Motivation and literature review

Origin of radiocarbon

For dating of wooden objects, radiocarbon dating plays an important role (Němec, Wacker, & Hajdas, 2010). Measurements on the isotope are also crucial for Greenhouse Gas and other atmospheric science research. The key measurement is the ratio between the radioactive carbon isotope ^{14}C and the non-radioactive ^{12}C. This ^{14}C isotope makes up about one out of every trillion carbon atoms (Morlan, 2021). It is produced in the upper atmosphere by various reactions, the most important between thermalized neutrons (n) from cosmic radiation and nitrogen atoms (see below). The neutrons come from collisions of particles called 'cosmic rays' with atomic nuclei such as oxygen and nitrogen (Bronk Ramsey, 2008).

$$n(slow) + {}^{14}N \rightarrow {}^{14}C + {}^{1}H$$

The process in the reaction shown can be explained as follows: a neutron hits a nitrogen atom and this results in the elimination of a proton and an electron. The proton and electron together form a hydrogen atom. The remaining entity of 8 neutrons, 6 protons and 6 electrons together form a carbon isotope with 2 more neutrons than the regular ^{12}C, called ^{14}C (Choppin, Rydberg, & Liljenzin, 2013). The ^{14}C that is produced in the upper atmosphere then rapidly forms ^{14}CO and $^{14}CO_2$ (carbon dioxide) by reaction with oxygen (Bronk Ramsey, 2008).

The carbon dioxide formed will spread through the whole of the earth's atmosphere. In this way, trees and other plants can take up CO_2 and convert it to all their organic tissues. Now, plants are assumed to have about the same $^{14}C/^{12}C$ ratio as is present in the atmosphere. When the plant dies, its carbon compounds can either be released through the metabolic process of plant decay or remain fixed when the plant is well preserved. In the latter case, the radiocarbon concentration starts to exponentially decay with a half-life of 5730 years and form back ^{14}N again. The older the organic material, the less ^{14}C is present. Because the decay rate of the radiocarbon is known, it's age can be calculated from the difference between its original ^{14}C content and the content at the time of measurement (Bronk Ramsey, 2008).

The assumption made in this is that the ratio of $^{14}C/^{12}C$ in the atmosphere has remained constant during the past. This is not actually true. Reasons for the ratio change are the exchange of carbon between ocean and atmosphere, changes in the cosmic ray flux, and thus production, and changes in the earth's magnetic field that shields the earth from the radiocarbon forming particles. It can partially be corrected for by additional calculations, making the precision of radiocarbon dating for single samples at best a century or so.

One especially important factor in dating is the anthropogenic production of radiocarbon during atmospheric nuclear bomb tests in the 1960s. The bomb tests produced free neutrons on a large scale, resulting in large increases in radiocarbon production. In the graph below it can be seen that the $^{14}C/^{12}C$ ratio in the atmosphere almost doubled between 1955 and 1965. This is known as 'the Bomb spike'. The ratio from then slowly lowers by the dissolution of radiocarbon into the oceans. Other factors are the natural drawing down of radiocarbon from the atmosphere through photosynthesis and the adding of fossil CO_2 to the atmosphere. The fossil fuels that are burned contain virtually no radiocarbon because of their age (Bronk Ramsey, 2008).

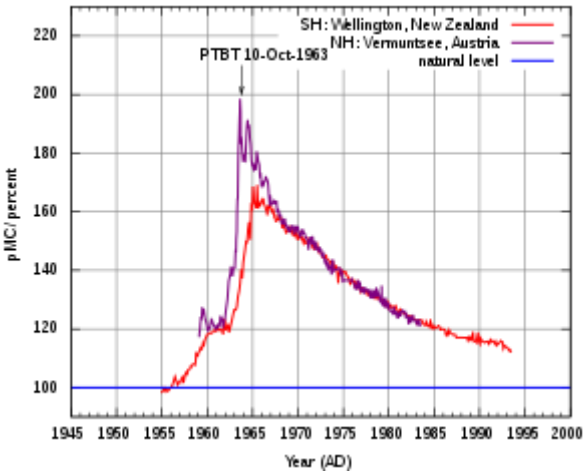


Figure 1 Atmospheric 14C concentration in the atmosphere from 1965 till 1993 (Universiteit Utrecht, sd)

From 2030, the concentration of radiocarbon is expected to have fallen below the pre-bomb level. This causes problems in radiocarbon dating, because carbon containing material will then be lower in ^{14}C than pre-bomb material. Thus, at the time, the very modern material might be mistaken for old material that has already lost carbon due to radioactive decay. It severely threatens the use of radiocarbon measurements as a dating technique (Graven, 2015).

Wood and radiocarbon

This report focusses on the radiocarbon dating and analysis of wood and the problem of sample contamination that arises in the preparation process. Wood on its own has many different constituents, including waxes, resins, oils, lignin, hemicellulose and cellulose. For trees, these constituents make up tree rings, with a new ring growing each year. After a tree ring has grown, the lignin, resins, hemicellulose and extractives tend to migrate throughout the wood. Therefore, a year ring will contain constituents from another year and therefore also its ^{14}C level is altered by constituents from another year. The part of the wood that doesn't migrate is the so-called α-cellulose. It is also the component that is the most chemically inert. Other wood components are more easily chemically modified by other compounds in the tree or in the ground if the wood has been buried underground for a while. Therefore α-cellulose is the best representation of the carbon isotope ratio's in the atmosphere at the growth period. (Němec, Wacker, & Hajdas, 2010).

To measure the $^{14}C/^{12}C$ ratio from the specific year in which the tree ring grew, the cellulose is removed from the rest of the wood components. This is regularly done in three steps: an optional cleaning step in a Soxhlet system, the oxidative removal of lignin and the removal of hemicellulose to obtain the pure alpha-cellulose (Southon & Magana, 2010).

This stepwise plan is based on the Jayme-Wise method described by J.W. Green, which used acidified sodium chlorite to delignify the wood (Green, 1963). Many modern α-cellulose extraction methods are therefore said to be based on the Jayme-Wise method (Loader, Robertson, & Barker, 1997), (Fogtmann-Schulz, Kudsk, & Adolphi, 2020). Commonly used methods are the Loader method, the Brendel method and the ABA method. All three methods are described below.

Loader method

The Loader method consist of three main steps: a Soxhlet extraction step to remove resins and non-polar constituents, an oxidation step to remove the lignin and a hydrolysis procedure to remove hemicellulose. The product is α-cellulose.

In the Soxhlet extraction step toluene, ethanol, hexane, water and acetone are used as common solvents. The removed extractives are organic non-structural 'smaller' components present in wood: fats, waxes, simple sugars, oils, etc (Rowell, Pettersen, & Han, 2005). For the delignification step, chlorite is used as an oxidant. Acetic acid was traditionally added to weaken the bonds between the lignin units and thereby speed up the process (Loader, Robertson, & Barker, 1997). In more recent research, the acetic acid is thought to react with OH-groups in cellulose, giving cellulose acetate, which has a different ^{14}C level than the cellulose itself and is therefore a contaminant. To prevent this, the acetic acid is replaced by hydrochloric acid (HCl) (Kudsk, Olsen, & Nielsen, 2018). The product of this second step is a mixture of hemicellulose and cellulose called holocellulose.

To remove the hemicellulose, a hydrolysis step at 75-80°C, in which either 10 or 17% NaOH or both sequentially are added to the holocellulose is carried out. Carbon dioxide from the atmosphere can dissolve in such an alkaline solution, providing an extra carbon source and thus possible contamination. Therefore most protocols provide a last step in which HCl is added to the solution in order to raise the pH and eliminate the carbon dioxide (Fogtmann-Schulz, Kudsk, & Adolphi, 2020), (Leavitt & Danzer, 1993), (Rinne, et al., 2005).

Brendel method

In the Brendel method, a mixture of acetic acid and nitric acid is used to remove the lignin from the sample. In this case, nitric acid is the oxidant. Hemicellulose is removed using a NaOH- and HCl-solution (Gaudinski, Dawson, & Quideau, 2005) or a solvent wash with ethanol, water and acetone (Anchukaitis, Evans, & Lange, 2008).

ABA method

The ABA in ABA method stands for Acid Base Acid. In this procedure an optional first step is again the Soxhlet solvent wash to remove non-polar extractives. This is done using an organic solvent such as hexane, acetone, toluene or ethanol and water. Next step is the alternative use of acidic (HCl) and basic (NaOH) solutions to remove lignin and hemicellulose. The solutions generally contain about 4-5 w/w% HCl or NaOH. An oxidation step using chlorite before or after the ABA process is optional (Michczynska, et al., 2018), (Hajdas, Hendriks, & Fontana, 2017), (Capano, Miramont, & Guibal, 2018), (Staff, Reynard, & Brock, 2014).

Other methods

A not so frequently used method is the ionic liquid procedure using [BMIM]Cl as a solvent instead of the organic solvents to remove unwanted fractions. Another method is the ABOX method, using potassium chromate in combination with sulfuric acid as an oxidizing agent. It is more aggressive and is only used for shorter time periods and lower temperature on older material or chemically treated wood that needs a more elaborate treatment (Němec, Wacker, & Hajdas, 2010), (Hajdas, Hendriks, & Fontana, 2017).

Groningen method

At the Centre for Isotope Research (CIO) in Groningen, a protocol very similar to most other academic ^{14}C laboratories is used. This protocol combines the Loader and ABA methods in one treatment (Dee, et al., 2020). Depending on the nature of the material (bone, collagen, wood, etc.) or acquired level of accuracy in the measurements, parts of the protocol are slightly adjusted or left out. The steps are as follows:

In the organic solvent pretreatment, the sample is refluxed for several hours in a series of organic solvents. This is done to remove additives such as glues, preservatives or extractives. If a sample isn't suspected to contain these components, a solvent pretreatment is left out. The solvents used are THF, chloroform, petroleum ether, acetone and methanol. The organic solvent pretreatment is done either in a Soxhlet apparatus for a small number of samples or in heat block (45 min. per solvent, 45°C) for bigger numbers.

For the aqueous pretreatment, an ABA protocol is applied to the material. The steps of the protocol are shown in Table 1 below: a 4% HCl-solution is added for minimum of 30 min to the sample, followed by 3 rinses with demineralized water (DW), the addition of 1% NaOH-solution, another 3 rinses with DW, again 4% HCl and another last rinse with DW. The exact temperature and duration of the treatment are tailored to the nature of each sample.

Table 1 The generic ABA protocol of the Groningen method (Dee, et al., 2020). RT = room temperature

Step	Acid	Rinse	Base	Rinse	Acid	Rinse
Reagent	HCl	DW (3x)	NaOH	DW (3x)	HCl	DW (3x)
Conc (w/vol)	4%	—	1%	—	4%	—
Time (min)	> 30	—	> 30	—	~ 30	—
Temp (°C)	RT-80	RT	RT-80	RT	RT-80	RT

Groningen α-cellulose treatment

For projects requiring high precision, a special α-cellulose treatment is applied (see Figure 2). This procedure also has an optional solvent rinse, but follows an intensified aqueous pretreatment protocol and an oxidation step using chlorite (Dee, et al., 2020). In this research, a slightly altered version of the Groningen α-cellulose treatment is used, with two different applied solvent rinses to see their effect. The final sample is not freeze-dried, but dried by air.

The solvent rinse sequence (if applied) consists:

- 1A: Either of a shorter series of rinses: acetone (45°C, 45 min), methanol, (45°C, 45 min), chloroform (45°C, 45 min), dry overnight.
- 1B: Or a Soxhlet extraction consisting of the same series of rinses: acetone (45 min), methanol (45 min) and chloroform (45 min), but now at their respective boiling temperatures.

The aqueous pretreatment starts with addition of an 5.47% (1.5 M) HCl-solution at 80°C for 20 min, followed by 3 rinses with DW. Then a 17.5% NaOH-solution is applied at room temperature for 60 min under N_2 atmosphere. The supernatant fluid is decanted off and the samples rinsed five times with DW, before addition of a 5.47% HCl-solution at 80°C for 20 min again. The treatment is finished with 3 DW rinses. Note that this pretreatment follows the same steps as the procedure in Table 1, but is stronger. The product is holocellulose.

In the final step, the chlorite oxidation, 1.5% $NaClO_2$ in 0.06M HCl is added to the samples. They are left at 80°C for 16h. Then a new batch of the acidified oxidant is added for 4h to form the α-cellulose. The samples get 3 rinses with DW and are dried by air.

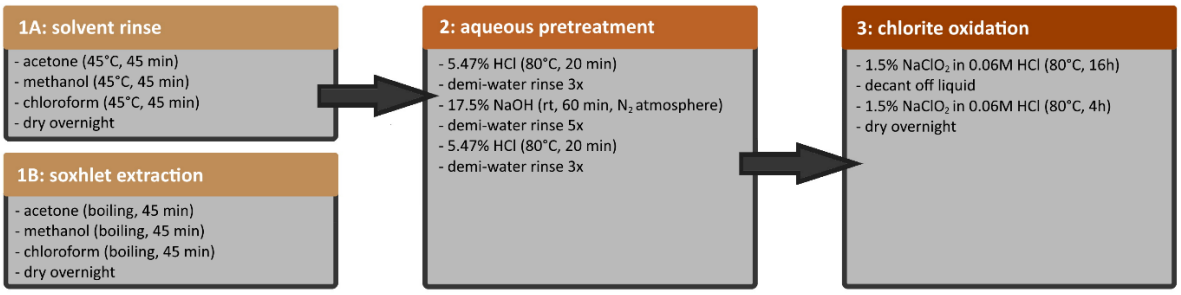


Figure 2 α-cellulose pretreatment according to the Groningen method

Note that in the α-cellulose treatment the ABA protocol is applied first, followed by an aqueous oxidation step. This is the reverse of what is the case in the Loader method (Loader, Robertson, & Barker, 1997). A reason the procedure is reversed is because sometimes archeological wood covered with mud is measured. The ABA procedure removes carbonates from the ground and humic acids built up in the wood before the oxidative removal of lignin is attempted (Dee, personal communication).

Whether all the wood components need to be removed from α-cellulose before ^{14}C dating is still part of discussion. In some treatments, hemicellulose is remained and the measured sample is then called holocellulose. At least there is consensus in the radiocarbon community that extractives need to be removed prior to ^{14}C dating of tree rings.

The carbon dating process: LSC and AMS

^{14}C measurements used to be done by a technique called Liquid Scintillation Counting (LSC), in which a radiocarbon-containing sample was converted to CO_2 gas. This gas was chemically converted to benzene (C_6H_6) and added to a benzene/toluene solvent mixture of known amount. Next, a scintillator (or photon emitter) was added to the mixture. If a ^{14}C-atom of the benzene radioactively degrades to ^{13}C, a beta particle is released. When beta particles hit the scintillator, photons are emitted. These photons can be electronically measured and the electronic signal is proportional to the amount of radiocarbon present (Hogg, 2002). Because the new AMS method takes a smaller sample size, less time and has a higher precision, LSC is outdated and no longer the standard method for ^{14}C measurement (Beta Analytic Testing Laboratory, 2020).

The now used Accelerator Mass Spectrometry (AMS) method uses samples that are also converted to CO_2 gas, but subsequently reduced to solid graphite (pure C) form. This takes place by using a metal catalyst. Next, a few milligrams of the graphite are pressed onto metal cathodes with a face and are ready for AMS-measurement. In Groningen, a MICADAS AMS is currently in use (Dee, et al., 2020). A schematic overview of such a system is shown in Figure 3 and will be explained below.

The metal cathodes are put into the spectrometer, where ions are fired at the sample to produce negatively charged atoms (1. in Figure 3) . Now the negatively charged atoms (or ions) pass through the focussing device (2) and an injection magnet to reach an accelerator where they are speed up by attraction towards a positive terminal. Atoms of the same mass as ^{14}C are unstable in a negative state, and thus cannot reach the terminal. The stable carbon atoms go through a gas or metal foil called the stripper (3), where they lose their electrons and become triple positively charged atoms (or single positively charged in the MICADAS). Molecules of the same mass as ^{14}C that may be present are eliminated because they cannot exist in this triple or single charged state. The carbon ions are pulsed away from the positive charge in the middle of the terminal, and pass through another set of focusing devices (4) where mass analysis occurs.

In mass analysis, a magnetic field is applied to these moving charged particles, which causes them to deflect from the path they are traveling. If the charged particles have the same velocity but different masses, as in the case of the carbon isotopes, the heavier particles are deflected least. Detectors at different angles of deflection then count the particles.

At the end of an AMS run, and also in Groningen, data (5) gathered is not only the number of ^{14}C atoms in the sample but also the quantity of ^{12}C and ^{13}C. From these data, the concentration ratio of the isotopes can be known (Purdue University Department of Physics and Astronomy, 2021) (Beta Analytic, 2021).

