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# **Identification of Fruit Parameters Related to Calyx Cavity Incidence for the Evaluation of Non-destructive Equipment as Segregation Tools**

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Horticultural Science

at Massey University, Palmerston North,  
New Zealand.

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2022

# Abstract

Calyx cavity is a major physiological disorder in persimmon that hinders exports and correlates with reduced fruit quality during storage. Postharvest quality changes, including increases in softening and colouration, have been related to the presence of calyx cavity. This thesis investigated the impact of calyx cavity on persimmon quality using both destructive and non-destructive methods of evaluation and assessed the ability of these tools, combined with modelling techniques, to segregate affected fruit.

Seven batches of 'Fuyu' persimmon were evaluated at the time of harvest and following a nine-week storage period in modified atmosphere packaging (MAP) at 1 °C. Quality parameters assessed were fruit size, texture, colour, sweetness, and severity of calyx cavity. Non-destructive methods used included near infrared spectroscopy (NIR), acoustic vibration and non-destructive compression; the destructive methods used were flesh firmness and soluble solids content (SSC). A visual scale was created to score the severity of calyx cavity from no separation (grade 1) to minor (2), moderate (3), and major separation (4).

After storage, fruit firmness decreased, and SSC and external colouration increased compared to at harvest values. Large differences in fruit maturity, in terms of firmness, colour and SSC were observed between fruit from different harvest dates. The presence and severity of calyx cavity was correlated with greater colouration and greater weight. On average, a 40 g increase in weight and 1 – 2 unit increase in colour index was observed with each level (1 – 4) of calyx cavity severity. Using colour and weight data, binary classification of calyx cavity by linear discriminant analysis (LDA) had a total accuracy of 74.1% correct segregation. Random forest (RF) segregation of calyx cavity using NIR data had a performance metric of 0.655.

The implementation of non-destructive calyx cavity classification based on the evaluation of changes in quality parameters would provide the ability to segregate healthy and affected fruit. However, the relatively low accuracy of these models creates limitations including yield losses and failed identification of fruit with calyx cavity. Further work is required to improve model performance including optimisation and validation of calyx cavity segregation models.

# Acknowledgements

I would like to express my gratitude to my primary supervisors, Sebastian Rivera-Smith, and Dr. Mo Li, who guided me throughout this project and provided invaluable advice, feedback, patience, and support. Thank you for your insightful comments and suggestions, and for believing in me. I would also like to extend my appreciation to Prof. Andrew East for his coordination and direction of the project. I could not have imagined having better mentors for my study. It has been an honour and great pleasure to work under their guidance.

My sincere thanks also go to Peter Jeffery and Sue Nicholson for help and support with laboratory work. Many special thanks for providing advice and expertise that allowed me to successfully carry out my experiments. I wish to thank my fellow students in the postharvest group at Massey University for making me feel welcome and part of the team. I would like to acknowledge Massey University for providing me with scholarship funding and my main sponsor, Lois Turnbull, for supporting me throughout the two years of my master's studies. I would also like to share how grateful I am to First Fresh NZ for supplying the experimental materials and contributing their expert knowledge to the project.

Thanks must also go to my family for the endless love and support they have given me throughout my life. I would like to give my biggest thank you to my mother, Karin Longuet-Higgins, for her ongoing patience, tolerance, and sacrifices throughout my studies. I would not have been able to achieve the success I have without all the great individuals mentioned.

# List of Abbreviations

ACC	1-Aminocyclopropane-1-Carboxylic Acid
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
$\beta$ -gal	Beta-Galactosidase
BPANN	Back Propagation Artificial Neural Network
CA	Controlled Atmosphere
CI	Chilling Injury
CO <sub>2</sub>	Carbon Dioxide
DAFB	Days After Full Bloom
DM	Dry Matter
EMSC	Extended Multiplicative Scatter Correction
FI	Firmness Index
FW	Fresh Weight
GA3	Gibberellic Acid
GLM	General Linear Model
iPLSR	Interval Partial-Least Square Regression
IQR	Inter Quartile Range
LDA	Linear Discriminant Analysis
LDPE	Low Density Polyethylene
LSD	Least Significant Difference
1-MCP	1-Methylcyclopropene
MA	Modified Atmosphere
MAP	Modified Atmosphere Packaging
MSC	Multiplicative Scatter Correction
N01	Normalization Between 0 and 1
NIRS	Near Infra-Red Spectroscopy
NA	Non-astringent
NZD	New Zealand Dollars
O <sub>2</sub>	Oxygen

OSC	Orthogonal Signal Correction
PC1	Principal Component 1
PC2	Principal Component 2
PC3	Principal Component 3
PC	Pollination Constant
PCA	Principal Component Analysis
PCNA	Pollination Constant Non-Astringent
PE	Polyethylene
PG	Polygalacturonase
PLS	Partial-Least Squares
PLS-DA	Partial-Least Squares Discriminant analysis
PME	Pectin Methyl Esterase
PV	Pollination Variant
PVA	Pollination Variant Astringent
PVNA	Pollination Variant Non-Astringent
R	Correlation Coefficient
R <sup>2</sup>	Coefficient Of Determination
RF	Random Forest
RH	Relative Humidity
RMSE	Root Mean Square Error
RMSEC	Root Mean Square Error of Calibration
RMSEP	Root Mean Square Error of Prediction
RPD	Ratio Of Performance to Deviation
SD	Standard Deviation
SEP	Standard Error of Prediction
SG	Savitzky–Golay Smoothing
SNV	Standard Normal Variate
SSC	Soluble Solids Content
SSOM	Supervised Self-Organising Map
TA	Titrateable Acidity
TCR	Temperature Controlled Room

TSS Total Soluble Solids

ULO Ultra-Low Oxygen

# Table of Contents

<b>Abstract</b> .....	<b>i</b>
<b>Acknowledgements</b> .....	<b>ii</b>
<b>List of Abbreviations</b> .....	<b>iii</b>
<b>Table of Contents</b> .....	<b>vi</b>
<b>Appendices</b> .....	<b>ix</b>
<b>List of Figures</b> .....	<b>x</b>
<b>List of Tables</b> .....	<b>xvi</b>
<b>Chapter 1. Introduction</b> .....	<b>1</b>
<b>Chapter 2. Literature Review</b> .....	<b>5</b>
2.1 Industry statistics & commercialisation.....	5
2.2 Persimmon taxonomy.....	6
2.3 Fruit biology & growth .....	6
2.4 Astringency in persimmon .....	9
2.4.1 Physiological disorders.....	12
2.4.1.1 Calyx separation.....	12
2.4.1.2 Chilling injury .....	15
2.5 Persimmon maturity & quality .....	18
2.5.1 Ripening physiology .....	19
2.5.2 Colour changes.....	20
2.5.3 Colour measurements.....	23
2.5.4 Textural changes .....	26
2.5.5 Texture measurements.....	29
2.5.5.1 Destructive firmness .....	30
2.5.5.2 Non-destructive firmness .....	31
2.5.5.3 Correlation between texture measurements .....	32
2.5.6 Sugar metabolism .....	33
2.5.7 Soluble solids measurements .....	35
2.5.8 Acidity & pH .....	36
2.5.9 Correlation between quality characteristics.....	37
2.5.10 Grading.....	38
2.6 Postharvest technologies.....	39

2.6.1	Storage environment .....	39
2.6.2	Atmosphere composition management.....	40
2.6.2.1	Modified atmosphere packaging .....	40
2.6.2.2	Controlled atmospheres .....	42
2.6.2.3	Detrimental effects of CA & MAP .....	42
2.7	Near infrared technology.....	43
2.7.1	Overview .....	43
2.7.2	Modelling .....	44
2.7.3	Quality predictions.....	45
2.7.4	Internal disorder detection by NIR .....	46
2.7.5	Cavity detection by NIR technology.....	50
2.7.6	NIR devices.....	53
2.8	Conclusions .....	54
<b>Chapter 3.</b>	<b>Materials &amp; Methodology .....</b>	<b>56</b>
3.1	Plant materials & storage .....	56
3.2	At harvest evaluations .....	58
3.2.1	Non-destructive measurements .....	59
3.2.1.1	Near infrared spectroscopy .....	59
3.2.1.2	Weight.....	60
3.2.1.3	Skin colour.....	60
3.2.1.4	Acoustic firmness .....	61
3.2.1.5	Non-destructive compression.....	62
3.2.2	Destructive measurements.....	62
3.2.2.1	Calyx cavity.....	62
3.2.2.2	Flesh firmness .....	63
3.2.2.3	Soluble solids content.....	64
3.3	Storage measurements.....	64
3.3.1	Gas, temperature & RH monitoring.....	64
3.4	After storage measurements .....	65
3.4.1	Water loss, rots & chilling injury.....	65
3.5	Statistical analysis .....	66
<b>Chapter 4.</b>	<b>Non-destructive Indicators &amp; Calyx Cavity .....</b>	<b>68</b>
4.1	The relationship between destructive and non-destructive evaluations.....	68
4.1.1	Maturity indicators at harvest time.....	68

4.1.2	Postharvest changes .....	71
4.1.2.1	Atmospheric CO <sub>2</sub> and O <sub>2</sub> composition in MAP .....	71
4.1.2.2	Skin colouration .....	74
4.1.2.3	Flesh firmness .....	76
4.1.2.4	Soluble solids content.....	78
4.1.2.5	Non-destructive firmness .....	80
4.1.3	Weight loss & postharvest disorders.....	81
4.2	Calyx cavity detection .....	83
4.2.1	Cavity incidence .....	83
4.2.2	Principal component analysis .....	86
4.2.3	Calyx cavity & quality variable relationships .....	87
4.2.3.1	Fresh weight.....	87
4.2.3.2	Peel colouration.....	91
4.2.3.3	Destructive & non-destructive firmness.....	92
4.2.3.4	Soluble solids content.....	94
4.2.3.5	The effect of calyx separation on quality changes during storage.....	95
4.2.4	Prediction of calyx cavity .....	96
4.3	Conclusions and future directions .....	100
<b>Chapter 5.</b>	<b>Near Infrared Spectroscopy .....</b>	<b>102</b>
5.1	Spectral data .....	102
5.2	Regression model summation and comparisons.....	106
5.2.1	Model accuracy & robustness .....	107
5.2.2	Pre-processing techniques.....	110
5.2.3	Wavelength selection .....	111
5.2.4	Sampling.....	112
5.2.4.1	Model Validation.....	114
5.3	Classification models.....	114
5.3.1	Model results .....	114
5.3.2	Application of NIRS to detect calyx cavity .....	117
5.4	Conclusions .....	118
<b>Chapter 6.</b>	<b>Conclusions &amp; Recommendations.....</b>	<b>119</b>
<b>References</b>	<b>.....</b>	<b>121</b>
<b>Appendices</b>	<b>.....</b>	<b>140</b>

# Appendices

Appendix A: Australian 'FreshSpec' grade standards .....	140
Appendix B: The random forest algorithm .....	141
Appendix C: Simple binary logistic regression .....	143
Appendix D: NIR cavity & quality predictions .....	144

# List of Figures

Figure 2.1 A labelled diagram showing the anatomy of a persimmon fruit longitudinal section. .....	7
Figure 2.2 Changes in fruit diameter and weight of non-astringent fruit during development in stages I, II and III (Testoni, 2002). .....	8
Figure 2.3 Fresh weight and longitudinal diameter of persimmon fruit ‘Rojo Brillante’ [RB], ‘Giombo’ [Gi], ‘Fuyu’ [Fu] and ‘Hana Fuyu’ [HF] at seven consecutive maturity phases during growth stage III. Adapted from Tessmer et al. (2016). .....	9
Figure 2.4 Images of the effect of pollination on ‘Rojo Brillante’, a PVA persimmon cultivar. Non-pollinated parthenocarpic fruit showing no seeds or discolouration (left) and pollinated fruit with seeds present and darkening of flesh resulting in no marketable fruit. Adapted from Blasco et al. (2020). .....	11
Figure 2.5 Images of ‘Fuyu’ persimmons with calyx cavity. Calyx separation disguised by the calyx lobes (left); severe calyx separation encircling the whole calyx (right) visible after removal of the calyx lobes. ....	12
Figure 2.6 The visual scale of calyx cavity created by Akagi et al. (2020) which scored fruit from levels 0 to 4 based on the worsening degree of separation between the calyx and the fruit flesh.....	14
Figure 2.7 Photos of the advancement of CI symptoms in ‘Fuyu’ fruit after cold storage. Symptoms range from level 0 (no chilling injury) to level 5 (total gelling of flesh). Adapted from Woolf and Ben-Arie (2011). .....	17
Figure 2.8 Respiration rate (left) and ethylene production (right) in NZ grown ‘Fuyu’ persimmons after storage at 0 °C for 4 weeks, colour graded based on the Japanese colour charts: ● 4.5 – 5; ○ 5 – 6; ▲ 6 – 6.5. Adapted from MacRae (1987). .....	19
Figure 2.9 Ethylene production of ‘Youhou’ sweet persimmon during storage at 1 °C for 10 weeks. Adapted from Zhao et al. (2020). .....	20

Figure 2.10 Colour index of persimmon fruit ‘Rojo Brillante’ [RB], ‘Giombo’ [Gi], ‘Fuyu’ [Fu] and ‘Hana Fuyu’ [HF] in seven maturity stages, showing increases in colouration as fruit matures from underripe to overripe (Tessmer et al., 2016). .....	21
Figure 2.11 Effect of storage temperature on the colour index of ‘Rojo Brillante’ after storage, CO <sub>2</sub> treatment and 3 days at 15 °C. Temperature: (◇) 1 °C, (■) 8 °C, (▲) 11 °C, (□) 15 °C (Arnal & Del Río, 2004).....	23
Figure 2.12 Relationship between hue angle and colour chart values of different coloured persimmon cultivars (Asakuma & Shiraishi, 2017) (left), and Korean colour chart for determining maturity of ‘Fuyu’; optimal colour rating on this scale is considered to be 5 (Bignell et al., 2017) (right). .....	25
Figure 2.13 (left) Activity levels of PME and PG enzymes in ‘Rojo Brillante’ fruit at different maturity stages (after destringency treatment). Values with the same letter in each column do not differ at the 5% significance level using the LSD (least significant difference) test. Adapted from (Salvador et al., 2007). (right) PME activity of fruit ‘Qiandaowuhe’ persimmon stored at 20 °C. ■ , control; ●, 3 ml/l 1-MCP (Luo, 2007). .....	27
Figure 2.14 Flesh firmness of persimmon fruit ‘Rojo Brillante’ [RB], ‘Giombo’ [Gi], ‘Fuyu’ [Fu] and ‘Hana Fuyu’ [HF] across seven different maturity stages from green, underripe to orange-red, overripe fruit (Tessmer et al., 2016). .....	28
Figure 2.15 SSC of persimmon fruit ‘Rojo Brillante’ [RB], ‘Giombo’ [Gi], ‘Fuyu’ [Fu] and ‘Hana Fuyu’ [HF] at seven consecutive maturity stages ranging from immature, green fruit (S1) to overripe, orange-red fruit (S7) (Tessmer et al., 2016). .....	34
Figure 2.16 Titratable acidity (cmol L <sup>-1</sup> ) of ‘Fuyu’ persimmon fruit in different types of packaging materials, at different periods under 1 °C and 90% RH + 5 days at 25 °C and 70% RH (Cia et al., 2006). .....	37
Figure 2.17 Firmness loss of ‘Rojo Brillante’ persimmon fruit during cold storage at 1 °C, 10 °C and 14.5 °C and 90% RH. ▲ = 1 °C, ■ = 10 °C, ○ = 14.5 °C (Orihuel-Iranzo et al., 2010). .....	39

Figure 2.18 Flesh firmness of ‘Fuyu’ persimmon fruit in different packaging types during storage at 1 °C and 90% RH (plus 5 days at 25 °C and 70% RH) over 12 weeks (Cia et al., 2006). .....	41
Figure 2.19 Raw NIR spectra of tangerine fruit comparing the five classes of granulation. Adapted from Theanjumol et al. (2019). .....	48
Figure 2.20 Images of the sequential development of brown core symptoms (Han et al., 2006). The level of the brown core was segregated into four grades (1: no brown core; 2: slight; 3: moderate; 4: severe).....	49
Figure 2.21 Average spectral composition of the light transmitted through pears with and without brown core illustrating differences exhibited between fruit with varying degrees of the disorder (Han et al., 2006).....	49
Figure 2.22 An example of the most severe degree of hollowness in white radish (A) and mean (semi-transmittance) spectra of white radishes with various degrees of hollowness (levels 0 – 4) (B). Sample sizes; level 0 = 105, level 1 = 30, level 2 = 53, level 3 = 66, level 4 = 43. Adapted from Pan et al. (2017). .....	51
Figure 2.23 Transverse sections of normal pickling cucumbers versus watery and hollow fruit (A), and mean spectra (and SD) of healthy compared to defective pickling cucumbers (B). Sample sizes; normal cucumbers = 300, defective = 300 (slight = 150, severe = 150). Adapted from Cen et al. (2013). .....	52
Figure 2.24 Spectra acquired from the bottom side of chestnuts displaying differences in absorbance between healthy and infested fruit, n = 952 (Moscetti et al., 2014). ...	53
Figure 3.1 Flow diagram of experimental procedures indicating the destructive and non-destructive evaluations completed at harvest and post storage. ....	57
Figure 3.2 ‘Fuyu’ persimmons individually labelled and laid out in an 18-count plastic fruit tray for at harvest measurements (left). Fruit packed on an industry standard 20-count cardboard tray inside a 60 µm MA bag after sample balancing before heat sealing (right). .....	58

Figure 3.3 Locations of the destructive and non-destructive measurements indicated by coloured areas on the equator (shown in purple) and shoulders (shown in yellow) of the persimmon fruit.....	59
Figure 3.4 A chromaticity diagram showing the colour coordinates (a, b and hue angle) and indicating colour directions (sourced from Konica Minolta Sensing). .....	61
Figure 3.5 Diagram of the visual scale created to segregated different levels of calyx separation in ‘Fuyu’ persimmon ranging from grade 1 (no cavity present) to grade 4 (severe cavity present). The scale can also be interpreted as a binary where grade 1 = absence of calyx cavity (0), and grades 2 – 4 = presence of calyx cavity (1). .....	63
Figure 3.6 ‘Fuyu’ fruit with various chilling injury symptoms (left) and common puncture and apex rots ( <i>Penicillium</i> spp., <i>Rhizopus</i> spp.) and black spot ( <i>Alternaria</i> spp.) (right). Images adapted from (Woolf & Ben-Arie, 2011) (rot) Bignell et al. (2017) (chilling injury).....	65
Figure 4.1 Correlations between each of main maturity indicators including both at harvest and after storage measurements. (A) represents colour index vs soluble solids; (B) colour index vs flesh firmness; and (C) soluble solids vs flesh firmness. ....	69
Figure 4.2 The average gas concentrations in the headspace of the modified atmosphere packages over the 9 weeks cold storage at 0 °C. CO <sub>2</sub> and O <sub>2</sub> readings were taken from each package once per week and averaged across packages and harvest batches. 71	
Figure 4.3 The final concentrations of CO <sub>2</sub> (A) and O <sub>2</sub> (B) for the seven harvest batches. Each batch was split between four separate MAPs – the bars represent the averages of the final O <sub>2</sub> and CO <sub>2</sub> readings for each MAP. ....	73
Figure 4.4 The average colour indices of fruit at harvest compared to after storage for each harvest batch of fruit. ....	74
Figure 4.5 The flesh firmness of fruit at harvest and after storage for each harvest batch of fruit. ....	77
Figure 4.6 The SSC levels of fruit at harvest and post storage split by harvest batch.....	79

Figure 4.7 The acoustic firmness of persimmons against the flesh firmness (A) and the non-destructive compression as a % of whole fruit deformation compared to the flesh firmness (B). Data includes both at harvest and after storage measurements, coloured by harvest batch. ....	81
Figure 4.8 Images of persimmon after 9 weeks MAP storage at 0 °C. Fruit with symptoms of chilling injury (a), (b) and (c); fruit with areas of fungal growth and rot (d) and (e); a close-up of mealy bugs in a calyx cavity (f). ....	83
Figure 4.9 Stacked bars representing the percentage of each calyx cavity grade across the different harvest batches after visual evaluation and scoring of the severity of calyx separation. ....	85
Figure 4.10 Loading (A) and score (B) plots of principal components 1 and 2, representing the relationship between seven postharvest response variables of ‘Fuyu’ persimmons. For the score plot (B), each point represents one individual fruit, coloured by harvest batch. ....	86
Figure 4.11 Raincloud plots displaying the distribution of persimmon weights at harvest based on the presence or absence of calyx cavity. ....	89
Figure 4.12 Raincloud plot comparing the distribution of weights at harvest based on the visual scale severity of the cavity, where grade 1 represents fruit without a cavity, and grades 2 – 4 fruit have cavities of increasing size. ....	89
Figure 4.13 The average weight of fruit for each of the different harvest batches prior to storage. n = 840 ....	90
Figure 4.14 The distribution of at harvest persimmon skin colour of fruit with a calyx cavity and without. Colour measurements were taken at the fruit shoulders and equator and averaged. The colour index was then calculated from the equation $(1000a)/(Lb)$ . ....	91
Figure 4.15 Comparison of the at harvest colour indices between persimmon with different degrees of calyx separation. ....	92

Figure 4.16 Flesh firmness (kgf) of 'Fuyu' fruit at harvest separated by the presence and absence of calyx cavity. ....	93
Figure 4.17 Raincloud plots showing the at harvest results of non-destructive compression (A) and acoustic firmness (B) separated by calyx cavity severity. ....	94
Figure 4.18 A comparison of at harvest Brix values (as an average of shoulder and equator readings) separated by the presence or absence of calyx cavity. ....	95
Figure 4.19 Flowchart showing an example of use of the classification model to segregate persimmons including potential commercial implications. ....	99
Figure 5.1 (A) The set of raw NIR spectra, and (B) the spectral data pre-treated by log and second order derivation using a polynomial number of 2 (with an 11-point window) within the range of 740 – 1070 nm. Line colours in (B) represent varying levels of SSC. ....	104
Figure 5.2 The variation in at harvest SSC (%Brix) (A) and flesh firmness (kgf) (B) between the 7 batches of persimmons harvested consecutively over the picking season. ....	112

# List of Tables

Table 2.1 A summary of the different persimmon classifications and the effect of each kind of pollination, and hence seed formation, on fruit astringency at harvest. ....	10
Table 2.2 Comparison between sensory, instrumental destructive and instrumental non-destructive methods used to estimate textural properties in persimmon fruit (excluding NIR-based techniques). ....	29
Table 4.1 Final or average gas concentrations reported by comparable studies on MAP in persimmon, including a variety of packing film thicknesses and cultivars. ....	72
Table 4.2 The presence and severity of calyx cavity given as a percentage of the total sample for each batch based on visual assessment scores. ....	84
Table 4.3 Eigenvectors for each parameter for the first three principal components and the proportion of variance accounted for by each component. ....	87
Table 4.4 The averages of quality characteristics for cavity and no cavity fruit before and after storage. ....	96
Table 4.5 Classification table of predictions based on linear discriminant analysis of at harvest weight and colour index data where calyx cavity data is categorised by severity....	97
Table 4.6 Classification table of predictions based on linear discriminant analysis of at harvest weight and colour index data where calyx cavity data is used as a binary (no = calyx cavity severity of 1; yes = calyx cavity severity of 2 - 4). ....	98
Table 5.1 Overview of the wavelengths at which typical absorption bands are seen for certain chemical groups in NIR spectra of various fruit samples reported by various studies, adapted from Wang et al. (2015). ....	103
Table 5.2 Summary of the PLS regression models across various quality attributes using 10-fold cross-validation. Pre-processing methods of log, averaging, and SNV (standard	

normal variate) were used. Models created in the SCIO™ online application can be found in Appendix D. ....	106
Table 5.3 Summary of comparative research on NIRS to predict quality characteristics in persimmon fruit. All models utilised PLS regression; other qualities were predicted in these papers but not included in the table. ....	108
Table 5.4 Summary of the classification models using the random forests (RF) algorithm to predict the presence or absence of calyx separation, and the severity of calyx cavity. ....	115
Table 5.5 The normalised confusion matrix prediction values of the presence (Y) or absence (N) of calyx separation and the level of separation from grades 1 (no separation) to 4 (severe separation) compared to the observed scores at harvest.....	116

# Chapter 1. Introduction

Persimmon is a member of the genus *Diospyros* which originated in central China; the commercial persimmon is the fruit of the Oriental or Japanese persimmon (*Diospyros kaki* L.) (Mowat & George, 2018). The fruit has an orange to red surface colouration, a rounded shape, and is typically consumed from firm to soft, depending on levels of astringency (Bignell et al., 2017). The New Zealand persimmon industry's focus is on producing high-quality export fruit, of the popular, non-astringent 'Fuyu' variety (Woolf & Ben-Arie, 2011). Persimmon exports have been growing year on year; in 2020 New Zealand persimmon exports were valued at over \$10 M (fob), with the main markets being Asia and Australia (Aitken & Hewett, 2021). Persimmon fruit are popular, particularly in Asian countries, for their subtle, sweet flavour and high nutritional value (Altuntas et al., 2011; Zhou et al., 2011). Current consumer preferences favour persimmons with bright skin colouration and minimal blemishes, and soft, juicy flesh which is high in flavour and sweetness (Ban et al., 2010; Mitani et al., 2015).

There are various defects which can render fruit unmarketable. In persimmon, calyx cavity is one of the major physiological disorders hindering fruit quality, particularly in non-astringent (or "sweet") cultivars like 'Fuyu' (Bignell et al., 2017). The formation of calyx cavity occurs during the later stages of fruit growth (phase III) when fruit expansion occurs more rapidly (Woolf & Ben-Arie, 2011). This rapid growth can result in a separation (varying degrees) between the calyx and the fruit flesh, forming a cavity (George et al., 2005). This cavity often harbours quarantine pests which can lead to rejection of the fruit by importing countries, and it can provide a favourable environment for pathogen growth causing spoilage of fruit and losses to industry (Bignell et al., 2017; Lay-Yee et al., 1997). The commercial recommendation is to remove fruit with calyx cavity during packing, along with prevention strategies (Bellini & Giordani, 2002; George et al., 2005). Severe instances of the disorder can be identified visually during grading, but detection of mild symptoms is more challenging due to obstruction by the calyx lobes (Woolf & Ben-Arie, 2011). Therefore, there is potential to develop a rapid, non-destructive approach to detect the presence (and severity) of calyx cavity.

The development and retention of good quality fruit follows correct ripening and maturation of fruit pre- and postharvest (Altuntas et al., 2011; Beever, 1990). Selection of the correct harvest maturity and storage conditions play a large part in fruit quality (Testoni, 2002). Harvest timing in persimmons is based on well-established external colour indices which are linked to fruit maturity (Asakuma & Shiraishi, 2017; Bignell et al., 2017). Commercial storage of persimmons generally entails modified atmosphere packaging (MAP) at 0 °C for up to 9 weeks (Woolf & Ben-Arie, 2011). During storage, changes to the physical and physicochemical properties of the persimmon occur. These changes are characterized by the advancement of flesh softening, augmentation of skin colour from yellow-orange to orange-red and an increase in soluble solids content (SSC) (Candir et al., 2009). The prevalence of calyx cavity is higher in larger fruit and has been related to greater fruit softening and chilling injury (CI) (Woolf & Ben-Arie, 2011). Fruit with the disorder may also exhibit increased external colouration near the cavity (Akagi et al., 2020). It has been reported that MAP can be used to minimise or delay the prevalence of chilling injury (CI) symptoms and the expression of rots in persimmon postharvest (Cia et al., 2006; MacRae, 1987; Zhao et al., 2020).

Fruit quality attributes can be measured by various non-destructive and destructive methods (Magwaza & Opara, 2015). Sufficient levels of key qualities, including colour, sweetness, firmness, maturity, and astringency for persimmons, is essential for consumer satisfaction (Chen & Opara, 2013; Shewfelt, 2014). Standard measurement techniques are frequently destructive, however, there is a range of non-destructive alternatives which offer rapid, easy and continuous evaluations without creating waste (Chen & Opara, 2013). For textural measurements, common non-destructive approaches include acoustic firmness and non-destructive compression (Ubierna et al., 2005); for SSC and colour estimation, near infrared (and Vis/NIR) systems have achieved good results (Walsh et al., 2020). The correlation between destructive and non-destructive measurements has been evaluated across a range of horticultural commodities. For example, Li et al. (2016) reported that in 'Gold3' and 'Hayward' kiwifruit the relationship between standard flesh firmness and acoustic firmness was strong ( $R^2$  of 0.889 and 0.936 respectively). There are limited studies on destructive and non-destructive evaluations in persimmon fruit which leaves space to investigate these relationships in terms of firmness, colour, and SSC.

The use of NIR (and related) technologies for quality assessment has been fairly well explored in persimmon with upwards of 26 publications. Previous studies in persimmon have reported high accuracies of prediction in SSC, and moderate to strong success in firmness prediction (Altieri et al., 2017; Ar et al., 2019; Hemrattrakun et al., 2021; Jannok et al., 2014; Wei et al., 2020). For other fruit, a fair amount of work has also been completed on NIR-based detection of quality defects, such as granulation in citrus fruit (Theanjumol et al., 2019; Xu et al., 2020) and brown core in pears (Cruz et al., 2021; Fu et al., 2007; Han et al., 2006; Sun et al., 2016) among others. However, research into the detection of disorders involving the development of cavities or hollows is comparatively limited. Such examples include internal hollowness in radishes (Pan et al., 2017; Takizawa et al., 2014) and pickling cucumbers (Ariana & Lu, 2008a, 2008b; Cen et al., 2013), and insect damage in chestnuts (Bedini et al., 2020; Moschetti et al., 2014). The classification of calyx cavity by NIR spectroscopy has not yet been attempted – this provides an opportunity to evaluate the usefulness of this technology to predict the presence and severity of the disorder.

The main aim of this research was to investigate the relationships between quality attributes and calyx separation using non-destructive methods of evaluation. The specific objectives of the present work are as follows:

**For Chapter 4**

- 1) To assess the influence of maturity on persimmon quality after long-term postharvest storage in MAP at 0 °C. The effect of postharvest changes was described by maturity indicators including SSC, firmness, and colouration.
  
- 2) To determine the strength of the relationship between destructive and non-destructive evaluations and investigate the potential for these non-destructive evaluation methods to be used to classify and segregate fruit with calyx cavity.

**For Chapter 5**

- 1) To investigate the potential for NIR spectroscopy evaluation to be used to classify and segregate fruit with calyx cavity. Classification models were developed from the NIR data collected at harvest and after cold storage.

The experimental objectives have been explored through the two research chapters in this study. The relationships between destructive and non-destructive evaluations and the post-harvest changes in maturity indicators are reported in Chapter 4. The use of NIRS to segregate fruit with calyx cavity is analysed in Chapter 5. With these investigations, it may be possible to develop a rapid, non-destructive method of classification which would allow the segregation of affected fruit from export quality fruit and minimize the chance of pests and rotten fruit in export consignments (Bignell et al., 2017). Outcomes from this research could benefit the postharvest processing and storage of commercially produced persimmon fruit.

# Chapter 2. Literature Review

This chapter provides relevant details on persimmon fruit biology and common physiological disorders, with a focus on the symptoms, causes, control, and classification of calyx separation. The ripening physiology of persimmon is discussed, and the mechanisms behind pre- and postharvest changes in fruit attributes including colour, firmness, sweetness, acidity, and dry matter are examined. The main methods of quality assessment, in terms of destructive and non-destructive approaches, are reviewed for the key quality characteristics of persimmon. In addition, the effects of the storage environment on postharvest changes are also considered. Finally, there is a brief overview of NIR technology and its use in the detection of cavity-based disorders in the horticulture industry.

## 2.1 Industry statistics & commercialisation

In New Zealand, persimmons have been grown since the 1870s. The main growing regions are Gisborne, Auckland, Northland, and Waikato, with fruit produced almost exclusively of the popular, non-astringent 'Fuyu' variety (MacRae, 1987). The industry focus is on export fruit; persimmon exports to Asia have increased from \$6 M to \$7 M (NZD) from 2019 to 2020. In 2020, New Zealand produced 1,700 tonnes of persimmon which returned \$1.3 M in domestic sales and \$10.7 M in total export sales. The biggest markets for New Zealand persimmon are Australia, Singapore, Thailand, and Vietnam which take a total of 75% of exports (Aitken & Hewett, 2021).

As New Zealand is located far from its main markets, there are implications for postharvest storage. Fruit requires prolonged storage to reach export destinations (Bignell et al., 2017). Container shipping from New Zealand to Asia requires 21 to 27 days of sea freight (Sankaran, 2000). Accounting for additional logistic requirements, a minimum timeframe of 42 storage days (6 weeks) are required to reach export markets (Rupavatharam et al., 2013). As the length of time required to reach the main markets is significant, modified atmosphere packaging and 0 °C cold storage is used to ensure fruit arrives in good condition and spoilage

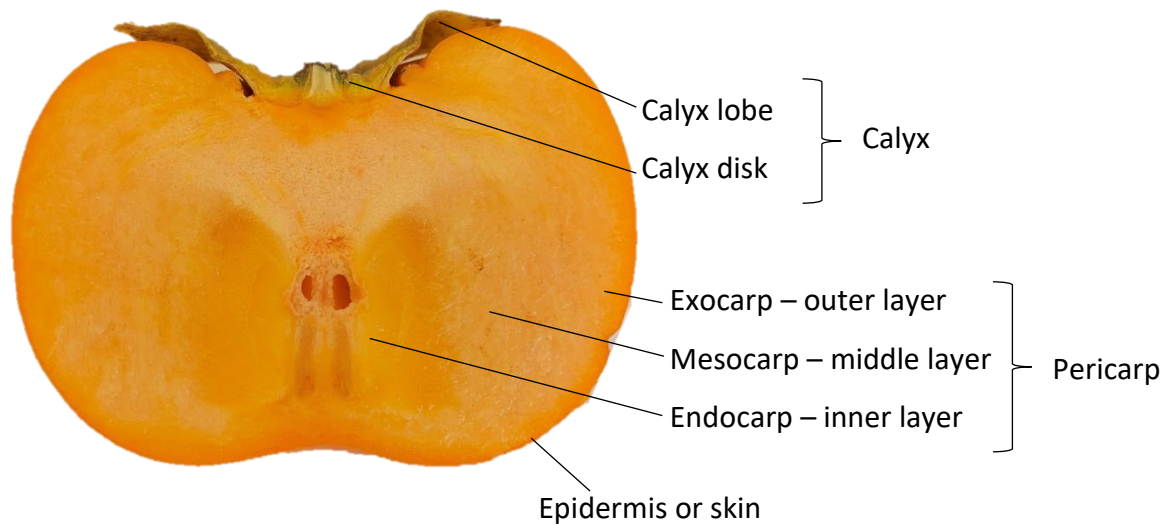
is minimised (Ben-Arie & Zutkhi, 1992). 1-Methycyclopropene (1-MCP) is often used in combination with MAP to delay fruit softening during prolonged sea freight transport periods. The extended storage time also allows for in-transit cold disinfestation of leaf roller which is approximately 30 days at 0 °C (Bignell et al., 2017).

## 2.2 Persimmon taxonomy

Persimmon is a member of the genus *Diospyros* which originated in central China and is native to Japan, Korea, Burma, and Nepal (Woolf & Ben-Arie, 2011). The *Diospyros* genus contains over 400 species which grow mainly in tropical or subtropical environments (Mowat & George, 2018). The commercial persimmon is the fruit of the Oriental or Japanese persimmon (*Diospyros kaki* L.). Cultivation began in China and extended to other parts of Asia, then California and southern Europe, and, subsequently, to New Zealand (Woolf & Ben-Arie, 2011). During the thousands of years of cultivation, over 2000 cultivars had been selected, with fewer than 100 being of importance to persimmon production today (Morton, 1987). The commercial cultivars grown vary between countries – popular cultivars include ‘Fuyu’, ‘Jiro’, ‘Youhou’ and ‘Rojo Brillante’ which have been selected for high yield and quality (del Mar Naval et al., 2010).

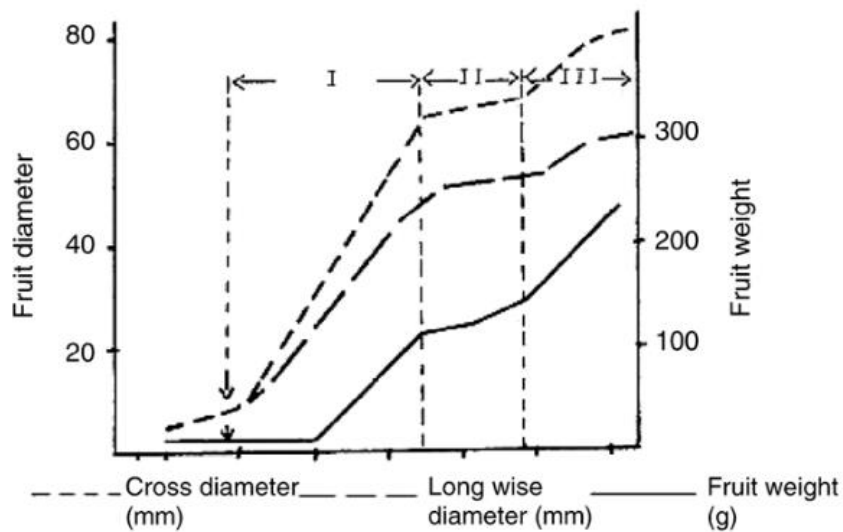
## 2.3 Fruit biology & growth

Persimmon fruit are botanically classified as true botanical berries (Mowat & George, 2018). A true berry is a simple fleshy fruit derived from a single pistil where the entire ovary wall ripens into an edible, relatively soft, fleshy pericarp with the exocarp forming a thin skin (**Figure 2.1**) (Suntudprom, 2014). Persimmon fruit forms from a superior ovary with the leafy calyx remaining at the stem-end; the calyx is made up of the calyx disk in the centre and surrounding calyx lobes (Nakano et al., 2003). The flesh consists of a dense parenchymatous cell structure which may contain large seeds in the inner section of each of the approximately eight carpels, but fruit may develop parthenocarpically (Woolf & Ben-Arie, 2011).



**Figure 2.1** A labelled diagram showing the anatomy of a persimmon fruit longitudinal section.

During fruit development, persimmon exhibits a double sigmoidal growth pattern consisting of two phases where the growth rate is rapid (stages I and III) with a period where the growth rate is slow in between (stage II) (Woolf & Ben-Arie, 2011). The double sigmoid growth pattern is visualised well by the increase in fruit diameter and weight over the growing season (**Figure 2.2**). The duration of fruit development ranges from 120 to 190 days, depending on the cultivar and environment. Typically, stage I lasts for 60 – 100 days; stage II for 20 – 40 days; and stage III for 40 – 50 days (Mowat & George, 2018). Growth stage I corresponds to cell division and differentiation, and growth stage III is associated with cell expansion and maturation (George et al., 1997). In 'Fuyu' fruit, cell division in the pericarp generally ends 30 DAFB (days after full bloom), with stage I of growth completed 70 DAFB (Mowat & Ah Chee, 1990). In contrast, 'Fuyu' fruit grown in NZ normally finalise stage I growth between 112 and 140 DAFB – the longer growth period is caused by the cooler growing conditions (Mowat & Ah Chee, 1990).



**Figure 2.2** Changes in fruit diameter and weight of non-astringent fruit during development in stages I, II and III (Testoni, 2002).

As fruit mature, size increases and therefore the potential to develop calyx cavity also increases. The formation of calyx cavity occurs during phase III of fruit growth (Woolf & Ben-Arie, 2011). During phase III, fruit size increases rapidly; the fruit tissue expands faster than the calyx which can result in the fruit flesh separating from the calyx tissue to form a cavity (Bellini & Giordani, 2002). George et al. (1994) first observed calyx cavity in Australian-grown 'Izu' and 'Fuyu' persimmons between 4 and 6 weeks prior to harvest. They also reported that the size of the cavity was moderately correlated with fruit weight for both cultivars ('Izu',  $r = 0.42$ ; 'Fuyu',  $r = 0.51$ ;  $P = 0.05$ ).

Persimmon fruit growth, in terms of mass and size, continues until harvest (Candir et al., 2009). As displayed in **Figure 2.3** below, most cultivars (including 'Fuyu') showed slight growth in fruit size throughout stage III as a result of cell enlargement. Small increases in the (longitudinal) diameter also occur until harvest. In Spanish-grown 'Fuyu', Tessmer et al. (2016) observed an increase in weight from approximately 100 g to 140 g over seven maturity phases from green to over-ripe fruit. Complete growth cessation coincides with harvest timing, occurring as late as 190 DAFB (Mowat & George, 2018). Weight gain in persimmon is influenced by the number of cells and cell size which is determined by the availability of

assimilate reserves during fruit growth (George et al., 1997). Positional effects of fruit within the tree canopy can have a greater effect on final fruit size in cooler regions. In NZ grown 'Fuyu', increases in size of more than 35% have been reported in fruit exposed to light compared to non-exposed fruit (Mowat & Ah Chee, 1990). The larger size of fruit was attributed to differences in flesh temperature up to 0.5 °C between exposed and shaded fruit.

