Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS) 2 (1): 132-136
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jeteas.scholarlinkresearch.org

Application of Near Infrared Spectroscopy in Chemometric Modeling of Tannin Content and Stiasny Number of Pinus Caribaea Bark

[1,2]Derkyi, N.S.A., [2]Adu-Amankwa, B., [1]Sekyere, D., [3]Darkwa, N.A

[1]CSIR-Forestry Research Institute of Ghana
[2]Department of Chemical Engineering, KNUST
[3]Faculty of Renewable Natural Resources, KNUST
Corresponding Author: Derkyi, N.S.A

Abstract
Tannin content and Stiasny number of pine bark are related to bond strength in tannin-formaldehyde adhesives. This study was to develop standard calibration models for rapidly determining tannin content and Stiasny number of pine bark by using Fourier transform near infrared spectroscopy. Total of 110 spectra were collected and true values of tannin content and Stiasny number, obtained using the hyde powder test and Stiasny reaction respectively. Partial least squares method was used to develop calibration models for predicting tannin content and Stiasny number. Predicted values were highly correlated with costly measured values of tannin content and Stiasny number

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Keywords: spectra pre-processing, partial least squares regression, hyde powder test, stiasny reaction, calibration models, rapid prediction

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INTRODUCTION

Tannins from pine bark, like all the condensed tannins, consist of flavonoid units with varying degrees of condensation (Pizzi 1983), which can be used for the preparation of bio-adhesives for bonding wood. In the past, there has been considerable interest worldwide in the development of tannin wood adhesives as substitutes for wood adhesives derived from non- renewable resources, and in particular phenol and resorcinol which are derivatives from the petrochemical industry. Tannins from two hardwoods, wattle and quebracho, have been produced and used commercially for many years, but production of pine bark tannins has generally not been successful on a commercial scale (von Leyser 1990). Pine bark, however, is a good source of natural polyphenolic compounds for wood adhesives. Many attempts have been made to utilize it as a wood adhesive (Pizzi et al. 1993; Yazaki 1985). The earliest attempt to produce pine bark tannins for wood adhesives was reported in 1958 by Australian researchers (Booth *et al*. 1958). Two years later, New Zealand scientists tested pine bark tannins for bonding particleboards (Hall *et al*. 1960).

Near infrared reflectance spectroscopy (NIRS) is a rapid, cheap, environmentally-friendly (Norris et al 1976, Templeton et al 1983, Norris, 1989**)** and non-destructive technique offering the potential for accurate and repeatable measurements of chemical constituents in organic materials (Norris et al., 1976; Williams, 1975). The most widely used method of calibration is Partial Least Squares (PLS) regression (Haaland and Thomas 1998). Traditionally, NIRS has been used to determine the composition of agricultural products, but today, it is also used in other areas, such as the oil industry, pharmaceuticals, polymers, food industry, and clinical laboratories (Hall and Pollard, 1992). The Association of Official Analytical Chemists (1995) recommends use of this technique for the analysis of phenolic compounds.

In spite of the appearance of numerous research reports, bark tannins have not seen worldwide commercial applications. One of the main reasons is the tedious and time-consuming nature of determining the chemical properties of bark samples through wet chemical methods. The aim of this study therefore was to develop and fit regression models to tannin content and Stiasny number of *P. caribaea* bark. These models would be an easy tool for the rapid determination of tannin content and Stiasny number of *P. caribaea* bark. The chemical analysis was performed at CSIR-Forestry Research Institute of Ghana, whilst the near infrared spectra analysis was performed at CIRAD Foret, France.

MATERIALS AND METHODS

Materials

Pinus caribaea bark, obtained from 110 trees in plantation stands at the moist and dry semi deciduous forest zones of Ghana, were dried at 40°C for 48 h in a convection oven, ground in a Wiley mill to 100 - 250 µm particle size, sealed in a plastic bag, and stored at room temperature until use. All chemicals used were of analytical grade, obtained from commercial suppliers.

