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Manipulating harvest maturity and ethylene to extend storage life of feijoa

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

In New Zealand feijoa (*Acca sellowiana*) are harvested by touch-picking and can be stored at 4 °C and 90% RH for up to 4 weeks with a subsequent shelf life of 5-7 d at 20 °C. Extending the storage potential of feijoa can enable export to new marketplaces through sea freight. The objective of this work was to extend the storage life of feijoa. Harvest maturity, variability within batch and ethylene all have the potential to influence postharvest storage performance.

Harvesting feijoa earlier not only makes touch-picking irrelevant but will necessitate changes to the present grading standards of feijoa. Harvesting feijoa 2 weeks prior to touch-picked maturity consistently enabled storage life extension for up to 6 weeks at 4 °C with a subsequent shelf life of 5 d at 20 °C. However, these fruit were low in SSC with high TA suggesting that their taste profiles may be altered and hence a consumer taste acceptance investigation is recommended. There is also a need to identify a ripening index for feijoa while redefining grading criteria suitable for both local and export markets.

This work demonstrates that feijoa having higher °hue (> 122) at the time of harvest possessed longer storage potential than those with lower °hue (< 122) that are ready-to-eat. Blocking ethylene responses of touch-picked or early harvested feijoa by postharvest 1-methylcyclopropene treatment had minimal effect on physiology (ethylene production and respiration rates) or quality after storage. Feijoa were also insensitive to exogenous ethylene application while CA technology stimulated surface injury. However preharvest Aminoethoxyvinyl glycine (AVG) application reduced fruit drop and delayed maturity (retaining firmness) of feijoa at the time of commercial harvest. AVG suppressed ethylene production during storage without altering quality attributes (firmness, SSC, TA

or flesh colour). A conceptual model was developed to summarise feijoa responses to ethylene manipulations. Postharvest ripening in feijoa appears to be substantially ethylene-independent.

Storage life of feijoa was extended by harvesting fruit earlier than current touch-picking maturity. Segregating feijoa by skin colour change would allow sea freight of less mature fruit. However, taste acceptance of these fruit and commercial trials of preharvest AVG are required before practical recommendations can be made to the industry.

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List of Abbreviations and Symbols

ACC	aminocyclopropane-1 carboxylic acid
ACO	ACC oxidase
ACS	ACC synthase
Ag	silver
AgNO ₃	silver nitrate
AIN	ACC insensitive
ANOVA	analysis of variance
AOA	aminooxyacetic acid
Au	gold
AVG	aminoethoxyvinylglycine
°C	degree Celsius
C	carbon
C ₂ H ₄	ethylene
CA	controlled atmosphere
<i>cnr</i>	colourless non-ripening
CNT	controls
CO ₂	carbon dioxide
CoCl ₂	cobalt chloride
CT	computed tomography
Cu	copper
d	day (s)
DACP	diazocyclopentadiene
DMCP	3,3-dimethylcyclopropene
DNP	2,4-dinitrophenol

EIN	ethylene insensitive
ERS	ethylene response sensor
ETO	ethylene overproducing
ETR	ethylene receptor
FDP	fruit development period
g	grams
GDD	growing degree days
GLM	general linear model
H	hydrogen
h	hour
H ₀	harvest at touch-picking maturity
H ₋₁	harvest at one week before touch-picked maturity
H ₋₂	harvest at two weeks before touch-picked maturity
H ₋₄	harvest at four weeks before touch-picked maturity
HCN	hydrogen cyanide
HP	hewlett packard
HU	hounsfield unit
<i>I</i> _{AD}	interactance spectrum
kg	kilogram
kPa	kilo Pascal
L	litre
μL	micro-litre
L*	lightness
lb	pound force
LEACS	<i>Lycopersicon esculentum</i> ACS

MCP	1-methylcyclopropene
min	minute
mL	millilitre
MRI	magnetic resonance imaging
MTA	5'-methylthioadenosine
N	newton
N ₂	nitrogen
NAI	normalised anthocyanin index
NBD	2,5-norbornadiene
NDVI	normalised difference vegetation index
NE	no effect
Ni	nickel
NIR	near infrared
nL	nano-litre
nmol	nano-mole
<i>nor</i>	non ripening
NS	not significant
NZFGA	New Zealand Feijoa Growers Association
O ₂	oxygen
PAL	Phenylalanine ammonia-lyase
PLP	pyridoxal-5'-phosphate
pmol	pico-mole
POD	peroxidase
PPO	polyphenol oxidase
PVC	polyvinylchloride

R2E	ready to eat
(r_{CO_2})	carbon dioxide production rate
RH	relative humidity
<i>rin</i>	ripening inhibitor
s	second
SAM	S-adenosylmethionine
SRS	space resolved spectroscopy
SSC	soluble solids content
STR	storage
STS	silverthiosulphate
TA	titratable acidity
TRS	time resolved spectroscopy
UK	United Kingdom
USA	United States of America
v/v	volume / volume
w/w	weight / weight
%	percent

1 Introduction

Feijoa (*Acca sellowiana* [Berg] Burret) is an aromatic fruit belonging to Myrtaceae family that is commercially grown in New Zealand (Thorp, 2008). Feijoa can be stored for up to 4 weeks at 4 °C with a subsequent 5-7 d at 20 °C (Klein & Thorp, 1987). Feijoa is consumed predominantly in the local market (Aitken & Hewett, 2013), with export of feijoa by refrigerated sea-freight from New Zealand curtailed by storage quality losses beyond 4 weeks. Sea freight is the most economical way to transport fresh fruit (Cirad, 2006). Due to the geographical isolation of New Zealand, at least 6 weeks of consistent storage quality is needed to enable sea freight of feijoa to distant markets in Europe, North America and Asia (Al-Harthy, 2010).

Feijoa is currently harvested in New Zealand by a ‘touch-picking’ method that relies on using low force to detach fruit from its stalk (Thorp & Bielecki, 2002). Fruit harvested with greater force are considered to be immature (Thorp & Klein, 1987). Downs et al. (1988) suggests that feijoa harvested using high force had the same eating appeal as that of touch-picked fruit after 4 weeks of storage and suggested that touch-picking was adopted by growers to provide ready-to-eat fruit for immediate consumption rather than to withstand longer storage. These reports lead to the hypothesis that harvesting earlier than ‘touch-picked’ maturity may result in fruit possessing more storage potential leading to extended postharvest life.

Postharvest treatments to extend storage life of feijoa using low temperature storage (Klein & Thorp, 1987), hot-water treatments (Woolf et al.,

2006), calcium application (Ramírez et al., 2005) or controlled atmosphere (CA) storage (Al-Harthy et al., 2010a; Al-Harthy et al., 2010b; East et al., 2009) have had limited success. Feijoa demonstrates climacteric behaviour with a rise in ethylene production and respiration rate during ripening (Reid, 1975). Controlling ethylene perception and its synthesis has been found to extend storage life of many climacteric fruits (Watkins, 2006). Ethylene action blocker 1-methylcyclopropene (1-MCP) was found to retain firmness and skin colour for up to 30 d in storage at 4 °C in Brazilian feijoa (Amarante et al., 2008) while Velho et al. (2008) reported no effect. Ethylene synthesis inhibitor aminoethoxyvinylglycine (AVG) is applied preharvest on climacteric fruit to delay at-harvest maturity and extend storage life (Lurie, 2008). It is hypothesised that inhibiting synthesis and ethylene action in feijoa can delay ripening and extend storage life. There are no published works evaluating effects of postharvest 1-MCP or by preharvest AVG application on New Zealand feijoa. Feijoa's responses to ethylene manipulations including application of ethylene perception blocker (1-MCP) and preharvest ethylene inhibition by AVG and application of exogenous ethylene during storage are further investigated in this research.

Fruit maturity at the time of harvest is the most important factor that determines quality during storage (Kader, 1999b). The present grade standard introduced by Plant and Food Research, New Zealand in 2004 relies on destructive testing and visual assessment (Schotsmans et al., 2011). The feijoa industry is challenged by variation within fruit batches that are complicated by the touch-picking method (Al-Harthy, 2010). Earlier efforts to non-destructively estimate maturity at harvest using fruit density (Clark et al., 2005), acoustic

impulse response (Gaddam et al., 2005) or compression firmness (Wiryawan et al., 2005) were inconclusive. Identifying a non-destructive and instrumental indicator of harvest maturity will contribute to controlling variability among fruit batches. This research explores use of skin colour to segregate feijoa at harvest time.

The feijoa cultivar 'Unique' was chosen in this research as it is a popular variety in New Zealand and also more extensively researched than any other variety (Al-Harthy, 2010; Al-Harthy et al., 2010a; East et al., 2009; Thorp & Bielecki, 2002). The goal of this research is to extend storage life of feijoa which will enable export of fresh fruit by sea freight to distant markets through the manipulation of harvest maturity or ethylene. Variability of product maturity was conducted by harvesting feijoa earlier than current commercial touch picking time.

The major objectives of this research are:

- To determine the role of harvest maturity on quality and storage life of feijoa
- To determine the role of ethylene on maturation and ripening of feijoa
- To evaluate the potential for use of skin colour as an indicator of feijoa maturity for sorting at harvest

All of these objectives contribute to the body of knowledge aimed at providing solutions to extend the storage life of feijoa and facilitate the development of a New Zealand feijoa export industry.

2 Literature review

2.1 Background

It has been estimated that 20-60% of all fresh produce is lost between production and consumption (Yu & Nagurney, 2013). Postharvest losses of fresh produce is high at the production and distribution side in developing countries while in developed nations losses are high at the consumer's end (Parfitt et al., 2010). Improved understanding of postharvest biology contributed to the advancements in postharvest technologies for maintaining quality in fresh supply chains (Kader, 2002). The very best in the knowledge of postharvest technologies can only achieve a reduced rate of product deterioration which is normal during ripening and senescence. Understanding preharvest factors that influence quality attributes of harvested produce impacts postharvest deterioration (Hewett, 2006).

New Zealand horticulture accounted for \$6.7 billion in 2013 where kiwifruit and wine industries dominated exports while feijoa contributed approximately \$0.2 million (Aitken & Hewett, 2013). The New Zealand feijoa growers association (NZFGA), constituted in 1983 (Morton, 1987) is active in marketing feijoa to local markets. Feijoa are harvested during autumn from March to June in New Zealand. Feijoa is a good source of fibre, vitamin C and used in processed foods like juice mixes, wine, jam and ice-cream. Fruit possess antioxidant activity and are considered to be useful source of bioactive compounds for nutraceutical and pharmaceutical industries (Bontempo et al., 2007; Weston, 2010). Traditional knowledge associated in rural communities of Brazil support medicinal properties of feijoa (Dos Santos et al., 2009).

Earlier research efforts focussed on postharvest treatments to extend storage life of feijoa indicated the complexity of mixed maturity at the time of harvest and grading standards relying upon external features such as size, weight and freedom from diseases and pests (Al-Harthy, 2010). Feijoa research has been limited due to the size of the industry. Increasing storage life from present 4 weeks at 4 °C by 2 weeks can open potential new market opportunities for feijoa and become stimulator for the industry in New Zealand.

2.2 Feijoa

2.2.1 Origins

Feijoa (*Acca sellowiana* [Berg] Burret) has its origin in the mountainous region bordering southern Brazil, Paraguay, Argentina and Uruguay (Thorp & Bielecki, 2002). There are two distinct populations in feijoa namely the 'Brazilian' type which has large hard seeds and the 'Uruguayan' type having small soft seeds. Feijoa is also known as 'goiabeira do mato' or 'goiabeira serrana' or 'goiabo do campo' in Brazil; pineapple guava in USA and 'guayabo' or 'guayabo del pais' in Uruguay. Berg (1855-1856) was first to use the name 'feijoa' in English. French botanist Dr. Edouard André was the first to introduce feijoa (Uruguayan type) into Europe in 1890s, later feijoa was brought to New Zealand in 1908 (Morton, 1987). It was initially grown as a hedge tree in backyards with the first commercial plantings made in California during 1910 (Popenoe, 1912).

2.2.2 Botany

Feijoa is closely related to two other species namely *Acca lanuginosa* and *Acca macrostema* while the genus *Acca* is related to *Myrrhinium* (Thorp &

Bieleski, 2002). Feijoa is a small perennial shrub tree growing up to 6 m high with shallow root system. Leaves are leathery green in colour about 1.5-4 cm wide and 3-7 cm long with a glabrous or with hairs. Flowers are bisexual but anthers are located 7 mm below the stigma forcing cross pollination for fruit set (Ducroquet & Hickel, 1997). The flower has 4 to 6 conspicuous purple crimson coloured fleshy petals that attract birds for pollination (Patterson, 1989). Fruit is an aromatic berry with shapes ranging from ovoid to spheroid, weighing 40-150 grams, measuring 2.5-6 cm wide and 2.5-10 cm long. Fruit skin is smooth green in colour having granular flesh enclosing 4-6 translucent locules (Fig. 2.1) with jellied pulp containing numerous seeds (Harman, 1987). The pericarp portion encloses vascular tissues which enable flow of nutrients and sugars during growth (Thorp & Bieleski, 2002). A pleasant aroma is emanated by feijoa during ripening which is described as a combination of pineapple, guava and strawberry.

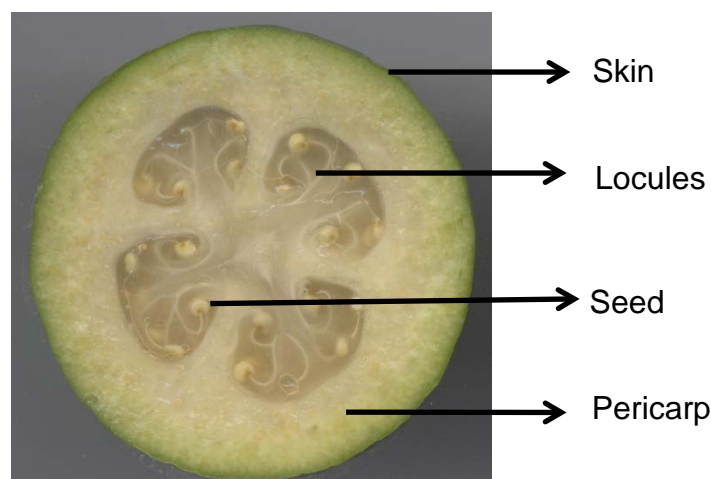


Fig. 2.1 Cross section of feijoa fruit

2.2.3 Cultivars

All the modern New Zealand cultivars of feijoa originate from a single plant introduced into Europe by Dr. André (Thorp & Bielecki, 2002). Feijoa varieties of high quality fruit trees and their selection is more advanced in New Zealand when compared to the rest of the world (Thorp, 2008). 'Choiceana', 'Superba' and 'Coolidge' were initial varieties released during 1920s, later Hayward Wright, the selector of 'Hayward' kiwifruit was responsible for the selection of 'Triumph' and 'NZ Superba' varieties of feijoa in 1942 (Endt, 1978). In 1954, 'Mammoth' was released by Department of Scientific and Industrial Research, New Zealand. During late 1980-1990s many cultivars were released in New Zealand by a selection programme involving Dr. K.J. Patterson and Dr. Grant Thorp (Thorp & Bielecki, 2002). In the last decade Anatoki, Kaiteri and Kakariki that produce large fruit are becoming popular in New Zealand. Some of the popular cultivars presently available in New Zealand are shown below (Table 2.1). Soil, climate, orchard practices of propagation, fertilization and irrigation are detailed by researchers (Al-Harthy, 2010; Thorp & Bielecki, 2002).

Heterogeneity in fruit population is considered to be a major challenge for feijoa industry in New Zealand which is presently managed by using selection of cultivars having consistency in fruit shape and size (Schotsmans et al., 2011). High genetic variability was reported in the germplasm of feijoa (Nodari et al., 1996) and only recently a map of the genome was released by South American researchers (Quezada et al., 2014). There are major inconsistencies within the fruit populations (size, shape and internal quality) due to improper selection of trees even in well managed orchards (Patterson, 1990). Variation in fruit quality was also attributed to the existence of unevaluated cultivars used by some growers in New Zealand (Al-Harthy, 2010).

Table 2.1 Commercial feijoa cultivars common in New Zealand (Anon, 2014a; Thorp & Bielecki, 2002)

Cultivar	Season	Pollination	Fruit size	General remarks
Kakariki	Very early	Self-sterile	Very large	New variety, matures 4 weeks before Unique
Kaiteri	Very early	Self-sterile	Large	New variety, smooth, matures 2-3 weeks before Unique
Anatoki	Very early	Self-sterile	Good size	New variety, smooth fruit with sweet, mild flavour and matures 2-3 weeks before Unique
Robert	Very early	Self-sterile	Medium	Leaves russet, hollow fruit set
Sweethart	Very early	Self-sterile	Large	New variety with good taste
Unique	Very early	Self-fertile	Small to Medium	Rough skin, prolific producer with good storage life, thinning of fruit recommended.
Pounamu	Early	Self-sterile	Medium	Dark green, smooth, pleasant flavour, with moderate vigour and good storage life.
Gemini	Early	Self-sterile	Small to medium	Smooth, dark green fruit with good storage life.
Apollo	Early-mid season	Part self-fertile	Large	Sweet, long with light green and rough skin.
Dens Choice	Midseason	Self-sterile	Medium	Sweet, smooth, mild aroma and juicy
Marion	Midseason	Self-sterile	Large	Low fruit set with little pulp
Huia	Midseason	Self-sterile	Large	Heavy and regular, flowers early and matures at same time as Apollo.
Cultivar	Season	Pollination	Fruit size	General remarks
Kakapo	Midseason	Self-sterile	Medium to large	Mild, sweet, refreshing taste, blocky oval. Open tree habitat so easy for picking.
Wiki Tu (Anilvinko ru)	Mid to Late season	Partially self-fertile	Very large	Dwarf, meaty, good taste with smooth texture.
Opal Star	Midseason	Self-sterile	Medium to large	Smooth, dark green skinned, very aromatic. Heavy cropping and good storage life, requires fruit thinning.
Triumph	Late	Self-sterile	Large	Oval shape, firm fruit with rough skin, excellent flavour and good storage life.
White Goose	Late	Self-sterile	Medium to Large	Oval shape, thick dark skin, good flavour.

In this research early season feijoa variety 'Unique' was used. 'Unique' fruit are small to medium in size with a green to light green skin colour. As fruit set is high in this variety, thinning can improve size of the fruit. Fruit are ovoid with a split calyx on one side, flesh is smooth and juicy with 4-5 locules (Thorp & Bielecki, 2002).

2.2.4 Pollination and fruit set

Flowering extends for 4-6 weeks during November / December months in New Zealand (Thorp & Bielecki, 2002). Feijoa flower is bisexual but self-sterile due to incompatibility between pollen and ovary (Dettori & Di Gaetano, 1991). Feijoa flower do not produce nectar and hence are not visited by bees. However, pollination is ensured by birds that are attracted to juicy crimson coloured petals (Ducroquet & Hickel, 1997). Blackbirds and mynas are active pollinators of feijoa in New Zealand (Stewart, 1987). Fruit set is restricted to 30% through open pollination by birds (Thorp & Bielecki, 2002).

Pollination of flowers effects fruit set, shape and size has been well established in many fruits (Hopping & Hacking, 1983; Volz et al., 1996). Patterson (1990) found a correlation between the number of seeds and shape of the feijoa and suggested that fruit size is influenced indirectly because of the effects of pollination on seed numbers. Hand pollination resulted in improvement of fruit set and yields in feijoa orchards when compared to pollination by birds (Patterson, 1989). To ensure good pollination for fruit set, it is recommended to plant more than one cultivar in an orchard to overcome self-sterility (Stewart & Craig, 1989).

2.2.5 Fruit growth and ripening

The first visible signs of fruit growth in New Zealand occur during January (Thorp & Bielecki, 2002). Growth of feijoa follows a double sigmoid shape displaying a linear but rapid growth during 20 to 70 d after anthesis. From 70 to 90 d after anthesis there is a decline in growth rate which picks up after 90 d. Fruit size almost doubles during the final growth phase and from fruit set it takes 120-140 d to grow up to the point of dropping from the tree (Harman, 1987). Rodriguez et al. (2010) found that cellular tissues do not change from floral bud stage up to the full development stage of fruit suggesting that the size of feijoa is determined by the rate of multiplication and expansion of cells during the course of development.

Feijoa is harvested during autumn season from March to June in New Zealand with 2-3 weeks of picking window depending on cultivar. In contrast, feijoa is harvested year round in Colombia with peak production for 6-8 months every year (Fisher et al., 2003). An orchard of feijoa trees in New Zealand approximately yields 22 tons per ha (30-40 kg per tree) and only 30% were considered as commercial grade standard (Gibson, 1986). Some regions in South America produce up to 144 tons per ha in an year (Fisher et al., 2003). Increasing feijoa yields is possible by hand pollination or reducing crop loads by thinning of fruitlets in some varieties (Thorp & Bielecki, 2002).

Ripening was defined as “a composite process occurring at the latter stages of development of a fruit through to the early stages of senescence resulting in changes in composition, texture, colour or other sensory attributes” (Kader, 1999b). Feijoa ripening starts from the inside of the fruit and spreads towards the outer areas by emanating strong aroma from the fruit (Thorp &

Bieleski, 2002). A ripe feijoa is considered edible at the stage where the locular jelly is clear and the fruit is soft enough to be eaten by a spoon. An overripe fruit is recognised by browning of the flesh and loss of flavour (Thorp & Bieleski, 2002). Aroma of feijoa is mainly due to high concentration of volatile methyl benzoate (Shaw et al., 1990). At natural fruit drop 'Unique' and 'Apollo' are best consumed immediately while 'Triumph' and 'Gemini' varieties are best eaten after few days, however over-ripe fruit are reported in the above cultivars at the time of harvest (Thorp & Bieleski, 2002). Feijoa demonstrates climacteric rise in respiration and ethylene production during ripening (Biale et al., 1954). The climacteric rise in ethylene production was observed by Reid (1975) as mature feijoa reaches up to $470 \text{ pmol kg}^{-1} \text{ s}^{-1}$ 12 d after harvest (Fig. 2.2).

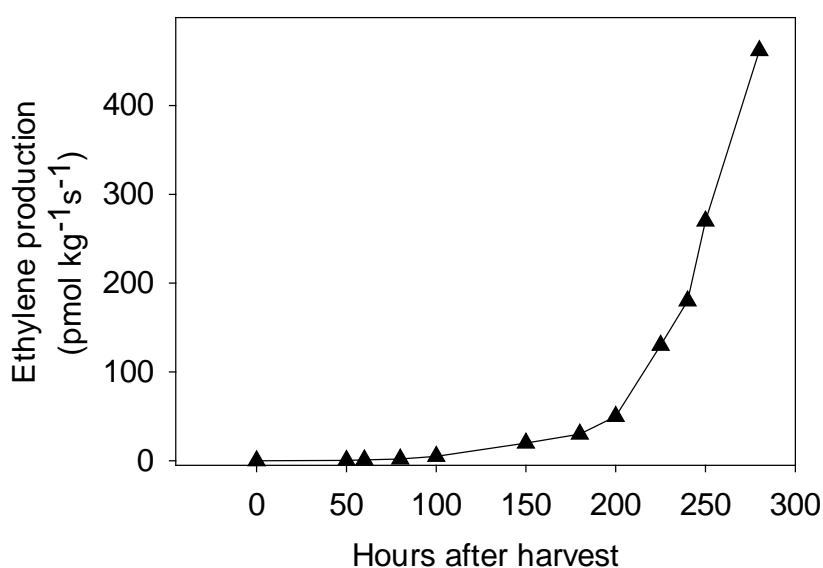


Fig. 2.2 Ethylene production rates of a mature feijoa after harvest (redrawn from Reid, 1975)

Harman (1987) reported low respiration rates in early harvested feijoa while it increased with later harvests. Field observations suggest differences

among cultivars on ripening rates can influence harvest timing (Thorp & Bielecki, 2002).

2.2.6 Fruit composition

Feijoa is rich in fibre and vitamin C (Belous et al., 2014); A detailed composition of feijoa fruit is provided by Romero-Rodriguez (1994) and Leterme et al. (2006). Unlike apple or banana, feijoa does not store high levels of starch (< 0.8% of dry weight) throughout its growth period (Thorp & Bielecki, 2002). Feijoa accumulates sugars (sucrose) along with an increase in organic acids during the final phase of growth and maturation (Harman, 1987). Accumulation of calcium and magnesium has been reported in feijoa until their natural fall from tree (Thorp & Bielecki, 2002).

Aroma of feijoas are generated by over 85 volatile compounds that develop during the later stages of development (Binder & Flath, 1989). Al-Harthi (2010) suggested that methyl benzoate, ethyl butyrate and ethyl benzoate concentrations increased during ripening of feijoa. Feijoa has been reported to have antioxidant and antibacterial benefits (Basile et al., 1997; Vuotto et al., 2000). Additionally phenolic compounds (Tuncel & Yilmaz, 2013) and bioflavonoids extracted from feijoa were found to benefit human immune system (Ielpo et al., 2000) and anti-cancer properties in fruit skin extracts were reported (Bontempo et al., 2007).

2.3 Feijoa maturity

2.3.1 Harvest maturity

Horticultural maturity is defined by Watada et al. (1984) as “the stage in development at which a plant part possesses the features that the consumer

wants for a particular purpose". Krammer and Twigg (1970) stated that harvesting of each and every fruit at the appropriate specific time being the "fleeting moment of perfection". Harvest maturity stage influences storage life and post-storage quality in fruit crops (Bulens et al., 2012; Reichel et al., 2010; Serrano et al., 2009; Singh & Singh, 2013). A feijoa internal visual grading scale developed by Plant & Food Research Institute, New Zealand (Anon, 2004) is a destructive measure that is based on locular development and change in flesh colour of fruit (Fig. 2.3). Maturity scale of rating 1 represents immature fruit, 2 and 3 as mature fruit for fresh consumption while 4 has darkened pulp area suitable for processing and scale above this is late senescence fruit (Schotsmans et al., 2011). Feijoa at touch-picking maturity (2-3 on maturity scale) are storable for up to 4 weeks at 4 °C with a subsequent 5 d at 20 °C. It is hypothesised that early harvested feijoa may possess more storage potential than those picked at commercial time.

Popenoe (1912) first reported that feijoa are best suitable for consumption when they are harvested just prior to falling from the tree. Later, Bailey (1952) reported that ripe feijoa that fall on the ground are picked up daily by growers. Harvesting of feijoa just prior to falling by pulling the fruit gently is mentioned by Endt (1978) and Fleming (1986) who reported 'touch-picking' of feijoa being used as a maturity standard by commercial growers in New Zealand. Touch picking stage corresponds with the natural maturity immediately prior to drop of feijoa from the tree and involves pulling the fruit gently from its stalk (Thorp & Klein, 1987). Feijoa picked using greater force are considered to be immature and remain unripe while feijoa left for longer time on tree will fall on the ground and bruised fruit are not attractive to customers (Thorp & Bielecki,

2002). However, Downs et al. (1988) found that feijoa picked at a substantially higher force than touch-picking had the same eating appeal after 4 weeks of storage.

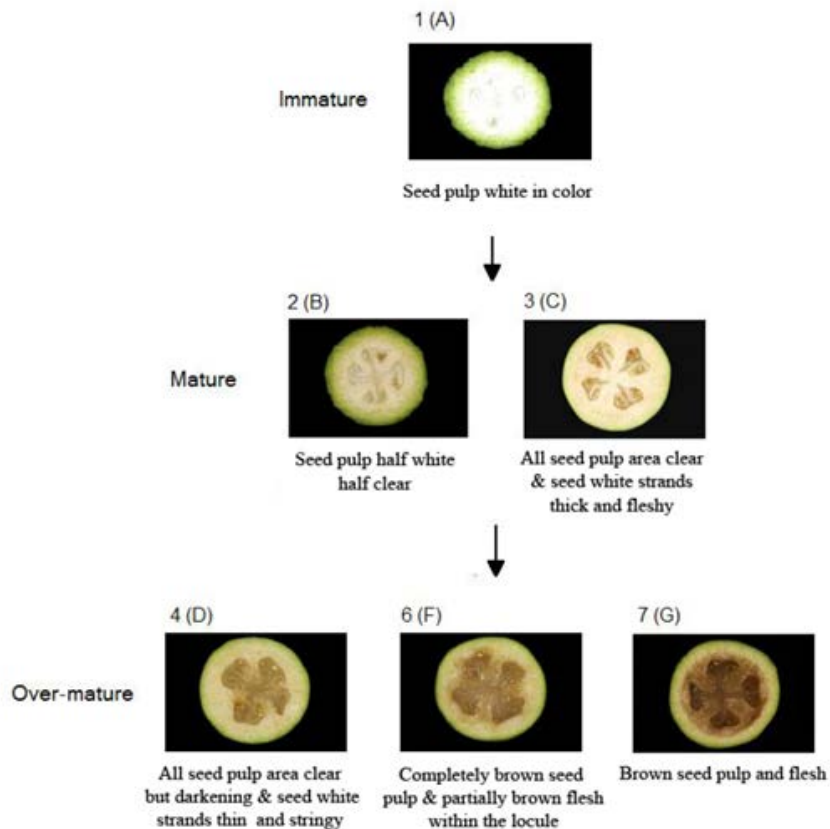


Fig. 2.3 Internal maturity rating scale developed by Plant & Food Research, New Zealand, 2004 (image used with permission)

An alternative to touch-picking method has been developed in the Poverty Bay region of New Zealand where feijoa are collected after shaking trees with suspended nets that protect fruit from bruising (Thorp, 1988). Feijoa harvested by nets may not be different in maturity status from those that are touch picked as both methods are performed at a time just before their natural drop.

2.3.2 Maturity index

Maturation is defined as a “stage of development leading to the attainment of horticultural or physiological maturity” (Watada et al., 1984). Maturity is defined as “ the stage of physiological development giving minimum acceptable quality to the ultimate consumer” (Reid, 2002). Harvesting fruit at optimal maturity determines its storage potential and final quality (Shewfelt, 2014). Maturity indices are developed specifically for each fresh product. The main objective of a maturity index is to enable uniform ripening and storage behaviour (Reid, 2002). Traditional maturity indices are determined by size, colour, density, weight, firmness, soluble solids or acid content (Ferrer et al., 2005). Maturity index should be an objective measurement rather than a subjective evaluation (Reid, 2002). A non-destructive measure of maturity is preferred for economic reasons by the industry (Butz et al., 2005). A list of maturity indices used for some popular fruit are summarised in Table 2.2. Colour of skin is the most commonly used indicator of maturity for a majority of fruit. Thorp & Bieleski (2002) reported that feijoa is mature for harvest just prior to their natural fruit drop. Maturity index of feijoa is reported as the time of abscission formation by Reid (2002) while Kader (Kader, 1999a) mentions ease of retention force and change in colour of skin from a dark green to light green colour as indices of maturity. In this work skin colour was used to segregate feijoa maturity at-harvest.

