The Non-Invasive Assessment of Avocado Maturity and Quality

Thesis submitted by

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and the School of Marine and Tropical Biology

James Cook University

Supervisors: Professor Ron White and Professor Paul Gadek

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institute of tertiary education. Information derived from the published and unpublished work of others has been acknowledged in the text, and a list of references is given.

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Brett Wedding

Date: 28/02/2018

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ABSTRACT

Horticultural products in today's modern market must have high quality standards. Consumer demand for consistent quality agricultural produce remains strong and continues to increase, this will lead to the development and subsequent increased availability of sophisticated techniques, sensors, and user-friendly non-invasive systems for measuring product quality indices. The inability to consistently guarantee internal fruit quality is a major factor not only for the Australian avocado industry but also the entire horticulture sector. Poor fruit quality is seen as a key factor affecting consumer confidence and impacts on supply chain efficiency and profitability. Removing fruit quality inconsistencies while providing the consumer with a consistent quality product is a vital commercial consideration of the Australian avocado industry for both domestic and export markets.

Many fruit quality attributes affecting consumer acceptance are assessed using traditional methods that are generally subjective, labour intensive and costly. Commercially, avocado maturity is measured destructively by the determination of dry matter (DM) content, moisture content (MC) or oil content, all of which are highly correlated. Maturity is an important component in avocado fruit quality and a prime factor in palatability. A rapid, non-destructive measurement system that can accurately and simultaneously monitor external and internal attributes of every avocado fruit either in the field or in an in-line setting, is highly desirable for ensuring consistent product quality over an extended season, increasing industry marketability and profitability.

The utility of near infrared (NIR) spectroscopy was investigated as a non-invasive assessment tool for estimating avocado maturity and thereby eating quality based on dry matter content of whole intact fruit primarily for the avocado variety 'Hass'. The technique was also assessed for detecting bruises and for predicting rot susceptibility as an indication of shelf-life for possible implementation in a commercial in-line application. The project also investigated the importance of the calibration model development process to incorporate seasonal and geographical variability to ensure model robustness.

NIR spectroscopy 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. The commercially available NIR spectroscopy systems assessed in this project highlighted the potential of NIR spectroscopy and its suitability for application in a commercial in-line setting for predicting avocado maturity and palatability of whole intact avocados, based on DM content.

With horticultural products, the major challenge of implementing NIR spectroscopy is to ensure that the calibration model is robust, that is, that the calibration model holds across growing seasons and potentially across growing districts. The present project represents the first study to investigate the effect of seasonal variation on model robustness to be applied to avocado fruit. It found that seasonal variability has a significant effect on model predictive performance for DM in avocados. The robustness of the calibration model, which in general limits the commercial application for the technique, was found to increase across seasons when more seasonal variability was included in the calibration set. Across the seasons it achieved predictive performances in this case in the range of: validation coefficient of determination (R_v^2) of 0.76 – 0.89, root mean square error of prediction (RMSEP) of 1.43 - 1.97%, and standard deviation ratio's (SDR) of 2.0 to 3.1.

Similarly, 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. As with seasonal variability, this can be addressed by incorporating multiple geographical growing regions into the calibration model to account for the biological variability to improve model robustness as demonstrated in this study (i.e., R_v^2 of 0.89, RMSEP of 1.51%, and SDR of 3.6). Furthermore, when models are constructed to include both season and geographical variability, model performance can be more robust when dealing with a broader range of future sample variability. This was demonstrated with calibration models constructed to incorporate 3 years of seasonal variability and encompassing 3 geographical regions, obtaining predictive performances ranging from R_v^2 0.87 - 0.89; RMSEP of 1.42 - 1.64% and SDR of 2.7 - 3.1 across the various geographical regions.

NIR spectroscopy shows great promise for the application in a commercial, in-line setting for the non-destructive evaluation of impact damage (bruising) and rot susceptibility of whole avocado fruit, although optimisation of the technology is required to address speed of throughput and environmental issues. The adoption of a rapid, non-invasive method to identify fruit that are less prone to rots and internal disorders would allow selection of fruit that could be sent to more distant markets with greater confidence that it will arrive in acceptable quality, thus ensuring maximum yield and higher returns for the producer and marketer.

The ability of the NIR classification models to accurately predict rot development of hard green avocado fruit (stage 0 ripeness) into two classes, ≤10% and >10% of flesh affected, ranged from 65-84% over the three growing seasons. When the rot classes were defined as ≤30% and >30% the accuracy ranged from 69%-77%. In relation to impact damage (bruising), trials conducted over three growing seasons using an NIR spot assessment technique found hard green fruit at

stage 2 ripeness, that were deliberately bruised could be correctly detected with 70-79% accuracy after 2-5 hours of impacting and with 83-89% accuracy after 24 hours. For eating ripe (stage 4) fruit, the accuracy was 60-100% after 2-5 hours of impacting and 66-100% after 24 hours across the three growing seasons. This indicates that in a commercial situation it would be an advantage to hold the fruit for 24 hours before undertaking NIR scanning.

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ABBREVIATIONS

ABS	Raw absorbance
ANN	Artificial neural network
ANOVA	An analysis of variance
CCD	Charge-couple device
CDA	Conical discriminative analysis
C-H	Methine group
CI	Confidence interval
cm^{-1}	Wavenumber
DM	Dry matter
EIS	Electrical impedance spectroscopy
Eqn.	Equation
FDA	Factorial discriminative analysis
FT	Fourier transform
h	Hour
H+	Hydrogen ion
Hz	Hertz
LDA	Linear discriminative analysis
MC	Moisture content
MHz	Mega-hertz
MLR	Multiple linear regression
mm	millimetres
ms	millisecond
MSC	Multiplicative scatter correction
MWIR	Mid wavelength infrared
NH	Neighbourhood H (Mahalanobis distance)
N-H	Amine group
NIR	Near infrared
NIRS	Near infrared spectroscopy
nm	nanometres
NMR	Nuclear magnetic resonance
O-H	Hydroxy functional group
^oC	Degrees Celsius
PC	Principal component
PCA	Principal component analysis
PCR	Principal component regression
PLS	Partial least squares
PLS-DA	Partial lest squares-discriminative analysis
PME	Pectinmethylesterase
R (or r)	Correlation coefficient
R^2	Coefficient of determination
R_c^2	Calibration model coefficient of determination
R_v^2	Validation coefficient of determination
ROC	Receiver operating characteristics
RI	Refractive index
RF	Radio frequency
RGB	Red Green Blue
RH	Relative humidity
RMSEC	Root mean square error of calibration
RMSECV	Root mean square error of cross validation
RMSEP	Root mean square error of prediction
RPD	Ratio performance deviation
SD	Standard deviation

SDR	Standard deviation ratio
SEC	Standard error of calibration
SECV	Standard error of cross validation
SEP	Standard error of prediction
SEV	Standard error of validation
SG	Savitsky-Golay transform
SIMCA	Soft independent modelling by class analogy
S/m	Siemens per metre
S/N	Signal-to-noise
SNV	Standard normal variate
SSC	Soluble solids content
SVM	Support vector machines
SWIR	Short wavelength infrared
TA	Titratable acidity
TSS	Total soluble solids
USA	United States of America
UV	Ultra Violet
ver.	Version
Vis	Visible
Vis-NIR	Visible-Near infrared
%	Percent

LIST OF PUBLICATIONS

The following is a list of publications / presentations derived from work by the candidate during the period of this thesis.

Refereed Journal Publications

Wedding, B.B.; Wright, C; Grauf, S.; Gadek, P.A. and White, R.D. (submitted 2018) The application of FT-NIRS for the detection of bruises and the prediction of rot susceptibility of Hass avocado fruit. *Journal of Food Science and Agriculture*.

Wedding, B.B.; Wright, C; Grauf, S.; Gadek, P.A. and White, R.D. (submitted 2018) Depth of penetration of Near Infrared radiation in Hass avocado fruit. *Postharvest Biology and Technology*

Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2013) Effects of seasonal variability on FT-NIR model robustness for the prediction of dry matter content for whole Hass avocado fruit. *Postharvest Biology and Technology*.

Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) Near Infrared Spectroscopy as a rapid non-invasive tool for agricultural management with special reference to avocado and Sandalwood industries. *Desalination and Water Treatment* **32**, pp 365-372.

Wedding, B.B., White, R.D.; Grauf, S.; Wright, C; Tilse, B.; Hofman, P. and Gadek, P.A. (2011) Non-destructive prediction of 'Hass' avocado maturity via FT-NIR spectroscopy. *Journal of the Science of Food and Agriculture* **91**, pp 233-238. (Published online in Wiley OnLine library: September 2010).

Refereed Conference Publications

Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) Non-invasive assessment of avocado quality attributes. In: *The Proceedings of the VII World Avocado Congress 2011*, Cairns – Australia, 5-9 September 2011.

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Non-refereed Conference Publications / Presentations / Posters / Abstracts

Fox, G. and Wedding, B.B. (2013) The Australian Institute of Food Science and Technology Convention 2013, 14-16 July. Presentation: Emerging non-invasive rapid assessment technologies (smart sensing technologies) and minimally processed convenience fruit and vegetables.

C. Wright, B. Wedding, S. Grauf, R. White and P. Gadek. (2012) Australasian Applied Statistics Conference, 4-7 December Queenstown, New Zealand. Presentation: Perfection Detection: Non-invasive assessment of avocado maturity.

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Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) *Impact assessment and prediction of rot susceptibility of Hass avocado fruit using FT-NIRS*. The 15th International conference on Near Infrared Spectroscopy, Cape Town, 16-20 May 2011.

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Wedding, B.B.; White, R.D.; Grauf, S.; Wright, C; Tilse, B; Fitzsimmons, J.; Hofman, P and Gadek, P. (2009) *NIRS technology for determining maturity in avocados*. Presentation and manuscript: At the 4th Australian and New Zealand Avocado Growers Conference, Cairns 21-24 July 2009.

Wedding, B.B., White, R.D.; Grauf, S.; Tilse, B.; Hofman, P. and Gadek, P.A. (2008) Presentation: At the *prediction of fruit quality by non-invasive assessment* at the 'Innovative supply chains- predicting, measuring and monitoring quality in supply chains' workshop at The National and Trans-Tasman Horticultural Science Conference, 21-23 July 2008, Gold Coast, Queensland.

Wedding, B.B., White, R.D.; Grauf, S.; Tilse, B.; Hofman, P. and Gadek, P.A. (2008) Poster*: Near Infrared Spectroscopy as a rapid non-invasive tool for assessing internal quality attributes of whole avocado fruit*. The National and Trans-Tasman Horticultural Science Conference, 21-23 July 2008, Gold Coast, Queensland.

Wedding, B.B., White, R.D.; Grauf, S.; Tilse, B.; Hofman, P. and Gadek, P.A. (2008) Presentation of the RAU project: *"Prediction of fruit quality by non-invasive assessment, with special reference to avocado"*. At the Avocados Australia annual Avocado R&D workshop, 7th July, Brisbane.

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Book Chapters

Brett B. Wedding, Carole Wright, Steve Grauf and Ron D. White. (2012) The application of near infrared spectroscopy (NIRS) for the assessment of avocado quality attributes. In: *Infrared Spectroscopy*. Intech Open Book Access.

CHAPTER 1

INTRODUCTION

With economic development and improvement of living standards, consumer expectations have grown with respect to quality of agricultural products. Many fruit quality attributes affecting consumer acceptance are assessed using traditional methods that are generally subjective or labour intensive. Most commercial quality classification systems for horticultural products, either manual or automated, are based on external features of the product (i.e., colour, size, shape, blemishes or defects). Currently, internal quality of horticultural produce is either judged on: i) subjective non-destructive attributes such as appearance, firmness and volatiles; ii) destructive, subjective measures such as internal flesh colour; or iii) destructive, objective measures such as sugar content, DM content, oil content, MC and titratable acidity (TA) which are applied to a small number of samples to represent an entire shipment (Magwaza and Tesfay 2015; Clark et al. 2003; Mizrach and Flitsanov 1999). It is therefore not surprising that there has been a significant increase in consumer driven research towards non-destructive, rapid techniques to measure horticultural produce internal quality attributes.

As with most horticultural produce, avocado quality characteristics are often variable resulting in large differences within a consignment. Maturity is an important component in avocado fruit quality and a prime factor in palatability. Eating quality is compromised if fruit are harvested too early. Commercially, avocado maturity is measured destructively by the determination of %DM, MC or oil content, all of which are highly correlated (Magwaza and Tesfay 2015; Clark et al. 2003; Mizrach and Flitsanov 1999). Since oil determination is quite expensive, the industry tends to use DM determination as the industry standard, with some countries using MC (Magwaza and Tesfay 2015; Clark et al. 2003).

Poor fruit quality is seen as a key factor affecting consumer confidence that impacts on supply chain efficiency and profitability. Avocado fruit with internal defects of ≥10% have a dramatic negative impact on consumer repurchasing (Petty and Embry 2011; Embry 2009). Bruising has been identified by consumers as the major defect and a significant barrier to purchasing other than price, followed by body and stem end rots (Harker 2009). Unfortunately, poor flesh quality cannot be determined until the fruit is cut open which is mostly discovered by the unsuspecting consumer after product purchase. Providing the consumer with a quality and consistent product is a critical factor in retaining and expanding markets. Avocados are highly perishable compared to other produce, as: fruit are ethylene sensitive and have a short shelf-life once ripening has been initiated; fruit are sensitive to physiological disorders associated with chilling injury; and fruit are

susceptible to latent infections during ripening (Gamble et al. 2010). As much as 80% of fruit displayed at retail outlets can have some degree of internal quality issues, mainly flesh bruising (Joyce et al. 2015).

In relation to export markets, reliable export of avocados from Australia requires 2-6 weeks sea freight, depending on destination. The biggest risk during transport is quality loss due to the development of rots and flesh disorders occurring once the fruit ripen and age. Long transit times and short shelf-life are significant obstacles for exporting avocados. Additionally, the fruit have to arrive at the destination with a suitable shelf-life for a successful integration into the buyer's marketing chain. At present, there is no commercially available non-invasive inline grading system that can reliably predict avocado flesh disorder development in relation to shelf-life to ensure that the fruit will arrive at the consumer with an acceptable quality. A rapid, non-invasive method to identify fruit that are less prone to rots and internal disorders would allow selection of fruit that could be sent to more distant markets with greater confidence that it will arrive at market having acceptable quality, thus ensuring maximum yield for the producer and retailer.

The inability to consistently guarantee internal fruit quality is a major factor for not only the Australian avocado industry but also the entire horticulture sector. A rapid, non-destructive measurement system that can accurately and simultaneously monitor external and internal attributes of every avocado fruit either in the field, or in an in-line setting, is highly desirable for ensuring consistent product quality over an extended season, increasing industry marketability and profitability. Various technologies have been investigated for the potential to meet the task of assessing internal attributes of fruit and vegetables, including machine vision (Li et al. 2015; Cubero et al. 2010; Blasco et al. 2003), acoustic techniques (De Ketelaere et al. 2006; Chen and Sun 1991), spectroscopic techniques (Butz et al. 2005), X-rays (Butz et al. 2005; Abbott 1999), microwaves and electronic noses (Butz et al. 2005). Among the potential technologies, NIR spectroscopy has received considerable attention because of its significant advantages, including: i) being non-invasive; ii) requiring minimal to no sample preparation; iii) accuracy and precision; and iv) multi-analytic allowing determination of several attributes simultaneously.

1.1 Rationale

There is an industry driven need to apply a rapid, non-destructive technology in a commercial setting to determine external and internal quality attributes of whole avocado fruit to ensure consistent product quality, marketability and industry profitability. The basis of this Thesis is to demonstrate that NIR spectroscopy may be a suitable non-invasive technology to accurately and rapidly monitor internal quality attributes of whole 'Hass' avocado in a commercial in-line setting.

NIR spectroscopy as a non-invasive technique to assess internal quality attributes of intact horticultural produce is well established in literature. However, the robustness of calibration models with respect to biological variability from different seasons has been disregarded and therefore these calibration models may be over-optimistic with respect to prediction accuracies on future samples. Robustness of calibration for seasonal and geographical variability is consequently a critical issue that needs to be addressed.

There has been limited investigations of the use of NIR spectroscopy to determine avocado maturity based on %DM (Olarewaju et al. 2016; Blakey et al. 2009; Walsh et al. 2004; Clark et al. 2003; Schmilovitch et al. 2001). These limited studies have not investigated the importance of the calibration model development process to ensure models future predictive performance or robustness is achieved by incorporating geographic and seasonal variability. To the best of the authors knowledge, there has been no research work identified in literature utilising NIR spectroscopy as a non-invasive technique to detect bruises or to predict the risk of developing flesh disorders (i.e., rot susceptibility) as an indication of shelf-life, in whole avocado fruit.

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

1.2 Research objectives

The aim of this research is to develop a non-invasive tool based on NIR spectroscopy to predict avocado internal quality attributes for possible at-line and in-line applications. This is achieved by addressing the following research objectives:

- To utilise NIR spectroscopy to provide a non-invasive assessment tool for predicting 'Hass' avocado maturity and thereby principal eating quality attributes based on %DM content;
- To develop calibration models from NIR spectral data to predict %DM content of 'Hass' avocado taking into account inter- and intra-seasonal variation;
- To determine predictive model robustness across different geographic/growing regions or districts and over several growing seasons (time);

- To develop non-invasive assessment methods and to select significant wavelength ranges for developing rapid non-invasive avocado grading systems required for commercial use in various settings;
- Evaluate the ability of NIR spectroscopy to predict export potential of individual avocados based on the risk of developing external and internal defects (i.e., flesh rots and disorders resulting in browning of the flesh);
- Evaluate the ability of NIR spectroscopy to detect bruises as a result of impact damage in intact avocados.

This project was a component of the Australian Research Council (ARC) Linkage Project: 'Prediction of fruit quality by non-invasive assessment with special reference to avocado' (Project ID LP0562294) completed in 2010. The outcomes of this project are expected to contribute to the field of NIR spectroscopy and its application to a range of high moisture horticultural products for rapid spectral assessment of internal quality attributes for both at-line and commercial high speed automated in-line situations. From an economic perspective, this project will provide a framework for the enhancement of commercially available non-invasive in-line automated horticultural grading equipment. Spectral assessment information gained will provide a solid basis for the implementation of grower best practices to ensure high quality fruit. This will enable producers to increase productivity, provide greater opportunity to access high volume export markets and to meet consumer expectations through guaranteeing product quality and safety.

1.3 Thesis organisation

This thesis is presented as a series of eight chapters, with the majority of chapters representing a manuscript/published paper or a series of papers/manuscripts at various stages of publication linked by a common theme. Where possible, the thesis is written to essentially present each manuscript(s)/paper(s) (or parts thereof) as written. A summary is provided at the beginning of each chapter to ensure cohesion and flow when reading each chapter in sequential order.

The work reported in this thesis was done by the author Brett Wedding and has included discussions, technical assistance, statistical guidance with supervisors, experts and colleagues as evident in the publications. Dr Carole Wright provided statistical knowledge and guidance for: data analysis, error associated with traditional DM methods and calibration model development. Professor Ron White provided advice in the area of spectroscopy and electromagnetic radiation penetration into biological materials. Stephen Grauf provided technical assistance throughout the experimental work.

Chapter 1

This chapter provides the aim, objectives and rational for this research project.

Chapter 2

In this chapter, the current state of the Australian avocado industry, along with industry issues associated with fruit maturity, fruit flesh disorders and defects affecting consumer purchasing are presented in *Section 2.1*. *Section 2.2* provides an overview of avocado fruit physiology and quality attributes relating to maturity indices and defect issues associated with flesh disorders (i.e., rots) and bruises. Traditional methods of assessing maturity indices (i.e., DM, MC and oil content) are also reviewed and described. An overview of non-destructive technologies suitable for assessing avocado fruit maturity for commercial in-line applications is provided in *Section 2.3*. The application of NIR spectroscopy for assessing avocado maturity based on DM content is reviewed in *Section 2.4*. The reader is deferred to the author's Master's thesis (Wedding 2007) for a complete description of the fundamentals of NIR spectroscopy and associated chemometrics. In *Section 2.5* the effects of geographical and season variation on NIR calibration model performance and robustness is discussed. *Section 2.6* reviews the application of NIR spectroscopy technology as a tool for prediction of storage disorders as an indication of shelf-life. *Section 2.7* explores potential non-destructive technologies for the detection of bruises in fruit, for possible application to avocado fruit in a commercial in-line setting.

Chapter 3

This chapter introduces the fundamentals in the development of the utility of NIR spectroscopy as a non-invasive tool for estimating DM content in whole avocado fruit. *Section 3.1* presents an overview of determining and measuring sources of error associated with traditional DM methods and the effects associated with the development of NIR calibration models. *Section 3.2* presents the results from investigations into the penetration ability of NIR radiation into avocado fruit. A manuscript has been submitted for review for publication detailing this work: *Wedding, B.B.; Grauf, S.; Wright, C; Gadek, P.A. and White, R.D. (submitted 2018) Depth of penetration of near infrared radiation in 'Hass' avocado fruit. Postharvest Biology and Technology*.

In *Section 3.3*, studies of DM variation within Australian avocado fruit to determine a suitable sampling point on the avocado that represents whole fruit DM content for NIR assessment are provided. This work, plus investigations to assess the potential of FT-NIR spectroscopy as a tool to determine the maturity and eating quality of Australian 'Hass' avocado fruit, based on DM content with the exocarp (skin) removed and on whole fruit, are presented in the following publications: I) *Wedding, B.B., White, R.D.; Grauf, S.; Wright, C; Tilse, B.; Hofman, P. and Gadek, P.A. (2010) Non-destructive prediction of 'Hass' avocado maturity via FT-NIR spectroscopy. Journal of the Science of Food and Agriculture* ***91****, pp 233-238;* and II) in *Appendix*

A providing a brief summary of results and discussion of: *Wedding, B.B., White, R.D.; Grauf, S.; Tilse, B.; Hofman, P. and Gadek, P.A. (2009) Non-invasive Assessment of Internal Quality Attributes of Whole Avocado Fruit by NIRS. SABRAO Journal of Breeding and Genetics Vol. 41, Special Supplement August 2009 ISSN 1029-7073.* The studies also examine the effects of intra-seasonal variation and orchard conditions on NIR calibration models as development for commercial applications by using a fruit population from one growing district over a single growing season.

Chapter 4

The robustness of calibration models with respect to biological variability from different geographic regions and seasons has been widely overlooked in literature. Therefore these calibration models may be over-optimistic with respect to prediction accuracies on future samples in practical applications. This chapter, presents a publication by the author that reviews the biological variability from two different geographic regions over a single season as part of developing robust NIR spectroscopy calibration models for determining DM content in Australian 'Hass' avocados, with results published in: *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) Near Infrared Spectroscopy as a rapid non-invasive tool for agricultural management with special reference to avocado and Sandalwood industries. Desalination and Water Treatment* ***32****, pp 365-372.*

Chapter 5

This chapter, investigates the influences of biological variability from different seasons (three consecutive seasons/years) that effect the robustness of NIR calibration models for determining DM content in avocados. Calibration models results based on different pre-processing techniques and these effects on model performance are presented in two publications provided in *Section 5.1:* I) *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2013) Effects of seasonal variability on FT-NIR model robustness for the prediction of dry matter content for whole Hass avocado fruit. Postharvest Biology and Technology 75, pp 9-16;* and II) in *Appendix B* providing a brief summary of results and discussion of: *Wedding. B.B.; Wright, C.; Grauf. B.; White, R.D. and Gadek, P. (2010) Prediction of Hass avocado maturity via FT-NIRS. In: NIR 2009 - Breaking the Dawn, Proceedings of the 14th International conference on Near Infrared Spectroscopy, Bangkok , Thailand, 7-16 November 2009, pp 260-272 IM Publications West Sussex.*

Section 5.2 presents further investigations of seasonal variability effects on model robustness to predict DM content on avocado fruit, based on a separate data set from a different growing area than what is presented in *Section 5.1*, with data collected over three consecutive growing seasons/years as published in: *Wedding, B; White, R; Grauf, S.; Wright, C; Tilse, B; Fitzsimmons,*

J.; Hofman, P and Gadek, P. (2009) NIRS technology for determining maturity in avocados. In: 4th Australian and New Zealand Avocado Growers Conference, Cairns 21-24 July 2009.

Chapter 6

This chapter, reports on the effects of biological variability influences from both geographical and seasonal variability that effect the robustness of NIR calibration models for determining DM content in avocados. In *Section 6.1*, avocado fruit were obtained from two different growing districts within Queensland, Australia over three consecutive growing seasons/years to determine the influences of both geographical and seasonal variability in relation to the robustness of the calibration model. A comparative study of two different commercially available NIR systems (a line scan camera based system and a spectrophotometer based system) was undertaken for assessing DM content in avocado fruit which are presented in the publication: *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) Non-invasive assessment of avocado quality attributes. In: The Proceedings of the VII World Avocado Congress 2011, Cairns – Australia, 5-9 September 2011.*

Further investigations of geographical and seasonal variability influence on calibration model robustness for the determination of DM in avocado fruit, using fruit obtained from two different growing regions (Note: a different region selection than presented in *Section 6.1)* over three consecutive seasons/years was also undertaken in *Section 6.2*, as published in: *Brett B. Wedding, Carole Wright, Steve Grauf and Ron D. White. (2012) The application of near infrared spectroscopy (NIRS) for the assessment of avocado quality attributes. In: Infrared Spectroscopy. Intech Open Book Access.*

Elements of these papers that correspond to initial investigations for detecting bruises and rots/shelf life have been extracted and deferred to *Chapter 7* and the associated *Appendix C* and *Appendix D.*

Chapter 7

This chapter, reports on studies that review the potential of NIR spectroscopy as a non-invasive tool to detect bruises in whole avocado fruit and to predict fruit susceptibility to rots as an indication of potential shelf-life. *Section 7.1* investigates the potential of FT-NIR spectroscopy as a non-invasive assessment tool to detect bruises in whole avocado fruit over three growing seasons. Likewise, to predict susceptibility of whole avocado fruit to future flesh disorders ('pre-disorder' development) at different stages of ripeness as an indication of potential shelf-life, as submitted for review in: *Wedding, B.B.; Wright, C; Grauf, S.; Gadek, P.A. and White, R.D.*

(submitted 2018) The application of FT-NIRS for the detection of bruises and the prediction of rot susceptibility of Hass avocado fruit. Journal of Food Science and Agriculture.

Section 7.1 also presents a summary of preliminary research findings from the initial assessment of developing a non-invasive NIR spectroscopy assessment tool for detecting bruises and rot susceptibility as an indication of shelf-life in fruit from two growing districts based on three publications presenting similar data and a variation of statistical methods as presented in: Appendix C: I) *Brett B. Wedding, Carole Wright, Steve Grauf and Ron D. White. (2012) The application of near infrared spectroscopy (NIRS) for the assessment of avocado quality attributes. In: Infrared Spectroscopy. Intech Open Book Access;* II) *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) Non-invasive assessment of avocado quality attributes. In: The Proceedings of the VII World Avocado Congress 2011, Cairns – Australia, 5-9 September 2011;* and Appendix D: III) *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2012) Impact assessment and prediction of rot susceptibility of Hass avocado fruit using FT-NIRS. In: The Proceedings of the 15th International conference on Near Infrared Spectroscopy, Cape Town, 16-20 May 2011.*

The preliminary work presented in Appendix C and Appendix D is a first step towards shelf-life prediction and bruise detection for avocado fruit using NIR spectroscopy.

Chapter 8

Concluding remarks and recommendations for future work are provided in this chapter.

CHAPTER 2

LITERATURE REVIEW

This chapters provides scientific background for the research presented in this thesis.

Section 2.1 gives a succinct overview of the current state of the Australian avocado industry in relation to production and industry issues associated with fruit maturity, fruit flesh disorders and defects affecting consumer purchasing. Fruit quality consistency is a major challenge to the industry which impacts on consumer confidence and end product sales. Consumers are often dissatisfied with avocado quality with the major defects affecting purchasing being bruising, body and stem rots (Harker 2009). Reducing the quality inconsistencies is a key commercial consideration of the avocado industry to successfully expand both domestic and international markets.

Section 2.2 provides an overview of avocado physiology and quality attributes relating to maturity indices and defect issues associated with flesh disorders (i.e., rots) and bruises. Various fruit quality assessment techniques, particularly methods of assessing maturity indices (i.e., DM, MC and oil content) are described. Fruit maturity is one of the most important factors determining quality, ripening, internal defect development and sensory acceptance of avocados (Embry 2006). There are only minor visible changes to the external appearance of avocado fruit that may be used to determine maturity which are discussed.

Section 2.3 gives a summary of some non-destructive technologies suitable for application in commercial in-line settings, with specific reference to ultrasonic systems, nuclear magnetic resonance (NMR) and NIR spectroscopy. Non-invasive technologies in a commercial inline setting offer the potential to test every piece of fruit passing through a pack-house. This would enable the avocado industry to assess for quality attributes and ensure product consistency and quality to the consumer.

Section 2.4 reviews the application of NIR spectroscopy as a non-invasive technology for assessing avocado fruit quality attributes for commercial in-line settings. In particular, for the measurement of fruit maturity based on DM content. Although there has been significant developments in the knowledge and the application of NIR spectroscopy as an analytical technique in the agriculture industry, particularly to estimate %DM content of various horticultural products (Xiaobo et al. 2006; Hartmann and Bijning-Pfaue 1998; McGlone and Kawano 1998; Birth et al. 1985), there has been limited investigations using NIR spectroscopy to

determine %DM content in avocado fruit (Olarewaju et al. 2016; Walsh et al. 2004; Clark et al. 2003; Schmilovitch et al. 2001).

Section 2.5 reviews the application of NIR spectroscopy technology as a tool for prediction of storage disorders (rot development) as an indication of shelf-life. There has been limited investigations reported in literature in relation to the prediction of storage disorders (Pérez-Marín et al. 2011; Sánchez et al. 2009; Camps et al. 2007; Nicolai et al. 2006; Clark et al. 2004; McGlone et al. 2003; McGlone et al. 2002). The majority of the studies relating to storage disorders address the potential of NIR spectroscopy for discriminating between products on the basis of postharvest storage duration as a means of estimating product shelf-life and not to predict future disorders.

Section 2.6 explores non-destructive technologies for the detection of bruises in fruit, for potential application to avocado fruit in commercial in-line settings. There has been various non-invasive technologies investigated to identify bruising in fruits, including NIR spectroscopy (Nagata et al. 2006; Nagata et al. 2002; Upchurch et al. 1991). The studies identified in literature were carried out on thin skin fruits and not thick skinned fruits such as avocado. Thick skin fruits provide substantial challenges for the application of NIR spectroscopy due to the reduced penetration depth of NIR radiation within the tissue compared to other non-destructive methods..

Section 2.7 investigates the effects of geographical and seasonal variation on NIR calibration model performance and robustness. Calibration model robustness is a critical issue for future application of NIR spectroscopy. The validity of calibration models for future predictions depends on how well the calibration set represents the composition of new samples. Composition of horticultural produce in relation to model development is subject to large physiological, seasonal and geographical variability. Even though NIR spectroscopy has been well established in literature to assess horticultural products for various quality attributes, calibration model robustness in relation to biological variability has been limited.

2.1 Introduction

The Australian avocado industry comprises of more than 1300 growers and produced over 66,700 tonnes of avocados in 2015/16 with a total Gross Value of Production estimated at $460 million and retail value of approximately $920 million (Avocados Australia Limited 2016). World production of avocados is over four million tonnes and steadily growing (Barnard and Goucher 2014). In the world avocado market, Australia is considered a small player producing less than 2% of global production of avocados (Avocados Australia Limited 2016). Currently, less than 3% of Australian avocado production is exported, mainly to Malaysia, Singapore and Hong Kong (Horticulture Innovation Australia Limited 2017; Avocados Australia Limited 2016).

Australia is able to produce avocados all year around due to the range of growing climates (Avocados Australia Limited 2016). Key production areas include the north, central and south-east areas of Queensland; central and northern New South Wales; the tristate area (Victoria, South Australia and south western New South Wales) and Western Australia (Avocados Australia Limited and Primary Business Solutions 2005). 'Hass' avocado harvested from April to February is the dominant variety, representing 80-85% of production, while 'Shephard' avocado harvested from February to April, represents 10-15% of production (Avocados Australia Limited 2016). Australian avocados are predominately grown for the fresh market (Horticulture Innovation Australia Limited 2017) with Queensland and Western Australia currently being the highest production states, with an average production of 49% and 37%, respectively, over 2014 to 2016 (Avocados Australia Limited 2016). New Zealand is currently the only exporter of avocados to Australia, with imports arriving during the Australian summer (Horticulture Innovation Australia Limited 2017).

Avocado has gained popularity over the years due to an increasing consumer awareness of the dietary values. Australian avocado consumption in 2015/16 was approximately 78,276 tonnes (Horticulture Innovation Australia Limited 2017) with an estimated consumption of 3.2 kg per capita (Avocados Australia Limited 2016). Avocado production in Australia is expanding rapidly to keep up with increasing domestic demand and there are strong financial incentives to increase sales both domestically and internationally. The Australian avocado industry is also facing increased international competition with major exporting countries, such as Mexico, Chile, and Peru; and emerging exporting countries, such as Brazil and Argentina increasing production and seeking more alternate markets for their product (Allen 2009). These factors combined with Australia's cost of production, long shipping distances and lack of market access all provide challenges for the industry.

Fruit quality consistency continues to be another major challenge for the industry, particularly with second grade fruit having a negative impact on domestic prices and consumer confidence (Horticulture Innovation Australia Limited 2017). The Australian industry must supply a quality product all year-round to the domestic market to reduce the need for imports (Horticulture Innovation Australia Limited 2017). Consumer and retail surveys conducted over the last two decades in relation to avocado quality have shown that consumers are often dissatisfied with avocado quality, particularly with poor flesh quality which is only discovered once the fruit has been purchased and cut open (Embry 2009; Gamble et al. 2008; Harker et al. 2007; Hofman and Ledger 1999). The major internal defects identified by consumers throughout these surveys, that influence future purchases include: bruising, body and stem end rots (Harker 2009). The risk of fruit quality loss before consumption is increased for international markets as the additional time and distance associated with travel to reach these markets results in increased duration from harvest to purchase. Thus, a key factor impacting on industry profitability is fruit quality reliability because consumer repeat purchasing is influenced by bad eating experiences. Removing fruit quality inconsistencies and providing the consumer with a consistent quality is a vital commercial consideration of the Australian avocado industry for both domestic and export markets (Avocados Australia Limited and Primary Business Solutions 2005).

Consumers currently judge internal quality of fruit based on appearance, firmness and smell. Most commercial quality grading systems are based on assessing external appearance such as colour, shape, size and blemishes. As the external features of fruit are generally not an accurate indication of internal quality, the horticulture industry utilises destructive measures including juice sweetness (^{o}Brix), DM content, or flesh colour applied to a small number of fruit to represent an entire consignment. In the case of avocados, fruit maturity is a primary determinant of avocado eating quality and is currently commercially assessed by destructively assessing the DM content of a few fruit to represent an entire batch or consignment (Harker et al. 2007; Brown 1984). Methods that rely on testing a few samples to represent a large batch pose a potential risk that the few fruit selected to be tested will not necessarily represent the whole batch due to biological variations within the batch being tested. A non-destructive inline system that can rapidly and accurately monitor all avocado fruit going through a pack-house for internal quality attributes such as DM content, bruising and rots, would allow the industry to provide a more consistent quality fruit to meet consumer expectations. This would assist to improve industry competitiveness, expansion of export markets and increase industry profitability. Despite the obvious commercial need for the development of rapid non-invasive assessment techniques to determine quality attributes of avocados there has been limited progress in this area.

2.2 Avocado physiology and quality attributes (maturity indices, rots and bruises)

The avocado (*Persea americana* (Mill.)) is a single seeded berry with a high oil bearing edible flesh ('mesocarp') and thin or thick skin ('exocarp') depending on variety (Lewis 1978). The fruit is climacteric displaying a rise in respiration with time, reaching a peak, after which the ripening and softening process occurs (Milne 1997). Depending on variety, the seed comprises 10-25% of the total fruit weight, the mesocarp contributes 50-80% and oil accumulation can reach as high as 30% of the fruit weight (Lewis 1978; Barmore 1976). The chemical composition of the edible flesh includes water 65-80%; oil 3-30%, protein 1-4% and sugar at approximately 1% (Ozdemir and Topuz 2004; Sinyinda and Gramshaw 1998). Avocado is also rich in vitamin B_6, vitamin E, β-carotene, ascorbic acid, and potassium with moderate amounts of vitamin A and D (Sinyinda and Gramshaw 1998).

Fruit maturity is one of the important factors determining quality, sensory acceptance, ripening characteristics, and incidence of rots and disorders (Embry 2006; Johnston et al. 2006). A unique characteristic of avocado biology is that mature fruit do not ripen on the tree, but soften several days after being harvested (Schmilovitch et al. 2001). This allows growers the ability to delay harvesting if needed to meet varying market demands. However, it is important to harvest mature fruit, so as to ensure that fruit will ripen properly and have acceptable eating quality. If the fruit are harvest too early then the eating quality will be compromised. For example, harvesting an immature fruit of any variety will likely result in the fruit shrivelling as it ripens and having a watery, bland, or even "grassy" flavour (Bergh et al. 1989). Embry (2006) reports that early season fruit often ripen slower making them more susceptible to rots and they are also susceptible to developing external chilling injury, shrivel stringiness, vascular browning and mesocarp discolouration. If the fruit is harvested at an over mature stage, it will have a short shelf life and is more prone to postharvest physiological disorders and diseases, including premature softening, vascular browning, mesocarp discolouration, body rots and stem end rots (Embry 2006; Flitsanov et al. 2000). Many of these maturity related disorders are aggravated by long-term storage (Embry 2006). Due to the importance of maturity to the industry, interest in improving various maturity methods, particularly for rapid, non-invasive tests exists today.

The "commercial" maturity of avocados can be defined as the stage of fruit development where the fruit following detachment from the tree will undergo normal ripening and result in good eating properties (Lee et al. 1983; Lewis 1978). In comparison, "physiological" maturity is the stage of development when avocado fruit will continue ontogeny even after it has been detached from the tree (Lee et al. 1983). There are only minor visible changes in the external appearance of avocado fruit that can be used to determine maturity. This may include loss of skin glossiness, fruit size, and increase in surface russeting, while some cultivars change in external appearance

from green to black or purple with increasing maturity (Bergh et al. 1989; Lewis 1978). This colouration can confuse customers as the fruit visually appears to be ripe, however it is not soft (Cox et al. 2004). Picking dates based on fruit size has been extensively used to improve the market acceptability, with larger fruit generally having higher flavour ratings than small fruit early in the season (Bower and Cutting 1988; Lee 1981). However, the picking date method is affected by varietal differences, geographic, and seasonal influences (Lee 1981). Sugar content of avocados has been related to fruit maturity and is dominated by the seven carbon (C7) sugar D-mannoheptulose, whereas sucrose, glucose and fructose are present in lower concentrations (Meyer and Terry 2008; Liu et al. 1999). As reported by Liu et al. (1999), as 'Hass' avocado fruit grow in size, the flesh accumulates proportionally increased levels of total soluble solids (SS) coinciding with the increase in dry weight. This accumulation stopped in fruit tissues when fruit reached a minimum maturity of 20.8% DM, where the flesh begins to accumulate oil (Liu et al. 1999). As accumulation of sugar stops after reaching a minimum maturity of 20.8% DM, this parameter cannot be used on a commercial basis to quantify maturity beyond 20.8% DM.