**Figure 2.3 Fresh weight and longitudinal diameter of persimmon fruit 'Rojo Brillante' [RB], 'Giombo' [Gi], 'Fuyu' [Fu] and 'Hana Fuyu' [HF] at seven consecutive maturity phases during growth stage III. Adapted from Tessmer et al. (2016).**

## **2.4 Astringency in persimmon**

*Diospyros kaki* L. has two significant characteristics which differ between cultivars – the astringency of the fruit and the variability of pollination. There are two distinct forms of astringency in persimmon fruit: astringent (A) and non-astringent (NA) (Mowat & George, 2018). Astringency is acquired due to tannin synthesis and accumulation in the fruit flesh during growth (Ozdemir et al., 2020). These tannins include catechin, catechin-3-gallate,

gallo catechin, and gallo catechin-3-gallate (Salunkhe & Kadam, 1995). At harvest, the concentration of water-soluble tannins in astringent fruit will be considerably higher than that of non-astringent fruit, where natural astringency loss occurs pre-harvest at growth stage II or III, depending on the astringency type. Astringency causes a dry sensation to be felt in the mouth when soluble tannins from the fruit bind to salivary proteins, preventing the desired lubricating feeling that usually occurs (Woolf & Ben-Arie, 2011). Hence, non-astringent persimmons can be eaten when firm and crisp, but astringent fruit must be left until fully soft and ripe, or alternatively, be treated artificially with CO<sub>2</sub> to remove the astringency (Munera et al., 2017a; Yamada et al., 2002).

Persimmon astringency is controlled by the pollination events that produce seeds and modify the levels of tannins present in fruit (Blasco et al., 2020). The pollination variability of the cultivar influences seed formation; persimmons may be classed as either pollination constant (PC) or pollination variant (PV) (Sato & Yamada, 2016). In PC varieties, the level of astringency is not impacted by the presence or absence of seed formation. In PV cultivars, the level of astringency at harvest is regulated by the presence or absence of seeds (Blasco et al., 2020). Combinations of the different astringency and pollination traits allows persimmon cultivars to be divided into four groups: pollination constant astringent, pollination variant astringent (PVA-type), pollination constant non-astringent (PCNA-type), and pollination variant non-astringent (PVNA-type) (Woolf & Ben-Arie, 2011). A summation of the differences between astringency and pollination types is provided in **Table 2.1**.

**Table 2.1 A summary of the different persimmon classifications and the effect of each kind of pollination, and hence seed formation, on fruit astringency at harvest.**

	Astringent (A)	Non-astringent (NA)
Pollination Constant (PC)	<b>Pollination constant astringent</b> astringent regardless of seed presence	<b>PCNA</b> non-astringent regardless of seed presence
Pollination Variant (PV)	<b>PVA</b> astringent apart from around seeds if present	<b>PVNA</b> non-astringent when seeds are present

**PCNA = pollination constant non-astringent; PVA = pollination variant astringent; PVNA = pollination variant non-astringent.**

The different pollination types also affect fruit flesh colouration (Blasco et al., 2020). In fruit produced from PC varieties, flesh does not exhibit any colour change when seed formation occurs whereas, in fruit produced from PV varieties, flesh darkens when seed formation occurs (Mowat & George, 2018). In the PVA, PVNA and pollination constant astringent type fruit, the loss of astringency is related to the ability of seeds to produce acetaldehyde. This production causes the observed browning around seeds (**Figure 2.4**) which interferes with deastringency treatments and leads to unmarketable fruit (Blasco et al., 2020). Hence, in pollination constant astringent and PVA, pollination is avoided, and instead parthenocarpic, non-pollinated fruit are produced (and astringency is removed by postharvest treatment). Distinguishing between classes is important as each class tends to exhibit certain characteristics and susceptibilities, and have different requirements (Yamada et al., 2002).

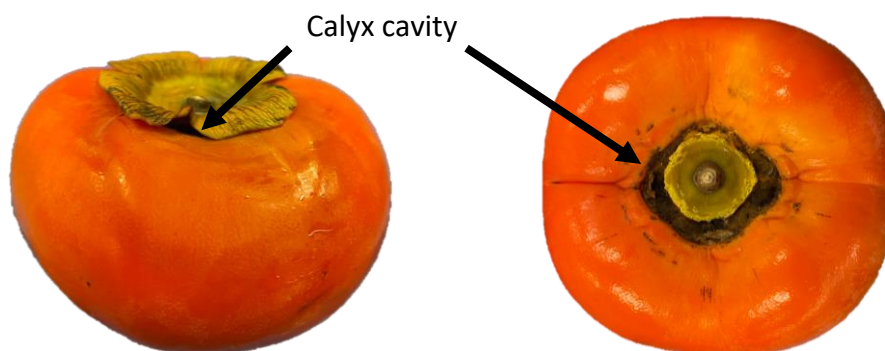


**Figure 2.4 Images of the effect of pollination on ‘Rojo Brillante’, a PVA persimmon cultivar. Non-pollinated parthenocarpic fruit showing no seeds or discolouration (left) and pollinated fruit with seeds present and darkening of flesh resulting in no marketable fruit. Adapted from Blasco et al. (2020).**

## 2.4.1 Physiological disorders

### 2.4.1.1 Calyx separation

Calyx cavity is a common physiological disorder seen in persimmon fruit. It is classified as a distortion disorder and is considered of major importance in persimmon production (George et al., 2005). Calyx cavity may also be referred to as ‘calyx-end cracking’ or ‘calyx separation’ (Akagi et al., 2020). However, calyx-end cracking is different from ‘apex cracking’ or ‘fruit cracking’ where physical failure of the peel leads to fractures or splits occurring on the fruit surface (Akaura, 2008; Khadivi-Khub, 2015). The disorder results in a space, or cavity, developing around or beneath the calyx of the fruit (**Figure 2.5**) – in severe cases, this gap may encircle the entire calyx (Woolf & Ben-Arie, 2011).



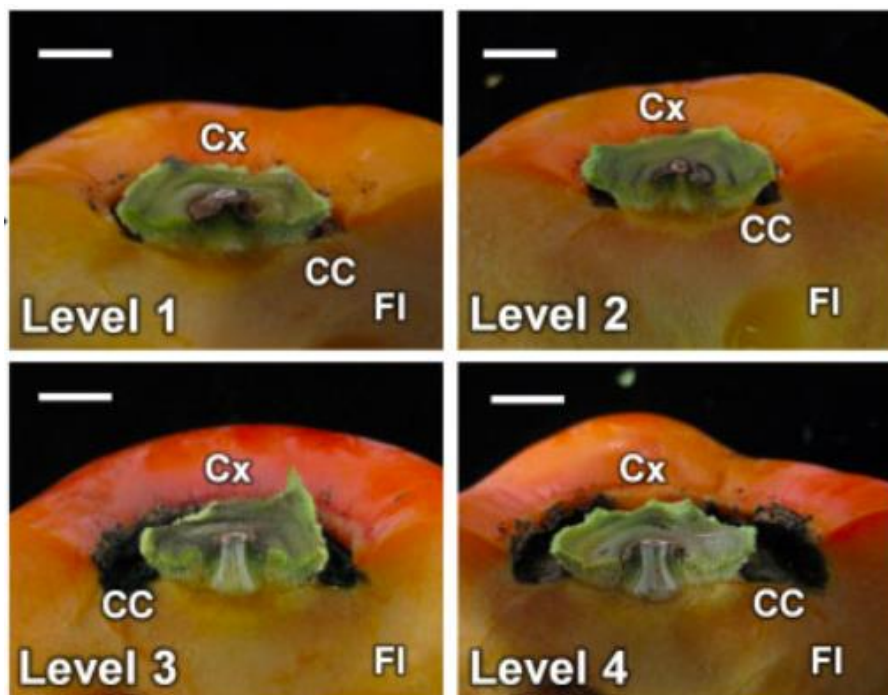
**Figure 2.5** Images of ‘Fuyu’ persimmons with calyx cavity. Calyx separation disguised by the calyx lobes (left); severe calyx separation encircling the whole calyx (right) visible after removal of the calyx lobes.

The calyx cavity acts as a refuge for unwanted pests including a common ‘hitchhiker’ pest *Pseudococcus longispinus* (longtailed mealy bug) which is classified as a quarantine pest in many importing countries (Lay-Yee et al., 1997). When a certain pest is not already present in a region, it presents an economic and environmental risk upon establishment of a population. If establishment occurs, the importing country may face costly, ongoing control measures and major produce losses (including persimmon and other horticultural commodities) (Hallman, 2011). When pests are identified on arrival, fruit entry is often conditional on fumigation with methyl bromide which harms fruit quality and storability

(Woolf & Ben-Arie, 2011). Calyx separation also provides a desirable environment for fungal pathogens to develop in the calyx cavity leading to fruit spoilage (Bignell et al., 2017). The presence of calyx cavity can also promote rapid softening of fruit before storage and a greater incidence of chilling injury during cold storage (Woolf & Ben-Arie, 2011). These consequences ultimately lead to significant quantities of unmarketable fruit, resulting in losses for the industry.

Calyx cavity can cause fruit to be unmarketable in both local and international markets. Tolerance of calyx cavity varies between markets; domestic markets tend to allow minor calyx cavity whereas export markets reject the disorder altogether (Bignell et al., 2017). Generally, fruit is deemed unmarketable for export if it has a cavity width or depth >3 mm (George et al., 1994). In Australia, industry grade standards ('FreshSpec') classify calyx cavity as a major defect; for class 1 (premium) exports 'slight' calyx cavity is acceptable (but total major defects must not exceed 2% of a consignment) (Bignell et al., 2017).

In previous literature, the severity of the disorder is often evaluated by degree of separation using arbitrary scales. Yamada et al. (1987) rated the degree of separation on a scale from zero to ten where the latter is the most severe. Akagi et al. (2020) categorised the disorder into five grades (levels 0-4) based on visual observation of calyx separation (**Figure 2.6**). In another work (George et al., 1994), the volume of the cavity was calculated to assess severity.



**Figure 2.6** The visual scale of calyx cavity created by Akagi et al. (2020) which scored fruit from levels 0 to 4 based on the worsening degree of separation between the calyx and the fruit flesh.

Calyx cavity formation is generally attributed to rapid growth of fruit during the final phase of growth (George et al., 2005). It is thought that the fruit expands faster than the calyx which causes the fruit flesh to partially detach from the calyx tissue, often resulting in significant apertures (Bellini & Giordani, 2002). As a result, fruit size has been linked to calyx cavity where larger fruit tend to have a higher prevalence of the problem compared to smaller fruit. In addition, there is a correlation with fruit shape, and flatter fruit are generally more prone to calyx cavity than round fruit (Bellini & Giordani, 2002). The age of the fruiting trees is also believed to influence the formation of calyx cavity; young trees tend to have rapid fruit growth which can prompt cavity development. In trees over 15 years, the incidence of calyx cavity usually declines (Woolf & Ben-Arie, 2011). The disorder may accompany poor early calyx growth which can be due to vigorous shoot growth and stress during fruit development (Bignell et al., 2017). Hence, fruit with a relatively large calyx at the time of flowering are less likely to develop a cavity (Sarkhosh et al., 2020).

Calyx separation has been connected to cultivar susceptibility as the development of the disorder varies between cultivars. The problem is more frequently seen in 'Fuyu' but occurs less in other cultivars such as 'Triumph', 'Jiro', and 'Suruga' (Woolf & Ben-Arie, 2011). Calyx separation is typical of the PCNA cultivars, and hence cultivars with small-sized calyx and distinctly large fruit are more greatly subjected to calyx cavity (Bellini & Giordani, 2002). All PCNA cultivars are prone to calyx cavity disorder while few cultivars from the other classes exhibit a tendency toward calyx cavity formation (Bellini & Giordani, 2002). Genetic variability within the class is limited which hinders elimination of the problem through breeding strategies (Yamada et al., 2002). However, studies conducted in Japan have shown that a predisposition to calyx cavity is genetically inherited (Yamada et al., 1988; Yamada et al., 2002).

The commercial recommendations are to remove fruit with calyx cavity during packing (Bellini & Giordani, 2002). While instances of severe cavity may be easily identified visually, detection of moderate and mild cases of calyx cavity can be arduous as the view of potential cavities is obstructed by the leafy calyx lobes. In fruit destined for high-value markets, like Japan, packhouses may employ the strategy of air-blasting beneath the calyx lobes to remove any hidden pests (Woolf & Ben-Arie, 2011). Prevention strategies are also used to reduce development of the disorder. Calyx cavity can be minimised through crop load management by pruning and fruit thinning to avoid the formation of excessively large fruit. Correct nutrition and irrigation practices can prevent intervals of stress during fruit development (George et al., 2005). Excessive nitrogen fertilization should be avoided as it has been linked to increased frequency of affected fruit (Bellini & Giordani, 2002). Additionally, early season thinning can also enhance calyx growth and help to prevent a cavity from establishing (Bignell et al., 2017).

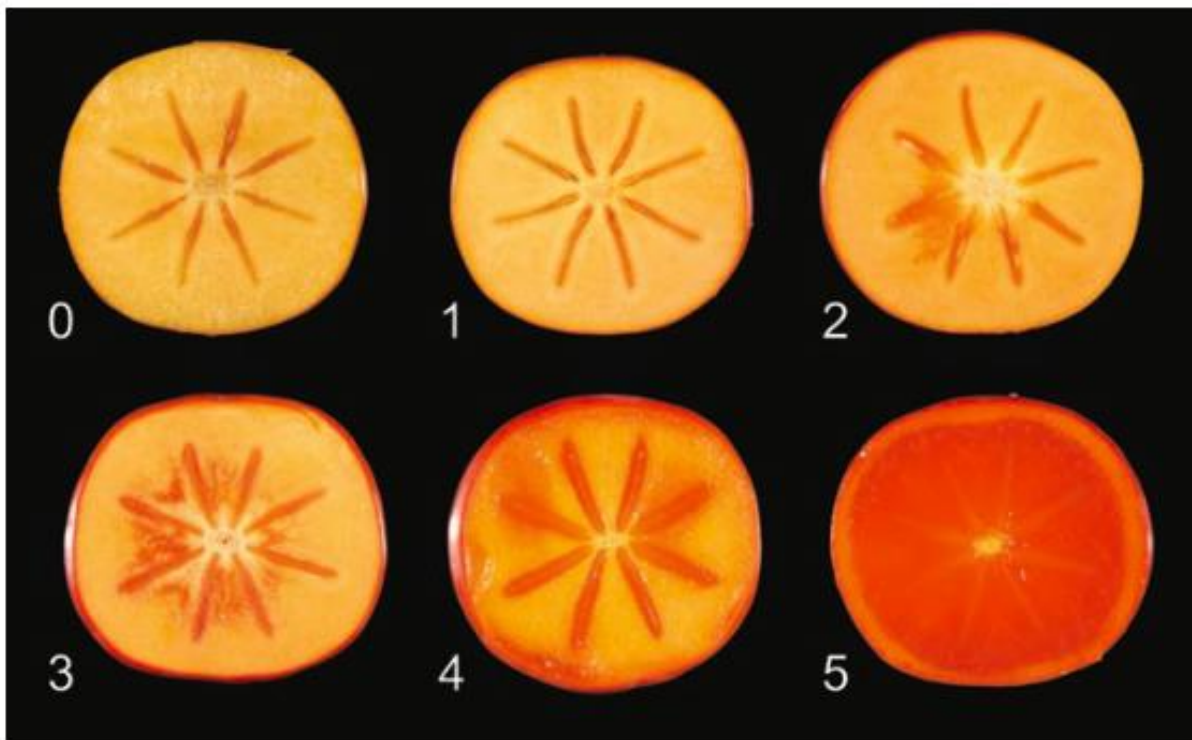
#### **2.4.1.2      *Chilling injury***

Cold storage can induce numerous storage-related disorders including chilling injury (CI) which is the primary storage problem in persimmon (Woolf & Ben-Arie, 2011). Symptoms of CI in persimmon are a gelatinous consistency in the flesh, large reductions in fruit firmness

and development of a deep red colouration. Chilling injury may also prompt the development of a range of other symptoms such as mealiness, loss of free juice, browning of skin or flesh, pitting, water-soaking, incomplete ripening, and off-flavours or odours (MacRae, 1987; Wills et al., 1998). The induction of CI also results in weakening of the tissues which renders the fruit very vulnerable to decay by postharvest pathogens (Wang, 1989). In its most severe form, chilling injury causes a rubbery texture, external mottling, and complete transformation of the flesh to be into a firm, dark, translucent gel (Grant et al., 1992). The progression from healthy fruit to severely CI-affected is visualised in **Figure 2.7** below. In CI-affected fruit, quality often critically declines to below acceptable levels, leading to losses for the industry (Wills et al., 1998).

Tropical and subtropical fruit like persimmon are particularly susceptible to CI as they are accustomed to warm climates and temperatures (Collins & Tisdell, 1995). Exposing fruit to sub-optimal storage temperatures for certain periods can induce a series of physiological disorders which are collectively referred to as CI. Many physiological and biochemical changes occur during cold storage and have been linked to CI (Wang, 1989). The disorder is associated with metabolic imbalances which affect membrane permeability and causes loss of cellular compartmentalisation. This leads to metabolites (including ions, amino acids, sugars, and pigments) leaking from cells and contributing to the development of CI symptoms (Grant et al., 1992). The severity and frequency of CI symptoms are connected with storage temperature, duration, cultivar, and maturity (Arnal & Del Río, 2004). Additionally, fruit with other pre-existing disorders (such as calyx cavity) may be predisposed to chilling injury (Woolf & Ben-Arie, 2011).

**Figure 2.7** Photos of the advancement of CI symptoms in ‘Fuyu’ fruit after cold storage. Symptoms range from level 0 (no chilling injury) to level 5 (total gelling of flesh). Adapted from Woolf and Ben-Arie (2011).



The full expression of CI is not evident until after removal from cold storage, which makes detection difficult (Lay-Yee et al., 1997). Chilling injury symptoms can be induced in persimmons fruit after a minimum of 14 days of cold storage at temperatures below 15 °C (Collins & Tisdell, 1995; Orihuel-Iranzo et al., 2010). In ‘Fuyu’ fruit, chilling injury can develop rapidly after exposure to storage temperatures of 0 – 4 °C (Woolf et al., 1997). A dark-coloured ‘star’ may be noticed at the distal end of the persimmon fruit during storage where CI is severe. When fruit is affected to a lesser extent, symptoms are not usually apparent until fruit are placed under ambient conditions for 3 days (Woolf et al., 1997). The expression of CI symptoms on removal from storage is associated with the stimulation of ethylene production and an increased respiration rate (MacRae, 1987).

There are many potential strategies to control CI in sensitive persimmon cultivars. Recently investigated solutions include heat treatments, controlled atmospheres (CA), modified atmospheres (MA), and 1-MCP (1-methylcyclopropene) (Besada & Salvador, 2018). Treatment with 1-MCP is commonly used to delay ripening processes induced by ethylene in

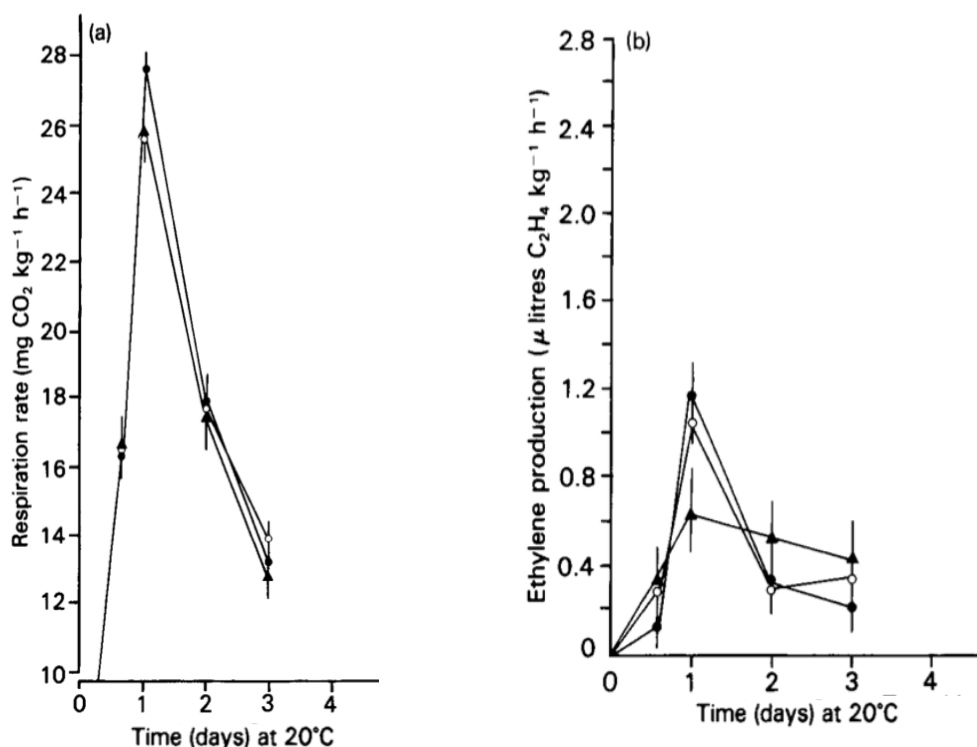
climacteric fruit by binding to and blocking ethylene receptors (Oz & Kefalas, 2010). Luo (2007) observed impedance of the softening process in 'Qaindaowuhe' persimmon with the application of 1-MCP. This study reported a reduction in the increase of water-soluble pectins in less depolymerisation of other pectic substances, as well as inhibition of Polygalacturonase (PG) and pectin methyl esterase (PME) activities. The reduction of these processes likely lessens the softening that is produced as a result of CI. However, the response to 1-MCP is dependent on harvest maturity and cultivar (Salvador et al., 2006).

## **2.5 Persimmon maturity & quality**

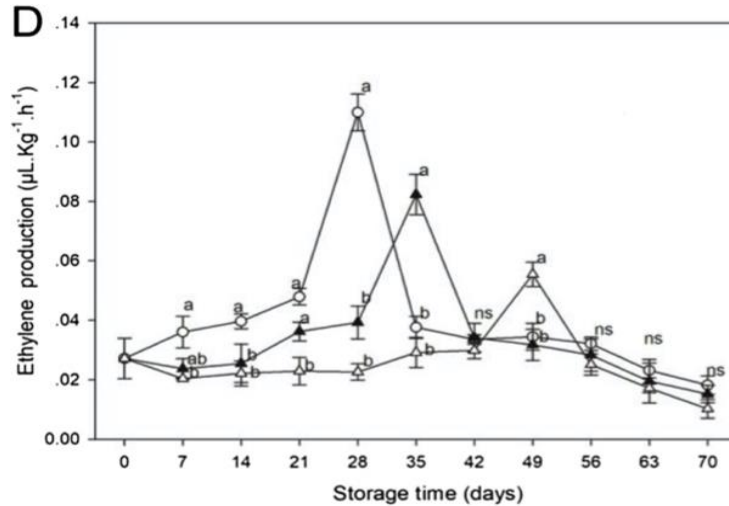
Persimmon fruit have a subtle, sweet flavour and low acidity which gives it high appeal in Asian countries. They are often consumed for their perceived health benefits (Woolf & Ben-Arie, 2011). The fruit flesh is comprised of predominantly water, sugars, pectins and tannins (Salunkhe & Kadam, 1995). In addition, persimmons contain a myriad of nutrients and phytochemicals such as organic acids, vitamin A, calcium, potassium, antioxidant polyphenolic compounds, dietary fibres and carotenoids (Altuntas et al., 2011; Zhou et al., 2011). According to current consumer preferences, a high-quality persimmon possesses the following traits: bright, even skin colouration with minimal blemishes, and soft, juicy flesh which is high in sweetness (and flavour) (Ban et al., 2010; Mitani et al., 2015). The development of these features follows the correct ripening and maturation of fruit pre- and postharvest.

### 2.5.1 Ripening physiology

Persimmon fruit are climacteric, and consequently, can be harvested when mature but before ripening has begun (Oz & Kefalas, 2010). The fruit exhibit a dramatic increase in respiration rate during ripening; the typical 'climacteric rise'. The rise in the respiration rate occurs simultaneously with a surge in ethylene production (Paul & Pandey, 2014). The classic increase in ethylene production can be seen in 'Youhou' fruit stored at 1 °C over 70 days where ethylene production peaked at 28 days (**Figure 2.9**) (Zhao et al., 2020). MacRae (1987) has also shown a substantial upsurge in respiration rate and ethylene production in NZ-grown 'Fuyu' stored for 4 weeks at 0 °C and subsequently brought up to 20 °C (**Figure 2.8**). The storage of fruit at low temperatures causes all respiratory activity to slow. The increased temperature after storage at 0 °C prompts a burst of respiration and ethylene production. In persimmon, the increases in colouration and softening that occur as a part of postharvest ripening follow ethylene synthesis (or exogenous application of ethylene) (Besada et al., 2010).



**Figure 2.8** Respiration rate (left) and ethylene production (right) in NZ grown 'Fuyu' persimmons after storage at 0 °C for 4 weeks, colour graded based on the Japanese colour charts: ● 4.5 – 5; ○ 5 – 6; ▲ 6 – 6.5. Adapted from MacRae (1987).



**Figure 2.9 Ethylene production of ‘Youhou’ sweet persimmon during storage at 1 °C for 10 weeks. Adapted from Zhao et al. (2020).**

Fruit respiration and ethylene production rates in persimmon may be affected by the maturity stage at harvest. (Kim et al., 2010) observed significantly higher respiration rates in early-picked ‘Fuyu’ fruit compared to late-picked fruit. The study also noted that fruit from later harvests had elevated weight, soluble solids content, pH, and surface colouration compared with earlier stage fruit. On the other hand, Itamural et al. (1991) reported no difference between ethylene and respiration rates for different maturity stages of 'Hiratanenashi'.

Additionally, the calyx lobes may play a part in the ethylene-associated ripening of persimmon. Potentially, softening can occur in the tissue surrounding the calyx after extended periods of storage as the calyx lobes lose water which prompts ethylene production (Woolf & Ben-Arie, 2011). This water stress signal triggers the expression of an ACC-synthase gene in the calyx; the resulting ethylene diffuses through the neighbouring tissues and activates autocatalytic ethylene production by inducing the expression of the ACC-synthase genes and two ACC-oxidase genes (Nakano et al., 2002).

### 2.5.2 Colour changes

Across most cultivars, persimmon external colour changes from green to yellow, orange and finally, orange-red (Besada & Salvador, 2018). Persimmon colouration continues to evolve

from early maturity stages preharvest until late in postharvest storage (**Figure 2.10**). Different cultivars may reach different levels of colouration e.g., 'Rojo Brillante' achieve a deep red colour whereas 'Taishu' remain closer to yellow at full maturity. This variation is controlled by the level of pigments, most notably carotenoids, present in the peel and flesh (Salunkhe & Kadam, 1995). Peel and flesh colour changes are a product of the synthesis of carotenoids and anthocyanins, and concomitant degradation of chlorophyll (Zhou et al., 2011). These changes involve the transformation of green chloroplasts (photosynthetic plastids) into chromoplasts (carotenoid-accumulating plastids), which is induced by endogenous phytohormones including gibberellins (Gross et al., 1984). Ebert and Gross (1985) reported that in unripe 'Triumph' fruit, the chloroplast-carotenoid pigments were predominant, such as b-carotene and lutein. As the fruit ripened, the levels of these constituents diminished and typical chromoplast carotenoids such as  $\beta$ -cryptoxanthin and zeaxanthin were continuously synthesized, becoming major pigments.

**Figure 2.10 Colour index of persimmon fruit 'Rojo Brillante' [RB], 'Giombo' [Gi], 'Fuyu' [Fu] and 'Hana Fuyu' [HF] in seven maturity stages, showing increases in colouration as fruit matures from underripe to overripe (Tessmer et al., 2016).**

In most persimmon cultivars,  $\beta$ -cryptoxanthin and zeaxanthin are the main carotenoids responsible for colouration (Ebert & Gross, 1985; Niikawa et al., 2007). According to Niikawa et al. (2007), these pigments accumulate to high levels in the fruit during the later stages of

maturity which accounts for the development of the deep orange-red hue. In agreement, Zhou et al. (2011) found  $\beta$ -cryptoxanthin was the pigment present at the highest quantities in both the flesh and the peel, constituting 20 – 30% of total carotenoid levels in cultivars ‘Xiaofangshi’ and ‘Youhou’. The study also described differences in total carotenoid levels in the flesh of 46 cultivars with amounts varying from 194.61  $\mu\text{g}/100\text{ g FW}$  (fresh weight) to 1,566.30  $\mu\text{g}/100\text{ g FW}$ . The combined amount of zeaxanthin and  $\beta$ -cryptoxanthin accounted for between 37.8% and 85.1% of the total carotenoids in the flesh. In the skin,  $\beta$ -cryptoxanthin is present at high levels; Breithaupt and Bamedi (2001) reported the peel of a fully ripe persimmon to contain 55  $\mu\text{g}$   $\beta$ -cryptoxanthin per 100 g FW. This matches previous research which reported increased  $\beta$ -cryptoxanthin levels with sampling time (Homnava et al., 1991).

Lycopene accumulation also plays a role in persimmon colour progression (Besada & Salvador, 2018). In many other red-coloured fruit such as tomatoes, watermelon, and pink grapefruit, lycopene is the main carotenoid that gives the characteristic ripe-red tones. In ripe cherry tomatoes, lycopene levels have been found to be upwards of 15,120  $\mu\text{g}/100\text{ g FW}$  (Raffo et al., 2002). In ‘Hana Fuyu’ persimmon, Homnava et al. (1991) reported that lycopene levels during on-tree ripening increased from  $<1$  to 167  $\mu\text{g}/100\text{ g FW}$ . However, the same study found that lycopene in ‘Sheng’ fruit first increased from  $<1$  to 45  $\mu\text{g}/100\text{ g FW}$ , then decreased to 20  $\mu\text{g}/100\text{ g FW}$  at harvest maturity. Zhou et al. (2011) detected lycopene in the flesh and peel of some cultivars but it was only a minor carotenoid present. There was also a fair amount of variation in lycopene levels between cultivars, and oftentimes lycopene content was below that which is detectable. One astringent cultivar, ‘Mantanhong’, had a comparably high lycopene quantity of 112.67  $\mu\text{g}/100\text{ g FW}$ . Overall, most carotenoids trend towards an increase in quantity as the fruit matures.

Temperature has an impact on persimmon both preharvest and postharvest ripening, particularly in terms of colour changes (Sugiura et al., 1991). Excessively high temperatures (above 30 °C) have been shown to greatly impeded fruit colouration by sustaining higher quantities of chlorophyll and a lower carotenoid content (Chujo, 1982). Storage temperature has a significant effect on colour development postharvest; lower temperatures have been correlated to a lesser increase in the colour index of ‘Rojo Brillante’ persimmons (**Figure 2.11**) as fruit metabolic processes are reduced more at lower temperatures. Although, it has been

noted by Woolf et al. (1997) that fruit stored at 0 °C can exhibit a decrease in colour index values as a consequence of CI. Additionally, the at harvest level of maturation can influence the ability for full colour development after harvest. Harvesting persimmons too early can result in the evolution of an undesirable pale orange with uneven, yellow discolouration (Testoni, 2002).

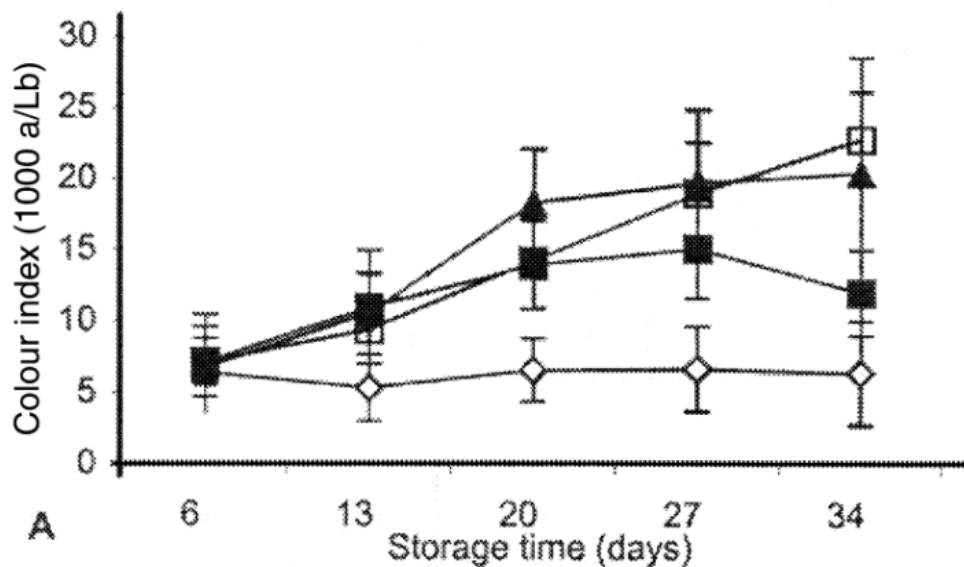


Figure 2.11 Effect of storage temperature on the colour index of 'Rojo Brillante' after storage, CO<sub>2</sub> treatment and 3 days at 15 °C. Temperature: (◇) 1 °C, (■) 8 °C, (▲) 11 °C, (□) 15 °C (Arnal & Del Río, 2004).

### 2.5.3 Colour measurements

Colour is one of the most important quality attributes of fruit. The appearance of fruit, especially surface colour, has a strong impact on consumer purchasing decisions (Costa et al., 2011). There tends to be a specific range of acceptable colours associated with each type (and often variety) of fruit which are linked to quality. Fruit with a colouration that falls outside of this range are often rejected as abnormal colours are indicators of undesirable traits including underripe or overripe fruit, and defects like bruising (Pathare et al., 2013). In persimmon, like many other fruit, the external colour is used as a maturity index to determine harvest timing (Forbus Jr et al., 1991). Skin colour is also the most reliable external parameter used to estimate storability in persimmon. The greener persimmons are mostly lower quality, but

longer-storing, while the redder fruit with superior eating and external quality are usually shorter-storing. Colour can also be connected to visual or non-visual defects – this provides potential to use colour readings to identify fruit disorders (Bignell et al., 2017).

As colour is a key feature in fresh produce, there are various ways in which it can be evaluated. Some of the common approaches include subjective methods such as visual assessment by colour charts, and objective methods such as colorimeters and chromatography (quantitative methods) (Pathare et al., 2013). Chromatography is a technique that separates a mixture into its individual components, allowing for the isolation of substances present e.g., carotenoid pigments in fruit (Hodisan et al., 1997). Compared to colour charts and colorimeter equipment, chromatography is useful for further in-depth analysis. However, is chromatography more time-consuming, expensive and outside the scope of this work. Consequently, this review will focus only on colour charts and colorimeter methodology.

Colorimeters use a standard colour coordinate system, being CIE  $L^*a^*b^*$  colour space the most often utilized in fruit quality characterization (Pathare et al., 2013).  $L^*$  (luminosity) is an approximation of the light or darkness of a colour;  $a^*$  represents reddish colours with positive values and greenish colours with negative values;  $b^*$  represents yellowish colours with positive values and bluish colours with negative values (Francis, 1995). Commonly, chroma ( $C^*$ ) and hue angle ( $h^\circ$ ) are used in the description of fruit colour; both are calculated from  $a^*$  and  $b^*$ . Chroma indicates colour saturation and intensity, and  $h^\circ$  represents how reddish or greenish a colour is (Pathare et al., 2013). In persimmon, colour is typically reported as a colour index value which is calculated from the colour coordinates (colour index =  $1000a/Lb$ ) as this represents all three colour parameters (Tessmer et al., 2016). The use of these colour coordinates allows for an objective and quantitative approach which helps to communicate colour measurements in a more exact manner (Lee, 2000).

Visual evaluation of peel colour in the persimmon industry is done mainly by comparison of fruit skin against standardised colour charts (Asakuma & Shiraishi, 2017). Persimmon colour guides are well developed in Japan and South Korea with charts created for each persimmon genotype (**Figure 2.12**). Based on the Japanese colour chart for 'Fuyu', fruit should be picked

at a rating of no lower than 4, with optimal colouration considered to be 5 (Bignell et al., 2017).

The correlation between colour scales and colour measurements is a strong; Asakuma and Shiraishi (2017) described a strong positive correlation between hue angle and a colour chart (Figure 2.10). They classified fruit into three groups; yellowish (hue angle  $\geq 60^\circ$ , colour chart value of 4 - 5); orangish (hue angle  $51^\circ - 59^\circ$ , colour chart value of 5 - 7); and reddish (hue angle  $\leq 50^\circ$ , colour chart value of 7 - 9). However, there are usually disparities in colour ratings between growers and locations (Bignell et al., 2017). Other challenges of visual assessment of colour include lack of accuracy and subjectivity of assessors' colour perception. Nevertheless, colour charts are inexpensive compared to instrumentation (Pathare et al., 2013).

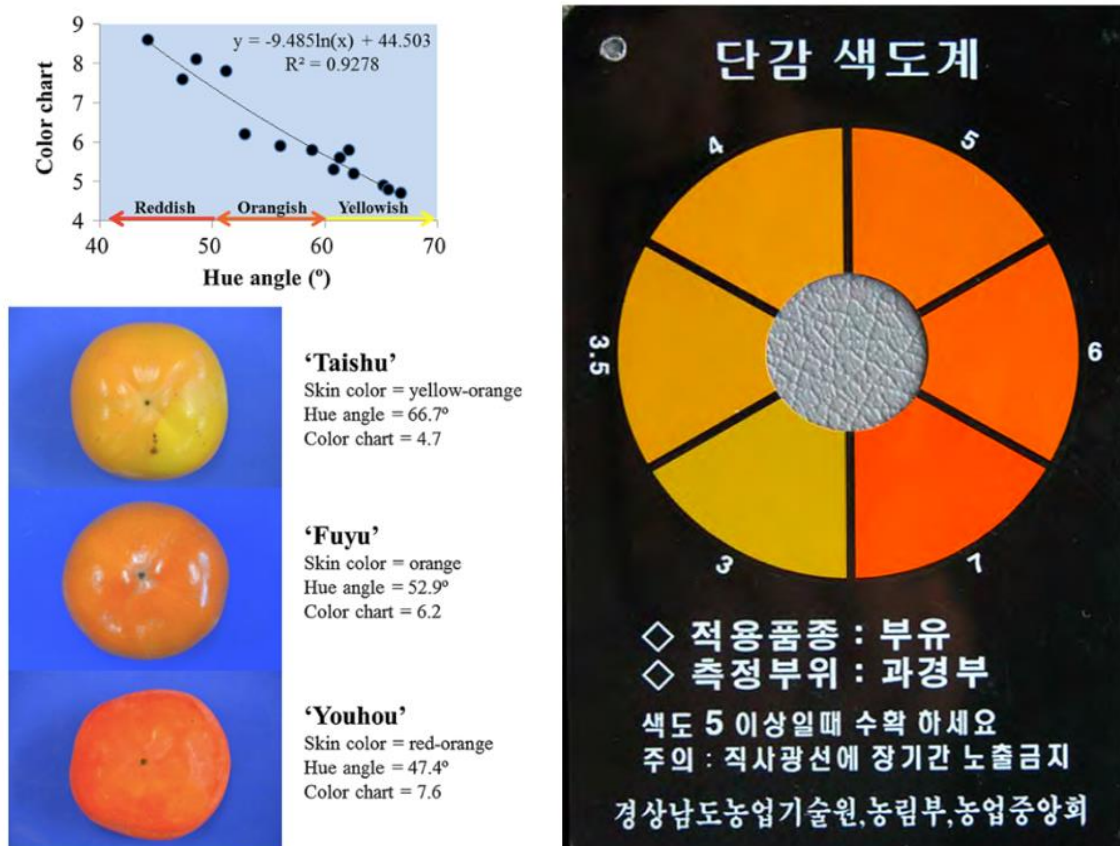


Figure 2.12 Relationship between hue angle and colour chart values of different coloured persimmon cultivars (Asakuma & Shiraishi, 2017) (left), and Korean colour chart for determining maturity of 'Fuyu'; optimal colour rating on this scale is considered to be 5 (Bignell et al., 2017) (right).

#### **2.5.4 Textural changes**

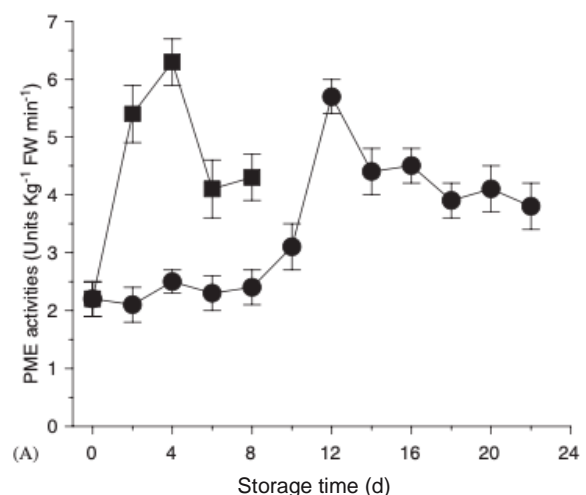
Textural changes, often quantified by a measurement of firmness, can be useful in fresh produce as it can indicate maturity and therefore handleability, storability, and resistance to mechanical damage (Magwaza & Opara, 2015). Texture is also very important as it is directly related to eating quality, and hence consumer acceptability (Chen & Opara, 2013).

Akin to colour changes, textural changes in persimmon continue from early maturity stages throughout postharvest storage. The textural changes that happen during ripening are brought about by degradation of the primary cell wall and middle lamella (Salvador et al., 2007). Previously reported studies have described the microstructural changes in persimmon during fruit maturation (Clark & MacFall, 2003; Tessmer et al., 2016) and storage (Salvador et al., 2007). During early maturity, the parenchyma cells that make up the pulp are compact cells with distinctly structured cell membranes and cell walls which is consistent with higher firmness. As the fruit matures, progressive degradation of the cell wall occurs and solutes leak into intercellular spaces (Tessmer et al., 2016). During the later stages of maturity, cell degradation becomes more pronounced and the adhesion bonds between cells are lost, and some cell separation takes place (Salvador et al., 2007).

