
INFRARED SPECTROSCOPY— LIFE AND BIOMEDICAL SCIENCES

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The Application of Near Infrared Spectroscopy for the Assessment of Avocado Quality Attributes

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1. Introduction

Quality and safety evaluation of agricultural products has become an increasingly important consideration in market/commercial viability and systems for such evaluations are now demanded by customers, including distributors and retailers. Unfortunately, most horticultural products struggle with delivering adequate and consistent quality to the consumer. Removing inconsistencies and providing what the consumer expects is a key factor for retaining and expanding both domestic and international markets. Most commercial quality classification systems for fruit and vegetables are based on external features of the product, for example: shape, colour, size, weight and blemishes. However, the external appearance of most fruit is generally not an accurate guide to the internal or eating quality of the fruit. Internal quality of fruit is currently subjectively judged on attributes such as volatiles, firmness, and appearance. Destructive subjective measures such as internal flesh colour, or objective measures such as extraction of juice to measure sweetness (°Brix) or assessment of dry matter (DM) content are also used, although obviously not for every fruit – just a sample to represent the whole consignment.

For avocado fruit, external colour is not a maturity characteristic, and its smell is too weak and appears later in its maturity stage (Gaete-Garreton et al., 2005). Since maturity is a major component of avocado quality and palatability, it is important to harvest mature fruit, so as to ensure that fruit will ripen properly and have acceptable eating quality. Currently, commercial avocado maturity estimation is based on destructive assessment of the %DM, and sometimes percent oil, both of which are highly correlated with maturity (Clark et al., 2003; Mizrach & Flitsanov, 1999). Avocados Australia Limited (AAL (2008)) recommend a minimum maturity standard for its growers of 23 %DM (greater than 10% oil content) for the 'Hass' cultivar, although consumer studies indicate a preference for at least 25 %DM (Harker et al., 2007).

The inability to consistently guarantee internal fruit quality is an important commercial consideration of the Australian avocado industry (HAL & AAL, 2005). Retail and consumer surveys over the last 15+ years have shown that consumers are not always satisfied with avocado quality, mainly because of poor flesh quality that can not be determined until the fruit is cut (Embry, 2009; Gamble et al., 2008; Harker et al., 2007; Hofman & Ledger, 1999). The surveys show that only 30% of the Australian population eat avocados and they expect to discard one in every four pieces of fruit they purchase because of poor internal quality (Avocados Australia Limited & Primary Business Solutions, 2005). Other reasons contributing to reduced consumption include concerns over spoilage, convenience, price and limited availability (Harker, 2009). The surveys revealed that consumers select bruising as the major defect, followed by body and stem end rots (Harker, 2009). Bruising was found to be a more important barrier to purchasing than price (Harker, 2009).

Fruit quality reliability is a key factor impacting on supply chain efficiency and related profitability since repeat purchasing by consumers is significantly affected by a bad eating experience. For example, avocados with internal defects of 10% or more have a dramatic negative impact on the consumer repurchasing (Embry, 2009; Petty & Embry, 2011). Research has shown that if a consumer is dissatisfied with the quality of fruit purchased, then that consumer will not purchase that commodity for another 6 weeks (Embry, 2009). Australian avocado quality surveys have shown that increased levels of purchase can be achieved by improving overall quality. For example, there is potential to increase consumer purchasing by 9% by reducing the average level of damage or defects by 15% (Embry, 2009).

Australian avocado production is expanding rapidly and there are strong financial incentives to increase sales domestically and to export product to increase returns directly. Reliable export of avocados from Australia by sea freight requires long storage times, typically 2 - 3 weeks to Asia and 5 - 6 weeks to the European Union (Hofman & Marques, 2009). The biggest risk during transport is the development of rots and flesh disorders resulting in a poor quality product. The additional time and distance associated with most export markets results in longer times from harvest to consumption which increases the risks of quality loss before the consumer receives the fruit. The key factor for retaining and expanding both domestic and international markets is removing inconsistency and providing what the consumer expects, i.e., a consistent quality product with suitable DM content and fruit free of bruises and flesh disorders. A rapid and non-destructive system that can accurately and rapidly monitor internal quality attributes would allow the avocado industry to provide better, more consistent fruit eating quality to the consumer, and thus improve industry competitiveness and profitability.

The development of automated technologies has enabled commercially feasible non-invasive methods for estimating internal quality attributes of agricultural products. These methods are generally based on one of the following properties: nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI), ultrasonics for vibrational characteristics, X-ray and gamma ray transmission, electrical properties, firmness, density, optical reflectance and transmission. Today, emphasis is put on the development of non-destructive methods for real-time in-line applications. Although several non-invasive techniques exist for this (Abbott, 1999; Butz et al., 2005; Chen & Sun, 1991; Gaete-Garretton et al., 2005; Mizrach, 2000; Mizrach & Flitsanov, 1999), NMR and near infra-red spectroscopy (NIRS) are the leading candidates for the application to fruit and vegetables. NMR has been demonstrated to have

the potential to measure the DM percentage in avocados (Chen et al., 1993; Kim et al., 1999), but the cost and challenges for in-line use in the sorting line means that it is not currently a commercially viable application for high volume, low value items such as fruit and vegetables (Clark et al., 1997; Clark et al., 2003).

