PEER REVIEWED

Rapid determination of cellulose in plantation eucalypt woods to predict kraft pulp yields

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ABSTRACT

The cellulose contents of plantation Eucalyptus nitens *woods were determined gravimetrically using three rapid methods for screening small-scale samples. All three methods involved acid-catalyzed organic solvolysis, and two of the procedures also utilized oxidizing agents. As a reference, cellulose content was determined chromatographically by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) of acid hydrolysates. The composition of cellulose residues was also examined by HPAEC-PAD to determine their purity. The solvolytic method involving treatment of wood meal in a boiling mixture of acetylacetone–dioxane–hydrochloric acid (the Seifert procedure) gave the most accurate cellulose values and produced the purest cellulose residues. The residues from the two oxidative procedures in which the wood meals were boiled with peroxyacetic acid or with nitric acid–acetic acid contained considerable amounts of xylan from hemicelluloses. Cellulose contents measured by the Seifert technique also gave a much better correlation with kraft pulp yield than did the other gravimetric methods examined.*

Application:

An evaluation of three rapid test methods for predicting kraft pulp yield for eucalypt woods.

PREVIOUS WORK HAS SHOWN THAT kraft pulp yields from hardwoods are positively correlated with the cellulose contents of the wood and more weakly correlated with hemicellulose contents *(1)*. For plantation eucalypt woods, kraft pulp yields are often strongly correlated with cellulose content *(2–5)* and weakly and negatively with hemicellulose contents *(5)*. The existence of a strong correlation between cellulose content and kraft pulp yield offers the possibility of screening large numbers of wood specimens for their pulp-yielding potential without having to resort to the laborious and expensive process of laboratory-scale pulping. An additional advantage of this strategy is that pulp yield could be estimated from cellulose contents of small wood samples taken from standing trees, thereby avoiding the need to destroy the tree in pulping trials. This would be particularly useful for tree breeders who seek to improve the value of future plantings by genetic selection of outstanding trees. The present approach involving chemical analysis is complementary to spectroscopic methods under development in our laboratory *(6–8)*.

One of the best available methods for determining cellulose in wood involves the hydrolysis of wood carbohydrates into their constituent sugars, followed by the separation and quantitation of the sugars by liquid chromatography *(9)*. Although this method yields important information about the composition of wood polysaccharides, there is a need for simpler, cheaper methods for cellulose determination because of the high cost and specialized nature of the liquid-chromatography equipment. The traditional method for determining cellulose in wood is a tedious two-step procedure involving delignification to give holocellulose, followed by an alkaline extraction to remove hemicelluloses *(10)*. The remaining alpha cellulose is then determined gravimetrically. Great attention to detail is required if reproducible results are to be obtained from this method *(11)*.

The need for more rapid methods of cellulose determination has led some researchers to develop alternative measurement techniques *(10)*. These methods usually involve an acid-catalyzed digestion of wood meal with an organic solvent, sometimes in the presence of an oxidizing agent *(12–16)*. The acid catalyzes the hydrolysis of the lignin and the hemicelluloses, rendering them soluble in the organic solvent. The resultant cellulose residue is then washed, dried, and weighed. Although some of these methods allow rapid determinations to be made on many samples, the correlations between kraft pulp yields and cellulose contents range from weak *(17)* to very good *(3)*, depending on the technique. This could be caused by the presence of impurities in the cellulose residues.

In the present report, three methods for determining gravimetric cellulose are critically evaluated and their suitability as indicators of kraft pulp yield is investigated. The techniques examined are:

- A peroxyacetic acid method described by Garbutt *(13)*
- A nitric–acetic acid method developed by Pereira *(14, 15)*, which is itself a modification of an earlier technique used by Kürschner and Hoffer *(12)*
- A solvolytic technique involving a mixture of dioxane–acetylacetone–hydrochloric acid described by Seifert *(16)*.

The first two methods combine both oxidative and solvolytic processes, whereas the Seifert method is solely an acid-catalyzed solvolysis.

