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Consequences of a Rapid Cellulose Extraction Technique for Oxygen Isotope and Radiocarbon Analyses

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We use infrared, radiocarbon, and stable isotope analyses to investigate the purity of cellulose extracted from wood using a rapid processing technique. Replicate laboratory standards processed using the standard Brendel method are not significantly different with respect to δ^{18}O from those prepared using traditional techniques, although the process does result in a slight acetylation of the wood samples. Radiocarbon comparisons, however, show significant differences. We conclude that the standard Brendel method is appropriate for developing stable isotope time series for high-resolution isotope dendroclimatology but must be used with caution for precision radiocarbon measurements.

Stable isotope analysis of tree rings for the study of paleoenvironmental and plant physiological processes has typically focused on the α-cellulose component of wood, necessitating the removal of resins, lignins, and noncelluloic polysaccharides, or hemicellulose, prior to measurement. α-Cellulose is by definition the components of a cellulosic material that are insoluble in a 17.5% solution of NaOH at 20 °C. This is primarily cellulose but also includes small amounts of lignin, hemicellulose, and extractives that cannot be removed even under these conditions.[1−3] The goal of extracting cellulose for stable isotope analysis is not, however, to arrive at α-cellulose simply by processual definition, but rather to remove as much of the unstable, translocated, or exchangeable noncellulosic components as possible in order to consistently isolate the most stable component of the wood.

The use of specific wood components is often preferred because the noncellulosic compounds can be deposited after the time the ring itself is formed, may be radially translocated, and have different isotopic signatures.[4,5] While recent studies suggest that for both stable carbon and oxygen isotopes the climate signal is preserved in whole wood.[6−11] analysis of specific compounds is still desirable or necessary in some applications, including radiocarbon analysis,[12] analyses of fossil wood that may have experienced heterogeneous decomposition,[13,14] and those instances where mechanistic models of α-cellulose synthesis will be used to interpret the climatic influence on stable isotope ratios.[15,16] Analyses using α-cellulose may also be preferable to avoid potential biases in isotope time series as a function of spatiotemporal changes in the relative contribution in whole wood from lignins, hemicellulose, and α-cellulose.

Here, we investigate a procedure for rapidly producing α-cellulose for use in high-resolution stable isotope dendrochronology. The Brendel method[17,18] allows on average 100 samples/day to be extracted to α-cellulose and prepared for analysis the following

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day. In its modified form,[18] it also permits the use of very small initial wood samples (~400 μg). Furthermore, the Brendel method also uses smaller quantities of less toxic reagents, reducing both chemical waste (less than 1.5 mL of waste per sample) and the potential hazards to laboratory personnel. Unlike traditional extraction procedures, however, it uses a hot nitric−acetic acid step to simultaneously remove both lignins and hemicellulose from wood samples. It is important to understand how cellulose extracted using the Brendel procedure compares to samples prepared using traditional techniques[1,2,19] and to determine the efficacy of the procedure in removing extractives, lignins, and hemicellulose. Potential biases introduced by alternative chemical preprocessing protocols could include a difference in means or a reduction in variance.[3] The former could complicate intercomparison with other studies, while the latter might lead to underestimation of environmental variability as reflected in isotope time series.

METHODS

Sample Preparation. We used three laboratory wood and α-cellulose standards in this study. We prepared our Arizona/Peruvian *Prosopis* (APP) laboratory wood standard by repeatedly grinding a radial cross section collected in 2001 from a living *Prosopis sp.* near Piura, Peru, until the wood powder could pass through a 125-μm sieve. The Alaska White Spruce (AWS) standard was previously prepared by grinding (to 250 μm) a cross section (UA-BCLV-132) of *Picea glauca* with a total of 172 annual rings collected in the early 1990s. We used Sigma-Aldrich (Batch 024K0329) Alpha Cellulose (SAC) for our α-cellulose control standard.

Samples (8 mg) of the whole wood standards (APP and AWS) were homogenized and separated using a microsplitter (ACS Scientific, Jamestown, RI) and sealed in commercial digestion pouches (ANKOM Technology, Boston, MA). These were processed to holocellulose in a Soxhlet apparatus[12,20] using a modified Jayme-Wise procedure (called Leavitt-Danzer).[1,2] All standards (APP, AWS, and SAC) were progressively extracted with toluene/ethanol, ethanol, and boiling deionized water. They were then delignified in a sodium chlorite−acetic acid solution at 70 °C and thoroughly rinsed in deionized water. The resulting holocellulose samples were then converted to α-cellulose by treatment in a 17% NaOH solution,[1,21] rinsed thoroughly, and dried in a warm oven.

