*African Journal of Rural Development*, Vol. 8 (3): July-September 2023: pp.242-249 ISSN 2415-2838



# Near-Infrared Reflectance Spectroscopy as Tool for Chemometric Analysis in Sweet Potato Vines and Cassava Foliage

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## ABSTRACT

The study was conducted to evaluate near-infrared reflectance spectroscopy (NIRS) as a non-invasive and environment-friendly technique for predicting nutrients content and digestibility of sweet potato vines (SPV) and cassava foliage (CSF). Dried vines from 13 sweet potato with 65 NIRS spectra data captures and foliage from 16 cassava varieties with 80 NIRS spectra data captures were used for the study. In the SPV, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), non-fibre carbohydrates, *beta*-carotene (250 ± 22.2 µg/100 g) and vitamin A (416 ± 37.1 IU/100 g) contents were well-predicted ($p < 0.05$) using NIRS with coefficients of determination ($R^2$) ranging from 0.52 to 0.81 and root mean squared errors of prediction (RMSEP) varying from 1.42 to 89.05 in SPV. There were no significant correlations ($p > 0.05$) between the beta-carotene content of the SPV and the recorded nutrient contents like DM, CP, NDF, ADF, non-fibre carbohydrates (NFC) and total digestible nutrient (TDN) values. Similarly, in the CSF, mean (± s.e.) recorded *in-vitro* 48 hr digestibility of DM (IVDMD) was 57.9 ± 1.47% and NDF (IVNDFd) was 80.4 ± 1.50%. Nutrient contents and *in-vitro* 48 h digestibility fitted well with $R^2$ varying from 0.57 for NDF to 0.94 for ADF while, RMSEP was about 0.35 for DM, NDF and IVDMD predictions. The IVDMD was predictable from ADF and TDN contents while, IVNDFd was predictable from NDF, ADF and TDN contents. The results suggest that NIRS technique can be developed as a rapid and accurate tool to predict the chemical composition and nutrient digestibility of samples of sweet potato vines and cassava foliage cultivars.

**Keywords:** Beta-carotene, cassava foliage, *in-vitro* digestibility, Near-infrared reflectance spectroscopy, Nutrient contents, sweet potato vines.

## RÉSUMÉ

L'étude a été menée pour évaluer la spectroscopie proche infrarouge (NIRS) comme technique non invasive et respectueuse de l'environnement pour prédire la teneur en nutriments et la digestibilité des vignes de patate douce (SPV) et du feuillage de manioc (CSF). Des vignes séchées de 13 patates douces avec 65 captures de données spectrales NIRS et du feuillage de 16 variétés de manioc avec 80 captures de données spectrales NIRS ont été utilisées pour l'étude. Dans le SPV, les teneurs en protéines brutes (CP), fibres au détergent neutre

**Cite as:** Etela, I., Paliwal, J., Afuape, S.O., Ingweye, J.N. and Iriso, B.V. 2023. Near-Infrared Reflectance Spectroscopy as Tool for Chemometric Analysis in Sweet Potato Vines and Cassava Forage. *African Journal of Rural Development* 8 (3): 242-249.

(NDF), fibres au détergent acide (ADF), glucides non fibreux, bêta-carotène (250 ± 22,2 µg/100 g) et vitamine A (416 ± 37,1 UI/100 g) ont été bien prédites ($p < 0,05$) en utilisant le NIRS avec des coefficients de détermination (R2) allant de 0,52 à 0,81 et des erreurs quadratiques moyennes de prédiction (RMSEP) variant de 1,42 à 89,05 dans le SPV. Il n'y avait pas de corrélations significatives ($p > 0,05$) entre la teneur en bêta-carotène du SPV et les teneurs en nutriments enregistrées comme DM, CP, NDF, ADF, glucides non fibreux (NFC) et les valeurs de nutriments totaux digestibles (TDN). De même, dans le CSF, la digestibilité in-vitro moyenne (± s.e.) de 48 heures de la DM (IVDMD) était de 57,9 ± 1,47% et celle de la NDF (IVNDFd) était de 80,4 ± 1,50%. Les teneurs en nutriments et la digestibilité in-vitro de 48 h correspondaient bien avec des R2 variant de 0,57 pour NDF à 0,94 pour ADF, tandis que le RMSEP était d'environ 0,35 pour les prédictions de DM, NDF et IVDMD. L'IVDMD était prédictible à partir des teneurs en ADF et TDN, tandis que l'IVNDFd était prédictible à partir des teneurs en NDF, ADF et TDN. Les résultats suggèrent que la technique NIRS peut être développée comme un outil rapide et précis pour prédire la composition chimique et la digestibilité des nutriments des échantillons de vignes de patate douce et de feuillage de manioc.

