# Non-invasive assessment of avocado quality attributes

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## Abstract

Avocado fruit maturity and quality characteristics are often variable resulting in variation within a shipment in ripening rates, shelf-life and quality. Inferior fruit quality is seen as one of the key factors impacting on supply chain efficiency and profitability (Margetts 2009). Consumer surveys show that only 30% of Australian's eat avocados and that they expect to discard one in every four pieces of fruit they purchase because of poor internal quality (Avocados Australia Limited and Primary Business Solutions 2005). Surveys reveal that consumers prefer avocado fruit with at least 25% dry matter (DM) (Harker et al. 2007) and select bruising as the major defect, followed by rots (Harker 2009). Research has shown that if a consumer is dissatisfied with fruit quality then that consumer will not purchase that commodity for another 6 weeks (Embry 2009). To expand domestic and international sales the industry must be able to supply the discerning and demanding consumer with a consistent high quality product. Therefore a rapid non-destructive system that can accurately and rapidly monitor avocado quality attributes would allow the industry to provide better, more consistent eating quality fruit to the consumer, thus improving industry competitiveness and profitability.

This paper presents the current research findings of developing a non-invasive near infrared spectroscopy assessment tool which uses optical light for detecting bruises and for predicting both avocado DM content and rot susceptibility as an indication of shelf-life.

# Evaluación de calidad del fruto mediante métodos no destructivos en aguacate

## Resumen

La madurez y atributos que definen la calidad del fruto de aguacate varían dentro de un mismo envío y es común encontrar frutos con diferentes grados de maduración, períodos de calidad postcosecha y calidad final de consumo. El deterioro de la calidad de fruto es uno de los factores más importantes que afectan negativamente la eficiencia de la cadena de transporte y el retorno económico. Encuestas a consumidores australianos indican que sólo el 30% consumen aguacates y que los consumidores anticipan descartar un fruto de cada cuatro que compran, debido a la baja calidad interna de fruto. Encuestas también indican que los consumidores prefieren frutos que posean un mínimo del 25% en materia seca y señalan daños externos en el fruto como defecto principal, seguido por podredumbres internas. Investigación previa reveló que el consumidor se abstendrá de comprar frutos por un período de seis semanas cuando no está satisfecho con el fruto. La industria de aguacate debe poder entregar una calidad alta y consistente al consumidor discriminativo y demandante con el objetivo de incrementar las ventas en el mercado nacional e internacional. Para ello, un método no destructivo y que sea capaz de monitorear la calidad de aguacate en forma precisa y rápida hará posible que la industria suministre frutos con mayor y más consistente calidad de consumo. A su vez, el método incrementará la competitividad de esta industria y la rentabilidad.

Este trabajo presenta resultados obtenidos en la corriente investigación y desarrollo de un método no destructivo de fruto que se puede emplear para estimar la calidad postcosecha del aguacate. El método emplea espectroscopía cercana al infrarrojo y utiliza luz óptica para la detección daños, y para predecir materia seca del fruto y susceptibilidad a podredumbres.

**Key words:** non-invasive assessment; near infrared spectroscopy; fruit, avocado; maturity; eating quality; dry matter; bruising; rots; flesh disorders.

## Introduction

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. Many commercial quality classification systems for fruit and vegetables are based on external features of the product, for example: shape, colour, size, weight and blemishes. For avocado fruit, external colour is not a maturity characteristic, and its smell is too weak and appears later in its maturity stage (Gaete-Garretón 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 (Mizrach and Flitsanov 1999; Clark et al. 2003). Avocados Australia Limited (AAL 2008) recommend a minimum maturity standard for its growers of 23 %DM (greater than 10% oil content) for all cultivars, although consumer studies for 'Hass' 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 and 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 (Hofman and 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 and 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). Thus, fruit quality reliability is a key factor impacting on supply chain efficiency and related profitability.

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 requires two to four weeks sea freight depending on destination. 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.