2.2.1 Quality assessment techniques

Fruit firmness has been used as a method for assessing fruit maturity and ripeness (Magwaza and Tesfay 2015; Harrison 2003; Howarth et al. 2003; Hung et al. 1999). Firmness has been shown to correlate with maturity measured by other parameters such as oil content and eating quality (White et al. 1999). Avocado fruit firmness changes gradually as the fruit ripens and this has been used as an index for maturity and postharvest ripening stage (Magwaza and Tesfay 2015). However, immature fruit often ripen and soften slower, while over mature fruit often are susceptible to premature softening (Embry 2006). Firmness is typically utilised by the consumer as a measure of postharvest ripening stage. From harvest the firmness declines at a moderate rate as the fruit ripens, this decline in firmness increases, falling close to zero as the fruit approaches full ripening (White et al. 2001). Impact techniques have given good results in firmness evaluation of various fruits (Howarth et al. 2003). However, local variations in surface texture can limit the accuracy of firmness prediction by impact testing (Howarth et al. 2003). This is a limiting disadvantage of the method, since impact force is a measure of local properties in the impact zone, rather than properties of the overall intact fruit (Howarth et al. 2003).

Electrical impedance may offer a promising area of testing of avocados, as impedance in biological materials depends on several factors including concentration of ionic substances in the cells, the capacitive effects of cell membranes, discrete particles within the cells (i.e., oil droplets) and on characteristics of measuring signals (Bean 1962). Factors in avocados that may contribute to a change in impedance include: oil content is increasing resulting in greater capacitive effect due to the interface between the oil droplet and the aqueous phase; concentrations of dissolved

substances in the cytoplasm vary with age and other factors; and membranes in the cells change with some degree with maturation (Bean 1962). In the study of Bean (1962), impedance throughout avocado maturation was measured. The author reported that there was a tendency for the impedance to drop near maturation, however the variation among individual fruit showed a wide range of resistances to be reliable. The method also was not temperature compensated and resistance varied greatly with changes in temperature. Furthermore, the procedure required the insertion of two electrodes into the fruit resulting in small injury and potential loss of fruit for future sales.

Montoya et al. (1994) also investigated electrical conductivity as a quality index for 'Hass' avocado fruit during cold storage and ripening. Respiration intensity and pulp firmness was determined to estimate the time course of ripening and relate to electrical conductivity signals. The authors reported an increase to a maximum and decrease slightly in the conductivity as the fruit ripens. A conductivity rise was also associated with the appearance of physiological disorders including chilling injury and senescence. While a pre-climacteric stage with optimum pulp firmness for marketing could be identified based on a conductivity threshold value of 0.24 S/m, as the conductivity curve resembled the ethylene production curve (Montoya et al. 1994). Softening is discernible approximately two days following the climacteric peak (Barmore 1976). Cellulase enzyme has been reported as playing a part of the softening process, since cellulose is a major constituent of avocado cell walls and is found to increase associated with the respiratory climacteric and ethylene (Bower and Cutting 1988). The enzyme pectinmthylesterase (PME) also decreases with maturity of the avocado fruit (Barmore 1976).

Specific gravity and seed coat colour have also been used as a maturity index with limiting reliability (Lee 1981). Typically, seed coats of mature fruit will be thin and brown, while seed coats of immature fruit will be fleshy and white (Bergh et al. 1989). Whereas fruit specific gravity has been reported to have a downward trend from ~1 to ~0.9 as they mature (Lewis 1978). Clark et al. (2007) investigated the potential of density (specific gravity) as an index for DM content based on the hypothesis that flesh density should decrease with increasing oil content and the avocado should show a greater tendency to float in water. The authors developed a generic multi-term expression for a whole-fruit system where the density of each component (i.e., exocarp, mesocarp and seed) was characterised with regard to its water, air and DM content. Poor correlation was reported ($r = -0.29$; $P<0.0001$) on a population of 1000 New Zealand 'Hass' avocado fruit with a DM range of 23 to 43% (Clark et al. 2007). Similarly, lenticel corking, pressure, heat capacity and light transmission tests have shown little value as indices of maturity (Chen et al. 1993; Lee 1981). Unfortunately, many fruit characteristics which show a trend with maturity are not applicable on a commercial basis.

2.2.2 Avocado dry matter, oil and moisture content

Currently, for commercial purposes destructive methods are utilised to determine avocado maturity through assessing a number of samples in a batch to represent an entire consignment. These destructive methods include, %DM, mesocarp oil content and MC, which are all highly correlated with maturity (Clark et al. 2007; Clark et al. 2003; Mizrach and Flitsanov 1999). However, it is impossible to apply a destructive method to a large number of fruit to determine the true index of maturity for any given consignment, as each test would result in a loss of sale for the assessed fruit. This is also further complicated since there is considerable variation among and within individual cultivars.

Both %DM and mesocarp oil content have been shown to increase with fruit development (Clark et al. 2007; Hofman et al. 2002). As the DM and mesocarp oil content increases, the water content decreases by the corresponding amount (Clark et al. 2007; Ozdemir and Topuz 2004; Fuchs et al. 1995; Ranney et al. 1992; Lee and Coggins 1982). DM content increases during growth but does not change after harvest (Hofman et al. 2002). The majority of avocado producing countries set minimum maturity standards to achieve standardisation of fruit quality across the industry and prevent poor quality immature fruit entering the market. For Australian avocado growers, Avocados Australia Limited (2008) recommends a minimum maturity standard of 23% DM (>10% oil content) for the 'Hass' cultivar (previously 21% DM) and 21% DM for the 'Shepard' cultivar. Although, consumer studies indicate a preference for at least 25% DM for 'Hass' (Harker et al. 2007). Research has shown that consumer acceptance of avocado quality declined from approximately 95% to 70% if the %DM of 'Hass' avocados was below 23% (Petty 2011). Furthermore, internal quality defects of greater than 10% impact negatively on consumers future purchase intent (Petty 2011).

Destructive measurement of oil content is slow and expensive when compared to %DM or MC assessment. Different analytical methods have been employed to determine the oil content of avocado fruit. For example, the standard method for lipid extraction is the Soxhlet technique, which commonly utilises petroleum-ether or hexane solvents at high temperatures and gravimetric determination of oil content from dehydrated avocado mesocarp tissue (Meyer and Terry 2008). Alternative oil extraction methods such as homogenisation with a solvent (e.g., petroleum-ether) or supercritical carbon dioxide have also been used for avocado fruit oil content determination (Meyer and Terry 2008). The accuracy of refractive index (RI) determination of an oil extract with chloronaphthalene has been deemed questionable (Ranney et al. 1992; Lewis et al. 1978). It is for these reasons that %DM or MC are employed by industry to indicate avocado maturity. DM and MC are measured by taking a known weight of avocado flesh tissue with skin

and seed coat removed, and drying it to a point of constant weight (Woolf et al. 2003), Eqns. 2.1 and 2.2 are used to calculate DM and MC, respectively, i.e.,

$$\%\text{Dry Matter content} = \frac{C-A}{B-A} \times 100 \qquad \textbf{Eqn. 2.1}$$

$$\%\text{Moisture content} = \frac{(B-A)-(C-A)}{B-A} \times 100\,, \qquad \textbf{Eqn. 2.2}$$

where A is mass of empty dish, B is total mass of sample and dish, and C is total mass of dry sample and dish.

The test can be done by using a drying oven, conventional oven, freeze drying, food dehydrator or a microwave. The microwave drying method is faster with drying times ranging between 15 to 40 minutes, depending on power output and settings, however it is less reliable due to the increased risk of burning the samples (Wang et al. 2012; Lee et al. 1983). Various ranges in temperatures and drying times are reported in literature for the oven drying method, from 55°C to 105°C with drying times from 3 hours to 7 days, or until constant mass (Carvalho et al. 2014; Gamble et al. 2010; Ozdemir and Topuz 2004; Clark et al. 2003; Flitsanov et al. 2000; Lee et al. 1983). There is a risk of burning the sample and oil at temperatures greater than 70°C which will negatively affect the final %DM result (Woolf et al. 2003). Conversely, with freeze drying there is no risk of burning the sample or oil.

2.2.3 Avocado defects - rots and bruises

Poor flesh quality is a major factor influencing avocado consumer dissatisfaction, particularly as it cannot be determined until the fruit is cut open. Australian consumers expect to discard one in four avocados they purchase as a result of poor flesh quality (Avocados Australia Limited and Primary Business Solutions 2005). Consumer surveys have highlighted that Australian consumers select bruising as the major defect, followed by body and stem end rots which have a highly negative impact on consumer repurchasing (Harker 2009). The causes of bruising are predominately post farm-gate as avocado fruit become susceptible to impact and compression damage resulting in bruise development once the fruit has started to soften as part of the ripening process. The ripening process continues as the fruit is dispatched from farm to retail outlets and the consumer. During this time the fruit continues to soften and becomes more susceptible to compression and impact damage. Bruising severity is also increased with a lower DM content at harvest and longer exposure to a compression or impact event (Joyce et al. 2015). Furthermore,

the presence of bruises increases the risk of fungal contamination as damaged tissue is more prone to infection (Luo et al. 2012; Xuan et al. 2012).

A major risk to transporting avocados, particularly over long distances, is the potential development of rots and flesh disorders (Hofman and Marques 2009). This is a significant consideration for export of avocados from Australia, as sea freight typically takes two to six weeks depending on destination (Hofman and Marques 2009). 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. Inferior fruit quality is seen as one of the primary factors impacting on supply chain efficiency and profitability (Margetts 2009). The key consideration of the Australian avocado industry for retaining and expanding both domestic and international markets is removing inconsistency and providing what the consumer expects, i.e., a consistent quality product that is free of bruises and flesh disorders and has a suitable DM content. Consumer response has shown that 60% of consumers would be more willing to buy fruit with a guarantee of quality, particularly when guaranteeing it has an absence of internal defects (Harker et al. 2010).

Currently, there is no reliable system to predict if avocado fruit will arrive at the consumer in an acceptable quality. Fruit with physical defects are typically visually sorted during packing on the farm, however, many of the flesh disorders only appear once the fruit ripen and age. Assessing fruit internal attributes at harvest may identify fruit that are less prone to rots and internal disorders. These fruit can be sent to more distant domestic markets and exported with greater confidence that the fruit will arrive at market in acceptable quality. A rapid and non-destructive system that can accurately and rapidly monitor internal quality attributes and predict flesh disorder development in relation to shelf-life would allow the avocado industry to provide better, more consistent fruit eating quality to the consumer, and thus improve industry competitiveness and profitability.

2.3 Non-destructive technologies suitable for assessing maturity and quality attributes for commercial in-line applications

The purpose of this literature review is not to provide a comprehensive, scientific review, rather to demonstrate the potential of the most commercial ready non-invasive technologies applicable to the avocado industry. In particular, for the measurement of fruit maturity based on DM content, bruise detection and potential to predict shelf-life based on flesh disorders (mainly rots) development during storage. Automated technology advancement has enabled the development of commercially feasible non-destructive techniques for estimating internal quality attributes for agricultural products. These techniques have involved the application of real-time monitoring

and non-destructive testing for various horticultural quality parameters. However, few of the proposed techniques seem to provide all the information necessary to characterise fruit quality. Various non-invasive techniques have been reported in literature by a number of researchers for evaluating numerous internal quality attributes of fruit and vegetables, examples include: magnetic resonance imaging (MRI) (Butz et al. 2005; Létal et al. 2003; Gonzalez et al. 2001; Abbott 1999; Clark et al. 1997); NMR (Butz et al. 2005; Létal et al. 2003; Abbott 1999; Kim et al. 1999; Chen et al. 1993; Barry et al. 1983); acoustic sensors (De Ketelaere et al. 2006; De Belie et al. 2000; Chen and Sun 1991; Bean 1962); ultrasonic excitation (Butz et al. 2005; Gaete-Garreton et al. 2005; Mizrach 2000; Abbott 1999; Mizrach and Flitsanov 1999; Mizrach et al. 1999; Chen and Sun 1991); optical (Butz et al. 2005; Garcia-Ramos et al. 2005; Abbott 1999; Chen and Sun 1991; Zachariah and Erickson 1965; Bean 1962); electrical properties (Chen and Sun 1991; Zachariah and Erickson 1965; Bean 1962; Bean et al. 1960); electronic nose (Butz et al. 2005), firmness (Garcia-Ramos et al. 2005; Harrison 2003; Hung et al. 1999); light emission (Zachariah and Erickson 1965); X-ray and gamma ray transmission (Butz et al. 2005; Throop et al. 2005; Abbott 1999; Barcelon et al. 1999; Chen and Sun 1991). Some techniques, however, have limited commercial capabilities as a result of high cost, slow speed for in-line setting applications, as with sonic and optical methods these may have limited penetration depth in many horticultural tissues (Kim et al. 1999).

2.3.1 Spectroscopic techniques

Spectroscopic techniques are based on the interactions between electromagnetic radiations and the sample atoms or molecules to supply qualitative and quantitative chemical and physical information (Butz et al. 2005). The electromagnetic spectrum spans from gamma-rays, through to X-rays, ultraviolet, visible, infrared, microwaves and finally radio waves. The most commonly used wavelength ranges for determining fruit quality characteristics include: ultraviolet (4 to 400 nm), visible light (approximately 380 to 770 nm) and NIR (750 to 2500 nm) (Bureau 2009). When electromagnetic radiation enters a fruit the majority is absorbed by the fruits constituents which occur at different wavelength regions depending on the fruits constituents and a percentage is scattered due to the tissue characteristics (Bureau 2009). The absorbed radiation at certain wavelengths can be utilised to assess horticultural quality attributes. Fluorescence is restricted to the non-destructive evaluation of chlorophyll, anthocyanin and total flavonoids (Bureau 2009). Multispectral and hyperspectral imaging provide both spectral and spatial information in the visible and NIR ranges which is feasible for in-line applications and mapping of attributes within samples (Lee et al. 2014; Opara and Pathare 2014; Van Zeebroeck et al. 2007).

2.3.2 X-ray technology

Considerable research has been applied to X-ray radiography as a technology with potential application in-line to assess various quality attributes of fruit based on the relative density of the scanned material compared to the density of water (Tollner et al. 1989). During the maturation and ripening process the density of fruit tissue is subject to change as a result of physiochemical properties that take place at different times within the fruit (Barcelon et al. 2000). Some applications of X-ray technology for assessment of fruit quality include: the detection of voids, splits and density variation (Bowers et al. 1988); detection of seed weevil in mango (Thomas et al. 1994); determining bruising, density and water content in apples (Schatzki et al. 1997; Tollner et al. 1992; Bowers et al. 1988; Diener et al. 1970); the detection of hollow heart in potato (Finney and Norris 1978); finding defects in watermelons and cantaloupes (Tollner 1993); the detection of split pit in peaches (Han et al. 1992); stones in apricots (Zwiggelaar et al. 1997); determining internal changes (MC, density and TA) in peaches during ripening (Barcelon et al. 2000); establishing maturity in green tomatoes (Brecht et al. 1991) and lettuce heads (Lenker and Adrian 1971). However, the application of X-ray and gamma radiation to fruit are subject to safety concerns due to potential radiation hazards that may result from leakage in an industrial situation (Arendse et al. 2018). As the method primarily depends on tissue density and not the chemical composition, the selectivity of application is limited (Butz et al. 2005). Additionally, data acquisition and lengthy processing times for data analysis currently limits the technology as a tool for rapid in-line or real-time non-destructive assessment of internal attributes of horticultural produce (Arendse et al. 2018).

2.3.3 Firmness techniques

A large number of devices and techniques have been developed to determine firmness of fruits and vegetables, most are based on compression, puncture or impact; and more recently impulse response ultrasonic techniques. There are several commercially available in-line impact testers utilised to assess mechanical properties such as fruit firmness, including a system used to measure avocado firmness. These include the Sinclair IQ™ non-destructive firmness tester (Harrison 2003) and the Aweta™ impact firmness and the Aweta™ or Autoline™ acoustic firmness tester (Harrison 2003). The Aweta™ impact firmness tester applies a large wheel equipped with multiple sensor tips to impact fruit moving on the conveyor lines at a speed of seven items per second (Lu and Cen 2013).

The Sinclair non-destructive firmness tester utilises four low mass impact sensors, which touch each fruit in an in-line setting at speeds of up to ten fruit per second depending on fruit size (Harrison 2003; Howarth et al. 2003). The in-line system makes four independent measurements around the fruit to provide a combined estimate of firmness (Howarth et al. 2003). This

technology has been applied to a range of fruit including, apples, kiwifruit, stone fruit, mango and avocado (Harrison 2003; Howarth et al. 2003). Howarth et al. (2003) reports correlations between using a trained sensory panel to determine firmness, and a parallel-plate compression test to determine apparent elastic modulus and tissue strength by destructive conical indenter plunger (correlation coefficient (R) = 0.866, 0.902 and 0.828 respectively). In a similar study, Shmulevich et al. (2003) compared the Sinclair low mass impact (firmness index (FI)) and acoustic response (internal quality index (IQ)) against destructive compression and penetration tests to evaluate 'Fuerte' avocado firmness. The authors report correlations between the destructive tests of R = 0.943 between the Sinclair low-mass impact firmness and modulus of elasticity and cone penetration (R = 0.953 and R = 0.955, respectively). Correlations of the acoustic technique (FI) to the elastic modulus and cone penetration, were reportedly lower with an R = 0.68 and R = 0.695, respectively (Shmulevich et al. 2003).

Estrada-Flores (2003) investigated the use of acoustic measurements (firmness tester by Aweta™, Netherlands) for the determination of firmness in 'Hass' avocados at varying temperatures. The authors reported that the acoustic measurements were more appropriate for fruit that had entered the ripening process than for unripe, and full green fruit. The variability of fruit had most significant impact on the mean acoustic FI, followed by storage time and then variation due to storage temperature (Estrada-Flores 2003). Vursavus et al. (2015) reports combining three non-destructive sensors to apply a 'fusion' technique to explore whether a combination of sensors would give better results than a single sensor for classification of peach firmness. The sensors included: i) a benchtop acoustic firmness sensor by Aweta; ii) a long-mass impact sensor by Madrid Polytechnic University Physical Properties Laboratory; and iii) a micro-deformation impact sensor (quality firmness tester (SIQ-FT) by Sinclair International). The fusion of the three non-destructive sensors decreased the error rate to 13% for firmness classification, while it varied from 25% to 19% for each individual sensor.

Although, several non-invasive techniques exist, current emphasis is for real-time in-line applications with NIR spectroscopy and NMR being leading candidates for the application to fruit and vegetables. However, there has been considerable work on ultrasonic measurement systems used for fruit evaluation during various stages of pre- and postharvest process, particularly with avocado maturity (Mizrach 2000; Mizrach and Flitsanov 1999).

2.3.4 Ultrasonic Systems

The potential for ultrasound and its application in the food industry has been steadily progressing since the 1970's (Mizrach 2008). The technique has not progressed as rapidly in the fresh fruit sector due to the lack of appropriate equipment sufficiently powerful enough to penetrate fruit

and vegetables, while at the same time sufficiently gentle enough to avoid damage to tissue (Mizrach 2008). Garcia-Ramos et al. (2005) report that it is difficult to use ultrasound greater than >20,000 Hz for fruit and vegetable quality determination, as it is strongly attenuated when travelling through plant tissues. In addition, Chen (1996) indicates that these waves cannot penetrate deeply into the fruit. However, the advances of equipment design have enabled the progression of research into ultrasonic attenuation of fruit and vegetables (Mizrach 2008). Of particular interest is the research into ultrasonic applications to avocado fruit for determination of quality attributes.

Mizrach (2008) report that the energy attenuation of the ultrasound beam and speed of propagation depends on the structure and nature of the material. Changes in the attenuation and velocity of the propagated beam is caused by physical or chemical changes in the material. Ultrasound systems have been investigated on avocado fruit for growth, maturation and shelf-life (Mizrach 2008). Mizrach and Flitsanov (1999) evaluated the use of a high-power, low frequency ultrasonic system for characterising avocado fruit (‘Fuerte‘ cultivar) tissue properties during ripening. The authors report that avocado firmness could be predicted satisfactorily with separation between hard (unripe), medium (firm ripe) and soft (overripe) fruit and that firmness correlated well with fruit maturity. The ultrasonic attenuation was found to be influenced by the oil content at harvest and by the dry weight percentage at full ripening. The ultrasonic wave attenuation diminished with increasing dry weight during fruit growth and increased during postharvest ripening and softening process. The authors suggested that measurements provided an indication of oil content in the avocado fruit since the dry weight of the avocado, measured destructively on the seventh day correlated closely with the ultrasonic attentuation measured on the fruit on the same day ($R = 0.8127$). Similar results were obtained in a study by Mizrach et al. (2000) using a ultrasonic technique for monitoring the ripening process in avocado fruits at low temperatures based on fruit firmness. The authors reported that the method successfully utilised ultasonic attenuation in the fruit flesh by means of ultrasonic probes in contact with the fruit peel to measure fruit firmness at a given temperature as an index of ripening stage.

In a study by Mizrach et al. (1999) an ultrasonic technique was evaluated to determine maturity of two cultivars of avocado fruit, ‘Fuerte‘ and ‘Ettinger‘. The authors reported a direct relationship bewteeen ultrasonic attenuation and dry weight content suggesting that pre-harvest avocado maturity could be determined by ultrasonic technology. The ultrasonics attenuation and dry weight measurememts showed scatter, which Mizrach et al. (1999) reported may be reduced by repeated measurements. Gaete-Garreton et al. (2005) investigated the feasibility of using an ultrasonic method to assess avocado ripening by measuring the acoustic surface properties of the exocarp. The authors reported a good correlation (coefficient of determination (R^2) = 0.97)

between firmness and acoustic absorption coefficient to evaluate the maturity degree of avocado fruit. Currently, the ultrasound technique remains an efficient research tool and is not yet ready for commecial use, with a lot to be done in relation to fruit contact and speed for inline applications to bring the technique to a point of being utilised widely as a grading tool for fruit and vegetable quality attibutes (Bureau 2009; Mizrach 2008).

2.3.5 Nuclear Magnetic Resonance (NMR)

NMR techniques have been used for examining various agricultural products since the mid-1950's (Kim et al. 1999). However, most of these investigations have been undertaken with commercial instruments designed for medical purpose, plus the small sample sizes and slow data processing has limited the practicality of these studies in terms of in-line sorting (Kim et al. 1999). NMR measures internal features based on the magnetic properties of the nuclei of the atoms within a product (Garcia-Ramos et al. 2005). The sample features extracted from an NMR signal can be related to chemical characteristics (e.g., moisture distribution) and internal structure (McCarthy 1994). Garcia-Ramos et al. (2005) report that the technique induces transitions between proton energy levels to establish a state of equilibrium of imbalance and then observes the return of energy. Signal intensity is recorded over time providing information about the environment of the nucleus. Typically, the nuclei excited are H+ and, thus, the information is primarily linked to the water content of the sample, the water mobility, and the hydrogen bonds present in the tissues (Garcia-Ramos et al. 2005; Simoneau et al. 1993). MRI is an extension of standard two-dimensional NMR techniques that allows the measurement of the NMR properties as a function of spatial position (Kim et al. 1999; Simoneau et al. 1993). MRI has been applied to horticultural products particularly for internal defect sorting, such as browning in apples and bruise detection (Gonzalez et al. 2001; Simoneau et al. 1993).

NMR has been demonstrated to have the potential to measure the DM and oil content in avocados (Kim et al. 1999; Chen et al. 1993; Barry et al. 1983). Barry et al. (1983), using a low resolution NMR spectrometer established a significant correlation (R = 0.98) between percentage oil of dehydrated avocado samples against the Soxhlet oil extraction method. Chen et al. (1993, 1996) investigated differences between NMR, spectroscopy and relaxometry as potential indices for maturity for avocado fruit. Significant correlations in the frequency spectrum were established between signal intensities for oil and water peaks. However, it is unlikely that these peaks could be resolved at the low proton frequencies application to in-line settings (Chen et al. 1996). Kim et al. (1999) developed an on-line NMR system to measure the oil/water ratio of avocado fruit which was correlated to %DM content. The correlation coefficient varied between 0.97 and 0.89, and decreased with increasing speed on a conveyor belt system. Similarly, Pathaveerat et al. (2001) reported using a single pulse NMR system on a group of five 'Hass' avocados with varying

maturity and obtained satisfactory correlation (R = 0.8575) between the NMR technique and dry weight. Nevertheless, the cost and challenges for in-line use of NMR technology has limited its current commercial application for high volume, low value fruit and vegetable commodities (Gaete-Garreton et al. 2005; Garcia-Ramos et al. 2005; Clark et al. 2003; Clark et al. 1997).

2.3.6 Near infrared (NIR) spectroscopy

Although, several non-invasive techniques have been developed for the prediction and detection of avocado maturity parameters, NIR spectroscopy is considered the most advanced non-invasive techniques in relation to applications, instrumentation, software packages, throughput speed and adaptability to a commercial setting (Nicolaï et al. 2007). The theory of NIR spectroscopy, equipment and chemometrics has been extensively covered in the author's Master's thesis (Wedding 2007) and the reader is referred to that document for comprehensive details. There are also a number of other authors who have written reviews including: Sandarfy et al. (2007), Cen and He (2006), Heise and Winzen (2002), Siesler (2002), Boyworth and Booksh (2001), Ciurczak (2001), Shenk et al. (2001), Bokobza (1998), Osborne et al. (1993), Scotter (1990b), Williams and Stevenson (1990), Birth and Hecht (1987), and Hruschka (1987).

NIR spectroscopy has been demonstrated to be an accurate, repeatable, rapid and non-invasive technique for providing information about relative proportions of C-H (methyl, methylene, aromatic, methoxyl, carbonyl), O-H (hydroxyl) and N-H (amides, amines) bonds within samples (Osborne et al. 1993). The technique requires minimal or no sample preparation in some instances, requires no reagents, has very low labour costs, is multi-analytical, allowing estimate of several properties simultaneously and is rapid (milliseconds (ms) to seconds) having the potential to assess every piece of product in an in-line setting (McClure and Tsuchikawa 2007; Williams 1987). However, as NIR spectroscopy is a secondary method of determination, spectral data must be calibrated against reliable primary reference methods to develop a predictive model for future sample assessment (Workman and Shenk 2004). The predictive ability of calibration models depends on how well the calibration data set represents the composition of the future samples being assessed. A major challenge with horticultural products is to ensure the calibration model is robust and updated for every different condition, including: variety, temperature, growing seasons and geographical regions (Zerbini 2006).

NIR sample spectra can be collected in three different modes: reflectance, transmission or interactance (see *Figure 2.1*). In the case of reflectance, the NIR light source and detector are located on the same side of the sample. In transmission mode, the NIR incident light illuminates one side of the sample and the NIR light transmitted through the sample is detected from the opposite side of the sample. Interactance mode measures the unabsorbed NIR light (i.e., internally

reflected) from a sample of the same side as the incident NIR light source. The transmittance method is very desirable for detecting internal information of matter with a larger volume, whereas the optical information from diffusely reflected spectra is confined to the subsurface layer of samples (Tsuchikawa and McClure 2007).

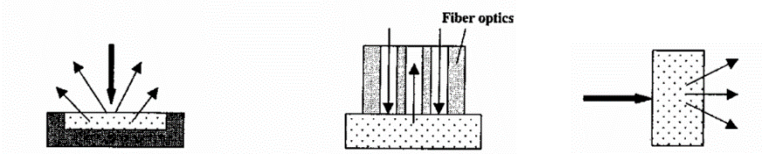


Figure 2.1. Representation of reflection, interaction and transmission (Modified from Kawano (2002)).

Most absorption bands in the NIR region are overtones or a combination of overtones of the fundamental absorption bands in the infrared region caused by vibrational and rotational transitions (Cozzolino et al. 2011; Inc. 2007). Different chemical bonds vary in strength and thus the amount of energy required for the bond vibration to move from one level to the next (Davies 2005). The variation in energy will be seen in a spectrum as a series of absorptions at different wavelengths. Inc. (2007) reports that each fundamental absorption in the mid-infrared region has several corresponding overtone and combination bands, many of which overlap and are broadened, NIR spectra are characterised by much broader features than FT-Infrared (FTIR) spectra and it is often not possible to make direct bond assignments to particular wavelengths.

2.3.6.1 Model development and performance

Multivariate statistical techniques (chemometrics) are required to extract the relevant characterising attribute which is incorporated into the NIR spectrum. The goal of multivariate analysis is to develop a calibration model which correlates the spectral information from the set of known sample measurements, to the desired attribute of interest (i.e., DM). Spectral pre-processing techniques are used in the process to remove any information that is considered irrelevant which cannot be handled by the regression techniques (Cozzolino et al. 2011). Data pre-processing techniques can eliminate effects such as baseline shifts (i.e., first and second derivatives); multiplicative and additive effects caused by different particle sizes (multiplicative scatter correction (MSC)); information not relevant spectrally to the attribute being measured (wavelength selection); and wavelength shifts and slope variation in a spectrum (standard normal variance (SNV) transformation) (Bouveresse and Massart 1996; Shenk and Westerhaus 1991; Barnes et al. 1989).

The calibration model development and evaluation methods are well documented by Williams (2013). The basic steps involved in the calibration model development procedure is outlined as follows:

1. Selection of calibration samples. Ideally, the calibration set must not only uniformly cover an entire constituent range, they should also be composed of a uniformly distributed set of sample types consisting of a considerable number of samples (Workman and Shenk 2004).
2. Selection of a suitable reproducible sample preparation and presentation method to capture the NIR spectra.
3. Recording of the NIR spectra by subjecting the calibration sample set to analysis by the NIR instrument at the required wavelengths.
4. Accurate analyse of the sample against the appropriate reference method for the constituent of interest (i.e., DM content, °Brix, oil content). The predictive performance of any NIR spectroscopy calibration model is dependent on the accuracy, error and/or bias that is inherent to the reference samples. Errors in the reference method will perpetuate through the NIR spectroscopy predictive models.
5. Development of a calibration equation using statistical modelling techniques such as multiple linear regression (MLR) and partial least squares (PLS) to establish a correlation between the NIR spectra data and the chemical parameters of the sample set. Simply, the calibration equation of the NIR and chemical loadings combine mathematically to yield the calibration for analysis of unknown samples. The calibration coefficients define the weights given to the different wavelengths in the linear equation, which is a simple formula that will therefore be used as a common way of expressing the analytical results from all the different linear calibration methods treated (Martens and Naes 1987).
6. Validation of the model to ensure that the model accurately predicts the property of interest in samples not subjected to the calibration process.
7. Predicting future unknown samples. A robust, accurate model can be used to predict rapidly the property of interest in new, unknown samples.

Several statistical criteria are used to describe the performance of NIR spectroscopy models to predict the required chemical parameter of the unknown sample, these include:

- The *coefficient of determination* (R^2) (see *Eqn. 2.3*) measures the extent to which the fitted straight line relationship explains the variability in the *y*-values. This parameter has values between 0 and 1e, and is the proportion of the total variance in the *y*-values explained by the fitted line (Osborne et al. 1993).
- The *root mean standard error of calibration* (RMSEC) (see *Eqn. 2.4*) is an estimation of the variation of the reference and predicted values of the calibration population, that is, how well the calibration model fits the calibration set (Boyworth and Booksh 2001).

- The *root mean square error of prediction* (RMSEP) (see *Eqn. 2.5*) is used to indicate the predictive performance of the calibration model, that is, an estimation of the variation of the reference and predicted values of the validation population (Lin et al. 2004).
- For *root mean square error of cross-validation* (RMSECV) (see *Eqn. 2.6*), a leave-one-sample-out cross-validation is performed: the spectrum of one sample of the training set is deleted from this set and a PLS model is built with the remaining spectra of the training set (Xiaobo et al. 2006). The left out sample is predicted with this model and the procedure is repeated with leaving out each of the samples of the training set. Cross-validation of a calibration model makes it possible to select the optimum number of latent variables (LV) or factors, that is, the number giving the minimum prediction error for the calibration set (Christy and Kvalheim 2007).
- The '*bias*' (see *Eqn. 2.7*) corresponds to the average difference between measured and predicted values. If there is no such difference, the bias will be zero. If the bias values are negligible, Buning-Pfaue (2003) suggest that the *standard error of prediction* (SEP) value can be equated with the *standard deviation* (SD) and therefore, in the case of a statistical certainty of 0.95, the maximum error range can be specified as ±2 (1.96) SEP, called the 'maximum error range'.
- The *standard deviation ratio* (SDR) (see *Eqn. 2.8*) is the ratio of the SD of the population divided by the RMSEP or the RMSECV and is the measurement of the ability of a calibration model to predict a constituent and enables comparison of model performance across populations with different standard deviations (Golic and Walsh 2006; Baillères et al. 2002). McGlone and Kawano (1998) reports that for large normally distributed data sets the SDR is equal to, and indicates more directly than either R^2 or RMSEP separately can, the relative predictive performance of a model; the higher the value, the greater the power. For 'difficult' applications, such as high moisture materials including fruit and vegetables, SDR values between 2.0 and 2.4 are regarded as adequate for rough screening; values between 2.5 and 2.9 are regarded as adequate for screening; values between 3.0 and 3.4 are regarded as satisfactory for quality control; values between 3.4 and 4.0 are regarded as very good for process control; values above 4.1 are excellent for any application (Williams 2008; Nicolaï et al. 2007; Schimleck et al. 2003). McGlone and Kawano (1998) suggest that an SDR of >3 is adequate to support sorting/grading into 3 classes, while Golic and Walsh (2006), report that an SDR of 2.5 allows sorting into two grades. For NIR spectroscopy to be commercially useful in fruit grading, Guthrie et al. (1998) suggest that the technique must be capable of sorting fruit into at least two grades (i.e., above and below an acceptable level) with approximately 80% accuracy. This requirement involves attainment of a validation correlation coefficient of at least 0.65.

- The term *ratio of (standard error of) performance deviation* (RPD) is similar to the SDR except the RPD uses a bias corrected RMSEP or RMSEC (Williams 1987). If the SEP is close to the SD of the reference data of the validation samples set (whether cross-validation or a test set is used), then the calibration model is not efficiently predicting and is of no practical value in analysis (Williams 2007; Baillères et al. 2002). If SEP = SD (i.e., RPD is of 1.0), the calibration is essentially predicting the population mean (Williams 2007; Baillères et al. 2002). An RPD below 2 cannot give a relevant prediction, while an RPD value of 2.0 - 3.0 is regarded as adequate for rough screening. RPD values of 3.0 and above are regarded as satisfactory for screening (Williams 2007), values of 5 and above are suitable for quality control analysis, and values of above 8 are excellent, and can be used in any analytical situation (Baillères et al. 2002).
- The following are some analytical expressions for the terms commonly used:

$$R^2 = \frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} = 1 - \left[\frac{RMSEC}{SD}\right]^2 \qquad \textbf{Eqn. 2.3}$$

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{n}(x_i - y_i)^2}{n - f - 1}} \qquad \textbf{Eqn. 2.4}$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{m}(y_i - \hat{y}_i)^2}{m}} \qquad \textbf{Eqn. 2.5}$$

$$RMSECV = \sqrt{\frac{\sum_{i=1}^{n}(x_i - y_i)^2}{n}} \qquad \textbf{Eqn. 2.6}$$

$$BIAS = \sqrt{\frac{\sum_{i=1}^{n}(x_i - y_i)}{n}} \qquad \textbf{Eqn. 2.7}$$

$$SDR = \frac{SD}{RMSEP\ or\ RMSECV\ or\ RMSEC} \qquad \textbf{Eqn. 2.8}$$

Where x_i is the reference (actual) concentration value of the sample in population i;

y_i is the predicted concentration of the samples i out of the calibration set;

n is the number of samples in the training/calibration set;

f is the number of factors (PC's) used in the calibration model;

$\hat{y}_i$ is the estimated (predicted) result for the test sample I;

m is the number of samples in the prediction/validation set;

$\bar{y}$ is the mean of the reference results for all samples in the training and test sets.

All calibration models should be evaluated by analysis of unknown samples. The highest R^2 and lowest SEP will be the optimum combination of wavelength and pre-processing treatment (Williams 1987). Ideally, the bias and the SEP should both be equal to zero with a small difference between SEP and *standard error of calibration* (SEC) (Gomez et al. 2006; Williams 1987). Thus, a low SEP and bias with a high R^2 means that the NIR results are accurate over the anticipated range, and likely to remain so, provided the statistics were based on a sufficient number of observations.

Guthrie (2005), reports that the R_c^2 (calibration model coefficient of determination) is a function of SD and RMSEC, and the R_v^2 is a function of SD and RMSEP (when bias = 0),

$$R^2 = 1-(SEP/SD)^2 \qquad \textbf{Eqn. 2.9}$$

The R^2 can be improved by increasing the SD of the calibration population. Therefore, an evaluation of a models performance using the R^2 statistic should be considered in conjunction with knowledge of the SD (which should be equivalent to that of the population to be predicted).

A low R^2 between NIR and reference data means that the NIR analysis has not been successful, and it is possible that NIR spectroscopy may not be applicable to that particular analysis. In general, as the R increases the SEC decreases; and provided the R and R^2 are high (>0.9), the accuracy can always be fine-tuned by a slope/bias adjustment, despite an apparently high initial SEP (Hruschka 1987; Williams 1987). If the R is ≤ 0.8, the SEP may be capable of improvement, however, it is not possible to obtain really high accuracy unless the R is high, generally ≥ 0.90 (Williams 1987).

2.3.6.2 Applications of NIR spectroscopy to fruit

NIR spectroscopy was largely pioneered by Karl Norris and co-workers in 1968 for the analysis of agricultural products, particularly grains (Shenk et al. 2001). There had been limited use of NIR spectroscopy in horticultural crops due to the high heterogeneity of samples; irregular shape resulting in different path lengths; thick exocarps limiting NIR penetration and the large amount of water (>80%) contained in fruit and vegetables (Buning-Pfaue 2003; Williams and Stevenson 1990). Water is an extremely strong absorber in the NIR region (i.e., overtone bands at 760, 970 and 1450 nm and combination band at 1190 and 1940 nm) and thus dominates the NIR absorption spectrum, complicating the prediction of other compounds (Nicolaï et al. 2007; Siesler 2002; Bokobza 1998; Osborne et al. 1993). NIR spectroscopy does have several other limitations, including: i) time-consuming and laborious calibration procedures and the complexity in the

choice of data treatment; ii) absorption in the NIR spectral region is extremely weak, therefore, the identification of macro constituents is the most common use of this technology as the sensitivity limit is about 0.1% for most constituents (Cen and He 2007; Buning-Pfaue 2003); iii) localised nature of the scanning (spot assessment) may not accurately predict the average internal quality due to spatial variability of the horticultural product. The majority of applications of NIR spectroscopy described in literature, essentially have applied single-point (or spot) measurements and spatial distribution information of chemical constituents in the sample are not identified. Depending on the uniformity of the quality attribute being measured within the fruit, it may be necessary with a single-point technique to capture several measurements around the fruit. In recent years the advancement of NIR charged couple devices (CCD) or cameras has started to address these spatial variability issues through line scan assessment across the sample or through multispectral and hyperspectral imaging of the whole sample providing both spectral and spatial information.