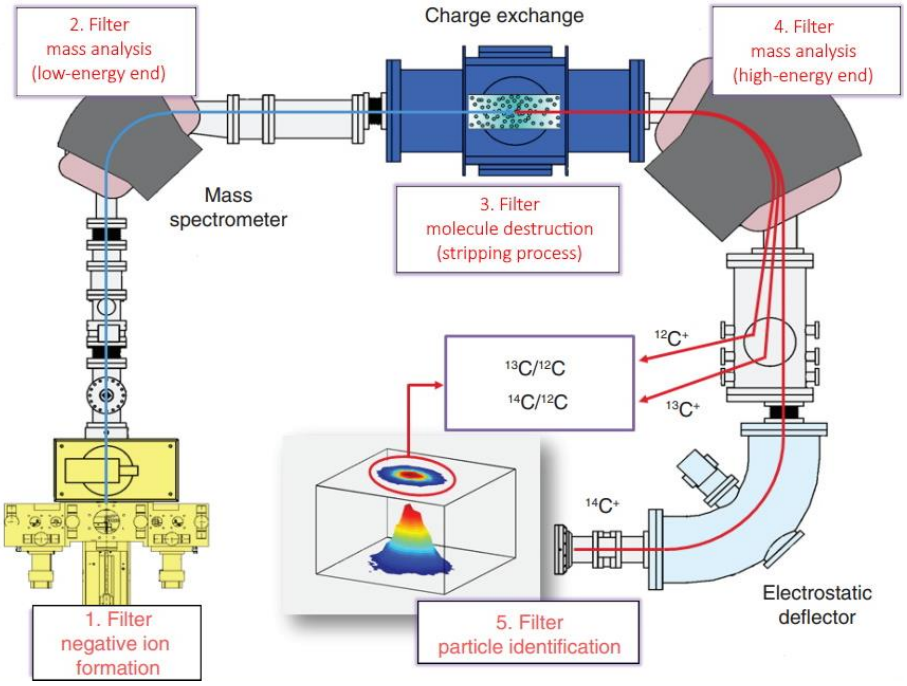


Figure 3 Schematic overview of a MICADAS accelerator mass spectrometer, used at the University of Groningen (Vuong, Song, Lee, & Roffel, 2016)

In Figure 3 it is shown that both the $^{14}C/^{12}C$ and $^{13}C/^{12}C$ ratio are measured. This is done because isotopic fractionation must be taken into account: certain biochemical processes alter the equilibrium between the carbon isotopes. These processes, such as photosynthesis, favour one isotope over another. To take this into account, the $^{14}C/^{12}C$ ratio must be adjusted by the $^{13}C/^{12}C$ ratio. If no isotopic fractionation factor is taken into account, a sample might be considered too young or old based on an inaccurate calculation of the original ^{13}C and ^{14}C content.

The extent of isotopic fractionation of the $^{14}C/^{12}C$ ratio, which must be measured accurately, is approximately double that for the measured $^{13}C/^{12}C$ ratio. If isotopic fractionation occurs in natural processes, a correction can be made by measuring the $^{13}C/^{12}C$ ratio in the sample being dated (Beta Analytic, 2021).

Uncertainties in AMS

In the measurement of uncertainties in AMS dating, different sorts of error are present (see **Fout! Verwijzingsbron niet gevonden.**). It is important to make a difference between external and internal errors. The external error is the error in the mean between different series of measurements. The internal error deals with the error within one single set of measurements. In a system with no systematic error, the internal error must be equal to the external error: all measurements are equally precise or imprecise, so to say (Beta Analytic).

Another view on errors deals with accuracy and precision. Accuracy refers to the established radiocarbon date being a true estimate of the age of the sample within its statistical limits. Precision deals with the size of the statistical limits. If the uncertainty around a date is high, the result is imprecise (Beta Analytic).

Summary; The accuracy of radiocarbon dates (modified from Polach, H.A. 1976). Sources of Error	**Effect upon Age Determination**	**Measures to minimize the error incurred**
1. Precision of age determination	Statistical; Typically ±1%Modern or less	Big samples, longer count times, repeat sample assays
2. Inherent a. ^{14}C half-life	Libby half life 3% too low	Multiply CRA's by 1.03 if necessary
b. $^{12}C/^{13}C$ fractionation	Variable, up to 450 yr for shell.	Stable isotope analyses using Mass Spec.
c. ^{14}C Modern standard	Variable > 80 yr	International crosscheck of secondary standards.
d. Variation in past ^{14}C production rates	0-800 yr, beyond *ca*12 ka not determined	Tree ring calibration; otherwise interpret results in radiometric timescale.
e. Distribution of ^{14}C in nature	Surface ocean latitudinal dependence -400 to -750 yr. Deep ocean -1800 yr.	Interpretation of results.
f. Changes of ^{14}C concentration in the atmosphere.	Industrial effect *ca* -2.5% and atom bomb effect +160% in atmosphere	Interpretation of results
3. Contamination	Nil to 300 yr up to 15 ka; >20 ka possible beyond 25 ka.	Interpretation of results, analysis and dating of extracted pretreated fractions.
4. Biological age of material	<10 yr to>1000 yr	Identification of species of material in the case of wood and charcoal to short lived samples only.
5. Association of sample and event	Intermediate	Interpretation of results
6. Human	Intermediate	Care in field and laboratory
7. Interpretation of results	Intermediate	Care in interpretation, interdisciplinary approach and collaboration

Figure 4 different sources of error in radiocarbon dating (Beta Analytic)

Problems with measurements in the ECHOES project

A problem in the ECHOES study, as in many research projects, is the accuracy of the results. With older AMS equipment, the number of counted carbon ions primarily determined the uncertainty limitation of the measurements: a small number of carbon ions gives a high standard deviation and thus a high uncertainty. This is called the error due to counting statistics. A big innovation in the results came with the introduction of the aforementioned MICADAS AMS. Now the number of counts is much higher and thus the uncertainty arising from counting statistics is much lower. This however has the consequence that other contributions to the final uncertainty are no longer negligible and must be taken into account (Aerts-Bijma, Paul, Dee, Palstra, & Meijer, 2020).

The other contributions to uncertainty come from various sources. An important source is the heterogeneity and thus variable carbon levels in the used sample material. This is hard to quantify. The chemical pretreatment is another source that can add to the uncertainty because of contamination with foreign carbon during sample handling or incomplete removal of already present contamination. The CO_2 production from the right carbon fraction can give rise to uncertainty by incomplete combustion, memory effect (carry over) from one sample to the next, contamination from reagents during sample handling and exchange from CO_2 between samples. In the graphitization systems, where the produced CO_2 is reduced to elemental carbon, again exchange of CO_2 can take place, together with contamination present in the glassware, iron powder, in insufficiently cleaned reactors or by carbon uptake from air (Aerts-Bijma, Paul, Dee, Palstra, & Meijer, 2020).

The above mentioned errors, among unknown other factors, form the 'dark uncertainty'. This is the error that must be added to the error in the AMS measurement. Research has shown that in the case of α-cellulose measurement the actual uncertainty is 1.4 times the uncertainty arising from the AMS measurement alone (Aerts-Bijma, Paul, Dee, Palstra, & Meijer, 2020). Doing measurements on the exact contaminants and later adjustment of the protocols if necessary can diminish the dark uncertainty.

Carbon contamination: impact and analysis

Diminishing the dark uncertainty is necessary because carbon contamination of a sample can have a serious impact on its measured age. For example, if a sample is 20,000 BP in age, and only 10% of the dating extract consists of a contaminant that is 100% modern, the final date estimate would be ~14,000 BP. The opposite situation can also be severe, even though the distortion is linear in nature. That is, if a dating extract contains 10% depleted carbon contamination, then the date will be too old by 846 yr, regardless of the sample's true age (Dee, Brock, Bowles, & Ramsey, 2011).

The contamination, as said before, is the focus of the sample pretreatment, where it is either added by mistakes or it is not fully removed. Testing if contamination is sufficiently removed is difficult. This is because many materials, each having their own contaminations, are selected for dating. Also, detecting small concentrations of contaminants is difficult and a detecting solution must be economically viable (Dee, Brock, Bowles, & Ramsey, 2011).

Testing the effectiveness of ^{14}C pretreatment is done in two ways: the first uses a known-age material as a substrate. The substrate is pretreated as is or deliberately contaminated using a specific impurity and then pretreated. Biggest disadvantage of this method is that it takes large numbers of samples to distinguish between results of different methods in a statistically secure way. The number of samples needed is even enlarged by the vast range of contaminants and pretreatment protocols that exist (Dee, Brock, Bowles, & Ramsey, 2011).

The second approach includes high-precision compositional analysis to monitor the concentration of the contaminant. These techniques include x-ray diffraction of shells and FTIR analysis of bone and charcoal. Such approaches are generally case-specific, referring to specific forms of contaminations and specific sample types (Dee, Brock, Bowles, & Ramsey, 2011). The FTIR analysis is currently a subject of research (Fogtmann-Schulz, Kudsk, & Adolphi, 2020). It will also be the technique used in this research project.

The term FTIR stands for Fourier Transform InfraRed. It is a technique which makes use of a beam (set of wavelengths) of infrared radiation. A sample is placed into the beam and molecular bonds absorb some of the radiation. The intensity of each of the remaining radiation wavelengths is measured and put into a spectrum. Each peak in the spectrum corresponds with a particular bond or set of bonds. In this way it is possible to show changes in molecular structure, and thus bonds, during chemical processes.

Peaks are assigned to typical molecules present in wood in various studies (Stewart, Wilson, Hendra, & Morrison, 1995), (Michczynska, et al., 2018), (Rinne, et al., 2005) (Brookman & Whittaker, 2012). For each of the wood components cellulose, hemicellulose and lignin the absorption regions are explained:

For cellulose, the absorbance around 900 cm^{-1} is caused by the C-O-C β-linkage between the sugar units, whereas the peaks around 1000-1200 cm^{-1} are caused by the C-O, C-C and C-O-C linkages in the sugar units themselves. The C=O ester bond in cellulose (open chain glucose) absorbs around 1260 cm^{-1}. In the regions between 1300-1500 cm^{-1}, the C-H bonds in cellulose, as well as in lignin and hemicellulose absorb.

For hemicellulose, the absorption between 1200-1300 cm^{-1} is caused by O-H groups and C-O vibrations. The absorption between 1300-1500 cm^{-1} comes from the C-H vibrations. The exact cause for the peak at 1750 cm^{-1} is not easily characterizable, and often remains unassigned.

For lignin, the origin of the peak at 863 cm^{-1} is not mentioned in literature. The peaks between 1220-1270 cm^{-1} are caused by aromatic C=C bond and C-O bonds. The peaks in the 1300-1500 cm^{-1} region come from the lignin side chains and methoxyl (CH_3-O) groups. The peaks in the 1500-1510 and 1600 cm^{-1} region are again caused by aromatic (C=C and C-C) ring vibrations, and C=O stretching.

An general overview constructed by combining this data is given in Table 2.

Table 2 wavenumber regions corresponding to molecular structures in wood

wavenumber (cm-1)	900	1000	1100	1200	1300	1400	1500	1600	1700	1800
cellulose	900		1000-1200		1260	1300-1500				
hemicellulose					1200-1500					1750
lignin	863				1220-1270	1300-1515		1600		
resin									1600-1700	

In the aforementioned research (Fogtmann-Schulz, Kudsk, & Adolphi, 2020), FTIR was used to monitor the stepwise pretreatment of wholewood to form α-cellulose (see Figure 5). A spectrum of microcrystalline cellulose (blue line) is added as a reference for the end product.

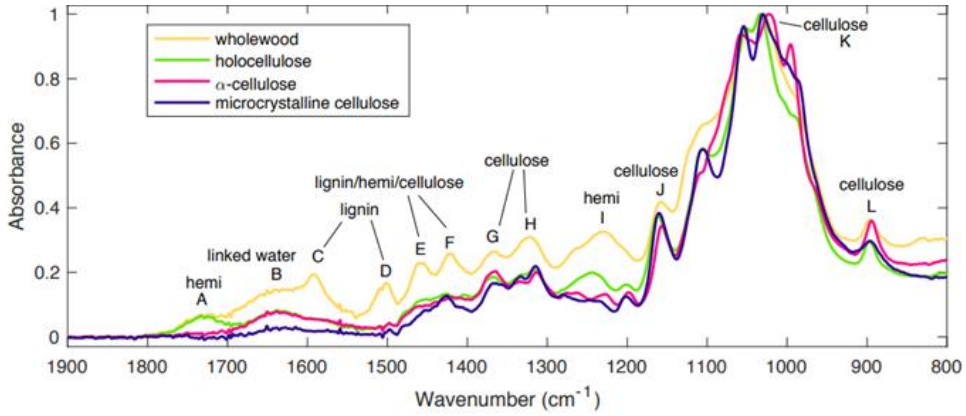


Figure 5 Absorbance spectra of three samples in different stages of pretreatment and a microcrystalline cellulose reference. Figure reproduced from (Fogtmann-Schulz, Kudsk, & Adolphi, 2020).

As can be seen in Figure 5, peaks are assigned to typical structural components of lignin, hemicelluloses and celluloses. As the pretreatment proceeds from whole wood (yellow line) to holocellulose (green) to α-cellulose (pink), typical peaks of lignin (C,D and partially E,F) and hemicellulose (A,I and partially E,F) disappear.

The cellulose formed ideally has the blue line shape of the microcrystalline cellulose reference. Any additional peaks rise from either a completely foreign contaminant or the remains of lignin or hemicellulose. The identity of these peaks might be determined by FTIR-analysis of resin fractions removed from the whole wood by organic solvent extraction. They might also be determined by FTIR analysis of possible contaminations such as glues, adhesives or external material added to the sample during the pretreatment itself (Aerts-Bijma, Paul, Dee, Palstra, & Meijer, 2020). Once the identity of these contaminants is known, pretreatment protocols might be adjusted to remove the contaminants (more) efficiently.

Solar storms

One of the applications of radiocarbon dating of trees lies in the exact chronology of climatic events. In most cases, the difference in ^{14}C concentration between years is only in the order of 0.1-0.2%. Largely because of this small difference between years, the traditional limit of AMS dating lies in the decadal range: a date could be determined with a precision in the tens of years at best (Kuitems, Panin, & Scifo, 2020).

A breakthrough in precise dating came with the implication of extreme cosmic radiation events. In such events the sun is more active than in other periods, causing a bigger cosmic ray flux to be sent to earth. Such a flux can have a massive detrimental impact on, for example, earth's satellite communication. The cosmic radiation produces radiocarbon by a series of reactions, which gets incorporated in organic materials such as trees. This event is therefore visible as a sudden increase in radiocarbon concentration in tree rings that grew during such storms (Scifo, Kuitems, & Neocleous, 2019).

Recent ^{14}C measurements on series of known-age tree rings from dendrochronological archives have revealed that sudden increases have occurred in the past on, at least, two occasions. Specifically, an increase within 1 y of about 1.2% (which manifests as a decrease in age of ~100 ^{14}C yrs) in 775 CE and 0.9% (decrease of ~70 ^{14}C yrs) in 994 CE. These increases, or "Miyake events," have been identified in known-age tree rings of different species from all around the world (Kuitems, Panin, & Scifo, 2020).

A so-called wiggle-match process is applied, in which different tree-ring sequences are matched to each other by looking at patterns of ^{14}C concentrations in the rings. This will enable precise matches to be made between known-age and unknown-age samples (Bronk Ramsey, Plicht, & Weninger, 2001). The spikes in radiocarbon concentration from the Miyake events can thereby assist a great deal in this process by acting as clear anchor points.

This whole process of year-to-concentration wiggle matching only works if the different concentration levels are distinguishable enough so the right years are matched. If a tree ring sample has a wrong ^{14}C-concentration due to contamination, years might become indistinguishable and incorrectly matched. This could lead to assigning a wrong date to an object.

Knowing whether a sample is contaminated and, if so, whether the contamination is properly removed during the pretreatment is key. Therefore the principle objective of this research project is as follows: **development of a protocol for checking the purity of alpha-cellulose product by running FTIR spectra on the wood, extracted cellulose, intermediates and the waste products obtained. The samples will be pretreated by using the Groningen α-cellulose treatment protocol and by experiment with a Soxhlet solvent rinse.**

Materials and procedures

In order to give a good overview of possible contaminants and their removal, different approaches for the pretreatment of wood samples have been made. These approaches differ in wood type, way of sample preparation and organic solvent wash. All options are discussed below.