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. Analysis of NIRS absorption spectra aids in the qualitative and quantitative determination of many constituents and properties of horticultural produce, including oil, water, protein, pH, acidity, firmness, and particularly soluble solids content or total soluble solids of fresh fruits (Abbott, 1999; Butz et al., 2005; Scotter, 1990). Of particular importance for the current study, NIRS has been used to estimate %DM in various horticultural products (Birth et al., 1985; Hartmann & Bijning-Pfaue, 1998; McGlone & Kawano, 1998; Sivakumar et al., 2006; Xiaobo et al., 2006) including avocados (Clark et al., 2003; Schmilovitch et al., 2001; Walsh et al., 2004). The technique requires minimal or no sample preparation, and avoids wastage and the need for reagents. Furthermore, it is multi-analytical, allowing estimates of several characteristics simultaneously and has the potential to test every piece of product in an in-line setting.

NIRS is a secondary method of determination and therefore must be calibrated against a primary reference method to develop a calibration model. However, to develop these predictive models requires many samples, many hours of work and many computer calculations to develop a statistical model which can be used to predict future samples (Davies, 2005). The validity of the calibration models for future predictions depends on how well the calibration set represents the composition of new samples. With horticultural products, the major challenge is to ensure that the calibration model is robust, that is, that the calibration model holds across growing seasons and potentially across growing districts.

NIR as a tool to assess internal quality attributes of intact horticultural produce is well established in literature. In general however, the robustness of calibration models with respect to biological variability from different seasons has been neglected and therefore these calibration models may be optimistic with respect to prediction accuracies on future samples in practical applications, such as grading lines (Nicolai et al., 2007). Nicolai et al. (2007) report that model prediction error in general may easily double when a calibration model is applied to a spectral data set of a different season or orchard. This lack of robustness often translates into bias (Golic & Walsh, 2006; Nicolai et al., 2007). Robustness of calibration is consequently a critical issue (Nicolai et al., 2007; Sánchez et al., 2003) and there has been recent work on fruit that considers the effect of different seasons (Peiris et al., 1998; Peiris et al., 2003; Miyamoto and Yoshinobu, 1995; Liu et al., 2005; Guthrie et al., 2005). These studies generally found that incorporating data from multiple growing seasons in the calibration model improved the predictive performance, compared with those calibration models developed using an individual season. Peiris et al. (1998) studied model robustness for the determination of soluble solids (SS) content of peaches and reported that a calibration developed on a population from three consecutive growing seasons had an improvement in prediction performance on a combined season validation set (standard error of prediction (SEP) of 0.94 - 1.26 %SS, and bias 0.17 - 0.38 %SS) over that developed from an individual season population (SEP of 0.90 - 1.36 %SS and bias 0.17

- 2.08 %SS). Peirs et al. (2003) studied the robustness of calibration models for SS content ($^{\circ}$ Brix) of 'Golden Delicious' apples, with respect to the effects of orchard, season and cultivar. It was found that the largest source of spectral variation between measurements on different fruit was due to seasonal effects. When more seasonal variability was included in the calibration set, for example the model based on the data of all three seasons, the predictive error reduced by approximately 10 to 60%. Similar studies on the seasonal effects for various fruit report similar outcomes (Guthrie, 2005; Liu et al., 2005; Miyamoto & Yoshinobu, 1995).

There have been limited investigations of avocado maturity based on %DM using NIRS. Schmilovitch et al. (2001) used a dispersive NIR spectrophotometer in reflectance mode to assess the 'Ettinger' and 'Fuerte' cultivars (both relatively thin-skinned) in the range 1200 - 2400 nm. Preliminary results identified standard errors of prediction for both 'Ettinger' and 'Fuerte' as 0.9 and 1.3%, respectively, over a 14 - 24 %DM range. Clark et al. (2003) investigated the use of a fixed polychromatic/diode array (PDA) spectrophotometer for estimating %DM in whole New Zealand 'Hass' avocado fruit using both reflectance and interactance modes. They concluded that interactance mode was a better predictor of %DM compared with reflectance. Reflectance models required high numbers (12 to 20) of latent variables (LV), indicating the models struggled against spectral noise and so required incorporation of many small spectral features to improve accuracy. Clark et al. (2003) reported interactance validation statistics of R^2 (coefficient of determination) prediction >0.83, and root mean square error of prediction (RMSEP) <1.8 %DM, over a range of 20 - 45 %DM, while the corresponding reflectance results were <0.75 and >1.9 %DM, respectively. Walsh et al. (2004), using a fixed PDA spectrophotometer (Ziess MMS1/NIR-enhanced spectrometer, Germany) in the 300 - 1100 nm range, reported calibration results of r (correlation coefficient) = 0.89, root mean square error of cross-validation (RMSECV) = 1.14, with a standard deviation ratio (SDR = standard deviation of the data set divided by the RMSECV or RMSEP) = 2.2, for %DM of avocado fruit of unspecified cultivar. The SDR statistic is the measurement of the ability of an NIRS model to predict a constituent and enables comparison of model performance across populations with different standard deviations (Baillères et al., 2002; Golic & Walsh, 2006). The higher the SDR statistic the greater the power of the model to predict the chemical composition accurately (Cozzolino et al., 2004). 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; a value between 2.5 and 2.9 are regarded as adequate for screening; a value 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; values above 4.1 are excellent for any application (Nicolai et al., 2007; Schimleck et al., 2003; Williams, 2008).

This study assessed the potential of FT-NIR diffuse reflectance spectroscopy as an objective non-invasive method for determining internal quality attributes of whole 'Hass' avocado fruit. These include: (a) to predict maturity and thereby eating quality based on %DM; (b) to predict the risk of developing internal rot disorders (i.e., rot susceptibility) as an indication of shelf-life; (c) to detect bruises. The study also demonstrates the importance of the calibration model development process to incorporate seasonal and geographical variability to ensure model robustness.

