Determination of holocellulose and alpha-cellulose contents in *Acacia* spp. using NIR spectroscopy

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Introduction

Acacia spp is one of the most important species used in temperate plantation forestry for the production of pulp for paper. The wood is characterised by high cellulose content, and low extractives and lignin levels, which are conducive to high pulp yield. Although, the wood properties of *Acacia* spp are generally favourable, much variation has been found in pulp yield. To be able to breed for high cellulose level trees, it is essential to be able to screen large numbers of individual trees. Measuring holocellulose and alpha-cellulose content by traditional chemistry is costly and time-consuming. The use of an indirect method for determining the holocellulose and alpha-cellulose content of wood, such as near infrared reflectance (NIR) analysis, could provide a cheaper and quicker alternative, which would allow the determination of these traits in a larger number of samples, and their incorporation in breeding programs. Near infrared (NIR) spectroscopy[1] has become a popular method for simultaneous chemical analysis, and is being studied extensively in a number of different fields such as process monitoring,[2] biotechnology,[3] and the pharmaceutical industry[4] because of the potential for on-line, non-destructive, and non-invasive 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.[5–8]

The aim of this investigation was to develop calibrations for predicting Holocellulose and Alpha-cellulose content in *Acacia* spp using near infrared spectroscopy (NIR) and partial least squares regression (PLS) as tools.

Experimental

Materials

Wood samples of 5–8 year-old *Acacia* spp plantation trees were obtained from two forestry farms with different sites located within Guangxi, China. Sixteen trees from five families of average size were cut. For each tree, 4–6 disks approximately 50 mm thick were cut at regular

intervals up each stem: at 1.5, 3, 4.5, 6 meters and further, with a total of 78 trees for this study. The samples were ground into wood meal (mesh 40–60) and then stored under controlled conditions.

Holocellulose and alpha-cellulose contents determination

Holocellulose preparation

Wood meal, 2 g oven-dried weight, was suspended into a 250 mL Erlenmeyer flask, to which 65 mL of 75°C deionised water was then added, followed by 0.5 mL of acetic acid and 0.73 g of 80% $NaClO_2$. The flask was kept at 75°C for 60 min, at which time an additional 0.5 mL of acetic acid and 0.73 g of 80% $NaClO_2$ were added. The 60 min cycle was repeated over the course of 4 hr. At the end of the 4 hr period, the flask was 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.

α-Cellulose preparation

1.5 g of the holocellulose (outlined above) was reacted with 23 mL of 17.5 g L^{-1} sodium hydroxide for 45 min, then diluted with 23 mL of deionised water and the fibre suspension was filtered with a coarse crucible, washed thoroughly with 50 mL 95 g L^{-1} sodium hydroxide and 400 mL DI water, and soaked in 2.0 M acetic acid for 5 min. The neutralised α-cellulose was then washed with deionised water. The yield was calculated after drying at 105 °C.

Near infrared spectroscopy

NIR spectra were collected by a Bruker MPA spectrometer within 4000–12500 cm^{-1} wavenumbers, using a standard sample cup. The samples/spectra were split into calibration and prediction sets. Equations were developed using partial least squares (PLS) regression and cross validation for multivariate calibration in this study.

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

Table 1. Chemical analysis results of calibration and prediction samples.

	Calibration		Prediction	
	Holocellulose	Alpha-cellulose	Holocellulose	Alpha-cellulose
N	58	58	20	20
Mean %	79.66	48.2	79.28	47.76
Maximum %	84.26	54.22	83.47	50.84
Minimum %	74.60	44.79	75.91	44.86
SD	1.76	2.17	1.70	1.74

