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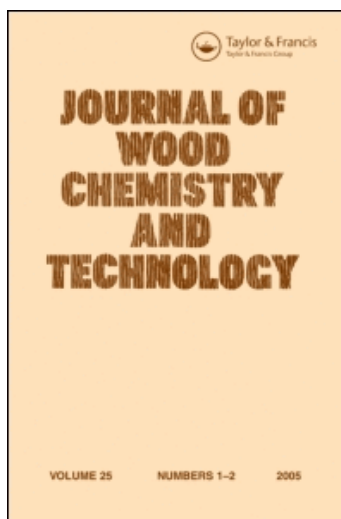
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Predicting Extractives, Lignin, and Cellulose Contents Using Near Infrared Spectroscopy on Solid Wood in *Eucalyptus globulus*

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Abstract: Near infrared reflectance (NIR) spectroscopy can be used to reliably predict both the physical and chemical wood properties of *Eucalyptus*. However, studies have been based on ground wood, which is costly and time-consuming to obtain. Predicting wood traits from NIR spectral data taken from solid wood would greatly increase the speed and cost-effectiveness of this procedure. Existing ground wood calibrations were evaluated for the prediction of wood chemistry from NIR spectral data taken from solid wood. Extractives, acid-soluble lignin, and Klason lignin contents were poorly predicted. Total lignin and cellulose contents showed moderate relationships between laboratory values and the NIR predicted values. NIR calibrations were further developed specifically for predicting wood chemistry from solid wood. All calibrations had high R^2 values from 0.72 to 0.88, and standard errors of calibration were less than 1.37%. Calibration validation produced high correlation coefficients between predicted and laboratory values for extractives, Klason lignin, total lignin, and cellulose contents with R^2 values ranging from 0.67 to 0.87. Acid-soluble lignin content was poorly predicted. This study showed that NIR analysis on solid wood of *E. globulus* could be reliably used to predict extractives, lignin, and cellulose

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contents. It also determined that existing ground wood calibrations, although they could give crude estimates of the wood chemistry values, would need to be re-developed for accurate predictions from solid wood.

Keywords: Calibration, ground wood, Klason lignin, acid-soluble lignin

INTRODUCTION

Eucalyptus is grown throughout the world for pulp and solid wood products. In temperate regions, *Eucalyptus globulus* is one of the major species grown for pulp production. Its wood is ideally suited to this purpose, with a high cellulose content, and low lignin and extractives contents, which contribute to a high pulp yield.^[1] Breeding programs have been established for this species with the objective of further improving some of these characteristics. However, these programs require assessing large numbers of trees for multiple traits. For this purpose, techniques have been developed for increasing the efficiency of this process. Near infrared reflectance (NIR) spectroscopy has been shown to be a reliable predictor of wood chemistry from increment cores in *Eucalyptus*.^[2–9] NIR calibrations developed for the prediction of pulp yield, cellulose content, lignin content, lignin composition, and extractives content have all been successful in predicting these traits in larger data sets with considerable accuracy. This technique has provided an excellent alternative to using traditional wet chemistry methods, which are costly and time-consuming, and has become a well used tool for the rapid and cost-effective prediction of important wood chemistry traits in tree breeding programs.

Tree breeding programs require large numbers of trees to be screened in the most rapid and cost-effective way. NIR analysis has certainly helped with this, making it possible to predict a number of traits from a single increment core. Improvements can still be made, however, to increase the efficiency of using NIR analysis as a prediction tool for wood chemistry. Existing calibrations for *Eucalyptus* (mentioned earlier) were developed using NIR spectroscopy on ground wood, a processing requirement that is quite costly and time-consuming. Increment cores must be ground to wood meal using a Wiley mill with a 1-mm screen, for a total of 3 min per sample.^[8] The mill must also be cleaned between samples, therefore making it possible to process only 12 samples per h. This grinding step can become quite costly when looking at sampling large field trials. Using NIR spectroscopy on solid wood could allow the removal of this step from wood chemistry evaluation, increasing the ease, cost-effectiveness, and rapidity of NIR as a prediction tool.

