

Research Article

Rapid Determination of Total Nitrogen, Ammonium and Nitrate Nitrogen in Vinegar Residue Growing Substrates using Near-Infrared Spectroscopy

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Abstract: The objective of this study was to investigate the feasibility of using Near-Infrared Spectroscopy (NIRS) to determine the total nitrogen, ammonium and nitrate nitrogen in growing substrates of vinegar residues. Samples of 106 substrates were collected from the cultivation experiments of vinegar residue substrates. Spectra of the visible and near infrared region (350-2500 nm) were obtained for all samples after drying and grinding (variant A) or in a moist state (variant B). Across validation was carried out using a partial least square method which was based on the entire spectra. For parameters TN, NO_3^- and NH_4^+ , precisions of the calibration and prediction models were inconsistent between variant A and B. For variant A, the prediction coefficient (r), Root Mean Squared Error of Cross-Validation (RMSECV) and the ratio of standard deviation and RMSECV (RSC) were 0.97, 0.64 g/kg and 4.4 for TN, 0.58, 15.4 mg/kg and 1.2 for NO_3^- , 0.79, 0.75 g/kg and 1.5 for NH_4^+ , respectively. For moist samples, the r, RMSECV and RSC were 0.79, 1.81 g/kg and 1.6 for TN, 0.85, 9.95 mg/kg and 1.9 for NO_3^- , 0.92, 0.45 g/kg and 2.5 for NH_4^+ , respectively. The results indicate a marked usefulness of NIRS in the nitrogen assessment and management of growing substrates, especially for TN and NH_4 . However, further research is needed to improve the prediction precision of calibration models by enlarging the number of samples and using other growing substrates.

Keywords: Detection, growing substrate, near-infrared spectroscopy, nitrogen

INTRODUCTION

In greenhouse horticulture, one of increasing interests was showed on looking for good quality and local available low cost substitutes of peat. Furthermore, the need for recycling wastes and increasing environmental pressures against peat extraction leads to the increasing interest. A number of composts obtained from different organic materials have been applied as the potential alternative substrates and had proved to be very promising (Garcia-Gomez *et al.*, 2002; Guo, 2005; Ribeiro *et al.*, 2007). In substrate cultivation, the fertility was an important agronomic characteristic. It was needed to detect the nitrogen nutrition parameters (including total-N and available-N) timely and accurately. However, conventional chemical methods usually require numerous reagents, skilled labor and longer measurement period in order to determine these parameters.

Near Infrared Spectroscopy (NIRS) is a sensitive, fast and non-destructive analytical technique providing simplicity in the preparation of samples. NIRS was applied to predict the organic C and total N contents, the stage of transformation of organic matter during the composting process of sewage sludges, green and yard wastes (Smidt and Meissl, 2007; Albrecht *et al.*, 2008).

NIRS was indicated as a useful tool to assessment the quality and the maturity of cattle wastes, sewage sludge during composting processes (Nakatani *et al.*, 1996; Malley *et al.*, 2005; Fan *et al.*, 2006; Grube *et al.*, 2006). Ludwig *et al.* (2006) revealed that NIRS could predict well the chemical parameters of peat and growing media such as pH and contents of salt, P and K for samples both after drying and grinding or in a moist state, NH_4^+ content in that after drying and grinding, but nitrate contents were predicted unsatisfactorily. Malley *et al.* (2005) and Huang *et al.* (2010) reviewed that it was feasibly and efficient using NIRS for determination Total Organic Carbon (TOC), Total Nitrogen (TN), pH and EC of animal manure compost.

NIRS was indicated as a useful tool to monitor the composting process of sewage sludge with wood chips and maturity and to evaluate the degradation rate and thus determine the maturity (Grube *et al.*, 2006). However, few reports were found in the literature using this tool to study the cultivation substrates obtained from different organic materials. The objective of this study was to determine the accuracy of NIRS for the changes of Total Nitrogen (TN) and available-N (NO_3^- , NH_4^+) contents in growing substrates and to determine the optimum spectral pretreatment for the substrates samples.