**Mots clés** : Bêta-carotène, feuillage de manioc, digestibilité *in-vitro*, spectroscopie proche infrarouge, teneurs en nutriments, vignes de patate douce

## Introduction

Conventional tools (*in-vivo* or *in-situ*, *in-sacco* or nylon bag, and *in-vitro* or test-tube gas production techniques) for determining forage and crop residue quality in agriculture are expensive, time consuming, and require large quantities of feed samples and laboratory chemical use. Near-infrared reflectance spectroscopy (NIRS) promises to be a better option because it is rapid, accurate, non-invasive and does not require the use of hazardous chemicals. Application of NIRS technique in crop improvement programmes for cassava (*Mannihot esculenta* Crantz) could boost its cultivation effort by the Nigerian Government under the Cassava Value Chain Project and promote the cultivation of orange-fleshed sweet potato (Mbanjo *et al*., 2022). The use of sweet potato vines (SPV) and cassava foliage (CSF) as livestock feed is also well reported.

The genetic basis of *beta*-carotene (a vitamin A precursor) in sweet potato and cassava suggests that the NIRS technique could support food-based approach to vitamin A supplementation against early child mortality and high risk factor for pregnant women and lactating mothers in Africa. Studies have shown that NIRS is well-adapted to the conditions in developing countries because, it is a low-cost technique and can be used for accurately screening a large number of samples per time (Etela *et al*., 2009;Lebot *et al*., 2009). However, only limited information about the application of NIRS technique on sweet potato vines and cassava foliage exist. Thus, the study investigated the possibility of using NIRS technique for detecting *beta*-carotene and other quality attributes in sweet potato and cassava forage from different clones.

## Materials and Methods

**Sample collection and management**. Vine samples from 13 white- and orange-fleshed sweet potato cultivars were obtained from scientists at the National Root Crops Research Institute (NRCRI), Umudike, Nigeria while, root and foliage samples from 16 cassava cultivars were obtained from the International Institute of Tropical

Agriculture (IITA), Onne sub-Station, Nigeria. The samples were then oven-dried at 60 °C for 72 h and later milled to pass through a 1.0 mm screen using a laboratory hammer mill and stored in air-tight whirl packs for subsequent analyses.

**In-vitro studies**. A portion of the 1.0 mm size sweet potato and cassava forage samples was later analysed for crude protein (CP) according to the micro-Kjeldahl method (AOAC, 2002). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined according to the procedures described by Van Soest and Robertson (1985). A second portion of the sweet potato vine samples was used to determine *beta*-carotene and vitamin A contents while, the second portion of the 1.0 mm size cassava forage samples was used for *in-vitro* 48 h digestibility of DM (IVDMD) and neutral detergent fibre (IVNDFd) as additional data to calibrate and validate the NIRS predictions.

**NIRS Analysis**. Finally, a third portion of the dried 1.0 mm forage samples was scanned using an Optiprobe System (NIRSystem Model 6500 Spectrophotometer) at the Advanced IR Laboratory of the Institute for Biodiagnostics, National Research Council, Canada. Spectra were collected in the wavelength range of 1100 nm to 2500 nm at 2 nm intervals and recorded as the absolute absorbance, which was defined as:

$$A = \log(\frac{1}{R}) \qquad (1)$$

where: A denotes the absolute absorbance, and *R*the reflectance signal of sample. The absorbance values were later transformed to the second derivative spectra to remove sloping and curving baseline effects. The transformed data were then analyzed using the PLSplus/IQ module of GRAMS AI software (Thermo Corp., Woburn, MA) to calibrate linear prediction models for different samples. Cross-validation was used to determine the model complexity. The data were randomly split into two groups: 80% for calibration set (52 SPV samples; 64 CSF samples), while the remaining 20% (13 SPV samples; 16 CSF samples) were used as validation/testing set. Linear regression and correlation calculations were then performed to establish the relationship between the reference values (wet chemistry) and the NIR predictions.

**Statistical analyses. W**ere done using PROC Means and PROC Corr procedures of the SAS software package (SAS Institute Inc., 2002). Where significant Pearson correlation coefficient ($r^2$) was observed between *beta*-carotene, IVDMD, IVNDFd and nutrient contents, linear regression equations were developed for their relationship.

