Rapid Prediction of Solid Wood Lignin Content Using Transmittance Near-Infrared Spectroscopy

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A rapid transmittance near-infrared (NIR) spectroscopic method has been developed to characterize the lignin content of solid wood. Using simple, multiple regression, and partial least-squares statistical analysis the lignin contents of wood wafers, taken from increment cores, and synthetic wood, prepared by blending milled wood lignin and holocellulose, were compared and quantified. Strong correlations were obtained between the predicted NIR results and those obtained from traditional chemical methods. In addition to the experimental protocol and method development, NIR results from wood samples with different particle sizes and various lignin contents are discussed.

KEYWORDS: Loblolly pine (*Pinus taeda*); transmittance near-infrared spectroscopy (NIR); increment cores; wood wafer; lignin content; prediction

INTRODUCTION

Increasing global population is placing substantial pressures on the forest industry. The demand for forest products is increasing. However, environmental concerns and the loss of available land for forestation are making the ability to meet that demand more challenging. In order for the U.S. forest industry to meet future demand and maintain global competitiveness, more wood with targeted characteristics will have to be produced more efficiently on less land. To accomplish this, the majority of the softwood pulpwood supply in this country will have to come from intensively managed fast-growing plantation forests. This will result in a substantial increase in the proportion of juvenile wood entering the pulp mill, significantly affecting both production cost and product quality. Genetic improvement must focus not only on the quantity but also on the quality of the raw material and must develop raw materials uniquely suited for particular processes and products.

The variation in most wood properties within a species is under a relatively high degree of genetic control (*1*). Wood properties, which are related to product quality, include density, fibril angle, tracheid diameter and length, cell wall thickness, and chemical composition. Paper properties such as burst, tear strength, and tensile strength are closely related to fiber morphology (*2*), whereas processing costs and resultant profitability are more significantly affected by chemical composition (*3*). Despite this, most operational breeding programs have placed limited emphasis on wood quality traits. This is largely due to the high costs of sampling and assessment. As the global market places more emphasis on wood properties, rapid, precise, and cost-effective assessment tools need to be developed.

Near-infrared spectroscopy (NIR) is a rapid, nondestructive technique that has been extensively used both quantitatively and qualitatively in the forest products industry (*4−10*). However, this work has been done using reflectance NIR spectroscopy. Reflectance measurements suffer from several limitations, the most serious being the small penetration depth (1−4 mm) into the sample. For nonhomogeneous samples such as wood, this limited penetration leads to a large variation in results and a strong dependence on sample size and preparation technique. Thus, large sample sizes are typically utilized to better represent the sample of interest. In contrast, transmittance techniques, which penetrate fully through the sample (*11*), are less sensitive to sample preparation and homogeneity and permit the analysis of smaller quantity samples. For whatever reason, the utilization of transmittance NIR in the analysis of wood has been limited. Here, we describe the use of transmittance NIR spectroscopy for characterizing the lignin content in wood. Using synthetic wood, produced by mechanically mixing milled wood lignin and holocellulose, and wood increment core microtome sections, regression models have been developed and are discussed.

MATERIALS AND METHODS

Materials. Wood meal samples were from the mature section (sapwood) of a 33-year-old loblolly pine (*Pinus taeda*). The wood was chipped and subsequently ground using a Wiley mill (Thomas standard model 3) into wood meals. The wood meals were further screened into fractions of different particle size using a stack of various mesh screens. Milled wood lignin (MWL) was prepared from the same batch of wood meals according to a previously published method (*12*).

Wood increment core samples were collected from 12-year-old loblolly pine received from the Tree Breeding Program, Department of Forestry, North Carolina State University. The increment wood cores were extracted by acetone as described previously (*13*). The extractives-free wood cores were soaked in deionized water overnight, and the

third-year-ring springwood was sectioned into thin wafers using a microtome. The wet thickness was 200 μm. The wood wafers were dried under vacuum over P_2O_5 overnight prior to NIR measurement. All NIR spectra were recorded prior to lignin content determination (see below).

Holocellulose Preparation. Wood meal, 5 g of oven-dried weight, was weighed into a 500-mL Erlenmeyer flask, to which 200 mL of 90 °C deionized water was then added, followed by 10 mL of acetic acid and 2.5 g of 80% (w/w) $NaClO_2$. The flask was kept at 90 °C for 30 min, at which time an additional 10 mL of acetic acid and 2.5 g of 80% (w/w) $NaClO_2$ were added. The 30-min cycle was repeated over the course of 2 h. At the end of the 2-h period, the flask was stoppered and cooled with cold water to stop the reaction. The reaction mixture was then filtered using a course crucible and dried at 105 °C until the crucible weight was constant, and the holocellulose yield was calculated.