Repeat purchasing by consumers is significantly affected by a bad eating experience. With avocado, internal defects of 10% or more has a dramatic negative impact on the consumer repurchasing (Embry 2009). 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 purchase by 9% by reducing the average level of damage or defects by 15% (Embry 2009). The key factor for retaining and expanding both domestic and international markets is removing inconsistency and providing what the consumer expects. That is 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 quality attributes of horticultural products. The Rapid Assessment Unit (RAU), a collaboration between the Department of Employment, Economic Development and Innovation (DEEDI, formally the Queensland Department of Primary Industries) and James Cook University (JCU) has been developing a non-invasive assessment tool based on near infrared spectroscopy (NIRS) to predict avocado internal quality attributes. These quality attributes include the prediction of maturity and thereby principal eating quality attributes based on %DM; the detection of bruises and the prediction of 'export potential' of avocados based on the risk of developing external and internal defects (i.e., flesh rots) as an indication of potential shelf life.

NIRS is a non-invasive method of measuring internal/external quality and safety attributes of horticultural produce using the near infrared part of the light spectrum to determine chemical composition. 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. The technology offers the advantage of being non-destructive, plus low cost analysis, fraction of a second per test, simplicity in sample preparation, no chemical agent requirements, good repeatability and has the potential to test every piece of product in an in-line setting for multiple internal attributes simultaneously including: sweetness, ripeness, maturity, acidity and chemical characteristics. NIRS is a secondary method of determination and therefore it must be calibrated against a primary reference method. 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.

This paper presents the current research findings of developing a non-invasive NIRS assessment tool for detecting bruises and for predicting both avocado maturity based on %DM content and rot susceptibility as an indication of shelf-life.

## Materials and methods

### *Avocado fruit samples*

*a) Avocado fruit for dry matter calibration development on a Bruker Matrix-F Fourier Transform (FT)-NIR spectrophotometer.*

'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' S, Longitude: 152 21' E) and Toowoomba, South East Queensland (Latitude: 27° 33' 0" South, Longitude: 151° 58' 0" 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. Approximately 100 fruit were collected at each harvest giving a total of around 900 individual fruit for each growing region.

*b) Avocado fruit for instrument comparisons (Matrix-F and HyperVision™ systems).*

'Sheppard' avocado fruit were obtained during the 2009 growing season (January to May) from a single farm in the production region of Mareeba, North Queensland (Latitude: 17° 0' 0" South, Longitude: 145° 26' 0" East). Similarly, Hass avocado fruit were collected during the 2009 growing season from a single farm near Ravenshoe on the Atherton Tablelands, North Queensland (Latitude: 17° 38' 0" South, Longitude: 145° 29' 0" East). 'Sheppard' avocado fruit were harvested at three maturity stages corresponding to early, mid and late season harvests, while Hass avocado fruit were harvested at two maturity stages of early and mid season harvests due to availability. Approximately 100 fruit were collected at each harvest.

*c) Avocado fruit for impact and rot assessment trials.*

'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' 0" South, Longitude: 145° 29' 0" East) and the second farm is located in the major production district of Toowoomba, South East Queensland (Latitude: 27° 33' 0" South, Longitude: 151° 58' 0" 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.

### *NIR data collection*

*a) NIR data collection of avocado fruit for dry matter calibration development on the Bruker Matrix-F FT-NIR spectrophotometer.*

The spectra of whole, intact 'Hass' 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 780–2500 nm range. Spectra were obtained in diffuse reflectance mode, using a 4 x 20 watt tungsten light source fibre-coupled emission head. A path-length of approximately 170 mm from the external measurement 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; 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.

