An examination of postharvest techniques to enable seafreight export of feijoa (*Acca sellowiana* [O.Berg.] Burret)

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Abstract

Export of feijoa (*Acca sellowiana* [O.Berg.] Burret) to the main markets in Europe, Asia and North America is currently by airfreight that is not only expensive but rather unsustainable as the industry expands. With the ongoing breeding works and expansions of plantings, growers will eventually have to seek for an economic mode of transport. As is the case with kiwifruit, apples, avocadoes, and squash, seafreight will provide an alternative option that is both cheaper and accommodates large fruit volumes. The short storage life of feijoa, however, is likely to pose a challenge to seafreight (that requires at least 6 weeks of storage) if appropriate postharvest techniques are not identified to extend storage life. Feijoa stores for about 4 weeks at 4 °C after which it becomes overripe, loses flavour, and develops chilling injury and internal browning. This study was undertaken to examine potential postharvest techniques that could extend storage life and maintain quality of feijoa. The postharvest techniques investigated were temperature and relative humidity management, harvest timing, step down conditioning, intermittent warming, chlorophyll fluorescence and development of non-destructive grading tools. The varieties used in this study were 'Kakariki', 'Wiki Tu' and 'Triumph' that were stored for 8 weeks under various conditions.

To assess the effects of temperature and relative humidity in storage, 'Kakariki', 'Wiki Tu' and 'Triumph' were stored at 1 °C (85% RH) and 4 °C (88% RH). These conditions were set to result in equal water vapour pressure deficits at both temperatures. The effects of RH on feijoa quality during 8 weeks storage were

tested by using a polyethylene liner (polyliner) to cover the fruit in each tray, for half of the treatments. Despite good retention of some attributes indicating quality (firmness and skin colour) for up to 8 weeks at 1 °C, many fruit developed chilling injury making it unsaleable and therefore causing huge losses. At both 4 °C and 1 °C the use of a polyliner resulted in reduced water loss, suggesting polyliners may be beneficial for feijoa storage. Given the chilling injury results, it is imperative to consider treatments that may reduce chilling injury and yet maintain fruit quality.

To alleviate chilling injury and extend storage life of 'Kakariki', 2 harvesting times (early (H1) and commercial (H2)), 2 storage temperatures (2 °C and 4 °C) and three conditioning treatments (single step down, [6 d at 9 °C then moved to 2 °C or 4 °C], double step down [3 d at 9 °C , 3 d at 6 °C then moved to 2 °C or 4 °C] and 'no conditioning' control [stored direct to 2 °C or 4 °C]) were established. Results showed that early harvested fruit had lower chilling injury incidence and retained more quality attributes thereby providing a possibility of extending Kakariki' feijoa storage life. There was no evidence for a difference in quality arising from storage at 2 °C or 4 °C but it was evident that single or double step down conditioning simply allowed an extended period of postharvest ripening because of the 6 d delay in reaching the more appropriate storage temperature of 2 °C or 4 °C. This led to faster deterioration of fruit. Therefore, it is advisable to rapidly cool feijoa soon after harvest to reduce metabolism and ripening; but then sell the fruit before they develop CI.

To assess the effects of intermittent warming (IW) on improving quality of 'Triumph' fruit. Three (3) intermittent warming conditions were tested (IW from

4 °C to 20 °C for 1 d after every 6 d storage, IW from 4 °C to 20 °C for 1 d after every 10 d storage and control) and stored at 4 °C for 6 weeks. Chlorophyll fluorescence was used as a non-destructive tool to assess quality. The results showed that intermittent warming just like conditioning treatments, accelerated ripening leading to faster deterioration. A decline in quantum yield (*Fv*/*Fm*) was observed during storage in the absence of CI. This suggests that it is linked to loss of chlorophyll content and chloroplast membrane injury associated with photosystem II (PSII) as feijoa ripened. The continuous decline in quantum yield (*Fv*/*Fm*) offers potential for a non-destructive technique to assess feijoa ripeness and could therefore be used in a cool store to detect batches of fruit that are ripening more quickly for immediate sales or those ripening slowly that may be more suited to export or long storage.

To re-evaluate the internal maturity/ripeness scale developed by scanned images of 'Kakariki', 'Wiki Tu' and 'Triumph' varieties from at harvest through storage were assessed against the PFR scale. The results showed that the PFR scale worked well for maturity assessment of 'Kakariki', 'Wiki Tu' and 'Triumph' varieties at harvest, despite their quite different internal anatomy. The same scale was also appropriate for each variety as a post-storage ripeness indicator. Evidence also suggested that one new step was required an internal maturity rating of 1.5. The problem was that fruit at stage 1 could be immature (and not ripen during storage) or mature. This new stage was used to describe fruit showing the first signs of locular gel clearing, suggesting that ripening was definitely underway. Firmness (non-destructively assessed) at harvest was correlated with quality after storage and therefore showed potential to predict fruit ripening behaviour in storage. This implies, that firmness could be used non-destructively in sorting lines to select firm fruit for long storage or soft fruit for immediate consumption.

Based on these findings', storage life of 'Kakariki', 'Wiki Tu' and 'Triumph' feijoa could not be reliably extended beyond 4 to 6 weeks and therefore seafreight export is still risky. The wide range of maturity variation within any batch of harvested feijoas accounts for much of this risk. Future research should focus on finding a rapid, non-destructive technique that can detect the new internal maturity/ripeness rating 1.5. This would assist growers to grade early harvested fruit and select mature but longer-storing fruit for export.

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Dedication

This work is dedicated to my lovely Hilary, Hellen and Leonard

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List of Abbreviations and Symbols

ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	analysis of variance
APX	ascorbate peroxidase
AVG	aminoethoxyvinylglycine
AW	water activity
°C	degree Celsius
C*	chroma
CA	controlled atmosphere
$CaCl_2$	calcium chloride
CAT	catalase
ChF	chlorophyll fluorescence
CI	chilling injury
cm	centimetre
CO_2	carbon dioxide
CRD	completely randomised design
d	days
DM	dry matter
EDTA	ethylene diamine tetraacetic acid
EMAP	equilibrium modified atmosphere packaging
FDA	American Food and Drug Administration
FFDCA	Federal Food, Drug and Cosmetic Act
Fig	figure
F_m	maximum fluorescence
F_o	minimum fluorescence

fob	free on board
F_v/F_m	PSII quantum yield
FW	fresh weight
g	gram
GLM	general linear model
GRAS	generally regarded as safe
°hue	hue angle
h	hours
H1	harvest fruit between 7-10 d before touch picking
H2	harvest fruit at touch picking
H_2O_2	hydrogen peroxide
HSD	Tukey's honest significant differences
HWD	hot water dipping
IAA	indole-3-acetic acid
IM	internal maturity
IR	internal ripeness
IW	intermittent warming
K_2CO_3	potassium carbonate
KCl	potassium chloride
kg	kilogram
KOH	potassium hydroxide
kPa	kilo Pascal
L	litre
L*	lightness
LiCl	lithium chloride
MA	modified atmosphere
MAP	modified atmosphere packaging

1-MCP	1-methylcyclopropene
MDA	malonyldialdehyde
MeJA	methyl jasmonate
$MgCl_2$	magnesium chloride
$MgNO_3$	magnesium nitrate
min	minutes
mL	millilitre
mm	millimetre
$	New Zealand dollar
N	newton
NaOH	sodium hydroxide
NIR	near infrared
nL	nano litre
nm	nano metre
nmol	nano mole
NO	nitric oxide
NZFGA	New Zealand Feijoa growers association
O_2	oxygen
O_2^-	superoxide
OH^-	hydroxyl radicle
PAs	polyamines
PB	peel browning
PCA	principal component analysis
PFR	New Zealand Institute of Plant and Food Research
PLA_2	phospholipases A2
PLD	phospholipases D
POD	peroxidases

ppi	pixels per inch
PPO	polyphenol oxidase activity
PSII	photosystem II
PVPP	polyvinyl polypyrrolidone
Q_{10}	temperature quotient
RH	relative humidity
ROS	reactive oxygen species
RQ	respiration quotient
s	second
SA	salicylic acid
SAM	S-adenosylmethionine
SBO	Southern Belle Orchard
SDC	step down conditioning
SOD	superoxide dismutase
SSC	soluble solid content
t	tonnes
TA	titratable acidity
TPA	texture profile analysis
USA	United States of America
VPD	vapour pressure deficit
WVP	water vapour permeance
ZR	zeatin riboside

Chapter 1 Introduction

1.1 Background to the study

Consumer demand for a wide range of fresh fruit and vegetables has continued to increase in recent times, partly driven by public interest in health benefits associated with their consumption (Artés et al., 2009). The demand has led to increased sales and new market opportunities; although the overriding challenge is maintaining products in their fresh state and in acceptable quality until they are consumed (Wills and Golding, 2016). Feijoa (*Acca sellowiana* [O.Berg.] Burret) is widely grown in New Zealand and has potential for increased exports if new markets can be reached (Amarante et al., 2017) Feijoa has carved a niche as a 'sociable fruit' because the fruit does not store well, and people share their feijoas with friends and neighbours during the months of April through to July (Prichard, 2017). Popularity of feijoa in New Zealand is attributed to its unique aroma and flavour, and a high level of nutritive value (Al-Harthy et al., 2008; Schotsmans et al., 2011). The fruit, originally from South America and a member of the Myrtaceae family, has also found its way into several other parts of the world including France, Italy, USA, Colombia, and Russia (Belous et al., 2014; Borsuk et al., 2017).

Feijoa as a minor commercial fruit in New Zealand is mostly traded in the local markets largely due to its short storage life of about 4 weeks, however potential for export does exist (Sharpe et al., 1993; Thorp and Bieleski, 2002). In 2019, published information records 225 feijoa growers who produced 1,200 t of fruit from 175 ha worth $ 4.0 M (domestic) and $ 0.2 M (export [fob] (Aitken and Warrington 2019). Growers' currently exporting feijoa use airfreight, which is fast but very expensive. Their main export markets are in Europe, Asia, and North America, which could easily be accessed by sea. Transportation by sea from New Zealand to such distant markets can take about 2-6 weeks depending on locality. For all shipments it is important to factor in delays in delivery because of time taken to load and fill a container, ship, unload, distribute and finally display product at retail stores (Beaudry, 1999). Seafreight of feijoa to a market that requires more than three weeks of storage life has a high risk of significant product losses due to over ripeness and decay. The recent attempts by some members of the New Zealand feijoa growers association (NZFGA) to ship feijoa to Singapore attests to the seafreight challenges. In 2017, some feijoa growers gathered their fruit within a day and filled a refrigerated container to ship to Singapore. After 2.5 weeks at sea, the container arrived on shore with product of widely varying quality. Some fruit had a few days left of storage life (okay for consumption); some were overripe while others were already rotting (Frans de Jong, personal communication, January 23, 2019). Apart from the challenge of seafreight, the issue of variation in quality points to problems in harvesting fruit at the right maturity. This clearly shows that more work still needs to be done to achieve premium quality for the feijoa industry to be competitive (Aitken and Warrington, 2017). Especially now that the NZFGA are adopting zeijoa as a growers brand with an aim of "taking New Zealand feijoas to the rest of the world" (Frans de Jong, personal communication, January 23, 2019).

Selection and breeding of new feijoa varieties are ongoing in Brazil, Colombia, New Zealand, and Uruguay (Schotsmans et al., 2011; Amarante et al., 2017; Parra-Coronado et al., 2017b; Urraburu et al., 2018). In New Zealand feijoa was first introduced in 1908 with minimum attention. In 1920s 'Choiceana' and 'Coolidge' seeds arrived under Hayward Wright, although it was not until 1942 that 'Triumph' was released and thereafter several other minor varieties (Thorp and Bieleski, 2002). In 1983 'Apollo' and 'Gemini' were released by Kevin Patterson, while 'Opal Star' was released in 1989 by Grant Thorp. Since the early 1990s Roy Hart has released several varieties including his early ones

‘Kakapo’ and ‘Pounamu’ in 1992. More recent varieties released by Hart that are now under commercial production mainly due to their bigger size include ‘Anatoki’, ‘Kakariki’ and ‘Wiki Tu’. Studies to confirm how these varieties respond to storage conditions have not been conducted. Therefore, in this study ‘Kakariki’ and ‘Wiki Tu’ (the new varieties) and ‘Triumph’ (an older variety) were used to reassess feijoa storage conditions (Thorp and Bieleski, 2002).

Low temperature and high relative humidity retain quality by reducing fruit metabolism. However, in industrial practice the two factors can be difficult to control unlike in laboratory situations where all factors can be closely regulated. Application of low temperature by Klein and Thorp (1987), Al-Harthy et al. (2008), and (Amarante et al., 2013) to extend storage life of feijoa was able to achieve only 4 weeks of storage. East et al. (2009) and Rupavatharam (2015) also applied low temperature in addition to controlled atmospheres and their success in extending storage life was also minimal.

Growers who export feijoa traditionally package the fruit in corrugated fibre boxes without using polyliners because the growers believe polyliners lower fruit storage life. Since feijoa are airfreighted, the requirement for optimal temperature and RH control is not stringent as for longer-term storage and fruit generally arrive at their destination in good condition. However, if seafreight becomes an alternative then optimising temperature and relative humidity control during transit needs revisiting. Paull (1999) argues that in large storage facilities control of relative humidity (RH) is difficult. A small change in RH could lead to condensation and fungal growth or excessive weight loss, and these is possibly the worry of feijoa growers. This study will revisit temperature and relative humidity conditions for emerging new varieties ('Kakariki', 'Wiki Tu') that are becoming popular with increased planted areas.

Long storage life of feijoa implies extended periods when the fruit is constantly at low temperature. Feijoa, being a sub-tropical fruit suffers from chilling injury at 4 °C, which is the recommended storage temperature of feijoa (Thorp and Bieleski, 2002). However, kiwifruit another subtropical fruit can be stored for up to 6 months at 1-2 °C despite a few cases of chilling injury. Chilling injury (CI) affects fruit quality, which directly affects growers' profits but unfortunately has received little attention. Woolf et al. (2006) outlined symptoms of chilling injury in feijoa as reddening of the flesh and skin browning; however, it is possible that other signs do exist (Al-Harthy et al., 2010a; Rupavatharam, 2015). For instance, symptoms of CI as documented for other horticultural crops include pitting, discoloration, internal breakdown, loss of flavour, and failure to ripen. These responses are possibly due to changes in cell membranes and an accompanying production of reactive oxygen species (ROS) (Wang, 1993; Pan et al., 2017; Heyes, 2018). Often these symptoms will not be visible until after the product is transferred to 20 °C (Klein and Thorp, 1987). Implications of these effects would be unacceptable especially to consumers if the product has been shipped overseas and after offloading and distribution, it is found to have deteriorated in transit. This study will evaluate step down conditioning and intermittent warming as ways to control CI in feijoa. In addition, chlorophyll fluorescence will be used to as a tool to evaluate the quality of feijoa.

The long flowering period of feijoa that takes about 4-6 weeks, leads to an equally longer fruit setting period that results into a mixture of maturities at harvest. Touch picked fruit seems to provide ready to eat fruit which complicates storage (Rupavatharam et al., 2015c). Touch picking as a harvesting technique has been attempted to standardise maturity, however, other researchers in literature have already shown it not to be accurate. Fruit harvested at touch picking can have fruit with locular gel area at different stages of maturities and therefore there is a need to find a way to optimise harvest time to extend storage life. In this study, the author will evaluate harvesting time as a technique that can extend feijoa storage life.

Another issue of importance in the feijoa industry is the lack of a well-developed maturity index. Currently the industry relies heavily on the internal maturity scale developed in 2004 by the New Zealand Institute of Plant and Food Research (PFR). This scale depends on cutting the fruit transversely and visually assessing it internally (Schotsmans et al., 2011). While the scale is referred to as the 'internal maturity', it is important to note that it has traditionally been used to assess ripeness after storage of feijoa (Duan, 2015; Rupavatharam, 2015). The internal maturity ratings 4 to 7 are not find at harvest because fruit fall from the tree at around internal rating 3, since the pedicel-fruit retention force becomes weak. Ever since the scale was developed, it has not been revisited to ascertain if it is till adequate for all varieties including the recently released ones. In addition, there has been little success with attempts to correlate internal maturity ratings with objectively assessed fruit attributes such as firmness, skin colour or sugar concentration. In this study, the author will check the suitability of the PFR scale for assessing harvest maturity for all tested varieties; assess its usefulness for ripening of the tested varieties after storage and determine correlations with objective measures to allow non-destructive harvest maturity assessment.

1.2 Thesis outline

Although feijoa is a popular fruit, it does not come close to what kiwifruit is to the New Zealand horticulture industry. Remarkably, feijoa and kiwifruit were introduced in New Zealand at the same time, and currently kiwifruit exports are top on the list for horticultural products bringing in high foreign exchange (Skinner et al., 2013; Aitken and Warrington, 2017). In attempts to improve feijoa quality there has been development of new feijoa varieties based on size (big) and harvesting time (season). The large commercial plantings of these new varieties without appropriate knowledge about their storage potential is a problem thus the need for this study.

This thesis is presented in eight chapters summarised as follows: -

Chapter 1 gives an overview of the research problem for this study. It has covered aspects of feijoa storage temperature and humidity, chilling injury development, internal maturity assessment, harvest timing and objective non-destructive assessment.

Chapter 2 reviews existing literature where the research problem of short storage life was articulated. The chapter starts by looking at the origins of feijoa, its botanical description and the ecological conditions that favour its growth and development. Quality is what determines if feijoa sells or not and therefore, the review has evaluated some postharvest factors, influencing quality in which focus was on storage temperature and humidity. The critical analysis of these factors and the preliminary results obtained from first year studies narrowed down the issue that affected quality to chilling injury development of feijoa. The remaining parts of the chapter expounded on what chilling injury is, how it affects the normal physiological function of tissues and techniques that can be used to alleviate it. Another issue that arises during the study is the need to find an objective, non-destructive assessment for harvest maturity, which eventually leads to re –evaluation of the internal maturity scale developed in 2004 by the New Zealand Institute of Plant and Food Research (PFR).

Chapter 3 provides an overall description of the generic materials and methods used in the thesis. Specific methods required for particular research questions are covered in the results chapters 4, 5, 6 and 7.

Chapter 4 focuses on reassessing temperature and relative humidity conditions suitable for storing feijoa for three varieties 'Kakariki', 'Wiki Tu', and 'Triumph'. 'Kakariki' and 'Wiki Tu' are popular varieties released recently whose cultivation has been widely adopted by growers and yet scanty information exists about their postharvest behaviour. 'Triumph' on the other hand is an older variety developed in the early 1940s and known to have a better storage life (Thorp and Bieleski, 2002).

Chapter 5 and 6 focus on some possible mechanisms to alleviate chilling injury. Those tested were stepdown temperature conditioning and intermittent warming. Application of chlorophyll fluorescence meter was tested as a non-destructive tool to assess quality of feijoa during storage.

In chapter 7 the author revisited the internal maturity-rating index that was developed by PFR in 2004. Internal maturity/ripening ratings of three varieties ('Kakariki', 'Wiki Tu', and 'Triumph') were assessed and distinct features in the locular area identified. Internal maturity/ripening ratings of individual fruit were further correlated with other fruit attributes to ascertain if they could be used for objective non-destructive assessment of harvest maturity or post-storage ripening.

Chapter 8 gives an overall discussion and recommendations. This includes the summarised outcomes and possible implications for the feijoa industry. This chapter also proposes opportunities for further research with an aim of extending feijoa storage life.

Chapter 2 Literature Review

2.1 Origin, taxonomy, botanical description and ecology of feijoa

Feijoa (*Acca sellowiana* [Berg] Burret synonym *Feijoa sellowiana*) is a subtropical fruit originally from South America specifically; areas of southern Brazil, north Argentina, Uruguay, and east Paraguay (García-Rivera et al., 2016). Other names used to identify feijoa include 'goiabeira serrana' in Brazil, 'guayobo del pais' in Uruguay guavasteen, pineapple guava and New Zealand banana, though the latter is not commonly used (Morton, 1987; Janick and Paull, 2008;). Since the late 1890s, feijoa has been introduced to New Zealand, USA (California, Florida), the Mediterranean region (Israel, Spain, France and Italy), Australia, Russia, Algeria, Libya, and South Africa (Sharpe et al., 1993; García-Rivera et al., 2016). Popular members in feijoa family (Myrtaceae) include guavas (*Psidium guajava* L.), eucalyptus (*Eucalyptus spp*), myrtle (*Myrtus communis*), pohutukawa (*Metrosideros excels*), clove (*Syzygium aromaticum*), allspice (*Pimenta dioica*), and bay rum tree (*Pimenta racemosa*).

Feijoa is a perennial tree that grows to a height of 6 meters. The leaves are evergreen, shiny and heavy, while flowers are a bright red and showy (Thorp and Bieleski, 2002; Schotsmans et al., 2011). Feijoa fruit is a berry, dark green and oval to round depending on the variety and can be either smooth or rough skinned. The fruit generally has 4-5 transparent locules in which the seeds are enclosed inside a white juicy gelatinous flesh that produces an aroma when ripe (Fig.2-1, (Amarante et al., 2008). The pericarp is gritty and has vascular bundles that become soft on ripening, while the skin is sour and bitter (Weston, 2010).

Feijoa can do well in acidic soils or soils that are not fertile, but for commercial farming it is best to provide well-draining sandy loam soils that are rich in humus (Thorp and Bieleski, 2002; Schotsmans et al., 2011). Feijoa grows in subtropical areas with average temperatures of between 16 -18 °C and annual rainfall of between 1350 -1700 mm (Thorp and Bieleski, 2002). Similarly, it can grow in warm dry summers if cool winters provide the chilling needed to initiate flowering (Sharpe et al., 1993). This therefore makes feijoa adaptable to different agro-ecological areas (Parra-Coronado et al., 2016). In Colombia (a tropical area), feijoa is harvested all year round. Peak harvesting periods are between February - August and in December, while low season is around September –November and in January. In temperate areas feijoa is ready for consumption during the autumn periods in both northern and southern hemispheres (Schotsmans et al., 2011). Morton (1987) reports that in warm regions, the fruit develops better flavour and for that reason has a better chance of being further developed commercially.

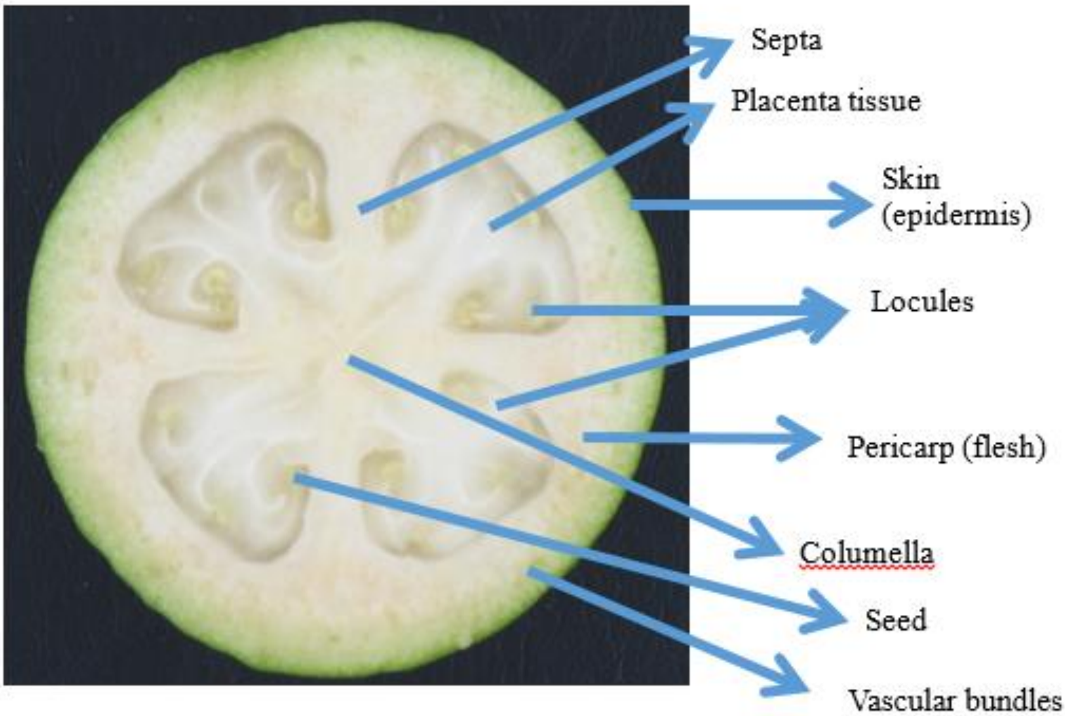


Figure 2-1 Fruit anatomy showing parts of a ripe feijoa fruit.

2.2 Uses and Importance (Economic and nutritional) of feijoa

Commercial production of feijoa has advanced in New Zealand, Colombia, California, Georgia and Azerbaijan (Schotsmans et al., 2011). Production in New Zealand is found around the North Island and the upper regions of the South Island. In New Zealand, an orchard of mature feijoa trees can yield around 22 tonnes of fruit/ha, which approximates to 30-40 kg of fruit/tree (Janick and Paull, 2008). The group further noted that commercial feijoa orchards produce about 950 tonnes of fruit for fresh consumption and 200 tonnes for processing; however, (Schotsmans et al., 2011) reported that active growers produced about 500 tonnes of fruit per year mainly sold locally and a few to Australia and the USA. Moreover, Aitken and Hewett (2016) and Aitken and Warrington (2019) estimates production of feijoa in New Zealand between 2015/2016 and 2018/2019 to be about 1,200 tonnes valued at $ 3.8 m (domestic) and $ 0.3 m (export fob). The confusion in figures from these commentators on the industry suggests at the need to harmonize information to give a truer picture of the feijoa industry in New Zealand.

Feijoa can be eaten fresh or processed and their aroma and flavour remain unaffected by processing. Some of the processed products include yoghurts, ice cream, chocolates, pies, wines, jams, liqueur, and juices (Morton, 1987; Thorp and Bieleski, 2002; Schotsmans et al., 2011). Even though the period for consumption of fruit is short, personal taste and preferences guide how fruit is consumed. Some consumers may prefer fruit when tart while others enjoy fruit that is sweet with the strong unique flavour and aroma (Schotsmans et al., 2011).

Biochemical analysis of the fruit suggests that it is rich in vitamins and minerals such as ascorbic acid (vitamin C), potassium, iodine, fibre, calcium, and magnesium (Weston, 2010). Numerous studies have also found antimicrobial properties against bacteria and fungi, antioxidant, anti-inflammatory, anti-cancer and anti-mutagenic properties (Weston, 2010; Amarante et al., 2017; Peng et al., 2018). Besides consuming the fruit, traditional communities in Brazil and Uruguay use the fruit for medicinal purposes; supporting the conclusion, that feijoa has nutraceutical properties (Janick and Paull, 2008; Schotsmans et al., 2011).

2.3 Cultivar availability

There is continuous private breeding work going on in New Zealand to provide growers with superior cultivars. New Zealand cultivars are all based on the Uruguay fruit type which has small seeds and are very juicy (Santos et al., 2017). Studies by (Amarante et al., 2008) to compare the two major fruit types (Uruguay and Brazilian) found that they exhibited similar behaviour. More studies on the physiology, and preharvest and postharvest behaviours of both Brazilian and Uruguayan types are needed for objective comparison to aid in selecting superior genotypes and scale up commercial production of feijoa (Amarante et al., 2008).

Variety/cultivar selection will depend on market demands. For instance, requirements for export fruit are different from that of local or processing fruit (Thorp and Bieleski, 2002). Selection of cultivars for export will ensure only top-quality fruit in terms of earliness, size, aroma, sweetness, and storability (Kader and Rolle, 2004). In New Zealand, apart from, 'Apollo', 'Unique' and 'Wiki Tu' which are partially self-fertile other commercial varieties are self-sterile and hence require planting of different cultivars in alternating rows to increase pollination (F. de Jong, personal communication, April 13, 2016).

Table 2-1 shows description of some commercially grown cultivars including recently bred varieties available in New Zealand. Information provided include fruit size, skin colour and rate of development to horticultural maturity. In the table, the cultivars are grouped depending on the harvest season.

Table 2-1 Examples of commercially grown including recently bred feijoa in New Zealand

Harvest season	Cultivar	Breeder	Breeding locality (former &/or current sites)	Pollination	Description of fruit
Very early Season	'Anatoki'	Roy Hart	Waimea Nurseries (New Zealand)	Self-sterile	Medium sized smooth fruit with sweet, mild flavour and matures 2-3 weeks before 'Unique'.
	'Kaiteri'	Roy Hart	Waimea Nurseries (New Zealand).	Self-sterile	Large smooth fruit, matures 2-3 weeks before 'Unique' and carries on for longer (Prolific).
	'Kakariki'	Roy Hart	Waimea Nurseries (New Zealand)	Self-sterile	Sweet and large fruit, matures 4 weeks before 'Unique'.
	'Unique'	HortResearch Tharfield Nursery	Kumeu Research Orchard New Zealand	Reliable self-fertile	Light green, medium to large size fruit, rough skin, juicy, mild flavour and aroma, prolific producer with good storage life, thinning of fruit recommended.
Early season	'Gemini'	HortResearch	Kumeu Research Orchard New Zealand	Self-sterile	Smooth, small to medium size fruit, dark green fruit with good storage life.

	'Pounamu'	HortResearch Tharfield Nursery	Kumeu Research Orchard New Zealand	Self-sterile	Medium, dark green, smooth, juicy with pleasant flavour, and good storage life.
	'Waitui'				Very uniform fruit size, good flavour.
Early season - mid season	'Apollo'	HortResearch, Tharfield Nursery	Kumeu Research Orchard New Zealand	Partially self-fertile	Large sweet, long with light green and rough skin.
	'Kawatiri'				Sweet fruit, good consistent size, 'waitui' suggested pollinator.
Mid-season	'Dens Choice'	Tharfield Nursery	Kumeu Research Orchard New Zealand	Self-sterile	Medium sized fruit, sweet, smooth mild aroma and juicy.
	'Kakapo'	HortResearch Tharfield Nursery	Kumeu Research Orchard New Zealand	Self-sterile	Medium to large, mild, sweet, refreshing taste, blocky oval, and good storage life.

	'Mammoth'	HortResearch	Kumeu Research Orchard New Zealand	Self-fertile	Selected from 'Choiceana', large round to oval wrinkled fruit, gritty flesh, good flavour, softer and doesn't ship well.
	'Manawatu'				Great flavour, medium to large fruit, self-sterile.
Mid to Late season	'Golden Goose'	Tharfield Nursery	New Zealand		Large to very large fruit, smooth skin, sweet flavour once picked, stores well.
	'karamea'	Tharfield Nursery	New Zealand		Consistent fruit size, tangy and tasty flavour, 'waingaro' the suggested pollinator.
	'Opal Star'	HortResearch	Kumeu Research Orchard New Zealand	Self-sterile	Medium to large, smooth, dark green skinned, very aromatic fruit. Heavy cropping and good storage life, requires fruit thinning.
	'Wiki Tu' ('Anilvinkoru')	HortResearch	Kumeu Research Orchard New Zealand	Partially self-fertile	Large fruit, lots of flesh, good taste with smooth texture. Stores well.
Late season	'Triumph'	HortResearch	Kumeu Research Orchard New Zealand	Self-sterile	Large, oval shaped firm fruit with rough skin, gritty, excellent flavour and good storage life. Selected from 'Choiceana' variety. Good pollinator of 'Mammoth'.

	'White goose'	Tharfield Nursery	New Zealand	Self-sterile	Medium to large oval shaped fruit, thick dark skin, and good flavour, juicy pulp.
	'Waingaro'				Heavy cropper, inconsistent fruit, very good flavour.

Source (Morton, 1987; Sharpe et al., 1993; Lim, 2012; Rupavatharam, 2015).

2.4 Feijoa maturity and harvesting

Feijoa fruit quality is influenced by maturity at harvest and harvesting method although genetic, environmental and agronomic factors (preharvest) are other influencers. Harvesting earlier or late can lead to decreased quality of fruit. Unlike many other fruit, feijoa skin colour does not change tremendously as it ripens, with the green colour only fading slightly and therefore making it difficult to visually determine maturity for harvesting (Duan, 2015). For this reason, feijoa maturity is identified horticulturally as the stage where the fruit naturally detaches with low retention force (Thorp and Bieleski, 2002). When the fruit is gently tilted sideways or forward and does not detach from the plant, then the fruit is not yet ready for harvest. This procedure is referred to as the 'touch-pick' method and orchards will employ trained pickers to apply this method (Downs et al., 1988; Thorp and Bieleski, 2002). Then again, some growers in New Zealand harvest feijoa by using nets suspended below the branches, but approximately 60 cm above the ground in order to avoid fruit bruises. During harvest, growers shake the trees and mature fruit drop into the net (Schotsmans et al., 2011). A recent assessment on maturity and storability between touch picked fruit and machine harvested fruit did not show noticeable differences (East et al., 2013).

Although touch picking is the currently recommended practice, recent studies suggest that earlier picking may have advantages for longer storage life. Rupavatharam et al. (2015c) found that harvesting 'Unique' feijoa two weeks earlier before touch picking showed potential for improving storage up to 6 weeks. However, low soluble solid content (SSC) and increased titratable acidity (TA) resulted at the end of storage and this could potentially affect eating experiences of consumer (acceptance). Thorp and Bieleski (2002) also reported that fruit harvested too early failed to develop the distinctive feijoa flavour and taste, while those harvested late suffered losses from bruising due to natural fruit drop.

Feijoa growers in New Zealand determine maturity by using a destructive visual grading scale that was developed by the New Zealand Institute of Plant and Food Research (PFR) (Fig. 2-2). In this scale 1 (A) represent a fruit that is immature, that means it was pulled early when too hard and the likelihood of the fruit ripening properly is negligible. Scale 2 (B) and 3 (C) are the right maturity stages for harvesting for export or local market respectively. At stage 2 (B) the jellied sections are half white/half clear and therefore not ready for immediate consumption, while at stage 3 (C) the jellied sections are clear and therefore it's at the start of optimum eating maturity. Stages 4 to 7 the locular area darkens and is overripe. Depending on taste and preferences stage 4 (D) maybe eaten although, it is best suitable for processing (F. de Jong, personal communication, April 13, 2016). Stage 6 (F) and 7 (G) are in the senescence stages and hence fruit are not fit for consumption. Note that the scale as designed by PFR has no rating 5 (E). This scale as developed is destructive since it involves cutting the fruit in half and comparing the internal maturity/ripeness with the PFR maturity index. According to the scale, fruit at best harvest maturity is said to be at touch pick which is usually at stage 2 (B) – 3 (C). At this stage the peduncle easily detaches from the stalk and the fruit can be expected to be stored for up to 4 weeks in cold storage (4 °C, 90 -95% RH) with subsequent 5 d storage at 20 °C (Thorp and Bieleski, 2002). This scale is also very subjective (visually assessing), which, coupled with the range of different maturities or ripeness on feijoa cultivars means that the maturity/ripeness index is not perfect and hence there are opportunities to improve it (Clark et al., 2005; Gaddam et al., 2005).

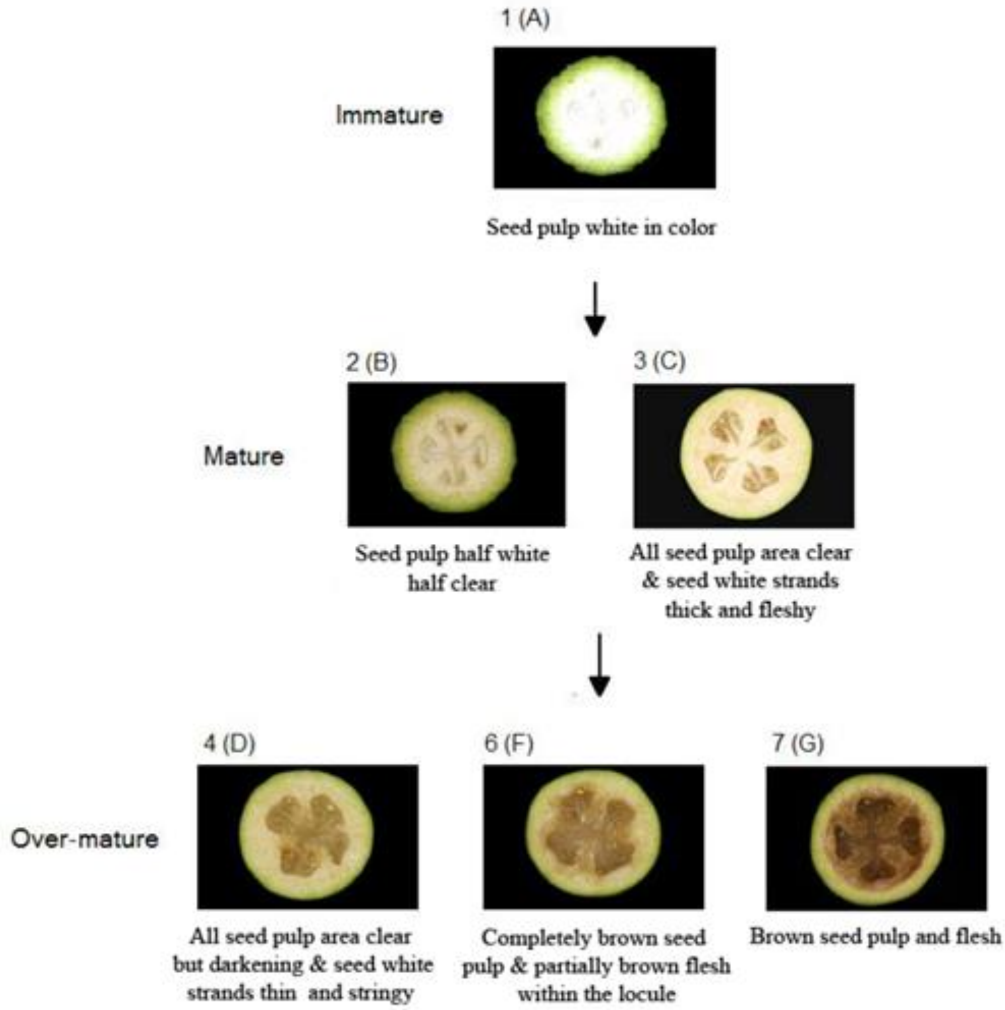


Figure 2-2 Plant & Food Research (PFR), New Zealand, 2004 Internal maturity rating scale, (image used with permission).

2.4.1 Non-destructive methods of segregating feijoa

Use of non-destructive methods such as near infrared spectroscopy (NIRS), in sorting lines helps to assess fruit maturity at harvest. NIR spectroscopy application aids in characterising and analysing fruit and vegetables by successfully developing accurate models that predict postharvest fruit quality characteristics (Nicolai et al., 2007). The essence is to form homogenous batches of fruit by using quality attributes such as firmness, soluble solids content (SSC) and dry matter (DM) of unripe fruit (Nordey et al., 2019). For instance, Harker et al. (2008) studied consumer acceptance of apples ('Red Delicious', 'Gala', 'Fuji', 'Golden Delicious' and 'Braeburn') in the USA and positively correlated consumers preferences with grading based on NIR-predicted post-storage SSC. Flores et al. (2009) in their feasibility studies of predicting internal quality parameters of individual tomatoes showed that NIRS technology was reliable with accurate prediction using SSC and TA. In another study, Magwaza et al. (2014) demonstrated the capabilities of visible to near infrared (Vis/NIR) spectroscopy of intact 'Nules Clementine' mandarin fruit at harvest to predicting postharvest rind physico-chemical properties (quality) and susceptibility to rind breakdown disorder (RBD).

Currently automated grading of feijoa relies only on sorting fruit by weight, however, several attempts to segregate feijoa maturity non-destructively have been made. Clark et al. (2005) attempted to sort 'Apollo' feijoa using fruit density but fruit could not clearly segregate into different maturity stages because the density-grading concept failed. Gaddam et al. (2005) attempted to segregate 'Unique' feijoa using non-destructive compression firmness, acoustic impulse response and near infrared (NIR) and found that complementing firmness with dry matter (DM) or sugar content measurements was necessary. On the other hand, Wiryawan et al. (2005) studied at harvest fruit qualities of 'Unique', 'Triumph', 'Apollo' and 'Mammoth' and observed big variations among fruit; although 'Apollo' were prominently softer and therefore they concluded that non-destructive (compression) firmness had potential to indicate maturity. Using the magnetic resonance imaging (MRI) technique Al-Harthy (2010) concluded that MRI could be used to determine feijoa maturity from the relationship between spin -spin relaxation time and compression firmness. In extending Al-Harthy's (2010) thoughts, Rupavatharam (2015) did studies on X ray computed tomography (CT) and observed that the technology could differentiate maturities in feijoa. However neither of these approaches is suitable for non-destructive use at grading speed.

Duan (2015) picked up the ideas of Wiryawan et al. (2005) and used varieties 'Anatoki', 'Barton', 'Kakariki', and 'Wiki Tu' to correlate compression firmness with internal maturity. Duan (2015) observed that 'Barton', 'Kakariki', and 'Wiki Tu' could be segregated using firmness at harvest because proportion of wrongly classified fruit was sufficiently low and thus had potential for commercial adoption although slow and expensive. Rupavatharam (2015) attempted to segregate 'Unique' feijoa using skin colour at harvest and suggested that colour had potential to segregate 'Unique'. From observations of Rupavatharam (2015), 100% 'Unique' fruit with °hue > 122 at harvest had a long storage life, low SSC, high TA and showed delayed over ripening whereas fruit with °hue < 122 had 42% of fruit unsaleable after 6 weeks of cold storage.