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MATERIALS AND METHODS

Substrate samples and preparation: Samples were obtained from different stages of the cultivation experiments with growing substrates of vinegar residue wastes. The collection lasted 4 months in order to obtain a sample number which was sufficient for the Near Infrared (NIR) study. All samples were stored at 4°C directly after collecting. There were 106 substrate samples obtained in this study. The sample was divided into two parts. One part was dried at 65°C in a forced-air drier to a constant weight for moisture measurement; the other was used for spectra analysis and chemical analysis for TN, NO₃⁻ and NH₄⁺. During storage, the characteristics of the growing substrates were determined (Table 1).

Chemical analyses: For TN measurement, 0.1 g portion of ground sample (<1 mm) was digested with 5 mL of sulfuric acid in an oven and then cooled. Once the sample had cooled to room temperature, the volume was made up to 100 mL with distilled water. The contents of soluble NO₃⁻ and NH₄⁺ were determined by adding 50 mL of 0.01 M CaCl₂ to 5 g of fresh samples (moisture content as received). Then the suspension was shaken for 0.5 h and filtered through a fine-pored filter. All the above soluble samples were stored at 4°C until analysis. The contents of Total Nitrogen (TN), NO₃⁻ and NH₄⁺ were measured by continuous flow analyzers (Seal analytical company, UK) and were calculated on dried weight basis.

NIRS spectral reflectance measurements: The samples were either measured after drying and finely grinding (1 mm, variant A) or in a moist state (variant B). The diffuse reflectance spectra analysis were recorded at 1 nm intervals in the Vis-NIR region (350-2500 nm) using a Field Spec 3 spectrometer (analytical spectral device company, USA). Samples were divided in two sets (66 for the calibration set and 40 for the prediction set). The number of spectral data was 2151 for each sample. The average of ten measurements from each sample was taken and the values transformed to absorbance spectra (log [1/reflectance]).

Statistical analysis: All spectra were manipulated and processed and all calibration equations were obtained using NIRSA software (the registry number 2007SR06801). Partial Least Square Regression

(PLSR) was used to relate the spectral data with the analyzed parameters.

The PLSR method is a multivariate linear calibration technique that reduces large sets of raw data into small numbers of orthogonal (non-correlated) factors so as to minimize the error sum of squares among the values to be predicted. The optimum number of PLS factors used was determined by cross-validation. Sixty six samples were used to calibrate the model and 40 samples were used for the cross validation of the model. Residuals give the Standard Error of Cross-Validation (SECV) on independent samples. The final model was then recalculated with all the samples to obtain the Standard Error of Calibration (SEC). Data analyses were conducted using the SPSS software system (Zhu *et al.*, 2011). Prior to calibration, typical spectroscopic pretreatment of the spectra was performed, including Moving Average Filter (MAF), Savitzky-Golay Filter (SGF), First Derivative (FD), Normalization (NM) and the combination of these methods.

RESULTS

Chemical composition and NIRS spectra analysis:

Table 1 shows the composition statistics in calibration sets and validation sets for nutrient contents on dried weight basis, which include the minimum, maximum, mean, standard deviation and coefficient of variance. For all samples, the means and standard deviations of nutrient content were 28.5±2.9 g/kg for TN, 29.2±19.5 mg/kg for NO₃⁻, 4.5±1.2 g/kg for NH₄⁺.

Figure 1 and 2 shows the NIRS spectra of fresh and dried samples, respectively. The two biggest peaks were observed at the wavelength of about 1450 and 1930 nm in the spectra of fresh samples, mainly due to the absorption of water.

Calibration and validation for TN, NO₃⁻, NH₄⁺:

Several pretreatment options were investigated. Moving Average Filter (MAF), Savitzky-Golay Filter (SGF), First Derivative (FD), Normalization (NM), FD after MAF (MAF+FD), FD after SGF (SGF+FD) and FD after NM (NM+FD) with a window size of 5 data points were tested respectively. Partial Least Square Regression (PLSR) with the pretreatment methods selected above was used to develop calibrations.