It is crucial to investigate a wide range of the different wood varieties, ages, types of extractives and thus types of possible contamination that need to be removed. Therefore, both resinous (cypress, sequoia, kauri) and non-resinous (oak) wood types are included in the research (see Table 3). A pure cellulose sample is included as a non-contaminated reference for the desired α-cellulose end product.

Table 3 used wood varieties in this research

Type	name	code
Cypress leylandii *(gymnosperm, resinous)*	Margot's Hedge	MH
Sequoiadendron giganteum *(gymnosperm, resinous)*	Sturt's 400 BCE sequoia	SS
Agathis australis *(gymnosperm, resinous)*	Kauri	KA
Quercus robur *(angiosperm, non-resinous)*	Esther's 400 BCE oak	EO
Quercus robur *(angiosperm, non-resinous)*	Margot Spike	MS
Quercus robur *(angiosperm, non-resinous)*	Windsor Castle	WC
Pure α-cellulose (Sigma)	Pure	Pure

To make the wood samples more accessible by the pretreatment chemicals, they are split up into smaller pieces. This is done in two ways: by using a knife to cut the wood into splinters and by using a Dremel® with a rotary head to make saw-dust like material (see Figure 6).

The cutting procedure takes less time. A drawback is that is gives bigger chunks of wood, which are thought to be less homogenous and permeable than the Dremel® saw dust. For each of the samples, an amount of 30-80 mg is taken. See Figure 7.

Figure 6 a Dremel with a rotary head to make saw dust (Dremel, 2021)

Figure 7 a used sample of wood dust (left) and cut wood (right) (own work)

The resinous wood types (cypress, sequoia and kauri) and the α-cellulose are given a solvent rinse using the standard Groningen unknown organic contaminant. This is procedure 1A in Figure 9. The solvent rinse is carried out in thick-walled 12 ml test tubes in a block heater, with the amount of solvent being 8-10 ml.

In addition, the most resinous sample cypress (MH) is given a Soxhlet extraction in attempt to remove enough resin so that an FTIR-spectrum can be taken. This is protocol 1B in Figure 9. After Soxhlet extraction, no further steps are taken.

The Soxhlet extraction is carried out in a special setup shown in Figure 8. Wood dust is added to the white thimble shown and solvent is added to the round bottom flask below. The solvent is heated, evaporates and then cools down in the water cooling unit in the top of the setup, where the liquid drips into the thimble. If the compartment of the thimble is full, the compartment drains itself into the round bottom flask again.

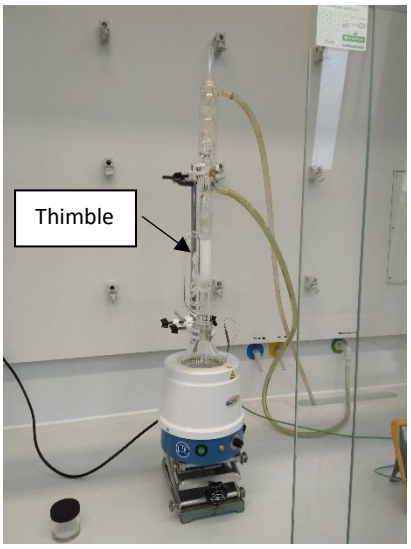


Figure 8 the used setup for Soxhlet extraction (own work)

The samples treated with protocol 1A get an aqueous pretreatment following the Groningen α-cellulose protocol as shown in Figure 9 (step 2 and 3).

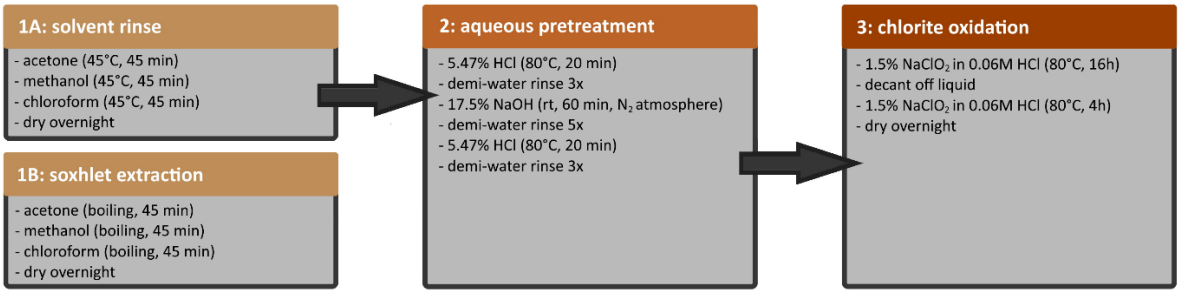


Figure 9 α-cellulose pretreatment according to the Groningen method

This altogether results in the project planning scheme in Appendix 1.

As stated in the previous section, the used FTIR spectral range is 500 – 4000 cm^{-1}. Because of this wide range and the difference in maximum peak height between spectra, differences between the spectra are hardly visible. In literature, these problems were overcome by normalisation (Fogtmann-Schulz, Kudsk, & Adolphi, 2020), (Brock, Dee, & Hughes, 2017). Also, the structural changes that happen to the material in the pretreatment process only show in a small part of the spectrum. Therefore, two adaptations to all the spectra are done:

- A smaller spectral range, equal to the range of the Fogtmann-Schulz research (Fogtmann-Schulz, Kudsk, & Adolphi, 2020) of 800 – 1900 cm^{-1}. This also eliminates the peaks below 600 cm^{-1} that cannot be identified and causes the least differences between the different baselines as possible.
- Enhancement of the absorbance ratio to a range of 0 till 1 to show smaller peaks and make the ratio equal to the Fogtmann-Schulz research.

The last step in the research scheme is performance of FTIR-spectra on the wood and product samples, and processing of the data in such a way that clear spectra are given as a result. In order to reproduce this process, a protocol for the FTIR measurements and normalization has been established. This protocol can be found in Appendix B (page 44).

Analysis and discussion of results

Overview

IR analysis using a Shimadzu IRTracer-100 at the RUG Chemical Engineering department was done on the following green coloured samples:

Table 4 samples used for FTIR-analysis

		Pure wood	After solvent wash (Groningen)		After solvent wash (Soxhlet)		After pretreatment	
Sample name	**Code**	**Cut + mill**	**Cut**	**Mill**	**Cut**	**Mill**	**Cut**	**Mill**
Margot's Hedge	MH							
Sturt's 400 BCE sequoia	SS							
Kauri	KA							
Esther's 400 BCE oak*	EO							
Margot Spike*	MS							
Windsor Castle*	WC							
Pure cellulose	Pure							

** = non-resinous samples, no solvent wash done*

Green = IR-spectra taken

Black = Steps are not carried out, so no IR-spectra taken

Red = IR-spectra could not be made due to material loss

The spectra are compared on the same graph in various ways to point out the differences between the different types of wood and the change in material composition during the pretreatment process. All combined spectra can be found below.

First all the untreated wood spectra are compared to cellulose (see Figure 10). The most distinct differences between all the spectra of wood and wood derivatives arise in the area between 800 and 1900 cm^{-1}. Therefore, only this area is shown in all the graphs below.

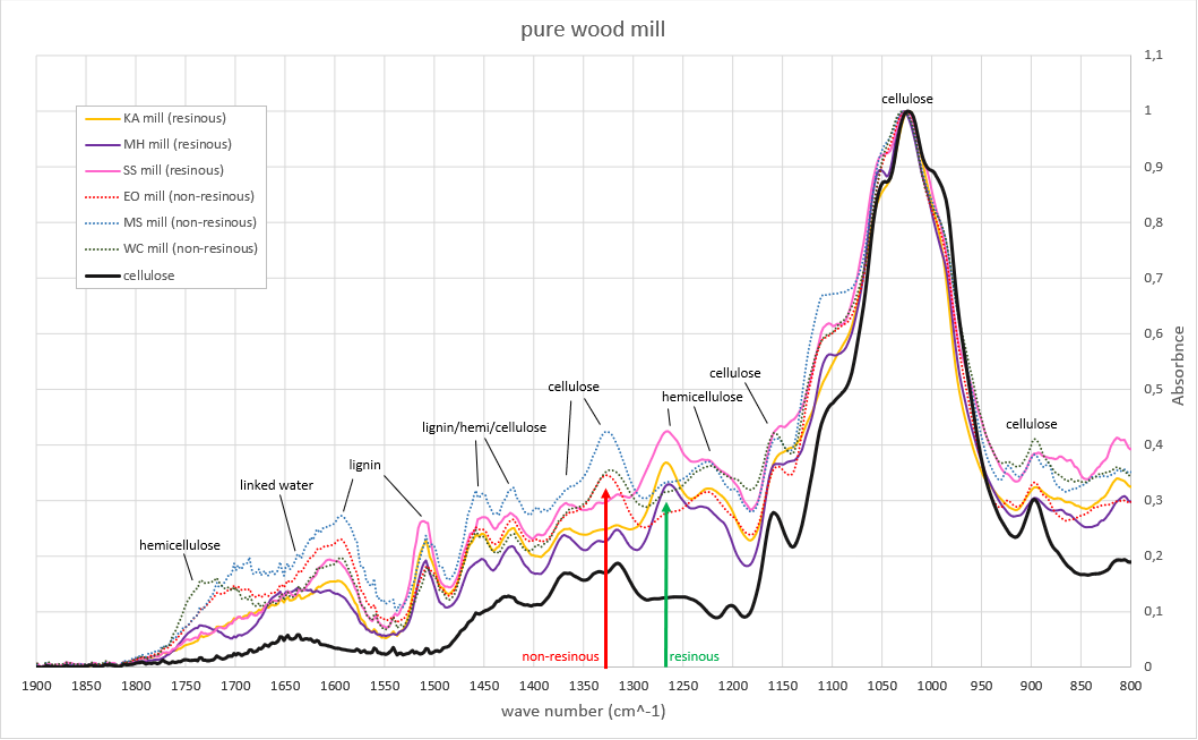


Figure 10 FTIR-spectra of all the used milled wood samples

From this spectra, it can be seen that the wood samples can be divided into two groups: the resinous species being the solid lines and the non-resinous species being the dashed lines.

All resinous samples KA, MH and SS seem fairly similar in the shape of their FTIR spectra and the position (wave number) of small distinct peaks. The other group is formed by the non-resinous Quercus robur (oak) samples. All the non-resinous species have a clear cellulose peak around 1330 cm^{-1} (red arrow in Figure 10), whereas the resinous species have a more distinct hemicellulose peak at 1270 cm^{-1} (green arrow).

Resinous samples

In the first pretreatment round, all resinous samples underwent treatment. IR-spectra of the samples were taken after solvent wash and after the full pretreatment. Pure cellulose was also analysed as a reference. The spectra taken after solvent wash are shown in Figure 11.

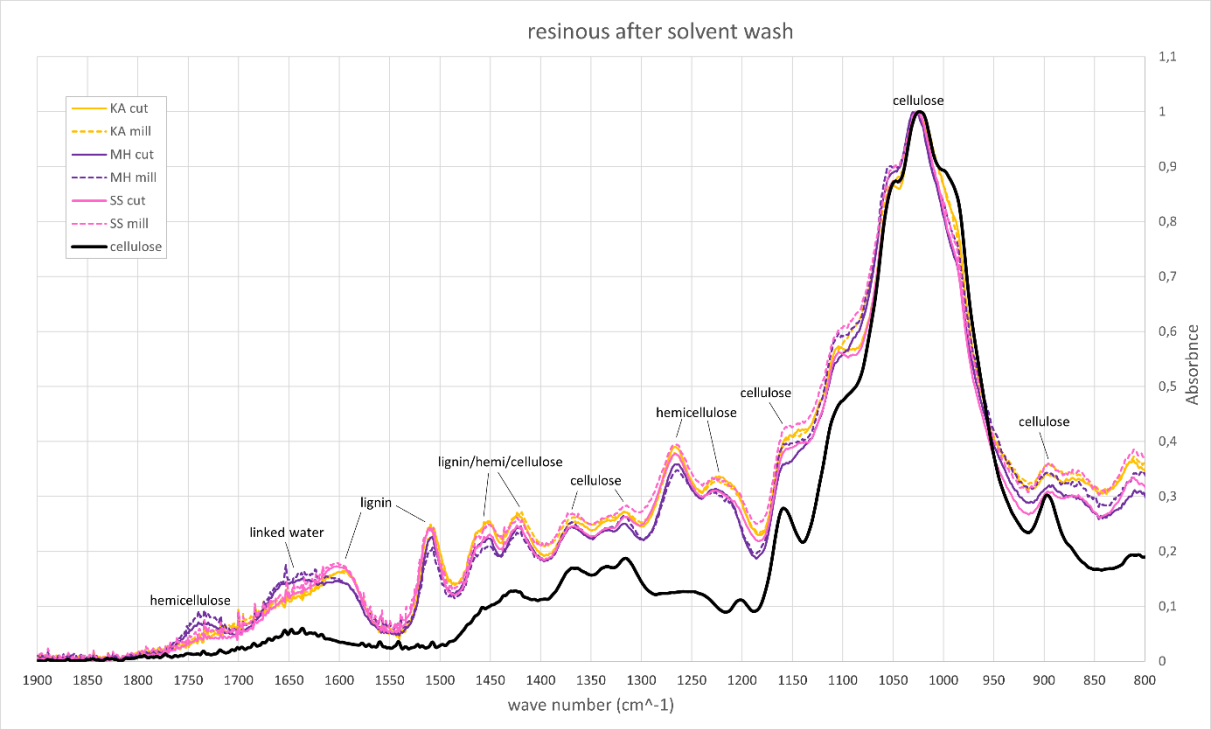


Figure 11 FTIR-spectra of the resinous wood samples after solvent wash

In Figure 11, it is visible that the resinous wood samples show only small differences from another. A peak around 1740 cm^{-1} shows that the MH samples contain (more prominent) hemicellulose than the SS and KA samples. The peaks around 1510 and 1600 cm^{-1} show that the MH samples might contain less lignin than SS and KA.

The last step of the pretreatment is the aqueous ABA pretreatment and the chlorite oxidation (step 2 and 3 in Figure 9). The spectra of all the resinous samples resulting from this step are shown in Figure 12.

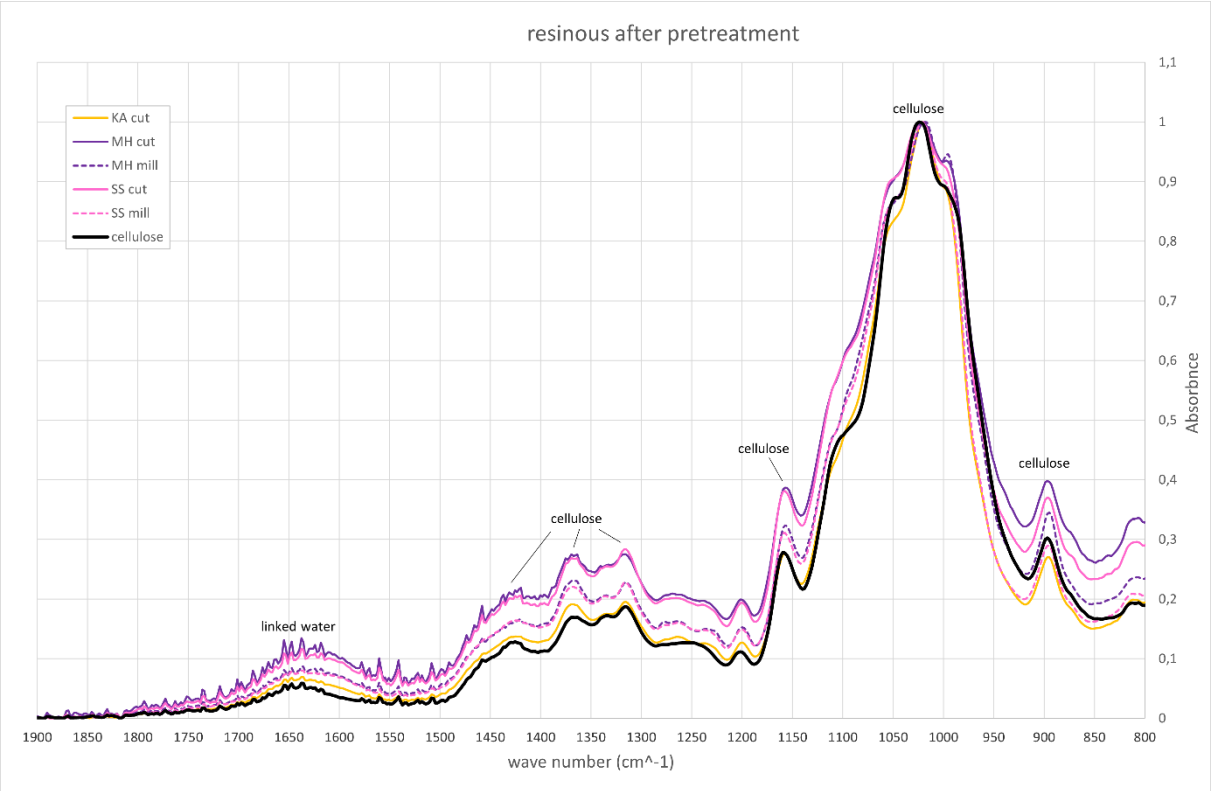


Figure 12 FTIR-spectra of the resinous samples after all pretreatment steps. Insufficient sample material was left after the pretreatment of KA mill, so no spectrum is taken.