Hyde powder test

Tannin content was determined by the method of Roux (1951): For each sample, a mass of 800 mg were dissolved in 200 ml distilled water. Slightly chromated hyde powder (6 g) previously dried in vacuum for 24 hours over $CaCl_2$ was added and the mixture stirred for 1 hour at ambient temperature. The suspension was filtered without vacuum through a sintered glass filter. The weight gain of the hyde powder expressed as a percentage of the weight of the starting material was equated to the percentage of tannin in the sample. All samples were analysed in triplicate.

Stiasny Reaction

Reactive tannin content was determined by the method of Hillis and Urbach (1959): For each sample, a mass of 200 mg were dissolved in 20 ml distilled water. 2 ml of 10M HCl and 4 ml of formaldehyde (37%) were added and the mixture heated under reflux for 30 min. The reaction mixture was filtered whilst hot through a sintered glass filter. The precipitate was washed with hot water (5 x 10 ml) and dried over $CaCl_2$. The yield was expressed as a percentage of the starting material. All samples were analyzed in triplicate.

Near Infrared Spectra Aquisition

Each of the 110 powdered un-extracted bark samples was placed in a NIRSystem micro sample cup. Near-infrared (NIR) spectra were recorded on a Bruker FT-IR spectrometer to measure diffuse reflected light in the range 800 nm to 2,600 nm from the sample held in the spinning sample holder. With a spectral resolution of 20 cm^{-1} (678 data points), sixty four scans per spectrum were acquired and averaged. After the spectrum had been obtained, the sample cup was emptied, repacked with the next sample and the spectrum obtained. The background was taken with a Spectralon reference standard.

Chemometric Modeling

Chemometric modelling was performed with the OPUS software package (version 5.5). For each sample, one reflectance measurement of monochromatic light was made from 800 to 2600 nm to produce a spectrum with 678 data points at 2-nm intervals over this range. Reflectance (R) was converted to absorbance (A) using the following equation: $A = \log(1/R)$.

The relative reflectance spectra were smoothed using the Savitsky-Golay method at a gap of 25 data spectra. Once these preprocessing procedures were completed, partial least squares method (PLS) was used to develop calibration models for predicting the tannin content and Stiasny number. For each model, the standard error of calibration (SEC) was calculated for the calibration samples and the standard error of cross validation (SECV) was calculated for the predicted samples. The coefficient of determination (r^2) for each model was also calculated.

RESULTS AND DISCUSSIONS

Because of its high reactivity and slow solubility, the processing of pine tannin for commercialization has been a slow process. Pine tannins are considerably more reactive toward hardeners than mimosa or quebracho because of their more reactive phloroglucinolic A-ring in their monomeric flavonoid structure (von Leyser 1990). Pine tannins have a much higher average degree of polymerization than mimosa tannins. The adhesive property of a tannin compound depends on the reactive tannin groups present. The higher the number of reactive tannin groups, the greater the crosslinking capacity and therefore the greater the strength of the cured glue line.

The Stiasny reaction gives an indication of the number of reactive tannins. In this study, the values of tannin content and Stiasny number of *P. caribaea* bark determined through wet chemical analysis are shown in Table 1.

Table 1: Descriptive statistics for tannin content and Stiasny number of *P. caribaea* bark samples. N: total number of samples statistically analysed.

Parameter	N	Range	Mean	Standard deviation
Tannin content (%)	110	9.2 – 15.4	11.82	0.73
Stiasny number	110	64.36 – 86.12	74.97	4.91

Any interpretation of calibration results depends greatly on the precision of the wet chemical analysis of the samples. The tannin content of *P. caribaea* bark varied from 9.2 % to 15.4 %, with a mean and standard deviation of 11.8% and 0.73% respectively. The tannin content measurements (*N*=110) were fairly normally distributed around the mean. Similarly, the Stiasny number of *P. caribaea* bark varied from 64.36% to 86.12% with a mean and standard deviation of 74.97 and 4.91 respectively. The Stiasny number measurements (N =110) were also fairly normally distributed around the mean. The values determined for both tannin content and Stiasny number of the samples covered a large enough range, which was helpful for developing the models.