Table 2.2 Maturity indices of selected fruits summarised (Kader, 1999b) and (Anon, 2014b)

Fruit	Maturity indices
Apple	Starch pattern, SSC above 10.5 to 12.5 and firmness 18-23 lb force based on cultivars
Apricot	Skin colour from green to yellow (>1/2 yellow)
Avocado	19-24.2% dry weight depending on cultivar
Banana	Degree of fullness of finger, disappearance of angular cross section
Cantaloupe	Clear abscission from vine, some cultivars change in appearance of netting on fruit surface
Cherimoya	Change in skin colour from dark to yellowish green, appearance of cream colour between segments
Cherry	Skin surface red and SSC 14-16%
Durian	Fruit abscission and skin colour change from olive green to yellowish-green
Feijoa	Touch picking, ease of retention force just before abscission
Fig	Skin colour change depending on cultivar
Grape	SSC 14-17.5%, SSC/ TA is >20, minimum colour for some cultivars
Grape fruit	>2/3 of skin colour shows yellow colour, SSC/TA is 5.5-6
Guava	Skin colour changes from dark to light green
Jack fruit	Large size and colour change from green to yellow-brown
Kiwifruit	SSC >6.5%, minimum firmness of 14 lb force
Lemon & Lime	Juice content > 30% by volume
Longan	Skin becoming smooth and colour changes to yellowish brown, SSC 20%
Loquat	Skin colour change from green to yellow-orange
Lychee	Red coloured skin
Mandarin/ Tangerine	75% of fruit surface changes colour to yellow/orange or red, SSC/TA of >6.5
Mango	Fullness of cheeks, skin colour change in some cultivars
Mangosteen	Skin colour changes to red-purple
Nectarine & Peach	Skin colour changes from green to yellow
Orange	SSC/TA >8, or colour change to 25% yellow
Papaya	Skin colour change from dark to light green-yellow (25%)
Passion fruit	Skin colour change to yellow-purple depending on cultivar
Pear	SSC >13%, Skin colour yellowish green, firmness 23 lb force
Persimmon	Skin colour changes from green to orange
Pineapple	Skin colour changes from green to yellow
Pitaya	27-33 d after flowering, skin colour is intense red or yellow
Plum	Skin colour and flesh firmness depending on cultivar

Fruit	Maturity indices
Pomegranate	Red juice colour with TA <1.85%
Rambutan	Fruit colour becomes red, SSC >16%
Sapotes	Skin colour changes to dark brown, flesh colour from green to reddish brown
Starfruit	Skin colour changes from green to yellow
Strawberry	2/3 fruit surface becomes red or pink
Tamarillo	Skin colour becomes full red or yellow colour, SSC >10%
Watermelon	SSC >10% at the centre of melon, wilted vine tendril at stem end

2.3.3 Postharvest storage

New Zealand grown feijoa can be stored at 4 ± 1 °C and 90% relative humidity for up to 4 weeks with a further 5-7 d at 20 °C before losing their edibility (Al-Harthy et al., 2008; Klein & Thorp, 1987). Likewise Amarante et al. (2013) found storage temperatures of 4 ± 1 °C suitable for Brazilian feijoa while Parra-Coronado & Fischer (2013) recommended 4-10 °C for Colombian feijoa. During storage feijoa loses flavour and softens with a decline in soluble solids concentration and titratable acidity content. Flesh browning occurs rapidly with unacceptable flavours even after 3 weeks of storage (Thorp & Bielecki, 2002).

Postharvest treatments using hot water dips at 50 °C for 2.5 or 5 min before storage were not effective in extending storage life of feijoa (Klein & Thorp, 1987). Woolf et al. (2006) applied low temperature acclimatization of 3 cultivars of feijoa ('Unique', 'Opal Star' and 'Apollo') at 12, 9 or 6 °C for 3-6 days prior to storage, resulting in no effect and suggested that at-harvest maturity and cultivar differences may contribute to the complexity of postharvest life. Klein and Thorp (1987) applied calcium chloride (2-4% w/v CaCl_2) for 1-2 min at 25 °C and Ramírez et al. (2005) applied 5, 10 and 25% of CaCl_2 dips for 45 min at 18 °C with both reporting no effect on storage life extension of feijoa.

2.3.4 Chilling injury

Temperatures below 4 °C (at 0 °C) are considered to make feijoa susceptible to chilling injury which is characterised by browning of pericarp region and the stem end of the fruit (Thorp & Klein, 1987). Development of chilling injury may occur during exposure to low temperature while symptoms might appear after transfer to warmer temperatures (Cheng & Shewfelt, 1988). The complex problem of chilling injury was divided into two events where temperature dependent primary phenomenon occurs due to a fall in temperature for a period of time resulting in metabolic dysfunction. Later, secondary symptoms develop as a consequence of the primary event (Wills et al., 2007).

Incidence of chilling injury is not solely dependent on the storage temperature and length of storage. In particular, fruit maturity; heat treatments and delays prior to cooling; and the rate of cooling have all been attributed to influencing susceptibility to chilling injury. Chilling injury symptoms were found to be dependent upon a number of preharvest factors and harvest maturity in peach and nectarine (Lurie & Crisosto, 2005). Burdon et al. (2014b) found that prediction of chilling injury was not possible at the time of harvest using non-destructive flesh colour, firmness, SSC or dry matter estimations in 'Hort16A' kiwifruit, demonstrating that environmental conditions and orchard management practices have an influence on chilling injury development. Postharvest heat treatments using hot water, hot air and vapour heat were useful in controlling chilling injury in some fruit crops (Lurie, 1998). Development of chilling injury in tomato was found to be affected by temperature manipulations conducted before storage (Lurie & Sabehat, 1997). Usually rapid cooling is commonly used

commercially to remove field heat and hence enable storability of fruit. Step-down cooling of fresh produce has been adopted to reduce chilling injury in many horticultural products. Zhao et al. (2014) suggested that gradual cooling of kiwifruit allows fruit to acclimatise to low temperature storage.

There is limited literature available on effects of maturity, delayed cooling and rate of cooling on chilling sensitivity of feijoa. East et al. (2009) and Al-Harthy (2010) reported no chilling injury symptoms on 'Unique' feijoa after 10 weeks of storage at 4 °C. Two cultivars of Colombian feijoa were reported to be unaffected when stored at 1.67 °C (Valderrama et al., 2005). More work on determining factors that influence chilling injury development are required for feijoa.

2.3.5 Modified atmosphere conditions

Modifying the atmosphere around stored fresh produce in addition to temperature and RH control can reduce postharvest losses and preserve quality (Kader, 1993). Generally ethylene production and respiration rates of produce decline under low O₂ or high CO₂ atmospheres (Kader, 1995). Fresh fruit have varied tolerance levels to the concentration of O₂ and CO₂ in the atmosphere with potential consequence on their physiology being adversely affected (Kader et al., 1989). Responses of fruit to manipulation of atmospheric O₂ or CO₂ is dependent upon fruit maturity stage (Beaudry, 2010). A detailed range of O₂ and CO₂ concentrations at specific temperatures are provided for flowers (Reid, 1997), fruit and vegetables (Ben-Yehoshua et al., 2005; Kader, 2003; Kader et al., 1989), however finding optimal level of modified atmospheres can be limited by natural variability existing in horticultural production systems (Saltveit, 2003).

In feijoa low oxygen atmospheres (2.1-4.8 kPa O₂ + 0 kPa CO₂) was found to delay ripening during storage and at shelf life (Thorp & Bielecki, 2002). East et al. (2009) found that CA conditions of low O₂ (1.15-3 kPa) and low CO₂ (0-0.2 kPa) provided highest benefit in reducing weight loss and blemishes when compared to air storage. Al-Harthy et al. (2010a; 2010b) reported that 'Unique' and 'Opal Star' feijoa stored in CA (2-5 kPa O₂) were effective in retaining quality when compared to air stored fruit. Pesis et al. (1991) found that exposure of feijoa before storage to acetaldehyde or to anaerobic conditions of 98% N₂ for 24 h was effective to increase volatiles and maintain appearance of fruit after 13 d at 20 °C. Reduced oxygen atmospheres can also have negative responses like fermentation leading to off flavours in fruit and vegetables (Beaudry, 2000). In guava fruit reduced O₂ atmospheres lead to browning of skin (Singh & Pal, 2008b). Effects of reduced oxygen atmospheres on storage quality of feijoa are investigated to verify the appropriateness of this technology in devising a postharvest strategy for feijoa export.

2.4 Grading of feijoa

Monitoring produce enables decision making for conducting a harvest or at a later stage for grading in a packhouse (Abbott et al., 1997). Non-destructive methods are engaged by companies in commercial sorting lines to segregate the product based on pre-defined quality attributes (Chen & Sun, 1991). The critical role played by the sensors in the identifying of specific product properties have evolved as a dynamic research area over the last 50 years (Ruiz-Altisent et al., 2010). The present grading scale used by feijoa growers is a destructive measure and this research explores use of non-destructive methods to determine at-harvest maturity of feijoa.

Feijoa can be bruised easily, so extreme care is taken while handling the fruit at all stages right from picking up to their display on the retail shelf. Packhouses are vigilant while grading feijoa destined for export by air blasting fruit to remove mealy bugs, mites and other small insects (Thorp & Bielecki, 2002). Feijoa with no blemish are graded mechanically and placed in a single layered commercial corrugated fibreboard trays containing plix and some growers use polyliner (to prevent weight loss and shrivel) in New Zealand (Schotsmans et al., 2011). Large variation of maturity within fruit batches can be observed for most quality attributes in touch-picked feijoa (Al-Harthy, 2010).

To reduce variation in a fruit population, it is critical to sort fruit accurately by assessing maturity at-harvest (Tijssens et al., 2007). Segregating feijoa at-harvest using density was unsuccessful as Clark et al.(2005) found no meaningful relationships between density and physicochemical measurements dry matter, soluble solids or locular clearing index. Gaddam et al. (2005) found that feijoa sorted by non-destructive acoustic firmness had a similar dry matter, TA and SSC. Wiryawan (2005) suggested non-destructive firmness as a potential indicator of maturity for feijoa. Identification of maturity in feijoa has been a major challenge that hinders the expansion of industry in New Zealand (Schotsmans et al., 2011). Kader (1999a) suggests change in skin colour of feijoa from dark green to light green as an indicator of harvest maturity, however there are no publications to confirm. In this study, use of skin colour to discriminate maturity at the time of harvest was investigated. The outcome of this work will facilitate grading of fruit into homogenous groups based on storage potential and reduce variability caused by human errors of touch-picking feijoa. It becomes pertinent to develop a suitable non-destructive

maturity index for maintaining uniform quality after storage before exploring new export markets.

2.4.1 Non-destructive grading

Quality is viewed as the degree of excellence (Shewfelt, 1999). Distributors of fresh produce lean towards product-oriented-quality while consumer-oriented-quality arising from the needs of consumers are better suited for the distribution systems (Shewfelt, 2014). Fruit quality is largely based on visual appearance (size, shape, defects and damage) and physicochemical attributes like soluble solids content, titratable acidity and texture which are a function of at-harvest maturity (Shewfelt, 2014). Consumers may like a commodity based on the perceived nutritional content (vitamins, dietary fiber, minerals and antioxidants) of the product (Kader, 2008). Beaudry (1999) suggests that flavour, aroma and taste are the least discussed in postharvest quality.

2.4.1.1 Background colour

A number of non-destructive optical evaluation methods exist using wavelengths within the visible light range (400- 700 nm). Visual spectroscopy using skin colour ($^{\circ}$ hue, L^* , a^* , b^* and c^*) was useful in determining maturity of pomegranate (Manera et al., 2013; Schwartz et al., 2009), mango (Jha et al., 2007) and guava (Mercado-Silva et al., 1998). Pinheiro et al. (2013) reported use of colour parameter $^{\circ}$ hue as a standard index of tomato quality during storage. Feijoa is green in colour without any visually obvious changes during ripening and harvesting fruit is relied on discretion of individual pickers while touch-picking contributes to variation within fruit batches (Al-Harthy, 2010;

Thorp & Bielecki, 2002). It is hypothesised that non-destructive skin °hue can be useful in segregating maturity of feijoa at the time of harvest.

Reflectance spectrum at wavelengths in the regions of 425-705 nm were useful to estimate content of chlorophyll, carotenoid and anthocyanin in apple skin (Merzlyak et al., 2003). Maturity index in dragon fruit was generated using light reflectance (Wanitchang et al., 2010). In this work reflectance spectra of feijoa skin will be explored in understanding maturity at-harvest.

2.4.1.2 Firmness assessment

Cell wall metabolism and water status (turgor pressure) play a vital role in determining firmness of fruit (Baritelle et al., 2001). Firmness at the time of harvest has an inverse relationship with maturity stage in many fresh products and hence used as an indicator of maturity during sorting and grading (Valero et al., 2007). Non-destructive firmness of fruit is measured using force deformation, Impact analysis of force, rebound technique, acoustic response to vibrations and even nuclear resonance imaging (García-Ramos et al., 2005). De Ketelaere et al. (2006) suggested that sorting operations of fresh produce should be executed immediately after harvest.

Rebound technique has been used with some success to quantify firmness of fresh product after an impact with surface (Gan-Mor & Galili, 2000). Ragni et al. (2010) developed an impact measuring device for prediction of firmness on-line in a kiwifruit pack house. Lien and Ting (2014) in guava predicted maturity by using an automated sorting machine by analysing the impact response of dropped fruit. Gutierrez et al. (2007) reported 80% repeatable accuracy for sorting peaches through firmness assessments.

Measurement of force necessary for causing a pre-set deformation using digital firmness meter was suitable to grade mango and tomato fruit (Macnish et al., 1997). Compression firmness is sensitive to influences by cell wall material and water status (turgor) of fruit tissue. Compression firmness represents a combination of fruit properties like strength and toughness of skin, pericarp tissue, turgor pressure, contents of locular gel and size of the fruit (Paniagua et al., 2014). Al-Harthy (2010) used a non-destructive compression firmness analyser and found it more suitable than Sinclair firmness tester for feijoa. Wiryawan et al. (2005) suggests potential use of compression firmness as an indicator of feijoa maturity at-harvest.

Gaddam et al. (2005) used acoustic firmness to sort feijoa but found it unsuitable to differentiate maturity at-harvest. This report suggests that feijoa differentiated by firmness had similar quality attributes of dry matter, SSC and TA. Al-Harthy (2010) found that non-destructive compression firmness using a textural analyser was more reliable than acoustic firmness in determining feijoa maturity. In this study textural analyser was limited to determining firmness of feijoa at-harvest and after storage.

2.4.1.3 Other grading technologies

NIR spectroscopy is used to assess variation in absorption and scattering of radiation in the region of wave lengths 760-2500 nm. Changes in spectrum depend on the content of water, chlorophyll, carotenoids, sugars and anthocyanin in fruit tissues. NIR instruments have been used to measure at-harvest and storage quality in apple (McGlone et al., 2002), kiwifruit (Burdon et

al., 2014a), oranges (Wang et al., 2014), citrus (Lu et al., 2007) and nectarines (Giné Bordonaba et al., 2014).

Time and space resolved reflectance spectroscopy (TRS/SRS) where detectors calculate the distribution of time-of-flight of photons from a fixed distance of a detector (Torricelli et al., 2008). Absorption properties are related to chemical composition of tissues while microstructural features of cell size, shape and intercellular spaces related to scattering properties (Nicolai et al., 2014). TRS technique has been useful in determining maturity at harvest in nectarines (Eccher Zerbini et al., 2009), peaches (Shinya et al., 2013) and apple (Vanoli et al., 2014). Changes on skin colour of fruit are monitored rapidly to aid in determination of harvest maturity. On tree monitoring of chlorophyll content on fruit skin is non-destructively performed using a DA-meter (Infante et al., 2011). Absorbance spectrum at 670 nm is subtracted from 720 nm to calculate interactance spectrum (I_{AD}). Apple industry monitors optimal harvest maturity ranges using a DA-meter (DeLong et al., 2014).

A pigment analysing spectrophotometer is used to capture reflectance measurements in the region of red (670 nm) and at near infrared region (780 nm). A normalised difference vegetation index (NDVI) is then calculated using reflectance measurements $NDVI = (R_{780nm} - R_{670nm}) / (R_{780nm} + R_{670nm})$ which correlated with chlorophyll content (Kuckenberg et al., 2008). Likewise normalised anthocyanin index (NAI) = $(R_{780nm} - R_{570nm}) / (R_{780nm} + R_{570nm})$ is used to estimate lycopene content in tomato (Schouten et al., 2014).

There are advances in spectroscopy to monitor biospeckle and backscattering activity that evaluate particle movements at cellular level

(Kurenda et al., 2014). Remittance spectra (re emission) is captured by a device in ranges 400-1100 nm wavelengths. In apple biospeckle activity decreased linearly with higher chlorophyll content (Zdunek & Herppich, 2012).

Machine vision using digital cameras was useful in prediction of lycopene in tomato (Arias et al., 2000). Hyper-spectral and multispectral imaging using blocks of data in 3D were found useful in determining quality of fresh produce (Sun, 2010). Chlorophyll fluorescence imaging uses high resolution microscopes and field level remote sensing is used to determine photosynthetic activity of horticultural products (Gorbe & Calatayud, 2012).

Magnetic resonance imaging (MRI) was found useful in measuring fruit maturation and ripening in tomato (Musse et al., 2009) and kiwifruit (Taglienti et al., 2009). Al-Harthy (2010) found that time of spin-spin relaxation (T_2) and half-height peak width (ΔH_2) of pulp and flesh reasonably correlated with compression firmness of feijoa. This report suggests that MRI technology has potential to indicate maturity stage of feijoa, however measuring individual feijoa took long time (30 minutes) for getting a stable MRI signal which is impractical in a commercial scenario. Hence MRI investigation was not undertaken in this study.

X-ray visualises internal structures of a fruit noninvasively and used in detection of pest infestation in horticultural products (Lammertyn et al., 2003). Maturity of green tomato was determined using X-ray CT (Brecht et al., 1991). Barcelon et al. (1999a) used X-ray CT to determine maturity of mango and found that CT number was related with moisture content, density and titratable acidity. Herremans et al. (2014) used X-ray CT to identify water-core disorder in

apple. In this study feijoa will be examined using X-ray 3D to understand internal structure of the fruit.

Advanced gas and mass spectrometry techniques are useful in determining volatiles for a variety of fruit (Cappellin et al., 2012). Electron nose (e-nose) is a complex aroma perception device simulating human olfactory system to determine volatile aroma of many fruit. E-nose was useful in determining the optimal harvest date in maturing apples (Saevels et al., 2003) and monitoring storage life of tomato (Gómez et al., 2008). Non-destructive techniques for determining quality of fruit and vegetables are provided by Shewfelt (2014) , Abbott et al. (2010) and Nicolaï et al. (2014).

2.5 Ethylene

The biological activity of gaseous hormone ethylene was first discovered by Neljubov (1901) with Denny (1924) later discovering that ethylene influenced the colour change of lemons. Ethylene is involved during growth and development of higher plants by invoking diverse events including the germination of seeds, abscission of leaves and fruit, ripening and senescence (Abeles et al., 1992). Changes related with fruit colour, firmness, volatiles, starch, sugars and organic acids during ripening are influenced in some way or the other by ethylene (Barry & Giovannoni, 2007; Cherian et al., 2014; Pech et al., 2012).

The biochemical synthesis of ethylene was describe by Yang and Hoffman (1984). Methionine is converted into S-adenosylmethionine (SAM) by the enzyme SAM synthetase (Fig. 2.4). The conversion of SAM into 1-aminocyclopropane-1carboxylic acid (ACC) and 5'-methylthioadenosine (MTA)

is mediated by the enzyme ACC synthase (ACS) which is considered as the rate limiting step in the biosynthetic pathway (Kende, 1993). ACC is oxidised in the presence of ACC oxidase to produce ethylene, CO₂ and hydrogen cyanide (HCN). MTA is recycled back to produce methionine through the Yang cycle. The poisonous HCN gas is detoxified by the enzyme β -cyanoalanine synthase (Van de Poel et al., 2012).

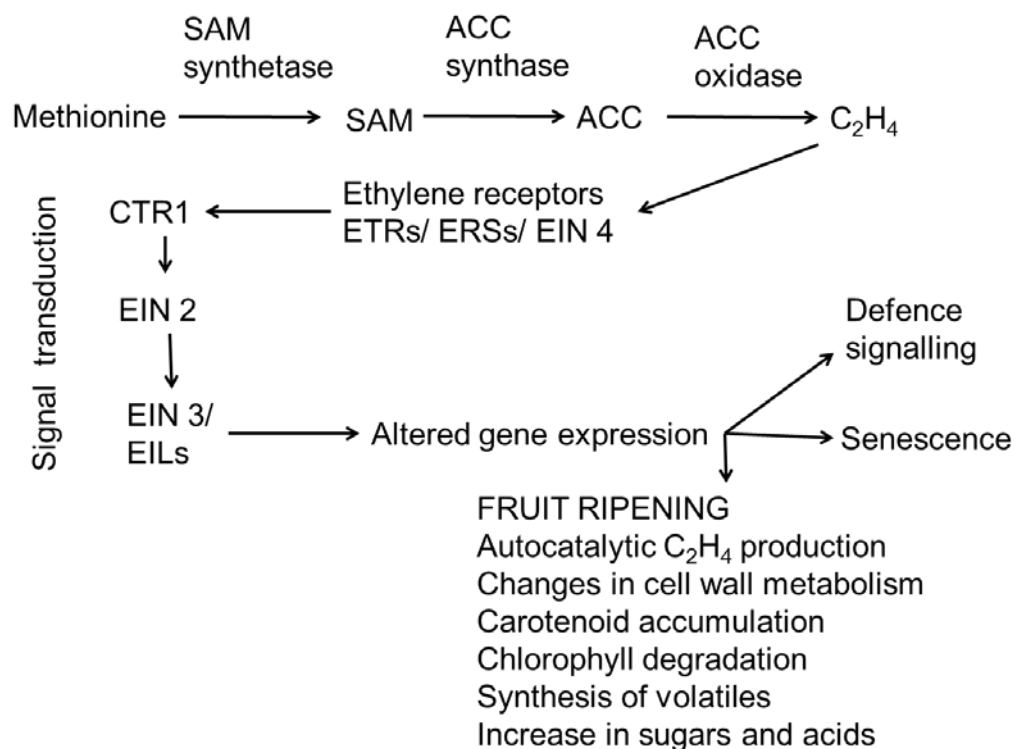


Fig. 2.4 Schematic diagram of ethylene biosynthesis and signalling pathway (Alexander & Grierson, 2002; Binder et al., 2012)

Fruit are classified broadly into two groups as climacteric and non-climacteric. 'Climacteric' was termed by Kidd & West (1930's) for a transition stage in development just prior to senescence marked by a sudden rise in respiration without any external influence (Biale, 1950). Non-climacteric ripening is considered to be without rise in physiology even when ethylene appears to

influences some ripening events like pigmentation change in orange (Alonso et al., 1995).

McMurchie et al. (1972) proposed that there exists a common system of ethylene production in all fruit referred to as 'system 1' while an additionally 'system 2' is present in climacteric fruit where rapid increase in production rates occur during ripening. Regulation of ethylene is auto-inhibitory during system 1 in immature climacteric fruit as tissue in perception of ethylene inhibits further synthesis of ethylene (Barry & Giovannoni, 2007; Vendrell & McGlasson, 1971). However, when mature climacteric fruit are exposed to external ethylene or propylene a system II response is observed with a dramatic rise in auto catalytic production of ethylene (McMurchie et al., 1972). After the fruit attains a maximum of ethylene production then there is a termination of system II ethylene production which delays senescence as observed in tomato ripening (Van de Poel et al., 2012). However Barry and Giovannoni (2007) speculated that ethylene played a regulatory role during ripening of fruit while there may be complex interactions with other developmental factor during ripening and senescence. Velho et al. (2008) and Al-Harthy (2010) reported no response by feijoa when exposed to exogenous ethylene treatments. Only Akerman et al. (1993) found rise in ethylene and respiration rates when feijoa were exposed to ethylene.

In addition to the acceleration of ethylene during the climacteric, ethylene production can also be dramatically induced due to stress. Stress induced ethylene production is caused by both abiotic and biotic factors like light, wounding, insects and pests, pathogens, temperature, radiation, drought, chemicals, carbon dioxide, ozone, nitric oxide and other pollutants (Minas et al.,

2014; Singh et al., 2009; Yang & Hoffman, 1984). Metabolites like polyamines (spermine, spermidine and putrescine) (Mondal et al., 2008), salicylic acid (Hong et al., 2014) and sugars (Jia et al., 2013; Yanagisawa et al., 2003) also influence ethylene pathway during fruit ripening. Other plant hormones like indole-3-acetic acid (Cohen, 1996), jasmonic acid (Zapata et al., 2014), abscisic acid (Hong et al., 2002), cytokinins (Kushwah et al., 2011) and brassinosteroid (Symons et al., 2012) also influence ethylene synthesis during ripening.

Ripening of fruit is genetically programmed with complex process involving changes in texture, colour, aroma and flavour (Alexander & Grierson, 2002). *Arabidopsis* and tomato fruit have been studied extensively to understand the role played by ACS and ACO enzymes in the synthesis of ethylene (Pech et al., 2012). Nine ACS genes (*ACS1 A*, *ACS1 B*, and *ACS2-ACS8*) and six ACO genes (*ACO1-6*) were identified in tomato during ripening (Lin et al., 2009). Nakatsuka et al. (1998) suggested that during system 1 pre-climacteric stage of tomato, ethylene production is mediated by low levels of *ACS1 A* and *ACS3* while high levels of *ACS6* controlled negative feedback mechanism. Barry et al. (2000) proposed a model where at the transition phase from system I to system II expression by *ACS4* increases which in turn activates *ACS2* that initiates autocatalytic ethylene production. Argueso et al. (2007) demonstrated that in addition to the expression of ACS genes there is a complex mechanism of stability of ACS proteins controlling ethylene production. ACS activity is found to be effected by other plant hormones like auxins (Yu & Yang, 1979).

ACO genes are expressed differently during ripening of tomato as Barry et al. (1996) found *ACO1* and *ACO3* active during senescence of fruit while

ACO1 was expressed during wound induction. In some crops ACO genes were found to be effected by other plant hormones like auxins (Chae et al., 2003) and gibberellic acid (Calvo et al., 2004). There is no published work on feijoa, however we assume that the mechanism of ripening will not be different from other climacteric fruit.

2.5.1 Ethylene perception

Isolation of *Arabidopsis thaliana* mutants that either do not respond to exogenous ethylene (ethylene insensitive *EIN*) and ACC insensitive (*AIN*) or those that show triple response and are ethylene overproducing (*ETO*) were useful in revealing the ethylene sensing mechanisms (Zarembinski & Theologis, 1994). Tomato phenotypes that exhibited no system 2 ethylene, like non-ripening (*nor*), colourless non-ripening (*cnr*), ripening-inhibitor (*rin*) were useful to understand ethylene signalling pathway in climacteric fruit (Barry & Giovannoni, 2007).

Detailed reviews on ethylene receptors are described by Binder et al. (2012) and Merchante et al. (2013). The ethylene molecule binds to ethylene receptors (ETRs) bound on membranes of endoplasmic reticulum and this perception generates a response (Chen et al., 2002). There are 2 subfamilies within a family of 5 receptors namely: subfamily I (ethylene receptor 1 [ETR1], ethylene response sensor 1 [ERS1]) and subfamily II (ETR2, ERS2 and ethylene insensitive [EIN4]) encoding these receptors in *Arabidopsis* (Hall et al., 2000). Tomato has seven ethylene receptors (*LeETR1-7*) with similar binding capacities to ethylene (O'Malley et al., 2005). Transition metal copper (Cu) present in the binding sites of receptors have high affinity to ethylene (Hirayama

et al., 1999) and this changes the chemistry of copper which then is responsible for transmitting a signal to downstream elements.

Shutting down (or loss) of the ethylene receptors results in increase of ethylene response and sensitivity while conversely an increase in the expression of receptors shows decrease in sensitivity (Tieman et al., 2000). Most of the studies focussed on documenting receptor level transcripts based on responses to stimulus (Binder, 2008). However Kevany et al. (2007) found that ethylene receptor protein levels decreased during ripening of tomato even when there was an increase in the transcripts. Hence ethylene receptor turnover is now considered to be controlling ethylene sensitivity.

Details on molecular mechanisms and signalling are described in Gapper et al. (2013), (Merchante et al., 2013) and Xu and Zang (2014). Binder and Bleeker (2003) suggested an inverse antagonistic model to explain the mechanism of ethylene binding on receptors leading to make them inactive. This model is based on the function of the ethylene receptors which act as negative regulators after perception. When there is no ethylene bound to the receptor then the receptors are in an active stage. When ethylene molecule binds to the receptor, then there is an intermediate stage of the receptor during its change from an active stage (on) to an inactive stage (off). This intermediary stage provides explanation of why receptors can be in an active stage even in the presence of saturating concentrations of ethylene (Wang et al., 2006). Inactivation of multiple receptors results in the formation of a negative response signal termed as constitutive triple response (CTR1), a protein complex associated with shutting down of the ethylene signal pathway (Huang et al., 2003). Downstream ethylene signal from CTR1 is passed on to EIN2 located on

the endoplasmic reticulum (Fig. 2.4). Activated EIN2 then moves to the nuclear membrane and passes on the signal to EIN3 or EIN3-like (EILs) proteins located in the nucleus that act as positive regulator of the signal (Alonso et al., 1999; Gapper et al., 2013).

Recent findings suggest that ethylene-independent mechanisms are also involved in the transmission of the signal in the downstream of receptors (Osorio et al., 2012). A cascade of ethylene signals are triggered through ethylene response factors (ERFs) which regulate complex genetic and molecular signals (Fig. 2.4). A wide range of mechanisms related to ripening, senescence and defence systems have been recorded (Alexander & Grierson, 2002; Cherian et al., 2014; Xu & Zhang, 2014).