Following on from the work of Karl Norris, there has been considerable NIR spectroscopy research undertaken on the determination of fruit quality attributes over the years by various researchers. This has been largely attributed to the advancement of powerful computers combined with the use of multivariate data techniques to extract the relative spectral information about the attribute of interest (Cozzolino et al. 2011). The opportunity for NIR analysis of plant biological materials is varied and immense and has been covered extensively in literature. The analysis of the NIR spectroscopy spectra enables both qualitative and quantitative determination of attributes of fruit and vegetables, including: protein, oil, pH, acidity, firmness, water, SS content, %DM, sensory properties and shelf-life (Butz et al. 2005; Abbott 1999; Scotter 1990a). Numerous horticultural products, mainly thin skinned, have been investigated over the years for quality parameters using NIR spectroscopy, including: peaches (Ying et al. 2005; Slaughter 1995; Kawano et al. 1992); nectarines (Slaughter 1995); apples (Liu et al. 2006; Zou and Zhao 2006; Zude et al. 2006; Lu 2004; Lammertyn et al. 1998; Choi et al. 1997; Moons et al. 1997); tomatoes (Hong 1998); mango (Sivakumar et al. 2006; Ueno et al. 2005; Saranwong et al. 2004; Schmilovitch et al. 2000); grapes (Cozzolino et al. 2004; Jarén et al. 2001); cherries (Lu 2001); kiwi fruit (Valero et al. 2004; McGlone et al. 2003); strawberries (Nagata et al. 2004; Nagata et al. 2002) and pears (Liu and Ying 2004) to name a few. Commercial application of NIR spectroscopy to fruit sorting was first implemented by Japanese companies Fantec and Mitsui Metals and Mining in 1990 (Kawano 1994). Other companies have since followed suit.

Application of NIR spectroscopy has been largely limited to thin skin fruits due to the reduced penetration depth of NIR radiation within the tissue compared to other non-destructive methods such as X-ray (Arendse et al. 2018). The thick skin or rind has been reported to interfere with the

light path for internal quality measurements by changing the optical path length or optical density due to varying thickness or shape of the rind (Arendse et al. 2018). The penetration depth has been reported to be wavelength dependent and that light intensity to detect fruit quality decreases exponentially with increased distance from the NIR light source (Greensill and Walsh 2000; Lammertyn et al. 2000). The limited penetration depth of NIR radiation restricts the potential of the technique for detecting internal attributes of thick skinned fruits and may decrease the accuracy of NIR measurements (Nicolai et al. 2007). Despite the challenges associated with thick skinned fruits, NIR spectroscopy has been successfully used to measure various quality attributes of fruits with thick skins including: citrus (Gomez et al. 2006; Kawano et al. 1994), pomegranates, melons (Guthrie et al. 1998; Dull et al. 1992; Dull et al. 1989), pineapple (Guthrie et al. 1998; Guthrie and Walsh 1997) and avocados (Olarewaju et al. 2016; Clark et al. 2004).

With particular interest to this project, in relation to maturity characteristics and potential application to avocado fruit, NIR spectroscopy has been used to estimate %DM content in various horticultural products (Sivakumar et al. 2006; Xiaobo et al. 2006; Hartmann and Bijning-Pfaue 1998; McGlone and Kawano 1998; Birth et al. 1985), including avocados (Olarewaju et al. 2016; Walsh et al. 2004; Clark et al. 2003; Schmilovitch et al. 2001). NIR spectroscopy has also been investigated for determination of fruit storage disorders (Pérez-Marín et al. 2011; Sánchez et al. 2009; Camps et al. 2007; Nicolai et al. 2006; Clark et al. 2004; McGlone et al. 2003; McGlone et al. 2002) and bruise detection (Nagata et al. 2006; Nagata et al. 2002; Upchurch et al. 1991).

2.4 NIR spectroscopy for assessing avocado maturity based on dry matter content

Although there have been significant developments in the knowledge and application of NIR spectroscopy as an analytical technique in the agricultural industry, there has been limited investigations using NIR spectroscopy to determine the maturity of avocado fruit. The application of NIR spectroscopy to determine %DM content in avocado fruit has been demonstrated by Schmilovitch et al. (2001), utilising a dispersive NIR spectrophotometer in reflectance mode in the spectral range 1200-2400 nm. The authors assessed relatively thin-skinned cultivars including 'Fuerte' and 'Ettinger' from several orchards located on the coastal plain of Israel, over one season. NIR spectra were collected at the equator of the fruit and DM was determined by two methods, i) taking a cylindrical core perpendicular to the NIR scanned area and drying the mesocarp; ii) cutting the fruit in half and taking a 10 g sample of the mesocarp from the whole exposed mesocarp ring. Both samples were oven dried at 65^{o}C for 24 hours. The authors suggested that the cylindrical core method perpendicular to the scanned area was the more accurate method of determining DM for the NIR predictions, reducing the error by an average of 0.3% DM. Schmilovitch et al. (2001) reported the SEP as 1.3% for 'Fuerte' and 0.9% DM for 'Ettinger' over a single season with a population range of 14-24% DM.

Blakey et al. (2009) explored using NIR spectroscopy in reflectance mode to predict MC to relate to ripening physiology and maturity parameters of South African produced 'Hass' avocados over two seasons. The authors reported using a bench top research monochromator NIRS6500 instrument (Foss NIRSystems, USA) in the 400-2500 nm spectral range for developing calibration models from the 1100-2000 nm region for the prediction of MC. Water content is used in South Africa for maturity indexing, and determining the start and end of the packing period (Blakey et al. 2009). The authors reported calibration statistics of R^2 of 0.92, SEC of 1.8% over a population range of 55.4-87.4% water (n = 1200) with a standard error of validation (SEV) of 2%.

In another study, Clark et al. (2003) investigated estimating %DM of whole New Zealand 'Hass' avocado fruit using a fixed polychromatic/diode array (PDA) spectrophotometer (Zeiss MMS1-NIR, Germany) in the range of 300-1140 nm for both reflectance and interactance modes. Mature 'Hass' avocados were obtained from three commercial orchards within close proximity of each other, on four occasions during a single growing season. On each of the four occasions, 80 fruit were collected from each orchard. Clark et al. (2003) obtained reflectance (~24 mm sample area, 50 W halogen light source, 200 ms integration time) and interactance (12 mm sample area, 650 W projector bulb, 1.5 seconds integration time) spectrum from two opposing points at the equator of the fruit. From each of the NIR spectrum collection sites, a 17.3 mm core of tissues, collected perpendicular to the fruit surface was excised and %DM determined following the removal of the exocarp, seed coat and plug tissue cut into slices to facilitate drying in an oven at 65°C to constant weight. The authors trialled four separate spectral windows, including: i) standard 500-1050 nm; ii) visible 500-750 nm; iii) NIR1 750-1050 nm; iv) NIR2 800-1000 nm. The authors concluded that the PLS interactance mode calibration model using the NIR1 window (750-1050 nm) was superior at predicting %DM content with a R^2 prediction >0.83 and RMSEP <1.8%. In comparison, to the best reflectance mode PLS calibration model using the standard window (500-1050 nm) obtaining validation statistics of R^2 prediction <0.75 and RMSEP >1.9%, over a population range of 20-45% DM. Clark et al. (2003) also reported that the reflectance calibration models required a large number (12 to 20) of LV's, suggesting that the models struggled against spectral noise and to improve model accuracy required the incorporation of many small spectral features.

Clark et al. (2003) reported that the predictions for the interactance data for the fourth harvest (late season) were more scattered compared to earlier season harvests and attributed this to changes in the fruit attributes unrelated to DM, such as fruit texture, that affect optical density and make NIR prediction of DM more difficult later in the season. The authors suggested

inspection times for on-line grading need to be in the order of 100 ms integration time and that selecting two- or three-wavelength detection systems would further reduce the necessity for long integration times. Clark et al. (2003) determined that the relevant spectral information for %DM determination in the developed models is primarily obtained from the carbohydrate/lipid CH absorbance band in the 900-920 nm range, with a minor role from water related absorbance's. Interactance illumination and detection at lateral points on the sample surface is suitable for detecting diffusely reflected energy from deep within a sample (Saranwong and Kawano 2007); and does not require correction for path length differences for fruit with varying sizes (Williams and Norris 2001). However, interactance mode in a commercial setting may prove difficult as the emitter and detector would need to be in contact with the fruit (Williams and Norris 2001).

In a similar study, Walsh et al. (2004) using a fixed PDA spectrophotometer (Zeiss MMS1-NIR enhanced spectrometer, Germany) in the 300-1100 nm range for reflectance mode assessed a population of avocado fruit (n = 100) of unknown variety to determine %DM. The authors reported calibration results of R of 0.89, RMSECV of 1.14, with an SDR of 2.2, for %DM. More recently, Olarewaju et al. (2016) utilised a XDS bench top research monochromator spectrophotometer (Foss NIRSystems, USA) in the 700-2500 nm range to evaluate a population (n = 155) of whole South African 'Hass' avocados, collected over two seasons, for MC, %DM and oil content. The robustness of the models were explored by grouping data based on seasonal and orchard location. Olarewaju et al. (2016) reported spectral peaks at 970 and 1200 nm associated with H-O-H stretching related to water; wavebands around the 920, 930, 1200, 1750 and between 2200 and 2400 nm were associated with C-H_2 stretching and combinations related to oil; wave bands around 900-920 were associated with DM and possibly sugar. The authors reported that that calibration models from one season (2013) did not effectively predict the following season (2014). The 2013 season data set produced the best PLS calibration model with an R^2 of 0.91 for both %DM and MC and corresponding RMSECV of 2.27. While the calibration model for oil content produced R^2 of 0.53 and RMSECV of 5.16. The model developed from combining both seasons had significantly better predictive performance as more biological variability was included into the model. Reported predictive statistics for the combined seasons included an R^2 of 0.84 for both DM and MC, and 0.58 for oil content and corresponding RMSEP of 2.49 and 5.40 respectively.

However, Olarewaju et al. (2016) reports a high number of LV (seven in most cases) for a small number of samples (32-60) utilised in the development of the DM, MC and oil content models. The performance of these models may be over optimistic due to potential over fitting. For instance, as more LV's are included in the calibration model, the model begins to fit random errors embedded in the spectra and reference method data. The RMSEC will decrease as more factors

are added; and when extra factors that mostly describe random errors are included in the model, these factors will not fit the errors in the future samples and the RMSECV and RMSEP may increase (Boyworth and Booksh 2001).

2.5 The effects of geographical and seasonal variation on NIR calibration model performance and robustness

NIR calibration models are termed robust when the prediction accuracy is relatively unaffected by unknown changes due to external factors (Nicolaï et al. 2007). Factors that affect calibration model robustness include: i) transferring calibration models developed on one instrument (termed 'Master' instrument) to a second instrument (termed 'Slave' instrument) that produces instrumental responses that differ from responses obtained on the 'Master' instrument; ii) the instrumental response measured on a single instrument drift due to electronic drift, temperature fluctuations, and changes in detector stability over time; and iii) the samples belong to different batches and have significant biological variability between batches (Nicolaï et al. 2007). Many standardisation procedures have been developed in an attempt to eliminate the need for full recalibration and to preserve the valuable spectral information (Cen and He, 2007).

Composition of horticultural produce is subject to within tree, vine or crop variability (i.e., plant age, fruit position within tree, light effects, production load); within orchard or planting variability (i.e., plant location, light effects); and intra-orchard or intra-crop variability (i.e., soil type, weather conditions, nutrient availability, season variability) (Cozzolino et al. 2011; Marques et al. 2006; Peirs et al. 2003b). Due to this biological variation, harvest maturity will vary from year to year (McCarthy 2005). Even though NIR spectroscopy has been well established in literature to assess various internal attributes of horticultural products, calibration model robustness in relation to biological variability has been limited. Not taking into account seasonal and geographical variability within calibration models may result in over optimistic prediction accuracies of future samples (Nicolaï et al. 2007).

In general, the lack of model robustness often translates into bias and model prediction error may easily double when predicting on a data set from a different season or geographic location (Golic and Walsh 2006; Nicolaï et al. 2007). Prediction bias for new populations can be corrected by model updating or direct bias adjustment (Golic and Walsh 2006; Fearn 2001). However, the validity of the calibration models for future predictions depends on how well the calibration set represents the composition of new specimens. It is expected that the model prediction statistics for horticultural produce of an independent population will be poorer than the calibration statistics (i.e., RMSEP>RMSECV, bias>0). The calibration model may therefore require frequent re-calibration.

As calibration model robustness is a critical issue for future application of NIR spectroscopy technology there has been some work reported in literature that considers the effect of different seasons (Penchaiya et al. 2009; Golic and Walsh 2006; Guthrie et al. 2006; Guthrie et al. 2005b; Liu et al. 2005; Peirs et al. 2003b; McGlone et al. 2002; Peiris et al. 1998; Miyanoto and Yoshinobu 1995). 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. Nicolaï et al. (2007) reports that a typical RMSEP for %SS on fruit appears to be approximately 0.5% SS, however, in the few applications where validation sets from different seasons or orchards were externally used to calculate the RMSEP, it is considerably higher (1-1.15% SS).

Peiris et al. (1998) investigated NIR spectroscopy method using an acousto-optic spectrometer (800-1050 nm range) for non-destructive determination of SS content of four varieties peaches ('Blake', 'Encore', 'Red Haven' and 'Winblo'). The authors reported that a calibration model developed on a population from three consecutive growing seasons had an improvement in prediction performance on a combined season validation set with an SEP of 0.94-1.26% SS, and bias 0.17-0.38% SS, over that developed from an individual season populations (SEP of 0.90-1.36% SS and bias 0.17-2.08% SS). Peiris et al. (1998) report that individual cultivar calibrations were not good predictors when the cultivar was different. However, an overall combined calibration model consisting of all cultivars and seasons, predicted well for SS content of individual cultivar and season data sets.

Peirs et al. (2003) investigated the effect of biological variability (orchard, season and cultivar) on the robustness of NIR spectroscopy calibration models for SS content (°Brix) of 'Golden Delicious' apples. The authors reported that season (31%) and cultivar (17%) were responsible for a major amount of the spectral variability in the calibration model, whereas the influence of the orchard was low and only appeared for certain cultivars during specific seasons. Similarly, Liu et al. (2005) investigated the effect of the biological variability on the robustness of models for sugar content of three pear cultivars ('Xueqing', 'Xizilu' and 'Cuiguan') using a benchtop Nexus FT–NIR spectrometer (Nicolet, USA) in the spectral range of 800-2400 nm. The authors report that the largest source of spectral variation between different pear fruit measurements was caused by the seasonal effect. While McGlone et al. (2002) investigated NIR spectroscopy for estimating pre- and post-storage quality indices (background colour, starch pattern index, SS content, penetrometer firmness, quantitative starch and TA) for 'Royal Gala' apples. The authors noted orchard, region and seasonal variation was considered important to include in the

experimental design as different growing seasons, in different places, under different conditions could affect fruit chlorophyll levels with contaminant effects on Vis-NIR predictions.

The study of Miyanoto and Yoshinobu (1995) developed a calibration model over three consecutive years to predict total %SS content of 'Satsuma' mandarins. The authors report that the models performed well against the prediction set of the same harvest season (SEP of 0.55-0.58, bias of 0.01). However, had the calibration models had a reduced performance against a different harvest season prediction set (SEP of 0.51-0.68, bias of ≤0.40). When a calibration model was developed incorporating data from all three years, the model predicted well against each individual season with a reported SEP of 0.5-0.59 and bias of <0.09. Similarly, Guthrie et al. (2005a) reports that calibration model predictions for total SS content of intact 'Imperial' mandarin fruit were more variable and less robust across seasons than across harvest days or location. The authors demonstrated that model updating to the calibration model data set of relatively a small number of samples (<20) from a new population was a successful strategy to maintain model robustness. Guthrie et al. (2005a) concluded that model performance was to be more sensitive to year than to time within a growing season or growing location.

In another study Guthrie et al (1998) investigated model robustness in intact melons across multiple populations, varieties and seasons using a NIRSystems 6500 spectrophotometer (Foss NIRSystems, USA) equipped with reflectance fibre optic probe to collect reflectance spectra in the 400-2500 nm range. Model performance of the MLR models for total SS's was reported to be reasonable across some varieties, but not all, and model performance deteriorated across data sets of the same varieties harvested at different times throughout the same season. In a similar study on intact melons, Guthrie et al. (2006) using a Zeiss MMS1 spectrometer (306-1130 nm range) in partial transmission mode investigated model robustness across 22 populations over three years. PLS calibration model statistics varied across the 22 populations (RMSECV ranging from 0.63 to 1.2% total SS; with the SDR from 1.1 to 2.7). Prediction performance of independent data sets varied in model performance with a range of RMSEP of 0.7-2.1% total SS and bias -1.60 to 0.92.

Golic and Walsh (2006) developed calibration models for three types of stone fruit (peach, nectarine and plum) of various varieties, obtained over three seasons and incorporating temperature variations. The Zeiss MMS1 spectrometer (306-1150 nm range) was mounted in a non-contact interactance mode (partial transmittance) above a conveyor system as in an in-line setting. Model robustness to temperature was achieved by including samples scanned at two temperatures (5 and 20°C) into the calibration set. The authors report that less than 5% of total population was required to be treated in this way and that temperature difference between

calibration data sets and validation data sets were largest in terms of prediction bias. Combined nectarine-peach calibration models and separate plum models were successfully used to predict nectarines and peaches, and plums, respectively. However, it was found that separate multi-variety plum model was required to improve model performance. Model performance was reported to be stable over several seasons in terms of R^2 (typically $R^2>0.8$), with bias corrected SEP varying in portion to population standard deviation. Prediction bias was corrected by model updating or by direct bias adjustment (Golic and Walsh 2006).

2.6 NIR spectroscopy technology as a tool for prediction of storage disorders as an indication of shelf-life

In relation to the prediction of storage disorders in fruit by NIR spectroscopy technology, there have been limited investigations reported in literature. However, there have been several studies addressing the potential of NIR spectroscopy for discriminating between products on the basis of post-harvest storage duration, as a means of estimating product shelf-life.

Camps et al. (2007) reported satisfactory results when using NIR spectroscopy for determining storage type (cooled room at 2°C and 95% relative humidity (RH) versus shelf-life conditions at ~20°C and 40% RH) and storage duration for three apple cultivars ('Gala', 'Elstar' and 'Smoothee'). The authors report classifications of apples according to the duration of storage (cooled room up to 120 days and shelf-life conditions up to 28 days in storage) "global model" for all three varieties using factorial discriminative analysis (FDA) and achieved 75% correct classification for cooled room stored fruit and up to 85% for fruit stored under shelf-life conditions (Camps et al. 2007). The efficiencies of the FDA models were increased when applied to individual varieties. Camps et al. (2007), highlighted that the wavelengths 680, 935, 1000, 1400, 1870, 2070 and 2400 nm related to biological changes in the apple fruit during storage and played an important role in grading of the fruit.

A study by Clark et al. (2004) reported successful results on the use of visible (Vis)-NIR to predict storage disorders of kiwifruit at harvest by separating into categories of 'sound' fruit and chill-injured 'affected' fruit during a 24-week cold (-1.5 to 1.5°C) storage period. Conical discriminant analysis (CDA) classification and sorting was used to estimate overall incidence of disorders that have already occurred. The authors estimated that by employing discriminative Vis-NIR spectroscopy techniques the overall incidence of disorders could have been reduced from 33.9 to 17.9% at their early harvest, and from 14.7 to 8.5% at their second harvest. Where the fruit were categorised as 'sound' and those affected by a single disorder (chill injury) indicated a potential reduction in disorder incidence from 13.7 to 6.8%. Paz et al. (2009b), evaluated three commercially available spectrophotometers for the determination quality parameters including

shelf-storage duration. Two varieties of apples ('Fuji' and 'Golden Delicious') were stored at 20°C, 40% RH and analysed after day 8 and 14. PLS–discriminative analysis (PLS-DA) correctly classified 86.1% of the mixed cultivar group and 86.6% of samples from the single-cultivar groups.

Pérez-Marín et al. (2011) demonstrated that NIR spectroscopy could be used to classify nectarines in post-harvest storage, as a function of pre-harvest irrigation strategies applied and post-harvest cold storage duration. Samples were assessed after 7, 14, 21 and 28 days of refrigerated storage (0°C and 95% RH). The authors report that the PLS2-DA models correctly classified between 86 and 96% of samples (Pérez-Marín et al. 2011). Similarly, Pérez-Marín et al. (2010) compared the performance of two NIR instruments for the measurement of quality-related parameters including post- harvest storage duration (0, 6 and 9 days) under refrigeration in intact plums. PLS-DA models correctly assigned 94.5% of samples to their refrigerated storage day as an approach for estimating shelf-life (Pérez-Marín et al. 2010).

Sánchez et al. (2009) report successfully using NIR spectral data to classify intact asparagus stored in refrigeration under controlled atmosphere, both by storage time and by post-harvest treatments applied. Samples were assessed after 7, 14, 21 and 28 days of refrigerated storage (2°C and 95% RH), under three controlled atmosphere treatments and evaluating two different spectrophotometers. Models developed using PLS2-DA correctly classified 81-100% of samples by post-harvest storage time and 72-85% of samples for post-harvest treatment, depending on the spectrophotometer used (Sánchez et al. 2009). Investigations by Di Egidio et al. (2009) indicate positive results when using NIR spectroscopy to support conventional techniques (chemical and microbial) in studying shelf-life (as a loss of freshness) of fresh-cut pineapple stored at different temperatures.

Typically, these studies evaluating the post-harvest storage duration as a means of estimating product shelf-life, assess the fruit at certain points in time while in storage to then develop calibration models to predict relative shelf-life. Hence, the analysis is very much 'after the fact', i.e., the system for segregating fruit on the premises of post-harvest storage duration was developed following actual post-harvest storage and disorder development. A major benefit to the Australian avocado industry would be the development of a non-invasive assessment tool to predict susceptibility to future flesh disorders ('pre-disorder' development) as an indication of potential shelf-life for transport and storage to distant markets.

2.7 Non-destructive technologies for bruise detection

The development of innovative smart sensing technologies has enabled commercially feasible non-invasive methods for the rapid in-line assessment of various internal quality attributes and defects of horticultural products. Non-destructive technologies detailed in literature are investigated with particular reference to bruise detection.

As previously mentioned, MRI and X-ray imaging offer great potential for internal quality assessment, however, these methods have not been readily commercially implemented as with NIR spectroscopy due to costs and methodological problems (Baranowski et al. 2009). With this said, Shahin et al. (2002) applied line-scan- X-ray imaging to detect new (24 hour after damage) and old (1 month after damage) bruises on 'Red Delicious' and 'Golden Delicious' apples. For old bruises, an accuracy of 90% and 83% (93% after threshold adjustment) was achieved for 'Red Delicious' and 'Golden Delicious', respectively using artificial neural network (ANN) classifiers (Shahin et al. 2002). New bruises were not adequately separated by this method and achieved an accuracy of approximately 60% for both varieties (Shahin et al. 2002). Using a linear discriminative analysis (LDA), an overall accuracy of 82% and 52% was obtained for old bruises and new bruises respectively for the 'Red Delicious' validation set. The authors concluded that old bruises could be successfully detected using X-ray line scan imaging, while new bruises were difficult to detect due to low contrast between the bruise features and the fruit tissues in the image (Shahin et al. 2002).

Thermography has also been investigated with some success for the detection of bruises in apples, where the temperature of the bruised surface is different to that of found in a sound surface (Baranowski et al. 2009). Varith et al. (2003), using treatments that warmed cold fruit at 3^{o}C by using air at 26^{o}C during thermal imaging, successfully detected 100% of bruises on 'Fuji' and 'McIntosh' apples within 180 seconds. Only up to 66% of bruises were detected for 'Red Delicious' apples and deep bruises were reported to be more easily detected than shallow ones (Varith et al. 2003). Practicality of this technique would require further investigation before application in a commercial in-line setting requiring high throughput.

Electrical impedance spectroscopy (EIS) has also been used to assess the extent of tissue damage of apple fruit resulting from bruising with quite positive results (R^2 of up to 0.71) (Jackson and Harker 2000). However, Jackson and Harker (2000) report that influence of cultivar and temperature on electrical impedance may cause difficulties when implementing EIS measurements in a commercial situation. Mechanical damage effects photosynthetic activity, which results in a reduction of fluorescence of the cells (Yi-Chich et al. 2015). With this in mind, Yi-Chich et al. (2015) employed chlorophyll fluorescence imagery to detect bruises on 'Golden

Delicious' apples. The authors reported obtaining 86.7% correct classification of bruised fruit after 30 minutes following impact and 100% correct classification after 1 hour following impact.

Machine Vision using conventional CCD cameras have been used extensively in grading fruit for size, shape, colour and some external defects with varying success and reliability (Van Zeebroeck et al. 2007). In some commodities bruises may be recognised as skin discolouration's caused by enzymatic browning reactions in the injured tissue (Zwiggelaar et al. 1996). The scale of the skin discolouration may be utilised by machine vision systems to detect bruises. Surface discolouration differences vary depending on the extent of damage to the tissue, plus natural colour variations in the surface of the fruit can cause difficulties with machine vision systems in separating bruised fruit from sound fruit (Tallada et al. 2006; Zwiggelaar et al. 1996). Leemans et al. (2002) when using machine vision for grading external defects on 'Golden Delicious' and 'Jonagold' reported that errors in classification mainly came from bruises. Classification of 'Jonagold' was particularly less accurate when the bruises were located in the blush area (Leemans et al. 2002).

Zou et al. (2010), reported that machine vision using three colour cameras could not distinguish different defect types (i.e., bruising, scab, fungal growth, and disease) and that they are treated the same when classifying sound and defective 'Fuji' apples. The authors reported that the classification error of unjustified acceptance of blemished apples reduced from 21.8% to 4.2%, for a single camera and for a three camera system, respectively (Zou et al. 2010). Lu et al. (2011) used a method combining fractal theory and support vector machines (SVM) to detect bruises on red bayberry's from red, green, blue (RGB) camera images. Total classification accuracy of 100% was reported for SVM and principal component (PC)-SVM models on fractal parameters, with 85.29% accuracy achieved with RGB intensity values (Lu et al. 2011). Similarly, Zheng et al. (2011) applied an adaptive neural-fuzzy inference system (ANFIS) model to detect bruises in Chinese bayberries as a function of fractal dimension and RGB intensities from camera images. Classification accuracy of 100% and 78.57% was reported for sound and bruised fruits, respectively, with a total classification rate of 90%.

The application of Vis-NIR spectroscopy has proved accurate in the detection of bruised surfaces (Opara and Pathare 2014). Xing et al. (2005b) using a Vis-NIR spectrometer in the 400-1700 nm range for spot assessment (or single point measurement) obtained a classification accuracy of more than 90% for separating bruises from healthy tissue for 'Jonagold' apples one day after tissue damage. Xing et al. (2006) reported effective wavebands of 545 ± 25 and 1200 ± 50 nm, plus 745 and 905 nm for detecting old bruises. Classification accuracy improved with increased time following injury with error rates varying from 22.8% following 3 hours after injury and as

little as 2% for 21 days after injury (Xing et al. 2006). Xing and De Baerdemaeker (2007) reported that correct classification of more than 95% could be achieved for both sound and freshly bruised fruit based on softening index (<45 minutes following impact) for 'Golden Delicious', 'Jonagold', and 'Braeburn' apple varieties using a Zeiss Corona spectrophotometer in the wavelength range of 400-1700 nm.

Similarly, Bennedsen and Peterson (2005) applied a machine vision system for sorting eight different apple varieties ('Gala', 'Empire', 'Jonagold', 'Golden Delicious', 'Pink Lady', 'Red Delicious', 'Fuji' and 'Rome') for surface defects (caused by blister spot, early frost damage, powdery mildew, russet and sunburn) and bruises. Images were acquired through two optical filters at 740 and 950 nm and defects were detected using a combination of different threshold segmentation routines and a routine based on ANN and PC's. The accuracy of the routines to find individual defects and measure the area ranged from 77 to 91% for the number of defects detected, and from 78 to 92% of the total defective area (Bennedsen and Peterson 2005). Luo et al. (2012), applied a spectrometer in the 380-1000 nm range and selected effective wavelengths for bruise detection in four cultivars of apples by receiver operating characteristic (ROC) analysis. In most cases, predictive accuracy exceeded 90% utilising the following wavelengths 808-760 nm, 832-772 nm, 834-762 nm, 788-742 nm for 'Fuji', 'Jonagold', 'Otin' and 'Sinano' varieties, respectively (Luo et al. 2012).

Geoola et al. (1994) investigated using a spectrometer in the 400-840 nm wavelength range to detect bruises on 'Golden Delicious' apples. The authors reported that the 750 to 800 nm wavelength region produced the best classification with results of 96.1%, 88.4% and 93% for refrigerated apples that were unbruised, bruised and left for 90 minutes and 24 hours, respectively. Similarly, the classification performance for non-refrigerated apples was 92.9% and 91.8% for unbruised, and bruised and left for 24 hours, respectively (Geoola et al. 1994). Guillermin et al. (2005b), evaluated NIR reflectance spectroscopy using a spectrometer at wavelengths ranging from 800 nm to 2200 nm to detect bruises on two apple varieties, 'Elstar' and 'Gala'. The authors reported good classification accuracy (95-100%) to distinguish between bruised and sound fruit. However, it was noted that the ability of NIR spectroscopy to detect bruises on apples and the many discriminative spectral absorbance bands for bruising varied according to the cultivar and the stage of development (Guillermin et al. 2005b).

Wu and Wang (2014) successfully applied NIR spectroscopy in the 600-1600 nm region to assess the effect of simulated transport vibration on tissue damage of tomatoes obtaining a prediction performance of: R = 0.984, RMSEP = 0.137 and bias of 0.003 for damage degrees. In another study, Jimenez-Jimenez et al. (2012) reported obtaining R^2 of 0.87-0.90 to predict the damage to

olive fruits by absorbed impact energy and to quantify the degree of bruise damage as bruise volume using Vis-NIR spectroscopy. It was reported that the unbruised and bruised olive fruit could be distinguished throughout the visible region (435-670 nm), with greater differences occurring in the 670-740 nm region (Jimenez-Jimenez et al. 2012).

Zwiggelaar et al. (1996) explored using machine vision to detect bruises on apricots and peaches using narrowband spectral filters on a wide band monochrome CCD camera. The effective wavelengths selected for apricots were 930 nm and 970 nm while a single wavelength of 750 nm was selected for peaches. The reported success rate for bruise detection was approximately 65% (Zwiggelaar et al. 1996). Similarly, Miller and Delwiche (1991), reported a three wavelength combination centred around 650 nm, 720 nm and 815 nm gave good separation of surface defects on peaches. In another study, Li et al. (1993) applied NIR reflectance measurements over the wavelength range of 800-940 nm and 1140-1400 nm that yielded more than 5% difference in spectral reflectance between sound and damaged peaches.

In more recent times, considerable research has been reported on using hyperspectral imaging for quality and safety inspection of various agricultural products. Hyperspectral imaging is a technique that combines spectroscopy and imaging to acquire both spectral and spatial information from the sample (Lee et al. 2014; Opara and Pathare 2014; Van Zeebroeck et al. 2007). The three dimensional hyperspectral cube containing the data consists of two dimensional spatial images with additional spectral information. Each spatial pixel contains the spectral information relating to the chemical attributes of substances at the corresponding spot on the hyperspectral image providing an opportunity for a more detailed image analysis of the whole product and detection of localised effects (Lee et al. 2014; Ariana et al. 2006a). However, continuous spectral analysis used in hyperspectral imaging require extensive time for image acquisition and analysis, as a result the technique has not readily been implemented into commercial on-line systems for agricultural product sorting (ElMasry et al. 2008; Ariana et al. 2006a; Xing et al. 2005a). In comparison, multispectral imaging is based on discrete spectral analysis of a few wavelengths resulting in a faster technique than hyperspectral imaging (Ariana et al. 2006a; Xing et al. 2005a). The disadvantage of hyperspectral and multispectral systems is the large accumulation of data which requires significant time for image acquisition and relatively complicated procedures for offline image analysis (Arendse et al. 2018).

The hyperspectral imaging system of Ariana et al. (2006b) was capable of detecting bruises from normal tissue on pickling cucumbers with accuracies of up to 95% two hours following mechanical injury and decreasing to 75% after 6 days after bruising. This decrease in accuracy over time was attributed to the self-healing of the bruised tissue following mechanical injury. The

authors report that the wavebands 988 and 1085 nm had detection accuracies between 93 and 82%, while the combination of 1346 and 1425 had accuracies between 89 and 84% (Ariana et al. 2006b). Xing and De Baerdemaeker (2005) applied a hyperspectral imaging system for detecting bruises on 'Jonagold' apples within the wavelength range of 400-1000 nm reporting a correct classification rate of 84.6 % for non-bruised apples and 77.5% for 1-day-old bruised apples. Similarly, ElMasry et al. (2008) investigated hyperspectral imaging in the region between 400-1000 nm for early (1 hour after impact) and old (> 3 days after impact) detection of bruises on 'McIntosh' apples, identifying three effective wavelengths (750, 820 and 960 nm) to successfully discriminate between bruised and sound apples. Xing et al. (2005a) selected four wavebands centred at 558, 678, 728 and 892 nm for multispectral imaging of 'Golden Delicious' apples for bruise detection. Classification accuracy of 86% was achieved for detecting bruises, and 93% of the non-bruised apples were recognised as sound (Xing et al. 2005a)

A feasibility trial conducted by Lee et al. (2014) showed great promise using hyperspectral line-by-line imaging in the range 950-1650 nm, to detect bruise areas from sound surface of 'Shingo' pears with the optimal wave band ratio of 1074 nm and 1016 nm obtaining an accuracy of 92%. Similarly, Hong-Quan et al. (2012) reported detecting bruises on pears after 1 hour following impact using hyperspectral imaging in the 948 to 1666 nm range. In another study, Lu (2003) trialled hyperspectral NIR imaging in the spectral region 900-1700 nm to detect both new and old bruises on two varieties of apples ('Red Delicious' and 'Golden Delicious') over a period of 47 days after bruising. The system was reported to be able to detect both new and old bruises, with a correct detection rate from 62% to 88% for 'Red Delicious' and 59% to 94% for 'Golden Delicious' (Lu 2003). Lu (2003), reported that the spectral region between 1000 nm and 1340 nm as the most suitable for bruise detection.

Upchurch et al. (1994) using NIR imaging observed significant influence of time, bruise-type, and severity on the NIR reflectance from bruised regions on both 'Red Delicious' and 'Golden Delicious' apples. Contrast profiles from bruised regions was greatest at day one and then decreased as a result of dehydration of the bruised tissue, meaning the reflectance increased (Upchurch et al. 1994). The multispectral vision system of Kleynen et al. (2005) successfully classified 94.3% and 84.6% of sound and defective bicolour 'Jonagold' apples, with less than 2% of defective fruits being classified into the sound group. The 750 and 800 nm wavebands provided good contrast between the defect with internal tissue damage (i.e., hail damage, bruises etc) and sound fruit, while 450 nm spectral band brought significant information to identify slight surface defects like russet (Kleynen et al. 2005).

Baranowski et al. (2012) successfully applied both hyperspectral imaging cameras (Vis-NIR, 400-1000 nm) and short wavelength infrared (SWIR, 1000-2500 nm) and a thermal imaging camera in the mid wavelength infrared (MWIR, 3500-5000 nm) to detect early bruises and depth of bruises in five apple varieties ('Champion', 'Gloster', 'Golden Delicious', 'Idared' and 'Topaz'). The authors report that the combination of all wavelength ranges (Vis-NIR, SWIR and MWIR) performed better in all cases than each individual range. Best accuracies for detection of early bruises from sound fruit obtained using LDA was 95%, using SVM was 92% and using soft independent modelling class of analogy (SIMCA) was 67% for models incorporating all Vis-NIR, SWIR and MWIR ranges.

Qing et al. (2007), employed hyperspectral imaging in the 408-1117 nm range to detect bruises on kiwifruit and reported a detection error rate of 14.5%. In another hyperspectral imaging study, Qiang et al. (2011) selected multiple wavelengths of 682, 723, 744, 810 and 852 nm to detect bruises on kiwifruits reporting a total detection error rate of 12.5%. In a study on strawberries, Nagata et al. (2002) used NIR spectroscopy and spectral imaging to detect bruises in the range of 600-1000 nm. Wavelengths critical for bruise detection were determined (945 to 975 nm), and spectral images were acquired for bruised and sound fruit with filters at 860 and 960 nm, respectively. Bruises were detected using simple image subtraction methods (Nagata et al. 2002). Using a similar approach, Nagata et al. (2006) detected 86.5% of bruises in strawberry fruit by NIR hyperspectral imaging using two key wavelengths (825 nm and 980 nm). Nagata et al. (2006) report that NIR light is readily absorbed by water and when strawberry fruit are bruised, cellular materials and water are forced out into extracellular spaces increasing the light absorption in the NIR region, particularly around the 960-980 nm.

All the previously mentioned studies using various non-destructive technologies to identify bruising are based on thin skinned fruits and not on thick skinned fruits such as 'Hass' avocado. Thus, there is a challenge to apply non-invasive technologies to detect bruises on the thick skinned 'Hass' avocado fruit, and this is a key area of investigation for this study.

CHAPTER 3

FUNDAMENTALS FOR THE APPLICATION OF NIR SPECTROSCOPY AS A TOOL FOR PREDICTING DRY MATTER CONTENT OF WHOLE 'HASS' AVOCADOS

In this chapter, several fundamental aspects were investigated for developing the utility of FT-NIR spectroscopy as a non-invasive technique for estimating %DM of whole intact 'Hass' avocado fruit.

Section 3.1 provides a succinct overview of determining and measuring the sources of error of the reference DM method and its effect on the developed calibration models.

Section 3.2 investigates the penetration ability of NIR electromagnetic radiation into 'Hass' avocado fruit. 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 (Saranwong and Kawano 2007; Guthrie et al. 2004). It has also been identified that NIR in diffuse reflectance mode has limited penetration into fruit, in comparison to transmission or interactance modes (Saranwong and Kawano 2007; Lu 2004; Krivoshiev et al. 2000). This section closely follows an article that has been submitted for publication by the author detailing depth of NIR penetration into 'Hass' avocado fruit: *Wedding, B.B.; Grauf, S.; Wright, C; Gadek, P.A. and White, R.D. (submitted 2018) Depth of penetration of near infrared radiation in 'Hass' avocado fruit. Postharvest Biology and Technology.*

Section 3.3 - with fruit variability in mind, the application of NIR spectroscopy technology to avocado fruit requires an understanding of the DM distribution within the fruit. It is well known that physiological gradients of DM exist within 'Hass' avocados (Woolf et al. 2003; Schroeder 1985), though there were no known published studies for Australian 'Hass' avocado fruit. Consequently, this section investigates the %DM variation within Australian 'Hass' avocado fruit to identify a suitable sampling point on the avocado that represents whole fruit %DM. This section closely follows a component of an article that has been published by the author detailing DM distribution within Australian 'Hass' avocado fruit: *Wedding, B.B., White, R.D.; Grauf, S.; Wright, C; Tilse, B.; Hofman, P. and Gadek, P.A. (2010) Non-destructive prediction of 'Hass' avocado maturity via FT-NIR spectroscopy. Journal of the Science of Food and Agriculture* ***91****, pp 233-238.* Additional components on the analysis of variance (ANOVA) and the confidence interval (CI) with regards to the variation from the two opposing sides of the fruit assessed; plus,

a comparison of the spectral features between the NIR spectra of the avocado sample without-skin (flesh only) and with skin has been included for further clarification.