EXPERIMENTAL

Woods, pulps, and chemicals

Wood chips were obtained from 21 seven-year-old *Eucalyptus nitens* trees from Traralgon, Gippsland, Victoria. The wood chips were prepared for analysis by Wiley milling according to Australian Standard AS1301.002s.91. Kraft pulping of the wood samples was carried out with a liquor of 25% sulfidity using an *H*-factor of 2000. Samples were cooked with varying alkali charges to give pulps with a range of kappa numbers and yields. Pulp yields at kappa no. 15 were obtained by interpolation of the laboratory data. Glacial acetic acid, hydrochloric acid (36%), and hydrogen peroxide (30%) were supplied by Rhône–Poulenc. Nitric acid (70%), sodium perborate (trihydrate), and 1,4-dioxane were obtained from BDH Chemicals. Acetylacetone (2,4-pentanedione) was purchased from Aldrich Chemicals.

Peroxyacetic acid cellulose

Cellulose was obtained from wood meal by digestion in peroxyacetic acid according to the method of Garbutt *(13)*. Air-dried *E. nitens* wood meal [1.0 g ovendry (o.d.)] was added to a solution of sodium perborate trihydrate (5.6 g) in glacial acetic acid (25 mL) and 30% hydrogen peroxide (25 mL) in a 250-mL round-bottomed flask, and the mixture was refluxed for 4 hours. After cooling, the cellulose residue was filtered under vacuum into preweighed, sintered porous crucibles and was washed with hot water (500 mL) followed by ethanol (25 mL). The sintered crucible containing the residue was dried overnight under vacuum at 50°C. When cool, the crucible and residue were weighed and the cellulose content was calculated as a percentage of starting material. All samples were run in duplicate.

Wood sample no.	CELLULOSE CONTENT, % on o.d. wood			
	Peroxyacetic acid method	Nitric–acetic acid method	Seifert method	HPAEC-PAD method
1	47.4	45.1	37.0	35.5
2	47.4	43.7	37.2	36.8
3	49.7	45.2	37.8	38.5
4	49.1	45.9	38.5	40.0
5	48.9	45.1	37.7	37.8
6	50.0	45.1	38.1	39.0
7	48.4	46.1	37.4	38.6
8	49.0	45.8	38.4	38.1
9	48.3	47.0	38.7	39.3
10	49.7	48.2	40.4	41.1
11	49.6	48.0	39.3	41.2
12	49.0	46.9	40.2	40.4
13	49.3	48.2	40.3	41.6
14	51.4	47.7	40.7	40.9
15	50.0	47.7	39.6	41.5
16	49.0	46.5	40.0	41.1
17	50.1	48.1	40.2	41.1
18	50.0	47.6	40.6	41.6
19	47.4	46.6	39.6	40.4
20	50.1	48.2	40.9	41.5
21	50.5	48.4	41.8	44.0
σ^2	0.966	1.851	1.915	3.823
Mean	49.3	46.7	39.3	40.0

I. Cellulose contents of E. nitens woods determined by gravimetric and chromatographic methods

Nitric acid cellulose

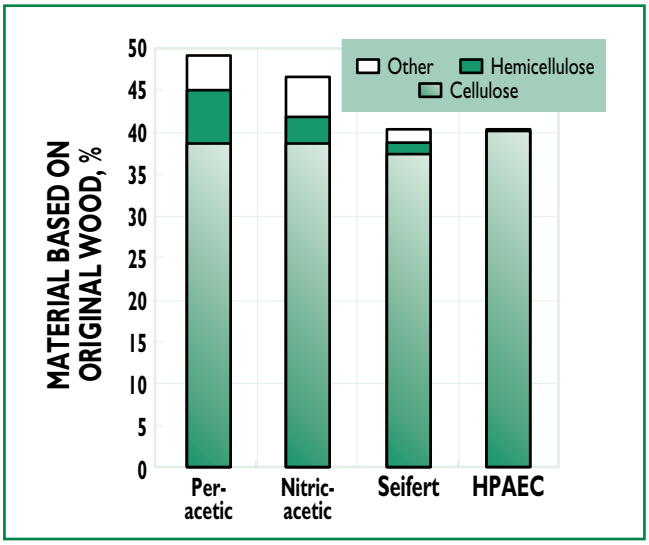
The method for cellulose purification using nitric acid and acetic acid was based on the method described by Pereira *(14, 15)*. Air-dried *E. nitens* wood meal (1.0 g o.d.) and nitric acid–acetic acid (25 mL)—prepared by diluting nitric acid (90 mL) and acetic acid (732 mL) to 1 L with water—was placed in a 50-mL round-bottomed flask, and the mixture was refluxed for 25 min. After cooling, the residue was filtered under vacuum into preweighed sintered porous crucibles and was washed with hot water (500 mL) and then ethanol (25 mL). The yield of cellulose was determined using the previously described method for peroxyacetic acid-derived cellulose.