Microstructural changes that occur during ripening and maturation are due to the breakdown of cell wall polymers including pectic compounds, glycans and hemicelluloses (Cutillas-Iturralde et al., 1993). In general, the process of softening involves the depolymerization of glycans, the solubilization and depolymerization of pectins and the loss of neutral sugars from pectin side chains (Paniagua et al., 2014). Pectic polysaccharides, particularly polyuronides, are major components of the middle lamella, which are degraded during maturation, contributing to the collapse of cell adhesion and tissue strength (Wakabayashi, 2000). These cell wall and middle lamella modifications are a consequence of changes in enzyme activities that accompany ripening. The action of cell wall hydrolases increases; the enzymes typically involved are pectin methyl esterase (PME) polygalacturonase (PG),  $\beta$ -galactosidase ( $\beta$ -gal), and cellulase (He et al., 2018; Woolf & Ben-Arie, 2011).

There is uncertainty around the exact roles each enzyme plays in the ripening of persimmon. Cutillas-Iturralde et al. (1993) reported that PG is not crucial in the fruit softening process in persimmon. This is in agreement with Salvador et al. (2007) who observed that in persimmon PG activity remained relatively stable throughout the ripening stages evaluated, rather than increasing (**Figure 2.13**). However, studies on other fruit suggest that PG plays a more crucial role in the later stages of maturity when fruit is in the advanced phases of softening (Bonghi et al., 1996; Choi et al., 2002). Salvador et al. (2007) also noted that decreasing firmness was not correspondent with PME activity; PME activity peaked and subsequently decreased at the later stages of maturity. Luo (2007) observed spikes in both PG and PME activities in ‘Qiandaowuhe’ persimmon stored at 20 °C (**Figure 2.13**).

Maturity stage	PME activity (meq-g/min g)	PG activity (nmol/mg min)
I	8.0E-03 abc	27.56 a
II	7.9E-03 abc	21.59 a
III	8.9E-03 c	27.40 a
IV	8.5E-03 bc	27.31 a
V	7.1E-03 a	26.45 a
VI	7.6E-03 ab	22.34 a



**Figure 2.13 (left)** Activity levels of PME and PG enzymes in ‘Rojo Brillante’ fruit at different maturity stages (after deastringency treatment). Values with the same letter in each column do not differ at the 5% significance level using the LSD (least significant difference) test. Adapted from (Salvador et al., 2007). (right) PME activity of fruit ‘Qiandaowuhe’ persimmon stored at 20 °C. ■ , control; ● , 3 ml/l 1-MCP (Luo, 2007).

Different persimmon cultivars have expressed variation in the extent of softening that happens during maturation (Salvador et al., 2006). Tessmer et al. (2016) observed that the cultivars ‘Giombo’ and ‘Fuyu’ tended to retain higher flesh firmness levels (50 N) better than ‘Rojo Brillante’ which were significantly softer (30 N) (**Figure 2.14**). The difference in firmness preservation may be due, in part, to slight differences in structural changes. In ‘Giombo’ and ‘Fuyu’ fruit, cell wall and membrane integrity was preserved up to late maturity, whereas

'Rojo Brillante' fruit had lost some cell structure and adhesion. Luo (2007) reported that during ripening, flesh firmness decreases by up to 90% of initial firmness depending on the cultivar.

In persimmon, the degree of firmness at harvest is a strong factor in determining how well quality is preserved postharvest. Presently, no minimum recommended degree of firmness has been established (Testoni, 2002), although persimmons are usually harvested while still relatively firm to minimise bruising and injury (Salunkhe & Kadam, 1995). Some research has proposed that values less than 10 N post-storage are unfit for commercialisation, while others suggest that 20 N is the cut-off for acceptable firmness levels in persimmon (Besada et al., 2010; Salvador et al., 2007). Fruit with high firmness at harvest tends to store better, although the optimum firmness for storage may be different depending on the storage conditions used (Salvador et al., 2007). Testoni (2002) argues that firmness cannot be considered a reliable maturity index since the decrease in firmness during the harvest window is insignificant in many persimmon cultivars.

**Figure 2.14 Flesh firmness of persimmon fruit 'Rojo Brillante' [RB], 'Giombo' [Gi], 'Fuyu' [Fu] and 'Hana Fuyu' [HF] across seven different maturity stages from green, underripe to orange-red, overripe fruit (Tessmer et al., 2016).**

## 2.5.5 Texture measurements

There are numerous destructive and non-destructive technologies than can be implemented to quantify textural changes, especially changes in firmness in horticultural commodities (Table 2.2). Each of these methods has certain advantages and disadvantages when it comes to evaluating texture. Firmness has traditionally been evaluated using destructive procedures which are well established, relatively simple and inexpensive, but are also time-consuming and labour intensive (Jie et al., 2014). Non-destructive techniques have advantages over destructive methods including the potential for repeat measurements of the same fruit before, during, and after storage or treatment, as well as the estimation of multiple attributes simultaneously (Døving et al., 2005). Many non-destructive methods also assess the whole fruit, and retain the ability to control whether a specific part of the fruit has a significant effect on the measurement made (Li et al., 2016).

**Table 2.2 Comparison between sensory, instrumental destructive and instrumental non-destructive methods used to estimate textural properties in persimmon fruit (excluding NIR-based techniques).**

Method	Instrumentation	Settings	Property	Reference
Destructive	Material test device	7.9 mm diameter probe 10 mm min <sup>-1</sup> down-speed 8 mm puncture depth	Flesh firmness (N)	(Altuntas et al., 2011)
	Texture device	8 mm probe 5 - 10 mm min <sup>-1</sup> speed	Flesh firmness (N, kg)	(Arnal & Del Río, 2004) (Salvador et al., 2007) (Salvador et al., 2006) (Harker & Forbes, 1997) (Tessmer et al., 2016) (Sanchis et al., 2015)
	Hand-universal pressure tester	5 mm diameter 10 mm probe	Flesh firmness (kg)	(Asakuma & Shiraishi, 2017)

	Penetrometer	8 mm probe, 11 mm probe	Flesh firmness (N, kg)	(Celik & Ercisli, 2008) (MacRae, 1987) (Forbus Jr et al., 1991) (Zhao et al., 2020)
Non- destructive	Electrodynamic shaker (+ other equipment) Piezoelectric sensor (+ other equipment)	0 – 2 kHz  1 mm thickness 10 mm diameter	Elasticity index  Texture index	(Taniwaki et al., 2009)  (Taniwaki et al., 2009)
Sensory	Sensory analysis	6 panellists Sensory scale of 1 – 5	Sweetness juiciness thickness hardness fragrance appearance	(Taniwaki et al., 2009)

### **2.5.5.1 Destructive firmness**

The most common destructive methods used to describe texture in fruit are sensory analysis and instrumental flesh firmness (Chen & Opara, 2013). The standard method in many fruit is the measurement of flesh using a penetrometer – this approach most commonly measures the peak amount of force required to penetrate to a certain depth at a defined speed (Li et al., 2016). It may also be measured by how deep the plunger can be pressed into the fruit at a specific speed and force (Døving et al., 2005). In persimmon, flesh firmness is the main method used to evaluate firmness. Flesh firmness measurements are most commonly carried out by removing 1 – 2 mm of skin and taking a reading on each of the fruit cheeks (Orihuel-Iranzo et al., 2010).

The main variables that affect penetrometer readings are probe diameter, penetration speed, and distance (Abbott, 1999). In kiwifruit, Li et al. (2016) and Feng et al. (2011) observed greater flesh firmness values with greater penetrometer speeds, particularly in softer kiwifruit

(5 to 20 mm s<sup>-1</sup>, and 4 to 40 mm s<sup>-1</sup>, respectively). For most fruit, a probe size of 7.9 mm with a measurement depth is commonly used. Kader et al. (1978) found that in tomato fruit, smaller diameters tend to produce greater variation in firmness values; larger probes tend to compress and the results may be influenced by the size or shape of the fruit (Larsen & Watkins, 1995). As with SSC, the location of measurement can also affect firmness values. Generally, the equator is used to produce a representative measurement (Døving et al., 2005).

Another destructive method used to evaluate fruit texture (and flavour) is sensory analysis. Sensory evaluations assess food qualities in terms of the human response of sense of taste, smell, touch, sight, and hearing to the properties of foods (Chen & Opara, 2013). This approach uses groups of panellists trained in sensory evaluation methodologies; panels usually consist of 10 members and can range in experience from 'semi-trained' to 'expert' (Albert et al., 2011). Sensory analysis also facilitates the description of multiple aspects of texture simultaneously – for example crispness (the quality of being firm yet easily broken), hardness (the amount of force required to bite through) and chewiness (the amount of sustained, elastic resistance to chewing action). Generally, each property is rated on a scale, usually based on the intensity of the property (Salvador et al., 2009). The main benefit of using sensory analysis over instrumental procedures is the insight it provides into human perception of fruit quality. However, this method is subjective, expensive, and relies on the expertise of the panellists (Chen & Opara, 2013).

#### **2.5.5.2      *Non-destructive firmness***

Non-destructive texture measurements encompass a variety of different techniques including non-destructive compression and acoustic firmness (**Table 2.2**). Compression tests are often used to describe various textural aspects of food such as stiffness, elasticity, crispiness, and many others (Chen & Opara, 2013).

For fresh produce, compression can be used as a non-destructive approach to measure firmness when the compression deformation distance is in the elastic compression phase

(below a certain deformation). This technique requires pressing fruit with a flat probe to produce a force-deformation curve (Ubierna et al., 2005). Firmness is estimated by either the amount of deformation given by a fixed compression force for a certain time or by the force required to reach a uniaxial deformation (Macnish et al., 1997). Compression firmness values are regularly expressed by the peak force or the percentage of deformation (Jarimopas & Kitthawee, 2007). The main parameters are the force, compression distance, crosshead speed, and probe size (Chen & Opara, 2013). It has also been suggested that the size of fruit can affect compression readings; larger fruit may have greater resistance to compression as the contact area between the probe and the fruit is increased (Sirisomboon et al., 2000).

Acoustic sensors are a purely non-destructive method used to estimate produce texture. The fruit receives a small impact by a piston which causes it to vibrate and the signal produced is picked up by a microphone (Ubierna et al., 2005). The main resonant frequency, along with the fruit weight, is then used to calculate firmness (Johnson & Dover, 2004; Molina-Delgado et al., 2009). Acoustic methods are useful for the measurement of firmness in apples (Johnson & Dover, 2004), tomatoes (De Belie et al., 2000) and kiwifruit (Li et al., 2016). Acoustic methods assume that the object being measured is mostly spherically-shaped (Ubierna et al., 2005). For persimmon, this may present a problem as fruit, especially larger ones, often deviate from this shape and have irregularities. In “Jonagold” apples Chen and DeBaerdemaeker (1993) found that resonate frequency measured at various parts on the fruit surface can produce considerably different results, and this difference tends to increase with the divergence of the fruit shape from that of a sphere. To reduce errors in acoustic firmness readings, multiple measurements along the fruit equator are required (Ding et al., 2021). It has also been reported that acoustic firmness may be more sensitive for firmer samples rather than softer fruit (De Ketelaere et al., 2006).

### **2.5.5.3      *Correlation between texture measurements***

Each method of evaluation provides a slightly different perspective on fruit texture as different techniques correlate better with different properties. Li et al. (2016) suggested the relationship between destructive flesh firmness, non-destructive compression, and acoustic

firmness in kiwifruit is strong. This research reported a tighter association between acoustic firmness measurements and penetrometer values, compared to compression. They also reported similar values for compression and acoustic firmness. In agreement, Hertog et al. (2004) showed a linear relationship between flesh firmness and non-destructive compression within a range of 0 - 30 N in 'Hayward' kiwifruit. Conversely, Schotsmans and Mawson (2004) noted that this correlation was not strong ( $R^2 = 0.61$ ). In general, flesh firmness is thought to have the best correlation with sensory firmness (Chen & Opara, 2013), and with consumer perception of texture (Abbott, 1999).

In persimmon, the use of non-destructive vibrational technology was found to be a good predictor of optimum eating quality. Taniwaki et al. (2009) reported a strong correlation between the elasticity index (measured by acoustic vibrational methods) and sensory index in 'Taishuu' and 'Fuyu' persimmon. Acoustic firmness has also been correlated with ripeness and storability in persimmon (Woolf & Ben-Arie, 2011). Persimmon flesh firmness has been found to have a weaker relationship with maturity compared to colour (Del Bubba et al., 2009; Forbus Jr et al., 1991).

Overall, there is limited information on the relationship between different textural measurements in persimmon fruit. This provides an opportunity to investigate the correlation between destructive and non-destructive methods of evaluation in persimmon.

### **2.5.6 Sugar metabolism**

As ripening advances, soluble solids content (SSC) rises resulting from the conversion of starch to sugar. In most persimmon cultivars, sucrose, glucose and fructose are the most abundant sugars in the flesh (Del Bubba et al., 2009). During ripening, both fructose and glucose levels increase, while sucrose content peaks and then gradually decreases. Del Bubba et al. (2009) attributed these changes to strong invertase activity which hydrolyses sucrose into glucose and fructose. This is in agreement with other studies (Hirai et al., 1986; Zheng & Sugiura, 1990) which observed elevated invertase activity during ripening in persimmon. Plaza et al.

(2012) reported a significant rise in SSC during the ripening process of persimmon; the astringent cultivar 'Rojo Brillante' increased from 15.8% to 17.2%.

On the other hand, Tessmer et al. (2016) observed no significant changes in SSC in the astringent cultivars 'Rojo Brillante' and 'Giombo' throughout maturity, while non-astringent 'Hana Fuyu' and 'Fuyu' exhibited an increase in SSC (**Figure 2.15**). These astringent cultivars also obtained higher SSC values than non-astringent ones during the earlier maturity stages, but the non-astringent cultivars reached relative levels by the more advanced stages. The study noted that the SSC measurement included not only sugar content but also soluble tannins. Thus, they concluded that in non-astringent fruit SSC was a fair predictor of sugar content but should be avoided in astringent cultivars.

**Figure 2.15** SSC of persimmon fruit 'Rojo Brillante' [RB], 'Giombo' [Gi], 'Fuyu' [Fu] and 'Hana Fuyu' [HF] at seven consecutive maturity stages ranging from immature, green fruit (S1) to overripe, orange-red fruit (S7) (Tessmer et al., 2016).

In general, persimmon fruit should reach an SSC of 14% to 18%, depending on cultivar differences and climactic conditions (Celik & Ercisli, 2008; Tessmer et al., 2016). Differences in SSC is often seen between fruit of the same cultivar grown in different locations; for example, 'Fuyu' fruit grown in New South Wales, Australia is harvested at 15% SSC while 'Fuyu' grown in Japan reaches up to 18% SSC (Tessmer et al., 2016). While external colour is

used as the non-destructive harvest index for persimmon, Testoni (2002) suggested that SSC could be suitable as a destructive maturity index due to the significant increase of SSC found between the two harvesting dates (about 2% increase for 10 days between each harvest date) in 'Hachiya' fruit.

### **2.5.7 Soluble solids measurements**

Sweetness is an important and desirable attribute in fresh produce, especially fruit, that is largely determined by sugar levels (Magwaza & Opara, 2015). Greater sweetness is generally connected to maturity, internal quality, and to consumer satisfaction and purchasing decisions (Chen & Opara, 2013; Shewfelt, 2014). This holds true for persimmon as its sweet flavour is the primary characteristic in its consumption (Chen et al., 2016).

Sweetness is usually quantified destructively by the instrumental measurement of the sugar concentration of the fruit juice, or by sensory assessment. In addition, near infrared (NIR) spectroscopy methods may also be used to determine SSC. The SSC of most fruit, including persimmon, is measured using a refractometer (Shewfelt, 2014). A refractometer uses the principle of light refraction to estimate the quantity of soluble solids (as degrees Brix) which is equivalent to the percentage of dry substance content of the solution (Harrill, 1998). The SSC is comprised of mainly sugars (mostly sucrose, glucose, and fructose) plus dissolved vitamins, minerals, fructans, phenolics, and pigments (Kader, 2008). In most fruit, soluble solids consist of 85% sugars, but compositions can vary greatly between and within fruit type and cultivar (Magwaza & Opara, 2015).

The soluble solid content is the predominant quality parameter used to evaluate sweetness in research, and industry, and to develop marketing standards. Low SSC in many horticultural products, including persimmon, is associated with poor taste and quality (Magwaza & Opara, 2015). In astringent persimmon fruit, tannins make up a significant proportion of the SSC which can lead to an inaccurate estimation of sweetness (Tessmer et al., 2016). A low correlation between SSC and sweetness has been observed in strawberry and blueberry fruit. These fruit contain high quantities of anthocyanin and phenolic compounds which strongly

refract light, contributing up to 32% of SSC values (Kader et al., 2003). This suggests that SSC may not be the best indicator of sweetness in highly pigmented fruit as refractometers read the sum total of soluble solids, including pigments and organic acids. In some cases, SSC values can be adjusted using a factor based on the percentage contribution by sugars (Magwaza & Opara, 2015).

### **2.5.8 Acidity & pH**

In contrast to soluble solids, acid levels in fruit tend to diminish during maturation due to their use in respiratory metabolism (Kays & Paull, 2004). In persimmon, the levels of titratable acidity are about 2% in ripe fruit (Altuntas et al., 2011; Celik & Ercisli, 2008), this is comparable to many other fruit such as kiwifruit which contain 0.9 – 2.5% total acidity at harvest (Marsh et al., 2004). The typical pH at harvest for persimmon is 5.5 (Altuntas et al., 2011) compared to approximately 3.5 in kiwifruit (Marsh et al., 2004) and 3.3-4.6 for peaches (Moing et al., 1998). In many fruit, pH increases with ripening, while in other fruit such as tomato (Kaur et al., 2006), cherries (Serrano et al., 2005), and persimmon (Plaza et al., 2012), pH remains relatively stable. During ripening, Salvador et al. (2007) found only very slight differences in pH across a range of maturity stages for 'Rojo Brillante'. Although, the fruit displayed a significant increase in pH after destringency treatment; this has been linked to the process of tannin insolubilisation as tannins are acid components. It has been noted that the levels of acidity of persimmon usually increase during the beginning of storage, then continuously diminish as ripening progresses (**Figure 2.16**) (Cia et al., 2006; Senter et al., 1991).

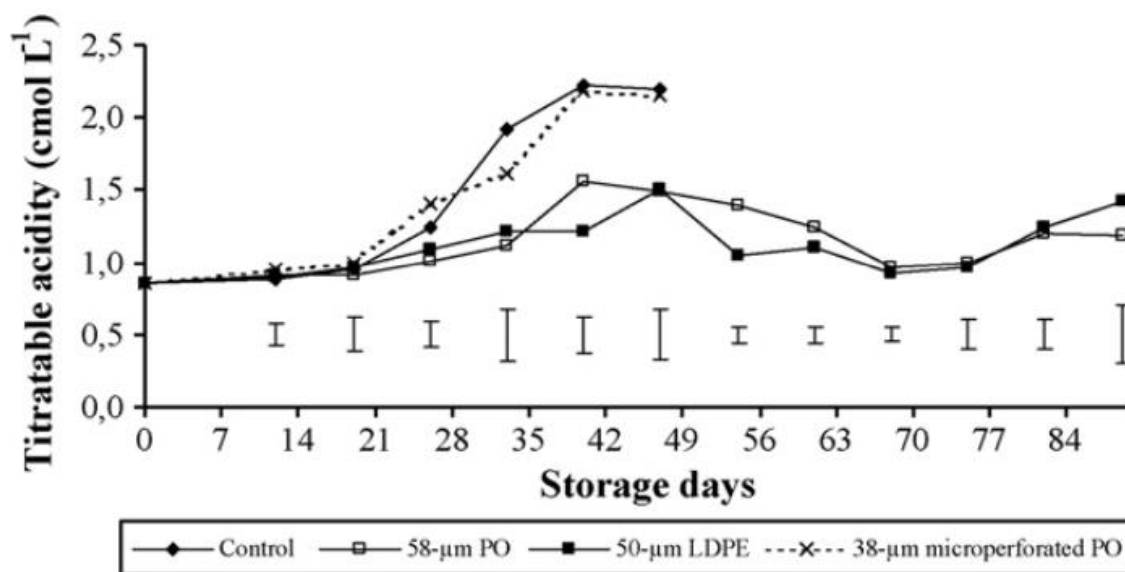


Figure 2.16 Titratable acidity (cmol L<sup>-1</sup>) of 'Fuyu' persimmon fruit in different types of packaging materials, at different periods under 1 °C and 90% RH + 5 days at 25 °C and 70% RH (Cia et al., 2006).

### 2.5.9 Correlation between quality characteristics

There is a notable relationship between colour changes and the softening that accompanies ripening (Del Bubba et al., 2009; Salvador et al., 2007; Salvador et al., 2006; Tessmer et al., 2016). A strong, negative correlation has been characterised between postharvest colour and firmness values (Tessmer et al., 2016). This work showed a negative slope for peel colour and flesh firmness indicating that increased colour corresponds to reduced firmness, across the astringent cultivars 'Giombo' and 'Rojo Brillante' and the non-astringent 'Hana Fuyu' and 'Fuyu'. The Salvador et al. (2006) study established that during ripening of 'Rojo Brillante' persimmons, more intense colour was accompanied by lower firmness levels. This relationship between colour and firmness allowed for flesh firmness to be predicted accurately from peel colour measurements.

Choi et al. (2019) reported a strong negative correlation between persimmon size and firmness – larger fruit tended to have lower firmness values. This study also found a positive

correlation between soluble solid contents and fruit size, but this relationship was not significant. Concurrently, Asakuma and Shiraishi (2017) did not report any relationships between fruit weight, skin colour, and SSC for a variety of different persimmon cultivars. On the other hand, parallel increases in fruit weight, colour, and soluble were observed in 'Fuyu' fruit when exposed to greater light levels during development (Takano et al., 1991).

### **2.5.10 Grading**

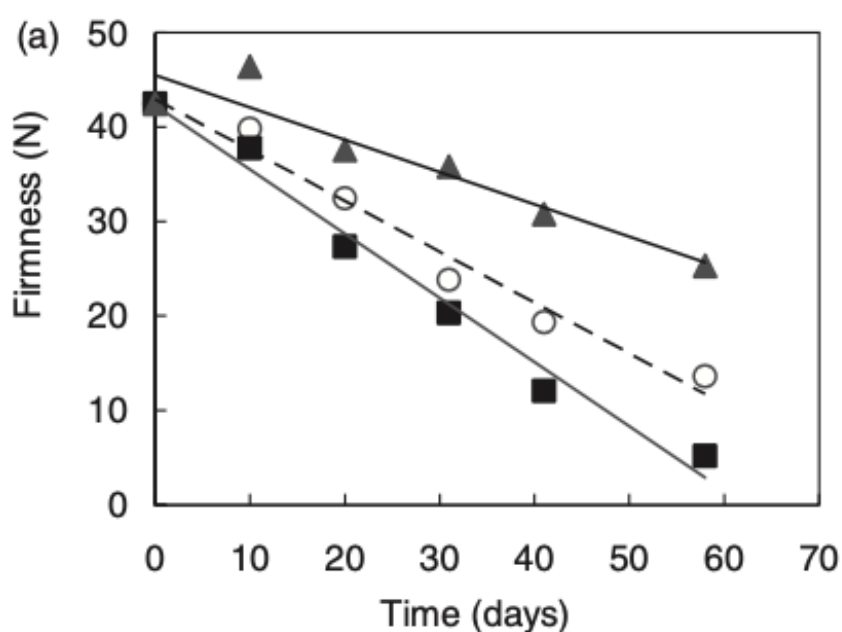
In the industry, the standard procedures and processes used to pack fruit vary between persimmon pack houses. The general order of operations is: initial sorting for blemishes, brushing or polishing (optional), weight grading, final quality grading, and finally, packing (Bignell et al., 2017). The main grading technologies are grading by sight, electronic in-line weight grading, and circular electronic weight graders (Kitinoja & Kader, 2002). Most commonly, any fruit with prominent defects or blemishes are discarded in the field during picking followed by packhouse segregation of fruit into first and second classes (and simultaneous rejection of undersized, blemished, and infested fruit) (Bignell et al., 2017).

Persimmons are graded foremost by size, although grading takes size, shape, firmness, degree of blemish and colour into account. A desirable size for 'Fuyu' ranges from 230 to 250 g, with a minimum marketable size of 150 g (Broadley et al., 2005). By Australian industry standards, persimmon fruit must be 'sound, clean, well-formed, not shrivelled, mature but not overripe, of one cultivar, free from broken skins, and reasonably free from skin blemishes' (Bignell et al., 2017). Additionally, supermarket chains usually also have their own set of grade standards for persimmon that must be met. Fruit which fails to meet these standards is classified as 'Second Class' and can still be sold to other markets (such as farmers' markets) (Bignell et al., 2017). See **Appendix A** for the Australian 'FreshSpec' and supermarket grade standards.

## 2.6 Postharvest technologies

### 2.6.1 Storage environment

Fresh produce is generally stored at low temperatures to slow down the respiration rate and senescence processes that happen after harvest. The highly perishable nature of fruit is associated with the increase in metabolic reactions that occur with increasing temperature. Hence, low-temperature storage is implemented to delay further ripening throughout postharvest storage (Arnal & Del Río, 2004; Wills et al., 1998). In persimmon, the optimum storage conditions are usually 0 °C and 85 – 95% relative humidity (RH) to preserve firmness and minimise weight loss (Bignell et al., 2017). Although, storage at temperatures below 15 °C has been shown to induce CI, temperatures above 1 °C tend to result in greater rates of softening (**Figure 2.17**) (Orihuel-Iranzo et al., 2010). Optimum storage temperature often varies between countries and cultivars – this has been attributed to differences in soluble solid levels (Gross et al., 2004). Storage duration also differs with maximum cold storage timeframes of between 12 and 16 weeks at 0°C. This length of long-term storage is normally accomplished through various strategies including optimised temperature, 1-MCP, and MAP. Further extension of persimmon storage is limited by excessive softening, disease and skin discolouration (Woolf & Ben-Arie, 2011).



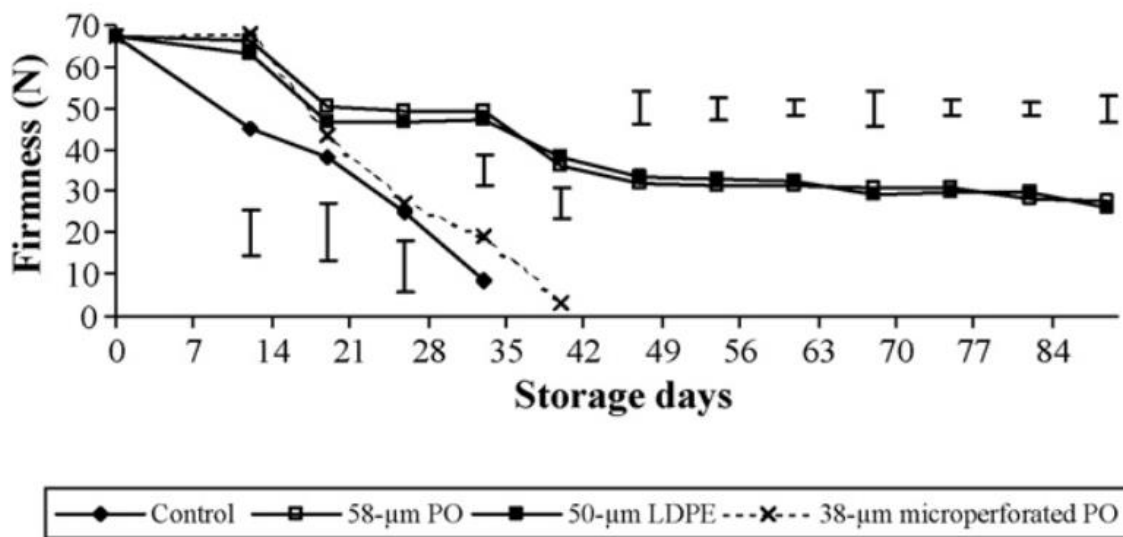
**Figure 2.17** Firmness loss of 'Rojo Brillante' persimmon fruit during cold storage at 1 °C, 10 °C and 14.5 °C and 90% RH. ▲ = 1 °C, ■ = 10 °C, ○ = 14.5 °C (Orihuel-Iranzo et al., 2010).

## 2.6.2 Atmosphere composition management

### 2.6.2.1 *Modified atmosphere packaging*

Modified atmosphere packaging (MAP) is a popular postharvest technology used in combination with cold storage to lengthen the storage life and maintain quality for a range of commodities (Kader et al., 1989). MAP generally achieves atmosphere management using packaging to generate a specific atmosphere of raised carbon dioxide (CO<sub>2</sub>) and lowered oxygen (O<sub>2</sub>) around produce. The packaging material used – typically polyethylene (PE) films – allows O<sub>2</sub> to permeate out while retaining CO<sub>2</sub> within the package. In this case, the desired atmospheric composition is attained by passive modification where respiration of the fruit inside the package alters the atmosphere (Cia et al., 2006). It can also be created by active modification where the atmosphere is entirely replaced by the desired gas mixture with the potential addition of absorbent materials to scavenge O<sub>2</sub>, CO<sub>2</sub> and ethylene to manage gas levels (Kader et al., 1989).

The creation of a low O<sub>2</sub> and high CO<sub>2</sub> environment suppresses respiration, ethylene production, and water loss which subsequently delays ripening and senescence (Ben-Arie & Zutkhi, 1992). Lower respiration rates are connected to reduced cellular reactions and enzyme activities (such as the degradation of cell wall polymers) which helps to retain fruit quality. In persimmon, the main effects of MAP are the alleviation of CI and reduction of softening during storage (**Figure 2.18**) (Cia et al., 2006). Ben-Arie and Zutkhi (1992) reported a diminished softening rate and a significant reduction in decay incidence, peel browning and flesh breakdown with fruit stored in 60 µm and 80 µm polyethylene MAP. Additionally, MAP maintains high RH levels which minimises water loss; this reduces the rate at which CI develops by hampering the collapse of epidermal and underlying cells (Forney & Lipton, 1990; Wang, 1993). Other advantages include less surface abrasion, better sanitation, and minimised pathogen spread (Kader et al., 1989).



**Figure 2.18** Flesh firmness of ‘Fuyu’ persimmon fruit in different packaging types during storage at 1 °C and 90% RH (plus 5 days at 25 °C and 70% RH) over 12 weeks (Cia et al., 2006).

The relative success of MAP varies between cultivar, maturity stage, atmospheric composition, and packaging type (Besada & Salvador, 2018). Oxygen levels must be below 8% to markedly slow ripening with lower concentrations of O<sub>2</sub> having greater effects. Elevated CO<sub>2</sub> concentrations (greater than 1%) also retard ripening, with these effects occurring in addition to the benefits of a reduced O<sub>2</sub> atmosphere (Kader et al., 1989). The thickness of the packaging film used affects the permeability of the MAP which, in turn, influences the gas concentrations at equilibrium. Ben-Arie and Zutkhi (1992) reported that average O<sub>2</sub> and CO<sub>2</sub> levels at equilibrium in ‘Fuyu’ stored in 60 µm thick bags were 13.8% and 3.8%, respectively compared to 5.8% and 7.0% in 80 µm thick MA packages (at 0 °C). Thicker films are less permeable and as a result, more CO<sub>2</sub> is retained within the package (and more O<sub>2</sub> is prevented from entering the package) than thinner films. The quantity of fruit per package (fill weight) and the free volumes also affect the performance of MAP. Greater amounts of fruit and less headspace reduces the time taken to achieve a steady-state atmosphere but puts fruit at a higher risk of anaerobic metabolism (Mangaraj et al., 2014).

In persimmon, passive MAP is used commercially in New Zealand, Japan, and Korea to extend the storage window of fruit and suspend the onset of CI symptoms (Woolf & Ben-Arie, 2011). Typically, in the persimmon industry, 4 – 10 kg trays of fruit are heat-sealed in 60 µm polyethylene bags to establish atmospheres of 4 – 8% CO<sub>2</sub> and 0.5 – 1.5% O<sub>2</sub> (and almost 100% RH) with storage at 0°C (Kim & Lee, 2004; MacRae, 1987).

### **2.6.2.2      *Controlled atmospheres***

Cold storage under controlled atmosphere (CA) conditions can extend the storage life of many fruit and vegetables in similar ways to MA approaches. Controlled atmosphere methods involve actively altering the atmospheric composition around produce to create an environment that differs from that of air (Besada & Salvador, 2018). Most commonly O<sub>2</sub> levels are decreased, and CO<sub>2</sub>, N<sub>2</sub>, and RH levels are increased (in combination with low temperatures), reducing respiration, ethylene production, and ripening. The effect of CA on persimmons has been widely studied in some cultivars, like ‘Fuyu’, but research into others is limited (Besada & Salvador, 2018). It is generally accepted that CA of 2 – 5% O<sub>2</sub> delays ripening, and 5 – 10% CO<sub>2</sub> restricts softening and can minimise CI symptoms (Burmeister et al., 1997; Gross et al., 2004). An interesting study on ‘Fuyu’ by Besada et al. (2015) found short-term high CO<sub>2</sub> treatment alleviated CI symptoms by maintaining cell integrity. Ultra-low oxygen (ULO) atmospheres of 1.3 – 1.8% O<sub>2</sub> have also been trialled in ‘Rojo Brillante’ fruit but failed to affect the incidence of CI (Orihuel-Iranzo et al., 2010).

### **2.6.2.3      *Detrimental effects of CA & MAP***

In addition to the benefits provided by CA and MAP, there are various disadvantages to the use of these technologies in persimmon storage. A significant factor that restricts longer storage life under MAP is the potential emergence of off-flavours and tissue browning that can result from accumulation of ethanol and acetaldehyde (Ben-Arie et al., 1991). This has been attributed to fruit being exposed to CO<sub>2</sub> concentrations above their tolerance limits (between 10% (Gross et al., 2004) and 20% (Haginuma, 1972) depending on the cultivar)

which can cause an increase in anaerobic respiration (Woolf & Ben-Arie, 2011). Another challenge is the potential for MA conditions to be lost by a puncture to the packaging. In addition, problems like calyx abscission and 'glassy ring' may arise where packages are stored above the optimal temperature, which can lead to damagingly high CO<sub>2</sub> levels and low O<sub>2</sub> levels (Woolf & Ben-Arie, 2011). The main limitation to storing 'Fuyu' in CA storage is the prevalence of skin and flesh disorders. Many fruit express external or internal browning as a consequence of O<sub>2</sub> concentrations being too low (rather than CO<sub>2</sub> levels not being high enough) (Park & Lee, 2008).

## **2.7 Near infrared technology**

### **2.7.1 Overview**

Near infrared spectroscopy (NIRS) can be used to non-destructively measure quality attributes, particularly SSC, in horticultural commodities. In NIRS, fruit is irradiated with electromagnetic radiation within the wavelength range of 780 and 2500 nm and the reflected or transmitted radiation is measured as a spectrum (Nicolai et al., 2007a). The incident radiation may be absorbed, reflected, or transmitted by the sample, with the amount of each depending on the chemical composition and physical characteristics of the fruit (Walsh et al., 2020). Vibrations within certain chemical bonds contribute to the absorption bands in NIR spectra; as fruit consists largely of water and sugar, the absorption bands of the O-H and C-H bonds dominate the spectrum (Nicolai et al., 2007a). Unique absorption bands for each fruit are formed by combination and overlapping of the absorptions that result from differences in chemical composition (Magwaza et al., 2012a).

The complex chemical composition and microstructure of fruit (as well as additional sources of variability) cause NIR spectra to be highly convoluted (Nicolai et al., 2014). As a result, it is hard to attribute specific bands to specific components within the fruit. Instead, multivariate statistical techniques including pre-processing of spectral data and regression (referred to as chemometrics) is used to elicit useful information on fruit quality parameters from the

spectrum (Nicolai et al., 2007a). Pre-processing techniques such as normalisation, derivatives and smoothing can be used to reduce the influence of noise and baseline shifts in spectra (Magwaza et al., 2012a).

## 2.7.2 Modelling

Standard destructive methods are used to assess the quality characteristics of the fruit and calibration models are then developed to relate the spectral data with the quality characteristics. The most commonly used calibration method is partial least squares regression (PLS) which allows for quality traits to be modelled by correlating the spectral data with the related physical and chemical data (Magwaza et al., 2012a). The accuracy of these NIRS models is most often described by the correlation coefficient ( $R$ ), coefficient of determination ( $R^2$ ), and the root mean square error of calibration/prediction (RMSEC/RMSEP). A strong model should have a higher  $R$  or  $R^2$  (corresponding to a greater proportion of explained variance) and ratio of performance to deviation (RPD), and a lower RMSE (Bobelyn et al., 2010). A good NIR model will have an RMSE lower than its standard deviation (SD), and an  $r$  or  $R^2$  near 0.9 and an RPD of close to or above 2.0 (Pissard et al., 2013; Wang et al., 2015).

Model robustness – the ability of a model to make accurate predictions regardless of changes in external factors – is also important. Often, models are not applicable to fruit sourced from separate locations or seasons, even within a single variety and cultivar, because models are highly specific and sensitive to the nature of the calibration dataset (Magwaza et al., 2012a). The major physical elements affecting model performance have been described by Nicolai et al. (2007b) as within-tree variability (crop load, fruit position, light effect, and tree age), within-orchard variability (tree location and light effects), fruit age, orchard variability, and seasonal variability. The main experimental factors include wavelength selection, light penetration depth, dataset size, methods of statistical analysis chosen, instrumentation, and temperature (Nicolai et al., 2007a). The type of sensor used often affects the precision of a NIR model, with performance varying between instruments (Nicolai et al., 2007a). Kaur et al. (2017) observed unsatisfactory performance when using hand-held NIR devices for DM

predictions in kiwifruit and apples when compared to similar studies using benchtop instrumentation.

### **2.7.3 Quality predictions**

NIR data can be used to predict many quality traits in fruit including sweetness (soluble solids), flesh firmness, DM, acidity, maturity and ripeness (Li et al., 2018). Of these attributes, NIR tends to give fairly accurate predictions of SSC as illustrated by many studies across a range of fruit such as apples, mandarins, melons, and peaches (Nicolai et al., 2007a). Good models have also been developed for DM, but acidity may not be predicted particularly well by NIR spectroscopy. This has been attributed to the relatively small quantity of acid present in fruit which is likely too little to have a detectable or significant influence on the spectrum. Additionally, there is disagreement on the utility of NIR to predict textural attributes (Walsh et al., 2020). NIR estimations of firmness tend to be imprecise because changes in tissue structures associated with texture cause differences in light scattering which are harder to tease out of spectra (Cen & Lu, 2009).

McGlone and Kawano (1998) reported good accuracy in the prediction of both SSC and DM in kiwifruit with an overall  $R^2$  of 0.90 and RMSEP of 0.42% for DM and 0.39° for SSC. However, this work also found that firmness was not predicted well. Guthrie et al. (2005) displayed encouraging results for SSC and DM prediction in mandarin fruit ( $R^2$  of > 0.75 and 0.90, and RMSEP of < 0.40° and 0.60%, respectively), but predictions of acidity and juiciness were unsatisfactory. In tomatoes, He et al. (2005) described excellent performance of vis-NIR models in predicting SSC, firmness and pH. Soluble solids content has also been predicted fairly accurate in apples (McGlone et al., 2002), pears (Nicolai et al., 2008), and grapes (Jarén et al., 2001) among other fruit.

In persimmon, NIR technologies have been used to estimate a variety of qualities, including SSC, firmness, colour, acidity, astringency, vitamin C, and ripeness, with varying levels of success. Jannok et al. (2014) reported good SSC prediction with an  $R^2$  of 0.88 when using NIR spectra of 400 – 1100 nm and PLS regressions. Similarly, Ar et al. (2019) predicted SSC and

firmness of 'Rendeu' persimmon using (1000 – 2500 nm) NIRS data and PLS to develop a model with an R value of > 0.75. The study also found that the prediction of vitamin C and total acid levels were inaccurate, likely due to the small levels being difficult to detect. There is potential to use NIR to detect calyx cavity if presence of the cavity is linked to a difference in quality attributes, particularly SSC.