The second component of *Section 3.3* was to assess the potential of FT-NIR spectroscopy to provide a non-invasive assessment of Australian 'Hass' avocado maturity and thereby eating quality based on %DM of both the avocado flesh (endocarp) without the skin (exocarp) in comparison to the whole fruit (with exocarp on). Removing the skin gives an indication of the maximum potential accuracy of FT-NIR spectroscopy in predicting %DM on whole (with exocarp) fruit. The irregular surface and thickness of the 'Hass' skin may reduce accuracy because of decreased light penetration and increased noise and specular reflectance. Two articles have been published by the author detailing NIR spectroscopy assessment of 'Hass' avocado maturity based on %DM of flesh only and of whole fruit (see *Section 3.3* and *Appendix A*). These two publications also examine the effects of intra-seasonal variation and orchard conditions on NIR spectroscopy calibration models as a tool to predict %DM in Australian 'Hass' avocados. This information is essential in ascertaining the stability or robustness of NIR calibration models by incorporating intra-seasonal variation as further development for commercial implementation.

These two publications by the author detailing NIR spectroscopy assessment of 'Hass' avocado maturity based on %DM of whole fruit present the same data set based on a 'Hass' avocado fruit population from the Bundaberg growing district in Queensland, Australia, produced over a single growing seasons (2006). However, each publication presents *calibration models based on different pre-processing techniques and software packages* as a result of knowledge developed throughout the studies. The outcome of these different techniques and software packages on overall model performance are presented in full for the publication provided in *Section 3.3* and a short summary of results and discussion of the second publication is provided in *Appendix A*.

The research provided in the second component of *Section 3.3* details studies on the ability of NIR spectroscopy to assess 'Hass' avocado maturity based on %DM of whole fruit from a single growing district in Queensland, Australia, produced over a single growing season (2006). This section is as published in: *Wedding, B.B., White, R.D.; Grauf, S.; Wright, C; Tilse, B.; Hofman, P. and Gadek, P.A. (2010) Non-destructive prediction of 'Hass' avocado maturity via FT-NIR spectroscopy. Journal of the Science of Food and Agriculture* ***91****, pp 233-238.*

Section 3.3 study data analysis was conducted using the commercially available chemometric software package 'The Unscrambler™' version 9.8 (CAMO, Oslo, Norway). PLS regression was used to build the prediction models of the diffuse reflectance spectral data. Final models for individual harvest populations presented in this study, for both avocado flesh and for whole

avocado, were based on a combination of a 25 point Savitzy Golay (SG) spectral smoothing (2nd order polynomial) and a SG second derivative transformation over 25 points (2nd order polynomial). The final model for all three harvests combined, for whole avocado, was based on a 25 point SG spectral smoothing (2nd order polynomial), followed by a SG first derivative transformation over 25 points (2nd order polynomial).

Appendix A provides a summary of the results and brief discussion of using the same 'Hass' avocado population as described in *Section 3.3*, utilising a different chemometric software package and different pre-processing techniques to develop suitable calibration models. *Appendix A* is as published in: *Wedding, B.B., White, R.D.; Grauf, S.; Tilse, B.; Hofman, P. and Gadek, P.A. (2009) Non-invasive Assessment of Internal Quality Attributes of Whole Avocado Fruit by NIRS. SABRAO Journal of Breeding and Genetics Vol. 41, Special Supplement August 2009, ISSN 1029-7073*. In this study PLS regression was used to build the prediction models of the diffuse reflectance spectral data. This was achieved using the commercially available chemometric software package OPUS™ QUANT (version 6.0 and 6.5). The OPUS™ QUANT automatic selection process was used to identify spectral regions and pre-processing treatments for improved model development. Straight line subtraction was selected as the optimal mathematical pre-treatment for the two spectral regions selected for model development.

3.1 Dry matter reference method and associated error estimates

3.1.1 Introduction

NIR spectroscopy has received considerable attention for determining quality attributes in fruit and vegetables. Of particular importance for the current study, NIR spectroscopy has been used to estimate %DM in various horticultural products (Sivakumar et al. 2006; Xiaobo et al. 2006; Hartmann and Bijning-Pfaue 1998; McGlone and Kawano 1998; Birth et al. 1985) including avocados (Walsh et al. 2004; Clark et al. 2003; Schmilovitch et al. 2001). However, NIR spectroscopy has been criticised as a result of errors associated with the predicted value for chemical attributes. These errors can be defined as the difference between the true traditional analytical reference value and the NIR predicted value. It is well known that all methods have errors associated with them (Fassio and Cozzolino 2004). As the NIR spectroscopy method is a secondary method of determination, the error of the reference method used for the wet chemistry is inherently carried into the calibration model development procedure, plus other errors that could occur through sample presentation, scatter effects, temperature, and instrumental disturbances to name a few (Zeaiter et al. 2006; Fassio and Cozzolino 2004). Therefore, it is important to determine and measure the sources of error of the reference method and its effect on the calibration model. Some of the sources of error for the avocado DM method include: DM distribution within an individual fruit, sample preparation, poorly dried sample containers, poor use of desiccators, individual sample container/tray contamination, drying method and drying temperature, insufficient drying time, balance error, weighing sample containers/trays to hot and of course, operator error.

The drying method can be achieved by the use of microwaves, dehydrators (65°C) or ovens (65°C) (Woolf et al. 2003). The key issue is not by what method the flesh sample is dried, but that the tissue is dried to a constant weight. Woolf et al (2003) report no significant difference between these three methods as long as the temperature is kept at 65°C or below to prevent sample burning and loss of non-water components. This component of the study investigated some of the errors associated with the DM reference method which would be inherently carried through to the NIR calibration model. Areas of specific interest were identifying the optimal drying time based on the sample reaching a constant weight when dried at 65°C and the associated error.

3.1.2 Materials and methods

'Hass' avocado fruit were obtained during the mid season (mid July) harvest of the 2006 growing season (May to August) from a single farm in the major production district of Bundaberg, Queensland (Latitude: 24 52' S, Longitude: 152 21' E). The %DM reference sample was obtained from a population of 106 avocados by taking a core perpendicular to the surface of the fruit with

a radius equal to the NIR spectroscopic sampling area on opposing sides of the fruit using a 50 mm diameter steel corer, and excising both skin and underlying flesh to a depth of approximately 10-20 mm. The skin (2-4 mm) was removed from the edible flesh, and the flesh core was finely cut into pieces to facilitate drying and then dried in a fan-forced oven at 60-65°C to enable determination of %DM by percentage weight difference. The DM content of the avocado samples were assessed at days 0, 2, 5, 7 and 10. Data analysis was conducted using the commercially available statistical software package GenStat for Windows 11th Edition (VSN International, Hemel Hempstead).

3.1.3 Results and discussion

The relationship between the DM (water loss) and drying time can be explained by an exponential curve (see *Figure 3.1*). The avocado samples have the same rate of change in the %DM and it is only the asymptote which differs between samples. The asymptote represents the %DM which could be obtained if the samples were left to dry for an extended period (10+ days). However, the asymptote is never actually achieved, but the %DM approaches this value. Using the parameters from the fitted exponential model drying time at a designated temperature (i.e., 65°C) can be calculated based on how long the avocados would need to be dried to obtain a %DM within a given percentage from the asymptote.

Figure 3.1 shows that the longer the drying time the flatter the individual avocado sample DM data plots become and hence the closer the %DM gets to the asymptote (essentially the minimum achievable %DM).

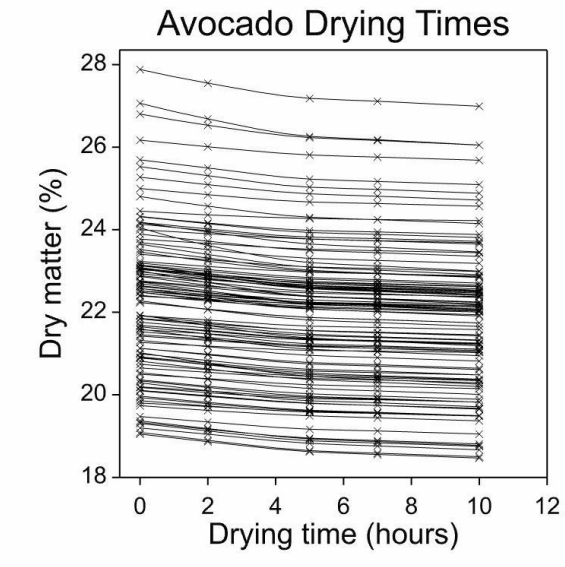


Figure 3.1. Avocado %DM drying times.

As shown in *Figure 3.2*, if the avocado samples are left to dry for 4 days, the DM would be approximately 0.3 greater than the minimum %DM (asymptote), while after 5 days this difference is approximately 0.26. From *Figure 3.2*, the length of drying time to be within an acceptable level of the asymptote can be determined. For example, to accept a %DM that was within 0.2 of the minimum DM, then the drying time should be no less than approximately 6.5 days.

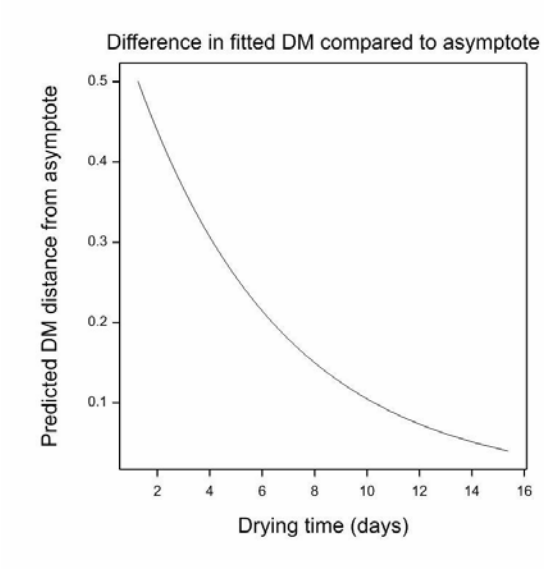


Figure 3.2. Difference in fitted %DM compared to asymptote.

Currently, maturity determination based on DM for commercial purposes is drying at a 65°C for approximately 2 days (Woolf et al. 2003). On this basis, assuming the actual %DM is the asymptote and the measured %DM is given by the fitted model, the avocado samples dried for 2 days at 65°C would be approximately 0.45% (< 0.5%) DM distance from the asymptote. Using the asymptote as the actual %DM and the measured %DM as recorded at day 2, 89% of the avocados are within the 0.5% DM distance from the asymptote. Reviewing the %DM recorded at day 5, all avocados have a DM within 0.3% of their predicted asymptotes.

In terms of differences between the five drying times, *Figure 3.3* presents the differences between the %DM at day 0 and at day 2, 5, 7 and 10. The more negative the value, the greater the decrease in %DM over time. Leaving the avocado samples to dry for up to 10 days can result in a decrease in %DM of over 1% compared to day zero, however for the majority of avocado samples the decrease is less than 0.6%.

The boxplots in *Figure 3.3* are slightly negatively skewed. Given this skewness it is appropriate to use percentiles as a summary statistic. The 5th percentile represents the difference in %DM values for which 5% of the data lies below and for the 95th percentile 5% are greater than this value.

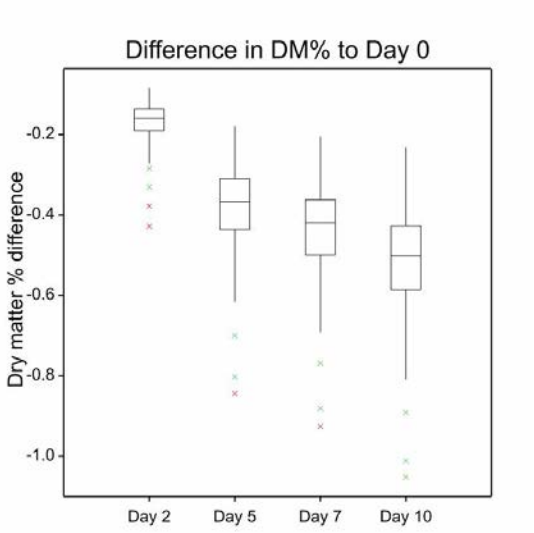


Figure 3.3. Difference in %DM between day 0, 2, 5, 7 and 10.

Table 3.1 provides the 5th and 95th percentiles respectively for all %DM differences over time. As would be expected the biggest difference in %DM occurs between time 0 and time 10 where the 5% percentile is 0.325 and the 95% percentile is 0.755. This indicates that 90% of the %DM differences lie between 0.325 and 0.722 for day 0 to day 10. The decrease in %DM from day 7 to day 10 is less than 0.116 for 95% of the avocado samples. As can be expected, the longer the avocados are left to dry the smaller the reduction in DM between consecutive occasions.

Table 3.1. 5th and 95th percentiles in %DM differences for drying at 65°C at day 2, 5, 7 and 10.

	Day 0	**Day 2**	**Day 5**	**Day 7**
Day 2	(0.099, 0.258)			
Day 5	(0.241, 0.572)	(0.131, 0.318)		
Day 7	(0.273, 0.642)	(0.171,0.382)	(0.031, 0.074)	
Day 10	**(0.325, 0.755)**	(0.223,0.497)	(0.084, 0.191)	(0.048, **0.116**)

3.1.4 Conclusion

The most appropriate drying time is dependent on the maximum feasible drying time and how close to the absolute minimum %DM is required. For commercial purposes DM is obtained by drying at 65°C for approximately 2 days. This investigation showed that after 2 days drying at 65°C, 89% of the avocados were within 0.5% of the predicted absolute minimum DM. Therefore all DM trials will be based on a drying time of a minimum of 48 hours to ensure %DM data obtained is within 0.5% of the asymptote.

3.2 Depth of penetration of Near Infrared radiation in 'Hass' avocado fruit

3.2.1 Introduction

When a beam of NIR radiation is incident on the surface of a fruit or vegetable, a fraction is reflected at the surface (known as specular reflectance) while the rest penetrates into the fruit. Upon entering the fruit tissue, photons scatter before being absorbed or exiting from the fruit at various distances from the incident point (diffuse reflected light). The scattering of light is dependent on the density, composition, cell structure, and extra- and intra-cellular matrices of fruit tissue (Lu 2004). Few investigations have been made on the optical properties and the depth of penetration of NIR radiation of intact fruit. The depth of penetration, also known as the information depth, is defined as "the maximum distance for NIR light traveling inside a sample matrix along the depth direction" (Shi and Anderson 2010). Understanding the depth of penetration in various wavelength regions for transmittance and reflectance modes would greatly enhance calibration model development and subsequent model interpretation.

Hruschka (1987) explains that NIR measurement can introduce a sampling error, because the radiation penetrates less than 2 mm into a sample, so only a portion of the sample is actually being measured. Greensill and Walsh (2000) report that the intensity of the detected light in a fruit decreases exponentially with the distance from the source, in accordance with the Beer-Lambert law. The rate of exponential decay is related to the scattering and absorption properties of the tissue and are influenced by the skin and other features in the fruit, such as the core and segment boundaries (Fraser et al. 2003). While there have been some theoretical/simulation studies of the light path within the fruit (Saeys et al. 2008; Lu 2004; Fraser et al. 2003; Wilson et al. 1985), very little is known about the actual path that light takes inside an intact fruit, and the attenuation it experiences in different regions of that fruit. Knowing the light distribution in the fruit may enable the selection of the most effective NIR mode of measurement for a given fruit type or shape (Fraser et al. 2003) and hence optimise the non-invasive assessment method.

In this study the depth of penetration into 'Hass' avocado fruit of NIR radiation in both transmission and reflectance modes were investigated.

3.2.2 Materials and Methods

Whole hard green 'Hass' avocado fruit were cut in half (longitudinally), the seed removed and the cut surface trimmed to remove the seed cavity providing a flat surface. The fruit were then scanned in both transmission and reflectance modes as detailed below.

3.2.2.1 Transmission mode

For transmission mode, a Bruker Matrix F FT-NIR spectrophotometer which measured in the 12820-4000 cm^{-1} (780–2500 nm) range, linked with an external fibre-coupled emission head utilising a 4 × 20 W tungsten halogen light source was employed. The fibre optic probe in the emission head used to relay the spectral signal to the Matrix F spectrophotometer was removed from the emission head and placed directly under the emission head light source at a distance of 170 mm to provide a transmission configuration (see *Figure 3.4*). For spectra collection, the avocado half with a known thickness was placed onto the fibre optic head with the cut side to the fibre optic head and the skin side to the light source.

3.2.2.2 Reflectance mode

For reflectance mode, the Bruker Matrix F F-T NIR spectrometer and emission head was again employed (see *Figure 3.4*). The cut flat surface of the avocado half was placed onto a mirror to provide a reflective surface and the skin side of the avocado was presented to the light source. A template was placed at a path-length of 170 mm from the light source to the skin surface of the thickest section of the fruit to provide a spectral scan area on the avocado sample of 30 mm in diameter. In obtaining each sample spectrum 8 scans at a resolution of 16 cm^{-1} were collected and averaged.

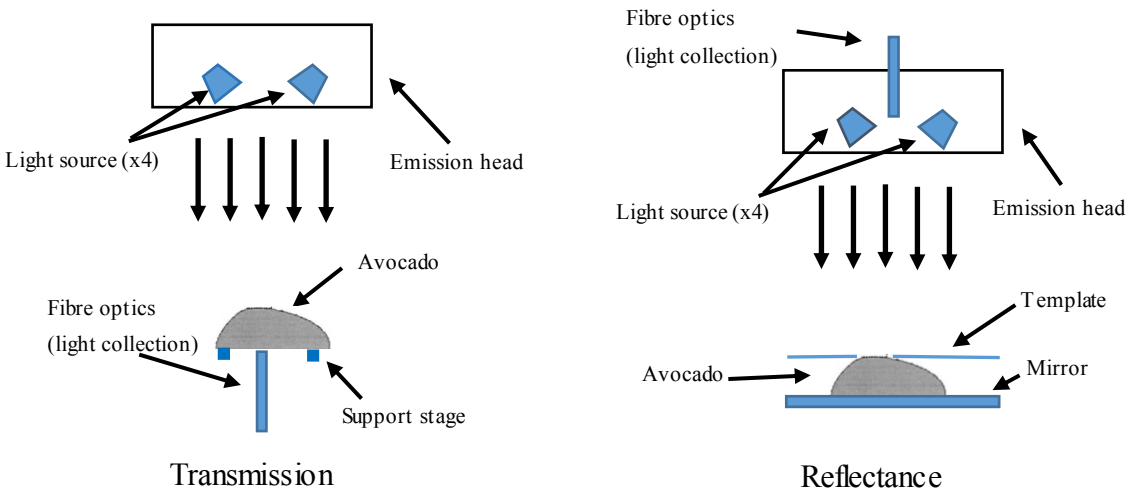


Figure 3.4. Schematic of reflectance and transmission measurement configurations.

For both transmission and reflectance modes, the sample depth at the spectral collection point (skin side to cut side) was recorded. Once the spectra had been collected for the known thickness, a thin longitudinal slice (1-2 mm) of the avocado fruit from the cut side was removed using a V-slicer and the thickness of the now trimmed sample recorded using Vernier callipers before subsequent spectral collection. This process was repeated until the sample was reduced to

approximately 1-2 mm in thickness. One spectra was captured for the sample at each trimmed thickness. For both transmission and reflectance modes, five whole fruit cut in half, providing 10 halves were used for spectral collection. Typical transmission and reflectance (absorbance) mode spectra for an avocado fruit at varying thicknesses are shown in *Figure 3.5*. The error associated with the thickness measurements were estimated to be less than 1mm, which accounts for nodule sizes and curvature of the fruit.

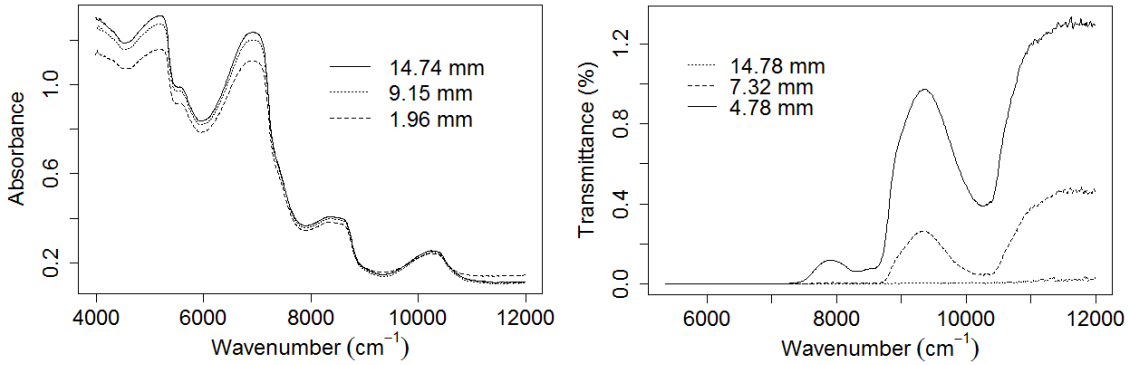


Figure 3.5. Typical reflectance (left) and transmission (right) spectra for an avocado at varying thicknesses.

3.2.3 Data Analysis

In transmission mode, the transmitted spectrum, $T(\lambda,\theta)$ was measured as a function of the fruit thickness θ. The transmitted spectrum is related to the incident spectrum $T(\lambda)$ via Beer-Lambert law:

$$T(\lambda,\theta) = T(\lambda)\exp(-\beta_1(\lambda)\theta),$$

where $\beta_1(\lambda)$ represents the wavelength-dependent absorption coefficient. The depth of penetration is then defined to be the thickness at which the transmitted intensity falls to 1/e of its incident value, where e is Euler's irrational number - 2.71828. A non-linear least squares model was used to estimate the absorption coefficient at each wavelength. The variation of the absorption coefficient with wavelength then gives a measure of the wavelength dependence of the depth of penetration of the NIR radiation.

In reflectance mode, a similar procedure was followed to that adopted by Lammertyn et al. (2000). The spectrum obtained from the thickest part of the whole avocado half was utilised as the reference spectrum, $R_{ref}(\lambda)$. For each trimmed avocado slice of thickness θ, the following function was calculated using the reflected spectrum $R(\lambda,\theta)$ and the reference spectrum:

$$g(\lambda,\theta) = \frac{log_{10}\,(R_{ref}(\lambda))}{log_{10}\,(R(\lambda,\theta))} \qquad \textbf{Eqn. 3. 1}$$

A non-linear model was then fitted to $g(\lambda,\theta)$ assuming the functional form (Lammertyn et al. 2000):

$$g(\lambda,\theta) = \delta(\lambda) + \alpha(\lambda)e^{-\beta_2(\lambda)\theta}, \qquad \textbf{Eqn. 3.2}$$

where δ, α and β_2 are estimated from the model fit.

The estimated depth of penetration was then obtained using the following equation:

$$g(\lambda,\theta) = \frac{log_{10}[se(\hat{\delta})*2.576/\hat{\alpha}(\lambda)]}{-\hat{\beta_2}(\lambda)}, \qquad \textbf{Eqn. 3.3}$$

where 2.576 is the critical value associated with a 99% confidence interval for a standard normal distribution and $se(\hat{\delta})$ is the standard error associated with the parameter estimate of δ. An example of the fitted model $g(\lambda,\theta)$ and estimated penetration depth is shown in *Figure 3.6* for three sample wavelengths.

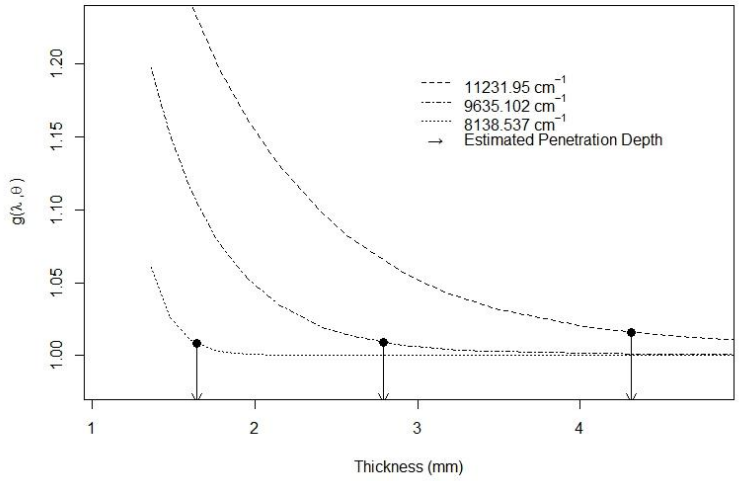


Figure 3.6. Variation of $g(\lambda,\theta)$ for various wavelengths, and the associated estimate of the penetration depth for reflectance mode.

The software package 'R' was utilised for the analysis of the depth of penetration data for both transmission and reflectance modes.

3.2.4 Results and discussion

In this study both transmission and reflectance methods have different penetration depth classifications. In transmission mode, as detailed above, the penetration depth is defined as the depth at which the light intensity drops to 1/e of the initial intensity. In reflectance mode, the depth of penetration is based on the classification developed by Lammertyn et al. (2000). It

should be highlight that the differences in definitions of penetration distance between transmittance and reflectance will result in differences in the absolute measures. Aside from these differences, physically the two processes also differ. In transmission mode, the radiation passes through skin and flesh, and each will have different absorption and scattering characteristics. In reflectance mode, the measurable radiation (excluding specular reflection) must pass through the skin twice. Furthermore, the wavelength variation of penetration depth between the two techniques may be quite different if the scattering of light within the fruit is highly anisotropic in nature.

It is noted that, for both transmission and reflectance modes, signal variability was introduced by repositioning the sample for subsequent spectra collection following removal and slicing of the sample. The sample could not be placed in the exact same position each time which resulted in a slight change in the optical properties for each spectra. The 10 avocado halves ranged in initial thickness from 16.53 to 22 mm with final depth thickness ranging from 0.97 to 1.88 mm at the spectral collection point (see *Table 3.2*). Each avocado half had a minimum of 10 thicknesses assessed and the skin thickness at each assessment decreased by approximately 1-2 mm.

Table 3.2. Initial and final depth of avocado halves for both reflectance and transmittance modes.

Mode	Fruit	Initial thickness (mm)	Final thickness (mm)	Number of thickness intervals assessed
Reflectance	1– side b	20.66	1.74	11
	2– side a	19.46	1.60	10
	2– side b	18.83	1.76	12
	3– side a	16.53	1.63	12
	4– side a	18.25	1.82	15
	4– side b	18.50	1.96	13
	5– side a	16.72	1.48	12
	5– side b	17.53	1.36	15
Transmission	1– side a	17.28	2.24	10
	1– side b	16.92	0.97	11
	2– side a	18.12	1.88	10
	2– side b	21.78	1.20	14
	3– side a	22.00	2.47	14
	3– side b	21.40	2.88	13
	4– side a	17.63	1.56	11
	4– side b	18.13	1.78	12
	5– side a	20.71	1.23	13
	5– side b	18.74	1.56	13

Figure 3.7 is a plot of the representative depth of penetration for each wavelength for both transmission and reflectance modes. Representative depths of penetration are only reported where the estimates were conclusive and deemed reliable. Calculated depths of penetration for the reflectance mode that were based on estimates of $\hat{\delta}$ with a standard error of more than 50% were deemed unreliable and are not reported. For transmission mode depths of penetration were less than 3 mm for wavelengths from 5369 to 7413 cm^{-1} (1862–1348 nm). Maximum depths of penetration of approximately 12 mm occurred around wavelengths 11293 to 11987 cm^{-1} (885-834 nm). Two other wavelength regions were identified with significant depth of penetration, one peak occurring around 9272 cm^{-1} (1078 nm) and the second at approximately 7899 cm^{-1} (1265 nm) with approximately 10 mm and 4.5 mm penetration respectively. At certain wavelengths, a very low signal to noise ratio caused much uncertainty on the calculated depths of penetration and these were removed. A similar trend for wavelength regions and depth of penetration was seen with the reflectance mode, with the highest depth of penetration of approximately 4.8 mm occurring in the 11625 to 11987 cm^{-1} (860-834 nm) region and a 3.3 mm penetration depth around the 9295 cm^{-1} (1076 nm) region (see *Figure 3.7*).

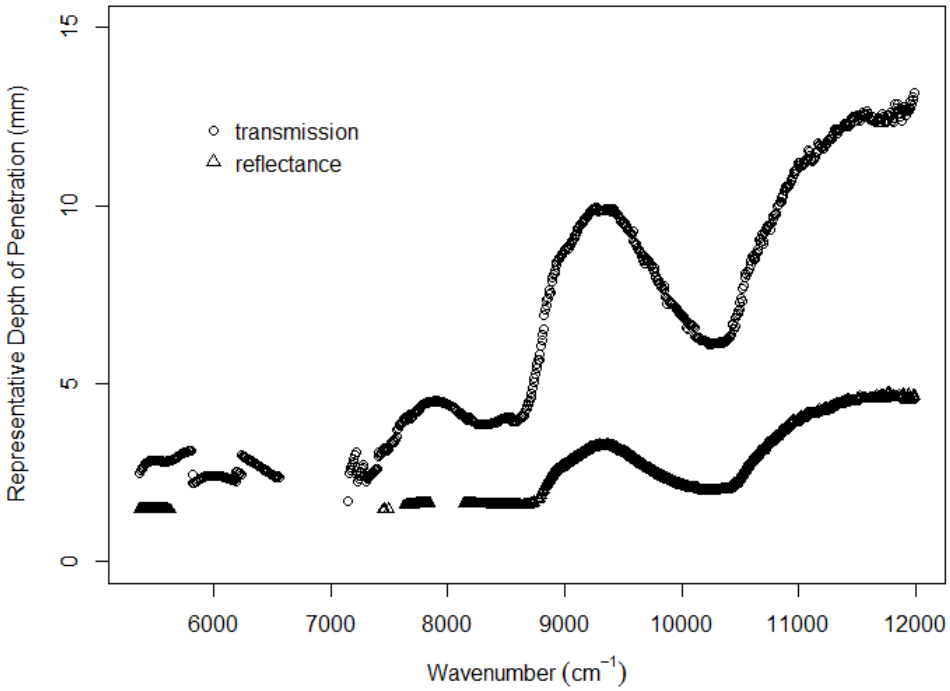


Figure 3.7. The representative depth of penetration for each wavelength for both transmission and reflectance modes in avocado fruit.

The depth of penetration for transmission and reflectance modes in this study compare favourably with other studies found in literature. The depth of penetration is strongly dependant on tissue

matrix and composition, and on wavelength (Wilson and Adam 1983). Peirs et al. (2002) report penetration depths for 'Jonagold' apple vary between 1 and 5 mm, depending on the wavelength, the instrument and ripeness stage of the fruit. Similarly, Lammertyn et al. (2000) report that penetration depth of NIR into apples was wavelength dependent, where the authors obtained up to 4 mm penetration in the 700-900 nm range and between 2 and 3 mm in the 900-1900 nm range. Lammertyn et al. (2000) suggest a penetration depth of 1-5 mm for a typical reflectance setup.

For diffuse reflectance trials conducted on rockmelons, Greensill (2000) reports that approximately 25% of the diffuse light at 10 mm depth is expected to re-emerge from the fruit surface. Thus, in Greensill (2000) trials the detected light will have primarily originated within the upper 10 mm of the fruit. Fraser et al. (2003) assessed light distribution through mandarin fruit using a laser light at a wavelength of 808 nm. Results showed that there was a rapid reduction in light level across the thick skin (3-5 mm), a less rapid but still high reduction in light in the flesh due to light scattering, with some perturbation through the central pith and air gap, and a rapid drop in light across the distal skin (Fraser et al. 2003). Investigations by Scotter (1990b) with fruit, fish and meat products indicate that good reflectance data can be achieved with a sample thickness of between 0.5 and 2 cm, depending upon the absorbance of the sample material.

3.2.5 Conclusion

This study measured the depth of penetration of NIR radiation in avocado in both transmission and reflectance modes, thus sampling different optical paths within the fruit. The results obtained in this study show that NIR radiation in both transmission and reflectance modes have qualitatively similar wavelength dependencies in penetration. However it is noted that the transmission and reflectance methods have different threshold classification of penetration depth. The study highlighted some spectral windows where the depth of penetration in transmission mode was as high as 12.5 mm in the region around 11500 cm^{-1} (869 nm) and 10 mm in the region around 9400 cm^{-1} (1063 nm). At certain wavelengths a very low signal to noise ratio caused much uncertainty on the calculated depths, and further study is required to enable an estimate of the penetration distance in these regions.

3.3 Non-destructive prediction of 'Hass' avocado dry matter via FT-NIR spectroscopy

3.3.1 Introduction

Quality and safety evaluation of agricultural products has become an increasingly important consideration in market/commercial viability and systems because such evaluations are now demanded by customers, including distributors and retailers. Unfortunately, many internal quality factors are difficult or impossible to assess during sorting, processing, and marketing by visual-type observations. The inability to consistently guarantee internal fruit quality is an important commercial consideration of the Australian avocado industry (HAL and AAL 2005). A non-destructive system that can accurately and rapidly monitor internal quality would allow the avocado industry to provide a better, more consistent fruit to the consumer, and thus improve industry competitiveness and profitability. Despite the obvious need, there has been limited progress in the investigation of non-invasive rapid assessment techniques to determine fruit quality of avocados.

Maturity at harvest is a major determinant of avocado eating quality (Harker et al. 2007; Brown 1984) and can influence outturn quality after storage (Hofman et al. 2002). However, without a reliable method of predicting maturity through external indicators (Hofman and Jobin-Decor 1999), such assessments are currently undertaken destructively through either oil analysis or %DM (Clark et al. 2003; Mizrach and Flitsanov 1999; Fuchs et al. 1995; Ranney et al. 1992; Lee and Coggins 1982). %DM is accepted internationally as an avocado maturity index for commercial purposes since the oven drying method of determining %DM is faster and more economically feasible than determining oil content by solvent extraction. In California (USA), minimum standards for %DM range from 19 to 25%, depending on cultivar (Kader and Arpaia 2000). The recommended minimum DM standard for Australia is 23% DM (approximately 10% oil content) for 'Hass' and 21% for all other cultivars (Harker et al. 2007; McCarthy 2001), although consumer studies for 'Hass' indicate a preference for at least 25% DM (Harker et al. 2007).

The development of automatic technologies has enabled commercially feasible non-invasive methods for estimating internal quality attributes of agricultural products. Although several non-invasive techniques exist for this (Butz et al. 2005; Gaete-Garreton et al. 2005; Mizrach 2000; Abbott 1999; Mizrach and Flitsanov 1999; Chen and Sun 1991), NIR spectroscopy in particular has received considerable attention for determining quality attributes in fruit and vegetables. Analysis of NIR spectroscopy 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 SS content or total SS (TSS) of fresh fruits (Butz

et al. 2005; Abbott 1999; Scotter 1990a). Of particular importance for the current study, NIR spectroscopy has been used to estimate %DM in various horticultural products (Sivakumar et al. 2006; Xiaobo et al. 2006; Hartmann and Bijning-Pfaue 1998; McGlone and Kawano 1998; Birth et al. 1985) including avocados (Walsh et al. 2004; Clark et al. 2003; Schmilovitch et al. 2001). 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.

There have been limited investigations of avocado maturity based on %DM using NIR spectroscopy. 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 and 2400 nm. Preliminary results identified SEP 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 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 LV's, 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 (prediction) >0.83, and 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 (Zeiss MMS1/NIR-enhanced spectrometer, Germany) in the 300-1100 nm range, reported calibration results of R = 0.89, RMSECV = 1.14, with an SDR = 2.2, for %DM of avocado fruit of unspecified cultivar. The higher the SDR statistic the greater the power of the model to predict the chemical composition accurately (Cozzolino et al. 2004) as discussed in *Section 2.3.6.1* and *Section 2.4*.

The above studies report calibration statistics with NIR spectroscopy for avocado %DM prediction, but there has been limited progress in testing potential commercial systems for avocados. The present study used diffuse reflectance mode FT-NIR spectroscopy to study %DM of 'Hass' avocados for the first time. This system is commercially available and is currently used in other industries, such as the sugar industry, in a commercial in-line setting. The proposed advantages of an FT-NIR spectrophotometer have been detailed in literature (Liu et al. 2007; Yande et al. 2006; Ying et al. 2005). In this study, the FT-NIR spectroscopy results obtained against both the original reflectance NIR spectroscopy study, and the enhanced accuracy of the interactance mode of Clark et al. (2003) were compared. Clark et al. (2003) also suggested that further investigation of reflectance and interactance NIR spectroscopy in an in-line setting was warranted, providing further motivation for the current study. It must emphasize however, that 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. Therefore, this study only compares the obtained calibration results to those found in literature as a general comparison.

This study assessed the potential of FT-NIR spectroscopy to provide a non-invasive assessment of 'Hass' avocado maturity and thereby eating quality based on %DM. It assessed the ability of FT-NIR spectroscopy to determine the %DM of avocado flesh (endocarp) without the thick skin (exocarp). Removing the skin gives an indication of the maximum potential accuracy of FT-NIR spectroscopy in predicting %DM on whole (with exocarp) fruit. The study also assessed the ability of FT-NIR spectroscopy to predict %DM of whole fruit, and included fruit from three harvests across the commercial harvesting season.

3.3.2 Materials and methods

3.3.2.1 Reference avocado selection

'Hass' avocado fruit were obtained during the 2006 growing season (May to August) from a single farm in the major production district of Bundaberg, Queensland (Latitude: 24 52' S, Longitude: 152 21' E). Avocado fruit were harvested from the same trees in the orchard at three maturity stages corresponding to early (late-May), mid (mid-July) and late (late-August) season harvests, to allow for the variability in the %DM range and other seasonal factors to be included in the calibration procedure. Approximately 100 avocados were collected at each harvest.

3.3.2.2 Dry matter distribution within 'Hass' avocado

Application of NIR spectroscopy technology to avocado fruit requires an understanding of the DM distribution within the fruit. It is well known that physiological gradients of %DM exist within 'Hass' avocados (Woolf et al. 2003; Schroeder 1985), though there are no known published studies for Australian fruit. The first component of our study was to investigate the %DM variation within these fruit to identify a suitable sampling point on an avocado that represents whole fruit %DM.

To estimate the %DM variation in Australian 'Hass' avocado's three 16 mm diameter cores were taken from each fruit, one from the 'top' (stem end), one from the 'middle' and one from the 'bottom' (blossom end) of eleven 'Hass' avocado fruit (see *Figure 3.8*). The core taken from the 'stem end' of the fruit was divided into four 10 mm long sections (i.e., Outer, Middle 1, Middle 2 and Inner sections), whilst the cores from the middle and blossom were divided into three 10 mm long sections (i.e., Outer, Middle and Inner sections). The skin was removed and the %DM of all the flesh sections was determined through the standard technique of fruit weight difference

following drying in an oven at 60-65°C to constant weight (approximately 48 hours). Data analysis was conducted using the commercially available statistical software package GenStat for Windows 11th Edition (VSN International, Hemel Hempstead).

3.3.2.3 Reference percentage dry matter analysis

Because of the %DM variations within avocado fruit (see *Figure 3.8*) the same area of the fruit was used for both NIR spectroscopy and %DM measurement. The %DM reference sample was obtained by taking a core perpendicular to the surface of the fruit with a radius equal to the NIR spectroscopy sampling area on opposing sides of the fruit using a 50 mm diameter steel corer, and excising both skin and underlying flesh to a depth of approximately 10 mm. This ensured that the same flesh was used for both NIR spectra and %DM determination. The skin (2-4 mm) was removed from the edible flesh, and the flesh core was cut into quarters to facilitate drying and dried in a fan-forced oven at 60-65°C to constant weight (approximately 48 hours) for determination of %DM by percentage weight difference.

