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# Use of Visible and Near Infrared Spectroscopy to Predict Klason Lignin Content of Bamboo, Chinese Fir, Paulownia, and Poplar

Anmin Huang, Gaiyun Li, Feng Fu, and Benhua Fei

Research Institute of Wood Industry, Chinese Academy of Forestry, Beijing, China

**Abstract:** The use of calibrated visible and near infrared (NIR) (400–2500 nm) spectroscopy to predict the klason lignin content of Moso bamboo (*Phyllostachys pubescens*), Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.), Paulownia (*Paulownia elongata*), and Poplar (*Populus nigra var.*) samples was investigated. For bamboo, Chinese fir, and Paulownia, the lignin content predicted by means of chemical methods and that predicted by NIR are similar. The ratio of performance to deviation (RPD) of bamboo, Chinese fir, and Paulownia was 3.33, 2.53, and 1.77, respectively. However, for the poplar, the RPD was 1.07 only. The original models were constructed using a full spectrum ranging from 400 nm to 2500 nm. If the spectral range is reduced to the range of 400–1050 nm or 1100–2500 nm, a slight decrease in the quality of the models will occur. However, this decrease is minor considering the advantages of using a reduced spectral range. The results demonstrated that NIR could predict Klason Lignin Content of bamboo, Chinese fir, Paulownia, and Poplar.

Keywords: Bamboo, lignin content, near infrared spectroscopy, wood

# INTRODUCTION

Wood is a complex and non-uniform material, with respect to both its anatomical and its chemical properties. The main components of wood cell walls are cellulose, hemicelluloses, and lignin, which make up more than 90% of wood mass.<sup>[1]</sup> The chemical composition of wood may vary considerably in different wood species and indeed within an individual tree, which has greatly affected

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Address correspondence to Prof. Benhua Fei, Research Institute of Wood Industry (CRIWI), Chinese Academy of Forestry (CAF), Beijing 100091, P.R. China. E-mail: feibenhua@woodguide.com.cn

the utilization of wood resources.<sup>[1]</sup> Lignin is an undesirable component in the conversion of bamboo and wood into pulp and paper. Lignin removal is a major step in the papermaking process.<sup>[2]</sup> Lignin is an important determinant with respect to cellulose fiber extraction from bamboo and wood. Lignin subunit composition influences cellulose accessibility.<sup>[2]</sup> The analysis of the lignin of wood has traditionally involved laboratory-based wet chemical techniques that are difficult, time-consuming, and costly, and also require skilled personnel to achieve accurate and reproducible results. So, new, fast, and cost-effective methods are needed. And now, it has been shown that near infrared (NIR) spectroscopy and partial least squares (PLS) regression can be used to estimate the lignin content much more rapidly and conveniently, although with lower accuracy. Moreover, NIR with PLS require minimal sample preparation, and non-contact, non-destructive spectral acquisition. The ability of using NIR to analyze the wood lignin has been demonstrated by several authors.<sup>[1,3-9]</sup> Schimleck<sup>[4]</sup> found that NIR could be used to predict cellulose, lignin, hemicellulose, glucose, xylose, and acetyl content. His work<sup>[5]</sup> shows that NIR models for a property of interest, for example, pulp yield, pulpwood quality of standing trees, can be determined with a small number of samples, and then this model can be used as a predictive tool to predict a large number of samples for unknown properties. Garbutt et al.<sup>[3]</sup> found that NIR could be used to predict lignin and cellulose content in wood samples of Eucalyptus. Together these previous works have illustrated the potential for using NIR to measure the wood chemical compositions using several different statistical techniques.<sup>[3,7,8,10–16]</sup>

In China, Moso bamboo (*Phyllostachys pubescens*), Chinese fir (*Cunning-hamia lanceolata* (Lamb.) Hook.), Paulownia (*Paulownia elongata*), and Poplar (*Populus nigra var.*) are the four most important forest products. Wang<sup>[17]</sup> indicated that Moso bamboo could grow up to more than 20 meters in height and 60 to 150 mm in diameter in a single growing season. Chinese fir, Paulownia, and Poplar are all major plantation species and have always been used in construction, furniture, and paper-making in China and other countries. In this study, lignin in bamboo, Chinese fir, Paulownia and Poplar were detected using a lightweight, portable, and battery-driven NIR spectrometer. The objective was to develop and evaluate calibrations for predicting lignin content in Moso bamboo, Chinese fir, Paulownia, and Poplar using NIR analysis. Comparisons are made between different species of bamboo, Chinese fir, Paulownia, and Poplar.