Li et al. (2018) used near infrared spectroscopy to provide spectral and quality information on 'Kakariki' feijoa. The authors observed that the regression model for °hue with a spectral wavelength of (740-1070 nm) would potentially classify 84% of fruit correctly as mature, 44% as immature and 67% as over mature fruit. However, 56% and 33% were false negative and further studies are needed to confirm these results. The problem of large biological variations among individual fruit and between different batches of fruit makes it difficult to explore non-destructive methods in attempts to segregate mature from immature feijoa. It is important to note that a method that works well in one season or one orchard may fail to give an economically justifiable result in another orchard or season, and this implies that a recalibration maybe required every season or orchard and this would impose additional costs for testing (Duan, 2015; Li et al., 2018).

In other fruit, several researchers have used other methods incorporating colour, SC, TA or starch to develop maturity indices. Choi et al. (1995) used skin colour in tomato, while in apples there are 3 indices developed that uses a combination of firmness, SSC, T.A. and starch to determine maturity (Musacchi and Serra, 2018). Just like apples, feijoa when harvested too early can remain firm for longer than the typical 4 weeks (current storage duration), however the fruit may have low SSC and high TA. If feijoa harvest is delayed fruit drops to the ground, becomes over ripe, and has a short storage life (Blanpied and Silsby, 1992; Rupavatharam, 2015). Therefore, determining a "harvest window" for feijoa would revolutionise the industry because picking fruit at peak quality time will satisfy consumers and extend storage life thereby improving foreign exchange earnings to the New Zealand horticulture industry in the end.

2.5 Feijoa fruit physiology in relation to ethylene

Feijoa is classified as climacteric, however its physiological responses when exposed to ethylene and postharvest quality changes (especially with respect to soluble solids content and titratable acidity) suggest the contrary. Ethene, or ethylene as it is commonly referred to, is a ripening/aging hormone that can be manipulated to extend storage life of horticultural crops (Binder, 2008). Ethylene is a simple unsaturated hydrocarbon gas involved in plant processes from seed germination through to ripening and finally senescence (Wang et al., 2002; Argueso et al., 2007). In having a better understanding of how ethylene affects horticultural crops, it is important to briefly understand its biosynthesis, action and how it can be regulated. Ethylene can be produced endogenously (naturally) in plants or exogenously from exhaust of diesel engines, bacterial and fungal fermentation, and other air pollutants (Saltveit, 1999; Scariot et al., 2014). This calls for careful consideration by maintaining hygiene around stored fresh horticultural products (Wills and Golding, 2016).

2.5.1 Ethylene biosynthesis

Ethylene biosynthesis is a complex pathway requiring oxygen and respiratory energy (Fig. 2-3). Ethylene biosynthesis is one area that has been widely studied and documented by several authors (Yang and Hoffman, 1984; Zarembinski and Theologis, 1994; Saltveit, 1999; Argueso et al., 2007; Van de Poel et al., 2012; Rupavatharam, 2015). Basically, ethylene cycle starts with conversion of methionine (amino acid) to *S*-adenosyl-L-methionine (SAM) by S-adenosylmethionine synthetase. SAM is then converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by 1-aminocyclopropane-1-carboxylase synthase (ACS) and the conversion of ACC to ethylene is carried out by ACC oxidase (Yang and Hoffman, 1984; Saltveit, 1999; Argueso et al., 2007; Van de Poel et al., 2012). For fruit ripening or seed germination to occur, ethylene must be perceived by its receptors (ETR1, ETR2, ERS1, ERS2, and EIN4) which are located in the endoplasmic reticulum (ER) membrane. CTR1 is a negative regulator of ethylene located downstream from the receptors. When ethylene binds to a receptor, CTR1 becomes inactive and the ethylene pathway is no longer inhibited (Alexander and Grierson, 2002; Prange and Delong, 2003; Scariot et al., 2014).

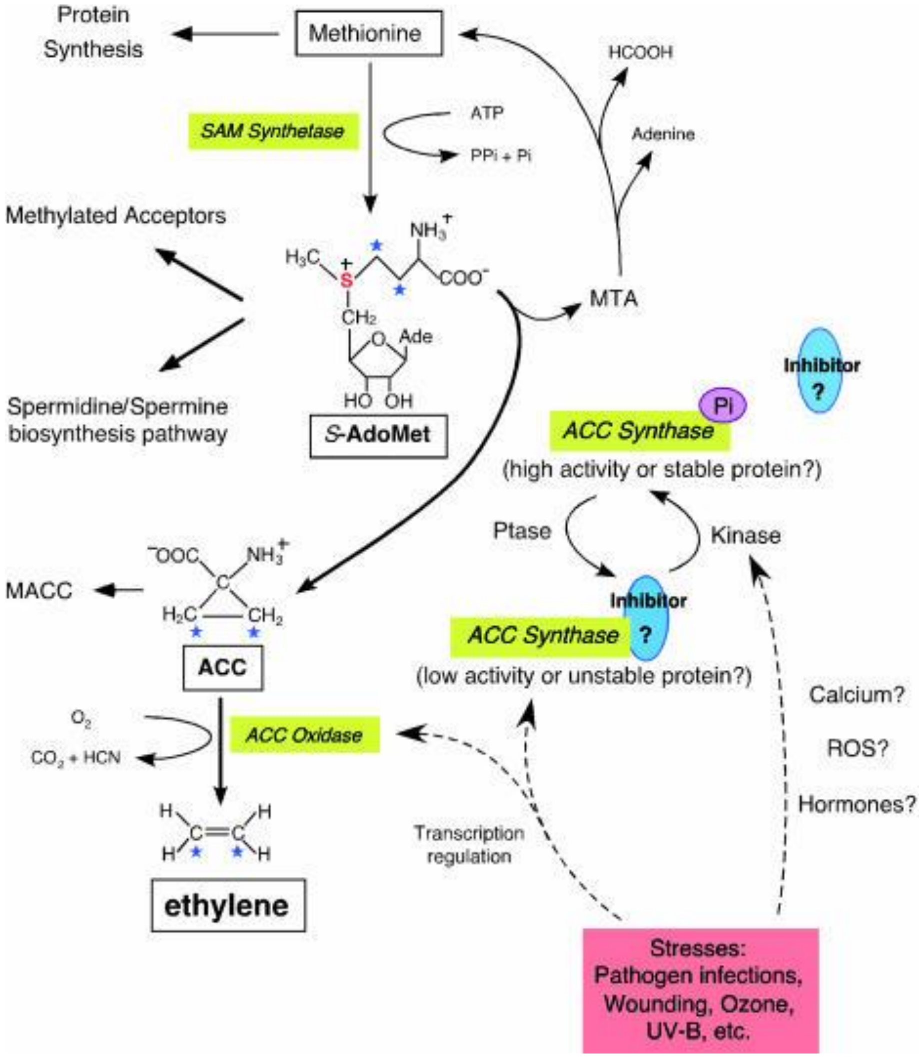


Figure 2-3: Ethylene biosynthesis pathway and regulation (Wang et al., 2002). Image used with persmission from the American Society of Plant Biologists (ASPB) as copyright owner.

In climacteric fruit a rise in respiration and ethylene production trigger the ripening process, while in non-climacteric fruit there is increase in ethylene production associated with ripening (Wills and Golding, 2016). A more precise definition suggests that the hallmark of climacteric fruit ripening is autocatalytic ethylene production (McMurchie et al., 1972) but more recent literature suggests there is a spectrum of ripening responses between these two extremes (Paul et al. 2012). In climacteric products such as apples, plums, bananas, pears, and tomatoes during the pre-climacteric stage, respiration and ethylene production are low. When respiration increases then the climacteric stage begins and autocatalytic ethylene production occurs accelerating ripening (Alexander and Grierson, 2002). Non climacteric products such as pineapple, citrus, grapes, capsicum, berries, and pumpkin do not ripen after harvest because they do not produce their own ethylene after harvest. Instead, exposure of such products to exogenous ethylene may accelerate senescence and shortens storage life (Kader and Rolle, 2004).

2.5.2 Ethylene regulation

There have been extensive studies on how to manipulate the effect of ethylene on horticultural crops (Scariot et al., 2014). Broadly these falls into three categories: raising or lowering ethylene concentration in the storage environment; reducing its production by fresh products; and reducing its activity on fresh products. Ethylene concentrations in storage areas can be altered to degreen citrus (Jomori et al., 2016; Correia- Mendes et al., 2018) and pineapple fruit by accelerating loss of chlorophyll (Guadalupe-Luna et al., 1991) as well as ripen bananas (Liu et al., 1999). In persimmon apart from degreening, ethylene is also used to soften and remove astringency (Yakushiji and Nakatsuka, 2007).

During ripening of climacteric fruit there is high internal ethylene production rate that may exceed rate of diffusion. To eliminate the ethylene from storage environments it is important to adequately ventilate with clean air from outside. However, if ventilating is not possible i.e. in controlled atmosphere situations then use of materials with adsorption properties to either oxidise or absorb ethylene are recommended. Some of the oxidising/absorption materials include use of zeolite, ozone, silver thiosulfate (although there are environmental concerns associated with their use) and potassium permanganate (also referred as ethylene scrubbers) (Saltveit, 1999; Serek et al., 2006; Scariot et al., 2014).

Controlled or modified atmospheres (CA/MA) is another way in which postharvest technologists can reduce the activity of ethylene on fresh products. For instance, use of modified atmosphere packages (MAP) lowers O_2 levels and this will limit ethylene action (remember O_2 is needed in ethylene biosynthesis [Fig. 2-3]). Inside CA or MAP bags raised CO_2 levels inhibits activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase; a key enzyme in ethylene biosynthesis. Other benefits of CA or MAP are reduced respiration rate and protection of individual products from internal and external ethylene production (Zhuang et al., 2011). For example Galvis-Venegas (2003), used MA packages flushed with 8 kPa O_2 and 5 kPa CO_2 to store feijoa at 6 °C and observed potential to extend storage life.

Interfering with increased ethylene production through blocking of ethylene biosynthesis pathway components has been successful especially in the flower industry. The ethylene biosynthesis inhibitors studied have included use of chemicals such as aminooxyacetic acid [AOA] (Baker et al., 1982; Reid and Wu, 1992) and aminoethoxyvinylglycine [AVG] (Baker et al., 1977), where both chemicals inhibit conversion of *S*-adenosyl-L-methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) (Serek et al., 2006; Scariot et al., 2014). In a study Rupavatharam et al. (2016) used a preharvest application of AVG to feijoa and observed retention of fruit firmness and flesh hue angle (colour), reduced fruit drop (fruit remained on shrubs longer) and inhibited postharvest production of ethylene. The reduction in fruit drop implied that application of AVG delayed the development of the abscission layer between the stalk and the fruit.

Blocking ethylene effects at the receptor level may prove effective as it protects fruit against both endogenous and exogenous ethylene. This thinking has led to another important area of concentration in reducing ethylene activity studies using 1 methylcyclopropene (1-MCP). I-MCP is extensively documented as being non-toxic with high efficacy of inhibiting ethylene action thereby making it easier for adoption on commercial use and trades as EthylBloc® and SmartFresh™ (Serek et al., 2006). 1-MCP is active at low concentrations and occupies or binds the ethylene receptors there by keeping CTR1 in its active state as an inhibitor (Blankenship and Dole, 2003; Prange and Delong, 2003; Rupavatharam et al., 2015a). 1-MCP effectively combines with controlled atmosphere storage (CA) and modified atmosphere packaging (MAP) to increase storage life of fresh products such as apple (Watkins et al., 2000) and green bananas (Jiang et al., 1999) as well as reducing enyzmatic browning. The effectiveness of I -MCP depends on species, cultivar, maturity, temperature, timing and storage conditions (Watkins et al., 2000). Ku and Wills (1999) observed that use of 1-MCP on broccoli increased storage life by over 50% at higher concentrations and when the treatment time was extended.

Results from other studies that manipulated ethylene during feijoa ripening and storage have showed varied responses. Akerman et al. (1993) applied exogenous ethylene to feijoa and observed increased respiration and ethylene production, while Rupavatharam et al. (2015c) working on 'Unique' and Velho et al. (2008) applied 1 methylcyclopropene (1-MCP) to feijoa and found no effect on production of ethylene or on respiration rate. Amarante et al. (2008) applied 1-MCP to a Brazilian type feijoa and observed delay's in ripening when 500 or 1500 nL L^{-1} was applied for 8 h. Rupavatharam et al. (2015a) further established that application of 1-MCP delayed ripening of 'Unique' feijoa harvested early but not in fruit harvested at touch pick maturity implying that application of 1-MCP had little influence on locular development. Observations from the above responses on various ethylene related treatments does not automatically classify feijoa as a climacteric fruit as earlier claimed (Harman, 1987). Since touch picked feijoa does not show, any response when ethylene treatments have been applied it is possible that the climacteric nature of feijoa fruit is complete preharvest. Another possible explanation would be that feijoa ethylene receptors are fully saturated at harvest thereby preventing any acceleration from surplus ethylene supply. All these studies have provided data, yet no practical solutions to extend postharvest life of feijoa has been offered. Further investigations to demystify the climacteric classification and get a reasonable explanation for the lack of responses maybe useful to allow use of metabolic levers to slow down ripening. Although ethylene is important in any postharvest studies, the current study will not evaluate its effects since ethylene manipulation (Akerman et al., 1993; Velho et al.,2008; Al-Harthy, 2010; Castellanos et al., 2016), pre -or postharvest (Amarante et al., 2008; Rupavatharam, 2015) has not yet allowed for significant extension of feijoa storage life. This study will try a completely different approach but integrate the previous findings into the discussion of results chapters within this study.

2.6 Feijoa fruit storage and quality

Feijoa has no documented standard procedure to follow harvest to final consumption, however different markets provide specifications on how the fruit is to be handled. For example, some markets may request that fruit remain with the natural wax while others may insist on brushing to enhance glossiness (Akerman et al., 1993; Thorp and Bieleski, 2002). Similarly, a market like Japan may require special packaging that costs much more than other markets that take feijoa as packaged by the orchardist (F. de Jong, personal communication, April 13, 2016).

Storage life of horticultural crops can be affected by factors such as temperature, relative humidity, atmospheric gas composition, pathogen attack and physiological disorders (Wills and Golding, 2016). More work is needed for a better understanding of feijoa and how it responds when any of the above factors are deployed to extend storage life. Ordinarily, increasing storability of any crop allows for regular supply of good quality produce and the ability to manipulate or manage supply and in turn translates to better profits to growers.

2.6.1 Effect of modified and controlled atmospheres on feijoa fruit quality

There have been several studies applying postharvest treatments, to extend storage life of feijoa, although the responses have been limited. Use of modified or controlled atmospheres (MA/CA) for increasing storage life of feijoa have either shown no positive responses or provided minimal benefit (East et al., 2009; Al-Harthy et al., 2010b). Both Al-Harthy et al. (2010a) and Rupavatharam et al. (2015a) revealed that increased levels of carbon dioxide in combination with midrange oxygen levels caused skin damage/surface injury and an increase in overripe fruit compromising storage life for 'Opal Star' and 'Unique' feijoa. Castellanos et al. (2016a) developed models that would best describe response of feijoa postharvest storage life to rate of respiration, ethylene production, and rate of transpiration in a modified atmosphere environment. They validated the models using modified atmosphere packaging (MAP) and asserted that the models could also be used to predict weight loss of other products. This assertion is very unlikely because it is not possible to develop a model for one crop and apply it to another crop, however further work may be needed to investigate their claims.

Castellanos et al. (2016c) further used the equilibrium modified atmosphere packaging (EMAP) concept to develop models that would best describe response of feijoa firmness and colour to changes in temperature, oxygen, and carbon dioxide concentrations. Results from their studies showed that storing fruit at 6 °C with headspace gas equilibrium of 8.2 kPa of O_2 and 5.8 kPa of CO_2 extended storage life of feijoa from 17 d to 38 d. Another achievement of Castellanos et al. (2016c) study was the observation of how well the models developed described changes in fruit firmness and colour when fruit were stored inside an EMAP. Their success would be attributed to how best they were able to match film permeation rates for O_2 (8.2 kPa) and CO_2 (5.8 kPa) with those of fruit respiration rates (RQ = 1.16) under the low temperature (6 °C) to reach the equilibrium that maintained freshness of feijoa (Castellanos et al., 2016b). There is a need to conclusively confirm these results before giving a recommendation.

2.6.2 Effect of temperature and relative humidity on feijoa fruit quality

Temperature and relative humidity affect storage life of horticultural crops. The management of these two factors is therefore crucial in maintaining product quality. Deterioration of fresh produce is highly influenced by temperature, increasing by two to three time with every 10 °C rise in temperature (Kader and Rolle, 2004). Storage of fresh produce in low temperatures can extend storage life by reducing respiration rate, ethylene production, microbial activity, transpiration and, consequently, rate of ripening. Velho et al. (2011) stored feijoa at 23 °C, which lasted only for a week, with a decline in fruit quality that included an increase in skin browning and decay incidences, unlike fruit stored at 4 °C, which lasted 4 weeks. Feijoa has recommended storage conditions of 4 °C and 90-95% RH that gives most varieties a storage life of 4 weeks, allowing for an additional 5 d at 20 °C (Schotsmans et al., 2011). Unfortunately, after this storage period fruit may look good externally but internally be over-ripe, with an internal browning discolouration and an unacceptable flavour (Thorp and Bieleski, 2002). Storing fruit at low temperature for a long time may also lead to development of chilling injury.

Relative humidity (RH) is a ratio, expressed as a percentage, of moisture that can be retained by air (moisture holding capacity) at a specific temperature and pressure without condensation (Maguire et al., 2001). Low relative humidity increases weight loss, reduces firmness and leads to desiccation, whilst humidity that reaches saturation causes moisture condensation, decay and potential loss of product (Paull, 1999). Unfortunately, in distribution chains, control of temperature is prioritised, but control of relative humidity is often not conducted (Paull, 1999; Kader and Rolle, 2004).

Transpiration is affected by both temperature and relative humidity; constant management of both can reduce weight loss (Mahajan et al., 2008). Feijoa fruit like most horticultural crops are sold by weight. This means that any loss of water leads to weight loss and henceforth loss of income. Transpiration is a continuous normal process by which fruit or vegetables lose water. Once a fruit or vegetable is harvested, transpiration process needs to be slowed or controlled, if not the product will lose a lot of water, which eventually leads to shrivelling making the product unsaleable. As Bovi et al. (2016) recaps postharvest storage life is usually a race against time for the produce handlers and therefore novel measures to slow down product deterioration are desirable.

According to Maguire et al. (2001) water loss depends on three factors, water vapour permeance (barrier properties of the surface of a fruit), fruit surface area and the driving force for water vapour transfer. The rate at which water evaporates from a product depends on the difference between the water vapour pressure of air and the fruit (this is the driving force). Water loss therefore can be minimized by either enhancing the barrier properties of fruit, or by reducing the driving force for water loss. Storing or transporting fruit in lower temperatures as well as increasing relative humidity (use of polyliners), are ways to reduce the driving force for water loss (Maguire et al., 2001).

2.6.2.1 Use of polyliners on feijoa fruit quality

Use of polyliners to reduce weight loss in fresh fruit and vegetables is a common postharvest practice. In feijoa, weight loss of 0.9 to 1.75% after 8 weeks of storage has been reported in experiments where flow- through systems were used (East et al., 2009; Rupavatharam, 2015). Polyliners are used to increase humidity around packaged fruit, other practices that achieve the same thing could be use of edible coatings/waxes, or use of MAP bags (Wills and Golding, 2016). Ben-Yehoshua et al. (1983) reported on various benefits (e.g. double storage life, fresh appearance, less water loss and firmer fruit) when individual fruit were wrapped in plastic film, working with tomatoes, citrus fruit, and bell pepper. They further attribute the benefits to water saturated atmosphere inside the seal of individual fruit. Although use of polyliners is recommended for feijoa packaging, unfortunately the practice is not widely practised by growers for fear of condensation and rots (Thorp and Bieleski, 2002).

Studies where feijoa are packed according to the common practice of not using polyliners report high weight losses of between 2.4 and 19% for up to 10 weeks of storage (Al-Harthy et al., 2008; Al-Harthy et al., 2010b; Amarante et al., 2017). Since most growers' store feijoa for short periods they find it unnecessary to use polyliners (Schotsmans et al., 2011). The principle behind use of polyliners is to ensure that transpiration water from fresh product is not lost to the storage environment but remains within the package and thus increases water vapour pressure. However, it is also important to manage the water vapour inside a package because when saturation point is reached, condensation occurs which may lead to microbial growth and decay (Paull, 1999). Combining use of polyliners and lowered temperature in a storage environment is desirable and needs further investigation.

Hoffmann et al. (1994) studied the effect of wrapping feijoa in polyethylene and storing them in two different temperatures. Their results showed minimal differences in soluble solid contents (SSC) and weight loss after 28 d. For SSC they recorded 9.56% (at 0 °C) and 8.83% (at 2 °C) when stored with polyethylene, whereas weight losses after same period (28 d) were 1.01% (0 °C) and 0.75% (2 °C) when wrapped in polyethylene. Even though their studies showed benefit, they did not report on how they monitored the RH, which ranged from 85-90%. Since the blanket recommendation of using 90-95% RH for most horticultural crops is hardly monitored, it then becomes difficult to ascertain the RH effect on feijoa storage. Another challenge would be suggesting an optimal RH for feijoa considering the high variabilities reported among varieties, orchards and localities.

2.7 Chilling injury in feijoa

Feijoa, being a subtropical fruit, will get chilling injury (CI) symptoms when exposed to critical low temperatures above their freezing point for a long period. Chilling injury is a physiological disorder which reduces fruit or vegetable marketability (Luengwilai et al., 2012). Klein and Thorp (1987) and (Ramírez et al., 2005) studied chilling injury in feijoa by applying a pre storage dip of calcium chloride ($CaCl_2$) solutions and found the treatment ineffective in controlling CI, although $CaCl_2$ increased fruit firmness. They recorded symptoms of chilling injury, which manifested itself both externally and internally once the fruit was transferred to warmer temperatures. Externally feijoa skin developed sunken patches that turned from green to brown and finally to black, while internally the vascular bundles became brown and the flesh developed a pink to brown colour with mild off flavours. Symptoms of CI depend on species and cultivar, maturity, storage duration, type of tissue, and the environmental factors such as storage humidity (Schotsmans et al., 2011). Moreover, feijoa cultivar 'Apollo' was reported to be less likely to develop chilling injury than cultivar 'Opal Star' when stored for five weeks' at 0 °C (Schotsmans et al., 2011).

2.7.1 Mechanism for Chilling Injury

Chilling injury has been studied for over a century and unfortunately, the exact mechanism of how it occurs is not well understood (Sevillano et al., 2009). Like many other authors in the field, they have proposed the approach of looking at the physiological, molecular, and hormonal level as possible mechanisms of chilling injury. At the molecular level, cell membrane alterations are believed to be primary effects of chilling injury. Whether this affects membrane lipids or membrane proteins, or an interaction of both lipids-proteins is still not clear (Sevillano et al., 2009; Heyes, 2018). However, low temperature of chilling sensitive tissues changes the composition of cell membranes by preventing lipid inclusion. The low temperature will degrade the phospholipids together with the galactolipids and this will increase the unsaturated (free) fatty acids. As free fatty acids increase in the cell membranes the protein structure becomes disorganized lowering the membrane fluidity (Marangoni et al., 1996; Lukatkin et al., 2012).

The transition in membrane lipids (from liquid to gel phase) leads to increased membrane permeability and leakage of ions (Mirdehghan et al., 2007; Promyou et al., 2008). Peel pitting of citrus fruit is an example of phospholipid membrane degradation. Others are water-soaked areas in tissues, increased decay susceptibility and accelerated senescence (Saltveit and Morris, 1990). Although phospholipid membrane degradation is a molecular mechanism, there are suggestions that phospholipase enzyme (PLA_2 and PLD) activity is involved and they are also regulated by genes encoding PLA_2 and PLD (Cronjé et al., 2017). This therefore implies that chilling injury mechanisms should not be considered in isolation since all three mechanisms are intertwined.

Physiological effects of chilling injury (CI) include oxidative stress from excess reactive oxygen species (ROS), which is a secondary response to CI after damage to cell membranes has occurred (Aghdam and Bodbodak, 2014). ROS are incomplete forms of oxygen, for example hydroxyl radicle (OH^-), hydrogen peroxide (H_2O_2) or superoxide (O_2^-) which are caused by an imbalance between antioxidants and ROS (Sevillano et al., 2009; Vicente et al., 2009). The antioxidant enzymes include superoxide dismutase (SOD) which is released to detoxify superoxide (O_2^-), catalase (CAT) and peroxidases (POD) that detoxify/reduce hydrogen peroxide (Valenzuela et al., 2017; Heyes, 2018). Normally these antioxidant enzymes would protect the plant/fruit from the effect of ROS. However, in circumstances where tissue is under stress, production of ROS (e.g. hydroxyl radicle) increases that then leads to oxidative damage of lipids. The oxidative damage of lipids eventually leads to formation of malonyldialdehyde (MDA) (Imahori et al., 2016; Heyes, 2018). Other secondary responses include changes in metabolism, accumulation of toxic compounds, change in ripening, ion leakage and loss of flavour (Wang, 1993; Marangoni et al., 1996).

Apart from enzymatic systems, tissues produce non-enzymatic detoxification systems to protect cells from oxidative stress. An example of non-enzymatic detoxification system is melatonin, which acts as an endogenous signalling molecule (Azadshahraki et al., 2018). Melatonin will scavenge and detoxify tissue from hydroxyl radical. Melatonin use is reported to reduce chilling injury in peaches (Gao et al., 2016), strawberries (Aghdam and Fard, 2017). and tomatoes (Azadshahraki et al., 2018). In peaches Gao et al. (2016) reported on slowed senescence, decreased decay incidence and maintenance of quality after applying a melatonin treatment of 0.1mmol L^{-1}. In the same study, Gao et al. (2016) observed an increase in antioxidant enzyme activities of POD, SOD, CAT and APX. The melatonin treatment reduced chilling injury by activating the fruit defence and this led to maintenance of membrane integrity.

In attempts to understand mechanisms that relate to CI Sevillano et al. (2009) further suggested that ethylene biosynthesis, polyamines (PAs), and cellular abscisic acid (ABA) increased in fruit suffering from chilling injury and this would possibly be at the hormonal level. Plants naturally produce ethylene to regulate growth and development. These regulatory roles are wide ranging from germination, abscission, ripening and finally senescence. Oxidative stress affects membrane integrity similarly to how over ripening would. It therefore means ethylene biosynthesis maybe implicated in development of chilling injury as observed in grapefruit (Schirra, 1993) among other fruit.

Polyamine (PA) compounds include putrescine, spermine, and spermidine, which are phytohormone-like aliphatic amine compounds that are physiologically released to protect tissue against stress related events such as drought, salinity and chilling injury (Abu-Kpawoh et al., 2002). Just like ethylene, S-adenosylmethionine (SAM) is also a precursor for PA although their interactions are competitive (Sevillano et al., 2009; Valenzuela et al., 2017). PA concentrations are reported to be higher than ethylene and therefore affect the availability of 1 aminocyclopropane-1-carboxylic acid (ACC). Since PAs compete with ethylene, they therefore retard ripening and thus stabilize cell membranes. Abu-Kpawoh et al. (2002) reported that 45 and 50 °C for 35 and 30 min was an effective treatment for 'Friar' plums to accumulate polyamine levels effective against chilling injury and decay.

2.7.2 Postharvest technologies to alleviate chilling injury

Several postharvest technologies have been employed to alleviate chilling injury in horticultural crops. The technologies used may help to avoid exposure in the first instance, increase tolerance of tissue, or reduce/inhibit the impact of chilling injury. Since it may not be practical to avoid CI, treatments to minimize effects have been sought and can be grouped either as physical, biotechnological or chemical in nature. Of the three groups named, use of biotechnology, to alleviate CI is beyond the scope of this study and thus will not be discussed. In the short-term storage chilling injury is reversible if the stress is removed, however long-term exposure leads to irreversible CI and this will also be the focus of this project.

Though use of chemicals has been effective in some cases, consumer concern about their use on food products has led to postharvest technologists seeking other alternatives, which have seen an increase in use of physical treatments. The physical treatments include temperature conditioning, heat treatments, intermittent warming, and use of controlled atmospheres (Sevillano et al., 2009). Some of the generally regarded as safe (GRAS) chemicals currently applied to alleviate chilling injury are methyl jasmonate (MeJA), salicylic acid (SA), calcium chloride dips and plant regulators/hormones (Lurie, 2008; Shewfelt et al., 2009; Tonutti, 2012; Aghdam and Bodbodak, 2014).

2.7.2.1 Physical treatments for alleviating chilling injury

Heat treatments can be used to reduce fruit susceptibility to chilling injury, even though in postharvest they are designed primarily for disinfestation of quarantine pests (Lay-Yee et al., 1997; Fallik, 2004). Heat treatment can be applied as hot water (dipping, rinsing or brushing), hot air or vapour heat (Fallik, 2004; Lurie, 2008; Aghdam and Bodbodak, 2014). Time and temperature combinations used are dependent on cultivar, maturity, and preharvest conditions for the product in question. All these treatments will affect tissue metabolism through some combination of increased antioxidant activity, increased heat shock proteins or enhanced membrane integrity (Aghdam and Bodbodak, 2014; Heyes, 2018). For instance, reduction in chilling injury for grapefruit was effective when hot water brushing at 59 °C or 62 °C for 20 seconds was applied (Porat et al., 2000). Ghasemnezhad et al. (2008) and Promyou et al. (2008) applied hot water dipping at 50 °C for 2 min and 42 °C for 15 min respectively on 'Satsuma' mandarins and Banana cv 'Gros Michel' and 'Namwa' and observed a reduction in chilling injury. Similarly, Woolf et al. (1995) applied hot air at 38 °C for 3, 6, or 10 h and 40 °C for 0.5 h to 'Hass' avocados and observed a reduction in chilling injury. In another study, Woolf et al. (1997) applied hot air at 47 °C for 0.5 -3 h to 'Fuyu' persimmon and observed a reduction in chilling injury. In an initial study of applying heat treatments to feijoa, Woolf et al. (2006) applied hot water dipping for up to 60 min to slow ripening and reduce chilling injury in cultivar 'Opal Star', 'Unique', and 'Apollo' but instead observed external (skin) browning. Skin browning due to heat damage differed among the three cultivars used and it is possible that feijoa sensitivity to heat treatment is cultivar specific and may also depend on fruit maturity.

Temperature conditioning is another non-chemical method that can be used to alleviate chilling injury by allowing tissue to acclimatize to CI stress. Temperature conditioning involves a gradual reduction of storage temperature, which can be either single step or multi step. Wang (1993) reports multistep conditioning as more effective than single step. Low temperature conditioning maintains high levels of phospholipid in membranes, and increases unsaturated fatty acids, polyamine, and long chain aldehyde concentrations. Zhang et al. (2017) studied low temperature conditioning of mango 'Guifei' at 12 °C for 24 h followed by 5 °C for 25 d and observed a decrease in chilling injury with increased soluble solids content, proline and softening. Additionally, Zhang et al. (2017) observed a reduction in electrolyte leakage, reactive oxygen species (ROS), and malondialdehyde (MDA) contents indicating maintained integrity of mango membrane.

Mirdehgan and Rahemi (2003) reported a reduction in chilling injury for pomegranate when fruit was pre stored with high temperature conditioning at 38 °C for 24 h and 36 h followed by storage at 1.5 °C (RH 83 ± 3%) for 4.5 months. Spalding and Reeder (1983) conditioned Tahiti limes for 1 week at 7.2 °C, 10 °C, 12.8 °C, 15.6 °C, and 21.1 °C, then stored them at 1.7 °C for 2 weeks and observed reduced chilling injury on conditioned fruit compared to the control. Bassal and El-Hamahmy (2011) observed reduced chilling injury in 'Navel' & 'Valencia' oranges when using hot water dipping (HWD) at 41 °C for 20 min in combination with temperature conditioning (6 d at 16-18 °C and 45-65% RH). Sapitnitskaya et al. (2006) used hot water rinsing (62 °C for 20 s) with pre-storage conditioning (16 °C for 7 d) on grapefruit 'Star Ruby' and found the treatments synergistically reduced chilling injury when fruit was stored at 2 °C. Likewise Lurie and Sabehat (1997) applied gradual cooling to tomato 'Daniella' (or 'Rehovot 144') by varying periods the tomatoes were hold at 38 °C before storing them at 2 °C for up to 4 weeks. Their results showed that application of temperature manipulations before storing at 2 °C reduced development of chilling injury. Yang et al. (2013) applied low temperature conditioning of 12 °C for 3 d on kiwifruit 'Hayward' and observed reduced chilling injury, with increased antioxidant enzyme activities, endogenous abscisic acid (ABA), indole-3-acetic acid (IAA), and zeatin riboside (ZR).

Further studies on feijoa by Woolf et al. (2006) reported no response when step down conditioning and hot water treatments were applied to feijoa varieties 'Triumph', 'Apollo', 'Gemini', 'Opal Star' and 'Unique' to reduce chilling injury. Woolf et al. (2006) started with 4 °C and dropped 1 °C each week (i.e. 4 °C, 3 °C, 2 °C,1 °C, & 0 °C) for 5 weeks and did not eliminate CI, although results indicated cultivar differences. The step down temperatures in this study were all within the chilling temperatures and therefore not sure they would achieve the stated objective. Therefore, finding temperature and time combinations that work for feijoa would be beneficial to the industry. Wills and Golding (2016) conclude by suggesting that if no other method is found to reduce chilling injury, then produce can be held at a temperature of between 3 to 5 °C, though an over mature/ripeness problem occurs. Alternatively, it is better to store chilling sensitive products for only a short time in low temperature and sell them while quality is still good.

Another physical method of alleviating chilling injury is intermittent warming. This method involves alternating shorter warm periods with longer cold periods to allow longer storage durations of chilling sensitive products. The essence of alternating cool and warm temperatures is to avoid irreversible chilling injury from occurring and instead develop chilling tolerance in the sensitive tissues (Wang, 1993; Biswas et al., 2016). Warming is believed to heal damaged membranes; for example, 'Nanguo' pears develop peel browning (PB) when continuously exposed to chilling temperatures, however Wang et al. (2017) observed that PB was alleviated when the pears were stored at 0 °C for 120 d with intermittent warming for 1 d 20 ± 1 °C after every 20 d of cold storage. Liu et al. (2015) applied intermittent warning to pepper and found that they effectively retained cell membrane integrity of pepper thereby reducing chilling injury. While studying chilling injury of tomato Biswas et al. (2012) used intermittent warming and found that its effectiveness depended on preharvest factors and also the cultivar used. Although intermittent warming yields positive results, it is difficult to practically apply it industrially on large fruit volumes and thus further investigation on its practicability is still needed (Biswas et al., 2016; Heyes, 2018).

As discussed earlier modified or controlled atmosphere (MA/CA) can be used to increase storage life of fresh fruit and vegetables. Similarly, they can be used to alleviate chilling injury (Wang, 1993; Heyes, 2018). Feijoa has responded poorly to a range of oxygen and carbon dioxide concentrations, and storage temperatures, so chances of alleviating chilling injury are close to zero. Studies by Al-Harthy et al. (2010a), Al-Harthy et al. (2010b) and Rupavatharam (2015) have shown that CA as a postharvest technique in feijoa causes skin damage/surface injury and increases the number of overripe fruit, thereby compromising storage life. However, success has been reported in other fruit like Japanese plums (Singh and Singh, 2013), mango (O'Hare and Prasad, 1992), avocados (Pesis et al., 1994), zucchini squash (Serrano et al., 1998), papayas (Chen and Paull, 1986), pineapple (Paull and Rohrbach, 1985), peaches and nectarines (Wang and Anderson, 1982). The possible explanation for CA/MA success is that low oxygen concentrations will limit ROS production thereby delaying oxidative stress that reveals itself as chilling injury or senescence (Singh and Singh, 2013).

2.7.2.2 "Safe" chemical treatments to alleviate chilling injury

Chemical treatments have been used for a long time and in most cases they have been effective. However, concerns with food safety have now shifted focus and consumers are demanding quality and safer products. As postharvest technologists we have also been forced to adapt to this complicated situation if we have to make meaningful change in the food industry. Advocacy in the use of GRAS products has been adopted widely and at times it's good to evaluate them critically. Generally regarded as safe, (GRAS) chemical/product is a term originally from American Food and Drug Administration (FDA) that has now been widely adopted (Barraj et al., 2016). GRAS products are determined scientifically or have been in use before January 1, 1958 and as such are exempted from other groups as outlined in Federal Food, Drug and Cosmetic Act (FFDCA) (Burdock and Carabin, 2004). These include inorganic ions such as calcium (Ca) or natural chemicals synthesised by horticultural crops such as nitric oxide (NO), methyl jasmonate (MeJA), salicylic acid (SA), abscisic acid (ABA), ethylene, and other hormones. The role of these natural chemicals affecting CI that have not been discussed previously with respect to feijoa and therefore more details will be discussed briefly in the next paragraphs.

Salicylic acid, jasmonic acid and its volatile methyl ester, methyl jasmonate are endogenous plant regulators that influence growth and development in a wide range of higher plants (Cao et al., 2009; Sayyari et al., 2009). Preharvest calcium (Ca) spraying of 'Hass' avocado to alleviate CI leads to an increase in Ca concentrations on the exocarp and a decrease in CI (Barrientos-Priego et al., 2016). Saba et al. (2016) applied postharvest calcium sprays on apricot and observed increased calcium concentrations in fruit with decreased production in ethylene. The observed increase in calcium concentrations helped in maintaining fruit quality and preventing fruit ripening. Other encouraging observations on use of calcium sprays have also been reported in storage of tomato (Morteza, 2013). A combination of salicylic acid, gibberellic acid, and calcium chloride were found to be most effective in preserving honey peaches while calcium dip alone improved fruit quality (Chengcheng et al., 2015; Li et al., 2018b). Mirdehghan and Ghotbi (2014) working on pomegranate, applied salicylic acid, jasmonic acid, and calcium to reduce chilling injury. They observed significant reduction of chilling injury in the pomegranate fruit. Measuring calcium concentration of stored pineapple, Hewajulige et al. (2003) observed reduced chilling injury in areas where calcium concentration was higher. On feijoa, only effects of Ca alone have been documented and there was no response in CI but extension of storage life, reduction in weight loss and reduction of disease as reported (Ramírez et al., 2005). There is considerable literature on the benefits of salicylic acid and jasmonic acid on other fresh products and this therefore opens avenues for more research on their possible impact on feijoa. Since this study is time bound, it might not be possible to apply all the above treatments. The focus of this study will be stepdown conditioning and intermittent warming.

2.8 Assessment of chilling sensitivity by use of chlorophyll fluorescence

Internal chilling injury of feijoa is determined destructively by cutting fruit before assessment is done. This method reduces quantity of good fruit, can be time consuming and impractical in grading/sorting lines. There is need to identify a non-destructive technique that is easy, faster, reliable and adaptable to sorting lines. Chlorophyll fluorescence (*ChF*) is a simple non-destructive technique that indirectly indicates the physiological status of a green tissue. *ChF* is widely used preharvest in low oxygen studies or salinity studies and gives information on how the green tissues utilise light energy. In stressed tissues, it can detect cellular changes before they become visible. When a green tissue absorbs light energy, competition occurs on whether to use the energy to drive photosynthesis, to dissipate as heat or to re-emit as fluorescence. Fluorescence originates from excitation of chlorophyll molecules in photosystem II (PSII) (Cascia et al., 2010). Since thylakoid membranes (sensitive to oxidative stress) in chloroplasts are related to protein chlorophyll complexes, fluorescence can indicate fluidity, stability or organization of membranes (Urbano et al., 2004; Arafat, 2005). In a CI tissue production of ROS due to oxidative stress will eventually lead to membrane disintegration thereby indicating a tissue response to stress (Tijskens et al., 1994; Baghbani-Arani et al., 2017).

When using a chlorophyll fluorescence meter several parameters can be studied. Minimum fluorescence (F_o) is measured using far-red light in a dark-adapted tissue when all the PSII reaction centres are open (fully oxidized). Maximum fluorescence (F_m) is measured when a high intensity saturation pulse reduces the reaction centres of PSII by closing them. Variable fluorescence is then calculated by subtracting the minimum fluorescence (F_o) from the maximum fluorescence (F_m). The ratio of variable fluorescence to maximum fluorescence (F_v/F_m) is used to indicate stress and for unstressed dark-adapted plants the value is around 0.8 (Arafat, 2005). F_v/F_m is the PSII quantum yield that measures the electron transfer efficiency and the chloroplast activity (Huang et al., 2012).

During postharvest storage of 'Golden Delicious', 'Starking Delicious' and 'Law Rome' apples the quantum yield has been shown to reduce in low O_2 (Song et al., 1997). Chlorophyll fluorescence has been used in 'Golden Delicious', 'McIntosh' and 'Delicious' apples to detect low O_2 stress before associated disorders occur (De Ell et al., 1998). Tian et al. (1996) showed that hot water treatment of 47 °C for 7.5 min maintained green colour of 'Shogun' broccoli and treated broccoli had a low F_v/F_m ratio. In another study Kosson (2002) stored green pepper at 1 °C, 4 °C and 8 °C for 18 d and observed F_v/F_m decrease from 0.85 to 0.55 after 3 d of storage at 1 °C and 0.45 after 18 d. If this technique works in detecting CI in feijoa, then it will offer growers, a chance to sell fruit quickly before irreversible CI occurs or to move fruit from a chilling environment by applying intermittent warming. Using chlorophyll fluorescence has advantages over other techniques because it is fast, sensitive, non-destructive and able to detect injury before symptoms are visible (Meir et al., 1997). Chlorophyll fluorescence does not need specialized skills since the equipment comes with pre-programmed protocols making the use of the equipment easier (De Ell and Toivonen, 2012). However, it would be important for chlorophyll fluorescence technique to be compared to other non-destructive approaches, such as NIR spectroscopy, by studying spectral response at the wavelength of chlorophyll absorption, to assess feijoa quality.