3.3.2.4 NIR spectra collection

The spectral reflectance characteristics of whole, intact 'Hass' avocado fruit and avocado flesh only samples were measured using a commercially available bench-top, Matrix-F, FT-NIR spectrophotometer (Bruker Optics, Ettlingen, Germany; operating software: OPUS™ version 5.1 & 6.0) in the 780-2500 nm range. All trials were conducted in a room where interference from external light was minimised. Spectra were obtained in diffuse reflectance mode using a 4 x 20 watt tungsten light source fibre-coupled measurement head. A path-length of approximately 170 mm from the external measurement head light source to the surface of the avocado 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. Standard Matrix-F calibration procedures were performed before the determination of each set of sample spectra. The signal-to-noise ratio, wavelength accuracy, repeatability and photometric response for the NIR spectroscopy system were routinely benchmarked prior to spectra collection. A white background reference spectrum was obtained using a white spectralon reference prior to the collection of each set of sample spectra.

Spectra were acquired after sample temperature equilibration in an air-conditioned laboratory at approximately 22-24°C, and within two days of harvest. The intact fruit were directly exposed to the focal point of the emission head. The spectral characteristics of the fruit were measured midway between the peduncle and base for each opposing half (i.e., two spectra per fruit including one spectra from side 'A' and one spectra from the opposing side 'B'). Due to the large variability

in %DM within a fruit as shown by this study (see *Figure 3.8*) and literature (Woolf et al. 2003; Schroeder 1985), the observed data from both sides (A and B) of the fruit were used in predictive calculations. For NIR spectroscopy assessments of the avocado flesh, spectra were obtained using the same procedure except the fruit skin (2-4 mm) was removed just prior to spectral measurement.

3.3.2.5 Calibration modelling and validation

Data analysis was conducted using the commercially available chemometric software package 'The Unscrambler™' version 9.8 (CAMO, Oslo, Norway). Spectral data was analysed by principal component analysis (PCA) and obvious spurious spectra eliminated. PLS regression was used to build the prediction models of the diffuse reflectance spectral data. A manual selection process was used to identify spectral regions and pre-processing treatments for improved model development. Among all spectra collected, significant noise was found within spectral ranges 780-843 and 2414-2503 nm.

The shorter wavelength portion of the NIR spectrum (<1100 nm) allows better penetration into biological material (Saranwong and Kawano 2007; Guthrie et al. 2004), and if the chemical constituent of interest is located deep in the flesh of biological material, this short wavelength region would provide more reliable information. Alternatively, the long wavelength region (>1100 nm) will be useful if the chemical constituent is located close to the surface. It is well known that spectra of samples with high water content (>80%), such as fruit, may cause difficulties in the calibration process, as they are strongly dominated by water absorption and are sensitive to temperature (Buning-Pfaue 2003; Williams and Stevenson 1990). The short wavelength NIR region has an advantage in this respect in that water peaks are localised to main regions (i.e., centred on ~760 and 970 nm) leaving the remainder of this region available for analysis (Greensill 2000). 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 range, and 930 nm (overtones of CH_2 stretching) (Guthrie et al. 2004; Clark et al. 2003; Osborne et al. 1993). The 900-920 nm absorbance band is often cited as the most important band for %DM and/or sugar determination, as it is located away from the water absorbance peaks that typically dominate spectra of fruit (Clark et al. 2003). Clark et al. (2003) concluded that their MLR model results for the prediction of %DM of intact avocados confirmed that the relevant spectral information is obtained primarily from the carbohydrate/lipid CH absorbance band in the 900-920 nm range, with a minor input from water related absorbance's.

The %DM for each sample was used as the constituent data, and the pre-processed spectra were used as the spectral data for PLS calibrations. A segmented cross validation procedure was

employed in the PLS regression analysis (Lin et al. 2004). Final models for individual harvest populations presented in this study (*Table 3.5* for avocado flesh harvested mid season and *Table 3.6* for whole avocado harvested at early, mid and late in the season) were based on a combination of a 25 point SG spectral smoothing (2^{nd} order polynomial) and a SG second derivative transformation over 25 points (2^{nd} order polynomial). The final model for all three harvests combined (see *Table 3.7*) for whole avocado was based on a 25 point SG spectral smoothing (2^{nd} order polynomial), followed by a SG first derivative transformation over 25 points (2^{nd} order polynomial).

3.3.3 RESULTS AND DISCUSSION

3.3.3.1 Dry matter distribution

In Queensland 'Hass' avocados, higher %DM was recorded at the stem end compared with the blossom end, and a gradient at the stem end of higher to lower %DM moving from the outside to the inside of the fruit was identified (see *Figure 3.8*). The middle and blossom end of the fruit had a gradient of lower to higher %DM, moving from the outside to the inside of the fruit. These results are similar to those reported for New Zealand (Woolf et al. 2003) and Californian (Schroeder 1985) 'Hass' avocados.

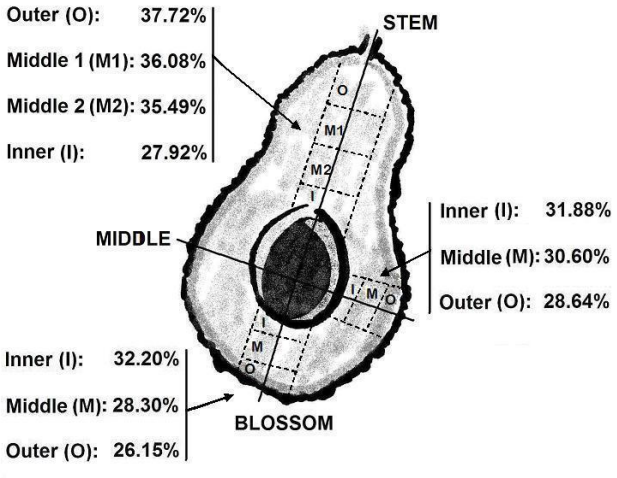


Figure 3.8. Diagram of average dry matter distribution for Queensland 'Hass' avocado fruit.

A more in depth review with regards to the variation from the two opposing sides of the fruit (i.e., Side A and Side B). An ANOVA suggests there are no significant differences ($p = 0.355$) between the mean absolute difference in the DM for the three sections (inner, middle and outer) from each of the cores taken from opposing sides of the avocado fruit as outlined in *Table 3.3*. There appears to be an increasing difference in the mean values from the inner (0.697), middle (1.013), to the outer section (1.107) of the cores with the following parameters: replication = 10; degrees of

freedom = 18; standard error of the difference = 0.290; 95% least significant difference 0.609. Thus, there is more variability in the DM for the outer section of the cores compared to the inner sections, however this difference is not significant.

3.3.3.2 Confidence intervals

A two-sided 95% CI for the mean absolute difference in the DM for each section was calculated as well as a 95% CI for the mean absolute difference in DM for the whole core. The difference in DM for the 'whole core' CI is calculated using the mean DM for whole cores. Thus, the CI for 95% of the time the true difference in DM between cores from opposing sides of an avocado is between, for example, 0.19% and 1.20% for the inner section of the fruit core; 0.3868% and 1.640% for the middle section; 0.5544% and 1.660% for the outer section; 0.3397% and 1.188% for the whole core.

Table 3.3. Summary of avocado fruit statistics for a two-sided 95% confidence interval (CI) for the mean absolute difference in the DM for each section (inner, middle, outer, whole core).

Sample	Fruit (n)	Mean	Variance	SD	SE of mean	95% CI for mean
Inner	10	0.6972	0.4886	0.6990	0.2210	(0.1972, 1.197)
Middle	10	1.013	0.7669	0.8757	0.2769	(0.3868, 1.640)
Outer	10	1.107	0.5967	0.7725	0.2443	(0.5544, 1.660)
Whole core	10	0.7637	0.3513	0.5927	0.1874	(0.3397, 1.188)

Note: SD = standard deviation; SE = standard error; CI = confidence interval.

A one-sided 95% CI for the mean absolute difference in the DM for each section was calculated as well as a 95% CI for the mean absolute difference in DM for the whole core as shown in *Table 3.4*. These results suggest that 95% of the time the true mean difference in DM between cores on the same fruit is less than 1.6% for all sections. Note that increasing the confidence level to 99% for all the CI results in the upper confidence limits, the true mean difference is still less than 2%.

Table 3.4. Summary of avocado fruit statistics for a one-sided 95% confidence interval (CI) for the mean absolute difference in the DM for each section (inner, middle, outer, whole core).

Sample	Fruit (n)	Mean	Variance	SD	SE of mean	95% upper CI limit
Inner	10	0.6972	0.4886	0.6990	0.2210	1.102
Middle	10	1.013	0.7669	0.8757	0.2769	1.521
Outer	10	1.107	0.5967	0.7725	0.2443	1.555
Whole core	10	0.7637	0.3513	0.5927	0.1874	1.107

Note: SD = standard deviation; SE = standard error; CI = confidence interval.

On this basis and noting that there is a non-uniform distribution of DM around the fruit, the comparison studies between the Californian method of two longitudinal slices of tissue which are grated and subsequently dried and the New Zealand plug or core method using flesh from the equator of the fruit of the two opposing sides of the fruit, all results have shown extremely high correlations (r^2>0.92) (Woolf et al. 2003). The NIR spectroscopic and actual %DM assessment of the fruit for DM assessment was done at the equatorial position midway between the peduncle and base and assumed to be representative of the %DM of a whole fruit.

3.3.3.3 Avocado flesh (skin removed)

To estimate the maximum potential for the FT-NIR spectroscopy system to predict %DM of a whole avocado, NIR spectroscopy spectra were obtained for the avocado flesh only (skin removed). The irregular surface and thickness of the 'Hass' skin may reduce accuracy because of decreased light penetration and increased noise and specular reflectance.

A comparison of the spectral features between the NIR spectra of an avocado sample without-skin (flesh) and with-skin in comparison to a pure avocado oil sample ('Melrose' Organic Avocado oil, unrefined, cold pressed from Queensland avocados) are shown in *Figure 3.9*. The two avocado spectra in general show similar tendencies over the entire spectral range of 11995-4000 cm^{-1} (834-2500 nm). The main difference occurring around the spectral regions 5931-5474 cm^{-1} (1686-1827 nm) and 4350-4210 cm^{-1} (2296-2375 nm), where the flesh sample spectrum has several distinct peaks which are not as pronounced in the skin-on sample. These similarities potentially indicate that the feature wavelength ranges for the flesh and skin-off 'Hass' avocado samples may be very similar over the spectral range of 11995-4000 cm^{-1} (834-2500 nm).

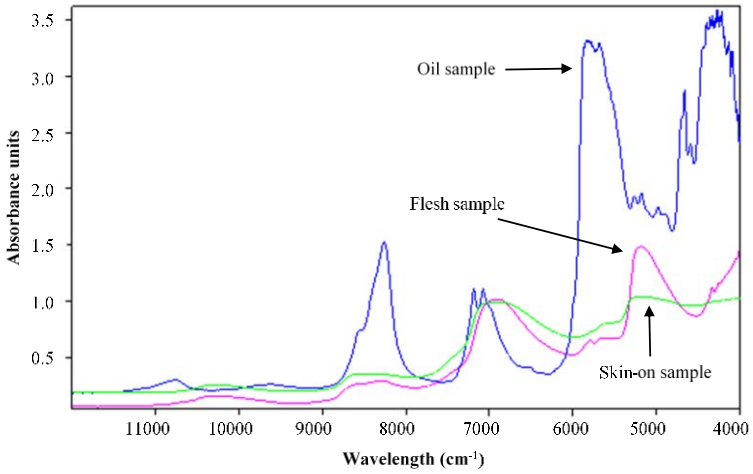


Figure 3.9. Comparison of reflectance spectra of 'Hass' avocado sample with-skin and with-out skin (flesh), plus a pure avocado oil sample.

A PLS calibration model was developed for the estimation of %DM for 'Hass' avocado flesh from the mid season harvest population based on a data set of 90 spectra (see *Table 3.5*). The major regression (β) coefficients for the flesh model occurred around 930, 962, and 2142 nm. The 930 nm peak is consistent with the presence of the third overtone of C-H stretch (oil) (Guthrie et al. 2004; Clark et al. 2003; Osborne et al. 1993), the 962 nm peak is related to the second overtone bonds of O-H stretch and NH_2 stretch, and 2142 nm peak is related to a combination of C-H stretch, C=C stretch and N-H stretch combination bonds (Fassio and Cozzolino 2004).

Table 3.5. Calibration statistics for the determination of %DM of 'Hass' avocado flesh for the mid season harvest.

Spectra n	% DM range	Mean	SD	Spectral region (nm)	R^2	RMSECV	LV	SDR	BIAS
90	21.3-31.2	27.3	2.15	928-935; 960-963; 2132-2153	0.86	0.84	2	2.56	0.008

Despite the small number of calibration samples and the small SD (2.15), the calibration statistics were encouraging in terms of an R_c^2 of 0.86 and an RMSECV of 0.84% DM, using only 2 LV's. Since only 2 LV's were used to construct the model, there are still 45 times more samples than variables. This is in agreement with a statistical rule of thumb, which says that the ratio of the number of samples to the number of variables should be equal to or larger than 10 (Lammertyn et al. 2000; Hruschka 1987). An SDR value above 2.5 allows for sorting into two grades (Golic and Walsh 2006), while a value of 3 enables the data set to be split into three groups (of width equivalent to RMSEP)(McGlone and Kawano 1998). Thus, the calibration model SDR of 2.56 indicates that the PLS model has the potential to sort/grade avocado flesh samples into two %DM groups (Golic and Walsh 2006; McGlone and Kawano 1998).

3.3.3.4 Whole avocado

The PLS calibration model statistics presented in *Table 3.6* for whole 'Hass' avocado fruit corresponds to each individual harvest date. The sample mean for %DM increased steadily over the season. The calibration results for determining %DM within individual harvest dates were relatively poor. The early and late season harvests were the worst for both calibration and predictive performance (SDR). The early and late season harvest dates also yielded the narrowest %DM range, resulting in considerably lower SD's. These results suggest that the fruit obtained from these two harvest dates possibly did not include a sufficiently broad variability in %DM to develop a suitable calibration model, although other biological or environment effects may have contributed.

Table 3.6. PLS calibration results for %DM for whole 'Hass' avocado fruit harvested at early, mid and late stage in the season.

Harvest	Spectra n (outliers removed)	% DM range	Mean	SD	R_c^2	RMSECV	LV	SDR
Early	207 (2)	21.1-29.9	25.0	1.57	0.71	0.82	12	1.92
Mid	205 (3)	18.2-31.7	26.5	2.70	0.86	0.98	10	2.75
Late	217 (1)	25.1-35.0	30.7	1.86	0.73	0.97	8	1.92

Of the three individual harvests, the mid season harvest performed the best with an R_c^2 of 0.86 and SDR of 2.75. The increased performance of the mid season harvest model may be attributed to incorporating a larger variability in %DM as indicated by the SD. Due to this DM variability between individual harvests, all three harvests were subsequently combined to develop a model incorporating all seasonal DM variability to ensure model robustness and predictive performance.

The PLS calibration and prediction statistics for the combined harvests using FT-NIR spectroscopy on the whole fruit are displayed in *Table 3.7*. The data set of 629 spectra were separated into a calibration set (n = 199) and a prediction set (n = 430). The validation statistics of the combined harvests calibration model delivered an $R_v^2 = 0.76$ with an RMSEP = 1.53 and SDR of 2.03. As expected the performance of this combined model for whole avocado fruit was lower than the upper bounds of accuracy determined from the flesh samples as presented in *Table 3.5* ($R_c^2 = 0.86$, RMSECV = 0.84, SDR = 2.56). The error (RMSECV) of the combined model was as expected considerably larger than that for the flesh samples, most likely due to the irregular thick skin.

Table 3.7. PLS calibration and prediction statistics with outliers removed, for the determination of %DM of whole 'Hass' avocado fruit for the combined harvests using FT-NIR spectroscopy.

Combined Harvests Data Set	Spectra n (outliers removed)	R^2	Range	Mean	SD	RM SECV	RM SEP	LV	SDR	BIAS
Calibration	199 (2)	0.81	18.2-35.0	27.34	3.40	1.47		5	2.31	-0.010
Prediction	430 (2)	0.76	19.4-34.2	27.53	3.12		1.53	5	2.03	-0.148

There is good agreement between the RMSECV and RMSEP values for the combined harvests calibration and prediction sets, indicating that the model has been robustly constructed (McGlone et al. 2002). The spectral regions selected in the whole avocado calibration were similar to those employed in the flesh calibration model. The largest regression (β) coefficients for the whole avocado calibration model occurred around the 914, 930 and 957 nm, with smaller peaks occurring in the vicinity of 962, 2117 and 2142 nm. The 914 nm band is consistent with the

presence of the third overtone of the carbohydrate CH absorbance band that normally occurs in the 900-920 nm range (Guthrie et al. 2004; Clark et al. 2003; Osborne et al. 1993). The 930 nm peak corresponds to oil (third overtone of C-H stretching) (Guthrie et al. 2004; Clark et al. 2003; Osborne et al. 1993), and the 957 nm peak relates to the second overtone of OH bending. The three smaller peaks in the vicinity of 962, 2117 and 2142 nm relate to the second overtone bonds of O-H stretch and NH_2 stretch, second overtone of CH_2 stretch and C=C stretch (Pereira et al. 2008), and a combination of C-H stretch, C=C stretch and N-H stretch combination bonds (Fassio and Cozzolino 2004), respectively.

It is illustrative at this stage to compare the results obtained with those currently available in the literature, although direct comparisons are difficult to interpret as both configurational and instrument effects are inherently present in all platforms. It would be necessary to compare the exact configurations, in reference to the angle between detector, light source and sample of the other spectrometer platforms identified in literature in order to make a definitive statement of the superiority or otherwise of the one spectrometer over the other. To illustrate the potential of the current techniques it is preferable to highlight and compare model accuracies of the present technique compared with other techniques. The combined harvests prediction results compare favourably with the models developed from data obtained from a PDA spectrometer (Clark et al. 2003). The reflectance mode results of Clark et al. (2003) reported as having an RMSEP of 2.6% DM over a 20-45% DM range and an R_v^2<0.75, as compared with an RMSEP of 1.53% DM and R_v^2 of 0.76 obtained in the present study. The reflectance mode models of Clark et al. (2003) required more LV's (12 to 20) than the current study which only required 5, possibly indicating more interference from spectral noise in Clark et al. (2003) study. In fact, the current FT-NIR spectroscopy reflectance based model was similar to the accuracy of the NIR spectroscopy interactance mode of Clark et al. (2003) (R_v^2 of 0.88 and an RMSEP of 1.8% DM) indicating reflectance FT-NIR spectroscopy may be a better alternative for in-line and at-line environments. In relation to other avocado studies, the current study results compare favourably, Walsh et al. (2004) using a PDA spectrometer reported calibration results of R_c^2 = 0.89, RMSECV = 1.14, SDR = 2.2, for %DM on raw absorbance data for whole avocado fruit (unspecified variety). Schmilovitch et al. (2001) using a dispersive NIR spectrophotometer in reflectance mode on the relatively thin-skinned 'Ettinger' and 'Fuerte' avocado reported preliminary results of errors of prediction (SEP), based on a PLS model with six factors using first derivative pre-treatment as 0.9 and 1.3% respectively, for a 14-24% DM range. Consequently, it is likely that the thin-skin cultivars would not suffer to the same extent from the experimental limitations experienced with the thick rough skin of 'Hass'.

3.3.4 Conclusion

The potential of FT-NIR spectroscopy in diffuse reflectance mode as a rapid and non-invasive technique for assessing %DM of the 'Hass' avocado for the use in a commercial in-line/at-line application has been investigated. The study found that combining three harvests (early, mid and late harvests) from a single farm in the major production district of Central Queensland, calibration models developed could predict %DM of whole 'Hass' avocado to within 1.53% with an R_v^2 of 0.76 and SDR >2.0. This indicates an ability to sort the fruit into two categories (i.e., above and below an acceptable %DM value) with approximately 80% accuracy (Guthrie et al. 1998). Furthermore, the results indicate that reflectance FT-NIR spectroscopy can achieve higher accuracy than dispersive or PDA spectrometers in reflectance mode (Clark et al. 2003; Schmilovitch et al. 2001) and approach the accuracy of the NIR spectroscopy in interactance mode (Clark et al. 2003). The current study used a Matrix-F, FT-NIR spectrophotometer which is routinely used in automated process control in both in-line and at-line settings. Thus, FT-NIR reflectance spectroscopy is an alternative to standard dispersive systems and PDA spectrometers in reflectance mode for the commercial, in-line, non-destructive %DM evaluation of avocado fruit. However, further work is required to optimise this technology, with calibration development and speed of throughput for an in-line setting being required for commercial adoption. Also, the calibration models would need to be assessed over several seasons and growing locations to confirm their predictive performance and overall robustness.

CHAPTER 4

EFFECTS OF GEOGRAPHICAL VARIABILITY ON MODEL ROBUSTNESS FOR THE PREDICTION OF DRY MATTER CONTENT FOR 'HASS' AVOCADO FRUIT

This chapter, is based on a publication by the author that investigates the biological variability from different geographic regions as part of developing robust NIR spectroscopy calibration models for commercial applications. The effects from geographic variation is examined in relation to the calibration models prediction performance for determining DM content in Australian 'Hass' avocados. Publication: *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) Near Infrared Spectroscopy as a rapid non-invasive tool for agricultural management with special reference to avocado and Sandalwood industries. Desalination and Water Treatment* ***32****, pp 365-372.*

In general, the robustness of calibration models with respect to biological variability from different geographic regions and seasons has been disregarded and therefore these calibration models may be over-optimistic with respect to prediction accuracies on future samples in practical applications, such as grading lines (Nicolaï et al. 2007). Robustness of calibration models is consequently a critical issue that needs to be addressed (Nicolaï et al. 2007; Sánchez et al. 2003). McGlone et al. (2002) reported that orchard, region and seasonal variations are important to include in experimental design when developing a calibration model for fruit as different growing conditions in different places and seasons could result in biological variability and effect NIR predictions. The inclusion of using 'Hass' avocado fruit from two growing regions (Bundaberg and Childers in Queensland, Australia) is to determine if a robust calibration model can be developed for farms that are geographically close to each other (approximately 60 kilometres apart) within the same growing district; or if there is a need to develop separate calibrations models for each individual farm. If separate calibration models are required then this would suggest that there is substantial variability for individual farms even within the same growing district and within close proximity to each other.

In the presented publication by the author in this chapter, the author had the opportunity to additionally discuss the application of NIR spectroscopy as a diagnostic tool in agricultural and environmental applications through the successful assessment of NIR spectroscopy to predict α-santalol in sandalwood chip samples. The sandalwood component of the publication has been removed for this chapter, however, the theme of the introduction reflects the focus of the publication on agricultural and environmental management.

4.1 Near Infrared Spectroscopy as a rapid non-invasive tool for agricultural management with special reference to avocado

4.1.1 Introduction

NIR spectroscopy 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 (Batten 2004). Climate change and nutrient pollution are currently the top two global environmental changes most rapidly increasing in their negative impact on the ecosystem (Malley and Williams 2005). This increases the need for reliable and rapid analytical information to achieve more environmentally friendly and sustainable practices. NIR spectroscopy 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 NIR spectroscopy relies on calibrations in known data sets, which utilize absorbance's at many wavelengths, to predict the composition of a sample (Moron and Cozzolino 2003; Batten 1998). However, to develop these calibrations requires many samples, many hours of work and many computer calculations (Davies 2005). The time-consuming collection of reference samples and the lack of reliable, precise chemical information may be a factor limiting the adoption of NIR spectroscopy (Malley and Williams 2005; Batten 2004).

The advantage of NIR spectroscopy 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.

NIR spectroscopy has received considerable attention over the years for analysis of plant material for many constituents including: oil, moisture, pH, acidity, SS, protein, lignin and cellulose (Tandy et al. 2010; Shepherd and Walsh 2007; Butz et al. 2005; McClure et al. 2002; Reeves et al. 2000; Abbott 1999; Ciavarella et al. 1998; Scotter 1990b). NIR spectroscopy 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 (Batten 2004), and is utilized in plant breeding programs (Shepherd and Walsh 2007). NIR spectroscopy has become accepted by the international standards committees in some agricultural applications within the feed and food sectors (Shepherd and Walsh 2007). More recently, there has been increasing interest in soil NIR spectroscopy 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 (Reeves et al. 1999). For example, NIR spectroscopy 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 (Tandy et al. 2010; Albrecht et al. 2008; Christy and Kvalheim 2007; Shepherd and Walsh 2007; Moron and Cozzolino 2003; Cho et al. 1998), with prospects for predicting soil fertility, soil erosion, soil infiltration capacity and plant growth (Shepherd and Walsh 2007).

Shepherd and Walsh (2007) 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. NIR spectroscopy assessment in the laboratory or in the field has the potential to allow many samples to be tested rapidly and economically (Malley and Williams 2005; Batten 2004). Pollution of water resources by domestic and/or industrial discharges has increased considerably in urban centres as a consequence of population growth (Sousa et al. 2008). The application of NIR spectroscopy 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 NIR spectroscopy is emerging for the detection of organic pollutants such as chlorinated hydrocarbons, pesticides and endocrine disrupting compounds (Shepherd and Walsh 2007). NIR spectroscopy has also been demonstrated as a promising alternative for determination of chemical oxygen demand in domestic waste water (Sousa et al. 2008).

The application of NIR spectroscopy in industrial process and waste water monitoring holds great potential as an on-line, real-time monitoring tool. The real-time monitoring capacity of NIR spectroscopy 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 (Dias et al. 2008). NIR spectroscopy 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 (Kawai et al. 2009; Suehara et al. 2007). Preliminary work by Dias et al. (2008) supports the use of NIR spectroscopy 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 NIR spectroscopy as a tool in agriculture, waste water and environmental management. Despite this fact NIR spectroscopy is not yet widely adopted commercially for environmental applications (Malley and Williams 2005).

The aim of this study was to assess the potential of FT-NIR spectroscopy 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-NIR spectroscopy to predict 'Hass' avocado maturity based on dry organic matter content and the *effects of geographic location on model performance*.

4.1.2 Materials and Methods

4.1.2.1 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 broad DM 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.

4.1.3 NIR method and data collection

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 6.5) in the 780-2500 nm range NIR spectral data collection on avocado fruit was as per *Section 3.3.2.4*.

4.1.4 Avocado dry matter analysis

The %DM reference measurement was obtained from the same area of the fruit that was used to obtain the NIR spectrum as per *Section 3.3.2.3*. The flesh was diced to facilitate drying in a fan-forced oven at 60-65°C to constant weight (approximately 72 h). 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.

4.1.5 NIR data analysis

Statistical analysis was conducted using the commercially available chemometric software package 'The Unscrambler™' version 9.8 (CAMO, Oslo, Norway). PCA was performed before 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 pre-treatment and smoothing for all avocado models in this study were 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). Significant noise was found within spectral ranges 780-843 and 2414-2503 nm for all spectra. *Figure 4.1* depicts representative avocado raw spectra from the sample population.

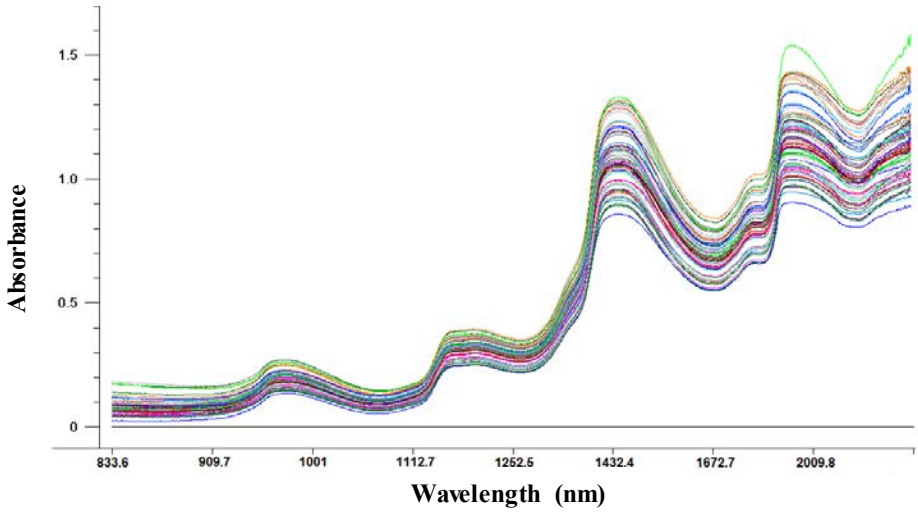


Figure 4.1. Representative raw avocado spectra from the sample population.

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 R^2 of the calibration (R_c^2) and prediction (R_v^2) data sets; RMSECV and RMSEP in relation to the bias (average difference between predicted and actual values) (Buning-Pfaue 2003). The 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) as presented in *Section 2.3.6.1* and *Section 2.4*.

4.1.6 Results and discussion

With horticultural products, a major challenge with NIR spectroscopy 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 (Marques et al. 2006; Peirs et al. 2003b). 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 4.1*. 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_v^2 = 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 (Williams 2008; Nicolaï et al. 2007; Schimleck et al. 2003), and would allow for grading into three groups (McGlone and Kawano 1998). 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_v^2 = 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_v^2 = 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 4.1. 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	L V	R^2	RM SECV	RM SEP	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 *Figure 4.2*). 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.

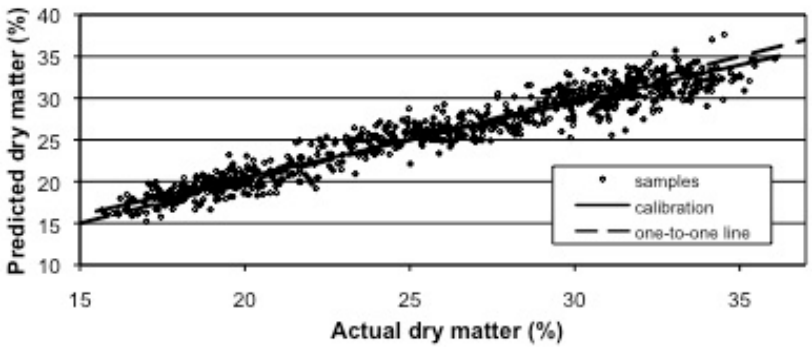


Figure 4.2. Test set validation for predicted vs. actual dry matter content (%) in avocado samples.

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.

4.1.7 Conclusion

NIR spectroscopy 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 NIR spectroscopy. 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, NIR spectroscopy 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, NIR spectroscopy has the potential to be used as a major decision making tool for many agricultural and environmental applications.

CHAPTER 5

THE EFFECT OF SEASONAL VARIABILITY ON MODEL ROBUSTNESS FOR THE PREDICTION OF DRY MATTER CONTENT FOR AUSTRALIAN 'HASS' AVOCADO FRUIT

This chapter, represents three publications by the author that investigate the biological variability influences from different seasons (time – over 3 consecutive years) as part of developing robust FT-NIR calibration models for commercial applications in an inline setting. The effects from seasonal variability (time) is examined in relation to the calibration models prediction performance for determining DM content in Australian 'Hass' avocados.

As identified in literature, seasonal variability has a significant effect on model predictive performance for horticultural produce (Penchaiya et al. 2009; Golic and Walsh 2006; Guthrie et al. 2006; Guthrie et al. 2005a; Liu et al. 2005; Peirs et al. 2003b; McGlone et al. 2002; Peiris et al. 1998; Miyanoto and Yoshinobu 1995). 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. Peirs et al. (2003b) report that season variability was responsible for approximately 31% of the spectral variability in the calibration model developed for SS in apples. If the calibration model is not robust in design it will limit the application of the technique, particularly in a commercial setting.

The first two publications by the author relating to the topic covered in this chapter, present the same data set and associated calibration models based on a 'Hass' avocado fruit population from the Childers growing district in Queensland, Australia, produced over three consecutive growing seasons 2006, 2007 and 2008. However, each publication presents calibration models based on different pre-processing techniques and the outcome of these different techniques on overall model performance. For this reason one paper is presented in full in *Section 5.1* while a short summary of results and discussion of the second publication is provided in *Appendix B*.

Section 5.1 is based on the publication: *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2013) Effects of seasonal variability on FT-NIR model robustness for the prediction of dry matter content for whole Hass avocado fruit. Postharvest Biology and Technology 75, pp 9-16.* All full spectrum models presented in this study were based on a combination of a 25-point SG spectral smoothing (2^{nd} order polynomial) and a MSC transformation.

Appendix B summarises the results and discussion from the publication: *Wedding. B.B.; Wright, C.; Grauf. B.; White, R.D. and Gadek, P. (2010) Prediction of Hass avocado maturity via FT-NIRS. In: NIR 2009 - Breaking the Dawn, Proceedings of the 14th International conference on Near Infrared Spectroscopy, Bangkok , Thailand, 7-16 November 2009, pp 260-272 IM Publications West Sussex*. All models presented in this study were based on a combination of a 25 point SG spectral smoothing (2^{nd} order polynomial) and a first derivative transformation (25 point SG smoothing and 2^{nd} order polynomial).

The third publication by the author in *Section 5.2*, presents a data set and associated calibration models based on a 'Hass' avocado fruit population from the Bundaberg growing district in Queensland, Australia, produced over three consecutive growing seasons 2006, 2007 and 2008. Thus, providing research into the effects of geographic variation on model robustness from two separate growing districts. *Section 5.2* is based on the publication: *Wedding, B; White, R; Grauf, S.; Wright, C; Tilse, B; Fitzsimmons, J.; Hofman, P and Gadek, P. (2009) NIRS technology for determining maturity in avocados. In: 4th Australian and New Zealand Avocado Growers Conference, Cairns 21-24 July 2009.*

5.1 Effects of seasonal variability on FT-NIR prediction of dry matter content for whole 'Hass' avocado fruit

5.1.1 Introduction

Most manual and automatic commercial quality grading systems for fruit and vegetables are based on external features of the product, for example: shape, colour, size, weight and blemishes (Cubero et al. 2010; Kondo 2010; Blasco et al. 2003). For avocado fruit, external colour is not a maturity characteristic, neither is smell as it is too weak and appears later in its maturity stage (Gaete-Garreton et al. 2005). There are only minor visible changes in the external appearance of the fruit that can be used in determining maturity. For example, some loss of skin glossiness, surface russeting increases, and the appearance of some cultivars change from green to black or purple with increasing maturity (Bergh et al. 1989; Lewis 1978). Selection of picking dates based on fruit size and weight within a variety has been extensively used in Florida (Lewis 1978). Studies have shown that, in general, larger fruit have higher flavour ratings than small fruit when tested early in the season at the time of minimum market acceptability (Bower and Cutting 1988; Lewis 1978). However, as the season progresses, differences between large and small fruit become less pronounced (Bower and Cutting 1988; Lee 1981). Unfortunately, many of these characteristics that show a trend with maturation are not applicable for determining maturity on a commercial basis. As 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. Mature avocado fruit do not ripen on the tree, but soften several days after being picked (Schmilovitch et al. 2001).

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 and Flitsanov 1999). Avocados Australia Limited (2008) recommend a minimum maturity standard for its growers of 23% DM (greater than 10% oil content) for 'Hass' avocados, although consumer studies indicate a preference for at least 25% DM (Harker et al. 2007). A rapid and non-destructive system that can accurately and rapidly monitor internal quality attributes (in this case %DM) would allow the avocado industry to provide better, more consistent eating quality fruit 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 and emphasis is put on the development of these methods for real-time in-line applications. Although several non-invasive techniques exist for this, NMR and NIR spectroscopy are leading candidates for the application to fruit and vegetables. NMR has been demonstrated to have the potential to measure

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

The potential of NIR spectroscopy to assess internal quality attributes of intact horticultural produce is well established in literature. However, in the majority of publications, the robustness of calibration models with respect to biological variability from different seasons has been neglected and therefore these calibration models may be ambitious with respect to predicting on future samples in practical applications, such as grading lines (Nicolaï et al. 2007). For example, Nicolaï et al. (2007) reports that a typical RMSEP for %SS on fruit seems to be around 0.5% SS, but in the few applications where validation sets from different orchards or seasons were externally used to calculate the RMSEP it is considerably higher (1-1.15% SS). The authors report that model 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 (Nicolaï et al. 2007; Golic and Walsh 2006). Prediction bias for new populations can be corrected by model updating or direct bias adjustment (Golic and Walsh 2006; Fearn 2001). Robustness of calibration is a critical issue and an active area of research (Nicolaï et al. 2007; Sánchez et al. 2003). Some of the published work on fruit that considers the effect of different seasons includes: Peiris et al. (1998), Peirs et al. (2003b), Miyanoto and Yoshinobu (1995), Liu et al. (2005) and Guthrie et al. (2005a). These studies generally found that incorporating data from multiple growing seasons in the calibration model improved the predictive performance in comparison to calibration models developed using an individual season. The published study of Peiris et al. (1998) on model robustness for the determination of %SS content of peaches 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 (SEP of 0.94-1.26% SS, and bias 0.17-0.38% SS) than that developed from an individual season population (SEP of 0.90-1.36% SS and bias 0.17-2.08% SS). Using 'Golden Delicious' apples, Peirs et al. (2003b) studied the robustness of calibration models for % SS content with respect to the effect of orchard, season and cultivar. The authors reported that the largest source of spectral variation between different fruit measurements was caused by seasonal effect. The validation errors for the calibration models based on the data of three individual seasons for %SS content varied from 1.09 to 2.92% SS. When more variability was included in the calibration set, for example the model based on the data of all three seasons, the predictive error reduced to 0.9% SS.

Miyanoto and Yoshinobu (1995) report the use of a calibration model developed over three consecutive years to predict total %SS content of 'Satsuma' mandarins. As expected, the models performed well against the prediction set of the same harvest season (SEP of 0.55-0.58, bias of

0.01) with a reduced performance against a different harvest season prediction set (SEP of 0.51-0.68, bias of ≤0.40). Prediction statistics for the model combining data from all three production years predicted well against every season (SEP of 0.5-0.59, bias of <0.09). Also using mandarins, Guthrie et al. (2005a) reports that model predictions for total SS of intact 'Imperial' mandarin fruit were more variable and less robust across seasons than across harvest days or location. Similarly, the study by Liu et al. (2005) looking at the effect of the biological variability on the robustness of models for sugar content of three pear cultivars ('Xueqing', 'Xizilu' and 'Cuiguan') reports that the largest source of spectral variation between different pear fruit measurements was caused by the seasonal effect.

The application of NIR spectroscopy to determine DM content in avocados has been demonstrated in the studies of Schmilovitch et al. (2001) and Clark et al. (2003), utilising a dispersive NIR spectrophotometer in reflectance mode and a fixed PDA spectrophotometer, respectively. While full transmittance mode is not possible for this fruit, reflectance and interactance modes have been studied, producing equivalent accuracies (Wedding et al. 2010; Clark et al. 2003; Schmilovitch et al. 2001). For commercial inline applications requiring commercial speeds, reflectance is the preferred technique. Schmilovitch et al. (2001) assessed %DM of both 'Fuerte' and 'Ettinger' cultivars during a single season, while Clark et al. (2003) measured %DM of 'Hass' avocados harvested at discrete intervals in a single growing season. Similarly, Wedding et al (2010), used FT-NIR spectroscopy to assess DM content in 'Hass' avocados of a single farm over a full growing season. However, no reported studies have investigated model robustness for avocado fruit over several seasons.

The validity of the calibration models for future predictions depends on how well the calibration set represents the composition of future samples (Liu and Ying 2005). Fruit composition (i.e., sugar, acid, oil, cell number, size and structure, and the amount of intercellular spaces) is subject to within tree variability (i.e., tree age, crop load, position within the tree, light effects); within orchard variability (i.e., geographical variation, light effects); and intra-orchard variability (i.e., soil characteristics, nutrition, weather conditions, fruit age and season variability) (Marques et al. 2006; Liu and Ying 2005; McCarthy 2005). 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 present study represents the first study to investigate the effect of seasonal variation on model robustness to be applied to avocado fruit.