*b) NIR data collection of avocado fruit for instrument comparison (Bruker Matrix-F and HyperVision™ systems).*

The spectra of whole, intact ‘Hass’ avocado fruit were collected using a commercially available Bruker Matrix-F, FT-NIR spectrophotometer as discussed in section ‘a’ of ‘NIR data collection’. Spectra of the same fruit were then captured on a commercial HyperVision™ in-line grading system (Optical Measuring Systems and Produce Sorters International, United States of America; operating software: Camdisp Version 2.1). The HyperVision™ system optics utilises a line scan camera based system (charge couple device or CCD) operating in the visual and shortwave NIR regions with the spectral range of 450-1150 nm. With the HyperVision™ system, all avocado fruit are placed on a conveyor belt and passed through the Visible/NIR light source for image and spectra collection. Spectra were obtained in diffuse reflectance mode using 6 x 300 watt tungsten halogen lights, with a path-length of approximately 650 mm from the light source to the surface of the conveyor belt and approximately 800 mm from the CCD to the conveyor belt. A conveyor belt speed of 50 cm/second was used for the trial. The system was programmed to capture a circular scan area from the middle of the avocado of approximately 50 mm in diameter and average these spectra to produce one spectra for the 50 mm diameter scan area. This was done to allow direct comparison against the Bruker Matrix-F research system but resulted in an increased spectral capture time. Therefore only one spectra was obtained from only one side of each fruit on the HyperVision™ system for these research trials.

*c) NIR data collection of avocado fruit for impact and rot assessment trials.*

With impact (bruise) and rot assessment trials, diffuse reflectance spectra of whole, intact ‘Hass’ avocado fruit were collected in the 780–2500 nm range using a Bruker Matrix-F, FT-NIR spectrophotometer as discussed in section ‘a’ of ‘NIR data collection’. Spectra for rot susceptibility prediction were collected from each opposing half of the hard green fruit prior to fruit being placed into 20$^{o}$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$^{o}$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. 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. The 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 a 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$^{o}$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.

***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 $^{o}$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.

***NIR data analysis***

*a) NIR data analysis of avocado fruit for dry matter calibration development on the Bruker Matrix-F FT-NIR spectrophotometer.*

Statistical analysis was conducted 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. Partial least squares (PLS) regression was used to build the prediction models of the diffuse reflectance spectral data, using segmented cross validation. Before calibration model development, the variation of the spectral data was analysed by PCA, and obvious spurious spectra eliminated. The cross-validation was performed using 20 segments. Data pretreatment and smoothing for the individual Bundaberg and Toowoomba 'Hass' avocado %DM models for the Bruker Matrix-F FT-NIR spectrophotometer in this study were based on a combination of a 25 point Sovitsky-Golay (SG) spectral smoothing (2$^{nd}$ order polynomial) and a second derivative transformation (25 point SG smoothing and 2$^{nd}$ order polynomial). For the combined Bundaberg and Toowoomba model, data pretreatment and smoothing was based on a combination of a 25 point Sovitsky-Golay (SG) spectral smoothing (2$^{nd}$ order polynomial) and a standard normal variate (SNV) transformation which removes scatter effects from the spectra. 'Significant noise was found within spectral ranges 780 – 843 and 2414 - 2503 nm for all spectra captured on the Bruker Marix-F FT-NIR spectrophotomer and was subsequently removed before model development.

Model performance was based on the coefficient of determination ($R^2$) of the calibration ($R_c^2$) and validation/prediction ($R_v^2$) data sets; root mean square error of cross validation (RMSECV); root mean square error of prediction (RMSEP) in relation to the bias (average difference between predicted and actual values) (Buning-Pfaue 2003), and 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) (Walsh et al. 2004). The higher the SDR statistic the greater the power of the model to predict the chemical composition accurately (Cozzolino et al. 2004). 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; 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 (Schimleck et al. 2003; Nicolaï et al. 2007; Williams 2008).

*b) NIR data collection of avocado fruit for instrument comparison (Bruker Matrix-F and HyperVision™ systems)*

Statistical analysis was conducted using the commercially available chemometric software package 'The Unscrambler™' version 9.8 (CAMO, Oslo, Norway) as described in 'a' of section 'NIR data analysis'. For the Bruker Matrix-F FT-NIR spectrophotometer data pretreatment and smoothing for 'Sheppard' avocado %DM models were based on a combination of a 25 point Sovitsky-Golay (SG) spectral smoothing (2$^{nd}$ order polynomial and 3$^{rd}$ order detrend) and a second derivative transformation (25 point SG smoothing and 2$^{nd}$ order polynomial). 'Hass' avocado %DM models were based on a combination of a 25 point Sovitsky-Golay (SG) spectral smoothing (2$^{nd}$ order polynomial) and a second derivative transformation (25 point SG smoothing and 2$^{nd}$ order polynomial).

