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Near Infrared Spectroscopy for Quality Evaluation of Root Crops: Practical Constraints, Preliminary Studies and Future Prospects

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Abstract

Near Infrared Spectroscopy (NIRS) is a rapid and non-destructive technique, which does not use chemicals but can simultaneously determine numerous constituents in root crops. Spectrophotometers are simple to manipulate and the simple spectral collection reduces the risks of operator errors. However, NIRS is not a stand-alone technology and its performance depends upon the relationships between the spectral information and an accurate chemical method to measure the constituents of interest. The major constraint is, therefore, the need to build a reliable equation of prediction, also called a calibration model. This paper reviews the practical steps involved in the development of a model, including: 1. the collection of highly variable samples for calibration and validation sets; 2. the production of chemical data for these samples; 3. the collection of spectra on samples from the calibration and validation sets; 4. the development of the multivariate model; 5. its validation using descriptive statistics and 6. the monitoring of the model performance. The paper also reviews recent studies conducted on six root crops, including four tropical species (cassava, sweet potato, yam and taro) and two temperate (potato and sugar beet). Their results and the perspectives for further studies are discussed.

Key words: Near infrared spectroscopy, partial least squares regression, cassava, sweet potato, yam, taro, potato, sugar beet

Introduction

The tropical root and tuber crops (cassava, sweet potato, yam and aroids) produce underground organs rich in major constituents (starch, sugars, proteins, minerals and cellulose) and secondary metabolites (carotenoids, anthocyanins and vitamins). A few anti-nutritional compounds (cyanogens, trypsin inhibitors, alkaloids and oxalate crystals) exist in the wild forms but have been considerably reduced through domestication. Their quality has been improved by farmers' traditional selection over millennia and more recently by breeding programmes (Bradshaw, 2010).

The chemistry of tropical root crops has been the objective of numerous studies involving different analytical techniques, all increasingly sophisticated and able to detect and quantify accurately the most important molecules (Bradbury and Holloway, 1988; Lebot, 2009). When routine check ups are needed for quality evaluation, the complexity of their chemical composition leads to various technical difficulties. Chemical analyses are expensive, labour intensive, time consuming and require well equipped laboratories, which are often beyond the financial means of research teams located in the tropical countries, where these crops are important to farmers and consumers alike.

In many countries, these crops are processed to satisfy the needs of local industries. The absence of a rapid but accurate assessment of the raw material quality is, therefore, a serious constraint for buyers and farmers.

Root crop breeders aim to produce numerous hybrids but are also constrained by the quality evaluation process, a situation somehow similar to the one faced by breeders working on other crops. Cereal chemists in charge of providing grain quality data in cereal breeding programmes were the first to identify NIRS (Near Infrared Spectroscopy) to replace complex laboratory tests for the quality evaluation of their large number of samples. This technique has now been used in cereal breeding programmes since the late 1970's (Osborne, 2006).

NIRS is a rapid, non-destructive technique which does not use chemicals, or generate chemical wastes requiring disposal, but it can simultaneously determine numerous constituents (Huang et al., 2008). NIR instrumentation is simple to manipulate, it operates without fume hoods, drains, or other installations and there are no moving parts in spectrophotometers, reducing maintenance needs. The simple spectral collection reduces the risks of operator errors and improves the transferability of methods between countries. However, NIRS is not a stand-alone technology and its performance depends upon the accuracy of the chemical method used to produce the reference data. The major constraint is the need to build a reliable calibration model. The model is dependent on accurate laboratory analyses and a large and diverse sample set is used to develop the calibration equations. Separate calibrations are required for each constituent and unknown samples must periodically be analyzed by the chemical method to verify that the calibrations remain reliable over time.

These attributes make NIRS an interesting technique for developing countries, either for quality control or for breeding programmes. This paper presents the practical constraints that have to be considered for adequate NIRS application for quality evaluation of tropical root crops. The approach used for model development and different applications or preliminary studies across different species are described.

Practical constraints for adequate NIRS applications

Near infrared spectroscopy

Near infrared refers to the region of light immediately adjacent to the visible range, between 800 and 2500 nanometers (nm) in wavelengths. Under a source of light, the vibration of a molecule has a fundamental wavelength as well as a series of overtones. The vibration depends on the nature of the atoms and liaisons composing the molecule (Williams and Norris, 2001; Bertrand and Dufour, 2006). Most organic materials have excellent reflectance or absorbance properties at NIR wavelengths. When the raw material, a flour sample for example, is exposed to light, infrared spectrophotometers can translate the light absorbance or reflectance in a spectrum. The spectrum is a curve produced by their respective values measured every nm or every two or four nm, depending on the spectrophotometer characteristics (Fig. 1). The spectral appearance for any material results from its molecular structure. Since the molecular structure of most constituents is complex, their spectra are the result of many overlapping peaks