The aim of the current study was to assess the potential of FT-NIR diffuse reflectance spectroscopy as an objective non-invasive method to assess 'Hass' avocado maturity and thereby

eating quality based on %DM and its ability to predict over several growing seasons for possible implementation in a commercial in-line application.

5.1.2 Materials and Methods

5.1.2.1 Sample selection

'Hass' avocado fruit were obtained over the 2006, 2007 and 2008 growing seasons (harvest months: May to August) from a single farm in the major production district of Childers, Queensland (Latitude: 25° 14' S, Longitude: 152° 16' E). Avocado fruit were harvested from the same trees in the orchard within an individual year, at three maturity stages corresponding to early, mid, and late season harvests over the three growing seasons. The three harvests obtained throughout each individual season were to ensure an extensive range of DM was collected to cover within seasonal variability. A minimum of 100 fruit were collected at each harvest giving a total of 925 individual fruit across the nine harvests. All fruit were harvested at the hard green stage of ripeness.

5.1.2.2 Spectral acquisition

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 780-2500 nm range NIR spectral data collection on avocado fruit was as per *Section 3.3.2.4*. Due to the large variability in %DM within a fruit (Wedding et al. 2010; Woolf et al. 2003; Schroeder 1985), data from both sides of the 925 fruit were used in the development of the model giving a total of 1850 fruit spectra.

5.1.2.3 Chemical analysis

The %DM reference measurement was obtained from the same area of the fruit that was used to obtain the NIR spectrum as per *Section 3.3.2.3*. The flesh was diced to facilitate drying in a fan-forced oven at 60-65°C to constant weight (approximately 72 h). This laboratory reference method for %DM estimation was determined to have a repeatability error of approximately 0.5%. 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.

5.1.2.4 Data analysis

Data analysis was carried out using 'The Unscrambler' Version 9.8 (Camo, Oslo, Norway). 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 the development of the calibration model, the variation of the spectral data was investigated by PCA and obvious atypical spectra eliminated. Among all spectra collected, significant noise was found at the extremities of the

spectral range (830-843 and 2414-2500 nm). Therefore all the raw spectra used for analysis were truncated to a range of 843-2414 nm. A typical absorbance spectrum for 'Hass' avocado fruit is shown in *Figure 5.1*. All full spectrum models presented in this study were based on a combination of a 25-point SG spectral smoothing (2nd order polynomial) and a MSC transformation. Model performance was based on the R^2 of the calibration (R_c^2) and validation/prediction (R_v^2); RMSECV; RMSEP in relation to the bias (average difference between predicted and actual values); SDR/RPD (Walsh et al. 2004) and SDR/RPD (Williams 2008).

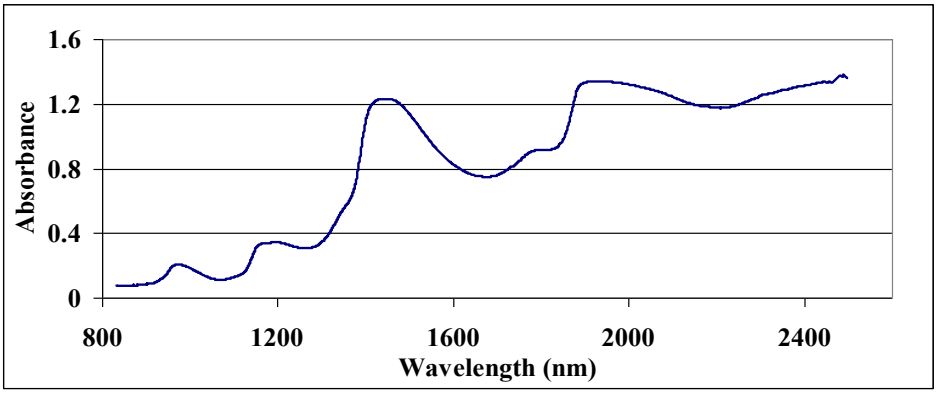


Figure 5.1. Typical absorbance spectrum for whole 'Hass' avocado fruit from the Childers region.

Calibration models were developed for each individual season, two seasons combination and for a combined data set encompassing all three seasons. The sample spectra for each data set were separated into a calibration (CAL) set and prediction (PRE) set (see *Table 5.1*). Fruit were assigned to the calibration sets from the PCA to provide global representation of the attributes of the entire fruit populations while eliminating repetition. All remaining fruit were used in the validation sets.

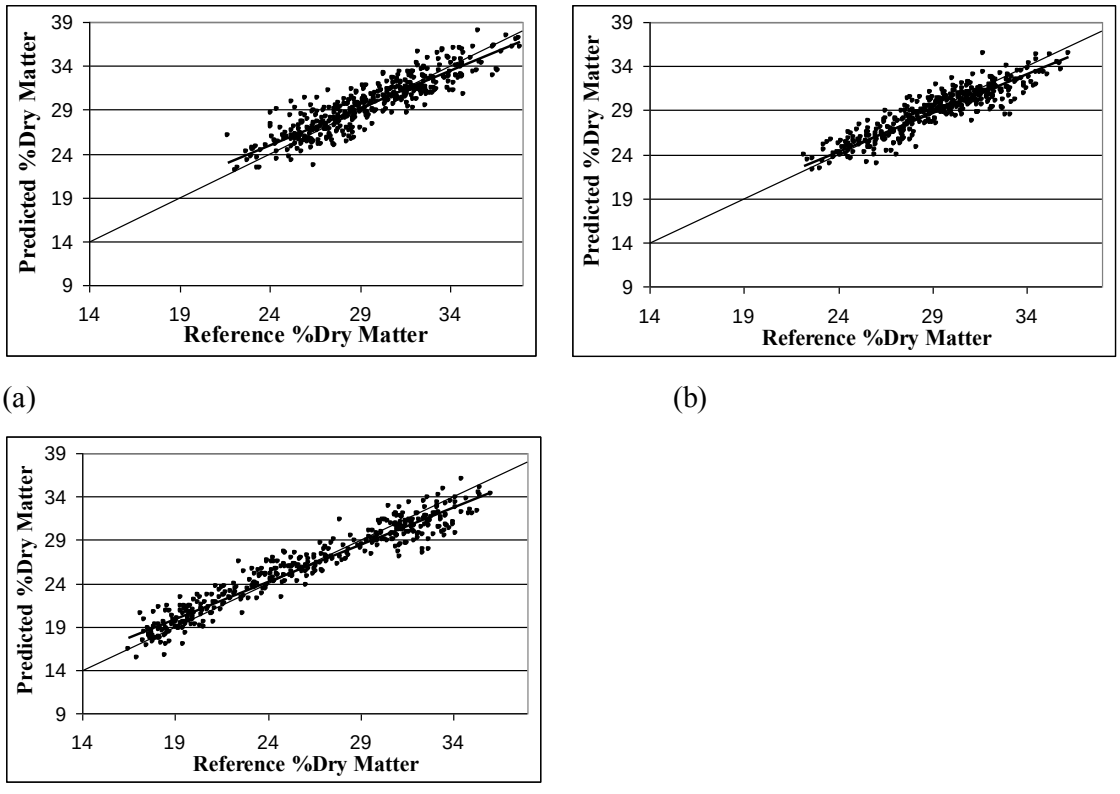
5.1.3 Results and Discussion

The calibration and prediction model statistics for each individual year (see *Table 5.1*) indicate that FT-NIR spectroscopy in diffuse reflectance has potential as a screening tool to predict %DM on whole 'Hass' avocado fruit. The 2006 (n = 632) and 2007 (n = 609) harvest seasons had lower SD than the 2008 season (n = 608). The 2008 harvest season calibration and prediction statistics were the best in terms of regression (R^2) and SDR/RPD due in part to the larger SD for this population. The RMSEP for each harvest season varied between 1.29 to 1.49% DM. The number of LV's are within an acceptable range for the number of samples for all models (Lammertyn et al. 2000; Hruschka 1987). Scatter plots of the NIR predicted values against the reference DM values for each individual season are shown in *Figure 5.2*.

Table 5.1. PLS calibration and prediction statistics for % dry matter for whole 'Hass' avocado fruit harvested over the 2006, 2007 and 2008 seasons.

Year	Spectra n (OR)	% DM range	Mean	SD	LV	R^2	RM SECV	RM SEP	Bias	Slope	SDR (RPD)
2006		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 (2.4)
PRE	425 (0)	21.7-37.9	29.5	3.3	9	0.8		1.47	0.0761	0.850	2.2 (2.2)
2007		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 (2.4)
PRE	400 (1)	22.2-36.2	29.2	3.0	8	0.81		1.29	-0.2867	0.835	2.3 (2.3)
2008		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 (3.8)
PRE	399 (0)	16.5-36.1	26.0	5.4	7	0.92		1.49	-0.1594	0.858	3.6 (3.5)

Note: OR = outliers removed; n = sample size.



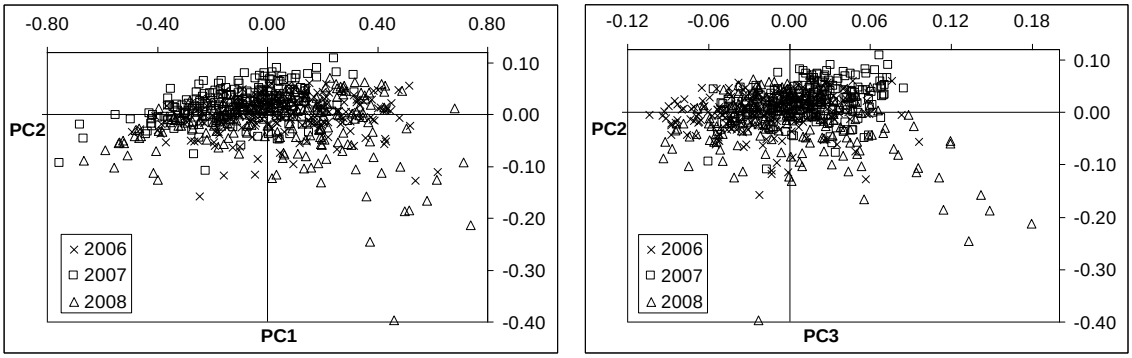
(a) (b)

(c)

Figure 5.2. Individual season model predictions plotted against reference values for % dry matter as presented in *Table 5.1* for (a) the 2006 season, (b) the 2007 season, and (c) the 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 in general be predicted (Peirs et al. 2003b). The influence of seasonal variability was subsequently investigated

over the individual years and by combining all three years. Each individual year calibration model in *Table 5.1* was used to predict the other two individual years. Three calibration models were developed by combining two individual years, which were then used to predict the remaining year. A combined calibration set of 2006, 2007 and 2008 seasons (n = 624) was used to predict a validation set of samples drawn from all 3 years (n = 1224). The score plots for the first three PC's (see *Figure 5.3*) displays the population distribution of the three seasons combined PLS model and shows no clear separation among the three harvest seasons. *Table 5.2* displays the summary statistics of the PLS calibration and prediction models for these combinations.



(a) (b)

Figure 5.3. Score plots of the principal components for the combined 2006-08 seasons PLS calibration model: (a) PC1 versus PC2, and (b) PC3 versus PC2.

As expected the application of a single-season calibration to a population from another growing season was not as successful as multi-season calibrations. For example, the 2006 calibration model could not be used to predict either the 2007 or 2008 season populations (see *Table 5.2*). The R_v^2 and RMSEP for the single season prediction models in *Table 5.2* ranged from 0.09 to 0.61 and 2.63 to 5.00, respectively, while for the two-season models, they ranged from 0.34 to 0.79 and 2.18 to 2.50, respectively. The bias for single-season models predicting an independent season was as high as 4.429 but ≤1.417 for the two-season combined models. The combined 2006, 2007 and 2008 calibration model was sufficiently more robust to predict %DM of whole 'Hass' avocado for the selected validation population (see *Figure 5.4*) to within 1.43% with an R_v^2 = 0.89 and SDR/RPD of 3.0. This indicated an ability to sort the fruit into three categories with approximately 80% accuracy (Guthrie et al. 1998). These results suggest that the issue of model robustness for predicting new seasons requires major consideration. The inclusion of further seasonal biological variability needs to be addressed to assist in the development of a robust model in order to adequately predict on future populations.

Table 5.2. PLS calibration and prediction statistics for % dry matter for whole Hass avocado fruit for individual seasons, two seasons combined, and all seasons combined (2006-08) models.

Harvest		Spectra n (OR)	SD	LV	R^2	RM SECV	RM SEP	Bias	Slope	SDR (RPD)
Calibration	Prediction									
2006		207 (2)	3.7	9	0.82	1.57		0.006	0.829	2.4 (2.4)
	2007	609 (0)	3.1	9	0.14		2.84	1.601	0.328	1.1 (1.1)
	2008	608 (0)	5.3	9	0.12		5.00	4.429	0.6538	1.1 (1.1)
2007		209 (0)	3.3	8	0.83	1.36		-0.010	0.842	2.4 (2.4)
	2006	632 (0)	3.4	8	0.42		2.63	-1.201	0.533	1.3 (1.3)
	2008	608 (1)	5.3	8	0.61		3.32	2.722	0.879	1.6 (1.6)
2008		209 (2)	5.2	7	0.93	1.39		0.010	0.934	3.8 (3.8)
	2006	632 (2)	3.4	7	0.09		3.28	-1.734	0.296	1.0 (1.0)
	2007	609 (0)	3.1	7	0.22		2.71	-1.599	0.608	1.1 (1.1)
2006 & 07		415 (1)	3.5	12	0.82	1.49		0.003	0.830	2.4 (2.4)
	2008	609 (0)	5.3	12	0.79		2.45	-0.547	0.863	2.2 (2.2)
2006 & 08		380 (3)	5.1	8	0.88	1.77		0.003	0.882	2.9 (2.9)
	2007	608 (0)	3.1	8	0.34		2.50	-1.417	0.482	1.2 (1.2)
2007 & 08		368 (2)	4.9	8	0.89	1.66		0.003	0.891	2.9 (3.0)
	2006	632 (0)	3.4	8	0.60		2.18	0.552	-0.672	1.6 (1.6)
Combined 2006-08		624 (1)	4.6	10	0.88	1.62		-0.001	0.879	2.8 (2.9)
	Combined 2006-08	1224 (0)	4.3	10	0.89		1.43	-0.021	0.857	3.0 (3.0)

Note: OR = outliers removed; n = sample size.

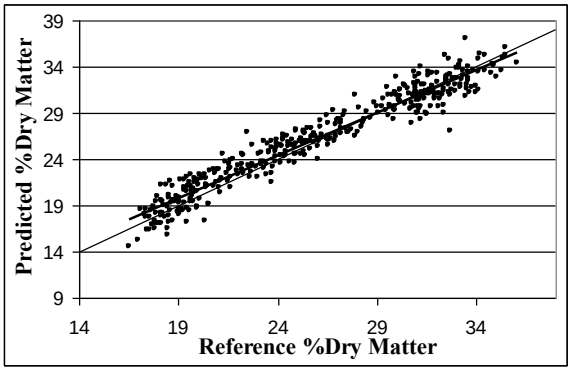


Figure 5.4. Model prediction for the combined 2006-08 calibration model predicting on the combined 2006-08 prediction set plotted against reference values for % dry matter.

This study is in agreement with the findings of Peiris et al. (1998), Peirs et al.(2003b), Miyanoto and Yoshinobu (1995), Liu et al. (2005) and Guthrie et al. (2005a) that incorporating data from multiple growing seasons in the calibration model will improve the predictive performance, in

comparison to calibration models developed using an individual season. As more biological variability is taken into account, the prediction accuracy becomes less sensitive to unknown changes of external factors (Bobelyn et al. 2010). However, in some cases, incorporation of 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).

It can be very difficult to interpret NIR models in terms of how various fruit components contribute to a model. Spectral co-linearity can mean that information in a model may not necessarily be carried by just a few independent wavelengths, but could well be due to the combined effect of many wavelengths with each contributing only relatively little information (McGlone and Kawano 1998). 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 (Saranwong and Kawano 2007; Guthrie et al. 2004). Models based on the short-wavelength NIR region only were also developed for the individual and combined seasons and are presented in *Table 5.3* and *Table 5.4*. These models required fewer latent variables and in general resulted in an increased RMSEP and decreased R^2 and SDR.

Table 5.3. PLS calibration and prediction statistics for % dry matter for whole 'Hass' avocado fruit harvested over the 2006, 2007 and 2008 seasons in the short-NIR region (<1100 nm) using a combination of a 25 point SG spectral smoothing (2nd order polynomial) and a first derivative transformation (SG 25 point spectral smoothing).

Year	Spectra n (OR)	% DM range	Mean	SD	LV	R^2	RMSECV	RMSEP	Bias	Slope	SDR (RPD)
2006		21.4-39.7	29.8	3.4							
CAL	207 (4)	21.4-39.7	30.2	3.7	4	0.80	1.62		0.004	0.809	2.3 (2.3)
PRE	425 (2)	21.7-37.9	29.5	3.3	4	0.74		1.66	0.024	0.807	2.0 (2.0)
2007		21.9-36.8	29.2	3.1							
CAL	400 (1)	21.9-36.8	29.2	3.3	4	0.79	1.53		0.003	0.787	2.1 (2.1)
PRE	400 (1)	22.2-36.2	29.2	2.9	4	0.75		1.54	-0.168	0.785	1.9 (1.9)
2008		16.1-36.2	25.8	5.3							
CAL	209 (0)	16.1-36.2	25.6	5.2	4	0.91	1.58		-0.007	0.912	3.3 (3.3)
PRE	399 (0)	16.5-36.1	26.0	5.4	4	0.91		1.63	-0.008	0.877	3.3 (3.3)

Note: OR = outliers removed; n = sample size; CAL – calibration; PRE = prediction.

Table 5.4. PLS calibration and prediction statistics for % dry matter for whole Hass avocado fruit for individual seasons, two seasons combined, and all seasons combined (2006-08) models for the short-NIR region (<1100 nm) using a combination of a 25 point SG spectral smoothing (2nd order polynomial) and a first derivative transformation (SG 25 point spectral smoothing).

Harvest		Spectra n (OR)	SD	LV	R^2	RM SECV	RM SEP	Bias	Slope	SDR (RPD)
Calibration	Prediction									
2006		207 (4)	3.7	4	0.80	1.62		0.004	0.809	2.3 (2.3)
	2007	608 (0)	3.1	4	-		3.47	-2.578	0.369	0.9 (1.3)
	2008	607 (0)	5.3	4	0.73		2.76	1.346	0.662	1.9 (2.2)
2007		209 (0)	3.3	4	0.79	1.53		0.003	0.787	2.1 (2.1)
	2006	632 (0)	3.4	4	-		8.99	-8.39	0.172	0.4 (1.1)
	2008	607 (0)	5.3	4	0.08		5.11	-4.29	0.584	1.0 (1.9)
2008		209 (0)	5.2	4	0.91	1.58		-0.007	0.912	3.3 (3.3)
	2006	632 (0)	3.4	4	0.25		2.99	-1.531	0.384	1.2 (1.3)
	2007	609 (0)	3.1	4	0.48		2.22	0.116	0.429	1.4 (1.4)
2006 & 07		415 (0)	3.5	5	0.74	1.80		0.007	0.745	2.0 (2.0)
	2008	607 (0)	5.3	5	0.76		2.62	-1.415	0.761	2.0 (2.4)
2006 & 08		380 (1)	5.1	5	0.89	1.72		0.003	0.888	3.0 (2.9)
	2007	608 (0)	3.1	5	0.33		2.50	-1.314	0.532	1.2 (1.4)
2007 & 08		403 (0)	4.7	6	0.87	1.71		0.002	0.877	2.8 (2.8)
	2006	632 (0)	3.4	6	0.25		2.98	-1.479	0.386	1.2 (1.3)
Combined 2006-08		624 (1)	4.6	6	0.85	1.80		-0.002	0.849	2.6 (2.6)
	Combined 2006-08	1223 (0)	4.3	6	0.84		1.75	-0.019	0.824	2.5 (2.5)

Note: OR = outliers removed; n = sample size.

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. In this instance the long-wavelength region appears to provide some relevant information relating to avocado maturity. For example, in avocado, the exocarp or skin, endocarp, and the seed contain lipids (Lewis 1978). A relatively thin cuticle forms a wax-like film over the surface of the fruit (Cummings and Schroeder 1942). This cuticular wax contains fatty acids, alcohols, and paraffin's and has been studied as a measure of maturity (Lewis 1978; Erickson and

Porter 1966). Erickson and Porter (1966) report that the cuticle wax on 'Hass' avocados increased in amount per unit surface area during the entire period of fruit development and that cuticular wax concentrations determined by infrared spectroscopy related with flesh oil levels.

In this study, the regression (β) coefficients for the individual season DM calibration models in *Table 5.1* had many similar peak positions over the 850-2250 nm range. However, as expected, there were slight differences in the wavelength selection from one year to another that can be attributed to seasonal variability. Relevant spectral information for all calibration models was obtained primarily from oil, carbohydrate, and water absorbance bands clustered in the 890-980, 1005-1050, 1330-1380 and 1700-1790 nm regions. The β coefficients for the combined 2006-2008 calibration model are displayed in *Figure 5.5*. This is consistent with the findings of Guthrie et al., (2004); Clark et al., (2003); Osborne et al., (1993) and Williams and Norris (1987). For example, 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 930 nm (overtones of CH_2 stretching) (Guthrie et al. 2004; Clark et al. 2003; 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).

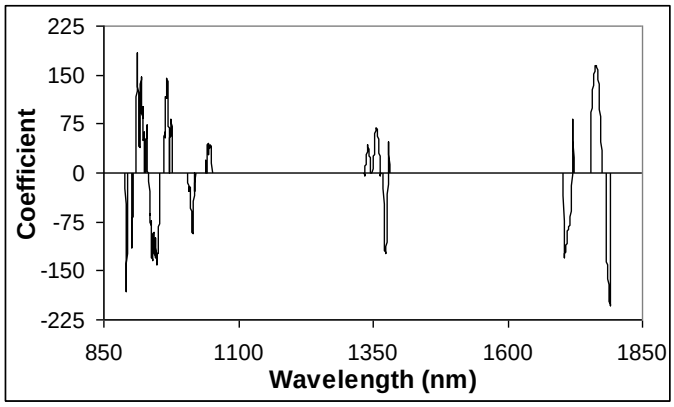


Figure 5.5. β coefficients for the 2006-2008 combined model.

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_v^2 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-NIR spectroscopy reflectance combined model compares well with the model accuracy obtained by Clark et al. (2003) (R_v^2 of

0.88 and an RMSEP of 1.8 %DM) using a PDA spectrometer in interactance mode. This indicates that reflectance FT-NIR spectroscopy may be a suitable alternative for in-line and at-line environments. Other comparative data are those of Schmilovitch et al. (2001) in which two relatively thin skin cultivars, 'Ettinger' and 'Fuerte', were investigated during a single season. The authors 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. It must be 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 techniques or with the geometry of the configurations used.

5.1.4 Conclusion

The present study 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. The results indicate the potential of FT-NIR spectroscopy 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 variation in the calibration model. FT-NIR reflectance spectroscopy therefore shows promise for application in a commercial, in-line setting for the non-destructive %DM evaluation of avocado fruit, although optimisation of the technology is required to address speed of throughput and environmental issues. Incorporating fruit physiological variability over future seasons will be essential to further increase model robustness and ensure predictive performance. The ability to develop calibration models valid across various growing districts remains an issue to be addressed.

5.2 NIR spectroscopy technology for determining maturity in avocados

5.2.1 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. 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 a fruit is 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 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, smell is too weak and appears later in its maturity stage (Gaete-Garreton et al. 2005). There are only minor visible changes in the external appearance of the fruit that can be used in determining maturity. As 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 and Flitsanov 1999). The recommended minimum maturity standard for Australia (legally enforced in Western Australia) is 21% DM (approximately 10% oil content) for all cultivars (McCarthy 2001), although consumer studies for 'Hass' indicate a preference for at least 25% DM (Harker et al. 2007). A rapid and non-destructive system that can accurately and rapidly monitor internal quality attributes (in this case %DM) 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: NMR and 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, NMR and NIR spectroscopy are leading candidates for the application to fruit and vegetables. NMR has been demonstrated to have the potential to measure the DM percentage in avocados

(Kim et al. 1999; Chen et al. 1993), but the cost and challenges of in-line use in the sorting line means that commercial use is some time off (Clark et al. 2003).

NIR spectroscopy is a non-invasive method of measuring internal/external quality and safety attributes of horticultural products using optical light to determine chemical composition. The technology offers the advantage of being non-destructive, fraction of a second per test, and has the potential to test every piece of product in an in-line application for various internal attributes simultaneously including: sweetness, ripeness, maturity, acidity and chemical characteristics. Such technologies may also be utilised as tools for quality control within manufacturing plants or in the production environment, and within breeding programs.

The potential of NIR to determine DM content in avocado has been demonstrated in the studies of Schmilovitch et al. (2001) and Clark et al. (2003). The validity of the calibration models for future predictions depends on how well the calibration set represents the composition of new samples (Liu and Ying 2005). Fruit composition (i.e., sugar, acid, oil, cell size, number of cells and amount of intercellular spaces) is subject to within tree variability (i.e., tree age, crop load, position within the tree, light effect); within orchard variability (i.e., geographical variation, light effects); and intra-orchard variability, such as soil characteristics, nutrition, weather conditions, fruit age and season variability (Marques et al. 2006; Liu and Ying 2005; McCarthy 2005; Peirs et al. 2003b). 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. The aim of this project was to assess the potential of FT-NIR diffuse reflectance spectroscopy as an objective non-invasive method to assess 'Hass' avocado maturity and thereby eating quality based on %DM and its ability to predict over several seasons. This project looked to develop robust calibration models over several growing seasons for possible implementation in a commercial in-line application.

5.2.2 Materials and methods

5.2.2.1 Avocado selection

'Hass' avocado fruit were obtained over the 2006, 2007 and 2008 growing seasons (Harvest months: May to August) from a single farm in the major production district of Bundaberg, Queensland (Latitude: 24 52' S, Longitude: 152 21' E). Avocado fruit were harvested from the same trees in the orchard for an individual year at three maturity stages 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.

5.2.2.2 Near infrared spectra collection

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 780-2500 nm range NIR spectral data collection on avocado fruit was as per *Section 3.3.2.4*.

5.2.2.3 % Dry matter analysis

The %DM reference measurement was obtained from the same area of the fruit that was used to obtain the NIR spectrum as per *Section 3.3.2.3*. The flesh was diced to facilitate drying in a fan-forced oven at 60-65°C to constant weight (approximately 72 h). 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.

5.2.2.4 Calibration modelling and prediction

PLS regression was used to build the prediction models of the diffuse reflectance spectral data as per *Section 3.3.2.5*. Among all spectra collected, significant noise was found within spectral ranges 2331-2503 nm. Therefore all the raw spectra used for analysis were truncated to a range of 834-2330 nm. The model performance was based on the R^2 of the calibration (R_c^2) and validation/prediction (R_v^2); RMSECV; RMSEP in relation to the bias, and the SDR used to determine the predictive ability of the calibrations. As presented in *Section 2.3.6.1* and *Section 2.4*, McGlone and Kawano (1998) suggest that an SDR of >3 is adequate to support sorting or grading into 3 classes. Golic and Walsh (2006) report that an SDR of 2.5 allows sorting into two grades, while Guthrie et al. (1998), suggest that for NIR spectroscopy to be commercially useful in fruit grading, the technique must be capable of sorting fruit into at least two grades (i.e., above and below an acceptable level) with approximately 80% accuracy. This requirement involves attainment of a validation correlation coefficient of at least 0.65.

5.2.3 Results

Calibration model development with segmented cross validation for %DM was conducted for each individual year and for a combined data set encompassing all years. The sample spectra for each data set were randomly separated into a calibration set and prediction set to develop the calibration and prediction models respectively. A combination of spectral smoothing and a second derivative pre-processing treatment was selected as the optimal mathematical pre-treatment for the spectral regions selected for model development. Summary statistics of the preliminary calibration and prediction models for individual years are depicted in *Table 5.5*.

Table 5.5. Preliminary PLS calibration and prediction results for % dry matter for whole 'Hass' avocado fruit harvested over the 2006, 2007 and 2008 seasons.

Year	Spectra n (outliers)	% DM range	Mean	SD	LV	R^2	RM SECV	RM SEP	SDR
2006		18.23-35.03	27.5	3.2					
Calibration	222 (2)				7	0.75	1.76		1.8
Prediction	407					0.75		1.51	2.1
2007		14.1-34.3	25.7	2.71					
Calibration	225 (2)				8	0.75	1.39		1.9
Prediction	384					0.70		1.41	1.9
2008		15.2-35.46	25.7	5.66					
Calibration	209 (1)				6	0.90	1.77		3.2
Prediction	397					0.88		1.94	2.9

Note: LV = latent variables.

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. 2003b). The influence of seasonal variability was subsequently investigated over the three years and by combining all three years. The 2006 calibration model was used to predict on the 2007 season population. The randomly selected calibrations sets from 2006 and 2007 seasons were combined to develop a calibration model which was then subsequently used to predict on the 2008 season population. A combined calibration set of 2006, 2007 and 2008 seasons was used to predict over all 3 years. A combination of spectral smoothing and a second derivative pre-processing treatment was selected as the optimal mathematical pre-treatment for the spectral regions selected for model development. Table 5.6 displays the summary statistics of the preliminary PLS calibration and prediction models for these combinations.

Table 5.6. Preliminary PLS calibration and prediction statistics for % dry matter for whole 'Hass' avocado fruit for 2006, 2006-7 and 2006-08 (Combined) seasons predicting on 2007, 2008 and 2006-08 (Combined) seasons respectively.

Harvest		Spectra n (outliers)	SD	LV	R^2	RMSECV	RMSEP	SDR
Calibration	Prediction							
2006		222 (2)	3.2	7	0.75	1.76		1.8
	2007	609	2.71		-		5.07	0.5
2006 & 07		426	3.1	9	0.75	1.60		1.9
	2008	606	5.66				4.1	1.4
Combined		595 (10)	4.14	8	0.80	1.78		2.3
	Combined	1250	4.14		0.83		1.75	2.4

5.2.4 Conclusion

The potential of NIR spectroscopy to assess internal quality attributes of intact horticultural produce is well established in literature. However, in the majority of publications, the robustness of calibration models with respect to biological variability from different seasons and years is neglected and therefore these calibration models may be ambitious with respect to predicting on future samples in practical applications, such as grading lines (Nicolaï et al. 2007; Peirs et al. 2003b). For example, Nicolaï et al. (2007) reports that a typical RMSEP for °Brix on fruit seems to be around 0.5% Brix, but in few applications where external validation sets from different orchards or seasons were externally used to calculate the RMSEP it is considerably higher (1-1.15% Brix). The calibration and prediction statistics for each individual year (see *Table 5.5*) indicate that FT-NIR spectroscopy diffuse reflectance has potential as screening tool to predict %DM on whole 'Hass' avocado fruit. The 2006 and 2007 harvest seasons had the lower SD's. This suggests that the fruit obtained from these two years 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 2008 harvest season calibration and prediction statistics were the best in terms of regression (R^2) and prediction (SDR). The RMSEP for each harvest season varied between 1.41 to 1.94% DM.

As expected, the application of these seasonal calibrations to populations involving other growing seasons was not as successful. As shown in *Table 5.6*, the 2006 calibration model could not be used to predict the 2007 season population. Similarly, the combined 2006 and 2007 calibration model struggled to predict on the 2008 season. However, the combined 2006, 2007 and 2008 calibration model was sufficiently robust to predict %DM of whole 'Hass' avocado to within 1.75% with an $R_v^2 = 0.83$. This indicates an ability to sort the fruit into two categories (i.e., above and below an acceptable %DM value) with approximately 80% accuracy (Guthrie et al. 1998). These results are slightly more favourable than those obtained by (Clark et al. 2003) using a fixed PDA spectrometer in reflectance mode (RMSEP of 2.6% DM over a 20-45% DM range and an $R_v^2<0.75$). In fact, the current FT-NIR spectroscopy reflectance based model was equivalent to the accuracy of the NIR spectroscopy interactance mode of Clark et al. (2003) (R_v^2 of 0.88 and an RMSEP of 1.8% DM) indicating reflectance FT-NIR spectroscopy may be a better alternative for in-line and at-line environments. In relation to other avocado studies, Walsh et al. (2004), using a PDA spectrometer reported calibration results of $R_c^2 = 0.79$, RMSECV = 1.14, SDR = 2.2, for %DM on raw absorbance data for whole avocado fruit (unspecified cultivar). Schmilovitch et al. (2001) used a dispersive NIR spectrophotometer in reflectance mode in the 1200-2400 nm range on 'Ettinger' and 'Fuerte' avocado (both relatively thin-skinned cultivars) and reported

preliminary results of errors of prediction (SEP) of 1.3%, $R^2 = 0.8$, for a 14-24% DM range, based on a PLS model with six factors using a first derivative pre-treatment. It is likely that the thin-skin cultivars would not suffer to the same extent from the experimental limitations experienced in the thick rough skin of Hass.

It was found that the models robustness increased across years when including more variability in the calibration set. However, in some cases, incorporation of more data into the calibration set increases the chance of adding atypical data, which may result in reduced model accuracy (Liu and Ying 2005; Peirs et al. 2003b). Overall, FT-NIR reflectance spectroscopy provides an alternative to standard dispersive systems and PDA spectrometers in the commercial, in-line, non-destructive %DM evaluation of avocado fruit. Further work is required to optimise this technology, with calibration development and speed of throughput for an in-line setting being required for commercial adoption. As demonstrated, the calibration models need to be assessed over several years to increase their robustness and ensure their predictive performance. This approach incorporated into automatic sorting equipment would provide high-speed and accurate measurements to enhance the competitive advantage of Australian avocado producers and processors by guaranteeing a high-quality consistent product. Determining the %DM/oil content of fruit at harvest with non-invasive systems will reduce the risk of the consumer buying low eating quality fruit, and significantly improve consumer confidence in the fruit.

CHAPTER 6

COMPARISION OF THE COMBINED SEASONAL AND GEOGRAPHICAL VARIABLITY ON MODEL ROBUSTNESS FOR THE PREDICTION OF DRY MATTER CONTENT FOR AVOCADO FRUIT

This chapter, presents two publications by the author that investigate the biological variability influences from both: i) geographical variability, by incorporating three different growing districts within Queensland, Australia; and ii) in combination with seasonal variability (time – over 3 consecutive growing seasons (2006, 2007 and 2008)), as part of developing robust FT-NIR calibration models for commercial applications in an inline setting. The effects from geographic and seasonal variation is examined in relation to the calibration models prediction performance for determining DM content in Australian 'Hass' avocados. The two publications also include preliminary research findings of developing a non-invasive NIR spectroscopy assessment tool for detecting bruises in whole Australian 'Hass' avocado fruit and for predicting rot susceptibility as an indication of shelf-life. These components of the two publications (including these elements in the introduction) have been removed from *Section 6.1* and *Section 6.2* and are presented in *Chapter 7* which is focussed on rots, bruises and shelf-life.

Section 6.1 is based on the publication: *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) Non-invasive assessment of avocado quality attributes. In: The Proceedings of the VII World Avocado Congress 2011, Cairns – Australia, 5-9 September 2011.* This study investigated:

a) The influences of both geographical and seasonal variability in relation to the calibration model robustness for determining DM content in Australian 'Hass' avocado fruit. Fruit were obtained from two different growing districts within Queensland, Australia (Toowoomba and Bundaberg) over three consecutive growing seasons (2006, 2007 and 2008).

b) NIR instrument comparison for assessing %DM in whole avocados.
Two commercially available NIR systems were investigated, including a Bruker Matrix-F FT-NIR spectrophotometer (780-2500 nm range) for single point assessment and a HyperVision™ in-line grading system using a line scan camera based (CCD) system operating in both the visible and NIR spectrum (450-1150 nm range) over a moving conveyor system using both 'Hass' and 'Shephard' cultivars.

Section 6.2 is based on the publication: *Brett B. Wedding, Carole Wright, Steve Grauf and Ron D. White. (2012) The application of near infrared spectroscopy (NIRS) for the assessment of*

avocado quality attributes. In: Infrared Spectroscopy. Intech Open Book Access. 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 the influences of both geographical and seasonal variability in relation to the calibration model robustness to predict maturity and thereby eating quality based on %DM of 'Hass' avocado fruit. Avocado fruit were obtained from two different growing districts within Queensland, Australia (Childers and Bundaberg in Queensland, Australia) over three consecutive growing seasons (2006, 2007 and 2008).

6.1 Non-invasive assessment of avocado quality attributes

6.1.1 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-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 and Flitsanov 1999). Avocados Australia Limited (AAL (2008) recommends 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 cannot 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). Thus, fruit quality reliability is a key factor impacting on supply chain efficiency and related profitability.

Repeat purchasing by consumers is significantly affected by a bad eating experience. 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. 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 Agriculture and Fisheries (DAF, formally the Queensland Department of Primary Industries) and James Cook University (JCU) has been developing a non-invasive assessment tool based on NIR spectroscopy 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.

NIR spectroscopy is a non-invasive method of measuring internal/external quality and safety attributes of horticultural produce using the NIR part of the light spectrum to determine chemical composition. NIR spectroscopy 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. NIR spectroscopy 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 as published presents the current research findings of: a) developing a non-invasive NIR spectroscopy assessment tool for detecting bruises and for predicting both avocado maturity based on %DM content and rot susceptibility as an indication of shelf-life; b) a comparative study of two different commercially available NIR systems for assessing DM content in avocado fruit. As discussed above, the rot susceptibility and bruise detection components are investigated and are presented in *Chapter 7*.

6.1.2 Materials and methods

6.1.2.1 Avocado fruit samples

6.1.2.1.1. Avocado fruit for dry matter calibration development on a Bruker Matrix-F 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.

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

'Shepard' 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). 'Shepard' 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.

6.1.2.2 NIR data collection

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

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

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 780-2500 nm range NIR spectral data collection on avocado fruit was as per *Section 3.3.2.4*.

6.1.2.2.2. 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 3.3.2.4*. Spectra of the same fruit were then captured on a commercial HyperVision™ in-line grading system (Optical Measuring Systems and Produce Sorters International, USA; operating software: Camdisp Version 2.1). The HyperVision™ system optics utilises a line scan camera (CCD) based system 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 Vis-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.

6.1.2.3 Avocado dry matter analysis

The %DM reference measurement was obtained from the same area of the fruit that was used to obtain the NIR spectrum as per *Section 3.3.2.3*. The flesh was diced to facilitate drying in a fan-forced oven at 60-65°C to constant weight (approximately 72 h). 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.

6.1.2.4 NIR data analysis

6.1.2.4.1 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) as per *Section 3.3.2.5*. 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 PCA results to provide a global representation of the attributes of the entire fruit population while eliminating repetition. 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 pre-treatment 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 SG spectral smoothing (2nd order polynomial) and a second derivative transformation (25 point SG smoothing and 2nd order polynomial). For the combined Bundaberg and Toowoomba model, data pre-treatment and smoothing was based on a combination of a 25 point SG spectral smoothing (2nd order polynomial) and a 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 Matrix-F FT-NIR spectrophotometer and was subsequently removed before model development.

Model performance was assessed 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 was used to determine the predictive ability of the calibrations.