## Results and Discussion

**Sweet potato vine**. There were wide variations ($p < 0.05$) in nutrients (crude protein, CP; neutral detergent fibre, NDF; acid detergent fibre, ADF; non-fibre carbohydrates, NFC; total digestible nutrients, TDN), *beta*-carotene and vitamin A contents recorded for the vines from the varieties used (Table 1). The variations in nutrient contents suggest that the sweet potato varieties could be selected as livestock feeds to supplement low quality feeds based on their nutrient contents predicted using the NIR technique. The variations in chemical composition of sweet potato vine varieties could, largely, be attributed to differences in genotype.

Linear regression equations indicating the relationship between the actual and the NIR predicted vine quality parameters are shown in Table 2.The high root mean squared error of prediction (RMSEP) for CP, ADF, NFC, *beta*-carotene and vitamin A indicate the reliability of the NIR prediction. However, the results did not detect any correlation ($p > 0.05$) between *beta*-carotene and the other vine nutrients (Table 3)

Table 1**.** Variations in nutrient, *beta*-carotene, and vitamin A contents in the vines from 13 sweet potato varieties

| **Chemical composition** | **Min.** | **Max.** | **Mean ± s.e.** |
|---|---|---|---|
| Nutrient content (%): | | | |
| Dry matter (DM) | 93.3 | 94.4 | 93.8 ± 0.10 |
| Crude protein (CP) | 11.7 | 27.5 | 18.6 ± 1.23 |
| Neutral detergent fibre (NDF) | 38.9 | 47.9 | 43.5 ± 0.75 |
| Acid detergent fibre (ADF) | 33.2 | 42.6 | 38.2 ± 0.75 |
| Non-fibre carbohydrates (NFC) | 18.4 | 38.6 | 27.0 ± 1.71 |
| Total digestible nutrients (TDN) | 56.9 | 61.6 | 59.1 ± 0.38 |
| *Beta*-carotene (µg/100 g) | 117 | 370 | 250 ± 22.2 |
| Vitamin A (IU/100 g) | 200 | 620 | 416 ± 37.1 |

Table 2. Linear regression and correlation between actual (wet chemistry) nutrient , beta carotene and Vitamin A contents and NIR predictions in the vines from 13 sweet potato varieties

| | **Mean ± s.d.* (n = 65)** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Forage quality parameter** | **Actual (Y)** | **Predicted (X)** | **Regression equation** | **$R^2$** | **SEC** | **SEP** | **RMSEP** |
| Nutrient content (%): | | | | | | | |
| Dry matter (DM) | 93.8 ± 0.38 | 93.7 ± 0.28 | Y = 0.85X + 13.84 | 0.41 | 0.10 | 0.08 | 0.288 |
| Crude protein (CP) | 17.5 ± 4.19 | 17.3 ± 4.05 | Y = 0.93X + 1.40 | 0.81 | 1.16 | 1.12 | 1.777 |
| Neutral detergent fibre (NDF) | 40.8 ± 2.55 | 41.4 ± 1.81 | Y = 1.21X – 9.19 | 0.74 | 0.71 | 0.50 | 1.415 |
| Acid detergent fibre (ADF) | 35.8 ± 2.56 | 36.5 ± 1.42 | Y = 1.29X – 11.32 | 0.52 | 0.71 | 0.40 | 1.878 |
| Non-fibre carbohydrates (NFC) | 25.4 ± 5.75 | 25.0 ± 5.12 | Y = 1.01X + 0.10 | 0.81 | 1.59 | 1.42 | 2.428 |
| Total digestible nutrients (TDN) | 55.4 ± 1.28 | 55.1 ± 0.53 | Y = 1.51X – 27.80 | 0.40 | 0.35 | 0.15 | 1.049 |
| *Beta*-carotene (µg/100 g) | 250 ± 80.1 | 232 ± 85.3 | Y = 0.75X + 76.81 | 0.63 | 22.2 | 23.7 | 54.23 |
| Vitamin A (IU/100 g) | 416 ± 133.8 | 386 ± 143.5 | Y = 0.75X + 126.85 | 0.65 | 37.1 | 39.8 | 89.05 |

*s.d. = standard deviation; $R^2$ = coefficient of determination; SEC = standard error of calibration; SEP = standard error of prediction; RMSEP = root mean squared error of prediction.