2.5.2 Inhibitors of ethylene

Controlling the start and rate of ripening in fruit has been a major goal of researchers as this would enable extension of storage life and reduce spoilage (Bleecker & Kende, 2000). Abeles et al. (1992) reports many inhibitors of ethylene that are not popular in the present day. Chemical compounds like Rhizobitoxine, silver nitrate (AgNO_3), silver thiosulphate (STS), n-propyl gallate, sodium benzoate, ethylene oxide, Cobalt chloride (CoCl_2), 2,4-dinitrophenol (DNP), polyamines, aminooxyacetic acid (AOA) and aminoethoxyvinylglycine (AVG) were found to inhibit ethylene production. Inorganic ions like Cobalt (Co^{++}) and Nickel (Ni^{++}) effectively inhibit ethylene synthesis (Lau & Yang, 1976). Sisler and Serek (1999) demonstrated blocking of ethylene action at the receptor level by 2,5-norbornadiene (NBD), 3,3-dimethylcyclopropene (DMCP), diazocyclopentadiene (DACP) and multiple-cyclopropenes like 1-methylcyclopropene (1-MCP). Ethylene action is also blocked by transition

metals like copper (Cu), silver (Ag) and gold (Au) that bind to the receptors of ethylene (Binder et al., 2007). In this study response of feijoa to the application of postharvest 1-MCP and preharvest AVG to extend storage life was investigated.

2.5.3 1-Methylcyclopropene (1-MCP)

Cyclic olefin (C_4H_6) 1-methylcyclopropene is a non-toxic gaseous compound at room temperature that is an effective inhibitor of ethylene action (Blankenship & Dole, 2003). Sisler and Blankenship (1996) patented 1-MCP which is commercially marketed as EthylBloc™ for floral application, SmartFresh™ for postharvest use in edible horticultural crops and as Harvista™ for preharvest application (AgroFresh Inc., USA). There is tremendous impact of 1-MCP use as a postharvest technology tool in not only preventing quality losses of fruit and vegetables but also to understand physiological processes during ripening. Extensive reviews of use of 1-MCP on horticultural products are available (Blankenship & Dole, 2003; Reid & Celikel, 2008; Schotsmans et al., 2009; Watkins, 2006).

A high affinity of 1-MCP to ethylene receptors (greater than 10 times to that of ethylene) has been reported (Sisler & Serek, 2003). 1-MCP treatment declines expression of ACS and ACO enzymes by reducing their activity in many fruit (Dal Cin et al., 2006). 1-MCP treatment has no effect on system I ethylene production but blocks autocatalytic synthesis in system II (Pathak et al., 2003). The consequence of blocking ethylene response at the receptor level leads to inhibition of ethylene production. Kevany et al. (2007) found that receptor levels were high before the onset of ripening in tomato but declined

during ripening, 1-MCP keeps the receptors in an active stage during ripening and prevents ethylene induced degradation of receptors.

Efficacy of 1-MCP depends upon multiple factors like cultivar, time of application from harvest, maturity stage in development, endogenous ethylene concentrations (Jung & Watkins, 2014). A maximum treatment concentration of $1\mu\text{L L}^{-1}$ was established for postharvest application of 1-MCP in USA while it is effective even at very low concentration of 2.5 nL L^{-1} (Watkins, 2006). Optimal concentration of 1-MCP specific to fruit species have been reported (Blankenship & Dole, 2003; Watkins, 2006). Treatment concentration interacts with time as lower concentration of 1-MCP require longer times of application with similar results obtained by higher concentration for lesser duration (Blankenship & Dole, 2003). Temperature also has a direct effect on efficacy of 1-MCP with lower temperatures requiring higher duration of treatment. Blankenship & Dole (2003) suggested that 1-MCP binding is incomplete at low temperatures or tissues might be regenerating new receptors due to cold stress. Rapid application of 1-MCP immediately after harvest enhanced storage life in apple when compared to delayed application (Watkins & Nock, 2012). 1-MCP is differently absorbed by fresh produce and this variation in rate of sorption was found to depend on the physical size, fresh weight and dry matter content of the commodity (Nanthachai et al., 2007). In this research feijoa was exposed to SmartFresh™ (0.14%) water soluble powder formulation of 1-MCP application at $1\mu\text{L L}^{-1}$ concentration for up to 12 h at 20 °C.

In some crops, the effect of 1-MCP has been transient due to synthesis of new binding sites (Cameron & Reid, 2001). Recovery of tomato ripening after application of 1-MCP was associated with increased expression of ethylene

receptors ETR1 and ERS1 (Tassoni et al., 2006). Fruit tissue can recover their sensitivity to ethylene. Multiple exposures with 1-MCP delays ripening when compared to single dose as observed in apple (Lu et al., 2013; Nock & Watkins, 2013) and pear (Ekman et al., 2004).

1-MCP was found to enhance ethylene production in parsley as the tissue was in auto-inhibition stage (Ella et al., 2003). In persimmon fruit where biphasic ethylene production is reported, 1-MCP had no effect on the first increase as it is not ethylene-driven and considered to be the result of water stress, however the second phase of autocatalytic ethylene rise was inhibited by 1-MCP (Nakano et al., 2003). Feijoa demonstrates climacteric behaviour with a postharvest rise in ethylene production (Reid, 1975) and we expect that 1-MCP will inhibit ethylene response and delay ripening.

2.5.3.1 Effects of 1-MCP on ripening

Ripening was delayed in 'Brazil (242)' feijoa when 1-MCP was applied at 500 or 1500 nL L⁻¹ for 8 h by retaining firmness and losses in skin colour (°hue) after 30 d of storage at 4 °C (Amarante et al., 2008). Contrastingly Velho (2008) reported no effect of 1-MCP applied at 1000 nL L⁻¹ for 24 h on ethylene production or respiration of Brazilian feijoa. An unpublished report by White and Woolf suggested no influence of 1-MCP on 'Apollo' feijoa (Schotsmans et al., 2011). A wide range of effects have been recorded using 1-MCP that are specific to fruit species and cultivar are described by Huber (2008), Watkins (2006; 2008), and Schotsmans et al. (2009).

Application of postharvest 1-MCP delayed ripening by inhibiting ethylene responses in many climacteric fruits like apple (Jung & Watkins, 2014; Yang et

al., 2013); tomato (Mir et al., 2004); avocado (HersHKovitz et al., 2005); pear (Chiriboga et al., 2013); peaches and nectarines (Liguori et al., 2004); banana (Golding et al., 1998); guava (Singh & Pal, 2008a); apricots and plums (Dong et al., 2002). A major effect of 1-MCP is in retaining firmness (Watkins, 2006) by retarding the activity of cell wall degrading enzymes (Botondi et al., 2003). Volatile production is lowered by 1-MCP treatment in apple (Kondo et al., 2005) and guava (Harb & Hasan, 2012). The metabolism of starch degradation, SSC, TA is differentially affected by 1-MCP specific to species (Blankenship & Dole, 2003; Watkins, 2006). However, Sigal-Escalada (2006) suggested that SSC and TA may be independent of ethylene action during apple ripening. Chlorophyll degradation is delayed with reduced expression of gene chlorophyllase (Gong & Mattheis, 2003). There are reports of 1-MCP delaying discolouration of flesh by reducing the activity of polyphenol oxidase (PPO) and peroxidase (POD) (HersHKovitz et al., 2005). Carotenoid synthesis was delayed during tomato ripening (Moretti et al., 2005) and at high concentrations 1-MCP completely inhibited lycopene and carotenoid accumulation (Opiyo & Ying, 2005). 1-MCP treatments can delayed anthocyanin accumulation in some fruits like grape and strawberry while having no effect in pear and sweet cherries (Schotsmans et al., 2009). Ripening of feijoa is characterised by softening and browning of flesh along with a decline in SSC and TA (Klein & Thorp, 1987) and postharvest treatment with 1-MCP may delay ripening.

Enzymatic browning was reduced by 1-MCP treatment by delaying ascorbic acid loss in pineapple (Budu & Joyce, 2003; Selvarajah et al., 2001). 1-MCP was found to lower losses of vitamin C in pineapple (Selvarajah et al., 2001), peaches (Liu et al., 2005) and guava (Singh & Pal, 2008a); anti-oxidant

properties were retained in apple (MacLean et al., 2003). Aroma volatiles were reduced by 1-MCP in apple (Kondo et al., 2005), avocado (Pereira et al., 2013), plums (Abdi et al., 1998) and peach (Ortiz et al., 2010). Lowering of alcohols and rise in esters contribute to aroma changes during ripening of apple can be influenced by 1-MCP treatment (Da-Peng et al., 2006).

When a disorder occurs during senescence then 1-MCP was found to be beneficial through inhibition of ethylene action as seen in reduction of core browning in 'Redcor Cortland' apples (DeLong et al., 2004). Singh and Pal (2008a) found that 1-MCP reduced symptoms of chilling injury and decay during storage of guava. Guillén et al. (2005) found that in some cultivars of tomato 1-MCP had beneficial effect in preventing decay as a result of enhanced activity of PAL, PPO and POD enzymes. Physiological disorders like superficial scald in apple is reduced by 1-MCP (Watkins et al., 2000) by influencing accumulation of α -farnesene and conjugated trienols (Lu et al., 2013). Chilling injury related browning in avocado declined after 1-MCP treatment (HersHKovitz et al., 2005) and Woolf et al., (2005) found that chilling injury symptoms on skin were not reduced by 1-MCP but internal symptoms in flesh discolouration were reduced in avocado.

Undesirable effects of 1-MCP were reported in some fruits like induction of severe cold storage disorders in nectarines (Dong et al., 2001). Porat et al. (1999) found oranges more susceptible to chilling injury after 1-MCP treatment. Fruit susceptibility to fungal rots increased by 1-MCP treatments in tomato (Díaz et al., 2002) and avocado (Adkins et al., 2005).

In this research further examination of postharvest 1-MCP application effects on ripening of feijoa are studied. 1-MCP treatment gives us the opportunity to understand the physiology of ripening in feijoa. As 1-MCP efficacy depends upon maturity stage of fruit, it will be interesting to study effects of 1-MCP on feijoa harvested at different maturities. There are no research publications on effects of 1-MCP on New Zealand grown feijoa.

2.5.3.2 Combining postharvest 1-MCP and CA

Combining postharvest treatments can have a synergistic effect on quality and extend storage life of fresh produce. Combining 1-MCP with CA has been found to be effective in maintaining quality during storage of apple (Cocci et al., 2014; Watkins & Nock, 2012); litchi (Sivakumar & Korsten, 2010); plums (Singh & Singh, 2012) and persimmon (Besada et al., 2014). When applying standalone controlled atmosphere storage to feijoa, Al-Harthy et al. (2010a; 2010b) found that 'Unique' feijoa were over ripe while 'Opal Star' developed external discolouration after 6 weeks of CA storage. In this research pre-storage 1-MCP in combination with reduced oxygen atmospheres is tested as a method to extend the storage life of feijoa.

2.5.4 Aminoethoxyvinylglycine (AVG)

Plant growth regulator aminoethoxyvinylglycine (AVG) is commercially available as ReTain™ (Valent BioSciences Corp., USA) in a w/w 15% formulation used as a preharvest spray for inhibiting ethylene production in fruit crops. AVG interferes with the synthesis of ethylene through inactivation of ACS enzyme in the biosynthetic pathway (Yu & Yang, 1979). AVG inhibits ethylene production rates many fruit crops like apple, nectarines, pears and peaches

(Cetinbas et al., 2012; D'Aquino et al., 2010; Halder-Doll & Bangerth, 1987; McGlasson et al., 2005).

Recommended application of Retain™ is at 125 mg L⁻¹ for apple and stone fruit having a withholding time of 7 d after application (Jobling et al., 2003; Phan-Thien et al., 2004). Schupp and Greene (2004) and Cline (Cline, 2006) found that higher concentration of AVG increased responses in apple and peach respectively. Effects of AVG can vary relative to the time of application in apple as 2-4 weeks before harvest was considered optimal (Schupp & Greene, 2004). In pear AVG is applied 1-2 weeks before harvest (Clayton et al., 2000). Efficacy of AVG depends upon environmental conditions in a location as observed in differences in fruit drop control in apple orchards located at Chaplain and Hudson regions (Stover et al., 2003). In this work feijoa were treated with AVG sprays at 4 or 2 weeks before the anticipated commercial harvest time.

Uptake of AVG is possible through fruit cuticle or through leaf surfaces and very low through bark. Increase in temperature and humidity increased penetration of AVG (Greene & Krupa, 2000). Organosilicone surfactants and hydroscopic salts like CaCl₂ lower surface tension enabling better penetration of AVG through stomata and lenticels located on surface of leaf and fruit (Knoche & Petracek, 2013).

2.5.4.1 Effects of AVG on yields

Ethylene was found to be responsible for fruit development process during maturation and ripening (Lelièvre et al., 1997). Ethylene is a promoter of abscission zone during fruit development (González-Carranza et al., 1998).

Preharvest application of AVG reduced abscission zone development in fruit resulting in over 50% reduction of fruit drop in apple (Greene, 2006). Likewise AVG reduced fruit drop in pear (Sánchez et al., 2011) and nectarine (Torrigiani et al., 2004). Walsh (1977) and Greene et al. (2014) reported that fruit abscission was associated with higher internal ethylene concentration in dropped apple.

AVG treatment increased seed number with reduced parthenocarpy in apple (Williams, 1980). Fruit set and fruit yields (numbers) were higher in AVG treated (preharvest) nectarines (Rath & Prentice, 2004) and pears (Sánchez et al., 2011). McGlasson et al. (2005) and Cetinbas and Butar (2013) found that AVG treatment increased fruit weight in nectarines and sweet cherry respectively. AVG had no effect on weight even when fruit set was higher in 'Williams' pear (Lafer, 2008). Greene (2014) reported no relationship between fruit drop and their weight in apple. Fruit drop in feijoa indicates harvest maturity for start of touch-picking in orchards of New Zealand (Thorp & Bieleski, 2002); in this work effect of preharvest AVG on fruit drop and yields is being investigated.

2.5.4.2 AVG effects on maturity and storage

AVG application resulted in maturity delay at harvest and extended storage life in some fruit (Table 2.3). Preharvest AVG application delayed maturity at the time of harvest by retaining higher firmness in apple (Yildiz et al., 2012), pear (Clayton et al., 2000), peach (Cline, 2006) and plums (Jobling et al., 2003). Starch degradation and colour development was delayed by AVG in apple (Byers, 1997a; Phan-Thien et al., 2004) and pear (Clayton et al., 2000).

AVG treated plums with delayed harvest possessed increasing SSC when compared to untreated fruit (Jobling et al., 2003).

During storage AVG retained firmness and lowered ethylene production in apple (Drake et al., 2006), pear (Clayton et al., 2000), and nectarines (Torrighiani et al., 2004) and plum (Jobling et al., 2003). SSC in peach and plum increased during storage by AVG (Bregoli et al., 2002; Jobling et al., 2003) while in apple and pear Yildiz et al. (2012) and D'Aquino et al. (2010) reported decline in SSC. AVG treated apple and peach had higher TA (Cetinbas & Butar, 2013; Ozturk et al., 2013) while reducing volatiles in apple (Halder-Doll & Bangerth, 1987; Mir et al., 1999). AVG treatment benefits pear by reducing core breakdown during storage (Clayton et al., 2000).

Ozturk et al. (2012) found negative impact of AVG on phenolic compounds in plum. Undesirable effects of AVG include inhibiting root elongation (Riov & Yang, 1989), somatic embryos (Meijer & Brown, 1988), PAL activity (Chappell et al., 1984), induction of female flowers in cucumbers (Takahashi & Jaffe, 1983) were reported. Decay caused by fungus *Botrytis cinera* increased in AVG treated plants (Chagué et al., 2006). AVG effects appear to be variable and dependent upon cultivars in many fruit crops are described by Lurie (2005; 2008).

It is hypothesised that inhibiting ethylene synthesis prior to touch picking maturity using AVG may result in delaying maturity at harvest and in extension of storage life. There are no published reports on inhibition of ethylene synthesis either by AVG or other inhibitors during the final stages of feijoa development.

Table 2.3 Summary of AVG effects in some fruit crops. Symbols ↓ indicates reduced, ↑ as increased and ↔ as delayed next to an attribute

Reference	AVG effects
Apple	
(Halder-Doll & Bangerth, 1987)	C ₂ H ₄ production and respiration rate ↓, firmness loss and TA loss ↓ at harvest but volatile production ↓ during storage
(Byers, 1997a)	Fruit drop ↓, colour development and firmness losses ↓, starch losses and maturity ↔ at harvest
(Greene, 2002),	Fruit drop ↓, SSC ↓, watercore ↓, ripening ↔
(Amarante et al., 2002)	Fruit drop ↓, colour development ↔
(Stover et al., 2003)	Fruit drop ↓, firmness loss ↔, location effect
(Phan-Thien et al., 2004)	Firmness loss, colour loss and starch conversion ↔ at harvest
(Schupp & Greene, 2004)	Fruit drop ↓, Firmness loss and colour loss ↔, internal C ₂ H ₄ concentration ↓
(Byers et al., 2005)	Fruit drop ↓, firmness loss, starch conversion and colour development ↔
(Drake et al., 2005)	Firmness loss and maturity ↔, flavour ↓
(Greene, 2005)	Fruit drop ↓, firmness loss, starch conversion and colour development ↔
(Drake et al., 2006)	Starch conversion and C ₂ H ₄ production ↓, firmness loss and colour development ↔ at harvest and during storage
(Moran, 2006)	Firmness loss ↔, scald ↓
(Rath et al., 2006)	C ₂ H ₄ production and fruit drop ↓, firmness loss, starch conversion and SSC loss ↔
(Fallahi, 2007)	Firmness loss and starch conversion ↔, C ₂ H ₄ production and respiration ↓, maturity and storage life ↔
(Whale et al., 2008)	Internal C ₂ H ₄ concentration ↓, firmness loss and colour development ↓
(Yildiz et al., 2012)	Fruit drop and C ₂ H ₄ production ↓, firmness loss and starch conversion ↓ at harvest and SSC ↓ during storage
(Ozturk et al., 2013)	Anthocyanin ↓, fruit mass, fruit diameter ↑, firmness loss and colour development ↓ TA ↑,

Reference	AVG effects
Other fruit Pear	
(Clayton et al., 2000)	Firmness, starch conversion and skin °hue ↔ at harvest and C ₂ H ₄ production, firmness loss, core breakdown and TA ↓ during storage
(Dussi et al., 2002)	C ₂ H ₄ production and fruit drop ↓, fruit set ↑
(Lafer, 2008)	Fruit set and yield ↑
(D'Aquino et al., 2010)	Firmness loss and colour loss ↓ at harvest, and during storage C ₂ H ₄ production, respiration rates, SSC and decay ↓
(Sánchez et al., 2011)	Fruit set and fruit yield ↑, fruit drop ↓
Peach & nectarine	
(Byers, 1997b)	C ₂ H ₄ production and firmness loss ↓
(Belding & Lokaj, 2002)	C ₂ H ₄ production and firmness loss ↓
(Bregoli et al., 2002)	C ₂ H ₄ production and firmness loss ↓, SSC ↑
(Rath & Prentice, 2004)	Fruit yield ↑, firmness loss ↓
(Torrighiani et al., 2004)	fruit drop ↓, C ₂ H ₄ production and firmness loss ↓ at harvest and during storage
(McGlasson et al., 2005)	C ₂ H ₄ production, fruit weight and firmness loss ↓ at harvest and internal bleeding ↑ during storage
(Bregoli et al., 2006)	C ₂ H ₄ production ↓ but no effect on storage
(Cline, 2006)	Firmness loss ↓ at harvest
(Hayama et al., 2008)	C ₂ H ₄ production and firmness loss ↓
(Cetinbas et al., 2012)	Weight loss ↑ at harvest, C ₂ H ₄ production and firmness loss ↓ during storage
Plum	
(Jobling et al., 2003)	C ₂ H ₄ production, colour and firmness loss ↓ and SSC ↑ at harvest and during storage
(Ozturk et al., 2012)	After storage firmness and phenolic compounds ↓
(Altuntas & Ozturk, 2013)	After storage firmness ↑ and phenolics ↓
(Karaman et al., 2013)	During storage weight loss and firmness loss, total phenolics and antioxidant ↓

2.6 Summary

This chapter reviewed the importance of harvest maturity of feijoa and its impact on quality during storage. Available literature suggests that feijoa is harvested at an advanced stage of maturity and harvesting them early may enable extension of storage potential. Role of ethylene during fruit ripening was examined in detail so that application of ethylene inhibition technologies like 1-MCP and AVG may result in delaying ripening of feijoa. Importance of combining postharvest treatments like 1-MCP with CA is likely to result in synergistic improvement of after storage quality of feijoa. In addition, high variability in harvested feijoa contributed to the limited success of earlier applied postharvest technologies, hence it is indispensable to explore non-destructive indicator of maturity at the time of harvest to enable uniformity in feijoa batches. This research is part of the continued search to devise a strategy and technology to extend storage life of feijoa which is a prerequisite to develop a strong commercial feijoa industry in New Zealand.

2.7 Thesis overview

Chapter 1 introduces the major issue faced by feijoa industry for providing longer storage that will enable expansion and growth and Chapter 2 reviews the research areas highlighting the opportunities to investigate storage life extension in feijoa. Importance of harvest maturity and ethylene's influence during ripening was identified as key research areas that effect storage life of feijoa. In addition, the review highlights the challenges to address variability at harvest time and envisages use of skin colour to identify maturity of feijoa.

Chapter 3 evaluates whether storage life of touch-picked feijoa can be extended by inhibition of ethylene perception by pre-storage 1-MCP treatment in combination with CA storage.

Chapter 4 examines to re-evaluate effects of harvest timing on storage quality of feijoa. Effects of postharvest application of 1-MCP and exogenous ethylene treatments on feijoa harvested at different maturities are studied.

Chapter 5 inspects the effects of inhibition of ethylene synthesis by preharvest AVG on maturity at-harvest and on fruit drop. This chapter also evaluates effect of AVG on storage performance of feijoa. Additionally effect of preharvest AVG on early harvested feijoa is investigated.

Chapter 6 assesses the use of non-destructive skin colour of feijoa as an indicator of maturity at harvest. This chapter further examines whether °hue and spectral wavelength can segregate feijoa at the time of harvest based on their storage potential.

Finally in chapter 7 the general discussion and conclusion is presented based on the outcomes of this research. At the end of the thesis, future recommendations based on the knowledge gained on feijoa is listed.

3 Combined effects of pre-storage 1-methylcyclopropene application and controlled atmosphere storage on 'Unique' feijoa quality

Acknowledgement:

Material from this chapter is included in the paper:

Rupavatharam, S., East, A.R., & Heyes, J.A. (2015). Combined effects of pre-storage 1-methylcyclopropene application and controlled atmosphere storage on 'Unique' feijoa quality. *New Zealand Journal for Crop and Horticultural Sciences*, 43(2), 134-143.

This chapter differs from the publication in that it includes an additional figure (Fig 3.1).

3.1 Introduction

Feijoa can presently be stored for up to 4 weeks at 4 ± 1 °C and relative humidity of $90 \pm 5\%$ (Thorp & Klein, 1987). New Zealand is a relatively large commercial producer of feijoa (500 T) which is largely consumed in the local market. Export of feijoa fruit by refrigerated shipping from New Zealand is limited by the inability to maintain fruit quality consistently for a period of 6 weeks that would allow transport to distant markets in North America, Asia and Europe (Al-Harthy, 2010).

Extending the postharvest storage life of feijoa has earlier been attempted using low temperature storage (Klein & Thorp, 1987), calcium chloride

application (Ramírez et al., 2005) and pre-storage hot water treatments (Woolf et al., 2006) with limited success. Previously, controlled atmosphere (CA) storage has been effective in improving storage performance of 'Unique' and 'Opal Star' feijoa (Al-Harthy et al., 2010a; Al-Harthy et al., 2010b). Reduced O₂ atmospheres of 1.15-3 kPa combined with 0-0.2 kPa of CO₂ were considered optimal to reduce weight loss and colour change during storage of 'Unique' (East et al., 2009). Despite the potential for improvements in storage life through application of CA, Al-Harthy et al. (2010a) reported that fruit were over ripe after 6 weeks. Hence despite the statistical improvement in storage life, applying CA as a stand-alone postharvest treatment is unlikely to be commercially applicable to extend storage life of feijoa, thus necessitating the search for adoption with other storage technologies.

Ethylene action inhibitor 1-methylcyclopropene (1-MCP) not only impacts postharvest life by maintaining quality but also alleviates physiological disorders in many fruit crops (Blankenship & Dole, 2003; Watkins, 2006). 1-MCP interacts by binding to ethylene receptors and prevents responses related to ethylene (Sisler & Serek, 1997). Application of 1-MCP (1500 nL L⁻¹ for 8 h at 20 °C) was found to maintain firmness and external skin colour during storage of 'Brazil (242)' feijoa for up to 30 d in refrigerated storage at 4 °C (Amarante et al., 2008) while Velho et al. (2008) reported that 1-MCP application had no effect on ethylene production during storage of Brazilian feijoa. Unpublished work by White and Woolf (Plant and Food Research, NZ) found that 1-MCP had no effect on ripening of 'Apollo' feijoa (Schotsmans et al., 2011).

Feijoa fruit demonstrate climacteric behaviour with increased respiration rate and ethylene production during the course of development (Biale et al.,

1954; Reid, 1975). However the role of ethylene in ripening of feijoa is uncertain given that the exogenous application of ethylene or the analogue propylene does not stimulate quality changes (Al-Harthy, 2010). This climacteric like rise in ethylene production coupled with a lack of ethylene response is similar to that observed for guava (*Psidium guajava*) (Azzolini et al., 2005) suggesting physiological similarities of these closely related fruit. Ethylene production influences fruit softening and skin colour development in guava fruit (Reyes & Paull, 1995). A number of studies with guava have observed extended storage life with application of 1-MCP. Bassetto et al. (2005) and Singh & Pal (2008a) delayed ripening and extended the storage life of 'Pedro Sato' and 'Allahabad Safeda' guava respectively. Despite the relative lack of response observed for feijoa to date, the similar physiological responses and observation of positive results for the closely related guava, warrant continued investigation of other cultivars of feijoa.

Given that 1-MCP is an at-harvest treatment and CA is a storage treatment, there is potential for both to be used in combination to achieve extended storage life outcomes. Combining pre-storage 1-MCP application along with CA storage ensured better retention of quality of apples than in air storage (Bai et al., 2005; Watkins et al., 2000). Overall quality of litchi fruit was best retained when 1-MCP was used in combination with CA (Sivakumar & Korsten, 2010). To the author's knowledge, there is no previous work combining 1-MCP application and controlled atmospheres in an attempt to further extend the storage life of feijoa. The aim of this research was to investigate if storage of feijoa fruit could be extended through the combined application of 1-MCP after harvest followed by reduced oxygen atmospheres at 4 °C.

3.2 Materials and Method

3.2.1 Fruit sample

Feijoa cv. 'Unique' were sourced from a commercial grower located near Matamata, New Zealand in the first week of April, 2011. Export grade fruit (approximately 1800) representing various sizes (averaging 66.4 g) were harvested by the touch picking method (Fleming, 1986) by experts. Fruit with a weight of less than 45 g were excluded from the sample as they are considered too small in commercial practice. Fruit were packed into commercial, single layer, corrugated fibreboard trays containing plix about 12 h after harvest in ambient temperature ($\approx 20\text{ }^{\circ}\text{C}$). Fruit were transferred by road to the postharvest laboratory at Massey University, Palmerston North. A commercial van with air conditioner was used to transport fruit in a cool condition ($15\text{ }^{\circ}\text{C}$). It took 12 h from the time of packing to reach Massey University laboratory. On arrival fruit were randomly divided into groups of 12, labelled and placed in mesh bags to create a total of 144 bags. Fruit were first treated with 1-MCP at $20\text{ }^{\circ}\text{C}$ and then transferred to a continuous flow through system of controlled atmosphere conditions at $4\text{ }^{\circ}\text{C}$ and 90% relative humidity for storage. The experiment treatments consisted of a full matrix of three 1-MCP and three controlled atmospheres treatments (including controls of air for both). Each gas treatment combination was replicated 4 times. Fruit was removed from storage fortnightly from 4 to 10 weeks for quality assessments either immediately after storage or after a further 7 d at $20\text{ }^{\circ}\text{C}$.

3.2.2 1-MCP treatment

The three pre-storage 1-MCP treatments were 500 nL L⁻¹ for 3 h; 1000 nL L⁻¹ for 12 h and air as a control. These doses were chosen to represent a wide range of the possible 1-MCP application treatments. Treatments were applied approximately 26 h after harvest. Water soluble powder of 0.14% 1-MCP (SmartFresh™, AgroFresh Inc., USA) was used to create atmosphere treatments. A predetermined quantity of powder was placed in an airtight 100 mL plastic syringe. Approximately 2 mL of deionised milli-Q water was dispensed through a septum into the syringe. The syringe was immediately closed and the contents shaken rigorously for 2 minutes to allow release of the gaseous vapour into the head space of the syringe. Care was taken to ensure that no trace of powder remained in the syringe. The syringe head space was injected through an inlet valve into an airtight 56 L high density polyethylene barrel containing 12 bags of fruit represented a treatment replicate. Barrels containing fruit treated with 1-MCP at 500 nL L⁻¹ were ventilated after 3 h of treatment for 30 minutes and then re-sealed for the remaining 8.5 h. The control treatment fruit were sealed in 56 L barrels without atmospheric modification (air). During 12 h period CO₂ accumulation ranged between 5-10% in barrels. Previously, anaerobic conditions for 24 h in feijoa had no impact on quality after storage (Pesis et al., 1991). After 12 h from initial treatment all barrels were vented in open air for 30 minutes before transferring the bags of fruit to controlled atmosphere storage.

3.2.3 Controlled atmosphere storage

After removal from the barrels, bags of fruit were transferred to 36 PVC containers (volume of 0.0135 m³). From each barrel 12 bags of fruit were

transferred to 3 PVC containers, one for each atmosphere. Each PVC container held 4 mesh bags (48 fruit) and was used to maintain constant atmosphere conditions of either 2 kPa O₂ with 0 kPa CO₂; 5 kPa O₂ with 0 kPa CO₂ or air (21 kPa O₂ with 0.04 kPa CO₂) as a control, using a flow through system. The 2 kPa O₂ and 5 kPa O₂ gas mixes were each created as a single gas flow that was generated by mixing dry air (as an O₂ source) with N₂ using a gas mixer. Gas mixes were then distributed to the individual PVC containers using a manifold. Gas flows were humidified to create 90% RH by bubbling through water mixed with glycerol at a constant flow rate of 0.2 L min⁻¹. Weekly checks of atmosphere conditions in the PVC containers were performed to ensure uniform conditions. Gas samples were collected using a 100 µL gas tight syringe. Two samples from each PVC container were drawn on each occasion and analysed as described below.