6.1.2.4.2 NIR data analysis 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 *Section 3.3.2.5*. For the Bruker Matrix-F FT-NIR spectrophotometer data pre-treatment and smoothing for 'Shepard' avocado %DM models were based on a combination of a 25 point SG spectral smoothing (2nd order polynomial and 3rd order detrend) and a second derivative transformation (25 point SG smoothing and 2nd order polynomial). 'Hass' avocado %DM models were based on a combination of a 25 point SG spectral smoothing (2nd order polynomial) and a second derivative transformation (25 point SG smoothing and 2nd order polynomial).

The HyperVision™ data pre-treatment and smoothing for 'Hass' avocado models were based on a combination of a 25 point SG spectral smoothing (2nd order polynomial) and a second derivative transformation (25 point SG smoothing and 2nd order polynomial). 'Shepard' avocado %DM models were based on a combination of a 9 point SG spectral smoothing (2nd order polynomial) and a second derivative transformation (9 point SG smoothing and 2nd order polynomial).

6.1.3 Results and discussion

6.1.3.1 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. 2003a). The influence of seasonal variability was investigated for the Bundaberg region over three years (*Table 6.1*). 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 6.1. PLS calibration and prediction statistics for % dry matter 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 6.1*, 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 (DM 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 (Marques et al. 2006; Peirs et al. 2003a). 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 6.2*. 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 (Williams 2008; Nicolaï et al. 2007; Schimleck et al. 2003). 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 6.2. PLS calibration and prediction statistics for % dry matter 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	RM SECV	RM SEP	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.

6.1.3.2 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 (HF) has had demonstrated potential in an in-line setting and was utilised for this purpose and assessed alongside the Bruker Matrix-F FT-NIR research system (MF). A population of 'Hass' and 'Shepard' 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 6.3*. 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 'Shepard' 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 'Shepard' 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 6.3. PLS calibration and prediction model statistics for both Matrix-F and HyperVision™ instruments and avocado varieties ('Hass' and 'Shepard').

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-Shepard		144(1)	12.7-36.3	6.04	4	0.91	1.78		3.4
	MF-Shepard	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-Shepard		144	12.7-36.3	6.04	7	0.86	2.26		2.7
	HV-Shepard	145	13.6-36.6	5.63		0.88		1.91	2.9

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

6.1.4 Conclusion

The present study demonstrated the potential of FT-NIR spectroscopy 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 results from the comparative study of two different commercially available NIR systems demonstrated that the in-line HyperVision™ system produced similar predictive performance on the limited trial samples ('Hass' and 'Shepard' cultivars) 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.

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

6.2.1 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 (^{o}Brix) or assessment of 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 and Flitsanov 1999). Avocados Australia Limited (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, particularly since repeat purchasing by consumers is significantly affected by a bad eating experience (Avocados Australia Limited and Primary Business Solutions 2005). Hence, fruit quality reliability is a crucial factor impacting on supply chain efficiency and related profitability. With Australian avocado production expanding rapidly, there are strong financial incentives to increase sales domestically and to export product to increase returns directly. 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: NMR and 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 (Butz et al. 2005; Gaete-Garreton et al. 2005; Mizrach 2000; Abbott 1999; Mizrach and Flitsanov 1999; Chen and Sun 1991), NMR and NIR spectroscopy 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 (Kim et al. 1999; Chen et al. 1993), 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. 2003; Clark et al. 1997).

NIR spectroscopy 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 NIR spectroscopy 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 SS content or TSS of fresh fruits (Butz et al. 2005; Abbott 1999; Scotter 1990b). Of particular importance for the current study, NIR spectroscopy has been used to estimate %DM in various horticultural products (Sivakumar et al. 2006; Xiaobo et al. 2006; Hartmann and Bijning-Pfaue 1998; McGlone and Kawano 1998; Birth et al. 1985) including avocados (Walsh et al. 2004; Clark et al. 2003; Schmilovitch et al. 2001). 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.

NIR spectroscopy 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 spectroscopy 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 (Nicolaï et al. 2007). Nicolaï 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 (Nicolaï et al. 2007; Golic and Walsh 2006). Robustness of calibration is consequently a critical issue (Nicolaï et al. 2007; Sánchez et al. 2003) and there has been recent work on fruit that considers the effect of different seasons (Guthrie 2005; Liu et al. 2005; Peirs et al. 2003b; Peiris et al. 1998; Miyanoto and Yoshinobu 1995). 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 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 (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. (2003b) studied the robustness of calibration models for SS content (°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; Miyanoto and Yoshinobu 1995).

There have been limited investigations of avocado maturity based on %DM using NIR spectroscopy. 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 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 LV's, 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 prediction >0.83, and 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 (Zeiss MMS1/NIR-enhanced spectrometer, Germany) in the 300-1100 nm range, reported calibration results of R = 0.89, RMSECV = 1.14, with SDR = 2.2, for %DM of avocado fruit of unspecified cultivar. As described in *Section 2.3.6.1* and *Section 2.4*, the higher the SDR statistic the greater the power of the model to predict the chemical composition accurately (Cozzolino et al. 2004).

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. However, for flow and ease of reading part (b) and (c) are presented in *Chapter 7*. The study also demonstrates the importance of the calibration model development process to incorporate seasonal and geographical variability to ensure model robustness.

6.2.2 Materials and methods

6.2.2.1 Avocado fruit samples

'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.

6.2.2.2 NIR data collection

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 780-2500 nm range NIR spectral data collection on avocado fruit was as per *Section 3.3.2.4*.

6.2.2.3 Dry matter analysis

The %DM reference measurement was obtained from the same area of the fruit that was used to obtain the NIR spectrum as per *Section 3.3.2.3*. The flesh was diced to facilitate drying in a fan-forced oven at 60-65°C to constant weight (approximately 72 h). 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.

6.2.2.4 Data analysis

Data analysis was carried out using the commercially available chemometric software package 'The Unscrambler™' version 9.8 (CAMO, Oslo, Norway) as per *Section 3.3.2.5*. The sample spectra for each data set were separated into a calibration set and prediction set to develop the calibration and prediction models respectively as per *Section 6.1.1.4.1*. PLS regression was used to build the prediction models of the diffuse reflectance spectral data, using segmented cross validation (20 segments in this case). Data pre-treatment and smoothing for the individual %DM models for each growing location in this study were based on a combination of: a 25 point 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 MSC transformation for the Childers models. For the combined Bundaberg and Childers model, data pre-treatment 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 6.1*. 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.

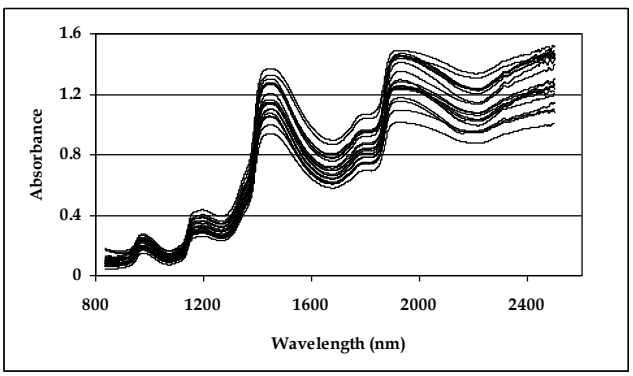


Figure 6.1. Typical absorbance spectra for whole 'Hass' avocado fruit.

6.2.3 Results and discussion

The calibration and prediction model (see *Figure 6.2*) statistics for each individual year (see *Table 6.4*) for both harvest locations indicate that FT-NIR spectroscopy 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 SD's 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 LV's are within an acceptable range for the number of samples for all models (Lammertyn et al. 2000; Hruschka 1987).

Table 6.4. PLS calibration (CAL) and prediction (PRE) statistics for % dry matter for whole 'Hass' avocado fruit harvested from Bundaberg (Bu) and Childers (Ch) over the 2006, 2007 and 2008 seasons.

Location -Year	Spectra n (OR)	% DM range	Mean	SD	L V	R^2	RM SECV	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

Note: OR = outliers removed; LV = latent variables; n = number of samples.

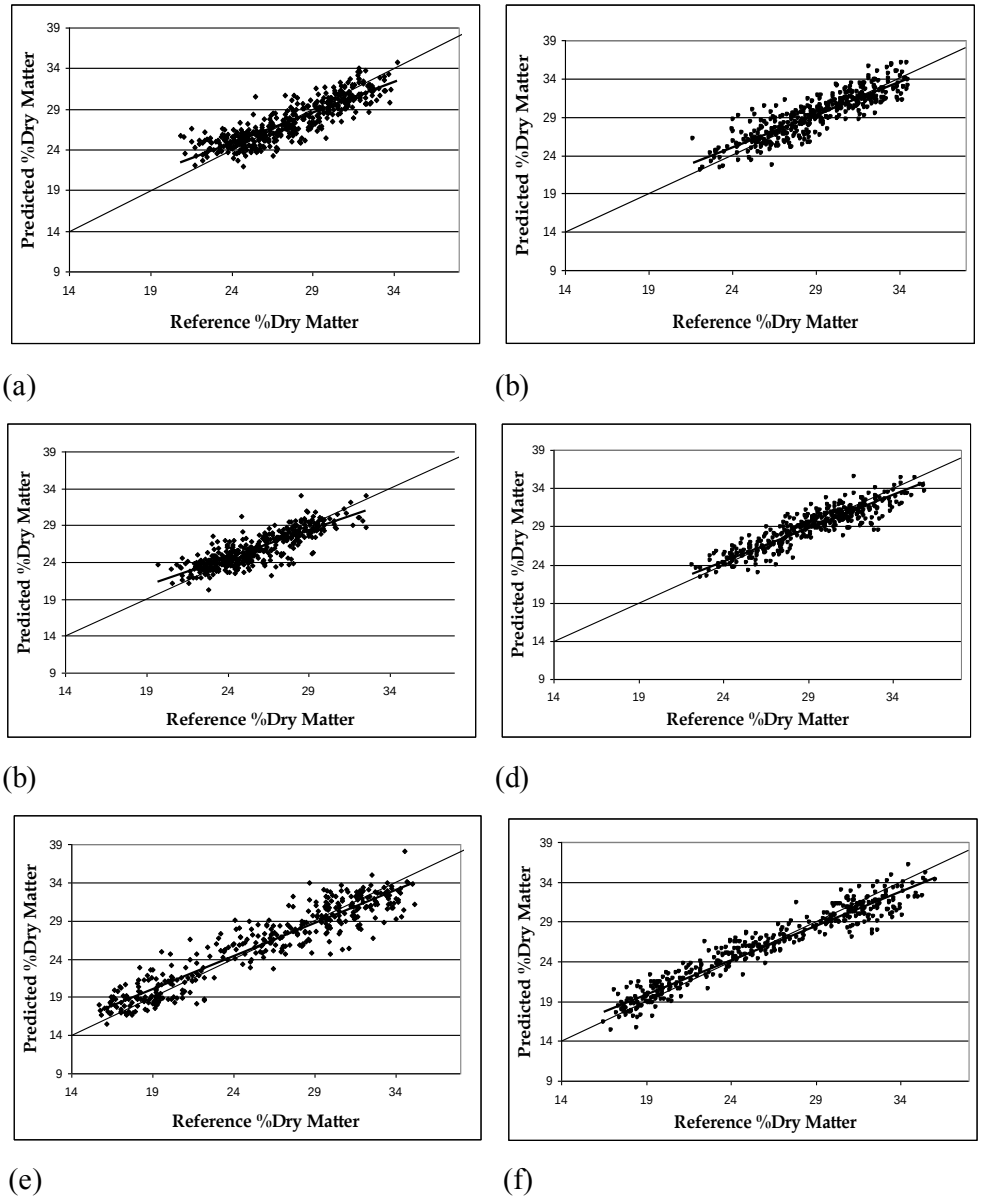


Figure 6.2. Model predictions plotted against actual constituent values for % dry matter for (a) Bundaberg 2006 season, (b) Childers 2006 season, (c) Bundaberg 2007 season, (d) Childers 2007 season, (e) Bundaberg 2008 season, and (f) Childers 2008 season.

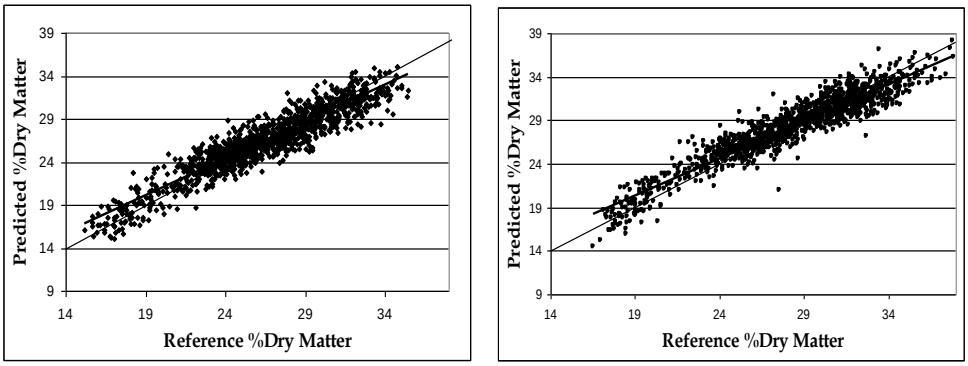
Large seasonal effects have a major consequence for calibration models for horticultural produce, since the spectral deviations due to biological variability of future samples cannot be predicted (Peirs et al. 2003b). The influence of seasonal variability was investigated for the Bundaberg and Childers 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 6.5* displays the summary statistics of the PLS calibration and prediction models for these combinations.

Table 6.5. PLS calibration (CAL) and prediction (PRE) statistics for % dry matter 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.

Location-Year		Spectra n (OR)	% DM range	SD	LV	R^2	RM SECV	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

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 (see *Figure 6.3*) 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 and 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).



(a) (b)

Figure 6.3. 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 (Guthrie et al. 2005a; Liu et al. 2005; Peirs et al. 2003b; Peiris et al. 1998; Miyanoto and Yoshinobu 1995). 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; Peirs et al. 2003b). 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 6.6*. 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 (Williams 2008; Nicolaï et al. 2007; Schimleck et al. 2003). 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).

Table 6.6. 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.

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

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 (see *Figure 6.4*). 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.

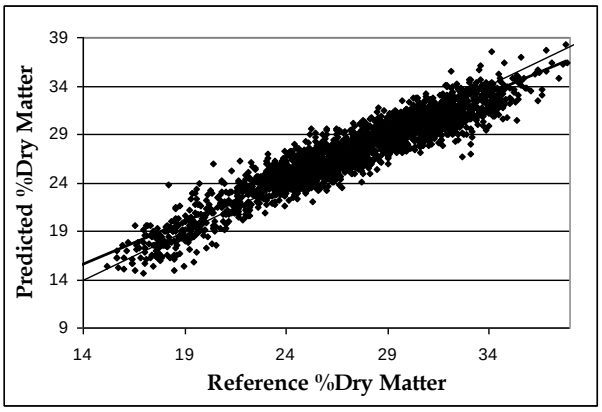


Figure 6.4. Model prediction for the Bundaberg and Childers combined 2006-08 calibration model predicting on the Bundaberg and Childers combined 2006-08 prediction set plotted against actual constituent values for % dry matter.

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 and 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 930 nm (overtones of CH_2 stretching) (Guthrie et al. 2004; Clark et al. 2003; Osborne et al. 1993) 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 (Saranwong and Kawano 2007; Guthrie et al. 2004). 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 DM 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 here-abouts 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_v^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-NIR spectroscopy reflectance combined models for both Bundaberg and Childers compare well with the model accuracy obtained by Clark et al. (2003) (R_v^2 of 0.88 and an RMSEP of 1.8% DM) using a PDA spectrometer in interactance mode, indicating reflectance FT-NIR spectroscopy 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. It must be emphasized 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.

6.2.4 Conclusion

NIR spectroscopy 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-NIR spectroscopy 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 (i.e., data input over three seasons increased model stability and performance). 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.

Overall, FT-NIR reflectance spectroscopy shows promise for the application in a commercial, in-line setting for the non-destructive evaluation of %DM, 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 NIR spectroscopy in industry and it needs to be implemented on a geographical growing area basis. 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, NIR spectroscopy 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.

CHAPTER 7

BRUISE DETECTION AND PREDICTION OF ROT SUSCEPTIBILITY OF 'HASS' AVOCADO FRUIT USING FT-NIR SPECTROSCOPY

This chapter, presents four publications by the author that review the potential of FT-NIR spectroscopy as a non-invasive tool to predict the susceptibility to rots as an indication of potential shelf-life and to detect bruises in whole avocado fruit.

Section 7.1 investigated the potential of FT-NIR spectroscopy as a non-invasive assessment tool to detect bruises in whole avocado fruit over three growing seasons. Furthermore, to predict susceptibility of avocado fruit to future flesh disorders ('pre-disorder' development) at different stages of ripeness (sprung stage and at eating ripe) as an indication of potential shelf-life, as submitted for review in: *Wedding, B.B.; Wright, C; Grauf, S.; Gadek, P.A. and White, R.D. (submitted 2018) The application of FT-NIRS for the detection of bruises and the prediction of rot susceptibility of Hass avocado fruit. Journal of Food Science and Agriculture*.

Appendix C presents a summary of preliminary research findings based on two publications presenting the same data: i) *Brett B. Wedding, Carole Wright, Steve Grauf and Ron D. White. (2012) The application of near infrared spectroscopy (NIRS) for the assessment of avocado quality attributes. In: Infrared Spectroscopy. Intech Open Book Access;* and *ii*) *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) Non-invasive assessment of avocado quality attributes. In: The Proceedings of the VII World Avocado Congress 2011, Cairns – Australia, 5-9 September 2011.*

It should be considered that the preliminary work presented here is a first step towards shelf-life prediction and bruise detection for avocado fruit using NIR spectroscopy. To incorporate geographic biological variability fruit were obtained from two growing districts (Toowoomba and Ravenshoe in Queensland, Australia) over one season (2008). Fruit from Ravenshoe were used for the impact assessment (bruising), while Toowoomba fruit were used for rot susceptibility (shelf-life) trials.

Appendix D provides a summary of key results and discussion on studies that investigated the potential of FT-NIR spectroscopy as a non-invasive tool to detect bruises in whole avocado fruit and to predict susceptibility to rots as an indication of potential shelf-life. The same fruit population is utilised for this study as presented in *Appendix C*. However, a revised statistical method targeted as more applicable for commercial applications is applied, as published in:

Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2012) Impact assessment and prediction of rot susceptibility of Hass avocado fruit using FT-NIRS. In: The Proceedings of the 15th International conference on Near Infrared Spectroscopy, Cape Town, 16-20 May 2011.

7.1 The application of FT-NIR spectroscopy for the detection of bruises and the prediction of rot susceptibility of 'Hass' avocado fruit

7.1.1 Introduction

Avocado production in Australia is expanding rapidly and there are strong financial incentives to increase sales both domestically and internationally. However, avocado maturity and quality characteristics including ripening rates and shelf-life are often highly variable within an individual shipment (Magwaza and Tesfay 2015; Blakey et al. 2008). This inability to consistently guarantee internal fruit quality is a major factor for not only the Australian avocado industry but also the entire horticulture sector. Inferior fruit quality is seen as one of the key factors impacting on supply chain efficiency and profitability (Margetts 2009). As such, removing inconsistencies and providing what the consumer expects is a critical factor for retaining and expanding both domestic and international markets.

As highlighted in retail and consumer surveys spanning the last 15+ years, consumers are not always satisfied with avocado quality (Embry 2009; Gamble et al. 2008; Harker et al. 2007; Hofman and Ledger 1999). The surveys show that poor flesh quality that cannot be determined until the fruit is cut is the main reason for this dissatisfaction. As a result, the consumers that eat avocados expect to discard 25% of fruit they purchase due to poor internal quality (Avocados Australia Limited and Primary Business Solutions 2005). Consumers select bruising as the major defect which was found to be a more important barrier to purchasing than price, followed by body and stem end rots (Harker 2009). Bruising is the most common type of postharvest mechanical injury for many fruit types and is the reason for rejecting the highest percentage of fruit in sorting lines (Opara and Pathare 2014; Luo et al. 2012; Baranowski et al. 2009). Bruising can be defined as fruit tissue damage as a result of external forces which cause physical changes of texture and/or chemical changes of colour, taste and odour (Baranowski et al. 2009). Bruising in avocado fruit are typically seen as defined areas of dark grey flesh and light coloured discolouration often associated with hairline cracking of the flesh (Hofman 2002). Depending on the extent and type of physical damage, fruit type, ripening stage, temperature, and fruit pre- and post-harvest conditions, it may take quite some time for the bruise to become visible and may not be detected until the point of purchase or consumption (Opara and Pathare 2014; Lu 2003).

Consumer surveys indicate that avocados with internal defects of ≥10% have a dramatic negative impact on the consumer repurchasing (Petty and Embry 2011; Embry 2009). Retail surveys of avocado quality conducted in 2001 showed that up to 80% of fruit were bruised to some extent, and that 20% of ripe fruit had at least 25% of the flesh volume that is unusable mainly due to rots and bruising (Mazhar et al. 2011; Hofman and Ledger 2001). Consumer survey's conducted in

2008 indicated that 63% of avocado fruit had flesh defects (i.e., bruising, stem end or body rots, diffuse flesh, vascular browning or other flesh defects) and that 29% of these fruit had more than 10% of the flesh affected by these defects (Mazhar et al. 2011).

Avocado fruit become susceptible to impact damage resulting in bruising once the fruit has reached the sprung stage of ripeness (stage 2). The sprung stage of ripeness is where the flesh deforms by 2-3 mm under extreme thumb pressure (Wedding et al. 2012a). Avocados are frequently subjected to various types of mechanical forces during harvesting, storage, transit and handling that may result in fruit damage. Baryeh (2000) reports that avocado fruit can withstand higher impact and compression loads in the first seven days following harvest, then after this time they are more susceptible to bruising by impact or compression forces as they ripen and become softer. Severity of bruising is also increased with a lower DM content at harvest and with longer exposure to an impact or compression event (Joyce et al. 2015). The fruit ripening process continues to progress as the fruit travels from orchard dispatch to retail outlets and finally to the consumer. During this time fruit softening continues and susceptibility to impact damage increases. As a results, much of the bruising of avocados occurs at retail outlets from the consumer squeezing the fruit by hand to see if it is ripe prior to purchase.

Bruise detection in the horticulture industry is commonly performed using manual visual inspection which is subjective, inconsistent and labour intensive. For avocados it is difficult to visually see the discolouring or tissue damage as much of it is internal and it is therefore only discovered when the product is cut open.

Export of avocados from Australia typically takes anywhere from two to six weeks by sea freight depending on destination (Hofman and Marques 2009). The time and distance associated with most export markets results in longer times from harvest to consumption which increases the risk of quality loss before the consumer receives the fruit. The biggest risk during transport involving long storage times is the development of rots and flesh disorders. The presence of bruises also increases the risk of bacterial and fungal contamination as damaged tissue is more prone to infection (Luo et al. 2012; Xuan et al. 2012). Avocado rot severity is dependent on both mineral concentration of the fruit and the amount of disease inoculum (Everett et al. 2003; Hofman et al. 2002). Research has shown that avocados with a lower prevalence of rots and flesh disorders are associated with higher calcium and lower potassium concentrations (Hofman et al. 2002; Willingham et al. 2001).

Unfortunately, at present there is no commercially available non-invasive inline grading system that can reliably predict avocado flesh disorder development in relation to shelf-life to ensure that

the fruit will arrive at the consumer with an acceptable quality. Currently, fruit with visible external defects are manually sorted on farm during pack-out. However, many of the flesh disorders only appear once the fruit ripen and age during storage. A rapid and non-destructive in-line grading system that can rapidly and accurately assess individual fruit for internal quality attributes would allow the industry to provide a more consistent fruit quality to the consumer. Assessment of fruit for internal quality attributes in the pack-house or at Distribution Centres, may identify fruit that are less prone to rots and internal disorders. These fruit can be sent to more distant markets with greater confidence that the fruit will arrive at market in acceptable quality, thus ensuring maximum yield for the producer and retailer.

The development of innovative smart sensing technologies has enabled commercially feasible non-invasive methods for the rapid in-line assessment of various internal quality attributes and defects of horticultural products. These smart sensing technologies include: X-ray (Shahin et al. 2002), thermography (Baranowski et al. 2009); Varith et al. (2003)), electrical impedance spectroscopy (Jackson and Harker 2000); MRI (Gonzalez et al. 2001; Kim et al. 1999; Simoneau et al. 1993) and machine vision using CCD's (Van Zeebroeck et al. 2007). Of the smart sensing technologies, NIR spectroscopy has received particular attention for horticulture applications.

Vis-NIR spectroscopy has become increasingly more widely used for quality and safety attributes of agriculture/food products because of its rapid non-invasive and cost effective application. Conventionally, spectral information relating to biochemical information is generally collected from a single area on a sample using a spectrophotometer (termed spot or single point assessment), which does not contain spatial information and thus limits its use for defect detection of whole horticultural produce (Opara and Pathare 2014; Ariana et al. 2006a; Nagata et al. 2006). However, the application of Vis-NIR spectroscopy has proven accurate in the detection of bruised surfaces (Opara and Pathare 2014), with the majority of published research and applications targeting thin skinned fruits such as: apples (Luo et al. 2012; Xing and De Baerdemaeker 2007; Xing et al. 2006; Bennedsen and Peterson 2005; Guillermin et al. 2005b; Xing et al. 2005b; Geeola et al. 1994); pears and strawberries (Nagata et al. 2002); tomatoes (Wu and Wang 2014); olives (Jimenez-Jimenez et al. 2012); apricots (Zwiggelaar et al. 1996) and peaches (Zwiggelaar et al. 1996; Li et al. 1993; Miller and Delwiche 1991). For example, correct classification rates of more than 90% for separating bruised and healthy apples was obtained by Xing et al. (2005b) for 'Jonagold' one day after bruising using a Vis-NIR spectrometer in the 400-1700 nm range, by Luo et al. (2012) for 'Fuji', 'Jonagold', 'Otin' and 'Sinano' using a spectrometer in the 400-840 nm range and by Guillermin et al. (2005) for 'Elstar' and 'Gala' apple varieties in the 800-2200 nm wavelength region. In another study Xing and De Baerdemaeker (2007) reported that correct classification of more than 95% could be achieved for both sound and freshly bruised fruit based

on a softening index (<45 minutes following impact) for 'Golden Delicious', 'Jonagold', and 'Braeburn' apple varieties. In that study a spectrophotometer in the wavelength range of 400-1700 nm was used.

Bennedsen and Peterson (2005) applied a machine vision system for sorting eight different apple varieties for bruises and surface defects (caused by blister spot, early frost damage, powdery mildew, russet and sunburn). The accuracy of the routines to find individual defects and measure the area, ranged from 77 to 91% for the number of defects detected, and from 78 to 92% for the total defective area. Geoola et al. (1994) reported correct classification rates of 96.1%, 88.4% and 93% for refrigerated 'Golden Delicious' apples that were unbruised, bruised and left for 90 minutes and 24 hours, respectively, using a spectrometer in the 400-840 range. Similarly, they reported the classification performance for non-refrigerated apples was 92.9% and 91.8% for unbruised, and bruised and left for 24 hours, respectively.

All the previously mentioned studies are on thin skinned fruits with only the preliminary studies by Wedding et al. (2012a,b) being identified as targeting fruit with thicker skins. The preliminary investigations by Wedding et al. (2012b) to detect bruises on thick skinned 'Hass' avocado fruit at the sprung stage of ripeness (stage 2) using an NIR spectrophotometer (780-2500 nm) indicated great potential. The single point assessment technique correctly classified more than 85% of the population based on two categories of percentage bruising ((i) ≤10%; (ii) >10%) of the area scanned after 1-2 hours following fruit injury. Classification rates increased to more than 90% following 24 hours after injury allowing sufficient time for bruise development to occur in the sprung fruit.

In relation to the prediction of storage disorders in fruit by NIR spectroscopy technology, there have been limited investigations reported in literature (Clark et al. 2004). However, there have been several studies addressing the potential of NIR spectroscopy for discriminating between products on the basis of post-harvest storage duration, as a means of estimating product shelf-life (Pérez-Marín et al. 2011; Pérez-Marín et al. 2010; Paz et al. 2009a; Sánchez et al. 2009; Camps et al. 2007).

Camps et al. (2007) report satisfactory results when using NIR spectroscopy for determining storage type (cooled room at 2°C and 95% RH versus shelf-life conditions at ~20°C and 40% RH) and storage duration for three apple cultivars. The authors report classifications of apples according to the duration of storage (cooled room up to 120 days and shelf-life conditions up to 28 days in storage) and achieved 75% correct classification for cooled room stored fruit and up

to 85% for fruit stored under shelf-life conditions. The accuracies of the models were increased when applied to individual varieties.

A study by Clark et al. (2004) reported using of Vis-NIR spectroscopy to predict storage disorders of kiwifruit at harvest by separating into categories of 'sound' fruit and chill-injured fruit during a 24-week cold (-1.5 to 1.5°C) storage period. The authors estimated that by employing discriminative Vis-NIR spectroscopy techniques the overall incidence of disorders could have been reduced from 33.9 to 17.9% at their early harvest, and from 14.7 to 8.5% at their second harvest.

Paz et al. (2009a) evaluated three NIR spectrophotometers for the determination of apple quality parameters including shelf-storage duration. Two varieties of apples were stored at 20°C, 40% RH and analysed after day 8 and 14. Classification models correctly classified 86.1% of the mixed cultivar group and 86.6% of samples from the single-cultivar groups. Similarly, Pérez-Marín et al. (2010) compared two NIR instruments for the measurement of quality-related parameters including post-harvest storage duration (0, 6 and 9 days) under refrigeration in intact plums. Classification models correctly assigned 94.5% of samples to their designated refrigerated storage time (day) as an approach for estimating shelf-life (Pérez-Marín et al. 2010). A further study by Sánchez et al. (2009) reported using NIR spectral data to classify intact asparagus stored in refrigeration under controlled atmosphere, both by storage time and by post-harvest treatments applied. Classification models correctly classified 81-100% of samples by post-harvest storage time and 72-85% of samples for post-harvest treatment, depending on the spectrophotometer used.

Other studies have shown that NIR spectroscopy can be used to classify nectarines as a function of the pre-harvest irrigation strategies applied and post-harvest cold storage duration (Pérez-Marín et al. (2011) and in studying shelf-life (as a loss of freshness) of fresh-cut pineapple stored at different temperatures (Di Egidio et al. 2009). Preliminary investigations by Wedding et al. (2012a) predicted the percentage rot development of whole 'Hass' avocado fruit using a spectrophotometer in the 780-2500 nm wavelength range. The technique correctly classified 92.8% of the test population into two categories, above and below 30% rot development for the area scanned. The percentage slightly decreased to 86.8% when the classification was reduced to above and below 10% rot development of the scanned area (Wedding et al. 2012a).

Typically, these studies evaluating the post-harvest storage duration as a means of estimating product shelf-life, assess the fruit at certain points in time while in storage to then develop calibration models to predict relative shelf-life. Hence, the analysis is very much 'after the fact',

i.e., the system for segregating fruit on the premise of post-harvest storage duration was developed following actual post-harvest storage and disorder development. In this study the potential of FT-NIR spectroscopy as a non-invasive assessment tool to detect bruises in whole avocado fruit and to predict susceptibility to future flesh disorders ('pre-disorder' development) as an indication of potential shelf-life is investigated.

7.1.2 Materials and Methods

7.1.2.1 Sample selection

'Hass' avocado fruit were obtained over the 2010, 2011 and 2014 growing seasons from three farms located in the major production district of Toowoomba, South East Queensland, Australia (Latitude: 27° 33' 0" South, Longitude: 151° 58' 0" East). All fruit were harvested at the hard green stage of ripeness.

7.1.2.2 Spectral acquisition and reference analysis

Diffuse reflectance spectra of whole avocado fruit were collected in the 780-2500 nm range using a Bruker Matrix-F FT-NIR spectrophotometer (Bruker Optics, Ettlingen, Germany; operating software: OPUS™ version 6.5) as described in *Section 3.3.2.4*. In obtaining each sample spectrum 16 scans at a resolution of 8 cm^{-1} were collected and averaged.

For impact assessment, hard green fruit were stored at 20°C and 85-95% RH until fruit reached the sprung stage of ripeness (stage 2) or eating ripe (stage 4). Stage of ripeness was subjectively assessed by hand, based on deformation of the fruit as rated in the Avocare Assessment Manual (White et al. 2001). Approximately 100 fruit were assessed in each year for both stage 2 and stage 4. Individual spectra were collected from each opposing half of the fruit on reaching the required stage of ripeness, that is, stage 2 or stage 4. Following initial spectra collection, half the fruit population at each stage of ripeness were impacted against a slate paver (height: 400 mm, length: 400 mm, width: 40 mm) placed upright and supported by concrete blocks to simulate impact damage at a height equivalent to 100 cm and 20 cm for stage 2 and stage 4 fruit, respectively. Impact was imposed on each half of the fruit by placing individual fruit into a cotton mesh bag which was firmly suspended by two cords attached to the laboratory ceiling. The fruit were positioned so that the scanned area would impact against the paver. The fruit in the mesh bag was pulled away from the slate paver and released to swing in a pendulum-like 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.

For all fruit, the impacted area was re-scanned after 2-5 hours and again after 24 hours. Following the final scan at 24 hours, stage 2 fruit were placed back into 20°C storage at 85-95% RH and assessed for bruises at eating ripe (approximately 5 days following impact). Stage 4 fruit were assessed for bruises immediately after the final scan at 24 hours. Bruise assessment was based on the presence or absence of bruising of the flesh within the scanned area.

A separate population of hard green fruit were used for the rot susceptibility trials than what was used in the impact trials. Approximately 100 fruit were assessed in 2008, 2010 and 2011. Spectra for this trial were collected from each opposing half of the hard green fruit prior to fruit being placed into 20°C storage at 85-95% RH. At eating ripe (stage 4) fruit were then assessed for rots based on a weight percentage of the flesh volume affected in each opposing half of the fruit.

7.1.2.3 NIR data analysis

'The Unscrambler' Version 10.3 (Camo, Oslo, Norway) chemometric software was used for discriminative analysis to separate the avocados into categories based on presence or absence of bruising and the percentage rot development within the scanned area.

Three classification techniques were used to discriminate between bruised and unbruised avocado fruit and for separating the rot development fruit into two groups, a) above and below 10% and b) above and below 30%. The techniques were principal components linear discriminant analysis (PC-LDA), SVM and PLS-discriminant analysis (PLS-DA).

PC-LDA is a classification technique where the number of groups and the samples that belong to each group are pre-defined (Naes et al. 2002; Otto 1999). This technique produces a number of orthogonal linear discriminant functions that maximise the separation between the groups, yet minimises the variance within groups (Naes et al. 2002). To overcome the requirement of LDA that the number of samples in the calibration set is larger than the number of variables, the data dimensionality is reduced using PCA prior to running the LDA. Three separation methods within LDA were investigated: linear, quadratic and mahalanobis distance.

SVM is a classification technique where a function that describes a hyperplane for maximum separation of groups is identified (Cortes and Vapnik 1995). By selecting an appropriate kernel function SVM has the ability to model linear and non-linear classification problems. Four kernel functions were investigated: linear, polynomial, radial basis function and sigmoid. A standard deviation weighting process was employed in all SVM models.

PLS-DA is the use of PLS regression for discriminating between multiple groups (Cozzolino et al. 2003; Osborne et al. 1993). Each sample in the calibration and validation set is assigned a dummy variable as a reference value indicating which group the sample belongs to. One group, for example bruised fruit, were assigned a numeric value of '1' and the other group, for example unbruised fruit, assigned a numeric value of '0'. The PLS-DA model is then developed by regressing the spectral data against the assigned dummy variable. A cut-off of 0.5 was used to determine class assignment.

For all models selected wavelengths were in the range of 1012.74 nm (9874.24 cm^{-1}) to 846.70 nm (11810.52 cm^{-1}). Spectral pre-processing transformations were applied where necessary to enhance the spectral features and included SG smoothing, SG first and second derivatives, MSC and SNV. Segmented cross-validation was used for the models combining data from all years (20 segments) and full cross validation was used for individual growing season models. The data sets were divided into approximately equal sized calibration and validation sets. Both calibration and validation sets contained spectra from each of the two groups being discriminated. The validation set was used to evaluate the accuracy of the models to classify samples into the two groups. Model selection was based on obtaining a high percentage correct for both sample groups which may not necessarily correlate to the highest overall percentage correct, particularly when there are unequal sample numbers in each group. The classification method which achieved the highest correct classification for discriminating between bruised and unbruised fruit is presented, and that which was best at discriminating between the rot classes is presented. These two classification methods were not restricted to being the same.

7.1.3 Results and Discussion

7.1.3.1 Impact assessment

This study found that the PLS-DA and PC-LDA classification methods produced similar correct classification rates for bruise detection which tended to be higher than those obtained using SVM. For simplicity only the PLS-DA results are presented. PLS-DA classification statistics for the prediction of bruising following impact of fruit at stage 2 and stage 4 ripeness, based on the presence or absence of bruising of the flesh within the scanned area are presented in *Tables 7.1* and *7.2*, respectively. Prediction models were developed for each growing season separately as well as a model that combined the scans from the three years.

As expected all models based on fruit scanned prior to impact were very poor at determining the correct classification of the fruit. These results are confirmation that the calibration models could not predict 'impacted' fruit when fruit had 'not' been impacted at this stage. This can be considered a check that the model was not predicting some other attribute in the fruit, unrelated

to bruising that may result in discrimination between the population. The validation results for fruit at stage 2 ripeness (see *Table 7.1*) scanned 2-5 hours following impact show that 70.3%, 77.6% and 79.3% were correctly classified for growing seasons 2010, 2011 and 2014, respectively. The percentage correctly classified increases when the fruit are scanned 24 hours following impact to 83.2%, 88.9% and 88.0% for years 2010, 2011 and 2014, respectively. Combining the populations from all three growing seasons showed similar classification rates and trend between 2-5 hours and 24 hours following impact as per the individual models. The validation results for fruit at stage 2 ripeness for the combined years shows an increase in correct classification over time from 71.5% at 2-5 hours after impact up to 87.7% at 24 hours following impact.

Table 7.1. PLS-DA summary statistics for stage 2 ripeness for years 2010, 2011, 2014 and all years combined.

Stage 2			Correct Classification % (number of samples)					
			Calibration			Validation		
Year	Scan (trans)	LV	No Impact	Impact	Total	No Impact	Impact	Total
2010	Initial (raw)	3	51.0 (102)	56.4 (101)	53.7	53.9 (102)	55.4 (101)	54.7
	2-5 hr (s)	6	80.0 (102)	74.3 (101)	79.3	75.5 (102)	65.0 (100)	70.3
	24 hr (raw)	5	87.3 (102)	78.2 (101)	82.8	87.2 (102)	79.0 (100)	83.2
2011	Initial (raw)	2	23.9 (46)	80.3 (61)	56.1	2.2 (46)	98.4 (62)	57.4
	2-5 hr (raw)	6	80.4 (46)	74.2 (62)	76.9	80.0 (45)	75.8 (62)	77.6
	24 hr (raw)	4	93.5 (46)	83.9 (62)	88.0	100 (46)	80.6 (62)	88.9
2014	Initial (raw)	3	69.0 (58)	46.0 (50)	58.3	72.7 (55)	49.1 (53)	61.1
	2-5 hr (raw)	5	75.4 (45)	67.8 (59)	71.6	82.5 (57)	76.3 (59)	79.3
	24 hr (raw)	4	94.8 (58)	88.0 (50)	91.7	96.4 (55)	79.2 (53)	88.0
All Years	Initial (raw)	2	36.2 (152)	72.1 (172)	55.2	23.0 (152)	79.8 (173)	53.2
	2-5 hr (s+snv)	6	74.7 (154)	69.0 (171)	71.7	77.1 (153)	66.5 (170)	71.5
	24 hr (s+snv)	4	95.4 (153)	78.9 (171)	86.7	94.2 (154)	81.8 (170)	87.7

Note: trans = the pre-processing data transformation. These are defined as: s = 25 point Savitzy-Golay smooth; snv = standard normal variate; raw = no transformation applied. LV = number of latent variables. Times represent time of scans since initial impact.