Table 1: Statistics for contents of TN, NO₃-N and NH₄-N in substrates of vinegar residue

	Samples	Min.	Max.	Mean	S.D.	C.V. %
TN (g/kg)	Calibration (n = 66)	23.0	35.6	28.4	3.0	10.5
	Validation (n = 40)	23.7	34.9	28.6	2.8	9.6
NO ₃ ⁻ (mg/ kg)	Calibration (n = 66)	4.3	80.7	30.2	20.5	67.8
	Validation (n = 40)	8.7	79.8	27.5	17.7	64.4
NH ₄ ⁺ (g/kg)	Calibration (n = 66)	0.1	6.0	4.4	1.2	28.2
	Validation (n = 40)	1.3	5.8	4.6	1.1	24.3

TN: Total nitrogen; Min.: Minimum; Max.: Maximum; S.D.: Standard deviation; C.V.: Coefficient of variance

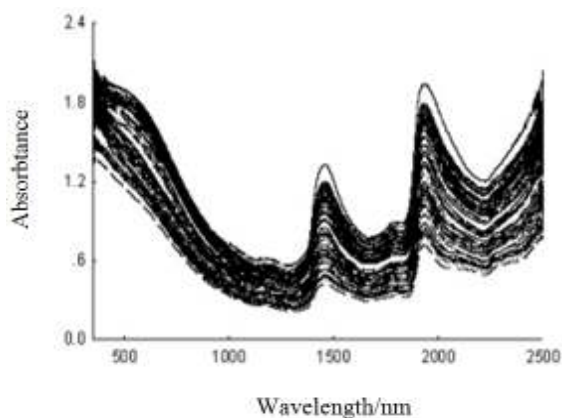


Fig.1: Typical reflectance spectra of calibration set of fresh samples

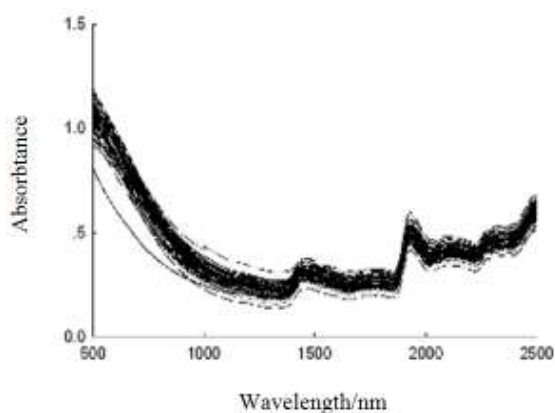


Fig.2: Typical reflectance spectra of calibration set of dried and ground samples

Table 2 shows statistics parameters of calibrations and validations of TN, NO_3^- and NH_4^+ based on the best pretreatment method choose. The treatment of the samples, either measuring the NIR spectra after finely grinding them (variant A) or in moist state (variant B), did not have a consistent effect on the statistical parameters: prediction of TN was more successful for variant A, whereas that of NO_3^- and NH_4^+ , variant B gave more accurate predictions (Table 2).

For TN, R, r and RSC values of variant A were 0.98, 0.97 and 4.4, respectively. But for variant B, they were 0.95, 0.79 and 1.6, respectively. For NH_4^+ , the r and RSC values of variant B were 0.92 and 2.5, which were higher than that of variant A.

Comparing with TN and NH_4^+ , the precisions of the calibration and prediction model for NO_3^- were lower. The r was 0.58 for variant A and B and 0.85 for variant B, respectively and the two RSCs were lower than 2.0.

Scatter plots of predicted vs. actual values for these 3 parameters are presented in Fig. 3. It represented

better validations of calibration models for the 3 parameters between the variant A and B.

DISCUSSION

There were two biggest peaks at the wavelength of about 1450 and 1930 nm in the NIRS spectra of fresh samples, which were mainly due to the absorption of water. The peak at 1450 nm may be caused by the first overtone with O-H stretching. A combination band of O-H stretching and O-H deformation may cause the peaks at near 1940 and 1440 nm (Lu, 2007). Comparing Fig. 1 with Fig. 2, the spectra of fresh samples showed the strong influence of water.

Pretreatment of spectral data was very important because it either fully or partly eliminates the systematic error, which could be caused by various factors. Several pretreatment options were investigated and contrasted. The choose best pretreatment method was showed in Table 2. For TN, NO_3^- and NH_4^+ , the best method was different between the variant A and B, respectively.