The recorded lack of correlation between *beta*-carotene and the other nutrients in the SPVs suggests that sweet potato varieties could be selected for higher *beta*-carotene contents by plant breeders without sacrificing other nutrients inbreeding programmes. Increasing sample size could detect possible relationship. Earlier studies have demonstrated that, the successful use of NIRS technique for predicting starch physiochemical quality and pasting properties in sweet potato (Lu *et al*., 2006).

**Cassava foliage**. Table 4 indicates the range in nutrient contents and *in-vitro* 48 h digestibility of DM (IVDMD) and NDF (IVNDFd). The variations in nutrient contents of CSF may be attributed to varietal differences in the leaf:stem proportion. The NIR predicted nutrient contents and *in-vitro* digestibility were positively correlated with the actual nutrient contents and *in-vitro* digestibility values as shown in Table 5. The higher RMSEP of 3.06 recorded for NDF and IVDMD predictions are indicative of the reliability of their predictions using the NIRS technique in CSF.

Table 3. Correlation coefficient between *beta*-carotene and nutrient contents in the vines from 13 sweet potato varieties (n = 65)

| Description of relationship | Correlation coefficient ($r^2$) | Significance |
|---|---|---|
| *Beta*-carotene vs. DM | 0.32 | Non-significant |
| *Beta*-carotene vs. CP | 0.12 | Non-significant |
| *Beta*-carotene vs.NDF | 0.052 | Non-significant |
| *Beta*-carotene vs. ADF | - 0.070 | Non-significant |
| *Beta*-carotene vs. NFC | - 0.11 | Non-significant |
| *Beta*-carotene vs. TDN | 0.070 | Non-significant |

The correlation coefficients between IVDMD and IVNDFd versus CSF nutrient contents are presented in Table 6. There were negative correlations between IVDMD versus NDF ($r^2$ = - 0.64; $p$ = 0.05) and ADF ($r^2$ = - 0.78; $p$ = 0.01) while, there was a positive correlation with TDN ($r^2$ = 0.76; $p$ = 0.01). Except for CP, which did not record any relationship with IVNDFd, there were negative correlations between IVNDFd versus DM ($r^2$ = - 0.71; $p$ = 0.05), NDF ($r^2$ = - 0.76; $p$ = 0.01) and ADF ($r^2$ = - 0.94; $p$ = 0.01). There was a significant positive correlation between IVNDFd versus NFC ($r^2$ = 0.70) and TDN ($r^2$ = 0.94) at $p$ = 0.01.

Table 4. Variations in nutrient contents and digestibility in the foliage from 16 cassava varieties

| Chemical composition | Min. | Max. | Mean ± s.e. |
|---|---|---|---|
| Nutrient content (%): | | | |
| Dry matter (DM) | 90.5 | 92.7 | 91.8 ± 0.15 |
| Crude protein (CP) | 21.9 | 30.6 | 25.2 ± 0.62 |
| Neutral detergent fibre (NDF) | 33.6 | 51.8 | 42.4 ± 1.20 |
| Acid detergent fibre (ADF) | 29.7 | 47.3 | 38.1 ± 1.27 |
| Non-fibre carbohydrates (NFC) | 15.3 | 29.9 | 21.9 ± 1.23 |
| Total digestible nutrients (TDN) | 53.9 | 63.4 | 59.0 ± 0.68 |
| Nutrient digestibility (%): | | | |
| *In-vitro* 48 h DM (IVDMD) | 47.5 | 67.6 | 57.9 ± 1.47 |
| Neutral detergent fibre (IVNDFd) | 68.3 | 89.9 | 80.4 ± 1.50 |

Similarly, the relatively high coefficients of determination ($R^2$) recorded for DM, ADF, NFC, TDN and IVNDFd further corroborate the usefulness of the NIRS technique for application in rapid feed analysis for CSF. In their study on cassava, Ikeogu *et al.* (2017) demonstrated the effectiveness of NIRS technique to rapidly analyse DM and carotenoids that, could be used for nutritional and breeding purposes. Furthermore, the results in Table 6 indicate that both IVDMD and IVNDFd were better predicted from ADF and TDN contents in the CSF studied.