2. Materials and methods

2.1 Avocado fruit samples

2.1.1 Fruit for dry matter model development

'Hass' avocado fruit were obtained over the 2006, 2007 and 2008 growing seasons (Harvest months: May to November) from two commercial farms in the major production districts of Bundaberg, South East Queensland (Latitude: 24° 52' South, Longitude: 152° 21' East) and Childers, South East Queensland (Latitude: 25° 14' South, Longitude: 152° 16' East). Avocado fruit were harvested at three maturity stages through each season, corresponding to early, mid and late season harvests over the three growing seasons. This allowed for sufficient variability in the %DM range and other seasonal factors to be included in the calibration procedure. A minimum of 100 fruit were collected at each harvest giving a total of a minimum of 900 individual fruit for each growing region. All fruit were harvested at the hard green stage of ripeness.

2.1.2 Fruit for impact and rot model development

'Hass' avocado fruit were obtained over the 2008 growing season from two farms in Queensland, Australia. The first farm is located near Ravenshoe on the Atherton Tablelands in North Queensland (Latitude: 17° 38' South, Longitude: 145° 29' East) and the second farm is located in the major production district of Toowoomba, South East Queensland (Latitude: 27° 33' South, Longitude: 151° 58' East). Fruit from Ravenshoe were used for the impact assessment trials (n = 102), while Toowoomba fruit (n = 125) were used for rot susceptibility (shelf life) trials. All fruit were harvested at the hard green stage of ripeness.

2.2 NIR data collection

2.2.1 Dry matter NIR data

The spectra of whole, intact avocado fruit were collected using a commercially available Matrix-F, FT-NIR spectrophotometer (Bruker Optics, Ettlingen, Germany; operating software: OPUS™ version 5.1 - 6.5) in the 830 - 2500 nm range. Spectra were obtained in diffuse reflectance mode, using a standard 4 x 20 watt tungsten light source fibre-coupled emission head fitted to the spectrometer. The external emission head was placed directly above the avocado fruit (0° configuration). A light reducing box with a 60 mm diameter cut out window was used to hold the fruit, so that the fruit skin was directly exposed to the focal point of the emission head. A path-length of approximately 170 mm from the external emission head light source to the surface of the fruit provided a spectral scan diameter on the avocado of approximately 50 mm. In obtaining each sample spectrum, 32 scans at a resolution of 8 cm⁻¹ were collected and averaged. Due to the large variability in the %DM within a fruit (Schroeder, 1985; Wedding et al., 2010; Woolf et al., 2003) two NIR spectra were collected from each fruit, one spectra from each opposing side midway from the peduncle and base (i.e., equatorial region). A white spectralon standard was used as the optical reference standard for the system prior to the collection of each set of sample spectra. Fruit spectra were acquired after sample temperature equilibration in an air-conditioned laboratory at approximately 22 - 24 °C, and within two days of harvest.

2.2.2 Impact and rot assessment NIR data

For both impact (bruise) and rot assessment trials, diffuse reflectance spectra of whole, intact 'Hass' avocado fruit were collected in the 830 – 2500 nm range using a Bruker Matrix-F, FT-NIR spectrophotometer as discussed in section 2.2.1. Spectra for rot susceptibility prediction were collected from each opposing half of the hard green fruit prior to fruit being placed into 20 °C storage at 85 - 95% relative humidity. At eating ripe fruit were then assessed for rots based on a weight percentage of the flesh volume affected.

For impact assessment, hard green fruit were stored at 20 °C and 85 - 95% relative humidity until fruit reached the sprung stage of ripeness. The sprung stage of ripeness is where the flesh deforms by 2 - 3 mm under extreme thumb pressure (White et al., 2001). Individual spectra were collected from a single side of the fruit on reaching the sprung stage of ripeness. Following initial spectra collection, fruit were dropped from a height of 100 cm against a slate paver (height: 400 mm, length: 400 mm, width: 40 mm) placed upright and supported by concrete blocks to simulate impact damage. Individual fruit were placed into a cotton mesh bag which was firmly suspended by two strings attached to the laboratory ceiling. Each fruit was positioned so that the scanned area would impact against the paver. The fruit in the mesh bag was pulled backwards away from the slate paver and released to swing in a pendulum motion to impact against the slate paver. Fruit were only allowed to impact the paver once. The height from the ground to the middle of the fruit was measured with the fruit sitting freely against the slate paver. The drop height was measured as the difference between the height at the top of the arch, and the height at the bottom of the arch where the fruit hit the paver.

The impacted area was re-scanned after 1 - 2 hours (maximum of 4) and again after 24 hours. Fruit were then placed back into 20 °C storage at 85 - 95% relative humidity and assessed for bruises at eating ripe (approximately 5 days following impact). Bruise assessment was based on visual estimate of percentage bruise development of the flesh within the scanned area.