With the near-infrared reflectance technique, the sample to be analyzed is subjected to NIR radiation; the sample surface layers absorb part of the energy, while the rest is dispersed in all directions. The dispersed light gives information about the composition of the test sample and the resulting spectrum is used in qualitative and quantitative analysis (Kubelka and Munk 1931; Kubelka 1948). The best frequency intervals are in the region of the spectrum in which the reflected light intensity best correlates with the concentrations of the analyzed chemical species. Figures 1 and 2 show typical relative reflectance spectra of highest, lowest and two other intermediate values of tannin content and Stiasny number respectively.

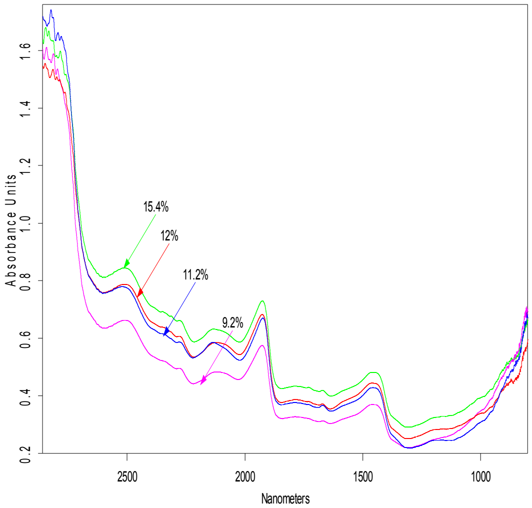

Fig 1: NIR spectra of four *P. caribaea* bark samples with different tannin contents indicated.

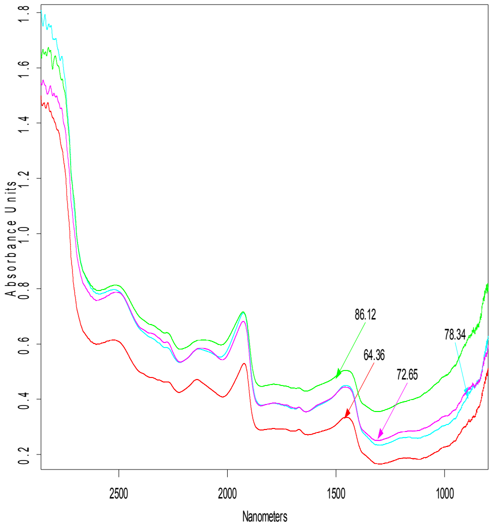

Fig 2: NIR spectra of four *P. caribaea* bark samples with different Stiasny numbers indicated.

Since the spectral data below 800 nm and above 2600 nm contained considerable noise, the wavelengths of 800 nm to 2600 nm were used in this study. Several bands in these regions showed variations in absorbance that could be attributed to a general trend of increasing or decreasing Stiasny number as observed by Schimleck and Yazaki (2003).

The flexibility of NIRS makes it possible to manipulate a set of samples and to choose the best mathematical regression for obtaining the calibration equation (Mark**,** 1989). The most appropriate mathematical model is used to predict the composition of samples that needs to be determined. In this study, the 'leave-one-out' cross validation of the PLS algorithm automatically yielded regression models. Table 2 presents the results obtained in the calibration process and summary statistics for both models using the partial least-squares method. These results show that, for both tannin content and Stiasny number, the coefficient of determination was high. These values represent a reduction in the standard error of calibration (SEC) and the standard error of cross validation (SECV). These figures can thus be used as an indicator of validation (Stone 1974).

Table 2: PLS regression calibration developed for tannin content and Stiasny number.

Model	N	Rank	SEC	SECV	SEC/SD	SD/SECV	SECV /SEC
TC (%)	110	9	0.12	0.14	0.16	5.21	1.16
SN	110	9	1.25	1.26	0.25	3.89	1.00

TC: Tannin content; SN: Stiasny number; SD: standard deviation; N: number of samples

The coefficient of determination, r^2, (Fig. 3 and 4) and the minimum value for the standard error of cross-validation, SECV, as well as the standard error of calibration (Table 2), were found. And for each model, the slope of the calibration curve was close to 1.0 (Fig. 3 and 4) indicating a good calibration.

