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Near infrared spectroscopy as a rapid non-invasive tool for agricultural and industrial process management with special reference to avocado and sandalwood industries

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ABSTRACT

Near infrared spectroscopy (NIRS) can play a vital role as a cost effective, rapid, non-invasive, reproducible diagnostic tool for many environmental management, agricultural and industrial waste water monitoring applications. In this paper we highlight the ability of NIRS technology to be used as a diagnostic tool in agricultural and environmental applications through the successful assessment of Fourier Transform NIRS to predict α-santalol in sandalwood chip samples, and maturity of 'Hass' avocado fruit based on dry matter content.

Keywords: Near infrared spectroscopy; Avocado maturity; Sandalwood oils; Non-invasive assessment

1. Introduction

Near infrared spectroscopy (NIRS) is a non-invasive method of measuring internal/external quality and safety attributes of agricultural products using optical light to determine chemical composition. The technology offers the advantage of being non-destructive, fraction of a second per test, with the potential to test every sample in an in-line application for various internal/external attributes simultaneously. Such technologies may also be utilized as tools for quality and sustainable management in the production environment. Field applications for soil and crop management would enable the primary producer to readily monitor individual plants and orchard/crop quality regularly for breeding programs, assist in fertilizer management, water and waste water monitoring and allow the primary producer to make informative decisions to achieve final product specifications and long term sustainable practices.

Science-based approaches to agricultural and environmental management are needed to assist with the impact of an increasing population and the demand to produce more food from the same amount of land and water without causing ecological damage [1]. Climate

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change and nutrient pollution are currently the top two global environmental changes most rapidly increasing in their negative impact on the ecosystem [2]. This increases the need for reliable and rapid analytical information to achieve more environmentally friendly and sustainable practices. NIRS has been demonstrated to be an accurate, precise, rapid and non-invasive alternative to wet chemistry procedures for providing information about relative proportions of C–H, O–H and N–H bonds which form the backbone of all biological material NIRS relies on calibrations in known data sets, which utilize absorbances at many wavelengths, to predict the composition of a sample [3,4]. However, to develop these calibrations requires many samples, many hours of work and many computer calculations [5]. The time-consuming collection of reference samples and the lack of reliable, precise chemical information may be a factor limiting the adoption of NIRS [1,2].

The advantage of NIRS over wet chemistry analyses lies in the fact that it is generally non-destructive and may be used in-situ, allowing determination of the chemical composition of the sample in its environment. The technique requires no or minimal sample preparation and avoids wastage and the need for reagents. Furthermore, the technique is multi-analytic allowing several simultaneous determinations. As a result, these techniques have developed into indispensable tools for academic research and industrial quality control, and a wide range of field applications from chemistry to agriculture, and from life sciences to environmental analysis.

NIRS has received considerable attention over the years for analysis of plant material for many constituents including: oil, moisture, pH, acidity, soluble solids, protein, lignin and cellulose [6–13]. NIRS has also supported the production of high crop yields through direct analysis of shoot tissues as an aid to appropriate fertilizer management in Australia and South Africa [1], and is utilized in plant breeding programs [6]. NIRS has become accepted by the international standards committees in some agricultural applications within the feed and food sectors [6]. More recently, there has been increasing interest in soil NIRS for precision agriculture, soil mapping and remote sensing. The technology has the potential to generate the extensive data bases on soil properties needed for generating spatial structure maps for soil properties that can be used for site-specific management of agricultural lands [14]. For example, NIRS has been used to determine a wide variety of soil parameters such as organic matter, pH, total nitrogen, electrical conductivity, extractable nutrients, heavy metals, microbial biomass, decomposition characteristics, salinity and soil quality indicators [3,6,13,15–17], with prospects for predicting soil fertility, soil erosion, soil infiltration capacity and plant growth [6].

Shepherd and Walsh [6] report that mineral forms of elements and plant constituents that occur in small concentrations can often be detectable indirectly, because of interactions or associations with other constituents that occur in measurable amounts. NIRS assessment in the laboratory or in the field has the potential to allow many samples to be tested rapidly and economically [1,2]. Pollution of water resources by domestic and/or industrial discharges has increased considerably in urban centres as a consequence of population growth [18]. The application of NIRS in the past was very limited for direct water quality assessment due to the strong water absorption of NIR by water. With the development of new optical sensors, water quality monitoring by NIRS is emerging for the detection of organic pollutants such as chlorinated hydrocarbons, pesticides and endocrine disrupting compounds [6]. NIRS has also been demonstrated as a promising alternative for determination of chemical oxygen demand in domestic waste water [18].