2.9 Conclusion and opportunity for research

Synthesis of literature indicates that potential for feijoa industry growth exists and with ongoing breeding work, the local market may not be sufficient to absorb the volumes produced thus the need for an alternative market outside New Zealand. This, however, can only succeed if appropriate techniques are identified to enhance quality and storage life for seafreight export. In view of this, the study aims at determining potential postharvest techniques that can extend storage life of 'Kakariki' 'Wiki Tu', and 'Triumph'. Key areas of focus include temperature and relative humidity management, harvest timing, stepdown conditioning, intermittent warming, and development of non-destructive grading tools such as chlorophyll fluorescence as briefly outlined below.

From the available literature, effects of temperature on feijoa are documented, however, the effects of relative humidity on feijoa are unknown since the growers are sceptical of using polyliners (during packing) in fear of condensation and rot problems. Besides, the combined effects of temperature and relative humidity are also unknown. This review has also shown that touch picking as a harvesting technique provides near ripe fruit that reduces the potential storage life of feijoa. It is therefore important to determine harvest timing in order to avoid harvesting immature or over mature fruit that may cause serious postharvest losses. Available literature has also shown the possibility of using stepdown conditioning and intermittent warming techniques to alleviate chilling injury and extend storage life. In addition, intermittent warming technique, if beneficial, could be combined with non-destructive chlorophyll fluorescence to identify an optimal regime. Another important aspect highlighted in this literature is the presence of mixed maturities at harvest due to long fruit setting period of 4-6 weeks that affects quality in storage. In order to create homogenous batches of fruit, there is need to find a more objective non-destructive assessment for harvest maturity.

Specific objectives for the study are: -

1. To reassess temperature and RH storage conditions for variety 'Kakariki', 'Wiki Tu' and 'Triumph' for extended storage life.

2. To assess effect of feijoa maturity at harvest in extending storage life of ‘Kakariki’ feijoa.
3. To determine effects of applying step down conditioning and intermittent warming in improving feijoa fruit quality.
4. To evaluate ability of Chlorophyll fluorescence (*ChF*) in predicting and detecting ripening of feijoa.
5. To re-evaluate suitability of current maturity/ripeness scale based on 3 varieties (‘Kakariki’, ‘Wiki Tu’ and ‘Triumph’).
6. To examine the relationship between maturity/ripeness at harvest and other fruit quality attributes (firmness, sugar and colour) and objectively develop a non-destructive grading tool.

Chapter 3 General laboratory methods

3.1 Overview

This section will describe the fruit source, and the common laboratory methods used in several experiments. Where specific methods were applied, a detailed description will be provided in those chapters. Table 3-1 provides information on source of fruit used during experimental setup; fruit of each cultivar were handled separately to avoid mixing of cultivars thus minimize (data) variation within cultivars.

Table 3-1 Fruit source information for each setup.

Year	Harvest date	Variety/ Cultivar	Orchard location	Fruit size (count/tray)	Quality
2016	15/04/2016 05/05/2016 12/05/2016	'Kakariki' 'Wiki Tu' 'Triumph'	Matamata Matamata Matamata	28-30 30-33 30-36	Export
2017	23/03/2017 31/03/2017	Immature 'Kakariki' Mature 'Kakariki'	Matamata Matamata	25-33 25-33	Export Export
2018	27/03/2018 27/04/2018	'Kakariki' 'Triumph'	Matamata Matamata	28-33 28-33	Export Export

In most cases, fruit were harvested and graded by experts from the orchard. The fruit were held in an on-farm cool store after harvest, transported in a non-refrigerated truck overnight, and received at Massey University postharvest laboratory, Palmerston North early in the morning (approximately 24-48 h after harvest). Experiments were set up immediately. Before transportation, all fruit were already graded and packed into corrugated fibreboard trays with polyethylene single layer plix from the grower. On reception at Massey University postharvest laboratory, fruit were randomly re-allocated to labelled trays as required for each experiment.

On the day of receiving fruit, the first set of data to be collected was harvest information from a set of 3 trays (90 fruit) picked randomly; fruit weight, compression firmness, internal maturity, skin colour (L*, C* and °hue), soluble solids content (SSC) and titratable acidity (TA). After storage of 4, 6- and 8-weeks fruit were brought to the 20 °C postharvest lab to allow temperature equilibration overnight. Measurements were made over a 3-6 d period at 20 °C on fruit weight, compression firmness, internal ripeness, skin colour (L*, C* and °hue), soluble solids content (SSC) and titratable acidity (TA). Additional data was sometimes collected as specified in chapters.

3.2 Fruit quality measures

3.2.1 Weight loss

Individual fruit weight was measured using an electronic balance Mettler Toledo (PG503s, Greifensee, Switzerland), to an accuracy of ± 0.001 g. Whole tray weights were measured using an electronic balance Precisa (30000D SCS, Zurich, Switzerland) to an accuracy of ± 0.1 g. Weight loss of feijoa was then calculated as a percentage of the initial weight as shown in the equation below.

$$\text{weight loss}(\%) = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100 \qquad \textbf{Eq. (1)}$$

3.2.2 Compression firmness

Compression firmness (N) was measured using a texture analyser (TA-XT2, Stable Microsystems, Surrey, UK) by obtaining a peak force (N) to achieve a 2 mm compression at the fruit equatorial region using a flat plastic cylindrical probe at 1 mm s^{-1} (Fig 3-1). An average peak force (N) from two adjacent sides of the fruit along the equatorial region was recorded.

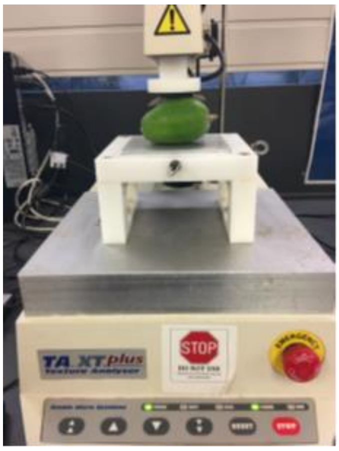


Figure 3-1 Compression firmness measurement of feijoa.

3.2.3 Skin colour

Skin colour was determined by using a spectrophotometer (CM -2600d, Konica Minolta, Sensing Inc., Osaka, Japan), with an observer at 10° and a D65 illuminant, fitted with an 8 mm diameter measuring head (Fig. 3-2). An average of three readings around the fruit equator were used to determine lightness (L*; colour intensity changing from light to dark), chroma (C*; intensity of colour) and hue angle (°h; actual colour). Spectral component excluded (SCE) was selected for this study.

Figure 3-2 Skin colour measurement of feijoa.

3.2.4 Internal maturity

Internal maturity was assessed subjectively by cutting fruit across the equatorial region and scanning the halved fruit (Fig. 3-3) with a flatbed scanner Epson (Perfection 1260, California, USA). Later the images were ranked according to a modified maturity scale (Fig. 3-4 that the author developed using variety 'Kakariki') based on the scale developed by Plant & Food Research, New Zealand (Schotsmans et al., 2011).

Figure 3-3 Scanning stem end view of halved feijoa.

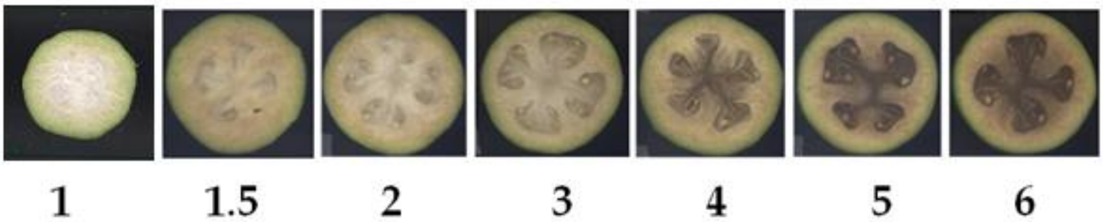


Figure 3-4 Modified internal maturity and ripeness scale for feijoa.

In this scale 1 represents an immature fruit that has been pulled too early. The inside of the fruit is all white and the likelihood of the fruit ripening properly is negligible. In short, with such a fruit it is simply difficult to tell if it is mature or not. Stages 2 and 3 in the scale is normally the desirable harvesting stages that the industry advocates for. However, attempts by growers to achieve this is not straight forward as observed by the recommended touch picking harvesting method. During the study period, it became apparent that many fruit were in between scale 1 and scale 2. At this point, the author introduced a 1.5 scale. Most of the fruit in scale 1.5 have a high chance of ripening and therefore a success in the feijoa industry. As explained above scale 2 and 3 represents the right maturity stage for harvesting. The seed pulp area is clearing, and white strands of seeds thicken. Stage 4 shows the seed pulp area starting to darken and the seed strands getting thin. At stage 4 it is considered over-ripe for eating, although mostly the fruit naturally drops to the ground. Stages 5 and 6 have the seed pulp area including the seeds and flesh darker. Mostly these stages are seen in fallen fruit or fruit after storage.

3.2.5 Internal chilling injury

Before scanning, the halved fruit was immediately scored for internal chilling injury by visually observation. Internal chilling injury manifests itself by the vascular bundles becoming brown and flesh developing a pink to brown colour (Fig. 3-5). Fruit showing even a slight symptom of internal chilling injury were recorded and incidence of injury calculated (Eq. 2). Incidence is the proportion (0 to 1) or percentage (0 to 100) of affected fruit by chilling injury within a sampling unity (Jongsri et al., 2016). Fruit that deteriorated above 60% of incidence were not used when analysing data on quality.

$$\text{Fruit CI incidence}(\%) = \frac{\text{Number of affected fruit}}{\text{Number of total fruit}} \times 100 \qquad \textbf{Eq. (2)}$$

Figure 3-5 Internal chilling injury of 'Triumph' feijoa.

3.2.6 Surface injury/rots and/or external chilling injury

Surface injury/rots and/or external chilling injury were scored by visually assessing the injury, estimating the area affected and assigning to 5 classes developed during the study period as shown below (Fig. 3-6) (the scale used 'Wiki Tu', 'Kakariki' and 'Triumph'). Severity is the term used which demonstrates the area/proportion of affected fruit within a sampling unit (Seem and Gilpatrick, 1980). All fruit showing < 5% surface area affected were still considered saleable. In addition, fruit showing even a slight symptom of external chilling injury were recorded and the incidence of injury calculated (Eq. 2).

Figure 3-6 Severity scale developed for feijoa showing percentage of surface area affected during cold storage.

3.2.7 Total soluble solids

Total soluble solids (SSC) measured as °Brix was determined by using the blossom end of the earlier halved fruit for scanning (Fig. 3-7). Two to four (2-4) drops of juice from single fruit were squeezed by hand onto a digital refractometer (PAL -1, Atago Ltd, Tokyo, Japan) for each reading. The refractometer was zeroed using distilled water.

Figure 3-7 Blossom end view of feijoa used to determine SSC (°Brix).

3.2.8 Titratable acidity

Titratable acidity (TA) was determined initially by using an automatic titrator (Titroline easy, Schott Instruments, GmbH, Mainz, Germany). In one season this equipment was being repaired and manual titration was required. The pulp (a combination of locule and pericarp) of 5 randomly picked fruit per replicate was mashed and placed into plastic bags. The samples were then frozen in liquid N_2 and stored at -30 °C. On the day of titration, the frozen block was crushed into paste while still frozen and mixed to obtain a representative sample before allowing full defrosting. Juice (1 mL) was extracted from each sample bag and diluted with 50 mL of distilled water. Phenolphthalein indicator (2-3 drops) was added into the solution and this was titrated against 0.1 N NaOH to reach an endpoint of pH 8.2 as specified for a fruit in which malic acid predominates. Using the method described in Sadler and Murphy (2010) acid strength (in g malic acid/100ml juice) was then calculated as follows: -

% acid (wt/vol) = ([mls NaOH used] x [0.1 N NaOH] x [milliequivalent factor of 0.067] x [100])/volume of sample x 1000).

3.2.9 °Brix/acid ratio

The °Brix to acid ratio for each sample was calculated by dividing the mean °Brix value for the fruit in that batch by the measured TA (% acidity).

3.3 Physiological fruit measures

3.3.1 Respiration rate

Respiration rate of fruit was determined fortnightly using the static method at cold storage temperature e.g. 2 °C or 4 °C. Fifteen (15) fruit of known weight per replicate were each sealed in an airtight glass jar (550 mL) with rubber septum. Gas samples were collected twice from the headspace using a 1 mL syringe. The initial CO_2 sample was collected immediately after sealing the jar while the final CO_2 sample was collected after 1.5 hours or 2 hours (ensuring accumulated CO_2 did not exceed 0.5%). Gas samples were then analysed using a CO_2 infrared transducer (Analytical Development Company, Hoddesdon, United Kingdom) that used N_2 as a carrier gas. The area under curve (output signal) was determined with an integrator (HP3396A, Hewlett Packard, USA). Respiration rates were then calculated based on fruit weight, free volume of glass jar, and temperature; which was expressed in nmol kg^{-1} s^{-1}. Calibration of the gas analyser was performed using commercially obtained β-standard 0.5 % CO_2 (BOC, Palmerston North, New Zealand).

Chapter 4 Reassessing temperature and humidity storage conditions for maintaining quality of feijoa cultivars

Acknowledgement:

Materials from this chapter is included in the paper:

Oseko, J., East, A. R., & Heyes, J. A. (2019). Reassessing temperature and humidity storage conditions for maintaining quality of 'Kakariki' feijoa. *Acta Horticulturae, 1256*, 157-162. DOI 10.17660/ActaHortic.2019.1256.22.

This chapter differs from the publication in that it includes data on 'Wiki Tu' and 'Triumph'; data on achieved conditions inside storage rooms, titratable acidity, soluble solids concentrations and °Brix/Acid ratio.

4.1 Introduction

Most feijoa studies have noted that its rapid decline in postharvest quality during storage, transport, and marketing is largely a factor of its rapid ripening and susceptibility to physiological disorders. In fruit supply chains, temperature and relative humidity (RH) are important environmental factors that influence quality and eventually consumer acceptability. For instance, to maintain high humidity, which in turn reduces water loss some horticultural products in New Zealand and the world over use polyliners that are wrapped around packed product inside each box (Burdon et al., 2014; Bovi et al., 2016; Lufu et al., 2017). Current recommended storage conditions for feijoa are 4 ± 1 °C and 90 ± 5% RH that gives a storage of up to 4 weeks plus another 5 days at 20 °C (Thorp and Bieleski, 2002). Exporting to markets in Europe and USA, growers are using airfreight which is faster but expensive compared to sea freight that requires at least 6 weeks of storage life.

Even though temperature and relative humidity play an instrumental role in determining the quality of feijoa, there influence has not been fully understood. Most of the studies so far have focussed mainly on low temperature with limited information on relative humidity (Klein and Thorp, 1987; Al-Harthy et al., 2008; Velho et al., 2011). Since little information exists on the combined effects of temperature and RH on feijoa, this study was undertaken with an objective of reassessing temperature and RH storage conditions for variety 'Kakariki', 'Wiki Tu' and 'Triumph' for extended storage life.

4.2 Materials and methods

4.2.1 Fruit material and experimental design

Three (3) varieties of (count 28-36) feijoa; namely 'Kakariki' (early season variety), 'Wiki Tu' (mid to late season variety) and 'Triumph' (late season variety) were harvested, graded and packaged by experts from Southern Belle Orchard (SBO) in Matamata, New Zealand in the months of April and May 2016 (as highlighted in Table 3-1). Fruit were later transported overnight in a non-refrigerated truck and received at Massey University, Palmerston North approximately 24 h after harvest. As soon as a variety was received, factorial arrangement of a completely randomised experiment was established. Fruit were randomly allocated into pre labelled trays that defined subsequent treatments and measurement (Table 4-1). Storage duration had 3 levels (4, 6 and 8 weeks), assessment time at 20 °C had 2 levels (3 d and 6 d), storage temperature had 2 levels (1 °C and 4 °C) and humidity had 2 levels which was achieved through the absence (no polyliners) and presence of a polyliner (with 10 μm thickness of high density polyethylene) wrap around fruit in trays. For each variety half of the fruit population (half of the fruit trays) were wrapped with polyliners and the other half was left unlined. Three replicate trays of 30 fruit were established for each treatment and measurement time combination. Using psychrometric charts, cool storage conditions were determined and set a week earlier at 1 °C, 85% RH and 4 °C, 88% RH resulting in equal water vapour pressure deficits at both temperatures for fruit in unlined trays (Paniagua et al., 2014). Actual temperatures and humidity inside trays were logged using I-buttons (DS1923 Maxim Integrated logger, Texas, USA) every 30 minutes. For each fruit batch/variety received, four (4) I-buttons were placed inside 4 trays labelled week 8 (2 for unlined and 2 for lined boxes) giving a total of twelve (12) I-buttons used for the whole experiment.

Table 4-1: Summarised illustration of treatment description for reassessing temperature and humidity storage conditions for 3 feijoa varieties.

Treatment	Tray Code	Description
T1	VxT1WyNLRz	Variety 'Kakariki' (or 'Wiki Tu' or 'Triumph'), 1 °C 85 %RH, week 4 (or 6 or 8) unlined, Rep 1 (or 2 or 3)
T2	VxT1WyLRz	Variety 'Kakariki' (or 'Wiki Tu' or 'Triumph'), 1 °C 85 %RH, week 4 (or 6 or 8) lined, Rep 1 (or 2 or 3)
T3	VxT4WyNLRz	Variety 'Kakariki' (or 'Wiki Tu' or 'Triumph'), 4 °C 88 %RH, week 4 (or 6 or 8) unlined, Rep 1 (or 2 or 3)
T4	VxT4WyLRz	Variety 'Kakariki' (or 'Wiki Tu' or 'Triumph'), 4 °C 88 %RH, week 4 (or 6 or 8) lined, Rep 1 (or 2 or 3)

4.2.2 Storage conditions and fruit quality assessment

On the day of fruit arrival for each variety, 3 random trays were used to collect at harvest data on individual fruit weight, non-destructive compression firmness, internal maturity, skin colour, soluble solids concentration (SSC), and titratable acidity (TA) as described in chapter 3. For initial fruit tray weights, fruit were weighed inside trays and the actual weight determined by subtracting the weight of box, plix and polyliner (where it was used). Inside the cold room as fruit was stored, empty trays were placed on top of each stack to maintain similar conditions for all treatments during the storage period.

Fruit were assessed after three storage durations (4, 6, and 8 weeks) and differences in fruit quality compared. At the end of each cool storage time, weighing of fruit was done inside the cold room in the plix tray but without fibreboard (since the boxes absorbed water unevenly during storage) and 3 d later at 20 °C. Weight loss was then calculated as a percentage of the initial weight as described in the general laboratory methods in section 3.2.1. The fruit were then transferred to 20 °C for equilibration for 3 d before quality assessments were carried out on half of the tray. The remaining half of the tray was left at 20 °C for a further 3 d and quality assessments were conducted again. At 20 °C, the polyliners were left tucked in and thus never tampered with. Fruit quality measurements (as described in section 3.2) were skin colour, non-destructive compression firmness, soluble solids concentration, titratable acidity, internal maturity, internal chilling injury and surface injury (which included rots and external chilling injury).

4.3 Data analysis

General linear model (GLM) procedure of ANOVA was performed using Minitab (Version 17.3.1, Minitab Inc, State College, Pennsylvania, USA) on all quality parameters to determine significance effects of treatments. Once differences were detected, treatment means were separated using Tukey's honest significant differences (HSD) test at 95% confidence level.

4.4 Results and discussion

4.4.1 Temperature and RH conditions

Temperature and relative humidity (RH) achieved inside boxes were within the expected range (Fig. 4-1, Table. 4-2). In this study lined boxes on average had slightly higher RH (94.58% and 95.83%) than unlined boxes (93.03% and 92.91%) and therefore. A RH of between 90-95% is generally regarded as safe for fresh horticultural products and may not cause shrivelling (when RH is too low) or favour rot development (when RH is too high). The data below are based on the corrected figures after the humidity sensors of the I-buttons were calibrated. The calibration was done using saturated salt solutions of magnesium chloride ($MgCl_2$), lithium chloride (LiCl), magnesium nitrate ($MgNO_3$), potassium chloride (KCl), and potassium carbonate (K_2CO_3) at -0.8 and at 20 °C for at least 2 d in a sealed container (Fig. 4-2). The equilibrium relative humidity of the air was measured using the water activity meter. (AW = RH when equilibrium of vapour and. and temperature was obtained). After calibration the corrected values for RH in lined boxes were 95 ± 1 and in unlined boxes 92 ± 1.

Table 4-2: Summarises of mean and range of achieved conditions inside lined and unlined trays.

	Lined boxes at 1 °C	Lined boxes at 4 °C	Unlined boxes at 1 °C	Unlined boxes at 4 °C
Mean Temp	1.27	4.36	1.40	4.62
SEM	0.05	0.03	0.06	0.02
Temp Range	0.54	0.86	0.76	1.06
Mean RH	94.58	95.83	93.03	92.91
SEM	0.02	0.02	0.03	0.02
RH Range	5.71	3.89	5.06	4.59

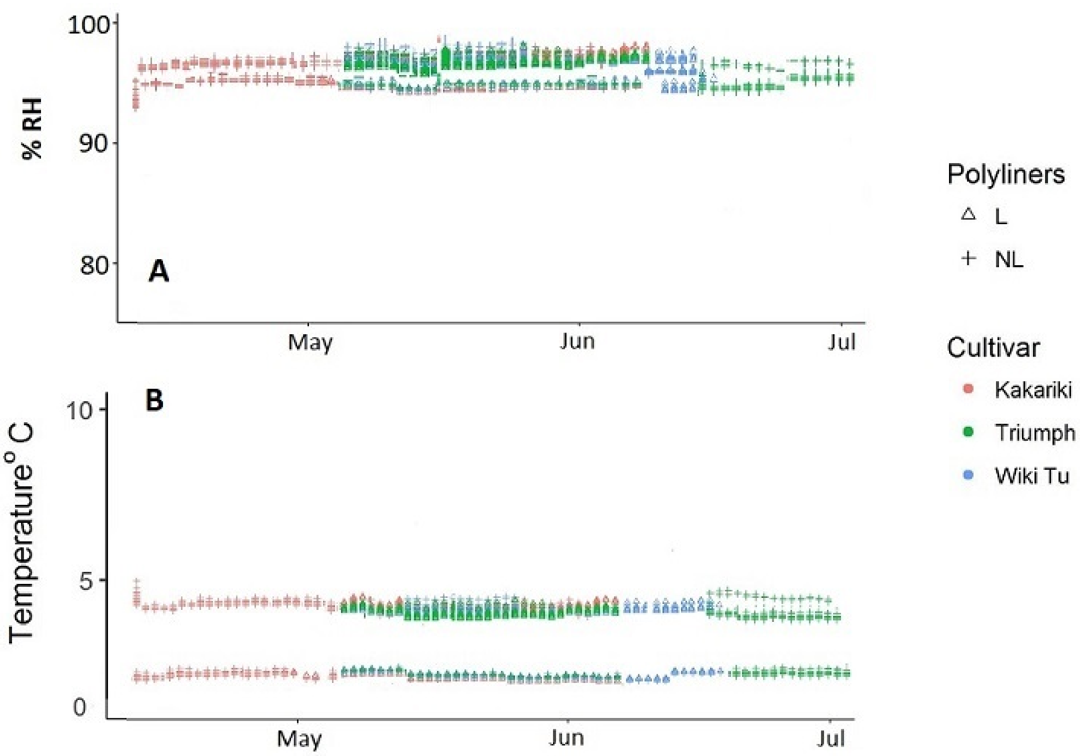


Figure 4-1 Average relative humidity (A) and temperature (B) attained inside fruit boxes from 11 I-buttons placed inside fruit trays whether lined or in unlined boxes, 1 I-button (kept in Lined boxes of 'Triumph' at 1 °C) malfunctioned and did not record any data.

Figure 4-2 Calibration of humidity sensors of I-buttons in potassium chloride solution at 20 °C.

4.4.2 At harvest fruit attributes

The three varieties received for this experiment were different in all aspects assessed (Table 4-2). In terms of weight 'Kakariki' were heavier with an average mass of 95.98 g whereas 'Wiki Tu' were light at an average mass of 81.35 g. 'Triumph' fruit were firmer than 'Kakariki' and 'Wiki Tu'. With respect to skin colour, 'Kakariki' were lighter green than 'Triumph' and 'Wiki Tu' as seen in the L*, C* and °h. 'Wiki Tu' with SSC of 10.35 °brix and TA of 1.02 g malic acid in 100 mL of juice were less sweet and slightly acidic. The subtle balance between sugar and sweetness in 'Triumph' in combination with internal maturity of 1.6 and high firmness of 28.53 N implied that 'Triumph' was actually immature. Just like the breeder descriptors 'Kakariki' were large fruited and in this experiment, they were big sized, more mature and somewhat sweet. Even though 'Wiki Tu' and 'Triumph' looked statistically different in terms of maturity, in truth they were both immature since they were below internal maturity rating 2 according to the PFR scale. Differences between varieties for at harvest fruit quality attributes were in agreement with findings of Wiryawan et al. (2005) who also observed variability within and between fruit batches in fruit mass, fruit firmness, soluble solids concentration, titratable acidity and °Brix to Acid ratio. Based on these at harvest fruit quality (mass, firmness soluble solids concentration, titratable acidity and °Brix to Acid ratio) the varieties can be grouped for different market outlets. For 'Kakariki' having IM of 2.5 reveals fruit almost ready for consumption and best suited for local markets and for 'Triumph' and 'Wiki Tu' with IM of about 1.6 gives slightly more time for consumption and thus best suited for export markets.

Table 4-3 Harvest date, individual fruit weight, firmness, skin colour, soluble solids concentration (SSC), titratable acidity (TA) and internal maturity of three feijoa varieties; 'Kakariki', 'Wiki Tu' and 'Triumph' soon after receiving fruit at Massey University postharvest lab. Values with different letters in a row indicate significant difference ($p < 0.05$).

Cultivar/Attributes		'Kakariki'(very early season)	'Wiki Tu' (mid to late season)	'Triumph'(late season)
Harvest date		15/4/2016	05/05/2016	12/05/2016
Individual Fruit weight (g)		95.98a	81.35b	82.71b
Firmness (N)		23.26b	21.35c	28.53a
	(L*)	47.83a	41.73b	40.92b
Skin colour	(C*)	32.43a	29.04b	26.38c
	(°hue)	110.56b	113.67a	114.02a
SSC (°Brix)		10.92b	10.35c	11.31a
TA (g malic acid in 100 mL of juice)		0.85c	1.02b	1.26a
°Brix/Acid ratio		12.85a	10.15b	8.98c
Internal maturity		2.5a	1.8b	1.6c

4.4.3 Surface injury including rots, external chilling injury, and other disorders

Development of rots and other disorders including chilling injury was observed after 4 weeks of storage and this increased over time (Fig. 4-3). Lining did not affect surface injury development, although development of surface injury was influenced by variety and storage conditions ($p < 0.05$). Statistically results showed that the quality of feijoa was acceptable for up to 4 weeks of storage although a translucent surface injury was observed especially for 'Kakariki' fruit stored at 1 °C. When fruit was kept at 20 °C for 3 d, the translucent surface turned black and a further 3 d later the surface injury became brown and, in some cases, hardening occurred (Fig. 4-4). As storage progressed especially in week 8, fruit became soft, rots and other forms of damage were also observed. From this experiment, storage of fruit up to 4 weeks could be regarded as satisfactory since fewer than 15% of fruit were adversely affected. As storage approaches week 6 damage incidence increased to about 25% and this amount of damage can be bad for a business. From this result (Fig. 4-3) it is obvious that there was no benefit in storing feijoa up to 8 weeks because surface damage reached 100%. Other defects that were observed especially at 1 °C included some fruit not ripening, and appearance of a reddish-brown colouration around vascular bundles implying that chilling injury had indeed affected the fruit (Thorp and Bieleski, 2002). It is possible that the high development of surface injuries was brought about by the high RH (Fig 4.1) that modified the physiological and biochemical processes associated with ripening (and eventually senescence) of feijoa.

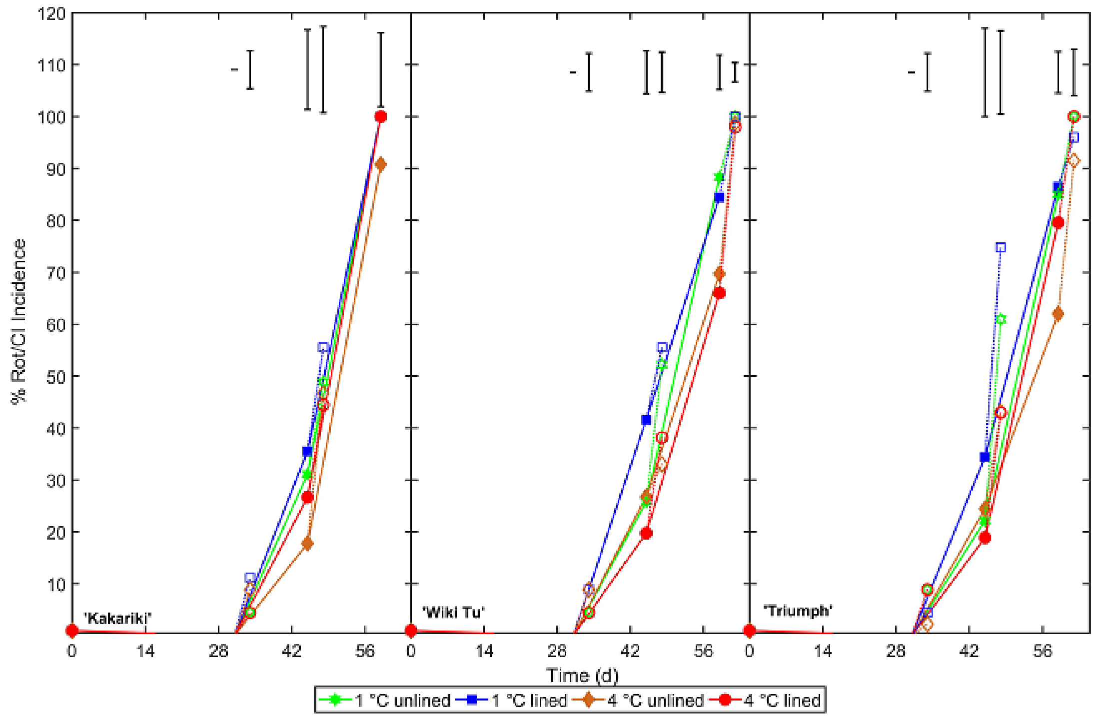


Figure 4-3 Variety differences for surface injury including rots and external chilling injury of 'Kakariki', 'Wiki Tu', and 'Triumph' stored at 1 °C and 4 °C and assessed 3 d after storage (Solid symbol) and after 6 d (hollow symbol) at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for 'Kakariki' week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey's).

Figure 4-4 Chilling injury as illustrated with the hardened surface browning of feijoa 6 weeks after storage at 1 °C.

4.4.4 Internal chilling injury

Development of internal chilling injury was worse compared to surface injury development especially for the 'Kakariki' variety stored at 1 °C (Fig. 4-5, & Fig.

4-6). Externally, irrespective of whatever variety, feijoa at week 4 of storage looked good with no damage but once it was cut transversely a pink-brown ring (different from browning of polyphenol oxidase [PPO]) was observed, and this finding was in agreement with observations of Amarante et al. (2017). At other times, internal browning along vascular bundles from the stem end was also observed. These observations were similar with the description of chilling injury of feijoa as outlined in literature (Thorp and Klein, 1987). It is possible that feijoa membranes had high proportion of saturated to unsaturated fatty acids that made it difficult to maintain membrane integrity and hence were sensitive to CI (Marangoni et al., 1996). Thorp and Bieleski (2002) suggested that external CI is observed as an outward indication of internal breakdown of vascular bundles or even of the entire fruit. An important inference from the results shows that by six weeks of storage greater than 25% of fruit developed CI and this makes storing these feijoa varieties for longer than 4 weeks uneconomic for growers and renders them unsuitable for seafreight.

Figure 4-5 Symptoms of internal chilling injury of 'Kakariki' (A) seen as internal breakdown of locular region and 'Triumph' (B) showing internal browning along vascular bundles 8 weeks after cool storage and 1 °C and 4 °C.

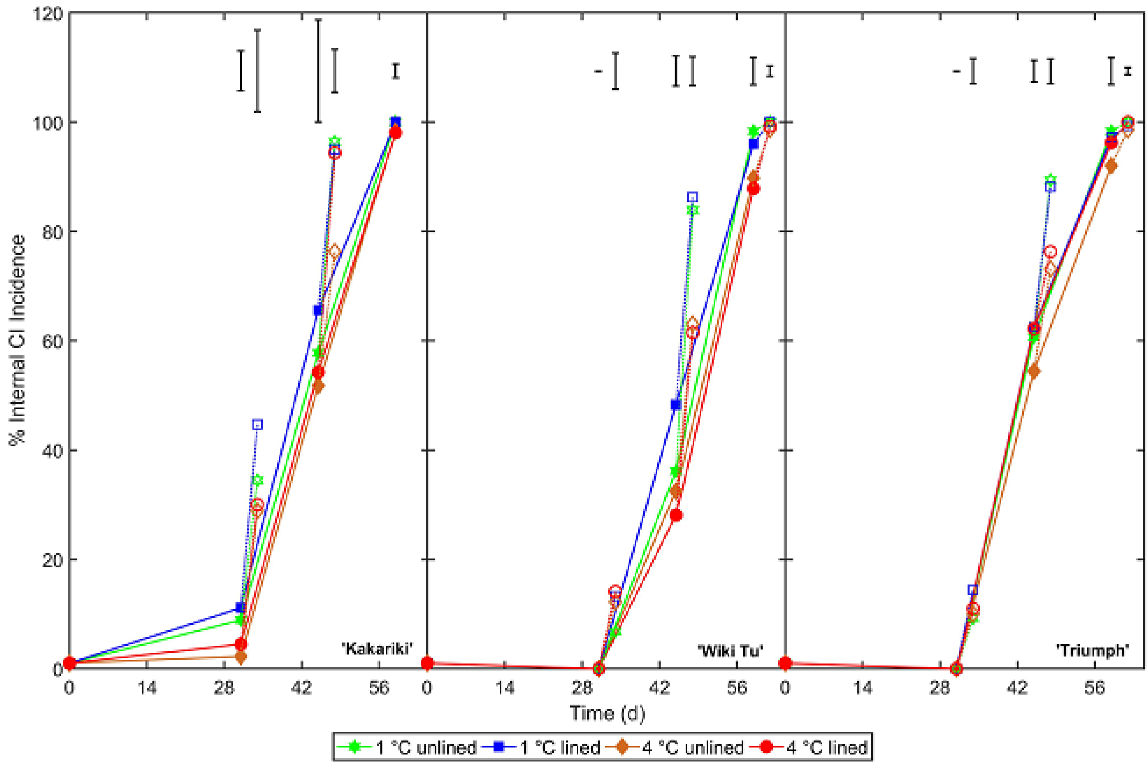


Figure 4-6 Variety differences for internal chilling injury of 'Kakariki', 'Wiki Tu', and 'Triumph' stored at 1 °C and 4 °C and assessed 3 d after storage (Solid symbol) and after 6 d (hollow symbol) at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for 'Kakariki' week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey's).

4.4.5 Internal maturity

Lining had no effect on development of internal maturity of the 3 varieties and as such data has been pooled together to represent only the temperature effect [that is 1 °C and 4 °C] (Fig. 4-7). There was gradual increase of fruit ripening particularly at 4 °C possibly due to the slightly warmer temperature that may have increased respiration (metabolism) as anticipated by the temperature quotient (Q10). Metabolism for 'Kakariki' particularly seemed to be high even from the at-harvest data, and this was perceived from how fast the variety progressed from one maturity scale to the next, particularly at 4 °C. An interesting observation though is seen in 'Wiki Tu' where the fruit progression from one scale to the next was faster at 1 °C than the other 2 varieties. Just as stated in literature 'Triumph' stores well and can be recommended for export or long storage unlike 'Kakariki' which growers should actually consider growing only for the domestic market. It is also important to differentiate the rapid darkening of flesh due to ripening from that of internal chilling injury development.

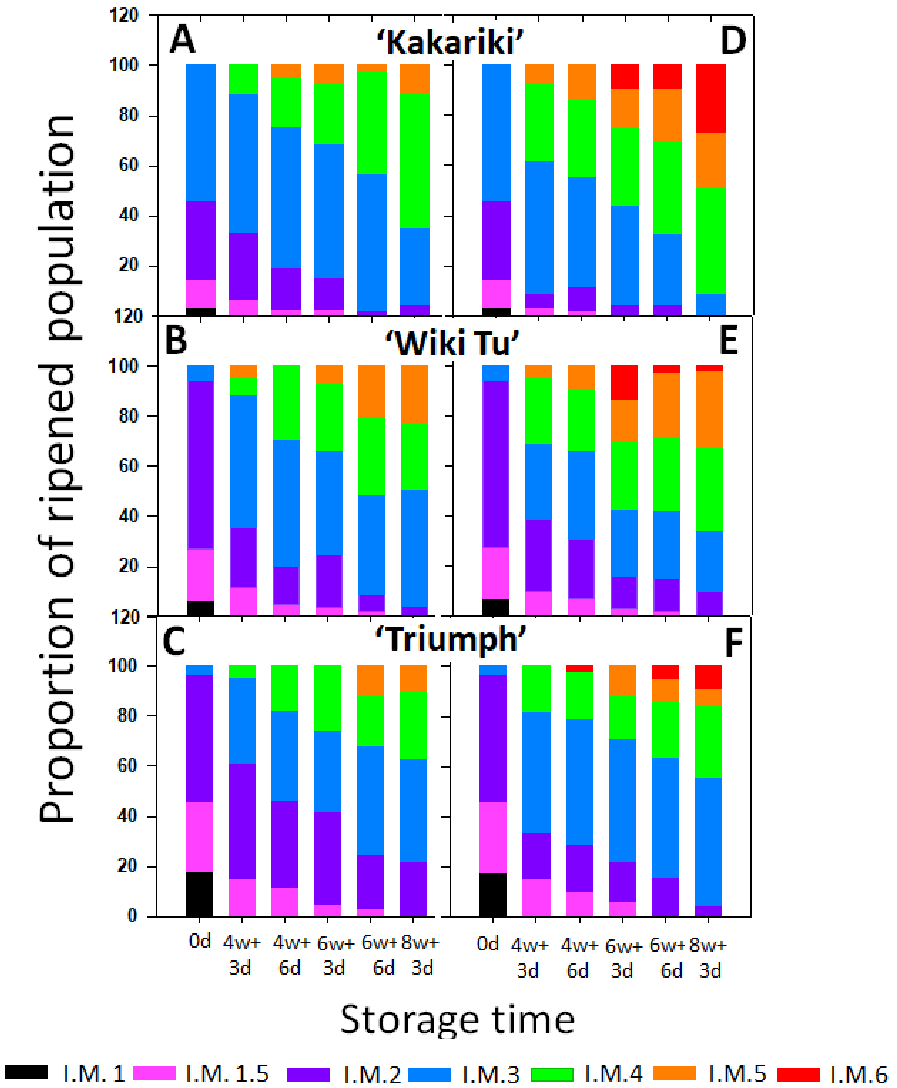


Figure 4-7 Variety differences for internal maturity of 'Kakariki', 'Wiki Tu', and 'Triumph' stored at 1 °C (A, B, & C) and 4 °C (D, E, & F) and assessed 3 d after storage and after 6 d at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for 'Kakariki' week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey's).

4.4.6 Weight loss

Weight loss increased over time and was influenced by variety, lining, and temperature (Fig. 4-8). 'Wiki Tu' fruit stored in 4 °C unlined tray lost the highest amount of water (11.7%) followed by 'Triumph' (9.8%) in 4 °C unlined tray and 'Kakariki' (7.1%) also in unlined tray at 4 °C. Even with weight loss of 11.7%, insignificant level of shrivel occurred and as earlier reported in literature (section 2.6.2.1) water loss is not a problem for feijoa. Unlined fruit lost more water than lined fruit at each temperature, whereas 4 °C in unlined trays lost the highest amount of water throughout the storage period. When fruit was kept at 20 °C for a further 3 d weight loss increased rapidly. The explanation for weight loss is largely by transpiration that occurs due to the vapour pressure deficit (VPD) between fruit and the surrounding fruit environment. Inside the polylined boxes (which in principle provides low permeability) the VPD was low resulting from low water loss caused by high RH Maguire et al. (2001), Where the VDP = vapour pressure at the fruit surface (P_{vs} – vapour pressure of the surrounding air (P_{va} [kPa]). So apart from temperature and relative humidity other factors that influenced this weight loss included air velocity, surface area to volume (or mass) ratio of fruit, fruit morphological characteristics (such as cuticular wax, cracks, stomata, or lenticels), and feijoa maturity (Maguire et al., 2001; Xanthopoulos et al., 2017). Another interesting argument fronted by Maguire et al. (2001) is the respiration contribution to weight loss. It is possible to calculate what proportion of weight loss is due to carbon loss (through respiration) by calculating the mass of carbon dioxide given off. And in future it would be interesting to use current data to simulate models of feijoa water loss in storage conditions. A 5% weight loss is documented to cause many fruit and vegetables to either wilt or shrivel, change colour, lose crispness or lose nutritional quality in severe cases. As for feijoa, even 11.7% weight loss does not cause any visible shrivel. Water loss as described by Wills and Golding (2016) has commercial significance for fruit and vegetables even after exposing them to warm temperatures for only a few hours. Clearly there is a large loss of economic value caused by not using a polyliner and it is the strongest conviction of the author that feijoa growers should adopt this practice.