2.3 Chemical analysis

The %DM reference measurement was obtained from the same area of the fruit that was used to obtain the NIR spectra. 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 hours). The %DM is defined by the percentage ratio of the weight of the dried flesh sample to the original moist flesh sample. It should be 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 Data analysis

2.4.1 Dry matter data analysis

Data analysis was carried out using the commercially available chemometric software package 'The Unscrambler™' version 9.8 (CAMO, Oslo, Norway). The sample spectra for

each data set were separated into a calibration set and prediction set to develop the calibration and prediction models respectively. Fruit were assigned to the calibration set from the principal component analysis (PCA) results to provide a global representation of the attributes of the entire fruit population while eliminating repetition. All remaining fruit were used in the validation sets. Partial least squares (PLS) regression was used to build the prediction models of the diffuse reflectance spectral data, using segmented cross validation (20 segments in this case). Before calibration model development, the variation of the spectral data was analysed by PCA, and obvious spurious spectra eliminated. Data pretreatment and smoothing for the individual %DM models for each growing location in this study were based on a combination of: a 25 point Savitsky-Golay (SG) spectral smoothing (2nd order polynomial) and a second derivative transformation (25 point SG smoothing and 2nd order polynomial) for the Bundaberg models; and a 25 point SG spectral smoothing (2nd order polynomial) and a multiplicative scatter correction (MSC) transformation for the Childers models. For the combined Bundaberg and Childers model, data pretreatment and smoothing was based on a combination of a 25 point SG spectral smoothing (2nd order polynomial) and a first derivative transformation (25 point SG smoothing and 2nd order polynomial). Among all spectra collected, significant noise was found at the extremities of the spectral range (830 - 843 and 2414 - 2503 nm). Therefore all raw spectra used for analysis were truncated to a range of 843 - 2414 nm before model development. Typical absorbance spectra for 'Hass' avocado fruit are shown in Figure 1. Model performance was based on the R^2 of the calibration (R_c^2) and validation/prediction (R_v^2) data sets; RMSECV; RMSEP in relation to the bias (average difference between predicted and actual values) (Buning-Pfaue, 2003), and the SDR.

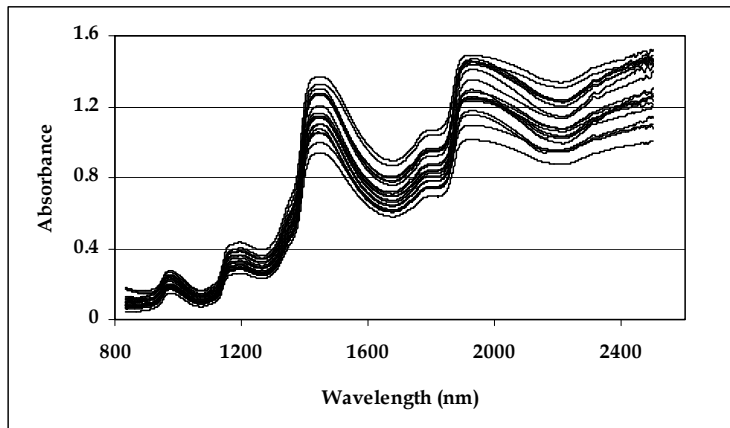


Fig. 1. Typical absorbance spectra for whole 'Hass' avocado fruit.

2.4.2 Impact and rot data analysis

'The Unscrambler™' version 10.1, (CAMO, Oslo, Norway) was used for discriminative analysis to separate the avocados into categories based on percentage rot and percentage bruise development of the scanned area. The 1 - 2 hours impact wavelengths were subjected to weighting by the standard deviation prior to analysis.

3. Results and discussion

3.1 Dry matter prediction

The calibration and prediction model (Figure 2) statistics for each individual year (Table 1) for both harvest locations indicate that FT-NIRS in diffuse reflectance has potential as a screening tool to predict %DM on whole 'Hass' avocado fruit. The 2006 and 2007 harvest seasons had lower standard deviations (SD) than the 2008 season for both the Bundaberg and Childers locations. For the two harvest locations the 2008 harvest season calibration and prediction statistics were the best in terms of regression (R^2) and SDR. The RMSEP for each harvest season varied between 1.29 to 1.49 %DM and 1.41 to 1.94 %DM for Childers and Bundaberg respectively. This suggests that the fruit obtained from the 2006 and 2007 harvest seasons possibly did not include a sufficiently broad variability in physiological attributes to develop a more suitable calibration model as seen with the 2008 harvest season, although other biological or environment effects may have contributed. The number of latent variables are within an acceptable range for the number of samples for all models (Hruschka, 1987; Lammertyn et al., 2000).

Location - Year	Spectra n (OR)	%DM range	Mean	SD	LV	R^2	RM SEC V	RM SEP	Bias	Slope	SDR
Bu-2006	629	18.2-35.0	27.5	3.2							
CAL	222(2)	18.2-35.0	27.2	3.5	7	0.75	1.76		-0.159	0.759	2.0
PRE	407	20.334.2	27.6	3.0	7	0.75		1.50	-0.582	0.818	2.0
Bu-2007	609	19.0-34.4	25.7	2.7							
CAL	211(0)	19.0-34.4	25.7	2.8	8	0.76	1.39		-0.0024	0.779	2.0
PRE	398(0)	19.7-32.5	25.7	2.6	8	0.70		1.41	0.112	0.754	1.8
Bu-2008	606	15.2-35.5	25.7	5.7							
CAL	209(1)	15.2-35.5	25.6	5.7	6	0.90	1.76		-0.0036	0.910	3.2
PRE	397(0)	15.6-35.1	25.8	5.7	6	0.88		1.94	0.1526	0.865	2.9
Ch-2006	632	21.4-39.7	29.8	3.4							
CAL	207 (2)	21.4-39.7	30.2	3.7	9	0.82	1.57		0.006	0.829	2.4
PRE	425 (0)	21.7-37.9	29.5	3.3	9	0.80		1.47	0.0761	0.850	2.2
Ch-2007	609	21.9-36.8	29.2	3.1							
CAL	209 (0)	21.9-36.8	29.1	3.3	8	0.83	1.36		-0.0098	0.842	2.4
PRE	400 (1)	22.2-36.2	29.2	3.0	8	0.81		1.29	-0.2867	0.835	2.3
Ch-2008	608	16.1-36.2	25.8	5.3							
CAL	209 (2)	16.1-36.2	25.6	5.2	7	0.93	1.39		0.0098	0.934	3.8
PRE	399 (0)	16.5-36.1	26.0	5.4	7	0.92		1.49	-0.1594	0.858	3.6