Lignin Content Determination. The lignin content was determined by Klason lignin method. Accordingly, 1 g (oven-dried) of wood meal was placed in a 100-mL beaker to which 15 mL of 72% H_2SO_4 was added. The mixture was left at room temperature for 2 h with occasional stirring. The solution was then transferred to a 1-L Erlenmeyer flask, diluted with 560 mL of deionized water to a H_2SO_4 concentration of 3%, and refluxed for 4 h. The solution was then filtered, and the acid insoluble lignin was determined gravimetrically. The filtrate was diluted to 1 L with deionized water, and the acid soluble lignin was calculated from the UV absorbance reading at 205 nm. The extinction coefficient used was 110 AU·L/g·cm (*14*). For the wood wafers the experimental conditions were reduced accordingly for a 0.1 g (oven-dried) sample weight. The pooled standard error for the Klason lignin content (laboratory error) of seven duplicate samples was 0.30.

NIR Sample Preparation. Wood meal samples for NIR analysis were prepared using a pellet method. The wood meals or holocellulose samples (100-mg OD weight) were put into a stainless steel capsule and vibrated in an amalgamator (Zenith, model Z-1A) for 1 min to produce a uniform particle size. The powder sample, 75-mg OD weight, was then pressed into a pellet, with a pellet diameter of 1.3 cm.

For the holocellulose and MWL blended samples, the amalgamated samples were separately weighed and mechanically mixed in different ratios using a mortar and pestle prior to being pressed into pellets. The holocellulose/MWL ratio was varied between 100/0 and 60/40.

For the wood wafer samples, the dried wafers were measured directly using a modified sample holder. The wafer diameter was the same as the increment core diameter, 1.2 cm.

NIR Spectroscopy. A Foss NIRSystems near-infrared spectrometer equipped with an InTact single-tablet module (NR-1650) and a monochromator (NR-6500-V/H) was used in this study. Absorbance spectra, 32 scans, were collected at 2.0-nm intervals over the range of 600−1900 nm.

Calibration Development and Statistics. All statistics, regressions, and predictions were performed using VISION software (version 2.51) from Foss NIRSystems. All spectra were converted to second-derivative spectra with a segment of 10 nm and a gap of 0 nm prior to any calibration development. Multiple correlation coefficients (R^2) and standard errors of calibration (SEC) were used to evaluate how well a calibration of simple or multiple linear regressions fit the data. SEC is the standard deviation for the residuals due to the difference between the actual laboratory values and the fitted values of samples within the calibration set (*15, 16*).

For the partial least-squares (PLS) regression, the regression models were developed with four cross-validation segments and a maximum of 16 factors. The best number of PLS factors for the model was determined by the prediction residual error sum of squares (PRESS) value, which is the sum of all squared differences between the laboratory and predicted values (*5*). The PLS factors that yielded the lowest PRESS value were used to establish each model (*5, 9*). In addition to R^2 and SEC, standard error of cross-validation (SECV) was also used to evaluate the PLS calibration performance. SECV can be an indication of how well an equation will predict samples that are not used to generate the calibration equation when there are insufficient external validation samples (*17*). In cross-validation, samples in the original calibration set are grouped into four subsets. One subset is withheld to simulate the external validation set, whereas a calibration equation is
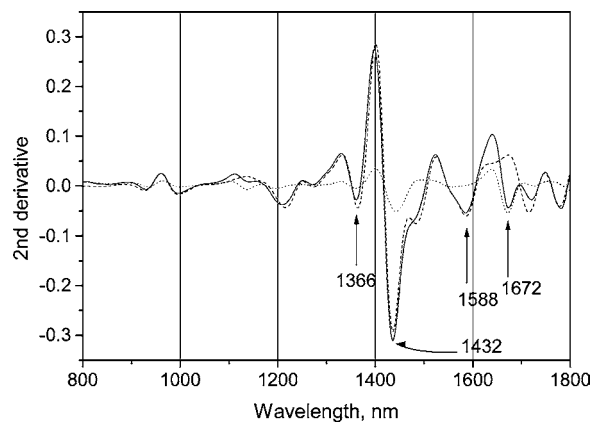