It was unknown if current ground wood calibrations could be used to predict traits based on spectral data from solid wood. There are also very few studies into the use of NIR analysis on solid wood for calibration development and trait prediction.^[11–14] The reason for this may involve the

limitation of using reflectance measurements on solid wood, which primarily includes the small penetration depth (1–4 mm) into the sample.^[10] For non-homogenous samples such as wood this may lead to large variation in the results, and a strong dependence on sample size and preparation technique.^[10] Despite this potential limitation, NIR analysis has been used on solid wood to develop calibrations for predicting physical traits such as density, stiffness (longitudinal modulus of elasticity), strength (modulus of rupture), and microfibril angle in *Eucalyptus delegatensis*, *Pinus radiata*, and *Larix decidua*, giving excellent results for both hardwoods and softwoods.^[11–13] However, the use of NIR analysis for the prediction of the chemical composition of solid wood is a relatively new area to be examined. Recently, this has been investigated in loblolly pine (*Pinus taeda*) with encouraging results.^[14] Kelley et al.^[14] successfully developed calibrations and predicted values for lignin, extractives, glucose, xylose, mannose, and galactose contents of solid wood. The calibrations for each trait had high correlation coefficients, and predicted and laboratory values were well correlated. To date this is the only published study detailing the prediction of chemical composition from solid wood using NIR, and so far this technique has not been examined in hardwoods such as *Eucalyptus*.

This study aimed to extend the findings of Poke et al.^[8] by exploring the possibility of using solid wood to increase the rapidity and cost-effectiveness of using NIR as a prediction tool for wood chemistry in *Eucalyptus*. The use of current ground wood calibrations for the prediction of wood chemistry based on solid wood NIR spectral data was evaluated in *E. globulus*. Further, new calibrations specifically for solid wood were developed for the prediction of wood chemistry traits. This would exclude the need for the grinding of increment core samples, and increase the speed and cost-effectiveness of screening samples for wood chemistry composition using NIR analysis. NIR spectra were collected for pith-to-bark strips, and extractives, acid-soluble lignin, Klason lignin, total lignin, and cellulose contents were predicted from current ground wood calibrations.^[8] Laboratory measurements were made for extractives, acid-soluble lignin, Klason lignin, total lignin, and cellulose contents on a subset of samples to evaluate these predictions. Further laboratory measurements were made for these traits to develop calibrations specifically for solid wood. Calibrations were used to predict these in the remainder of the data set, and were validated with further wet chemistry measurements.

MATERIALS AND METHODS

Field Sampling

Wood samples of *E. globulus* at 14 years of age were obtained from a base population field trial located at West Ridgley, Tasmania (Gunns Ltd). This trial was based on the CSIRO Australian Tree Seed Centre collection and is

composed of open-pollinated families.^[15,16] Ten trees were selected in pairs from the same family (five families from three different subraces in total), the same part of the trial (north or south), to span the range of NIR predicted lignin contents obtained using the calibrations of Poke et al.,^[8] and to be decay free (noted in the absence of decay in wood cores previously taken in 2002). Trees near the edges of the trial were also excluded as they may be wind damaged and contain tension wood. Due to these restraints, trees were selected to have a lignin content range from 26 to 30%. At the time of sampling tree 10 was found to be forked close to the base and therefore was excluded.

The nine trees were felled, total tree height was measured, and disks 15 cm deep were cut at each 10% increment of total tree height (or as close as possible to avoid branches and defects). The base represented 0% and disks were taken up to 70% of tree height, after which the stem generally became too small. From the disks, 20 mm wide by 15 cm deep bark-to-bark strips were cut through the pith in the same orientation for each tree using a band saw. This segment was then cut in half down the pith, and a 20-mm deep pith-to-bark strip was taken either from the bottom or top (to avoid defects) of a constant side for each disk. The 20 × 20 mm strip was air-dried and used for NIR analysis and wood chemistry measurements.

NIR Analysis

The NIR spectra were taken in increments along the transverse face of each pith-to-bark strip at CSIRO (Clayton, Victoria). The first increment was 40 mm in length (longer so the strip could be held in the machine) with the following increments 20 mm in length. Spectra were taken as described in Poke et al.^[8]

