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Silica content in reed bamboo (*Ochlandra travancorica* Gamble) and its rapid prediction using Fourier Transform Near-infrared Spectroscopy

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Abstract: Fourier Transform Near-infrared (FT-NIR) spectroscopy was successfully used to predict the silica content of *Ochlandra travancorica*. FT-NIR spectra were collected from powdered samples of reed bamboo with a fibre-optic probe in diffuse reflectance mode. NIR PLSR model of silica from the cross validation was developed by using spectra of 40 samples where silica content was determined by wet lab procedures. The precision and accuracy of the NIR PLSR model were analysed by comparing the statistical data of cross validation, calibration and test set validation. A good regression coefficient (r^2_{cv}=0.96) was obtained from the cross validation with FT-NIR spectroscopy for prediction of the silica in *O. travancorica*. The robustness of the PLSR model was proved with the similar values of coefficient of determination (r^2), RMSECV, RMSEE and RMSEP. The silica content of unknown samples was predicted using the PLSR model. The mean value of silica content varied from 3 to 5% among the populations from different geographic locations. However, there was no statistically significant difference in silica content between the populations.

Keywords:, Silica content, ash, FT-NIR spectroscopy, PLSR model, *Ochlandra travancorica*

INTRODUCTION

Bamboo being a member of grass family (Family: *Poaceace)*, silica content appears to be higher than in woody plants. Bamboo attracted worldwide attention as an alternative to timber for providing raw material to pulp and paper industries due to its long fibres, fast growth rate and short rotation of 2-3 years which is considered to be the maturity age of bamboos for harvesting (Hammett *et al*., 2001). Generally, presence of silica serves as a defensive feature of bamboos for protecting itself from external organisms and to withstand unfavorable environmental conditions (Hunt *et al*., 2008). Silica can be found in plants from 0.1 to 10% on a dry wet basis (Epstein, 1999). The presence of silica is not favoured in pulping as it is extremely alkali soluble and its

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presence in spent liquors inhibits conventional chemical recovery process especially in the evaporation of the waste liquor, combustion, causiticizing operations and in the rotary kiln and recovery units as well as tool knife wear during machining operations (Espiloy, 1982; Abd. Latif, 1993). Several authors have studied the silicon deposition in bamboo leaves and roots (Bennet and Sangster, 1981; Lux *et al*., 2003; Motomura *et al*., 2000, 2006, 2008). In bamboo roots, the deposition of Si is found only in endodermis cell walls. The silica contents show an increasing trend from stem, through branch, to leaves. The content of Si on dry weight basis was 7.6% in leaves and 2.4% in roots. The percentage of silica content by dry weight in bamboo culms varies from 0.3 to 7%, but almost the entire silica content is concentrated on the outer epidermal layers (Liese, 1985, 1992, 1998; Espiloy, 1982; Sathish *et al*., 1994) with hardly any silica in the rest of the cell wall. Ash content is mostly associated with the amount of silica (Abd. Latif *et al*., 1994). Silica and its distribution, both in temperate and tropical bamboo species, was reported to vary from 0.04 to 0.11% (Lybeer *et al.,* 2006), which is much lower than the values mentioned in previous studies.

The conventional analytical methods employed for wood, fibre and pulp evaluation are usually time consuming and expensive. Rapid and non-destructive analysis methods such as near infrared reflectance spectroscopy (NIRS) provide an opportunity to shorten analysis time, elevate the throughput and lower the costs (Greaves *et al.,* 1996; Schimleck *et al.,* 2000; Raymond and Schimleck, 2002; Tsuchikawa, 2007; Tsuchikawa and Schwnninger, 2013). Near-infrared (NIR) spectroscopy is a rapid non-destructive technique that has been employed to characterize chemical and physical properties of a wide range of materials within a short period. However, the reference measurements must be accurate and represent a wide range of values to achieve valid predictions although some researchers have worked on low precision reference values and noisy spectra (Rodrigues *et al*., 2006). NIRS technology is now being developed and calibrated to replace classical wet-chemical methods for wood physical, mechanical and chemical property studies in a variety of qualitative and quantitative applications (Schwanninger and Hinterstoisser, 2001; Gierlinger *et al*., 2002; Raymond and Schimleck, 2002; Schimleck *et al*., 2003, Kothiyal and Raturi, 2011; Kothiyal *et al*., 2012, Raturi *et al*., 2012). Rapid estimation of kraft pulp yield and lignin in Eucalyptus and Ipil-Ipil has been attempted by NIRS by Ramadevi *et al*. (2010). NIRS has been used to determine the nutrient composition of bamboos and monitor the physiological status of Giant Pandas (Wiedower, 2008). FT-NIR has been used very recently for estimation of the biomass composition of *Miscanthus giganteus* plants (Haffner *et al*., 2013).