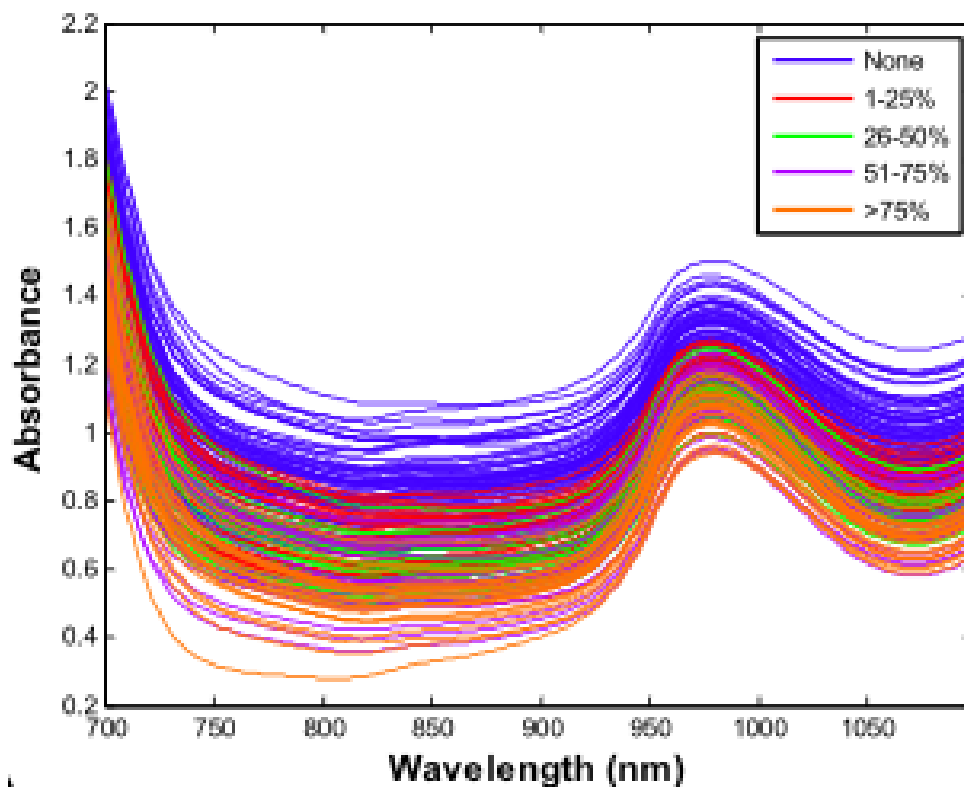
Most NIR applications are based on spot measurements and assume the data is representative of the whole fruit when these measurements are taken at the equator, while qualities tend to vary by location (Guthrie et al., 2005). In many fruit, differences in chemical composition have been observed between the stem and blossom ends, and the sun and shaded sides (Guthrie & Walsh, 1997). Sugiyama (1999) pointed out spatial variation in SSC of 5 – 10 °Brix in melon, and Long and Walsh (2006) found a 3° difference between the stylar and stem ends. This means for non-uniform quality attributes, measurements are required on several positions, and for disorders, the whole fruit surface may need to be scanned. In such cases, multispectral or hyperspectral imaging systems are often better suited (Nicolai et al., 2007a). Similarly, the acquisition of NIR spectral data using transmission mode (rather than reflectance or interaction setups) tends to be better at detecting internal disorders and less susceptible to surface properties (Schaare & Fraser, 2000). In persimmon, hyperspectral imaging has been shown to have potential in predicting astringency, ripeness, SSC, and firmness (Munera et al., 2017a; Munera et al., 2017b; Wei et al., 2020; Wei et al., 2014).

#### **2.7.4 Internal disorder detection by NIR**

Near infrared spectroscopy can be used to detect disorders in fruit; for example, the internal browning disorder 'brownheart' in apples. Clark et al. (2003) reported significant changes in the spectra of 'Braeburn' apples. Affected fruit had stronger absorbency in the red area of the spectrum (650 – 750 nm) due to the browned flesh, and weaker absorbency above 840 nm as a consequence of the drier, floury-textured flesh with much lower O-H levels. Although, the precision of the models generated in this experiment was inadequate to predict the extent of browning in mildly affected fruit with only 0 – 20% flesh browning. In kiwifruit, Clark et al. (2004) used Vis/NIR to sort fruit into 'sound' and 'affected' groups to reduce the

incidence of postharvest storage disorders. Fruit that developed storage rots and CI were generally less mature as illustrated by NIR profiles of greener flesh colour, lower SSC and reduced DM. NIR technologies have also shown potential for the detection of many other disorders like rind breakdown in mandarin (Magwaza et al., 2012b), bitter pit in apples (Nicolai et al., 2006), postharvest rind pitting in grapefruit (Ncama et al., 2018), and internal browning in pears (Cruz et al., 2021).

Another example of NIR-based disorder detection is a recent study by Theanjumol et al. (2019) in which 700 – 1100 nm NIRS was used to predict granulation in tangerine fruit. Granulation is a physiological disorder where the cell walls are thick and hardened with low soluble solids, which results in fruit with poor eating quality. The disorder is internal and so is difficult to detect prior to peel removal. Theanjumol et al. (2019) used a variety of classification models to predict the severity of granulation. The fruit was segregated into five classes based on the level of granulation: A (> 1%), B (1 – 25%), C (26 – 50%), D (51 – 75%), and E (> 75%). Differences in the raw spectra was observed between fruit with and without granulation (**Figure 2.19**). The best predictions were obtained from the supervised self-organising map (SSOM) algorithm which correctly classified fruit in the training and test datasets at a rate of 95.5% and 94.0%, and the external validation samples (from a different harvest season) at a rate of 78.4%. The success of these predictions largely depended upon the direct link between granulation and low moisture content and SSC which can be estimated to a high degree of accuracy by NIR spectroscopy (Nicolai et al., 2007a).



**Figure 2.19** Raw NIR spectra of tangerine fruit comparing the five classes of granulation. Adapted from Theanjumpol et al. (2019).

Visible-near infrared spectroscopy has also been utilised in the non-destructive detection of brown core in pears (Han et al., 2006; Sun et al., 2016). Symptoms of brown core consist of internal discolouration of pulp, followed by the potential development of cavities. Han et al. (2006) used discriminant analysis based on data from the 651 – 1282 nm region to discriminate between normal pears and those with brown core and separate the affected fruit into categories of no brown core, slight, moderate, and severe (**Figure 2.20**). The total accuracy rate for classification of these grades was 94.7%, and 95.4% for the identification of sound or brown core. The model had a prediction error of 5.3% for false positives (regular pears classed as brown core), and for false negatives, the error percentage was 4.3%. The strength of the model’s prediction abilities, in this case, are immediately related to differences in colouration due to the browning. The degree of absorption between 710 and 750 nm was dependent on the amount of browning, with more browning equating to greater absorption (**Figure 2.21**).



Figure 2.20 Images of the sequential development of brown core symptoms (Han et al., 2006). The level of the brown core was segregated into four grades (1: no brown core; 2: slight; 3: moderate; 4: severe).

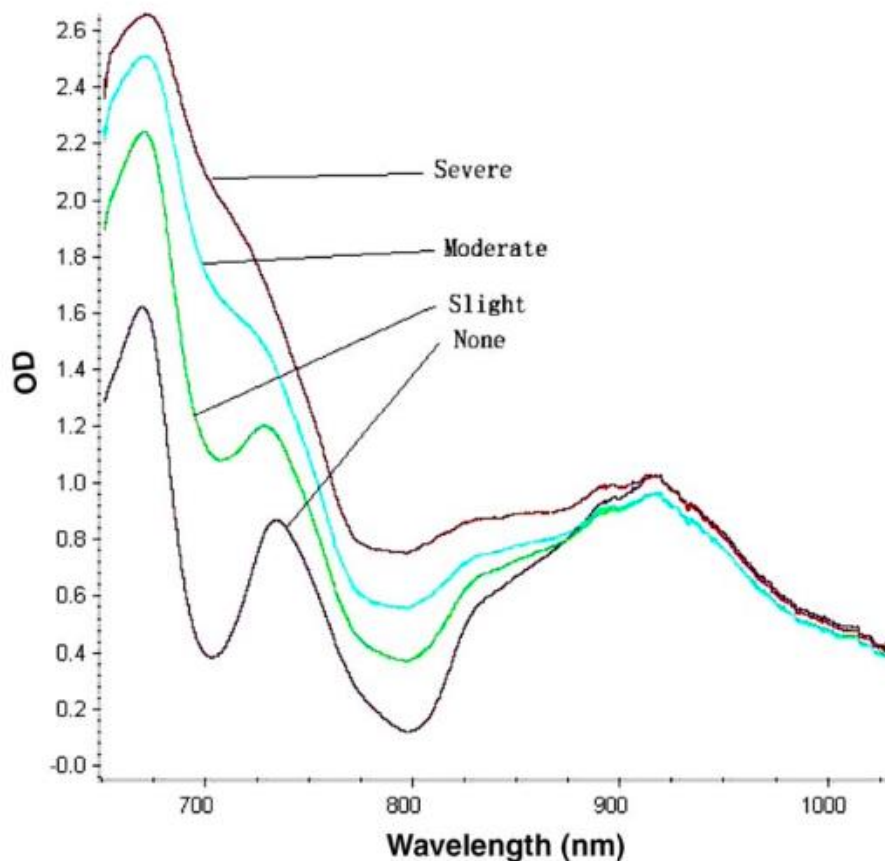
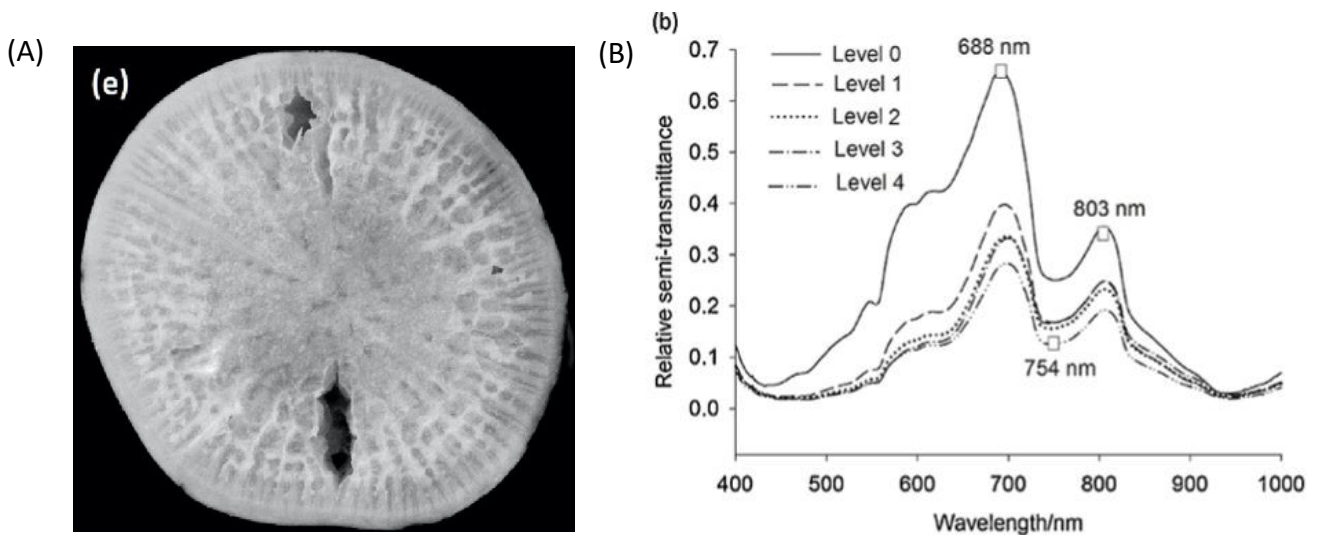


Figure 2.21 Average spectral composition of the light transmitted through pears with and without brown core illustrating differences exhibited between fruit with varying degrees of the disorder (Han et al., 2006).

### 2.7.5 Cavity detection by NIR technology

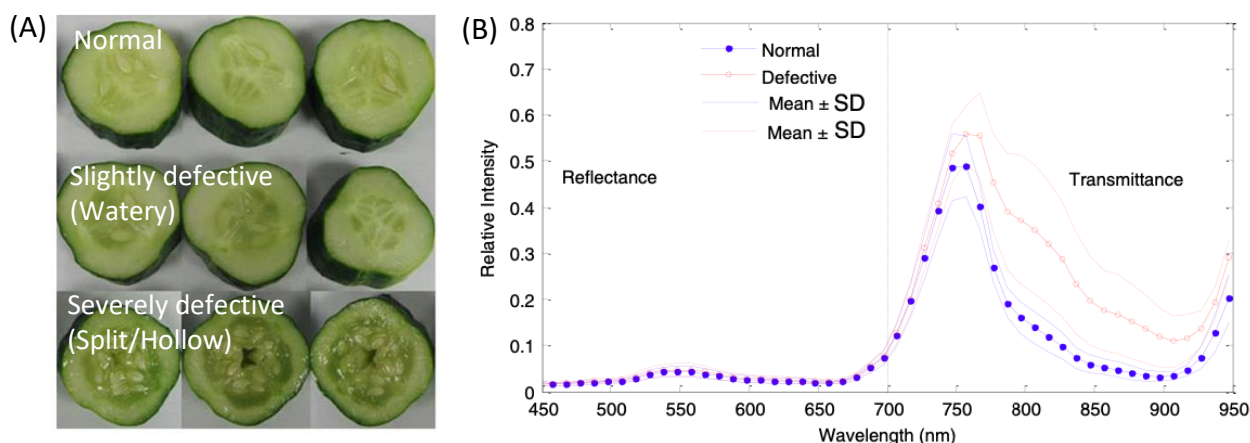
As discussed, there are numerous studies which utilise NIRS to predict disorders across a range of fruit – some additional examples are Clark et al. (2003); Clark et al. (2004); Nicolai et al. (2006); Magwaza et al. (2012b); Ncama et al. (2018); and Mogollón et al. (2020) among others. However, there has been a limited amount of work done on the detection of changes related to the presence of hollows or cavities. Such examples include internal insect damage in chestnuts (Bedini et al., 2020; Moscetti et al., 2014), hollowness in radishes (Pan et al., 2017; Takizawa et al., 2014), and split or hollow centres in pickling cucumbers (Ariana & Lu, 2008a, 2008b; Cen et al., 2013).

In white radishes (*Raphanus sativus* L.), hollowness, characterised by internal cavities and lignification, is a physiological disease that frequently occurs during growth and postharvest storage (Kano & Fukuoka, 1996) (**Figure 2.22 (A)**). The disorder is usually assessed destructively (by sensory panels), and has been linked to quality differences including reduced moisture content and crispiness, and increased crude fibre and lignin content (Pan et al., 2017). Using hyperspectral imaging (400 – 1000 nm, reflectance, transmittance, and semi-transmittance modes), Pan et al. (2017) found radishes with hollowness had higher absorbances in the region of 612 – 825 nm associated with the presence of fibre and lignin (**Figure 2.22 (B)**). The radishes with increasing hollowness severity (from levels 0 – 4) exhibited correlative heightened crude fibre (20.75% – 40.70%) and lignin (9.81% – 21.63%) contents. The study was able to classify the presence or absence of hollowness with good overall accuracy rates of between 84.8% and 98% using PLS-DA (partial least square discrimination analysis) and BPANN (back propagation artificial neural network) modelling techniques. They also reported difficulty in distinguishing between the levels of hollowness at both three-class (i.e., ‘normal’, ‘semi-hollow’, and ‘fully hollow’) and five-class (i.e., ‘normal’, ‘slightly hollow’, ‘moderately hollow’, ‘gravely hollow’ and ‘extremely hollow’) classifications.



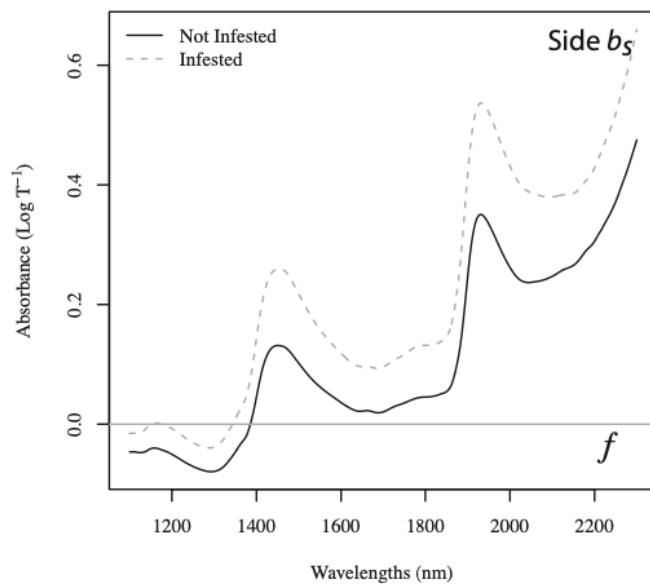
**Figure 2.22** An example of the most severe degree of hollowness in white radish (A) and mean (semi-transmittance) spectra of white radishes with various degrees of hollowness (levels 0 – 4) (B). Sample sizes; level 0 = 105, level 1 = 30, level 2 = 53, level 3 = 66, level 4 = 43. Adapted from Pan et al. (2017).

Similarly, hyperspectral-based imaging has been used to segregate internally defective pickling cucumbers. Common internal disorders in pickling cucumbers include a watery carpel and split or hollow centre (carpel separation) that occurs as a consequence of poor growing conditions or mechanical damage during harvest and transport (**Figure 2.23 (A)**) (Miller et al., 1995). The severity of internal splitting has been categorised into three classes: ‘normal’, ‘slightly defective’, and ‘severely defective’ (Ariana & Lu, 2008b). In affected fruit, cell rupture, tissue separation, carpel degeneration, and the emergence of watery carpel likely reduces the amount of backscattering. This probably leads to better light transmittance through the disordered fruit than through the healthy fruit (Cen et al., 2013). Cen et al. (2013) employed hyperspectral transmittance imaging (700 – 1000 nm) and observed higher transmittance in hollow cucumbers within the region of 780 – 900 nm (**Figure 2.23 (B)**). This study was able to predict normal versus defective fruit (two-class classification) with a highest accuracy of 95.1% (using optimum waveband selection and discriminant analysis with Mahalanobis distance classifier). They also attained a high classification accuracy of 82.8% for the three-class classifier. Previously, Ariana and Lu (2008a) found that hyperspectral transmittance data in the region of 675 – 1000 nm achieved the best detection accuracy (up to 99%) of internal defects in pickling cucumbers.



**Figure 2.23** Transverse sections of normal pickling cucumbers versus watery and hollow fruit (A), and mean spectra (and SD) of healthy compared to defective pickling cucumbers (B). Sample sizes; normal cucumbers = 300, defective = 300 (slight = 150, severe = 150). Adapted from Cen et al. (2013).

Additionally, NIR technology has shown potential for use in sorting chestnuts. In chestnuts (*Castanea sativa*, Miller), the larvae of insect pests (including various weevil and leafroller species) infiltrate fruit and feed on the flesh, diminishing fruit quality (Paparatti & Speranza, 2004). The larva exits through a 1 mm hole which also grants entry to other pathogens, often causing rot. The presence of a hole is a visual identifier of affected fruit, although the damage often remains hidden as many larvae remain inside fruit at harvest (Paparatti & Speranza, 1998). Moscetti et al. (2014) used 1100 – 2300 nm NIRS to segregate sound chestnuts from those with insects or related internal damage. They found changes in wavebands associated with altered water content (near 1450 nm and 1940 nm) (Figure 2.24) likely as in chestnuts insect damage has been linked to lower water content (Rajendran, 2005). Differences seen in absorption in protein/amino acid regions (of 1700 – 1800 nm and 2100 – 2250 nm) were suggested to be connected to bacterial growth and tissue repair after infestation. The researchers used feature selection and LDA to achieve prediction error rates as low as 16.81% false negative, 0.00% false positive, and 8.41% total error, and a 55.3% average improvement over the traditional flotation sorting system. Comparably, Bedini et al. (2020) reported a best result of 8% false positive, 14% false negative, and 11% total error rates in the classification of unsound chestnuts using a Vis/NIR hyperspectral imaging.



**Figure 2.24 Spectra acquired from the bottom side of chestnuts displaying differences in absorbance between healthy and infested fruit, n = 952 (Moscetti et al., 2014).**

### 2.7.6 NIR devices

There is a range of different types of NIR devices available for quality estimation and prediction. This includes multilane sensors, bench-top systems, handheld and pocket sensors (Li et al., 2018). Commercial grading lines can be equipped with multilane NIR sensors to grade fruit by internal qualities such as SSC. Many companies have these systems available such as the online multilane NIR InSpectra sensor (Compac, Auckland, NZ) (Tian & Xu, 2022). Bench-top systems are mainly used for research scale industrial and laboratory analysis. Handheld NIR sensors tend to be used for fieldwork assessments (Walsh et al., 2020). Pocket-sized devices, such as the SCiO™ molecular sensor used in this work, have recently been developed to be used on the consumer level. These sensors were created to be low-cost, highly portable and provide rapid, cloud-based information and analytics (Li et al., 2018). While such devices aim to increase accessibility and affordability, there is limited information available on the accuracy of pocket sensors in comparison to existing equipment. Li et al. (2018) found that the SCiO™ molecular sensor performed well in predicting the quality of ‘Zesy002’ kiwifruit, particularly for SSC, but less successful for the prediction of ‘Braeburn’ apple quality.

In summary, the use of near infrared technology to predict quality characteristics of various horticultural products is well established. Persimmon NIR studies have shown good results for the prediction of quality characteristics including astringency, SSC, and firmness. However, none of these studies have tried to classify disorders using spectral data. Overall, there has been a very limited amount of research completed on the detection of cavities in fruit using NIR technology. In the studies that have been carried out, model performance was generally strong with high levels of correct identification and low levels of error. This provides the opportunity to use NIR technology to detect the presence and classify the severity of calyx cavity in persimmon fruit.

## **2.8 Conclusions**

Calyx cavity is a preharvest physiological considered of major importance in persimmon production. The disorder results in a separation developing around the calyx of the fruit, which, in severe cases, may encircle the entire calyx. The cavity can hide quarantine pests which puts importing countries at risk, and it fosters the growth of fungal pathogens, leading to spoilage and postharvest losses. Fruit with calyx separation is also more susceptible to CI and excess softening during storage. The disorder is linked to the rapid enlargement of fruit during the final phase of growth; fruit flesh expands faster than the calyx causing the fruit flesh to partially detach from the calyx tissue creating a cavity. Calyx cavity is cultivar dependent with varieties like 'Fuyu' more severely affected by the disorder than others. Detection of affected fruit can be difficult because cavities are often obstructed by the calyx lobes. In the literature, the severity of separation is often evaluated by visual observation and subsequent segregation into levels using an arbitrary scale.

The climacteric ripening physiology of persimmons leads to the fruit undergoing pronounced changes in skin colouration and firmness after harvest. Colour changes are attributed to the accumulation of carotenoid pigments and simultaneous degradation of chlorophyll. In persimmon, colouration is commonly measured by a colorimeter and expressed as a colour index. The degradation of cell wall components causes coincident changes in flesh texture from firm to soft with maturity. Textural changes are frequently quantified by destructive

flesh firmness readings, although they may be described by non-destructive methods such as compression and acoustic firmness. Sweetness, generally measured by SSC, also increases with maturity. High quality in all of these traits is important for persimmons to be attractive to consumers. To preserve these qualities and extend shelf life, fruit are stored in modified atmosphere packaging and low temperatures.

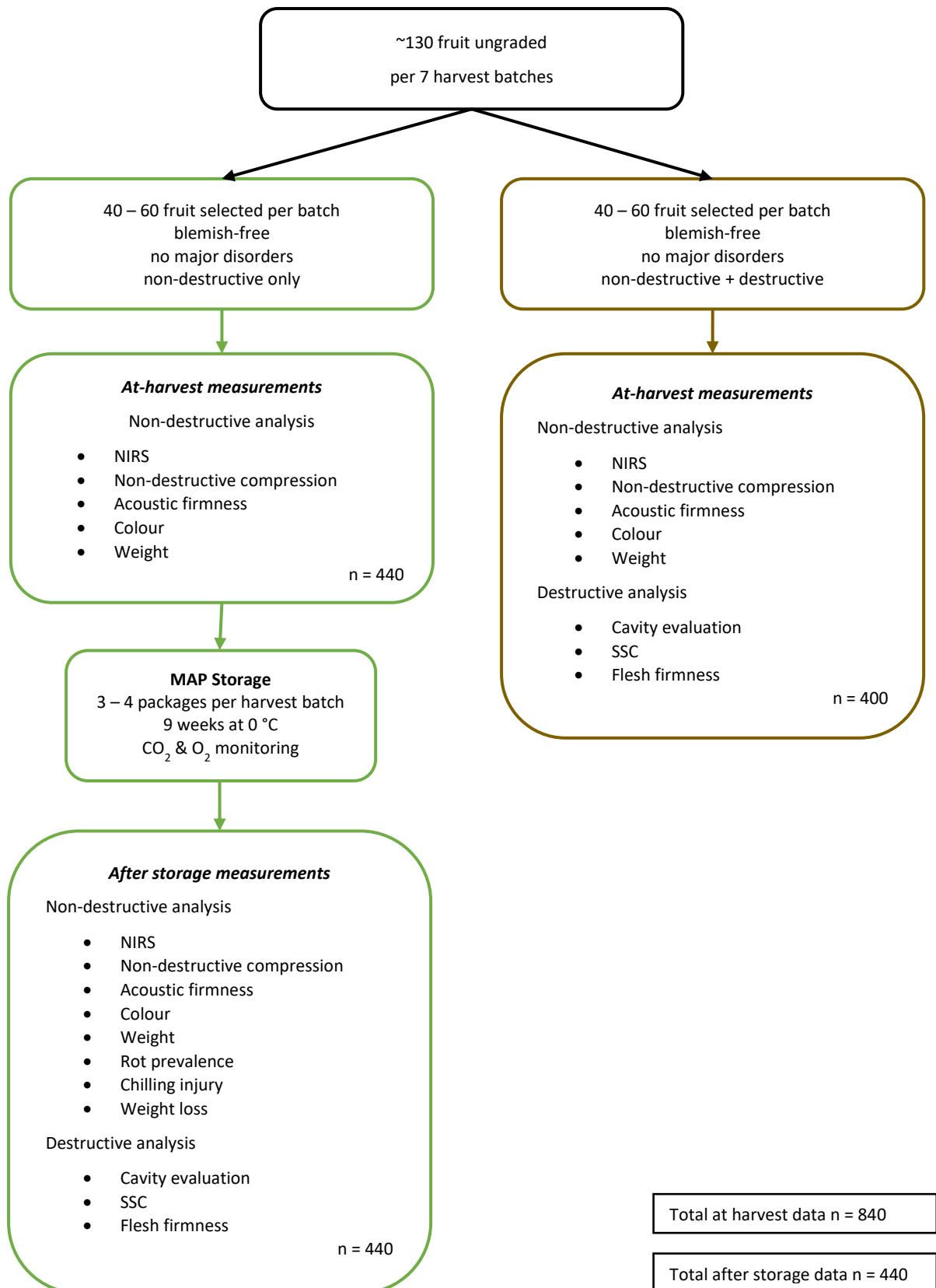
This study hypothesises that non-destructive evaluation of maturity or quality indicators (i.e., colour, size, SSC, firmness) can be used to detect persimmon with calyx cavity disorder. This study aims to determine the strength of the relationship between maturity indicators using destructive and non-destructive evaluations and investigate the potential for non-destructive evaluation methods to be used to classify and segregate fruit with calyx cavity.

# Chapter 3. Materials & Methodology

## 3.1 Plant materials & storage

Persimmon fruit (*Diospyros kaki*, cv. 'Fuyu') were supplied by a commercial orchard located in Gisborne, NZ (FirstFresh, NZ). Approximately 130 un-graded fruit from each of 7 different harvest timings were sent to Massey University Postharvest Lab during a 1-month time frame. Fruit arrived mixed together in a single, large cardboard box (rather than packed into individual trays). A sample of 100 – 145 persimmons free of major defects were selected per harvest batch. Fruit were considered acceptable if they showed no evidence of bruising, physical damage (such as splits or cuts), live insects or related damage, rots, or skin russetting ('FreshSpecs', Fresh Markets Australia). Fruit were labelled by correlative numbers and placed into plastic fruit trays inside cardboard fruit boxes (**Figure 3.2**). Of these fruit, half were used to perform destructive evaluations consisting of calyx cavity assessment, flesh firmness and SSC measurements. The remaining half of the sample were then evaluated using non-destructive techniques to measure quality properties including size, peel colour and firmness.

After completion of the non-destructive assessments, fruit was packed into boxes with cardboard fruit trays of different counts (from count 12 to 28) based on size. As each harvest contained fruit from a range of sizes (approx. 100 g to 400 g), sample balancing was done where the weight for each box was adjusted to 4.40 kg  $\pm$  0.56 kg using the extra fruit. Each MAP was comprised of 12 to 28 fruit with 3 to 4 MAP packages created per harvest batch (**Figure 3.2**). The fruit was sealed inside 60  $\mu$ m polyethylene MAP bags using a temperature sealer. MAPs were stored in a temperature-controlled room (TCR) at 0 °C for 9 weeks (Ahmed et al., 2011). After storage, the non-destructive and destructive evaluations were completed as previously with additional assessments to determine rot prevalence, chilling injury incidence, and water loss. An overview of the sequence of measurements and procedures carried out during the experiment is displayed in **Figure 3.1**.



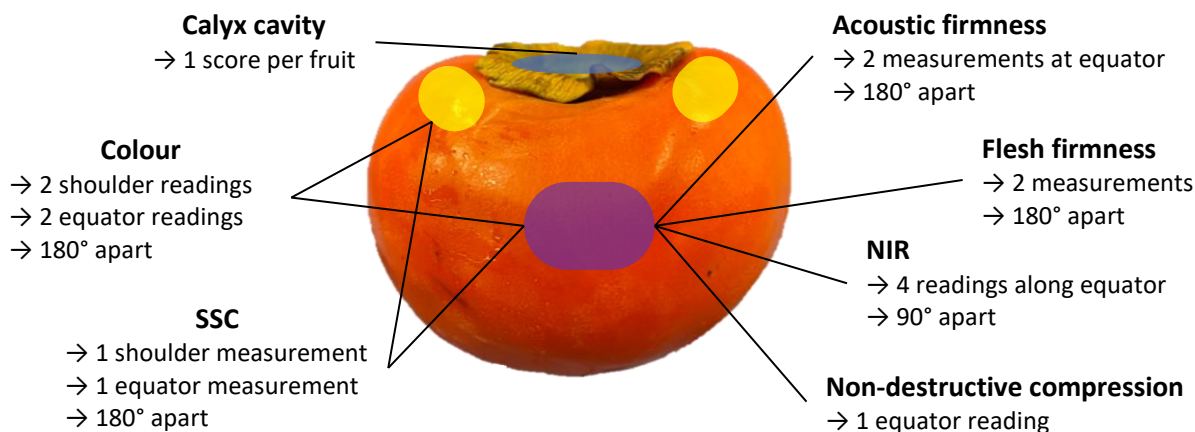
**Figure 3.1** Flow diagram of experimental procedures indicating the destructive and non-destructive evaluations completed at harvest and post storage.



**Figure 3.2** 'Fuyu' persimmons individually labelled and laid out in an 18-count plastic fruit tray for at harvest measurements (left). Fruit packed on an industry standard 20-count cardboard tray inside a 60  $\mu$ m MA bag after sample balancing before heat sealing (right).

### **3.2 At harvest evaluations**

The environmental conditions for all of the quality analyses was 20 °C as temperature can influence firmness, SSC, and NIR readings. A measurement of fruit flesh temperature was taken prior to measurement using a hand-held digital thermometer. The location of each evaluation on the fruit is displayed in **Figure 3.3**.



**Figure 3.3** Locations of the destructive and non-destructive measurements indicated by coloured areas on the equator (shown in purple) and shoulders (shown in yellow) of the persimmon fruit.

### 3.2.1 Non-destructive measurements

#### 3.2.1.1 *Near infrared spectroscopy*

Spectral data was collected using the handheld SCiO™ molecular sensor (v1.2, Consumer Physics Inc., Tel-Aviv, Israel) on whole fruit samples over a wavelength range of 740 – 1070 nm. The device was set up in interactance mode with a wavelength resolution  $< 10 \text{ cm}^{-1}$  and a sampling interval of 1 nm (Li et al., 2018). Calibration with the white reference was completed prior to the first measurement and repeated every 240 measurements. The SCiO™ Lab online application (Consumer Physics Inc., Tel-Aviv, Israel) was used for data collection, storage, and analysis. Readings were collected with the illuminator pointing towards the fruit skin at 4 separate locations, about 90° apart, around the equator of the fruit; uneven areas, spots, and blemishes on the fruit surface were avoided. To minimise background interference, provide a sufficient light seal, and maintain a consistent measurement distance, a 9 mm light shield was used with the sensor during measurements. The reflected spectrum from each specimen was sent through Bluetooth to a smartphone, and subsequently to the online SCiO™ cloud database for spectral analysis (Li et al., 2018).

### **3.2.1.2 Weight**

Individual fresh fruit weights were measured by digital balance (PR50003DR, Mettler Toledo, USA) with results expressed in grams (g) to 3 decimal places. The weight was the second measurement taken; it was recorded approx. 2 hours after the fruit arrived. Each batch of fruit was not graded by size prior to arrival; fruit of a range of sizes (from about 100 g to 400 g) were selected for assessment.

### **3.2.1.3 Skin colour**

The peel colour was evaluated using a spectrophotometer (CM-2000d, Konica Minolta Sensing, Osaka, Japan) in the CIE L\*a\*b\* space of 0.5 cm<sup>2</sup> area in 4 locations (two measurements on the shoulders and two measurements around the equator). A calibration was done against white and black standards prior to sample measurement. Measurements were taken first from the left shoulder followed by the right shoulder for each fruit, then measurements from the right and left equator were done. The fruit was held against the sensor, and two consecutive readings taken for each measurement location. The interval between shoulder (or equator) measurements was one second where the fruit was rotated to the opposing shoulder (or equator) from left to right. The 8 values per fruit were averaged, and colour results were recorded as L, a, b, chroma (C), and Hue angle (h°), as well as in the form of a colour index (Salvador et al., 2007). C, h° and the colour index were calculated from the L, a, and b values obtained using **Equation 3.1**, **Equation 3.2**, and **Equation 3.3** respectfully.

$$C = (a^2 + b^2)^{\frac{1}{2}} \quad \text{Equation 3.1}$$

$$h^\circ = \tan^{-1} \left( \frac{b}{a} \right) \quad \text{Equation 3.2}$$

$$\text{Colour index} = \frac{1000 a}{L b}$$

Equation 3.3

The L\*a\*b\* colour space coordinates express colour as lightness (L) which ranges from 0 to 100, where 0 is black and 100 represents white, and a and b which represent the four colours of human vision: red, green, blue, and yellow. Colour intensity and saturation are described by chroma and hue angle. The colour parameters are displayed in **Figure 3.4**.

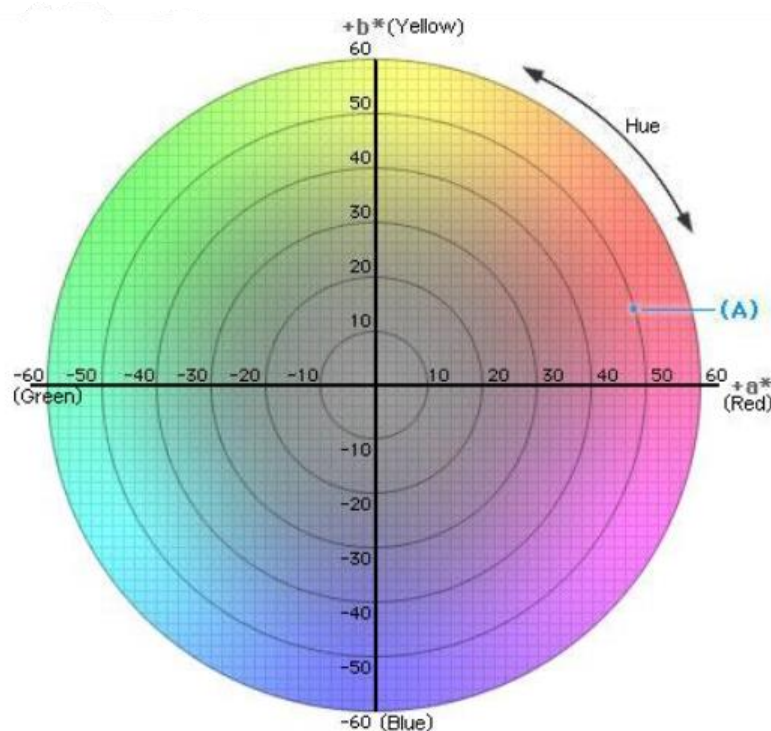


Figure 3.4 A chromaticity diagram showing the colour coordinates (a, b and hue angle) and indicating colour directions (sourced from Konica Minolta Sensing).

### 3.2.1.4 Acoustic firmness

The acoustic firmness sensor (AFS, Aweta, Nootdorp, The Netherlands) was used to measure the weight (in g) and the peak resonant frequency ( $f$  in Hertz, Hz). The frequency and weight are then used to estimate the acoustic firmness index (FI in  $\text{Hz}^2 \text{g}^{2/3}$ ) using **Equation 3.4** (Rowe et al., 2014). A tick power of 9 and microphone gain of 51 was used, and a weight calibration was carried out before measurement. Readings were taken on two opposite positions around

the equator of each fruit and the two values averaged. Results were reported in firmness index units.

$$FI = \frac{f^2 m^3}{10^6} \quad \text{Equation 3.4}$$

Where f is frequency in Hz and m is mass in g.

### **3.2.1.5 Non-destructive compression**

Firmness was also measured by non-destructive compression using a Texture Analyser (TA-XT2, Stable Micro Systems, UK) equipped with a 5 Kg cell and fitted with a 60 mm diameter compression plate probe. Calibration with a 5 kg standard was completed before sample measurement every 60 samples. Per fruit, a single compression to a target force of 4 N at a loading speed of 1 mms<sup>-1</sup> was done, following methods from (Schotsmans & Mawson, 2004) with modifications. Results were expressed as deformation strain (% of equatorial diameter).

## **3.2.2 Destructive measurements**

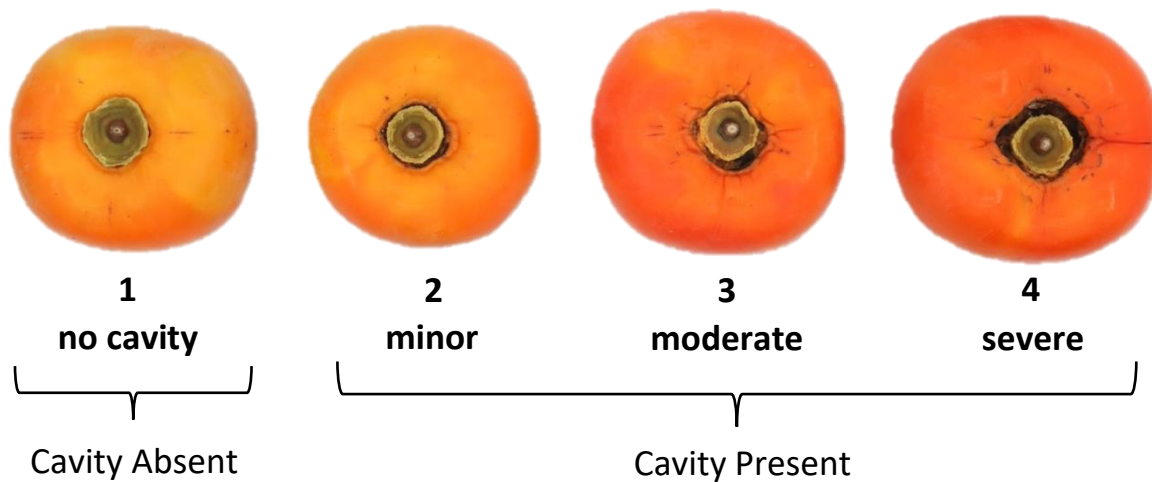
### **3.2.2.1 Calyx cavity**

To assess the presence and severity of calyx cavity, the calyx lobes of each fruit were carefully removed using tweezers, leaving only the calyx disk remaining. This was done to expose the area between the calyx and fruit tissue where separation occurs. The degree of separation was visually observed and then scored based on the size and depth of the cavity (Yamada et al., 1987). Each fruit was assigned a scale value where 1 = no cavity, 2 = minor, 3 = moderate, 4 = severe. This arbitrary scale was created using fruit with representative cavity sizes based on visual estimation (**Figure 3.5**) based on previous work by Akagi et al. (2020). Results are expressed as a binary (0 = cavity absent (score 1); 1 = cavity present (score 2, 3, and 4)) as well as within the severity categories (scale values 1 - 4). The incidence of calyx cavity (%) was calculated by the total number of fruit with calyx cavity levels 2, 3, and 4 using the individual

calyx cavity severity data (**Equation 3.5**). The incidence of each level of calyx cavity (%) was calculated similarly as shown in **Equation 3.6**.

$$\text{cavity incidence (\%)} = \frac{(\text{score 2} + \text{score 3} + \text{score 4})}{\text{total count}} \times 100 \quad \text{Equation 3.5}$$

$$\text{severity incidence (\%)} = \frac{\text{score } x}{\text{total count}} \times 100 \quad \text{Equation 3.6}$$



**Figure 3.5** Diagram of the visual scale created to segregated different levels of calyx separation in 'Fuyu' persimmon ranging from grade 1 (no cavity present) to grade 4 (severe cavity present). The scale can also be interpreted as a binary where grade 1 = absence of calyx cavity (0), and grades 2 – 4 = presence of calyx cavity (1).

### 3.2.2.2 *Flesh firmness*

Destructive firmness ( $\text{kg}_f$ ) was measured using an electric penetrometer (Willowbank Electronics, New Zealand) equipped with the standard 7.9 mm round tip Magness-Taylor probe. Prior to evaluation, 1 mm of fruit skin was removed at 2 positions  $180^\circ$  apart around the equator. Readings were taken of the peak force necessary to puncture the tissue at a speed of  $8 \text{ mm s}^{-1}$  to an 8 mm depth (Salvador et al., 2007). Flesh firmness ( $\text{kg}_f$ ) was expressed

as the average of the 2 values. Firmness evaluations were performed penultimately, ensuring fruit was able to reach 20 °C before readings were taken.

### **3.2.2.3 Soluble solids content**

SSC was measured by coring fruit through the equator (from the front of the fruit) to remove approximately 20 g of flesh, using a 12 mm diameter corer. The core was cut to size (to about 2 cm in length), skin removed, and placed in a garlic press with a cloth layer as a filter (Suntudprom, 2014). The extracted persimmon juice was measured as % Brix by a hand-held digital refractometer (Pocket PAL-1, Atago, Japan). A second core was taken from the left top shoulder of each fruit and the SSC reading replicated, providing separate SSC values for the shoulder and equator of each fruit. The average of the shoulder and equator readings was calculated to provide the SSC, expressed as % Brix. Calibration with distilled water was conducted prior to sample measurement, and every 120 samples.