Evaluation of Wood Chemistry Predictions Using Ground Wood Calibrations

Using the ground wood calibrations developed in Poke et al.^[8] the extractives, acid-soluble lignin, Klason lignin, and total lignin contents were predicted for each increment. Cellulose content was also predicted using a CSIRO calibration. Using these NIR prediction estimates, samples were selected for wood chemistry analysis so as to cover the range of predicted values, and in the majority of cases, so they would occur at the end of a pith-to-bark strip for easy sampling. Increments were cut from the strips and ground to wood meal as described in Poke et al.^[8] Extractives and lignin contents were measured according to the Appita Standards^[17,18] on 13 and 14 samples, respectively, for validation of the predictions. Crude cellulose content was measured in duplicate on nine samples for calibration validation using the

diglyme method of Wallis et al.^[19] Diglyme (10 mL) and concentrated hydrochloric acid (2 mL) were added to 1 g of wood meal in a 50 mL reaction bottle, which was sealed with a teflon coated cap, and placed in a shaking water bath at low speed at 90°C for 1 h. The residue was collected in a tared alundum crucible by vacuum filtration; washed with 50 mL of methanol, followed by 250 mL of boiling water; and dried overnight at 105°C. The cellulose content was determined from the mass of residue after drying, and reported as a percentage of the original wood sample after adjustment for moisture content. Regression relationships were determined between the predicted and laboratory values to evaluate the accuracy of the predictions.

Solid Wood Calibration Development and Validation

Extractives and lignin contents were measured on a further 27 and 29 increment samples, respectively, for calibration development. Crude cellulose content was measured in duplicate on a further 30 samples for calibration development. Calibrations were developed for extractives, acid-soluble lignin, Klason lignin, total lignin, and cellulose contents using the method detailed in Poke et al.^[8] at CSIRO (Clayton, Victoria). These calibrations were then used to predict the wood chemical composition of the remaining increments (253 increments for extractives content, 250 for lignin content, and 254 for cellulose content). Seven to nine of these increment samples, which spanned the range of predicted extractives, lignin, and cellulose values were then selected for laboratory measurements and regression relationships between predicted and laboratory values were determined.

RESULTS

Evaluation of the NIR Predictions from Ground Wood Calibrations

Ground wood calibrations were unsuitable for predicting extractives and acid-soluble lignin contents from solid wood spectral data, with many of the predicted values being negative numbers (data not shown). Predictions of Klason lignin content for 14 samples were poorly correlated with laboratory values with an R^2 of 0.27 (Figure 1). Predicted total lignin contents for 14 samples were moderately correlated with laboratory measurements with an R^2 value of 0.54 (Figure 2). Predicted and laboratory values for cellulose content for nine samples were moderately well correlated with an R^2 value of 0.63 (Figure 3). Despite the moderate correlation coefficients for total lignin and cellulose contents (Figures 2 and 3), the regression equations indicated that the predictions lacked accuracy with X multipliers of 0.44 and 0.48, respectively (should be close to 1 for accurate predictions).

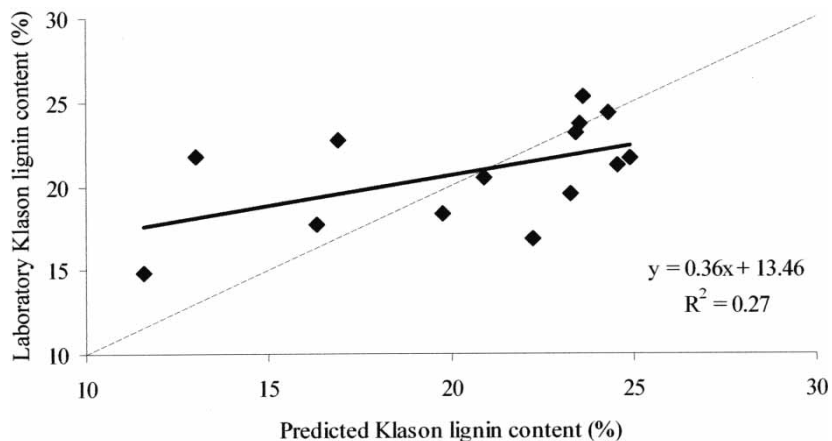


Figure 1. Relationship between NIR predicted and laboratory Klason lignin content for 14 samples of *Eucalyptus globulus*. Predictions were made for solid wood using ground wood calibrations.^[8]

Predictions for total lignin content were either under- or over-estimated (shown by the regression line crossing the 1:1 line), whereas cellulose content was generally overestimated. These regression lines were generally driven by outliers at one end of the extreme for both total lignin content and cellulose content (three and two points, respectively), and when these were removed multipliers on the X factor increased dramatically to 0.80 for