Figure 1. Second-derivative NIR spectra of wood and its chemical components: (–) wood meal; (- - -) holocellulose; (···) MWL.
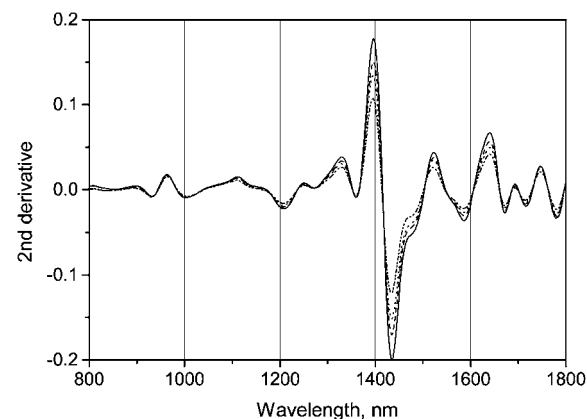

Figure 2. Second-erivative NIR spectra of the different mesh wood meals: (—) −60 mesh; (- - -) −35 +60 mesh; (···) 20 +35 mesh; (– ·· –) −10 +20 mesh.

created using the remaining samples. The resulting equation can then be used to predict the samples in the withheld subset. The standard error between the predicted and laboratory values is then calculated. The first subset is then returned to the calibration set, and the process is repeated for each group. The standard errors are then combined to produce the SECV (*18, 19*).

The standard error of prediction (SEP) was used in the increment core model to evaluate how well the calibration predicts the constituent value of interest for a set of unknown samples that are different from the calibration set (*20*).

RESULTS AND DISCUSSION

Chemical Components in the NIR Spectra. To utilize the simple regression method, which is based on the Beer−Lambert law, to build the prediction calibration line, it is of great importance to distinguish each of the component peaks in the NIR spectra. NIR spectra of mature pine wood meal, holocellulose, and MWL pellets were measured. The corresponding second-derivative NIR spectra are shown in **Figure 1**.

The second-derivative spectra are the inverse of the original absorbance spectra, in which the peaks project downward and are better resolved than the original spectra. From a comparison of these three spectra with a pure cellulose spectrum (spectra not shown), the peak at 1672 nm can be assigned to lignin and the peaks at 1366, 1432, and 1588 nm to carbohydrates. These peak assignments are similar to the previously reported using diffuse reflectance NIR (*21*).

Particle Size Effects. Figure 2 shows the results obtained for the NIR analysis of fractionated wood meal. Contrary to

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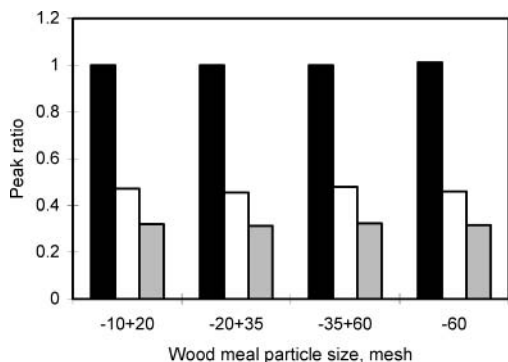*J. Agric. Food Chem.,* Vol. 52, No. 6, 2004 **1437**

Figure 3. Relative peak ratio of different particle size wood meals: (black bar) 1672/1366; (white bar) 1672/1432; (gray bar) 1672/(1366+1432).
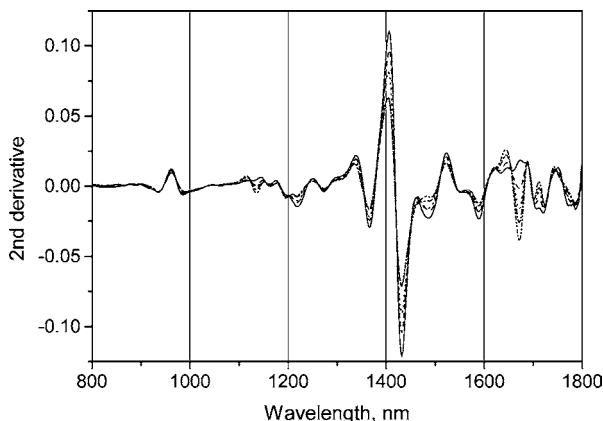

Figure 4. Second-derivative NIR spectra of different lignin content blending samples: (—) H100L0; (- - -) H90L10; (···) H80L20; (– · –) H70L30; (– · · –) H60L40.

reports in the literature wherein variations in particle size between 30 and 60 mesh did not have a significant effect on the NIR spectra (diffuse reflectance) (*15*), it can be seen that as the particle size changes from −10 to +20 mesh (pass through 10 mesh but retained by 20 mesh) to −60 mesh (pass through 60 mesh), the characteristic peak intensities increase.