In Figure 12 it can be seen that all spectra resemble the structure of α-cellulose (black dashed line) to a high extent. Analysis of the Kauri (KA) spectra before and after pretreatment showed that lignin peaks at 1500 and 1600 cm^{-1} as well as hemicellulose peaks at 1200-1300 and 1700-1800 cm^{-1} seem to have disappeared. The spectrum is very similar to the spectrum of pure cellulose after pretreatment. This indicates that relatively pure α-cellulose is obtained after pretreatment.

The only thing that needs to be clarified is a side peak at 1000 cm^{-1},most clearly visible in the spectrum of MH mill after pretreatment and to a smaller extent in the spectrum of MH cut after pretreatment. This peak is also shown in the spectrum from the Fogtmann-Schultz research (see Figure 5). The peak might indicate a contamination, cellulose degradation product or derivative formed in the degradation process, as it is not visible in the pure wood spectra of MH mill and cut in Figure 10.

Non-resinous samples

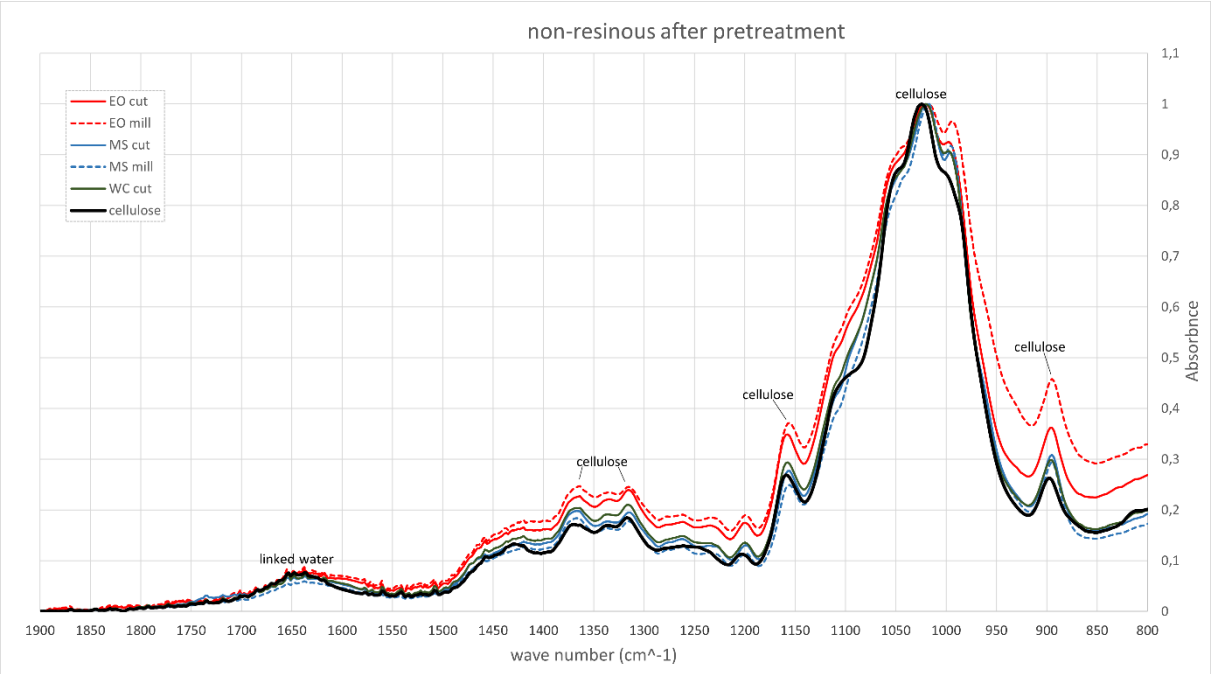


Figure 13 FTIR-spectra of the non-resinous samples after all pretreatment steps. Insufficient sample material was left after the pretreatment of WC mill, so no spectrum is taken.

In Figure 13 it can be seen that the structure of the samples after pretreatment resembles the structure of pure α-cellulose to a high extent. The biggest difference between the samples and pure cellulose is the side peak around 1000 cm^{-1}, which is also visible in the resinous MH-sample. This peak might indicate a contamination, cellulose degradation product or derivative formed in the degradation process, as it is not visible in the non-resinous pure wood spectra (Figure 11). The peak seems to be typical for cypress (MH) and oak (EO, MS, WC) species.

Spectra of the full process for each variety

To clearly point out differences in sample composition during the processes, all FTIR spectra for the various resinous woods are shown in separate graphs below.

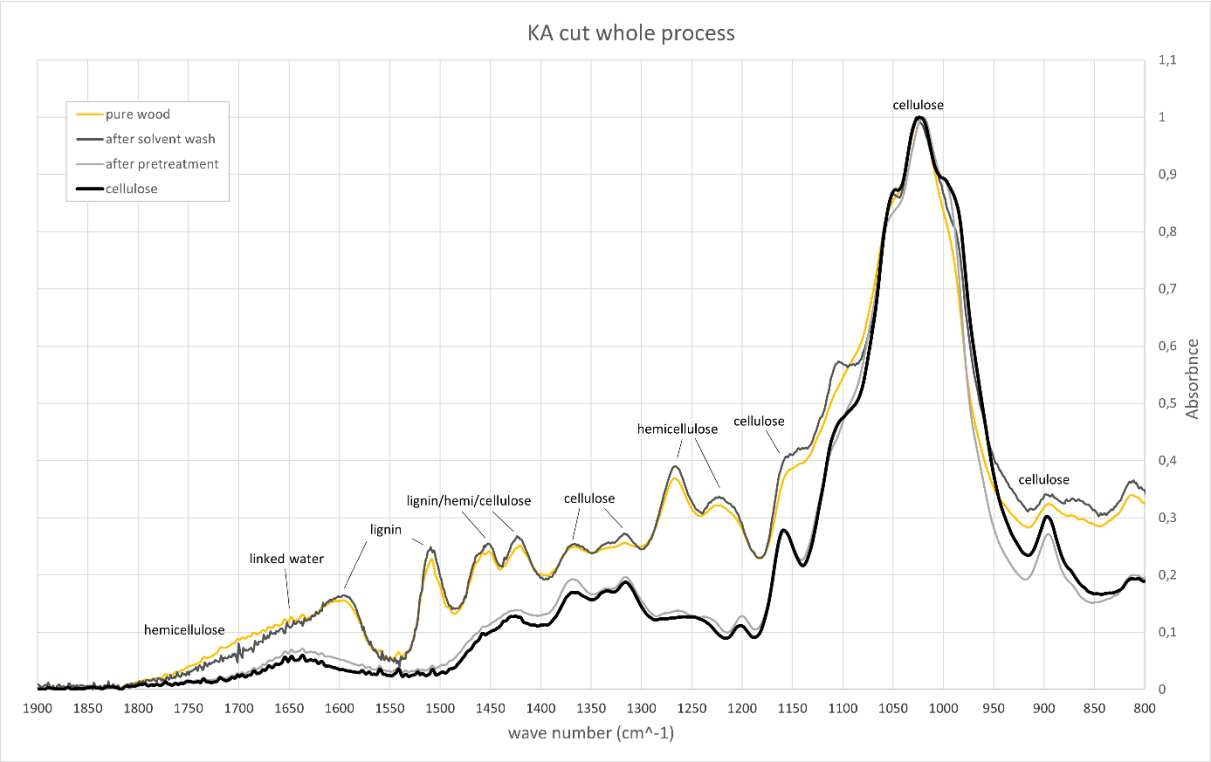


Figure 14 FTIR-spectra of KA cut for the whole process

After solvent wash, a clear peak at 1110 cm^{-1} becomes visible, but has disappeared after all pretreatment steps. This could be either a solvent residue or vulnerable cellulose part or derivative.

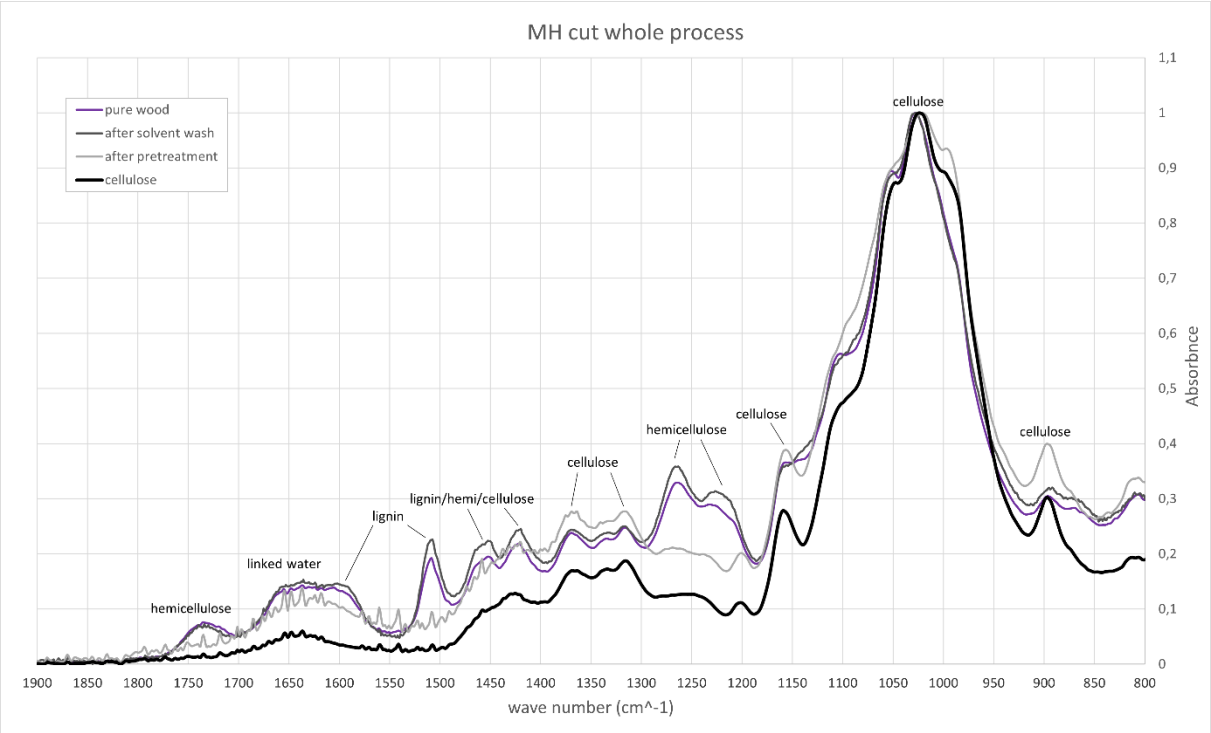


Figure 15 FTIR-spectra of MH cut for the whole process

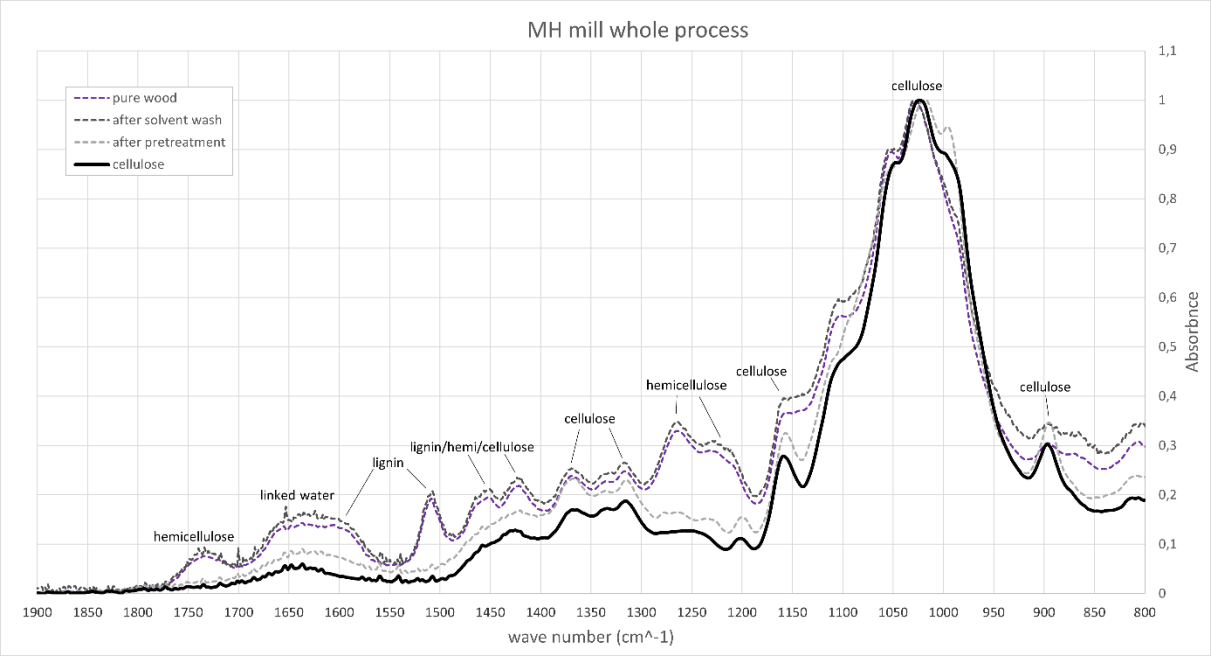


Figure 16 FTIR-spectra of MH mill for the whole process

After all pretreatment steps, the clearest difference between the MH cut and mill product and pure cellulose is a side peak of the biggest cellulose peak at 1000 cm^{-1}. This peak might indicate a contamination, cellulose degradation product or derivative formed in the degradation process, as it is not visible in the pure wood spectrum of MH.