Table 1. PLS calibration (CAL) and prediction (PRE) statistics for %DM for whole 'Hass' avocado fruit harvested from Bundaberg (Bu) and Childers (Ch) over the 2006, 2007 and 2008 seasons. Note: OR = outliers removed; LV = latent variables; n = number of samples.

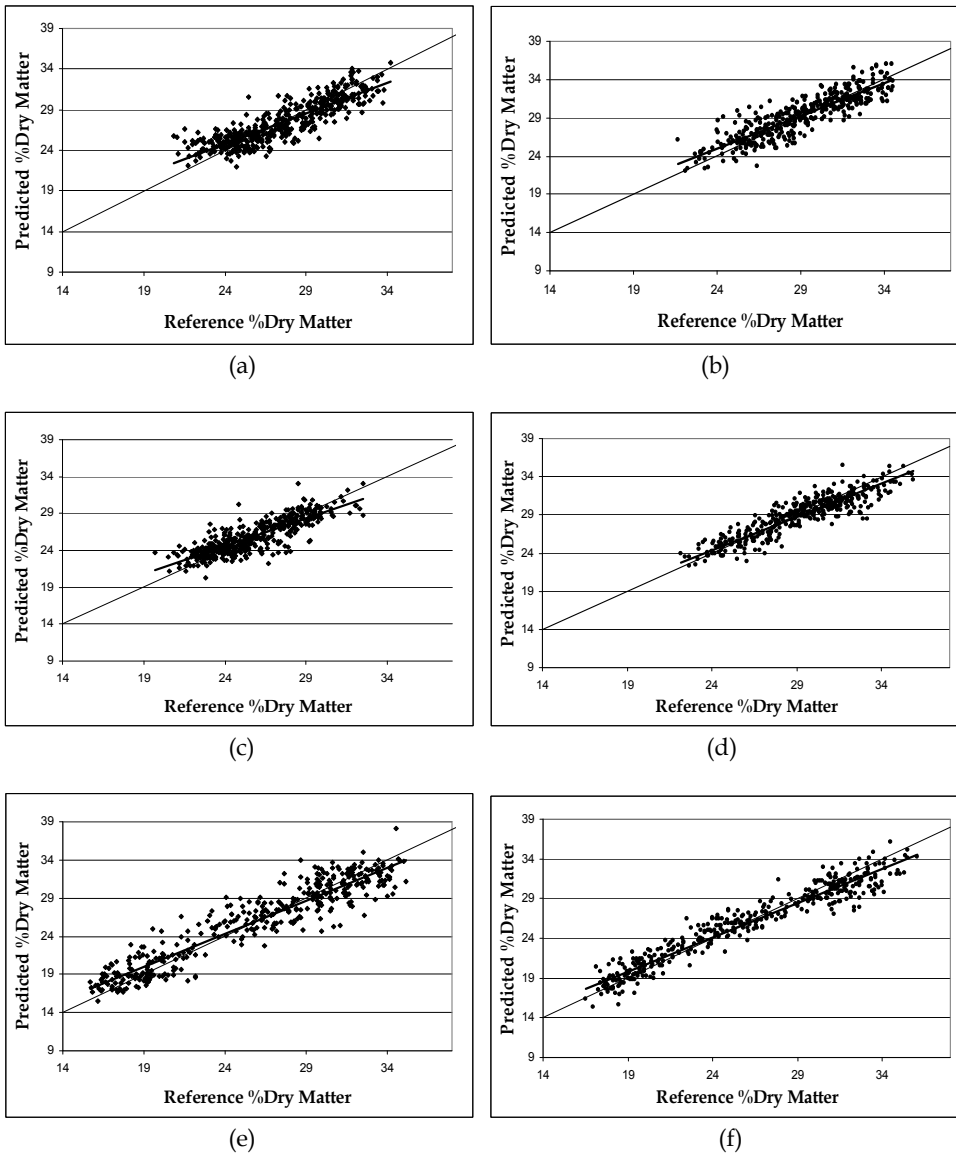


Fig. 2. Model predictions plotted against actual constituent values for %DM for (a) Bundaberg 2006 season, (b) Childers 2006 season, (c) Bundaberg 2007 season, (d) Childers 2007 season, (e) Bundaberg 2008 season, and (f) Childers 2008 season.

Large seasonal effects have a major consequence for calibration models for horticultural produce, since the spectral deviations due to biological variability of future samples cannot be predicted (Peirs et al., 2003). The influence of seasonal variability was investigated for the Bundaberg and Childers growing locations over three years. For both growing locations, the

2006 calibration model was used to predict on the 2007 season population. A combined calibration set using spectra from 2006 and 2007 seasons was used to develop a calibration model that was then subsequently used to predict the 2008 season population. A combined calibration set of 2006, 2007 and 2008 seasons was used to predict over all 3 years. Table 2 displays the summary statistics of the PLS calibration and prediction models for these combinations.