Replicate samples (1−1.5 mg) of the whole wood and industrial α-cellulose (APP, AWS, and SAC) were processed following the standard Brendel method[17] (hereafter "SBrendel") as modified for small sample processing.[18] Samples were heated at 120 °C in a 10:1 mixture of 80% acetic acid and 69% nitric for 30 min. Samples were then washed with 100% ethanol and Milli-Q water and subsequently dehydrated with consecutive additional washes of pure ethanol and acetone. Samples were dried for 30 min in a warm oven and then overnight in a vacuum desiccator.[18]

We also processed a set of 1−1.5 mg of wood and cellulose samples (SAC, APP, and AWS) using the "Modified Brendel" method (hereafter, "MBrendel"),[3] which adds a 10-min 17% NaOH extraction and several additional water rinses to the published Brendel procedure.[17,18]

Isotopic and Infrared Analysis. For stable oxygen isotope analysis, 100−150-μg cellulose samples were loaded in silver capsules and converted to CO online by pyrolysis in a ThermoFinnigan thermal conversion/elemental analyzer at 1450 °C. The stable isotopic composition of the CO was then analyzed on a ThermoFinnigan Delta Plus XL continuous flow isotope ratio mass spectrometer. Data are reported relative to Vienna Standard Mean Ocean Water (VSMOW). External precision for oxygen isotopic measurements during these experiments based on a Baker α-cellulose standard was 0.35‰ (VSMOW).

For radiocarbon analysis, carbon was extracted from the samples in the form of CO_2 using conventional combustion methods. Unextracted whole wood samples were pretreated with a weak acid−base−acid (ABA) wash to remove potential contamination.[12] The volume of the evolved and purified gas was measured, and the CO_2 was reduced to an iron carbide powder over hot zinc. Radiocarbon measurement was performed on a National Electrostatics accelerator mass spectrometer at the University of Arizona-NSF AMS Facility.[22] Instrument precision is 0.5% ($^{14}C/^{13}C$).[23]

Fourier transform infrared spectroscopy (FT-IR)[24,25] was performed using a Thermoelectron (Nicolet) Avatar spectrometer at a resolution of 4 cm^{-1}. The spectra were all obtained using a single-bounce diamond attenuated total reflectance (ATR) accessory. At least two duplicate spectra were obtained in every case to verify the results.

RESULTS

Stable Isotopes and Radiocarbon. Stable oxygen isotope measurements on the paired groups of replicate laboratory wood and cellulose standards show no statistically significant difference (two-tailed *t*-test, $\alpha = 0.05$) between those processed using the SBrendel method and those extracted using the Leavitt-Danzer procedure (Figure 1, Table 1). Significant differences are observed (Figure 2), however, in the fraction of modern radiocarbon (FMC) measured in replicate samples processed using SBrendel, MBrendel, and Leavitt-Danzer[26] procedure. Both the unprocessed whole wood and the cellulose samples prepared using Leavitt-Danzer have consistently higher FMC, whereas samples processed using SBrendel and MBrendel have reduced FMC, indicating contamination by radiocarbon-depleted sources. For these wood and cellulose standards, the FMC difference between extracted or industrial α-cellulose and SBrendel is consistently 0.06−0.08 (~6.0 ± 0.56% overall). Wood samples processed with MBrendel show FMC values inconsistently intermediate between SBrendel and Leavitt-Danzer and whole wood, with fractional modern carbon differences ranging from 0.05 to 0.14. The Sigma Alpha Cellulose processed with MBrendel actually shows further decreases in FMC relative to the raw standard.