The aim of the present work was to examine the silica content and distribution in reed bamboo, *Ochlandra tranvancorica* and its quantification by wet-chemistry methods to develop FT-NIR calibration model for the prediction of silica content of large number of samples. *O. travancorica* is endemic to Western Ghats of peninsular India and is a major long fibred raw material for pulp and paper and cottage industries

in the State of Kerala, India. This study was taken up as part of an all India coordinated research to look for and evolve low lignin pulpwood species for future breeding programmes in view of increased environmental concerns and the high cost involved in the bleaching process.

MATERIALS AND METHODS

Field collection

Reed bamboos of commercial importance are known to occur in the wild throughout the Western Ghats region in India, although *O. travancorica* is abundant in the semi-evergreen and moist deciduous forests all along the entire stretch of the Western Ghats in the central and southern parts of the State of Karnataka and Kerala, India. A total of 281 mature culms (2-year old) were collected from the wild for the present study which belongs to 66 populations covering 12 geographic locations of Kerala. All these wild populations have been assigned accession numbers and the same has been grown and maintained in the *Ochlandra* germplasm established at the KFRI Field Research Centre, Palappilly, Kerala, India for further studies. Geographic locations, coordinates and the details of germplasm accessions of species are presented in Table 1.

Wet laboratory analysis

Mature mid-internode culm was air-dried and powdered in a Wiley mill. The powder was sieved and retained in No.60 sieve (260 μm) to obtain uniform particle size and 1g powder was used for the analysis. The silica content of reed bamboo culm was determined in duplicate on oven-dry basis in accordance with the TAPPI test method T 245 cm-07 (wet ash method). The ashing temperature used in this method has been

Table 1. Geographic sources of sample origin for *O. travancorica*

Sl. No.	Geographic Location	District	Germplasm Accession No. and population	North latitude	East longitude	Altitude (masl)
1	Peechi	Thrissur	KFRI	10° 31' 46"	76° 20' 52"	121
2	Vazhachal	Thrissur	VAZ	10° 18' 42"	76° 34' 18"	327
3	Sholayar	Thrissur	SHO	10° 18' 30"	76° 45' 21"	484
4	Idamalayar	Idukki	IDA	10° 13' 02"	76° 43' 06"	59
5	Kuttampuzha	Ernakulam	KUT	10° 11' 08"	76° 47' 55"	335
6	Achenkovil	Kollam	ACK	09° 04' 31"	77° 11' '55"	1000
7	Goodrickal	Pathanamthitta	GUD	09° 19' 34"	77° 06' 02"	822
8	Trivandrum	Trivandrum	TVM	08° 53' 42"	80° 50' 59"	115
9	Neriamangalam	Idukki	NM	10° 04' 19"	76° 47' 09"	112
10	Peermedu	Kottayam	PER	09° 33' 55"	76° 59' 07"	835
11	Nilambur South	Malappuram	NBR	11° 16' 22"	76° 26' 04"	329
12	Aanakkulam	Idukki	ADI	10° 08' 333"	76° 54' 006"	349

changed from 575°C (T 245 om-88) to 525°C to prevent the degradation of calcium carbonate that can occur at higher temperature. The percent silica is determined by the formula,

$$\text{Silica \%} = \frac{weight\ of\ silica}{weight\ of\ overn-dry\ sample} x\,100$$