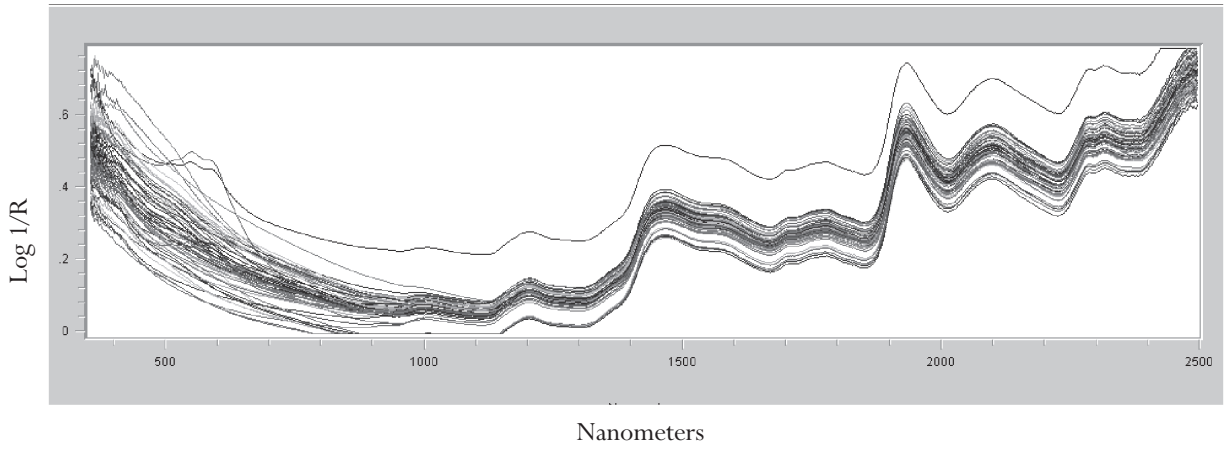

Fig. 1. Very near infrared (350-800 nm) and infrared (800-2500 nm) spectra of 214 *Dioscorea alata* accessions. Variation in the 400-800 nm regions, the visible range, corresponds to absorbance (*Log 1/R*) variability in colours of dry matter of flour samples. The peak at 1450 nm could be attributed to starch and the one at 1940 nm to water

and valleys corresponding to the excitation wave lengths from 800-2500 nm. The problem is to identify the numerous features of a spectrum.

The spectrum is used as a source of information to develop a relation to the chemical data. This relation is then used to predict the concentration. The chemical constituents of interest (major constituents and/or secondary metabolites) should have direct or indirect absorbance in the NIR region in order to be predictable. Also, the concentration of constituent in the sample should be above 1%. The same samples are submitted to spectral collection and chemical analysis to measure the constituents. The spectral and chemical data are then analyzed by means of statistical methods using commercially available chemometrics softwares that usually come with the spectrophotometer (i.e., GRAMS©, WINISI©). When a sufficient number of samples have been collected and analyzed, an equation of prediction (also called a "model") is constructed that describes the relationship between specific spectral characteristics and the constituent.

Robust models are developed by collecting spectra from samples displaying maximum variability of the constituents. Germplasm collections of root crop species hosting significant genetic variability are consequently very useful. In order to understand correlations between constituents, maximum variability and contrasting characteristics are required. Varieties collected from different locations and from different cropping seasons should be added to the calibration set to add maximum variability. A robust model might require hundreds of samples to represent the diversity that could be encountered when using the model. The more the samples in the calibration model, the more reliable are the prediction performance (Shiley, 2010). So, a good model represents a significant financial investment in chemical analyses.

Chemical or reference data

The chemical data should result from an analytical method that is reproducible with minimum laboratory error. In most cases, the reference data used to develop a calibration model is a chemical test but it can also result from a physical test, such as starch viscosity for example (Lu et al., 2006). The most efficient way of assessing the reproducibility of an analytical method is through the Standard Error of Laboratory (SEL). Verification of SEL is easily done by submitting the same sample (under different numbers) to successive analyses and by comparing results. For example, in most laboratories, nowadays, accepted mean coefficient of variation of major constituents are not more than ± 3% for starch, amylose, sugars, cellulose and residual moisture and ± 2% for proteins (equivalent N) and ashes (minerals). Throughout the model development process, it is important to use the same laboratory and analytical protocols and even later on, for regular model reliability tests (Williams and Norris, 2001; Bertrand and Dufour, 2006; Shiley, 2010).

One full fresh corm, root or tuber, is harvested when mature. It is then peeled and approximately 0.5-1 kg weight, corresponding to the central part is manually sliced into chips and oven dried at 60°C for 48 h. Dry matter samples are then split into two sub-samples: one sub-sample is used for chemical analysis and the other for collection of NIR spectra. Samples of approximately 50 g of dry chips are milled into flour just after oven drying and chips are ground in a stainless steel kitchen mill prior to spectra collection.

Collection of NIR spectra

The spectrophotometer performance is verified prior to collection of spectra and the wavelength stability is checked. This is usually possible through the software which comes with the spectrophotometer. Important variables controlling spectral acquisition should also be controlled: particle size of the sample, moisture and temperature. The samples should be milled to the same particle size and homogenized prior to scan collection. Dry matter samples are usually milled into flour and granule size is homogenized using four sieves with decreasing diameters until granules pass through the 106 μm sieve. If the form and particle size of the sample changes, then the model will not perform well. The sample illumination angles should also remain unchanged throughout the spectral collection. A reference reading (baseline) is taken when starting a session and another every 30 min. This reference is usually based on the reading of a standard material with high overall reflectance. All the spectra are then recorded in absorbance or reflectance with respect to this standard. It is often useful to convert reflectance spectra to absorbance format *Log (1/R)* when developing

calibration models as this conversion produces a more linear model (Shiley, 2010).

All samples in the calibration and validation sets should have their spectra collected on similar temperature and moisture content conditions. These should also remain identical for predictions. For dry matter (DM) determination, samples are dried again to remove residual moisture (measured as % of total dry weight) and the spectrum is collected for the oven dried sample. Moisture is therefore expressed as a measurement of the sample prior to drying. All constituent measurements are then expressed in % DM and the data are adjusted by the residual moisture following oven drying.