3.2.4 Respiration rate

Respiration rate was measured fortnightly during storage at 4 °C. Respiration rate was measured as rate of CO₂ production during storage while in the applied storage atmosphere, by using the static method in the PVC container. Gas flows used to establish the storage conditions were temporarily stopped and the container sealed. Gas samples were drawn using 100 µL gas syringes from sample ports and analysed as described below. Care was taken to ensure that accumulation of CO₂ in the PVC container was not above 0.5% (Hertog et al., 2004). Time between gas samples was 1 and 2 h depending upon storage conditions. Respiration rates (r_{CO_2}) were calculated based on fruit weight, free volume of PVC container and temperature and expressed in nmol kg⁻¹ s⁻¹.

3.2.5 Gas analysis

Gas samples were analyzed using a gas analyser with an O₂ electrode (Citicell, City Technologies, London, United Kingdom) in series with a CO₂ infrared transducer (Analytical Development Company, Hoddesdon, United Kingdom) that uses N₂ as a carrier gas with a flow rate of 35 mL min⁻¹. The output signal was analysed with HP integrators (model 3396A, Hewlett Packard, USA). Calibration of the gas analyser was conducted using commercially obtained β -standard 0.5% CO₂ (BOC, Palmerston North, New Zealand) or air for O₂.

3.2.6 Fruit quality measures

Fruit quality was measured at 20 °C after removal from storage. Weight loss, surface injury, compression firmness, colour of skin and flesh, titratable acidity, total soluble solids and internal maturity rating were used as measures of quality.

Fruit mass was measured using a balance (PG503-s, Mettler Toledo, Greifensee, Switzerland) to an accuracy of ± 0.001 g. Weight loss was measured as a percentage of loss from initial weight first measured prior to 1-MCP treatment.

Compression firmness was measured using a texture analyser (TA-Xt Plus, Stable Microsystems Ltd., Surrey, UK) by applying a non-destructive compression of 2 mm at the equatorial region using a flat plastic cylindrical probe at 1 mm s⁻¹. Average peak force (N) of 2 measures from opposite sides was used as fruit firmness.

Total soluble solids (SSC) content of the fruit was measured as °Brix, by halving the fruit at the equatorial region and squeezing the juice by hand on to a digital refractometer (PAL-1, Atago Ltd, Tokyo, Japan). Titratable acidity (TA) was measured by pooling juice from the locular gel of 6 fruits per sample. Juice was frozen with liquid N₂ and stored immediately in a freezer at -30° C. TA was later measured using an automatic titrator (TitroLine easy, Schott Instruments GmbH, Mainz, Germany). Feijoa juice (1 mL) was dispensed into 50 mL of de-ionised water and titrated against 0.1 N standard NaOH to an endpoint of pH 8.2 for malic acid. Acid strength was calculated using the method in Sadler and Murphy (2010).

Colour of the skin and flesh was measured using a reflectance spectrophotometer (CM-2600d, Konica Minolta Sensing Inc., Osaka, Japan). Reflectance measurements were noted on the skin at three positions on the equatorial region of the fruit. Flesh colour was measured immediately after halving the fruit at the equatorial region with a transverse slice and three measurements were taken randomly from a single side of the slice. Colour of skin and flesh was determined by average hue angle (°hue) of the three locations.

Severity of skin surface injury due to discoloration (browning) was recorded as the estimated percentage area coverage as determined by visual assessment (Fig. 3.1). Internal maturity ranking was conducted by cutting the fruit at the equatorial region and rating the maturity status as per the scale developed by Plant & Food Research, New Zealand (Fig. 2.3).

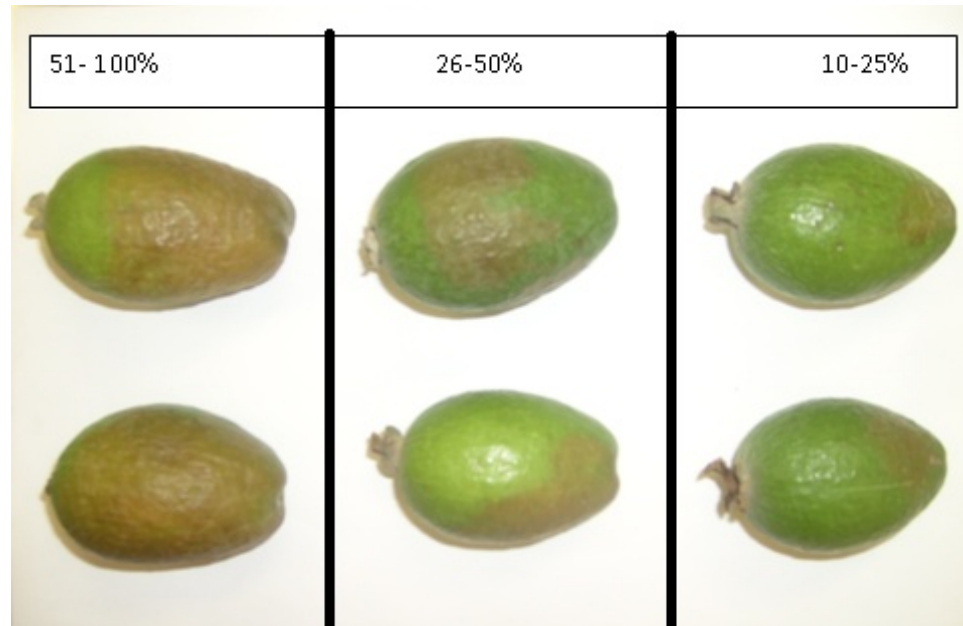


Fig. 3.1 Scale used to rate severity of external skin injury due to browning of fruit

3.2.7 Data analysis

With the exception of the surface injury incidence and maturity rating score data, the General Linear Model (GLM) procedure of ANOVA was conducted using Minitab (Version 16.1.0, Minitab Inc., State college, Pennsylvania, USA) to ascertain significant effects of treatments on quality parameters. Time in storage was considered as a factor and hence all data were analysed in a single ANOVA. Significant differences at 95% confidence were identified using Tukey's test.

Treatment effects on injury incidence and maturity rating scores were evaluated using a chi-square test on pooled sets of data from the range of storage lengths (i.e. 4 to 10 weeks).

3.3 Results

3.3.1 Fruit respiration

Respiration rate increased with storage time (Fig. 3.2). Rate of carbon dioxide production (r_{CO_2}) ranged from 60-200 $\text{nmol kg}^{-1} \text{s}^{-1}$ at 4 °C. The magnitude and increase of respiration rate observed was similar to that observed previously by East et al., (2009). Application of 1-MCP had no impact on respiration rate of feijoa in subsequent storage (data not shown). However oxygen partial pressure impacted the respiration rate (Fig. 3.2) with fruit kept at 2 kPa O_2 respiring less in comparison to the other 2 treatments. Respiration rate of fruit kept in air was higher than at 5 kPa O_2 only after 10 weeks of storage.

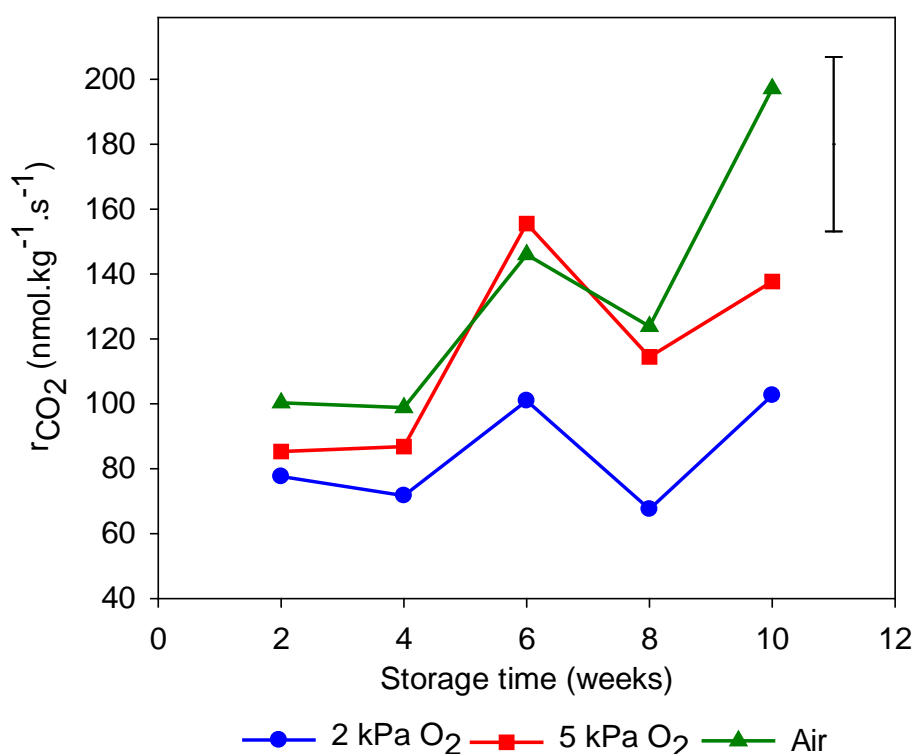


Fig. 3.2 Effect of oxygen partial pressure on respiration rate of 'Unique' feijoa stored at 4 °C. Each data point represents a mean of 12 samples across all 1-MCP treatments. Vertical bar represents $HSD_{0.05}$ (Tukey's)

3.3.2 Quality measures

Average weight loss ranged from 0.9 to 1.5% during storage, similar to the previous observations of East et al. (2009). This low rate of weight loss is likely to be the result of the high relative humidity established in the storage conditions due to the use of a flow through system. Fruit showed no visual symptoms of shrivel. Neither 1-MCP treatment nor controlled atmosphere storage had any effect on fruit weight loss (data not shown).

Compression firmness declined during storage from 27 N initially to approximately 11 N after 10 weeks of storage (Fig. 3.3). Subsequent periods at 20 °C for 7 d resulted in further rapid reduction in firmness. 1-MCP treatment prior to storage had no effect on firmness change (data not shown) while significant differences were caused by controlled atmosphere treatments (Fig. 3.3). Over the entire experiment mean compression firmness of 2 kPa O₂ (12.6 N) was significantly higher in comparison to 5 kPa O₂ (11.7 N) and air (10.8 N). Despite the effect of atmospheres across the whole experiment, only after 8 weeks of storage and 7 days at 20 °C was fruit stored in 2 kPa O₂ treatment significantly firmer than that stored in air.

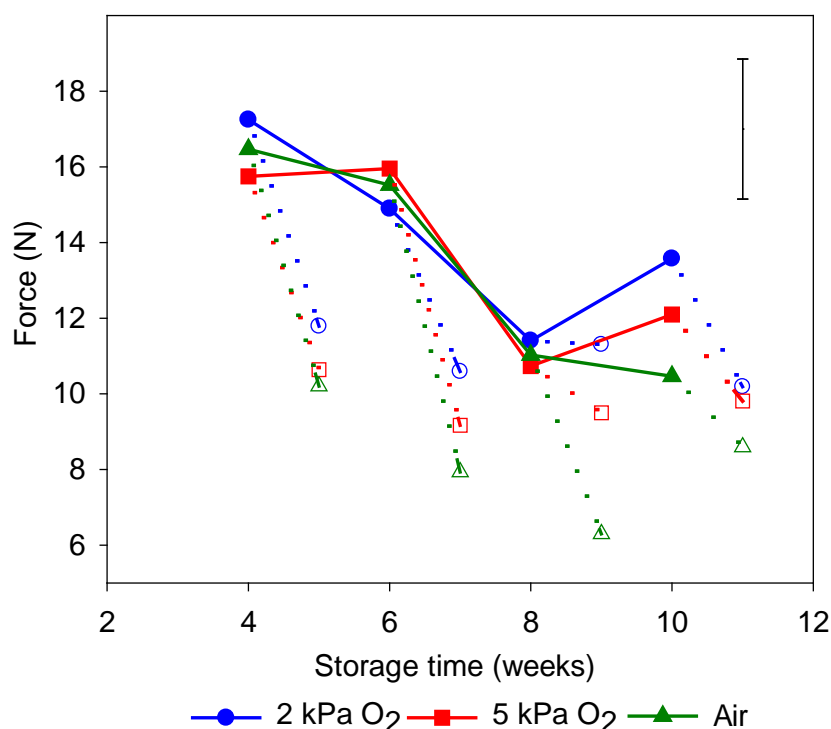


Fig. 3.3 Effect of atmosphere on compression firmness of 'Unique' feijoa at 4 °C and assessed after storage (solid symbols) and a subsequently 7 d at 20 °C (hollow symbols). Each data point represents the average of at least 37 fruit across all 1-MCP treatments. Average firmness was 27 N at harvest and vertical bar represents HSD_{0.05} (Tukeys)

Total soluble solids (SSC) were approximately 11.5 °Brix at harvest and decreased gradually to an average of 8.8 after 10 weeks storage (data not shown). Neither treatment with 1-MCP prior to storage nor controlled atmospheres influenced SSC change. Average TA was 0.89 g malic acid/100 mL of juice initially and subsequently declined during storage (Fig.3.4). A more rapid decline of TA during subsequent storage at 20 °C was observed. TA was observed to be influenced by storage atmosphere (Fig. 3.4) but not by 1-MCP treatment prior to storage (data not shown). Over the entire experiment, fruit in 2 kPa O₂ retained significantly higher TA in comparison to 5 kPa O₂ or in air. The increase in TA observed for the air treatment at the completion of the

experiment, may be a result of a physiological change related to advanced senescence that was not visually obvious during sampling.

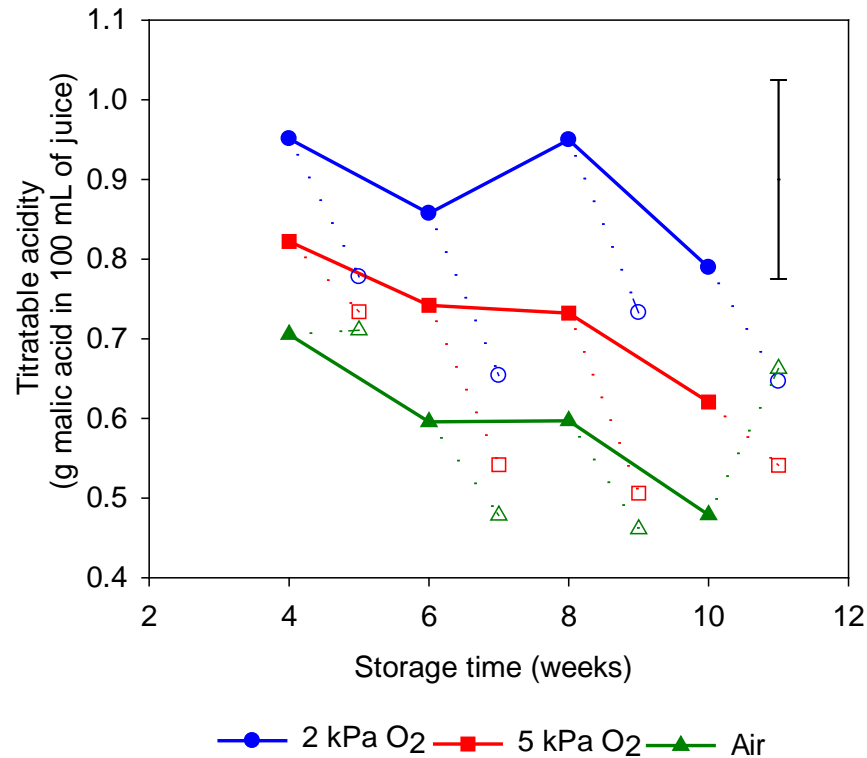


Fig. 3.4 Effect of atmosphere on titratable acidity of 'Unique' at 4 °C and assessed after storage (solid symbols) and a subsequent 7 d at 20 °C (hollow symbols). Each data point represents average of 12 pooled juice samples from 6 fruit across all 1-MCP treatments. Average TA was 0.89 g malic acid in 100 mL of juice at harvest and vertical bar represents $HSD_{0.05}$ (Tukey's)

Average skin colour decreased from a °hue angle of 112.8 to 110.5 in air stored fruit over the 10 weeks at 4 °C. Changes in skin colour were not influenced by 1-MCP treatment or storage atmospheres (data not shown). A brown surface injury was first observed after 4 weeks of storage in 2 kPa O₂. When fruit were assessed after a further 7 d at 20 °C, the surface injury was more severe. Incidence of this injury was found to be more prevalent after storage in 2 kPa O₂ than either of the other atmospheres (Table 3.1). After 6

weeks of storage at 4 °C and a further 7 d at 20 °C, surface injury was observed in 37.5% of fruit population in 2 kPa O₂ atmospheres while less than 5% of fruit had surface injury in 5 kPa O₂ or air (data not shown).

Table 3.1 Contingency table showing effects of storage atmospheres on surface injury incidence of ‘Unique’ feijoa at 4 °C. Data is pooled from both 1-MCP treated and non-treated fruit and from variable times in storage. Assessments made after removal from storage on 4, 6, 8 and 10 weeks and a further 7 d at 20 °C. Chi-square (X^2) = 56.262, $p < 0.001$. Values in parenthesis are contributions to chi square

		Surface injury incidence				Total
		<10%	10-25%	26-50%	>50%	
Controlled atmosphere	2 kPa O ₂	179 (7.75)	40 (11.9)	33 (6.86)	36 (6.88)	288
	5 kPa O ₂	253 (4.84)	13 (4.58)	10 (5.76)	12 (5.5)	288
	Air	229 (0.34)	17 (1.72)	20 (0.05)	22 (0.08)	288
	Total	661	70	63	70	864

At harvest flesh °hue was 88.1, which declined during storage. There was no influence of 1-MCP on internal flesh colour (°hue), while fruit stored at 2 kPa O₂ atmospheres reduced in °hue in comparison with air stored fruit (Fig. 3.5). Change in locular browning measured by the maturity rating scale did not reveal any effects due to 1-MCP or controlled atmospheres. Most fruit were ranked as over mature fruit (≥ 4) when assessed after 6 weeks of storage at 4 °C and a further 7 d at 20 °C (data not shown).

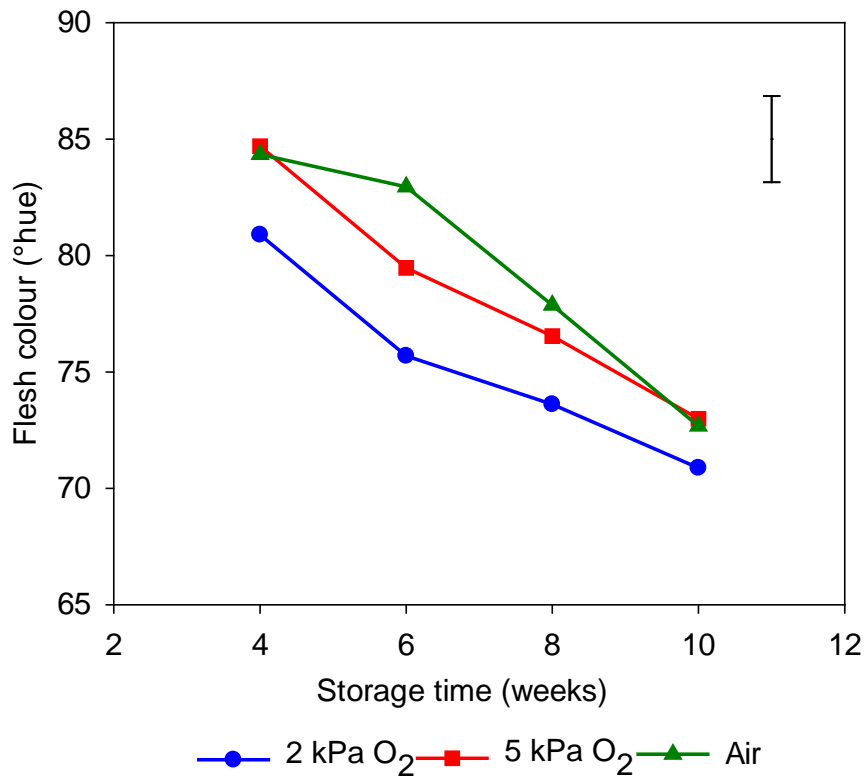


Fig. 3.5 Effect of storage atmospheres on flesh colour (°hue) of 'Unique' stored at 4 °C and assessed after removal from storage at 20 °C. Each data point represents average from at least 56 fruit across all 1-MCP treatments. Average flesh °hue was 88.1 at harvest time and vertical bar represents $HSD_{0.05}$ (Tukey's)

3.4 Discussion

Postharvest 1-MCP treatment applied to touch picked feijoa at either 500 nL L⁻¹ for 3 h or 1000 nL L⁻¹ for 12 h was found to impart no change in the physiology or postharvest quality changes during storage of 'Unique' feijoa. These results agree with Velho et al. (2008) and White and Woolf (Schotsmans et al., 2011) who reported that 1-MCP had no effect on ripening of 'Brazilian-type' and 'Apollo' respectively. The only contrasting report in which 1-MCP was observed to have a subsequent effect, is the work of Amarante et al. (2008) in which 500 or 1500 nL L⁻¹ for 8 h resulted in retention of firmness of flesh and

skin colour °hue of 'Brazilian (242)' feijoa after 30 d of storage. In agreement with our results, Amarante et al., (2008) found no effect of 1-MCP on either SSC or TA. Differences between the majority of results and the work of Amarante et al., (2008) may be attributable to harvest maturity, application timing or cultivar, although limited research that deliberately target these questions for feijoa currently exists. The relatively consistent observations of no effect of 1-MCP on feijoa, contrast with observation for the closely related guava, which displays 1-MCP-induced delays in ripening (Azzolini et al., 2005; Bassetto et al., 2005; Ding & Ong, 2010; Singh & Pal, 2008a).

In this work, touch picked feijoa were unresponsive to 1-MCP application despite being classified as climacteric (Reid, 1975). Ethylene perception is inhibited by 1-MCP, which irreversibly binds to ethylene receptors, however new binding sites can be synthesized by plants (Cameron & Reid, 2001). Ekman et al. (2004) in 'Bartlett' pears and In et al. (2013) in carnations, found transient effects of 1-MCP and suggested that plant tissue regained sensitivity to ethylene by continually generating new receptors. Tassoni et al. (2006) were the first to demonstrate that ethylene receptors regenerated after 1-MCP application in tomato. McArtney et al. (2009) also demonstrated that some apple cultivars are capable of generating new ethylene receptors rapidly during ripening. It is possible that new ethylene receptors may have been synthesised in feijoa after 1-MCP treatment, rendering the 1-MCP treatment ineffective. However, actual levels of ethylene receptors can diminish with the advancement of ripening as observed in tomato (Kevany et al., 2007).

A possible explanation for the lack of 1-MCP response in feijoa is that ethylene receptors could be saturated with endogenous ethylene at the time of

harvest. Sisler (2006) suggested that all the binding sites in plant tissue could be occupied by ethylene and hence prevent a response from an ethylene action inhibitor. In tomato and 'Bartlett' pears, high levels of internal ethylene at harvest time retard the efficacy of 1-MCP (Macnish et al., 2012; Zhang et al., 2009). Efficacy of 1-MCP is affected by the developmental stage of fruit at the time of application (Blankenship & Dole, 2003). Effectiveness of 1-MCP treatment was limited in apricots by the advancement of fruit maturity (Fan et al., 2000). Similar maturity affects have been observed for apple (Mir et al., 2001), fig (Sozzi et al., 2005), banana (Golding et al., 1998) and in 'Conference' pears (Chiriboga et al., 2013). In this work "touch-picked" feijoa were treated with 1-MCP. This maturity standard for harvest seems to originate to the recommendations of Popenoe (1912) who stated that "the best tasting feijoa were those that were harvested immediately prior to falling from the tree". Bailey (1952) reported that feijoa were allowed to fall on the ground and collected on a daily basis. Later, growers in New Zealand used touch picking of their fruit as the harvest maturity standard (Fleming, 1986). While in this research touch picked feijoa did not respond to at-harvest 1-MCP application this does not rule out that fruit harvested at an earlier maturity may exhibit a response. Further research may be needed to determine feijoa responses to 1-MCP over a range of maturities.

Storage temperatures subsequent to 1-MCP treatment can also influence the efficacy of response. Mir and Beaudry (2001) on apple and Bregoli et al. (2005) on nectarines found that the efficacy of 1-MCP declined as storage temperatures decreased. Conversely in tomato, Mostofi et al. (2003) found that 1-MCP was most effective in delaying ripening at 15 and not at 20 or 25 °C.

Efficacy of 1-MCP may not be dependent on storage temperature for feijoa as the lack of response observed in this study for fruit stored at 4 °C was earlier demonstrated in Brazilian-type feijoa stored at 23 °C (Velho et al., 2008).

A further possible explanation for the lack of 1-MCP response may be the existence of largely ethylene independent ripening and/or highly insensitive to ethylene processes (Ezura & Owino, 2008). Pech et al. (2008) found that some ripening features such as production of volatiles, respiration and de-greening of rind were regulated by ethylene while accumulation of sugars, acid loss and colour were independent of ethylene in melon fruit. Fruit ripening is complex and necessitates further investigations to understand the role of ethylene in ripening of feijoa that would enable application of appropriate technologies to extend the storage life.

In this work low oxygen atmospheres of 2 kPa O₂ significantly suppressed fruit respiration rates when compared with 5 kPa O₂ (Fig. 3.2) and subsequently resulted in retaining firmness (Fig. 3.3), titratable acidity (Fig. 3.4) and internal colour (Fig. 3.5). However this same atmosphere resulted in the greatest incidence of skin injury (Table 3.1). The observed decrease in respiration rates (r_{co_2}) as a result of low O₂ application are similar to those reported earlier for 'Unique' (East et al., 2009). Previously there was no additional storage life found for 'Unique' and 'Opal Star' after 6 weeks of storage in reduced oxygen atmospheres (Al-Harthy et al., 2010a; Al-Harthy et al., 2010b). 'Unique' fruit seem to be sensitive to low levels of O₂ (below 5 kPa) which is similar to the behaviour exhibited by guava cultivars (Singh & Pal, 2008b). However, East et al. (2009) reported that there is no impact on injury incidence in CA at levels of above 1.15 kPa O₂ in 'Unique' feijoa. Al-Harthy et al. (2010a) observed that CA

had no effect on skin colour which is in contrast to East et al. (2009) who reported that low O₂ atmospheres significantly reduced ripening-related change in skin °hue. Singh & Pal (2008b) observed a reduction in guava skin colour change in low oxygen atmospheres. The lack of influence of atmosphere on SSC development agrees with the previous findings on 'Unique' and 'Opal Star' (Al-Harthy et al., 2010a; Al-Harthy et al., 2010b). Biale (1961) attributed decline of SSC during storage to the utilisation of sugars for respiration. TA declined during storage in all treatments (Fig. 3.4), Mercado-Silva et al. (1998) ascribed this decline to the use of organic acids as respiratory substrates in guava fruit. Retention of TA during storage in reduced oxygen atmospheres is similar to the observation made by Singh & Pal (2008b) for guava fruit where TA was retained in CA when compared with air storage. There is paucity of research on impact of storage atmospheres on other cultivars of feijoa. The importance of cultivar is pronounced in highbush blueberries where some varieties have displayed extended storage life while others stored poorly in CA (Alsmairat et al., 2011).

In this study combining pre-storage 1-MCP application with controlled atmospheres did not yield any storage life extension, although in this case it is likely that this is a result of the ineffectual nature of 1-MCP treatment on feijoa storability. Combining 1-MCP treatments with CA resulted in better quality retention than standalone treatment in litchi fruit (Sivakumar & Korsten, 2010) and apple (Hoang et al., 2011; Watkins et al., 2000). Despite the poor result observed for feijoa, researchers should continue to explore the potential of combining postharvest treatments to get further extension of storage life.

3.5 Conclusion

Reduced oxygen atmospheres (2 kPa O₂) contributed to a slight improvement in some quality aspects, however by six weeks of storage, fruit had severe surface injury. CA technology may not be appropriate for storage quality retention in 'Unique' feijoa. Postharvest 1-MCP application had no impact on fruit respiration or in delaying ripening during storage of touch picked 'Unique' feijoa. Efficacy of 1-MCP is influenced by factors like fruit maturity and cultivar, and hence further studies at an earlier harvest or on a different cultivar may yield more positive results

4 Re-evaluation of harvest timing in ‘Unique’ feijoa using 1-MCP and exogenous ethylene treatments

Acknowledgement:

Material from this chapter is included in two papers:

Rupavatharam, S., East, A.R., & Heyes, J.A. (2015). Re-evaluation of harvest timing in ‘Unique’ feijoa using 1-MCP and exogenous ethylene treatments. *Postharvest Biology and Technology*, 99, 152-159.

All data presented in this chapter were presented in the journal publication.

Rupavatharam, S., East, A.R., & Heyes, J.A. (2013). Opportunities to manipulate harvest maturity of New Zealand feijoa (*Acca sellowiana*) to enable sea freight export. Conference paper presented at VI International conference on managing quality in chains, September, 2013 (in press).

Data of untreated fruit (controls) used in Table 4.1 and Figs 4.1, 4.4 and 4.5 were presented in the publication.

4.1 Introduction

Feijoa (*Acca sellowiana*) is an aromatic fruit of family Myrtaceae, a native of South America and commercially grown in small volumes in a number of countries (Schotsmans et al., 2011). Feijoa are harvested by touch-picking which relies on using low force to detach the fruit from its stalk. Fruit harvested with a greater force are considered immature (Thorp & Klein, 1987). This touch-picked maturity standard was first described by Popenoe (1912) who stated that

“the best fruit were those that were harvested just prior to falling from the tree”. Fleming (1986) reported touch picking of fruit as the maturity standard used by growers in New Zealand. Feijoa can be stored for 4 weeks at 4 °C and at 90% RH and retain good quality for a further 5-7 d at 20 °C (Klein & Thorp, 1987). Despite this, the most detailed work to date investigating harvest maturity effects for feijoa found that after 4 weeks of storage, fruit picked at a substantially higher force to touch picked fruit had the same eating appeal (Downs et al., 1988). These combined reports suggest that the current touch-picked harvest maturity leans towards providing ready-to-eat fruit at harvest rather than the optimum maturity suitable to withstand long periods in storage.

Biale et al. (1954) first demonstrated a climacteric increase in respiration and ethylene production during ripening of feijoa. Ethylene production rates at pre-climacteric minimum were in the range of 1.1-4.6 pmol kg⁻¹ s⁻¹ and can reach up to 462 pmol kg⁻¹ s⁻¹ at climacteric maximum (Reid, 1975). During climacteric fruit ripening autocatalytic ethylene production can be enhanced when exposed to exogenous ethylene (McMurchie et al., 1972). However previous application of ethylene on feijoa failed to produce this expected response. When Velho et al. (2008) applied 66 µL L⁻¹ ethylene for 24 h at 23 °C and Al-Harthy (2010) applied 10, 100 and 1000 µL L⁻¹ of ethylene or 1300 µL L⁻¹ of propylene for 24 h at 20 °C, both found no stimulatory ethylene production effect. Only Akerman et al. (1993) observed the expected physiological stimulation after applying 100 µL L⁻¹ ethylene for 96 h at 22 °C.

