DETERMINATION OF LIGNIN CONTENT IN *ACACIA* SPP. USING NEAR-INFRARED REFLECTANCE SPECTROSCOPY

Sheng Yao, Guofen Wu, Mian Xing, Shuke Zhou, and Junwen Pu*

Near infrared (NIR) spectroscopy method was introduced to measure the lignin content in *Acacia* species. Acid-soluble lignin, Klason lignin, and total lignin contents from 78 wood meal samples of *Acacia* spp. trees grown in Guangxi province with different ages, height, and families were measured by wet chemistry. NIR spectra were also collected using a Bruker MPA spectrometer within 4000-12500cm^{-1} of wavenumbers using a standard sample cup and split into calibration and prediction sets. Equations were developed using partial least squares (PLS) regression and cross validation for multivariate calibration in this study. High coefficients of determination (R^2) and low root mean square errors of cross-validation (RMSECV) were obtained for Klason lignin (R^2=0.94, RMSECV=0.398), acid-soluble lignin (R^2=0.87, RMSECV=0.144), and total lignin (R^2=0.91, RMSECV=0.448) from wood meal. High correlation coefficients were found between laboratory and predicted values for Klason lignin, acid-soluble lignin, and total lignin contents, with R^2 and RMSEP values ranging from 0.67 to 0.94, and 0.19 to 0.526, respectively. The study showed that NIR analysis can be reliably used to predict lignin content in *Acacia* spp.

Keywords: Near-Infrared spectroscopy (NIR); Acid-soluble lignin; Klason lignin; Total lignin; Acacia spp.

*Contact information: College of Material Science and Technology, Beijing Forestry University，100083,Beijing,China; *Corresponding author: pujunwen@126.com*

INTRODUCTION

Acacia is one of the most important wood sources used in temperate plantation forestry for the production of pulp for paper. The wood is characterized by high cellulose content, as well as low extractives and lignin levels, both of which are conducive to high pulp yield. Although the wood properties of *Acacia* spp. are generally favorable, much variation has been found in lignin content, cellulose content, and so on. One of the key economic drivers for kraft pulping is the yield of pulp per unit input of wood. The main factor influencing pulp yield is lignin content of the wood, which is negatively correlated with pulp yield. During kraft pulping, lignin must be chemically separated from the cellulose fibers to produce high quality pulp. To be able to breed for low lignin level trees, it is essential to be able to screen large numbers of individual trees. Measuring lignin content by traditional chemistry is costly and time-consuming. The lignin analysis in which the wood meal is first digested with 72% sulfuric acid 2h and subsequently allowed to degrade further in 3% sulfuric acid, takes 4h per sample to complete. The use of an indirect method for determining the lignin content of wood, such as near infrared reflectance (NIR) analysis, could provide a cheaper and quicker alternative, which would allow the determination of it traits in a larger number of samples and their incorporation in breeding programs. Near infrared (NIR) spectroscopy (Wesley 2001) has become a popular method for simultaneous chemical analysis and is being studied extensively in a number of different fields such as process monitoring (DeThomas 1994), biotechnology (Arnold 2000), and the pharmaceutical industry (Tran 2004) because of the potential for on-line, nondestructive, and noninvasive instrumentation. Near infrared (NIR) spectroscopy has the potential to provide the forest industry with a low cost and rapid tool for the non-destructive estimation of the chemical (Michell 1995; Raymond and Schimleck 2002; Schimleck et al. 2000; Wright et al. 1990)

NIR analysis has previously been used for the prediction of pulp yield (Wright 1990; Michel 1995; Schimleck, 1998), cellulose content (Wright 1990; Raymond 2002), and recently for extractives content, Klason lignin content, and lignin composition (ratio of syringyl to guaiacyl sub-units) (Baillères 2002). Schwanninger and Hinterstoisser (2002) examined the modifications to improve the precision of the standardized determination of Klason lignin by NIR spectroscopic method. Hodge and Woodbridge (2004) investigated the applicability of NIR spectroscopy to predict lignin content in five species of tropical and subtropical pine woods grown in Brazil and Colombia. Yeh et al. (2004) reported a rapid transmittance NIR spectroscopic method to characterize the lignin content of solid wood. Alves et al. (2006) investigated the calibration by NIR technique to assess lignin composition (H/G ratio) in maritime pine using analytical pyrolysis as the reference method. Strong correlations were obtained between the predicted NIR results and those obtained from traditional chemical methods. Their reported predictions were very good.