Depending on the type of infrared spectrophotometer used, different spectral acquisition devices exist. However, for root crops, flours and/or dry matter powders are the most common and these are usually placed in cups or cells. Some studies on the Irish potato (*Solanum tuberosum*) have shown that it is possible to collect spectra directly from the mashed fresh raw tubers (Haase, 2006; 2011). The resulting purée is also placed into cells. On an average, five to ten grams of homogenized flour are placed in an individual cell and compacted to eliminate air voids within the powder. Each spectrum is obtained by averaging repetitions of different cells containing the sample (usually three to five cups or cells) and with 25 scans for each. Then, for each sample, corresponding to individual accession (varieties or breeding lines), three sub-samples are scanned 25 times each and the resulting averaged spectrum is recorded for the accession. The number of cells per sample and the number of scans per cell are easily adjusted by the software managing the spectrophotometer.

Development of calibration models

Models are based on a calibration set and their performance is tested on a validation set. When the model is tested on the validation set, the predicted values are compared to those obtained with the chemical analyses. The number of samples in the validation set should represent approximately 20% of the number of samples in the calibration set and these samples must be removed from the calibration set. Proper selection of these samples is important as they must encompass the same variation in sample constituents as contained in the calibration set. These samples can be selected at random within the total number of samples available. For example, by ranking the samples by their decreasing constituent value and then, beginning with the first sample by marking every fifth sample for the validation set. Their selection should not be based on those samples which predict well on the calibration but should be done without knowledge of their predicted values. Consequently, samples should not be removed from the validation set because they do not fit the prediction.

The fitness of the model is assessed with various descriptive statistics. Thereafter, if the correspondence is good enough, it is possible to quickly measure that same constituent in a new sample by applying the model to the new spectra and samples. To avoid over fitting the model, it is suggested that 10 or more samples should be used in the calibration set for every factor (principal component) in the model (Workman, 2008).

Data pre-treatment is often necessary to minimize the number of factors that are used. Light scattering effects due to particle size differences can be corrected. Multiplicative Scatter Correction (MSC), Standard Normal Variate (SNV) (Barnes et al., 1989) or first derivative can help reduce the number of factors (Williams and Norris, 2001; Shiley, 2010).

Partial Least-Squares (PLS) is the most common regression technique used to develop a predictive model of the near infrared part of the spectra (Bertrand and Dufour, 2006). The optimum number of PLS factors (also called PLS terms) used for prediction is determined by full cross-validation and PRESS (Prediction Residual Error Sum of Squares). The PLS technique simplifies the spectral data by producing a series of descriptive vectors (factors or terms) and these are regressed against the chemical data to produce the model. The number of factors used in the model should not be more than one for 10-20 samples in the calibration set.

Principal Component Analysis (PCA) is first of all used to check for gross spectroscopic or chemical concentration outliers. Samples may be removed because their spectral characteristics are substantially different than the other samples (spectral outliers) or because their chemical data does not fit the relationship to the other samples (concentration outliers). The Mahalanobis distance of each spectrum to the mean spectrum of the group is calculated and the removal of outliers is usually

based on distance $H > 3$ from the average spectrum. The same approach is used to identify concentration outliers. In both cases, the objective is to remove as few samples as possible to produce the best calibration possible (Shiley, 2010).

Descriptive statistics

The descriptive statistics used to evaluate performance of models include:

- the standard error of cross validation (SECV)
- the standard error of calibration (SEC)
- the standard error of prediction (SEP)
- the determination coefficient for cross validation (r^2_{cv})
- the determination coefficient for prediction (r^2_{pred}) and
- the ratio of performance to deviation (RPD)

The Standard Error of Cross Validation (SECV) is a technique that produces an estimate of the calibration error on unknown but similar samples. In this technique, each sample is automatically removed and a calibration developed with the remaining samples and the error calculated on the removed sample. Then that sample is returned and the next removed. Finally, the error for each sample, while it was not part of the calibration, is calculated. Spectra and concentration outliers are then removed and PLS is run again until the highest r^2_{cv} (determination coefficient for cross validation) corresponding to the smallest SECV are obtained.

The SEC and SEP can be calculated using excel spreadsheet by squaring the differences of the actual minus the predicted concentrations for each sample in the calibration (SEC) and test (SEP) sets. These values are then summed and the sum is divided by the number of samples (n). The square root of this value is used for SEC and SEP. The SEC describes the calibration set and SEP describes the validation set.

If the modelling has been performed correctly, SECV should approximate the error of a true independent validation set as measured with the Standard Error of Prediction (SEP). If more than 10% of the samples are removed then the calibration might be forced to establish a relationship where one does not exist. If SECV and SEP values are found to differ significantly, this is an indication that too many samples (outliers) were removed during the modelling process or that the model has been over-fitted. In that case, when the validation set is predicted, the relationship of r^2_{pred} and SEP would not be similar to the r^2_{cv}, SECV and SEC of the model. The objective is to keep SECV, SEC and SEP at similar values.

Outliers can sometimes provide useful information and therefore they should not simply be discarded without additional investigation. Samples removed as outliers should be rechecked by chemical analysis and should also have new spectra acquired if possible. If the chemical data changes significantly following reanalysis, then the sample can be included back into the calibration set. Likewise, samples that are removed as spectral outliers should have new spectra collected as the cause might be improper spectral collection. If the spectra for the sample are recollected, yet still is marked as an outlier then additional samples of that type should be identified and added to the calibration set to better represent that type (Shiley, 2010).