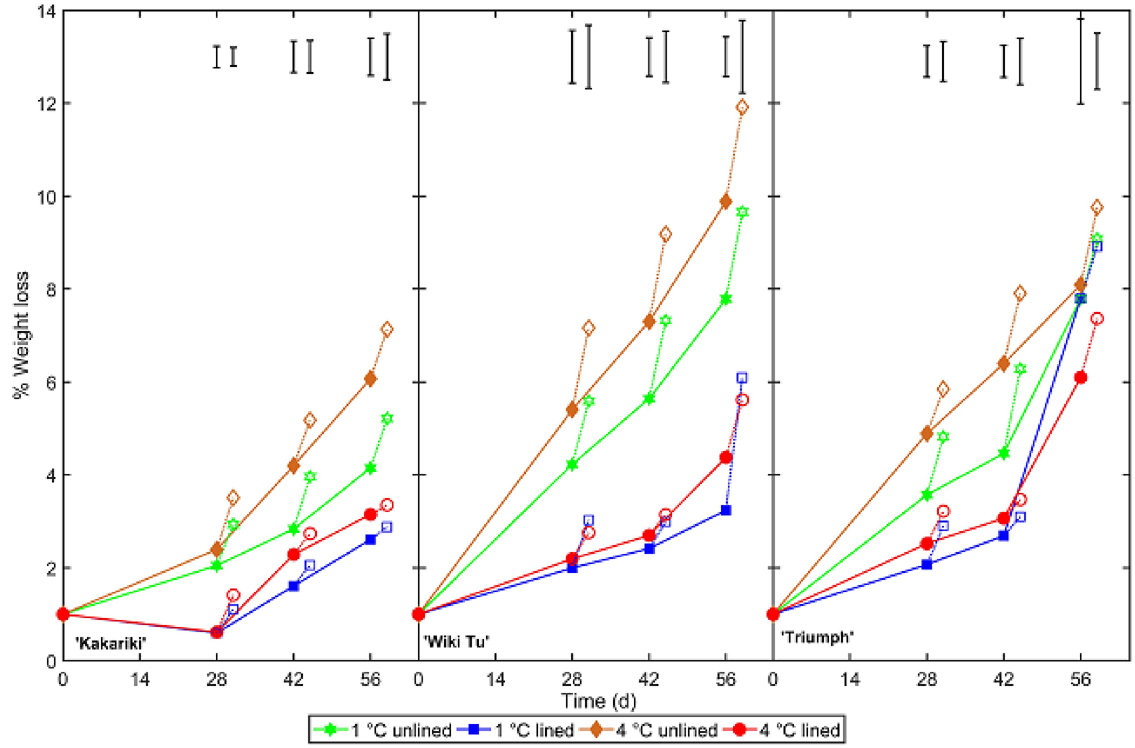


Figure 4-8 Variety differences for weight loss of 'Kakariki', 'Wiki Tu', and 'Triumph' stored at 1 °C and 4 °C and assessed 3 d after storage (Solid symbol) and after 6 d (hollow symbol) at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for 'Kakariki' week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey's).

4.4.7 Compression firmness

Compression firmness declined progressively over time, with further reductions occurring when fruit were kept at 20 °C for an additional 6 d (Fig. 4-9). This result replicates those of Al-Harthy et al. (2008) and Rupavatharam et al. (2015b) who observed a decrease in 'Unique' feijoa from about 27 N at harvest to 8 N after 10 weeks of storage. 'Triumph' consistently maintained firmer fruit in comparison to 'Kakariki' and 'Wiki Tu'. Likewise, 1 °C lined trays had firmer fruit whereas 4 °C unlined fruit were soft. Loss of firmness at 4 °C and in unlined trays could be related to low turgor pressure in cells resulting from high water loss caused by high temperature and low RH as earlier observed in section 4.4.6. Although the mechanism for feijoa softening is not well understood it is possible that alteration of cell walls (due to cell wall degrading enzymes) and fruit water status (associated with cell turgor) had some effects (Paniagua et al., 2014). The results of water loss and firmness elucidate that plastic film delayed deterioration as observed by Ben-Yehoshua et al. (1983) in relating water stress to physiological parameters of lemon and bell pepper in sealed and unsealed fruit. The driving force for water loss (VPD) in lined trays was lower than in unlined trays even though the RH was > 90% (Table 4.2}. The firmness is reflective of this Apart from transpiration fruit ripening is expected to contribute to the firmness loss. Little has been published on ripening related modifications to feijoa cell walls, but the development of locular gel demonstrates visible anatomical changes that contribute to loss of firmness. Heyes and Townsend (1992) also suggested that fruit softening of nectarines would be triggered by increased proton pumping across the plasma membrane, however, this idea is yet to be tested in feijoa.

In brief, different storage VPDs (temperature and RH) that affected weight loss also affected firmness of feijoa. At 1 °C, low temperature reduced water movement from fruit and possibly decreased activity of cell wall degrading enzymes and ethylene related enzymes to activate ripening thereby resulting in firm fruit (Abu-Goukh and Bashir, 2003). It is also important to note that polyliners delayed softening of feijoa however, on the final firmness the 3 varieties softened differently. For instance, lined 'Triumph' stored at 1 °C after 62 d had firmness of about 20 N while unlined 'Triumph' had about 8 N. In the case of lined ‘Wiki Tu’ stored at 1 °C had about 7 N while unlined ‘Wiki Tu’ was 5 N. Determining a firmness level that describes fruit for local or export consumption can help feijoa industry. For instance, the kiwifruit industry in New Zealand has identified firmness that defines the “eating window”. Unfortunately for feijoa this is unknown and maybe in future it would be a good idea to do a texture profile analysis (TPA) test to define the fruit texture features (gumminess, springiness, hardness, resilience, chewiness, and cohesiveness) and associated sensory analysis to identify the zone of “acceptable texture” for consumers (Singh et al., 2013).

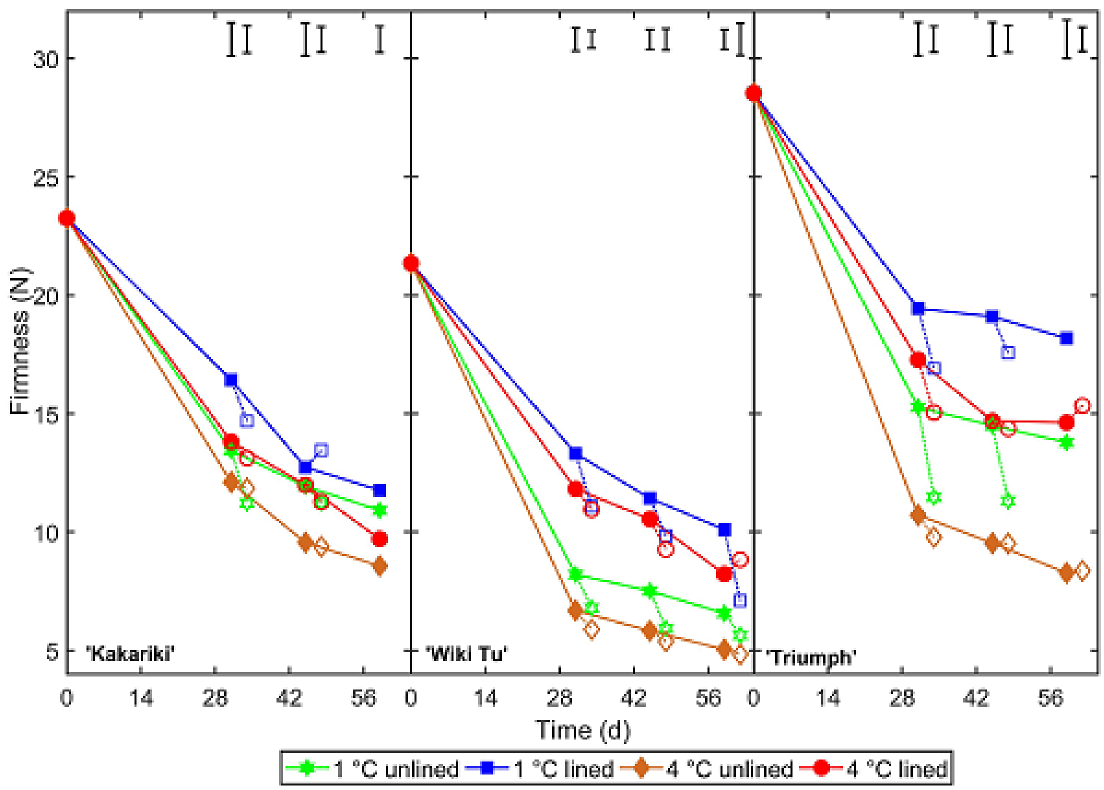


Figure 4-9 Variety differences for compression firmness of ‘Kakariki’, ‘Wiki Tu’, and ‘Triumph’ stored at 1 °C and 4 °C and assessed 3 d after storage (Solid symbol) and after 6 d (hollow symbol) at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for ‘Kakariki’ week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey’s).

4.4.8 Soluble solids concentration (SSC)

Soluble solids concentration was influenced by temperature and lining in all varieties during storage (Fig. 4-10). Fruit stored at 1 °C lost SSC more slowly than fruit stored at 4 °C. Possibly the low temperature delayed loss of sugars since respiration was slowed down and therefore conversion of glucose to carbon dioxide curtailed. Transferring fruit to 20 °C increased loss of sugars. These observations were similar to those of Klein and Thorp (1987) and Al-Harthy et al. (2010b) who reported a decline in SSC as feijoa ripened. During the entire storage time, 'Triumph' maintained relatively high SSC and stored better than 'Wiki Tu' that had the lowest SSC. Fruit with high SSC is thought to store well and to have a good taste (Meena et al., 2018). As much as feijoa is classified as a climacteric fruit, it does not have large starch reserves like most climacteric fruit and thus after harvest there is no evidence of SSC increasing as fruit ripens. Maybe its climacteric features are completed before or by harvest time. Unlike kiwifruit, SSC in feijoa has not been studied or proposed for use as a maturity index to determine when to harvest fruit (Burdon et al., 2016).

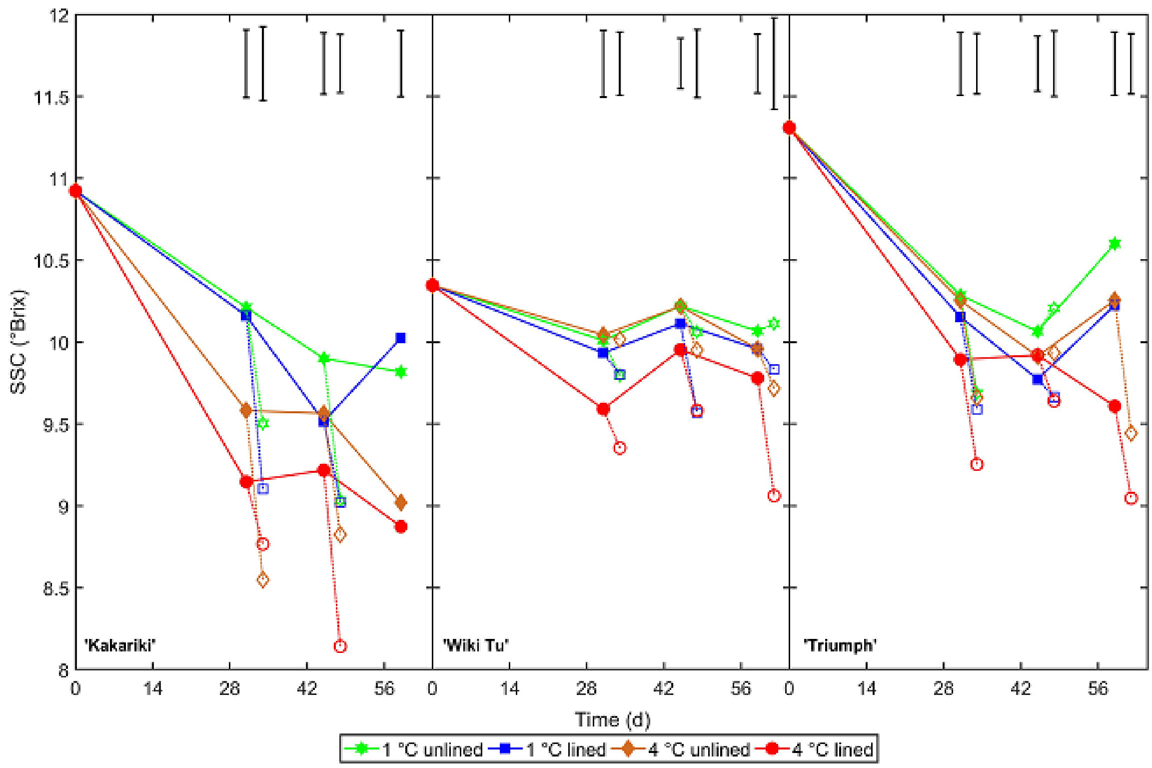


Figure 4-10 Variety differences for soluble solids concentration of 'Kakariki', 'Wiki Tu', and 'Triumph' stored at 1 °C and 4 °C and assessed 3 d after storage (Solid symbol) and after 6 d (hollow symbol) at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for 'Kakariki' week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey's).

4.4.9 Titratable acidity (TA)

Titratable acidity of the 3 feijoa varieties declined gradually over time (Fig. 4-11). Lining had no effect on TA, although temperature and storage time had some influence. TA for 'Kakariki' was almost constant but for 'Triumph' there was a steep decline right from harvest to end of storage period. TA together with SSC determines the sourness and sweetness of a fruit. Decrease in TA as fruit ripened is in agreement with findings in literature (Al-Harthy et al., 2010b; Rupavatharam, 2015). The main organic acids reported by Harman (1987) and reviewed by Schotsmans et al. (2011) are malic acid and citric acid with similar acidity strengths of 0.80–1.60 g malic acid 100 g^{-1} FW. Decrease in TA could imply that organic compounds are used during respiration and ripening (which affects flavour/taste). The decrease in TA implies that there as an alteration in organic acid levels, and this is likely to influence pH and, therefore, possibly sugar transformation and carotenoids in feijoa pericarp.

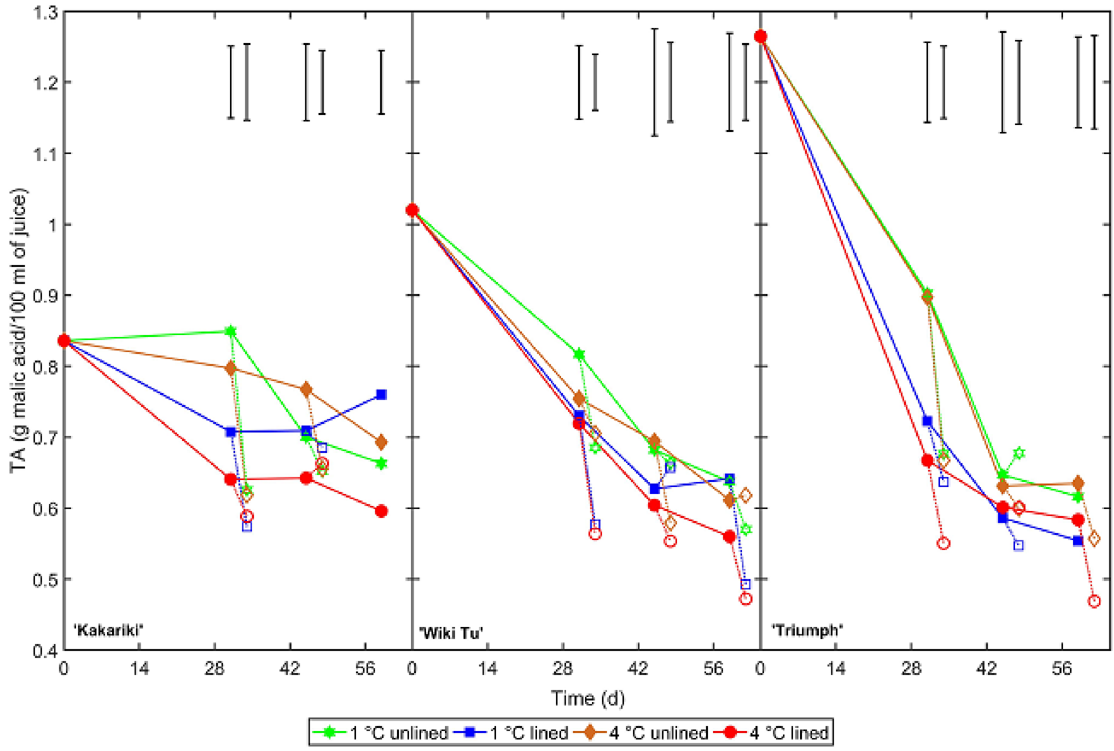


Figure 4-11 Variety differences for titratable acidity (TA) of 'Kakariki', 'Wiki Tu', and 'Triumph' stored at 1 °C and 4 °C and assessed 3 d after storage (Solid symbol) and after 6 d (hollow symbol) at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for 'Kakariki' week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey's).

4.4.10 °Brix/Acid ratio

The °Brix/Acid ratio ranged from 10.1 - 20.2 during storage and was influenced by temperature, lining, storage time and variety. (Fig.4-12). 'Kakariki' maintained constant values whereas 'Wiki Tu' and 'Triumph' had the ratio increasing suggesting loss of flavour (bland taste). Recalling the at harvest data, the °Brix/Acid ratio demonstrates the possibility of 'Triumph' being more immature than the other varieties. The decrease in SSC and TA in feijoa leads to deterioration of taste (Amarante et al., 2017). Determination of proper harvesting time reflective of an ideal feijoa would have a fruit that is balanced in sourness and sweetness and at the same time stores for a longer period of time. This ideal fruit maturity at harvest is yet to be established for feijoa.

Touch picking, a technique whereby fruit is tilted gently from one side to the other, is currently used. This method is subjective and if a reliable technique can be developed, then that would be a plus to the feijoa industry, because growers will get better commercial returns. In grapes, apples, and grapefruit studies have shown a direct relationship between SSC, TA and/or SSC/TA with consumer acceptability and these attributes can be used to determine fruit ripeness (Jayasena and Cameron, 2008).

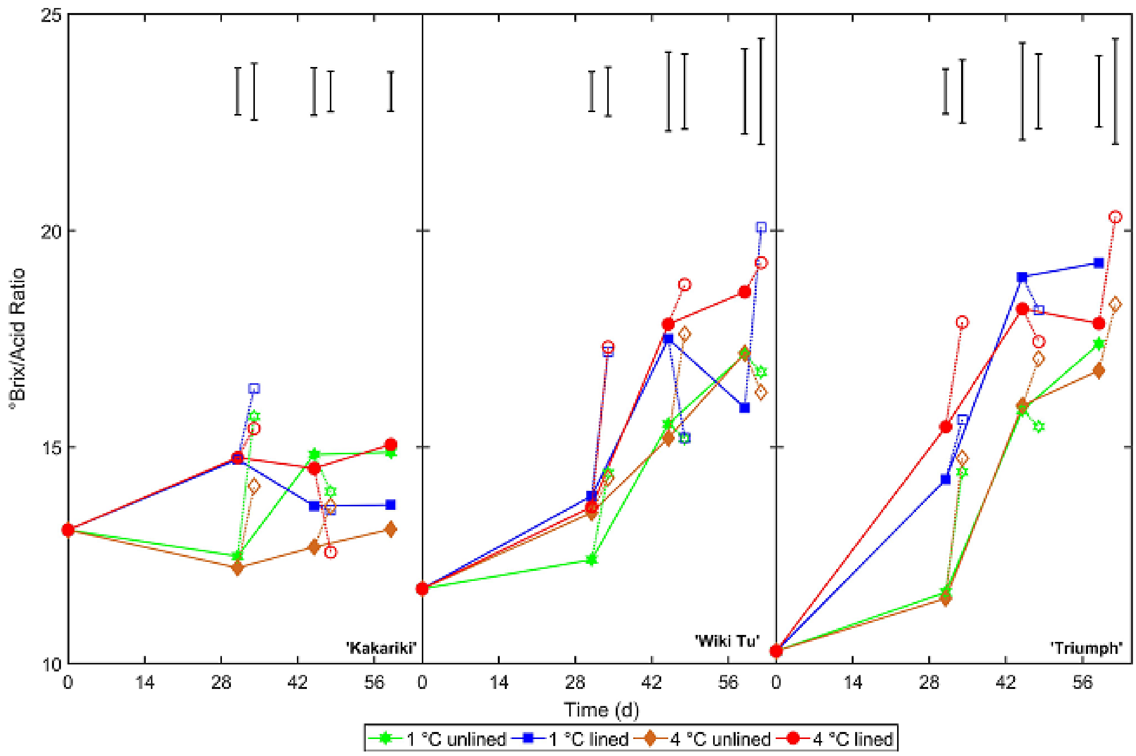


Figure 4-12 Variety differences for °Brix/Acid ratio of 'Kakariki', 'Wiki Tu', and 'Triumph' stored at 1 °C and 4 °C and assessed 3 d after storage (Solid symbol) and after 6 d (hollow symbol) at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for 'Kakariki' week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey's).

4.4.11 Skin colour

Lightness increased under the storage conditions (Fig. 4-13A). 'Kakariki' was distinctly different with a high value of 50 at harvest, while 'Triumph' and 'Wiki Tu' ranged from approximately 40 to 44. Throughout the experiment there were no differences between 'Triumph' and 'Wiki Tu' except for fruit stored at 1 °C for 8 weeks. Keeping the fruit at 20 °C for a further 6 d caused a further increase in lightness. Increase in lightness at 20 °C implies that rise in temperature did not preserve the dark green colour of feijoa therefore, implying loss of chlorophyll and revealing of carotenoids as fruit ripened (East et al., 2009).

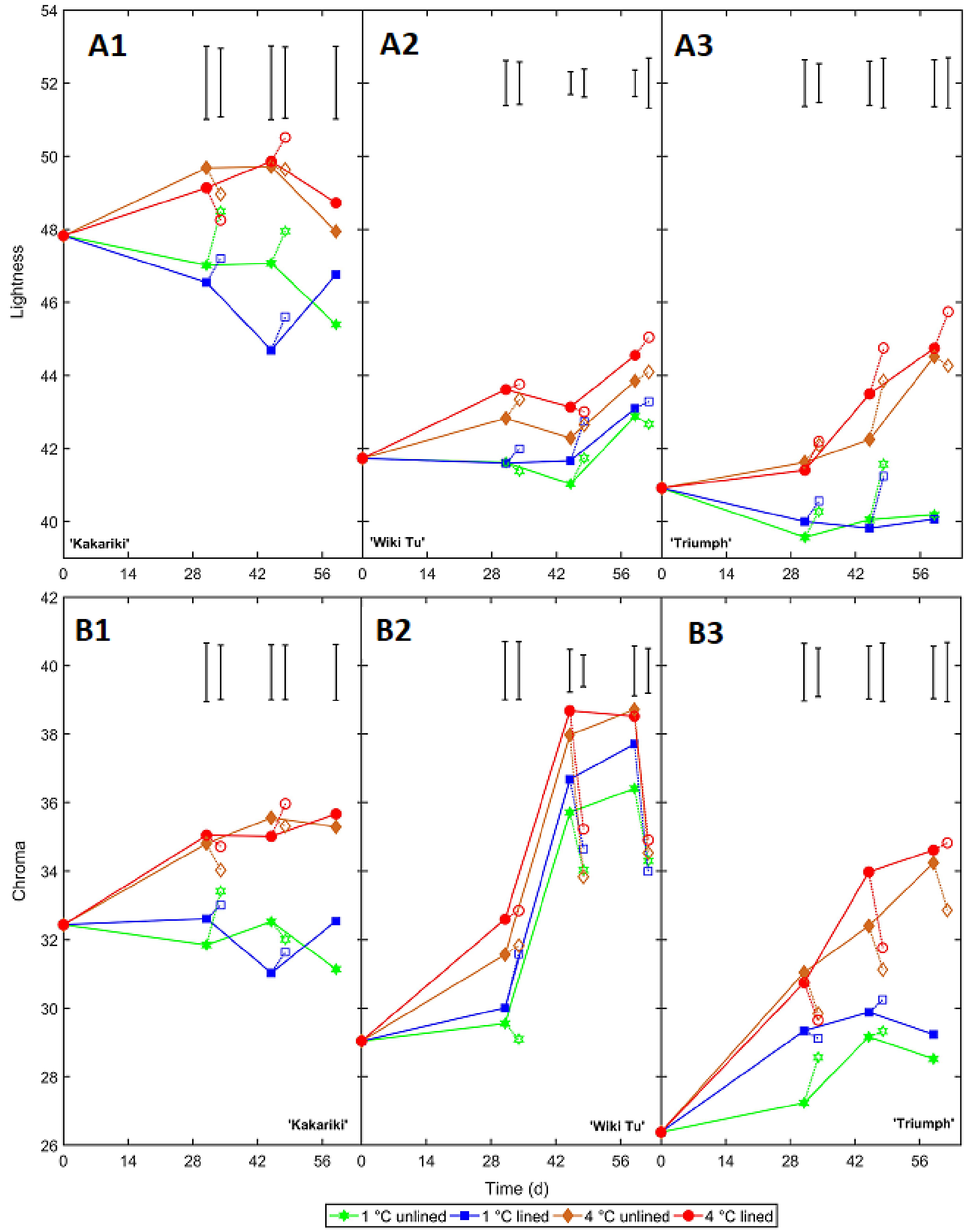


Figure 4-13 Variety differences for lightness (A) and chroma (B) of 'Kakariki', 'Wiki Tu', and 'Triumph' stored at 1 °C and 4 °C and assessed 3 d after storage (Solid symbol) and after 6 d (hollow symbol) at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for 'Kakariki' week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey's).

Observations on chroma generally showed an upward trend with a minimal average chroma of 25 for 'Triumph' and a high of 46 for 'Wiki Tu' (Fig.4-13B). Subsequent assessment at 20 °C for a further 3 d resulted in chroma declining. In both temperatures, 'Wiki Tu' developed high chroma in comparison to the other varieties (P < 0.05). At end of storage 'Triumph' and 'Kakariki' colour in terms of chroma were similar.

Variety and temperature both had an effect on colour development (Fig. 4-14). Average hue angle for the three varieties ranged from 114° at the start of the experiment and dropped to a low of about 106°. Decrease in hue angle indicates a loss of chlorophyll content. Similar results have also been reported by East et al. (2009); Duan, (2015) and Rupavatharam et al. (2015a). At harvest 'Triumph' and 'Wiki Tu' were slightly darker green with a hue angle of about 114°, while 'Kakariki' had hue angle of 110.5°. After 4 weeks of storage at 1 °C all treatments did not reduce in hue angle unlike fruit at 4 °C. The implication is that low temperatures reduced the rate of ripening, retaining the green colour. When transferred to 20 °C for a further 3 d the hue angle steeply declined. After 6 weeks in 1 °C plus 6 d at 20 °C, the hue angle increased, while for the 4 °C stored fruit only 'Wiki Tu' increased.

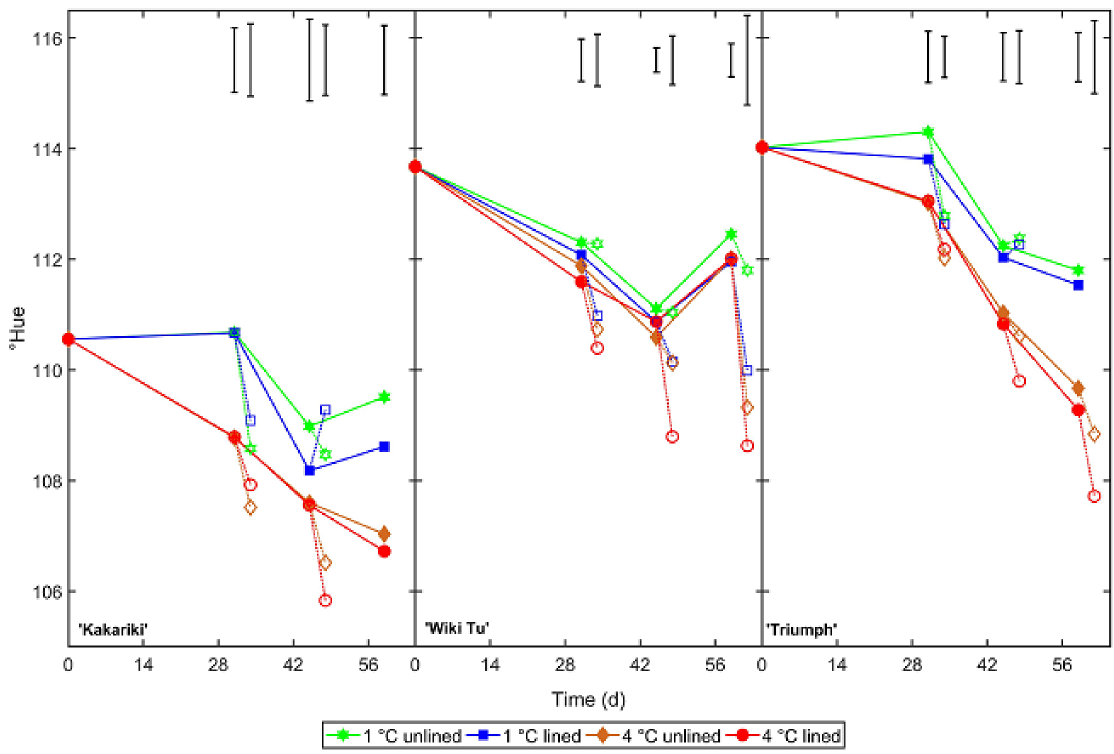


Figure 4-14 Variety differences for hue angle of 'Kakariki', 'Wiki Tu', and 'Triumph' stored at 1 °C and 4 °C and assessed 3 d after storage (Solid symbol) and

after 6 d (hollow symbol) at 20 °C. Each data point represents average of 3 replicates of 15 fruit except for 'Kakariki' week 8 that had 12 fruit. Vertical bars represent HSD 0.05 (Tukey's).

From the above result (Fig. 4-14), it is clear that all varieties were different in their colour development and this changed during storage. 'Triumph' and 'Wiki Tu' appeared darker green (°hue = 114) than 'Kakariki' (°hue = 110). Colour change was greater at warmer temperatures and lining (RH) was not important. Therefore, it is important to note that lower temperatures seem desirable because they retained skin greenness (colour), but the concomitant increased in chilling injury prevents this from being a useful strategy.

4.5 Conclusions

In conclusion, this study demonstrated how changes in temperature and humidity affected fruit quality attributes; and how the information helped identify significant varietal differences of feijoa in attempts to extend storage life. Spectrophotometer, texture analyser, titrator, weighing balance and refractometer were tools used to assess skin colour, compression firmness, titratable acidity, weight loss and soluble solids concentration of three feijoa varieties. Wrapping fruit in a polyliner led to lower water loss and firmer fruit after storage but did not affect fruit ripening. Weight loss was observed to increase linearly with storage time.

The first key finding was that varietal differences were found among the three varieties in all quality attributes assessed. This finding outlined pertinent issues of maturity, internal browning, chilling injury and how these attributes would assist in choosing either a domestic or export market to send a particular feijoa variety in order to break even or even make profits. From the above studies all varieties were not suitable for long storage/seafreight but can be consumed locally as has been the case. However, it is important to note that 'Kakariki' had the highest chilling injury incidence when stored both at 1 °C and 4 °C which also led to high incidences of fruit decay. Another factor that led to poor performance of 'Kakariki' was the fact that fruit at harvest was already in advanced ripening state and this lowered the fruit quality during storage.

Another finding was fruit stored at 1 °C had retained many of the fruit quality attributes, however, internal chilling injury was observed especially in variety 'Kakariki' and to a less extent 'Wiki Tu' even at 4 weeks of storage making the fruit unmarketable. There is significant expansion in plantings of 'Kakariki' and 'Wiki Tu' and these data are important because they suggest the industry should not plan for seafreight with these varieties. More studies including harvesting time should also be conducted on the varieties to ascertain their storage potential. There is urgent need for further studies to find ways of reducing chilling injury in feijoa 'Kakariki', 'Triumph' and 'Wiki Tu'. In addition, further selection of suitable varieties and development of technologies that would allow long-term storage if the feijoa industry is to successfully expand its exports by sea.

Chapter 5 Influence of harvest maturity and step down conditioning temperature on storage life of feijoa stored at different temperatures

5.1 Introduction

As noted earlier, a number of factors come into play in determining storage life of feijoa. In chapter 4, after reassessing the effect of temperature and relative humidity, the results showed the importance of chilling injury as a major constraint to storage life. This section examines the influence of harvest maturity and step down conditioning in attempts to alleviate chilling injury and extend storage life.

In horticultural production maturity is either classified as commercial or physiological. While market forces determine commercial maturity, physiological maturity on the other hand is attained when fruit fulfills its purpose i.e. contains mature seed. In feijoa, growers either attach catching nets to shrubs to harvest mature fruit by shaking trees or use the standard commercial maturity method of ‘touch picking’ technique. In this technique, growers will allow fruit to detach naturally from the stalk (pedicel) when little force is applied (Klein and Thorp, 1987).

Previous efforts to extend storage life of feijoa using touch picked fruit have not been successful. One reason may be that touch picking provides “ready to eat fruit” as opposed to fruit suitable for long storage (Klein and Thorp, 1987; Wiryawan et al., 2005; Al-Harthy et al., 2008; Amarante et al., 2008; East et al., 2009). Rupavatharam et al. (2015c) decided to break away from the touch pick tradition and tested early harvested feijoa (less mature fruit) to extend storage life. Even though the group succeeded in extending storage life to a period of

6 weeks, early harvested fruit had low soluble solids content (SSC) and high titratable acidity (TA) implying that fruit may have tasted underripe or less appealing. The researchers concluded that sensory testing would be required in new markets to see if these fruit were still deemed acceptable.

Step down conditioning is a technique that can be used for some fruit to allow time for acclimation to low temperature conditions (Wang, 1993). The technique involves exposing fruit to non-chilling temperature for a short period before transferring to low temperature for long-term storage. In relation to feijoa Woolf et al. (2006) exposed 'Gemini', 'Unique', 'Opal Star' and 'Apollo' feijoa to a 5 week step down temperature conditioning where 1 °C was dropped each week for 5 weeks i.e. from 4 °C, to 3 °C, to 2 °C, to 1 °C before storing at 0 °C. This group observed chilling injury disorders even in fruit stored at 4 °C and more fruit decay at 0 °C than at 4 °C. However, this experiment seems flawed in that the tested step down regime was entirely within the window for chilling injury. Moreover, no recent published work has tested the efficacy of feijoa storage in temperatures below 4 °C.

In other fruit however, authors have reported success in use of step down cooling. For instance, conditioning loquat fruit at 5 °C for 6 days before transferring them to 0 °C for storage reduced chilling injury by 10% (Cai et al., 2006). In another study, Sapitnitskaya et al. (2006) 'Star Ruby' grapefruit were pre-stored at 16 °C for 7 d before storing at 2 °C and the results showed markedly reduced CI symptoms and enhanced chilling tolerance. In kiwifruit step down conditioning at 12 °C for 3 days before storing at 0 °C reduced chilling injury and maintained firmness, reduced water loss and rate of respiration and increased activity of antioxidant enzymes (Yang et al., 2013).

As clearly illustrated in section 2.6, earlier research in New Zealand has focussed on older varieties grown in New Zealand, warranting revisiting of research that has potential to extend postharvest storage of fruit on a newly introduced and commonly planted feijoa cultivar such as 'Kakariki'. Equally for step down conditioning on feijoa only one combination that was within the chilling temperature range (4, 3, 2, & 1 °C) has been reported (Woolf et al., 2006).Consequently, more studies are needed to extend fruit quality at non-chilling temperatures while increasing fruit tolerance to avoid chilling injury. This study therefore undertook to assess the effect of maturity at harvest on storage life of 'Kakariki' feijoa and to investigate the effect of step down conditioning on reducing chilling injury in 'Kakariki' feijoa.

5.2 Materials and methods

5.2.1 Fruit sample

Fruit at two harvest maturities i.e. early (one week before touch picking) and commercial (at touch picking) of 'Kakariki' were collected on 23/03/2017 and 31/03/2017 respectively from Southern Belle Orchard (SBO) in Matamata (Table 3-1). With guidance from the orchardist's experience based on days after fruit set combined with appearance of dull fruit (not very glossy), the early harvested 'Kakariki' fruit of size that would equate to count 28-33 were removed with a slightly higher force. At touch picking time, a week later fruit equivalent to count 28-33 were again removed from the trees but this time using 'touch picking'. All the fruit for both experiments were randomly harvested from all blocks containing 'Kakariki' trees in SBO.

Two thousand seven hundred (2700) fruit were graded based on size (averaging 103 g) and in the 28-33 count range using a computerised weight grader (Frans de Jong, personal communication, April 14, 2016). The fruit were then packaged in single layered commercial corrugated fibreboard trays that contained plix. The fruit was then transported in a non-refrigerated truck overnight and received at Massey University, Palmerston North approximately 48 h after harvest. On arrival, fruit were randomly allocated into pre-labelled trays that defined subsequent treatments and measurement. Randomly three trays were picked for at harvest fruit quality measurements and the remaining trays were polylined with 10 µm thickness high density polyethylene to reduced water loss due to transpiration (Fig. 5-0).

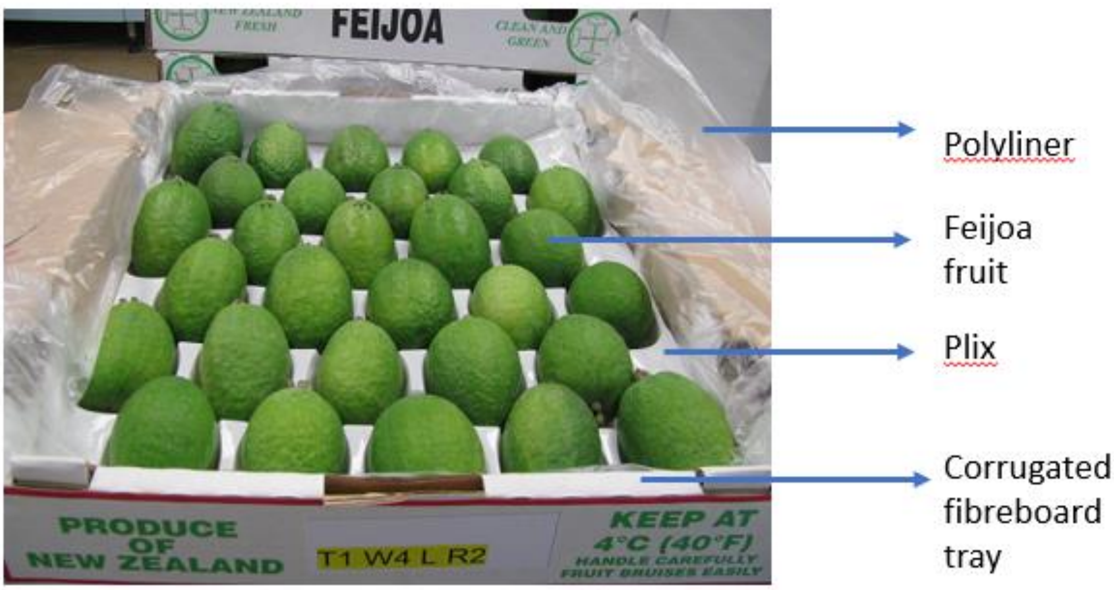


Figure 5-0 Fruit in plix with a liner inside a fibreboard tray.

5.2.2 Experimental design

A factorial arrangement comprising 2 harvesting times (early (H1) and commercial (H2)), 2 storage temperatures (2 and 4 °C) and three conditioning treatments was established. The three conditioning treatments were as follows:

1. Fruit initially stored at 9 °C for 6 days before transferring to 2 or 4 °C for 8 weeks (single step-down conditioning denoted by S6d,9→2 or 4).

2. Fruit stored at 9 °C for 3 days then moved to 6 °C for another 3 days before transferring to 2 or 4 °C for 8 weeks (double step-down conditioning denoted by S3d,9→6→2 or 4).
3. Fruit stored directly at 2 or 4 °C for 8 weeks (control denoted as C2 or C4).

Fruit for each treatment combination were assessed after four storage durations (6 d [soon after conditioning], and after 4, 6, and 8 weeks of cold storage). Each treatment by storage time combination had 3 replicate trays of 30 fruit. After cold storage fruit were allowed to equilibrate at 20 °C overnight and the following morning quality assessment was carried on half of the trays. The remaining half of a tray was left at 20 °C for a further 4 d before quality assessment was conducted.

5.2.3 Fruit quality, physiological and biochemical measurements

Fruit quality measurements were firmness, skin colour, soluble solids content, postharvest weight loss, internal and external chilling injury, and internal maturity. Physiological measurements to indicate the fruit physiology included respiration rate and biochemical measures were made of antioxidant enzyme activity. The antioxidant enzymes measured were peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). Measurements on fruit quality and respiration were used as described in the laboratory methods chapter 3, while the antioxidant enzyme assays are described below.

5.2.3.1 Antioxidant enzymes assay

For each treatment and storage time, the pulp (a combination of locule, septa and columella) of 6 random fruit per replicate was mashed and placed into plastic bags. The samples were then frozen with liquid N_2 and stored at -28 °C. On the day of enzyme analysis the frozen block was crushed into paste using a pre chilled mortar and pestle while still frozen and mixed to obtain a comp osite/representative sample purée. The samples were always placed in crushed ice to reduce browning (Fig 5-1).