# MATERIALS AND METHODS

#### **Collection of Bamboo and Wood Samples**

The bamboo, Chinese fir, Paulownia, and Poplar samples were collected in China from four different sites. Nine bamboos of 2–6 years of age were harvested from plantations in Hangzhou city, Zhejiang province, China  $(29^{\circ}50'-30^{\circ}23' \text{ N}, 120^{\circ}04'-120^{\circ}43' \text{ E})$ . Disks of bamboo were sawn at a height of 0.5–2.5 m and 4.5–6.5 m, and were left to air-dry in an environment-controlled room for several months. These were further broken down into matchsticks prior to milling in a Wiley mill equipped with 40 mesh and 60 mesh screen. A total of 54 powdered bamboo samples were prepared for analysis.

Eight Chinese firs of 29 years of age were cut from plantations in Huangshan city, Anhui province, China  $(29^{\circ}57'-31^{\circ}19' \text{ N}, 117^{\circ}58'-119^{\circ}40' \text{ E})$ . Three were sampled at an altitude of 800 m and the other five at an altitude of 400 m. The samples have an average diameter at breast height (dbh) of 402 mm and an average height of 30.3 m Three disks (10 cm) were sawn from the stems at heights of 1.3 m, 3.3 m, and 5.3 m. Two samples were taken from heartwood and sapwood area for each disc. A total of 48 powdered Chinese fir samples were prepared for analysis.

Eight Paulownias of 21 years of age were harvested from plantations in Lankao county, Henan province, China (34°24′–35°12′ N, 114°06′–114°54′ E) with average dbh of 387 mm and height of 12.6 m, respectively. Disks were cut from the stems at heights of 1.3 m, 3.3 m, 5.3 m, and 7.3 m. Two samples were taken from heartwood and sapwood area for each disc. A total of 64 powdered Paulownia samples were prepared for analysis.

Twenty-five Poplars 26 years of age were harvested from plantations in Anqing city, Anhui province, China  $(29^{\circ}42'-30^{\circ}54' \text{ N}, 116^{\circ}54'-117^{\circ}48' \text{ E})$  with average dbh of 307 mm and height of 24.4 m, respectively. Disks were cut from the stems at height of 0.5 m. Two samples were taken from heartwood and sapwood area for each disc. A total of 50 powdered Poplar samples were prepared for analysis.

These samples were broken into matchsticks and then ground in a Wiley mill equipped with a 2-mm screen. The 2-mm material was sieved for 5 minutes with a 40–60 mesh sieve. These powdered samples that passed the 40-mesh screen but did not pass the 60-mesh were prepared for chemical and NIR analysis.

#### **Chemical Analysis**

The content of lignin is usually characterized by the amount of acid-insoluble lignin. Acid-insoluble lignin can be measured by the Klason determination. In this method, one gram of air-dried, extractive-free sample is first treated with 72% sulfuric acid for 2 h at  $20 \pm 1^{\circ}$ C (first hydrolysis). Subsequently, the sample is diluted with water to reduce the sulfuric acid concentration to 3% and boiled for 4 h to hydrolyse the polysaccharides to soluble fragments, mainly monosaccharides (second hydrolysis). The solid residue (Klason lignin) is then washed, collected, dried, and weighed (ASTM D1106-96, 1996).

#### SPECTROSCOPIC METHODS

The NIR measurements were collected with an Analytical Spectral Devices (ASD) Lab Spec at 1-nm intervals between 4000 and 2500 nm. The powdered samples were transferred to a bottle cap, leveled, and rotated at 45 rpm to minimize specular interference and surface heterogeneity. A fiber optic probe oriented perpendicular to the sample surface was used to collect the reflectance spectra. A piece of commercial microporous Teflon was used as the white reference material. Thirty scans were collected and averaged into a single spectrum. During NIR scanning, the temperature was controlled at 22°C  $\pm$  1 in a laboratory environment with a mean relative humidity of 30%.

The data set was further reduced by averaging the spectra that were collected at intervals of 1 nm, to a spectral data set at intervals of 10 nm. Averaging the spectral data reduces the size of the spectra matrix and significantly reduces the time required to compute the PLS models without decreasing the quality of the models.