The factor loadings are then used to determine which wavelengths are important to correlate with concentrations in order to narrow down the region of the wavelengths (from 350-2500 nm to 1000-2400 nm, for example). The loading plots show which wavelengths are important to correlate with starch concentrations in yam (Fig. 2). The loading weights show how much each wavelength point contributes to

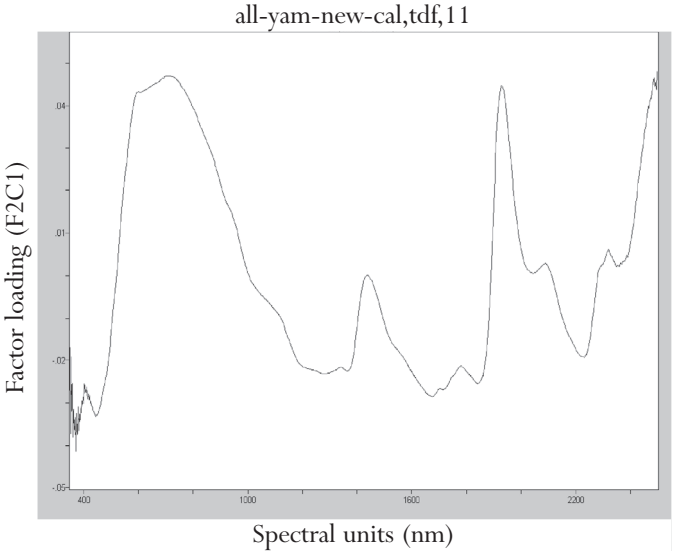

Fig. 2. Factor loadings for starch on 250 yam accessions measured in absorbance from 350 to 2500 nm. The peaks and valleys between 800 and 2500 nm indicate which wavelengths are important to correlate with starch concentration. In this particular case, the NIR region can be reduced to 1200 to 2400 nm in order to reduce the number of factors

explaining the response along each model component. Ragged areas or areas close to zero do not provide much useful information. Conversely, wavelengths that are important show up as smooth peaks with high amplitude. The PLS analysis is then conducted again on these new regions in order to obtain for each constituent better equations without increasing the number of PLS factors used.

The ratio of performance to deviation (RPD= SD/ SECV) is also used to evaluate performances of the models (with SD as the standard deviation of the original chemical data in the calibration set) (Williams, 2003). In terms of predictive performance, the equations with RPD parameters above 2 could be considered as good. Some authors claim that a RPD value of at least 3 is necessary for efficient NIR reflectance predictions (Williams and Norris, 2001).

Finally, new calibrations are computed again on the total number of samples by adding those from the calibration and validation sets in order to see if the models have potential for improvement.

Monitoring of model prediction performance

An NIR calibration needs to be updated on a frequent basis or when the prediction error rises beyond the acceptable limits. Most models need to be updated within the first year, then less frequently as more samples are added and more variability is explained by the model. Model performance can be continually verified using comparisons to the chemical analysis. If the spectral residuals are found to be increasing in the new samples, this might indicate that the samples are not well represented by the model and new samples will have to be added to the calibration to provide proper representation in the model. If the concentration residuals are found to be increasing in the samples, this might indicate that the samples were not adequately represented or that there had been a change in the chemical analysis protocol and it is no longer producing data similar to that used in the first model. Regular checks are therefore necessary.

To sum up, the steps involved in the development of a model are:

1. to collect highly variable samples for calibration and validation sets. These should come from germplasm collections assembling accessions with significant genetic diversity but also from accessions collected from different environments. The idea is to collect as much variability as possible within the two sets of samples.
2. to obtain the reference chemical data for these samples as well as to obtain an estimate of laboratory error (SEL).
3. to collect spectra on samples from the calibration and validation sets.
4. to develop the multivariate model.
5. to validate the model using the validation data set (and descriptive statistics).
6. to use the model and also to monitor its performance against the chemical analysis on a frequent basis to assure continued reliability and to improve the model as needed.

Preliminary studies on root crops

Compared to cereals (Osborne, 2006), root crops are under researched regarding the potential applications of NIRS. The most significant studies that have been conducted on root and tuber crops, including four tropical species: cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), yam (*Dioscorea* spp.) and taro (*Colocasia esculenta*) and two temperate species: the potato (*Solanum* spp.) and sugar beet (*Beta vulgaris*) are reviewed here.

Cassava

Surprisingly, despite the economic importance of this crop , very few studies have been conducted on cassava. A method for predicting pasting properties of cassava starch based on NIR has been developed in Thailand. Cassava starch was heat-treated for different time-temperature combinations in varying concentrations. The changes in the pasting properties of heat-treated starch were measured and correlated with associated NIR spectra. The prediction models had r^2_{cv} in the range of 0.81-0.96. It was concluded that NIR spectroscopy could be used as a rapid and non-destructive method for predicting pasting parameters of heat-treated cassava starch (Liplap and Jindal, 2006). NIRS has, however, been identified as a technology with great potential for cassava breeding and biofortification (Ceballos et al., 2010) but robust calibration models are yet to be developed. A preliminary study conducted with an insufficient number of samples has shown, however,

Table 1. Descriptive statistics for the calibration models on cassava

Constituents	Calibration (n=50)			Validation (n=12)		
	PLS terms	r^2_{cv}	SEC	r^2_{pred}	SEP	RPD
Starch	8	*0.83*	1.32	*0.82*	1.44	1.92
Sugars	6	*0.70*	0.92	*0.77*	0.58	1.46
Cellulose	0	*0.14*	-	-	-	-
Total nitrogen	7	*0.88*	0.28	*0.96*	0.14	2.14
Ash	6	*0.85*	0.20	*0.90*	2.62	2.00

Source: Lebot et al. (2009)

that the technique looked attractive for cassava, especially for starch, sugars, total N and total minerals (Lebot et al., 2009) (Table 1).

Sweet potato

Calibration models have been developed in China to predict sweet potato starch physico-chemical quality and pasting properties. Isolated starch samples, extracted from fresh roots one week after harvesting were scanned by NIRS. The results (Table 2) indicated that NIRS was reasonably accurate in predicting amylose, starch, protein, phosphorus, solubility, swelling power, granule diameter and viscosity (Lu et al., 2006). These authors concluded that NIR screening for starch quality parameters offer enough information to select or discard a breeding line. In order to further improve these calibrations, new sweet potato starch samples extracted from different genotypes should be added to the calibration set. It was however observed that the accuracy of the reference data was an important factor determining NIRS prediction and that some of the chemical analyses protocols should be improved further.