The antioxidant enzymes of feijoa were extracted according to methods described in Yang et al. (2013). About 3 g of fruit purée was homogenised with 7 mL of 100 mmol L^{-1} potassium phosphate buffer (pH 7.0), consisting of 1 mmol L^{-1} ethylene diamine tetraacetic acid (EDTA), 50 g L^{-1} insoluble polyvinyl polypyrrolidone (PVPP) and 10 mL L^{-1} Triton X-100. Each treatment was done in three (technical and biological) replicates. Biological replicates referred to the samples picked from different groups of fruit, whereas the technical replicates are the three sub-samples from each biological replicate taken and averaged to give a value for each biological rep. The homogenate was centrifuged at 4 000 × g for 20 min at 4 °C using a Heraeus Multifuge 1S-R refrigerated Centrifuge, (Thermo Fisher Scientific, Dreieich, Germany) and the supernatant collected as crude enzyme extract in capped centrifuge tubes for use in the following assays (Fig 5-1).

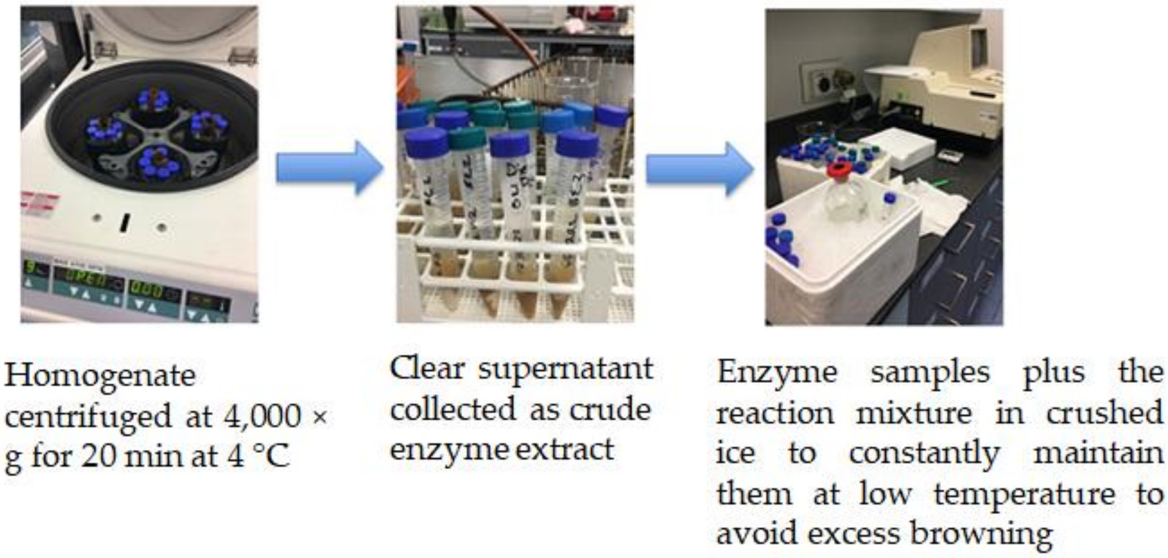


Figure 5-1 Key steps in antioxidant enzyme assay.

5.2.3.2 Peroxidase (POD)

Peroxidase (POD) activity was assayed according to the method of Hammerschmidt et al. (1982) with some modifications. Three (3) mL of the reaction mixture contained 50 mmol L^{-1} sodium phosphate buffer (pH 7), 10 mmol L^{-1} H_2O_2, 10 mmol L^{-1} guaiacol and 0.3 mL of enzyme extract (that was either the real enzyme extract, a blank [no enzyme], or a boiled enzyme extract as described below). The blank and boiled were used as controls in the experiment, to ensure that the procedure was working as expected. Boiling was achieved by pipetting 0.3 mL of enzyme extract and placing it inside a beaker containing boiling water on a hot plate for about 3 minutes. The increase in absorbance at 470 nm due to guaiacol oxidation was recorded automatically for 3 min using a spectrophotometer (Shimadzu UV 160A UV-Vis, Kyoto, Japan) in figure 5-2. One unit (U) of POD activity was defined as the amount of enzyme causing a change in absorbance of 0.01 min^{-1}. Specific POD activity was expressed as U g^{-1} FW min^{-1}.

Figure 5-2 Spectrometer showing a reading of a sample.

5.2.3.3 Catalase (CAT)

CAT activity was determined according to the method of Aebi (1984) with some modifications. Three (3) mL of the reaction mixture contained 50 mmol L^{-1} potassium phosphate buffer (pH 7), 10 mmol L^{-1} H_2O_2 and 0.3 mL of enzyme extract/blank (as describe in section 5.2.3.2 above). The decrease in absorbance at 240 nm due to the decomposition of H_2O_2 was automatically recorded for 3 min using a spectrophotometer (Shimadzu UV 160A UV-Vis, Kyoto, Japan) that had a UV transparent cuvette (quartz). One unit (U) of CAT activity was defined as the amount of enzyme causing a change in absorbance of 0.01 min^{-1}. Specific CAT activity was expressed as U g^{-1} FW min^{-1}.

5.2.3.4 Ascorbate peroxidase (APX)

Ascorbate peroxidase (APX) activity was assayed according to the method of Nakano and Asada (1981) with some modifications. Three (3) mL of the reaction mixture contained 50 mmol L^{-1} Hepes-KOH (pH 7.6), 0.1 mmol L^{-1}

EDTA, 0.5 mmol L^{-1} ascorbate, 1 mmol L^{-1} H_2O_2 and 0.3 mL of enzyme extract/blank. The decrease in absorbance at 290 nm due to ascorbate oxidation was automatically recorded for 3 min using a spectrophotometer (Shimadzu UV 160A UV-Vis, Kyoto, Japan). One unit (U) of APX activity was defined as the amount of enzyme causing a change in absorbance of 0.01 U min^{-1}. Specific APX activity was expressed as Ug^{-1} FW min^{-1}.

5.2.4 Data analysis

To determine significant differences caused by harvesting time, temperature and step down conditioning at 5% confidence level, analysis of variance (ANOVA) was performed using general linear model (GLM) procedures in Minitab (version 17.0, Minitab Inc., USA). Tukey's HSD test at 5% was used to separate means where statistical differences were found. For count data mean internal maturity, external chilling injury and internal chilling injury incidences were calculated before data were subjected to ANOVA.

5.3 Results and discussion

5.3.1 At harvest attributes

At both harvests, it is unmistakable that fruit were at different maturity stages based on the quality attributes measured (Table 5-1). Since harvesting of early and commercial fruit depended on the retention force, it was quite difficult to estimate precise maturity stages. This was evident from the average internal maturity rating of 1.25 and 1.89 for H1 and H2 respectively. Based on the PFR internal maturity-rating scale and the scanned images (Fig.5-3) these averages represented a range of immature to mature fruit of ratings 1 through to 3. For the 3 years of this study and working closely with SBO (In Matamata), it is clear that feijoa maturation is quite fast and difficult to assess in field. Feijoa fruit set is spread out necessitating multiple picks. The maturity marker used (formation of abscission layer) is either not easy to judge or is not well linked to fruit maturity (Patterson, 1990). For any particular feijoa variety, a market size fruit will only take about 3 weeks for that harvest season (initial harvest to final harvest) to be complete.

Despite the variable maturities found at each time of harvest, feijoa from H1 and H2 differed significantly in firmness (texture) at harvest. The high firmness of early harvested fruit (44.5 N) indicates that the fruit are significantly less mature compared to commercial harvested fruit (28.1 N). This result agrees with observations of Rupavatharam et al. (2015c) who reported that harvesting 'Unique' feijoa 4 weeks before touch picking had a firmness of 51.5 N whereas touch picked had 26.9 N. The similarity in early harvested firmness may indicate the potential for a non-destructive firmness measure that could be applied on the fruit grading line to separate fruit of differing internal maturities.

In this study (Table 5-1), early harvested fruit (H1) had slightly higher SSC than commercial harvested fruit (H2) implying that the normal off tree decrease in brix was due both to the cessation of loading the fruit with photosynthate and the onset of starch metabolism starch into sugars. This finding is in agreement with works of Downs et al. (1988) and Clark et al. (2005) who also reported higher SSC for early harvested fruit. Rupavatharam et al. (2015c) in their study observed similar SSC for the three harvests and all these three authors have dismissed use of SSC as a parameter to estimate fruit maturity. Although statistically the average soluble solids content showed a significant difference between the early and commercial harvested fruit, practically a 0.3 change between the two harvests is insignificant. The kiwifruit industry in New Zealand uses SSC of 6.2% as a minimum to predict harvest time, its SSC accumulates during harvest due to starch breakdown however, for feijoa its physiology especially of fruit composition is different. For example, in kiwifruit, there is a relatively abrupt transition to a period in which starch is mobilised into sugar in later phases of fruit development, coinciding with the approach of full maturity (Burdon et al., 2013). In feijoa, fruit do not accumulate as much starch as a kiwifruit (Thorp and Bieleski, 2002) and newly-imported sugar continues to contribute to fruit soluble solids content right up to harvest time, with no dramatic change in sweetness coinciding with harvest maturity (Harman, 1987).

There were no differences in the average fruit weight between the two harvests (Table 5-1), since the fruit was purposely sampled with fruit of count 28-30. Coincidentally this observation is comparable to observations of Rupavatharam et al. (2015c) who also reported no change in fruit weight of 'Unique' between H-2 (935.0 g/bag of 10 fruit) and H0 (935.5 g/bag of 10 fruit).

Table 5-1 Average at harvest quality attributes of early harvested (H1) and commercial harvested (H2) 'Kakariki' feijoa. Values with different letters in a column indicate significant differences ($p < 0.05$). n is the sample size and $HSD_{0.05}$ is the Turkey' Honest Significant Difference at $\alpha = 0.05$.

	Internal maturity rating	Firmness (N)	SSC (°Brix)	Weight (g)	L*	C*	°hue
H1	1.3b	44.5a	10.7a	103.6a	-	-	-
H2	1.9a	28.1b	10.4b	103.9a	39.5	27.4	111.6
n	90.0	90.0	90.0	90.0	90.0	90.0	90.0
$HSD_{0.05}$	0.4	6.7	0.2	0.4	-	-	-

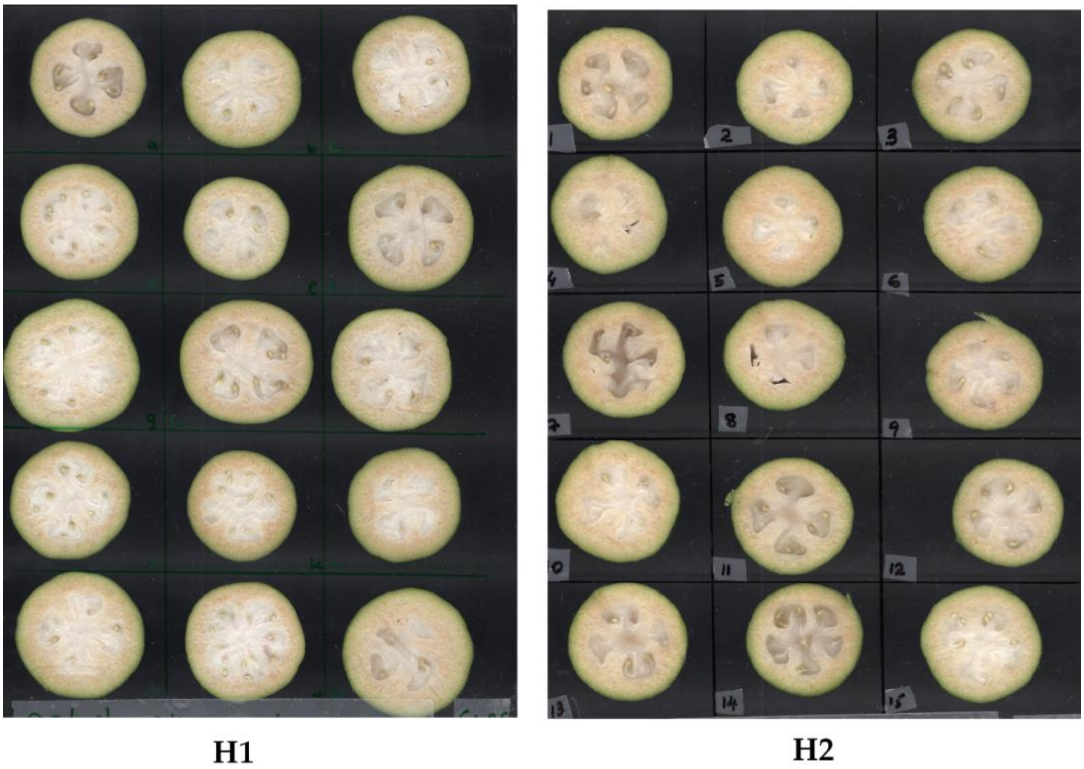


Figure 5-3 A sample of scanned images showing internal maturity of early harvested (H1) and commercial 'touch picked' (H2) 'Kakariki' fruit soon after harvest.

Due to a technical error in the spectrophotometer, colour for H1 was disregarded and after a recalibration of the spectrophotometer using different colour tiles and charts H2 data was collected at harvest (Table 5-1) and subsequent colour assessment had normal expected colour values.

5.3.2 Fruit response under conditioning

After conditioning, the fruit from the two times of harvest started to separate in terms of quality attributes (Table 5-2). The main effect of maturity is evident based on how fruit changed. Early harvested (H1) fruit were immature (maturity score of 1.5) and only a small amount ripened after the conditioning period. This is in comparison to when the fruit were initially received (maturity score was 1.3). Conversely maturity of touch picked (commercial harvested) fruit though immature soon after harvest (1.9) ripen during conditioning from 1.9 to 2.6.

The main effect of harvest maturity was greater than that of the storage conditions at this early stage of storage, but there was already evidence that step down conditioning was allowing fruit to continue to mature, whereas storing the fruit directly at low temperatures was retaining fruit attributes close to their at-harvest values (Table 5-2). Considering each attribute in turn firmness measurements showed that early harvested fruit remained firm with an average of 28.7 N as opposed to 17.5 N for commercially harvested fruit. Conditioned fruit either as a single or double step down (S6d,9→2 or 4 °C or S3d,9→6→2 or 4°C) were softer than fruit directly stored to 2 °C or 4 °C This demonstrates for the first time that fruit softening occurs rapidly during the first week of storage, especially if fruit cooling to the normal storage temperature of 2 - 4 °C is delayed.

The early harvested fruit registered a high SSC of 10.7 meaning they were sweeter compared to the touch picked fruit that now reached 9.4 °Brix. Storage conditions did not marketly effcet the main effects of harvest maturity on soluble solids Nevertheless there was a gradual decrease in SSC if low-temperature storage was delayed by conditioning, so that the highest SSC was found in early harvested fruit stored directly at 2 °C (11.5 °Brix) and the lowest SSC was found in touch-picked fruit exposed to 3d at 9 °C then 3 d at 6 °C (S3, 9 to 6 to 4 °C, at 8.9 °Brix).

In terms of water loss, early harvested fruit lost less weight (0.5%) than commercially harvested fruit (0.8%). The fruit exposed to conditioning lost considerably higher amounts of water (1.0% and 0.7%), than fruit stored directly at 2 or 4 °C. When assessing the interaction effects, the trend was very similar to SSC: the lowest weight loss was found in fruit transferred direcetly to either 2 or 4 °C (0.2%) and the highest weight loss came from fruit that spent the longest time at 9 and 6 °C.

In terms of colour (lightness) there were significant changes between early harvested (38.5) and commercial harvested fruit (40.4). It was also observable that storage temperature had little effect on lightness, apart from subtle differences that were not significant in a practical situation.

When assessing the intensity of colour (chroma) and °hue, early harvested fruit (25.4 & 112.7 °hue) were different from touch picked fruit (30.6 & 110.2 °hue). Storage temperature caused only subtle changes among fruit.

In summary, fruit that were more mature at harvest changed more dramatically during the conditioning period than less mature fruit; and these changes could be reduced by transferring the fruit immediately to low temperature storage. This applied to their internal maturity score, firmness, SSC and weight loss (Table 5-2). None of the three type of conditioning treatments (double step down, single step down and no step down) led to a differential effect on the three measured aspects of colour L*, C* & °h during the first 7 d of storage, perhaps because of the very small changes in colour that were noted across this time (Table 5-2).

Table 5-2 Main effects and the two way interactions of harvest time × step down conditioning treatment of average fruit quality attributes of early harvested (H1) and commercial harvested (H2) 'Kakariki' feijoa stored at 2 °C (C2), 4 °C (C4) and exposure to single step down conditioning (S6d,9→2 or 4 [6 d at 9 °C]) and double step down conditioning (S3d,9→6→2 or 4 [3 d at 9 °C+ 3 d at 6 °C])plus I d equilibration to 20 °C. Values with different letters in a column indicate significant differences ($p < 0.05$). n is the sample size and $HSD_{0.05}$ is the Tukey's Honest Significant Difference at $\alpha = 0.05$.

Factor	n	Value	I.M. rating	Firmness (N)	SSC (°Brix)	Weight loss (%)	L*	C*	°hue
Maturity (Mat)	166	H1	1.5a	28.7a	10.7a	0.5b	38.5b	25.4b	112.7a
	176	H2	2.6b	17.5b	9.4b	0.8a	40.4a	30.6a	110.2b
HSD (α=0.05)			0.13	1.36	0.24	0.05	0.85	0.93	0.50
Conditioning (TRT)	58	C2	1.9b	26.3a	10.9a	0.2c	39.6a	29.5a	110.9a
	58	C4	1.8b	28.1a	10.4a	0.3c	38.8a	27.1b	111.9a
	110	S6d,9→2 or 4	2.0a	20.9b	9.9b	1.0a	39.7a	28.4ab	111.3a
	116	S3d,9→6→2 or 4	2.2a	20.9b	9.6b	0.7b	39.3a	27.4b	111.6a
HSD (α=0.05)			0.28	2.92	0.51	0.12	1.81	1.97	1.04
Mat *TRT	28	H1 × C2	1.3c	31.7b	11.5a	0.2d	38.5ab	27.8bc	111.7abc
	28	H1 × C4	1.3c	37.5a	10.9a	0.2d	38.8ab	24.9cd	112.7ab
	54	H1× S6d,9→2 or 4	1.6c	25.5c	10.5bc	0.7c	39.1ab	25.6cd	112.6a
	56	H1 × S3d,9→6→2 or 4	1.5c	25.7c	10.3bc	0.6c	37.7b	24.2d	113.1a
	30	H2 × C2	2.4b	21.0d	10.2bc	0.2d	40.6a	31.2a	110.1cd
	30	H2 × C4	2.5b	18.6de	9.8cd	0.2d	38.9ab	29.2ab	111.0bcd
	56	H2× S6d,9→2 or 4	2.4b	16.4e	9.2de	1.3a	40.3a	31.1a	110.0d
	60	H2 × S3d,9→6→2 or 4	2.9a	16.2e	8.9e	0.8b	41.1a	30.5ab	110.1d
HSD (α=0.05)			0.48	5.02	0.87	0.21	3.07	3.35	1.77

5.3.3 Fruit response to storage

5.3.3.1 Internal maturity and ripeness

From the previous section 5.3.2, after the 6 d of conditioning plus 1 d equilibration to 20 °C (abbreviated as 7 d throughout the thesis for ease of reading) several points can be deduced. Exposure of fruit to temperatures of 6-9 °C during conditioning allowed ripening to continue and this has the potential to reduce feijoa quality after storage. Figure 5-4 and figure 5-5 shows the progression of internal ripening rating as affected by early harvesting and conditioning treatments. Early harvested fruit progressed gradually through the different ripeness ratings and still remained edible (rating 2-4) at the end of study to an average of 50% of the fruit when stored at 2 °C and 44% when stored at 4 °C (Fig. 5-4 H1 A - H1 F). Early harvested fruit had fruit at internal maturity rating 1 or 1.5 and some lingered at that low rating throughout storage but eventually the majority did ripen (Fig. 5-4). This result goes to illustrate the variations that exist within a particular fruit batch and confirm that harvesting decisions based on touch picking or early harvesting are inadequate to deliver fruit in tight maturity windows. The slow progression for early harvested fruit opens a window to market fruit over a long period; however, other fruit quality attributes must be checked to ascertain consumer acceptability. How then would one tell which fruit in internal rating 1 or 1.5 would ripen or fail to ripen? It is very likely that stage 1 fruit may include some immature fruit that cannot ripen; but it is evident that 1.5 fruit have embarked on the ripening process, the equivalent of 6.2 °Brix for a kiwifruit or mature green for tomatoes. Also due to variation in tastes and preferences, some consumers may not prefer fruit at internal rating 4 and this goes to emphasize the need to have sensory evaluations.

In commercially harvested batch, progression through internal ripeness ratings was higher in single step down conditioning than the other treatments (Fig. 5-5 H2 A and H2 D). This is evident with the percentage of fruit at internal ripeness rating 5 and 6 (about 70%) especially in fruit stored at 4 °C after 8 weeks of storage. For batches picked at conventional horticultural maturity, after 4 weeks of storage all fruit in stage 1 and 1.5 had all progressed to internal ripeness rating 2 and more unlike what was observed within early harvested fruit where some fruit lingered at 1 and 1.5 till end of the experiment. Fruit stored directly to 2 or 4 °C seemed to retain about 48% and 45% of fruit in edible state after 8 weeks of storage.

What also became apparent was that exposure to 6-9 °C temperatures during conditioning accelerated ripening and fruit progressed even faster when compared to the controls stored at 2-4 °C (C and F in both graphs). As stated by Rupavatharam et al. (2015c) the touch pick method provides 'ready to eat fruit' conversely from this study even early harvested batch did have about 10% of fruit at internal ripeness 3 which is the preferred consumption stage for feijoa. These observations show the difficulty in harvesting feijoa at the right stage. However, looking at the general progression through ripening on both harvests shows that growers are better off harvesting 'Kakariki' earlier than at touch picking (commercial harvest). When fruit were stored at 4 °C(or even more so, directly at 2 °C), this slowed ripening compared to SDC and allowed better retention of quality during storage confirming that step down conditioning is not a potential technique to apply to feijoa.

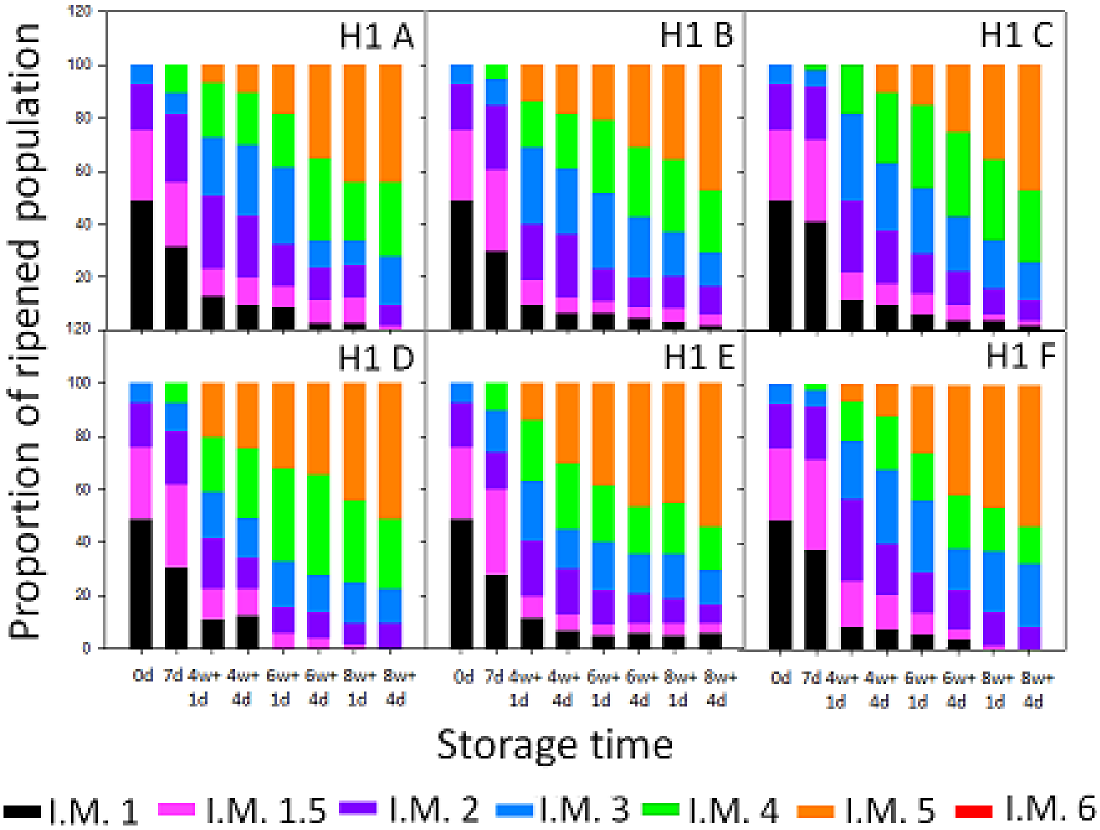


Figure 5-4 Proportion of early harvested 'Kakariki' feijoa at each internal maturity and progression of ripening as influenced by storage treatments over 8 weeks. At each storage condition, fruit were analysed after 1 d or 4 d at 20 °C. Treatments: S6d,9→2 (H1 A), S3d,9→6→2 (H1 B), 2 °C (H1 C), S6d,9→4 (H1 D), S3d,9→6→4 (H1 E) and 4 °C (H1 F) S6d,9→2 & S6d,9→4 = single step down treatment (6 d at 9 °C), S3d,9→6→2 & S3d,9→6→4 = double step down (3 d at 9 °C, 3 d at 6 °C). Each data point upto day 46 represents mean of 15 fruit per rep and for day 47 onwards n = 10 fruit per rep stored in polylined trays.

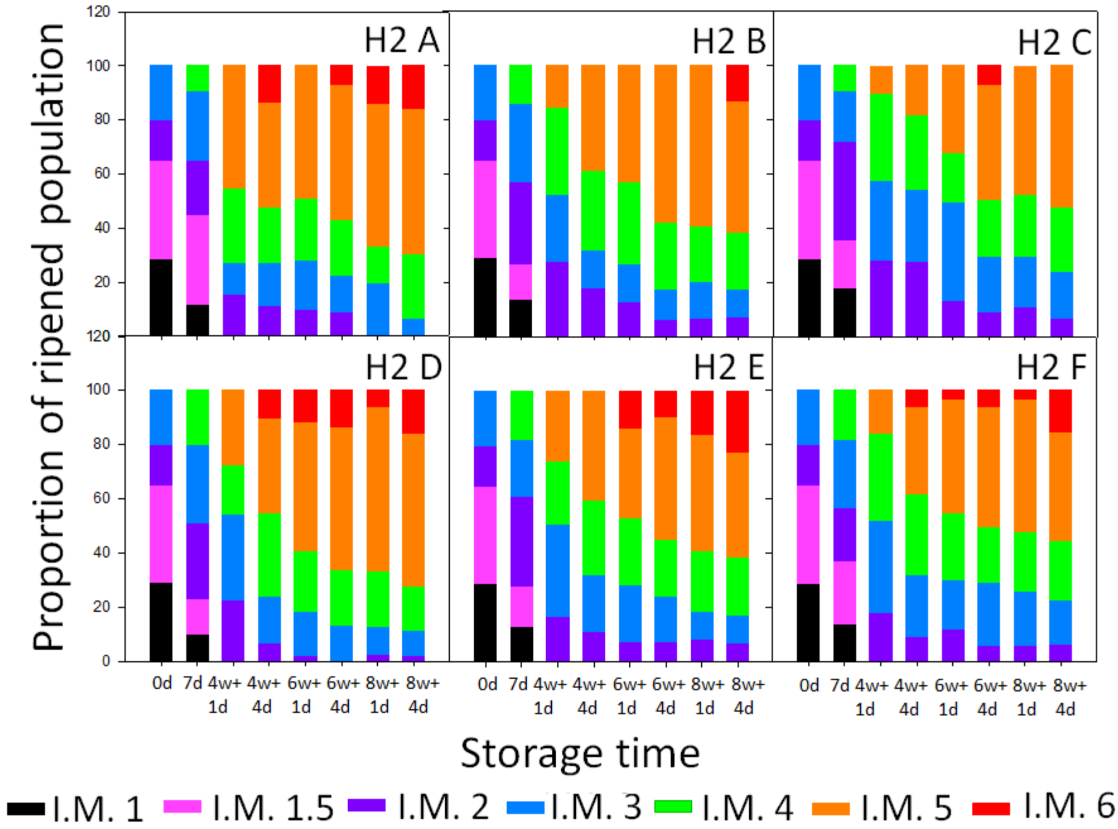


Figure 5-5 Proportion of commercially harvested 'Kakariki' feijoa at each internal maturity and progression of ripening as influenced by storage treatments over 8 weeks. At each storage duration, fruit were analysed after 1 d or 4 d at 20 °C. Treatments; S6d,9→2 (H2 A), S3d,9→6→2 (H2 B), 2 °C (H2 C), S6d,9→4 (H2 D), S3d,9→6→4 (H2 E) S6d,9→2 & S6d,9→4 = single step down treatment (6 d at 9 °C), S3d,9→6→2 & S3d,9→6→4 = double step down (3 d at 9 °C, 3 d at 6 °C). Each data point upto day 46 represents mean of 15 fruit per rep and for day 47 onwards n = 10 fruit per rep stored in polylined trays.

5.3.3.2 Respiration rate

Maturity, temperature and conditioning all affected respiration rate (Fig. 5-6). CO_2 production increased from about 20 nmol/kg s to 120 nmol/kg s for early harvested fruit (H1) and 60 nmol/kg s to 140 nmol/kg s for commercial harvested fruit (H2) after 8 weeks of storage. These values are within the range of respiration rates previously reported for feijoa (East et al., 2009; Rupavatharam et al., 2015c). The lower CO_2 production rate displayed by early harvested fruit early in storage (20 nmol/kg s) indicated low metabolic activity within the cells however later in storage this increased up to 140 nmol/kg s. The low rate of respiration in early harvested fruit suggested that some fruit were not yet ripening and thus may have longer storage life potential. This result shows that the values are not fixed and keeps on varying depending on fruit maturity, temperature and storage period. In the first three weeks of storage, early harvested fruit respiration rates increased sharply, while for commercial harvested fruit it was gradual. Higher rates reported by Rupavatharam et al. (2015c) of 300 nmol/kg s - 701 nmol/kg s (for early harvested fruit) are simply as a result of measurements being made at 20 °C instead of at 2 °C and 4 °C used in this present study. Although in another study where Rupavatharam et al. (2015a) investigated the effect of altering oxygen in 'Unique' feijoa storage atmosphere, they measured respiration at 4 °C and recorded about 60 nmol/kg s to 200 nmol/kg s for touch picked fruit which is close to results obtained for touch picked harvest in the current study. The increase in respiration that accompanies fruit ripening may have potential to use as a non-destructive tool to assess maturity although not practically applicable in a grading line considering time taken to measure rate of CO_2 production per fruit.

Harvesting fruit at stage 1.5, with a lower respiration rate, but truly embarking on ripening, has the most potential for storage because their limited sugar reserves will last for longer. As was to be expected, fruit stored continuously in 2 °C consistently maintained lower respiration rates than other treatments. This lower tissue metabolic activity correlates with slower fruit quality changes in comparison to other treatments in storage. Fruit stored at 2 °C ripened slowly especially in early harvested fruit (Fig. 5-4 H1C & Fig. 5-5 H2C). At day 42 in early harvested fruit, single step down fruit (S6d,9→2 °C & S6d,9→4 °C) decreased at the same rate whereas the double step down

(S3d,9→6→2 °C & S3d,9→6→4 °C) fruit increased at same rate as observed in Fig 5-6. In this case there was potential to expose fruit to conditioning to regulate fruit metabolism.

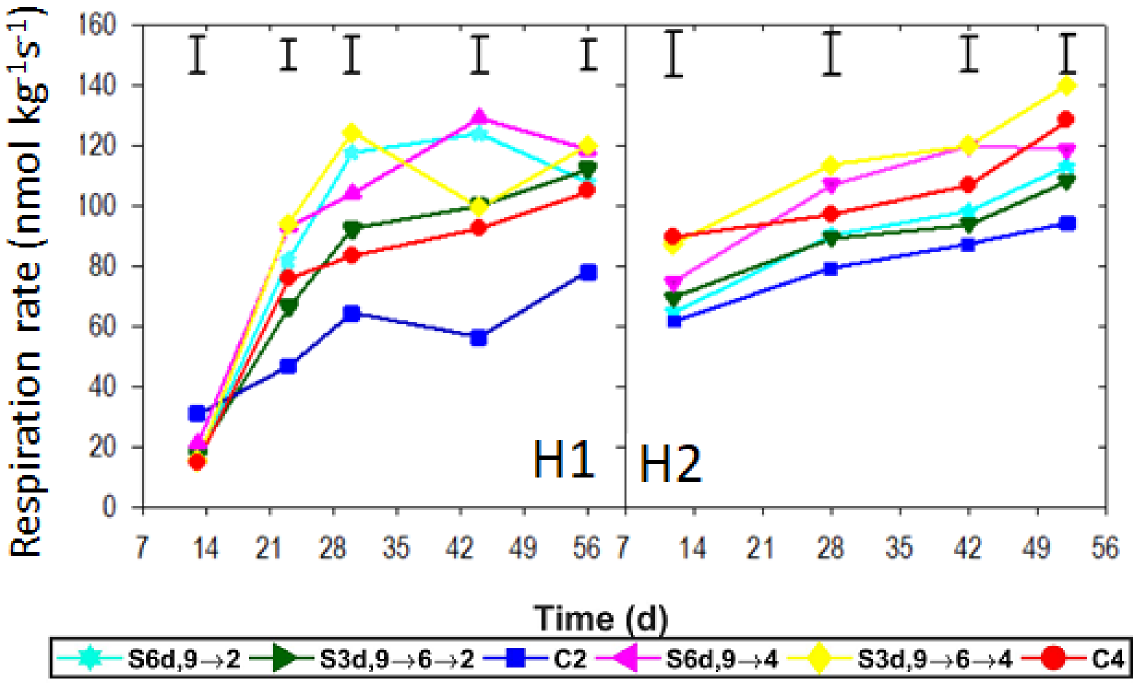


Figure 5-6 Effect of harvest time and step down conditioning on average respiration rate of early harvested (H1) and commercial harvested (H2) 'Kakariki' feijoa stored at 2 °C (C2), 4 °C (C4) and at 20 °C: S6d,9→2 & S6d,9→4 = single step down treatment (6 d at 9 °C), S3d,9→6→2 & S3d,9→6→4 = double step down (3 d at 9 °C, 3 d at 6 °C). Each data point upto day 46 represents mean of 15 fruit per rep and for day 47 onwards n = 10 fruit per rep stored in polylined trays measured at storage temperature either of 2 or 4 °C. The vertical bar represent Tukey's $HSD_{0.05}$.

5.3.3.3 Chilling injury

Maturity, temperature, step down conditioning, storage time and assessment time all had an effect in the development of external and internal chilling injury. Symptoms of external and internal chilling injury were initially noticed after 4 weeks of cold storage and intensity of the symptoms increased after 4 days at 20 °C (Fig. 5.7). Although at 4 weeks (28 d)after storage, the percentage of fruit affected was less than 20%, implications of this incidence level mean that growers can still sell some of their good fruit and somehow make profits. How much money the growers make will largely depend on how fast they are able to sell the fruit before it deteriorates completely. Increasing the storage time for both early and commercial harvests, increases the incidence of chilling injury. To growers whose main objective is to make profits, then storing feijoa for 8 weeks is uneconomical and a waste of resources, as stored fruit suffered from extensive chilling injury and that was often combined with rots.

Incidence of internal chilling injury was greater than external chilling injury (Fig. 5-7). For both harvesting times, after 8 weeks of cold storage and 4 d at 20 °C (shelf life) about 80% of the fruit were affected internally although externally about 60 % looked fine. This result is important in the context of marketing as internal injuries erode consumer confidence and hence repeat purchases. Therefore, finding means of eliminating internal injury from the supply becomes critical.

Generally, throughout the experiment, early harvested fruit (about 40 % at day 46 [end of week 6]) showed less internal chilling injury symptoms compared to commercial harvested fruit (about 65% at day 46 [end of week 6]). Previously on their study of revisiting maturity, Rupavatharam et al. (2015c) observed potential in harvesting feijoa early recorded about 1.7 % of surface injury incidence at end of 6 weeks (day 43) of storage. Storing fruit at 4 °C had some benefits since fewer fruit displayed internal chilling injury (about 48% for early harvested versus 65% for commercial harvested). Possible explanation for the low surface injury on their observations would be due to fact that the surface area injury was not what their main objective of study but a response resulting from the ethylene treatments. Alternatively surface area disorders including chilling injury is variety dependant and 'Unique' feijoa does not get affected as observed by the author in preliminary experiments in 2017 (data not shown). Normally immature fruit and vegetables including pears, kiwifruit, cucumber, tomato and courgettes among others became more susceptible to CI when harvested early. There is a need therefore to understand this paradox by having an in-depth study either through a genetic or cellular level study.

Double step down conditioning was slightly better than single step down conditioning and this result agrees with cited works (Wang, 1993). After 5 weeks of storage under 3 different temperature regimes Woolf et al. (2006) reported signs of chilling injury disorders at 2 °C, 4 °C and step down in 'Apollo', 'Gemini', 'Opal Star', and 'Unique' and this agrees with the authors result. However, on the contrary are the observations for loquat (Cai et al., 2006), lime (Spalding and Reeder, 1983), and pomegranate (Mirdehgan and Rahemi, 2003) where conditioned fruit display less chilling injury. Step down conditioning has no potential to extend storage life of feijoa looking at the chilling injury levels. In essence the fruit were supposed to acclimatise when moved to storage temperature however that did not happen. Exposing fruit to slightly warmer temperatures enhanced ripening and this may have altered the fruit physiology making it more vulnerable to chilling injury and increased rots. Since fruit and vegetable provide vitamin and minerals, damage by chilling injury may affect their nutritonal value. The unsightly appearance of fruit and maybe development of mycotoxins will not appeal to consumers and so chances of growers making any profits from sale of such fruit can be challenging (Fallik, 2004; Kader and Rolle, 2004).

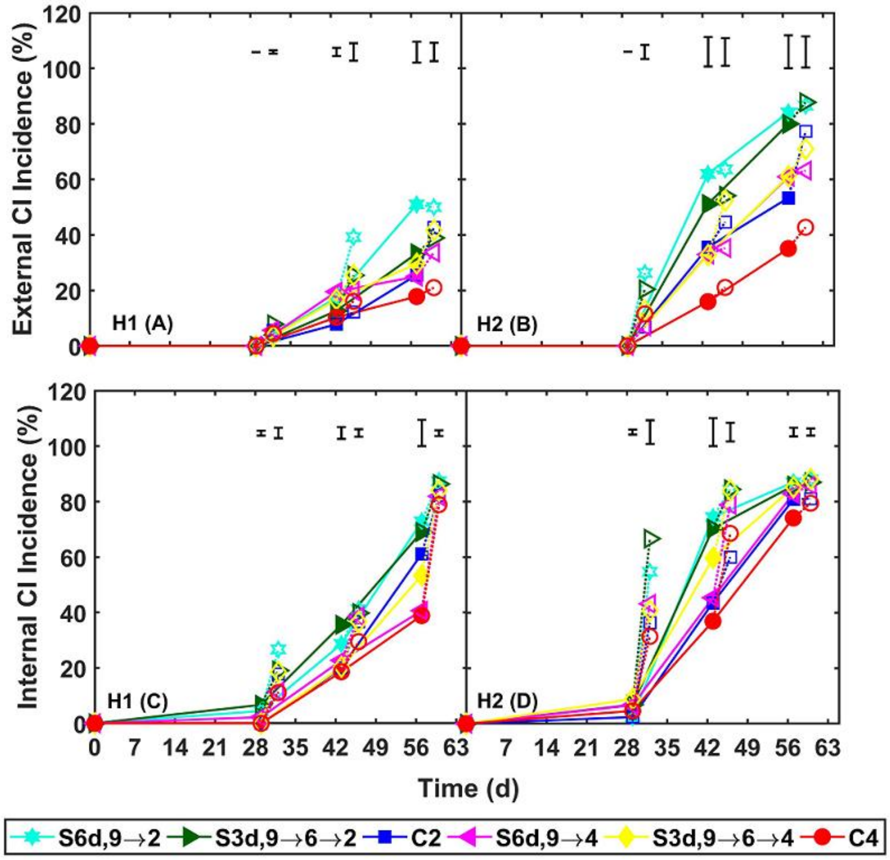


Figure 5-7 Effect of harvest time and step down conditioning on external (A & B) and internal (C & D) mean chilling injury incidence of early harvested (H1) and commercial harvested (H2) 'Kakariki' feijoa stored at 2 °C (C2), 4 °C (C4) and at 20 °C for 4 d after cold storage: S6d,9→2 & S6d,9→4 = single step down treatment (6 d at 9 °C), S3d,9→6→2 & S3d,9→6→4 = double step down (3 d at 9 °C, 3 d at 6 °C). Each data point upto day 46 represents mean of 15 fruit per rep and for day 47 onwards n = 10 fruit per rep stored in polylined trays. The vertical bar represent Tukey's $HSD_{0.05}$.

5.3.3.4 Firmness

There was a gradual decline of firmness in all fruit during storage (Fig. 5-8). Harvesting time, step down conditioning and storage temperature affected fruit softening. Early harvested fruit were firmer initially with a force of 45 N compared to 28 N of touch picked harvest. This result agrees with the results of Rupavatharam et al. (2015c) who reported a higher firmness value of 51.5 N and 38.7 N for 'Unique' feijoa harvested 4 and 2 weeks before touch picking.