The aim of this investigation was to develop calibrations for predicting acid-soluble lignin content, Klason lignin content, and total lignin content in *Acacia* spp. using near infrared spectroscopy (NIR) and partial least squares regression(PLS) as a tool.

EXPERIMENTAL

Materials

Wood samples of 5-8 year old *Acacia* spp. plantation trees were obtained from two forestry farms with different site indices located within Guangxi, China. Sixteen trees from five families of average size were felled, measured for total height, and sectioned. For each tree, 4-6 disks approximately 50 mm thick were cut above the base of the tree at regular intervals up each stem: at 1.5, 3, 4.5, 6 meters and so on with a total of 78 samples for this study. The samples were ground into wood meal (mesh 40-60) and then stored in a room under controlled conditions.

Near Infrared Spectroscopy

The near-infrared spectra of wood meal samples were recorded on a Bruker MPA equipped with an integrating sphere for sampling in diffuse reflectance mode using a spinning cup module in the 4000 cm^{-1}-12000cm^{-1} region. All the spectral data were collected with a 8cm^{-1} spectral resolution, and 64 scans were collected and averaged into a single average spectrum to ensure an adequate signal-to-noise ration. Figure 1 shows several spectra collected on the wood meal samples. All absorbance spectra had generally similar spectral patterns.

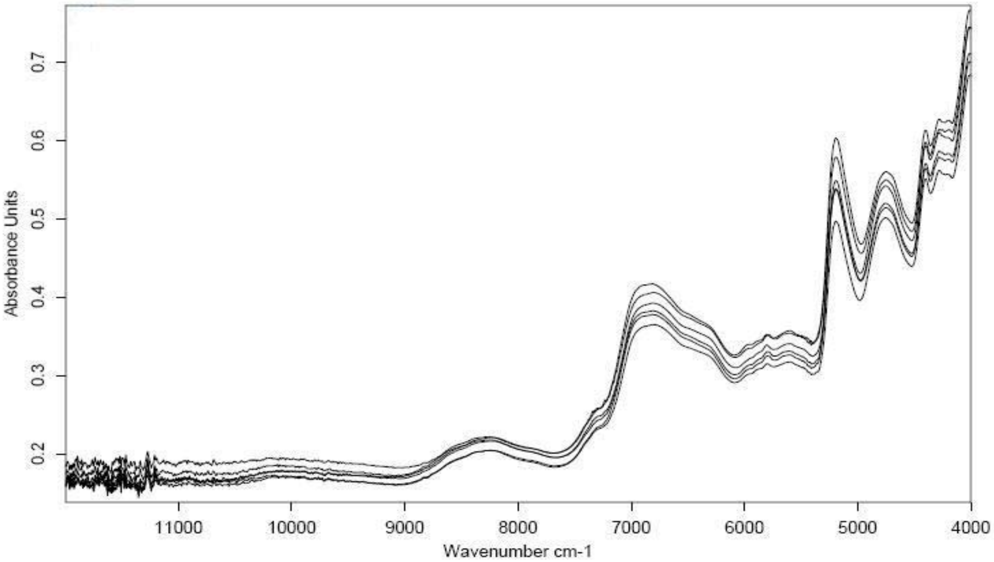

Fig.1. NIR diffuse reflectance spectra of wood samples

Lignin Content Determination.

The lignin content was determined by the Klason lignin method (GB/T 2677.8-94). Accordingly, 1 g of wood meal extracted with Benzene/Ethanol in a Soxhlet apparatus for 6 hr 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 (18-20^{o}C) for 2 h. 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 acid soluble lignin was calculated from the UV absorbance reading at 205 nm.

Calibration Development

All calibrations were developed using OPUS/QUANT6.5. Partial least squares (PLS) regression was used to create the calibrations with full cross validation (i.e., leave-one-out) and a maximum of 10 factors. The best number of PLS factors for the model was determined by the PRESS (Prediction Residual Error Sum of Squares) value, which is the sum of all squared differences between the lab and predicted values. The PLS factors that yielded the lowest PRESS value were used to establish each model. Pre-processing methods were applied prior to calculation of the PLS calibration. These methods include constant offset elimination, straight line subtraction, vector normalization, min-max normalization, multiplicative scattering correction, first derivative, second derivative, and the combination of first derivation with straight line subtraction, vector normalization, and multiplicative scattering correction. The quality of the calibration was also assessed by means of cross validation and prediction results: coefficient of determination (R^2), root mean square error of cross validation (RMSECV), and the root mean square error of prediction (RMSEP). RMSECV 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. RMSEP was used 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.