Fourier Transform –Near Infrared Reflectance (FT-NIR) spectroscopy

The reed bamboo samples for FT-NIR studies were prepared as described earlier (Bhat *et al.*, 2008 unpublished document). Solid samples could not be used for NIR analysis due to non-availability of adequate cross-sectional surface area for scanning with a fibre optic probe as the culm of reed bamboo is hollow inside and the wall thickness was less than 2.0 cm. Fixed quantity (3 g) of air-dried powder taken in a flat bottom glass vials (52 x 22 mm) was kept at 40°C for 2 days and reconditioned prior to NIR analysis for standardization and prediction of silica content. From the 12 geographic locations, 40 samples out of 281 were used for the analysis of silica by the laboratory method, T 245 cm-07. The spectral data of these samples were used for the development of the calibration, where the NIR spectra were cross validated to the known values of silica contents. Based on the model created by using FT-NIR cross validation (internal validation), the values of silica were predicted for the unknown 241 samples. It was ensured that the calibration represented the full scope of variation in the samples for silica being investigated for accurate prediction of large number of unknown samples.

Fibre optic probe (spot size 4 mm) of FT-NIR spectrophotometer (model MPA, Bruker Optik GmbH, Germany), was used for measuring the diffuse reflectance light as NIR spectra from the bamboo powder. The instrument was equipped with a TE-InGaAs detector with a measurement spectrum ranging between 12,500 and 4000 cm^{-1}. The Spectralon standard integrated in the fibre optic module served as the standard for background measurements. The spectra were collected after the fibre optic probe was placed directly over the fresh air-dried powder sample inside the glass vials. With a spectral resolution of 8 cm^{-1}, thirty scans were measured per sample and the mean spectra were used for the calibration.

Partial least square projection to latent structures (PLS)

Chemometric modeling using multivariate calibration was performed using the instrument software package OPUS/QUANT (version 5.5, Bruker Optik, Germany). This software includes the partial least squares projection to latent structures (PLS) algorithm. The QUANT software uses PLS 1 algorithm as it yields better results due to its capability to analyse each component separately rather than analysing simultaneously (PLS 2 algorithm). To set up the method, the calibration spectra of 40

samples and their respective reference values were read into the software. All of the FT-NIR spectra were combined into a single data matrix (*X*- matrix) while the chemical property data were combined into a response matrix (*Y*- matrix), and made projection to latent structures. The chemical properties were correlated with the NIR spectra using PLS models and the root mean square error of cross validation (*RMSECV*) and the value of the coefficient of determination (r^2_{cv}) were determined for the cross validation data sets. Cross- validation involves systematically removing a single sample from the calibration data set, constructs a model to predict the values of the *Y*-variables for the extracted sample. This process continued until each individual sample had been removed from the data set and a fully cross-validated model is constructed (Martens and Næs, 1991). The fully cross-validated model was then used to predict the chemical properties of the remaining unknown 241 samples. Data preprocessing techniques were applied and the instrument itself detects the spectral outliers in the calibration set and two outliers were excluded from the cross validation and test set validation to obtain the robust model for prediction. The First derivative and multiplicative scatter correction (MSC) was applied to obtain the best model. Outliers in the evaluation were detected through Mahalanobis distance calculations for each new spectrum. Thus, 38 samples were used for the final model constructed. All models were calculated to a maximum rank of 10 and the results of the calibration (r^2 and *RMSECV*) were compared.

The cross validation (internal validation) model was evaluated not only from its corresponding r^2_{cv} and RMSECV but also from the Test Set validation (external validation).The calibration data were divided into two groups, A and B. Each group was used for both cross validation (CV) and test set validation (TS). First, group A was used for CV and B for TS, and then vice versa to evaluate whether the model statistics were identical or at least very similar for the accuracy of the predictive model. A similar approach was used by Kelly *et al.* (2004) for predicting the chemical and mechanical properties of solid loblolly pine wood. The coefficient of determination (r^2), RMSECV, RMSEP, and rank were compared for the predictive ability of the model (Næs *et al.*, 2002). Comparison of the ranks determined by cross validation (CV) and test validation (TS) gives a first indication of the predictive ability of the model, because models with large differences between the ranks are usually not satisfactory (Gierlinger *et al.,* 2002).