The ethylene action inhibitor 1-methylcyclopropene (1-MCP) delays ripening of many horticultural crops (Watkins, 2006). Efficacy of 1-MCP depends on multiple factors like species, cultivar, maturity stage, concentration,

temperature and time of application (Blankenship & Dole, 2003). Results on touch-picked feijoa (section 3.3.2) and in a majority of cases, application of 1-MCP has resulted in no observed quality effects (Schotsmans et al., 2011; Velho et al., 2008). As an exception, application of 1-MCP at 1500 nL L⁻¹ for 8 h was found to retain firmness and skin colour during storage of 'Brazilian (242)' feijoa for 30 d (Amarante et al., 2008). A potential reason for the lack of observed 1-MCP response is that 'touch picked' feijoa may be in an advanced physiological maturity stage. Advanced maturity has previously been found to reduce 1-MCP efficacy in a number of other climacteric fruit crops including apple (Mir et al., 2001), pear (Chiriboga et al., 2013), banana (Golding et al., 1998; Pelayo et al., 2003), apricot (Fan et al., 2000), and fig (Sozzi et al., 2005). This study postulates that 1-MCP might be effective on feijoa harvested earlier than commercial touch-picked maturity, providing an opportunity to extend storage life. There is no previously published research on effects of 1-MCP in early harvested feijoa.

The aim of this study is to revisit the role of harvest timing on feijoa postharvest performance and investigate if 1-MCP and exogenous ethylene applied to early harvested fruit affects ripening and fruit deterioration under optimal storage conditions.

4.2 Materials and methods

4.2.1 Experiment design

The experiment comprised of a full matrix of three harvest times (4, 2 and 0 weeks before predicted commercial harvest) and three treatments: a 1-MCP treatment at 1000 nL L⁻¹ for 8 h prior to storage, an ethylene treatment of 1000

$\mu\text{L L}^{-1}$ during storage and air as a control. Each ethylene manipulation treatment (1-MCP, ethylene or air) had 3 replicates. All fruit were stored in a humidified flow-through atmosphere system of at 4 °C and 90% RH for 6 weeks.

Respiration and ethylene production rates were measured as indicators of physiological status. Weight, compression firmness, soluble solids content (SSC), titratable acidity (TA), internal maturity/ripening rating (locule development) and colour of skin and flesh were recorded as measures of quality.

4.2.2 Fruit sample

‘Unique’ feijoa (*Acca sellowiana* [Berg] Burret) were procured in three lots corresponding to the three harvest times of approximately 4 weeks before commercial harvest (H_{-4} , March 22nd), 2 weeks before commercial harvest (H_{-2} , April 3rd) and at commercial harvest (H_0 , April 16th). Early harvests (H_{-4} and H_{-2}) were performed using a higher force to remove the fruit, whereas in the third harvest (H_0), the ‘touch-picked’ method was used. Fruit were randomly harvested from the same block of ‘Unique’ trees located in a commercial orchard near Matamata, New Zealand. Fruit were packed in a single layered commercial corrugated fibreboard trays containing plix within 10 h after harvest at ambient temperature (≈ 20 °C). Later fruit were transported in an air conditioned commercial van (≈ 15 °C). Fruit were delivered to Massey University, Palmerston North on the day after harvest. It took 12 h to reach the laboratory in Massey University after packing the fruit.

At-harvest attributes were measured prior to any treatments were conducted (10 fruit per harvest). Fruit (405 fruit per harvest) of various sizes

(60.1 g average) were randomised between the ethylene treatments (135 per treatment) and labelled in mesh bags (15 fruit per bag) before treating (or not) with 1-MCP. Fruit (3 bags of 15 fruit per treatment replicate) were later placed in a PVC container (0.0135 m³) in a flow through system that delivered either air or ethylene (1000 µL L⁻¹) at 4 °C and 90% RH. One bag of fruit (15) from each PVC container (treatment replicate) was transferred to room temperature at two week intervals (2, 4 and 6 weeks) for quality assessment after storage (21 fruit per treatment) and a subsequent 5 d (24 fruit) at 20 °C. After removal from storage, fruit were allowed to equilibrate to 20 °C for 8 h before conducting any assessments. Fruit physiology and quality measurements were conducted on the same fruit.

4.2.3 1-MCP treatment

Pre-storage 1-MCP treatment used 0.14% water soluble powder 1-MCP (SmartFresh™, AgroFresh Inc., USA) applied to create 1000 nL L⁻¹ for 8 h. Treatment was conducted in a closed air tight 56 L high density polyethylene barrel at 20 °C. A predetermined quantity of 1-MCP was placed in an air tight plastic syringe (100 mL) to which 2 mL of deionised water (Milli-Q, Millipore Corporation, USA) was dispensed through a septum. The syringe was closed immediately and shaken vigorously for 2 minutes to allow the powdered 1-MCP to mix with water and release vapour into the head space of the syringe. Care was taken to ensure that there was no trace of powder left in the syringe. The syringe head space was then injected through an inlet valve into the plastic barrel with fruit (3 mesh bags). Three barrels were used as replicates. Bags of fruit for all other treatments were similarly sealed in other 56 L containers without 1-MCP. After 8 h, all barrels were ventilated for half an hour and fruit

were transferred to a flow-through storage system held at 4 °C and 90% RH. Bags (3) from each barrel were shifted into a single PVC container, representing a replicate.

4.2.4 Exogenous ethylene treatment

Ethylene was applied at 1000 $\mu\text{L L}^{-1}$ during storage by generating a gas mix from ripening gas having ethylene at 5% (BOC Ltd., Auckland, New Zealand) and dry air. The system was maintained at a constant flow rate of 200 mL min^{-1} using a digital flow meter (ADM 1000, J&W Scientific, Folsom, USA) and gas concentrations were checked on a weekly basis. Ethylene concentrations were checked by extracting gas samples from PVC containers using a 1 mL glass syringe and measured with a gas chromatograph (GC-8AIF, Shimadzu, Kyoto, Japan). The GC was fitted with a flame ionisation detector and a 3.2 mm diameter stainless steel column (Porapak Q 80/100, AllTech Associates, Auckland, New Zealand). Column temperature was 70 °C, injection temperature 120 °C and N_2 was used as a carrier gas at 35 mL min^{-1} . Hydrogen gas at 20 mL min^{-1} and air at 300 mL min^{-1} were used as fuel gases. The GC was calibrated using $10.1 \pm 0.5 \mu\text{L L}^{-1}$ ethylene β -standard (BOC Ltd., Auckland, New Zealand). Output signals were recorded using an HP integrator (HP3396A, Hewlett Packard, California, USA).

4.2.5 Fruit physiology

Respiration rate of the fruit was measured using the static method after removal from storage and after a subsequent 5 d at 20 °C. Fruit (6) of known weight were sealed in an air tight glass jar (1800 mL) with a rubber septum. Gas samples were collected twice from the headspace using a 1 mL syringe,

immediately after sealing the jar and again later after a known period of time. Incubation time was chosen to ensure that accumulation of CO₂ in the headspace did not exceed 0.5% (Hertog et al., 2004). Gas samples were analysed using a gas analyser fitted with a CO₂ infrared transducer (Analytical Development Company, Hoddesdon, UK) that uses N₂ as a carrier gas at a flow rate of 35 mL min⁻¹. The output signal was recorded on an integrator (HP3396A, Hewlett Packard, California, USA). Calibration of the gas analyser was conducted with commercially available 0.5% CO₂ β -standard (BOC Ltd., Auckland, New Zealand). Respiration rates representing carbon dioxide production rates (r_{co_2}) were calculated considering fruit weight and the resulting free volume in the jar and expressed in nmol kg⁻¹ s⁻¹.

Ethylene production rates were measured by flow-through method on a two week interval both after removal from storage and a subsequent 5 d at 20 °C. A laser-based ethylene detector (ETD-300, Sensor Sense B.V., Nijmegen, Netherlands) with a detection sensitivity of 0.3 nL L⁻¹ was used. Dry air at 83.3 mL min⁻¹ was circulated through an air-tight glass jar (980 mL) containing 6 fruit. Ethylene production rates were calculated with the method by Van den Dungen et al. (2011). Rate of ethylene gas produced (nL s⁻¹) was calculated by multiplying the measured concentration of ethylene (nL L⁻¹) with the known flow rate (L s⁻¹). The ethylene production rate (pmol kg⁻¹ s⁻¹) was derived using the ideal gas law and divided by the fresh weight (kg) of the fruit sample.

4.2.6 Fruit quality

One fruit bag (15 fruit) from each replicate was removed at two week intervals from storage (4 °C) for quality measurements at room temperature (20

°C). Fruit were allowed to equilibrate to room temperature for 8 h and quality was recorded on the same day for half the fruit (7). The remaining 8 fruit were left at 20 °C for a further 5 d prior to measuring quality. First, mass of each bag of fruit was measured to a precision of ± 0.001 g (PG503-S, Mettler Toledo, Greifensee, Switzerland). Weight loss was calculated as the percentage loss from the initial weight (measured prior to 1-MCP treatment).

Visual assessment of severity of skin injury due to browning (discolouration) was recorded as the estimated percentage of surface area covered (Fig. 3.1). Compression firmness was measured using a texture analyser (TA-Xt Plus, Stable Microsystems Ltd., Surrey, UK). A non-destructive compression of 2 mm was applied on an equatorial position using a 50 mm diameter flat plastic cylindrical probe at 1 mm s^{-1} . Average peak force (N) of 2 measures from opposite sides was used as firmness.

Skin and flesh colour was measured using a reflectance spectrophotometer (CM-2600D, Konica Minolta Sensing Inc., Osaka, Japan) with an 8 mm aperture diameter. Spectramagic NX software was used to calculate °hue and L^* . Skin colour was measured by averaged readings from three locations from the equatorial region and flesh colour was measured by halving the fruit at the equatorial region with a transverse slice and immediately taking three random measurements from a single side of the slice.

Visual assessment of maturity at harvest and internal ripening rating after storage was conducted by cutting the fruit at the equatorial region and ranking the status using the rating scale developed by Plant & Food Research, NZ (Schotsmans et al., 2011). Images of cut feijoas were scanned immediately by

placing them on top of a desktop flatbed scanner (Perfection 610 G750A, Epson, Singapore) at 600 dpi. Brightness and contrast were unchanged for uniform scanning during the course of the experiment. After at-harvest image capture, background light in the room was eliminated as a source of interference by covering the fruit sample on the scanner with a cardboard box.

Soluble solids content (SSC) in % was measured by squeezing the juice by hand onto a digital refractometer (PAL-1, Atago Ltd, Tokyo, Japan). Titratable acidity (TA) was measured by pooling juice extracted from the locular gel of at least 6 fruit per replicate. Juice was stored at -30 °C after freezing immediately with liquid N₂. Feijoa fruit juice (1 mL) was dispersed into 50 mL of de-ionised water and titrated against 0.1 N standard NaOH to reach an end point of pH 8.2 for equivalent malic acid using an automatic titrator (TitroLine, Schott instruments GmbH, Mainz, Germany). Titratable acidity was calculated using the method described in Sadler and Murphy (2010).

4.2.7 Data analysis

The General Linear Model (GLM) procedure was used to ascertain significant effects of treatments on measured attributes using Minitab (version 16.0, Minitab Inc., State College, Pennsylvania). As time in storage was considered as a factor, all data were analysed in a single ANOVA and significant differences caused by harvest timing, treatments and storage time were identified using Tukey's test at 95% confidence. Replicates analysed as a factor by ANOVA revealed no differences to influence the treatments of physiology/quality parameters. Internal ripening score was analysed using the chi-squared test on pooled data sets for each harvest timing.

4.3 Results

4.3.1 At harvest attributes

Fruit harvested at three different times were at diverse stages of maturity evidenced by differences in almost all quality variables (Table 4.1). During the last 4 week period of fruit growth, fruit weight increased by 12%, while firmness decreased by 50% and the surface colour became a lighter shade of green (decreased hue° and increased L*). Internally the fruit colour became less green (lower hue°) and darker (lower L*) which was reflected by a change in the internal maturity rating graded visually (Fig. 4.1). Given the current maturity standard harvests H₋₄ and H₋₂ would be considered immature at the time of harvest. Of all the attributes measured, only SSC did not change over the harvest period. Respiration rate of fruit showed a gradual increase with maturity of fruit.

Table 4.1 Average at harvest attributes of feijoa as influenced by harvest timing. Values with same letter in a row are not significantly different (p > 0.05)

Attribute	Units	H ₋₄	H ₋₂	H ₀	HSD _{0.05}	n
Relative harvest time	d	-25	-13	0		
Fresh weight (per bag)	g	835.1 ^b	935.0 ^a	935.5 ^a	32.1	27
Firmness	N	51.5 ^a	38.7 ^b	26.9 ^c	8.7	10
Skin colour	°hue	120.1 ^a	118.7 ^a	114.9 ^b	2.8	10
	L*	39.2 ^b	40.7 ^b	44.5 ^a	3.1	10
Flesh colour	°hue	92.2 ^a	90.9 ^{ab}	89.6 ^b	1.6	10
	L*	81.1 ^a	78.1 ^a	67.2 ^b	6.7	10
Maturity rating	-	1.0 ^b	1.0 ^b	2.6 ^a	0.3	10
SSC	%	10.7 ^a	11.6 ^a	11.6 ^a	1.1	10
Respiration rate (r _(CO₂))	nmol kg ⁻¹ s ⁻¹	309 ^b	315 ^{ab}	378 ^a	67	5

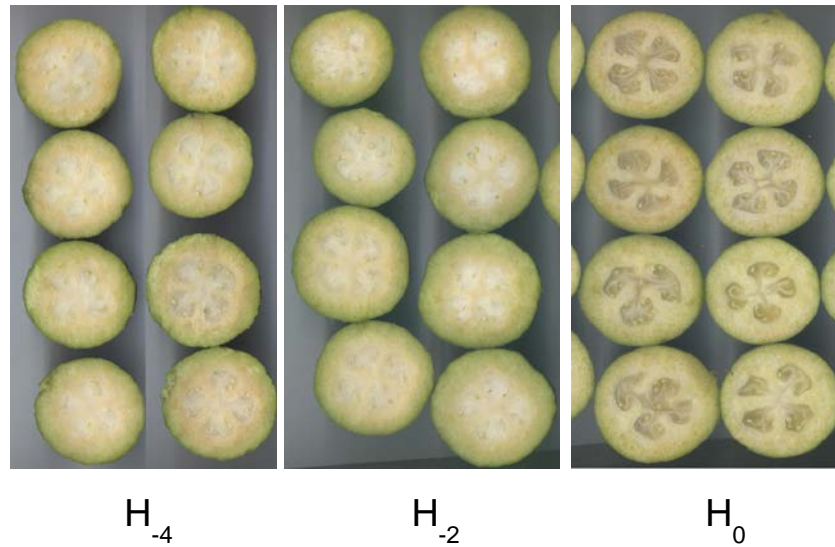


Fig. 4.1 Scanned images after equatorial halving of ‘Unique’ feijoa at harvest time before treatments

4.3.2 Fruit physiology

4.3.2.1 Respiration

Measured respiration rates (r_{CO_2}) after storage ranged between 315 to 701 $nmol\ kg^{-1}\ s^{-1}$ and generally increased with storage time and subsequent periods at 20 °C (Fig. 4.2B). However, in touch-picked fruit (H_0) respiration rates declined after 4 weeks of storage. In early harvested fruit (H_{-4} and H_{-2}) respiration rate was lowered by 1-MCP treatment in comparison to the respective controls while 1-MCP had no impact on touch picked fruit (H_0 ; Fig.4.2A). Exogenous ethylene had no effect on respiration rate irrespective of harvest timing.

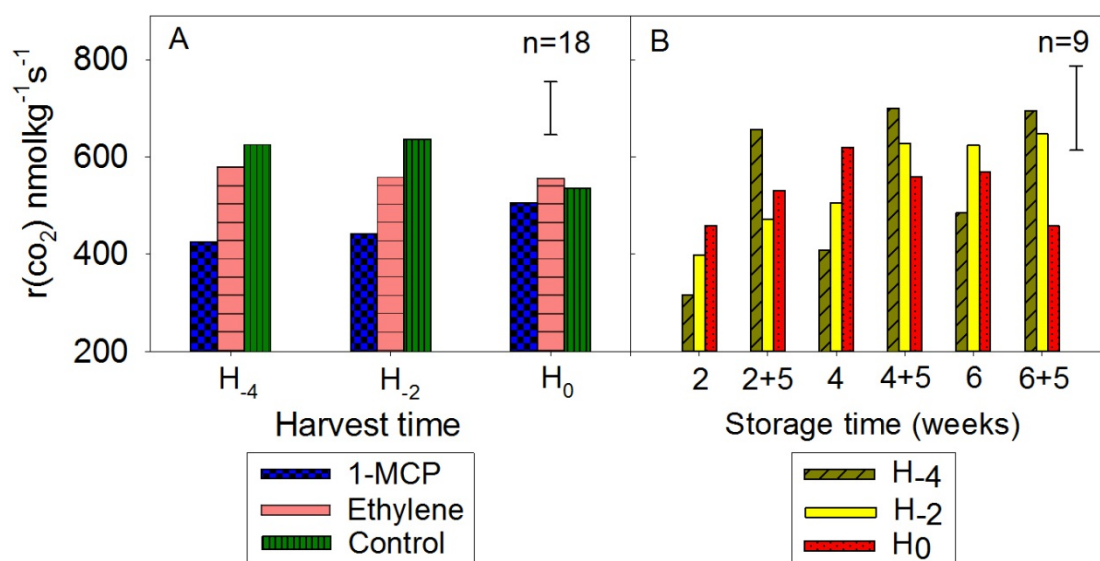


Fig. 4.2 Average respiration rate of 'Unique' feijoa after storage at 4 °C as influenced by (A) harvest timing and treatment and (B) time in storage and harvest timing. Fruit were assessed at two week intervals on removal and also after a subsequent 5 d at 20 °C. Data represent average measures either (A) for all storage times (n = 18) or (B) for all treatments (n = 9). The vertical bars represent HSD_{0.05} (Tukey's).

4.3.2.2 Ethylene production

Ethylene production rates similarly increased as a result of storage for all harvest times (Fig. 4.3B). Higher rates of ethylene production were observed when fruit were assessed after a further 5 d on removal from storage at 20 °C (Fig. 4.3B). Mean ethylene production rates of early harvested fruit (H₋₄ and H₋₂) after storage were lower (25-35 pmol kg⁻¹ s⁻¹) when compared to touch picked fruit (H₀; 57 pmol kg⁻¹ s⁻¹). Ethylene production rates were suppressed by 1-MCP treatment in H₀ but not in H₋₄ or H₋₂ (Fig. 4.3A). Exogenous ethylene treatment had no effect on ethylene production rates for any harvest time.

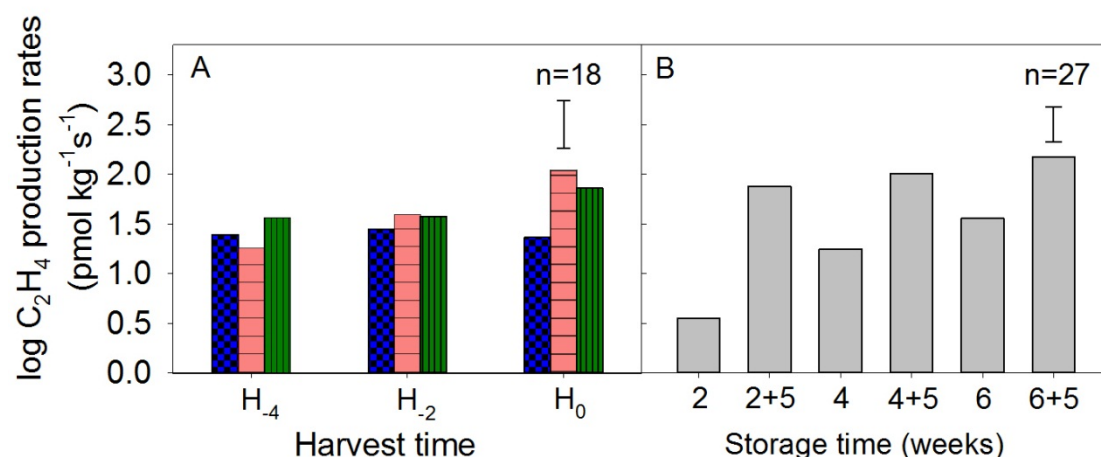


Fig. 4.3 Average ethylene production rates of 'Unique' feijoa after storage at 4 °C as influenced by (A) harvest timing and treatment, (B) storage time. Fruit were assessed at two week intervals on removal and also after a subsequent 5 d at 20 °C. Data represent average measures either (A) for all storage times (n = 18) or (B) for all treatments and harvest timings (n = 27). The vertical bars represent HSD_{0.05} (Tukey's)

4.3.3 Storage quality

There were no differences caused by ethylene treatment or harvest timing for weight loss during storage (data not shown), which was 1.2% of initial weight on average. Fruit showed no signs of shrivel after 6 weeks of storage probably due to the high relative humidity established in the flow through experimental system. At the end of 6 weeks of storage the incidence of surface injury (browning) was very low (1.7%) meaning that across the treatments statistical analysis of effects was unable to be conducted.

4.3.3.1 Firmness

Firmness declined progressively for all harvests and treatments during storage. Firmness of later harvests was lower not only at harvest (Table 4.1) but also after storage (Fig. 4.4A). After the period of 5 d at 20 °C fruit from H₂ were at a similar range of firmness (8.4-12.3 N) as H₀ (Fig. 4.4E). Treatment with 1-MCP retained firmness of early harvested fruit (H₄) when compared to the control fruit (Fig. 4.4A), but had no effect on either H₂ or H₀ fruit. Exogenous ethylene treatment during storage had no effect on firmness change for all 3 harvests.

4.3.3.2 SSC and TA

At harvest SSC was not different for the three maturities (Table 4.1), however the decline of SSC during storage was found to be influenced by harvest timing as H₄ lost SSC more rapidly than H₂ or H₀ (Fig. 4.4F). Neither 1-MCP nor exogenous ethylene had any effect on SSC during storage (Fig. 4.4B).

Mean TA of later harvests were lower after storage (Fig. 4.4G). TA after storage was not affected by either 1-MCP or exogenous ethylene treatments (Fig. 4.4C).

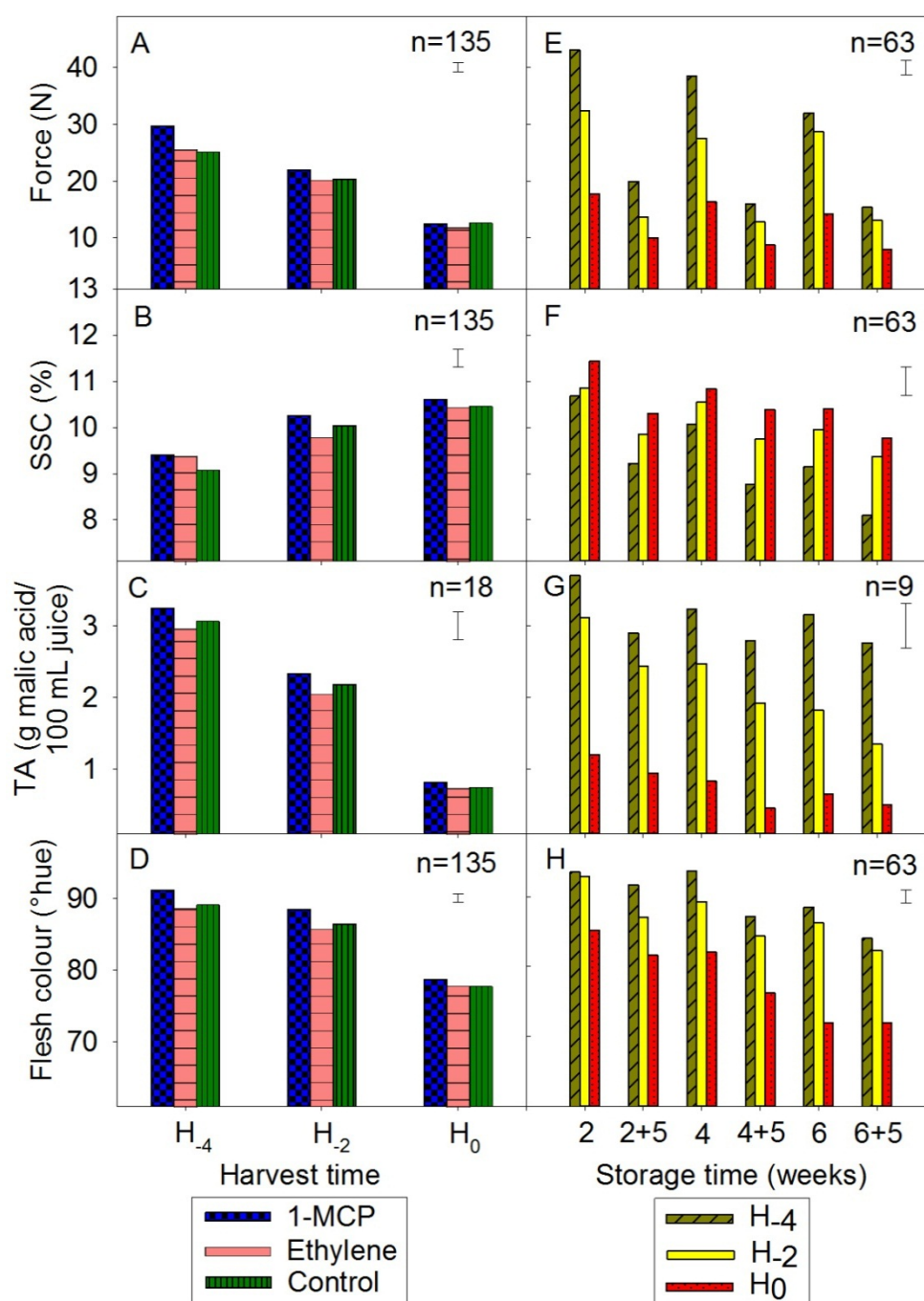


Fig. 4.4 Average compression firmness, SSC, TA and flesh colour of 'Unique' feijoa stored at 4 °C as influenced by (A-D) harvest timing and treatment; and (E-H) storage time and harvest timing. Fruit were assessed at two week intervals on removal and also after a subsequent 5 d at 20 °C. Data represent average measures either (A-D) for all storage times or (E-H) for all treatments. Number of independent measures per average is provided in each graph (n). The vertical bars represent HSD_{0.05} (Tukey's)

4.3.3.3 Skin colour

Neither harvest timing nor treatments (1-MCP or exogenous ethylene) resulted in differences of skin colour (°hue or lightness L^*) after storage (data not shown).

4.3.3.4 Flesh colour

Internal flesh colour (°hue) after storage was influenced by harvest timing. Both H_{-4} and H_{-2} were higher in °hue in comparison with H_0 fruit (Fig. 4.4D). Internal flesh colour (°hue) of H_{-4} and H_{-2} declined during storage, indicating maturation in the postharvest environment. For H_0 flesh colour change was more rapid as the internal flesh started to brown. H_{-2} after 6 weeks of storage reached the same °hue as H_0 after 2 weeks of storage potentially indicating an acceptable internal flesh colour (Fig. 4.4H). During storage 1-MCP treated fruit retained flesh colour when compared with respective controls in early harvested fruit (H_{-4} , H_{-2}) but 1-MCP treatment had no effect on H_0 fruit (Fig. 4.4D). Flesh colour (°hue) was not affected by exogenous ethylene treatment. Flesh lightness (L^*) was not influenced by either harvest timing or treatments during storage (data not shown).

Table 4.2 Contingency table of internal ripening scale of ‘Unique’ feijoa assessed at two week intervals after storage and a subsequent 5 d at 20 °C. Each count is the total from 135 assessed fruit. ‘ns’ indicates not significant at $p < 0.05$, value in parenthesis is contribution to Chi-square, χ^2 value of $H_{-4} = 34.676$, $H_{-2} = 17.802$ and $H_0 = 0.987$

(H ₋₄)	Internal ripening scale		Total
	< 2	≥ 2	
1-MCP	128 (4.88)	7 (17.32)	135
Ethylene	98 (0.51)	37 (1.81)	135
Control	90 (2.23)	45 (7.93)	135
Total	316	189	405

(H ₋₂)	Internal ripening scale		Total
	< 2	≥ 2	
1-MCP	93 (4.69)	42 (5.74)	135
Ethylene	71 (0.15)	64 (0.18)	135
Control	59 (3.16)	76 (3.88)	135
Total	223	182	405

(H ₀) ^{ns}	Internal ripening scale		Total
	≤ 3	> 3	
1-MCP	97 (0.2)	38 (0.44)	135
Ethylene	91 (0.03)	44 (0.07)	135
Control	90 (0.08)	45 (0.17)	135
Total	278	127	405

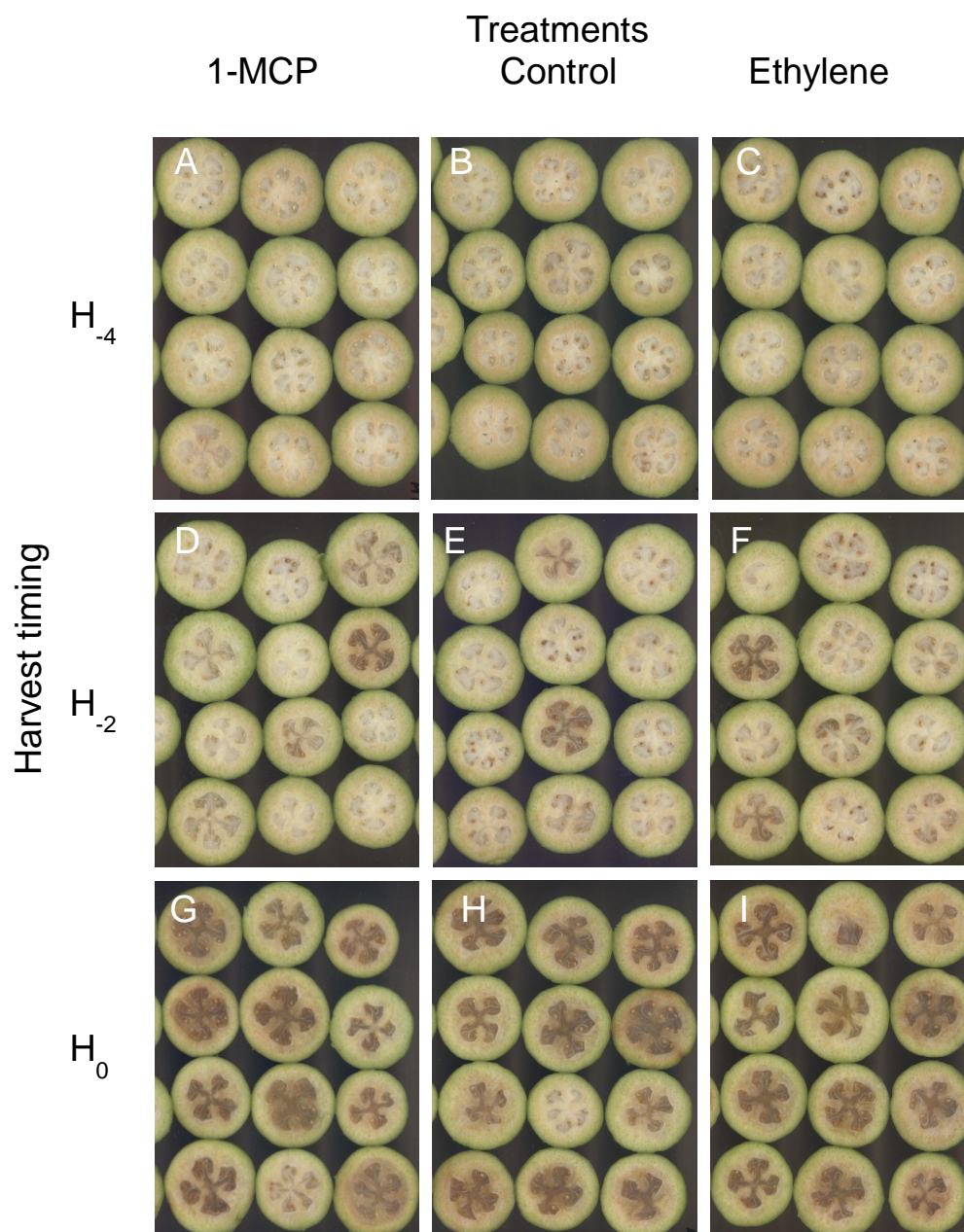


Fig. 4.5 Scanned images after equatorial halving of ‘Unique’ feijoa stored at 4 °C for 6 weeks and assessed after 5 d at 20 °C. Rows indicate harvest timing while columns represent treatments

Internal ripening rating of the fruit is based on visual assessment of locular development and flesh colour. Treatment with 1-MCP delayed ripening of early harvested (H_{-4} and H_{-2}) fruit whereas fruit harvested at H_0 were not affected (Table 5.2) confirming the flesh colour °hue findings (Fig. 4.4D). After 6 weeks

of storage and a subsequent 5 d at 20 °C, none of the assessed fruit were considered over ripe (internal ripening rating > 3, Table 4.2) from H₄ as internal browning of the locules was not observed (Fig. 4.5A-C). Fruit from H₂ displayed all the ranges from unripe to over ripe stages (Fig. 4.5D-F), and none of the fruit from H₀ were ranked unripe, with some considered as over ripe and unacceptable (Fig. 4.5G-I). Fruit treated with exogenous ethylene were ranked at a similar ripening rating as the control fruit in all three harvests (Table 4.2).