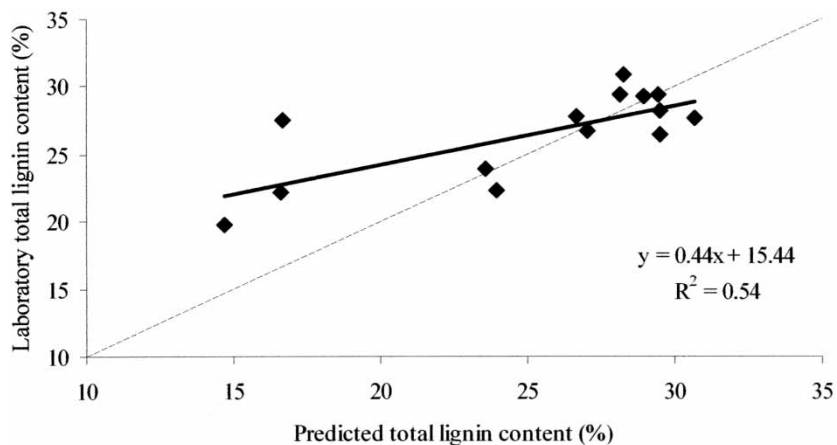


Figure 2. Relationship between NIR predicted and laboratory total lignin content for 14 samples of *Eucalyptus globulus*. Predictions were made for solid wood using ground wood calibrations.^[8]

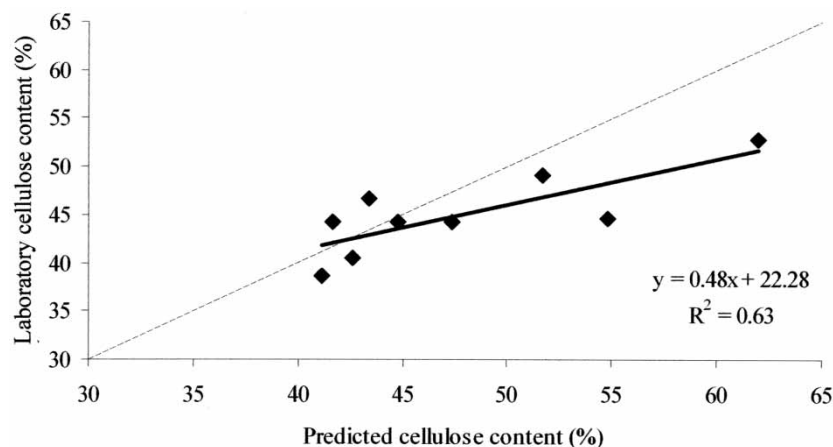


Figure 3. Relationship between NIR predicted and laboratory cellulose content for nine samples of *Eucalyptus globulus*. Predictions were made for solid wood using ground wood calibrations (CSIRO, Clayton).

total lignin content, and 0.69 for cellulose content, indicating that the majority of NIR predictions were quite close to the laboratory values (Figures 2 and 3).

NIR Calibrations for Solid Wood

Good calibrations were developed for predicting extractives, lignin, and cellulose contents from solid wood (Table 1). A high R^2 value of 0.84 and a SEC of 1.37 were obtained for the calibration for extractives content using measurements from 40 samples (Table 1). Calibrations developed for acid-soluble lignin, Klason lignin, and total lignin contents had R^2 values of 0.72, 0.78, and 0.76, respectively, and SECs of 0.41, 1.02, and 1.07 (Table 1)

Table 1. Statistics for the NIR calibrations for extractives, acid-soluble lignin, Klason lignin, total lignin, and cellulose contents developed for solid wood in *Eucalyptus globulus*

Wood component	Number of factors	R^2	SEC
Extractives content	6	0.84	1.37
Acid-soluble lignin content	6	0.72	0.41
Klason lignin content	4	0.78	1.02
Total lignin content	4	0.76	1.07
Cellulose content	5	0.88	1.14