The peak intensities in the spectrum of the finer particle size wood meal are stronger than that of the larger particle size wood meal. Therefore, erroneous results regarding lignin concentration or holocellulose content could result if individual peak heights from spectra of differently fractionated wood meals are utilized to determine component concentrations. This can be eliminated by utilizing the ratio of the component peaks, rather than the individual peak data. For the same wood meal, which has the same lignin content, the peak ratio of the lignin peak intensity to the carbohydrate peak intensities is the same and is independent of particle size (**Figure 3**).

Simple and Multiple Regression Methods. Figure 4 shows the second-derivative NIR spectra for different lignin content samples prepared by mechanically blending holocellulose with MWL. Distinct differences can be seen between the various bands associated with lignin and carbohydrates as the blend composition changes. For example, the intensity of the lignin peak at 1672 nm increases with increasing lignin content.

Similarly, changes in other bands and band ratios are observed with the change in lignin and carbohydrate concentration. A linear relationship was found between various other selected peak ratios for lignin and carbohydrates (**Figure 5**). Both the 1672/1366 and 1672/1432 band ratios show a linear relationship with increasing lignin content. The R^2 is 0.9964, and the SEC is 0.80 for the 1672/1366 band ratio.
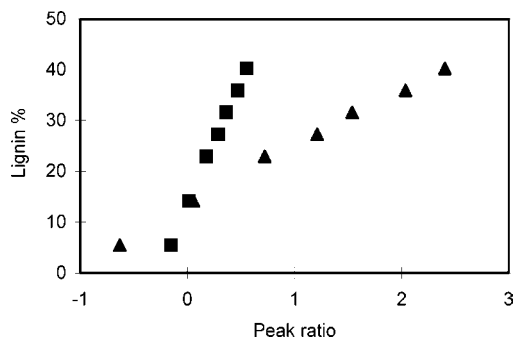

Figure 5. Linear relationships of some selected peak ratios: 1672/1366 (▲) $y = 11.3238x + 13.4941$ ($R^2 = 0.9964$, SEC = 0.80); 1672/1432 (■) $y = 48.738x + 13.435$ ($R^2 = 0.9986$, SEC = 0.50).
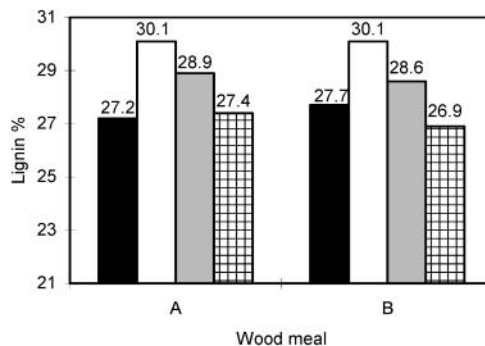

Figure 6. NIR-predicted lignin content by simple and multiple regression methods: (black bar) laboratory data; (white bar) 1672/1366; (gray bar) 1672/1432; (grid bar) (1672/1366) + (1672/1432).

These band ratios were then used to predict the lignin content of the mature pine wood meals, which are the same wood sources as the holocellulose and MWL used to build the models. The results are shown in **Figure 6**. The total lignin contents of the two wood samples as determined by Klason lignin are 27.2 and 27.7%, respectively. The predicted values from the 1672/1366 regression line are ∼30%, and those from the 1672/1432 regression line are ∼29%.

To increase the prediction accuracy, multiple linear regression analysis was applied. This model combines the two simple regression terms used in the previous section. An $R^2 = 0.9986$ with an SEC = 0.56 was obtained. The prediction results are also shown in **Figure 6**. The predicted values of 27.4 and 26.9% are much closer to the laboratory values of 27.2 and 27.7%, respectively, than the values obtained from the simple regression.

PLS Analysis. From the previous analysis, lignin content prediction depends on the peaks chosen and the regression method used. For a complex matrix material such as wood, the NIR spectra are composed of many overtones and combination bands. Therefore, if we can utilize the entire spectra in the regression, in which each individual piece of spectral information can be taken into consideration, a more powerful regression line can be obtained. Hence, the PLS method was used to obtain a calibration line. An additional 30 standard samples were utilized, varying in lignin content from 6.8 to 41.8%. The regression results obtained were explained by five PLS factors with $R^2 = 0.9948$, SEC = 0.74, and SECV = 1.05. The correlation between the standard laboratory values and the NIR fitted values of the calibration set is shown in **Figure 7**. The slope of this correlation is very close to 1. The lignin contents of the previous wood samples (27.2 and 27.7%) were predicted to be 27.4 and 27.9%, respectively, by this regression method.