For the calibrations, lower Root Mean Squared Error of cross-validation (RMSE), higher values and the highest stability index are considered better and more accurate (Windham *et al.*, 1987; Ludwig *et al.*, 2006). According to Ludwig *et al.* (2002) and Chang *et al.* (2001) the accuracy of NIR predictions can be described as follows: good predictions have a correlation coefficient greater or equal to 0.9, a regression coefficient in the range from 0.9 to 1.1 and a RSC (ratio of standard deviation of the laboratory results to standard error of cross validation) greater than 2. Satisfactory results are obtained for $0.8 \leq r \leq 1.2$, $r \geq 0.8$ and $1.4 \leq \text{RSC} \leq 2.0$ (Ludwig *et al.*, 2006).

The treatment of the samples, either measuring the NIR spectra after finely grinding them (variant A) or in moist state (variant B), did not have a consistent effect on the statistical parameters: prediction of TN was more successful for variant A, whereas that of NO_3^- and NH_4^+ , variant B gave more accurate predictions (Table 2).

Comparing with TN and NH_4^+ , the precisions of the calibration and prediction model for NO_3^- were lower. The r was 0.58 for variant A and B and 0.85 for variant B, respectively and the two RSCs were lower than 2.0. Similarly, Ludwig *et al.* (2006) reported for peat and growing media nitrate contents was predicted unsatisfactorily. However, the NO_3^- contents were predicted satisfactorily only for a less diverse subpopulation of samples which were all dried and ground growing media.

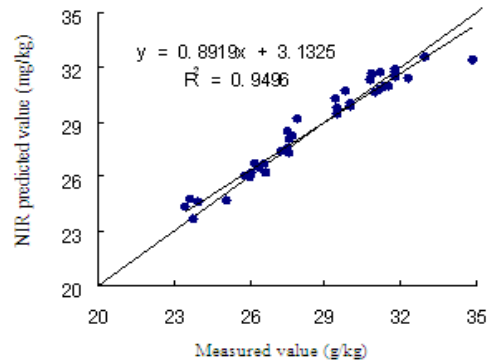
Such inconsistent results between variant A and B may come from the following reasons:

On the one hand NIR measurements of grinding samples should give more accurate results, because grinding reduces the effects of particle size on the

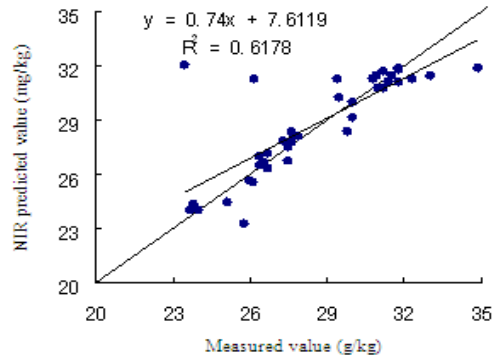
Table 2: Statistics of calibration and validation for the chemical parameters based on the choose best pretreatment method

Chemical parameter	Treatment ^a	Spectral treatment ^b	Spectra range (nm)	Calibration set			Validation set				
				f	R	RMSECC	r	RMSECV	RSC	Bias	Slope
TN (g/kg)	A	MAF	350-2500	10	0.98	0.48	0.97	0.64	4.4	0.90	3.13
	B	MAF+FD	350-2500	3	0.95	0.87	0.79	1.81	1.6	0.74	7.61
NO ₃ ⁻ (mg/N kg)	A	+FD	350-2500	15	0.86	9.95	0.58	15.40	1.2	0.39	19.50
	B	FD	350-2500	15	0.89	9.08	0.85	9.95	1.9	0.73	9.24
NH ₄ ⁺ (g/kg)	A	SGF	350-2500	13	0.98	0.22	0.79	0.75	1.5	0.76	1.30
	B	NM	350-2500	6	0.96	0.35	0.92	0.45	2.5	0.82	0.87