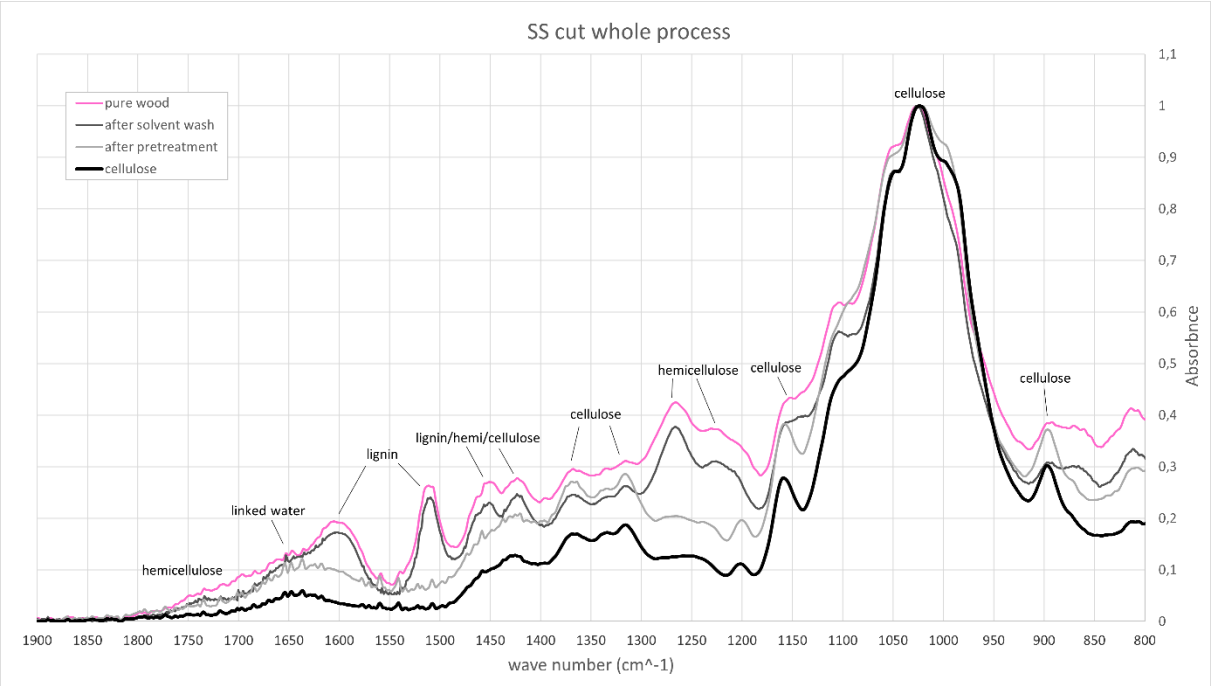


Figure 17 FTIR-spectra of SS cut for the whole process

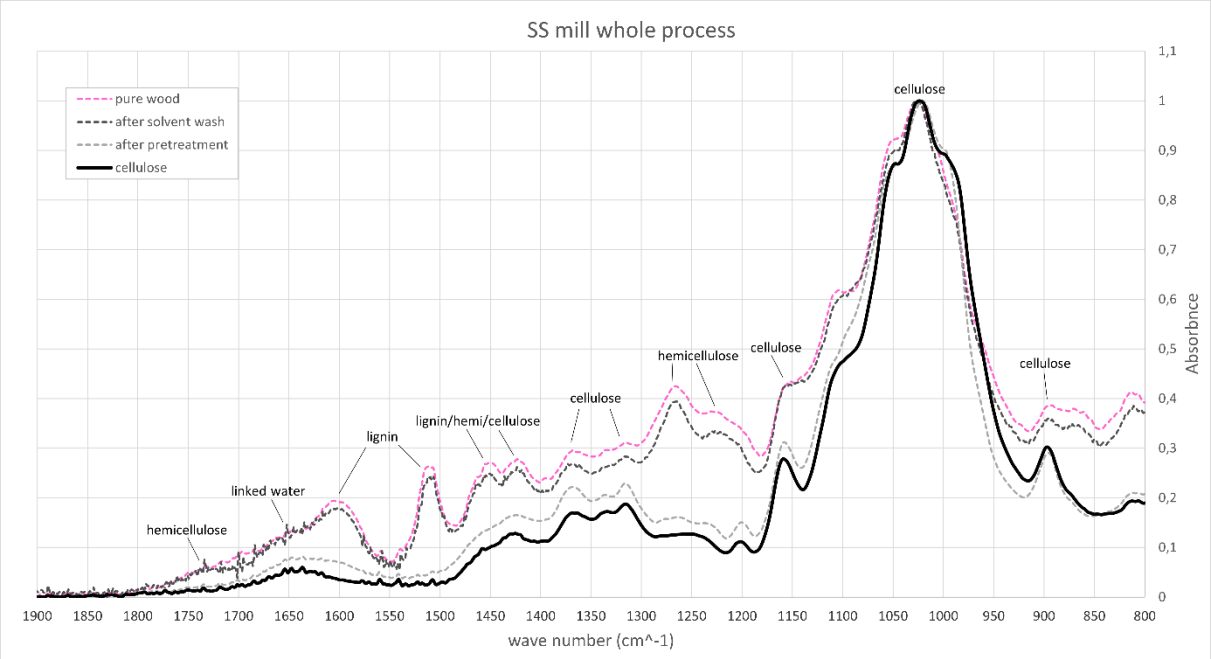


Figure 18 FTIR-spectra of SS mill for the whole process

Both the SS cut and SS mill samples look similar to pure cellulose. No obvious contaminations are shown.

All FTIR spectra for the various non-resinous woods are shown in separate graphs below.

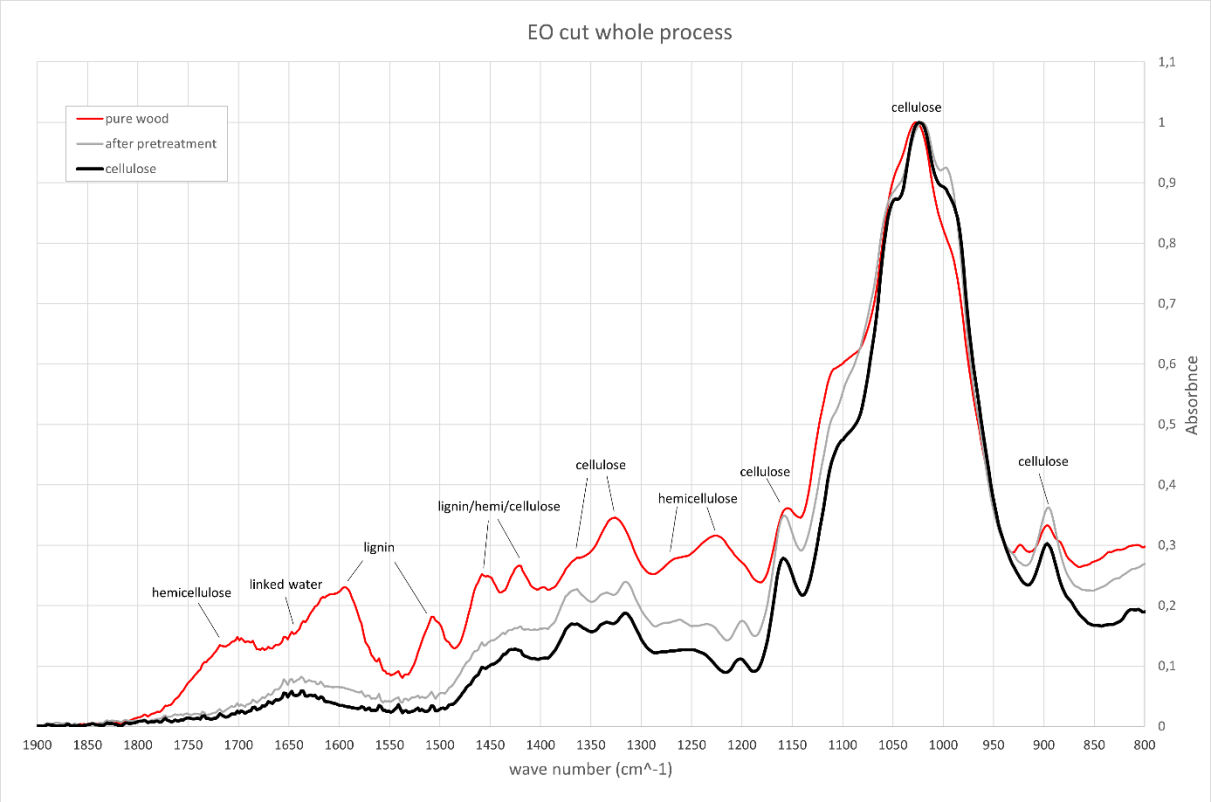


Figure 19 FTIR-spectra of EO cut for the whole process

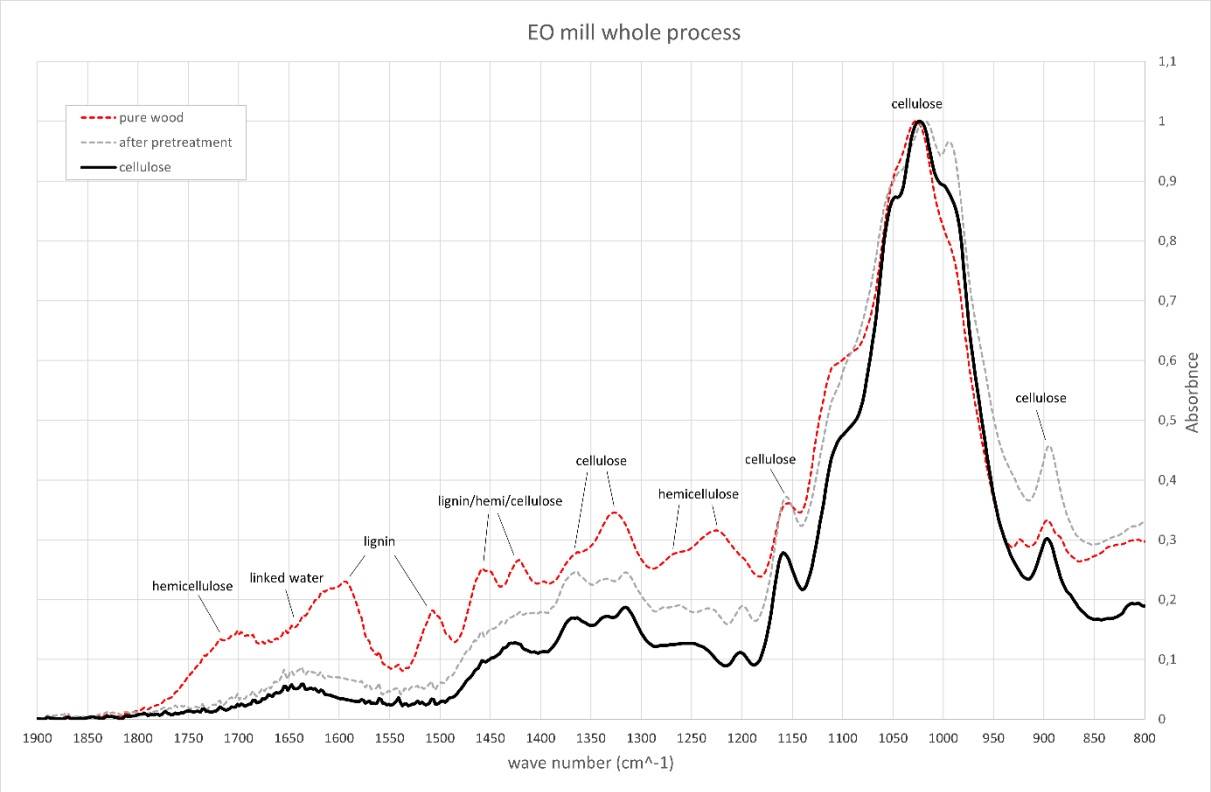


Figure 20 FTIR-spectra of EO mill for the whole process

The clearest difference between the MS cut and mill product after pretreatment and the pure cellulose is a side peak at 1000 cm^{-1}. This peak might indicate a contamination, cellulose degradation product or derivative formed in the degradation process. Also, the dip around 1400 cm^{-1} is not as visible in the product after pretreatment as it is in the cellulose. This also indicates some contaminant left behind.

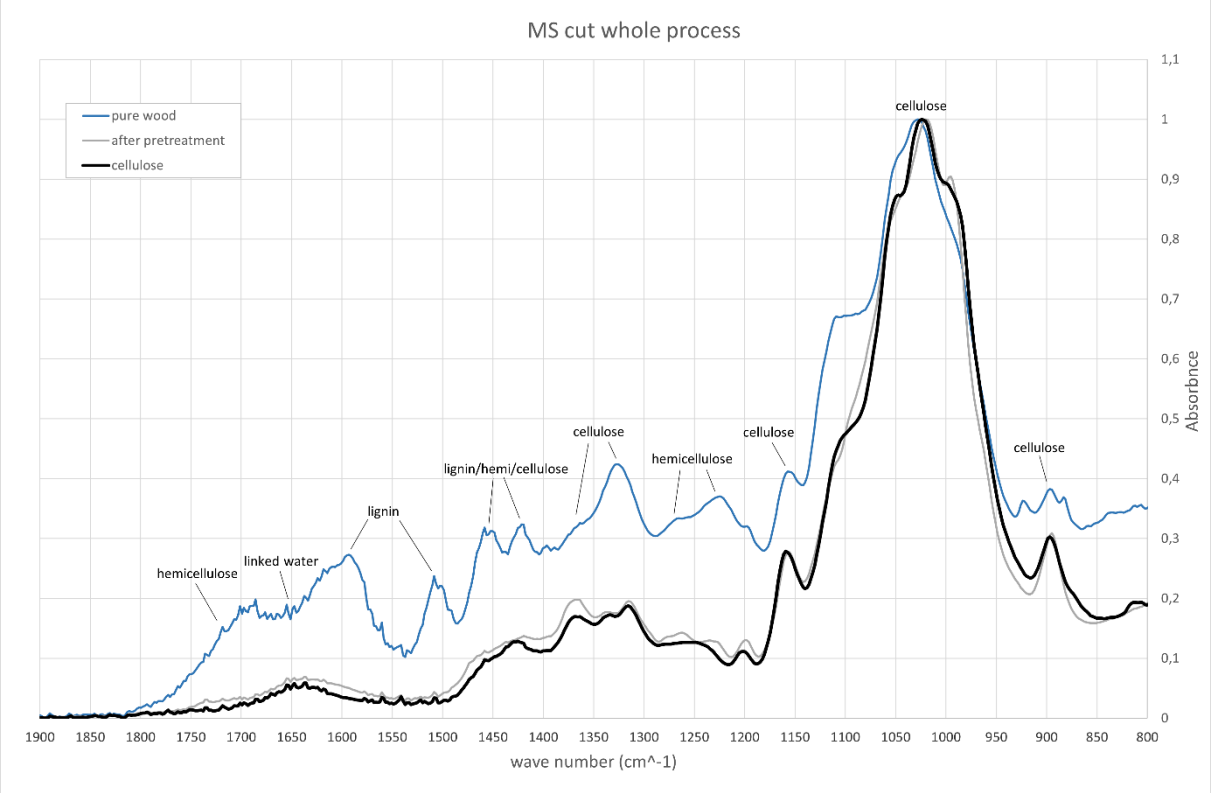


Figure 21 FTIR-spectra of MS cut for the whole process

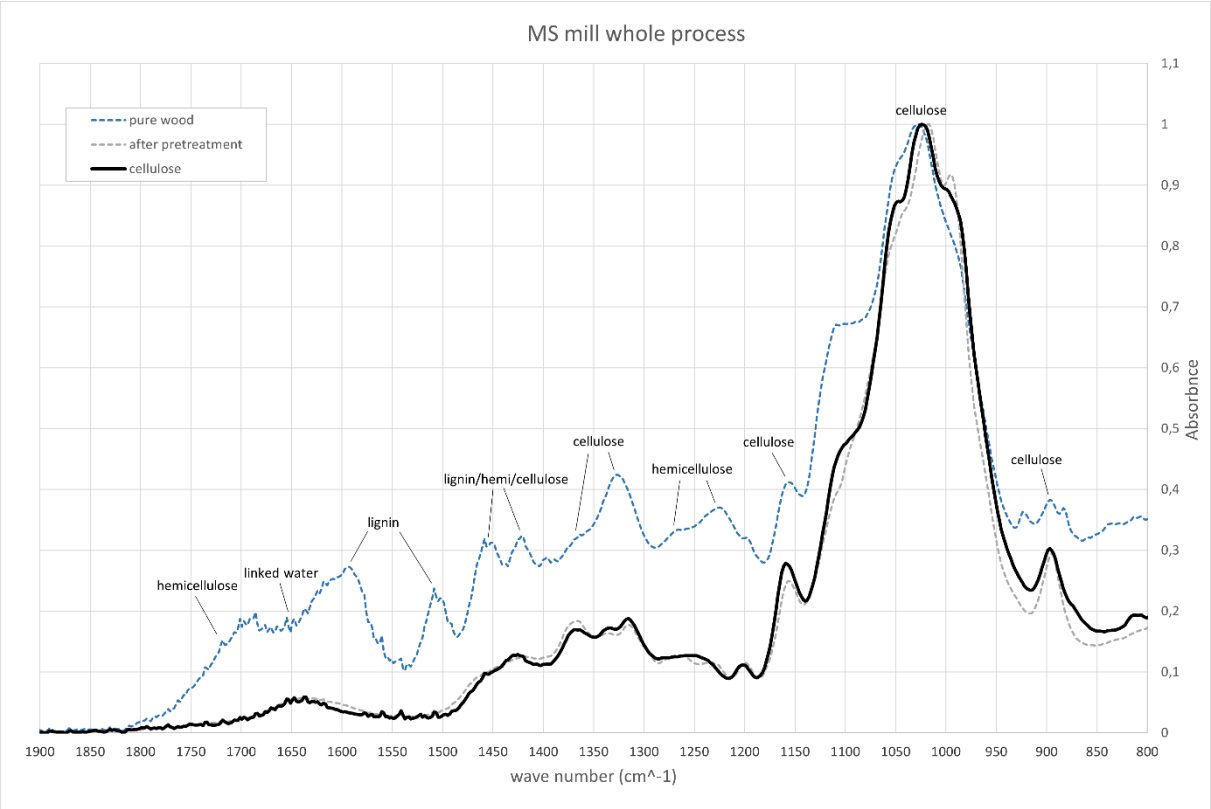


Figure 22 FTIR-spectra of MS mill for the whole process

The clearest difference between the MS cut and mill product after pretreatment and the pure cellulose is a side peak at 1000 cm^{-1}. This peak might indicate a contamination, cellulose degradation product or derivative formed in the degradation process. As a side mark, the dip around 1400 cm^{-1} is not as visible in the product after pretreatment as it is in the cellulose. This might indicate some contaminant left behind.