based on 43 samples. For cellulose content an R^2 value of 0.88 and a SEC of 1.14 were obtained for the calibration using 39 samples (Table 1). Based on the range of predicted extractives, lignin, and cellulose contents, laboratory measurements were made for additional increment samples to validate the calibrations. Predicted and laboratory extractives contents for nine samples were highly correlated with an R^2 of 0.87 (Figure 4). Predictions were found to be quite accurate with the regression equation giving a multiplier on the X factor close to 1 (1.14). Generally predictions were either over- or underestimated with the regression line crossing the 1:1 line (Figure 4). For acid-soluble lignin content a poor correlation was obtained between predicted and laboratory values for seven samples with an R^2 of 0.12 (Figure 5). Predicted and laboratory values for Klason lignin and total lignin contents for seven samples showed high correlations with R^2 values of 0.79 and 0.67, respectively (Figures 6 and 7). Regression equations had multipliers on the X factor of 1.04 for Klason lignin content, and 1.40 for total lignin content, indicating the predictions were quite accurate, particularly for Klason lignin content. Predictions for Klason lignin content were generally overestimated by half a unit (regression line lay parallel to the 1:1 line and was displaced to the right), whereas predictions for total lignin content were either over- or underestimated (regression line crossed the 1:1 line) (Figures 6 and 7). Predicted and laboratory cellulose contents for nine samples were well correlated with an R^2 of 0.69 (Figure 8). The regression equation indicated that the predicted values were quite accurate with a multiplier on the X factor of 0.96. Predicted cellulose contents were generally overestimated by a half unit, as indicated by the regression line being parallel to the 1:1 line and

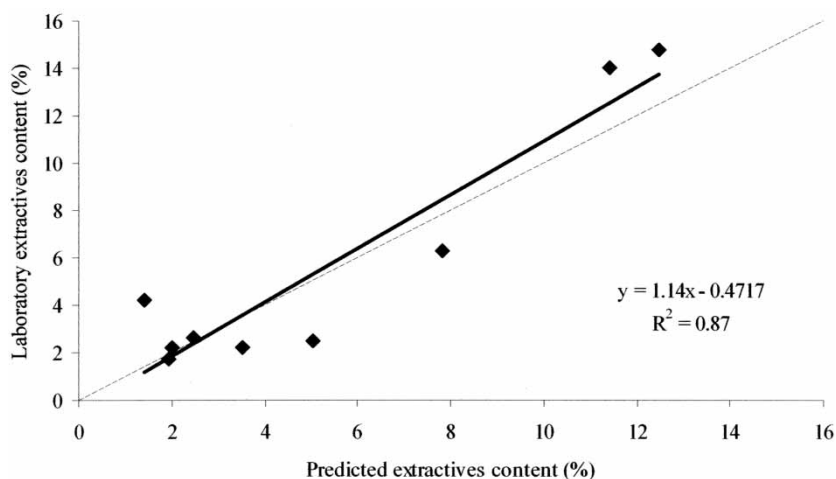


Figure 4. Relationship between NIR predicted and laboratory extractives content for nine samples of *Eucalyptus globulus* used to validate the calibrations.

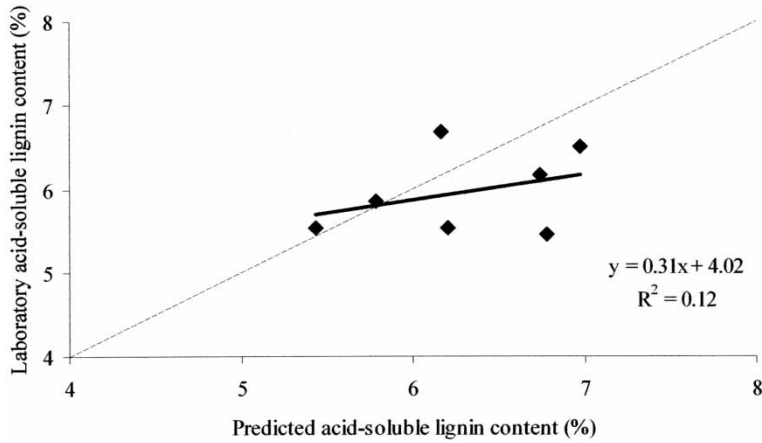


Figure 5. Relationship between NIR predicted and laboratory acid-soluble lignin content for seven samples of *Eucalyptus globulus* used to validate the calibrations.

displaced to the right (Figure 8). Two outliers were evident in this graph and when removed from the dataset the R^2 increased to 0.97.