The application of NIRS in industrial process and waste water monitoring holds great potential as an on-line, real-time monitoring tool. The real-time monitoring capacity of NIRS is a very important feature for the application to industrial process and waste water monitoring, prediction and control as it would allow fast evaluation of the state of the process [19]. NIRS has been shown to successfully measure oil, urea, methanol and glycerol concentrations and solids content of the waste water discharge from biodiesel fuel production processes [20,21]. Preliminary work by Dias et al. [19] supports the use of NIRS as an on-line quality monitoring tool for activated sludge reactors to detect changes in the feed influent. These are only a few of the many potential applications of NIRS as a tool in agriculture, waste water and environmental management. Despite this fact NIRS is not yet widely adopted commercially for environmental applications [2].

The aim of this study was to assess the potential of Fourier Transform (FT) NIRS as an objective and non-invasive tool for agricultural and environmental management. The concept of this technology to be used as an agricultural and environmental tool is demonstrated through the assessment of FT-NIRS to predict 'Hass' avocado maturity based on dry organic matter content and the effects of geographic location on model performance, plus its ability to predict essential oils (i.e., α-santalol) in sandalwood chip samples.

2. Materials and methods

2.1. Avocado fruit samples

'Hass' avocado fruit were obtained throughout the 2008 growing season (harvest months: March to September) from two commercial farms in the major production district of Central Queensland, Australia. The farms were located in the Bundaberg (Latitude: 25° 14′ S, Longitude: 152° 16′ E) and Childers (Latitude: 25° 15′ S, Longitude: 152° 16′ E) regions.

Avocado fruit were harvested at three maturity stages throughout the season, corresponding to early, mid and late season harvests to encompass a large dry matter range in the test population. For each of the three season harvests approximately 100 fruit were randomly collected from each farm providing a total of around 600 individual fruit. Fruit were transported immediately to the laboratory located in Cairns, North Queensland and maintained at 22–24°C in a controlled temperature room prior to analysis and measurements commenced within two days of harvest.

2.2. Sandalwood samples

Sandalwood core samples were collected from different regions of Cape York in Australia, and Vanuatu as detailed in Table 1. A total of 295 samples were randomly collected as part of a separate field survey of natural populations of sandalwood in Cape York for comparison of oil quality to sandalwood species in the Pacific islands.

2.3. NIR method and data collection

2.3.1. Avocado fruit samples

A commercially available bench-top, Matrix-F, FT-NIR spectrophotometer (Bruker Optics, Ettlingen, Germany; operating software: OPUS™ version 6.5) in the 780–2500 nm range fitted with a fibre-coupled emission head was used in reflectance mode to obtain NIR spectra of whole, intact 'Hass' avocados. A path-length of approximately 170 mm from the external emission head utilizing 4 × 20 watt tungsten light sources to the surface of the whole avocado fruit provided a spectral scan diameter of approximately 50 mm.

Due to the large variability in the percentage dry matter (%DM) within a fruit [22,23] two NIR spectra were collected from each fruit, one spectra from each opposing side midway between the peduncle and base (i.e., equatorial region). Each spectrum measurement, were recorded as an average of 32 scans at a resolution of 8 cm^{-1}. A white spectralon standard was used as the optical reference standard for the system prior to the collection of each set of sample spectra.

2.3.2. Sandalwood samples

Destructive gas chromatography — mass spectrometry (GC-MS) technique was used to analyze a representative portion of each of the 295 sandalwood core samples following collection. The remainder of each of the sandalwood core samples following GC-MS analysis were stored in paper envelopes within large sealed plastic containers at room temperature. The samples were cut into fine chips (2–5 mm diameter) after storage for up to 19 months and individually scanned in reflectance mode using the integrating sphere configuration on a Multi Purpose Analyser (MPA) FT-NIR instrument (Bruker Optics, Ettlingen, Germany). A spectrum of each core sample was captured over the spectral range of 780–2780 nm. The sandalwood chip samples were placed into glass vials and scanned on the MPA integrating sphere carousel. In obtaining each sample spectrum, 32 scans at a resolution of 8 cm^{-1} were collected and averaged for all samples. A total of 4 separate spectra were collected for each sandalwood chip sample, with individual samples being mixed between each spectrum capture. The 4 spectra from each of the 295 chip samples were then averaged to provide one averaged spectrum per sample.