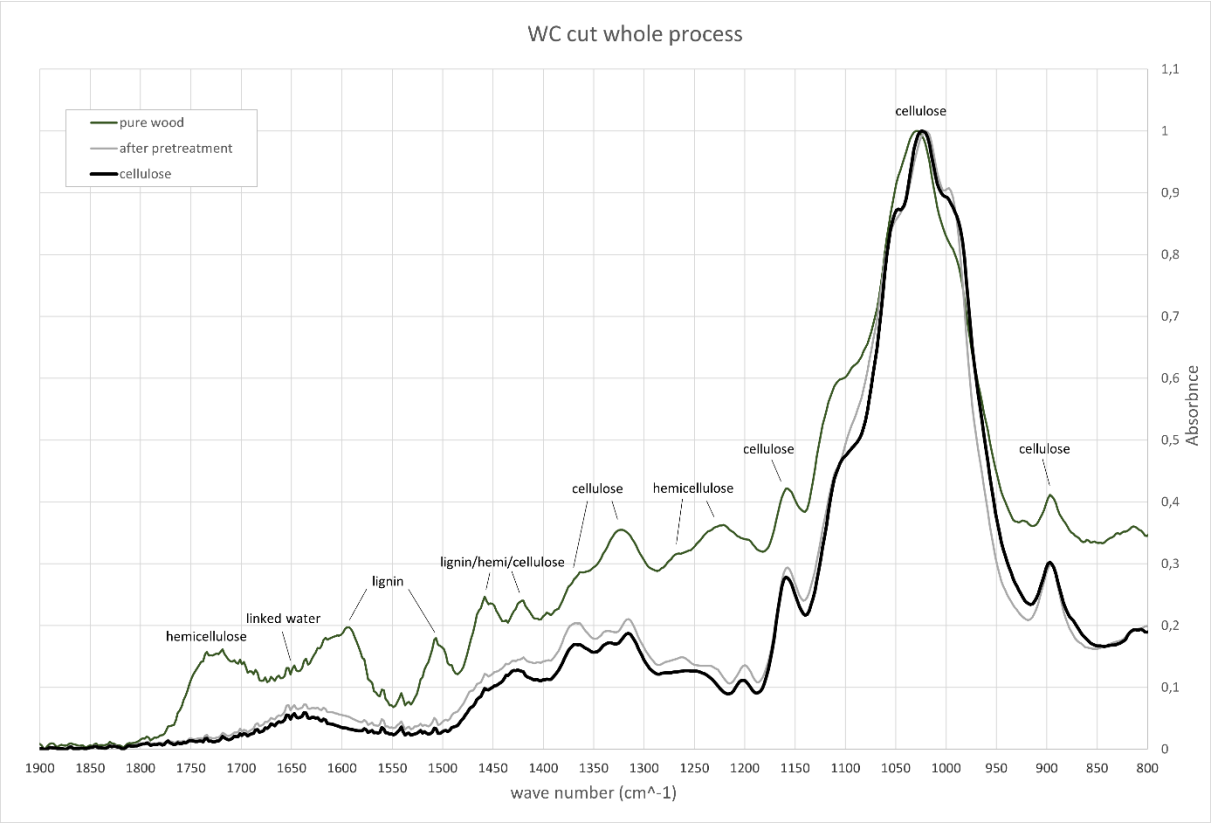


Figure 23 FTIR-spectra of WC cut for the whole process

The clearest difference between the WC cut product after pretreatment and the pure cellulose is a side peak at 1000 cm^{-1}. This peak might indicate a contamination, cellulose degradation product or derivative formed in the degradation process. Also, the dip around 1400 cm^{-1} is not as visible in the product after pretreatment as it is in the cellulose. This likely indicates some contaminant left behind.

Groningen solvent wash of pure α-cellulose

To see if any solvents remain in products after a solvent wash, the Groningen solvent wash protocol is performed on pure α-cellulose. This protocol consists of a 45 min. wash of the cellulose with acetone, followed by methanol and chloroform. Then the product is dried for approx. 90h in an open reaction tube in a fumehood. Then an FTIR spectrum of the cellulose is taken and compared with a pure cellulose spectrum. The result is shown in Figure 24.

To determine whether solvent residues are present, the spectra are compared with spectra of acetone, methanol and chloroform from the SDBS database (AIST, 2021). From these spectra it is shown that both an acetone and chloroform solvent residuals are present in the washed cellulose. As acetone is the first solvent in this step and should be washed away by both methanol and chloroform, its residue is unexpected.

It is hard to tell whether solvent residuals are also present in the wood samples after the solvent wash (Figure 11). At least no chloroform peak (750 cm^{-1}) is present in the wood samples. Because the acetone peaks overlap with (hemi)cellulose and lignin peaks, it is hard to tell whether acetone is present. However, multiple demi water washes and an oxidation step are performed after the solvent wash. Therefore it is unlikely that any solvent residuals will remain in the cellulose end product when it undergoes carbon analysis.

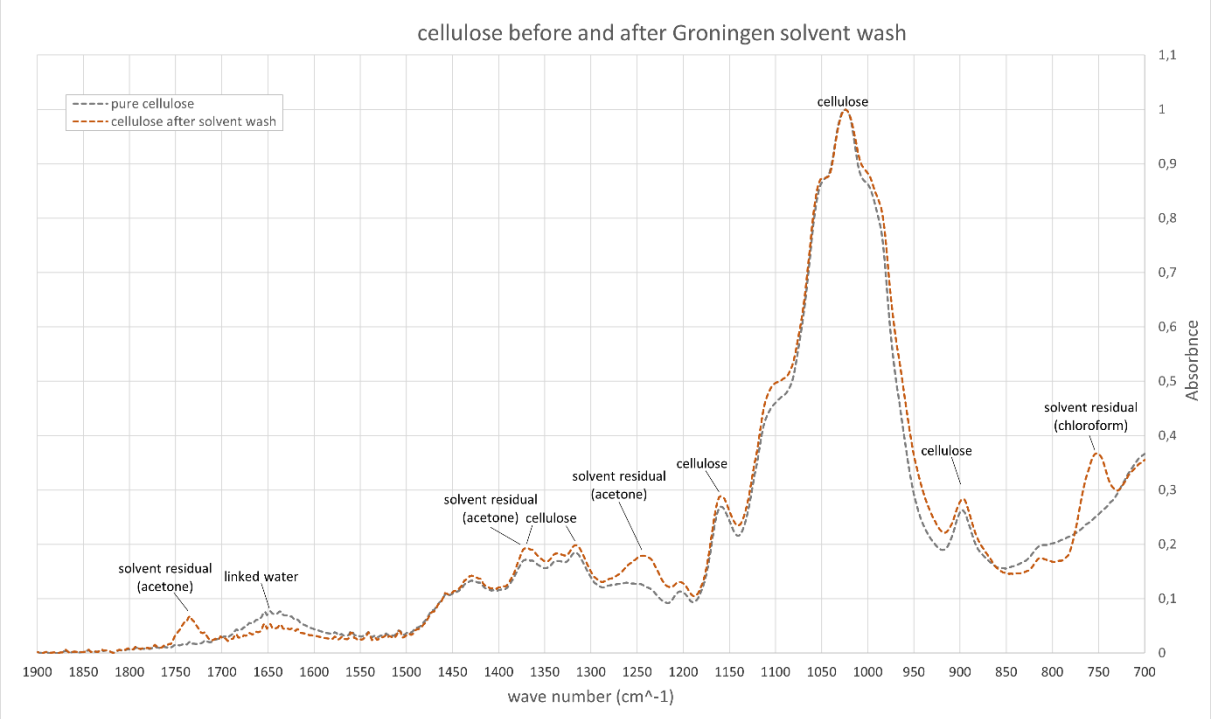


Figure 24 FTIR- spectra of cellulose before and after Groningen solvent wash

Soxhlet wash of resinous MH samples vs Groningen pretreatment

To find out if a Soxhlet procedure is more efficient to remove resinous material from samples than the standard Groningen solvent wash, Soxhlet procedures using acetone and both acetone, methanol and chloroform are carried out. The exact protocols were described in the materials and procedures section.

Afterwards, FTIR spectra of the Soxhlet solvent washed material were taken and compared with the standard Groningen solvent washed material. These are shown in Figure 25. Pure MH wood (starting material) and cellulose (as final product) are shown for reference.

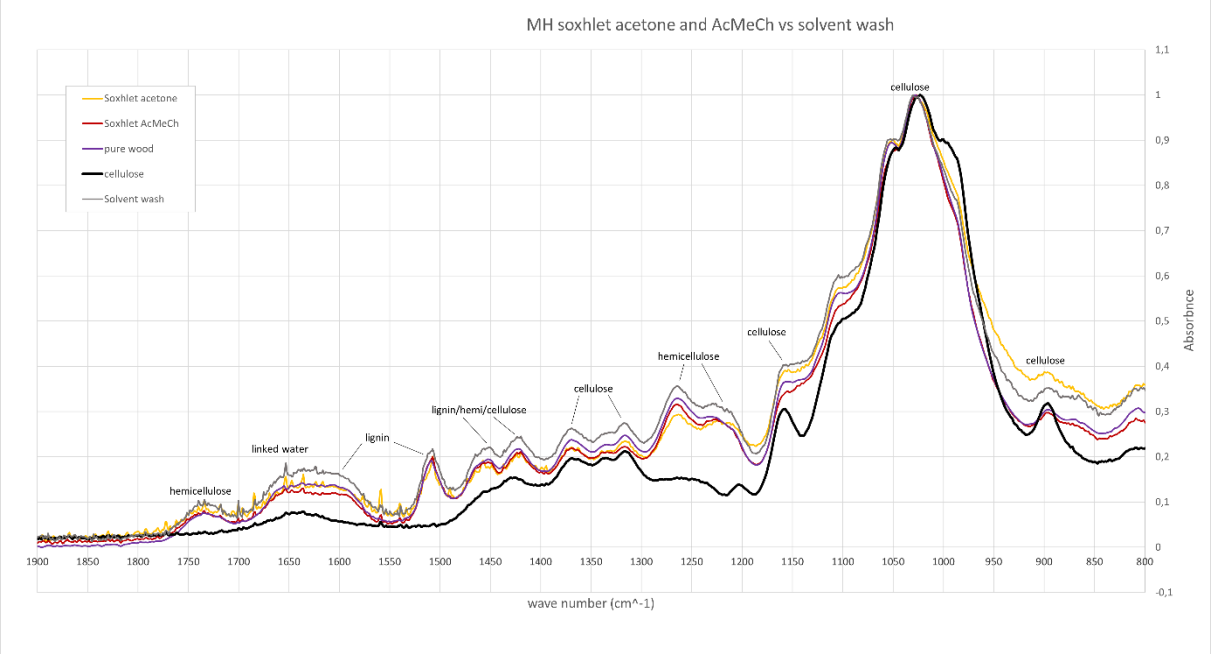


Figure 25 FTIR-spectra of MH wood samples after either Groningen solvent wash, Soxhlet acetone or Soxhlet acetone/methanol/chloroform (AcMeCh) procedure compared with starting material and cellulose

In Figure 25 it can be seen that the Soxhlet AcMeCh procedure seems to remove the peak around 1050 cm^{-1} slightly better than the Soxhlet acetone and standard Groningen solvent wash do. The exact nature of this contaminant is unknown. Apart from this peak, the difference between all procedures is not clearly visible. In the Groningen solvent wash procedure, the peak at 1050 cm^-1 is finally removed after oxidation (see Figure 15 and Figure 16) and therefore removing this peak at an earlier stage doesn't seem significant.

The difference in FTIR-spectra between the pure wood and all solvent-washed protocols also seems quite insignificant, as can also be seen in the spectra of the other resinous wood types (KA and SS, Figure 14, Figure 17 and Figure 18). It might therefore be good to investigate whether solvent wash is actually a necessary step in the protocol, by screening more resinous samples or more solvents.

To investigate what is actually removed in the acetone Soxhlet procedure, the acetone used as the solvent is left to dry in a beaker. A sticky brown layer with small brown particles remained after drying. This fraction is analyzed by FTIR and compared with the pure MH wood, the wood after the Soxhlet procedure and pure cellulose for reference. See Figure 26.

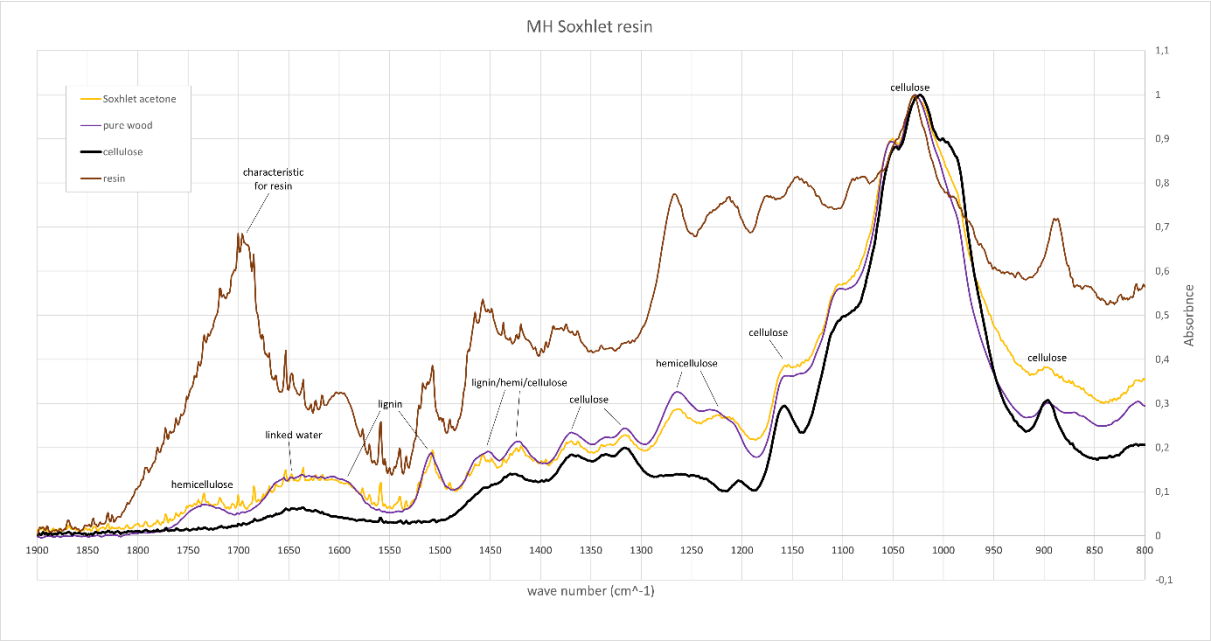


Figure 26 FTIR-spectra of MH wood resin compared with wood after Soxhlet solvent wash, starting material and cellulose.

In Figure 26 it can be seen that a mixture high in resin, lignin and hemicellulose seems to be extracted out of the wood by the acetone solvent. The mixture was dark compared to the wood starting material and seems to be lower in cellulose because non-cellulose peaks now are more prominent (except for the 890 cm^{-1} cellulose peak). It follows that the Soxhlet solvent wash procedure indeed removes some resin, although the overall composition of the wood does not significantly change, as can be seen in the FTIR-spectra in Figure 25.

Discussion and conclusions

The goal of this research project entailed the development of a protocol for checking the purity of alpha-cellulose product. This was done by running FTIR spectra on the wood, extracted cellulose, intermediates and the waste products obtained. The samples were pretreated by using the Groningen α-cellulose treatment protocol and by experiment with a Soxhlet solvent rinse.

The protocol for the purity check is fully described in in Appendix B.

In checking the purity of the starting materials, a difference between resinous and non-resinous woods was noted. All the non-resinous species in this research have a clear cellulose peak around 1330 cm^{-1}, whereas the resinous species have a more distinct hemicellulose peak at 1270 cm^{-1}. This might be an easy way to tell whether a wood sample is resinous or not. The resinous MH sample thereby has an unknown contaminant emerging at 1050 cm^{-1}. Except for this side peak, pure α-cellulose seemed to be delivered.

The procedure for attaining α-cellulose from wood started with cutting or milling the sample. No differences in quality between cutting or milling the sample were visible in the spectra. Because of easier sample preparation (handling the Dremel for milling is difficult) and less material loss during solvent washes and the oxidation step, cutting of the sample is preferred.

The second step in attaining α-cellulose was the solvent wash procedure. In this research, two Soxhlet procedures (acetone and acetone-methanol-chloroform) were performed on the most resinous wood sample (MH) as an alternative to the already in-use Groningen solvent wash procedure. One notable difference in the IR-spectra was the removal of a contamination side peak at 1050 cm^{-1}, which in the Groningen solvent wash procedure was removed in the oxidation step. Thus, in the end product, no clear differences were expected.