4.4 Discussion

Feijoa demonstrated climacteric behaviour (Reid, 1975); In this study feijoa harvested at different times and treated either with pre-storage 1-MCP or exogenous ethylene during storage had limited effect on ripening. Effects of harvest timing were more pronounced than those of postharvest ethylene manipulation for extending storage life.

4.4.1 Effects of harvest timing

Differences in physiological maturity at harvest were caused by harvest timing (Table 4.1). The decline in firmness with maturity was previously found by Al-Harthy (2010), Clark et al. (2005), Wiryawan et al. (2005) and Downs et al. (1988). There were small but statistically significant changes in skin colour between H₂ (118.7 °hue) and H₀ (114.9 °hue) suggesting the potential usefulness of this quality parameter in determining harvest maturity. Harman (1987) found climacteric rise in respiration 90 d after anthesis and in our study we observed gradual increase in respiration rates with fruit maturity (Table 4.1). SSC remained unchanged by harvest timing, in contrast to Downs et al. (1988) and Clark et al. (2005) who reported higher SSC in immature fruit.

Quality differences after storage were largely due to harvest timing. After six weeks of storage and a subsequent 5 d, H₄ fruit appeared unripe as per the internal ripening (Fig. 4.5 A-C; Table 4.2) and had low SSC with a high TA (Fig. 4.4F-G). At the same time, H₀ fruit appeared over ripe and inedible as fruit developed internal browning (Fig. 4.5 G-I; Table 4.2). Only H₂ fruit demonstrated ripening behaviour (locular clearing) and appeared saleable (i.e. a lack of browning) after 6 weeks of storage and a further 5 d (Fig. 4.4H, Fig. 4.5D-F). Our results indicate that H₂ may store for a longer time than fruit harvested at touch picked maturity (H₀). Similar findings on longer duration to ripen postharvest due to earlier harvest maturity have been reported for pears (Elgar et al., 1997). Firmness and flesh colour (Fig. 4.4E, H) appear suitable after 6 weeks of storage for H₂, as they are similar to H₀ after 2 weeks of storage. However at the same time, the lower SSC and higher TA than H₀ after 2 weeks storage (Fig. 4.4B-C) suggest that the H₂ fruit stored for 6 weeks may have a different taste profile. SSC and TA content play a critical role in determining the liking of fruit by consumers (Crisosto & Crisosto, 2001; Jayasena & Cameron, 2008). Therefore, while manipulating harvest timing for feijoa to 2 weeks prior to touch-picking offers potentially visually acceptable fruit that can withstand 6 weeks of storage, there is a need for investigating consumer acceptance of taste before widespread application.

4.4.2 Effects of ethylene manipulation

Mean ethylene production rates (3.8-178 pmol kg⁻¹ s⁻¹) for all harvests (Fig. 4.3B) were within the ranges reported earlier (Al-Harthy, 2010; Reid, 1975). However Velho (2011) found higher rates of up to 219 nmol kg⁻¹ s⁻¹ in 'Brazilian' feijoa during 14 d shelf life at 23 ° C. Respiration rates were within the

ranges (Fig. 4.2B) of earlier findings for feijoa (Al-Harthy, 2010; Biale et al., 1954). Results indicate that early harvested feijoa (H₄) responded to 1-MCP treatment by suppressing respiration rate (Fig. 4.2), softening (Fig. 4.4A) and loss of flesh colour (°hue; Fig. 4.4D). Internal ripening ratings (Table 4.2) confirm that 1-MCP delayed ripening in H₄ and H₂ (Fig. 4.1; Table 4.2). However for touch picked fruit (H₀) only ethylene production was observed to be influenced by 1-MCP application, with no observed effects on quality outcomes. This limited effect of 1-MCP on touch-picked (H₀) fruit mimics the results of our earlier findings (section 3.3.2). Velho et al. (2008) also found no change in firmness, SSC or TA by 1-MCP treatment. In contrast, Amarante et al. (2008) found that 1-MCP retained firmness and skin °hue in 'Brazilian (242)' feijoa.

Our results illustrate the importance of maturity stage at harvest on the efficacy of 1-MCP treatment (Blankenship & Dole, 2003). Applying 1-MCP to feijoa earlier than the present commercial touch picking maturity has a significant subsequent effect on ripening. Similar maturity effects have been observed in other horticultural crops like apple (Mir et al., 2001), apricots (Fan et al., 2000), banana (Golding et al., 1998) and pears (Chiriboga et al., 2013). However, effects on feijoa quality after storage were more heavily influenced by harvest maturity than 1-MCP treatment and hence 1-MCP is not recommended for commercial application.

Generally for mature climacteric fruit addition of ethylene is expected to trigger further ethylene production and potentially advance ripening. For feijoa, at the time of harvest fruit appears to be at a pre-climacteric stage as ethylene production rates were below detectable levels (<0.08 pmol kg⁻¹s⁻¹). Fruit harvested early (H₂) were immature and ripened during storage with rise in

ethylene production rate ($35 \text{ pmol kg}^{-1} \text{ s}^{-1}$). However, there were neither difference in fruit physiology (respiration, Fig. 4.2; and ethylene production, Fig. 4.3) nor in quality (Fig. 4.4, Fig. 4.5 and Table 4.2) during storage caused by exogenous ethylene treatment in comparison to the control treatment, despite being applied at very high concentration ($1000 \text{ }\mu\text{L L}^{-1}$) throughout the storage period. Our result is in agreement with those reported previously by Velho et al. (2008) and Al-Harthy (2010). Only Akerman et al. (1993) reported a dramatic increase in respiration rate and ethylene production with a reduction in TA by ethylene treatment. In closely related guava (*Psidium guajava*), Azzolini et al. (2005) observed insensitivity to exogenous ethylene. These results indicate that feijoa response to application of ethylene is in contrast to the conventional classification of climacteric fruit where system 2 ethylene production is expected to be stimulated (McMurchie et al., 1972). Pech et al. (2008) suggested that ripening in climacteric melons was regulated by both ethylene-dependent and-independent pathways. It is possible that endogenous ethylene within feijoa tissue may already saturate ethylene dependent processes making them insensitive to additional exogenous ethylene. Johnston et al. (2009) proposed a conceptual model for individual ripening attributes in apple that demonstrates that response curves saturate at a particular level of exposure to ethylene, making the tissue insensitive to higher concentrations. If this hypothesis for the lack of quality change response to exogenous ethylene is correct, questions still remain as to the purpose of the climacteric increase in ethylene production that occurs during feijoa ripening. It is possible that ethylene is required to stimulate the production of the complex and unique volatile profile that is known to develop during postharvest ripening (Al-Harthy, 2010).

Our results demonstrate that feijoa responded to inhibition of ethylene action at an early maturity stage but only very subtly at commercial touch picking time. However, after storage fruit harvested early were found to be low in SSC and high in TA with neither of these properties being affected by either 1-MCP or ethylene (Fig. 4.4B-C). This leads us to ponder what would result if ethylene production were inhibited during the final stages of fruit maturation on the tree. Potentially limiting ethylene production or action may assist in reducing maturation, (as measured by softening and loss of flesh colour) while enabling manipulation for the SSC and TA profiles after storage. It will be interesting to study fruit response to preharvest ethylene inhibition that may contribute to storage life extension in feijoa.

4.5 Conclusion

Commercially harvested fruit (touch-picked) can be stored for only 4 weeks whereas fruit harvested earlier by 2 weeks appear visually acceptable after 6 weeks of storage at 4 °C. For these earlier harvested feijoa, firmness and internal ripening appear suitable for edibility but lower SSC and higher TA may impact their taste. There may be potential to extend storage life of feijoa through earlier harvesting should the resulting product be found to be acceptable to consumers' taste.

During storage, feijoa demonstrated a rise in respiration and ethylene production rates as expected of a climacteric fruit. Efficacy of 1-MCP was limited to fruit harvested earlier than commercial touch-picking maturity, and tended to have less effect than harvest maturity itself on after-storage quality. Treatment with 1-MCP suppressed respiration rate, softening and losses in flesh colour (°hue). However skin colour, SSC and TA remained unaltered by 1-

Manipulating harvest maturity and ethylene to extend storage life of feijoa

MCP application irrespective of harvest timing. Feijoa were insensitive to exogenous ethylene application at all harvest times suggesting that endogenous production saturates ethylene responses. This lack of response to exogenous ethylene suggests that feijoa do not behave as expected of a fruit classified as climacteric. Further studies using preharvest ethylene inhibition may contribute in retaining quality at harvest and extend storage life of feijoa

5 Preharvest application of Aminoethoxyvinylglycine (AVG) to delay harvest maturity and extend storage life of ‘Unique’ feijoa

5.1 Introduction

Feijoa (*Acca sellowiana*) is a climacteric fruit with a storage life of 4 weeks at 4 °C and 90% relative humidity with a subsequent 5-7 d at 20 °C (Klein & Thorp, 1987). At least 6 weeks are required to sea freight feijoa to distant markets in Europe and North America in acceptable condition (Al-Harthy, 2010). Extending postharvest storage life of feijoa has previously been attempted subsequent to harvest using low temperature storage (Klein & Thorp, 1987), calcium treatments (Ramírez et al., 2005), temperature manipulations (Woolf et al., 2006), controlled atmospheres (Al-Harthy et al., 2010a; Al-Harthy et al., 2010b; East et al., 2009) or applying 1-MCP (Amarante et al., 2008; Velho et al., 2008) in our previous work (chapter 3 and 4) with limited success.

Ethylene plays a critical role in the life cycle of higher plants (Pech et al., 2012; Yang & Hoffman, 1984). Ethylene induced abscission poses problems in handling harvest of fruit, vegetable and flower (Reid, 1985). In apple, losses due to early fruit drop can be as high as 50% of crop yield (Greene, 2002). Ethylene production is inhibited by aminoethoxyvinylglycine (AVG) during climacteric fruit ripening (Lieberman, 1979). During the biosynthesis of ethylene, S-adenosylmethionine (SAM) is converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by the rate limiting activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) enzyme (Kende, 1993). AVG inhibits the activity of ACS and

restrains the production of ACC, the immediate precursor of ethylene (Yu & Yang, 1979). AVG is the most effective inhibitor of ACS enzyme among vinylglycine analogs like rhizobitoxine or methoxyvinylglycine (Yang & Hoffman, 1984). Huai et al. (2001) reported that AVG has specificity for ACS enzyme. This report identifies AVG binding close to cofactor pyridoxal-5'-phosphate (PLP) and suggests that α -amino and α -carboxylate groups may stimulate binding of substrate SAM to ACS. Although, there is no evidence of AVG inhibiting other enzymes in Huai et al. (2001) and it is not conclusive that AVG works solely on ACS as the only mechanism in which it affects fruit physiology. There are other inhibitors of ACS enzyme like polyamines (putrescine, spermidine and spermine) and vinylglycine which are not commercially popular (Jakubowicz, 2002).

Preharvest application of AVG 1 to 4 weeks prior to harvest inhibits biosynthesis of ethylene and influences abscission, ripening and senescence and reduces quality loss after harvest in many crops (Lurie, 2008; Martinez-Romero et al., 2007; Pech et al., 2012). Halder-Doll and Bangerth (1987) found that apple trees treated 4 weeks before harvest by AVG suppressed synthesis of autocatalytic ethylene production and retained higher firmness than control fruit. Application of AVG 14 or 7 d before harvest delayed maturity and ripening during storage of pears (Clayton et al., 2000). Likewise Cetinbas et al. (2012) found that AVG sprayed 7 d before commercial harvest of peach significantly delayed maturation and improved storage performance while in nectarines Torrigiani et al. (2004) found that ripening was delayed when AVG was applied 3 to 1 week before harvest date. Bregoli et al. (2002) found that peaches

treated 8 d before harvest by AVG inhibited ethylene production and increased SSC. Jobling et al. (2003) found that AVG treated plums allowed a delay in harvested timing, and resulted in retaining higher firmness and SSC in comparison to untreated fruit, suggesting that accumulation of soluble solids can take place when fruit are on trees for longer.

Previously, feijoa harvested earlier than touch picking maturity were found to store for longer, however this maturity resulted in low soluble solids content (SSC) and high titratable acidity (TA) after six weeks of storage, which is expected to impact their taste (Fig. 4.4). This difference in post-storage °brix was observed despite no differences in °brix being observed at the time of harvest. Treating feijoa with AVG and harvested at touch picking time are hence expected to have similar °brix to touch picked fruit but may be physiologically less mature due to the inhibition of ethylene. Hence, potentially, these feijoa may store for as long as early harvested fruit, and may have delayed the °brix losses of touch picked maturity fruit. To the best of our knowledge there is no publication to date on the effects of a preharvest ethylene inhibitor (AVG) on the subsequent harvest and post storage quality of feijoa.

The aim of the research is to investigate the impact of preharvest AVG on quality at harvest and evaluate storage performance of fruit stored under 4 °C and 90% relative humidity.

5.2 Materials and Method

5.2.1 Experiment design

The experiment comprises of three aminoethoxyvinylglycine (AVG) treatments applied 4 or 2 weeks before estimated “touch picked” harvest maturity (AVG₋₄ and AVG₋₂) with untreated fruit as a control (CNT). In addition fruit were harvested at two times, an estimated 2 weeks prior to (E) and at touch picking (T) maturity (Table 5.1).

Mature cv. ‘Unique’ feijoa trees of uniform canopy, located in a commercial orchard in Matamata, New Zealand were used for conducting the experimental trial. There were 79 trees (4 m x 3 m spacing) planted in 5 parallel rows in a continuous plot within the orchard. The layout was divided into a randomized complete block design of 3 blocks (approximately 26 trees per block) with each block representing a replicate of AVG treatment. A pair of trees was randomly selected in each block representing a treatment. Care was taken to ensure that each treatment within a block was not adjacent to another treatment with trees adjoining treatments used as buffers to limit the likelihood of cross contamination during AVG application.

Fruit drop was monitored by counting the fruit under each tree in the experimental plot on every second day. Fruit drop occurred only during the week before commercial harvest time which is widely noted as an indicator to initiate touch-picking by feijoa growers.

Table 5.1 Experiment matrix showing AVG treatment combination as influenced by AVG application and harvest timing

	AVG timing		
Harvest Timing	4 weeks before (AVG₋₄) (19 March))	2 weeks before (AVG₋₂) (2 April)	Not applied (CNT)
Early (E) (2nd April)	AVG ₋₄ E	N/A	CNTE
Touch Picking (T) (16th April)	AVG ₋₄ T	AVG ₋₂ T	CNTT

5.2.2 AVG application

AVG is commercially available as a water soluble powder formulation of 150 g kg⁻¹ (ReTain™, Valent BioSciences Corporation, Illinois, USA). AVG powder was applied at the rate of 0.83 g L⁻¹ of water (equivalent to 125 mg L⁻¹ of AVG) along with 1 mL L⁻¹ of 0.1% v/v organosilicone surfactant (Freeway™, Nufarm Ltd., Auckland, New Zealand). Application was conducted on March 19th (AVG₋₄) and on 2nd April (AVG₋₂) in the 2013 season. A knapsack sprayer (Model 475, Solo, Hamilton, New Zealand) was used to apply the chemical on fruit and leaves at the rate of 5 L per tree.

5.2.3 Fruit sampling

An initial fruit sample was drawn at the start of the experiment (4 weeks prior to touch picking maturity) before treating with AVG. Representative fruit were sampled from a randomly selected secondary branch of each treatment tree. All the fruit on a secondary branch were removed to maintain uniformity among treatment trees. Commercial sizing (> 45 g) of the fruit population was

not possible as average mass was lower (33.4 g). Two fruit from each tree (having highest mass) were used to assess quality (30 fruit) before AVG application.

Commercially feijoa are harvested by growers based on the at near to natural fruit drop, in a procedure known as 'touch-picking' the fruit with gently force (Thorp & Bieleski, 2002). Commercial harvest time was determined by an experienced orchard management team. At each harvest time, one tree per treatment per block was harvested by removing all fruit from the tree. Fruit numbers and weight were recorded to analyse effects of treatments on feijoa yield. Harvesting was conducted (Table 5.1) with all the fruit recovered from each tree at two times (early and touch-picked). Greater force was used to pick fruit during early harvest operation than at commercial touch-picking time. No harvesting tool was employed during picking of the fruit. At each harvest, individual fruit weighing > 45 g (the current minimal commercial grade standard) were segregated for use in the storage experiment. Fruit (55) drawn from each tree represented a sample replicate, resulting in each treatment comprising of 165 fruit (from 3 blocks of the orchard). At harvest assessments were performed on sample fruit (30 per treatment) and the rest of the fruit (135 per treatment) were stored under 4 °C and 90% relative humidity within single layered commercial corrugated fibreboard trays containing plix and a polyliner wrap of the fruit.

Fruit were assessed after 4, 6 and 8 weeks of storage on removal and after a subsequent 5 d at 20 °C. Upon assessment fruit (15 per replicate, 45 per treatment) were allowed to equilibrate to room temperature (20 °C) for 8 h after

which assessments of quality were conducted on the same day (21 fruit per treatment) and remaining fruit (24) were left for a further 5 d for later measurements. Fruit physiology (respiration and ethylene production rates) and quality assessments were performed on the same fruit.

5.2.4 Respiration rate and ethylene production

Fruit were assessed after removal from storage (4 °C) and allowing them to equilibrate to room temperature (20 °C) for 8 h and subsequently after 5 d. Respiration rate was measured as carbon dioxide production rate using the static method. Fruit (3) of known mass were placed in an air tight glass jar (980 mL) equipped with rubber septum to enable sampling of gas. Two gas samples were drawn, one immediately after closing the lid and later after a known time using 1 mL syringes. Care was taken to ensure that accumulation of CO₂ in the jar did not exceed 0.5% (Hertog et al., 2004). Gas samples were analyzed using a CO₂ infrared transducer (Analytical Development Company, Hoddesdon, United Kingdom) that uses N₂ as a carrier gas with a flow rate of 35 mL min⁻¹. The output signal was analysed by integrator (model 3396A, Hewlett Packard, California, USA). Calibration of the gas analyser was conducted using commercially obtained 0.5% CO₂ β-standard (BOC, Palmerston North, New Zealand). Respiration rates (r_{CO_2}) were calculated based on fresh weight and resulting free volume in the jar and expressed in nmol kg⁻¹ s⁻¹.

Ethylene production rates were measured by flow through method during storage at 4 °C on a weekly basis as well as after fruit removal and a subsequent 5 d at 20 °C. A laser-based ethylene detector (ETD-300, Sensor

Sense B.V., Nijmegen, Netherlands) with a detection sensitivity of 0.3 nL L⁻¹ was used. Ethylene free air was flowed through an air tight glass jar (980 mL) containing 3 fruit. Ethylene production rates were calculated with the method by Van den Dungen et al. (2011). Rate of ethylene gas produced (nL s⁻¹) is calculated by multiplying the measured concentration of ethylene (nL L⁻¹) with the known flow rate (L s⁻¹). The ethylene production rate (pmol kg⁻¹ s⁻¹) is derived using the ideal gas law and dividing by the fresh weight (kg) of the fruit sample.

5.2.5 Fruit quality

Fruit (15 per replicate) from each treatment (45) was removed every second week from 4 weeks of storage for subsequent quality measurement at 20 °C. Fruit were allowed to equilibrate to 20 °C for 8 h. Quality was recorded on the same day for half the fruit (21). The remaining 24 fruit were stored at 20 °C for a further 5 d prior to measuring quality. First, mass of each fruit (45) was measured to a precision of ±0.001 g (PG503-S, Mettler Toledo, Greifensee, Switzerland). Weight loss was calculated as the percentage loss from the initial weight (measured prior to storage).

Full details on quality measures are provided in section 4.2.6

5.2.6 Data analysis

The General Linear Model (GLM) procedure was used to ascertain significant effects of treatments on measured parameters using Minitab (version 16.0, Minitab Inc., State College, Pennsylvania). As time in storage was considered as a factor, all data were analysed in a single ANOVA. Significant

differences between treatments were identified using Tukey's test at 95% confidence. Orchard block effects analysed as a factor by ANOVA revealed no influence on the parameters studied, and hence results are presented as averages from all three replicates. Treatment effects on internal ripening index was evaluated using chi-square test on pooled sets of data from the range of storage lengths (i.e. 4 to 8 weeks).

5.3 Results

5.3.1 At harvest attributes

Natural fruit drop was only observed in the week before the touch picking treatment was conducted. Preharvest AVG treatment resulted in reduction of fruit drop in comparison to untreated trees (Table 5.2). Fruit treated with AVG₄T and AVG₂T had lower fruit drop (0.64 and 2.76% respectively) than untreated CNTT (7.87%). All the dropped fruit were of saleable size (> 45 g).

Table 5.2 Total preharvest fruit drops at commercial touch picking maturity (T) in ‘Unique’ feijoa trees treated with AVG₋₄ (4 weeks before commercial harvest) and AVG₋₂ (2 weeks before commercial harvest). Each count represents the sum of 3 trees. Pearson chi-square = 71.5 ($p < 0.001$), values in parenthesis are contributions to chi-square

Treatment	Dropped fruit	Remaining fruit	Total
AVG ₋₄ T	6 (23.41)	929 (0.89)	935
AVG ₋₂ T	29 (2.34)	1018 (0.09)	1047
CNTT	71 (43.21)	831 (1.65)	902
Total	106	2776	2884

Fruit growth increased in the last four weeks as fresh weight more than doubled from the first AVG treatment to those at touch picked time (Table 5.3). Mean fresh weight of early harvest (E) were approximately 30% lower than those picked at commercial harvest (T). Mean saleable fruit yield per tree (> 45 g) of the early harvest (3.8 kg) was substantially lower than those at touch picked time (11.6 kg). Average saleable fruit (> 45 g) per tree were 35% of total yield (by numbers) in early harvest which increased to 54.6% at commercial touch-picking time (data not shown). Feijoa yields in AVG treated trees were comparable to the controls at both harvest times ($p > 0.05$; Table 5.3)

Table 5.3 Mean attributes of 'Unique' feijoa treated with AVG and harvested early (E) at 2 weeks and (T) at 0 weeks before touch picking maturity. Mean values with the same letter in a row are not significant ($p > 0.05$)

Attribute	Units	Before treatment	Harvest (E)		Harvest (T)			HSD	<i>n</i>
			AVG _{4E}	CNTE	AVG _{4T}	AVG _{2T}	CNTT		
Relative treatment time	d	-	-28	-	-28	-14	-	-	3
Relative harvest time	d	-28	-14	-14	0	0	0	-	3
Fruit per tree	n	-	253 ^a	153 ^a	309 ^a	339 ^a	277 ^a	336	3
Fruit (> 45)	n	-	71 ^a	61 ^a	176 ^a	205 ^a	128 ^a	211	3
Fruit wt.	g	33.4 ^c	54.2 ^b	50.5 ^b	74.2 ^a	76.3 ^a	76.5 ^a	9.0	30
Firmness	N	62.2 ^a	45.6 ^b	46.7 ^b	40.2 ^c	38.2 ^{cd}	34.4 ^d	5.4	30
Skin colour	°hue	121.8 ^a	119.1 ^b	119.8 ^{ab}	116.0 ^c	114.5 ^c	116.1 ^c	2.1	30
	L*	40.4 ^c	42.6 ^b	43.3 ^{ab}	45.0 ^a	44.1 ^{ab}	45.4 ^a	2.1	30
Flesh colour	°hue	89.7 ^{cd}	92.4 ^a	91.9 ^{ab}	88.5 ^d	89.0 ^d	90.8 ^{bc}	1.3	30
	L*	78.5 ^a	78.8 ^a	78.4 ^a	72.4 ^b	70.9 ^b	71.5 ^b	3.8	30
SSC	%	11.0 ^b	11.5 ^{ab}	11.7 ^{ab}	11.7 ^a	11.8 ^a	12.2 ^a	0.7	30
TA	g malic acid/100 mL juice	1.9 ^b	3.9 ^a	4.6 ^a	2.6 ^b	2.2 ^b	2.2 ^b	1.1	3
Respiration rate	nmol kg ⁻¹ s ⁻¹	320 ^{bc}	260 ^c	350 ^{bc}	450 ^{ab}	410 ^{abc}	580 ^a	180	3

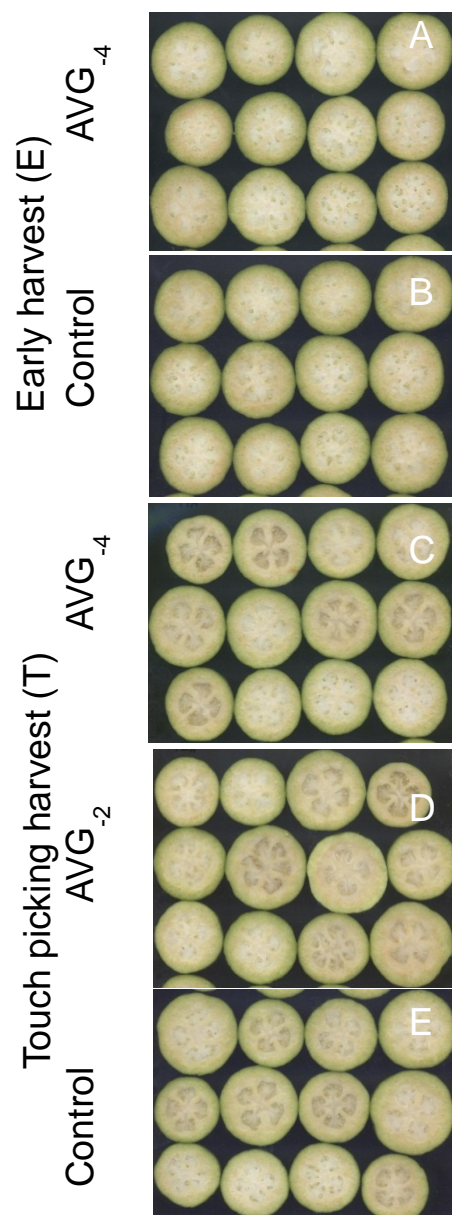


Fig. 5.1 At harvest scanned images after equatorial halving of ‘Unique’ feijoa harvested 2 weeks before (A-B) and at commercial touch-picking time (C-E).

Fruit were significantly firmer with more green skin colour (higher °hue) before applying AVG treatment (Table 5.3). Fruit harvested early (E) had higher firmness; a more green (higher °hue) and darker (lower L*) skin; more green (higher °hue) and lighter (higher L*) flesh; higher TA and lower respiration rate in comparison to fruit harvested at touch picking maturity (T). The only

measured attribute that did not change with harvest timing was SSC. Ethylene production rates at early harvest were below detectable levels ($< 0.08 \text{ pmol kg}^{-1} \text{ s}^{-1}$) while at touch picking maturity mean production rates were $< 1 \text{ pmol kg}^{-1} \text{ s}^{-1}$ with no differences between treatments observed (data not shown).

There were no changes in quality due to AVG treatments at early harvest (Table 5.3). However, at touch picking (T) harvest AVG treatment 4 weeks prior to harvest (AVG₋₄T) retained firmness and reduced flesh colour change (°hue). Skin colour, SSC and TA were not affected by AVG treatments. Irrespective of AVG treatment, all fruit were ranked immature (< 2) at early harvest, while a mix of both mature (2-3) and immature fruit were observed at touch picking time (Fig. 5.1).

5.3.2 Performance in storage

5.3.2.1 Fruit physiology

Ethylene production rates increased during storage (4 °C) in all treatments (Fig. 5.2A). Mean ethylene production rates at the climacteric maximum ranged between $10.8\text{-}14.9 \text{ pmol kg}^{-1} \text{ s}^{-1}$ in the control fruit for both harvests. Fruit harvested early produced ethylene at a lower rate in comparison to the touch picked harvest. Ethylene production rate was suppressed in fruit treated with AVG. The combined effects of harvest and AVG resulted in the AVG₋₄E treatment producing the least ethylene ($0.27\text{-} 5.33 \text{ pmol kg}^{-1} \text{ s}^{-1}$) throughout storage.