2.4. Reference methods

2.4.1. Avocado %DM analysis

The %DM reference measurement was obtained from the same area of the fruit that was used to obtain the NIR spectrum. To determine the %DM, a 50 mm diameter core equal to the NIR scan area was taken perpendicular to the surface of the fruit, at a depth of approximately 10–15 mm. The skin (2–4 mm) was removed from the avocado flesh, and the flesh was diced to facilitate drying in a fan-forced oven at 60–65°C to constant weight (approximately 72 h). The %DM is defined by the percentage ratio of the weight of the dried flesh sample to the original moist flesh

Table 1
Sandalwood core sample species and collection site details

Species	Location, Country	Sites
S. lanceolatum	Cape York/Australia	Injinoo, Lockhart River, Napranum, Kormpuraaw/Kowanyama, Coen, Aurunkun, Normanton/Delta Downs, Richmond/Basalt Walls, Hopevale.
S. austrocaledonicum	Efate, Vanuatu	Moso Island
	Erromango, Vanuatu	Rampunalvat, Pongkil Bay & South River
	Tanna, Vanuatu	Loanatit Point, Green Point, Imaru and Lomteuneakal
	Aniwa, Vanuatu	Entire island
	Malekula, Vanuatu	Wintua and Lawa
	North-west Santo, Vanuatu	Nokuku and Wusi

sample. It should be re-emphasized that fruit spectra and %DM were acquired after sample temperature equilibration in an air-conditioned laboratory at approximately 22–24°C, and within two days of harvest.

2.4.2. Sandalwood oil analysis

The GC-MS system consisted of a Shimadzu GC-14B with AOC-1400 auto-sampler, with 'Delta 5.0' software and Dataworx 'Datacenter 4000' interface controlling the computer. The GC parameters included an initial oven temperature of 60°C at 9°C/min for 3 min, and a final oven temperature of 200°C for 7 min with an inlet temperature of 200°C at 100 KPa and a flame-ionisation detector temperature of 300°C. Split injections of 50:1 were made by an autosampler. Sandalwood cores samples were thinly sliced and chipped in preparation for chromatography analysis. Where available, >1 g of sample was utilised in 10 ml of internal standard solution (1 mg/ml tetradecane in ethanol) and bought to the boil in a microwave. Samples were placed into GC vials following 48 h of extraction. Samples were analysed for a range of essential oils, but only α-santalol content data was used in this study. Tetradecane was used as an internal standard to determine the α-santalol (mg/g) content and oil concentration (mg/g) using α-santalol percentage from the chromatograph.

2.5. NIR data analysis

2.5.1. Avocado fruit samples

Statistical analysis was conducted using the commercially available chemometric software package 'The Unscrambler™' version 9.8 (CAMO, Oslo, Norway). Principal component analysis (PCA) was performed before partial least squares (PLS) regression models were developed and obvious spurious spectra removed. PLS regression with segmented cross-validation with 20 segments was used as the method for development of calibration models. Data pretreatment and smoothing for all avocado models in this study were based on a combination of a 25 point Sovitsky-Golay (SG) spectral smoothing (2nd order polynomial) and a first derivative transformation (25 point SG smoothing and 2nd order polynomial). Significant noise was found within spectral ranges 780–843 and 2414–2503 nm for all spectra. Fig. 1 depicts representative avocado raw spectra from the sample population.

Calibration models were developed for each farm/region and for a combination population encompassing both farms/regions. The sample population set for each farm and combination population was divided into calibration and prediction sets. Calibration sets were developed from PCA results providing a global representation of the attributes of the entire avocado population while eliminating repetition. Model performance was based on the coefficient of determination (R^2) of the calibration (R^2_c) and prediction (R^2_v) data sets; root mean square error of cross validation (RMSECV) and root mean square error of prediction (RMSEP) in relation to the bias (average difference between predicted and actual values) [24]. The standard deviation ratio (SDR) was used to determine the predictive ability of the calibrations (calculated as the ratio of standard deviation (SD) of the data set divided by the RMSECV or RMSEP) [25]. The higher the SDR statistic the greater the power of the model to predict the chemical composition accurately [26]. For example; SDR values between 2.0 and 2.4 for 'difficult' applications, such as high moisture materials including fruit and vegetables are regarded as adequate for rough screening; between 2.5