**Spectral Graphs.** A comparative evaluation of both sweet potato vines and the cassava foliage indicate some level of similarity in their absorbance levels (Figure 1). The similarity in the graphs suggest that, a single model could be developed for NIRS models for both crops for

Table 5. Linear regression and correlation between actual (wet chemistry) nutrient contents and digestibility, and NIR predictions in foliage from 16 cassava varieties

| Forage quality parameter | Mean ± s.d.* (n = 80) | | Regression equation | $R^2$ | SEC | SEP | RMSEP |
|---|---|---|---|---|---|---|---|
| | Actual (Y) | Predicted (X) | | | | | |
| Nutrient content (%): | | | | | | | |
| Dry matter (DM) | 91.8 ± 0.60 | 92.0 ± 0.41 | Y = 1.23X – 21.36 | 0.70 | 0.15 | 0.10 | 0.351 |
| Crude protein (CP) | 23.1 ± 2.32 | 22.7 ± 2.13 | Y = 0.88X + 3.12 | 0.66 | 0.58 | 0.53 | 1.413 |
| Neutral detergent fibre (NDF) | 39.0 ± 4.59 | 39.8 ± 3.57 | Y = 0.97X + 0.39 | 0.57 | 1.15 | 0.89 | 3.055 |
| Acid detergent fibre (ADF) | 35.0 ± 4.82 | 35.1 ± 4.41 | Y = 1.06X – 1.98 | 0.94 | 1.20 | 1.10 | 1.212 |
| Non-fibre carbohydrates (NFC) | 20.1 ± 4.39 | 19.8 ± 3.24 | Y = 1.14X – 2.41 | 0.71 | 1.10 | 0.81 | 2.368 |
| Total digestible nutrients (TDN) | 54.2 ± 2.26 | 54.2 ± 2.15 | Y = 0.97X + 1.48 | 0.86 | 0.57 | 0.54 | 0.834 |
| Nutrient digestibility (%): | | | | | | | |
| *In-vitro* 48 h DM (IVDMD) | 57.9 ± 5.86 | 57.2 ± 5.12 | Y = 0.98X + 2.15 | 0.73 | 1.47 | 1.28 | 3.06 |
| Neutral detergent fibre (IVNDFd) | 80.4 ± 6.00 | 79.9 ± 5.34 | Y = 1.05X – 3.82 | 0.88 | 1.50 | 1.33 | 2.10 |

*s.d. = standard deviation; $R^2$ = coefficient of determination; SEC = standard error of calibration; SEP = standard error of prediction; RMSEP = root mean squared error of prediction.

use as global NIRS models for nutrients prediction in sweet potato and cassava varieties. Thus, the results from the study would provide part of the basis for further developing a robust NIRS database and models for easier and faster evaluation of forage/feed quality of SPV vis-à-vis its suitability for feeding livestock under resource-poor crop and livestock farming systems in the Niger Delta area and other parts of Nigeria (Sánchez *et al*., 2014; Tilahun *et al*., 2018). It also has the potential of being scaled-up to other regions of sub-Saharan Africa (SSA), especially in areas where sweet potato forms a significant part of human diet. Breeding programmes for sweet potato designed to develop varieties that could be used as dual-purpose varieties should be supported. Such developed varieties must be capable of producing substantial quantities of tubers for human consumption and foliage for feeding livestock either as supplemental feeds or as sole diets.

## Conclusions

There should be improved funding for studies aimed at increasing understanding on the adoption of NIR technique in feed evaluation as a possible means for rapid and cost-effective method compared to conventional wet chemistry and other *in-vitro* and *in-vivo* feed evaluation studies. Ultimately, it is expected that the spectra measurements shall be carried out directly on-farm with the development of a portable system as more data are gathered. Also, the NIRS calibrations can be updated by adding new samples of cultivars to the calibration and validation sets to accommodate changes in nutrient contents and *in-vitro* digestibility of DM (IVDMD) and NDF (IVNDFd). This will enable plant breeders to, accurately, monitor the nutrient composition of new cultivars. A further step in the study would be to indicate these parameters per variety to understand how varieties differ in predictability of their feed value using the NIRS technique.

Table 6. Correlation coefficient ($r^2$) and linear regression equation between in-vitro 48 h digestibility of dry matter (IVDMD) and neutral detergent fibre (IVNDFd) versus nutrient contents in cassava foliage from 16 varieties (n=80)