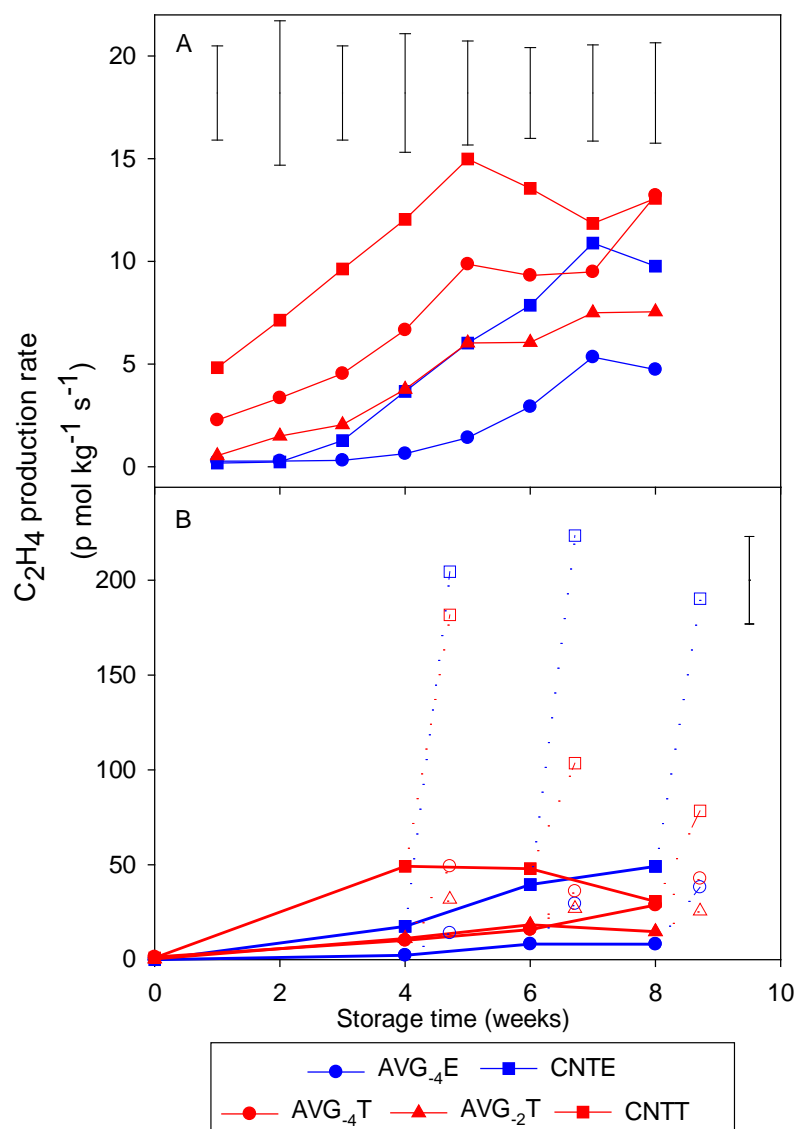


Fig. 5.2 Ethylene production rates of 'Unique' feijoa stored for 8 weeks and assessed (A) at 4 °C and (B) after removal (solid symbols) and a subsequent 5 d (hollow symbols) at 20 °C. Each data point represents mean from at least 3 measurements of 3 fruit. The vertical bars represent HSD_{0.05}

As to be expected, on removal from storage, ethylene production rates were higher, especially when assessed after a subsequent 5 d at 20 °C (Fig. 5.2B). Ethylene production rates were suppressed by AVG treatment when compared to the untreated fruit especially after a considerable time at 20 °C. Early harvest control fruit (CNTE) produced higher levels of ethylene than touch

picked fruit (CNTT) after 5 d at 20 °C. Similar to the behaviour during storage, the AVG₋₄E treatment continued to produce lowest ethylene rates.

Mean respiration rates of early harvested fruit (680-790 nmol kg⁻¹ s⁻¹) were higher (≈35%) than those of touch picked fruit (510-530 nmol kg⁻¹ s⁻¹). The mean respiration rate of fruit treated with AVG were lower than control fruit for the early harvest while AVG had no effect on respiration rate of touch picked fruit (Fig. 5.3A). After removal from storage respiration rates were higher when assessed subsequently after 5 d at 20 °C (Fig. 5.3B) after 4 and 6 weeks storage, but not after 8 weeks storage.

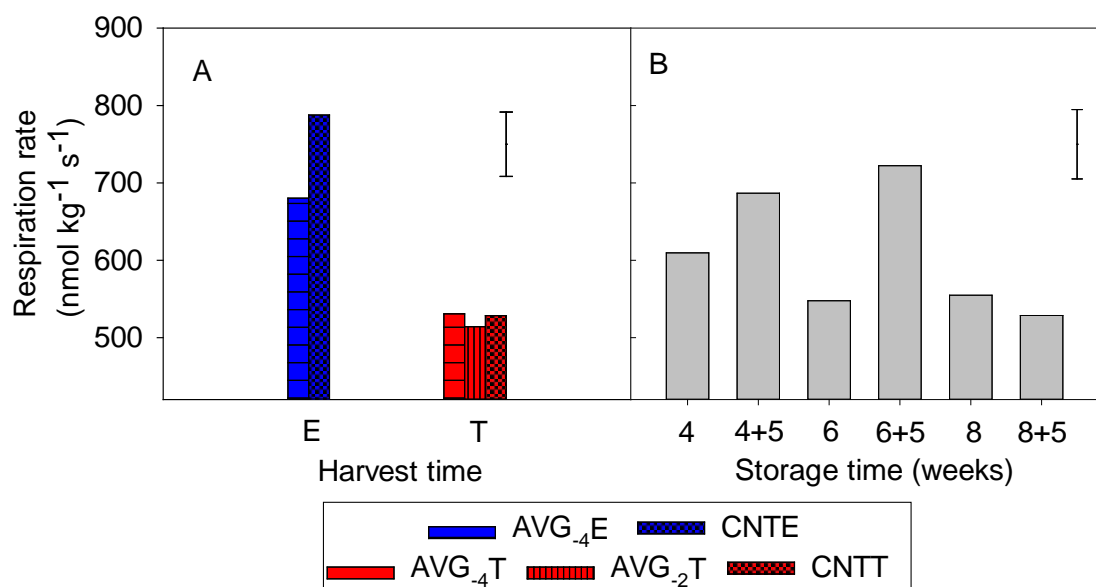


Fig. 5.3 Effects of AVG treatment (A) and storage time (B) on respiration rates of 'Unique' feijoa stored at 4 °C for 8 weeks and assessed after removal fortnightly from 4 weeks and subsequently after 5 days at 20 °C. Harvest times were at 2 weeks before (E) or at touch picking maturity. Each data point represents mean from at least 42 (A) or 30 (B) independent measurements of 3 fruit. The vertical bars represent HSD_{0.05}

5.3.2.2 Quality

There were no weight loss differences caused by AVG treatment or harvest timing during storage (data not shown), which ranged between 0.9-1.6% of initial weight on average after 8 weeks storage. Fruit showed no signs of shrivel after 8 weeks of storage. Quality (firmness, SSC, TA and flesh colour) declined during storage and was more rapid (except for TA) after removal during the subsequent 5 d at 20 °C (Fig. 5.4). Quality after storage was affected far more by harvest timing than by AVG treatment. Fruit harvested 2 weeks earlier than commercial harvest time remained firmer and retained flesh colour, were higher in TA (> 2.6 g malic acid/100 mL juice) and low in SSC (<9.0%) after 6 weeks of storage. AVG treatment had no effect on firmness, total soluble solids, titratable acidity and flesh °hue (Fig. 5.4). After storage, internal ripening index confirmed the differences due to harvest timing rather than by AVG treatment (Table 5.4).

Fruit harvested early (E) had a higher number of fruit that were ranked immature (<2) than those harvested at touch picking (T) time at harvest (Fig. 5.1). After 6 weeks of storage and a subsequent 5 d at 20 °C, early harvested fruit (E) appear visually mature and acceptable (Fig. 5.5 A,B) while those harvested at touch picked time (Fig. 5.5 C-E) have a significant proportion of the population with internal browning. Beyond 8 weeks of storage fruit of both harvests appear darkened and unacceptable (Fig. 5.5 F-J).

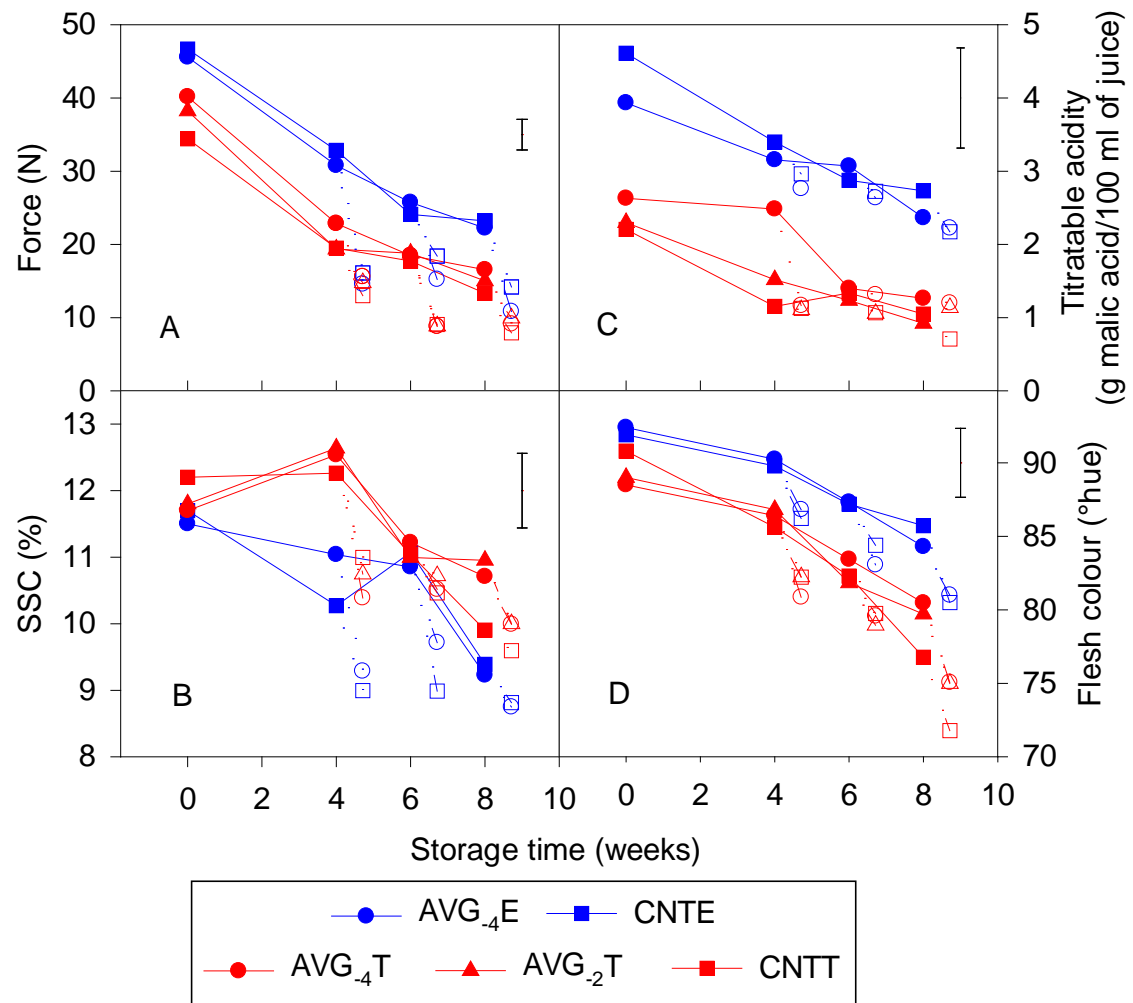


Fig. 5.4 Compression firmness (A), soluble solids content (B), titratable acidity (C) and flesh colour (°hue) of 'Unique' feijoa stored at 4 °C for 8 weeks and assessed fortnightly after removal (solid symbols) and subsequently on d 5 (hollow symbols) at 20 °C. Each data point represents mean from at least 21 fruit in A,B,D and mean from 3 independent pooled samples from at least 7 fruit in C. The vertical bars represent HSD_{0.05} (At-harvest values in week 0 are not included in HSD)

Table 5.4 Contingency table of internal ripening ranking of ‘Unique’ feijoa treated with AVG and stored at 4 °C. Fruit are pooled from all assessments after removal from storage (4, 6 and 8 weeks) and a subsequent 5 d at 20 °C. Columns represent internal ripening scale of unripe (< 2), ripe (2-3) and over ripe (≥ 4) fruit. Each count is an assessment from a total of 135 fruit. The chi-square (X^2) = 148.204 (p < 0.001), values in parenthesis are contributions to the chi-square

Treatment	Internal ripening index			Total
	<2	2-3	≥4	
AVG₄E	66 (46.38)	37 (0.35)	32 (16.75)	135
CNTE	53 (19.40)	36 (0.56)	46 (5.55)	135
AVG₄T	15 (6.91)	39 (0.08)	81 (3.94)	135
AVG₂T	7 (16.88)	45 (0.43)	83 (4.98)	135
CNTT	5 (20.06)	47 (0.94)	83 (4.98)	135
Total	146	204	325	675

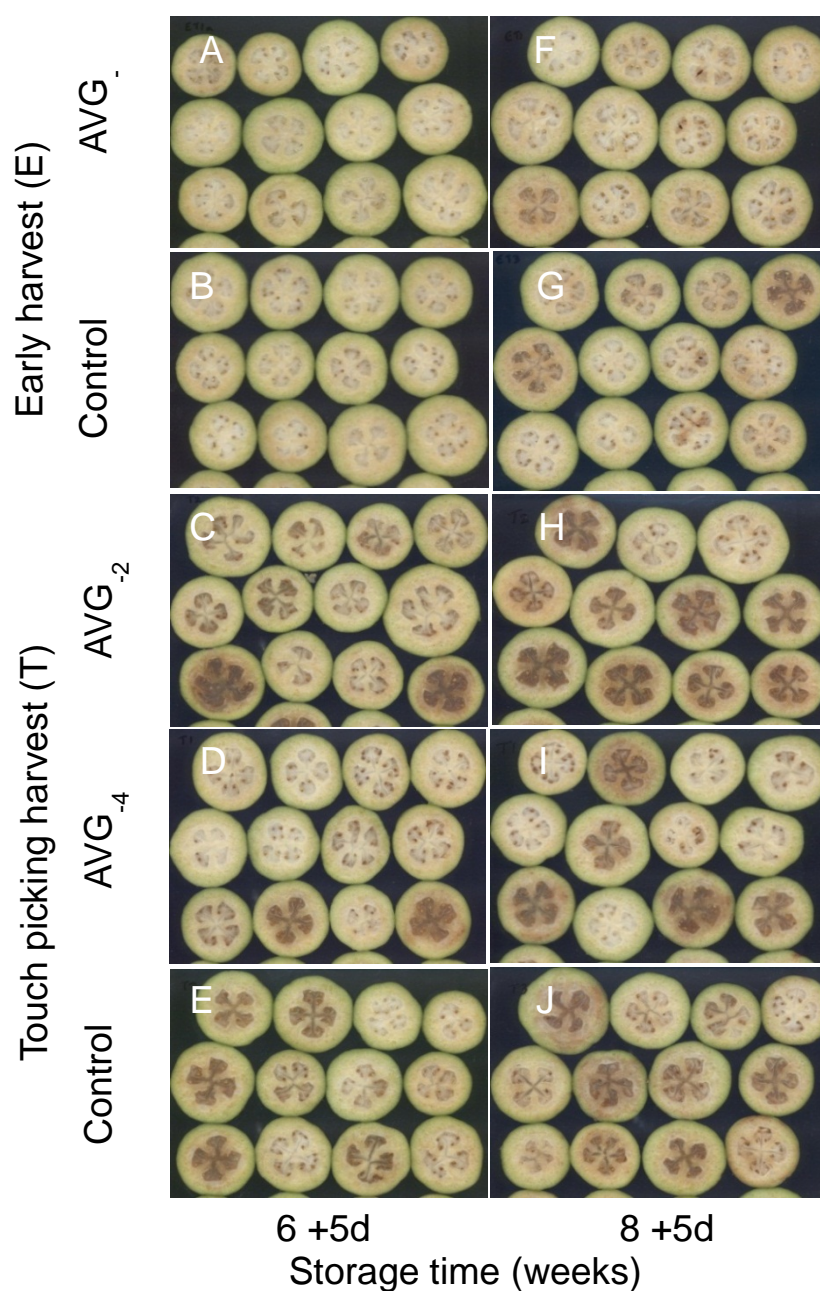


Fig. 5.5 Scanned images after equatorial halving of 'Unique' feijoa stored at 4 °C and assessed after 6 or 8 weeks of storage and a subsequent 5 d at 20 °C. Rows represent treatments and columns represent storage time

5.4 Discussion

Postharvest ethylene manipulation had minimal effect on delaying ripening of feijoa. In this study preharvest ethylene inhibition by applying AVG at 4 or 2

weeks before touch picking maturity was attempted. Application of preharvest AVG to feijoa resulted in reducing fruit drop and delaying maturity at touch picking time. Harvest time effected ripening delay during storage. AVG suppressed ethylene production rates without altering storage performance of feijoa.

5.4.1 Harvest time effects

Mean feijoa weights doubled in the last 4 week before commercial touch-picking time (Table 5.3) in agreement with Harman (1987) who found rapid growth in size during the last phase of fruit development. Mean fruit weight of early harvest and total fruit yield (>45 g) from early harvest (3.8 kg per tree) were substantially lower than those at commercial harvest (11.6 kg per tree). However lower yields in early harvests may be the result of harvesting method employed in our study where all the fruit were removed from trees on a single day while in a commercial orchard fruit are touch-picked over a period of 2-3 weeks (Thorp & Bielecki, 2002). AVG treated trees were not different from controls (Table 5.3) as variability was large between fruit yields of individual trees. Hence, this work needs to be further investigated with more replicate trees. Early harvested fruit were immature (Fig. 5.1A-B) while those harvested at touch picking time included both mature and immature fruit (Fig. 5.1C-E) which translated to an ability to store for a longer period before developing internal browning (Fig. 5.5). At the same time, a change to skin colour (°hue) occurs between the harvests (119.8-119.1 °hue to 116.1-114.5 °hue). While in this experiment harvest maturity treatments were created based on timing of the harvest, the change in skin colour between harvest dates observed both in this work and our previous (Table 4.1), suggests that external colour (°hue) may be

a useful attribute to non-destructively assess individual fruit maturity. Being able to segregate individual fruit by maturity at the time of harvest (potentially through the use of skin colour), may enable grading and segregation of fruit suitable for immediate consumption in local markets and those that could withstand extended storage for export market. Should this be achieved then timing of the harvest at a point which optimises yield with a proportion of long storing fruit could be investigated.

In this study, during storage fruit from early harvest were not only firmer (Fig. 5.4A) with higher flesh °hue (Fig. 5.4D) but internally appeared visibly acceptable after 6 weeks of storage (Fig. 5.5). However, after 8 weeks of storage fruit appear over ripe and unacceptable at both harvest times with locular browning (Table 5.4; Fig. 5.5). While maintaining firmness and visual quality early harvest also results in low SSC and high TA (Fig. 5.4B-C) which may impact the fruit taste profile after storage. These harvest timing effects mimic those found in earlier work (Fig. 4.4). The ratio of SSC and TA are important in determining the liking of fruit by consumers (Crisosto & Crisosto, 2001; Jayasena & Cameron, 2008) and hence before this strategy can be implemented, impacts on consumer acceptability need to be ascertained.

5.4.2 AVG effects

5.4.2.1 Fruit drops

Respiration rates were suppressed by AVG in early harvested fruit (Fig. 5.3) while ethylene production rates were suppressed for both harvests (Fig. 5.2). Similar inhibition of ethylene production and lowering of respiration rates by pre-harvest AVG has been observed in apple (Halder-Doll & Bangerth,

1987), pears (D'Aquino et al., 2010), peach (Cetinbas et al., 2012) and nectarines (McGlasson et al., 2005). Preharvest AVG reduced fruit drop in feijoa at commercial harvest time (Table 5.2). Our data adds to earlier reports of reduced fruit drop caused by AVG in apples (Byers, 1997a; Yildiz et al., 2012), nectarines (Torrighiani et al., 2004), pears (Sánchez et al., 2011) and longkong (Taesakul et al., 2012). Formation of abscission layer is a consequence of not only ethylene as a promoter but also by other growth hormones like auxins that inhibit its formation (González-Carranza et al., 1998). It would seem that AVG application reduced ethylene production and subsequently abscission zone development resulting in lowering of fruit drop in feijoa. All the fruit that dropped were >45 g, and if we take into account all the fruit dropped of untreated fruit (Table 5.2) then it appears that yields can be improved by up to 18.6% by AVG treatments.

Feijoa yields in fruit number appear higher in AVG treated trees than the untreated trees while having no effect on average fresh weights (Table 5.3). Rath and Prentice (2004) and Sanchez (2011) found that AVG not only increased fruit numbers but also their average size (weight) in nectarines and pears respectively. However Dussi et al. (2002) and Lafer (2008) found higher fruit numbers and a decline in average fruit weight due to AVG application in pears. McGlasson et al. (2005) and Cetinbas and Butar (2013) found that AVG treatment increased fruit weight for nectarines and sweet cherry respectively. Greene (2006) suggests that AVG treatment indirectly results in larger apple based on the fact that maturity delay allows treated fruit to remain longer on trees and accumulate size. The dropped feijoa are collected and sold at a lower price to processing units by growers as they are not saleable along with touch-

picked fruit. By applying AVG, growers can increase profits with fruit remaining longer on trees (because of reduced fruit drops), leading to accumulation of size and improving production volumes for fresh market consumption. Harvesting in our study was performed by removing all the fruit from tree at the same time, however yields can be substantially higher in a commercial scenario because saleable fruit (> 45 g) are touch-picked in a 2-3 week harvesting window (Thorp & Bielecki, 2002). The effect of AVG on feijoa yield numbers (> 45 g) may be greater in a commercial orchard.

5.4.2.2 At-harvest maturity

AVG_{4T} retained firmness and reduced flesh colour (°hue) at touch-picked (T) harvest (Table 5.3) while quality was unaltered by AVG at early harvest (E). Preharvest AVG applied 4 weeks rather than 2 weeks before commercial touch-picking time delayed maturity at the time of harvest in feijoa. Previously, AVG application resulted in higher firmness in apple (Whale et al., 2008; Yildiz et al., 2012), pear (Clayton et al., 2000), nectarine (Rath & Prentice, 2004; Torrigiani et al., 2004) and peach (Bregoli et al., 2002; Cline, 2006) at harvest. SSC and TA were not affected by AVG in feijoa (Table 5.3), replicating the lack of effect found in apple (Whale et al., 2008; Yildiz et al., 2012) and pear (Clayton et al., 2000; D'Aquino et al., 2010). There was no effect of preharvest AVG on skin colour (°hue) in feijoa, however AVG was found to retard development of red colour in many apple varieties (Byers, 1997a; Schupp & Greene, 2004). Phan-Thien et al. (2004) recommends partial treatment of orchard blocks of apple with AVG to delay maturity and stagger harvest time. AVG has potential to delay maturity of feijoa and hence a commercial trial is recommended for other varieties of feijoa.

5.4.2.3 Storage

Respiration rates increased during storage reaching a peak around week 6 and then started to decline in week 8 (Fig. 5.3B), ethylene production rates appear to decline during the same time (Fig. 5.2B). This is indicative of the post-climacteric stage during fruit development. Respiration rates declined in early harvested AVG treated fruit while ethylene production rates were suppressed at both harvest times (Fig. 5.2A). There was no consequent impact on quality during storage by AVG treatment (Fig. 5.4). Similarly, Bergoli et al. (2006) found for peach and nectarine, AVG treatment strongly inhibited ethylene production but quality of fruit remained unaltered during storage. Likewise, Munoz-Robredo et al. (2012) found that SSC, TA and colour were not affected by AVG treatment even when ethylene synthesis was inhibited in apricot. Firmness of feijoa in storage was not affected by pre-harvest AVG treatment (Fig. 5.4A), contrastingly firmness during storage was retained in apple (Drake et al., 2006; Yildiz et al., 2012), pear (Clayton et al., 2000; D'Aquino et al., 2010), plum (Jobling et al., 2003), nectarine (Torrighiani et al., 2004) and peach (Cetinbas et al., 2012). AVG treatment had no effect on SSC during storage of feijoa (Fig. 5.4B), contrastingly Bregoli et al. (2002) in peach and Jobling et al. (2003) in plum found higher SSC in fruit treated with AVG. However Yildiz et al. (2012) in apple and D'Aquino et al. (2010) in pear found that AVG treatment lowered SSC. AVG treatment had no effect on TA of feijoa (Fig. 5.4C) which has similarity to Clayton et al. (2000) and Drake et al. (2005) who found no effect in apple and pear. Contrastingly Ozturk et al. (2013) in apple and Cetinbas et al. (2012) in peach found that AVG treatment retained higher TA during storage. Flesh °hue was not affected by AVG in feijoa (Fig. 5.4D) while AVG treatment

retained flesh °hue in apple (Drake et al., 2005) and reduced internal browning in pears (D'Aquino et al., 2010). Skin colour (°hue) was not altered by AVG treatments in feijoa (data not shown). Skin colour (°hue) in pear was retained during storage in AVG treated fruit (Clayton et al., 2000; D'Aquino et al., 2010) while AVG reduced °hue (Cline, 2006) and darkened skin colour (Cetinbas et al., 2012) in peach. Red colour development declined in AVG treated apple during long term storage (Wang & Dilley, 2001; Whale et al., 2008). Quality after storage was not affected by preharvest AVG treatments in 'Unique' feijoa.

5.4.2.4 Application

Time of application of AVG significantly influences fruit drop and maturity in apple (Greene, 2002). Retain™ label recommends commercial application at 2-4 weeks before anticipated harvest in apple. Schupp and Greene (2004) and Greene (2005) found effectiveness of AVG in controlling fruit drop when applied 2 weeks before commercial harvest. Similarly, Clayton et al. (2000) found that preharvest AVG treatments conducted 1-2 weeks rather than at 3-4 weeks before harvest delayed maturity and ripening in pear. Our results suggest that AVG treatment appears to be more effective in reducing fruit drops and in delaying maturity when applied at 4 rather than 2 weeks before harvest for feijoa (Fig.5.2, Table 5.2, Table 5.3). In this research AVG concentration was applied as per the recommendations made on the label (Retain™, 83 g/100 L water) for apple and stone fruit (Jobling et al., 2003; Phan-Thien et al., 2004; Rath et al., 2006). However, Greene (2002, 2005) and Schupp and Greene (2004) found that fruit drop decreased linearly with increasing concentration of AVG in apple and likewise fruit responses increased with higher rates of AVG in

peach (Cline, 2006). Similar effect of increased response to AVG concentration may be possible for feijoa.

It has been demonstrated that ethylene plays a vital role not only in initiating ripening but also in the progression of softening in climacteric fruit like pears (Hiwasa et al., 2003); banana (Trivedi & Nath, 2004) and kiwifruit (Boquete et al., 2004). However, our study shows that climacteric rise in ethylene production occurred and AVG treatments suppressed biosynthesis of ethylene leading to influence on reducing fruit abscission and delaying maturity at harvest time; but quality was not altered during. Pech et al. (2008) found that some ripening events like sugar accumulation, TA and flesh colour during ripening of melon fruit were regulated independent of ethylene. Pech et al. (2012) also suggests that ripening processes can be altered in some fruit only when there is complete inhibition (>99%) of ethylene biosynthesis. This physiological state has been witnessed in genetically modified (anti-senescence) cultivars of apple, tomato and cantaloupe melons. Earlier studies show that exogenous ethylene had no effect on 'Unique' feijoa (Fig. 4.3 and Fig. 4.4;(Al-Harthy, 2010)). Combining this with the AVG effects of present study, we suggest that ethylene initiated ripening has a substantial ethylene-independent regulation during postharvest life of 'Unique' feijoa. Our results indicate potential benefits in using preharvest AVG in feijoa, however we suggest a robust commercial trial before recommending AVG for orchards. Additionally in apple effects of AVG were cultivar dependent (Byers, 1997a), it is entirely possible that preharvest AVG can contribute not only for delaying maturity at-harvest but also for storage life extension in other cultivars of feijoa.

5.5 Conclusion

Feijoa harvested early were storable for 6 weeks but with a low SSC and high TA. Application of AVG at 4 weeks before commercial harvest time was found to be effective for feijoa. Preharvest AVG inhibited ethylene production and resulted in reduced fruit drop. Fruit treated with AVG retained firmness and change in flesh colour ($^{\circ}$ hue) than untreated fruit at commercial touch-picking maturity. Storage life was not effected as quality (firmness, SSC, TA or flesh colour) during ripening was not altered by AVG treatments. Postharvest ripening in 'Unique' feijoa may be ethylene-independent but future investigation on other varieties of feijoa may lead to storage life extension.

6 Using skin colour to segregate feijoa by maturity at harvest

6.1 Introduction

Physiological maturity is defined as “the stage during development when a plant or its part would continue its ontogeny even after its detachment” while horticultural maturity is “the stage in development at which a plant part or plant possess the features that the consumer wants” (Watada et al., 1984). The feijoa industry in New Zealand wants a long storing and yet good tasting fruit to enable sea-freight export to markets in Europe, Asia and North America. Maturity at harvest is the most important factor that impacts ripening and quality during storage life of a product (Shewfelt, 2014). Maturity indices of fresh produce are essential in enabling supply chain decisions right from the field to the consumers’ plate. Maturity choices are also made to enable an industry to flourish. The primary objective of a maturity index is to enable predictable uniform ripening and storage behaviour.

Industry prefers non-destructive measures for economic reasons to predict harvest maturity by using unbiased indices (Reid, 2002). Various non-destructive methods are used to evaluate maturity in fruit and vegetable crops (Abbott et al., 1997). Measurement of a predefined quality attribute can be used as a non-destructive method in packhouses to sort products (Abbott, 1999). Grading of eggplant used multiple colour cameras which measure size, shape and colour in the packhouse (Kondo et al., 2007). Highly automated grading systems using robotic technologies with near infrared inspection are often employed to sort fresh produce by developed industries (Kondo, 2010).