RESULTS AND DISCUSSION

Lignin Content

The results for 16 trees from five families of *Acacia* spp. are shown in Table 1. The Klason lignin, acid-soluble lignin, and total lignin content of each tree are the average of samples taken at different heights of the tree. We found from Table 1 that there was no significant difference in acid-soluble lignin content among the different trees except for *A. melanoxylon* (3.01%).

There was a large variation in klason lignin content. *A. melanoxylon* had the lowest klason lignin content (16.74%); and the *A. crassicarpa* had the highest klason lignin, where the average klason lignin content of different years old trees reached 21.48%. The average klason lignin of *A. mangium* (19.67%) and *A. mangium* × *A. auriculiformis* (19.32) were lower. The klason lignin content of 5 years old *A. cincinnata* was 20.32%, which was 3.95% higher than 7 years old *A. cincinnata*, and that observation needs to be investigated further.

Table 1. Lignin Contents of Different *Acacia* spp.

Family name	age	Klason lignin /%	acid-soluble lignin /%	total lignin /%
A. melanoxylon	7	16.74	3.01	19.75
A. crassicarpa	5	21.23	1.82	23.05
A. crassicarpa	6	21.57	1.77	21.19
A. crassicarpa	7	20.92	1.55	22.47
A. crassicarpa	7	22.21	1.64	22.59
A. mangium	5	18.59	1.83	20.42
A. mangium	6	19.81	2.05	21.86
A. mangium	7	19.97	1.70	21.67
A. mangium	8	20.29	1.36	20.49
A. mangium × *A. auriculiformis*	5	19.85	1.80	21.65
A. mangium × *A. auriculiformis*	6	19.75	1.92	21.67
A. mangium × *A. auriculiformis*	7	17.73	1.89	19.61
A. mangium × *A. auriculiformis*	7	19.37	1.59	20.96
A. mangium × *A. auriculiformis*	7	19.89	1.59	21.48
A. cincinnata	5	20.32	1.89	22.21
A. cincinnata	7	16.37	2.37	18.73

NIR Calibration

The number of samples used in the calibration set, the test set, and statistical summary of laboratory determined lignin content for each set are given in Table 2.

Table. 2. Chemical Analysis Results for Calibration and Prediction Samples

	Calibration			Prediction		
	Klason lignin	acid-soluble lignin	total lignin	Klason lignin	acid-soluble lignin	total lignin
N	60	60	60	18	18	18
Mean (%)	19.83	1.83	21.68	19.91	1.80	21.66
Maximum (%)	22.59	3.20	24.88	22.96	2.71	23.91
Minimum (%)	15.99	1.20	17.91	16.27	1.32	19.11
SD (%)	1.60	0.40	1.47	1.82	0.31	1.33

SD=standard deviation of the reference method values

The calibrations of the Klason lignin, acid-soluble lignin, and total lignin content of laboratory vs. NIR calibration measured values are presented in the Table 3. As the table illustrates, the NIR calibration predicted values for Klason lignin were close to the laboratory results. The regression results obtained were explained with an R^2=0.9378 and RMSECV=0.398 when the original spectrum was expressed as the first derivative and the wavelength range between 7502.1cm^{-1} and 4597.7cm^{-1}. The regression results of the acid-soluble lignin were not as good as for the Klason lignin content (R^2=0.8721, RMSECV=0.144) when the original spectrum was the second derivative and the wavelength range was between 6102cm^{-1} and 4597.7cm^{-1}. The total lignin calibration results were reasonably good (R^2=0.9116, RMSECV=0.448), and the first derivative plus multiplicative scatter correction method was used to pretreat the original spectrum and the wavelength range was between 6102cm^{-1} and 5446.3, 4601.6cm^{-1}, and 4246.7 cm^{-1}.

The calibrations were used to predict lignin content for the 18 validation samples that had not been included in the calibration set, and the prediction results are shown in Table 3. Regression equations indicted a strong relationship between the predicted and laboratory of Klason lignin and total lignin. Thus, we concluded that NIR analysis appeared to be a reliable predictor of Klason lignin and of the total lignin content.