| Description of relationship | Correlation ($r^2$) | Significance† | Regression equation | $R^2$ |
|---|---|---|---|---|
| *In-vitro* 48 h dry matter digestibility (IVDMD): | | | | |
| IVDMD vs. dry matter | - 0.43 | NS | - | - |
| IVDMD vs. crude protein | 0.28 | NS | - | - |
| IVDMD vs. neutral detergent fibre | - 0.64 | * | IVDMD = – 0.79*NDF + 91.25 | 0.41 |
| IVDMD vs. acid detergent fibre | - 0.78 | ** | IVDMD = – 0.90*ADF + 92.05 | 0.60 |
| IVDMD vs. non-fibre carbohydrate | 0.45 | NS | - | - |
| IVDMD vs. total digestible nutrients | 0.76 | ** | IVDMD = 1.64*TDN – 38.66 | 0.57 |
| *In-vitro* 48 h neutral detergent fibre digestibility (IVNDFd): | | | | |
| IVNDFd vs. dry matter | - 0.71 | * | IVNDFd = – 7.09*DM + 731.53 | 0.51 |
| IVNDFd vs. crude protein | - 0.035 | NS | - | - |
| IVNDFd vs. neutral detergent fibre | - 0.76 | ** | IVNDFd = – 0.96*NDF + 120.91 | 0.58 |
| IVNDFd vs. acid detergent fibre | - 0.94 | ** | IVNDFd = – 1.11*ADF + 122.88 | 0.88 |
| IVNDFd vs. non-fibre carbohydrate | 0.70 | * | IVNDFd = 0.85*NFC + 61.71 | 0.49 |
| IVNDFd vs. total digestible nutrients | 0.94 | ** | IVNDFd = 2.08*TDN – 42.51 | 0.89 |

†NS = non-significant; *$P < 0.05$; **$P < 0.01$.

$$A = \log(\frac{1}{R})$$

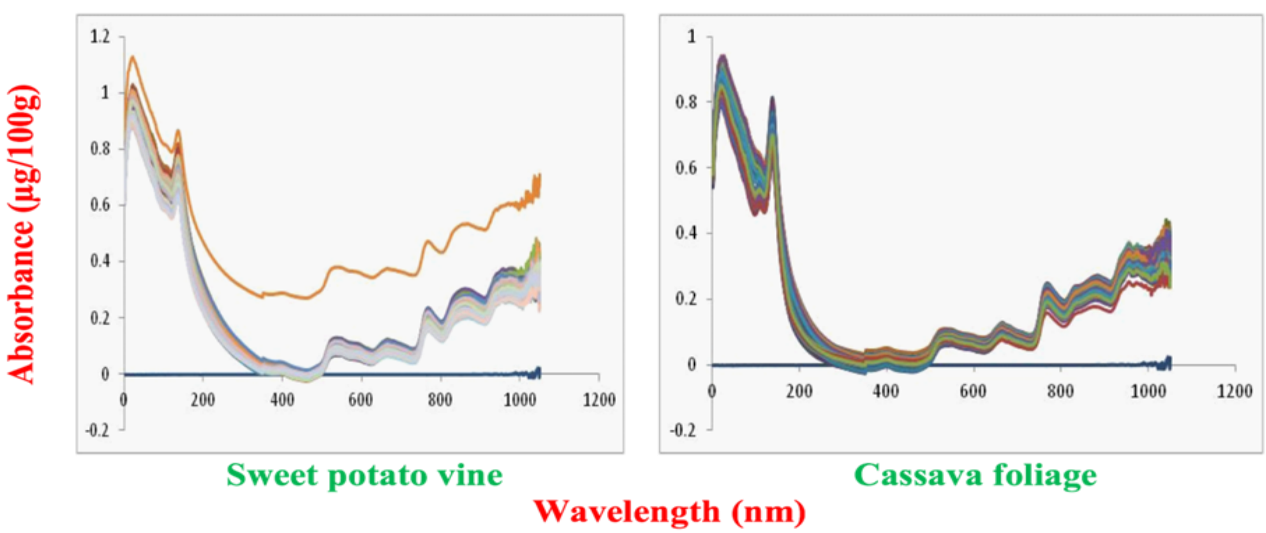


**Figure 1:** Comparative evaluation of nutrient predictability in sweet potato vine and cassava foliage using the NIRS technique

**Acknowledgement**

The authors acknowledge the Association of Commonwealth Universities (ACU), UK, London for the ACU Titular Fellowship (Ref: NGDF-2006-08) through George Weston Limited, Canada. Also acknowledged is Dr. J.C. Plaizier of the Department of Animal Science for allowing access to the Animal Science Nutrition Laboratory, University of Manitoba, Winnipeg MB, Canada. The technical support provided by Dr. Wenbo Wang (Biosystems Engineering, University of Manitoba), Dr. Larry Leonardi and Ms. Jeri Friesen (Institute for Biodiagnostics, National Research Council, Winnipeg, Canada) in NIR spectra data acquisition and analysis, is gratefully acknowledged. .

**Declaration of Conflict of interest**

The authors declare no conflict of interest in the paper.

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