DISCUSSION

Existing ground wood calibrations^[8] were found to be unsuitable for the prediction of extractives, acid-soluble lignin, and Klason lignin contents from solid wood. Moderate correlation coefficients were found between predicted

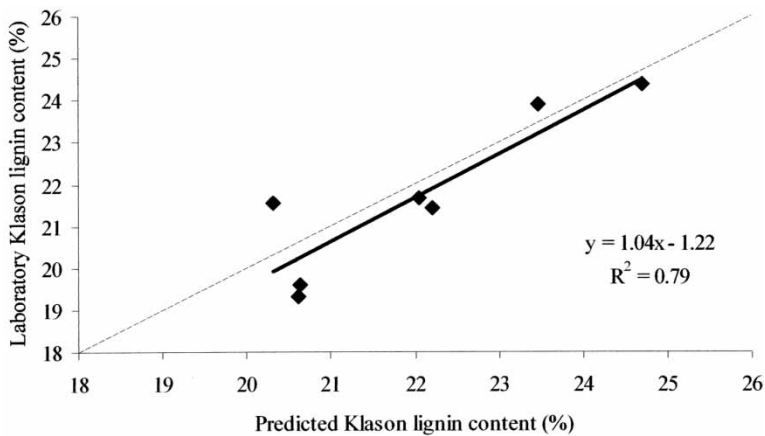


Figure 6. Relationship between NIR predicted and laboratory Klason lignin content for seven samples of *Eucalyptus globulus* used to validate the calibrations.

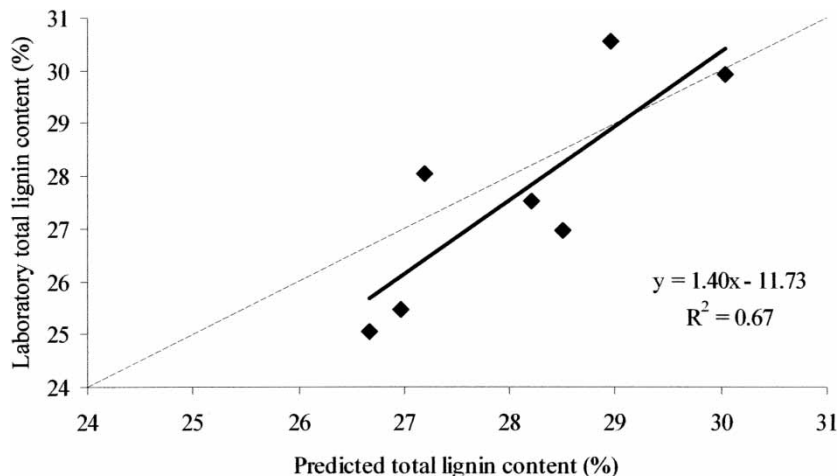


Figure 7. Relationship between NIR predicted and laboratory total lignin content for seven samples of *Eucalyptus globulus* used to validate the calibrations.

and laboratory values for total lignin content (R^2 value of 0.54) and cellulose content (R^2 value of 0.63), suggesting this method could be used to give crude estimates of these traits, particularly cellulose content. Although regression equations indicated that these predictions did not have high accuracy overall, the majority of predictions were close to laboratory values and therefore current ground wood calibrations may be useful for obtaining

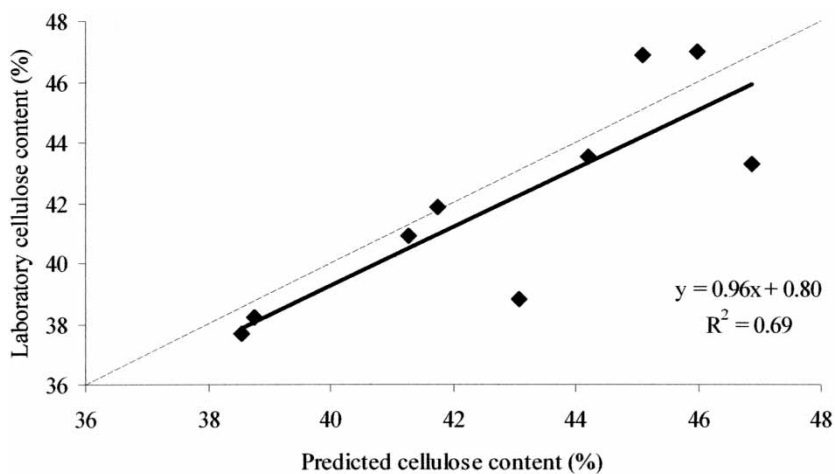


Figure 8. Relationship between NIR predicted and laboratory cellulose content for nine samples of *Eucalyptus globulus* used to validate the calibrations.

approximate values for these traits. Total lignin content was either under- or over-estimated, and cellulose content was generally overestimated. To obtain more accurate NIR predictions, calibrations for extractives, lignin, and cellulose contents will need to be redeveloped for solid wood. Ground wood calibrations may not work as well for solid wood because the spectra taken from ground wood involve the cell wall polymers being at varying angles incident to the NIR radiation within the one sample. For solid wood spectra the cell wall polymers will be at a consistent incident angle to the NIR radiation within the same sample. The spectra will differ between the two sample types as a result. Overall this study indicated that it is unlikely that existing wood chemistry calibrations, that have been developed using ground wood, can be used for accurate NIR prediction of these traits from solid wood.