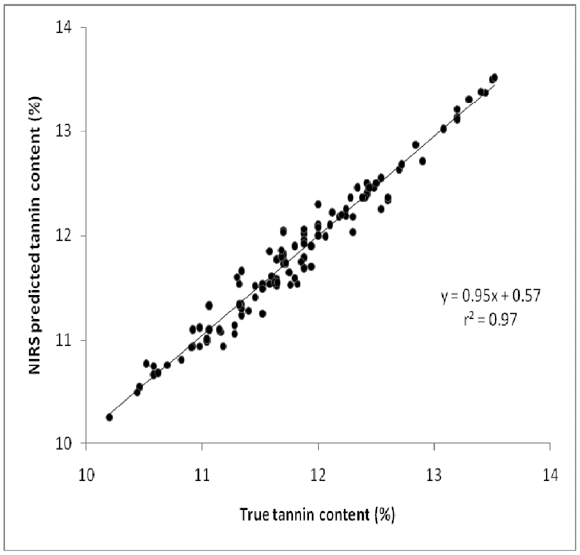

Fig 3: True versus predicted values for model estimating tannin content

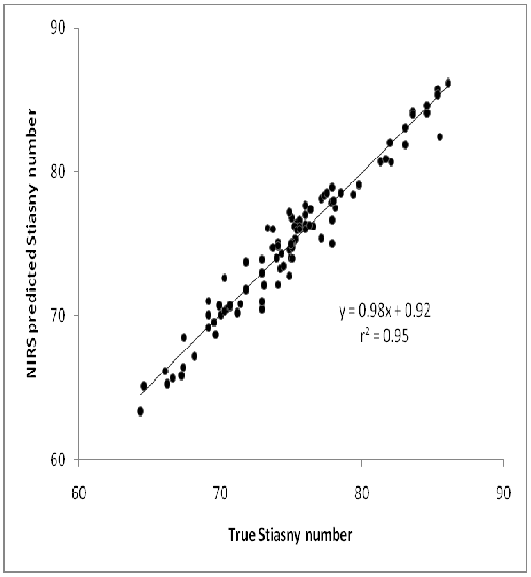

Fig 4: True versus predicted values for model estimating Stiasny number

The correlation between the measured and predicted values of the parameters was higher for tannin content than for Stiasny number. Thus the tannin content model had a better predictive ability than the Stiasny number model. Schimleck and Yazaki (2003) used NIR spectroscopy to estimate Stiasny number from the bark of pine reporting a correlation coefficient of 0.96 which is in close agreement to that obtained in this study.

CONCLUSION

With current environmental and health concerns on petroleum-derived phenols in adhesive manufacture, tannins play a very important role and have a wide range of applications. As a consequence of this, it is very important to provide very fast, more accurate and precise quantitative method for the analysis of tannins and Stiasny reaction in biomass samples.

By means of PLS multivariate calibration techniques, relationships were established between reflectance spectra and tannin content and Stiasny number of *P. caribaea* bark. The first derivative spectra with PLS regression were found to provide the best prediction of the tannin content and Stiasny number of *P. caribaea* bark with a SECV = 0.14 and 1.26 and r^2 = 0.97 and 0.95 respectively. The predicted values were highly correlated with destructively measured values of tannin content and Stiasny number.

Thus, NIRS can be used to predict the concentration of tannins and Stiasny number after calibration from sets with known values. However, for precise NIRS estimation, the composition of the new sample must be within the range of variation included in the calibration set. Application to other population samples, will probably require addition of new population variations to the former calibration set. During the calibration process, the scores- and loading-vectors were calculated, as well as the calibration function *b*. The resulting values, stored in databases, are available for the analysis of new samples.

ACKNOWLEDGEMENT

The first author expresses his gratitude to CIRAD Foret for the use of the near infrared spectrometer.

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