The HyperVision™ data pretreatment and smoothing for 'Hass' avocado models were based on a combination of a 25 point Sovitsky-Golay (SG) spectral smoothing (2$^{nd}$ order polynomial) and a second derivative transformation (25 point SG smoothing and 2$^{nd}$ order polynomial). 'Sheppard' avocado %DM models were based on a combination of a 9 point Sovitsky-Golay (SG) spectral smoothing (2$^{nd}$ order polynomial) and a second derivative transformation (9 point SG smoothing and 2$^{nd}$ order polynomial).

*c) NIR data analysis of avocado fruit for impact and rot assessment trials.*

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 1-2hr impact wavelengths were subjected to weighting by the standard deviation prior to analysis.

**Results and discussion**

*a) Dry matter calibration development on the Bruker Matrix-F research instrument.*

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 region over three years (Table 1). The 2006 calibration model was used to predict on the 2007 season population. The selected calibration sets from 2006 and 2007 seasons were combined 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 1. PLS calibration and prediction statistics for %DM for whole Hass avocado fruit for 2006, 2006-7 and 2006-08 (Combined) seasons predicting on 2007, 2008 and 2006-08 (Combined) seasons respectively.**

| Bundaberg harvest | | Spectra n (OR) | %DM range | SD | LV | $R^2$ | RM SECV | RM SEP | SDR |
|---|---|---|---|---|---|---|---|---|---|
| **Calibration** | **Prediction** | | | | | | | | |
| 2006 | | 222(2) | 18.2-35.0 | 3.2 | 7 | 0.75 | 1.76 | | 1.8 |
| | 2007 | 609 | 14.1-34.4 | 2.71 | | - | | 5.07 | 0.5 |
| 2006 & 07 | | 426 | 14.1-35.0 | 3.1 | 9 | 0.75 | 1.60 | | 1.9 |
| | 2008 | 606 | 15.2-35.5 | 5.66 | | | | 4.1 | 1.4 |
| Combined | | 595(10) | 14.1-35.5 | 4.14 | 8 | 0.80 | 1.78 | | 2.3 |
| | Combined | 1250(1) | 15.2-35.4 | 4.14 | | 0.83 | | 1.75 | 2.4 |

Note: OR = Outliers Removed; LV = Latent Variables.

As expected, the application of single seasonal calibrations to populations from other growing seasons was not very successful due to the seasonal biological variation. As shown in Table 1, the 2006 calibration model could not be used to predict the 2007 season population. Model predictive performance improved as more biological variability was included in the model, as seen when the combined 2006 and 2007 model was used to predict on the 2008 season. The combined 2006, 2007 and 2008 calibration model was sufficiently robust to predict %DM of whole Hass avocado to within 1.75% with a coefficient of determination of the validation set ($R_v^2$) =0.83 (meaning that 83% of the variance in the reference samples (dry matter results) can be explained) and SDR of 2.4. This indicated an ability to sort the fruit into three categories with approximately 80% accuracy (Guthrie et al. 1998).

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, such as soil characteristics, nutrition, weather conditions, fruit age and season variability (Peirs, Tirry et al. 2003; Marques et al. 2006). 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 Toowoomba regions collected over 3 years.