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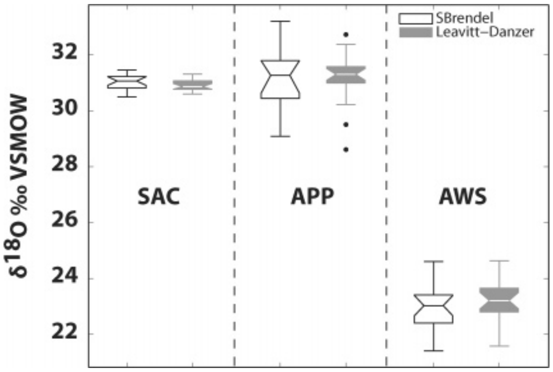

Figure 1. Box plots for $\delta^{18}O$ measurements on replicate Sigma Alpha Cellulose (SAC), Arizona/Peruvian *Prosopis* (APP), and Alaska White Spruce (AWS) laboratory standards prepared with Leavitt-Danzer and SBrendel techniques. Following Tukey,[72] boxes show the interquartile range, with the notch line indicating the median. Whiskers demarcate values within 1.5 times the interquartile range, and dots are outliers. In all three cases, there is no significant difference (two-tailed *t*-test, 95% significance level) between the samples processed by the two methods.

Table 1. Statistical Intercomparison of the $\delta^{18}O$ (‰ VSMOW) of Wood and Cellulose Prepared with SBrendel and Leavitt-Danzer Techniques[a]

standard material	$\bar{x}_B(\sigma_B)$, ‰	$\bar{x}_{LD}(\sigma_{LD})$, ‰	df	*t*-statistic	*p* value
SAC	30.91 (0.29)	31.03 (0.23)	34	1.35	0.184
APP	31.26 (1.11)	31.16 (0.95)	28	0.25	0.808
AWS	23.03 (0.84)	23.26 (0.79)	37	−0.88	0.387

[a] $\bar{x}_B/\sigma_B$ is the mean and standard deviation of the samples processed using the SBrendel technique, $\bar{x}_{LD}/\sigma_{LD}$ is the mean and standard deviation of those processed using Leavitt-Danzer. Twenty samples per standard per treatment were prepared and measured, and df is the number degrees of freedom, based on the samples that passed strict data quality control for minimum voltage and stable backgrounds. Also shown are the result of the Student's *t*-test and the significance level ($p > 0.05$ indicates no statistically significant difference in the means). Larger standard deviations for in-house wood standards partially reflect bulk sample heterogeneity,[6,19] since Baker α-cellulose measured at the same time are precise to ~0.45‰.

Infrared Analysis of Compounds. Figures 3 and 4 show the FT-IR spectra for in-house wood and α-cellulose samples. Consistent with previous studies,[27] SBrendel-processed whole wood shows removal of resins (Figure 3a, peak 2; ~1600 cm^{-1}), lignin (Figure 3a, peak 3; 1550−1450 cm^{-1}), and noncelluloic polysaccharides (Figure 3a, peak 5; 1230−1180 cm^{-1}). However, samples (both wood and α-cellulose) processed with the SBrendel method are characterized by infrared spectra with three peaks that are not present in wood or cellulose processed using Leavitt-Danzer at ~1720, 1245, and 1110 cm^{-1} (Figure 3, peaks 1, 4, and 6). Additionally, the peak near ~900 cm^{-1} (Figure 3a,b, peak 7) seen in all samples is less distinct in those processed using SBrendel.

The peak near 1720 cm^{-1} is reduced and shifted toward a maximum near 1710 cm^{-1} but is not fully removed by the MBrendel NaOH extraction (Figures 3b and 4, peak 1). This peak reduction and shift is consistently detected despite being close to instrument wavelength resolution. The NaOH wash does appear to sharpen the peak near 900 cm^{-1} but, interestingly, also removes the peak near 1430 cm^{-1} (Figure 3b and Figure 4, peak 8), which is present in both the unprocessed and SBrendel-processed SAC samples, and adds an additional peak near 3430 cm^{-1}, something that is also seen in the Leavitt-Danzer-processed samples (not shown). For the samples that were put through the additional 17% NaOH wash, the peaks at 1245 and 1110 cm^{-1} also disappear (Figure 3b, peaks 4 and 6).