Seifert cellulose

A cellulose residue was obtained from wood meal using the technique described by Seifert *(16)*. Air-dried *E. nitens* wood meal (1.0 g o.d.) was added to a mixture of acetylacetone (6 mL), 1,4-dioxane (2 mL), and hydrochloric acid (1.5 mL) in a 50-mL round-bottomed flask, and the mixture was refluxed for 30 min. After cooling, the solid material was filtered under vacuum in the same way as the previously described methods and was washed sequentially with methanol (100 mL), hot water (300 mL), methanol (100 mL), and acetone (100 mL). After drying overnight under vacuum (50°C), cellulose was determined in duplicate as a percentage of starting material.

Chromatography

The high-performance anion-exchange chromatography (HPAEC) system comprised a Waters 717 auto-sampler, a 626 pump, 464 pulsed amperometric detector (PAD), and an Alltech 301 LC pump for delivery of post-column alkali. All equipment was plumbed with polyethyl ether ketone (PEEK) tubing. Post-column alkali (350m*M*) was delivered to the detector at a rate of 0.7 mL/min. Separation



1. Composition of cellulose residues prepared by the peroxyacetic acid, nitric–acetic acid, and Seifert gravimetric procedures. HPAEC data are included for comparison. Results are for wood no. 12 in Table I.

of monosaccharides was carried out using a CarboPac PA1 analytical anion exchange column protected by a CarboPac PA1 guard column and an IonPac NG1 guard column. All columns were supplied by Dionex. The electrode potential sequence for the detector was: measuring potential E_1 = 150 mV (0.4 s); cleaning potential E_2 = 600 mV (0.2 s); electrode regeneration E_3 = –800 mV (0.3 s). Data were collected and analyzed using Waters Millennium software.

Analysis of cellulose by HPAEC-PAD

The method for measuring cellulose by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has been described in detail previously *(5, 9)*. Air-dried *E. nitens* wood meal (120 mg o.d.) or cellulose residue (60 mg o.d.) were hydrolyzed at 30°C for 1 hour in 72% sulfuric acid (1.5 mL). The solutions were quantitatively transferred to 100-mL conical flasks with purified water (41 mL), and a 5% internal standard solution (L-fucose, 1.0 mL) was added. The flasks were covered and subjected to a secondary hydrolysis treatment at 120°C for 1 hour in an autoclave. A cottonwood sample of known composition was run with each batch of unknowns to act as a check. Each hydrolysate was filtered through a 0.45-µm PTFE membrane, and a small volume of the filtrate (15 µL) was injected into the liquid chromatograph. Calibration curves relating peak area to sugar composition were prepared by running solutions of authentic wood sugars of known concentration as standards. The standard solutions were subjected to secondary hydrolysis only.

RESULTS AND DISCUSSION

The cellulose contents of *E. nitens* woods obtained both gravimetrically and by HPAEC are shown in **Table I**. All cellulose values are expressed as a percentage of original o.d. material. The cellulose contents determined by HPAEC were calculated from glucose contents (as anhydroglucose) after correction for noncellulosic glucose present as glucomannan. In eucalypt glucomannans, glucose-to-mannose ratios of 0.5:1 and 2.1:1 have been reported *(18, 19)*. Therefore, a 1:1 glucose:mannose ratio was assumed in this work, and noncellulosic glucose was estimated from mannose contents *(9)*.

For each wood in Table I, a range of cellulose contents is observed, and the cellulose contents vary with the method of measurement. Gravimetric cellulose prepared by digestion of wood meal in peroxyacetic acid gives the highest estimate of cellulose in every case, followed by cellulose obtained by digestion in nitric–acetic acid. The solvolytic method of Seifert gives the lowest estimate of cellulose content and produces very similar results to the chromatographic method, which we consider to be the most accurate technique. This is reflected in the mean values for cellulose content for the four methods, which were 49.3%, 46.7%, 39.3%, and 40.0%, respectively (Table I). The high values obtained for cellulose by the peroxyacetic acid and nitric–acetic acid gravimetric techniques suggest that substantial levels of impurities are present in the cellulose isolated by these methods. It has been noted previously that cellulose results from the nitric–acetic acid method give values in excess of the true cellulose content, and this method should only be regarded as approximate *(14)*.