The PLS calibration and prediction model statistics for both the Bundaberg and Toowoomba regions and combination of both regions are presented in Table 2. The Bundaberg data set of 1845 spectra was separated into a calibration set (n = 595) and a prediction set (n = 1250). The validation statistics of the calibration model were quite good and delivered an $R_v^2$ = 0.83 with an RMSEP = 1.75 and SDR of 2.3 for %DM. An SDR value between 2.0 and 2.4 is regarded as adequate for rough screening (Schimleck, Mora et al. 2003; Nicolaï, Beullens et al. 2007; Williams 2008). The Bundaberg PLS model was used to predict on the entire Toowoomba 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 undefined $R_v^2$, RMSEP = 5.48, SDR of 1.1. Similarly, the Toowoomba data set of 1652 spectra were separated into a calibration set (n = 526) and prediction set (n = 1126). The Toowoomba PLS model also produced reasonable validation statistics ($R_v^2$ = 0.76 with an RMSEP = 1.97 and SDR of 2.0), when predicting fruit from within the Toowoomba region. As with the Bundaberg model, the Toowoomba model did not perform as well when it was used to predict %DM of fruit from a different geographic location (i.e., the Bundaberg population). However, the combined Bundaberg and Toowoomba calibration model incorporating biological variability from both regions was sufficiently robust to predict %DM of whole 'Hass' avocado to within 1.64 % with an $R_v^2$ = 0.87 and SDR of 2.7.

**Table 2. PLS calibration and prediction statistics for %DM for whole 'Hass' avocado fruit harvested over three seasons for each region and combination of both regions.**

| Harvest | | Spectra n (OR) | %DM Range | SD | LV | $R^2$ | RMSECV | RMSEP | SDR |
|---|---|---|---|---|---|---|---|---|---|
| **Calibration** | **Prediction** | | | | | | | | |
| Bundaberg | | 595(10) | 14.1-35.5 | 4.14 | 8 | 0.80 | 1.78 | | 2.3 |
| | Bundaberg | 1250(1) | 15.2-35.4 | 4.14 | | 0.83 | | 1.75 | 2.4 |
| | Toow | 1652(1) | 16.4-41.6 | 4.07 | | - | | 5.48 | 1.1 |
| Toow | | 526(1) | 16.4-41.6 | 4.39 | 9 | 0.76 | 2.13 | | 2.1 |
| | Toow | 1126 | 16.9-40.1 | 3.96 | | 0.75 | | 1.97 | 2.0 |
| | Bundaberg | 1845 | 14.1-35.5 | 4.14 | | 0.33 | | 3.38 | 1.5 |
| Bundaberg & Toow | | 999(6) | 14.1-41.6 | 4.61 | 10 | 0.87 | 1.67 | | 2.8 |
| | Bundaberg & Toow | 2496 | 15.7-40.8 | 4.46 | | 0.87 | | 1.64 | 2.7 |

Note: OR = Outliers Removed; LV = Latent Variables; Toow = Toowoomba.

*b) Instrument comparison (Bruker Matrix-F and HyperVision™ systems).*

The high resolution Bruker Matrix-F FT-NIR system was used to determine if it was possible to predict %DM in whole intact 'Hass' avocado fruit. The system was then used to assess both seasonal and geographical location variability influences on model robustness. The next aim of the project was to apply the knowledge gained to the development of an in-line system suitable for assessing and grading all avocados for %DM before proceeding to market. Fruit inspection times for in-line grading need to be in the order of 100 ms. The commercially available HyperVision™ grading system has had demonstrated potential in an in-line setting and was utilised for this purpose and assessed along side the Bruker Matrix-F FT-NIR research system. A population of 'Hass' and 'Sheppard' avocado varieties collected were used to assess the predictive performance of both systems.

The PLS calibration and prediction model statistics for both instruments and avocado varieties are presented in Table 3. The calibration and validation statistics for %DM in 'Hass' avocados are comparable for both systems. The relatively poor SDR values (MF: 1.4; HV: 1.5) can be attributed to the narrow %DM range, resulting in a low SD (2.88). This suggests that the 'Hass' population did not include a sufficiently broad variability in %DM to develop a suitable calibration model, although other biological or environmental effects may have contributed. The calibration model statistics for the 'Sheppard' variety were better than the 'Hass' variety for both systems, with the Matrix-F ($R^2$=0.91; RMSECV=1.78; SDR=3.4) being slightly better than the HyperVision™ ($R^2$=0.86; RMSECV=2.26; SDR=2.7). These improved results compared to the 'Hass' population can be attributed to the larger SD of the samples and the thinner skin of the 'Sheppard' allowing further penetration of NIR light into the fruit. However, in saying this, the validation statistics for both systems were very similar with an $R^2$ of approximately 0.9, RMSECV of around 1.9 and an SDR of approximately 3. Further development of the calibration models for the HyperVision™ system are required to ensure enough biological sample variation has been included to enable the system to accurately and robustly predict future samples in a commercial situation.