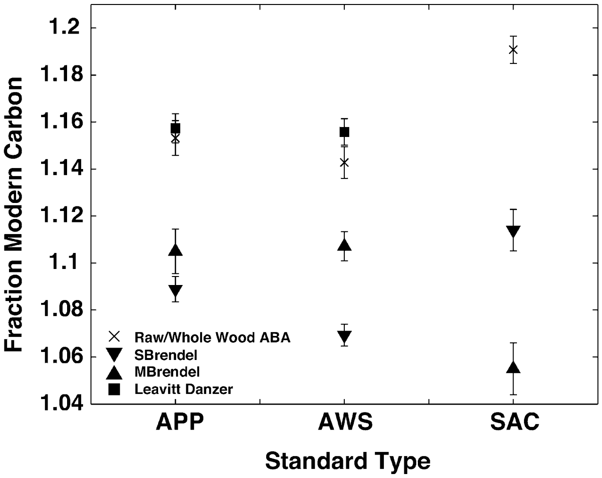

Figure 2. Fraction modern carbon determinations from AMS radiocarbon assays at the University of Arizona's Accelerator Mass Spectrometry Laboratory. SBrendel-processed samples are consistently offset from raw, ABA, and Leavitt-Danzer processed samples suggesting a 6.0 ± 0.56% contribution of ^{14}C dead carbon from a contaminant, most likely acetyl groups inherited for acetic acid. MBrendel-processed methods show an inconsistent difference across treatments.

ANALYSIS AND DISCUSSION

Stable Isotopes and Infrared Spectra. Statistical analysis of the paired stable oxygen isotope measurements on laboratory standard material reveals that no significant bias ($p < 0.05$) is introduced by the small-sample SBrendel method for $\delta^{18}O$ analysis. In agreement with the paired *t*-test reported above, an analysis of variance (ANOVA) on unprocessed, Brendel, and Leavitt-Danzer extracted SAC also shows no significant difference in the measured mean at the nominal instrument precision (0.3‰). These findings are consistent with previously reported results[18] for a small set of $\delta^{18}O$ samples, as well as for $\delta^{13}C$ as reported by Brendel et al,[17] but differs from the conclusions of Gaudinski et al.[3] This discrepancy may be the result of comparing different techniques across tissue types, (leaves, wood, roots), instead of considering xylem wood separately. Leaves and roots, in particular, may pose a particular challenge for cellulose extraction. In this respect, however, concerns raised by Gaudinski et al.[3] apply equally for any methods used to extract α-cellulose from these tissues. Boettger et al.[28] recently reported that interlaboratory offsets between $\delta^{18}O$ measurements on identical standard material, in some cases exceeding 1‰, could not be explained by systematic differences in sample preparation, suggesting that the determination of the absolute mean $\delta^{18}O$ value of cellulose remains difficult even when only traditional extraction methods are used.

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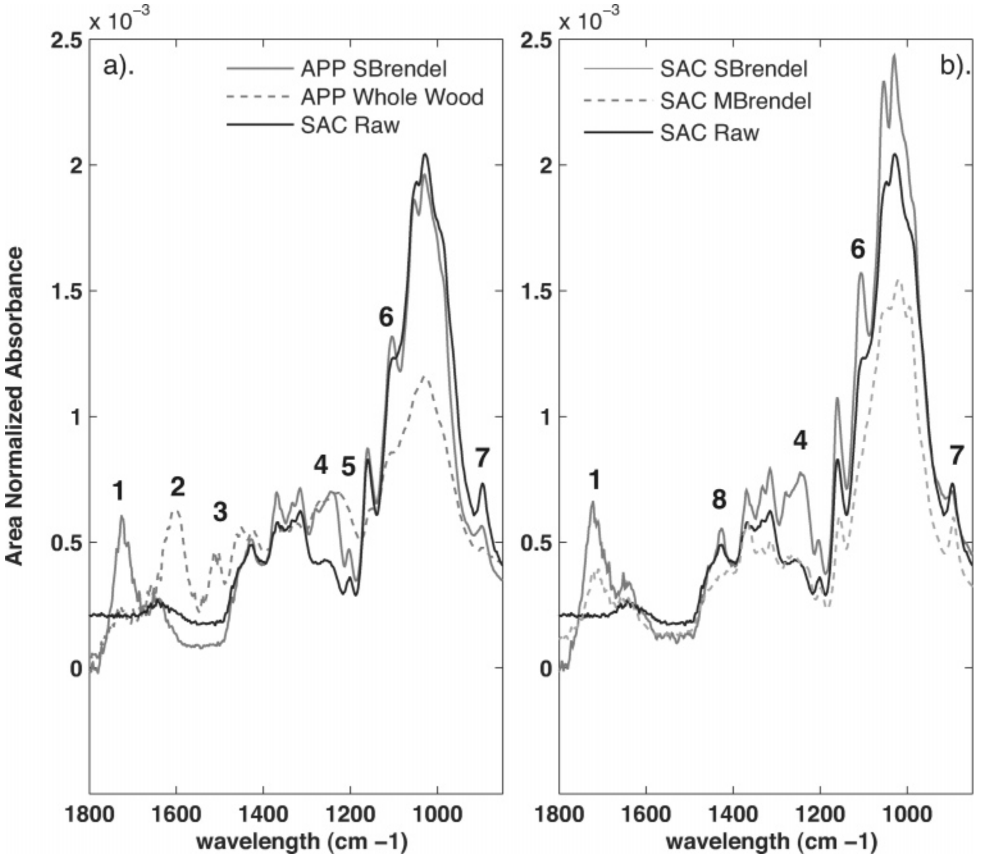