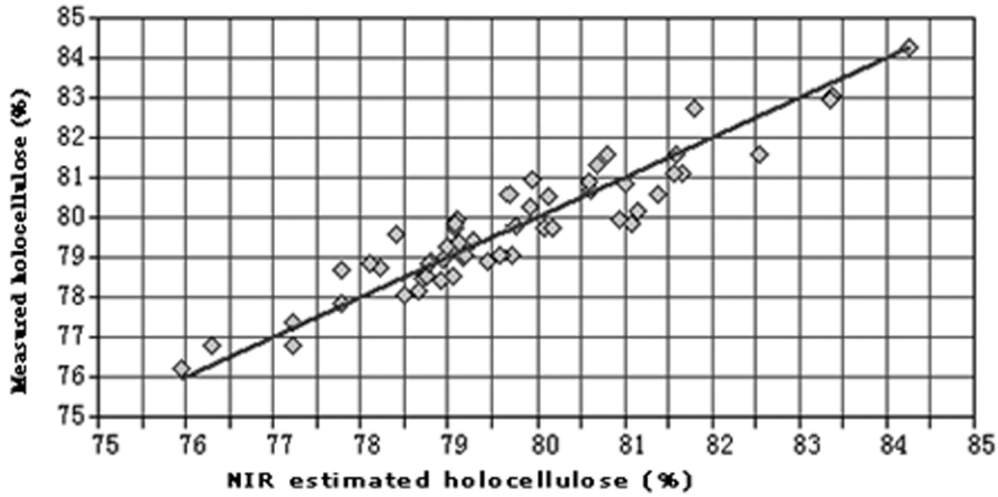

Figure 1. Result of holocellulose content for calibration model.

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. Pre-processing methods were applied prior to calculation of the PLS calibration. The quality of the calibration was also assessed by means of cross validation and prediction results: R^2, *RMSECV* and *RMSEP*.

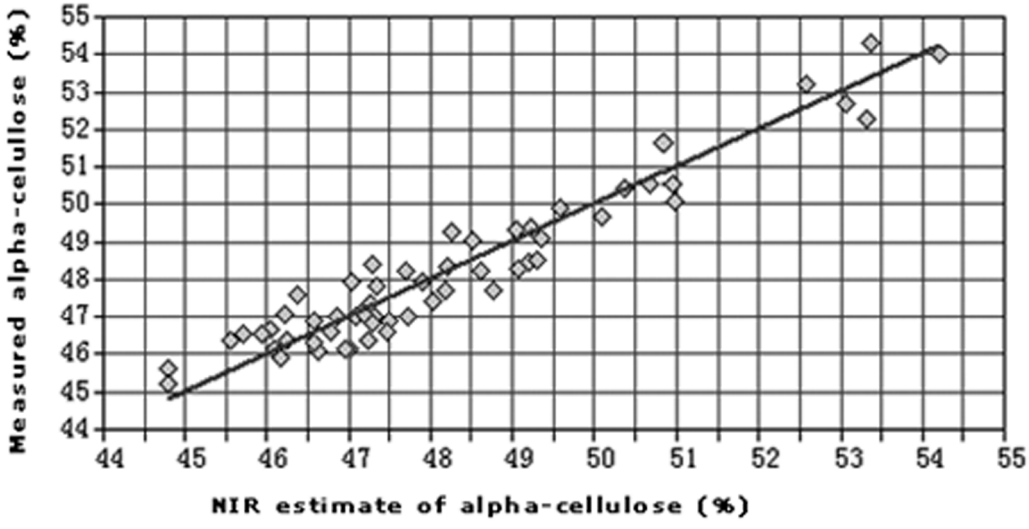

Figure 2. Result of alpha-cellulose content for calibration model.

Table 2. Main parameters, results of calibration and prediction of holocellulose and alpha-cellulose.

Chemical compositions	Pre-processing	Calibration			Prediction			
		R^2_{cv}	*RMSECV* %	*RPD*	R^2_{val}	*RMSEP* %	*RPD*	Bias
Holocellulose	MSC	0.881	0.567	2.9	0.8681	0.57	2.72	0.0384
Alpha-cellulose	1nd.Der	0.9184	0.615	3.5	0.9195	0.706	2.44	0.131

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

Results and discussion

All spectral date were split into the calibration and validation sets by PCA methods, which consisted of 58 and 20 samples, respectively. Sample set conditions are summarised in Table 1.

The calibrations of the holocellulose and alpha-cellulose contents of laboratory vs. measured values are presented in Figures 1 and 2.

High coefficients of determination (R^2) and low root mean square errors of cross-validation (*RMSECV*) were obtained for holocellulose ($R^2 = 0.881$, *RMSECV* = 0.567) and alpha-cellulose ($R^2 = 0.9184$, *RMSECV* = 0.615) from wood meal.

The calibrations were used to predict holocellulose and alpha-cellulose contents for the 20 validation samples. Prediction produced high coefficients of determination between laboratory and predicted values for holocellulose and alpha-cellulose contents, for which R^2 values are 0.8232 and 0.9195 and *RMSEP* are 0.699 and 0.706, respectively (Table 2).

This study showed that NIR analysis could be reliably used to predict holocellulose and alpha-cellulose content in *Acacia* spp.

Conclusions

In this study, we successfully developed NIR calibrations for predicting holocellulose and alpha-cellulose content in *Acacia* spp. The *RMSEP* of calibration and prediction were generally 0.7 or lower indicting that holocellulose and alpha-cellulose content can be reliably predicted from spectra.

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