Table 2. Calibration and validation for sweet potato starch quality

Constituents	Calibration (n=128)		Validation (n=70)	
	SEC	r^2_{cv}	SEP	r^2_{pred}
Amylose	0.647	*0.92*	0.882	*0.91*
Starch	1.274	*0.92*	1.772	*0.86*
Protein	0.028	*0.88*	0.042	*0.86*
Phosphorus	0.853	*0.94*	1.528	*0.89*
Solubility	0.653	*0.92*	0.936	*0.89*
Swelling power	0.767	*0.89*	0.937	*0.85*
Granule diameter	0.917	*0.87*	1.012	*0.87*
Peak viscosity	8.41	*0.92*	13.10	*0.91*

Source: Lu et al. (2006)

Another study was conducted in Vanuatu on dry matter samples obtained from 240 accessions representing varieties and breeding lines (Lebot et al., 2011a). Calibration equations, developed on 190 accessions, showed high explained variances (r^2_{cv}) for starch (0.82), sugars (0.91), proteins (0.89) and minerals (0.74) but no response for cellulose (0.21). The models were tested on an independent set of 50 randomly selected accessions. The r^2_{pred} values for starch, sugars and proteins were 0.71, 0.82 and 0.87 respectively, with ratios of performance to deviation (RPD) of 2.11, 2.29 and 2.93 respectively (Table 3). New calibration equations developed on 240 accessions revealed higher RPD values indicating that larger sets could improve prediction. The results indicated that NIRS could be used in sweet potato breeding programmes to predict starch, sugars and protein contents in the roots.

Yam

A preliminary study conducted on 260 accessions, belonging to seven different *Dioscorea* spp. and based on dry tubers, has permitted the establishment of equations of calibration for starch, sugars and proteins. The r^2_{pred} values for starch, sugars and proteins (0.75, 0.73 and 0.81 respectively) were high enough to allow good estimates of their contents. RPD of 4.05 and 3.64 for the sugars and protein models also allowed good quantitative predictions to be made. Amylose, cellulose and minerals could not be predicted precisely. A second calibration conducted by adding the calibration and validation sets revealed an improvement of the RPD values for starch, sugars and proteins indicating that the models could be improved (Table 4). Discriminant analysis conducted using 2151 wavelengths (nm) as variables was applied to a set of 214 accessions of *D. alata* and the results were

Table 3. Calibration for major constituents measured on dry matter of roots of sweet potato

Constituents	Calibration (n=190)				Validation (n=50)			New calibration (n=240)				
	PLS terms	r^2_{cv}	SECV	SEC	r^2_{pred}	SEP	RPD	PLS terms	r^2_{cv}	SECV	SEC	RPD
Starch	12	*0.82*	2.37	1.48	*0.71*	3.13	2.11	8	*0.82*	2.49	2.27	2.65
Sugars	11	*0.91*	1.55	1.20	*0.82*	2.31	2.29	10	*0.86*	1.77	1.77	2.75
Cellulose	3	*0.21*	1.26	-	-	-	-	6	*0.46*	1.26	1.17	1.58
Proteins	9	*0.89*	0.39	0.34	*0.87*	0.44	2.93	10	*0.91*	0.36	0.30	3.47
Minerals	10	*0.74*	0.32	0.27	*0.56*	0.73	1.1	9	*0.72*	0.38	0.10	2.34

Source: Lebot et al. (2011a)

Table 4. Statistical parameters of the calibration and validation sets on yam

Constituents	Calibration (n = 210)				Validation (n = 50)		New calibration (n = 260)			
	PLS terms	r^2_{cv}	SEC	RPD	r^2_{pred}	SEP	PLS terms	r^2_{cv}	SEC	RPD
Starch	8	*0.86*	2.56	2.86	*0.75*	1.23	11	*0.87*	2.33	2.92
Amylose (1)	4	*0.32*	4.69	1.39	*0.18*	4.93	-	-	-	-
Sugars	10	*0.93*	0.95	4.05	*0.73*	0.48	12	*0.94*	0.78	4.34
Proteins	10	*0.92*	0.91	3.64	*0.81*	0.54	11	*0.93*	0.82	3.86
Minerals	8	*0.54*	0.88	1.69	*0.22*	0.72	11	*0.54*	0.82	1.62
Cellulose	5	*0.72*	1.56	1.94	*0.10*	1.79	5	*0.72*	1.70	1.96

(1) Calibration set of 100 accessions and validation set of 25 accessions

Source: Lebot and Malapa (2012)

compared to the PCA of chemical data. Accessions could be classified according to the amylaceous fraction of the chemotype (Lebot and Malapa, 2012). The authors concluded that NIRS could be used in breeding programmes to characterise rapidly and at a low cost the numerous accessions and breeding lines.

Taro

Taro (*Colocasia esculenta*) corm quality is related to a certain chemical composition and different varieties are processed and cooked into various preparations throughout the World. In Vanuatu, blind panel testing had shown that good varieties had high DM (> 35%) and starch contents (> 80% DM). NIRS calibration equations, developed on a calibration set composed of 243 accessions, showed high explained variances in cross-validation (r^2_{cv}) for starch (0.89), sugars (0.90), proteins (0.89) and minerals (0.90) but poor response for amylose (0.44) and cellulose (0.61) (Lebot et al., 2011b). The predictions were tested on an independent set of 58 randomly selected accessions. The r^2_{pred} values for starch, sugars, proteins and minerals were 0.76, 0.74, 0.85 and 0.85 respectively with ratios of performance to deviation (RPD) of 3.41, 4.01, 3.78 and 3.64. New calibrations on 303 accessions confirmed good RPD values for starch, sugars, proteins and minerals (Table 5). Lebot et al. (2011b) concluded that NIRS could be used to predict starch, sugars, proteins and mineral contents in taro corms with reasonably high confidence.