Table 3. Main Parameters, Results of Calibration, and Prediction of Lignin

Chemical compositions	pre-processing	Calibration		Prediction	
		R^2_{cv}	RMSECV/%	R^2_{val}	RMSEP%
Klason lignin	1st.Der	0.94	0.398	0.94	0.531
acid-soluble lignin	2nd.Der	0.87	0.144	0.67	0.19
total lignin	1st.Der +MSC	0.91	0.448	0.88	0.526

R^2_{CV}=determination coefficient of cross validation; RMSECV=a root mean square error of cross validation; R^2val=determination coefficient of external validation; RMSEP=a root mean square error of prediction; MSC=multiplicative scatter correction

CONCLUSIONS

In this study, klason lignin, acid-soluble lignin, and total lignin content in *Acacia* spp. were measured. Klason lignin content showed quite large variation when comparing different families. *A. melanoxylon* had the lowest klason lignin; *A. crassicarpa* had the highest klason lignin; *A. mangium* and *A. mangium* × *A. auriculiformis*' klason lignin were intermediate in lignin content.

We successfully developed NIR calibrations for predicting Klason lignin, acid-soluble lignin, and total lignin content in *Acacia* spp. NIR analysis provided good calibrations for predicting Klason lignin and total lignin content. The RMSEP of calibration and prediction were generally 0.5 or lower, indicting that Klason lignin and total lignin content can be reliably predicted from spectra.

ACKNOWLEDGMENTS

The authors are grateful for the support of the National Science & Technology Pillar Program in the Eleventh Five-year Plan Period. No. 2006BAD32B03.

REFERENCES CITED

Alves, A., Schwanninger, M., Pereira, H., and Rodrigues, J. (2006). "Calibration of NIR to assess lignin composition (H/G ratio) in maritime pine wood using analytical pyrolysis as the reference method," *Holzforschung* 60(1), 29-31.

Arnold, S. A., Crowley, J., Vaidyanathan, S., Matheson, L., Mohan, P., Hall, J. W., Harvey, L. M., and McNeil, B. (2000). "At-line monitoring of a submerged filamentous bacterial cultivation using near infrared spectroscopy," *Enzyme Microb Tech.* 27(9), 691-697.

Baillères, H., Davrieux, F., and Ham-Pichavant, F. (2002). "Near infrared analysis as a tool for rapid screening of some major wood characteristics in a Eucalyptus breeding program," *Ann. For. Sci*. 59(5-6), 479-490.

DeThomas, F. A., Hall, J. W., and Monfre, S. L.(1994). "Real-time monitoring of polyurethane production using near infrared spectroscopy," *Talanta* 41, 425-431.

Hodge, G. R., and Woodbridge, W .C. ,(2004). "Use of near infrared spectroscopy to predict lignin content in tropical and sub-tropical pines," *Journal of Near Infrared Spectroscopy* 12(6), 381-390.

Michel, A. J. (1995). "Pulpwood quality estimation by near-infrared spectroscopic measurements on eucalypt woods," *Appita J*. 48(6), 425-428.

Raymond, C. A., and Schimleck, L. R. (2002). "Development of near infrared reflectance analysis calibrations for estimating genetic parameters for cellulose content in *Eucalyptus globulus*," *Can. J. For. Res*. 32(1), 170-176.

Schimleck, L. R., and Michel, A. J. (1998). "Determination of within-tree variation of kraft pulp yield using near-infrared spectroscopy," *TAPPI J*. 81(5), 229-236.

Schimleck, L. R., Raymond, C. A., Beadle, C. L., Downes, G. M., Kube, P. D., and French, J. (2000). "Applications of NIR spectroscopy to forest research," *Appita J.* 53(6), 458-464.

Schwanninger, M., and Hinterstoisser, B. (2002). "Klason lignin: Modifications to improve the precision of the standardized determination," *Holzforschung* 56(2), 161-166.

Tran, C. D., Oliveira, D., and Grishko, V. I. (2004). "Determination of enantiomeric compositions of pharmaceutical products by near-infrared spectrometry," *Anal. Biochem*. 325(2), 206-214.

Wesley, I. J., Larroque, O., Osborne, B. G., Azudin, N., Allen, H., and Skerritt, J. H. (2001). "Measurement of gliadin and glutenin content of flour by NIR spectroscopy," *J. Cereal Sci*. 34(2), 125-133.

Wright, J. A., Birkett, M. D., and Gambino, M. J. T. (1990). "Prediction of pulp yield and cellulose content from wood samples using near infrared reflectance spectroscopy," *TAPPI J*. 73(8), 164-166.

Yeh, T. F., Chang, H. M., and Kadla, J. F. (2004). "Rapid prediction of solid wood lignin content using transmittance near-infrared spectroscopy," *Journal of Agricultural and Food Chemistry* 52(6), 1435-1439.

Article submitted: Oct. 28, 2009; Peer review completed: Jan. 19, 2010; Revised version received and accepted: Feb. 5, 2010.