Location - Year		Spectra n (OR)	%DM range	SD	LV	R ²	RM SEC V	RM SEP	Bias	SDR
CAL	PRE									
Bu-2006		222(2)	18.2-35.0	3.5	7	0.75	1.76		-0.159	2.0
	Bu-2007	609	14.1-34.4	2.7		0.09		5.07	4.358	0.5
Bu-2006-07		426	14.1-35.0	3.1	9	0.75	1.60		0.112	1.9
	Bu-2008	606	15.2-35.5	5.7		0.45		4.3	0.161	1.4
Bu-2006-08		600(4)	15.8-35.4	4.2	6	0.86	1.55		-0.009	2.7
	Bu-2006-08	1244(1)	14.1-35.6	4.1	6	0.87		1.48	0.0104	2.8
Ch-2006		207(2)	21.4-39.7	3.7	9	0.82	1.57		0.006	2.4
	Ch-2007	609(0)	21.9-36.9	3.1	9	0.14		2.84	1.601	1.1
Ch-2006-07		415(1)	21.4-39.7	3.5	12	0.82	1.49		0.003	2.4
	Ch-2008	608(0)	16.1-36.2	5.3	12	0.79		2.45	-0.547	2.2
Ch-2006-08		624(1)	16.1-39.7	4.6	10	0.88	1.62		-0.001	2.8
	Ch-2006-08	1224(0)	16.5-37.9	4.3	10	0.89		1.43	-0.021	3.0

Table 2. PLS calibration (CAL) and prediction (PRE) statistics for %DM for whole 'Hass' avocado fruit from both Bundaberg (Bu) and Childers (Ch) for 2006, 2006-07 and 2006-08 seasons predicting on 2007, 2008 and 2006-08 seasons respectively. *Note: OR = outliers removed; LV = latent variables; n = number of samples.*

As expected, the application of single seasonal calibrations to populations from other growing seasons was not very successful due to seasonal biological variation. For example, the 2006 calibration models for both Bundaberg and Childers could not be used to predict the 2007 season population for the corresponding harvest location. Model predictive performance improved as more biological variability was included in the models, as seen when the combined 2006 and 2007 models was used to predict on the 2008 season. The combined 2006, 2007 and 2008 calibration models (Figure 3) was sufficiently robust to predict %DM of whole Hass avocado to within 1.48% with an $R_v^2 = 0.87$ and SDR of 2.8 for Bundaberg; and to within 1.43 %DM with an $R_v^2 = 0.89$ and SDR of 3.0 for the Childers harvest location. This indicated an ability to sort the fruit into three categories with approximately 80% accuracy (Guthrie et al., 1998).

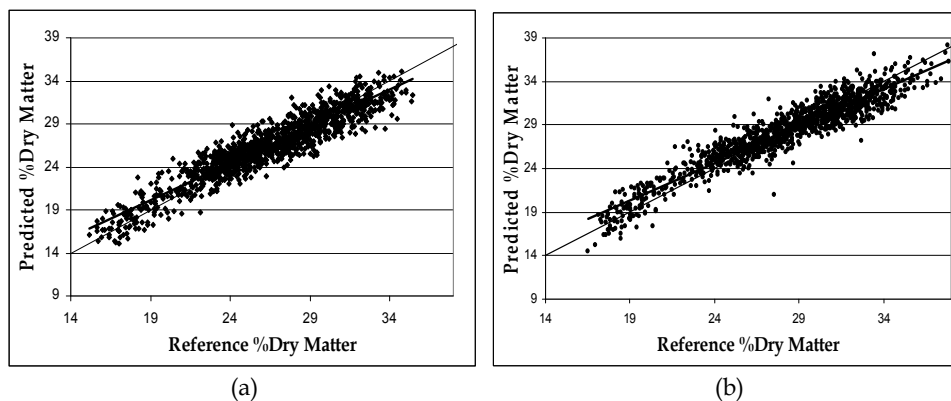


Fig. 3. Model prediction for the combined 2006-08 calibration model for both (a) Bundaberg and (b) Childers locations predicting on the combined 2006-08 prediction set plotted against actual constituent values for % dry matter.

This study demonstrated that including data from multiple growing seasons in the calibration model will improve the predictive performance, in comparison to calibration models developed using an individual season. This is in agreement with the previous studies on this topic (Peiris et al., 1998; Peiris et al., 2003; Miyanoto and Yoshinobu, 1995; Liu et al., 2005; Guthrie et al., 2005). As more biological variability is built into the model, the prediction accuracy becomes less sensitive to unknown changes of external factors (Bobelyn et al., 2010). However, in some cases, including more biological variability (at the risk of including atypical data) in the calibration set can significantly reduce the models prediction accuracy (Bobelyn et al., 2010).

Geographic location (growing regions) effects may also 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 (i.e., soil characteristics, nutrition, weather conditions, fruit age and season variability) (Marques et al., 2006; Peiris et al., 2003). The influence of geographic location variability on %DM for whole avocado fruit was subsequently investigated by assessing calibration model performance using avocado fruit obtained from Bundaberg and Childers locations collected over 3 years.