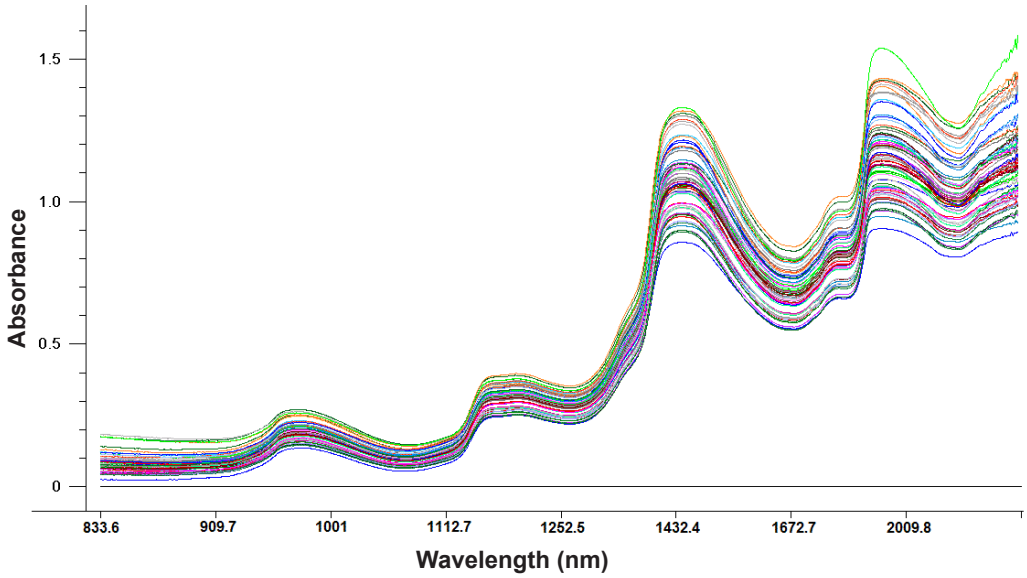

Fig. 1. Representative raw avocado spectra from the sample population.

and 2.9 are regarded as adequate for screening; between 3.0 and 3.4 is regarded as satisfactory for quality control; a value between 3.4 and 4.0 is regarded as very good for process control; while values above 4.1 are excellent for any application [27–29].

2.5.2. Sandalwood samples

OPUS™ QUANT (version 6.0 and 6.5) chemometric software was used to process all sandalwood spectra (wavelength region 834–2330 nm) and constituent data. PLS regression was used to build the calibration models of the diffuse reflectance spectral data with full cross validation. A range of multivariate statistical calibration techniques, including PLS and PCA together with various mathematical pre-processing, wavelength selection and outlier elimination were used to develop calibration models and determine the robustness of these models. The OPUS™ QUANT automatic selection process was used to select the best model. The selected model presented in this study was based on a combination of a 17 point spectral smoothing and a first derivative plus multiplicative scatter correction transformation. Sandalwood model performance was assessed as per the avocado models (see Section 2.5.1.), however, the residual prediction deviation (RPD) was reported by OPUS™ QUANT package instead of an SDR. RPD is similar to the SDR except the RPD uses a bias corrected RMSEP or root mean square error of calibration (RMSEC) [30].

3. Results and discussion

3.1. Avocado

With horticultural products, a major challenge with NIRS predictive models is to ensure that the calibration model is robust, so that the model holds across growing seasons and potentially across growing districts. Geographic location (growing district) effects may have a major consequence on model robustness as fruit composition is subject to within tree variability (i.e., tree age, crop load, position within the tree, light effects); within orchard variability (i.e., location of tree, light effects); and intra-orchard variability, such as soil characteristics, nutrition, weather conditions, fruit age and seasonal variability [31,32]. The influence of geographic location variability on %DM for whole avocado fruit was subsequently investigated over the 2008 growing season.