## **3.3 Storage measurements**

### **3.3.1 Gas, temperature & RH monitoring**

During storage, CO<sub>2</sub> and O<sub>2</sub> levels (as a percentage of the total gas concentration) in the MAP headspace were monitored every week using a hand-held CO<sub>2</sub>/O<sub>2</sub> analyser (0-10% CO<sub>2</sub> and 0-25% O<sub>2</sub>, CM-1000, GasLab Pro, USA) Gas concentrations were measured by inserting the needle connected to the CO<sub>2</sub>/O<sub>2</sub> analyser into the middle of each package. A small piece of tape was immediately placed over the puncture to minimise any potential alterations in the gas concentration caused by the procedure. The measurements were carried out in the TCR at 0 °C to avoid fluctuations in fruit temperature. The first reading of each MAP was done 2 days after fruit was placed at 0 °C and subsequent readings were taken from the same area of the package every week during the 9 weeks. Temperature (°C) and RH (%) logging within the package was also done with a dual (temperature and RH) logger (iButton®, Maxim Integrated, CA, USA).

### 3.4 After storage measurements

Fruit were removed from cold storage and placed in the laboratory at 20 °C for approx. 16 hours prior to evaluation to ensure fruit reached ambient temperatures at the time of measurement. All of the destructive and non-destructive analyses completed prior to storage were repeated after fruit were removed from MAP cold storage at 0 °C for 9 weeks using identical methods as described above.

#### 3.4.1 Water loss, rots & chilling injury

Fruit weights after storage were recorded as previously. Weight loss (WL) was calculated as the difference between initial weight ( $W_i$ ) at harvest and final weight ( $W_f$ ) after storage of individual fruit (**Equation 3.7**). Rot prevalence (%) was the number of fruit with rot symptoms out of the total fruit number. Chilling injury prevalence (%) was the number of fruit with chilling injury symptoms out of the total fruit number. Images of CI and common rots in persimmon fruit are shown in **Figure 3.6**.

$$WL (\%) = \frac{(W_i - W_f)}{W_i} \times 100 \quad \text{Equation 3.7}$$

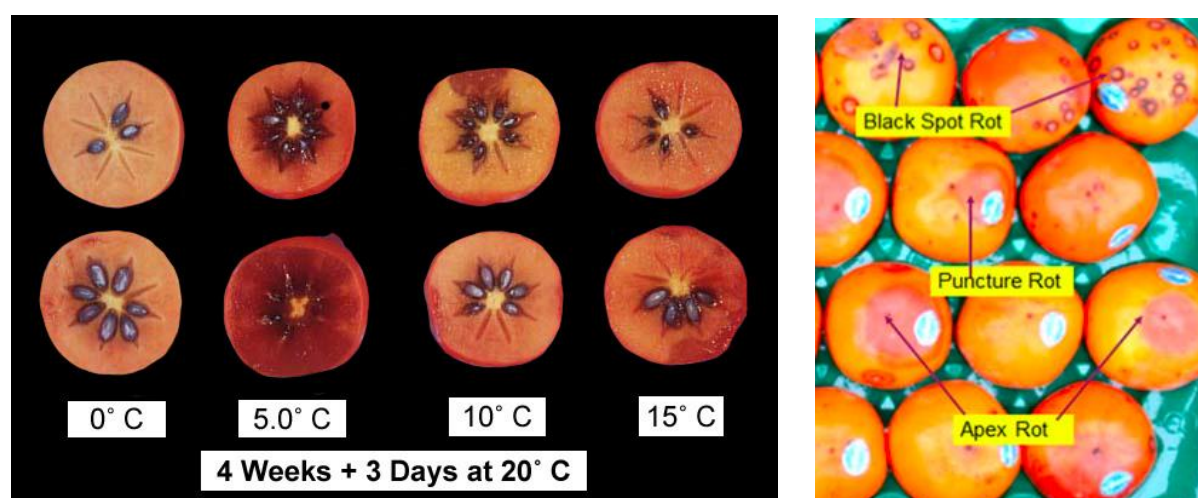


Figure 3.6 'Fuyu' fruit with various chilling injury symptoms (left) and common puncture and apex rots (*Penicillium* spp., *Rhizopus* spp.) and black spot (*Alternaria* spp.) (right). Images adapted from (Woolf & Ben-Arie, 2011) (rot) Bignell et al. (2017) (chilling injury).

### 3.5 Statistical analysis

For the persimmon fruit, each harvest batch of fruit were randomly separated into two groups; both groups were non-destructively evaluated at harvest and one group was also destructively assessed while the other was stored. One group containing 60 fruit from the same batch, either cold-stored or destructively measured at harvest was considered as the experimental unit. The influence of storage time and the presence of calyx cavity on fruit quality variables was analysed using a General Linear Model (GLM) in ANOVA. Significant differences in means were indicated by LSD Fisher (Least Significant Difference test) at P-value < 0.05. Pearson's correlation coefficient ( $r$ ) matrix was constructed to determine the linear association between response variables of weight, colour index, SSC, flesh firmness, non-destructive compression, and acoustic firmness. 120 observations acquired from the different harvest batches (seven batches assessed before and after storage at 0 °C in MAP) were considered for the 'Fuyu' cultivar.

A Principal Component Analysis (PCA) biplot was generated to display the relationship between response variables (Loading Plot) and the presence of calyx cavity (Score Plot). PCA was performed using the correlation matrix (rescaled values) of the response variables of fresh weight, colour, non-destructive compression, acoustic firmness, flesh firmness, and SSC. The response variables were acquired from seven observations at harvest. The Loading Plot was produced using the coefficients of each variable for the first two components. This was done to discern which response variables had the greatest effect on each principal component and the direction of each variable on the first and second principal components.

Simple binary logistic regression was used to determine whether response variables (quality attributes) of persimmon were related to the presence of calyx cavity. The response variables were used as continuous predictors with calyx cavity as the binary response (where 1 = presence and 0 = absence of calyx cavity). Linear discriminant analysis (LDA) was also used to determine how well certain response variables describe calyx cavity. Linear discriminant

analysis was done using the response variables of (at harvest) weight and colour index as continuous predictors of either calyx cavity presence or severity.

Statistical analysis and plotting of figures was accomplished using statistical software RStudio (R Foundation for Statistical Computing, Vienna, Austria). Error bars present represent standard errors.

Analysis of spectral data was performed using the SCiO™ Lab online interface. On this website, various pre-processing procedures including log transformation, standard normal variate (SNV), averaging, and second order derivation were used on the at harvest or after storage data. To construct a model, the data pre-processing methods were first selected, then the quality attribute of interest was designated followed by choosing 'Create Model' to produce the model. The partial least squares (PLS) algorithm was used on the cloud database to generate the models for quality predictions, and the random forest (RF) algorithm was used to predict the presence or the severity of calyx cavity. Further description of RF is provided in **Appendix B**. In this work, the spectral wavelength range (740 – 1070 nm) was selected for all measurements completed before and after harvest. After model creation, the predicted values were compared to the measured values in the quality models. The success of these models was also gauged by the  $R^2$  value (coefficient of determination), and the RMSE (root-mean-square error) provided by the SCiO™ Lab application. In the segregation models, the predicted classes were compared to the known classes, and performance evaluated by the rates of true positives, true negatives, false positives, and false negatives. The overall performance of the calyx cavity models was also determined by comparison of the F1 statistic.

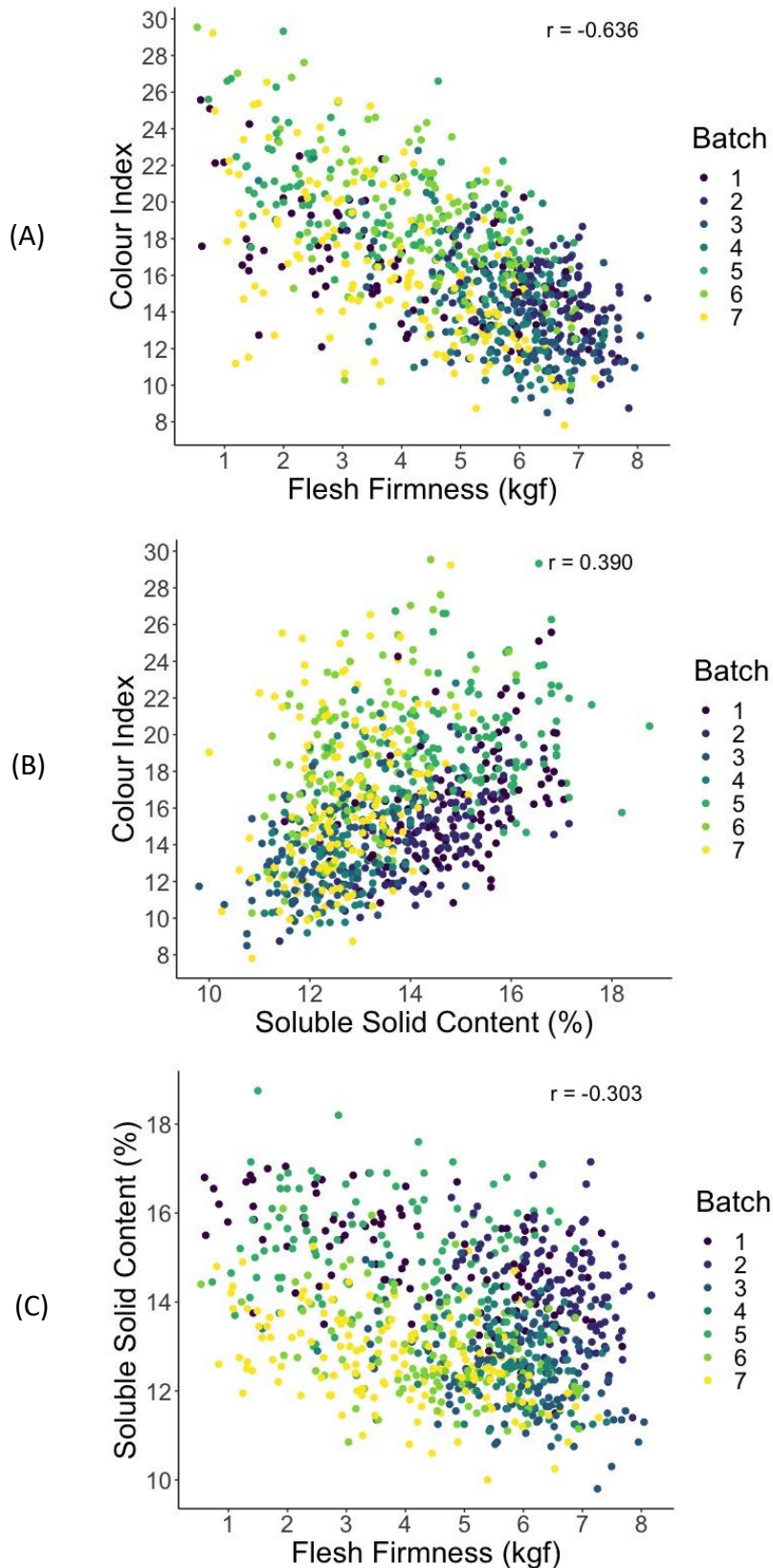
# Chapter 4. Non-destructive Indicators & Calyx Cavity

## 4.1 The relationship between destructive and non-destructive evaluations

### 4.1.1 Maturity indicators at harvest time

Correlations between maturity indicators were found as can be seen by the negative and moderate relationship between the colour index and flesh firmness ( $r = -0.636$ ) including both at harvest and after storage data (**Figure 4.1 (A)**). Fruit with greater orange colouration tended to have correspondingly lower firmness levels. The relationship between firmness and colouration in fruit is due to physiological changes – including chemical, physical, and enzymatic alterations – that occur during maturation (Bignell et al., 2017). An essential part of persimmon fruit maturation is augmentation of skin colour and simultaneous softening of fruit tissue (Clark & MacFall, 2003). It has been recognised that both colour and firmness changes also continue postharvest, rather than ceasing at harvest (Salvador et al., 2005).

While a moderate correlation was observed, the relationship between fruit colour and firmness in this experiment was lower compared to results that have been reported by other studies. Salvador et al. (2006) described a strong, negative association between flesh firmness and colour indices in 'Rojo Brillante' fruit where high colour values were associated with low firmness values. They used linear regression (GLM) to predict fruit firmness from external colour for fruit stored at 1 °C for 50 days and reported that firmness values decreased 0.99 units by colour unit increase (adjusted  $R^2 = 81.78$ ). For early harvested fruit, firmness values decreased 0.99 units by colour unit increase, with a prediction accuracy of 81.8%. In agreement, Tessmer et al. (2016) found a strong, negative correlation between peel colour and flesh firmness in 'Fuyu', 'Hana Fuyu', 'Giombo', and 'Rojo Brillante', and was able to predict mean firmness with an accuracy of over 80% for all the cultivars.



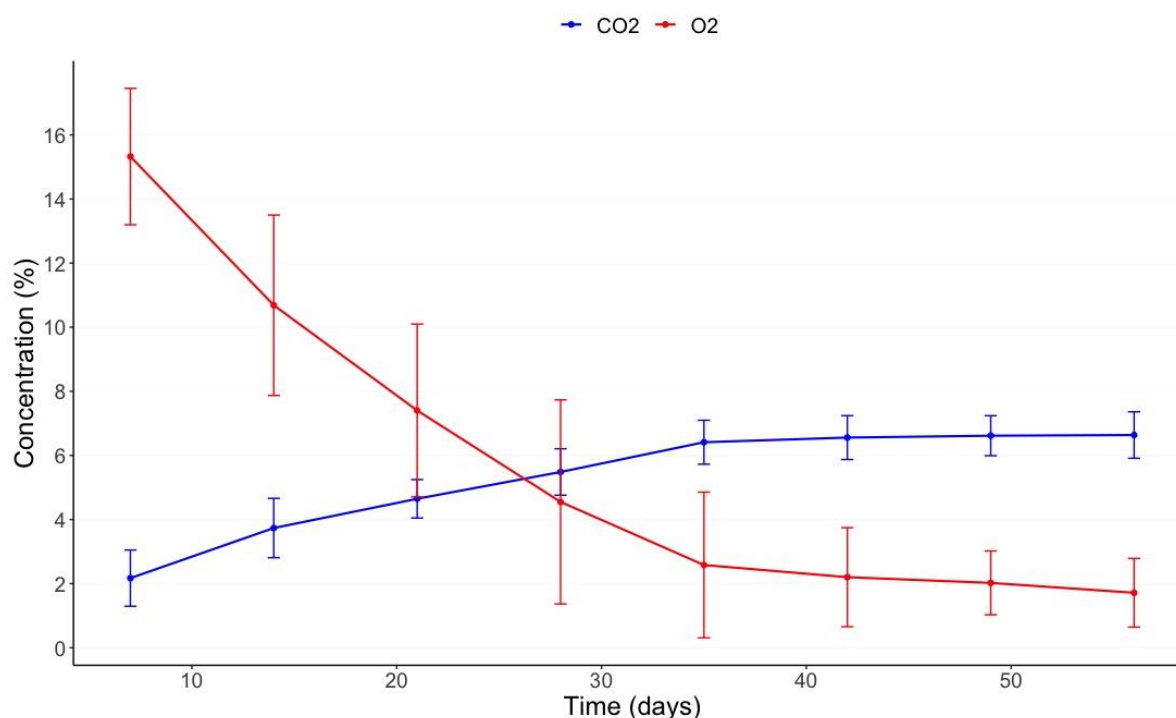
**Figure 4.1** Correlations between each of main maturity indicators including both at harvest and after storage measurements. (A) represents colour index vs soluble solids; (B) colour index vs flesh firmness; and (C) soluble solids vs flesh firmness.

In the present work, the correlation between maturity indicators was not as strong potentially due to factors including sample size, harvest timing, cultivar selection, and environmental conditions (Bignell et al., 2017). The spread of maturities in this work is highly variable in comparison to other studies. Novillo et al. (2016) reported a high negative correlation ( $r = -0.9$ ) between external colour and firmness in various cultivars when accounting for harvest stage (immature green, colour-break, and commercial maturity). They noted that the correlation was not strong for 'Tonewase' ( $r = -0.6$ ) and that differences were highly cultivar dependent. Salvador et al. (2006) showed that the spread of data was larger when both early and late harvest 'Rojo Brillante' fruit were assessed as the late harvest fruit were more highly coloured and softer. The colour index ranged from a value of approximately 5 to 33 after 50 days at 1 °C with both harvest dates, but separately early harvest fruit colour indices varied between about 5 and 20 and late harvest 21 to 33.

The relationship between the colour index and soluble solids was weak and positive ( $r = 0.390$ ) (**Figure 4.1 (B)**). Likewise, the correlation between flesh firmness and soluble solids was weak and negative ( $r = -0.303$ ) (**Figure 4.1 (C)**). Higher SSC was not necessarily associated with greater colouration, or with lower firmness. In persimmon, SSC can be a good indicator of maturity, however, this is not always the case (Salvador et al., 2006). SSC may be less closely associated with fruit maturity than colour and firmness as it can vary significantly depending on climatic conditions (Woolf & Ben-Arie, 2011). For instance, Australian-grown 'Fuyu' fruit has a recommended at harvest SSC of 15%, while 'Fuyu' fruit grown in Japan can reach SSC up to 18% (Tessmer et al., 2016). Another factor may be that changes in SSC postharvest tend to be less pronounced compared to postharvest changes in colouration and firmness (Salvador et al., 2007).

## 4.1.2 Postharvest changes

### 4.1.2.1 Atmospheric CO<sub>2</sub> and O<sub>2</sub> composition in MAP



**Figure 4.2** The average gas concentrations in the headspace of the modified atmosphere packages over the 9 weeks cold storage at 0 °C. CO<sub>2</sub> and O<sub>2</sub> readings were taken from each package once per week and averaged across packages and harvest batches.

Fruit were stored in 60 µm polyethylene modified atmosphere packages for approximately 56 days at 0 °C. Oxygen (O<sub>2</sub>) levels decreased with a concomitant increase in carbon dioxide (CO<sub>2</sub>) for the first 20 days. After 25 days in cool storage, the relative quantity of O<sub>2</sub> dipped below that of CO<sub>2</sub> (**Figure 4.2**). By 35 days, the gas concentrations had stabilised – the percentage of O<sub>2</sub> continued to decrease slightly over the successive 20 days. In comparison to similar studies, these MAPs required more time to reach a steady-state atmosphere (**Table 4.2**). Ahn et al. (2007) saw stable O<sub>2</sub> and CO<sub>2</sub> concentrations after approx. 8 days in ‘Fuyu’ stored under 0 °C conditions; MacRae (1987) reported stable O<sub>2</sub> and CO<sub>2</sub> levels of 4 – 6% and 6 – 8%, respectively, by 28 days at 0 °C in NZ grown ‘Fuyu’ persimmons stored in 60 µm MAP. In the present work, the relatively long time to reach stable gas concentrations could be due to the quantity of fruit per package. Some studies packed fewer or more fruit into the MAP e.g. Fahmy and Nakano (2012) included 10 kg of fruit per package which reached stable gas

concentrations in 3 days. Other contributory factors include at harvest maturity, metabolism, and growing environment (Ramin & Tabatabaei, 2003).

The average MAP CO<sub>2</sub> concentration after 7 days of cold storage was 2.2%; this level was elevated to 6.6% at the end of storage. The mean O<sub>2</sub> level was 15.3% at the beginning of storage which dissipated to 1.7% after 56 days (9 weeks) of cold storage. These levels are in accordance with other research on MAP in persimmon storage (**Table 4.1**). Comparable studies which used 'Fuyu' fruit and 60 µm polyethylene, reported gas concentrations of 7.8% CO<sub>2</sub> and 0.7% O<sub>2</sub> (Lee, 2004), and 5.5% CO<sub>2</sub> and 1.5% O<sub>2</sub> (Ahn et al., 2007). The relative concentrations of CO<sub>2</sub> and O<sub>2</sub> cause postharvest respiration and ripening to be reduced which limits postharvest changes in colouration, firmness and SSC (Kader et al., 1989).

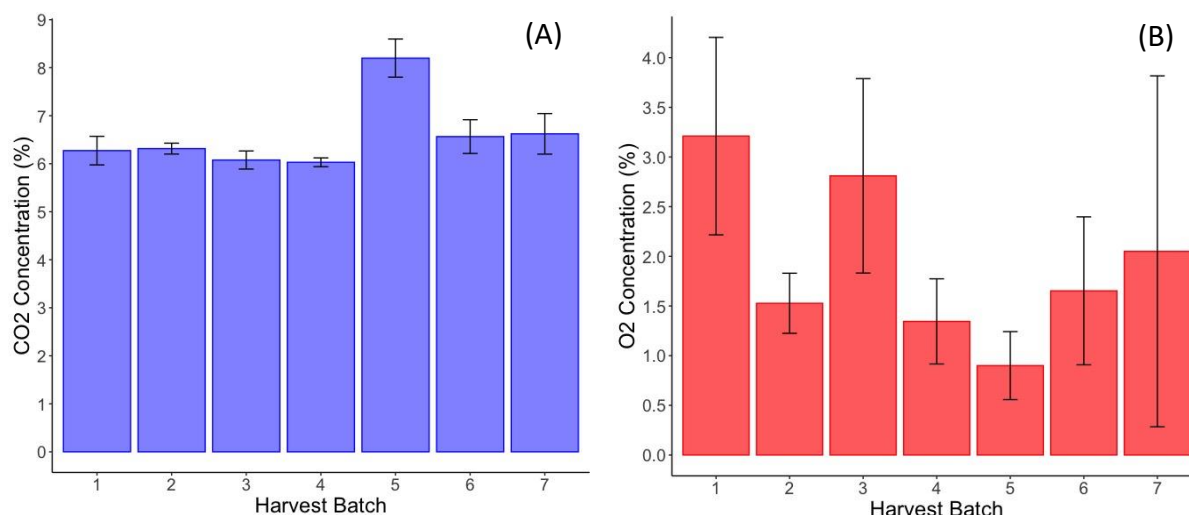
**Table 4.1 Final or average gas concentrations reported by comparable studies on MAP in persimmon, including a variety of packing film thicknesses and cultivars.**

Cultivar	Packaging (µm LDPE)	Temperature (°C)	Time to equilibrium (days)	CO <sub>2</sub> (%)	O <sub>2</sub> (%)	Source
'Triumph'	80	-1	2	8.0	3.0	(Prusky et al., 1997)
'Jiro'	40	2	3	6.19	4.18	(Fahmy & Nakano, 2012)
'Jiro'	40	2	2 - 5	5.41	6.63	(Fahmy & Nakano, 2016)
'Fuyu'	-	0	-	7.07	0.5	(Lee et al., 2008)
'Fuyu'	-	0	-	6.8	0.8	(Lee et al., 2010)
'Fuyu'	50	1	21	4.19	2.15	(Cia et al., 2006)
'Youhou'	50	1	7 - 21	6.96	9.96	(Zhao et al., 2020)
'Fuyu'	60	0	-	3.8	13.8	(Ben-Arie & Zutkhi, 1992)
	80			7.0	5.8	
'Fuyu'	65	0	-	8.7	0.5	(Lee, 2004)
	60			7.8	0.7	
	55			6.8	1.3	
	50			6.5	1.7	
	45			6.0	3.0	
'Fuyu'	70	0	8	6.2	1.1	(Ahn et al., 2007)
	60			5.5	1.5	

	52			4.7	2.7	
	40			3.6	6.2	
'Fuyu'	60	0	28	6 - 8	5 - 6	(MacRae, 1987)

LDPE: Low-density polyethylene; CO<sub>2</sub>: carbon dioxide; O<sub>2</sub>: oxygen.

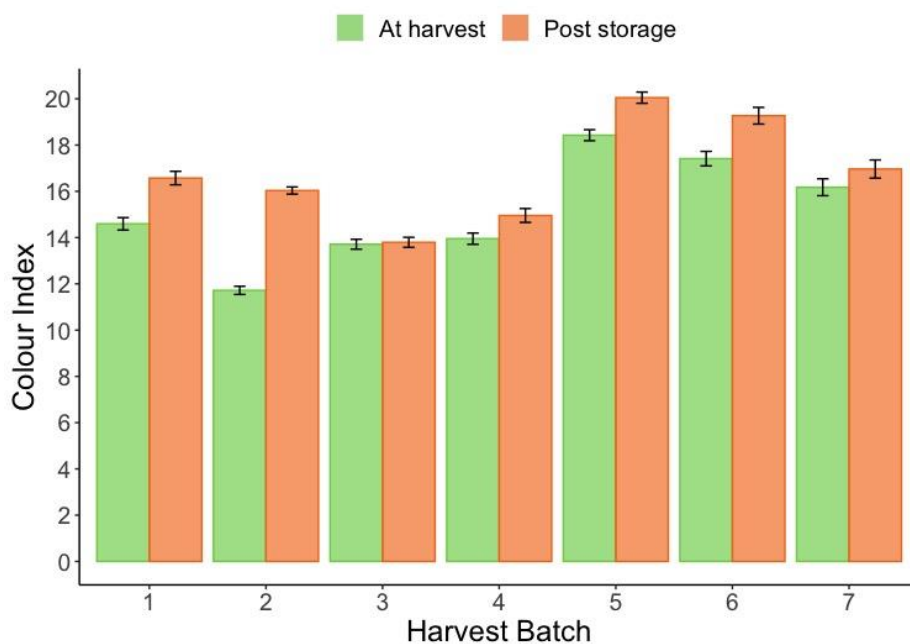
There were differences in CO<sub>2</sub> and O<sub>2</sub> concentrations between individual MAPs, and between harvest batches (**Figure 4.3**). The highest final CO<sub>2</sub> level observed was 9.1%, and the lowest was 5.5%. Final O<sub>2</sub> levels ranged from 0.3% to 7.4%, compared to the 1.7% average. In packages where CO<sub>2</sub> levels were particularly high, O<sub>2</sub> levels tended to be very low. The variance in gas concentrations between harvest batches may have been contributed to by differences in the metabolism of each batch. **Figure 4.3** shows that batch 5 had higher CO<sub>2</sub> and lower O<sub>2</sub> levels compared to the other batches, suggesting batch 5 persimmons had a higher rate of respiration. In addition, some MAPs had imperfect seals or developed punctures over the course of storage which may have promoted gas leakage, limiting the performance ability of the packaging (Woolf & Ben-Arie, 2011). The few MAPs with very high oxygen concentrations were caused by small perforations in the packaging material during storage which was a source of experimental error.



**Figure 4.3** The final concentrations of CO<sub>2</sub> (A) and O<sub>2</sub> (B) for the seven harvest batches. Each batch was split between four separate MAPs – the bars represent the averages of the final O<sub>2</sub> and CO<sub>2</sub> readings for each MAP.

#### 4.1.2.2 Skin colouration

The peel colouration of persimmon fruit increased during postharvest cold storage. In terms of colour index interpretation, higher values indicate greater orange-red fruit skin colouration and lower values indicate poorer colour. The average colour index value for all fruit pre-storage was 15.1 compared to 16.8 after storage, a mean increase of 1.7. These results are similar to those published by Salvador et al. (2005) who found that the colour index values for 'Rojo Brillante' fruit increased by 1.38 after 40 days stored at 1 °C from at harvest levels. The deepening of persimmon fruit colour after harvest is due to continued (although slowed) fruit metabolism (Besada & Salvador, 2018). During storage, chlorophyll levels continue to reduce as this pigment is degraded and carotenoid levels (including  $\beta$ -cryptoxanthin, zeaxanthin, and lycopene) continue to increase as these pigments are synthesised and accumulated (Salunkhe & Kadam, 1995).



**Figure 4.4** The average colour indices of fruit at harvest compared to after storage for each harvest batch of fruit.

There was variation in the colouration of fruit skin between batches of fruit (**Figure 4.4**). In many instances, there was a greater difference in the colouration between batches than the colour change that occurred during storage. Batch 5 had the highest mean colour index value of 18.4 and batch 2 had the lowest value of 11.7 at harvest. Many factors can affect persimmon colouration at harvest including the growing environment and harvest timing (Besada & Salvador, 2018). Salvador et al. (2006) reported a mean colour index of 4.87 in early harvested fruit compared to 23.94 in late harvested 'Rojo Brillante' fruit. The researchers suggested that the most significant factor in colour index differences at harvest was the harvest date, while the orchard was not as important of a factor.

Persimmon harvest timing is based on the skin colour of fruit which is strongly correlated to the maturity of fruit; greater colouration generally equates to greater maturity (Salvador et al., 2007). There is a colour range within which persimmon fruit can be harvested to meet market quality standards, based on fruit maturity (Bignell et al., 2017). Early harvested fruit may be less mature but still meet the lower end of this spectrum. The later harvested fruit is likely more mature, with greater colour due to carotenoids accumulating to high levels during the later stages of maturity (Niikawa et al., 2007). The level of light exposure (through on-orchard canopy management) can also influence persimmon skin colouration. Exposed New Zealand 'Fuyu' persimmons had higher colouration than unexposed fruit in the mid-canopy (Woolf & Ben-Arie, 2011).

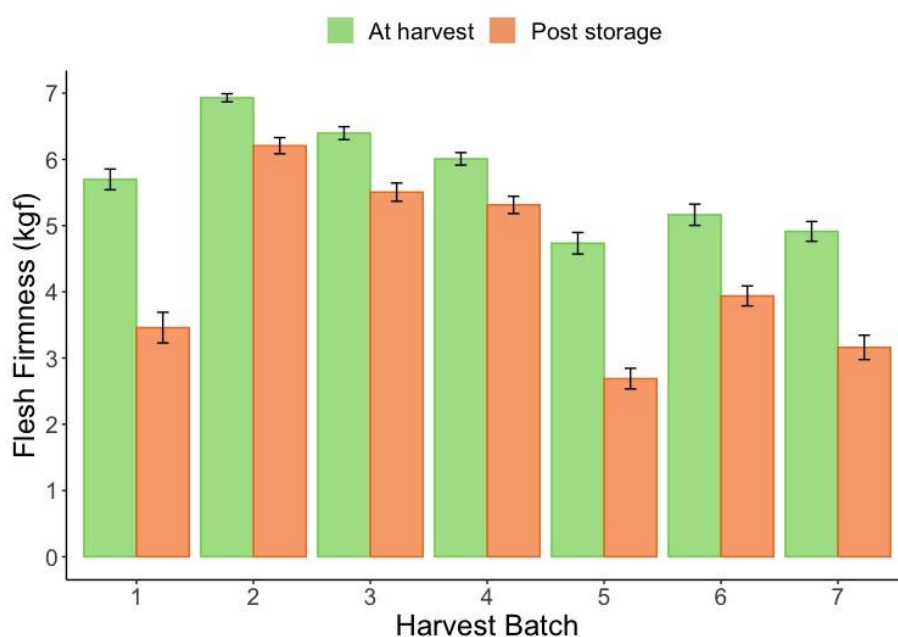
All batches showed increases in peel colouration post storage (**Figure 4.4**). Although, there were differences in the amount of colour change that occurred during storage. The largest increase in the average colour index value was 4.3 (seen in batch 2) compared to <0.1 (in batch 3). Postharvest colour development can be affected by the maturity of fruit at harvest; poor and uneven postharvest colour development has been shown to occur in persimmons picked too early (Testoni, 2002). For peel colouration to continue to develop postharvest, new pigment formation needs to occur which depends on the maturation stage at harvest. Batch 3 fruit may have been harvested at a slightly less mature stage than other batches, potentially leading to less colour development. Salvador et al. (2005) reported that colour index values increased by 2.6 in early harvested fruit, and by 3.5 in late harvested 'Rojo Brillante' fruit after 40 days of storage at 1 °C. The greater increase in colour index values in later season fruit

compared to earlier fruit, indicated harvest date was an influential factor in postharvest colour development.

The development of chilling injury can also affect the postharvest colour evolution in persimmon fruit. Salvador et al. (2005) reported a decrease in colour index values corresponding to external darkness in the peel of the fruit. This was only noticed in early harvested 'Rojo Brillante' fruit stored at 1 °C (not later season fruit), and attributed to cold damage. Gorini and Testoni (1988) found that during storage, a brownish colouration may develop as a consequence of cold damage related to early harvesting. A reduction in colour due to CI has also been observed in 'Fuyu' fruit stored at 0 °C, followed by 3 days at 20 °C (Woolf et al., 1997). This study observed a low incidence of CI so it is unlikely that this affected results.

#### **4.1.2.3      *Flesh firmness***

Analogous to the colour evolution observed, there was a change in fruit flesh firmness throughout cold storage. During the 63-day storage window, the average flesh firmness dropped by 1.4 kg<sub>f</sub>; the initial mean firmness was 5.7 kg<sub>f</sub> and after storage 4.3 kg<sub>f</sub>. A similar study noted average at harvest flesh firmness values of 4.4 to 7.3 kg<sub>f</sub> of NZ-grown 'Fuyu' persimmons (MacRae, 1987). A postharvest decrease in firmness, or softening, happens as a result of changes in enzyme activities that are associated with cell wall degradation (Clark & MacFall, 2003).



**Figure 4.5** The flesh firmness of fruit at harvest and after storage for each harvest batch of fruit.

The initial flesh firmness values varied between fruit from different harvest batches (**Figure 4.5**). Similar to colouration, the variation in initial SSC among batches is larger than the difference in SSC before and after storage, in many cases. The softest fruit were from batch 5 which had an average firmness of 4.7 kg<sub>f</sub>, compared to batch 2 which were the firmest fruit which had an average firmness of 6.9 kg<sub>f</sub>. According to (Ramin & Tabatabaei, 2003), the first harvest of persimmon fruit (early in the season) tend to have higher flesh firmness levels compared to subsequent harvests in which firmness declines considerably. This study reflects these results; the general trend is a decrease in firmness as the harvest season continues. The at harvest firmness is determined mainly by the maturity of fruit at harvest (which is characterised by colour), and the cultivar (Salvador et al., 2007). In ‘Fuyu’ persimmons, softening occurs at a high rate during the later stages of maturity until full orange-red colouration is reached (Tessmer et al., 2016).

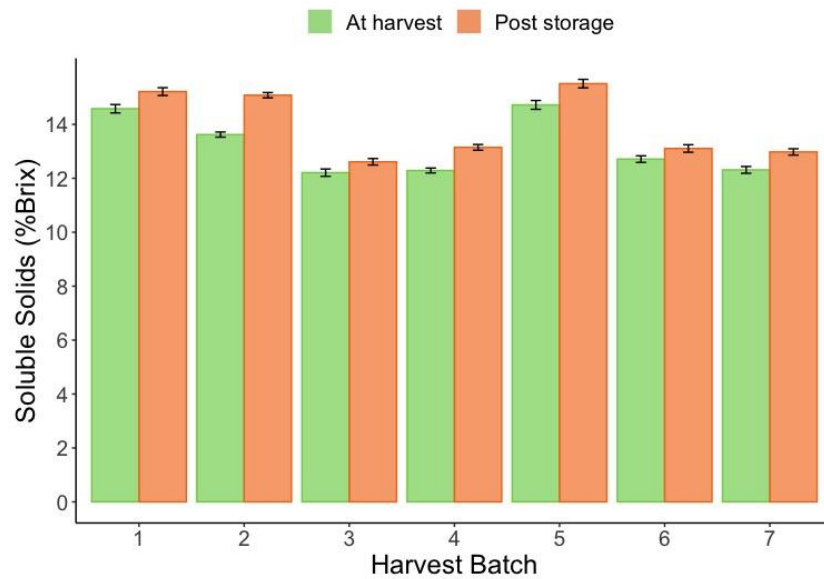
There was a reduction in flesh firmness before and after storage across all harvest batches (**Figure 4.5**). The decrease in firmness was greatest in harvests 1 and 5; both had an average loss of more than 2 kg<sub>f</sub>. Harvest 5 fruit were, on average, softer prior to cold-storage, but this

was not the case for harvest 1 which saw a similar degree of firmness loss. Harvests 2, 3, and 4 were initially firmer on average and showed less of a reduction in firmness compared to many of the other harvests. Similar research (Salvador et al., 2006) found early season 'Rojo Brillante' fruit (initial firmness 50.7 N) retained high levels of firmness of > 40 N up to 40 days of 1 °C storage, which then decreased to 30 N after 50 days. Late season fruit averaged 25.7 N at harvest which reduced gradually to 10 N (which is below acceptable firmness levels).

As displayed in **Figure 4.5**, batches that were on average less firm to begin softened more than fruit that are initially firmer. This is in agreement with Bignell et al. (2017) who noted that the degree of firmness at harvest has a strong influence on how well firmness is preserved postharvest. Potentially, fruit from batches harvested earlier in the season were less mature than the later season fruit.

#### **4.1.2.4 Soluble solids content**

In accordance with the colour and firmness changes, the SSC of persimmon fruit flesh was augmented over the duration of cold storage. At harvest, the average SSC of fruit was 13.2% which increased to 13.9% Brix after storage. A rise in SSC was expected from the postharvest increase in reducing sugars (fructose and glucose) from heightened invertase activity, and a coincident reduction in sucrose levels that continues with slowed fruit metabolism during cold storage (Del Bubba et al., 2009). In non-astringent persimmon, including 'Fuyu', SSC normally increases more during the later maturity stages, characterised by a colour change from orange to a more intense orange-red (Tessmer et al., 2016). Salvador et al. (2005) found only small changes during cold storage of 'Rojo Brillante' fruit over 50 days at 1 °C. Although, it has been noted that astringent cultivars (like 'Rojo Brillante') and non-astringent cultivars (like 'Fuyu') differ in SSC augmentation during maturity (Tessmer et al., 2016).



**Figure 4.6** The SSC levels of fruit at harvest and post storage split by harvest batch.

Similar to colour and firmness changes, all harvest batches exhibited different initial SSC values (**Figure 4.6**). NZ-grown ‘Fuyu’ SSC levels have been reported to vary between 12.0% and 15.1%, depending on harvest timing, yearly variations, and growing area (MacRae, 1987). Mason et al. (1989) observed NZ grown ‘Fuyu’ persimmons harvested from February to June had increasing SSC from 8% to 14%; the later season fruit displayed higher SSC compared to the earlier fruit. The study also found an average 1.5% difference between fruit grown in Kumeu and Gisborne, suggesting an environmental factor in SSC levels in persimmon. According to (Takano et al., 1991), greater sun exposure produces fruit with higher SSC, colour and size.

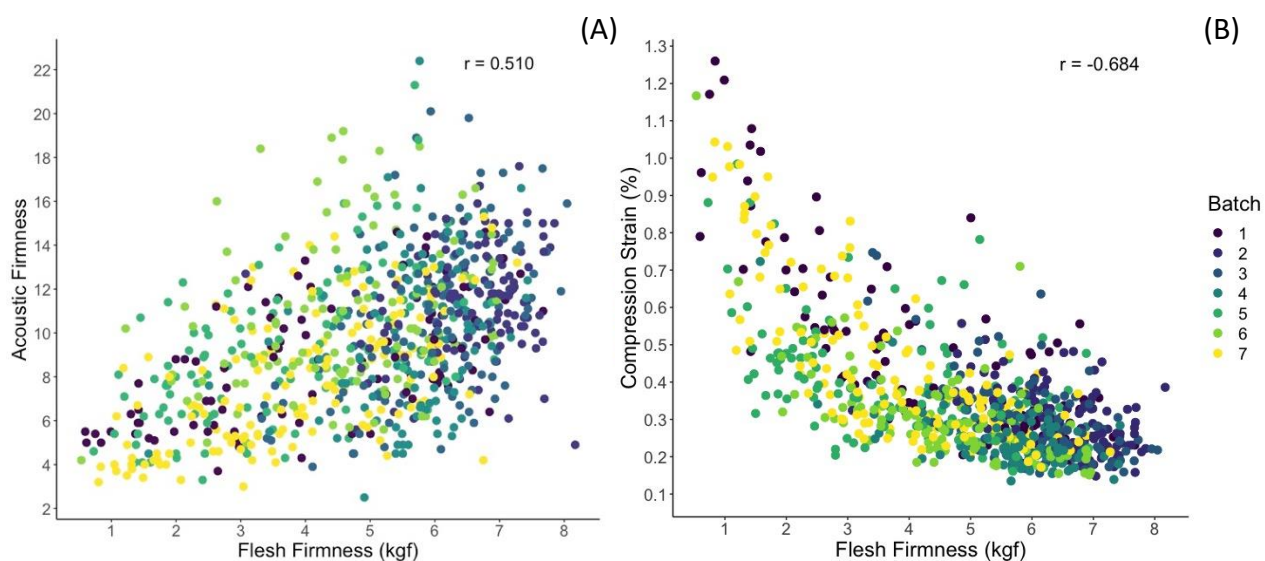
The amount of postharvest increase in SSC also differed between batches (**Figure 4.6**). Like colour and firmness variation, differences in harvest maturities between batches could have contributed to SSC differences. For example, batch 5 has the highest initial colour index, lowest flesh firmness and highest SSC which is usually associated with more mature fruit. However, this does not appear to be a general trend across at harvest batches. Salvador et al. (2005) detected little difference in SSC between early and late harvested ‘Rojo Brillante’ fruit (17.9% and 17.8%, respectively). It may be the case that SSC is not as closely related to maturity compared to colour and firmness. Findings by Clark and MacFall (2003) support this

idea; they reported that persimmons exhibited a notable deeper colouration and softening while only non-significant fluctuations in SSC in NZ grown 'Fuyu' fruit harvested between 25 and 30 weeks from fruitset.

#### **4.1.2.5 Non-destructive firmness**

During cold storage, the average amount of non-destructive compression per fruit increased from 0.25% to 0.42% during storage. The average acoustic firmness index of fruit lowered from 11.3 at harvest to 8.7 after storage. The greater percentage of fruit deformation and lower acoustic firmness post storage indicates softening. There was variation in the non-destructive firmness measurements between harvest batches before and after storage. These differences were seen in the other quality variables and are likely a result of harvest maturities, as discussed in previous sections.