The results indicate that a 24 hour time delay after impact allowed for the bruising to further develop, thus assisting with improved classification. This is consistent with other researchers who have reported that the discolouration, or the softening of the damaged tissue, is related to the time elapsed after damage, and therefore the efficiency and effectiveness of bruise detection during grading can be affected (Opara and Pathare 2014). Stage 2 avocados require substantial force to bruise and hence the bruise development may therefore be slower compared to bruise

development in ripe avocados (stage 4-5). Also, it is possible that some of the avocado samples may not have been fully at stage 2 (i.e., between stage 1 and 2) at the time of impact resulting in reduced, impact damage and slower and reduced bruise development.

The validation results for fruit at stage 4 ripeness (see *Table 7.2*) scanned 2-5 hours following impact for growing seasons 2010, 2011 and 2014 obtained correct classification rates of 59.7%, 100% and 72.4% respectively. The majority of samples scanned in this time frame in 2010 and 2014 were scanned at 2-4 hours following impact, whereas samples from the 2011 population were scanned 3.5-5 hours following impact, which may help to explain the higher correct classification obtained in 2011. Within each year the stage 4 ripeness correct classification rates improved when the fruit were scanned at 24 hours after impact (66.0%, 100% and 89.7% for 2010, 2011 and 2014 respectively).

The 2010 stage 4 sample population showed a much smaller increase in the correct classification as the time between impact and spectra collection increased compared with stage 2 results and the 2011 and 2014 stage 4 populations. Applying different wavelength regions for the stage 4, 2010 population improved the correct classification rates, however, these wavelength regions did not perform well for the 2011 and 2014 populations (data not presented). The 2010 population was sourced from a different farm in the Toowoomba region from those for the 2011 and 2014 populations. However, it would be expected that the same wavelength regions selected for bruising should remain similar for different populations as it is based on the same physiological reaction. That is, when bruising occurs, matrix, cell wall destruction and chemical changes within the fruit tissue may change the light scatter properties, leading to detectable differences in reflectance properties when compared to non-bruised fruit tissue (ElMasry et al. 2008; Upchurch et al. 1994). However, as the bruise development process of softening and discolouration of damaged tissue is related to time after injury, time is an important factor when developing machine vision for detection of bruises in commercial environments (ElMasry et al. 2008; Xing et al. 2006; Upchurch et al. 1994).

Combining the populations from all three years showed similar classification rates and trend between 2-5 hours and 24 hours following impact as per individual growing seasons. The validation results for fruit at stage 4 ripeness for the combined years shows an increase in correct classification from 73.7% to 84.3%, respectively. These results indicate that in a commercial situation it would be an advantage to hold the fruit for 24 hours prior to assessment to ensure improved detection of bruises.

Table 7.2. PLS-DA summary statistics for stage 4 ripeness for years 2010, 2011, 2014 and all years combined.

Stage 4			Correct Classification % (number of samples)					
			Calibration			Validation		
Year	Scan (trans)	LV	No Impact	Impact	Total	No Impact	Impact	Total
2010	Initial (raw)	2	54.2 (96)	56.3 (96)	55.2	58.3 (96)	43.8 (96)	51.0
	2-5 hr (s+snv)	2	54.1 (98)	58.5 (96)	56.3	64.9 (94)	54.6 (97)	59.7
	24 hr (s)	5	63.7 (102)	65.6 (90)	64.6	70.1 (77)	63.2 (114)	66.0
2011	Initial (raw)	3	36.6 (41)	73.3 (60)	58.4	22.0 (41)	81.7 (60)	57.4
	2-5 hr (raw)	4	100 (39)	98.4 (61)	99.0	100 (41)	100 (60)	100
	24 hr (raw)	4	100 (41)	100 (60)	100	100 (41)	100 (60)	100
2014	Initial (raw)	3	57.9 (57)	64.4 (59)	61.2	49.1 (57)	64.4 (59)	56.9
	2-5 hr (raw)	3	70.2 (57)	69.5 (59)	69.8	78.9 (57)	66.1 (59)	72.4
	24 hr (raw)	5	94.7 (57)	81.4 (59)	87.9	96.5 (57)	83.1 (59)	89.7
All Years	Initial (raw)	3	34.9 (149)	69.4 (170)	53.3	6.0 (149)	97.1 (170)	54.6
	2-5 hr (s+d1)	6	71.1 (149)	69.4 (170)	70.2	77.9 (149)	70.0 (170)	73.7
	24 hr (raw)	6	83.2 (149)	81.2 (170)	82.1	88.6 (149)	80.6 (170)	84.3

Note: trans = pre-processing data transformation. These are defined as: s = 25 point Savitzy-Golay smooth; snv = standard normal variate; d1=Savitzy-Golay first derivative (25 point smooth, polynomial order 2); raw = no transformation.

The correct classification rates achieved in this study for bruising of whole avocado fruit are similar to those reported in literature for thin skin fruits. For example Shahin et al. (2002) using X-ray line scan imaging of 'Red Delicious' apples obtained an accuracy of 82% and 52% for old (1 month after damage) and new (24 hours after damage) bruises, respectively. Xing et al. (2005b) reported achieving correct classification of more than 90% for separating bruised from sound fruit for 'Jonagold' apples using a single point Vis-NIR spectrophotometer in the 400-1700 nm range. Similarly, Xing and De Baerdemaeker (2007) reported 95% correct classification for both sound and bruised apple varieties ('Golden Delicious', Jonagold', and 'Braeburn') less than 45 mins following impact using a Vis-NIR spectrophotometer in the 400-1700 nm range. Luo et al. (2012) reported predictive accuracy exceeding 90% for the detection of bruises in four apple cultivars ('Fuji', Jonagold', 'Otin' and 'Sinano') utilising the following wavelengths 808-772 nm, 834-762 nm, 788-741 nm. Geoola et al. (1994), using a spectrometer in the 400-840 nm range reported obtaining best classification accuracies in the 750 to 800 nm wavelength region of 92.9% and 91.8% for unbruised and bruised non-refrigerated 'Golden Delicious' apples 24 hours following impact. Guillermin et al. (2005a) reported classification accuracy of 95-100% to distinguish between bruised and sound apples ('Elstar' and 'Gala') using a spectrometer in the 800 to 2200 nm range. Lu et al. (2011) using an RGB camera to detect bruises on red bayberry's reported

classification accuracy of 100% and 78.57% for sound and bruised fruit, respectively. Zwiggelaar et al. (1996) reported using a CCD camera with narrowband filters to detect bruises on apricots (using 930 nm and 970 nm) and peaches (using 750 nm) to obtain classification accuracies of 65%.

In preliminary investigations by Wedding et al. (2012b) to detect bruises on thick skinned 'Hass' avocados reported correct classification results of greater than 85%, 1-2 hours following impact and greater than 90%, 24 hours following impact. The bruise categories in that study were based on percentage bruise area, unlike the bruise classes in this study which are based on impacted and non-impacted fruit.

Avocados are frequently subjected to various types of mechanical forces during harvesting, storage, transit and handling that may result in fruit damage. It is therefore possible that fruit which were not deliberately impacted may still develop mild non-visible bruising from impacts during post-harvest processing and transportation. The chemical change due to the bruising may therefore be detected by FT-NIR, although it may not have been detected visually. Incorrect classification of impacted fruit may also be due to bruising on the edge and not the middle of the scanned area resulting in reduced detection of the impact damage. Consequently hyperspectral imaging, which can look at the entire sample and not just a spot (single point assessment) on the fruit, may improve the percentage correctly classified.

7.1.3.2 Rot assessment

The PC-LDA classification method produced higher correct classification rates for rot prediction than SVM and PLS-DA, and hence only the PC-LDA results are presented. Classification statistics for separation of the prediction of percentage rot development into a) above and below 10% and b) above and below 30% rot development for the scanned area for years 2008, 2010, 2011 and for all years combined are presented in *Tables 7.3* and *7.4* respectively. The percentage of fruit that were correctly classified as above or below 10% predicted rot development for 2008, 2010, 2011 and all populations combined were 65.3%, 69.1%, 84.4% and 73.3%, respectively (see *Table 7.3*). The 2011 population had the highest correct classification, possibly due to method refinement. In most instances the PC-LDA discriminative method correctly predicted more of the fruit with rot percentages ≥10%.

Similarly, the validation percentages correctly classified for above and below 30% rot development within the scanned area were 76.9%, 68.5%, 77.1% and 73.3% for the 2008, 2010, 2011 and all populations combined, respectively (see *Table 7.4*).

Table 7.3. PC-LDA classification statistics for prediction of percentage rot development of whole 'Hass' avocado fruit into two groups 0-10% and 11-100%.

0-10% / 11-100%		Correct Classification % (number of samples)					
		Calibration			Validation		
Year	Trans	0-10%	11-100%	Total	0-10%	11-100%	Total
2008	s+snv	97.1 (34)	68.1 (94)	75.8	75.0 (28)	62.4 (93)	65.3
2010	raw	75.9 (87)	81.7 (93)	78.9	64.6 (99)	74.7 (79)	69.1
2011	raw	77.0 (113)	94.9 (118)	86.1	74.3 (113)	94.1 (118)	84.8
All Years	raw	76.0 (296)	77.3 (214)	76.6	74.0 (289)	72.3 (242)	73.3

Note: Trans = pre-processing data transformation. These are defined as: s = 25 point Savitzy-Golay smooth; snv = standard normal variate; raw = no transformation applied. All models are based on 10 PC's.

Table 7.4. PC-LDA classification statistics for prediction of percentage rot development of whole 'Hass' avocado fruit into two groups 0-30% and 31-100%.

0-10% / 11-100%		Correct Classification % (number of samples)					
		Calibration			Validation		
Year	Trans	0-10%	11-100%	Total	0-30%	31-100%	Total
2008	s+snv	81 (21)	75.7 (107)	76.6	81.3 (16)	76.2 (105)	76.9
2010	s+d1	78.2 (101)	54.4 (79)	67.8	70.7 (99)	65.8 (79)	68.5
2011	raw	76.4 (140)	71.4 (91)	74.5	77.1 (140)	76.9 (91)	77.1
All Years	raw	75.4 (350)	71.3 (188)	74.0	73.4 (342)	73.0 (189)	73.3

Note: trans = pre-processing data transformation. These are defined as: s = 25 point Savitzy-Golay smooth; snv = standard normal variate; d1=Savitzy-Golay first derivative (25 point smooth, polynomial order 2); raw = no transformation applied. All models are based on 10 PC's.

The preliminary study of Wedding et al. (2012a) reported correct classification rates of more than 86% and 92% for above and below 10% and above and below 30% respectively, which are higher than those found in this study. Wedding et al. (2012a) did not report the correct classification rate of the individual rot categories making it difficult to compare the two studies. When allowing fruit to develop defects naturally, it is possible for the size classes to be highly unbalanced, therefore the overall correct classification rate may not be reflected by each of the individual categories.

7.1.4 Conclusion

The present study highlights the potential of FT-NIR spectroscopy as a non-invasive tool to predict impact damage of whole avocados, and to predict shelf-life based on rot development (susceptibility). The single point NIR assessment technique applied in this study correctly

classified 70% to 79% of the stage 2 maturity populations for bruising, 2-5 hours after impact. The correct classification rate increased to 83% to 89% when spectral data were collected 24 hours after impact allowing sufficient time for the bruise development to occur and to be detected. Avocado populations at stage 4 maturity achieved classification rates of 60% to 100% for bruising, by scanning 2-5 hours after impact and 66 to 100% 24 hours following impact. The 2010 stage 4 sample population did not follow the same pattern as the 2011 and 2014 stage 4 populations or the 2010, 2011 and 2014 stage 2 populations. The results indicate only a small improvement between the initial, 2-5 hour and 24 hours following impact for the stage 4, 2010 population. The study indicates that in a commercial situation it would be an advantage to hold the fruit for approximately 24 hours prior to scanning to allow bruise development to occur, particularly in hard fruit (i.e., stage 2) prior to bruise assessment.

The single point NIR assessment method correctly, classified up to 75% and 94% of the individual populations for separating percentage rot development as an indication of shelf-life into two categories, a) 0-10% and b) 11-100% rot development for the area scanned, respectively. Similar classification rates of up to 81% and 77% where obtained when separating into a) 0-30% and b) 31-100% rot development for the area scanned, respectively.

The classification models presented 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 impact damage and rot susceptibility of whole avocado fruit, although optimisation of the technology is required to address speed of throughput and environmental issues. Hyperspectral imaging options which can scan the entire sample and not just single point assessment on the fruit require investigation as the technology may improve on the percentage correctly classified compared with spot assessment and line scan imaging.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Poor and inconsistent avocado fruit quality is seen as a key factor affecting Australian consumer confidence and impacting on supply chain efficiency and profitability. Avocado fruit with internal defects of greater than 10% have a dramatic negative impact on consumer repurchasing (Petty and Embry 2011; Embry 2009). Bruising has been identified by consumers as the major defect and a significant barrier to purchasing other than price, followed by body and stem end rots (Harker 2009). Providing the consumer with a quality and consistent product is a critical factor in retaining and expanding markets. A non-destructive inline system that can rapidly and accurately monitor all avocado fruit going through a pack-house for internal quality attributes such as maturity based on DM content, impact damage (bruising) and rot susceptibility, would allow the industry to provide a more consistent quality fruit to meet consumer expectations. This would assist to improve industry competitiveness, expansion of domestic and export markets, and increase industry profitability.

Automated technology advancement has enabled the development of commercially feasible non-destructive techniques for estimating internal quality attributes for agricultural products. Although, several non-invasive techniques have been developed for the prediction and detection of avocado maturity parameters, NIR spectroscopy is considered the most advanced non-invasive techniques in relation to applications, instrumentation, software packages, throughput speed and adaptability to a commercial setting. The aim of this project was to investigate the potential of FT-NIR diffuse reflectance spectroscopy as an objective non-invasive tool for determining internal quality attributes of whole 'Hass' avocado fruit. These attributes include: prediction of maturity and thereby eating quality based on DM content; (b) to predict the risk of developing flesh disorders (i.e., rot susceptibility) as an indication of shelf-life; (c) to detect bruises. The project also aimed to demonstrate the importance of the calibration model development process to incorporate seasonal and geographical variability to ensure model robustness.

8.1 NIR spectroscopy prediction of avocado dry matter content

Currently, for commercial purposes traditional destructive methods are utilised to determine avocado maturity through assessing a number of samples in a batch to represent an entire consignment. These destructive methods include, DM content, mesocarp oil content and MC, all of which are highly correlated with maturity. The utility of FT-NIR spectroscopy was investigated for the first time as a non-invasive technique for estimating DM content of whole intact 'Hass' avocado fruit. The commercially available FT-NIR spectroscopy systems assessed

in this project highlighted the potential of FT-NIR spectroscopy and its suitability for its application in a commercial in-line setting for predicting avocado maturity and palatability of whole intact avocados, based on DM content. The results found in this project compared favourably against data from other NIR systems identified in literature which have been used in research applications on avocados.

8.2 Calibration model robustness

The importance of NIR spectroscopy in postharvest technology is evident from the literature, as well as the fact that some manufacturers of in-line grading equipment have implemented NIR systems to measure various quality attributes of horticultural products. However, the robustness of calibration models with respect to biological variability from different seasons has been disregarded and therefore calibration models may be over-optimistic with respect to prediction accuracies on future samples. Particularly in a commercial setting, if the calibration model is not robust in design it will limit the application of the technique. Robustness of calibration for season and geographical variability is consequently a critical issue that needs to be addressed.

Unfortunately, the process of calibration development is a major impediment to the rapid adoption of NIR spectroscopy. 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, NIR spectroscopy 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. This project represents the first study to investigate the effect of geographical variability (growing districts) and seasonal variability (time) on calibration model robustness to be applied to avocado fruit for the determination of DM content for possible implementation in a commercial in-line application.

The predictive ability of calibration models depends on how well the calibration data set represents the composition of the future samples being assessed. It is found that seasonal and geographic variability has a significant effect on model predictive performance for DM in avocados, since the spectral deviations due to biological variability of future samples cannot be predicted. This project demonstrated that by increasing seasonal variability into the calibration set through incorporating data from multiple growing seasons, predictive robustness (stability) of the calibration model can be increased across seasons to achieve predictive performances in this case in the range of: R_v^2 of 0.76 – 0.89, RMSEP of 1.43 - 1.97%, and SDR of 2.0 to 3.1.

The research also demonstrated 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. This can be addressed by incorporating multiple geographical growing regions into the calibration model to account for the biological variability as highlighted with season variability (i.e., R_v^2 of 0.89, RMSEP of 1.51%, and SDR of 3.6). Furthermore, when models are constructed to include both season and geographical variability model performance can be more robust when dealing with a broader range of future sample variability. This was demonstrated with calibration models constructed to incorporate 3 years of seasonal variability and encompassing 3 geographical regions, obtaining predictive performance ranging from R_v^2 0.87 - 0.89; RMSEP of 1.42 - 1.64% and SDR of 2.7 - 3.1. This study found that to produce robust calibration models suitable for application in a commercial setting, calibration models must contain a minimum of three seasons of data which incorporates a sufficiently broad range of biological variability for the attribute of interest to enable prediction of future samples.

This research provides positive results for the potential application of NIR spectroscopy as a rapid non-destructive technique that can accurately monitor avocado quality attributes. By further developing and implementing a commercial NIR based inline system, the avocado industry will be able to maximise sales in existing markets and to target new markets with a differentiated, more consistent quality product to meet the increasingly higher standards expected by domestic and overseas consumers. Calibration robustness is the key to successful use of the NIR spectroscopy technique and continual calibration maintenance is vital to ensure calibration model robustness. 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. This requires the calibration models to have sufficient biological variability incorporated into the model that represents future samples, thus maintaining predictive performance. 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, there is always some risk that by including more biological variability, a percentage of atypical data may be included in the calibration set which can significantly reduce the models prediction accuracy (Bobelyn et al. 2010). Thus calibration maintenance is an on-going process and an important consideration to commercial implementation.

8.3 NIR spectroscopy prediction of avocado rot susceptibility and shelf-life

The biggest risk during transit is quality loss due to the development of rots and flesh disorders occurring once the fruit ripen and age during storage. At present there is no commercially

available non-invasive inline grading system that can reliably predict avocado flesh disorder development in relation to shelf-life to ensure that the fruit will arrive at the consumer with an acceptable quality. To the best of the authors knowledge there has been no research work identified in literature utilising NIR spectroscopy as non-invasive technique to detect bruises or predict the risk of developing flesh disorders (i.e., rot susceptibility) as an indication of shelf-life, in whole avocado fruit. A rapid, non-invasive method to identify fruit that are less prone to rots and internal disorders would allow selection of fruit that could be sent to more distant markets with greater confidence that the fruit will arrive at market in an acceptable quality, thus ensuring maximum yield for the producer and retailer. An NIR spot assessment technique was investigated as a non-invasive tool to predict rot development as an indication of shelf-life in whole 'Hass' avocado fruit.

This research demonstrated that NIR spectroscopy was a suitable method for prediction rot susceptibility as an indication of shelf-life of whole 'Hass' avocado fruit, with great potential for commercial implementation. The ability of the NIR classification models to accurately predict rot development of hard green avocado fruit into two classes, ≤10% and >10% of flesh affected, ranged from 65-84% over the three growing seasons. When the rot classes were defined as ≤30% and >30% the accuracy ranged from 69%-77% over the seasons.

8.4 NIR spectroscopy to detect bruises in avocado fruit

An NIR spot assessment technique was investigated as a non-invasive tool to detect bruises in avocado fruit. Avocado fruit were assessed at the sprung stage of ripeness (stage 2) and at eating ripe (stage 4) for presence or absence of bruising. Trials conducted over three growing seasons found hard green fruit (stage 2 ripeness) that were deliberately bruised could be correctly detected with 70-79% accuracy after 2-5 hours of impacting and with 83-89% accuracy after 24 hours. For eating ripe (stage 4) fruit, the accuracy across the seasons was 60-100% after 2-5 hours of impacting and 66-100% after 24 hours. This would indicate that in a commercial situation it would be an advantage to hold the fruit for 24 hours prior to scanning. This research demonstrated that there is great potential to use NIR spectroscopy as a tool to predict impact damage (bruising) of whole avocados.

Overall, FT-NIR reflectance spectroscopy shows promise for the application in a commercial, in-line setting for the non-destructive evaluation of impact damage (bruising) and rot susceptibility of whole avocado fruit, although optimisation of the technology is required to address speed of throughput and environmental issues. Multispectral and hyperspectral imaging options which can look at the entire sample and not just single point assessment on the fruit require investigation as

the technology may improve on the percentage correctly classified compared to spot assessment and line scan imaging.

8.5 Recommendations for future work

There are still areas that need to be addressed relating to NIR spectroscopy in commercial settings, including: speed; laborious and complex calibration model development, degree of accuracy of calibration models; robustness of models over seasons and geographic regions, transfer of calibration models between instruments. NIR has proven to be a valuable tool in agriculture for over 40 years and there is great potential for its implementation into the avocado industry for maturity testing based on DM content, bruise detection, shelf-life prediction base on flesh disorder (i.e., rots) development during storage, and classification of ripening stage. While further research will be required to fully utilise the NIR technique in a commercial setting, the technology offers considerable advantages to industry.

The majority of applications of NIR spectroscopy described in literature, essentially used single-point (spot) measurements and spatial distribution information of chemical constituents in the sample are not identified. In recent years the advancement of NIR technology has started to address these spatial variability issues through line scan assessment, or through multispectral and hyperspectral imaging of the sample, providing both spectral and spatial information (Lee et al. 2014; Opara and Pathare 2014; Van Zeebroeck et al. 2007). These technological advancements require investigation for commercial applications to fruit as they offer distinct advantages of assessing the entire product in comparison to just assessing a single spot or combination of points on the product. Technology suitability for commercial applications in relation to 'fitness for purpose' (i.e., speed of throughput, pack-house environments, ease of use, hardware configurations, computing power, data storage etc) will need to be considered. A combination of several techniques may be useful for a better determination of fruit quality attributes. For example, the combination of NIR spectroscopy and acoustic technologies to provide a better prediction of internal quality attributes. However, costly methods will only be applicable where a corresponding return on investment (profit) is to be expected.

Over recent years, much progress has been made in developing rapid, non-destructive techniques for the assessment of fruit and vegetable quality parameters. Commercial application of these techniques will be beneficial for the grower, marketer and consumer. However, it is the consumer who buys the product and who is willing to pay the cost, thus it should be kept in mind that it is better to measure a parameter that is really important to the consumer, with the aim of improving both consumer satisfaction and product quality. With this said, an opportunity exists to utilise the technology to predict the number of days before the avocado fruit is ripe and ready-to-eat. These

fruit could then be categorised in a retail situation based on the number of days to ripe. Thus, for the consumer this would take the guess work out of selecting fruit that are ready-to-eat or that will be ripe in a certain number of days.

Similarly, Central Distribution centres face serious logistical problems with avocado fruit in the same consignment having a mix of ripening stages. If avocado fruit were graded accordingly to their ripening stage, these logistical problems could be resolved based on factual information, fruit quality could be maintained through limiting handling and operating costs could be reduced to maximise returns for the industry. With emphasis on consistent fruit quality by consumers, marketers and retailers, there is a need in the avocado industry for rapid, accurate, non-destructive in-line sorting of product into relative categories based on quality attributes and ripening stage. For the consumer, the implementation of such 'smart sensing' technology would significantly reduce the risk of buying poor-quality avocados, giving confidence in the product for future purchases.

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APPENDICES

Appendix A

Non-invasive Assessment of Internal Quality Attributes of Whole Avocado Fruit by NIRS

Summary of key results and discussion as published in: Wedding, B.B., White, R.D.; Grauf, S.; Tilse, B.; Hofman, P. and Gadek, P.A. (2009) Non-invasive Assessment of Internal Quality Attributes of Whole Avocado Fruit by NIRS. *SABRO Journal of Breeding and Genetics* Vol. **41**, Special Supplement August 2009 ISSN 1029-7073.

Abstract

The utility of FT-NIR spectroscopy was investigated as a non-invasive technique for estimating %DM of whole intact 'Hass' avocado fruit, and included fruit from three harvest across the commercial harvesting season. PLS regression models were developed from the diffuse reflectance spectra to predict %DM, taking into account effects of intra-seasonal variation and orchard conditions. The study found that combining three harvests (early, mid and late seasons) yielded a predictive model for %DM with $R_v^2 = 0.86$, RMSEP = 1.18% for the DM in the range 18.2-35.0%. These results indicate the potential of FT-NIR spectroscopy, in diffuse reflectance mode to non-invasively predict the %DM (and thus internal fruit quality) of whole 'Hass' avocado fruit.

Materials and methods

Avocado selection as per *Section 3.3.2.1*; %DM analysis as per *Section 3.3.2.3*; NIR spectra collection as per *Section 3.3.2.4*.

Calibration modelling and validation

PLS regression was used to build the prediction models of the diffuse reflectance spectral data. Before calibration model development, the spectra variation of the data was analysed by PCA and obvious spurious spectra eliminated. A range of multivariate statistical calibration techniques, including PLS and PCA together with various mathematical pre-processing, wavelength selection and outlier elimination methods were used to develop calibration models and determine the robustness of these models. This was achieved using the commercially available chemometric software package OPUS™ QUANT (version 6.0 and 6.5). The OPUS™ QUANT automatic selection process was used to identify spectral regions and pre-processing treatments for improved model development. Among all spectra collected, significant noise was found within spectral ranges 2331-2503 nm. Therefore all the raw absorbance (ABS) spectra used for analysis were truncated to a range of 834-2330 nm.

The %DM for each sample was used as the constituent data, and the spectra (with and without pre-processing) were used as the spectral data for PLS calibrations. A leave-one-out cross validation procedure was employed in the PLS regression analysis (Lin et al. 2004).

Results and discussion

To ensure intra-seasonal factors including variability in %DM range were incorporated into the calibration procedure, 'Hass' avocado fruit were harvested at discrete intervals during the 2006 growing season, representing three maturity stages (early, mid and late season harvests). Calibration model development (cross validation) for %DM was conducted on the entire noise reduced spectral region (834-2330 nm) with no pre-processing treatment. Summary statistics for each harvest date and a combined data set encompassing all three harvest dates (Combined) are depicted in *Table A.1*. The population mean for %DM increased steadily over the season.

Table A.1. PLS calibration results for % dry matter for whole 'Hass' avocado fruit harvested at early, mid and late stage in the season and with all three harvests combined. Calibration models are based on ABS spectral region 834-2330 nm with no pre-processing.

Harvest	Spectra n (outliers)	% DM range	Mean	SD	R^2	RMSECV	Terms	RPD
Early	207 (3)	21.1-29.9	24.9	1.6	0.51	1.1	16	1.43
Mid	205 (6)	18.23-31.71	26.5	2.7	0.81	1.13	17	2.32
Late	217 (6)	25.08-35.03	30.7	1.9	0.64	1.08	11	1.66
Combined	629 (11)	18.23-35.03	27.5	3.2	0.88	1.11	17	2.88

Table A.2 displays the PLS calibration models for the combined harvest using FT-NIR spectroscopy on the whole fruit. The data set of 629 spectra were randomly separated into a calibration set (n = 466) and prediction set (n = 163) to develop the calibration and prediction models respectively. Varying spectral ranges and mathematical pre-treatments result in different calibration performance. Straight line subtraction was selected as the optimal mathematical pre-treatment for the two spectral regions selected (834-1333 and 1639-1836 nm) for model development.

Table A.2. PLS calibration and prediction statistics with outliers removed, for the determination of % dry matter of whole 'Hass' avocado fruit for the combined harvests using FT-NIR spectroscopy.

Test Set	Spectra n (outliers)	Spectral Region (nm)	Pre-treatment	R^2	RM SECV	RM SEP	LV	RPD	BIAS
Calibration	466 (6)	834-1333 &	Straight line	0.85	1.19		11	2.6	-0.00015
Prediction	163 (0)	1639-1836	subtraction	0.86		1.18	12	2.7	-0.19

The calibration results based on the ABS spectral region for determining %DM within individual harvest dates were relatively poor. The early and late season harvests were the worst for both regression and prediction. These early and late season harvest dates yielded the narrowest %DM range, resulting in significantly lower SD's. These results suggest that the fruit obtained from these two harvest dates possibly did not include a sufficiently broad variability in physiological attributes to develop a suitable calibration model, although other biological or environmental effects may have contributed.

Two spectral regions were selected in the development of the calibration for %DM prediction of Hass avocado fruit. The selection of this broad wavelength region indicates that the information incorporated into the model is due to the combined effect of many wavelengths with each contributing only relatively little information. This selection of wavelengths is consistent with the presence of strong electromagnetic absorption for oil around 2200-2400 nm with weaker absorption around 930 and 1200 nm; and the presence of the third overtone of the carbohydrate CH absorbance band in the 890-920 range (Clark et al. 2003). The use of multiple window selection may lead to even better models but the problem is analytically more complex, requiring sophisticated window selection procedures not utilised in this study.

Chemometric models were developed that could predict %DM of whole Hass avocado to within 1.2% with an RPD>2.6, indicating an ability to sort the fruit into at least two categories (i.e., above and below an acceptable %DM value) with approximately 80% accuracy (Guthrie et al. 1998). The combined harvest prediction results of this study of an RMSEP of 1.18% DM and R_v^2 0.86 were similar to those obtained by Clark et al. (2003) using a fixed PDA spectrometer in reflectance mode (RMSEP of 2.6% DM over a 20-45% DM range and an R_v^2<0.75). In fact, the current FT-NIR spectroscopy reflectance based model was similar to the accuracy of the NIR spectroscopy interactance mode of Clark et al. (2003) (R_v^2 of 0.88 and an RMSEP of 1.8% DM) indicating reflectance FT-NIR spectroscopy may be a better alternative for in-line and at-line environments. In relation to other avocado studies, Walsh et al. (2004), using a PDA spectrometer reported calibration results of Rc = 0.89, RMSECV = 1.14, SDR = 2.2, for % DM on raw absorbance data for whole of avocado fruit (unspecified cultivar). Schmilovitch et al. (2001) used a dispersive NIR spectrophotometer in reflectance mode in the 1200-2400 nm range on 'Ettinger' and 'Fuerte' avocado (both relatively thin-skinned cultivars) and reported preliminary results of errors of prediction (SEP), based on a PLS model with six factors using first derivative of R, as 0.9 and 1.3% respectively, for a 14-24% DM range. It is likely that the thin-skin cultivars studied would not suffer to the same extent from the experimental limitations experienced in the thick rough skin of 'Hass'.

Appendix B

Prediction of Hass avocado maturity via FT-NIR spectroscopy

Summary of key results and discussion as published in: Wedding. B.B.; Wright, C.; Grauf. B.; White, R.D. and Gadek, P. (2010) Prediction of 'Hass' avocado maturity via FT-NIRS. In: *NIR 2009 - Breaking the Dawn, Proceedings of the 14th International conference on Near Infrared Spectroscopy*, Bangkok, Thailand, 7-16 November 2009, pp 260-272 IM Publications West Sussex.

Abstract

In this study FT-NIR spectroscopy was investigated as an objective non-invasive technique for estimating 'Hass' avocado maturity and thereby eating quality based on %DM and its ability to predict over several growing seasons. 'Hass' avocado fruit were obtained over the 2006, 2007 and 2008 growing seasons from a single farm in the major production district of Childers, Queensland. PLS calibration models were developed from the diffuse reflectance spectra to predict %DM, taking into account the effects of seasonal variation. The study found that the model's robustness increased across years (seasons) when including more variability in the calibration set. The calibration model encompassing fruit from all three years yielded predictive statistics of $R_v^2 = 0.89$, RMSEP = 1.52% for %DM using 10 latent variables, in the range 16.1-39.7% DM. These results indicate the potential of FT-NIR spectroscopy in diffuse reflectance mode to non-invasively predict the %DM of whole 'Hass' avocado fruit and the importance of calibration model development incorporating seasonal variation.

Materials and Methods

Avocado selection as per *Section 5.2.2.1*; %DM analysis as per *Section 3.3.2.3* where the flesh was diced to facilitate drying in a fan-forced oven at 60-65°C to constant weight (approximately 72 h); NIR spectra collection as per *Section 3.3.2.4*; data analysis as per *Section 3.3.2.5*. All models presented in this study were 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).

Results and Discussion

The influence of seasonal variability was investigated over three years (see *Table B.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 B.1. Preliminary PLS calibration and prediction statistics for % dry matter for whole 'Hass' avocado fruit for 2006, 2006-7 and 2006-08 (Combined) seasons.

Harvest		Spectra n (outliers removed)	SD	LV	R^2	RMSECV	RMSEP	SDR
Calibration	Prediction							
2006		207 (1)	3.4	10	0.83	1.56		2.2
	2007	609 (0)	3.1		0.20		2.73	1.1
2006 & 07		415 (0)	3.5	9	0.82	1.50		2.4
	2008	606 (0)	5.3		0.82		2.27	2.4
Combined		624 (1)	4.6	10	0.89	1.52		3.0
	Combined	1224 (0)	4.3		0.89		1.41	3.1

Note: LV = latent variables.

As expected, the application of single seasonal calibrations to populations from other growing seasons was not very successful. 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 1.41% with a $R_v^2 = 0.89$ and SDR of 3.1. This indicated an ability to sort the fruit into three categories with approximately 80% accuracy (Guthrie et al. 1998).

Appendix C

The application of FT-NIR spectroscopy for the detection of bruises and the prediction of rot susceptibility of 'Hass' avocado fruit

Summary of key results and discussion as published in:

i) *Brett B. Wedding, Carole Wright, Steve Grauf and Ron D. White. (2012) The application of near infrared spectroscopy (NIRS) for the assessment of avocado quality attributes. In: Infrared Spectroscopy. Intech Open Book Access;* and

ii) *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (2011) Non-invasive assessment of avocado quality attributes. In: The Proceedings of the VII World Avocado Congress 2011, Cairns – Australia, 5-9 September 2011.*

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% 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.

It should be considered that the preliminary work presented here is a first step towards developing a non-invasive NIR spectroscopy assessment tool for detecting bruises and for predicting rot susceptibility as an indication of shelf-life of avocado fruit.

Materials and Methods

'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. All fruit were harvested at the hard green stage of ripeness.

With impact (bruise) and rot assessment trials, diffuse reflectance spectra of whole, intact 'Hass' avocado fruit were collected 780-2500 nm range using a Bruker Matrix-F, FT-NIR spectrophotometer as discussed in *Section 3.3.2.4*. Method of fruit handling and spectra collection for impact assessment and rot susceptibility prediction was as per *Section 7.1.2*. The impact assessment method varied slightly in this study compared to *Section 7.1.2.2*, with the impacted area being re-scanned after 1-2 hours (maximum of 4 hours) and again after 24 hours. Bruise assessment was based on *visual estimate* of percentage bruise development of the flesh within the scanned area.

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

Results and Discussion

Classification statistics for the prediction of percentage rot development are presented in *Table C.1*. 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 C.1. 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 C.2 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 misclassified 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 samples, 4 (3.9%) were at the ambiguous change over point of the two defined classification categories at 10% bruising.

Table C.2. 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.

Appendix D

Impact assessment and prediction of rot susceptibility of 'Hass' avocado fruit using NIR spectroscopy

Summary of key results and discussion as published in: *Wedding, B.B.; Wright, C; Grauf, S.; White, R.D. and Gadek, P.A. (Submitted 2011) Impact assessment and prediction of rot susceptibility of 'Hass' avocado fruit using FT-NIRS. In: the Proceedings of the 15th International conference on Near Infrared Spectroscopy, Cape Town, 16-20 May 2011.*

Abstract

Avocado fruit maturity and quality characteristics are often variable. This results in variation within a shipment in ripening rates, shelf-life and quality. Reliable export of avocados from Australia requires 2-6 weeks sea freight depending on destination. The biggest risk during transport is the development of rots and flesh disorders due to disruption of cell structure and function. Internal defects of 10% or more has a dramatic impact on the consumer repurchasing, for this reason fruit quality reliability is seen as one of the key factors impacting on supply chain efficiency and related profitability. In this study FT-NIR spectroscopy was investigated as a non-invasive tool to predict which avocado fruit are more susceptible to rots as an indication of potential shelf life of fruit requiring extended storage.

The study found that by applying discriminative analysis techniques 83% of the population could be correctly classified into 2 categories, above and below 30% rot development; and 86% of the population could be correctly classified into 3 categories based on percentage bruise development (0%, 10-50%, 50-100%) following impact. The results of this study indicate there is great potential to use FT-NIR spectroscopy as a tool to predict impact damage of whole avocados based on percentage bruise development, and to predict shelf-life based on rot development.

Materials and Methods

Avocado selection as per *materials and methods in Appendix C*; NIR spectra collection, impacting of fruit and assessment was as detailed in *Section 7.1.2.2*. TQ Analyst™ chemometric software (Version 8.0.36, Thermo Fisher Scientific Inc. Madison, WI USA) was used for discriminative analysis to separate the avocados into categories based on percentage rot and percentage bruise development of the scanned area. The rot prediction models presented in this study were based on (i) a combination of spectral mean centring, with a 25 point SG spectral smoothing (second order polynomial) and a MSC transformation over selected wavelength regions (not shown) for the ≤30% and ≥31% rot model; (ii) a combination of spectral mean centring, variance scaling and a first derivative transformation (41 point SG smoothing, first order polynomial) over selected

wavelength regions (not shown) for the ≤10% and ≥11% rot model. A data normalisation technique of variance scaling was applied to all bruising assessment spectra used to develop the models presented in this study.

Results and Discussion

Classification statistics for the prediction of percentage rot development are presented in *Table D.1*. The preliminary study found that by applying discriminative analysis techniques, 84.8% 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 82.4% when the classification was reduced to above and below 10% rot development for the scanned area.

Table D.1. 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	15.2 (n=38)	84.8 (n=212)
	250	(i) 0-10; (ii) 11-100	9	17.6 (n=44)	82.4 (n=206)

Note: LV = Latent Variables.

Table D.2 depicts the classification statistics for the prediction of percentage bruise development. The results indicate that 85% of the population could be correctly classified into 2 categories based on percentage bruise development in the scanned area (≤10%, >10%) using scans conducted 1-2 h following impact. Of the 15 (14.7%) samples misclassified, 3 (2.9%) samples with bruising visually rated below 10% were placed into the >10% bruise category; 12 (11.8%) samples visually rated with bruising greater than 11% were placed into the <10% bruise category, with 5 (4.9%) of these samples being right on the ambiguous change over point of the two defined classification categories at 10% bruising.

These results improved significantly to >90% correctly classified when the fruit were rescanned 24 h following impact. It appears the 24 h 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 h prior to scanning. Of the 9 (8.8%) samples misclassified, 1 (1%) sample with bruising visually rated below 10% was placed into the >10 bruise category; 8 (7.8%) samples with bruising visually rated greater than 11% were placed into the ≤10% bruise

category, with 5 (4.9%) of these samples being at the ambiguous change over point of the two defined classification categories at 10% bruising.

Table D.2. 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	8	14.7 (n = 15)	85.3 (n = 87)
	24	102		8	8.8 (n = 9)	91.2 (n = 93)

Note: LV = Latent Variables.