RESULTS AND DISCUSSION

Wet chemical analysis

The results of the wet chemical analysis of *O. travancorica* samples are given in Table 2.

Table 2. The percentage silica of *O. travancorica* by wet lab analysis (*N=40*)

Component	Mean*	Standard deviation	Minimum	Maximum
Silica	3.76	0.76	2.08	5.7

*Pooled values of duplicate analysis

Calibration, cross-validation and test set validation

Cross validation showed linear relationships between NIR spectra and wet-chemistry data. The cross validation model of 40 samples based on the pre-processed spectra was in the range 4529 - 4116cm^{-1} with rank 3 showing a good coefficient of determination with r^2_{cv} value of 0.96 and RMSECV of 0.154% (Fig. 1).

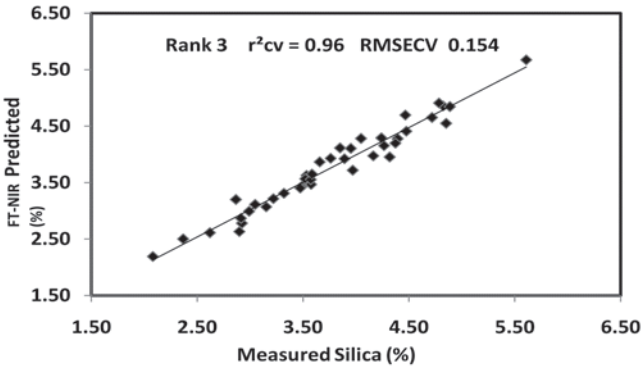

Figure 1. Correlation between NIR predicted and silica content for 40 samples of *O. travancorica* by cross validation

The suitability of the chosen data preprocessing methods, rank and of the frequency range for the given measurement task is evaluated during validation. The wave number range (4529-4116 cm^{-1}) and the pre-processing method (first derivative and multiplicative scatter correction, MSC) were taken for producing a good cross validation model for prediction of the silica (Table 3).

The coefficient of determination, r^2 and root mean square errors of calibration, RMSECV and TS for both samples A and B were analysed. All models led to almost identical model statistics. All models were calculated to a maximum rank of 10 and

Table 3. The PLSR model of silica by FT-NIR spectroscopy

Cross validation Model	Regression Coefficients (r^2_{cv})	RMSECV %	Wave number cm^{-1}	Data preprocessing	Rank
Silica	0.96	0.154	4529 - 4116	First derivative and MSC*	3

*MSC: Multiplicative Scatter Correction

Table 4. PLSR results of the calibration, cross validation (CV) and test set validation (TS) of the two sets (A and B)

Data set	Calibration			Cross validation			Test Set validation		
	RMSEE (%)	r^2	Rank	RMSECV (%)	r^2_{cv}	Rank	RMSEP (%)	r^2_p	Rank
CV all	0.140	0.96	3	0.154	0.96	3	-	-	-
Set A	0.106	0.98	3	0.150	0.96	3	0.184	0.94	3
Set B	0.162	0.96	3	0.194	0.94	4	0.155	0.96	3

CV all, cross validation of all samples (both A & B together)

the results of the calibration (r^2 and root-mean-square error of estimation, RMSEE), the cross-validation (r^2_{cv} and root mean square error of cross-validation, RMSECV) and the test set validation, (r^2_p and root-mean-square error of prediction, RMSEP) were compared (Table 4). The model statistics shows that no systematic difference between groups A and B exists. This proved the robustness of the model from the similarity results from calibration, cross validation and test set validation models.