Early harvested fruit when kept at 20 °C for shelf life assessment dropped its firmess more than the touch picked fruit especially at week 6 (32 N) and 8 (28 N) and these results were in agreement with Rupavatharam et al. (2015c) who also reported about 30 N after 6 weeks of storage. The firmness of early harvested fruit reported for week 6 and 8 is equivalent to at harvest firmness of touch picked fruit which implies that the early harvested fruit softened slowly and this would provide a window for longer storage as far as firm fruit were concerned. Softening of feijoa would largely be associated with changes in cell wall (pectin related enzyme activity) but this would need further studies.

Fruit subjected to step down conditioning and kept in cold storage for 8 weeks were softer. Fruit stored directly to 2 °C or 4 °C were firmer compared to conditioned fruit in all fruit and this result agrees with results of Woolf et al. (2006) who observed firmer fruit at 0 °C. However, as storage time increased, fruit became soft and chilling injury increased, potentially indicating that firmness loss could be linked to chilling injury development. During conditioning period, cellular changes occurred in all fruit and this affected firmness throughout storage as earlier stated. Due to the rapid firmness loss, step down conditioning does not provide potential for long storage life of feijoa.

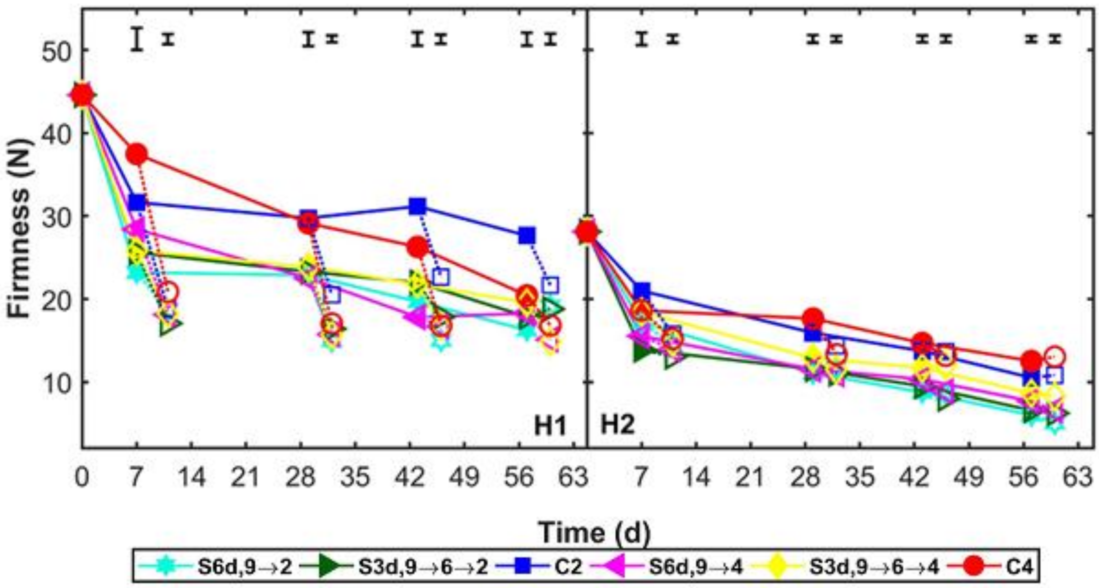


Figure 5-8 Effect of harvest time and stepdown conditioning on average compression firmness of early harvested (H1) and commercial harvested (H2) 'Kakariki' feijoa stored at 2 °C (C2), 4 °C (C4) and at 20 °C for 4 d after cold storage: S6d,9→2 & S6d,9→4 = single step down treatment (6 d at 9 °C), S3d,9→6→2 & S3d,9→6→4 = double step down (3 d at 9 °C, 3 d at 6 °C). Each data points up to day 46 represents mean of 15 fruit per rep and for day 47 onwards n = 10 fruit per rep (due to physiological disorders) stored in polylined trays. The vertical bar represents Tukey's $HSD_{0.05}$.

5.3.3.5 Weight loss

Weight loss increased over time in fruit from both harvest (Fig, 5-9 A & B). Maturity, temperature, storage time, assessment time and step down conditioning all had a significant effect ($p < 0.05$) on weight loss. For both harvest maturities, fruit stored in continuous cold storage lost the least amount of water. Fruit under conditioning treatments lost more water though not significantly by end of the study (8 weeks after storage). The average weight loss was about 4.2% (H1) and 3.8% (H2) and this agrees with observations in chapter 4 and of Rupavatharam et al. (2015c) who did not observe any significant difference with different harvest maturities. In chapter 4, lined 'Kakariki' stored at 4 °C lost about 3.2% of its weight. Other similar observations in recent feijoa studies are contained in Duan (2015), and Yue (2018) reports where the duo observed averagely a 4% water loss on polylined fruit. The use of polyliners plus close monitoring of storage rooms using Tiny tags and I-buttons throughout the study ensured that fruit were held at a high relative humidity. Low amounts of water loss imply that fruit cells should maintain high turgidity that leads to firmer fruit. With this kind of results (water loss of about 5%), growers should have confidence of making profits if temperatures oscillate around 4 °C since that water loss will not affect saleable weight.

5.3.3.6 Soluble solids concentration (SSC)

Soluble solid content of early harvested fruit held in continuous cold storage in the first 6 days increased before declining over the storage period (Fig 5.9 C & D). This initial increase in soluble solid content was not observed for the touch picked harvest. Although Rupavatharam et al. (2015c) did not have a data point on day 7 of storage, SSC for the 3-harvest time showed an increase of SSC from fruit harvested 4 weeks before touch picking (H_{-4}) and at touch pick (H_0). The possible explanation for this is that since the fruit had not reached commercial maturity then it is probable that there was some starch content, which was converted to sugar. And by end of storage at week 8 early harvested fruit had low SSC compared to commercial harvest which agree with Rupavatharam et al. (2015c) observations of low SSC of early harvested fruit.

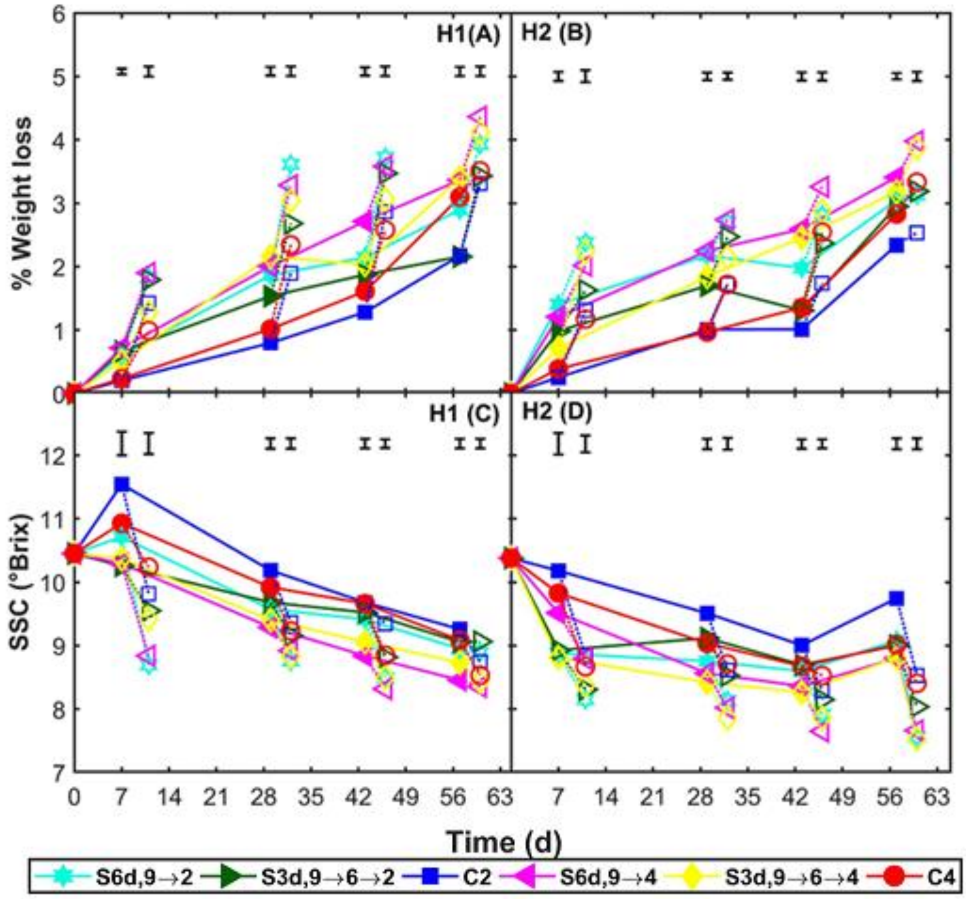


Figure 5-9 Effect of harvest time and stepdown conditioning on average %weight loss (A & B) and average SSC (C & D) of early harvested (H1) and commercial harvested (H2) 'Kakariki' feijoa stored at 2 °C (C2), 4 °C (C4) and at 20 °C for 4 d after cold storage: S6d,9→2 & S6d,9→4 = single stepdown treatment (6 d at 9 °C), S3d,9→6→2 & S3d,9→6→4 = double stepdown (3 d at 9 °C, 3 d at 6 °C). Each data points up to day 46 represents mean of 15 fruit per rep and for day 47 onwards n = 10 fruit per rep (due to physiological disorders) stored in polylined trays. The vertical bar represents Tukey's HSD$_{0.05}$.

Fruit subjected to step down conditioning lost soluble solids content during the conditioning treatment. As a result, fruit stored in continuous cold storage whether at 2 °C or 4 °C had a higher soluble solute content when compared to the fruit subjected to step down conditioning treatment. A further decline of SSC was observed when the fruit were held at 20 °C for 4 d after cold storage. All factors i.e. temperature, step down conditioning, storage time (week) and fruit assessment time had some effect at particular storage times during the experiment. From this study step down conditioning was not beneficial since fruit lost more SSC than fruit stored directly into 2-4 °C.

5.3.3.7 Skin Colour

Maturity, temperature, step down conditioning, storage time and assessment time as main effects had an effect on colour development. However, when the factors interacted little or no effect was observed. At the end of storage irrespective of conditioning treatment or harvest time skin lightness ranged from 35 to 42, chroma changed from 18 to 22 and hue angle ranged from 110 to 98, which practically showed minimal colour change. Moreover, this was the same case even when fruit were kept at 20 °C for 4 d (Fig. 5-10). Comparing colour development of early and commercially harvested fruit showed virtually no difference except in week 8 day 4, which had paler fruit mostly caused by ripening and subsequent senescence of fruit. Nevertheless, this has no value because fruit at week 8 is uneconomical for markets as earlier stated.

Fruit subjected to stepdown conditions (S6d,9→2 °C; S6d,9→4 °C; S3d, 9→6→2 °C & S3d, 9→6→4 °C) had higher lightness values, while control fruit; C2 and C4 had lower lightness values respectively. When Woolf et al. (2006) applied the 1 °C drop every week for their study on step down conditioning, they did not record colour as a quality attribute and thus it is hard to do a comparison. However, the changes observed in this study on conditioning treatment may be largely due to the slightly warmer temperature exposure at the start of storage which made the fruit ripen faster (as earlier stated in section 5.3.3.1). The almost constant C* and °hue observed throughtout storage could be an indication of chilling injury as observed in persimon fruit where suppression of colour has been suggested to be a sign of CI (Collins and Tisdell, 1995; Woolf et al., 1997). Since colour change is minimal in feijoa, looking at the literature and results obtained so far, it would be hard to develop grading machines with colour sorters or even develop colour charts to identify fully ripe fruit for consumpation as practiced in the tomato industry. With feijoa fruit being relatively new in markets, there is need to find other ways of grading needs to be sought to advance this industry.

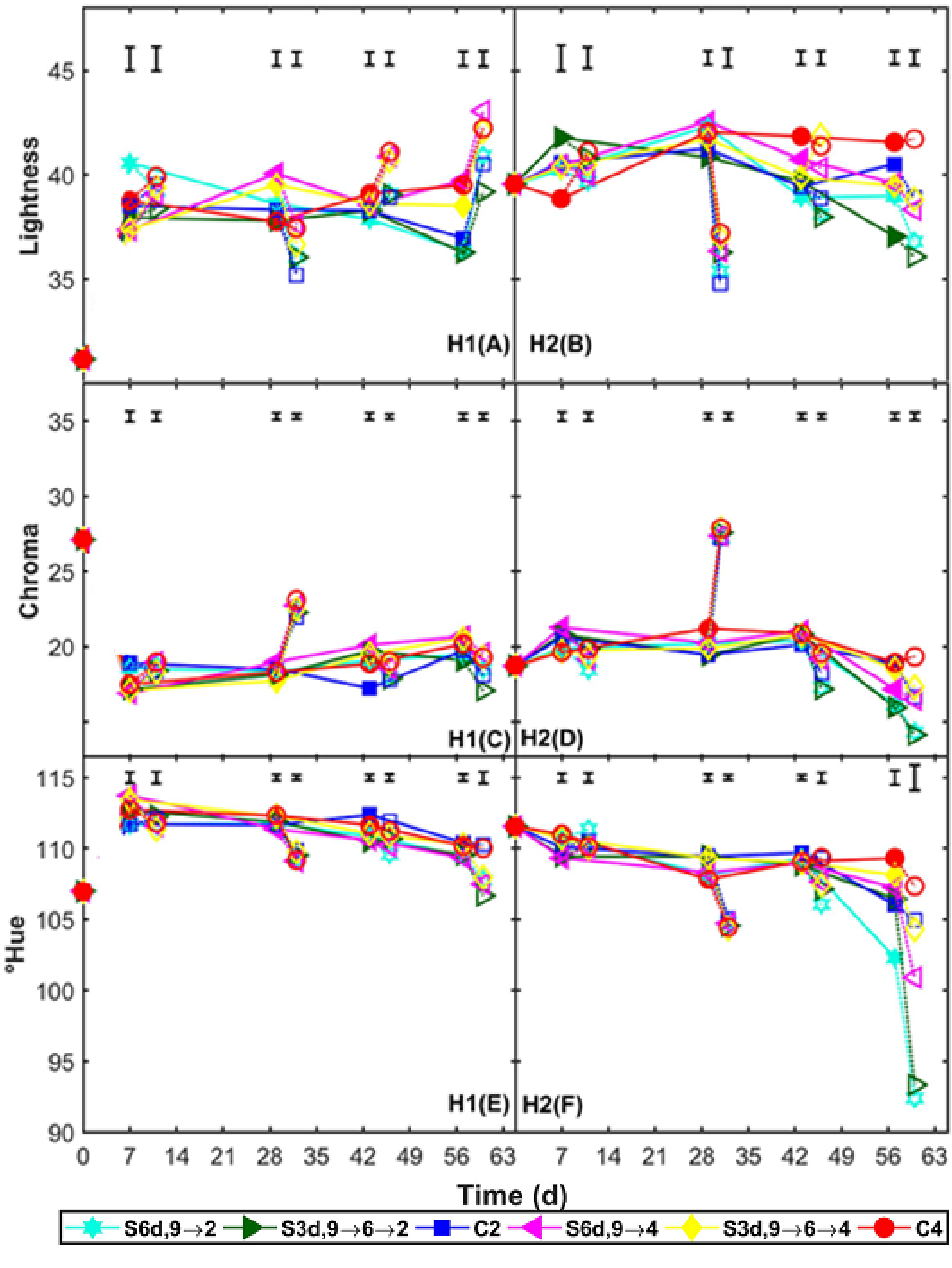


Figure 5-10 Effect of harvest time and stepdown conditioning on average skin colour (L*[A & B], C*[C & D] & °h[E & F]) of early harvested (H1) and commercial harvested (H2) 'Kakariki' feijoa stored at 2 °C (C2), 4 °C (C4) and at 20 °C; 4 d after cold storage: S6d,9→2 & S6d,9→4 = single step down treatment (6 d at 9 °C),

S3d,9→6→2 & S3d,9→6→4 = double step down (3 d at 9 °C, 3 d at 6 °C). Each data point upto day 46 represents mean of 15 fruit per rep and for day 47 onwards n = 10 fruit per rep (due to physiological disorders) stored in polylined trays. The vertical bar represent Tukey's $HSD_{0.05}$.

5.3.3.8 Antioxidant enzyme activity

Maturity, temperature, conditioning and storage time affected peroxidase enzyme activity (Fig. 5-11). Early harvested fruit had POD that ranged from 40 U g^{-1}FW min^{-1} to 85 U g^{-1}FW min^{-1} whereas touch picked fruit ranged from 22 U g^{-1}FW min^{-1} to 72 U g^{-1}FW min^{-1}. These results are within the range of figures measured by Beninca et al. (2018) although they recorded their results as absorbance, which they should have converted to POD activity to get the specific POD activity. Duong and Balaban (2014) also did some work on feijoa POD and measured residual enzyme activity, which they recorded as a percentage within the range of 69.9 ± 5% and 121.6 ± 1%. Since their work was to optimise hydrostatic pressure and carbon dioxide process on enzyme inactivation this makes it difficult to compare with present work.

Only the activity of peroxidase (POD) was enough to be detected. Ascorbate peroxidase (APX) and catalase (CAT) were either minimal or very close to the limit of detection (0.000 absorbance) from blank and boiled samples. Even in their studies Beninca et al. (2018) have reported only on peroxidase (POD), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) with the rationale that PAL generated substrates for PPO and POD. In their methods, they suggested to measure POD as UEA $min^{-1}g^{-1}$ protein but ended up reporting it as absorbance. They further state that the POD activity was low on all the five genotypes used. Since the POD was low in concentrations then it is possible to imagine it was due to an equilibrium between the oxidant/antioxidant where antioxidants such as vitamin C or tannins balanced the POD, APX, CAT and SOD. In other literature relating to feijoa antioxidants researchers have measured antioxidants using the 2,2-diphenyl-1-picrylhydrazil (DPPH) method and ferric reducing antioxidant power (FRAP) where results are expressed as effective concentration (EC_{50}) that shows the minimum extract concentration able to scavenge 50% of the total DPPH. Since other researchers measured antioxidants and enzymes in different ways this might explain the diversity of results found.

There was no potential in using conditioning to scavenge the free radicals produced after chilling injury stress. This is evident from the results obtained in the POD measured. In early harvested feijoa, the fruit stored continuously at 2 and 4 °C had low POD activity, while in touch picked fruit, continuous storage at 2 and 4 °C had the highest POD activity.

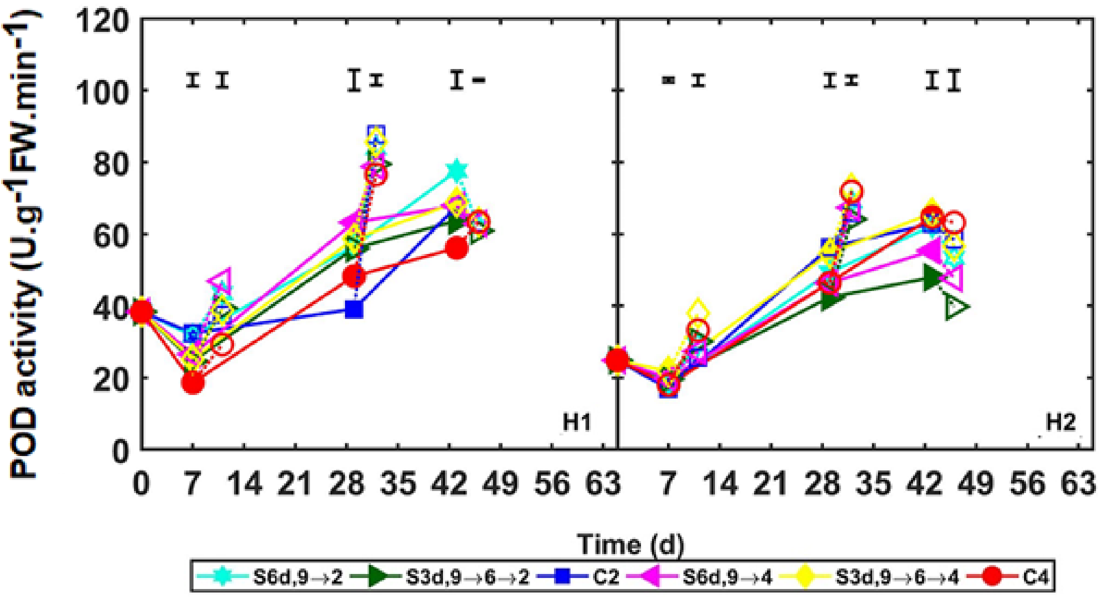


Figure 5-11 Effect of temperature and harvest maturity on average peroxidase (POD) activity of early harvested feijoa Kakariki' from Matamata stored at 4 °C (C4), 2 °C (C2) and at 20 °C for 4 days after cold storage: S6d,9→2 & S6d,9→4 = single step down treatment (6 d at 9 °C), S3d,9→6 →2 &S3d,9→ 6 → 4 = double step down (3 d at 9 °C, 3 d at 6 °C). Each data point represents mean of a composite of 6 fruit per rep (that had 3 technical and 3 biological reps) stored in polylined trays. The vertical bar represents Tukey's $HSD_{0.05}$.

5.4 Conclusions

In line with the set objectives of this chapter, the following conclusions can be deduced.

Early harvested fruit after 4 weeks (28 d) of cold storage slightly retained some quality attributes better than touch picked fruit in particular 60% of fruit remained at internal ripeness less than or equal to 4 after 6 weeks at 4 °C plus 4 d at 20 °C and about 20% of fruit displayed internal CI. This is considerably better than commercially harvested (touch picked) fruit at the same time (40% fruit at ≤ internal ripeness 4 while internal CI was at 60%). POD activity and skin colour in both harvesting times showed subtle changes after storage. Weight loss, and soluble solids concentration showed no difference between batches harvested early or touch picked. Early harvested fruit had increased respiration rate initially but after 4 weeks, it became constant, while touch picked fruit had gradual increase in respiration. Unfortunately, even this amount of chilling injury incidence is likely to be unacceptable to consumers and may seriously compromise profitability of the industry. It seems that early harvesting of fruit is not enough to extend feijoa storage life beyond 4 weeks for 'Kakariki' fruit, unlike the as observation of Rupavatharam et al. (2015c) in 'Unique' feijoa.

While studies have indicated that step down conditioning helps in reducing chilling injury, conclusions from this study are that storing fruit directly to 2 or 4 °C retained fruit quality better than step down conditioned fruit. The 2 °C stored fruit were able to retain firmness, reduce water loss and retain SSC although this temperature resulted in accelerated chilling injury development.

In the tested SDC treatments all it resulted was to allow fruit to ripen during the 7 d period and that SDC did not offer protection on chilling injury.

From the only tested small number of combinations, feijoa ripen quickly off tree and it seems therefore unlikely there will be a beneficial SDC treatment. The indifference in the conditioning treatments above (non-chilling) and previous studies (within chilling temperatures) imply that whereas longer conditioning treatments might be required to allow the tissue to acclimate to low temperatures, there will be an accompanying acceleration of postharvest ripening. This suggests that alternate temperature time combinations are unlikely to provide benefit for feijoa and therefore there is no need wasting resources to test other different temperature time combinations. This further implies that the industry needs to rapid cool fruit soon after harvest to minimize ripening and then maintain fruit at a safe low temperature if profits are to be realised.

Chapter 6 Effects of intermittent warming and chlorophyll fluorescence on quality and storage of 'Triumph' feijoa

Acknowledgement:

Materials from this chapter is included in the paper:

Oseko, J., East, A., & Heyes, J. (2020). Can changes in chlorophyll fluorescence be used to determine chilling injury of cold stored feijoa? *Acta Horticulturae, 1275*, 125-132. DOI: 10.17660/ActaHortic.2020.1275.18.

This chapter differs from the publication in that it includes additional figures 6.3 and 6.5c and table 6.1. The paper equally has extra figures 1, 2 and 5C.

6.1 Introduction

As demonstrated in chapter 4 and 5 chilling injury still remains a challenge to feijoa growers. The injury is more problematic when it occurs internally because (a) retailers and consumers cannot see it and (b) it affects flavour (Wang, 1989). Although attempts have been undertaken to reduce chilling injury, more effective methods need to be developed to safeguard quality and market. This chapter therefore evaluates the combination of intermittent warming and chlorophyll fluorescence in detecting and reducing chilling injury.

Intermittent warming (IW) involves storing fruit or vegetables at low temperature and alternating with short periods of warmer temperature usually at room temperature (20 °C) to reduce chilling injury and improve fruit quality. Available literature shows a range of fruit and vegetables that have responded positively to intermittent warming treatment with most authors concluding that its effectiveness depended on cultivar, maturity stage, storage temperature, and warming cycle (Biswas et al., 2016). The mode of action is not certain but could involve inducing cold tolerance by repairing membranes and organelles, in addition to removing toxins produced during the chilling period. This process maintains membrane integrity and makes fruit more tolerant thereby allowing it to be stored under chilling temperatures. Another way in which intermittent warming maintains membrane integrity may be by allowing the tissue to increase the proportion of unsaturated fatty acids in cell membranes, thereby reducing electrolyte leakage and malondialdehyde (MDA) production (Valenzuela et al., 2017). All this is only possible if warming treatments are applied before chilling injury symptoms become irreversible.

Intermittent warming mainly focuses on reducing chilling injury; however, it is not easy to identify when chilling injury is close to becoming irreversible so that IW can be applied at or before that time. Therefore, it becomes essential to incorporate a simple quick technique that can assist in predicting and/or detecting chilling injury thus the need for chlorophyll fluorescence (*ChF*) (Cascia et al., 2010; Urbano et al., 2004). Studies in chilling injury stress have identified F_v/F_m as an important component of *ChF*, which is the quantum yield of photosystem II (PSII) or the electron transfer efficiency of PSII (Baghbani-Arani et al., 2017). The reduction in quantum yield indicates how two processes e.g. photosynthesis and ripening are competing for available energy. Therefore, how fast the quantum yield (F_v/F_m) recovers when the stress is removed will indicate ability of tissue to repair or resynthesize the thylakoid membranes.

Several authors in postharvest have used quantum yield (F_v/F_m) as a diagnostic tool in apples (De Ell et al., 1998; Song et al., 1997), pears (Cascia et al., 2010;

Streif and Saquet, 2000), mango (Arafat, 2005; Lechaudel et al., 2010) and green pepper (Kosson, 2002) to predict chilling injury or ripening of the fruit. Therefore, combining intermittent warming and *ChF* techniques for feijoa would increase knowledge of how the two interact postharvest and this could help in detecting and predicting maturity, ripening, and chilling injury. This study therefore sought to evaluate intermittent warming as a technique to improve feijoa quality by reducing chilling injury and extending storage life. In addition, the study sought to establish the ability of *ChF* to predict chilling injury occurrence; and if *ChF* would actually detect when chilling injury occurred before it became irreversible.

6.2 Materials and methods

Experts from Southern Belle Orchard (SBO) in Matamata, New Zealand harvested and supplied export grade (count 28-33) feijoa (cv. 'Triumph') in April 2018. After packaging, a non-refrigerated truck transported fruit overnight to Massey University, Palmerston North approximately 48 h after harvest. On arrival, the experiment was established immediately. Allocation of fruit randomly into pre-labelled trays that defined subsequent treatments and measurements was done. Three intermittent warming treatments were established;

- Control,
- One day warming to 20 °C after every 6 d at 4 °C (IW 6 d),
- One day warming to 20 °C after every 10 d at 4 °C (IW 10 d).

Fruit were assessed after three storage durations namely 2, 4, and 6 weeks. Three replicate trays of 30 fruit were established for each treatment and measurement time combination. All the trays were lined with 10 μm thick high-density polyethylene to reduce water loss due to transpiration. On removal from cold storage fruit were equilibrated at 20 °C overnight and the following morning quality assessment on half of the tray was conducted. The remaining half of a tray was left at 20 °C for a further 4 d before quality assessment was conducted again. Fruit quality measurements taken as described in chapter 3 included firmness, internal maturity/ripeness, internal and external chilling injury, and skin colour. Physiological assessment included measuring respiration rate as described in chapter 3 and chlorophyll fluorescence (*ChF*) parameters.

Chlorophyll fluorescence measurements were made with a field portable pulse modulated chlorophyll fluorometer (FMS 2, Hansatech Instruments Ltd, UK), using the quantum yield (F_v/F_m) test at 20 °C. Fruit were dark-adapted using a black plastic sheet for 30 minutes before measurements around the fruit equator taken. For the 1st 15 fruit per rep, initial *ChF* measurements were taken immediately after removing fruit from cold storage rooms and the following day (1 d) a second measurement was again taken. For the remaining 15 fruit, a final *ChF* measurement data was taken after 4 d of storage at 20 °C. Changes in fluorescence emitted by feijoa were collected using an optic fibre that provided the following information; minimum fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence (F_v) and the quantum yield of PSII or the electron transfer efficiency of PSII (F_v/F_m).

6.3 Data analysis

The experimental setup was a completely randomised design (CRD) with three treatments replicated three times. Using the general linear model (GLM) method in Minitab (Version 17.3.1, Minitab Inc., State college, Pennsylvania, USA), analysis of variance (ANOVA) was performed on data to determine significance differences at 5% confidence level. Tukey's honest significant differences (HSD) at 95% confidence level separated means where statistical differences from ANOVA were found. External and internal chilling injuries, rots, maturity and ripeness scores were treated as count data and each calculated as either a mean or proportion of total fruit population. For chilling injury and rots, percentage incidence or severity level was calculated. while maturity and ripeness scores were assessed using the modified Plant and Food Research scale outlined in chapter 3. Data were expressed as a proportion of the total fruit population.

6.4 Results and discussion

6.4.1 At harvest fruit data

As earlier stated in chapters 4 and 5, at-harvest fruit attributes customarily gauge maturity of fruit. New Zealand feijoa industry is yet to define which particular fruit attributes can estimate maturity as is the case with kiwifruit. The strength of abscission layer during touch picking is the closest it gets to a maturity indicator in feijoa. Table 6-1 shows average at harvest fruit attributes for 'Triumph'. When the 2018 batch of 'Triumph' was compared to 2016 batch (see table 4-2) the fruit were found to be slightly more mature. This is obvious when comparing 2016 values for L* (40.92), C* (26.38), °hue (114.02), and internal maturity (1.6), though not so much with the firmness measurements. The data in table 6-1 show a decrease in green colour (hue angle) and in lightness (L*), while the internal maturity rating increased. Colour in feijoa does not change drastically like in oranges or tomatoes and therefore the little changes in L* and °hue together with IM are indicators that the 2018 fruit were slightly mature than the 2016 fruit.

Table 6-1 Average at-harvest fruit quality attributes of commercially harvested 'Triumph' in 2018, and n is the fruit sample size.

	L*	C*	°hue	Weight (g)	Firmness (N)	Maturity
Mean value	39.79	26.71	112.42	59.32	28.92	2.3
n	90	90	90	90	90	90

6.4.2 Development of chilling injury

In this trial feijoa (cv. 'Triumph'), fruit were stored for up to 6 weeks at 4 °C. These conditions were chosen because of previous studies (chapters 4 and 5) that showed storing feijoa below 4 °C or for longer periods was not practical. Unfortunately for this study, this batch of 'Triumph' fruit did not develop significant chilling injury during this period and therefore it is possible that seasonal variations do exist. Only three fruit started to show initial signs of CI after 6 weeks storage, and since they were minute and believed not to affect sales if they appeared in the market, the author recorded them as sound fruit. Thorp and Bieleski (2002) noted that 'Triumph' fruit had good storage traits. Nevertheless, this result was unexpected since figure 4-6 (chapter 4) showed 'Triumph' suffered serious CI when stored at 1 °C and 4 °C even after just 4 weeks. Therefore, it is reasonable to conclude that for this particular study due to seasonal variation 4 °C was not cold enough to cause chilling injury development in 'Triumph' within the six weeks storage in 2018.

6.4.3 Respiration rate

The rate of carbon dioxide production after 2 and 4 weeks of cool storage at 4 °C (control) was 40 $nmolkg^{-1}s^{-1}$ and 130 $nmolkg^{-1}s^{-1}$ while for IW 10 d it was 90 $nmolkg^{-1}s^{-1}$ (2 weeks) and 140 $nmolkg^{-1}s^{-1}$ (4 weeks) (Fig. 6-1). A clear indicator that storage period increases fruit metabolism by utilizing stored sugars. Schotsmans et al. (2011) and Rupavatharam (2015) have also reported similar observations for 'Unique' feijoa when stored under similar conditions. At 2 weeks of storage, fruit stored directly at 4 °C had significantly ($p < 0.05$) lower CO_2 production compared to fruit exposed to intermittent warming treatments. The brief exposure of fruit to high temperature increased metabolism whereby IW periods may have allowed some fruit ripening. Conversely, after 4 weeks of storage, there was no difference in storing fruit continuously at 4 °C or exposing them to intermittent warming treatments. Feijoa after 4 weeks are ripe and, in some cases, overripe. At 2 weeks of storage, a few fruit were ripening, slower tissue metabolism and thus rate of CO_2 production was also lower. However, after 4 weeks of storage some of the stored sugars would be spent and as tissues move towards senescence respiration increased. This agrees with the previous data on SSC (Fig. 4-10 and Fig. 5-9 C&D). While no chilling injury was recorded in this experiment, it would be important in future to have a deeper insight into feijoa fruit physiology before and after intermittent warming. This would provide a clearer picture of how fruit responds to different regimes of intermittent warming treatments.

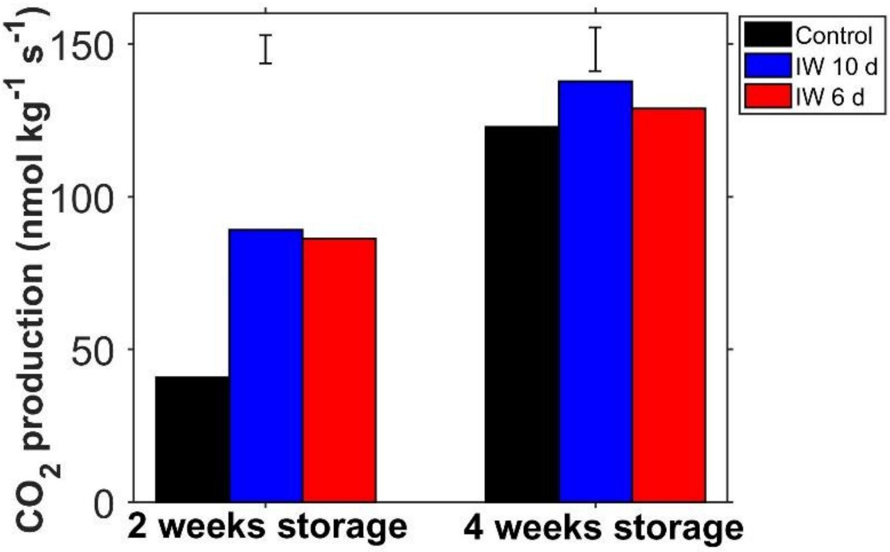


Figure 6-1 Effect of intermittent warming on respiration rate of feijoa 'Triumph' stored at 4 °C (control), one day warming to 20 °C after every 6 d at 4 °C (IW 6 d) and one day warming to 20 °C after every 10 d at 4 °C (IW 10 d) for 4 weeks. Each data point represents mean of 15 fruit. Vertical bars represent the HSD 0.05 (Tukey's).

6.4.4 Chlorophyll fluorescence

Quantum yield (*Fv*/*Fm*) for all fruit decreased during storage time in all storage conditions (Fig. 6-2). However, *Fv*/*Fm* for control fruit kept at 20 °C for 4 d after 4 weeks of cold storage had a slightly higher recording compared to fruit subjected to intermittent warming conditions. This implies the IW fruit were more stressed or riper indicating damage to their chlorophyll molecules. A similar trend continued into week 6 of storage. There was no recovery of *Fv*/*Fm* as expected when fruit were returned to 20 °C from the chilling temperature (data not shown). Although there is evidence of progressive decline in *Fv*/*Fm*, section 6.4.2 illustrates that fruit did not show irreversible chilling injury. The fact that *Fv*/*Fm* gets worse on removal from cold is consistent with damage occurring to the thylakoids. Even though this severe reduction in *Fv*/*Fm* would be classified as 'stress' in other studies, in this study it does not lead to development of CI symptoms. This therefore suggests *Fv*/*Fm* is not suitable as a predictor of CI. Similar results are supported by observations of Yue (2018) when using chlorophyll fluorescence to evaluate overall quality of 'Triumph' feijoa. Toivonen and De Ell (2001) observed a slight recovery after MAP bags of broccoli were opened and held in air at 1 °C for 4 d. Continuous decline in quantum yield (*Fv/Fm*) reflects aging of the fruit as ripening progressed. In an apple study Song et al. (1997) observed a decrease in quantum yield that was also linked to chlorophyll breakdown. That reflected a reduction in the PSII activity of the thylakoid tissue. For this study, the significant decline in *Fv/Fm* implies that it is useful as an indicator of storage time or ripening but unfortunately it is not possible to predict what it would show if the fruit had developed chilling injury.

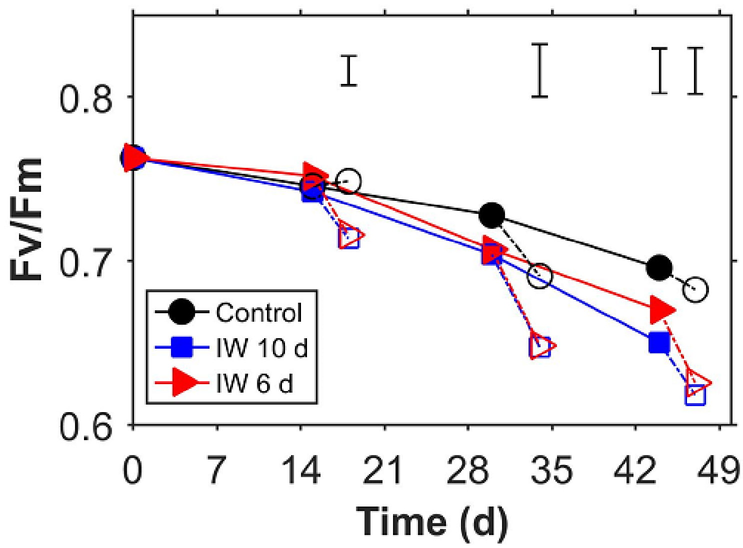


Figure 6-2 Effect of intermittent warming on quantum yield (F_v/F_m) during storage at 4 °C (control), one day warming to 20 °C after every 6 d at 4 °C (IW 6 d) and one day warming to 20 °C after every 10 d at 4 °C (IW 10 d) for 4 weeks. Fruit assessment done at 20 °C, 1 d after removal from cold storage (solid symbols) and 4 d at 20 °C (hollow symbols). Each data point represents average of 3 replicates of 15 fruit. Vertical bars represent the HSD 0.05 (Tukey's) and absence of bar indicates non-significance (ns).

6.4.5 Fruit quality attributes

From previous sections above, there are no signs of chilling injury development and the physiological measurements taken supported this observation. Therefore, in this section expectations are for fruit quality measures to correlate with these observations.

6.4.5.1 Internal maturity and ripeness

Maturity and ripeness of fruit increased gradually over time both at 4 °C and subsequently at 20 °C (Fig. 6-3). In all, the 3 treatments by end of week six resulted in a few fruit that were still at maturity rating scale 1 and 2, whereas a few fruit had reached ripening scale 5 with the majority of fruit being at rating 2, 3 and 4. This observation suggests major differences in maturity do exist within a batch and this is consistent with observations by previous feijoa researchers (Al-Harthy et al., 2010b; Duan, 2015; Rupavatharam, 2015; Schotsmans et al., 2011). The huge differences in maturity/ripeness within a batch are not obvious externally until fruit is cut and this is the biggest hindrance for growers to deliver consistent quality feijoa to markets and as a result the growth of feijoa industry is slow.

By end of week 6, there were hardly any rotten fruit observed in this study and this result agrees with Thorp and Bieleski (2002) who observed that 'Triumph' had a good storage life. In other fruit, high incidences of rot development can be indicators of CI symptoms. Therefore, the low incidence of rots further shows minimal CI development. From these results, over 95 % of the fruit were saleable and this goes to emphasise the need of choosing appropriate varieties for different markets. Simply varieties such as 'Kakariki' that ripen faster and suffer severely from chilling injury are better suited for local markets or sold soon after harvest. Likewise, growers should be encouraged to increase acreage of varieties such as 'Triumph' that can withstand almost 6 weeks of storage and further studies should be conducted to ascertain their resilience during seafreight, consumer preferences and commercial yield.