Figure 3. Area-normalized absorbance spectra derived from ATR FT-IR analysis of (a) unprocessed and SBrendel-processed Arizona/Peruvian *Prosopis* (APP), compared against SAC and (b) SBrendel- and MBrendel-processed as well as unprocessed SAC. Number labeled spectral peaks or bands show characteristic wavelengths[17,24,30,33,34,73−77] for (1) insoluble cellulose acetate, (2) resin, (3) lignin, (4 and 6) soluble cellulose acetate or acetic acid, (5) hemicellulose, and (7) cellulose. Peak 8 reflects cellulose crystallinity but is removed by the Leavitt-Danzer method, even though it is still present in SBrendel-processed and raw SAC (See Figure 4a,b.). Slight differences in the normalized area under the curve reflect between-sample differences in cellulose adsorbed water, whose bands (not shown) are disproportionately strong and vary with relative humidity.

Peaks in the FT-IR absorbance spectra for SBrendel samples at ~1720, 1245, and 1110 cm^{-1} are associated with carbonyl group $C=O$ and $C-O$ bond stretching and are most likely related to the presence of cellulose acetate or triacetate esters.[29−34] The appearance of FT-IR peaks in the regions associated with cellulose acetate was originally interpreted by Brendel et al.,[17] using DRIFT spectroscopy, as arising from modified noncellulosic polysaccharides (glucimannans) present in the wood of *Pinus sylvestris*. Our infrared analysis, however, indicates that it is a slight acetylation of cellulose that is causing the differences detected in the radiocarbon results and NMR spectroscopy in previous studies.[3] If it were instead simply residual acetic acid, as has been alternatively suggested,[3] we might expect the diagnostic peaks in the carbonyl band ($C=O$) near 1700 cm^{-1} and the $C-O$ band near 1250 cm^{-1}.[33,34] There could be some uncertainty in the expected position of a residual acid carbonyl peak, however, since potentially a combination of some free acid (near 1760 cm^{-1}) and the acetic acid dimer could result in a peak intermediate to these. However, samples of SAC processed using SBrendel but omitting the catalytic nitric acid show no peak in this range, further suggesting the carbonyl band peak is a result of acetylation.

Furthermore, it is highly unlikely that residual acid would persist following treatments in strong NaOH and subsequent repeated solvent washes. Differences in SAC processed with SBrendel and MBrendel (Figure 3b) might be partially related to residual acetic acid or perhaps to removal of acetylized acid-insoluble hemicelluloses, but the diagnostic peak near ~1720 cm^{-1} is almost certainly indicative of cellulose acetate formation. The shift of this carbonyl peak toward a maximum at ~1710 might indicate partial hydrolysis of the acetylized α-cellulose,[35] but it is clear that saponification is incomplete and a portion of the cellulose acetate or triacetate ester remains insoluble.[31] We believe that the acetylation found here is similar in nature to that reported previously for stable isotope analyses of pollen grains,[36,37] where