# **Multivariate Data Analysis**

Multivariate analysis was performed using The Unscrambler<sup>®</sup> version 9.2 (CAMO, Corvallis, OR). The package has the capability to perform partial least squares (PLS) analyses. All of the NIR spectra were combined into a single data matrix (X-matrix) whereas the lignin contents were combined into a response matrix (Y-matrix).

Calibration models were constructed with the samples using PLS regression. First or second derivatives of the data were analyzed. Over two-thirds of the samples were used for the calibration models whereas one-third were used for testing.

The standard error of cross validation (SECV) (determined from the residuals of each cross validation phase), the standard error of calibration (SEC) (determined from the residuals of the final calibration), and the co-efficient of determination ( $\mathbb{R}^2$ ) were used to assess calibration performance. The standard

Sample set			Lignin(%)		
	No. of samples	Minimum	Maximum	Mean	SD
Bamboo	53	19.67	28.48	23.87	2.46
Chinese Fir	46	32.36	35.28	34.11	0.64
Paulownia	64	15.52	20.48	18.11	1.81
Poplar	50	15.73	20.34	18.24	1.07

Table 1. Summary of lignin contents for bamboo and three woods

error of prediction (SEP) was used to measure how well a calibration predicts the parameter of interest for a set of unknown samples that are different from the calibration set. The predictive ability of calibrations was assessed by calculating the ratio of performance to deviation (RPD) (ratio of the standard deviation of the reference data to the SEP).<sup>[18]</sup> An RPD of greater than 2.5 is considered satisfactory for screening.<sup>[18]</sup>

# **RESULTS AND DISCUSSION**

#### Lignin Analyses of Bamboo, Chinese Fir, Paulownia, and Poplar

The results of Klason lignin of bamboo, Chinese fir, Paulownia, and Poplar with chemical analyses are shown in Table 1. The samples chosen cover different ranges of lignin content. Specifically, the lignin of the bamboo varies from 19.67 to 28.48, Paulownia varies from 15.52 to 20.48; however, the lignin of Chinese fir varies from 32.36 to 35.28, and Poplar varies from 15.73 to 20.34.

#### NIR Spectra of Bamboo, Chinese Fir, Paulownia, Poplar, and Lignin

Representative spectra of bamboo, Chinese fir, Paulownia, and Poplar are shown in Figure 1. Wood is a complex and non-uniform material, with respect to both



*Figure 1.* NIR spectra of moso bamboo (1), Chinese fir (2), Paulownia (3), and Poplar (4).

its anatomical and its chemical properties. It consists of different kinds of cells, performing the necessary functions of mechanical support, water transport, and metabolism. As a complex chemical polymer, wood is composed of polysaccharides (cellulose and hemicelluloses), lignin, extractives, and inorganic components. The main components of wood cell walls are holocellulose and lignin, which make up more than 90% of wood mass.<sup>[11]</sup> These organic compounds show absorption in near infrared region caused by overtone and combination bands of the fundamental stretching vibrations or deformations of C–H, N–H, and O–H bonds.<sup>[19]</sup> Figure 2 shows the visible and NIR spectra (400 nm–2500 nm) of lignin of Moso bamboo (1), Chinese fir (2), Paulownia (3), and Poplar (4). The differences between bamboo, Chinese fir, Paulownia, and Poplar are not obvious for regions of the NIR spectra, and there is also considerable overlap.

The major vibrations at 400 nm–700 nm are primarily due to the presence of lignin and extractives. It can be seen that the first overtone of cellulose and hemicellulose hydroxyls occurs between 1400 nm and 1660 nm, and the interactions between carbohydrate hydroxyls and water occur between 1890 nm and 2020 nm.<sup>[14,15]</sup> There is also a strong vibration in the region from 2020 nm to 2250 nm, which has been assigned to the cellulose hydroxyl vibrations. The first and second overtones of the lignin aromatic and aliphatic carbon/hydrogen vibrations are seen between 1635 nm and 1825 nm, and between 1075 nm and 1250 nm, respectively. Some of the lignin hydroxyl vibrations overlap with the cellulose hydroxyl vibrations, for example, the first overtone of the lignin



*Figure 2.* NIR spectra of lignin of moso bamboo (1), Chinese fir (2), Paulownia (3), and Poplar (4).