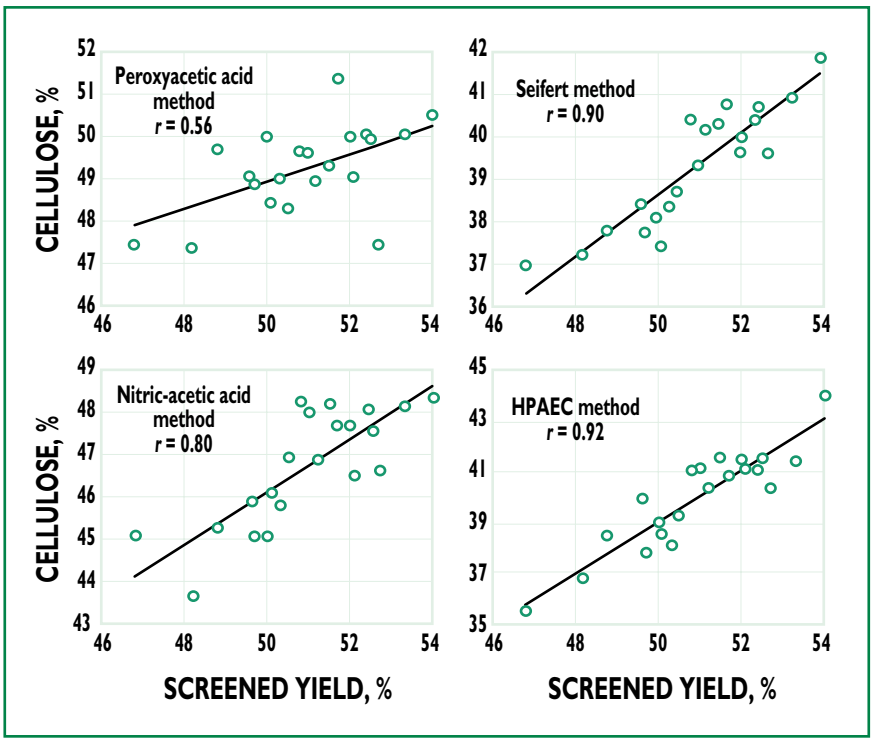
To investigate the purity of cellulose residues from the gravimetric procedures further, each residue was subjected to carbohydrate analysis by HPAEC-PAD. The composition of the cellulose residues obtained from a typical wood sample is shown in **Fig. 1**. It is clear that significant levels of impurities exist in the cellulose residues prepared by digestion in peroxyacetic acid and nitric–acetic acid. These impurities are present as hemicelluloses and other material, most likely oxidized hemicelluloses and lignin, which inflate the estimates of cellulose and reduce the accuracy of the analysis. The oxidizing nature of the peroxyacetic acid and nitric acid solvent systems produced cellulose residues that were significantly lighter in color than those prepared by the Seifert technique, although the Seifert residues were more pure. Figure 1 shows that cellulose prepared by the Seifert technique contains only low amounts of extraneous material and gives estimates of cellulose content that are not too different from the reference values obtained by HPAEC. The Seifert residues are particularly low in hemicellulose, which can be attributed to the relative severity of the acid hydrolysis conditions in the sol-

vent system. The cellulose residues in Fig. 1 all contain slightly less cellulose than is measured in the original wood by HPAEC. This is attributed to the degradation of minor amounts of lower-molecular-weight amorphous cellulose, probably by hydrolysis, during digestion.

In many cases, the accuracy of a method for measuring cellulose is not as critical as its ability to correctly rank samples. For example, accuracy is less important when establishing correlations between cellulose and other properties, or when screening trees for within-tree variation. An inaccurate gravimetric method may still be practical for screening or correlation purposes if the impurities in the cellulose residues remain at an approximately constant level. This ensures that differences in measured cellulose contents reflect real variations in the cellulose content, and not merely fluctuations in the levels of impurity. The data in **Table II** give an indication of the ranges of composition observed for the cellulose residues in this study. Each method of digestion produces cellulose residues with varying proportions of noncellulosic material. In all three methods, the amount of noncellulosic material was unrelated to the cellulose contents. Cellulose prepared by the peroxyacetic acid method showed a total variation of 2.6% in hemicellulose and 4.4% in other materials. This means that two peroxyacetic acid cellulose residues with identical cellulose contents may show a difference of up to 7% when measured gravimetrically. Such a "worst case" figure would decrease to about 5.5% for nitric–acetic acid cellulose, and would be around 2% for the "worst case" cellulose residue by the Seifert method. As a result, cellulose estimated by the Seifert method would suffer the least interference from impurities, and would be expected to yield the best data for monitoring relative changes in the cellulose content of wood samples.