Potato

Dry matter (DM) and sugar contents are important quality parameters for assessing the potential of potato (*Solanum tuberosum*) tubers to produce acceptable

Table 5. Statistical parameters of the calibration and validation sets for taro

Constituents	Calibration (n = 245)				Validation (n = 58)		New calibration (n=303)			
	PLS terms	r^2_{cv}	SEC	RPD	r^2_{pred}	SEP	PLS terms	r^2_{cv}	SEC	RPD
Starch	12	*0.89*	1.56	3.41	*0.76*	2.14	11	*0.89*	1.91	3.30
Amylose[(1)]	3	*0.44*	3.66	1.67	*0.15*	5.76				
Sugars	12	*0.90*	0.75	4.01	*0.74*	1.50	12	*0.90*	0.85	4.13
Cellulose	8	*0.61*	0.41	2.05	*0.37*	0.83	9	*0.56*	0.54	2.11
Proteins	9	*0.89*	0.57	3.78	*0.85*	0.57	11	*0.89*	0.58	3.61
Minerals	8	*0.90*	0.35	3.64	*0.85*	0.44	11	*0.90*	0.38	3.74

[(1)] Calibration set of 160 accessions and validation set of 40 accessions

Source: Lebot et al. (2011b)

processed products with good texture and colour. A study conducted to develop NIR calibration for DM and to screen tubers with high reducing sugar content has been conducted in Manitoba, Canada. The wavelengths that correlated well for specific gravity also correlated well for DM. Calibrations for DM were robust and were not rejected by time or conditions of storage or by change over from one crop year to another. NIR was however, unable to act as a reliable screening tool for unacceptable sugar content in raw tubers (Scanlon et al., 1999). In another study, conducted in the Netherlands, a total of 275 mashed potatoes were analysed to predict DM, starch, crude proteins and recoverable proteins. The equations for DM and starch presented an accuracy acceptable for routine analysis with RPD values of 4.2 and 3.1 respectively. However, the equations for proteins presented a low and unacceptable RPD (1.5) (Fernàndez-Ahumada et al., 2006). Other studies also confirmed that NIR can predict DM and starch contents in potatoes (Hartmann and Büning-Pfaue, 1998; Haase, 2006; Subedi and Walsh, 2009).

In Germany, calibration equations have been developed on very large sample sets (total n = 2517). A retrospective procedure combined all the data sets from 1999 to 2007 seasons to develop and test models on raw and mashed (fresh weight) tubers. The models presented very high r^2_{pred} and RPD values indicating that they were very robust and reliable (Table 6). More extensive sample sets and further external validation would have improved the results. Also, the level of crushing seems to have an influence upon the prediction performance (Haase, 2011). On the other hand, NIR prediction of low molecular weight carbohydrates (reducing sugars, sucrose and total sugars) was rather poor as shown by the low r^2_{pred} and RPD values. For potato products, quality prediction by NIR measurements of raw potatoes was on a qualitative level with rough determination. All RPD values were below 2 indicating poor performance but the r^2_{pred} values indicated limited applicability in predicting processing quality (Table 6).

A study to appreciate the potential of NIRS to identify cultivars of potato used in the production of crisps was conducted in New Zealand and has shown that discriminant analysis could separate the spectra into different classes corresponding to cultivars with 93% of success rate. This suggested that it was possible to use NIRS for identifying cultivars in single batches of potato crisps (Yee et al., 2006). At the International Potato Centre, (CIP, Peru), NIR calibration equations were developed to estimate total and individual carotenoid concentrations in *Solanum phureja* germplasm. The use of NIRS has permitted the identification of accessions with high total carotenoids at low cost and in reduced time compared to HPLC, the reference method. It was concluded that this technique can be applied for the management of potato germplasm collections and assessing the food value of present varieties as well as for the selection of parents in breeding programmes (Bonierbale et al., 2009).

Table 6. NIR models to predict constituents in fresh potato and quality in potato products

Constituents and products	Calibration				Validation			
	n	SEC	r^2_{cv}	SECV	n	SEP	r^2_{pred}	RPD
Dry matter	1401	0.34	*0.99*	0.38	935	0.39	*0.99*	8.49
Starch	384	0.32	*0.98*	0.43	223	0.47	*0.96*	5.41
Reducing sugars	694	39.7	*0.58*	46.0	426	38.9	*0.43*	4.48
Sucrose	691	110	*0.81*	121	531	106	*0.59*	2.37
Total sugars	717	116	*0.76*	138	450	135	*0.66*	1.76
Dehydrated potatoes	466	1.32	*0.65*	1.55	296	147	*0.52*	1.44
Potato crisps	813	2.75	*0.74*	3.09	556	2.80	*0.69*	1.92
French fries	615	0.54	*0.58*	0.55	395	0.57	*0.56*	1.40

Source: Haase (2011)

Sugar beet

In France, the most important parameters of sugar beet quality, DM and sucrose content, were well predicted by NIRS. Sucrose content determines the grower payments and its model had a very high RPD value (Table 7). Glucose, Na and K were not determined accurately by NIRS. It is possible that the NIR spectrum did not contain enough information on Na and K. Regarding glucose, the concentration was probably too low to be accurately determined by NIRS or the concentration range was too narrow to develop a robust calibration model. The "marc" value corresponded to the beet pulp and contained the cellulose and fibre contents. The model had a fairly high r^2_{pred} allowing robust prediction but the RPD was fairly low (2.42) (Roggo et al., 2004).