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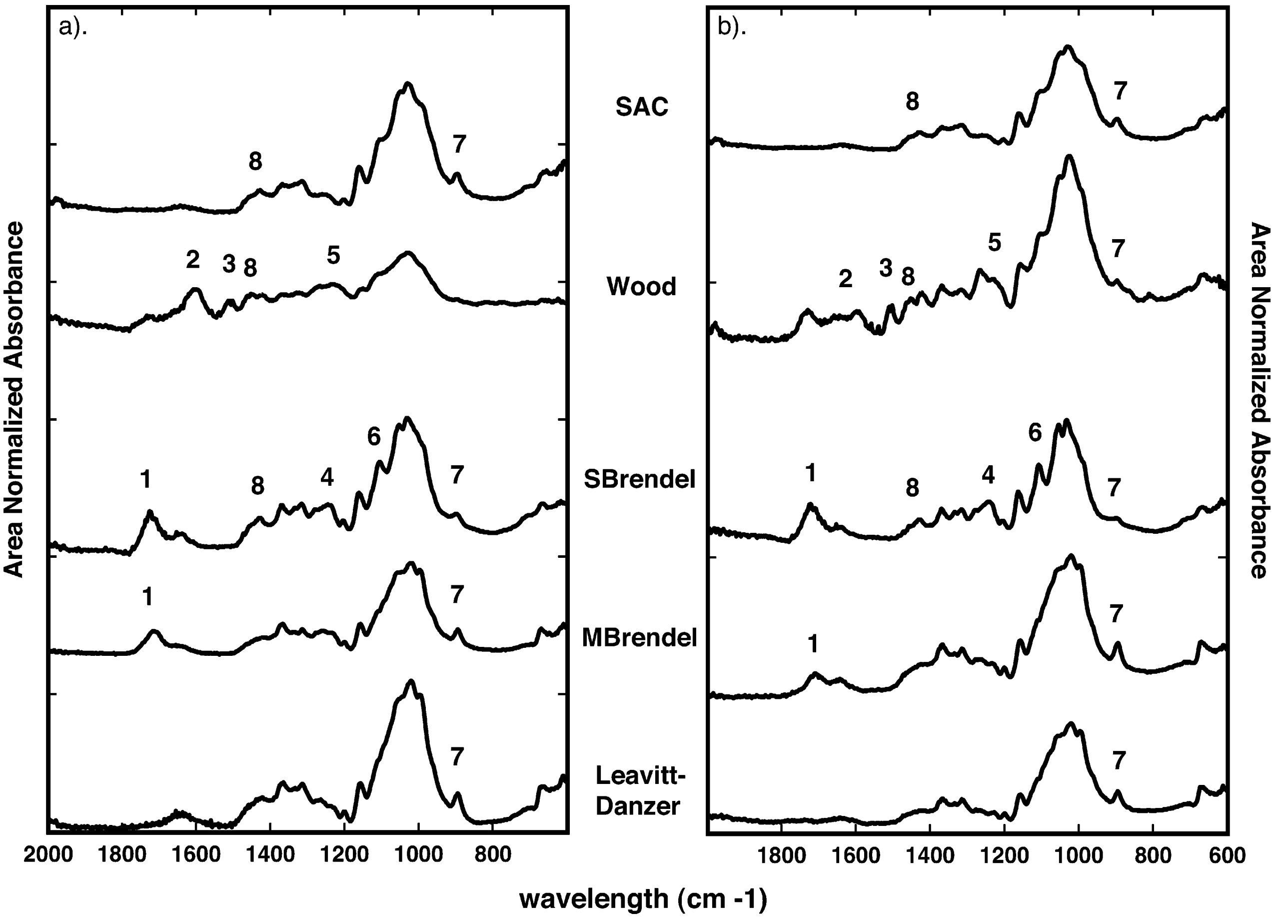

Figure 4. Area-normalized absorbance spectra derived from ATR FT-IR analysis of (a) APP and (b) AWS, processed using SBrendel, MBrendel, and Leavitt-Danzer. Spectra from the untreated wood and SAC are shown for comparison. Peak numbers are the same as described in Figure 3.

it occurs during standard acetolysis procedures for pollen extraction and preparation,[38] and in industrial chlorine-free pulping applications.[33]

Gaudinski et al.[3] found evidence of increased total nitrogen (up to ~1.5%) in all tissue types for samples processed with SBrendel, suggesting the presence of residual nitric acid. Brendel et al.,[17] however, found no change in nitrogen content of wood samples prepared using SBrendel. Our FT-IR spectra from samples processed with SBrendel show no apparent additional peaks that would be indicative of residual free nitric acid (for instance, near 1660 and 1420 cm^{-1}). Farquhar et al.[39] showed that even nitrogen content up to 10% had no significant influence on the δ^{18}O of the sample.