**Table 3.**

| Instrument - avocado variety | | Spectra n (OR) | %DM Range | SD | LV | $R^2$ | RMSECV | RMSEP | SDR |
|---|---|---|---|---|---|---|---|---|---|
| **Calibration** | **Prediction** | | | | | | | | |
| MF - Hass | | 101 | 17.1-31.8 | 2.88 | 6 | 0.59 | 1.85 | | 1.6 |
| | MF - Hass | 101 | 19.9-31.7 | 2.42 | | 0.46 | | 1.76 | 1.4 |
| MF - Sheppard | | 144(1) | 12.7-36.3 | 6.04 | 4 | 0.91 | 1.78 | | 3.4 |
| | MF - Sheppard | 145 | 13.6-36.6 | 5.63 | | 0.89 | | 1.85 | 3.0 |
| HV - Hass | | 101 | 17.1-31.8 | 2.88 | 4 | 0.57 | 1.89 | | 1.5 |
| | HV - Hass | 101(1) | 19.9-31.7 | 2.42 | | 0.54 | | 1.63 | 1.5 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| HV - Sheppard | | 144 | 12.7-36.3 | 6.04 | 7 | 0.86 | 2.26 | | 2.7 |
| | HV - Sheppard | 145 | 13.6-36.6 | 5.63 | | 0.88 | | 1.91 | 2.9 |

Note: MF = Matrix-F; HV = HyperVision™.

*c) Impact and rot assessment trials*

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.

**Table 4.** Classification statistics for prediction of percentage rot development (shelf life) of whole Hass avocado fruit**.**

| 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) |

Note: LV = Latent Variables.

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 (≤10%, ≥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 ≥11% bruise category. The 4 missclassified samples with bruising below 10% were all on the ambiguous change over point of the two defined classification categories at 10% bruising.

These results improved significantly 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. The 5 (4.9%) samples misclassified were all samples with bruising visually rated below 10% and placed into the≥11 bruise category. Of these sample s 4 (3.9%) were at the ambiguous change over point of the two defined classification categories at 10% bruising.

**Table 5.** Classification statistics for prediction of percentage bruise development in whole Hass avocado fruit.

| 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) |

Note: LV = Latent Variables.

## Conclusion

The present study demonstrated the potential of FT-NIRS in diffuse reflectance mode as a non-invasive method to predict the %DM of whole Hass avocado fruit, and the importance of calibration model development incorporating seasonal and geographical variation. As shown, the calibration models need to be assessed over several years to increase their robustness and ensure their predictive performance. The commercial in-line HyperVision™ system produced similar predictive performance on the limited trial samples as the Bruker Matrix-F research instrument. Further development of the calibration models for the HyperVision™ system are required to ensure enough biological sample variation has been included to enable the system to accurately and robustly predict future samples in a commercial situation. By further developing and implementing the commercial system the avocado industry will be able to maximise sales in existing markets and to target new markets with a differentiated product to meet the increasingly higher standards expected by domestic and overseas consumers.

As shown there is 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). The technique correctly classified >90% of the population based on two categories ((i) ≤10%; (ii) ≥11%) of percentage bruising using scans conducted 1-2 hours after impact. This improved to >95% if scans were conducted 24 hours after impact damage (bruising) allowing sufficient time for bruise development to be detected. This would indicate that in a commercial situation it would be an advantage to hold the fruit for 24 hours prior to scanning. It should be considered that the work here presented 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

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 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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