The PLS calibration and prediction model statistics for both the Bundaberg and Childers regions and combination of both regions are presented in Table 2. The Bundaberg data set of 607 spectra were separated into a calibration set (n = 209) and a prediction set (n = 397). The validation statistics of the calibration model were good and delivered an R^2_v = 0.93 with an RMSEP = 1.48 and SDR of 3.82 for %DM. An SDR value between 3.4 and 4.0 is regarded as very good for process control [27–29], and would allow for grading into three groups [33]. The Bundaberg PLS model was used to predict on the entire Childers population. As expected the application of the Bundaberg model to a population from another growing district was not as successful, providing a substantially reduced predictive performance with an R^2_v = 0.71, RMSEP = 2.68, SDR of 1.85 and bias of 1.99. Similarly, the Childers data set of 608 spectra were separated into a calibration set (n = 209) and prediction set (n = 399). The Childers PLS model also produced good validation statistics (R^2_v = 0.92 with an RMSEP = 1.55 and SDR of 3.48) when predicting fruit from within the Childers region. As with the Bundaberg model, the Childers model did not perform as well when it was used to predict %DM of fruit from a different geographic location.

Table 2
PLS calibration and prediction statistics for %DM for whole 'Hass' avocado fruit harvested over the 2008 season for each region and combination of both regions

Harvest 2008		Spectra n (OR)	%DM range	Mean	SD	LV	R^2	RMSECV	RMSEP	Bias	SDR
Calibration	Prediction										
Bundaberg		209 (1)	15.2–35.5	25.6	5.68	5	0.92	1.60		3.8e–7	3.55
	Bundaberg	397 (0)	15.6–35.1	25.8	5.66	5	0.93		1.48	0.063	3.82
	Childers	608 (0)	16.1–36.2	25.8	5.34	5	0.71		2.86	1.99	1.85
Childers		209 (2)	16.1–36.2	25.6	5.24	7	0.93	1.41		0.014	3.72
	Childers	399 (0)	16.5–36.1	26.0	5.40	7	0.92		1.55	–0.216	3.48
	Bundaberg	606 (1)	15.2–35.4	25.0	5.66	7	0.75		2.84	–0.163	1.99
Bundaberg & Childers		418 (3)	15.2–36.2	25.6	5.53	7	0.91	1.61		8.68e–7	3.43
	Bundaberg & Childers	796 (0)	15.7–36.1	25.9	5.52	7	0.93		1.51	–0.098	3.66

Note: OR = outliers removed; LV = latent variables

A generic calibration model was developed by combining both Bundaberg and Childers populations. Model predictive performance of the combined population was comparable to the individual regional models of Bundaberg and Childers, with an R_v^2 = 0.93, RMSEP = 1.51, and an SDR of 3.66 (see Fig. 2). These results demonstrate that there are spectral differences between growing districts and that each individual regional model does not incorporate the relevant spectral information enabling the model to successfully predict samples containing biological variability from a different growing district without reduced predictive performance. It is therefore important that calibrations be developed on populations representative in which sorting is to be attempted.

FT-NIR reflectance spectroscopy shows great promise for the application in a commercial, in-line setting for the non-destructive prediction of %DM of whole avocado fruit. Incorporating physiological variability from populations representative in which sorting is to be attempted is essential during calibration development to ensure model robustness and reliable predictive performance.

3.2. Sandalwood

The PLS calibration and prediction statistics for the sandalwood feasibility trial are summarised in Table 3. The data set of 295 spectra were randomly separated into a calibration set (n = 228) and a prediction set (n = 59). An R_c^2 of 0.93 was obtained for the calibration model, with an RMSECV of 3.67% and an RPD of 3.81 over a sample population range of 0.47–61.8% α-santalol. The validation statistics of the calibration model (see Fig. 3) delivered an R_v^2 = 0.87 with RMSEP = 4.71 and RPD = 2.72. These results were very encouraging and indicate that it is possible to use NIRS to predict α-santalol content in sandalwood chip samples.

The performance of a predictive model can be affected by many variables, for example temperature, geographic region, harvest time, cultivar, data pre-treatment and model algorithm [34]. It is therefore expected that model prediction statistics for a truly independent population will be poorer than the calibration statistics (i.e., RMSEP>RMSECV; |bias|>0) [34]. In the case for this sandalwood study an RPD of 3.81 indicated that the calibration model prediction would be suitable for process control purposes, while the RPD for the validation results indicated being only adequate for a screening tool. This decrease in RPD and the large prediction error of 4.71% may have also resulted from the combined effects of (i) the NIR spectrum was not obtained on the exact sandalwood sample that was tested by the GC-MS reference method and (ii) there was up to a 19 month time lapse