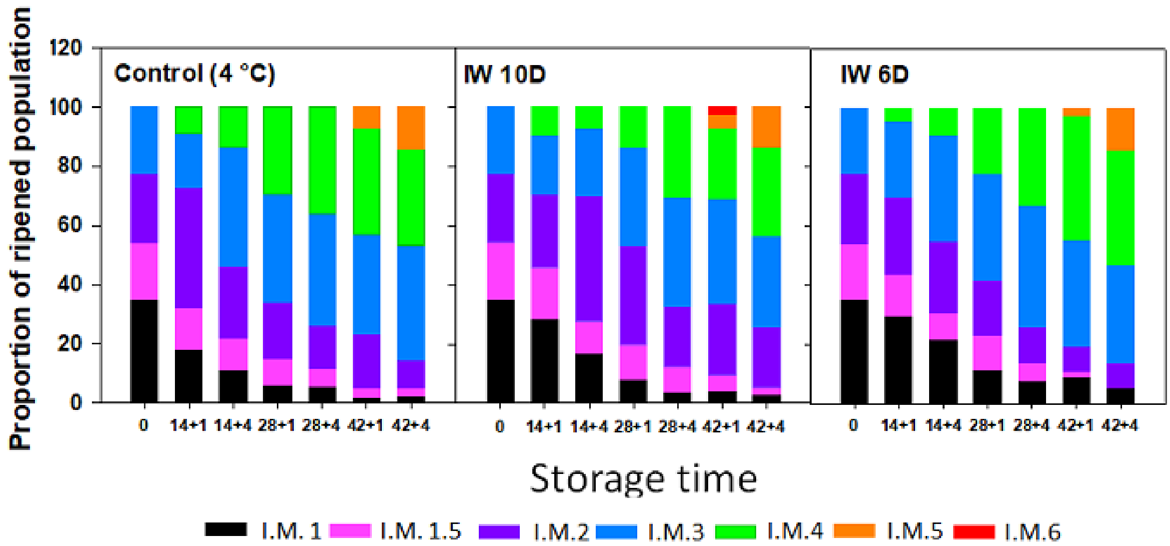


Figure 6-3 Proportion of 'Triumph' feijoa showing internal maturity and progression of ripening as influenced by intermittent warming treatments; 4 °C (control), one day warming to 20 °C after every 10 d at 4 °C (IW 10D) and one day warming to 20 °C after every 6 d at 4 °C (IW 6D). Assessment of fruit done at two-weekly interval, 1 d after removal from cold storage and subsequently after 4 d at 20 °C. Each data point represents average of 15 fruit per rep.

6.4.5.2 Fruit firmness and skin colour

During the experimental period, firmness declined over time from 29 N to about 14 N after 6 weeks of storage (Fig. 6-4 A). Fruit stored continuously at 4 °C (control) were significantly firmer than fruit subjected to intermittent warming up to week 4 and thereafter it did not matter because all fruit softened to 14 N. The fruit softening observed is normal during fruit ripening as recorded in the literature.

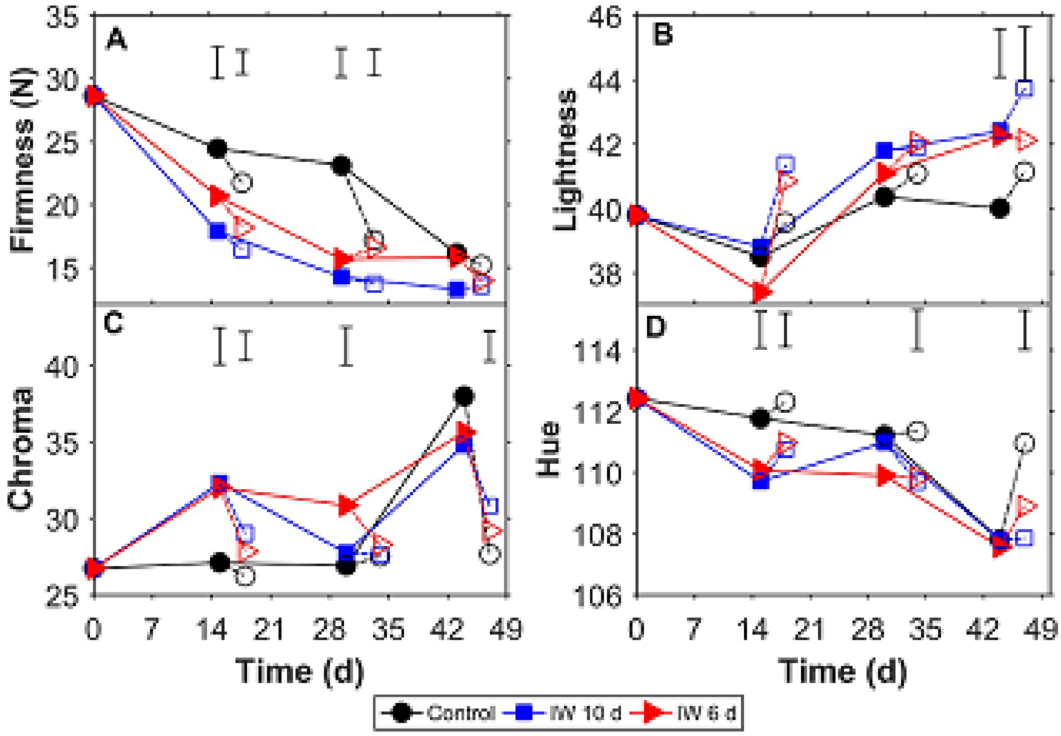
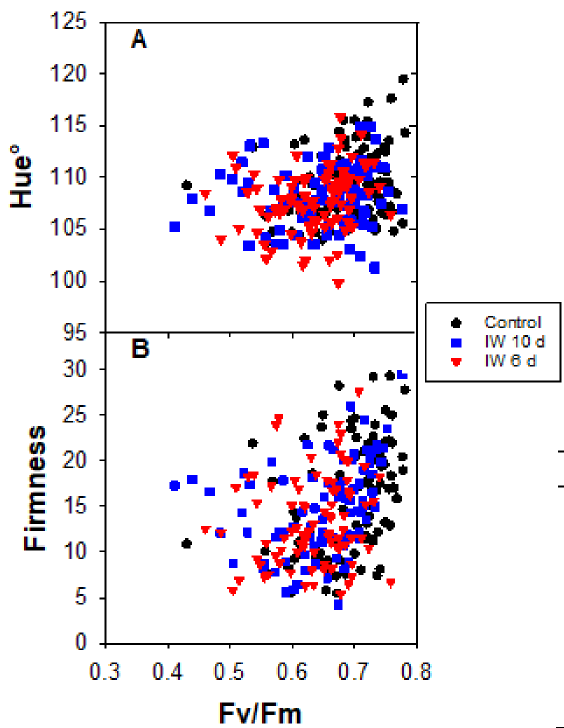
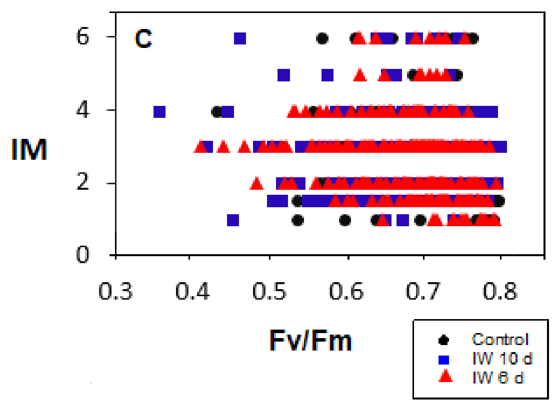


Figure 6-4 Effect of intermittent warming on firmness (A), lightness (B), chroma (C) and hue angle (D) during storage at 4 °C (control), one day warming to 20 °C after every 6 d at 4 °C (IW 6 d) and one day warming to 20 °C after every 10 d at 4 °C (IW 10 d). Fruit measurement occurred at 20 °C after 1 d (solid symbols) or 4 d (hollow symbols). Each data point represents average of 3 replicates of 15 fruit. Vertical bars represent the HSD 0.05 (Tukey's) and absence of a bar indicates non-significance (ns).

Skin colour in terms of lightness (*L**) increased gradually from 40 initially to 44 at the end of the experimental period (Fig.6-4 B). When the fruit was left at 20 °C for 4 d, the lightness value increased even more. After 6 weeks, fruit subjected to intermittent warming had higher lightness than fruit stored continuously at 4 °C. Chroma (*C**) increased from 26 soon after harvest to 37 by end of week 6 (Fig. 6-4 C). Conversely keeping fruit at 20 °C for another 4 d showed a decrease in chroma value. Hue angle decreased during storage regardless of treatment, implying gradual degradation of chlorophyll, which is an indicator of ripening process. At harvest, the hue angle was 112.5° and this decreased to 109.5° by end of week 6. An additional 4 d storage at 20 °C showed a further decrease in hue. Although quantitatively hue angle decreases, qualitatively the colour change is so minimal that fruit always remain green throughout storage. The colour change of 'Triumph' from dark green to light green is in agreement with observations of previous results (chapter 2, 4 and 5) which, simply shows ripening process taking place.

Correlating *Fv/Fm* with hue (Fig. 6-5 A), *Fv/Fm* with firmness (6-5 B) and *Fv/Fm* with internal maturity/ripeness (6-5 C) showed positive relationships. Internal ripening scores showed little impact of IW, with more fruit moving into score 2-3 than in control. This was consistent with the firmness and colour data. i.e. all IW fruit were softer and lighter in colour. The consistent trend is that IW accelerated ripening and therefore accelerated loss of *Fv/Fm.* Chlorophyll fluorescence seems to provide a way of estimating fruit ripening non-destructively.





	R²		
	Control	**IW 10 d**	**IW 6 d**
Fv/Fv vs Firmness	0.48	0.55	0.48
Fv/Fm vs Hue angle	0.33	0.45	0.31
Fv/Fm vs IM	0.016	0.048	0.05

Figure 6.5. Relationship between *Fv/Fm*, firmness, and internal maturity & ripeness.

6.5 Conclusions

The main purpose of this chapter was to evaluate intermittent warming and chlorophyll fluorescence in improving quality of feijoa. Although studies have shown intermittent warming to improve quality of some fresh produce, findings from the two IW regimes showed no improvement on feijoa quality. Instead fruit slightly accelerated in ripening, presumably because they spent more time in warmer temperatures. However, due to season to season variation, chilling injury did not occur at 4 °C in 2018 but did in 2016 and therefore it is difficult to recommend 'Triumph' for long storage even though literature states that 'Triumph' has good storage traits. As a recommendation it is important for this experiment to be repeated at 4 °C and also at lower storage temperatures e.g. 1 °C or 2 °C to see if IW can actually reduce the chilling injury that normally occurs at those temperatures.

In seeking to establish the ability of *ChF* to predict chilling injury occurrence before becoming irreversible, the results showed a decline in the quantum yield (*Fv/Fm)* but with no chilling injury. The decline in *Fv/Fm* over storage must have been responding only to ripening. Whether there could be a further decline as a result of CI is unknown but the values recorded here are already quite low and are comparable to stressed papaya (Urbano et al., 2004), green pepper (Kosson, 2002) and apples (De Ell et al., 1998). Using *ChF* as a non-destructive tool to monitor ripening during storage seems the most appropriate application for this technology.

Chapter 7 Re-evaluating feijoa maturity and ripeness indices

7.1 Introduction

Maturity at harvest has been identified as a key factor influencing quality of feijoa. As elaborated in section 2.4 and section 5.1 above feijoa is currently harvested at commercial maturity through the touch picking technique where fruit is removed just prior to natural drop due to abscission zone separation. During harvesting, some cultivars may naturally abscise earlier or later in relation to the development of the locular gel. This suggests a possibility that abscission zone on the pedicel and locule development within the fruit may have different timing between different feijoa varieties. For beginners in the industry, it is advisable for the growers to tune their harvesting technique by comparing their harvested fruit to the internal maturity scale developed by the New Zealand Institute for Plant and Food Research Ltd (Fig. 2.2). The PFR scale allows growers to rate fruit maturity based on destructive observation of locular development. If there were a fast and accurate non-destructive maturity evaluation technique, that would be a major step forward in segregating fruit into classes with different storage potential.

To optimise on feijoa consumption and extend storage life, it is important to have knowledge of reliable maturity indices (Kader and Rolle, 2004). Size, shape, firmness, and colour are maturity indices used in other fruit such as mango, apple, tomatoes and kiwifruit; regrettably feijoa maturity assessment depends on visualisation of locular development (Schotsmans et al., 2011; Wills and Golding, 2016). From chapters 4, 5 and 6 it was clear that the appearance of mature locules varies amongst 'Kakariki', 'Wiki Tu' and 'Triumph'. These differences in seed colour, locule number and the presence of a columella cast doubt on the universal applicability of the Plant and Food Research (PFR) scale for different varieties. It is possible that the scale may need modification so that it applies to each different variety. Similarly, during storage the industry still uses the PFR maturity index as a ripeness index. It is possible that previous studies that used the PFR scale as a maturity and ripeness scale were flawed in accurately describing fruit appearance after storage. It therefore becomes important to re-assess the scale in multiple varieties, both at harvest and after storage, and assess to what extent locular development correlates with other ripening-related processes like colour change and loss of firmness.

Ultimately the development of accurate maturity/ripeness indices may be a compromise between indices that ensure best eating qualities to consumers and those that provide elasticity in marketing. In apples researchers have combined some quality parameters to come up with better maturity indices applicable to different varieties and with advanced maturity. These indices are Streif, Perlim and Thiault; the indices are based on various combinations of SSC, firmness, TA and starch (Musacchi and Serra, 2018). Similarly, correlating some of these attributes with the ripening rating stage can assist in developing non-destructive indices to monitor ripening in storage (Abu-Goukh and Bashir, 2003). Considering all the above factors, comprehensive analysis of information regarding feijoa maturity and ripeness remain instrumental towards improving quality of fruit. This study therefore examined the following objectives:

a. To assess whether the PFR scale needs to be modified so that it applies to ‘Kakariki’, ‘Wiki Tu’ and ‘Triumph’ varieties.
b. To determine if the PFR scale is appropriate when used as a ripeness indicator after storage.

c. To examine if there is evidence that different varieties mature and ripen in different ways.

7.2 Materials and methods

For this experiment, data were collected between 2016 and 2018 using freshly harvested and stored fruit. As described in the previous chapters (4, 5 & 6), initial fruit quality data and stored fruit data from non-destructive measurements of firmness (section 3.2.2), skin colour (section 3.2.3), and external blemishes (section 3.2.6) were taken. Fruit were individually numbered in the storage trays and all data were captured for each fruit to allow accurate correlations to be assessed by individual fruit. Once the non-destructive measurements had been taken, fruit were cut transversely and immediately assessment of internal chilling injury done. This urgency ensured that correct assessment was done before the rapid internal browning occurred because of polyphenol oxidase activity (PPO) on phenolic compounds in the presence of oxygen (O_2). Other destructive measures conducted thereafter include scanning for internal maturity/ripeness/other disorders (rots, dry locular gel area) assessments and SSC.

As described in section 3.2.4, internal maturity was assessed by cutting fruit across the equatorial region and scanning the cut surface of the stem-end half (Fig. 3-3) with a flatbed scanner (Perfection 1260, Epson, California, USA). Fifteen (15) fruit were placed on a rectangular grid labelled 1-15 or a-o. The scanned images were coloured and at high-resolution (300 pixels per inch [ppi]). After scanning, the images were saved for later assessment. On the day of image assessment, each fruit was graded using a modified maturity/ripeness scale (Fig. 3-4 that the author developed initially using variety 'Kakariki') which was based on the scale originally developed by Plant & Food Research, New Zealand (Schotsmans et al., 2011).

7.3 Data analysis

Data from the scanned images showing locular development of each fruit were used to produce maturity, ripeness and disorder scales for each variety.

Using Hmisc package and FactoMineR package in RStudio (version 1.0.136, RStudio, Inc., Massachusetts, USA) correlations matrix plots and principal component analysis (PCA) images allowed for visualised summaries of at harvest and storage data.

7.4 Results and discussion

7.4.1 Development of maturity scale for 'Kakariki', 'Wiki Tu', and 'Triumph'

Comprehensive maturity scales developed for each of the varieties (Figs. 7-1, 7-2, & 7-3) replaced the term "seed pulp area" with "locular gel" to describe differences in anatomy as fruit matured, ripened and developed disorders. Classifying fruit to rating 1 or 2 was recognised as being too coarse a division and a new intermediate rating of 1.5 was developed for these scales. In this new rating (1.5) the locular gel was showing first signs of clearing, and for 'Wiki Tu' (Fig. 7-2) the anatomical development is slightly different. The locules initially appear like tiny holes unlike those of 'Kakariki' and 'Triumph' that display the typical feijoa locular development; this could probably be because of open pollination, which could be causing the variation (Patterson, 1990). At this stage, seed development is not well defined probably because the seeds are still embedded in the white locular gel and the testa is still white, therefore few can be observed in all the three varieties. In 'Wiki Tu' and 'Triumph', the septa and columella are distinct but in 'Kakariki' only the septa can be distinguished. In addition, as the fruit progresses to rating 2 this distinction becomes more pronounced.

Another observation was that fruit harvested at touch picking were of differing maturities between varieties and seasons (years). This observation suggested that touch picking criterion did not reliably deliver fruit of rating 2 as originally intended. For example 'Kakariki', 'Triumph' and 'Wiki Tu' touch picked in 2016 for this experiment were at average internal rating 2.5, 1.6 and 1.8 respectively (Table 4-2) suggesting that the abscission layer of 'Triumph' and 'Wiki Tu' developed earlier than that of 'Kakariki' and therefore at touch picking the fruit were likely to be immature inside. These results are in agreement with findings of Duan (2015) who observed differing maturities in attempts to segregate 'Kakariki', 'Barton', 'Anatoki', and 'Wiki-Tu'. These results implied that touch-picked 'Kakariki' fruit harvested at an advanced maturity rating 3 was suitable for domestic market and processing, whereas 'Wiki Tu' and 'Triumph' that were harvested at maturity rating 1.5 or 2 were more suitable for export markets via seafreight.

Figure 7-1 Developed maturity scale for 'Kakariki' feijoa. The term "locular gel" is preferred to seed pulp area as used in the original PFR internal maturity scale for feijoa. Introduced level include 1.5.

Figure 7-2 Developed maturity scale for 'Wiki Tu' feijoa. The term "locular gel" is preferred to seed pulp are as used in the original PFR internal maturity scale for feijoa. Introduced level include 1.5.

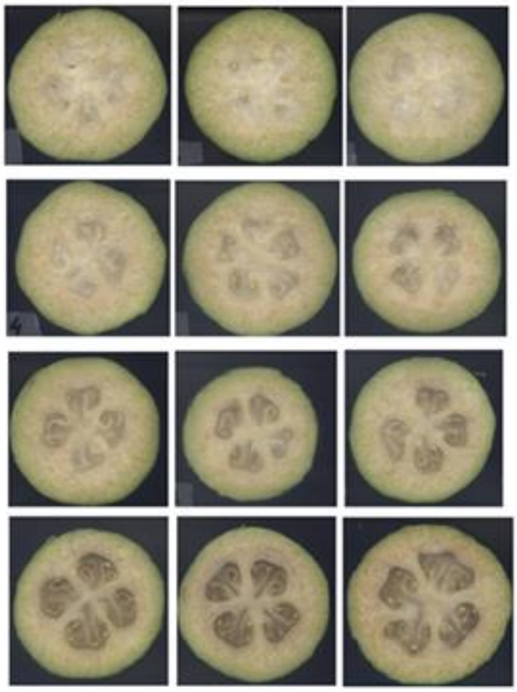


Figure 7-3 Developed maturity scale for 'Triumph' feijoa. The term "locular gel" is preferred to seed pulp area as used in the original PFR internal maturity scale for feijoa. Introduced level include 1.5.

In attempts to predict maturity non-destructively fruit quality attributes were correlated, and they showed diverse relationships (Figs. 7-4, 7-5). From the results of 'Kakariki' (Fig. 7-4) it was difficult to use firmness (r = NA) and brix (r = -0.03) to predict internal maturity, however skin lightness and chroma had stronger relationships (r = 0.46 and r = 0.4 respectively). Interestingly correlating lightness and chroma (r = 0.78), lightness and hue (r = -0.6) and chroma and hue (r = -0.8) had very strong relationships as expected and this agreed with observations of Rupavatharam (2015) who attempted to segregate feijoa 'Unique' using skin colour. Just like in 'Kakariki', for 'Triumph' the relationship between lightness and chroma (r = 0.7), lightness and hue (r = -0.49) and chroma and hue (r = -0.83) were very strong and this further agrees with results of Clark et al. (2005) and Rupavatharam (2015). Unfortunately, while scientifically interesting, these correlations amongst the various colour attributes do not take us any further towards developing a non-destructive maturity index. If any attribute had shown a strong correlation with IM it would have been a candidate for use as a non-destructive tool to segregate fruit at harvest for different markets. Even without such strong correlations, there are other ways to interpret the data. For example, it may be important to be able to identify a portion of the crop that has the best storage potential. For 'Triumph', there is one such opportunity. It is clear from Fig. 7-5 that 'Triumph' fruit with firmness greater than 25 N (as represented by the green vertical line on the figure) were found only at I.M. 1 and 2. It is likely that using a non-destructive device on a grader could separate out such firmer fruit that may be more suited for long storage e.g. for export.

The lack of relationship between internal maturity and soluble solids content agrees with observations of Gaddam et al. (2005) and Duan (2015) who could not use soluble solid content as a maturity index for feijoa. Although Gaddam et al. (2005) suggested that linking a firmness segregation tool (they developed) and SSC using a NIR could develop a maturity index for feijoa. Nevertheless, looking at the non-satisfactory result of Li et al. (2018) when using NIR to predict SSC of 'Kakariki' shows the need for comprehensive studies to be carried out. Although the group suggested that the complex internal structure of feijoa had contributed to the poor results, maybe attempting to revisit the same studies using different feijoa varieties may give satisfactory results.

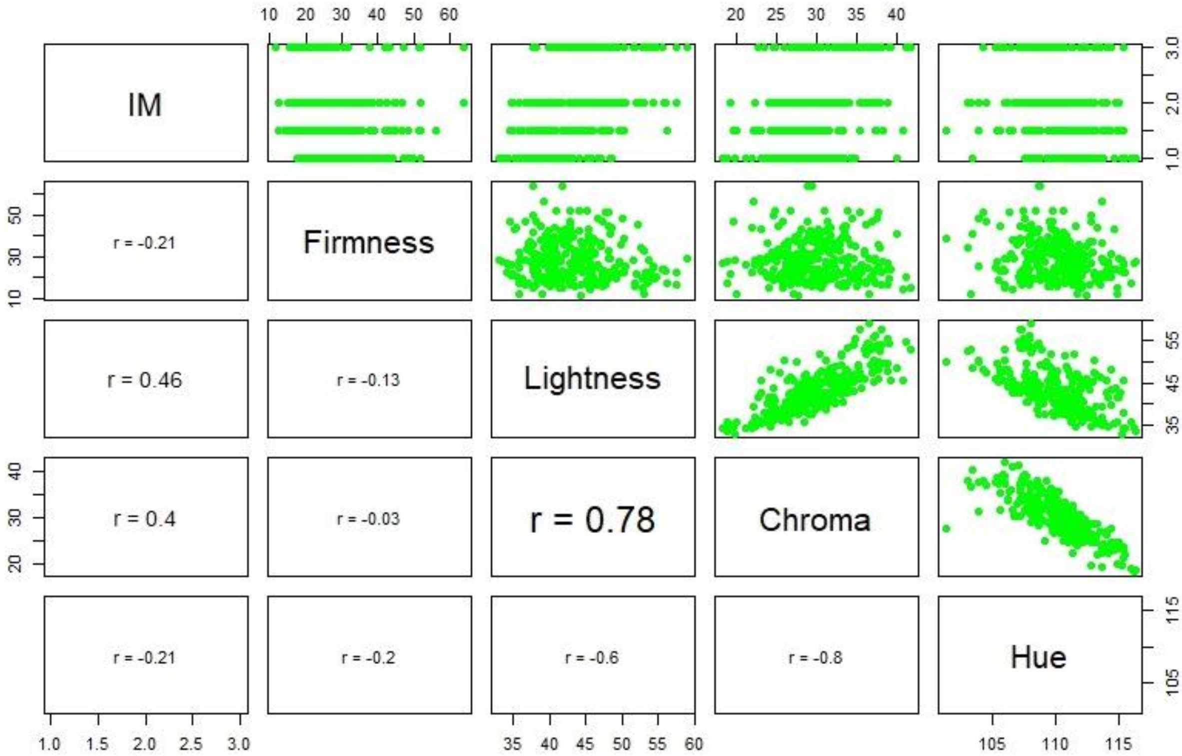


Figure 7-4 Relationships between internal maturity rating, firmness, lightness, chroma, hue angle, and brix of touch picked 'Kakariki', soon after harvest. Each data point represents individual fruit (n = 270).

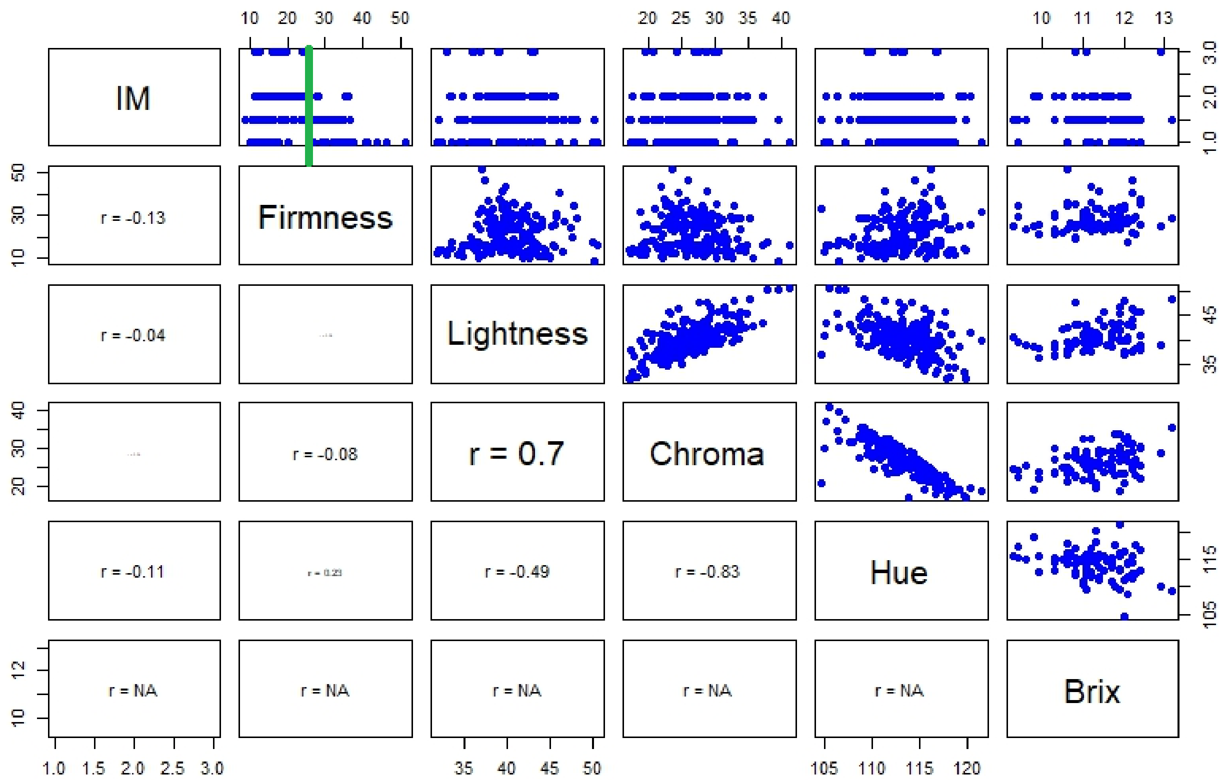


Figure 7-5 Relationships between internal maturity rating, firmness, lightness, chroma, hue angle, and brix of 'Triumph' feijoa, soon after harvest. Each data point represents individual fruit (n = 180). The green vertical bar on 25 N represents the threshold for segregating fruit for long storage (> 25 N) from those for short term storage (< 25 N).

The use of PCA sometimes provides a powerful way of distinguishing clusters of attributes that are not obvious from multiple one-way correlations. In this case, combining the at-harvest data for all four varieties studied, we find that there are no distinctive features that allow discrimination amongst the varieties (Fig. 7.6). The only cluster that separates from the others is composed of the early harvested (i.e. immature) 'Kakariki' fruit. These fruit were darker green and firmer than all other fruit and hence have segregated well. It is also interesting to see that the 'latent vectors' for the quality attributes of firmness and internal maturity are almost diametrically opposite; again, hinting that there may be potential to find a way of using fruit firmness as a non-destructive aid in making storage decisions based on fruit maturity.

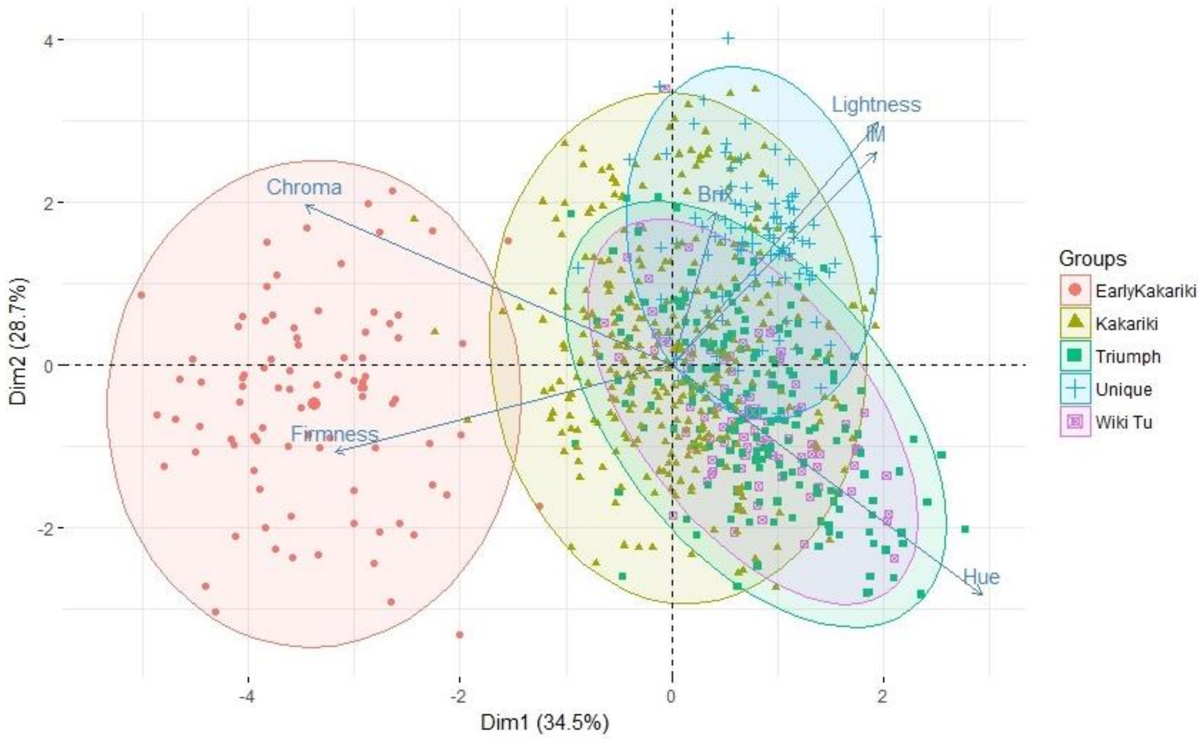
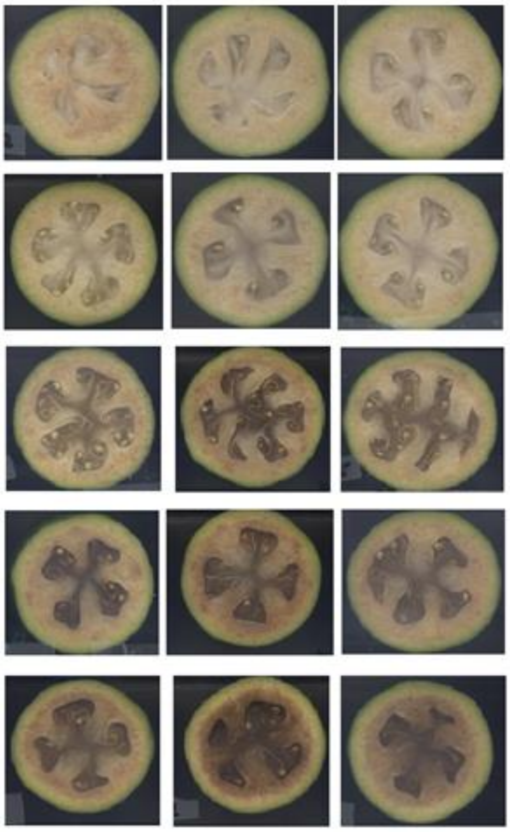


Figure 7-6 Principal component analysis (PCA) bi-plot of the at harvest data of early harvested 'Kakariki' ( ), touch picked (commercial) 'Kakariki' ( ), 'Triumph' ( ), 'Unique' ( ) and 'Wiki Tu' ( ), for all fruit varieties studied (including preliminary the study on 'Unique') in 3 seasons (7 batches of 90 fruit each of 2016, 2017 & 2018) n=630.

7.4.2 Development of ripeness scale for 'Kakariki', 'Wiki Tu', and 'Triumph'

The pericarp development for 'Kakariki', 'Wiki Tu' and 'Triumph' especially in maturity ratings 1, 1.5, 2, and 3 is distinct and these stages were indistinguishable from post storage of ratings 1, 1.5, 2 and 3 (Fig. 7.77-8, and 7-9,). Kakariki' has 4-6 locules, which are not in any particular orientation, whereas 'Wiki Tu' has 4-5 locules located quite regularly in a cross shape. As for 'Triumph', the locules are consistently four in a consistent orientation. As 'Kakariki' ripens (internal ripeness rating 4), the vascular strands remain thick and white and the seeds do not turn brown as described in the PFR scale (Fig 2-2) and the columella is non-existent. In internal ripeness 5 and 6 the brown locular gel becomes translucent and seeds can still be seen as white. The take home message here is that rating 4-6 agree with previous uses of the scale. The browning patterns are similar among varieties. Worth noting is that internal ripeness 5 and 6 are not fit for consumption. Fruit with internal ripeness 4 may still be saleable Mostly at internal ripeness 4, fruit is unacceptable for fresh consumption as reported by Al-Harthy et al. (2010b) but can be used in processing products such as wine, liquor, jams and yoghurts (Zhu, 2018).



Rating 2: Locular gel half white half clear,

Rating 3: All locular gel area clear, with clearly visible vascular strands

Rating 4: All locular gel area is clear but darkening & vascular strands thick & longer,

Rating 5: Translucent brownish locular gel with visible white seeds, but no columella

Rating 6: Brown locular gel and flesh

Figure 7-7 Developed ripeness scale for 'Kakariki' feijoa. The term "locular gel" is preferred to seed pulp that was used in original PFR internal maturity scale for feijoa. Introduced level include 5.

In 'Wiki Tu' seeds are already brown from the field after harvest (rating 1) and the vascular strands remain thick throughout its lifespan. Just like in 'Kakariki'

‘Wiki Tu’ internal ripeness, rating 4 and 5 some fruit may have columella while others lack it, possibly because of the open pollination that brings this variation. ‘Triumph’ is the only variety that closely follows the PFR scale descriptions, suggesting that ‘Triumph’ may have been one of the fruit used to develop PFR scale (Schotsmans et al., 2011). The only point where the ‘Triumph’ scale developed differs from the PFR description is at internal ripeness 5 where especially in fruit batch of 2016 the locular gel dried out as it darkened, however it is possible that this could be a physiological disorder (CI). With ongoing breeding of feijoa varieties and the results above (Fig. 7-1 to 7-3 and Fig. 7-7 to 7-9) on maturity and ripeness scales, the variety-based scales developed here have been shown to be appropriate and thus do not require modification apart from the introduction of rating 1.5 and improved terminology. The ripening patterns among the 3 varieties were comparable. The introduction of rating 1.5 clearly shows that growers have fruit with high chance of ripening than at rating 1 (section 5.3.3.1) which will include fruit that are physiologically immature. Rating 1.5 begun clearing the locule and thus fruit have embarked on the ripening pathway.

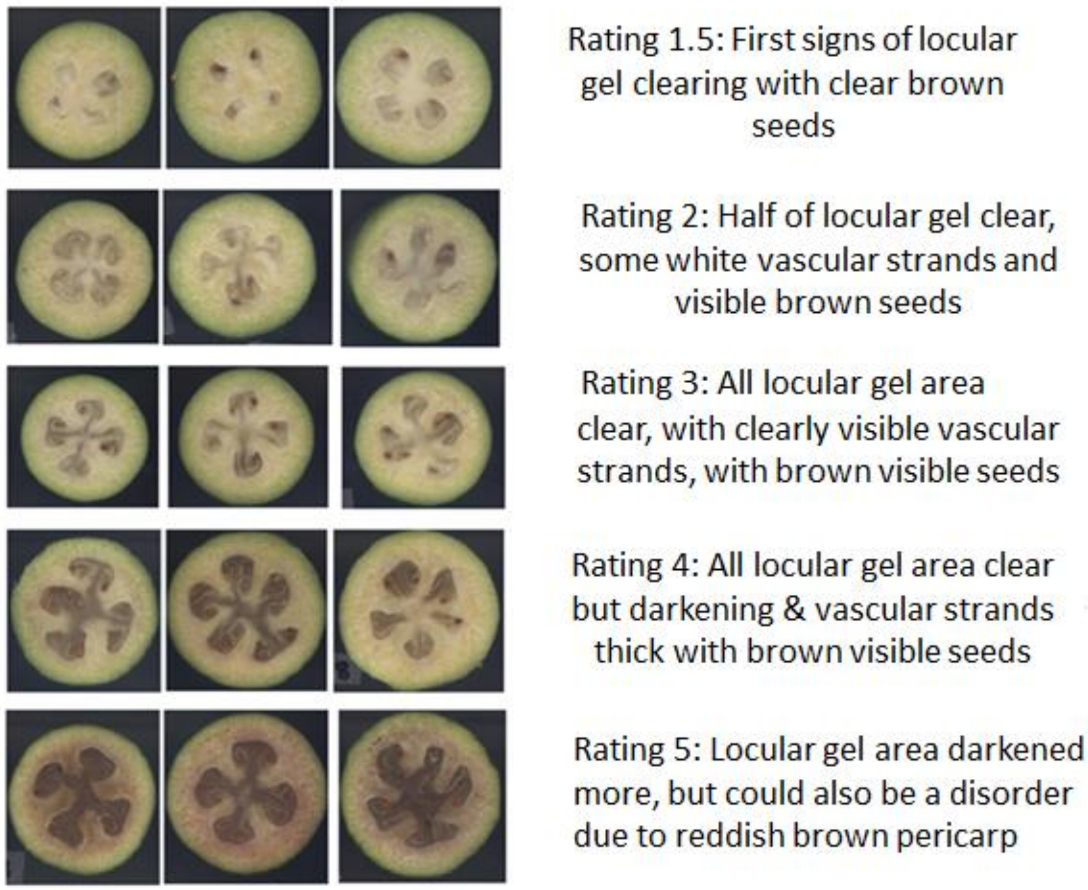


Figure 7-8 Developed ripeness scale for 'Wiki Tu' feijoa. The term "locular gel" is preferred to seed pulp as used in the original PFR internal maturity scale for feijoa. Introduced level include 1.5 and 5.

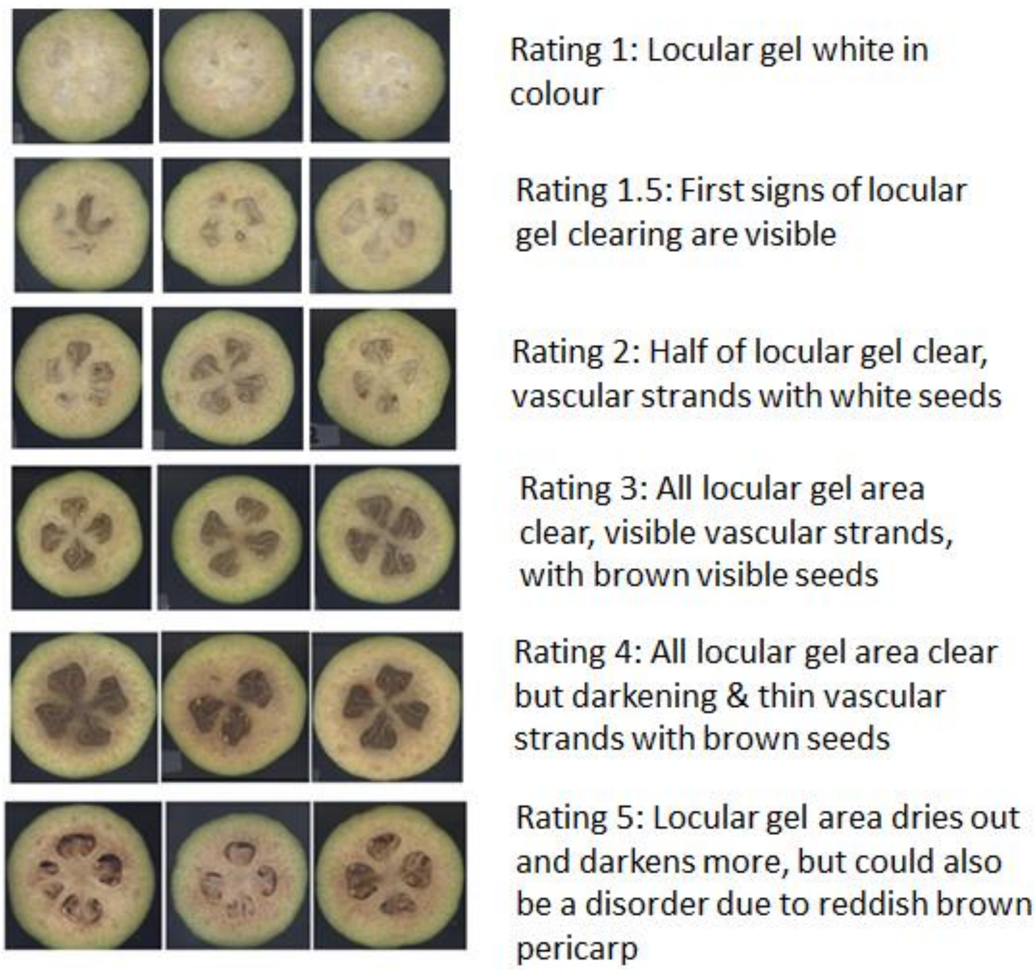


Figure 7-9 Developed ripeness scale for 'Triumph' feijoa. The term "locular gel" is preferred to seed pulp as used in the original PFR internal maturity scale for feijoa. Introduced levels include 1.5 and 5.