			Calibration set		Validation set		
Sample set	No. of samples	No. of factors	<b>R</b> <sup>2</sup>	SEC	<b>R</b> <sup>2</sup>	SEP	RPD
Bamboo	53	7	0.98	0.42	0.94	0.65	3.78
Chinese fir	46	9	0.96	0.13	0.81	0.28	2.29
Paulownia	64	6	0.90	0.40	0.74	0.61	2.97
Poplar	50	9	0.74	0.55	0.45	0.81	1.32

 Table 2.
 Summary of variation in measured wood chemical properties for the calibration and validation sets in the range of 350–2500

hydroxyl vibrations occurs between 1400 nm and 1520 nm. These assignments provide some insight into the chemical structures present in the material, but the overlapping bands limit the information available from simple, visual inspection of NIR spectra.<sup>[14,15]</sup>

# NIR Calibration and Test of Lignin of Bamboo, Chinese Fir, Paulownia, and Poplar

PLS regression was performed on the NIR spectra applying spectral pretreatments, as well as using different spectral ranges.

Table 2 shows the results of calibrations and tests of lignin for bamboo, Chinese fir, Paulownia, and Poplar. The calibrations developed for different species have similar coefficients of determination ( $\mathbb{R}^2$ ) and SEC. The relationships were good, except for Poplar, which has coefficients of determination ( $\mathbb{R}^2$ ) ranging from 0.83 for the Eucalyptus calibration to 0.89 for the Chinese fir calibration, but the  $\mathbb{R}^2$  for Poplar was 0.34. The calibration was developed using two factors for bamboo to four factors for the Chinese Fir calibration. The SEC ranged from 0.21 for the Chinese Fir calibration to 0.91 for the bamboo calibration.

For almost all calibrations, the SECV, which is considered to be a better measure of calibration error than the SEC, was considerably larger than the SEC. A high SECV indicates that the sample excluded in each cross validation phase was not well predicted by the corresponding calibration and that the SEC is overly optimistic.<sup>[20]</sup> Table 2 shows the SECV of PLS regression calibration model for bamboo, Chinese fir, Paulownia, and Poplar.

The current calibration was used to predict the lignin content of each sample.  $R^2$  of test was noticeably lower than the  $R^2$  of calibration. For the bamboo, the relationship between lignin determined by measurement and NIR prediction was excellent (Figure 3(A)). The  $R^2$  was higher and the SEP was very low. The RPD is more than 3.3. The regression line plotted in Figure 2 is very close to the line of equivalence indicate that there is very little bias and that

lignin contents predicted were very close to the experimental determinations of lignin. For Chinese fir and Paulownia, the relationship between the lignin amount determined by chemical and NIR-based lignin was good (Figure 3(A) and (B)). The R<sup>2</sup> were generally lower than that of bamboo, and the SEP was higher. For the Poplar, R<sup>2</sup> was the lowest and the SEP was the highest (Figure 3(D)). The RPD of Chinese fir, Paulownia, and Poplar is 2.53, 1.77 and



*Figure 3.* Relationships between analytical chemistry and NIR-estimated values for moso bamboo (A), Chinese fir (B), Paulownia (C), and Poplar (D).



Figure 3. Continued

1.27, respectively. From Figure 2 and Figure 3, we can see that there were not obvious differences between the NIR of Poplar and that of others species, and in the lignin NIR, there were similarities, too. The narrow range of lignin for the Chinese fir calibration relative to the other sets explains why it had a lower SEC, but the fact that the SEC for Poplar was highest indicated there were other reasons. The calibration and test statistics reported demonstrate that good calibrations for the estimation of lignin can be derived from NIR spectra of wood powder. Prediction error for Poplar was high and may be a consequence

of different cellular construction or the diverse origins of the samples in the test set.

Hence, if a larger calibration set was utilized in this study, stronger predictions may have been obtained. Further research with a large number of samples or by other Chemometrics, such as artificial neural networks (ANN), is required so as to determine whether prediction errors can be reduced. However, the high cost associated with standard analytical chemical analyses limits the number of samples that can be examined. The time and cost of analytical chemical methods emphasizes the importance of developing a rapid alternative method to routinely measure lignin contents on a large scale.

The current cost of chemistry methods is both an argument for using NIR spectroscope and the limiting factor in creating calibrations with large enough populations. This highlights the importance of identifying suitable samples prior to wood chemical content analysis. The results reported in this study demonstrate that it is possible to calibrate reflectance NIR spectroscopy for the estimation of lignin contents with bamboo and wood powder. The ability of NIR spectroscopy to estimate a wide range of properties makes it a valuable tool for wood property assessment.