Perspectives

The above preliminary studies indicate that NIRS has good potential for predicting the quality parameters of most root crop species. However, robust models have to be based on large data sets and this is demonstrated by the work conducted on the temperate root crops, sugar beet (Roggo et al., 2004) and potato (Haase, 2011). For the tropical root crops, the calibration sets are not large enough and further investment is needed. Determination coefficients (r^2_{pred}) generally improve as the working range increases. If more range is added in the same model then it could improve coefficient values. Additionally, when different samples are added, a larger spectroscopic diversity is described and some samples might actually be better spectrally described as the number of samples increases. For most studies, especially on cereals

Table 7. Statistical indicators for calibration and validation in sugar beet

Constituent	Calibration				Validation			
	n	PLS terms	r^2_{cv}	SEC	n	r^2_{pred}	SEP	RPD
Sucrose	2210	11	*0.99*	0.09	525	*0.99*	0.10	10.72
Glucose	994	13	*0.49*	0.01	1066	*0.31*	0.01	2.47
Dry matter (brix)	1025	9	*0.98*	0.17	955	*0.98*	0.19	7.16
Marc (insoluble dry matter)	218	10	*0.91*	0.11	198	*0.83*	0.13	2.42
Nitrogen	994	15	*0.74*	0.10	1066	*0.64*	0.11	2.24
Potassium	994	8	*0.58*	0.38	1066	*0.48*	0.41	1.61
Sodium	994	5	*0.42*	0.12	1066	*0.32*	0.14	1.63

Source: Roggo et al. (2004)

(Osborne, 2006) it has been shown that when the number of samples increased, the values improved. To a lesser extent, these preliminary studies also indicate the same trend for sweet potato, yam and taro with improved values with greater sets. For cassava, unfortunately, the paucity of the data does not allow for interpretation and further investigation is needed. For other crop species, errors of prediction values have been shown to have uncertainties and it is therefore recommended to be cautious while reporting prediction errors because they may change according to the validation set used (Sileoni et al., 2001).

For the root crop species presented here, the prediction values (r^2_{pred} and SEP) of the protein content are particularly interesting as they can probably be further improved. Protein content is usually estimated by multiplying the total N content by a standard conversion factor of 6.25 (Kjeldahl method). However, the N to protein ratio does vary according to the species considered and it changes with amino acid content and mineral N and non-protein N. In all studies reviewed in this paper, the results were measured as total N and presented as proteins. It would be of interest to improve the calibration models on the real protein content of these species which vary according to amino acids. Once known, the values obtained by the Kjeldahl method could be converted into more accurate measurements and therefore NIRS calibrations could be further improved.

The prediction values of starch and sugars are already high and will probably be improved with great number of samples analyzed. The situation for amylose is not clear. When measured on extracted starch, the r^2_{cv} and r^2_{pred} values are quite high (Lu et al., 2006). However, when amylose is measured directly on DM (Lebot et al., 2011b; Lebot and Malapa, 2012), the values are very disappointing. It is not for the first time that poor prediction performance of NIRS on amylose has been reported, but this may be related also to the chemical analysis protocol (De Alencar Figueiredo et al., 2006). NIRS prediction of cellulose (total fibres) has been shown to be unsuccessful and further work is needed.

All chemical analyses indicate that starch and dry matter contents are always positively correlated and that starch is always negatively correlated with other major constituents (sugars, proteins, minerals and cellulose). In breeding programmes of root crops, mass selection has to be complemented with efficient screening techniques of hundreds of hybrids generated in controlled crosses. Correlation coefficients between major constituents indicate that increased DM and starch levels will reduce sugars, proteins and minerals. These correlations do not present practical problems as poor quality varieties have been shown to present low DM and starch. However, NIRS could already assist breeders by predicting simultaneously the major constituents on a single sample.

For most species, the spectra are collected on dry matter samples. However, the prediction values obtained with spectra collected on raw tubers of potato are impressive (Haase, 2011). If transferable to other species, this technique might speed up the whole process and save preparation time of the samples. Although it is likely that the yam mucilage existing in the raw tuber will represent a significant technical constraint, for cassava, sweet potato and taro, this approach should be experimented.

Conclusion

NIRS is a rapid technique which does not use chemicals but can simultaneously determine numerous constituents. Spectrophotometers are simple to manipulate with limited maintenance needs. However, NIRS is not a stand-alone technology and its performance depends upon the availability of a large set of chemical data to build a reliable calibration model. The costs of developing robust models are therefore those resulting from the numerous accessions that have to be chemically analysed. The preliminary results presented in this paper indicate, however, that NIRS could be a very useful tool for root crop breeders and/or scientists to screen large sample sets for their studies. NIRS also represent a promising tool for quality control in the flour and starch industries.

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References

Barnes, R.J., Dhanoa, M.S. and Lister, S.J. 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.,* 45: 772-777.

Bertrand, D. and Dufour, D. 2006. *La spectroscopie infrarouge et ses applications analytiques*. 2nd Edn., Lavoisier, Paris, France, 660 p.

Bonierbale, M., Grüneberg, W., Amoros, W., Burgos, G., Salas, E., Porras, E. and Zum Felde, T. 2009. Total and individual carotenoid profiles of *Solanum phureja* cultivated potatoes: II. Development and application of near-infrared reflectance spectroscopy (NIRS) calibrations for germplasm characterization. *J. Food Comp. Anal.,* **22**: 509-516.