Good calibrations were developed using NIR analysis for predicting extractives, lignin, and cellulose contents for solid wood in *E. globulus*. Calibration coefficients were generally high and above 0.72, and SEC were below 1.37%. Validation of these calibrations produced good correlations between NIR predicted and laboratory values for all traits except acid-soluble lignin content (R^2 of 0.87 for extractives content, 0.12 for acid-soluble lignin content, 0.79 for Klason lignin content, 0.67 for total lignin content, and 0.69 for cellulose content). Klason lignin and cellulose contents were generally overestimated by a half unit, whereas total lignin content was either over- or under-estimated slightly. These results suggest that calibrations developed using NIR spectra from solid wood, could be reliably used to predict extractives, lignin, and cellulose contents with considerable accuracy. The good calibration for extractives content is particularly surprising as it is a composite trait comprised of a range of non-structural compounds that are soluble in organic solvents and water.^[18] Therefore, prediction of extractives content from the NIR spectra, particularly from solid wood, would be expected to be more difficult, unlike pure compounds such as cellulose. The less successful prediction of acid-soluble lignin content may be due to this trait having small values with less variation between them, and it may be possible that lignin composition is having an effect on this trait, which has been suggested previously when NIR was used to predict this trait from ground wood.^[8] Although acid-soluble lignin content could not be reliably predicted using this calibration, estimates for Klason lignin content and total lignin content were good, with Klason lignin content proving to be the most reliable prediction trait for lignin content from solid wood. Together these results suggest that calibrations developed for solid wood can be reliably used to predict wood chemistry, and will provide a rapid and cost-effective alternative to using ground wood calibrations.

The only published study detailing the development of NIR calibrations for chemistry prediction from solid wood is in loblolly pine.^[14] Kelley et al.^[14] developed good calibrations with high correlation coefficients for both lignin (0.81) and extractives (0.93) contents, which are higher than those established in this current study. A stronger correlation between

predicted and laboratory values for lignin content was also obtained, with a correlation coefficient of 0.76, and a similar correlation coefficient was found for extractives content of 0.85, which was slightly lower than that obtained here.^[14] The variation in results between these studies are likely to be due to differences between the genera used, *Eucalyptus* and *Pinus*, and to differences in the type of wood used, softwood versus hardwood. However, results from both studies suggest that NIR scans of solid wood for calibration development and trait prediction will be a viable option. Axrup et al.^[20] have also shown that NIR analysis can be used to predict the extractives and Klason lignin contents of wood chips moving along a conveyor belt. However, the root mean square errors of calibration and prediction were consistently higher than those reported by Kelley et al.,^[14] indicating this technique is not as reliable.

It has been suggested that the quality of NIR calibrations developed for solid wood may be reduced,^[14] which has also been noted here. Calibrations developed for ground wood of *E. globulus*^[8] had very high correlation coefficients compared to those established using solid wood. The accuracy of the predictions for ground wood were also excellent with regression relationships giving very high R^2 values for Klason lignin and total lignin contents (0.97 and 0.99, respectively), and high R^2 values for acid-soluble lignin and extractives contents (0.83 and 0.89, respectively). For cellulose content, Raymond and Schimleck^[7] developed a range of ground wood calibrations for different sites of *E. globulus*, and obtained moderate to very high correlation coefficients ranging from 0.78 to 0.94. Predicted and laboratory values were also highly correlated with R^2 values between 0.82 and 0.95.^[7] The values associated with ground wood calibrations are generally higher than those obtained for solid wood, however, the advantages to using solid wood calibrations may outweigh the slight decrease in accuracy for many applications. The increase in the ease and speed, and the decrease in the cost associated with the removal of the wood grinding step, is a major advantage for using NIR analysis on solid wood for wood chemistry prediction, in preference to ground wood. Solid wood NIR calibrations can provide a useful and cost-effective tool for the rapid screening of large numbers of trees for wood chemical composition in breeding programs.

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