The relationships between fruit quality attributes after storage were variable as shown in figure 7-10 ('Kakariki') and 7-11 ('Triumph'). Although at harvest data on firmness of 'Kakariki' could not predict internal maturity, the negative relationship between internal ripeness (IR) and firmness (r = -0.42) shows that feijoa at IR 5-6 had particularly low firmness. This could allow the elimination of over-ripe fruit if assessed after storage and before release to consumers. Furthermore, as fruit ripened, the hue angle, declined while skin lightness increased, and this indicated loss of chlorophyll content as revealing of carotenoids occurred.

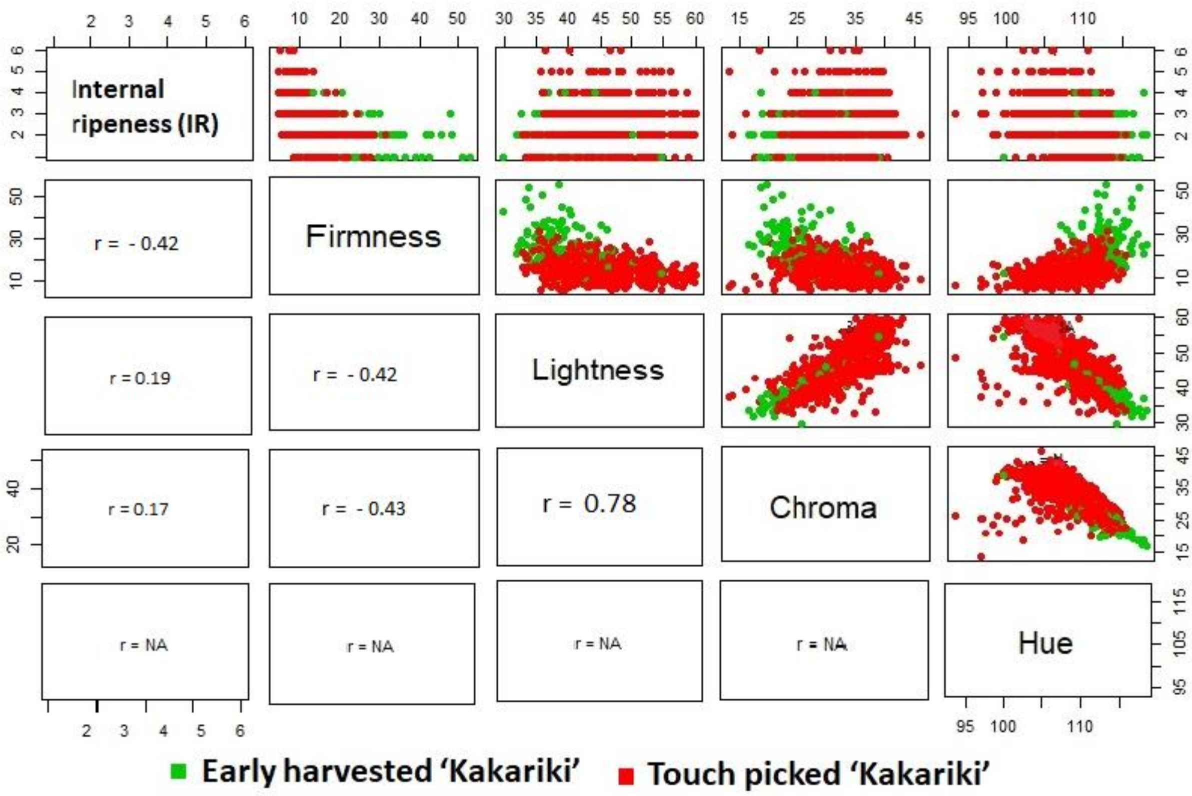


Figure 7-10 Relationships between internal ripeness (IR) rating, firmness, lightness, chroma, and hue angle of early harvested 'Kakariki', and touch picked 'Kakariki',) stored for between 1 - 8 weeks; either at 1 °, 2 °, 4 °, and at 20 °C when assessed after 1 d, 3 d, 4 d or 6 d after cold storage. Each data point represents individual fruit (n = 4090).

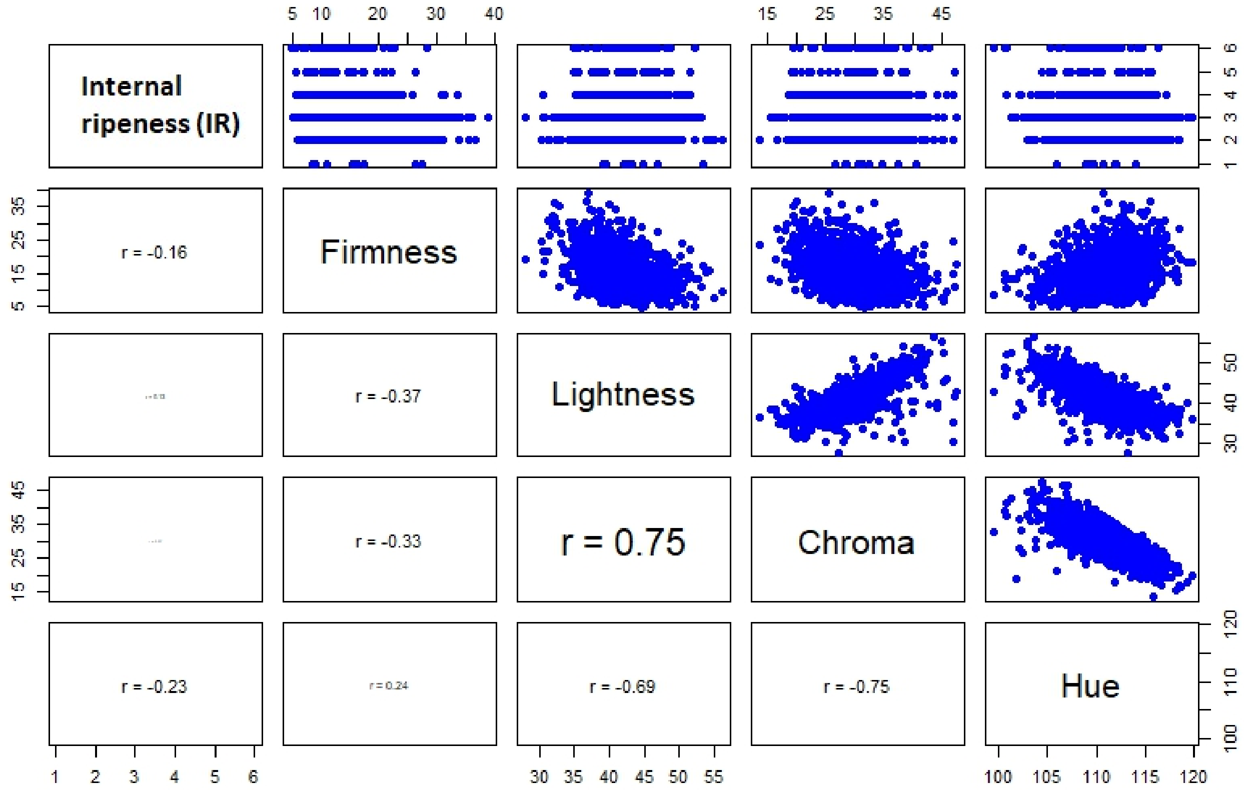


Figure 7-11 Relationships between internal ripeness (IR) rating, firmness, lightness, chroma, and hue angle of 'Triumph', stored for between 1 - 8 weeks; either at 1 °, 4 °, and at 20 °C when assessed after 1 d, 3 d, 4 d or 6 d after cold storage. Each data point represents individual fruit (n = 1520).

7.4.3 Development of disorder scales for 'Kakariki', 'Wiki Tu', and 'Triumph'

In the process of storing feijoa, whether at 1 °C, 2 °C, or 4 °C and subsequently assessing the fruit at 20 °C after 1 d, 4 d or 6 d, the fruit either developed chilling injury or even become rotten depending on storage duration and storage temperature. Figures 7-12 to 7-17 below illustrate some of the disorders observed in the course of the experimental period 2016-2018. The development of chilling injury among the three varieties as observed differs from the general description in literature and this emphasizes the need for variety specific descriptions (Schotsmans et al., 2011). What is common though as stated in previous results chapters is the delayed external chilling injury which was minimal when compared to internal chilling injury. For instance, 'Kakariki' internal chilling injury is characterised by a reddish-brown ring around the locular gel, whereas 'Triumph' has localised reddish brown around vascular bundles and 'Wiki Tu' has reddish brown around the pericarp. Among the varieties, there were fruit with empty locules (Fig. 7-13) but this would relate more to pollination, since each locule in feijoa has about 60 ovules, which normally abort after flowering. Other times few ovules are fertilised and these result in misshapen fruit (Thorp and Bieleski, 2002). Rot development among the three varieties is also distinct with 'Kakariki' having a complete tissue collapse (Fig. 7-14) while 'Triumph' and 'Wiki Tu' will generally be at a spot (Fig. 7-17).

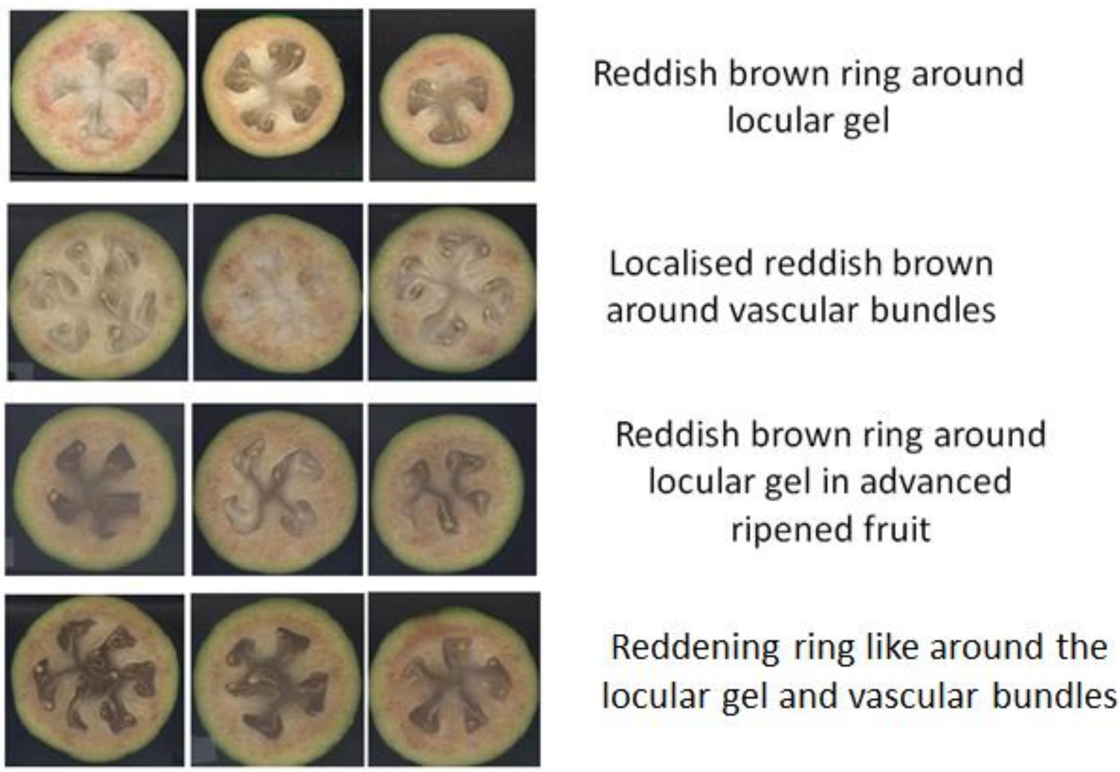


Figure 7-12 Images showing different disorders development in 'Kakariki' feijoa.

Figure 7-13 Another interesting fruit development (disorder) in 'Kakariki' maybe due to pollination.

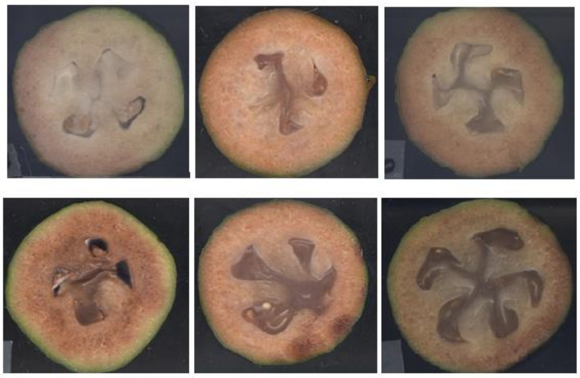


Figure 7-14 Rots development in 'Kakariki.

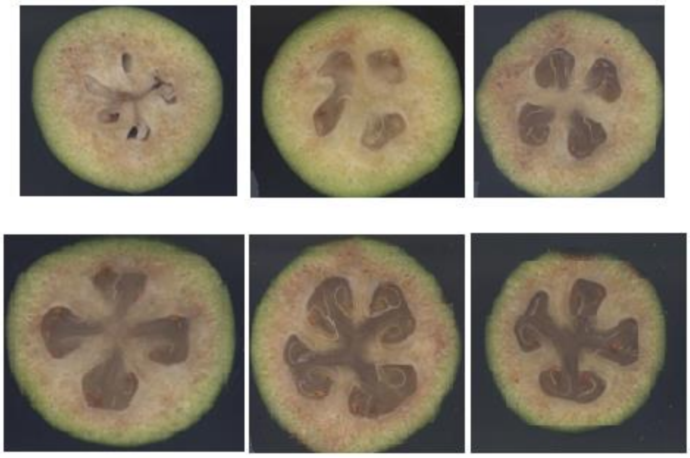


Figure 7-15 Disorder development in 'Wiki Tu' feijoa.

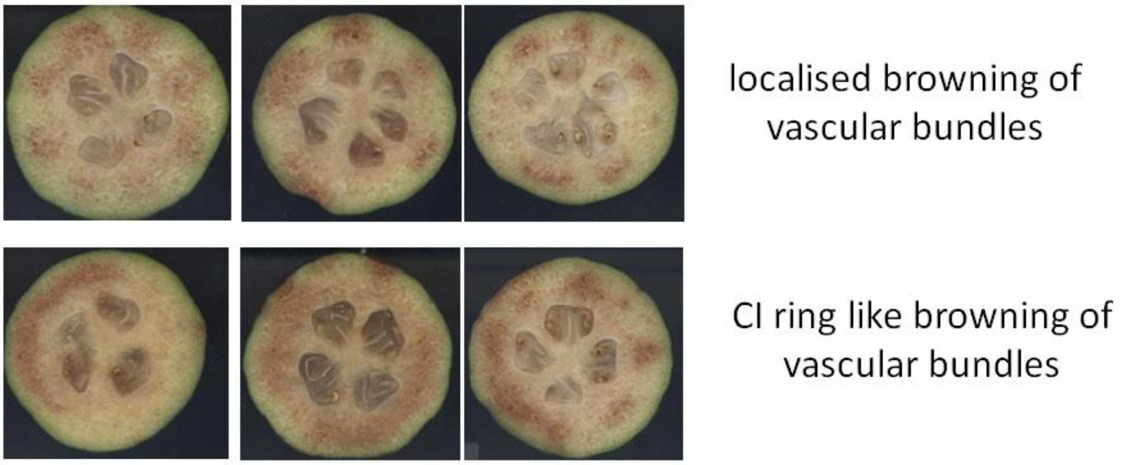


Figure 7-16 Disorder development in 'Triumph' feijoa.

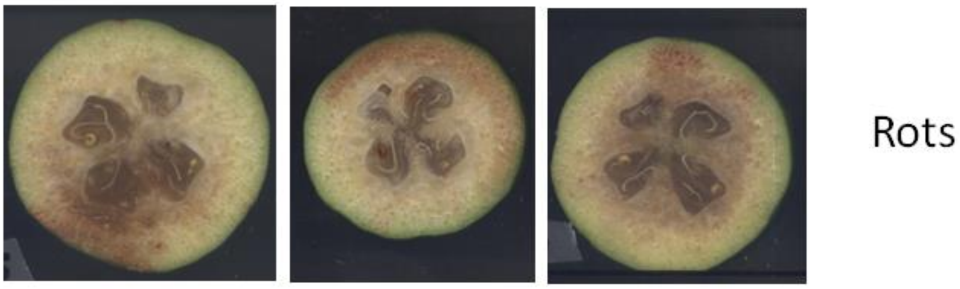


Figure 7-17 Rot development in 'Triumph' and 'Wiki Tu' feijoa.

7.5 Conclusions

No simple relationship could be established in any variety that would allow the use of a non-destructively assessed quality attributes to predict fruit maturity. However, from hypothetical post hoc analysis 'Triumph' fruit with firmness of > 25 N had IM of 1-2 and so could be suitable for long storage. As outlined in the results and discussion section above, the 3-maturity scales and the 3- ripeness scales developed for three varieties show minimal varietal differences. This implies that the PFR scale does not need modification except for the introduction of internal rating 1.5 and change of terminology from seed pulp area to locular gel. It seems that at rating 1.5 majority of feijoa are able to progress through with ripening since the locular clearing has started. It is possible that stage 1.5 would be the equivalent of the 6.2 °Brix of kiwifruit; a marker that ripening is underway.

Ripening of the three varieties is different especially after ripeness rating four (4). For 'Kakariki' the seeds do not turn brown as stated in the PFR scale, whereas for 'Wiki Tu', the seeds are already brown from harvest. 'Triumph' seemed to be the variety described in PFR scale, because the seeds turned brown after ripeness rating four (4).

Development of disorders whether chilling injury or those that eventually may lead to rots were distinct. 'Kakariki' developed chilling injury a reddish-brown ring along the locular gel, while the rots caused a total tissue collapse. 'Wiki Tu' had the reddish-brown colouration completely covering the pericarp (flesh), whereas 'Triumph' had localised reddish brown around vascular bundles. With these differences in mind and as the industry develops with the increased ongoing breeding programs, it is advisable to have in-depth study of new varieties so that specific postharvest techniques can apply. From the results above feijoa disorder development is associated with vascular bundles, implying sugar concentrations may be influencing the disorders. Alternative vascular bundles maybe regions of denser tissue, perhaps implying low oxygen as a problem. Further investigations might give insights into what influences the pattern that the disorder follows.

Chapter 8 Overall discussion and recommendations

8.1 Introduction

The main purpose of this study was to examine potential postharvest techniques of extending storage life of feijoa. This was informed partly because the present reliance on airfreight imposes significant volume restrictions on the industry and airfreight is expensive per carton, unlike seafreight. All large volume export crops from New Zealand (kiwifruit, apples, avocadoes, onion and squash) rely on seafreight. Detailed discussion of the results has been presented in relevant chapters.

This chapter, therefore, presents a general discussion of the results and recommendations for further research.

8.1.1 Extension of feijoa storage life

8.1.1.1 Temperature and relative humidity

Looking across the majority of feijoa storage literature, it appears South American authors (Colombia in particular) have often chosen to avoid chilling injury and accept short storage lives by storing fruit at 5-10 °C after harvest (Schotsmans et al., 2011). In New Zealand however, due to distance to main markets (usually USA and Europe) most studies are done at 4 °C aiming for longer storage lives, but thereby risking the development of chilling injury. Very few workers have tested whether even lower storage temperatures might paradoxically be better than 4 °C. Van der Plank and Davies (1938) showed that CI symptoms in plums and peaches were more visible at 3 °C than at 0 °C.

Following this logic, the use of 1 or 2 °C as the storage temperature was tested for the first time for the recently bred feijoa varieties ‘Kakariki’ and ‘Wiki Tu’ and similarly the same temperature was tried on an old variety ‘Triumph’ Unfortunately, these lower storage temperatures just led to accelerated chilling injury and so the search for a lower, ‘safe’ temperature was abandoned. The other important variable examined at the start of this thesis was RH: all previous PhD students at Massey had followed conventional postharvest wisdom and tested feijoas when stored with an enclosing polyliner, or in flow-through experiments maintained at very high humidity. Feijoa growers normally do not use polyliners and it was possible that, due to feijoa’s waxy skin, the fruit may perform better without polyliners. Again, careful experimentation simply demonstrated that, as expected, water loss is accelerated under lower RH, because of the greater driving force from a larger VPD. In this study weight loss reached a maximum of almost 12% in ‘Wiki Tu’ fruit stored at 4 °C in non-polylined trays after 8 weeks of storage, which would clearly make a serious impact on grower returns if they are paid by fruit weight at outturn.

Given that a wide range of storage manipulations has now been tried for extending feijoa storage life without success, it may be time for a fresh assessment of what anatomical or compositional factors matter the most for the storage life of feijoa. A wider study looking at a large number of varieties, including those like ‘Unique’ and ‘Triumph’ that have moderately longer storage potential, and ‘Kakariki’ that seems to have one of the shortest storage lives, might allow a future student/researcher to assess such factors as metabolic rate, pericarp thickness, locular gel volume, ethylene sensitivity, membrane lipid saturation index or even aroma production to try and identify a suite of ‘storage risk factors’ that apply across a number of genotypes. This could serve both to indicate a path for future research and offer indices that could be used as a screening tool in assessing progeny in a fruit breeding programme.

8.1.1.2 Harvest timing

As mentioned previously (section 2.4), the Massey University postharvest research group has been concerned that "touch picking" is designed to pick fruit just before they are fully ripe and fall to the ground. For many fruit, harvesting before the fully ripe stage has proven to be a technique that allows longer storage, for example tomato (Choi et al., 1995). Earlier work with feijoa showed that fruit harvested before the touch picking stage would ripen after harvest but risked never developing full flavour and aroma (Rupavatharam et al., 2015c). In this thesis, harvest timing entailed picking fruit a week before commercial harvest (early harvested 'Kakariki') and at commercial harvest (touch picked 'Kakariki'). The results indicated that these early harvested 'Kakariki' feijoa entered storage at a lower average internal maturity rating and were firmer with a higher SSC at harvest than 'touch-picked' fruit, and emerged from storage with a higher proportion of fruit still at an acceptable stage of ripeness (Table 5-1). Fruit picked more mature, i.e. at touch picking stage, became riper and softer during storage than fruit picked earlier (Fig. 5-8). A high proportion of fruit was observed to be overripe after storage for 6 weeks, i.e. between internal ripeness rating of 4 and 6. During the same time high incidence of chilling injury (Fig. 5-7) was observed demonstrating that feijoa sensitivity to chilling injury increased with maturity and thus lowered storage life. This finding demonstrates that for long storage life to be achieved then fruit need to be harvested earlier and CI interventions put in place. If interventions for CI alleviation are in place then it is possible to further studies of Rupavatharam et al. (2015c) who found that harvesting feijoa 2 weeks earlier before touch picking extended storage life of 'Unique' to 6 weeks even though the fruit were more acidic and less sweet after storage than fruit harvested at the touch picking stage. The interesting observation in this study was how 'Kakariki' fruit that had reached a maturity of 1.5 at harvest, appeared to retain a satisfactory SSC after 6 weeks in storage.

Close examination of the data however revealed that a proportion of fruit at both harvest dates was probably immature, with an internal maturity of 1; and this emphasised that a key problem for the industry is the wide range of fruit maturities found on the trees at any one time. This is itself a reflection of the long period of fruit set in feijoa (Clark et al., 2005; Wiryawan et al., 2005). Finding a way to segregate fruit non-destructively after harvest into maturity classes would allow identification of potentially longer-storing fruit at around stage 1.5-2 and fruit more suited to local marketing at score 3. This challenge has been explored previously, e.g. Gaddam et al. (2004) attempted to segregate 'Unique' feijoa into different maturity caterogies based on firmness and suggested a possibility of correlating firmness with sugar content or dry matter. Such practices are commonplace in other export-oriented fruit industries. For example, maturity indices for apple and kiwifruit are an essential tool for identifying orchard blocks that are ready for harvest and high dry matter (DM) kiwifruit are believed to deliver a superior eating quality after storage, hence qualify for premium payments. Musacchi and Serra (2018) have reported how the apple industry used TA, firmness, SSC and starch contents to develop Streif, Perlim and Thiault indices in approximating physiological maturity. Similarly, Burdon (2017) reported in how SSC and starch content have been used as indictors of maturity in kiwifruit industry.

The simple use of an earlier harvest date for feijoas does not deliver a less variable range of maturities than conventional touch-picking, so the idea of finding some other attributes that changed consistently across the harvesting period and could be adapted for use as a non-destructive assessment tool of fruit maturity was explored in detail. Because fruit at internal maturity 1.5 are clearly on the ripening path, i.e. the locular gel is beginning to clear, there needs to be even more effort invested in identifying non-destructive tests that can identify this stage unequivocally.

Another important aspect to discuss is the result of fruit quality assessments after 7 d (6 d of conditioning plus 1 d equilibration to 20 °C) of storage that has previously not been published. Findings of this study showed that some of the early harvested fruit ripened slightly (Table 5-2) and during the first 7 d after harvest the SSC of these early harvested fruit increased whereas in all other assessments SSC in feijoa just declined during storage (although this is contrary to an earlier published description, Thorp and Bieleski, 2002). The initial SSC increment in harvested fruit suggests that the fruit were metabolising starch to sugar. After the 7 d period feijoa resumed its normal physiology by using up sugars during ripening. This is consistent with the concept that, if feijoa are truly climacteric, the climacteric may be essentially complete by the time of 'touch-picking'. Feijoas do not have major starch reserves and so they quickly move from mature to senescing (Thorp and Bieleski, 2002). By contrast, 'Hayward' kiwifruit has large starch reserves and is able to store for about 6 months.

8.1.2 Alleviation of chilling injury

This study confirmed that chilling injury is a major limitation to 'Kakariki' fruit storage and has previously been identified as a major contributor to the short storage life of feijoa (along with over-ripening). Two possible treatments for alleviating CI were tested in this study, step down conditioning and intermittent warming.

After the 7 d conditioning period, fruit were stored for up to 8 weeks at either 2 or 4 °C. During this period fruit exposed to step down conditioning showed increased ripening (Figs. 5-4, 5-5), reduced firmness (Fig. 5-8), reduced SSC (Figs. 5-9 C & D) and increased weight loss (Figs. 5-9 A & B). Loss of firmness could have been attributed to water status of the fruit, ripening or even chilling injury. In an ideal circumstance however, the initial exposure of fruit to 9 °C (single step down) or 9 °C then 6 °C (double step down) temperatures for conditioning before storage in either 2 °C or 4 °C would allow fruit to acclimatize to low temperature, reducing chilling injury and extending storage life. But since that did not happen, it is therefore advisable to rapidly cool feijoa fruit soon after harvest to reduce metabolism and ripening; but then sell the fruit before they develop CI.

In the case of intermittent warming (IW), occasional exposure of some fruit species to warm temperatures after storing fruit at lower temperature can alleviate CI and extend storage life. However, in this study, IW was not found to be beneficial. It is important to note that this observation was confounded in this thesis, because of season to season variation in fruit storage performance. In 2016 'Triumph' performed poorly at 4 °C but in 2018 when IW was tested, 'Triumph" fruit had an extremely low incidence of CI and IW was therefore only responsible for a little accelerated ripening and weight loss. The use of IW for feijoa therefore still remains to be explored under conditions where control fruit show a high proportion of CI. Season to season variability in susceptibility to CI has been reported previously in many fruit species and is generally attributed to in-orchard factors such as exposure to heat or drought during growth or at the time of harvest or cultural practices (soil nutrients, water availability, pesticides and growth promoters, and stage of fruit maturity) during fruit growth (Weston and Barth, 1997). Another reason could be the variation of temperature and gas composition inside the fruit during storage in different seasons, as a result of inconsistent storage conditions (Wills and Golding, 2016).

Both step down conditioning and intermittent warming exposed fruit to warmer temperatures higher than the normal storage temperature of 4 °C and this exposure enhanced ripening and increased weight loss that led to faster deterioration of fruit. Since the essence is to extend storage life, clearly, feijoa does not benefit from this early exposure to warmer temperatures and therefore the best thing is to ensure that precooling of fruit occurs as fast as possible to retard fruit metabolism. Any delays in cooling have a great impact during the long storage phase of feijoa. Another way maybe by applying 'safe' chemical treatments, because physical interventions do not seem to hold much promise in a fruit that will ripen rapidly in the time it is held above 4 °C. Nevertheless, in a batch of fruit with a high propensity for CI, it is still possible that IW could be beneficial: this has simply not been adequately tested yet.

As reported in literature other ways of reducing CI and extending storage life could be by developing newer varieties either as selections from the wild or from breeding programmes (Burdon, 2017). For feijoa breeders, breeding programs for long storage, slower-metabolising and less chilling sensitive traits should be adopted rather than the present practice of selecting shape or size. This can be achieved by selecting genotypes that have lower respiration and ethylene production rates, slower softening rate, enhanced nutritional quality, and decreased susceptibility to chilling injury (Meena et al., 2018). The observation that Triumph' fruit can show better storage properties than 'Kakariki' in some seasons is an important reminder that breeders need to include 'long storage ability' as a desirable attribute in a breeding programme, but this is always hard to achieve in practice. In addition, a better understanding of the physiological and environmental factors associated with postharvest fruit senescence can be essential in developing various methods or treatments to further delay senescence and maintain best promising qualities to extend postharvest life of the fruit.

8.1.3 Chlorophyll fluorescence

In the quest for a non-destructive marker of the transition from reversible to irreversible chilling injury, the work reported in this thesis tested the use of chlorophyll fluorescence. Chlorophyll fluorescence uses *Fv/Fm* (the quantum yield of photosystem II [PSII]) parameter as a tool for assessing stress and damage in green tissues. The results showed that 'Triumph' feijoa stored for 6 weeks at 4 °C had a decline in *Fv/Fm* even though no chilling injury was observed (Fig. 6-2). The observed decline may have been linked with loss of chlorophyll content and chloroplast membrane injury associated with PSII as feijoa ripened (Fig. 6-3) and therefore chlorophyll fluorescence detected cellular injury due to natural ripening and senescence. Similar findings were reported by Song et al. (1997) and Urbano et al. (2004) while working on 'Starking Delicious' apples and 'Golden' papaya respectively. Since the results showed that 'Triumph' did not develop irreversible chilling injury during the storage period (Section 6.4.2) the sharp decline in *Fv/Fm* is clearly not associated with development of CI symptoms. The correlation between *Fv/Fm* and fruit firmness and hue angle during ripening is positive and moderate reinforcing the observation of some loss of chloroplast function with advancing senescence. The continuous decline in quantum yield (*Fv/Fm*) during storage is therefore just an indicator that fruit was aging /senescing. This offers potential for non-destructive technique in assessing feijoa maturity at harvest or ripeness in storage. It could be used in a cool store to detect batches of fruit that are ripening more quickly for immediate sales or those ripening slowly for export market or long storage. A similar thinking was portrayed by Prange et al. (2007) who developed and patented a dynamic controlled atmosphere (DCA) system that used chlorophyll fluorescence emission to determined safe lower oxygen limits for maintain apple fruit quality during storage. This invention provides a method to "detect stress in chlorophyll containing matter" by emitting a fluorescence signal when oxygen drops lower to dangerous concentrations that may lead to fermentation of fruit.

8.1.4 Re-evaluation of maturity and ripeness indices

Quality attributes at harvest can be reliable indicators of how fruit will behave after harvest. Attributes such as firmness and soluble solids content can be used non-destructively in sorting lines to segregate fruit into different maturities. In line with the above, this section sought to re-evaluate maturity and ripeness indices of feijoa.

Observed results showed that there was high variability in fruit maturity in each batch harvested at the same time (as a result of the long period of time over which fruit set occurs). Touch picking did not successfully minimise this maturity range and there are currently no other external ripeness cues suitable for reducing feijoa maturity variability. Results also indicated that touch picking (approximate time when abscission zone develops) occurred at a more advanced stage of ripening in 'Kakariki' than in 'Triumph', meaning that by the time 'Kakariki' fruit were harvested, many were already at rating 3 (sections 4.4.5, 5.3.3.1, 6. 4.5.1 and 7.3.1) and this observation is also evident in the work of Al-Harthy (2010).

During storage, fruit maturation was assessed by monitoring changes of the skin colour, firmness and soluble solids content. After 6 weeks of storage about 5% of the early harvested fruit (Figs. 5-4 & 6-3) had not ripened at all. The implication is that such fruit were not competent to ripen and therefore could not ripen at all off tree. From these results therefore, if an effort is made to harvest fruit earlier than at touch picking stage, it will be essential to find a way to grade out fruit that have not reached stage 1.5. This requires a non-destructive and preferably automated technique.

When ascertaining appropriateness of the current PFR maturity/ripeness scale based on 'Kakariki', 'Wiki Tu' and 'Triumph' varieties, the author found that apart from introducing rating 1.5 and using locular gel term instead of seed pulp area the PFR scale was generally suitable. By introducing rating 1.5, first signs of locular gel clearing were observed suggesting that ripening was underway. No direct and simple relationship between internal maturity/ripeness and quality attributes (firmness, SSC or colour) could be established. Nonetheless the potential to predict storage life based on a non-destructive test that can be used in sorting lines is good. Worth noting though is that for feijoa, the largest changes in measured attributes were always found for firmness, which generally correlated with chlorophyll fluorescence. Together these attributes still seem to hold the best potential for development into a non-destructive tool that could identify the less-mature fruit in a harvested batch. In theory these fruit could be stored for longer periods; but only if a way can be found to eliminate immature fruit from these batches. What is now needed is a way to reliably detect and exclude fruit that have not reached stage 1.5 and therefore not reached harvest maturity. Since this measure indicates the onset of locular gel clearing further work is required to allow external detection of this fundamental step in feijoa ripening. X-rays and

MRI have potential to detect this internal anatomical change but currently are too slow to segregate every fruit in a grading line.

In conclusion, this study has added to the knowledge base of feijoa research and will serve as a platform for future studies. The study has emphasized the importance of using polyliners during packaging of fruit to maintain saleable weight, the study has ruled out use of step down conditioning as a storage technology that can alleviate chilling injury and extend storage life. The potential to extend storage life can be seen in using harvest maturity and segregating fruit non-destructively using compression firmness and by using chlorophyll fluorescence to monitor quantum yield (Fv/Fm) during storage. Since intermittent warming (IW) was only studied at 4 °C with no effect, it is still worth doing further studies at temperatures lower than 4 °C in attempts to extend storage life. Besides IW other areas that can be studied in future include the need for non-destructive maturity tests, or for more control of feijoa flowering and pollination; or more fundamental understanding of ripening physiology and molecular biology.

8.2 Recommendations for future research

8.2.1 Development of non-destructive grading tool

As observed in chapters 2, 4, 5 and 7, there exists high variability among feijoa fruit at harvest that significantly affects quality in storage hence reduced profits. Although touch picking remains the traditional method followed in harvesting feijoa, this study together with previous studies indicate that it is not yet possible to optimize harvesting and achieve uniform quality. This has been attributed to the long flowering period that results in fruit setting occurring over a period of 4- 6 weeks. This is further compounded by the lack of external visual signals such as colour change to indicate maturity. Although previous studies have attempted to objectively determine maturity by using SSC, firmness, skin colour, and NIR the success has been minimal.

This study therefore recommends the development of a non-destructive grading tool that can objectively visualize the locular gel clearing and segregate fruit into different maturities. Some of the techniques may include magnetic resonance imaging (MRI) by advancing previous work by Rupavatharam (2015), and electronic nose (Enose) whereby 'low aroma' fruit might be detectable, allowing the elimination of immature fruit from an early-harvested batch. The process may require strengthening some of the already existing techniques mentioned above (X-ray, NIR). For instance, X-rays and MRI can be strengthened by using engineering skills that may provide a faster way to scan large volumes of fruit for some particular signal attribute rather than generating a processed image. Previous studied in the Massey postharvest group that may be advanced for feijoa include a wider range of 'visualisation' techniques that look at or inside fruit, e.g. other works of Li et al. (2018) on kiwifruit with micro-computed tomography (which also suffers from an inability to work quickly and non-destructively). Also, worth looking at are works of Cantre et al. (2014) who used X-ray micro computed tomography (μCT) to study pore structure, porosity and raphide features of kiwifruit tissue in detail. In the study X-ray μCT revealed differences in the cell/void networks near the skin of the 5 different cultivars ('Hayward', 'Hort16A', 'G3', 'G9' and 'G14') used. Even more promising is the faster non-destructive tool of laser fringe projection, which holds potential to detect the change in surface 'feel' of a feijoa from shiny and wrinkly to smoother and duller at harvest (Lai et al 2019). However, money, time for data collection and analysis and the big samples needed maybe a hindrance to explore this study.

8.2.2 Why are feijoa fruit hard to store

Is it possible for modern varieties of feijoa to store for more than 4 weeks? This arises from the current and previous studies that have applied several postharvest techniques to feijoa with minimal success or no benefit at all. This study therefore recommends a new way of thinking beyond the physiology of feijoa in order to overcome quality and storage challenges. This may include breeding or genetically engineering using recombinant DNA technique to develop a perfect feijoa that has a slower ripening phase. If CI is controlled, then this is possible in a variety like 'Triumph' that has a slightly longer storage life than the other varieties. It is important to note that currently there is a lot of ongoing private breeding work for feijoa although varieties released have not been tested for their postharvest performance (as outlined in chapter 2). To breed for a perfect feijoa, breeders therefore need to select genotypes with traits for earliness, sweetness, size, delayed softening, and chilling tolerance. As for 'Triumph' feijoa, in some seasons it stored well, although the fruit size is small with a moderate sugar-acid balance. If a breeder could combine traits of different varieties to produce larger sized fruit with a low metabolic rate then we can inch closer to a perfect feijoa.

The perfect feijoa should meet growers need for high yield and high quality, shippers' requirements for storage properties as well as consumer's desires for eating quality. Another important point is that most of the New Zealand varieties are based on the Uruguay fruit that normally are small sweet fruit. While the Brazilian type are known to be large fruit, which implies that a trait for big fruit can be selected and crosses-made with the Uruguay fruit. From this study, it is difficult to state a fruit with desirable traits although due to seasonal variation 'Triumph' seemed to indicate it was slightly better than 'Kakariki' and 'Wiki Tu' traits.

In the southern hemisphere feijoa flowers are set from November through until as late as February and as such a wide range of fruit maturities are present on the tree. This problem is compounded by poor pollination and therefore in storage it becomes complicated. Feijoa are cross-pollinated using blackbirds and mynah birds. Instead of using the birds, growers could control crosses by removing immature flowers and transferring pollen to mature flowers by hand. This may cut down on the long flowering period of fruit set. Multiple pollinations can be done in a short period to ensure successful fertilization and fruit set in a much narrower window.

8.2.3 Is feijoa more of a non-climacteric fruit?

Looking at the feijoa ripening behaviour it is hard to know if it is non-climacteric or climacteric. Non-climacteric fruit such as strawberries or citrus do not ripen after harvest and need to be picked at close to 'eating maturity'. Climacteric fruit such as tomato or apple can be picked less mature and ripen during storage after harvest and this will be accompanied by an increase in respiration and ethylene production. Ripening is a well-coordinated process with physiological and biochemical changes occurring. The process entails softening, accumulation of sugars, decline in titratable acidity and colour changes. Traditionally feijoa is classified as climacteric fruit; however, past data by Rupavatharam (2015) and Velho et al. (2008) shows that it is hard to modify the ripening behaviour of fruit at touch-picking stage with ethylene or 1-MCP. During this study, fruit harvested at internal maturity rating 1 may be physiologically immature and have sometimes failed to ripen; whereas by internal rating 1.5 they appear to be committed to a ripening path. The fruit has low starch of about 0.8% of fruit dry matter throughout. During ripening both sugars and titratable acids decline which is contrary to how climacteric fruit behaves. The respiration pattern of feijoa does agree to the climacteric pattern however when it comes to the ethylene production and responses it is contrary to a classical climacteric fruit. Previous studies by Rupavatharam (2015) and Schotsmans et al. (2011) have shown feijoa not responding to application of 1-MCP and its non-sensitivity to external ethylene treatments. Even with application of ethylene feijoa, skin colour will remain green and there was no effect on firmness. There remains a need for a molecular study to investigate the role of ethylene biosynthesis and ethylene response genes to identify the timing of the feijoa ripening pathways and the potential to use new inhibitors to delay over-ripening.

8.2.4 Development of models to explain changes as fruit ripens

From this study it is evident that seasonal variation existed among varieties and this can affect the industry negatively. This therefore calls for development of models that can assist growers to understand how the variations affect fruit quality in order to put in place better management practices. In other fruit species considerable effort is being expended to develop models that link in-orchard factors (such as orchard nutrition, growing season temperature and rainfall, or position on the tree) with harvest maturity factors and storage conditions (temperature, humidity, duration, atmospheric composition) to allow the prediction of fruit quality after any period of storage. From an industry point of view, embarking on large storage trials to develop the data for these models might be of more practical value than continuing to attempt to define the underlying physiological factors that influence chilling sensitivity or restrict storability. This kind of work requires large numbers of fruit to be assessed from a range of orchards and under a range of conditions, so would be expensive for a small industry. Nonetheless the current approach of exploring techniques aimed at mechanisms known to control storage life of other fruit has not yet led to a significant benefit; and hence a fresh approach may be required.

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For this publication, the main author did the experimental set up, data collection, analysis and writing the report. The co-authors edited the manuscript and provided valuable suggestions while designing and conducting the work.	
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Primary Supervisor's Signature:	Julian Heyes Digitally signed by Julian Heyes Date: 2020.09.01 08:11:48 +12'00'
Date:	01/09/2020

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)