The PLS calibration and prediction model statistics for both the Bundaberg and Childers harvest locations and combination of both regions are presented in Table 3. The Bundaberg data set of 1844 spectra was separated into a calibration set ($n = 600$) and a prediction set ($n = 1244$). The validation statistics of the calibration model were quite good and delivered an $R_v^2 = 0.87$ with an RMSEP = 1.48 and SDR of 2.8 for %DM. An SDR value between 2.5 and 2.9 is regarded as adequate for screening (Nicolai et al., 2007; Schimleck et al., 2003; Williams, 2008). 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 region was not as successful, providing a substantially reduced predictive performance with an $R_v^2 = 0.59$, RMSEP = 2.84 and SDR of 1.55. Similarly, the Childers data set of 1848 spectra were separated into a calibration set ($n = 624$) and prediction set ($n = 1224$).

Harvest Location		Spectra n (OR)	%DM Range	SD	LV	R ²	RM SECV	RM SEP	SDR
Calibration	Prediction								
Bundaberg		600(4)	15.8-35.4	4.2	6	0.86	1.55		2.7
	Bundaberg	1244(1)	14.1-35.6	4.1	6	0.87		1.48	2.8
	Childers	1847	16.1-39.7	4.4	6	0.59		2.84	1.55
Childers		624(1)	16.1-39.7	4.6	10	0.88	1.62		2.8
	Childers	1224(0)	16.5-37.9	4.3	10	0.89		1.43	3.0
	Bundaberg	1844(1)	14.1-35.5	4.2	10	0.74		2.14	1.96
Bundaberg & Childers		1224(4)	15.8-39.7	4.5	9	0.88	1.55		2.9
	Bundaberg & Childers	2468(1)	14.1-37.9	4.3	9	0.89		1.42	3.1

Table 3. PLS calibration and prediction statistics for %DM for whole 'Hass' avocado fruit harvested over three seasons for Bundaberg and Childers growing locations and combination of both regions. *Note: OR = outliers removed; LV = latent variables; n = number of samples.*

The Childers PLS model also produced reasonable validation statistics ($R_v^2 = 0.89$ with an RMSEP = 1.43 and SDR of 3.0) 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 such as the combined 2006-08 Bundaberg population ($R_v^2 = 0.74$ with an RMSEP = 2.14 and SDR of 1.96).

A calibration model was developed by combining both Bundaberg and Childers populations, incorporating biological variability from both regions over three growing seasons. Model predictive performance of the combined population was comparable to the individual regional models of Bundaberg and Childers, with an $R_v^2 = 0.89$, RMSEP = 1.42, and SDR of 3.1 (Figure 4). 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.

Interpreting NIR models in terms of the various fruit components is often difficult due to spectral co-linearity where information in a model may not necessarily be carried by just a few independent wavelengths, but is possibly a combined effect of many wavelengths with each contributing only relatively little information (McGlone & Kawano, 1998). For oil, strong electromagnetic absorption is reported around 2200 – 2400 nm (CH_2 stretch bend and combinations), with weaker absorption around 1750, 1200 and 900 – 920 nm ranges, and

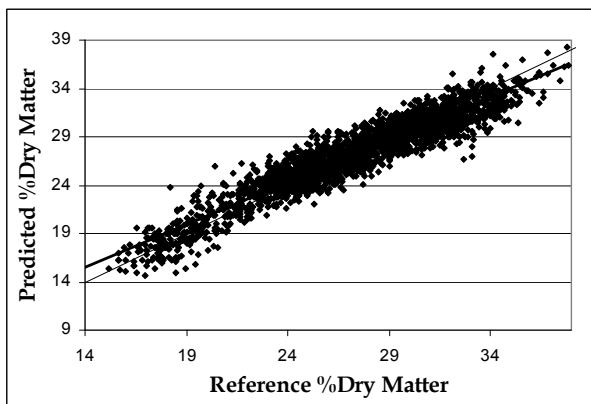


Fig. 4. Model prediction for the Bundaberg and Childers combined 2006-08 calibration model predicting on the Bundaberg and Childers combined 2006-08 prediction set plotted against actual constituent values for %DM.

930 nm (overtones of CH_2 stretching) (Clark et al., 2003; Guthrie et al., 2004; Osborne et al., 1993). Williams and Norris (1987) report that the 1300 - 1750 nm range is very fruitful for absorbers for use in the determination of protein and oil. The 900 - 920 nm absorbance band is often cited as the most important band for %DM and/or sugar determination, as it is removed from the troublesome interferences from the water absorbance peaks that typically dominate spectra of fruit (Clark et al., 2003). However, light penetration depth is wavelength dependent (Lammertyn et al., 2000). The 700 - 1100 nm short-wavelength NIR region allows better penetration into biological material, while wavelengths above 1100 nm (long-wavelength region) have limited penetration providing information only relatively close to the surface (Guthrie et al., 2004; Saranwong & Kawano, 2007). In some instances, there may be secondary correlations between skin properties and those of the bulk flesh and in these circumstances the long-wavelength region can provide relevant information.

The regression coefficient vectors β for the dry matter calibration models across all years in this avocado study had many similar peak positions over the 850 - 2250 nm range. However as expected, there were slight differences in wavelength selection from one year to another which can be attributed to seasonal variability. Relevant spectral information for the calibration models was obtained primarily from oil, carbohydrate and water absorbance bands clustered in the 900 - 980 nm region (second and third overtone), with further contribution from absorbance bands for oil in the vicinity of 1360, 1703, 1722 and 1760 nm. However, these assignments can only be tentative because of other peaks and troughs present hereabouts in the β vectors.