As can be seen in **Figure 4.7 (A)**, there is a moderate, positive correlation between acoustic and flesh firmness ( $r = 0.510$ ). The correlation coefficient between compression and flesh firmness was  $-0.684$ , signifying a moderate, negative relationship (**Figure 4.7 (B)**). Fruit with lower flesh firmness tended to have correspondingly lower acoustic firmness values and higher compression values. The association between compression and flesh firmness appears to be non-linear and stronger for softer fruit compared to firmer fruit. This is similar to findings reported in 'Gold 3' kiwifruit where the relationship between compression and flesh firmness was found to differ for firmer and softer fruit; softer fruit showed a greater change in compression and acoustic firmness readings (Li et al., 2016). The study suggested that there are different aspects of texture that change during softening which affect destructive and non-destructive measurements differently. The variation seen between the different firmness values (for the same fruit) is likely a result of measuring different aspects of texture. Schotsmans et al. (2008) proposed that change in outer pericarp tissue texture can influence flesh firmness measurements to a greater extent, whereas the degree of water loss will affect the non-destructive firmness measurements irrespective of tissue texture.



**Figure 4.7** The acoustic firmness of persimmons against the flesh firmness (A) and the non-destructive compression as a % of whole fruit deformation compared to the flesh firmness (B). Data includes both at harvest and after storage measurements, coloured by harvest batch.

In agreement with work by Li et al. (2016), this experiment found that softer fruit showed a greater difference in the level of compression achieved; softer fruit (1 – 3 kg<sub>f</sub> flesh firmness) ranged from approx. 0.2% to 1.3%, whereas firmer fruit (4 – 8 kg<sub>f</sub> flesh firmness) ranged from approx. 0.1% to 0.7% deformation. The compression firmness readings of softer fruit may have been associated with water loss, as suggested by (Schotsmans et al., 2008). Measurements taken after storage could have included the effects of water loss whereas the at harvest measurements would not, as weight loss (and hence water loss) occurred during storage. However, weight loss levels were low in fruit after MAP storage, so water loss may not have noticeably influenced non-destructive firmness values.

#### 4.1.3 Weight loss & postharvest disorders

The average weight loss was 1.6 g per fruit (or 0.67% of fresh weight) after 9 weeks of MAP storage. This is comparable to similar studies such as Cia et al. (2006) which reported <1% weight loss in 'Fuyu' persimmons after 90 days of storage (1 °C, 90% RH) in 50 µm polyethylene MAP. Weight loss in persimmons stored (at 1 °C, 90% RH) without packing film

can lose over 6% of their fresh weight in 5 weeks, mainly due to water loss (Cia et al., 2006). The high RH environment inside the MAPs decreases the rate of water loss during storage, and hence the weight loss (Forney & Lipton, 1990).

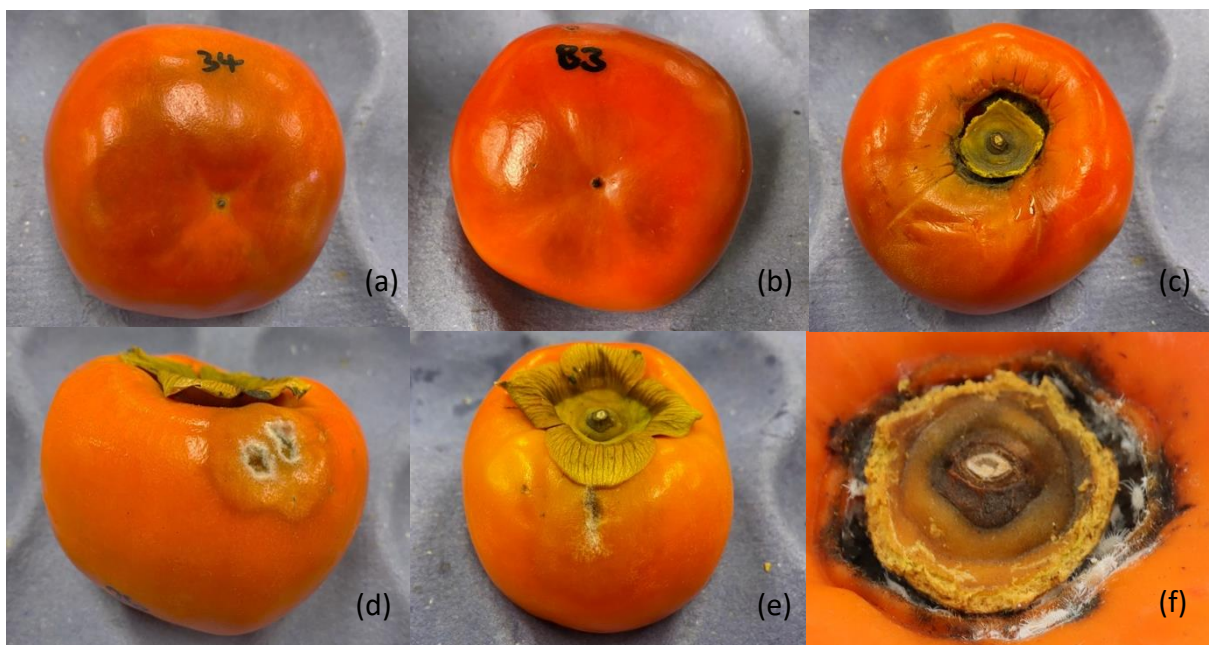
There was a difference in weight loss between fruit with or without a calyx separation. The average weight loss in cavity fruit was 0.64%; the average of unaffected fruit was 0.71%. This is likely more related to the difference in weight between the groups, rather than the presence of the cavity itself. The surface area to volume ratio is correlated to water loss; smaller fruit have a higher ratio and so tend to lose more water compared to larger fruit (Lownds et al., 1993).

The frequency of CI (**Figure 4.8 (a, b, c)**) observed after storage was low. In total, out of the 445 fruit that were stored and subsequently evaluated for CI, a total of 8 fruit (1.80%) showed symptoms of CI. Symptoms observed included parts of fruit with deep orange-red colouration (visible both externally on fruit skin and internally in fruit flesh), sections of very soft flesh, rubbery skin texture and discolouration in a star-shape on the bottom of fruit. The 'Fuyu' cultivar is particularly susceptible to CI; Collins and Tisdell (1995) observed 14 fruit of 30 fruit total, or 46.6%, 'Fuyu' with CI symptoms after storage in open trays at 0 °C for 56 days. The low level of CI observed in the current work could be attributed to the after-storage assessments being carried out after a shelf life (at 20 °C) of <1 day (approximately 16 hours). To best evaluate the level of CI present, assessment of symptom expression should have been done at 3 days shelf life (Woolf et al., 1997).

Of fruit with CI, seven fruit had calyx separation and one was without separation. The majority of the CI-affected fruit were categorised as grade 4 separation severity. Woolf and Ben-Arie (2011) indicated that there is a link between fruit with calyx cavity and a greater incidence of chilling injury. Salvador et al. (2006) remarked that early harvested fruit showed higher sensitivity to CI compared with late harvested fruit, and MacRae (1987) reported less damage as a result of CI in late season fruit. Half of the fruit with CI were from batch 5 which were harvested later in the season. MacRae (1987) also found a relationship between O<sub>2</sub> levels during cool storage and the development of CI; high O<sub>2</sub> levels of 14 – 20% allowed greater CI.

Additionally, the study suggested that the occurrence of CI in NZ-grown 'Fuyu' may be related to the effects of climatic conditions on fruit development.

Akin to weight loss and CI, the incidence of rot (shown in **Figure 4.8 (d, e)**) was low. The total amount of fruit with some rot or mould growth after 9 weeks of storage was 4 fruit (0.89%). The low level of rot is mainly a result of cold-storage which prevents pathogen growth, and MAP which minimises surface abrasion and pathogen spread (Kader et al., 1989). In fruit where rot was present, it was commonly observed in or around the cavity. The calyx detached from the fruit during calyx evaluation in these cases. Mealybugs were also observed in the cavities of some fruit (**Figure 4.8 (f)**).



**Figure 4.8** Images of persimmon after 9 weeks MAP storage at 0 °C. Fruit with symptoms of chilling injury (a), (b) and (c); fruit with areas of fungal growth and rot (d) and (e); a close-up of mealy bugs in a calyx cavity (f).

## 4.2 Calyx cavity detection

### 4.2.1 Cavity incidence

Approximately half of all persimmon fruit sampled had some degree of calyx separation. The presence of calyx cavity refers to instances where fruit exhibited any level of calyx separation

(grades 2 – 4); the absence of calyx cavity includes only grade 1. Grade 1 fruit did not exhibit any visible calyx separation (after calyx lobe removal), grade 2 had minor separation, grade 3 showed moderate separation and grade 4 had major separation. Out of 840 fruit, a total of 404 fruit (48%) had a calyx cavity (grades 2 – 4) compared to 436 (52%) of fruit without (grade 1). Grade 2 occurred at a rate of 28%, grade 3 at 13% and grade 4 at 7%.

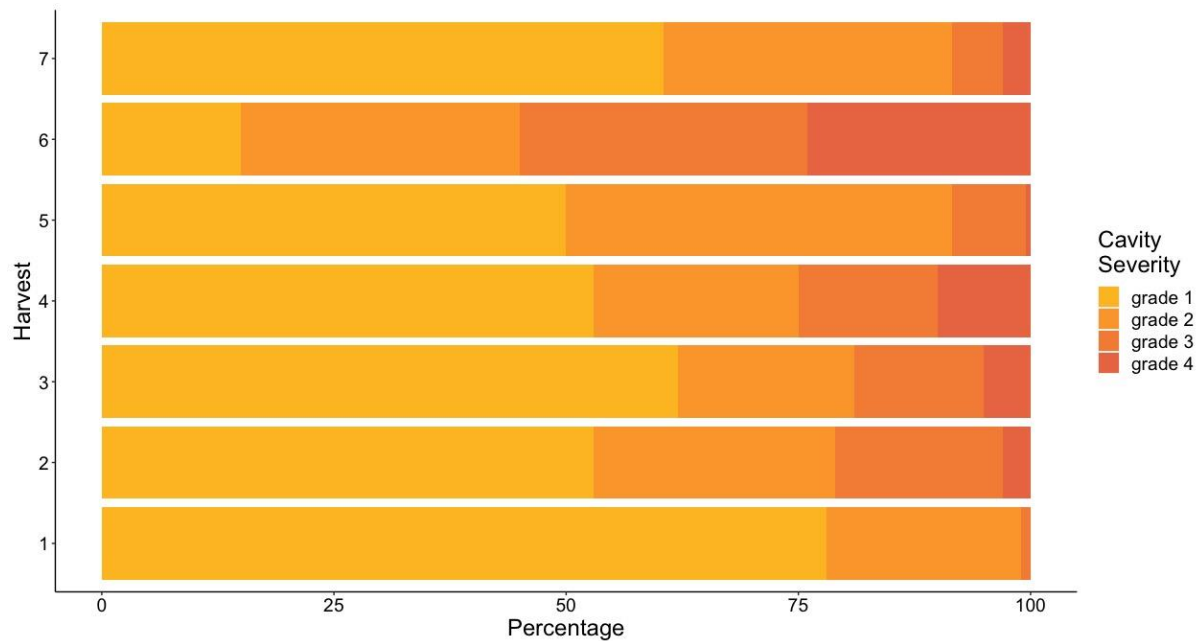
There are large differences in reported rates of calyx cavity; an incidence of 75% was noted in NZ ‘Fuyu’ (Glucina, 1987) compared to < 25% found in Japanese ‘Fuyu’ (Akagi et al., 2020). Yamada et al. (1988) observed an incidence between 5.4% and 56.2% in fruit from a variety of different cultivar crosses. Differences in the occurrence of calyx cavity have been attributed to size, cultivar, poor early calyx growth, stress during fruit development, fruiting tree age, genetic inheritance, and environmental conditions including excessive soil fertility and accentuated rainfall (Bellini & Giordani, 2002; Bignell et al., 2017; Woolf & Ben-Arie, 2011).

**Table 4.2 The presence and severity of calyx cavity given as a percentage of the total sample for each batch based on visual assessment scores.**

Batch	grade 1 (%)	grade 2 (%)	grade 3 (%)	grade 4 (%)	total prevalence (%)
1	78	21	1	0	22
2	53	26	18	3	47
3	62	19	14	5	38
4	53	22	14	11	47
5	50	42	7	1	50
6	15	30	31	24	85
7	61	31	5	3	39
Mean	53	27	13	7	47

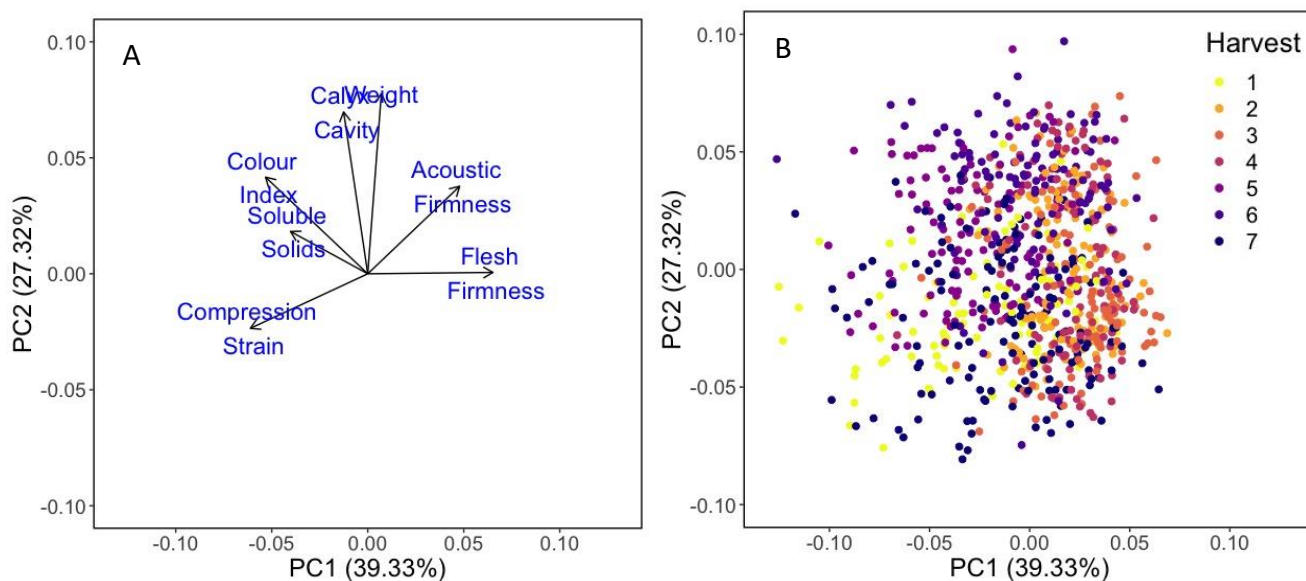
Variation in the severity of calyx cavity was observed between fruit from different harvest batches (**Figure 4.9**). In most batches, affected fruit was most commonly grade 2 (minor separation), followed by grade 3 (moderate separation), then grade 4 (major separation) (**Table 4.2**). A considerable deviation from this trend can be seen in Batch 6 where 85% of fruit had some amount of separation around the calyx. In this batch, more fruit were classified as grade 3 than grade 2, and there was a much higher number of severely affected fruit (grade

4) compared to all the other batches. This is likely related to the higher average weight of batch 6 fruit as discussed in subsequent sections. In comparison, Akagi et al. (2020) reported only 65 fruit (2.0%) with level 4 calyx cavity out of the 3,173 'Fuyu' persimmons visually evaluated. Yamada et al. (1988) found level 4 severity occurred in 0.0% - 6.7% of progeny fruit, depending on the severity of calyx separation in the parent varieties.



**Figure 4.9** Stacked bars representing the percentage of each calyx cavity grade across the different harvest batches after visual evaluation and scoring of the severity of calyx separation.

## 4.2.2 Principal component analysis



**Figure 4.10 Loading (A) and score (B) plots of principal components 1 and 2, representing the relationship between seven postharvest response variables of ‘Fuyu’ persimmons. For the score plot (B), each point represents one individual fruit, coloured by harvest batch.**

A PCA (principal component analysis) model was applied to the data to determine the most important components that explain the relationships between the quality attributes and the presence of calyx cavity. The first three principal components explained 78.23% of the overall variance (**Table 4.3**). The first principal component (PC1) accounted for 39.33% of the total variance. The variables that correlate the most with PC1 are flesh firmness (0.533), non-destructive compression (-0.500), colour index (-0.436), and acoustic firmness (0.391). PC1 is positively correlated with flesh firmness and acoustic firmness, and negatively correlated with fruit compression and peel colour index. As both colour and firmness are good predictors of maturity in persimmon fruit (Tessmer et al., 2016), it is likely that this component is primarily a measure of the maturity of the fruit.

The second principal component (PC2) explains 27.32% of overall variance. PC2 is more closely associated with fruit weight (0.635) and the presence of calyx separation (0.571), and both correlations are positive. Hence, PC2 is mainly a descriptor of fruit size. The third principal component (PC3) explains 11.58% of the overall variance. PC3 is positively associated with soluble solids (0.861), indicating that this component is largely a measure of fruit flesh SSC.

**Table 4.3 Eigenvectors for each parameter for the first three principal components and the proportion of variance accounted for by each component.**

Parameter	Eigenvector		
	PC1	PC2	PC3
Weight (g)	0.058	0.635	-0.048
Cavity presence	-0.103	0.571	-0.320
Compression strain (%)	-0.500	-0.192	0.015
Acoustic firmness index	0.391	0.309	0.330
Flesh firmness (kgf)	0.533	0.005	0.208
Soluble solids (%Brix)	-0.329	0.149	0.861
Colour index	-0.436	0.341	-0.046
	Proportion		
Proportion of variance	0.3933	0.2732	0.1158
Cumulative proportion	0.3933	0.6665	0.7823

As can be seen in **Figure 4.10**, various relationships among quality attributes were identified. Based on the position of the loadings, the main variables that correlated with calyx separation are the weight and the colour index. The acute angle between the loadings of calyx cavity and weight suggests a positive relationship between the two variables. This association suggests that heavier fruit more commonly had some degree of calyx separation. The angle between the loadings of calyx cavity and flesh firmness is near 90° which indicates null correlation. The acoustic firmness is located close to 180° part from both the non-destructive compression and the flesh firmness, showing a negative association between the attributes. This suggests persimmons with higher acoustic firmness or flesh firmness tended to have a higher percentage of compression (in agreement with the results displayed in previous sections). The close proximity of the soluble solids and colour index loadings also implies a relationship between the two variables.

### **4.2.3 Calyx cavity & quality variable relationships**

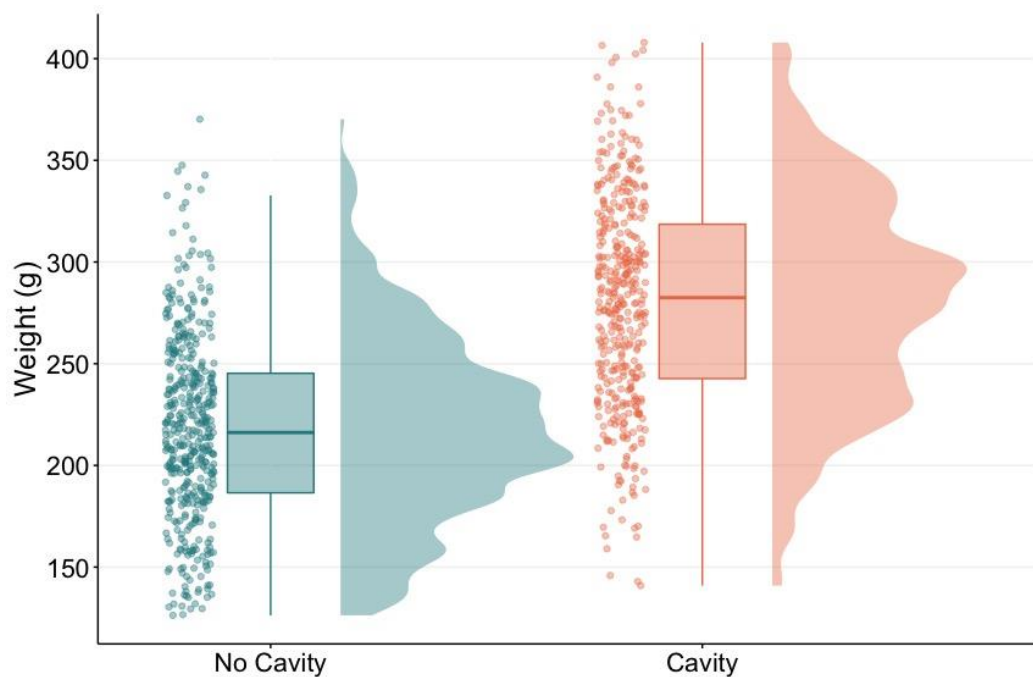
#### **4.2.3.1 Fresh weight**

As illustrated in **Figure 4.11**, the rate of calyx cavity was greater in heavier fruit. The average weight of fruit without a calyx cavity was significantly lower (218.0 g) than fruit with a cavity (280.6 g) ( $p$ -value  $< 2.2 \times 10^{-16}$ ). In the literature, fruit size has been linked to calyx cavity – larger fruit are more prone to the disorder than smaller fruit (Yamada et al., 1987). The development of calyx cavity occurs during the last phase of the double sigmoid growth curve (cell expansion) late in the season (Woolf & Ben-Arie, 2011). The persimmon flesh separates from around the calyx in one or more areas, forming one or more gaps. It is believed that this occurs when the fruit flesh expands more rapidly than the calyx often following an earlier period of stress during fruit development, which happens more often in younger, more vigorous trees (Bignell et al., 2017).

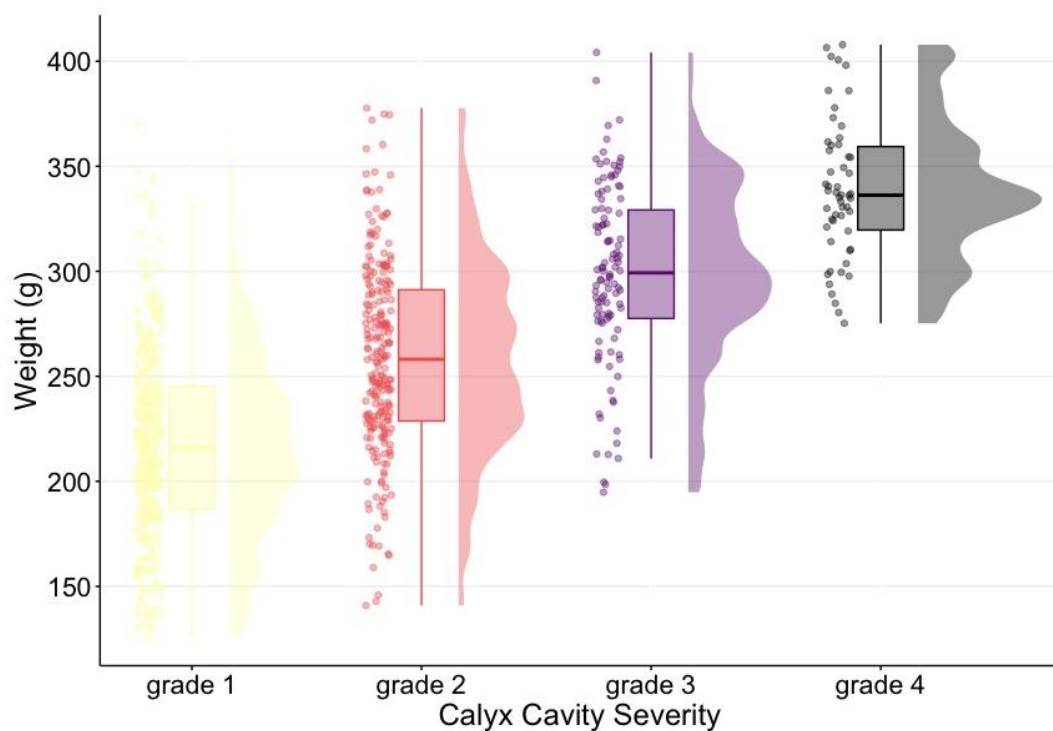
The severity of calyx separation also increased with fruit weight. Persimmons with higher weights often had a higher incidence of severe separation; medium-weight fruit tended to have moderate-sized cavities, and smaller fruit were mainly without a cavity (**Figure 4.12**). The average fresh weight for grade 2 fruit was 258.5 g, grade 3 was 298.8 g, and grade 4 was 338.8 g, with the difference between each grade approx. 40 g. This suggests that the severity of calyx separation tends to increase at a steady rate with fruit weight. Potentially, larger fruit at harvest had more severe calyx separation due to greater expansion (as persimmon fruit size increases up until harvest), allowing the degree of separation to worsen (Bellini & Giordani, 2002). Although, it has been noted that the magnitude of the disorder is highly environmentally influenced (Yamada et al., 1987).

The distribution of weights was large for all levels of calyx cavity. The overall spread of weights was 281.6 g, ranging from 126.3 g to 407.9 g at harvest. The IQR (interquartile range) was 75.6 g for fruit with calyx cavity and 58.8 g for fruit without calyx cavity. Within each level of calyx separation, there was also a large spread of fruit weights. The large spread suggests that the mean weight of each grade is not as representative of fruit within the grade as there were large differences in size between fruit. There was a large range of fruit sizes, likely because

the fruit obtained was not graded. Fruit weight would have been more uniform had fruit been through the grading process prior to assessment (Bignell et al., 2017).

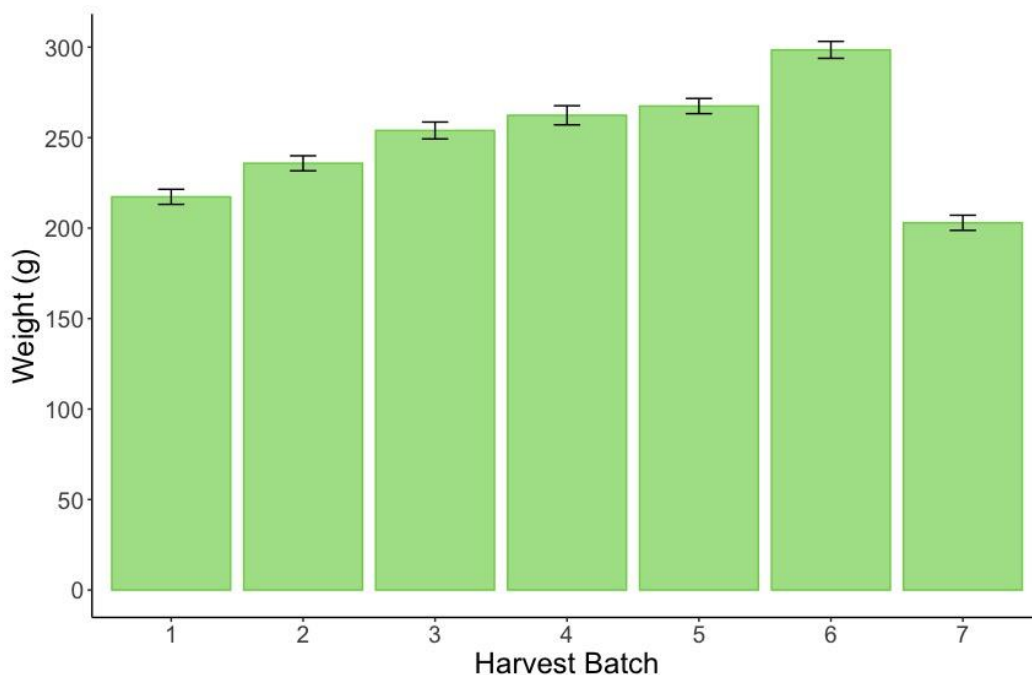


**Figure 4.11** Raincloud plots displaying the distribution of persimmon weights at harvest based on the presence or absence of calyx cavity.



**Figure 4.12** Raincloud plot comparing the distribution of weights at harvest based on the visual scale severity of the cavity, where grade 1 represents fruit without a cavity, and grades 2 – 4 fruit have cavities of increasing size.

The weight of fruit varied by harvest batch; the average fruit weight increased with batch number, with the exception of harvest 7 (**Figure 4.13**). The mean weight of batch 1 fruit was 217.3 g which steadily increased to 298.5 g by batch 6. The pattern of increased weight with batch number matches the general trend of greater rates of calyx separation with batch number. Persimmon fresh weight increases steadily with fruit maturity, with slight increases observed in 'Fuyu' up until late maturity or orange-red colouration (Tessmer et al., 2016). It is possible that batch 1 fruit were less mature than batch 6, with fruit maturity, and therefore size, increasing over the harvest window. As calyx separation is also linked to fruit size, the corresponding increase in calyx cavity incidence with harvest batch is consistent. The potential link between the incidence of calyx cavity and maturity implies that this disorder is also linked to colour and firmness. This could complicate the ability to attribute subsequent differences in quality characteristics to calyx cavity, rather than such difference being a result of variation in harvest maturity.

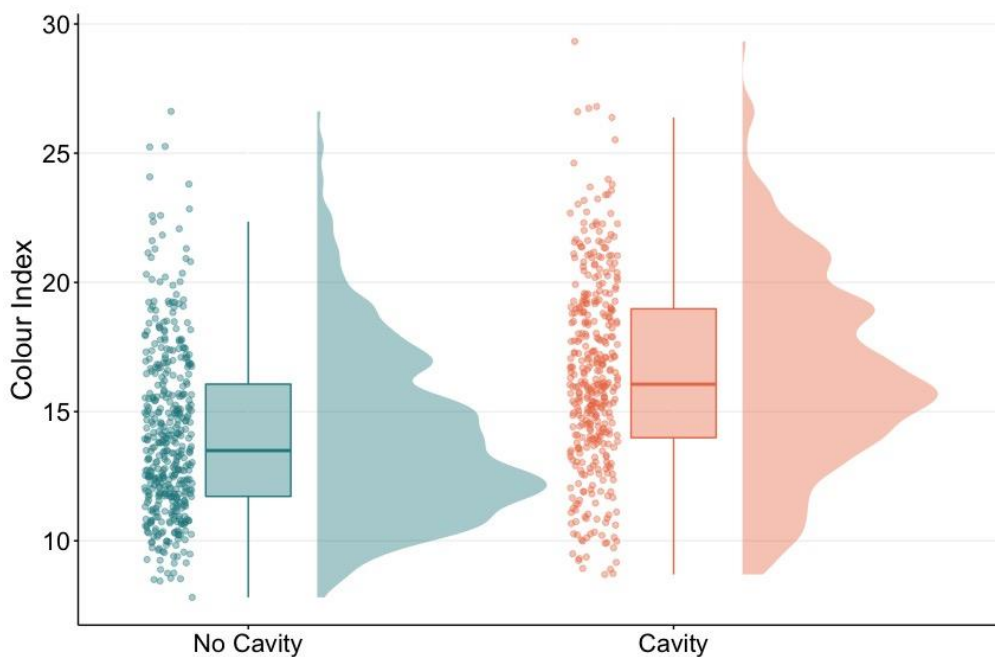


**Figure 4.13** The average weight of fruit for each of the different harvest batches prior to storage. n = 840

Batch 7 fruit deviated from the observed course with a lower mean weight (202.9 g) than previous harvests (**Figure 4.13**). However, batch 7 also had an equivalent lower cavity

incidence than the prior harvests. This batch, being the final harvest, likely included the remaining marketable fruit. The larger fruit were likely already harvested in previous batches, hence batch 7 would have had a lower percentage of export quality fruit (which tend to be the larger fruit).

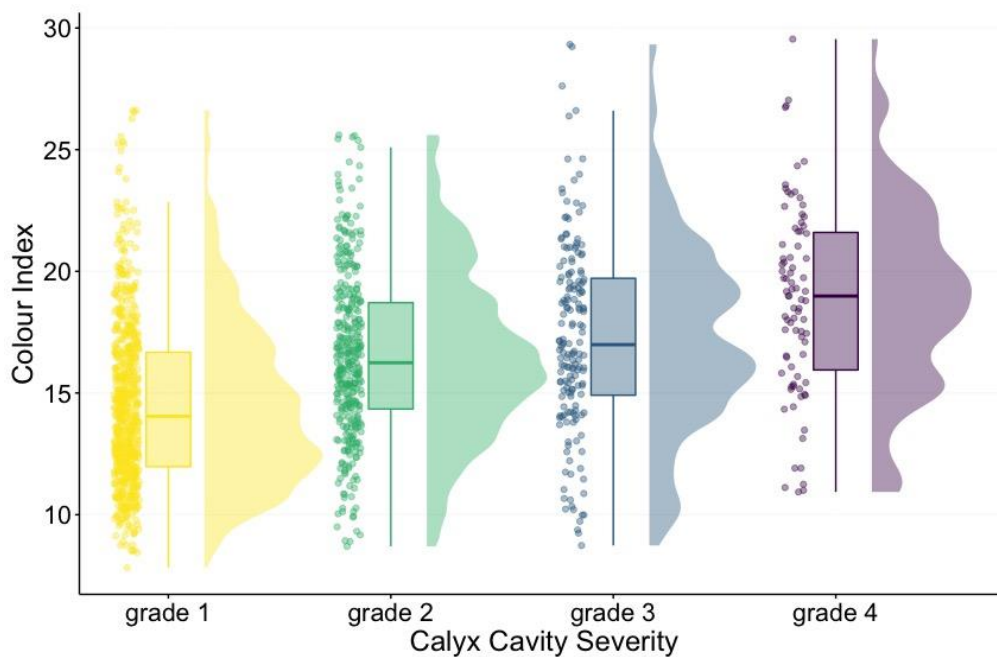
#### 4.2.3.2 Peel colouration



**Figure 4.14** The distribution of at harvest persimmon skin colour of fruit with a calyx cavity and without. Colour measurements were taken at the fruit shoulders and equator and averaged. The colour index was then calculated from the equation  $(1000a)/(Lb)$ .

Fruit with calyx separation had a greater average skin colouration than unaffected fruit (**Figure 4.14**). The mean colour index value was 16.4 in fruit with a cavity and 14.1 in those without, at harvest. This difference was found to be statistically significant ( $p$ -value  $< 2.2 \times 10^{-16}$ ). Parallel differences in skin colour between grades was observed – fruit with more severe cavities had higher colourations on average (**Figure 4.15**). The mean colour index value for a grade 2 persimmon was 17.6, compared to grade 4 fruit which averaged 18.1. The higher colour index values in cavity fruit may have been contributed to by the (probable) correlation between calyx separation and maturity. As a result, the observed differences could be due to

the presence of calyx separation or fruit maturity at harvest. On the other hand, Akagi et al. (2020) detected specific patterns of uneven colouration in fruit with the disorder. They noticed a darker reddish colouration in the pericarp from the point of separation between the calyx and fruit tissue in the more severe cases of calyx separation. This suggests the observed colour differences are a feature of calyx separation rather than maturity alone. Yamada et al. (1988) hypothesised that these colour changes are a form of stress response in the fruit.

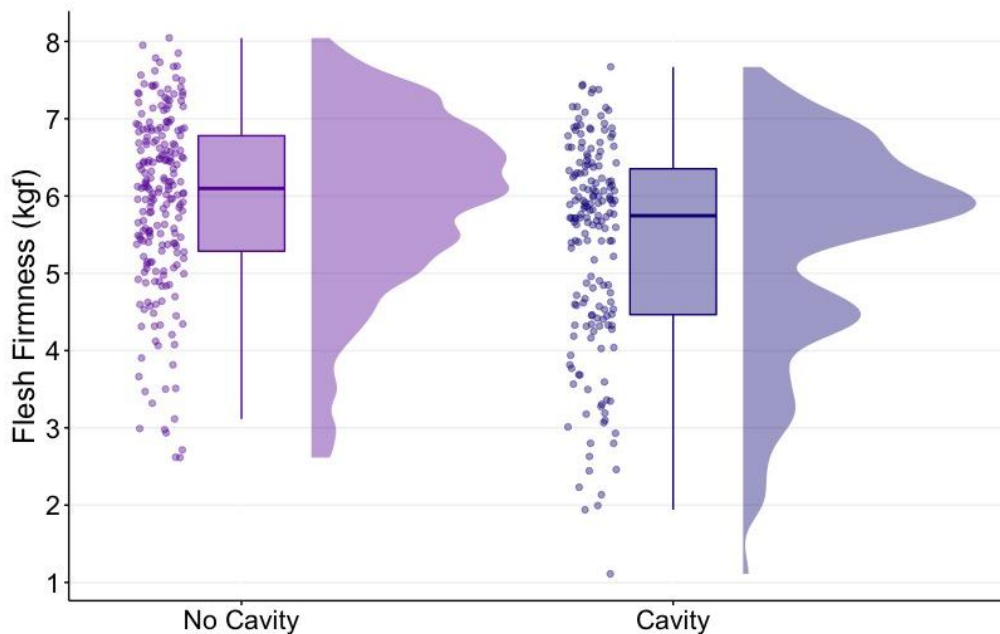


**Figure 4.15 Comparison of the at harvest colour indices between persimmon with different degrees of calyx separation.**

#### **4.2.3.3 Destructive & non-destructive firmness**

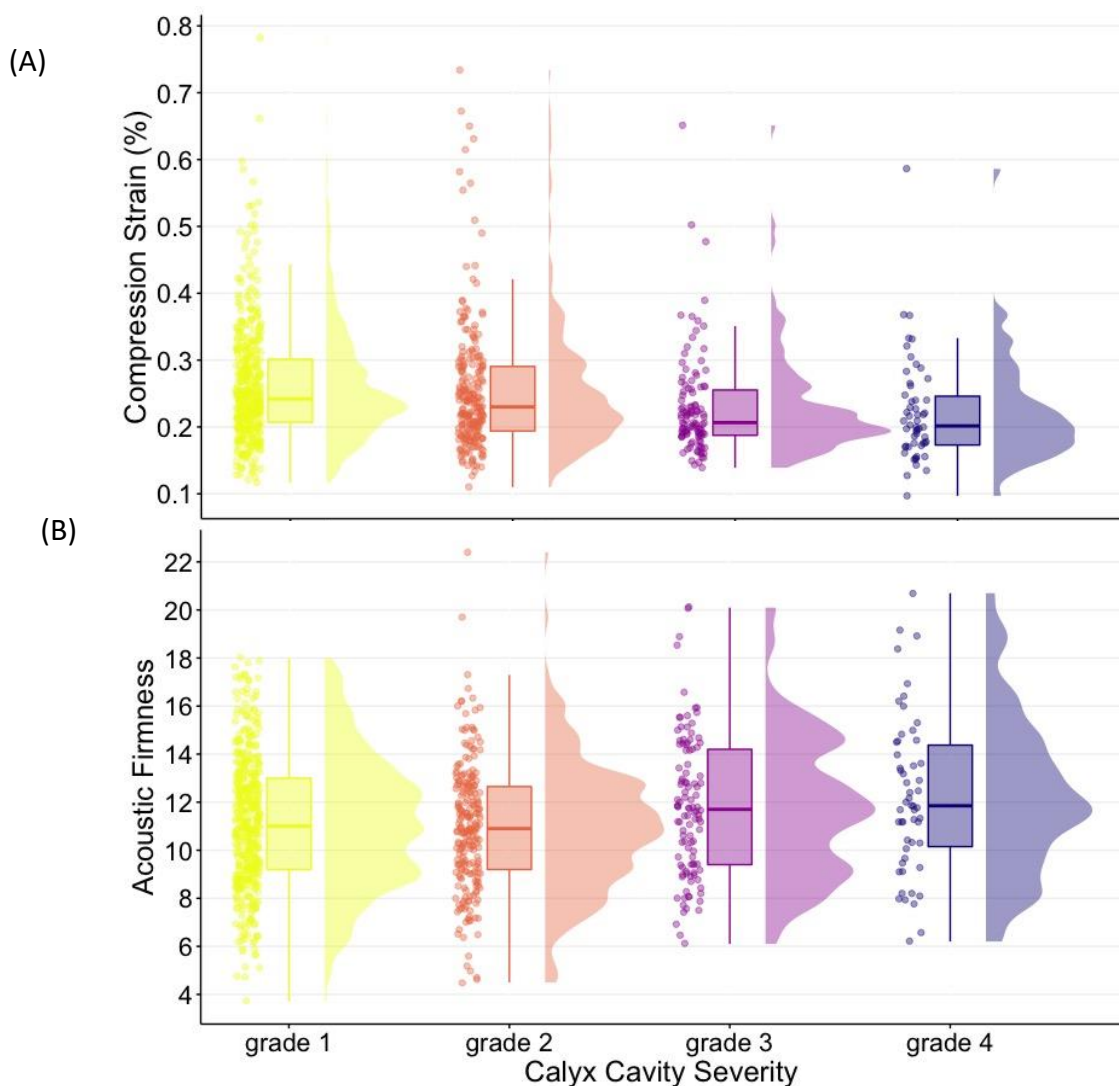
The average firmness of persimmons with calyx separation was lower than the firmness of fruit without separation based on the destructive firmness readings (**Figure 4.16**). The average flesh firmness of cavity persimmons was 5.4 kg<sub>f</sub> compared to 5.9 kg<sub>f</sub> in unaffected fruit ( $p$ -value =  $1.728 \times 10^{-5}$ ). According to Sarkhosh et al. (2020), the formation of a cavity on one side of the calyx can cause the fruit to ripen unevenly. This could have contributed to the lower flesh firmness exhibited by fruit with calyx cavity. Differences in harvest maturity

between fruit with and without calyx separation may also have influenced flesh firmness averages, as mentioned in previous sections.