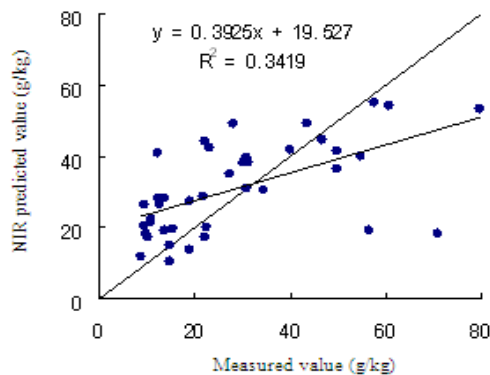
TN: Total nitrogen; a: Treatment A refers to the NIR measurement of dried and ground samples and treatment B to the measurement of samples with a moisture content as received; b: Pretreatment of the spectra, the number of gap between points used to calculate the difference or that of data points used to smooth the data is 5; f: The number of principal component factor; R: The correlation coefficient for calibration set; RMSECC: Root mean squared error of calibration; r: The correlation coefficient for prediction set; RMSECV: Root mean squared error of cross-validation; RSC: The ratio of standard deviation and RMSECV



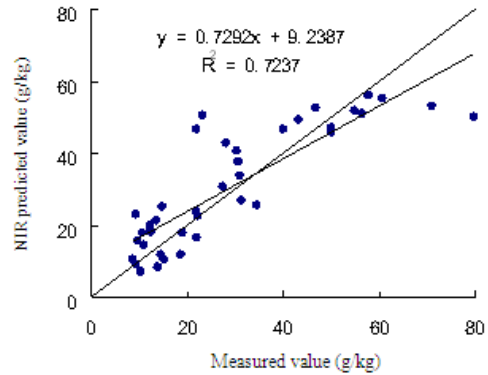
(a) TN (drying and grinding samples)



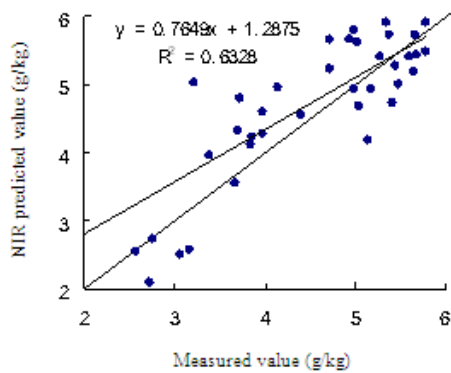
(b) TN (moist samples)



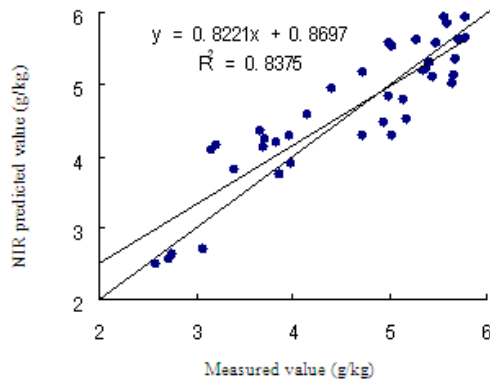
(c) NO₃⁻ (drying and grinding samples)



(d) NO₃⁻ (moist samples)



(e) NH₄⁺ (drying and grinding samples)



(f) NH₄⁺ (moist samples)

Fig. 3: Measured against predicted values for TN, NO₃⁻ and NH₄⁺ based on dry matter content

spectra (Foley *et al.*, 1998; Cen *et al.*, 2011) however, on the other hand. Drying may affect the composition of organic materials considerably. Varying degrees of the moist may have significant effect on the reflectance spectroscopy (Zhu *et al.*, 1984). Moreover, presence of water may complicate the calibration because of marked absorbance of water in some regions which may mask peaks of organic functional groups (Chang *et al.*, 2001). Thus, for moist samples a greater number of samples might be required for a calibration to reduce the effects of water absorbance and particle size.

It represented better validations of calibration models for the 3 parameters between the variant A and B (Fig. 3). Especially for TN and NH_4^+ , the results showed that NIRS is a useful method for quantitative analysis on drying-grinding and moist basis, respectively.

CONCLUSION

This study explored the usefulness of near infrared spectroscopy to predict the nitrogen nutrient content of cultivation substrates of vinegar residue. It was demonstrated that NIRS is a potential method to predict TN and NH_4^+ contents. Furthermore, NIRS is rapid and makes it possible to analyze a large number of samples in a practical and timely manner. In addition, because it is a cheap and non-destructive method, NIRS is an invaluable tool in analyzing the nutrients' change and for accurate fertilization. However, further research is needed to improve the prediction accuracy for NO_3^- .

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