The results of this study are very encouraging and compare favourably to the results obtained by Clark et al. (2003) (RMSEP of 2.6 %DM over a 20 - 45 %DM range and an R_c^2 of 0.75) and Walsh et al. (2004) ($R_c^2 = 0.79$, RMSECV = 1.14, SDR = 2.2, for %DM of an unspecified cultivar) using a fixed PDA spectrometer in reflectance mode. The current FT-NIRS reflectance combined models for both Bundaberg and Childers compare well with the model accuracy obtained by Clark et al. (2003) (R_c^2 of 0.88 and an RMSEP of 1.8 %DM) using a PDA spectrometer in interactance mode, indicating reflectance FT-NIRS may be a suitable

alternative for in-line and at-line environments. Another comparative study was conducted by Schmilovitch et al. (2001) for two relatively thin skin cultivars, 'Ettinger' and 'Fuerte', during a single season. They used a dispersive NIR spectrophotometer in reflectance mode in the 1200 - 2400 nm range, reporting errors of prediction for 'Ettinger' and 'Fuerte' of 0.9% and 1.3% respectively, for fruit having a 14 - 24 %DM range. It is likely that the relatively smooth to medium textured, thin-skin cultivars would not suffer to the same extent from the physiological limitations experienced in the thick rough skin of 'Hass', and prediction errors would certainly be expected to be lower. We must emphasize however, it is difficult to make a meaningful comparison of the various techniques as there is insufficient detail presented in these papers to establish if the differences are associated with the spectroscopic technique or with the geometry of the configurations used.

3.2 Impact and rot assessment

Classification statistics for the prediction of percentage rot development are presented in Table 4. The preliminary study found that by applying discriminative analysis techniques, 92.8% of the test population could be correctly classified into 2 categories, above and below 30% rot development for the area scanned. The percentage correctly classified decreased slightly to 86.8% when the classification was reduced to above and below 10% rot development for the scanned area.

Item assessed	Spectra (n)	Defined classification (%)	LV	Spectra misclassified (%)	Spectra correctly classified (%)
%Rots of scanned area	250	(i) 0-30; (ii) 31-100	8	7.2 (n=18)	92.8 (n=232)
	250	(i) 0 -10; (ii) 11 - 100	9	13.6 (n=33)	86.8 (n=217)

Table 4. Classification statistics for prediction of percentage rot development (shelf life) of whole 'Hass' avocado fruit. Note: LV = latent variables; n = number of samples.

Table 5 depicts the classification statistics for the prediction of percentage bruise development. The results indicate that 90% of the population could be correctly classified into 2 categories based on percentage bruise development in the scanned area ($\leq 10\%$, $\geq 11\%$) using scans conducted 1 - 2 hours following impact. Of the 10 (9.8%) samples misclassified, 6 (5.9%) samples visually rated with bruising greater than 11% were placed into the $<10\%$ bruise category and 4 (3.9%) samples with bruising visually rated below 10% were placed into the $\geq 11\%$ bruise category. The 4 samples misclassified with bruising below 10% were all on the ambiguous change over point of the two defined classification categories at 10% bruising.

These results improved to $>95\%$ correctly classified when the fruit were rescanned after 24 hours following impact. It appears the 24 hour time delay allowed more time for the bruising to develop assisting with classification. This would indicate that in a commercial situation it would be an advantage to hold the fruit for 24 hours prior to scanning. The 5 (4.9%) samples misclassified were all samples with bruising visually rated below 10% and placed into the $\geq 11\%$ bruise category. Of these samples 4 (3.9%) were at the ambiguous change over point of the two defined classification categories at 10% bruising.

Item assessed	Time after impact (hours)	Spectra (n)	Defined classification (%)	LV	Spectra misclassified (n)	Spectra correctly classified (%)
%Bruising of scanned area	1-2	102	(i) 0 - 10; (ii) 11 - 100	10	9.8 (n=10)	90.2 (n=92)
	24	102		8	4.9 (n=5)	95.1 (n=97)

Table 5. Classification statistics for prediction of percentage bruise development in whole 'Hass' avocado fruit. Note: LV = latent variables; n = number of samples.

4. Conclusion

NIRS has come to be extensively used in many applications for the non-invasive rapid assessment of a wide variety of products. These both include quantitative compositional determinations and qualitative determinations. The present study indicates the potential of FT-NIRS in diffuse reflectance mode to be used as a non-invasive method to predict the %DM of whole 'Hass' avocado fruit and the importance of incorporating seasonal and geographical variation in the calibration model. The results showed that the calibration model robustness increased when data from more than one season, incorporating a greater range of seasonal variation, was included in the calibration set. Also, that there are spectral differences between geographical regions and that, specific regional models may have significantly reduced predictive performance when applied to samples containing biological variability from a different growing region. It is therefore important that calibrations be developed on populations representative in which sorting is to be attempted.

As shown, there is also great potential to use FT-NIRS as a tool to predict impact damage of whole avocados based on percentage bruise development, and to predict shelf-life based on rot development (susceptibility). It should be considered that the preliminary work presented here is a first step towards shelf-life prediction and bruise detection for avocado fruit. However, this was only a preliminary study and the classification models require many more samples, incorporating seasonal and geographical biological variations, to enable the development of a robust model suitable for commercial use.

Overall, FT-NIR reflectance spectroscopy shows promise for the application in a commercial, in-line setting for the non-destructive evaluation of %DM, bruises and rot susceptibility of whole avocado fruit, although optimisation of the technology is required to address speed of throughput and environmental issues. Incorporating fruit physiological variability over future seasons and growing regions will be essential to further increase model robustness and ensure the predictive performance suitable for commercial use.

Unfortunately, the process of calibration development is a major impediment to the rapid adoption of NIRS in industry. 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 agriculture 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.

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