**Figure 4.16** Flesh firmness (kgf) of 'Fuyu' fruit at harvest separated by the presence and absence of calyx cavity.

In contrast to flesh firmness, both the non-destructive firmness measurements found that fruit with calyx separation was firmer. Based on the flesh firmness measurements, non-destructive compression would be expected to have a higher percentage of deformation in affected fruit, but the opposite was found. The average compression of fruit without separation was 0.26% compared to 0.22% in severely affected fruit (**Figure 4.17 (A)**). For acoustic firmness, the averages were higher rather than lower for fruit with calyx cavity. The average acoustic firmness index increased with the degree of separation; grade 1 fruit averaged 11.4 and grade 4 fruit averaged 12.3 (**Figure 4.17 (B)**). This indicates a lack of cohesion between firmness measurements. The skew towards lower firmness in the compression and flesh firmness data, and the low sample sizes in the more severe grade categories may have been contributing factors. The very high variability of the acoustic firmness values suggests that this parameter is not a useful measure of firmness in persimmon.

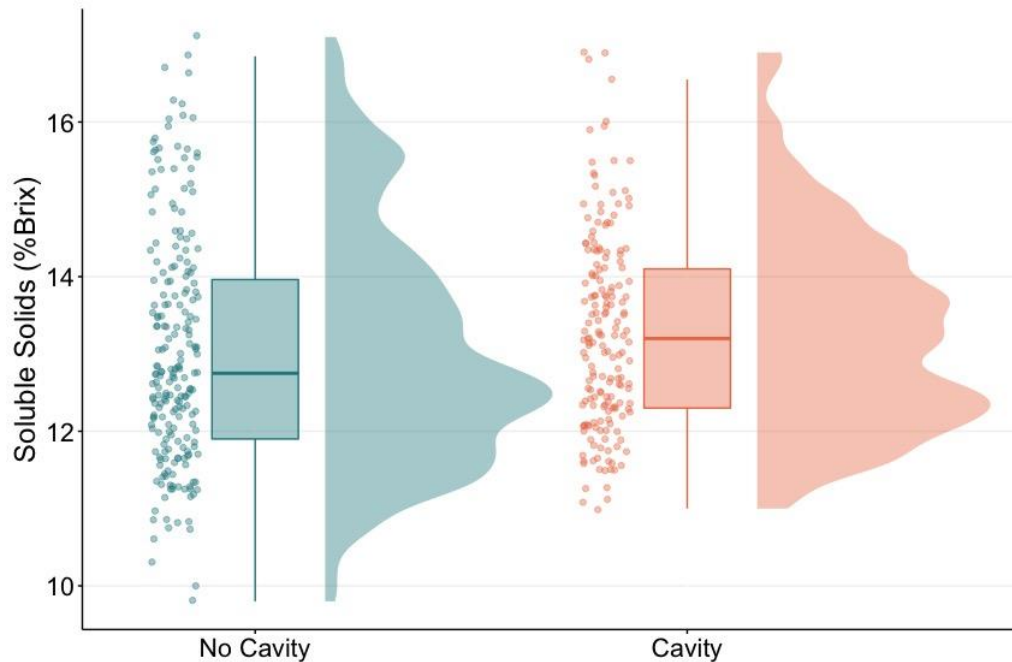


**Figure 4.17** Raincloud plots showing the at harvest results of non-destructive compression (A) and acoustic firmness (B) separated by calyx cavity severity.

#### 4.2.3.4 Soluble solids content

As visualised in **Figure 4.18**, persimmons with calyx separation had a greater average SSC compared to unaffected fruit at harvest, but this difference was not significant (p-value = 0.09657). The mean SSC calyx cavity fruit was 13.3% and 13.0% in those without separation. While colouration and firmness differences have been detected in calyx cavity fruit, there has not yet been a link between SSC and calyx separation established (Sarkhosh et al., 2020). The higher SSC of affected fruit may be related to at harvest maturity as suggested by the colour

and firmness differences that were found (Salvador et al., 2007). Maturity may have been higher in fruit with calyx cavity, in line with the colour and flesh firmness observations.



**Figure 4.18** A comparison of at harvest Brix values (as an average of shoulder and equator readings) separated by the presence or absence of calyx cavity.

#### **4.2.3.5**      *The effect of calyx separation on quality changes during storage*

The presence of calyx separation may have played a part in the changes in fruit quality changes during postharvest storage. Overall, quality characteristics were all significantly different before compared to after storage regardless of whether fruit were affected by calyx cavity. All fruit (both cavity and no cavity) were significantly less firm, more coloured, and higher in SSC after storage than at harvest (p-values <0.05, data not shown). However, some qualities changed more in fruit with calyx cavity than in fruit without calyx cavity (**Table 4.4**). This occurred with the SSC changes where the SSC of fruit was not significantly different at harvest but after storage fruit with calyx cavity had a higher average SSC than fruit without calyx cavity. In terms of colour, fruit with calyx cavity were significantly higher in colour both at harvest and after storage. Based on the flesh firmness, healthy fruit were firmer on average at harvest compared to fruit with calyx cavity. Although, after storage, there was no

significant difference between the firmness of fruit with or without calyx cavity. Overall, the results suggest that the presence of calyx cavity leads to accelerated ripening.

**Table 4.4 The averages of quality characteristics for cavity and no cavity fruit before and after storage.**

Quality parameter	Sampling time	No cavity	Cavity	difference	p-value
Colour Index	at harvest	14.1	16.4	2.3	$<2.2 \times 10^{-16}$
	after storage	15.5	18.2	2.7	$5.50 \times 10^{-15}$
Non-destructive compression (%)	at harvest	0.26	0.24	0.02	0.00627
	after storage	0.44	0.42	0.02	0.2995
Acoustic firmness index	at harvest	11.2	11.4	0.2	0.3267
	after storage	8.1	9.3	1.2	0.0003694
Flesh firmness (kgf)	at harvest	5.9	5.4	0.5	$1.73 \times 10^{-5}$
	after storage	4.3	4.2	0.1	0.3245
SSC (%)	at harvest	13	13.3	0.3	0.09657
	after storage	13.7	14.1	0.4	0.008704

#### 4.2.4 Prediction of calyx cavity

Linear discriminant analysis (LDA) was done using at harvest weight and colour index data as continuous predictors of calyx cavity severity. Of the quality variable evaluated, fresh weight and colour index were chosen as predictors to estimate the degree of calyx cavity due to the patterns of increase in these parameters observed between the levels of calyx separation. Both the weight and the external colour of fruit was found to be significantly higher in fruit with calyx cavity compared to fruit without calyx cavity (p-value  $< 2.2 \times 10^{-16}$  and 0.001172,

respectively). These parameters also increased with the increasing severity of each grade of calyx cavity. Fresh weight increased by approximately 40 g with each grade and the colour index value increased by 1 to 2 units with each grade.

The first model classified fruit as either grade of severity (1, 2, 3, or 4). As displayed in **Table 4.5**, the model segregated the absence of calyx cavity (grade 1) and the most severe form of calyx cavity (grade 4) more successfully than the intermediate grades. Classification of grade 2 and 3 separation was poor; only 32.8% of grade 2 fruit and 40.0% of grade 3 fruit was correctly categorised compared to precisions of 70.0% and 74.1% in grades 1 and 4, respectively. This is likely due to larger differences in weight and colour index in the least and most affected fruit; these differences were less pronounced between fruit in grades 2 and 3. Based on previous analysis of colour and weight data, fruit without calyx separation were on average smaller and lighter in colour whereas fruit with major separation were generally larger and more coloured. Akagi et al. (2020) carried out similar work that used colour differences to predict the degree of calyx separation and noted prediction problems stemming from imbalanced class weights (as group sizes were significantly smaller at higher severities).

**Table 4.5 Classification table of predictions based on linear discriminant analysis of at harvest weight and colour index data where calyx cavity data is categorised by severity.**

Cavity severity	1	2	3	4	Observed	Correct (N)	Correct (%)	Error (%)
1	310	96	31	6	443 (53%)	310	70.0	30.0
2	78	76	51	27	232 (27%)	76	32.8	67.2
3	11	17	44	38	110 (13%)	44	40.0	60.0
4	0	1	13	40	54 (7%)	40	74.1	29.6
Total					839	470	56.0	44.0

**Table 4.6 Classification table of predictions based on linear discriminant analysis of at harvest weight and colour index data where calyx cavity data is used as a binary (no = calyx cavity severity of 1; yes = calyx cavity severity of 2 - 4).**

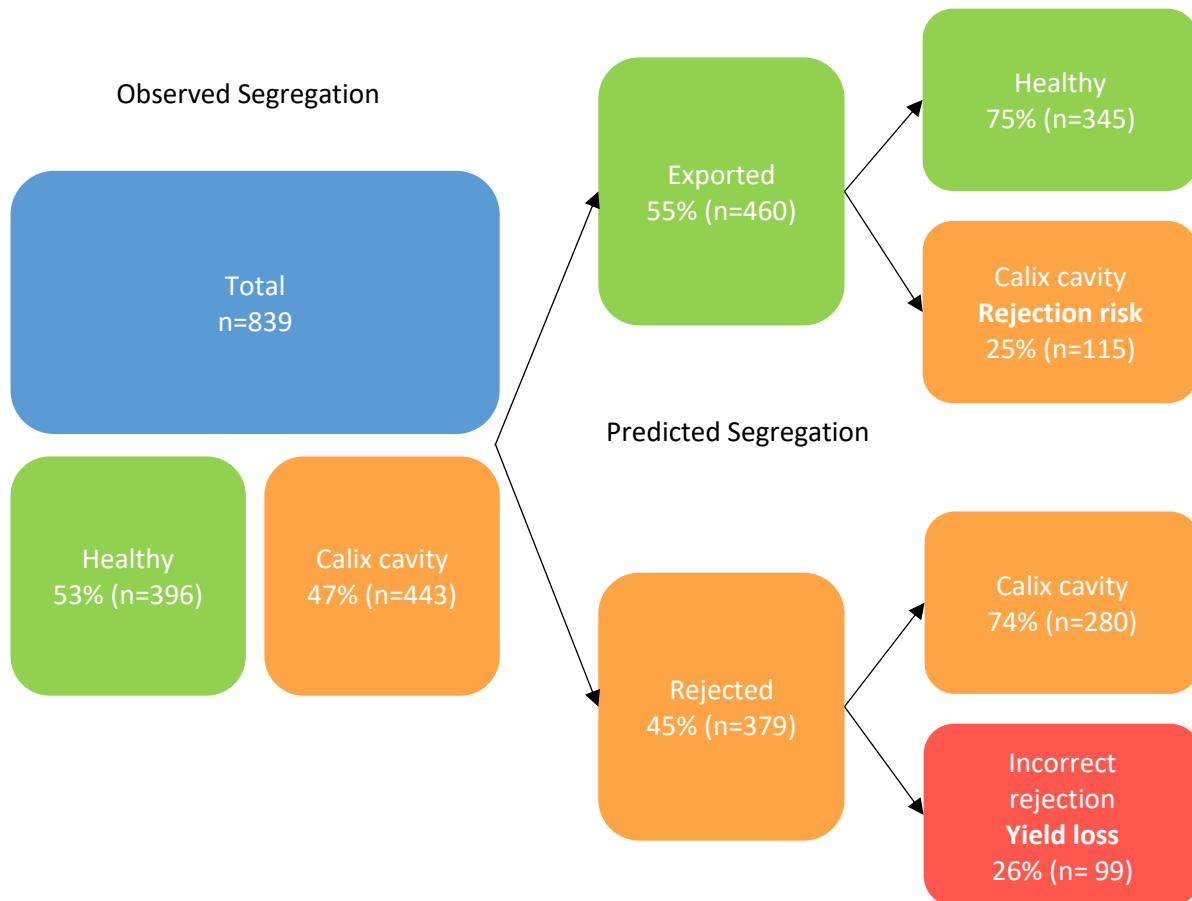
Cavity	No	Yes	Observed	Correct (N)	Correct (%)	Error (%)	Risk
No	343	100	443 (53%)	343	77.4	22.6	Rejection
Yes	117	279	396 (47%)	279	70.5	29.5	Yield loss
Total			839	622	74.1	25.9	

Another model was created to improve the prediction accuracy of calyx cavity, using at harvest weight and colour index data with calyx cavity as a binary. Model predictions were fairly accurate for the second model in terms of estimation of both fruit with calyx separation absence or presence. Healthy fruit were correctly identified 77.4% of the time, and fruit with calyx separation were correctly selected at a rate of 70.5% (Table 4.6).

The potential use of these classification models to segregate calyx cavity fruit in industry only reduces the problem by about half. As displayed in Figure 4.19, the amount of fruit with calyx cavity is 47%, and after segregation, the amount of fruit with calyx cavity in the exported sample is 25%. In this situation, unaffected fruit was also unnecessarily removed by misclassification, causing a 26% yield loss. Thus, the model leaves large potential for unaffected fruit to be removed, and for fruit with calyx separation to be left in with the healthy fruit. This would likely result in undesirable consequences for exports. When fruit with calyx separation are not identified and segregated, entire consignments of fruit could be put at risk of rejection from the presence of pests in the cavity. When healthy fruit are wrongly classified as calyx cavity fruit, yield loss is increased. Additionally, larger fruit are more likely to be sorted out by being wrongly categorised as calyx cavity when healthy, leading to high yield losses. The potential rejection risk and high yield losses mean the benefit to the industry is limited.

The use of a calyx cavity segregation model could provide several benefits to the persimmon export industry. Possible commercial advantages include increasing the quality of fruit by

minimising the amount of fruit with calyx cavity as this disorder is considered a disorder of major importance to importers (Bignell et al., 2017). Fewer fruit with calyx cavity in a consignment could reduce the amount of spoilage that occurs due to rots that develop inside the calyx cavity and spread to the surrounding fruit (George et al., 2005). Segregating affected fruit may also reduce the likelihood of shipments of fruit being rejected due to the presence of quarantine pests.



**Figure 4.19** Flowchart showing an example of use of the classification model to segregate persimmons including potential commercial implications.

For the segregation model to be implemented successfully, accuracy should be significantly improved. A more accurate model for the prediction of calyx cavity could be created by using fruit that has been through the grading process already, which would remove outliers and provide more uniform samples. The influence of harvest maturity on quality parameters suggests that using samples with more similar harvest maturities could reduce any effects due to more mature fruit rather than the presence of calyx cavity. Increasing the amount of

data collected could also help increase model performance, as well as considering the use of other non-destructive variables. Further study is required for the optimisation and validation of a segregation model of calyx cavity in persimmon.

### **4.3 Conclusions and future directions**

In the present work, the colour index was found to be negatively correlated with the flesh firmness as darker orange-red fruit were softer than lighter coloured. In terms of correlation between destructive and non-destructive evaluations, non-destructive compression was moderately related to flesh firmness, with a better relationship discovered for softer persimmon fruit. These correlations were found both at harvest and after storage. There was variation observed in the maturity indicator averages between harvest batches potentially due to differences in at harvest maturity. During the 9 weeks of MAP storage at 0 °C, the average CO<sub>2</sub> and O<sub>2</sub> concentrations stabilised at approximately 6% and 2%, respectively. The colour index and SSC were augmented during storage and the flesh firmness decreased. The use of MAP minimised water loss to below 1%, and CI to less than 2%.

The average incidence of calyx separation was 47%, with grade 2, or minor separation, the most common level of separation found in affected fruit. The presence and severity of calyx cavity was correlated to fruit weight and colour index. Fruit with calyx cavity were on average heavier and more deeply coloured compared to healthy fruit, and this trend increased with larger degrees of separation. As a result, there is potential for the combination of weight and colour index to assist calyx cavity segregation. Binary classification of calyx cavity had a total accuracy of 74.1% correct segregation, which is low for commercial use. Industry use would also be limited by the potential for incorrect identification to lead to possible rejection and yield losses.

Future work is required to improve the performance of the calyx cavity segregation model including model optimisation and cross-validation. The persimmon industry would benefit from the development of a standard scale for the evaluation of calyx cavity severity. From this scale, a commercial threshold for acceptability of calyx cavity could be established. A

definitive classification between levels of calyx cavity has the potential to improve segregation model performance. Further research could also explore the mechanisms that cause calyx cavity. This could include the use of growth regulators to increase cell division during flowering and fruit development to investigate the effect of calyx size on calyx cavity formation.

# Chapter 5. Near Infrared Spectroscopy

## 5.1 Spectral data

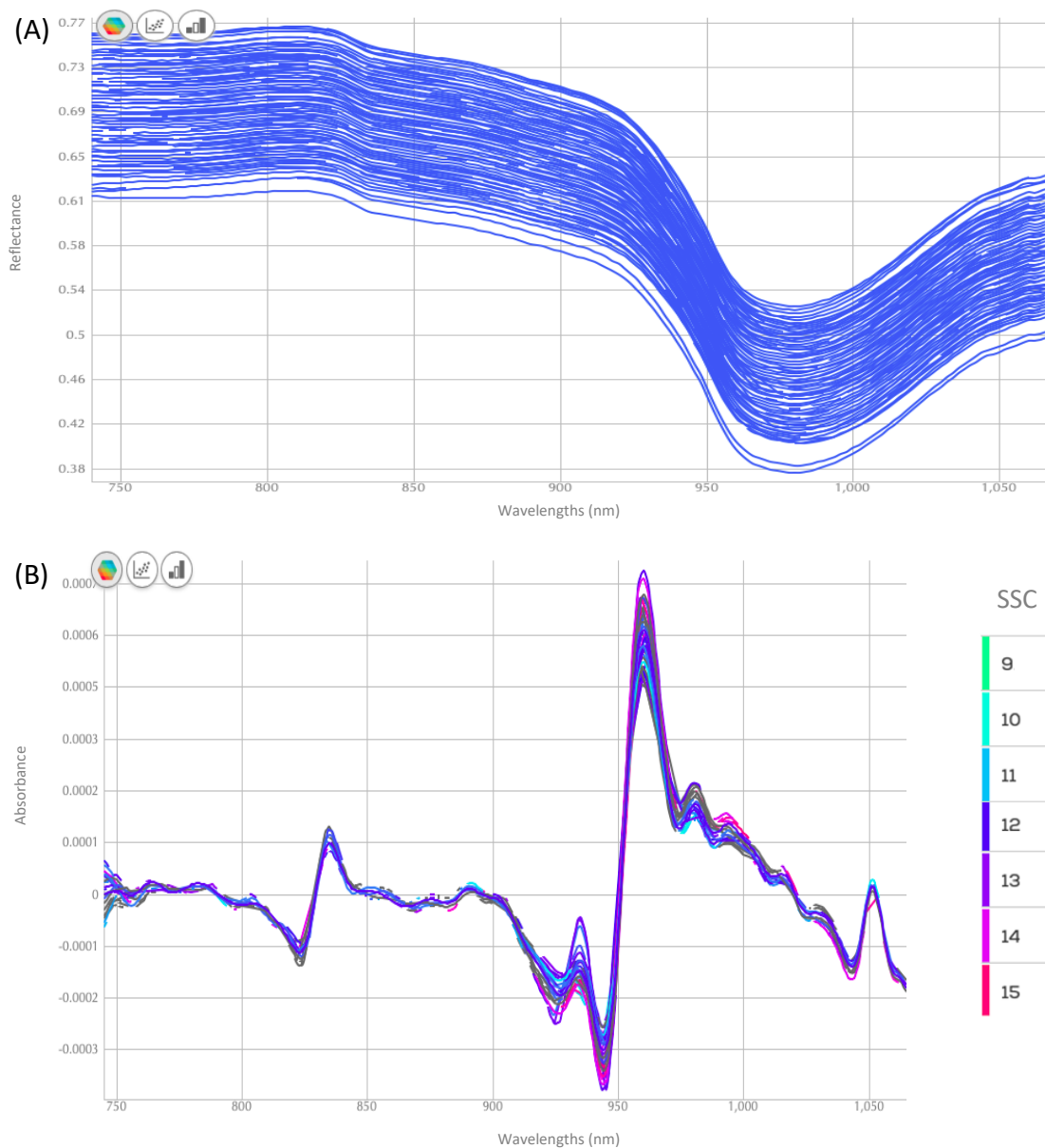
The reason near infrared spectroscopy (NIRS) is a good predictor of certain fruit quality characteristics, particularly SSC, is due to the wavelength at which the chemical constituents (and water molecules) absorb energy. The components of these quality attributes – including sucrose, glucose, fructose, organic acids, and pectins – contain many O-H and C-H groups within their chemical structures (Wang et al., 2015). The vibrations in the intermolecular hydrogen bonding between these molecules, and the interaction between molecules or absorbances, corresponds to specific patterns in spectra. Simeone et al. (2017) reported that the spectral regions of 1700 – 1850 nm are related to first overtone of C-H stretching, and between 2200 and 2500 nm are associated with C-H + C-H and C-H + C-C combination bands which have been attributed to vibrations of sugar molecules. There are also bands produced from the interaction of water and sugar absorbances. Bands within the range of 1100 – 1300 nm belong to the C-H second overtone and O-H combination, 1300 – 1600 nm belongs to O-H first stretch overtone and C-H combinations (Osborne, 2006). Reported wavelengths at which peak absorbances have been observed at differs between studies; the typical absorbances for some fruit qualities are summarised in **Table 5.1**.

Many of the aforementioned absorbance bands are in the upper range of wavelengths used for NIRS and outside of the wavelength scope of this experiment (740 – 1070 nm). However, this range covers a few wavelengths at which peak absorbances related to water and SSC are detectable. In a typical NIR spectrum of fruit, the overtone scope of water molecules' O-H bonds is present at 980 nm (Wei et al., 2020). In the raw spectra of this experiment, there was a distinct absorbance at approximately 980 nm, which is attributed to the water content in the persimmon (**Figure 5.1 (A)**). In a comparative study on the estimation of SSC in persimmon using 700 – 1100 nm NIRS, Jannok et al. (2014) reported raw spectra containing a strong absorption at 976 nm, owed to water.

**Table 5.1 Overview of the wavelengths at which typical absorption bands are seen for certain chemical groups in NIR spectra of various fruit samples reported by various studies, adapted from Wang et al. (2015).**

Quality Attribute	Chemical Group	Wavelength (nm)	Reference
Sugar	O-H	1190, 1400	(Kemps et al., 2010)
	C-H	910	(Omar, 2013)
SSC	O-H	975	(Shao & He, 2007)
	O-H	960, 1450	(Liu et al., 2010)
	C-H and O-H	1210	(Liu et al., 2010)
	C-H	963	
	O-H	960, 1180, 1450, 2000	(Zhang et al., 2012)
	Combination bands of C-H and O-H	2000–2500	
	O-H and C-H	950–1075	(Yu et al., 2008)
Acidity	O-H from carboxyl acids	1127	
	C=O from saturated and unsaturated carboxyl acid	1437	(Bennedsen et al., 2004)
	C-O from COOH	1607	
pH	C-H	768	(González-Caballero et al., 2010)
	O-H	986	

The NIR spectra of attributes of qualities such as SSC relate to bands which are characteristically broad and overlap, creating a ‘featureless’ spectra. Hence, second derivative transformation of the spectral data can be done to tease out differences (Guthrie et al., 2005). According to Delwiche et al. (2008), the presence of simple sugars associated with SSC modifies the downward peak caused by water, which was observed in their second derivative spectra of mango fruit. In **Figure 5.1 (B)** the transformed spectra show an alteration in the downward peak at 940 nm; this may be correlated to the SSC of the persimmon. There were also smaller peaks located between 800 and 850 nm, and at 1050 nm. These may also be associated with the SSC, or it could potentially be amplified noise with higher orders of derivation (Wang et al., 2015).



**Figure 5.1 (A) The set of raw NIR spectra, and (B) the spectral data pre-treated by log and second order derivation using a polynomial number of 2 (with an 11-point window) within the range of 740 – 1070 nm. Line colours in (B) represent varying levels of SSC.**

Delwiche et al. (2008) suggests that in NIR spectra of fruit, solutes affect spectral water absorption bands by changing the distribution of its hydrogen bands. These alterations can influence the absorption magnitude, and frequency of vibration. The sugar concentration determines the extent and direction of absorbance or wavelength shift. A shift toward lower wavelengths suggests an interference in the water molecules H-bonding; a shift toward higher wavelengths indicates augmentation of H-bonding. Low sugar concentrations promote

the disruption of water H-bonding, whereas high levels produce the opposite effect (Giangiacomo, 2006). The composition of the sugar within the fruit – relative glucose, sucrose, and fructose levels – can also affect any changes seen in this waveband (Delwiche et al., 2008). In this experiment, fruit with higher SSC (represented by the pink lines in **Figure 5.1 (B)**) showed less disruption/greater absorption in the 940 nm peak compared to lower SSC fruit, likely associated with the greater sugar concentration within the persimmon fruit.

The absorbances related to changes in firmness are harder to differentiate from spectra as there are many processes that occur both chemically and physically to fruit during softening (Guthrie et al., 2005). A loss of firmness can partially be attributed to degradation of important structural components such as pectins and celluloses, and generally occurs in tandem with an increase in soluble solid levels (Salvador et al., 2007). A study on the prediction of firmness using 400 – 1130 nm VIS-NIRS in mango (Mishra et al., 2020) suggested that changes in cell wall composition could affect NIR spectra. The research used interval partial-least square regression (iPLSR) to select the key wavelengths responsible for predicting firmness. The regions identified were 743 – 770 nm and 870 – 905 nm, which corresponded to the overtones of CH and CH<sub>2</sub>, and CH<sub>2</sub> and CH<sub>3</sub>, respectively. Although, it was concluded that these peaks could not be directly explained by any chemical parameter in particular, CH, CH<sub>2</sub>, and CH<sub>3</sub> bonds can be related to chemicals like sugar, hemicellulose, and pectin. However, the concentration of pectin in persimmon is 0.52 - 1.07% (per 100g of edible fresh weight) (Salunkhe & Kadam, 1995) which is below the level that is detectable by NIRS. In studies where reasonable results were acquired for texture attributes, it was often believed to be related to the scattering properties of the tissue which is better assessed by multi- and hyper-spectral techniques (Nicolai et al., 2007a).

In spectral data, absorbances correlated to colour can be detected by VIS-NIR spectroscopy (Nicolai et al., 2007a). The peak absorbances for colour pigments are within the visible spectrum (380 – 740 nm); the wavelength range for green is 500 – 565 nm; yellow 565 – 590 nm; orange 590 – 625 nm; red 625 – 740 nm (Walsh et al., 2020). Peaks within these ranges indicate fruit colouration in relation to pigment levels (e.g., chlorophyll and carotenoid quantities). A typical VIS-NIR spectra of ripe persimmon exhibits a rise in intensity between the orange- and red-associated wavelengths. Altieri et al. (2017) reported a peak from 550 –

700 nm related to the orange-red colouration of the ‘Rojo Brillante’ persimmon fruit. No peaks in the spectra of this experiment can be linked directly to colouration as these peaks lie outside of the scope of wavelengths measured.

## 5.2 Regression model summation and comparisons

The partial least squares (PLS) regression algorithm was used with the NIR spectral data and quality attribute evaluations to predict various fruit characteristics. PLS is a method for creating predictive models when there are many, highly collinear factors (Tobias, 1995). PLS works by extracting a smaller set of components that describes maximum correlation between the predictors and response variables. These components are usually underlying (latent) factors that account for most of the variation in the response. PLS tends to avoid over-fitting where a model fits the sampled data perfectly but fails to predict new data well (Tobias, 1995).

**Table 5.2 Summary of the PLS regression models across various quality attributes using 10-fold cross-validation. Pre-processing methods of log, averaging, and SNV (standard normal variate) were used. Models created in the SCIO™ online application can be found in Appendix D.**

Variable	Sampling time	n	RMSE	R <sup>2</sup>	RPD
SSC (%Brix)	At harvest	395	0.769	0.698	1.822
	After storage	445	0.785	0.728	1.922
Colour Index	At harvest	840	2.696	0.453	1.353
	After storage	445	2.711	0.489	1.400
Flesh firmness (kg <sub>f</sub> )	At harvest	395	1.03	0.336	1.229
	After storage	445	1.149	0.576	1.540
Non-destructive compression (%)	At harvest	840	0.086	0.151	1.045
	After storage	445	0.13	0.549	1.494
Acoustic firmness index	At harvest	840	2.567	0.154	1.086
	After storage	445	3.077	0.187	1.109

The predictive models were generated using PLSR, following the implementation of various pre-processing techniques on the raw spectra (**Table 5.2**). The models with the highest accuracy were those predicting SSC levels. An accurate and robust model should have a low RMSE, high  $R^2$ , and high RPD values. The best performing model was the estimation of SSC after storage. This model had a ratio of performance to deviation (RPD) of 1.922,  $R^2$  of 0.728, and RMSE of 0.785%. The fit of the at harvest SSC model was slightly lower than that of the after-storage model, with an RPD of 1.833,  $R^2$  of 0.698, and RMSE of 0.769%. The prediction of (destructive and non-destructive) firmness was considerably poorer than SSC; flesh firmness was estimated to a moderate degree of accuracy ( $R^2 = 0.576$ ), compression firmness less so ( $R^2 = 0.549$ ), and acoustic firmness substantially less so ( $R^2 = 0.187$ ) (at harvest). Colour index was approximated moderately to weakly with an  $R^2$  of 0.453 at harvest and 0.489 after storage.

### **5.2.1 Model accuracy & robustness**

Across the board, model prediction accuracy and performance was better post storage compared to at harvest. This difference is particularly pronounced in flesh and compression firmness where models were considerably better when built from after storage data. The  $R^2$  of flesh firmness models before and after storage was 0.336 and 0.576, respectively, and for non-destructive compression 0.151 and 0.549, respectively. This coincides with the drastic decrease in firmness that was observed after 9 weeks of MAP storage at 0 °C. The firmness of fruit likely affected model success; potentially, the softer fruit produced less scattering which could have contributed to better spectral data (due to less interruption of absorbance). There was not much difference observed between at harvest and after storage colour models. This was expected as colour is measured on the visible spectrum which was outside of the chosen wavelength range (Altieri et al., 2017).

**Table 5.3 Summary of comparative research on NIRS to predict quality characteristics in persimmon fruit. All models utilised PLS regression; other qualities were predicted in these papers but not included in the table.**

Paper	Cultivar	n	Acquisition mode	$\lambda$ range	Predicted attribute	Pre-processing	RMSE	R <sup>2</sup>	RPD
(Hemrattrakun et al., 2021)	'Hiratanenashi'	124	Interactance	310 – 1100 nm	SSC (%)	EMSC + OSC	0.58	0.82	1.73
					Firmness (N)	OSC	4.21	0.89	2.14
			Reflectance		SSC (%)	OSC + 2-Der	0.59	0.81	1.70
					Firmness (N)	EMSC + OSC	5.94	0.75 (r)	1.51
(Wei et al., 2020)	Not reported	100	Interactance	900 – 1700 nm	SSC (%)	Raw	1.592	0.744	1.866
						SG	1.595	0.733	1.862
						SNV	1.620	0.727	1.833
						MSC	1.657	0.703	1.792
					Firmness (kg/cm <sup>2</sup> )	Raw	0.278	0.871	2.802
						SG	0.279	0.870	2.792
						SNV	0.319	0.848	2.442
						MSC	0.372	0.871	2.094
(Ar et al., 2019)	'Rendeu'	147	Not reported	1000 – 2500 nm	SSC (%)	Raw	2.21	0.87	1.79
						N01	2.29	0.85	1.72
						1-Der	2.7	0.84	1.46
						MSC	2.21	0.86	1.79
						N01 + 1-Der	2.58	0.85	1.53
						1-Der + MSC	2.52	0.85	1.56
					Firmness (Kg $\ddot{r}$ )	Raw	0.1	0.82	2.09
						N01	0.07	0.94	2.9
						1-Der	0.1	0.89	2.09
						MSC	0.07	0.94	2.74
						N01 + 1-Der	0.09	0.92	2.35
						1-Der + MSC	0.1 (SEP)	0.9 (r)	1.94
(Altieri et al., 2017)	'Rojo Brillante'	481 473 738	Reflectance	470 – 1032 nm	SSC (%)	Normalisation	0.510	0.905	3.25
					Firmness (N)		3.469	0.963	5.18
					Colour index		1.735	0.961	5.09
(Jannok et al., 2014)	Not reported	120	Interactance	400 – 1100 nm	SSC (%)	Log + 2-Der	0.47 (SEP)	0.88	Not reported

**N01: normalization between 0 and 1, 1-Der and 2-Der: first and second derivatives, MSC: multiplicative scatter correction, EMSC: extended multiplicative scatter correction, OSC: orthogonal signal correction, SG: Savitzky–Golay smoothing, and standard normal variate (SNV).**

SSC was the only quality attribute predicted to a sufficient level of accuracy by NIR in this experiment. Other studies on persimmon have reported comparable results in terms of SSC, although much of the research points to greater outcomes for the predictability of firmness, unlike this study. In persimmon, colour has been estimated accurately by VIS-NIR; acidity and astringency are among other qualities that researchers have demonstrated success in using spectral data to predict (Cortes et al., 2017). An accurate NIR model will have an RMSE lower than its SD, and an  $r$  or  $R^2$  of above 0.75. A reliable and robust model should have a RPD of near or above 2.0; the higher the RPD values are the better the model can predict unknown samples (Wang et al., 2015). RPD values less than 1.5 suggest that the calibration model is not applicable; values from 1.5 to 2 imply that model discrimination of low and high values in the response variable is good (Saeyns et al., 2005). According to Pissard et al. (2013), a model with an RPD value of 2 to 2.5 can allow for coarse prediction, while an RPD value above 2.5 signals good to excellent prediction.

Of the papers outlined in **Table 5.3**, SSC model accuracy ranged from 0.703 to 0.963 ( $R^2$ ) and 1.657% to 0.510% (RMSE); the scope of model robustness was from 1.46 to 3.25 in terms of accuracy. The after-storage SSC model results from this experiment fall within these ranges, but the others do not. This model had a RMSE which was lower than the SD for the SSC measurements (0.785% compared to 1.508%, respectively), and a RPD value close to 2.0. It was, however, lacking slightly in terms of correlation with an  $R^2$  value just below 0.75. This suggests that the SSC of persimmon fruit can be predicted to a satisfactory level using spectral data collected after storage. Fruit exhibited higher SSC levels after post storage compared to at harvest; this likely affected the NIR readings as sugar concentration is linked to absorbance or wavelength shifts (in the main peak associated with water) (Delwiche et al., 2008).

On the other hand, the flesh firmness was not predicted accurately by the NIR data collected during this experiment. This is in opposition to other persimmon studies where firmness models performed well, or even outperformed the SSC models. In **Table 5.3**, the lowest reported  $R^2$  was 0.848, with some  $R^2$  values over 0.9 for flesh firmness prediction. In comparison to these other studies, the models created in this work for the estimation of firmness had significantly lower  $R^2$  and RPD statistics. However, in many other commodities the prediction of SSC tends to be markedly better compared to firmness. This is a result of

NIR measuring the sum of signals of absorption and scattering. Light scattering in biological materials is affected by structural properties of the tissue including density, particle size, and cellular structures (Cen & Lu, 2009). Therefore, the scattering signals provide information on the textural properties but are more difficult to extricate (Nicolai et al., 2007a). Walsh et al. (2020) suggests that there is not yet a consensus on whether firmness of horticultural products can be accurately predicted by NIR.

Modelling results obtained from NIR spectral data are affected by many factors. Some of the differences seen in performance ability may be attributed to variation in experimental factors such as wavelength selection, equipment, acquisition mode, and pre-processing techniques, among others.

### **5.2.2 Pre-processing techniques**

In this experiment, the pre-processing methods of log 1/R, averaging, SNV, and/or second derivation were applied to spectral data prior to model construction. Spectral data underwent logarithmic transformation to obtain the absorbance spectra which provides more information (Song et al., 2020). Averaging over wavelengths was used for the purpose of smoothing the spectrum. Average subtraction allowed for results to be centred so they could be interpreted in terms of variation from the mean. SNV and derivative spectra were used to remove any additive (baseline shift) and multiplicative (tilt) effects from light scattering (Nicolai et al., 2007a).

In comparative persimmon studies, numerous pre-processing techniques have been used, and in different combinations, which affected model performance. Different studies drew different conclusions on the best use of pre-processing methods to improve modelling results. Ar et al. (2019) compared the models generated from various pre-treatments consisting of N01, 1-Der, N01 combined with 1-Der, and 1-Der with MSC (full terms given in **Table 5.3**). The study reported the best model for SSC implemented MSC (although this was on par with the raw spectra for SSC), and the best prediction of firmness was attained by normalisation between 0 and 1. On the other hand, Wei et al. (2020) found models that used raw data as

the input had the best performance in SSC and firmness prediction. Substituting different pre-processing approaches was done to improve the performance of the models generated as part of this section, and the best performing models for main quality characteristics were selected.

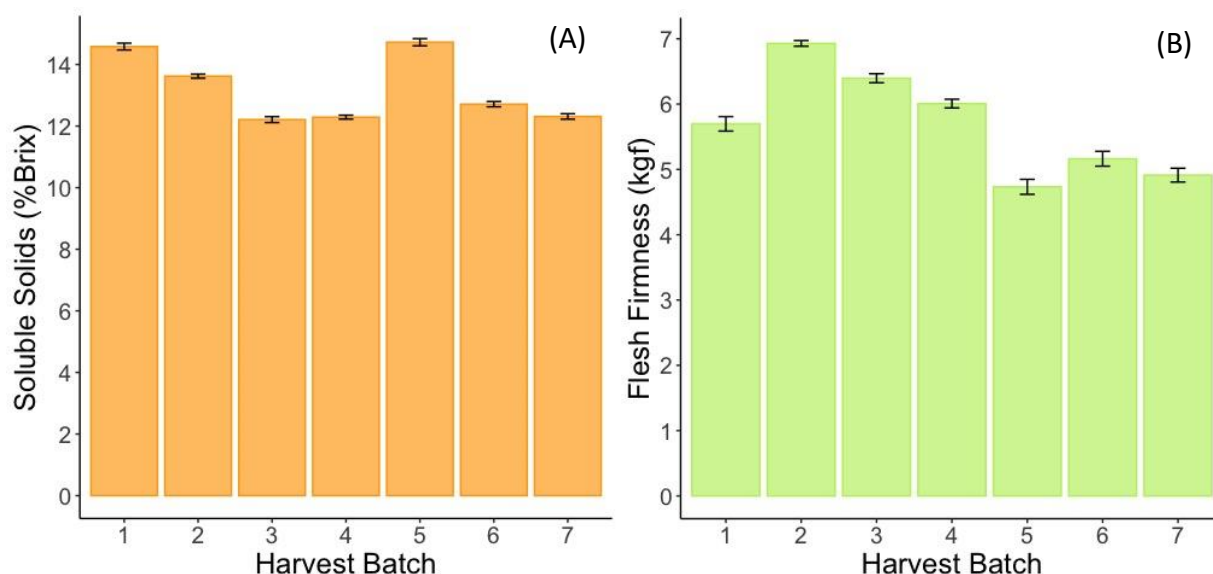
### **5.2.3 Wavelength selection**

Some NIR persimmon studies selected a higher wavelength range compared to others. Ar et al. (2019) used a region of 1000 – 2500 nm and found 5 peaks above 1150 nm which reflected the water content, carbohydrate content, and total acid content, and the interactions between these properties. Wei et al. (2020) chose a range from 900 – 1600 nm and detected three distinct absorbances at 980, 1150, and 1420 nm, associated with water, SSC and firmness. VIS-NIR research (Altieri et al., 2017; Hemrattrakun et al., 2021; Jannok et al., 2014) used wavelengths between about 300 – 1100 nm and reported only peaks associated with colour at around 600 nm and water at 980 nm. Despite this, model performance was equivalent between the different wavelength selections.

It may be the case that attributes like SSC and colour are more directly linked to absorbances at certain wavelengths, whereas more complex traits, such as firmness, are generally indirectly estimated. As the negative relationship between SSC and firmness is usually strong and linear, firmness predictions may be based on this correlation rather than changes more directly related to firmness loss. A moderate, negative correlation ( $r = -0.636$ , at harvest data) was observed between the colour index and the flesh firmness in this work. However, the subsequent firmness models constructed were not as good as in other similar research (where a correlation between colour and firmness was also observed). A contributing factor may have been the fact that the colour-firmness relationship was not as strong in this work as in others. Thus, if the relationship between SSC and firmness contributed to better the model prediction, it would be expected that a lower correlation would result in poorer firmness predictions.

## 5.2.4 Sampling

Differences in samples may be a contributing factor in the relative success of NIRS-based models. This includes variance in cultivar, sample size, homogeneity, harvest time, and environmental conditions, among others. A notable distinction between experiments is the sample size. This study analysed 840 samples, whereas most NIR papers in **Table 5.3** assessed between 100 and 150 fruit. The smaller sample sizes also came from single harvests, in most instances, and so the homogeneity of these populations was likely much higher. The persimmons used in this study showed significant differences in SSC, firmness, and colour and between many of the 7 harvest groups. The PCA score plot (in section 4.2.2) shows that Harvest Batch contributed to considerable variance in the data. The high variability observed in the current study is more representative of reality and therefore provides arguably more realistic assessment of the capability of NIR for quality predictions.



**Figure 5.2** The variation in at harvest SSC (%Brix) (A) and flesh firmness (kg<sub>f</sub>) (B) between the 7 batches of persimmons harvested consecutively over the picking season.

The total difference in the average SSC across the 7 batches was 2.5% and the variation in average flesh firmness was 2.2 kg<sub>f</sub> at harvest (**Figure 5.2**). The difference in average colour index values was also large between batches – values ranged from 11.7 (batch 2) to 18.4 (batch 5) at harvest. In general, persimmon fruit harvested at more mature stages have higher SSC, higher colouration and lower firmness, and less mature fruit display the opposite

qualities (Testoni, 2002). Hence, results indicate differences in the harvest maturity between batches; batches with higher average SSC and colour index values, and lower firmness were likely more mature at harvest compared to other batches. Batch 5 had the greatest SSC, colour index and flesh firmness values, suggesting that these fruit were on average more mature. Batches 3 & 4 had low average SSC and colour, and high flesh firmness, implying lower overall levels of maturity. The difference in fruit maturities may have affected the ability for the model to make accurate predictions (Walsh et al., 2020).