The SBrendel method does successfully delignify and remove most hemicelluloses from our whole wood samples. The method, however, slightly acetylates some of the nonextracted cellulose fraction. A similar conclusion about the efficacy of the cellulose extraction and the concomitant acetylation has been reached for industrial applications of the nitric/acetic acid delignification approach.[33,35,40-44] One alternative hypothesis concerning the shape of the peak near 900 cm^{-1} and the presence of additional bands near 1240 and 1110 cm^{-1} would be that they are related to incomplete removal of some residual (alkali-soluble) hemicellulose. However, since the SAC processed with the SBrendel method also has these features (Figure 3b), it must instead be predominantly related to the creation and presence of acetylized cellulose. The addition of an NaOH step,[27] or use of the MBrendel technique,[3] may indeed remove some remaining acid-insoluble hemicelluloses and any partially acetylized hemicelloses,[45,46] but still incompletely removes the acetylized α-cellulose. That the diagnostic peak near 1720 cm^{-1} largely remains even after the alkaline hydrolysis suggests perhaps that either a portion of the cellulose acetate cannot be deacetylized by saponification in NaOH, possibly because the ester group cannot be readily hydrolyzed, or that incomplete hydrolysis of the cellulose acetate does occur, but an additional solvent treatment is necessary to subsequently dissolve and remove the remaining acetate.

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Gaudinski et al.[3] concluded that the SBrendel process potentially leaves behind hemicelluloses, lipid, or waxes, as well as acetic acid contamination, which can potentially bias stable isotope measurements. As we have indicated here, however, the primary contaminating residue is most likely a small amount of insoluble cellulose acetate that persists even after the application of a strong alkali treatment and additional water washes in the MBrendel[3] protocols. The NMR spectra, which Gaudinski et al.[3] identified as diagnostic of lipids and waxes, is also potentially diagnostic for the presence of cellulose acetate,[47−51] which would also explain the apparent inconsistency between the higher δ^{18}O values they observed for Brendel samples in contrast to the expected lighter values that would result from incomplete removal of these compounds. Because the dominant contamination is from acetyl groups, with one oxygen atom and two carbon, δ^{18}O analyses utilizing wood processed with the SBrendel techniques should be less problematic than for δ^{13}C isotope ratios, assuming equilibrium fractionation due to acetylation. However, esterification is known to cause kinetic fractionation effects in carbon isotopes, particularly when there is an excess of the acetylating agent.[52] That there is apparently no difference in δ^{13}C or δ^{18}O ratios between SBrendel and traditional α-cellulose preparation methods despite this contamination suggests that the kinetic fractionation is relatively minor for both stable carbon and oxygen or that there are offsetting influences between the isotopic differences of the acetic acid and kinetic isotope effects. Previous results showing no significant difference between the δ^{13}C ratios of cellulose prepared with SBrendel and traditional methods[17] might also suggest that the carbon isotope signature of the contaminating acetyl groups could be similar to that of the cellulose of C3 plants.

Extraction procedures that use strong ($>$10%) NaOH (Leavitt-Danzer, MBrendel) appear to add a peak to the infrared spectra near 3430 cm^{-1} and remove one near 1430 cm^{-1}. The peak at 3430 cm^{-1} corresponds to hydroxyl group configuration changes[53,54] and is associated with the use of concentrated NaOH.[55] The band near 1430 cm^{-1} is associated with the relative degree of cellulose crystallinity.[55] Concentrated NaOH can alter the crystalline structure and other properties of cellulose[56,57] by changing it from cellulose I to cellulose II.[55,58] Differences in hygroscopicity as a consequence of differences in amorphous and crystalline cellulose have been suggested as a possible cause of differences in the δ^{18}O of standard wood and cellulose material during interlaboratory comparison exercises.[28]

Approaches to Deacetylation. Our experiments with a variety of pre- and post-treatments have failed to completely remove the small amount of acetylized cellulose as diagnosed from the 1710−1720 cm^{-1} peak. Our experimental goal was to either prevent acetylation during the delignification step or remove it post hoc following acid digestion. Approaches to solubilizing the refractory cellulose acetate using extended, hot alkali saponification with NaOH (0.05−4 N) in various combinations of water and ethanol[45,46] failed to further reduce or shift the diagnostic FT-IR peak near 1710 cm^{-1}. There was no detectable difference as a function of temperature, time, solvent, or alkali normality. Adding extended vortex mixing between NaOH washes likewise has no discernible effect. We also attempted to prevent acetylation using sulfolane as an additional solvent during delignification,[59] but FT-IR spectra indicated that acetyl group substitution still occurred. The addition of other solvents during the acid delignification step could in theory help reduce the eventual acetylation, but it may be impossible to prevent the conversion of some number of the hydroxyl groups to acetyl groups.[60,61] The overall inefficacy of these experimental methods may be a consequence of the low-order, heterogeneous or random nature of the minor acetylization of the cellulose structure, which could render the acetate fraction largely insoluble.[62,63]