Bradbury, J.H. and Holloway, W.D. 1988. *Chemistry of Tropical Root Crops: Significance for Nutrition and Agriculture in the Pacific*. ACIAR Monograph No. 6. Canberra, 201 p.

Bradshaw, J.E. 2010. *Root and Tuber Crops. Handbook of Plant Breeding* 7. Springer Science-Business Media, New York, USA, 295 p.

Ceballos, H., Okogbenin, E., Pérez, J.C., Augusto, L., Lopez-Valle, B. and Debouck, D. 2010. Cassava. In: *Root and Tuber Crops.* Bradshaw, J. E. (Ed.). Springer Science-Business Media, New York, USA, pp.53-96.

De Alencar Figueiredo, L.F., Davrieux, F., Fliedel, G., Rami, J.F., Chantereau, J., Deu, M., Courtois, B. and Mestres, C. 2006. Development of NIRS equations for food grain quality traits through exploitation of a core collection of cultivated sorghum. *J. Agric. Food Chem.*, **54**: 8501-8509.

Fernàndez-Ahumada, E., Garrido-Varo, A., Guerrero-Ginel, J.E., Wubbels, A., Van der Sluis, C. and Van der Mer, J. 2006. Understanding factors affecting near infrared analysis of potato constituents. *J. Near Infrared Spectrosc.*, **14**: 27-35.

Haase, N.U. 2006. Rapid estimation of potato tuber quality by near-infrared spectroscopy. *Starch/Stärke*, **58**: 268-273.

Haase, N.U. 2011. Prediction of potato processing quality by near infrared reflectance spectroscopy of ground raw tubers. *J. Near Infrared Spectrosc.*, **19:** 37-45.

Hartmann, R. and Büning-Pfaue, H. 1998. NIR determination of potato constituents. *Potato Res.*, **41**: 327-334.

Huang, H.B., Yu, H.Y., Xu, H.R. and Ying, Y.B. 2008. Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review. *J. Food Engng.*, **87:** 303–313.

Lebot, V. 2009. The *Tropical Root and Tuber Crops: Cassava, Sweet Potato, Yams and Aroids.* CABI Crop Production Science in Horticulture, CABI Publishing, Cambridge, UK. 420 p.

Lebot, V., Champagne, A., Malapa, R. and Shiley, D. 2009. NIR determination of major constituents in tropical root and tuber crop flours. *J. Agric. Food Chem.*, **57:** 10539–10547.

Lebot, V., Ndiaye, A. and Malapa, R. 2011a. Phenotypic characterization of sweet potato [*Ipomoea batatas* (L.) Lam.] genotypes in relation to prediction of chemical quality constituents by NIRS equations. *Pl. Breeding*, **130**(4): 457-463.

Lebot, V., Malapa, R. and Bourrieau, M. 2011b. Rapid estimation of taro quality by near infrared spectroscopy. *J. Food Agric. Chem.,* **59**(17): 9327-9334.

Lebot, V. and Malapa, R. 2012. Application of near infrared reflectance spectroscopy to the evaluation of yam (*Dioscorea alata*) germplasm and breeding lines. Communicated to *J. Sci. Food Agric.*

Liplap, P. and Jindal, V.K. 2006. Prediction of pasting properties of heat-treated cassava starch by near infrared spectroscopy. *Proceedings of the 7th Annual Conference of Thailand Society of Agricultural Engineering.* Mahasarakarm University, Thailand.

Lu, G., Huang, H. and Zhang, D. 2006. Prediction of sweet potato starch physico-chemical quality and pasting properties using near-infrared reflectance. *Food Chem.,* **94**: 632-639.

Osborne, B.G. 2006. Applications of near infrared spectroscopy in quality screening of early-generation material in cereal breeding programmes. *J. Near Infrared Spectrosc.*, **14**: 93-101.

Roggo, Y., Duponchel, L. and Huvenne, J.P. 2004. Quality evaluation of sugar beet *(Beta vulgaris)* by near-infrared spectroscopy. *J. Agric. Food Chem.,* **52**: 1055-1061.

Scanlon, M.G., Pritchard, M.K. and Adam, L.R. 1999. Quality evaluation of processing potatoes by near infrared reflectance. *J. Sci. Food Agric.,* **79**: 763-771.

Shiley, D.A. 2010. Quantitative prediction of material properties using reflectance spectroscopy: a multivariate chemometrics-based approach. ASD Inc. Boulder, Co, US, 15 p.

Sileoni, V., van den Berg, F., Marconi, O., Perretti, G. and Fantozzi, P. 2011. Internal and external validation strategies for the evaluation of long-term effects in NIR calibration models. *J. Agric. Food Chem.,* **59**: 1541-1547.

Subedi, P.P. and Walsh, K.B. 2009. Assessment of potato dry matter concentration using short-wave near infrared spectroscopy. *Potato Res.*, **52**: 67-77.

Williams, P. 2003. *Near-Infrared Technology: Getting the Best Out of Light.* A short course in the practical implementation of near-infrared spectroscopy for the user. PDK Projects, Inc.: Nanaimo, Canada.

Williams, P.C. and Norris, K.H. 2001. *Near-Infrared Technology in the Agricultural and Food Industries.* 2nd Edn., AACC, St. Paul, Minnesota, USA.

Workman, J.J. 2008. Spectroscopy calibration basics. In: *Handbook of Near Infrared Analysis.* Burns D.A. and Ciurczak, E.W. (Eds.). CRC Press, pp. 123-149.

Yee, N., Bussell, W.T. and Coghill, G.G. 2006. Use of infrared spectra to identify cultivar in potato (*Solanum tuberosum*) crisps. *New Zealand J. Crop Hort. Sci.,* **34**: 177-181.