Unfortunately, lignin content prediction of wood samples other than those used to build the method, that is, not the same
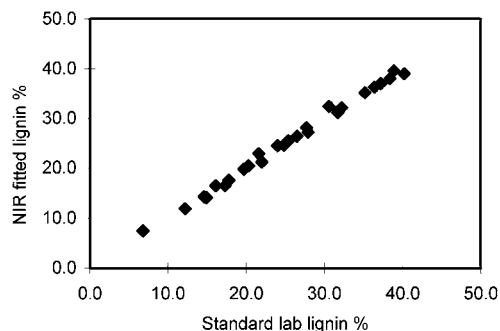
Figure 7. PLS correlation between the standard laboratory values and the fitted values of the calibration set from holocellulose and MWL blending samples ($y = 0.9948x + 0.1314$, $R^2 = 0.9948$, SEC = 0.74, SECV = 1.05).
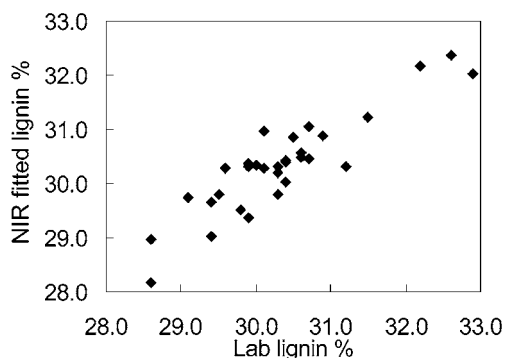

Figure 8. PLS correlation between the laboratory lignin values and the NIR fitted lignin values of the increment core model ($y = 0.8121x + 5.701$, $R^2 = 0.8121$, SEC = 0.47, SECV = 0.73).

source as the holocellulose and MWL, is not satisfactory, regardless of the regression model used. The calibration model is very specific and good only for the prediction of wood sources that are included in the model development. Therefore, the development of a universal calibration line for the prediction of lignin content through the utilization of *synthetic wood* is not feasible. Aside from the poor prediction of wood samples not included in the calibration set, the preparation of blending samples is a tedious process. The samples need to be ground, mixed, and pressed into pellets before any NIR analysis.

Increment Core Model. It is apparent that the utilization of NIR for the determination of lignin content depends on the sample set utilized to build the prediction model. Therefore, if a method is to be rapid, as required to screen the enormous number of wood samples produced by breeding programs, minimal sample preparation is critical. To address this, we investigated the use of wood wafers produced from increment wood cores traditionally used in wood density analysis. The increment core model was built using 32 different wood cores. Fifteen pieces of thin wood wafers were obtained from the third-ring springwood of each increment wood core. The dried wafers were scanned directly by transmittance NIR, and each set of 15 spectra was averaged to represent the third-ring springwood NIR spectrum. The total lignin content of each set of 15 wafers was determined by using the Klason method. The total lignin contents of the third-ring springwood of these 32 wood cores ranged from 28.6 to 32.9%. PLS analysis was applied to this model, and the correlation between the laboratory lignin values and the NIR fitted lignin values of the increment core model is shown in **Figure 8**. The regression results can be explained by six PLS factors with $R^2 = 0.8121$ and SEC = 0.47. The residual values (the difference between the predicted value and laboratory
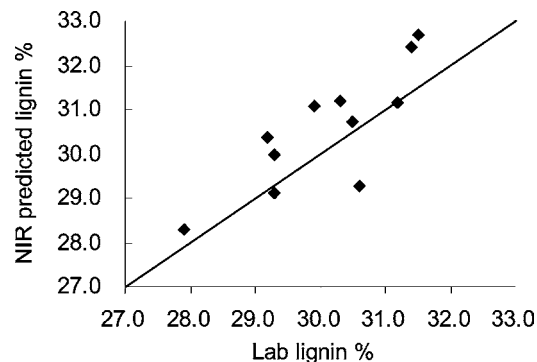

Figure 9. Prediction results of the external validation wood wafer samples by using the wood wafer calibration model ($R^2 = 0.7162$, SEP = 0.87).

value) of all of the calibration samples are within 1% differences. The SECV for this model is 0.73. The prediction results of some external validation samples are shown in **Figure 9**. The SEP is 0.87. These results indicate a good regression model can be obtained using the transmittance mode NIR with wood wafers taken from increment cores. Again, as with all NIR methods the model is dependent on the calibrations set used. However, using NIR transmittance, very little sample preparation is required; no grinding, screening, or other tedious preparation procedures are required for the wood meal regression models discussed above or the traditional reflectance NIR methods. This new procedure can reduce analysis time and chemical costs and provide a quick lignin assessment for tree-breeding programs.

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