On the other hand, it is known that a narrow range of reference values (small SD) can give rise to smaller RPD values (Cozzolino et al., 2011; Shah et al., 2010). Thus, the reliability or robustness of a model, in terms of RPD, is usually heightened by greater sample sizes and a more variable population. Altieri et al. (2017) evaluated a much larger and more diverse population compared to the other persimmon studies; fruit were sourced from different packhouse and at different maturities. Subsequently, they reported very high  $R^2$  values ( $>0.9$ ) for and significantly larger RPD values for SSC, firmness, and colour index estimation. Constructing a model from a small sample size is often reported as a limitation. Having a more robust model is important as it then has a greater chance of being able to reliably predict the qualities of a greater range of persimmon fruit, rather than being limited to the population from which it was derived (Nicolai et al., 2007a).

Of the regression models created in this experiment, most exhibited prediction performances that are considered below the ideal levels in terms of both accuracy and robustness. Results indicated that the models developed for firmness and colour parameters were not suitable to predict these qualities in a meaningful capacity. The models generated for the prediction of SSC, particularly after storage SSC levels, had sufficient statistical indicators whereby they may be judged as usable for rough screening applications (Altieri et al., 2017). Limitations of model performance may be contributed to by the measurement depth limitation of 3 – 4 mm beneath the skin stemming from a wavelength selection of 740 – 1070 nm (Lammertyn et al., 2000). Consequently, the spectral data collected was likely associated with the skin and outer fruit tissues, and this could have affected model accuracy and performance (Li et al., 2018).

### **5.2.4.1 Model Validation**

To assess the accuracy of a calibration model, validation procedures are applied (Magwaza et al., 2012a). Commonly, internal validation is done where the dataset is split into two datasets, one for calibration, and one for validation. The performance of the calibration model can then be observed on the validation dataset (Walsh et al., 2020). Cross validation, a form of internal validation, is often used where subsets of data are left out, and a calibration model is constructed on the remaining dataset (as in this work). The subset of removed samples are used to calculate the prediction residual, the difference between the predicted and observed results. This is repeated, leaving out different subsets each time, and the final variance of all prediction residuals is estimated (Nicolai et al., 2007a). There is also the method of external validation where the validation dataset is independent and collected from a different season or orchard. External validation is important as it indicates how well a model may perform when implemented in a real-world scenario (Ramspek et al., 2021).

In many cases, preliminary model results are promising, but when the model is applied to an independent dataset, performance is unsatisfactory (Nicolai et al., 2007a). The reference studies in **Table 5.3** showed good ability for models to predict fruit firmness, but these results have not been tested on external datasets to validate their performance. These studies only completed internal validation so it is unclear whether relative model success would be replicated on fruit from other growing regions or seasons.

## **5.3 Classification models**

### **5.3.1 Model results**

As part of this experiment, the random forest (RF) algorithm was used with the NIRS data collected from approximately 840 persimmon samples and the corresponding calyx cavity evaluations. Classification models were derived (from the at harvest data) to predict both the presence or absence of calyx separation in the persimmon fruit, as well as the severity of the separation that occurred. The pre-processing techniques that produced the best results was

the sequence of log transformation, averaging, second derivative, and SNV. The relative successes of the models are outlined in **Table 5.4** and **Table 5.5** below. These models are based on NIR detection of secondary chemical compositional changes as a result of cavity separation. It is likely that the NIRS data is correlated with differences in certain fruit quality parameters (particularly higher SSC and softening) related to the presence of calyx cavity.

Confusion matrices were created to visualise and summarise the performance of the calyx cavity classification models. In the confusion matrices (**Table 5.5**) the columns represent the true class that the persimmons were classified as during calyx cavity evaluation, and the rows display the classes predicted by the model. The values in the complementary cells constitute the percentage of times the model estimation matched the true class. For the prediction of calyx cavity, fruit were either identified as possessing a cavity (a positive result), or not possessing a cavity (a negative result). Where the model classifier correctly predicts the outcome of a cavity when it is present, this is termed a true positive. Where the classifier accurately predicts the outcome of no cavity when it is absent, this is termed a true negative. On the other hand, when the model incorrectly estimates the outcome of no cavity when it is present (and vice versa), this is termed a false negative (or false positive) (Chicco & Jurman, 2020).

**Table 5.4 Summary of the classification models using the random forests (RF) algorithm to predict the presence or absence of calyx separation, and the severity of calyx cavity.**

Variable	Sampling time	n	Pre-processing method	F1
Calyx cavity	At harvest	840	Log, averaging, SNV	0.655
	After storage	445	Log, averaging, SNV	0.673
Grade	At harvest	840	Log, averaging, SNV	0.370
	After storage	445	Log, averaging, SNV	0.405

**The original confusion matrices created in the SCIO™ online application can be found in Appendix D.**

**Table 5.5 The normalised confusion matrix prediction values of the presence (Y) or absence (N) of calyx separation and the level of separation from grades 1 (no separation) to 4 (severe separation) compared to the observed scores at harvest.**

Known class		N	Y	Known class				
				1	2	3	4	
Classified	N	<b>310</b>	151	1	<b>372</b>	172	64	27
	Y	128	<b>242</b>	2	58	<b>49</b>	19	2
				3	5	7	<b>26</b>	11
				4	0	0	0	<b>8</b>
Observed	443 (53%)	397 (47%)	443 (53%)	232 (28%)	111 (13%)	54 (6%)		
Predicted	461 (55%)	370 (44%)	635 (76%)	128 (15%)	49 (6%)	8 (1%)		
Rate of correct identification			66.4%				55.5%	

**Due to rounding errors in the SCIO™ online application, total fruit numbers and total percentages may not equal 100%.**

As can be seen in **Table 5.5**, fruit with calyx cavity present were correctly identified at a rate of 70% (true positives), and fruit without a cavity were correctly identified 61% of the time (true negatives). The rate of false positives was 29%, and the rate of false negatives was 38%. Thus, the model correctly segregated 66.4% of fruit, and incorrectly segregated 33.6% of fruit. For the prediction of severity, the majority of fruit classified as grade 1 were successfully forecasted as grade 1 (84%). However, this model also misclassified grades 2, 3, and 4 as grade 1 at a rate of 74%, 58%, and 50%, respectively. Fruit were only correctly identified as grade 2 at a rate of 21%, grade 3 at a rate of 23%, and grade 4 at a rate of 14%. This indicates that the model has a tendency to predict all fruit as grade 1 fruit. Additionally, models constructed from after storage data performed marginally better than those generated from at harvest data. This could be linked to the greater change in SSC, firmness, and colour that was exhibited by fruit with calyx cavity after storage compared to healthy fruit.

A metric of model performance is the F1 score supplied in **Table 5.4**. The F1 score is a summary statistic that provides a general idea of model performance. It is a combination of the precision (number of correctly identified members of a group divided by all of the times the model predicted that group) and the recall (number of correctly classified members of a class divided by the total members of the class) of the model (Liaw & Wiener, 2002). In cases where F1 is low, either both or one of these metrics are low. In the at harvest severity model,  $F1 = 0.370$  which is much lower than the at harvest calyx cavity model which had an F1 score of 0.655. Where F1 is low, like in the case of severity classification, the model is often classifying most fruit as part of a large group (Chicco & Jurman, 2020). More than half of all fruit were grade 1 severity (no cavity) compared to the other grades where the amount of fruit was lower with each successive grade. This is supported by the confusion matrix as described above. Better results may be achieved by evening out the number of samples in each level of severity to overcome class imbalance (Bekkar et al., 2013; Gu et al., 2009). However, this would be difficult to accomplish as only a minority of fruit have calyx cavity, and even less fruit have severe symptoms.

### **5.3.2 Application of NIRS to detect calyx cavity**

In terms of industry application, the potential to incorporate a method such as NIRS has the potential to non-destructively sort fruit. The model predicting the presence of calyx separation likely does not have a high enough degree of accuracy for use on a grading line. The fairly large levels of false negatives and false positives would mean that many fruit without a cavity would be rejected unnecessarily, and many fruit with a cavity would not be removed. This may allow fruit with pests and inadequate quality to be included in export consignments. Progress on the predictive ability of models could possibly be made by analysing bigger datasets which encompass multiple orchards, seasons, and environmental conditions.

## 5.4 Conclusions

Spectral data from NIR measurement of persimmon was used to develop models to predict various qualities of the fruit. Absorbance peaks observed in the spectra were similar to those reported by comparative research. Regression models, using the PLS algorithm, were constructed to estimate the properties of soluble solids, colouration, and firmness. The most successful estimations were that of SSC, followed by flesh firmness and colour. The SSC models showed sufficient performance levels to be considered for use in rough grading applications (RMSE = 0.769% and  $R^2 = 0.698$ ). However, even the better performing of the colour and firmness models (with  $R^2$  of 0.489 and 0.576 respectively) did not predict either of these characteristics to a satisfactory degree of accuracy to be considered usable. In comparison to similar persimmon studies, apart from the prediction of SSC, the performance ability of the models created in this experiment was substandard.

The classification models for calyx cavity detection produced using random forests exhibited some success. The predictive power of the calyx cavity classifier was moderate; 66.4% of fruit were segregated into the correct category, but 33.6% of fruit was misclassified. The grade model showed a poorer performance as it grouped 55.5% of fruit correctly and 45.5% incorrectly. Overall, the use of NIRS may have potential to identify calyx cavity in persimmon fruit in coarse screenings, although model performance would benefit from further research. This research could include more fruit of differing calyx separation levels and greater expression of postharvest rot and chilling injury.

## Chapter 6. Conclusions & Recommendations

This research aimed to determine the strength of the relationship between maturity indicators and destructive and non-destructive evaluations performed at harvest. It was found that colour index was negatively correlated with flesh firmness, indicating fruit with greater colouration tended to be softer, in agreement with other persimmon research (Salvador et al., 2007; Tessmer et al., 2016). The relationships between non-destructive firmness parameters (compression and acoustic firmness) and destructive flesh firmness were weak to moderate, and better for softer fruit compared to firmer fruit. This provides evidence that these methods obtain information on different textural aspects of fruit (Li et al., 2016). Due to very high variability, acoustic firmness does not appear to be a useful tool for measuring persimmon firmness.

The at harvest maturity of fruit influenced persimmon quality after long-term postharvest storage in MAP at 0 °C. Fruit that were determined to be at a later maturity stage at harvest generally showed greater changes in quality characteristics than those harvested at earlier maturities. Differences in the attributes of colour, firmness, SSC, and weight were also found between fruit with and without calyx cavity. This suggests that calyx cavity influences persimmon fruit quality.

The investigation into the use of non-destructive evaluation methods to classify and segregate fruit with calyx cavity yielded moderate success. The current work suggests that non-destructive methods have potential to be used to segregate healthy and affected persimmon fruit. Although, the classification models developed to identify calyx cavity presence were better in comparison to those created to distinguish between the severity of calyx separation. Similar challenges have been reported by other studies where the classification of different levels of a disorder is poorer than basic detection (Cen et al., 2013; Pan et al., 2017). The models for binary calyx segregation do not have a high enough degree of accuracy for use on a grading line. While the implementation of non-destructive calyx cavity classification would give packhouses the ability to eliminate many of the affected fruit during grading, it would also create certain risks. These risks include the potential for healthy

fruit to be removed, resulting in higher yield losses, and failed identification of fruit with calyx separation which could put entire consignments of fruit at risk of rejection from the presence of quarantine pests in the cavity. These factors suggest that the benefits of a segregation model in industry would at this stage be limited.

At present, the persimmon industry lacks a standardised method to evaluate the severity of calyx cavity. The development of a standardised scale would be beneficial in setting commercial thresholds and improving segregation models. Because of the potential benefits provided by calyx cavity segregation, further development of the calyx cavity classification model would be worthwhile. Further research would be required to increase the accuracy of prediction, optimise performance and complete model validation. The persimmon industry would also benefit from work exploring the mechanisms that cause calyx cavity. This could include the use of growth regulators to increase cell division during flowering and fruit development to investigate the effect of calyx size on calyx cavity formation.

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# Appendices

## Appendix A: Australian 'FreshSpec' grade standards

Effective: 1 November 2006



<b>PRODUCE:</b>	<b>PERSIMMON</b>
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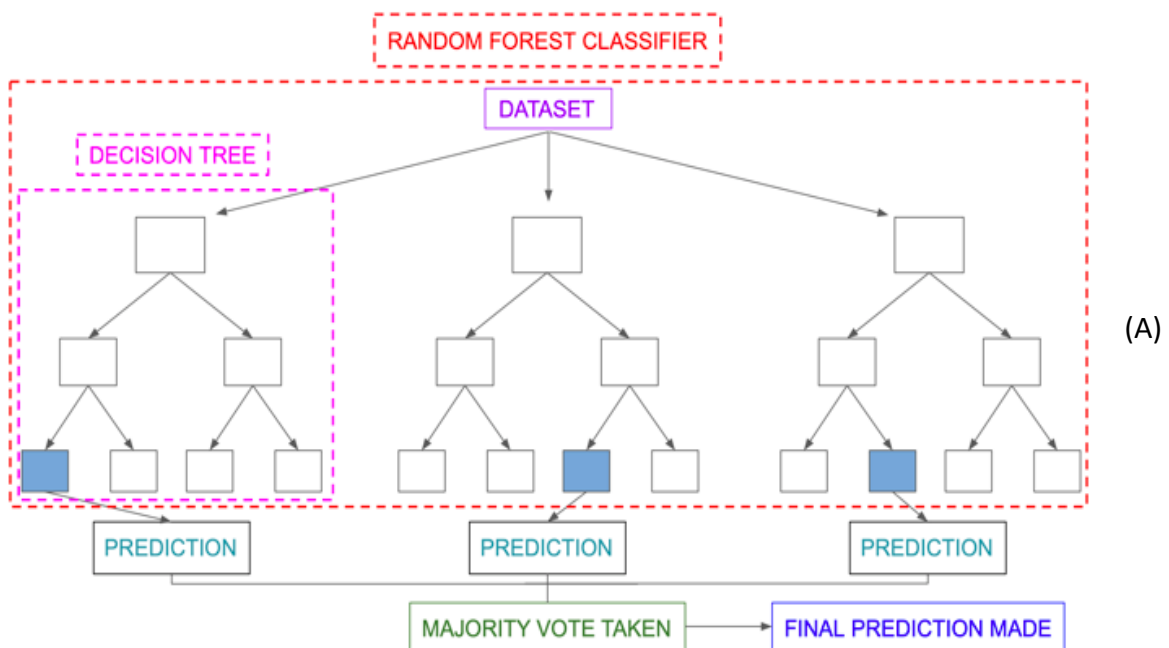
<b>TYPE</b>	<b>Sweet</b>	<b>VARIETY</b>	<b>Various</b>
<b>CLASS</b>	<b>Fuyu Fruit</b>	<b>NOTES</b>	

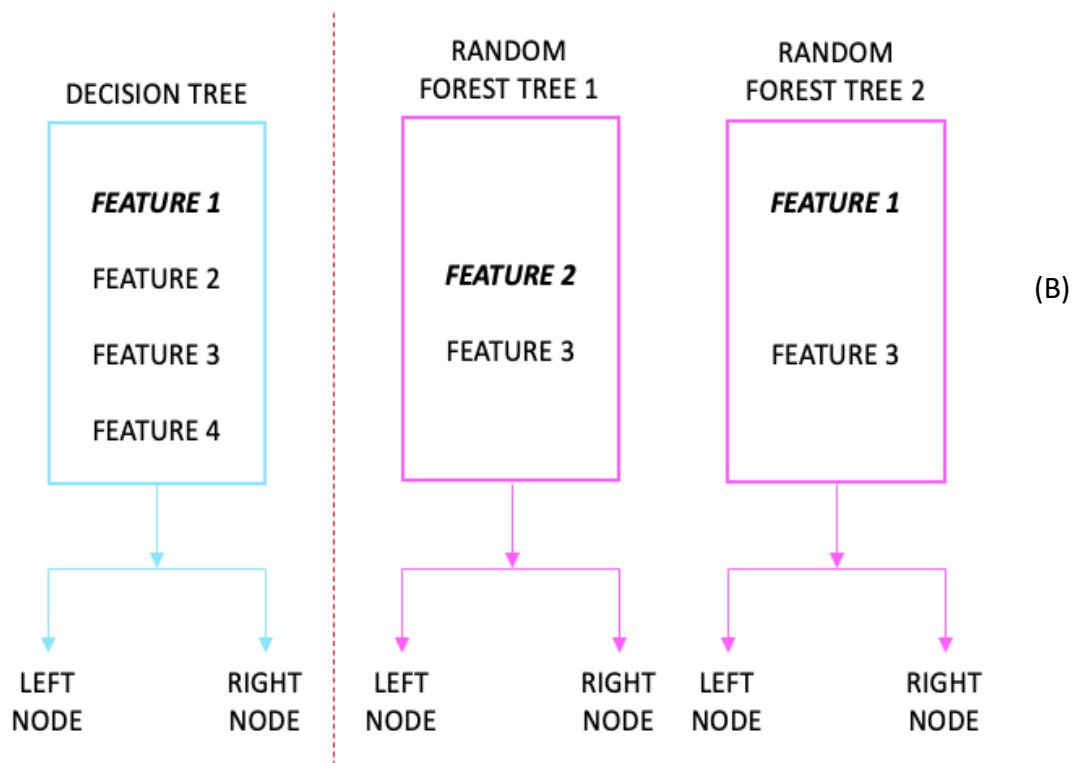
GENERAL APPEARANCE CRITERIA	
<b>Colour</b>	Bright orange to orange/red skin; bright orange flesh.
<b>Visual Appearance</b>	Thin skin; glossy, plump fruit; dry calyx lifted from the skin and surrounding a short stem; free from foreign matter.
<b>Sensory</b>	Smooth, slightly waxy skin; firm flesh softening to slightly crunchy; free from bitterness or astringency; free from foreign and 'off' smells or tastes.
<b>Shape</b>	Round to slightly squat fruit, often slightly pointed at apex.
<b>Size</b>	As per pre-ordered size requirements
<b>Maturity</b>	Firm, full coloured fruit; TSS >14° Brix .
MAJOR DEFECTS	
<b>Insects</b>	With evidence of live insects. (eg. fruit fly and mealy bugs)
<b>Diseases</b>	With evidence of fungal or bacterial rots.
<b>Physical/Pest Damage</b>	With unhealed cuts, holes or splits from physical or pest damage.
<b>Skin Marks / Blemishes</b>	With deep seated bruises.
<b>Physiological Disorders</b>	With evidence of skin russetting and calyx end cracking. With evidence of juice leakage or severe softening (over ripe) With presence of a cavity beneath the calyx
<b>Temperature Injury</b>	With flesh browning, softening and watersoaked appearance (chilling injury). With evidence of dark water-soaked areas (freeze damage).
MINOR DEFECTS	
<b>Physical/Pest Damage</b>	With superficial bruising >2 sq cm. With superficial cuts, scratches, marks > 2 sq cm.
<b>Skin Marks/Blemishes</b>	With healed scars >2 sq cm.
CONSIGNMENT CRITERIA	
<b>Tolerance Per Consignment</b>	Total minor defects (within allowance limit) to be < 2 defects per item Total minor defects (outside allowance limit) must not exceed 10% of consignment. Total major defects must not exceed 2 % of consignment. Combined Total not to exceed 10%.
<b>Packaging &amp; Labelling</b>	Packaging manufactured from new food grade materials or sanitised returnable crates. All labelling must meet the current legislative requirements. Labelling to identify grower's name/brand (plus growers name/code if via a packhouse), address, contents, class, size and/or minimum net weight. Produce to identify Country of Origin (eg. Produce of Australia) on outer container.
<b>Shelf Life</b>	Produce must provide not less than 14 days clear shelf life from date of receipt.
<b>Receival Conditions</b>	Compliance with Quarantine Treatments (if required) for Interstate Consignment. Stacked onto a stabilised pallet. Refrigerated van with air bag suspension, unless otherwise approved. Pulp Temperature 10 - 15 °C for Receipt.
<b>Chemical &amp; Containment Residues</b>	All chemicals used pre/postharvest must be registered and approved for use in accordance with the requirements of the APVMA regulatory system. Residues, Contaminants and Heavy Metals to comply to the FSANZ Food Standards Code ML's and MRL's.
<b>Food Safety Requirements</b>	Produce is to be grown and packed under a HACCP based food safety program that is subject to an annual third-party audit. A copy of current certification to be forwarded to receiver.

\*Specifications reviewable: e.g. to account for specific regional effects or adverse seasonal impacts on quality or early or late seasonal variances as agreed and communicated formally in writing.

## Appendix B: The random forest algorithm

Random forest (RF) is a classification algorithm that creates numerous decision trees and aggregates their results – it is a type of ensemble learning proposed by Breiman (2001). A decision tree is a map of possible outcomes which weighs various factors to predict the most likely outcome. RF uses bagging of classification trees to determine the final outcome of each classification (Liaw & Wiener, 2002). In bagging, or bootstrap aggregation, each individual decision tree randomly samples from the dataset with replacement. As decision trees are sensitive to the data they are trained on, this results in the independent creation of unique decision trees using a bootstrap sample of the dataset. A majority vote is then taken to decide the final prediction (Liaw & Wiener, 2002). An overview of a simple random forest classifier is visualised below in **Figure B.1 (A)**.

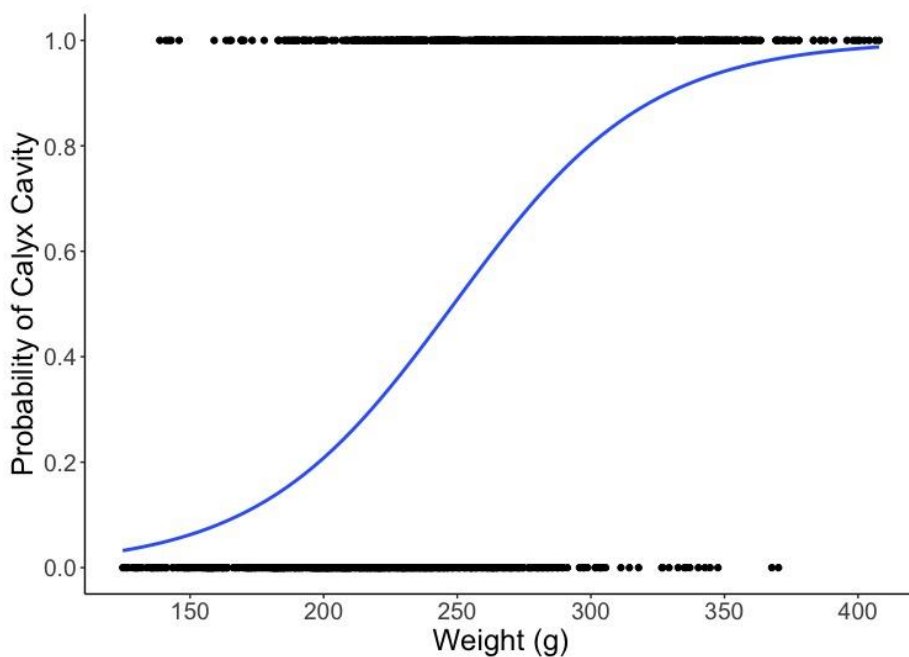




**Figure B.1** A basic diagram showing the overview of the processes involved in a random forest modelling (A), and a comparison of node splitting between regular decision trees and those utilising feature randomness (B).

Random forests also utilise feature randomness which alters the way the trees are constructed. In standard decision trees, each node is split by the feature that produces the most separation between observations (Liaw & Wiener, 2002). In a random forest, each node is split using the best feature from a random subset of these features (**Figure B.1 (B)**). This drives greater variation amongst the decision trees, resulting in more diverse, less correlated trees (de Santana et al., 2018). The combination of bagging and feature randomness means trees are both trained on different datasets and also use different features to make decisions. This helps to reduce errors and generate more accurate predictions compared to individual decision trees (Liaw & Wiener, 2002).

## Appendix C: Simple binary logistic regression



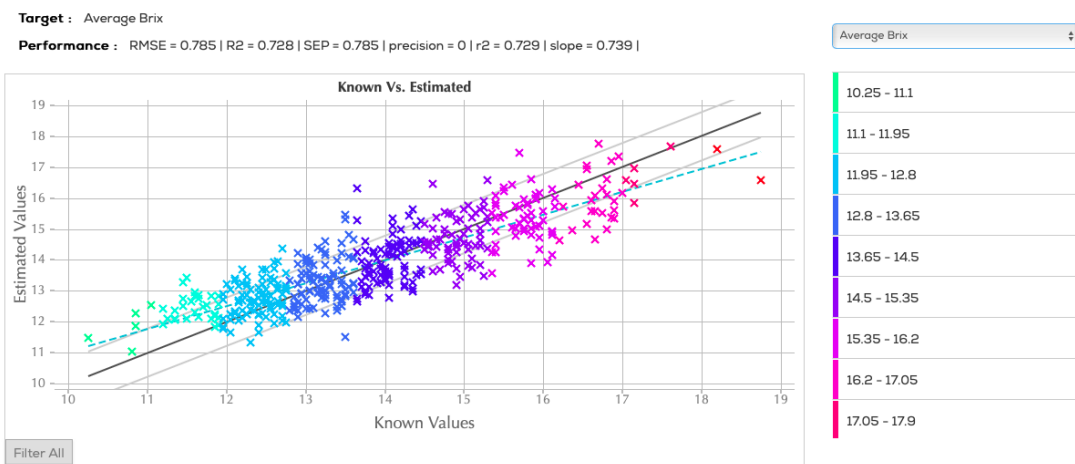
**Figure C.1** A binary fitted line plot; the binary response is the presence (1) or absence (0) of calyx cavity, and the continuous predictor is the weight.

Simple binary logistic regression was used to determine whether the weight of persimmon is related to the presence of calyx cavity. The response value of 1 on the y-axis represented the presence of calyx separation. **Figure C.1** shows that the probability of calyx separation increased as the weight increased. When the weights in the data were near 100 g, the slope of the line was not very steep, which suggested that the probability increased slowly as weight increased. The line was steeper in the middle portion of the weight data, which indicated that a change in weight of 1 g had a larger effect in this range. When the probability of calyx separation approached one at the high end of the weight range, the line flattened again. In the case of a good model fit, the high predicted probabilities should show where the event is most frequent. This was seen when the weights in the data were near 400, the response value of 1 was most common. As the weight decreased, the response value of zero became more common; as the weight increased, the response value of one became more common. The association between calyx separation and fruit weight was found to be statistically significant ( $p\text{-value} < 2 \times 10^{-16}$ ). However, the AIC (estimation of prediction error) of this model was 1296.3 which is high, indicating that the model does not fit the data well.

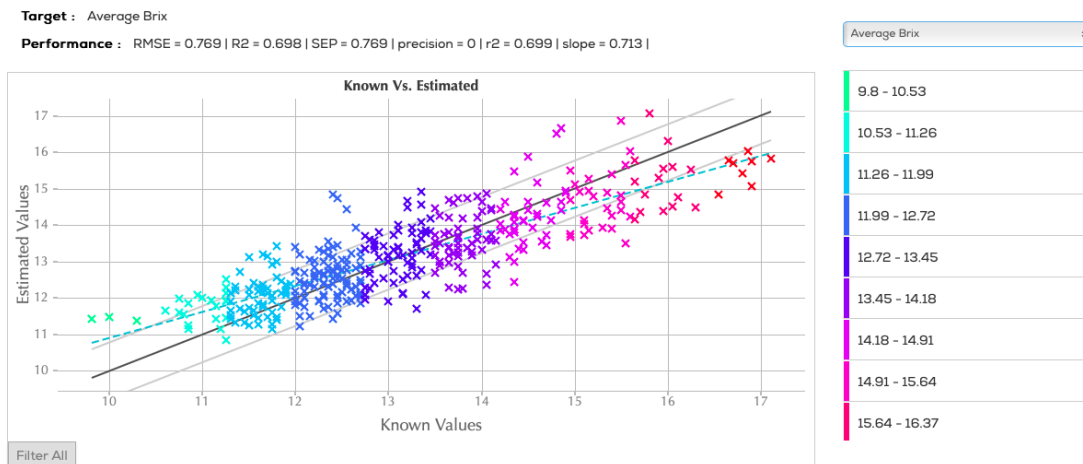
## Appendix D: NIR cavity & quality predictions

The following graphs were created by the SCIO™ online application showing the PLS regression model predictions compared to known values for select quality attributes, and the RF classification of the presence and severity of calyx separation versus the actual categorisation.

### SCC predictions at harvest



### SCC predictions after storage

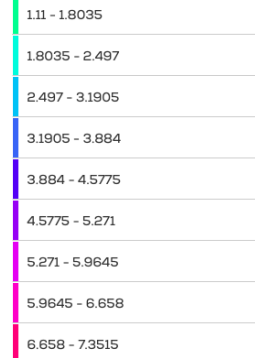
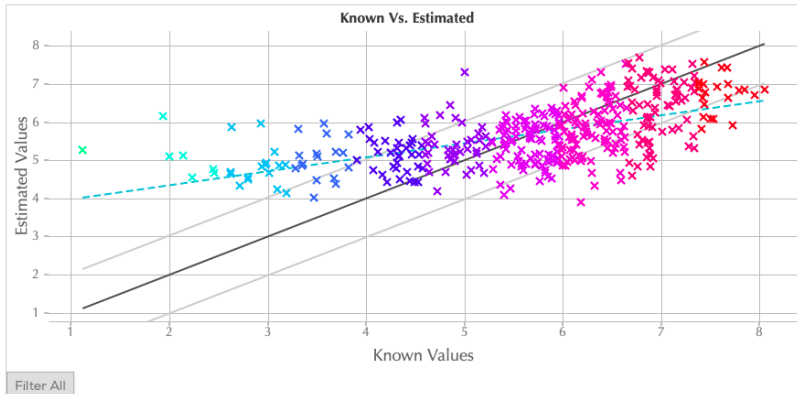


## Flesh firmness predictions at harvest

**Target :** Penetration Firmness

**Performance :** RMSE = 1.03 | R2 = 0.336 | SEP = 1.03 | precision = 0 | r2 = 0.338 | slope = 0.368 |

Penetration Firmness

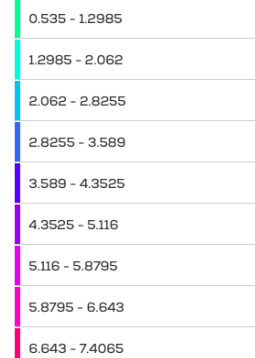
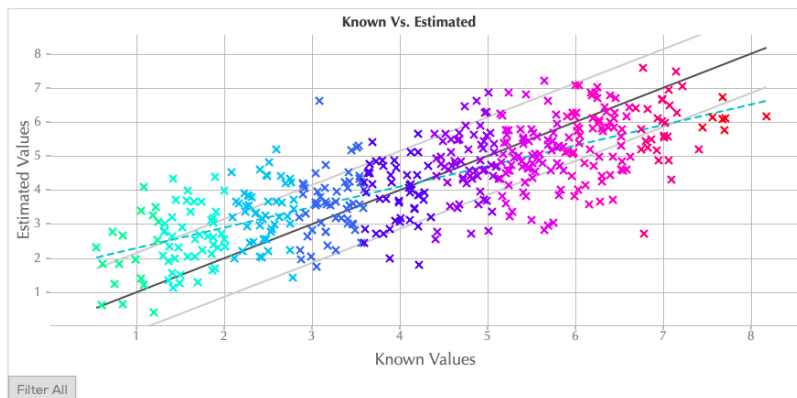


## Flesh firmness predictions after storage

**Target :** Penetration Firmness

**Performance :** RMSE = 1.149 | R2 = 0.576 | SEP = 1.149 | precision = 0 | r2 = 0.577 | slope = 0.602 |

Penetration Firmness

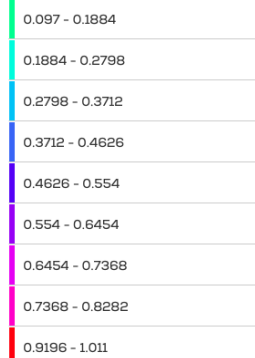
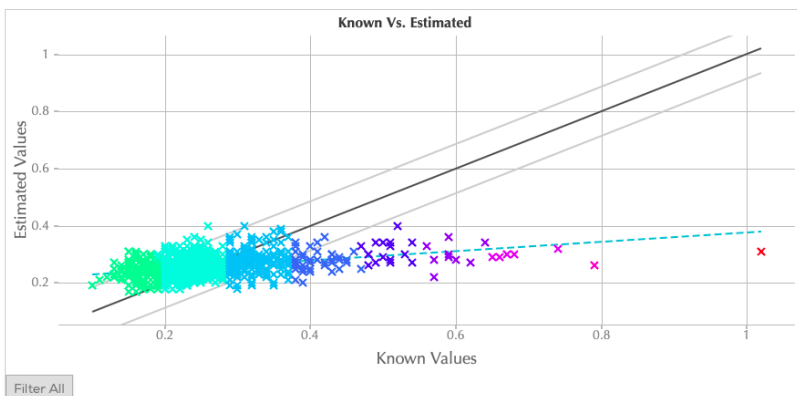


## Non-destructive compression predictions at harvest

**Target :** Compression Firmness - strain

**Performance :** RMSE = 0.086 | R2 = 0.151 | SEP = 0.086 | precision = 0 | r2 = 0.152 | slope = 0.164 |

Compression Firmness - strain

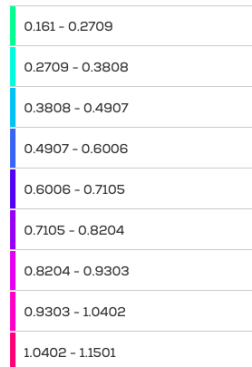
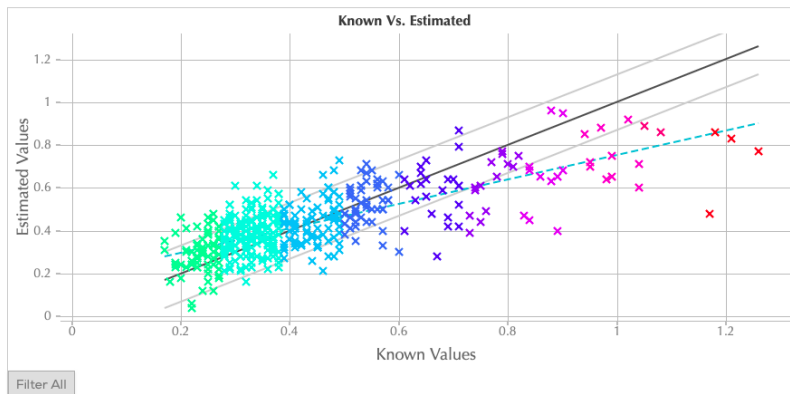


## Non-destructive compression predictions after storage

**Target :** Compression Firmness - strain

**Performance :** RMSE = 0.13 | R2 = 0.549 | SEP = 0.13 | precision = 0 | r2 = 0.55 | slope = 0.572 |

Compression Firmness - strain

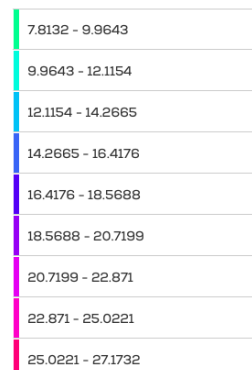
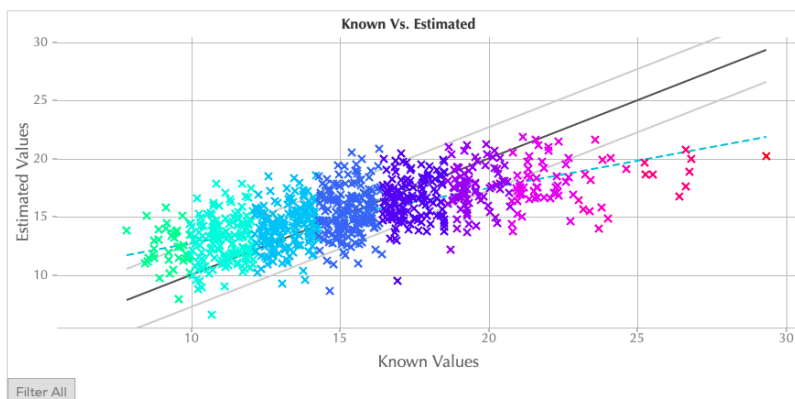


## Colour index predictions at harvest

**Target :** CI Average

**Performance :** RMSE = 2.696 | R2 = 0.453 | SEP = 2.696 | precision = 0 | r2 = 0.454 | slope = 0.474 |

CI Average

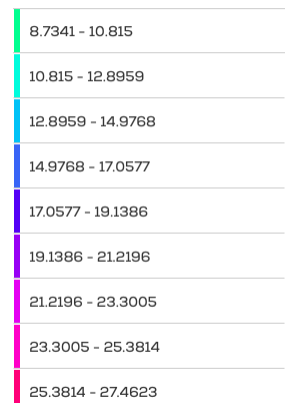


## Colour index predictions after storage

**Target :** CI Average

**Performance :** RMSE = 2.711 | R2 = 0.489 | SEP = 2.711 | precision = 0 | r2 = 0.492 | slope = 0.528 |

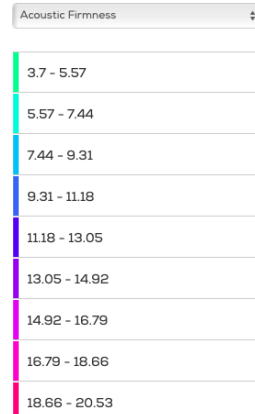
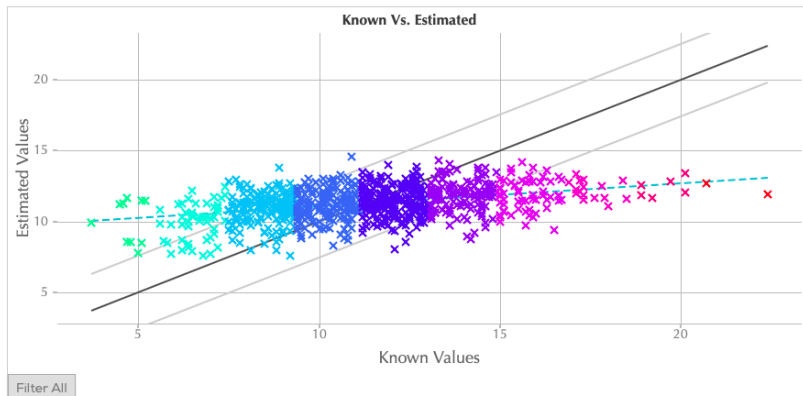
CI Average



## Acoustic firmness predictions at harvest

**Target :** Acoustic Firmness

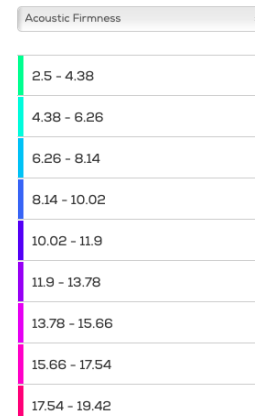
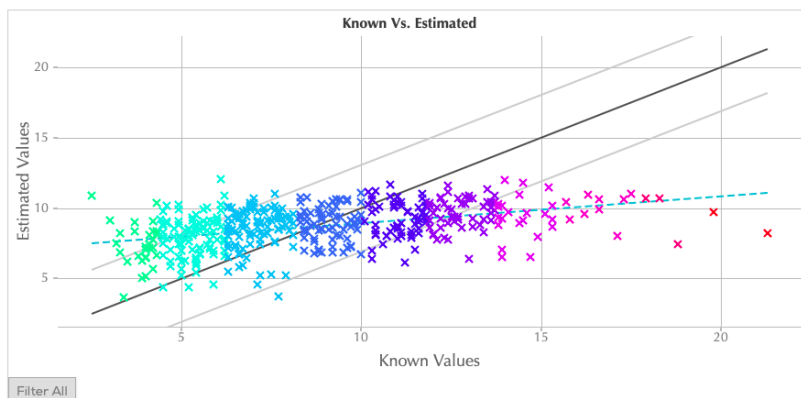
**Performance :** RMSE = 2.567 | R2 = 0.154 | SEP = 2.567 | precision = 0 | r2 = 0.154 | slope = 0.166 |



## Acoustic firmness predictions after storage

**Target :** Acoustic Firmness

**Performance :** RMSE = 3.077 | R2 = 0.187 | SEP = 3.077 | precision = 0 | r2 = 0.187 | slope = 0.189 |



## Calyx cavity confusion matrix at harvest

**Target :** Calyx Cavity

**Performance :** F1 = 0.655 |



## Calyx cavity confusion matrix after storage

Target : Calyx Cavity

Performance : F1 = 0.673 |

1	30%	66%
0	69%	33%
Classified Known Class	0	1

## Cavity severity confusion matrix at harvest

Target : Cavity Grade

Performance : F1 = 0.37 |

4	0%	0%	0%	14%
3	1%	3%	23%	21%
2	13%	21%	17%	14%
1	84%	74%	58%	50%
Classified Known Class	1	2	3	4

## Cavity severity confusion matrix after storage

Target : Cavity Grade

Performance : F1 = 0.405 |

4	0%	3%	0%	25%
3	2%	6%	41%	0%
2	9%	22%	12%	12%
1	87%	68%	45%	62%
Classified Known Class	1	2	3	4