For the solvent wash procedure as a whole, no clear differences between the wood before and after solvent wash showed in the FTIR spectra. The Groningen solvent wash procedure seemed to be adding a peak (1000 cm^{-1}) that might be solvent and therefore might contaminate the product. It is therefore recommended to do more research on the effects of a solvent wash on the purity of the α-cellulose final product.

One of the unresolved parts of this research project was the nature of the contamination of specifically the 1000 cm^{-1} peak on the MH sample as it could contain foreign carbon with different ^{14}C level than the wood. This peak might indicate a contamination, cellulose degradation product or derivative formed in the degradation process. A strategy to solve this might be to systematically take an FTIR (or look up spectra) of possible contaminations for all unknown peaks.

In the spectra of the pure wood, not all peaks are cleared up either (see Table 2). A good strategy (in addition to the 1000 cm^{-1} MH peak might be to look up or make spectra of wood components such as lignin and resin.

An unexpected result showed up in the FTIR-spectrum of α-cellulose after solvent wash to check whether solvents of the solvent wash procedure remain in the end product. From this spectrum it was shown that both an acetone and chloroform solvent residuals were present in the washed cellulose. It is recommended to check whether this is still the case after washing steps with water (as is the case in the oxidation step) and after longer drying times (> 90h).

As mentioned in the conclusion section, it might be worth the effort to look into a protocol with less spillage of solvent during handling steps than the current Groningen solvent wash procedure. This might be done in combination with the automatic pretreatment system that has been developed in the research group before. Another option to more thoroughly look at is the Soxhlet procedure, which was easier in material and solvent handling.

Lastly, the acquired FTIR spectra must be normalized and overlayed to make comparison between multiple spectra, for example between wood and α-cellulose end product. This was done using spectrographic software. A good option is SpectraGryph, which is free and allows for import of .txt files generated by the FTIR apparatus. It can overly and normalize spectra and finally copy data to clipboard so it is suitable for making a final graph in Excel, as was done for this report.

References

Let's Talk Science. (2019, May 28). *What are Cosmic Rays?* Retrieved from Let's Talk Science: https://letstalkscience.ca/educational-resources/backgrounders/what-are-cosmic-rays

Aerts-Bijma, A., Paul, D., Dee, M., Palstra, S., & Meijer, H. (2020). An independent assessment of uncertainty for radiocarbon analysis with the new generation high-yield accelerator mass spectrometers. *Radiocarbon*, 1-22.

AIST. (2021). *SDBS Compounds and Spectral Search*. Retrieved from Spectral Database for Organic Compounds SDBS: https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi

Anchukaitis, K., Evans, M., & Lange, T. (2008). Consequences of a Rapid Cellulose Extraction Technique for Oxygen Isotope and Radiocarbon Analyses. *Analytical Chemistry*, 2035-2041.

Beta Analytic. (2021). *Accelerator Mass Spectrometry (AMS) Dating*. Retrieved from Beta Analytic testing laboratory: https://www.radiocarbon.com/accelerator-mass-spectrometry.htm

Beta Analytic. (n.d.). *Introduction to Radiocarbon Determination by the Accelerator Mass Spectrometry Method.* Retrieved from AMS methodology: https://www.radiocarbon.com/PDF/AMS-Methodology.pdf

Beta Analytic Testing Laboratory. (2020). *Sample Suitability: AMS or Radiometric Dating?* Retrieved from radiocarbon.com: https://www.radiocarbon.com/radiometric-plus.htm

Brock, F., Dee, M., & Hughes, A. (2017). Testing the effectiveness of protocols for removal of common conservation treatments for radiocarbon dating. *Radiocarbon*, 1-16.

Bronk Ramsey, C. (2008). Radiocarbon dating: revolutions in understanding. *Archaeometry*, 249-275.

Bronk Ramsey, C., Plicht, J. v., & Weninger, B. (2001). 'Wiggle matching' radiocarbon dates. *Radiocarbon*, 381-389.

Brookman, T., & Whittaker, T. (2012). Experimental assessment of the purity of a-cellulose produced by variations of the Brendel method: Implications for stable isotope (13C, 18O) dendroclimatology. *Geochemistry Geophysics Geosystems*, 1-17.

Capano, M., Miramont, C., & Guibal, F. (2018). WOOD 14C DATING WITH AIXMICADAS: METHODS AND APPLICATION TO TREE-RING SEQUENCES FROM THE YOUNGER DRYAS EVENT IN THE SOUTHERN FRENCH ALPS. *Radiocarbon*, 51-74.

Choppin, G. R., Rydberg, J., & Liljenzin, J. E. (2013). *Radiochemistry & Nuclear Chemistry.* Oxford: Academic Press.

Dee, M., Brock, F., Bowles, A., & Ramsey, C. B. (2011). Using a silica substrate to monitor the effectiveness of radiocarbon pretreatment. *Radiocarbon*, 705-711.

Dee, M., Palstra, S., Aerts-Bijma, A., Bleeker, M., de Bruijn, S., Ghebru, F., . . . Meijer, H. (2020). Radiocarbon dating at Groningen: new and updated chemical pretreatment procedures. *Radiocarbon*, 63-74.

Dremel. (2021). *Dremel 26150225JA 225 Flexibele Verlengstuk voor Multitools met Comfort-Grip Handstuk, 220V, Meerkleurig, 1 stuk*. Retrieved from Amazon: https://www.amazon.nl/Dremel-26150225JA-Verlengstuk-Comfort-Grip-Meerkleurig/dp/B008ETGPOY

Fogtmann-Schulz, A., Kudsk, S. G., & Adolphi, F. (2020). Batch processing of tree ring samples for radiocarbon analysis. *Radiocarbon*, not submitted yet.

Gaudinski, J., Dawson, T., & Quideau, S. (2005). Comparative Analysis of Cellulose Preparation Techniques for Use with 13C, 14C, and 18O Isotopic Measurements. *Analytical Chemistry*, 7212-7224.

Graven, H. D. (2015). Impact of fossil fuel emissions on atmospheric radiocarbon and various applications of radiocarbon over this century. *PNAS*, 9542-9545.

Green, J. (1963). Methods of Carbohydrate Chemistry, III. *Academic Press New York*, 9-21.

Hajdas, I., Hendriks, L., & Fontana, A. (2017). Evaluation of preparation methods in radiocarbon dating of old wood. *Radiocarbon*, 727-737.

Hogg, A. (2002, May 16). *Liquid Scintillation Counting (LSC)*. Retrieved from http://www.c14dating.com/lsc.html

Kudsk, S., Olsen, J., & Nielsen, L. (2018). what is the carbon origin of early-wood? *Radiocarbon*, 1457-1464.

Kuitems, M., Panin, A., & Scifo, A. (2020). Radiocarbon-based approach capable of subannual precision resolves the origins of the site of Por-Bajin. *PNAS*, 14038-14041.

Leavitt, S. W., & Danzer, S. R. (1993). Method for Batch Processing Small Wood Samples to Holocellulose for Stable-Carbon Isotope Analysis. *Analytical Chemistry*, 87-89.

Loader, N., Robertson, I., & Barker, A. (1997). An improved technique for the batch processing of small wholewood samples to a-cellulose. *Chemical Geology*, 313-317.

Michczynska, D., Krapiec, M., Michczynski, A., Pawlyta, J., Goslar, T., Nawrocka, N., & Piotrowska, N. (2018). Different pretreatment methods for 14C dating of Younger Dryas and Allerød pine wood (Pinus sylvestris L.). *Quaternary Geochronology*, 38-44.

Morlan, R. (2021). *Radiocarbon dating principles*. Retrieved from Canadian Archeology: https://www.canadianarchaeology.ca/dating

Němec, M., Wacker, L., & Hajdas, I. (2010). Alternative methods for cellulose preparation for AMS measurement. *Radiocarbon*, 1358-1370.

Purdue University Department of Physics and Astronomy. (2021). *What is AMS?* Retrieved from Purdue University College of Science Department of Physics and Astronomy: https://www.physics.purdue.edu/ams/introduction/ams.html

Rinne, K., Boettger, T., Loader, N., Robertson, I., Switsur, V., & Waterhouse, J. (2005). On the purification of a-cellulose from resinous wood for stable isotope (H, C and O) analysis. *Chemical Geology*, 75-82.

Rowell, R., Pettersen, R., & Han, J. (2005). 3.3. Extractives. In R. Rowell, R. Pettersen, & J. H. Al, *Handbook of Wood Chemistry and Wood Composites* (p. 45). CRC Press LLC.

Scifo, A., Kuitems, M., & Neocleous, A. (2019). Radiocabon Production Events and their Potential Relationship with the Schwabe Cycle. *Nature research Scientific Reports*, 1-8.

Southon, J., & Magana, A. (2010). A Comparison of Cellulose Extraction and ABA Pretreatment Methods for AMS 14C Dating of Ancient Wood. *A Comparison of Cellulose Extraction and ABA*

Pretreatment Methods for AMS 14C Dating of Ancient Wood (pp. 1371-1379). California: Cambridge University Press.

Staff, R., Reynard, L., & Brock, F. (2014). Wood pretreatment protocols and measurement of tree-ring standards at the Oxford radiocarbon accelerator unit (ORAU). *Radiocarbon*, 709-715.

Stewart, D., Wilson, H., Hendra, P., & Morrison, I. (1995). Fourier-Transform Infrared and Raman Spectroscopic Study of Biochemical and Chemical Treatments of Oak Wood (Quercus rubra) and Barley (Hordeum vulgare) Straw. *Journal of Agricultural and Food Chemistry*, 2219 - 2225.

Universiteit Utrecht. (n.d.). *Radiocarbon Dating*. Retrieved from UU Universiteit: https://web.science.uu.nl/AMS/Radiocarbon.htm

Vuong, L., Song, Q., Lee, H., & Roffel, A. (2016). Opportunities in low-level radiocarbon microtracing: applications and new technology. *Future Science OA*, 1.

Yizhaq, M., Mintz, G., & Cohen, I. (2005). Quality controlled radiocarbon dating of bones and charcoal from the early pre-pottery neolithic B (ppnb) of Motza (Israel). *Radiocarbon*, 193-206.

Appendix A Project planning scheme

Sample Details					Whole Wood Stocks (ww)				Organic Solvent Washes (S)								Aqueous Pretreatment						
Type	Our Identification Name	Our Identification Code	Age (Years BP)	Age (BCE/CE)	Preparation Type	Total Stock Weight (mg)	Stock Code	Taken for FTIR (mg)	Method Name	Description	Subsample Code	Starting Weight (mg)	Yield Wood (mg)	Yield Resin (mg)	Taken for FTIR Wood (Y/N)	Taken for FTIR Resin (Y/N)	Method Name	Description	Subsample Code	Starting Weight (mg)	Yield (mg)	Taken for FTIR (Y/N)
Cypress leylandii (gymnosperm, resinous)	Margot's Hedge	MH	0	2020 CE	Mill with Dremmel	210	MH.Mill.ww	Y/N	Tubes (T)	Groningen unknown organic contaminant treatment	MH.Mill.S.T.a	> 30 mg	?	?	Y/N	Y/N	Alpha-cellulose	Groningen alpha-cellulose prep	MH.Mill.S.T.a.AC1	?	?	Y/N
											MH.Mill.S.T.b	> 30 mg	?	?	Y/N	Y/N			MH.Mill.S.T.a.AC2	?	?	Y/N
					Cut with scalpel	260	MH.Cut.ww	Y/N			MH.Cut.S.T.a	> 30 mg	?	?	Y/N	Y/N			MH.Cut.S.T.a.AC1	?	?	Y/N
											MH.Cut.S.T.b	> 30 mg	?	?	Y/N	Y/N			MH.Cut.S.T.a.AC2	?	?	Y/N
									Soxhlet 1 (X1)	Soxhlet toluene/methanol mix	MH.Mill.S.X1.a	> 30 mg	?	?	Y/N	Y/N			MH.Mill.S.X1.a.AC1	?	?	Y/N
											MH.Cut.S.X1.a	> 30 mg	?	?	Y/N	Y/N			MH.Cut.S.X1.a.AC1	?	?	Y/N
									Soxhlet 2 (X2)	Soxhlet acetone/water	MH.Mill.S.X2.a	> 30 mg	?	?	Y/N	Y/N			MH.Mill.S.X2.a.AC1	?	?	Y/N
											MH.Cut.S.X2.a	> 30 mg	?	?	Y/N	Y/N			MH.Cut.S.X2.a.AC1	?	?	Y/N
																			MH.Mill.ww.AC1	> 30 mg	?	Y/N
																			MH.Cut.ww.AC1	> 30 mg	?	Y/N
Sequoiadendron giganteum (gymnosperm, resinous)	Sturt's 400 BCE sequoia	SS	2.400	400 BCE	Mill	CIO Project 3592, Sample 76174 (or similar)	SS.Mill.ww	Y/N	Tubes (T)	Groningen unknown organic contaminant treatment	SS.Mill.S.T.a	> 30 mg	?	?	?	?	Alpha-cellulose	Groningen alpha-cellulose prep	SS.Mill.S.T.a.AC1	?	?	Y/N
											SS.Mill.S.T.b	> 30 mg	?	?	?	?			SS.Mill.S.T.a.AC2	?	?	Y/N
					Cut	CIO Project 3592, Sample 76174 (or similar)	SS.Cut.ww	Y/N			SS.Cut.S.T.a	> 30 mg	?	?	?	?			SS.Cut.S.T.a.AC1	?	?	Y/N
											SS.Cut.S.T.b	> 30 mg	?	?	?	?			SS.Cut.S.T.a.AC2	?	?	Y/N
									Soxhlet 1 (X1)	Soxhlet toluene/methanol mix	SS.Mill.S.X1.a	> 30 mg	?	?	?	?			SS.Mill.S.X1.a.AC1	?	?	Y/N
											SS.Cut.S.X1.a	> 30 mg	?	?	?	?			SS.Cut.S.X1.a.AC1	?	?	Y/N
									Soxhlet 2 (X2)	Soxhlet acetone/water	SS.Mill.S.X2.a	> 30 mg	?	?	?	?			SS.Mill.S.X2.a.AC1	?	?	Y/N
											SS.Cut.S.X2.a	> 30 mg	?	?	?	?			SS.Cut.S.X2.a.AC1	?	?	Y/N
																			SS.Mill.ww.AC1	> 30 mg	?	Y/N
																			SS.Cut.ww.AC1	> 30 mg	?	Y/N
Agathis australis (gymnosperm, resinous)	Kauri	KA	> 50,000	> 50,000 BCE	Mill	260	KA.Mil.ww	Y/N	Tubes (T)	Groningen unknown organic contaminant treatment	KA.Mill.S.T.a	> 30 mg	?	?	?	?	Alpha-cellulose	Groningen alpha-cellulose prep	KA.Mill.S.T.a.AC1	?	?	Y/N
											KA.Mill.S.T.b	> 30 mg	?	?	?	?			KA.Mill.S.T.a.AC2	?	?	Y/N
					Cut	120	KA.Cut.ww	Y/N			KA.Cut.S.T.a	> 30 mg	?	?	?	?			KA.Cut.S.T.a.AC1	?	?	Y/N
											KA.Cut.S.T.b	> 30 mg	?	?	?	?			KA.Cut.S.T.a.AC2	?	?	Y/N
									Soxhlet 1 (X1)	Soxhlet toluene/methanol mix	KA.Mill.S.X1.a	> 30 mg	?	?	?	?			KA.Mill.S.X1.a.AC1	?	?	Y/N
											KA.Cut.S.X1.a	> 30 mg	?	?	?	?			KA.Cut.S.X1.a.AC1	?	?	Y/N
									Soxhlet 2 (X2)	Soxhlet acetone/water	KA.Mill.S.X2.a	> 30 mg	?	?	?	?			KA.Mill.S.X2.a.AC1	?	?	Y/N
											KA.Cut.S.X2.a	> 30 mg	?	?	?	?			KA.Cut.S.X2.a.AC1	?	?	Y/N
																			KA.Mil.ww.AC1	> 30 mg	?	Y/N
																			KA.Cut.ww.AC1	> 30 mg	?	Y/N
Quercus robur (angiosperm, non resinous)	Esther's 400 BCE oak	EO	2.400	400 BCE	Mill	CIO Project 3592, Sample 76179 (or similar)	EO.Mill.ww	Y/N									Alpha-cellulose	Groningen alpha-cellulose prep	EO.Mill.ww.AC1	> 30 mg	?	Y/N
																			EO.Mill.ww.AC2	> 30 mg	?	Y/N
					Cut	CIO Project 3592, Sample 76179 (or similar)	EO.Cut.ww	Y/N											EO.Cut.ww.AC1	> 30 mg	?	Y/N
																			EO.Cut.ww.AC2	> 30 mg	?	Y/N
Quercus robur (angiosperm, non resinous)	Margot Spike	MS	3.500	1600 BCE	Mill	70	MS.Mill.ww	Y/N									Alpha-cellulose	Groningen alpha-cellulose prep	MS.Mill.ww.AC1	> 30 mg	?	Y/N
																			MS.Mill.ww.AC2	> 30 mg	?	Y/N
					Cut	40	MS.Cut.ww	Y/N											MS.Cut.ww.AC1	> 30 mg	?	Y/N
																			MS.Cut.ww.AC2	> 30 mg	?	Y/N
Quercus robur (angiosperm, non resinous)	Windsor Castle	WC	447	1503	Mill	100	WC.Mill.ww	Y/N									Alpha-cellulose	Groningen alpha-cellulose prep	WC.Mill.ww.AC1	> 30 mg	?	Y/N
																			WC.Mill.ww.AC2	> 30 mg	?	Y/N
					Cut	150	WC.Cut.ww	Y/N											WC.Cut.ww.AC1	> 30 mg	?	Y/N
																			WC.Cut.ww.AC2	> 30 mg	?	Y/N
Pure α-cellulose (Sigma)	Pure	Pure	-	-					Tubes (T)	Groningen unknown organic contaminant treatment	Pure.S.T	> 30 mg	?	?	Y/N	Y/N	Alpha-cellulose	Groningen alpha-cellulose prep	Pure.S.T.AC1	?	?	Y/N
									Soxhlet 1 (X1)	Soxhlet toluene/methanol mix	Pure.S.X1	> 30 mg	?	?	Y/N	Y/N			Pure.S.X1.AC1	?	?	Y/N
									Soxhlet 2 (X2)	Soxhlet acetone/water	Pure.S.X2	> 30 mg	?	?	Y/N	Y/N			Pure.S.X2.AC1	?	?	Y/N
																			Pure.AC1	> 30 mg	?	Y/N
																			Pure.AC2	> 30 mg	?	Y/N
																			Pure.1			Y/N
																			Pure.2			?