Radiocarbon Analysis. Radiocarbon assays demonstrate significant differences between samples processed using SBrendel and those prepared with conventional pretreatments. Furthermore, these differences are not resolved using the MBrendel procedure,[3] indicating that both SBrendel and MBrendel protocols introduce contamination from radiocarbon-dead sources, and should be used with considerable caution. The amount of contamination in samples processed using MBrendel,[3] as determined from radiocarbon assays, is not consistent across samples (Figure 2), confirming that it should not be used for ^{14}C measurements. The use of a weak NaOH wash by Brendel et al.,[27] without additional solvent washes, has a similar effect on the extracted cellulose as the MBrendel technique but also leaves a considerable alkali residue, which can be readily detected in FT-IR spectra (results not shown).

Using all available paired radiocarbon measurements (Figure 2, Table 2), a simple mass balance equation comparing samples processed using Leavitt-Danzer and SBrendel suggests that $\sim$6.0 ± 0.56% of the carbon in the SBrendel samples comes from the contaminating acetyl groups (Table 2). This assumes that petroleum-derived acetic acid possesses no detectable ^{14}C. This percentage is also consistent with preliminary tests of the Brendel method conducted by Poussart.[64] The similarly between the mass balance calculations of the percent of radiocarbon-dead contamination suggests that, for the delignification duration, temperature, and acid molarity used in the Brendel protocols described by Evans and Schrag,[18] the degree of acetyl group substitution is similar between samples. This finding leads us to believe that, in the

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Table 2. Radiocarbon Differences Associated with the Brendel Technique with No NaOH Step (SBrendel)[a]

sample ID	FMC_m	FMC_a	% C contam	ref
AA64893	1.114 (0.0088)	1.1908 (0.0058)	5.9 ± 0.6%	this study
AA64894	1.0693 (0.0047)	1.1558 (0.0056)	7.4 ± 0.4%	this study
AA64895	1.0889 (0.0054)	1.1573 (0.0062)	6.5 ± 0.5%	this study
Brendel-A02	1.5247 (0.0061)	1.6453 (0.0108)	5.0 ± 1.0%	Poussart et al.[64,69]
PG1−1	1.0941 (0.0043)	1.174 (0.0045)	6.8 ± 0.4%	Poussart et al.[64,69]
AA74380	1.0143 (0.0083)	1.0842 (0.0088)	6.4 ± 1.0%	Anchukaitis et al.[70]
mean (pooled σ)			6.0 ± 0.56%	

[a] FMC_m is the measured fraction modern carbon, FMC_a is the 'actual' fraction modern carbon measured on traditionally extracted α-cellulose.[2,71] Percent contamination is the calculation of the contribution from a radiocarbon-dead source (FMC = 0).

temporary absence of a procedural chemical solution to the acetylation bias, a statistical adjustment can be applied to develop a corrected radiocarbon date in those instances where SBrendel has been used to extract α-cellulose. Adjusting for the fractional contamination and its uncertainty range results in calibrated calendar ages[65] with a difference of less than 1 year during the post-1955 atmospheric ^{14}C peak that resulted from aboveground atomic weapons testing,[66−68] compared to radiocarbon dates on wood processed using the traditional Leavitt-Danzer technique.

CONCLUSIONS

Ultimately, the choice of α-cellulose extraction protocols for environmental and paleoenvironmental studies will depend rather specifically on the particular application. Wood and cellulose samples prepared using the standard Brendel methodology[18] have $\delta^{18}O$ values statistically indistinguishable from those processed using traditional Leavitt-Danzer-type protocols. More importantly, there is no indication that the Brendel method biases the range of $\delta^{18}O$ variability between samples. α-Cellulose prepared using this technique can therefore be securely used for paleoclimate and other time series applications, and absolute mean values can be reasonably compared between samples processed with either method. The Brendel method does result in a slight acetylation of the α-cellulose that is sufficient to bias radiocarbon measurements by ~6%, which can be corrected statistically if necessary but should be avoided when possible. MBrendel does appear to remove some of the acetyl group contamination we have identified in our analyses, but results in an inconsistent shift in the offset between measured radiocarbon ages on samples extracted with different methods. The SBrendel technique accomplishes the goal of rapidly extracting very small wood samples to α-cellulose, which is important for a range of stable isotope applications requiring fine-scale spatiotemporal resolution and massive sample replication.

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