#### Effects of Reduced Spectral Range

This article focuses on using the NIR spectral range (1100 nm–2500 nm), shortranged spectrum (400 nm–1050 nm) and both the visible and NIR spectral range (400 nm–2500 nm). The use of NIR spectra to predict the lignin content of wood has been reported by several groups.<sup>[4,5,21–25]</sup> Two recent studies have revealed that even the very subtle second overtone spectral signals contain information that can be used to predict wood properties of interest. Axrup and coworkers.<sup>[26]</sup> showed that the full chemical composition of wood chips—glucose, galactose, xylose, mannose, arabinose, and lignin—could be measured using the spectral information between 800 nm and 1100 nm. Malkavaara and Alén<sup>[27]</sup> measured the lignin content of kraft pulps subjected to different bleaching sequences using a spectral range between 360 nm–740 nm. In both cases the correlation

Sample set	No. of samples	No. of factors	Calibration set		Validation set		
			<b>R</b> <sup>2</sup>	SEC	<b>R</b> <sup>2</sup>	SEP	RPD
Bamboo	53	7	0.97	0.44	0.93	0.66	3.72
Chinese fir	46	9	0.85	0.24	0.67	0.37	1.73
Paulownia	64	6	0.90	0.40	0.70	0.68	2.66
Poplar	50	9	0.61	0.67	0.36	0.88	1.22

 Table 3.
 Summary of variation in measured wood chemical properties for the calibration and validation sets in the range of 1100–2500

Sample set	No. of samples	No. of factors	Calibration set		Validation set		
			<b>R</b> <sup>2</sup>	SEC	<b>R</b> <sup>2</sup>	SEP	RPD
Bamboo	53	7	0.94	0.62	0.86	0.93	2.67
Chinese fir	46	9	0.87	0.23	0.72	0.34	1.88
Paulownia	64	6	0.86	0.47	0.71	0.67	2.81
Poplar	50	9	0.52	0.74	0.24	0.98	1.09

**Table 4.** Summary of variation in measured wood chemical properties for the calibration and validation sets in the range of 350–1050

coefficients were generally above 0.90, indicating that large reductions in the spectral range did not have a significant negative effect on the quality of the models. One report on the use of a reduced spectral range (400 nm–1100 nm) for predicting the stiffness of wood showed a substantial decrease in the quality of the models.<sup>[28]</sup> The use of this reduced spectral range to produce high quality models is very significant because this reduced spectral range can be accessed with small, inexpensive, lightweight, handheld spectrometers that can acquire spectra in fractions of a second.

Table 3 and Table 4 highlight the impact of reducing the spectral range from 400 nm-2500 nm to 1100 nm-2500 nm and 400 nm-1050 nm. The results generally show a slight decrease or increase in the R<sup>2</sup>-value for both the calibration set and test set. For the sample of Moso bamboo, in the 1100 nm-2500 nm, the increase in the R<sup>2</sup> is between 0.02 and 0.05, and the decrease of SEC and SEP is 0.10 and 0.07. In the 400 nm-1050 nm range, the decrease in the R<sup>2</sup> is between 0.13 and 0.13, and the increase of SEC and SEP is 0.39 and 0.26. This is a slight decrease given the significant advantages of moving to a reduced spectral range. Similar results are seen for the PLS model of Chinese fir, Paulownia, and Poplar. Table 4 highlights the impact of reducing the spectral range from 400-2500 nm to 400-1050 nm. The result is similar to the impact of reducing the spectral range from 400-2500 nm to 1100-2500 nm. The decrease in the quality of the models is generally greater than that of the 1100–2500 nm range. These models are based on smaller data sets that include a narrower range of chemical composition. This is probably the cause of the greater decrease in model quality rather than some inherent limitation of these species.

# CONCLUSIONS

The calibration and test statistics obtained using four sets (bamboo, Chinese fir, Paulownia, and Poplar) suggest that NIR spectroscope may provide a useful tool for rapid assessment of lignin contents. These correlations were strong for both the calibration and the test samples except for Poplar. The original

models were constructed using a full spectral range between 400 and 2500 nm. Reducing the spectral range to 400–1050 nm and 1100–2500 nm can cause a slight decrease of the quality of the models; however, this decrease was minor considering the advantages of using a reduced spectral range. Further research with a large number of samples or by other chemometrics, such as artificial neural networks (ANN), is required to determine if prediction errors can be reduced.

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