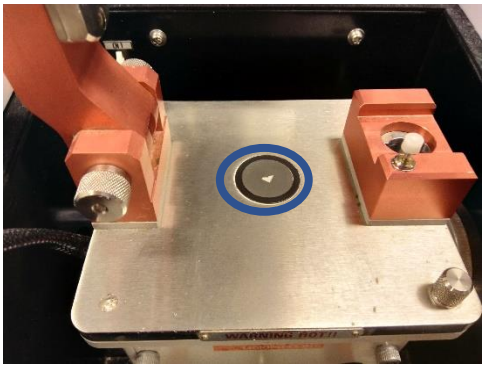
Appendix B FTIR and normalization protocol

FTIR measurements

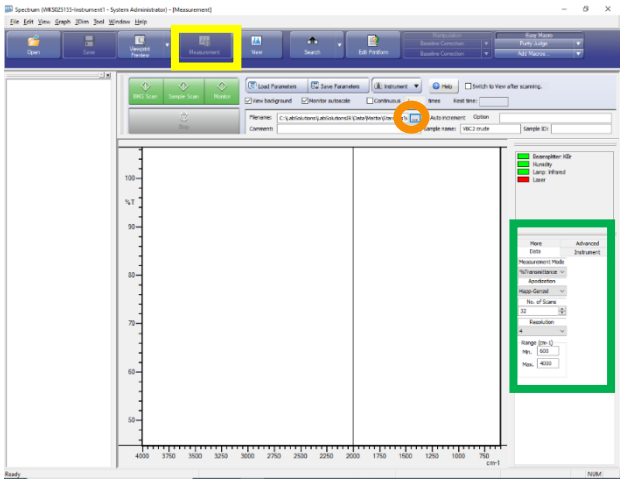
Location: lab 5118.0258 in the Nijenborgh 4 building)
Apparatus: Shimadzu IRTracer-100 + Specac Golden Gate^{tm}
Software: Shimadzu LabSolutions IR

- First open the press on the sample compartment by turning the small black hook (marked with red circle) on the right anticlockwise, if needed. Then the bridge (part with the bigger black button) can be turned up.

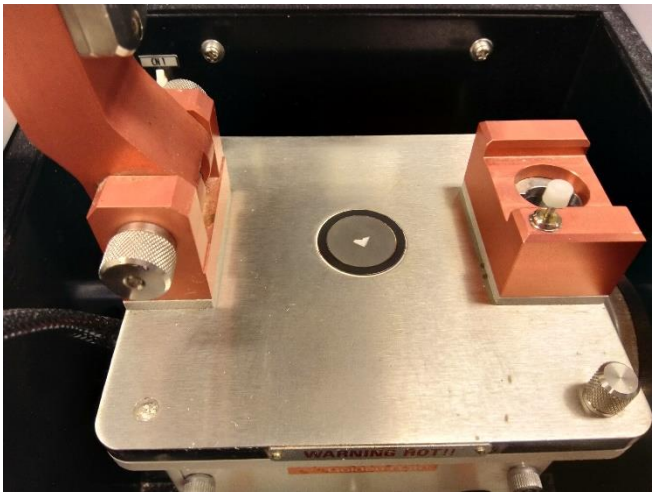
- The whole setup now looks like this:



- Put a bit of ethanol from the flask on a paper towel and use this to clean the laser place (blue circle)
- Leave the bridge in this position
- Insert a USB-stick in the right PC hardware thing under the desk
- Open programme LabSolutions IR on the desktop menu. Then click on ‘Spectrum’ in the menu that pops up.
- The following programme appears:



- Click on the ‘Measurement’ button in the above middle (if not selected). This button is marked yellow in above screenshot.
- Check on the panel ‘Data’ on the right (green marked in above screenshot) if the following settings are correct:
    - Measurement mode: Absorbance (change on the menu)
    - Apodization: Happ-Genzel (should already be correct)
    - No. of Scans: 32
    - Resolution = 4
    - Range (cm-1):
    - Min: 600
    - Max: 4000
- In the Filename box, select your USB-stick and the right folder by clicking the box with the three dots on the right (orange marked in above screenshot)
- Now change the last part of this destination (the sample name, in this case VBC2 crude) to your own sample name
- Also enter your sample name in the Sample name box
    - If all of above is correct, click on the green ‘BKG Scan’ button and press ‘OK’. The background check is being made. This may take a few minutes. In the left corner below the graph you can see how many of the 32 scans already have been made.
- When the background check is done (the BKG scan button turned from grey into green again), place a small amount of sample on the laser spot (see picture). Make sure the material covers the small glass-like area fully.



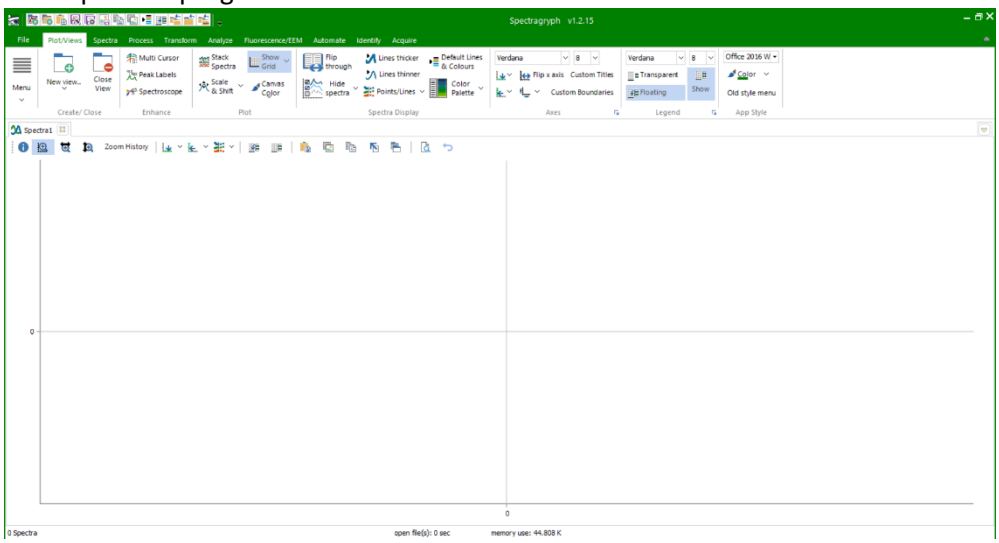
- Close the press and secure by turning the small black hook clockwise until you feel a ‘click’. Turn the top of the big black press clockwise until the sample is slightly stuck, but don’t so tight that you damage the sample and might break the laser crystal!)
- Press the ‘Sample scan’ button next to the ‘BKG scan’ button. **Now your spectra are being made**! This may take a few minutes.
- When the sample scan is done (the sample scan button turned from grey into green again), check if your spectrum ended up in the right folder on your USB-stick (it’s an IR spectrum data file, code name .ispd) . These can only be re-opened by specialistic software on your PC.
- Now you also want the text file (.txt) from the measurement. This includes the data points (wavenumbers and corresponding absorbances) in a table format so you can draw the graphs in Excel. To do this, go to the top left corner of the FTIR programme and click on ‘File’ and then ‘Export’. Then select the right folder on your USB-stick and press ‘Save’. This is a basic document

type that can be opened by your PC and with ctrl+A and ctrl+C, you can paste this in an Excel sheet. Well done!

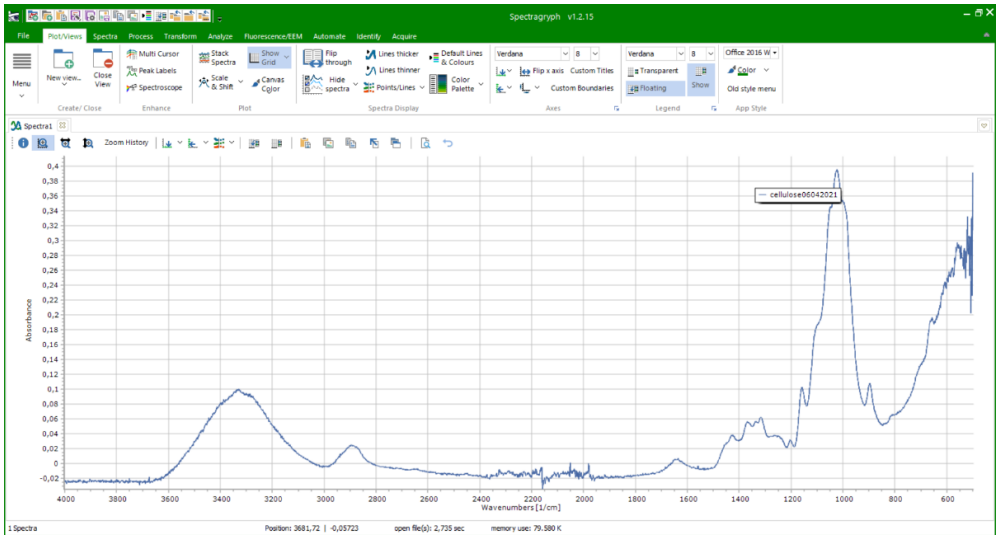
- Re-open the bridge by turning clean the big black press button anti-clockwise, turn the smaller black hook anti-clockwise and then open the bridge. Remove the sample and clean the laser with the towel and ethanol.
- If you need to do more measurements, then restart at ‘’In the Filename box..’’ and repeat.
- Are you done? You can leave the programme as it is now. Don’t forget your USB-stick!

How to normalize the data

- Download the free programme SpectraGryph 1.2 (or a newer version if applicable) by the following link: https://www.effemm2.de/spectragryph/down.html
- Open the programme. It should look similar to this:

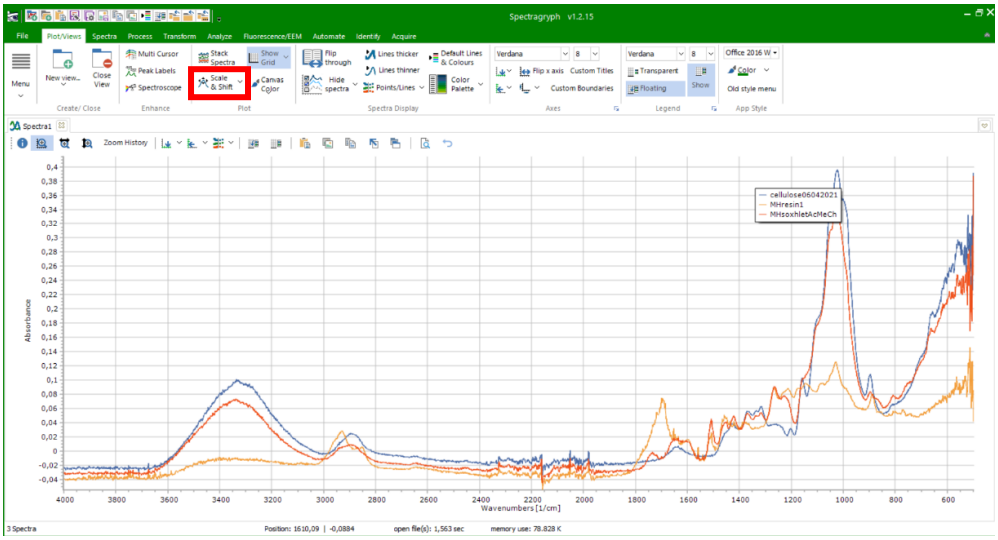


- Import your .txt files from the spectra by clicking on ‘Files’ (top left corner), ‘open/Import Data’ and ‘Open…’. Select your folder on the USB-stick and then the correct .txt file.
- A small menu opens (axis type query). Under X-Axis Type, choose Wavenumbers [1/cm]. Under Y-Axis Type, choose Absorbance.
- Now the programme looks similar to this:

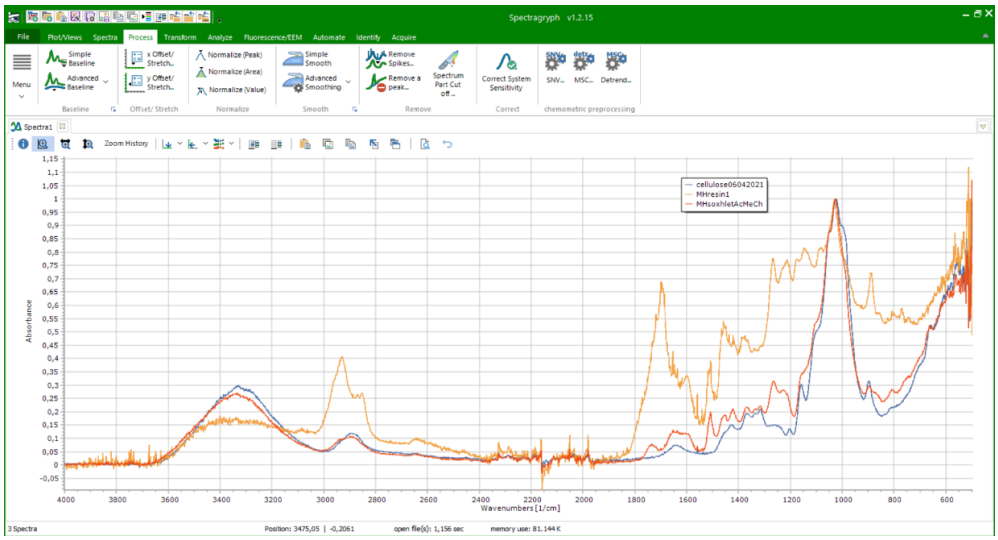


- If you want to compare multiple spectra, you can add more by repeating the previous 3 steps.

- If 3 graphs are added, the view looks similar to this:



- As you can see, the 3 graphs have a different base line. To make a good comparison between the peaks, it's a good idea to adjust this. You drag the spectra to let the base line (in this case the lines between 3700 – 4000 cm^{-1}) match 0 absorbance. This done by choosing Scale & Shift in the Plot/Views tab (in the red square in the screenshot above) and then drag the lines to the 0 absorbance by clicking on each line with the mouse, hold the mouse button (the line becomes thicker) and then drag them.
- Once this is done, save the changes by clicking 'Scale & Shift' again, but in the menu choose Plot changes => spectral data.
- To compare the spectra at best, the highest peak of each spectra must be set equal to absorbance = 1. This is called normalization and is done by selecting Normalize (peak) in the Process tab. !! If the spectrum is showing weird peaks out of the interpretation area, like in the above spectra for the lines below 800 cm^{-1}, the right area in the spectrum must be selected first by dragging with your mouse before clicking on Normalize (peak). The result looks similar to this:



- Now the spectra can be well compared. The result can be exported to an Excel sheet by clicking on 'Files' (top left corner), 'Save/ Export Data' and 'Copy Data to Clipboard'
- Open an Excel file and press Ctrl+V. Two columns (wave number and absorbance) appear. These can be turned into a graph if wished.