	COMPOSITION, %		
	Peroxyacetic acid method	Nitric–acetic acid method	Seifert method
Cellulose	35.9–41.9	35.3–42.1	34.6–38.5
Hemicelluloses	5.1–7.7	2.7–4.1	0.2–0.6
Other material	2.0–6.4	2.3–6.4	1.5–3.2

II. Composition range data for E. nitens cellulose residues



2. Scatter graphs illustrating the correlation of cellulose with pulp yield for four measurement methods

The advantages of selecting the appropriate technique for cellulose measurement are highlighted in the correlation of the cellulose data in Table I with the kraft pulp yields of the *E. nitens* series at kappa no. 15. It is clear from the correlation coefficients in **Fig. 2** that the method used to measure cellulose in wood has a marked effect on correlations with kraft pulp yield. In particular, cellulose measured by HPAEC and gravimetrically by the Seifert procedure give excellent correlations with kraft pulp yield.

The different methods of cellulose measurement encountered in the literature may account for some of the confusion over the influence of cellulose content on kraft pulp yield. For example, in a study of eucalypts in South Africa, Clarke and Wessels *(17)* reported a correlation coefficient of only 0.63 between pulp yield and cellulose measured by digestion of wood meal in peroxyacetic acid. On the other hand, duPlooy *(3)* measured the cellulose contents of 20 *E. grandis* trees using the Seifert procedure. When these data were correlated with pulp yield at constant kappa number, the much better correlation coefficient of 0.91 was obtained. The weak correlation found in the work of Clarke and Wessels *(17)* might be improved using more accurate measurement techniques.

For the screening of multiple wood samples, analysis time is another

KEYWORDS
Acetic acid, acetone, acetyl groups, acidolysis, catalysts, cellulose, cellulose content, chromatography, dioxane, Eucalyptus nitens, gravimetry, hydrochloric acid, kraft pulps, nitric acid, peroxy acids, plantations, procedures, samples, solvolysis, wood flour, yield.

important variable to consider when selecting an analytical method. Given a certain level of resources (analysts, equipment, etc.), analysis time directly affects the number of samples that can be processed in a day. With this in mind, the gravimetric methods most suited to high throughput are the nitric–acetic acid and Seifert methods, which take approximately 30 and 35 min, respectively, per analysis, including filtration and washing. The peroxyacetic acid method is considerably slower, taking a little over 4 hours per sample. For cellulose analysis by HPAEC-PAD, throughput is limited by the time taken to hydrolyze the samples. The analysis itself can be carried out unattended overnight. Sample preparation involving primary and secondary hydrolysis takes about 3 hours per sample; however up to ten samples can be hydrolyzed simultaneously. As a result, the measurement of cellulose by HPAEC-PAD gives approximately the same sample throughput as the quickest gravimetric methods.

CONCLUSIONS

Three methods for the gravimetric determination of cellulose have been evaluated as cheaper alternatives to high-performance anion-exchange chromatography (HPAEC). The most accurate gravimetric technique surveyed was the solvolytic method of Seifert *(16)*, which gave similar cellulose contents to those obtained by HPAEC. Cellulose residues obtained by the Seifert procedure also contained the lowest levels of noncellulosic impurities and, as a result, gave the best correlations with kraft pulp yield. More meaningful correlations with other properties would also be expected when cellulose is measured by the Seifert procedure.

Digestion of wood in a nitric–acetic acid mixture *(14, 15)* produced the next best estimate of cellulose content, although significant amounts of noncellulosic material were present in the cellulose residue. The peroxyacetic acid technique described by Garbutt *(13)* gave the most inaccurate estimate of cellulose content, and cellulose residues isolated by this technique were the most impure, resulting in poor correlations between cellulose content and kraft pulp yield due to statistical interference from the impurities.

It is concluded that the Seifert procedure is the best alternative to HPAEC-PAD for measuring cellulose. However, because hemicelluloses can be determined simultaneously, the latter technique can give much more chemical information about the total wood polysaccharides. **TJ**

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