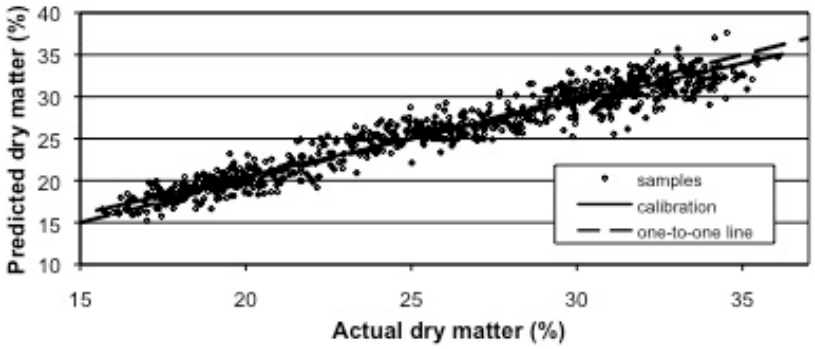

Fig. 2. Test set validation for predicted vs. actual dry matter content (%) in avocado samples.

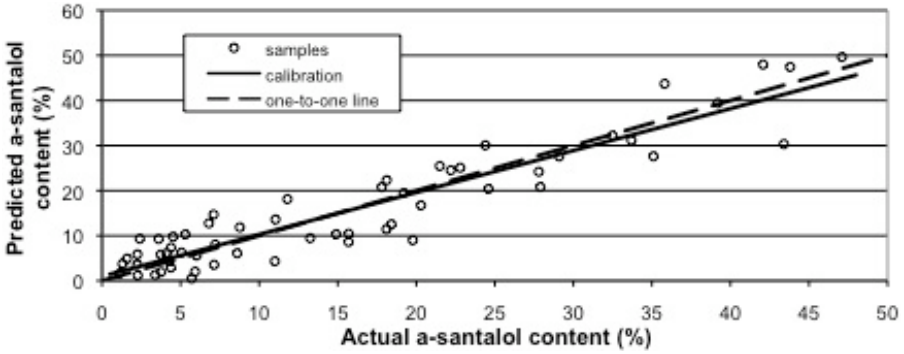

Fig. 3. Test set validation for predicted vs. actual α-santalol content in sandalwood chip samples.

Table 3
PLS calibration and prediction statistics for α-santalol content in sandalwood chip samples

Spectra *n* (OR)		α-santalol range (mg/g)	Mean	SD	LV	R2	RMSECV	RMSEP	RPD	Bias
Calibration	Prediction									
228 (8)		0.8–49	14.3	14.0	8	0.93	3.81		3.81	–0.046
	59 (1)	1.1–47.1	11.0	13.2	8	0.87		4.71	2.72	0.101

between the GC-MS and NIR sampling. This time lapse may have resulted in a decrease in santalol content due to the volatility of the oil in the samples. This was beyond the scope of the study which was focused on the ability of the technique to differentiate the samples.

Overall, FT-NIRS shows potential to be used as a rapid, non-invasive tool to assess sandalwood oil (α-santalol) content in chipped sandalwood samples. The technique of utilising NIRS technology for sandalwood quality and quantity determination needs to be further developed for utilisation as a tool in commercial applications. The technology offers the potential of being a rapid, non-invasive tool for assessing not only oil sample purity and quality of liquid oil samples, but also core wood samples in a processing plant situation, and seedlings and trees in a field environment. This has enormous possibilities for field selection of plants for processing and may be linked to selective breeding programs. This would enable genetic improvement programs to not only focus on quantity but also on the quality of the raw material, thus targeting the raw material to specific processes and products.

4. Conclusion

NIRS is now becoming readily adopted in many applications for the non-invasive rapid analysis of a wide variety of products. These include both quantitative compositional determinations, and qualitative determinations. However, as demonstrated in the application to %DM in avocados, it is important that calibrations be developed on population's representative in which assessment is to be attempted. Unfortunately, the process of calibration development is a major impediment to the rapid adoption of NIRS. The collection and precise analysis of the reference samples remains a time-consuming and a potentially costly exercise depending on the type of analysis. With this said, NIRS has an obvious place in agricultural and environmental applications with its core strength in the analysis of biological materials, plus low cost of analysis, simplicity in sample preparation, no chemical reagent requirements, simultaneous analysis of multiple constituents, good repeatability and high throughput capability. Also, laboratory based, on-site and in field NIR units can provide real-time information, enabling immediate decision making and problem solving. Thus, NIRS has the potential to be used as a major decision making tool for many agricultural, environmental and industrial waster water applications.

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