Cross-validation with one sample omitted (leave-out-one CV) was performed for the internal validation. The PLSR model obtained for all calibration samples was subjected to a further CV step by increasing the number of samples left out during CV, as full CV may give over-optimistic results (Næs *et al.*, 2002). When up to 35 samples were left out during CV, the r^2_{cv} value decreased from 0.96 to 0.76 and RMSECV increased from 0.15 to 0.37%. Leaving out more samples also led to an increase in the rank from 3 to 4. Overall, the results were very similar and the robustness of the model was proven (Table 5). It is concluded that the model is stable.

Prediction of silica content of *O. travancorica*

Silica content of *O. travancorica* samples from different geographic locations were predicted with the PLSR model (cross validation) by using FT-NIR. Similar pre-treatment was also adopted for the prediction of unknown samples. The spectra of

Table 5. Results for cross-validation of the calibration samples.

Leave out	Rank	r^2_{cv}	RMSECV (%)
01	3	0.96	0.15
05	3	0.95	0.17
10	3	0.95	0.17
15	4	0.90	0.24
20	4	0.95	0.17
25	4	0.93	0.21
30	4	0.76	0.37
35	4	0.85	0.30

The number of samples left out during the cross-validation was increased from 1 to 35

unknown powdered samples were taken by using the fibre optic probe of FT-NIR spectrophotometer as mentioned before. The Opus/Quant method, created from the PLSR model with r^2_{cv} of 0.96 and RMSECV of 0.15% was used for the prediction of each sample from their respective spectrum. A one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (*P*d"0.05) was used to compare the silica content between the different geographic locations (Table 6a & b). Since the number of culm samples represented in each geographic source was unequal, statistical analysis was carried out separately after necessary statistical transformations.

Table 6a. Summary of the results of ANOVA of silica with 65 populations

Source of variation	Sum of Squares	df	MSS	F-value	Significance
Between groups	61121.055	259	235.989	1.215	.383
Within groups	2136.000	11	194.182		
Total	63257.055	270			

Table 6b. Summary of the results of ANOVA of silica with 50 populations

Source of variation	Sum of Squares	df	MSS	F-value	Significance
Between groups	42606.108	244	174.615	1.348	.315
Within groups	1295.500	10	129.550		
Total	43901.608	254			

The silica content varies from 3 to 5% among the populations from the different geographic locations (Table 7). However, the difference was not statistically significant between populations (*P*=0.05; Table 6a, b). Li *et al.* (2007) reported that the Klason lignin and ash contents of *Phyllostachys pubescens* did not show significant difference with age and it stabilizes in 3 years period after a linear increase upto to 2 years.

Table 7. The values of silica of *O. travancorica* from different geographic sources (*N=241*)

Geographic sources	No of culms	Silica%			
		Mean	SD	Maximum	Minimum
IDA	56	3.9	0.7	5.7	3.1
KUT	47	3.5	0.5	4.8	2.2
VAZ	12	4.2	0.9	5.9	3.0
SHO	23	3.9	0.6	5.1	3.0
ACK	22	3.4	1.0	5.7	2.3
PER	6	3.0	0.5	4.0	2.6
GUD	35	3.8	0.8	5.7	2.3
NBR	15	5.0	0.7	5.8	3.5
NM	6	3.8	0.1	4.9	3.1
TVM	11	4.0	0.6	5.5	3.5
ADI	5	3.7	0.6	4.2	2.8
KFRI	3	2.9	0.3	3.1	2.5

CONCLUSION

FT-NIR spectroscopy coupled with multivariate statistical techniques has been used to predict the silica content of *O. travancorica*. A good predictive model with high coefficient of determination, r^2_{cv} and low RMSECV was obtained from cross validation. The accuracy and precision of the PLSR model were predicted by comparing the statistical data of r^2, RMSECV, RMSEE and RMSEP. The results obtained indicated its suitability for predicting the silica content with high degree of accuracy. The FT-NIR spectroscopy is considered reliable and offers an excellent alternative to standard techniques for rapid measurements, when a large number of samples have to be analyzed which is otherwise a cumbersome process. The method can be used as a routine, rapid and non-destructive method for estimating the silica content of wood samples. The silica content of reed bamboo varies from 3 to 5% among the populations and showed non-significant differences between geographic locations.

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