There are no obvious visible changes to the skin colour of feijoa during its development and fruit harvested as touch-picked can be at mixed maturities within a fruit batch (Thorp & Bielecki, 2002). During the previous experiments on feijoa harvested earlier than commercial touch picking maturity, a consistent decline in skin colour ($^{\circ}$ hue) was observed as fruit maturity progressed (Table 4.1 and Table 5.3). Feijoa harvested early were more green (higher $^{\circ}$ hue) and darker (lower L^*) than those picked at commercial touch picking maturity. Skin colour changes observed due to maturity differences created by harvest timing indicate that skin colour may be useful to determine harvest maturity. Change in external colour is used as a maturity index in many fruits including pomegranate (Manera et al., 2013), guava (Mercado-Silva et al., 1998), mango (Kienzle et al., 2011) and litchi (Reichel et al., 2010). The reflectance spectrum in the visible region (400-700 nm) was used to estimate maturity in dragon fruit (Wanitchang et al., 2010) and mango (Wanitchang et al., 2011). It is possible that skin colour ($^{\circ}$ hue) in feijoa may provide cues to identify maturity within fruit batches. This leads to the hypothesis that feijoa can be segregated into maturity categories non-destructively using skin colour. If individual feijoa are graded by maturity through skin colour at the time of harvest then fruit suitable for immediate consumption in local markets can be separated from those that can withstand longer storage for export market.

A destructive indicator of internal maturity is presently used by growers in New Zealand which was developed by Plant and Food Research (Fig. 2.3). Feijoa that are touch-picked were found to be either ripe or over ripe at the time of harvest (Al-Harthy, 2010). Feijoa maturity varies significantly due to a long flowering window of 4-6 weeks (Thorp & Bielecki, 2002). Variation within a fruit

batch at the time of harvest can be amplified by human error of inexperienced pickers in feijoa orchards. Time from fruit set is the most commonly used method for estimation of maturity, with feijoa taking 120-140 days from fruit set to full maturity (Harman, 1987). A number of previous studies have attempted to use non-destructive methods to segregate feijoa. Clark et al. (2005) used fruit density but found it ineffective due to high variability among fruit batches. Gaddam et al. (2005) used acoustic impulse response and Wiryawan et al. (2005) applied non-destructive compression firmness to segregate fruit at harvest with limited success. Al-Harthy (2010) suggested that a meaningful relationship between maturity and non-destructive quality measures like compression firmness or skin colour was not possible due to high variability in fruit batches. Thorp and Bielecki (2002) suggested that differences in postharvest storage potential of feijoa was probably due to the difficulties in maintaining uniform maturity at the time of harvest by touch-picking method. If feijoa are harvested early then touch-picking method becomes irrelevant and it may be necessary to grade feijoa non-destructively to identify storable maturity for suitable markets.

The aim of this study is to investigate if skin colour is useful in segregating feijoa by maturity at harvest. Colour segregation would be useful in grading fruit into homogenous groups representing different storage potential.

6.2 Materials and Methods

6.2.1 Fruit sample

Mature 'Unique' feijoa trees on a commercial orchard in Matamata, New Zealand were used to conduct the trial. Feijoa trees were randomly selected

and harvested at two weeks (H_{-2}), one week (H_{-1}) or no weeks (H_0) before predicted commercial touch-picking maturity in April, 2013. One tree was harvested at each time by removing all fruit. All fruit were weighed in the orchard and only feijoa weighing > 45 g were packed into a single layered commercial corrugated fibreboard trays containing plix. From the time of harvest, it took 5 h to pack the fruit. These fruit were transported by an air conditioned commercial van in cool conditions (≈ 15 °C). It took 6 h of transportation time to reach the postharvest laboratory at Massey University, Palmerston North on the same day.

Fruit weighing > 45 g (commercial standard) were used for assessments, resulting in selection of 52 fruit at H_{-2} , 115 at H_{-1} and 120 at H_0 for this research. Each fruit population was divided into four equal batches based on skin colour (°hue). Each batch of fruit represented the entire °hue range (108.3-130.3 °hue) that existed in the population at the time of harvest. It took 2-4 h to sort feijoa using a spectrophotometer. One batch of fruit was used to determine at-harvest quality and the remaining three batches of feijoa were stored at 4 °C and 90% relative humidity in single layered commercial corrugated fibreboard trays containing plix and a polyliner wrap. A batch of fruit from each harvest time was removed after 4, 6 and 8 weeks of storage and assessed after a further 4 d at 20 °C.

6.2.2 Respiration and ethylene production

Respiration was measured as carbon dioxide production rate (r_{CO_2}) using the static method after removal from storage (4 °C) and a subsequent 4 d at 20

°C. Each fruit of a known mass was placed in an air tight glass jar (580 mL) equipped with rubber septum to enable sampling of gas.

Ethylene production rates were measured by flow through method after removal and a subsequent 4 d at 20 °C. A laser-based ethylene detector (ETD-300, Sensor Sense B.V., Nijmegen, Netherlands) with a detection sensitivity of 0.3 nL L⁻¹ was used. Ethylene-free air was passed through an air tight glass jar (580 mL) containing a single fruit.

Full details on respiration and ethylene production are provided in section 4.2.5.

6.2.3 Quality

Skin colour was measured using a spectrophotometer (CM-2600D, Konica Minolta Sensing Inc., Osaka, Japan) with an 8 mm head diameter. Spectramagic™ NX (software) was used to calculate °hue, L* and the reflectance spectrum in the visible region (360 nm to 740 nm). Skin colour was measured by averaging readings from three locations around the equatorial of the fruit.

Details on flesh colour, compression firmness, visual maturity assessment, scanning of images, SSC and TA are provided in section 4.2.6.

6.2.4 Data analysis

The General Linear Model (GLM) procedure was used to ascertain significant effects of harvest time on measured parameters using Minitab (version 16.0, Minitab Inc., State College, Pennsylvania). As time in storage was considered as a factor, all data were analysed in a single ANOVA and significant differences between harvests or after *post hoc* analysis (section

6.3.3) were identified using Tukey's test at 95% confidence. Descriptive statistics were used to analyse at-harvest fruit population data and relationships between skin colour ($^{\circ}$ hue) grade at-harvest and quality attributes after storage were evaluated using Pearson's correlation coefficient.

6.3 Results and discussion

6.3.1 Maturity at harvest

The distribution of feijoa skin colour at the time of harvest for each harvest demonstrates the shift in colour from dark green to a light green colour on average. At the same time, skin $^{\circ}$ hue lowered and skin lightness (L^*) increased (Table 6.1). However the distribution also demonstrates that considerable overlap of colour profiles exists between each of the harvest timings (Fig. 6.1). More than 71% of the H_{-2} population, 14% of H_{-1} and 5% in H_0 had $^{\circ}$ hue above 122 (Fig. 6.1A-C). Only 4% of fruit appeared to have more than 44 (L^*) in H_{-2} while 50.4% of H_{-1} and 36.6% of H_0 were above 44 (L^*) (Fig. 6.1D-F). At-harvest qualities of fruit progressed with timing (Table 6.1) and yield of H_{-2} was lower when compared to H_{-1} or H_0 . Fresh weight increased by $\approx 15\%$ during the last two weeks of maturation on the tree.

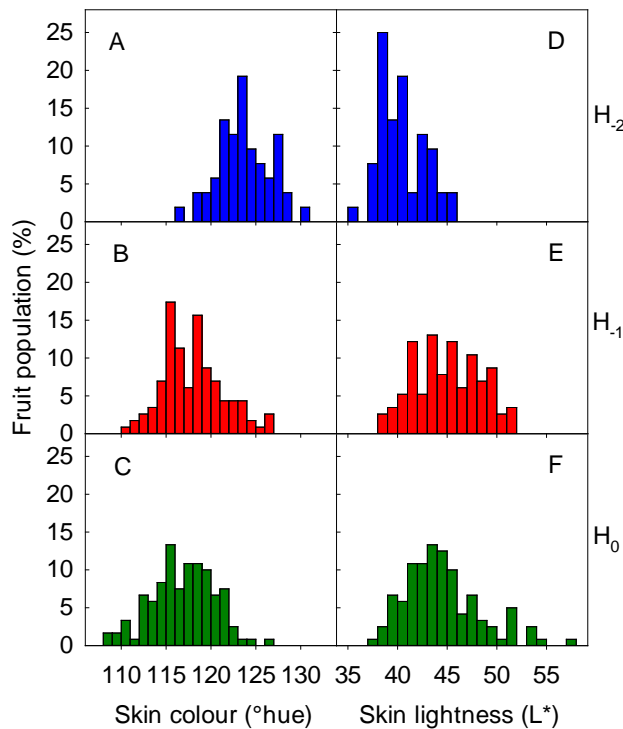
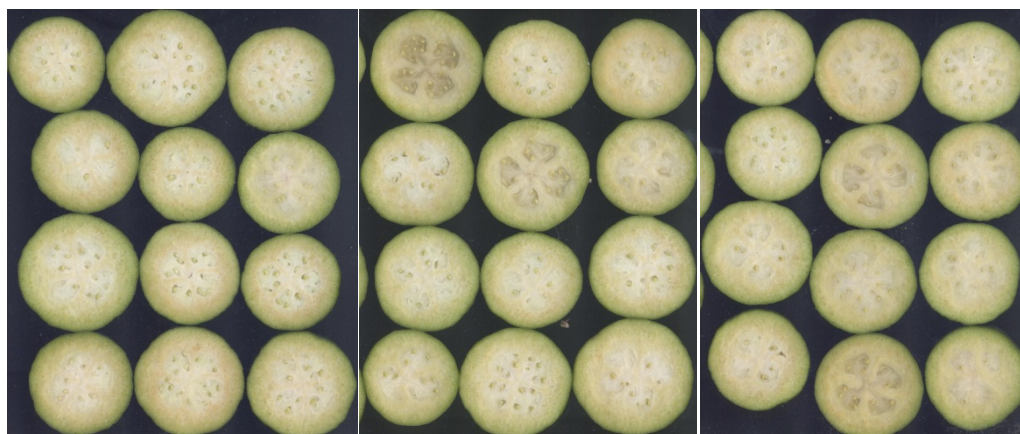


Fig. 6.1 Distribution of skin colour attributes of ‘Unique’ feijoas harvested at three different times. Harvests H₂, H₁ and H₀ are at 2, 1 and 0 weeks before commercial touch-picking maturity

Mean compression firmness and titratable acidity of H₂ were higher than H₁ or H₀ (Table 6.1). Flesh colour (°hue and L*) of H₀ (90.5 °hue and 71.8 L*) was lower than H₁ or H₂ (92.9-93.2 °hue and 75.6-77.0 L*) because of a more mature fruit due to locular clearing as confirmed by the scanned images (Fig. 6.2). All the fruit from H₂ appeared immature according to the internal maturity scale whereas H₁ and H₀ comprised a mix of both mature and immature fruit (Fig. 6.2). Internal maturity ratings confirm that fruit at H₂ and H₁ were less mature (mean score 1-1.2) than H₀ (mean score 1.5) fruit. SSC was the only quality variable that was not affected by harvest time (Table 6.1).



H_{-2}

H_{-1}

H_0

Fig. 6.2 At harvest scanned images after equatorial halving of 'Unique' feijoa harvested at different times. H_{-2} , H_{-1} and H_0 harvested at weeks 2, 1 and 0 before touch picking maturity

Table 6.1 Mean attributes at-harvest time of 'Unique' feijoa. $n(H_{-2}) = 13$, $n(H_{-1}) = 28$ and $n(H_0) = 30$. Values with same letter in a row are not significantly different ($p > 0.05$)

Attribute	Units	H_{-2}	H_{-1}	H_0	HSD _{0.05}
Relative harvest time	d	-14	-7	0	-
Fresh weight of saleable fruit	g	57.9 ^b	60.5 ^b	67.4 ^a	4.9
Skin colour	°hue	123.3 ^a	118.2 ^b	117.0 ^b	2.7
	L*	40.4 ^b	44.8 ^a	44.2 ^a	2.6
Flesh colour	°hue	92.9 ^a	93.2 ^a	90.5 ^b	1.2
	L*	77.0 ^a	75.6 ^a	71.8 ^b	4.6
Maturity index	-	1.0 ^b	1.2 ^b	1.5 ^a	0.3
Firmness	N	53.0 ^a	40.7 ^b	37.2 ^b	7.2
SSC	%	11.3	11.7	11.7	NS
Titrateable acidity	g malic acid/ 100 mL juice	4.6 ^a	3.4 ^b	3.0 ^b	0.5

Overall maturity differences due to harvest timing reported in this work (Table 6.1) mimic the previous findings (Table 4.1 and Table 5.3). A mix of °hue ranges (108.3-130.3 °hue) were observed across all harvest times which may be attributed to the variability in the maturity of the fruit due to the flowering window in feijoa being over 4 weeks (Thorp & Bielecki, 2002).

6.3.2 Storage quality

Differences in maturity at the time of harvest affected quality of feijoa after storage (Fig. 6.3). After 4 weeks of storage, mean firmness, titratable acidity, colour of skin and flesh of fruit harvested at H₂ was higher than that of fruit from H₁ or H₀ (Fig. 6.3A,C,E,F). However after 6 weeks of storage, differences due to harvest timing declined as fruit from H₂, H₁ and H₀ had similar ranges of firmness, titratable acidity and flesh colour. Mean SSC (%) was not different at the time of harvest but after 6 weeks of storage H₂ had lower SSC (8.2%) than H₁ (9.8%).

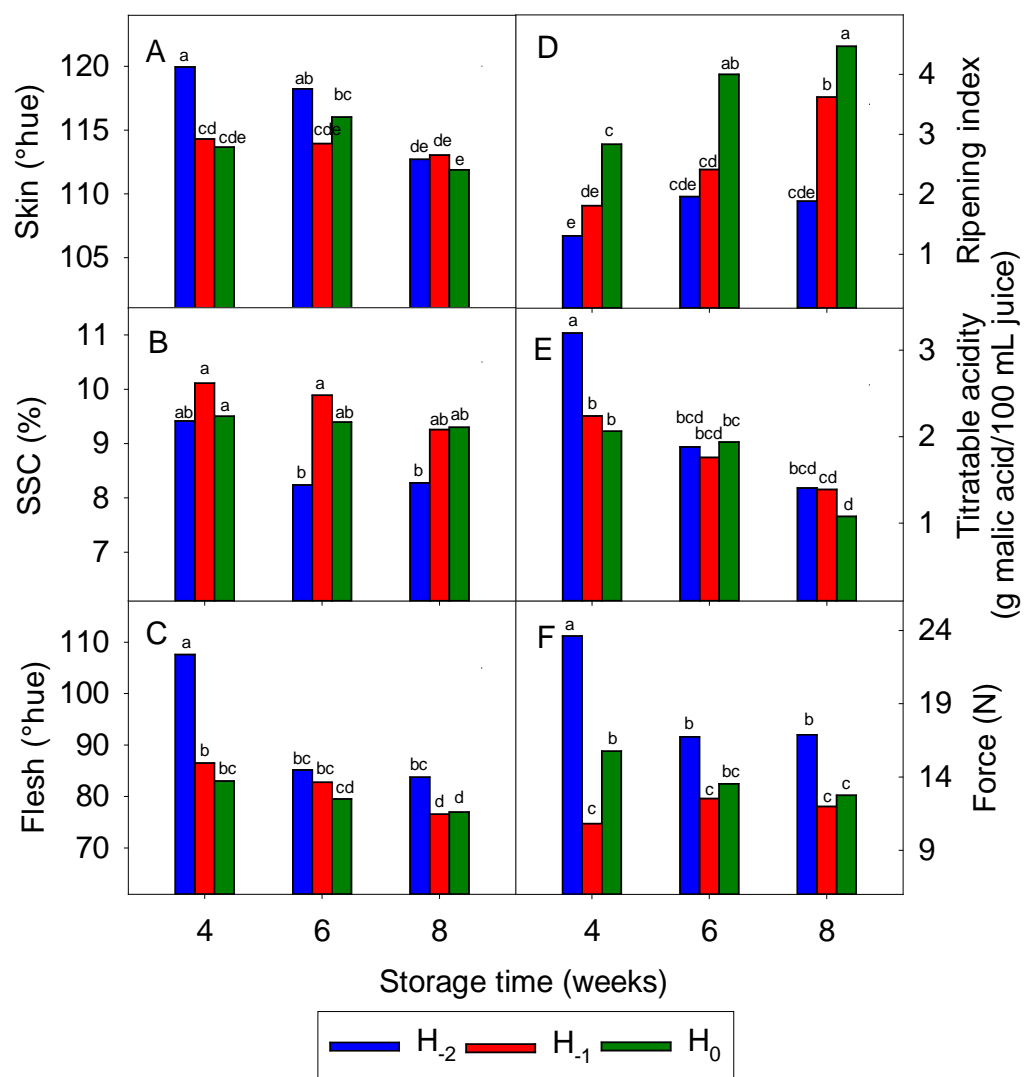


Fig. 6.3 After storage quality attributes of (A) skin colour (B) SSC (C) flesh colour (D) internal ripening (E) titratable acidity and (F) firmness of 'Unique' feijoa harvested at H₋₂, H₋₁ and H₀. Fruit assessed after 4,6 and 8 weeks of storage at 4 °C and a subsequent 4 d at 20 °C. n (H₋₂) = 13, n (H₋₁) = 29 and n (H₀) = 30. Same letters within a graph indicates no significant differences ($p > 0.05$)

After storage at every point of evaluation, average ripening index was lowest in H₋₂ when compared to H₀ (Fig. 6.3D). Visual assessment shows darkening of locules in some of H₀ (>4; Fig. 6.4K) fruit while H₋₁ and H₋₂ appear acceptable after 6 weeks of storage (Fig. 6.4C,G).

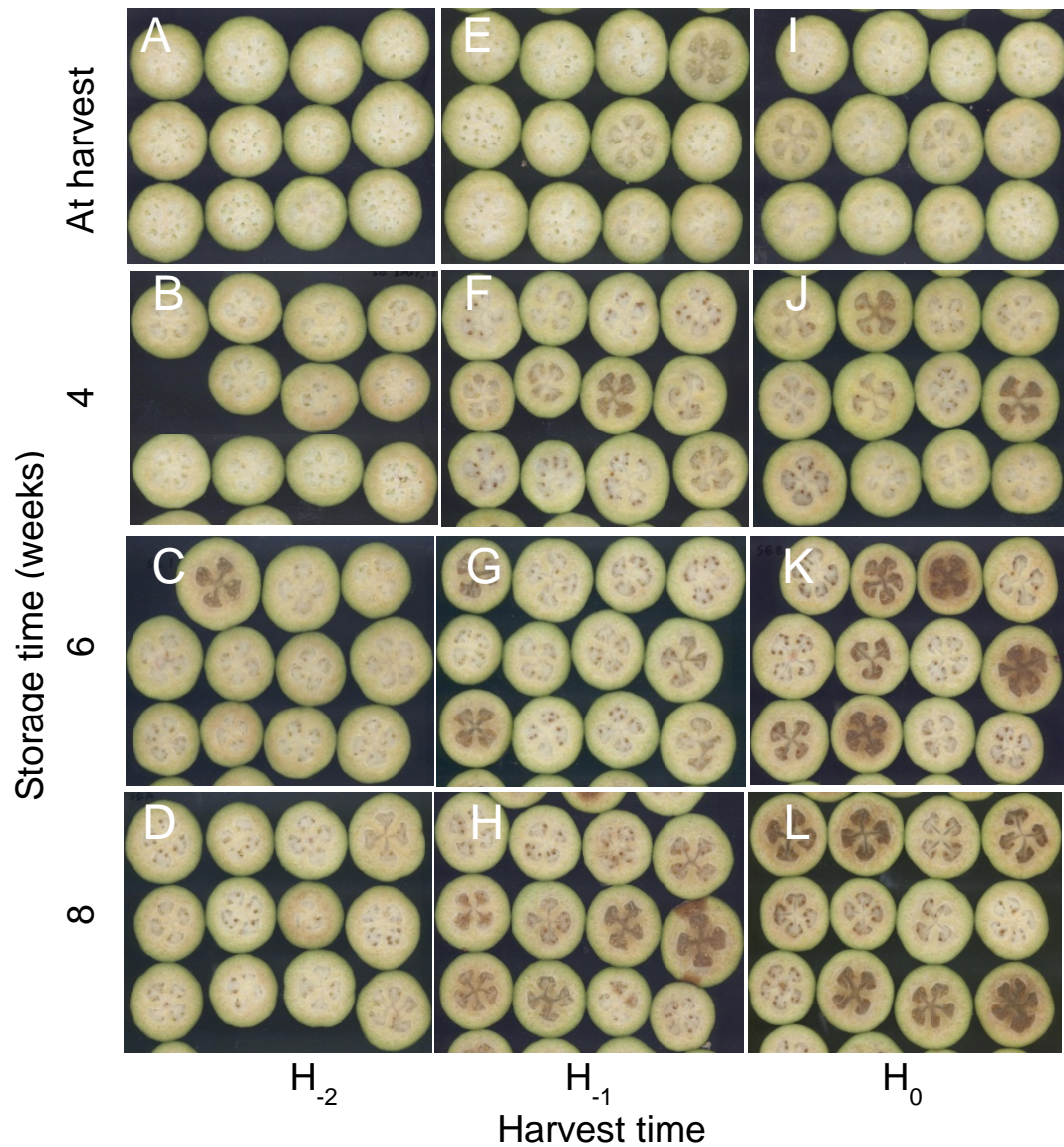


Fig. 6.4 Scanned images after equatorial halving of ‘Unique’ feijoa stored at 4 °C and a subsequent 4 d at 20 °C. Rows indicate storage time and columns indicate harvest time

Significant correlations between skin colour (°hue) at-harvest and quality attributes after storage (8 weeks) were observed (Fig. 6.5). Only compression firmness and ripening index showed stronger relationship when compared to other attributes. Fruit harvested with higher °hue tended to have higher firmness after storage (Fig. 6.5A) and lower ripening index (Fig. 6.5B). This high skin

°hue at harvest tended to result in high flesh °hue after storage ($r = 0.438$, $p < 0.001$; data not shown) and high skin °hue after storage ($r = 0.508$, $p < 0.001$; data not shown). A weak correlation was observed between skin °hue at harvest and respiration rate ($r = 0.347$, $p < 0.001$; data not shown) while no relationship was found between at harvest skin °hue and ethylene production rates or titratable acidity after storage (data not shown).

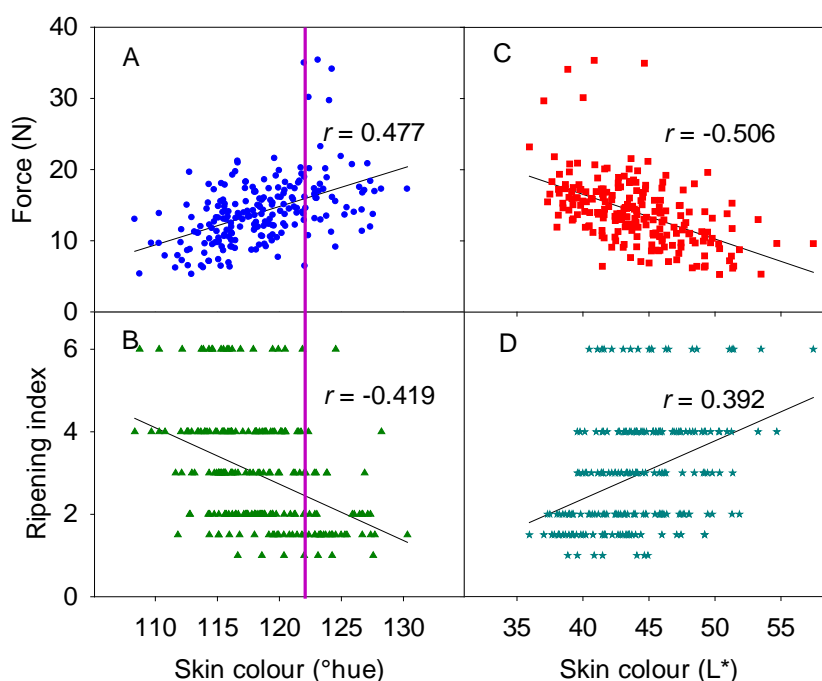


Fig. 6.5 Correlations between at-harvest skin colour (°hue and L*) of feijoa with compression firmness (A,C) and ripening (B,D) after storage (8 weeks). Each data point represents individual fruit ($n = 216$) and 'r' is Pearson's correlation coefficient. Vertical purple line at 122 °hue represents °hue threshold

At-harvest skin lightness (L*) showed inverse relationships to those exhibited by at-harvest skin °hue with the after storage quality attributes (Fig. 6.5). Low lightness after harvest related to high after storage firmness (Fig. 6.5C) while having a low ripening index (Fig. 6.5D). Lower lightness at-harvest tended to result in lowering of after storage skin °hue and flesh °hue ($r = -0.466$

and -0.407; $p < 0.001$; data not shown) while increasing SSC ($r = 0.425$, $p < 0.001$; data not shown). There was no relationship with titratable acidity, ethylene production rate or respiration rate (data not shown).

6.3.3 *Post hoc* analysis

In order to determine if skin °hue can predict maturity of feijoa, the experimental data was subjected to a *post hoc* analysis. The skin colour observed in the population ranged from 108.3-130.3 °hue at the time of harvest. Irrespective of harvest timing individual feijoa were segregated into two groups named “ready to eat” (R2E) and those that can withstand longer “storage” (STR). Segregation into either R2E or STR was based on °hue measured at harvest and was compared with the observed ripening index of feijoa after storage to determine the success of prediction. R2E fruit were expected to be unacceptable, while STR fruit were expected to be of acceptable quality after 6 weeks of storage.

Selection of the threshold (°hue) in which to segregate the population into the R2E and STR population was required. The entire batch of feijoa can be divided into two groups of equal size at a °hue of 118.5 (Fig. 6.6). The proportion of STR fruit segregated to the population decreases as the °hue threshold increases. Prediction success was considered best at the threshold of 122 °hue as STR had the lowest false STR (13.3%) drawn as a vertical purple line in Fig. 6.5. Hence feijoa having °hue greater than 122 were categorised as STR fruit and the remaining were considered as the R2E population. At 122 °hue only 20.8% of feijoa were categorised as STR at harvest. The percent of STR was about 71% in H_{-2} , 14% in H_{-1} and 5% in H_0 harvest times.

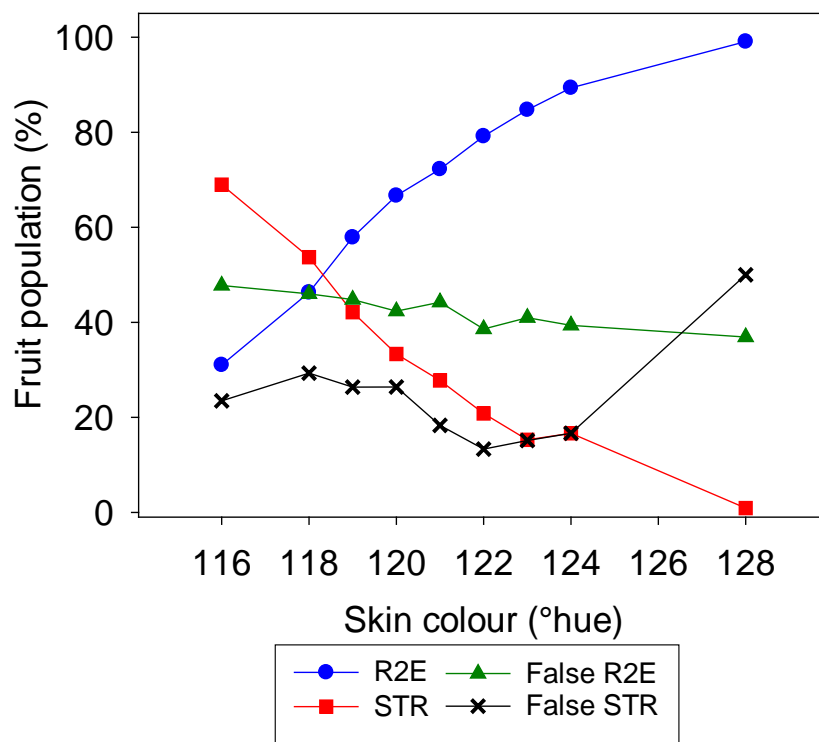


Fig. 6.6 Feijoa segregation results using different thresholds of skin colour (°hue) into two groups (R2E) and (STR) at-harvest. False R2E and False STR are based on ripening index score after storage time

6.3.4 Skin colour (°hue) based segregation effects

After *post hoc* segregation, at-harvest quality of STR fruit were dark green in colour in comparison to light green R2E (Table 6.2). STR was firmer with higher TA than R2E, however both flesh colour °hue and SSC of the segregated populations were not different (Table 6.2). Mean maturity index of STR (1.0) indicates that fruit were less mature than R2E (1.4; Table 6.2). These results suggest that using skin colour °hue can segregate maturity in a feijoa population at the time of harvest. STR fruit appear to be less mature and may possess more storage life than R2E.

Table 6.2 Mean attributes at-harvest of ‘Unique’ feijoa after *post-hoc* segregation as (A) STR (>122 °hue) and (B) R2E (<122 °hue). Values with same letter in a row are not significantly different ($p = 0.05$). n (STR) = 13; n (R2E) = 58

Attribute	Units	STR	R2E	HSD _{0.05}
Skin colour	°hue	124.7 ^a	117.2 ^b	1.8
	L*	40.2 ^b	44.6 ^a	1.9
Flesh colour	°hue	92.8	91.8	NS
	L*	78.3 ^a	73.3 ^b	3.5
Ripening index	Number	1.0 ^b	1.4 ^a	0.4
Firmness	N	52.3 ^a	39.0 ^b	5.6
SSC	%	11.5	11.6	NS
Titrateable acidity	g malic acid/ 100 mL juice	4.4 ^a	3.3 ^b	0.4

Feijoa with higher skin °hue (STR) at harvest had ripening delays when compared with those with lower °hue (R2E; Fig. 6.7). This ripening delay was evident after 6 weeks of storage (Fig. 6.7D) confirming that R2E fruit showed faster internal browning compared to STR fruit. After storage for 6 weeks, STR had higher mean firmness (17.3 N) than R2E (13.2 N) and STR had lower average ripening index (2.0) than R2E (3.2). This is supported by changes in flesh colour °hue of STR (90.3 °hue) which was higher than R2E (80.9 °hue). Skin °hue declined during storage but STR (117.2 °hue) remained higher than R2E (113.6 °hue). SSC of STR and R2E were at a similar range (11.5-11.6%) at harvest time (Fig. 6.7B), however mean SSC of STR (8.8%) after storage was lower than R2E (9.6%), however average TA of STR (2.2 g malic acid/100 mL juice) was higher than R2E (1.7 g malic acid/100 mL juice; Fig. 6.7E).

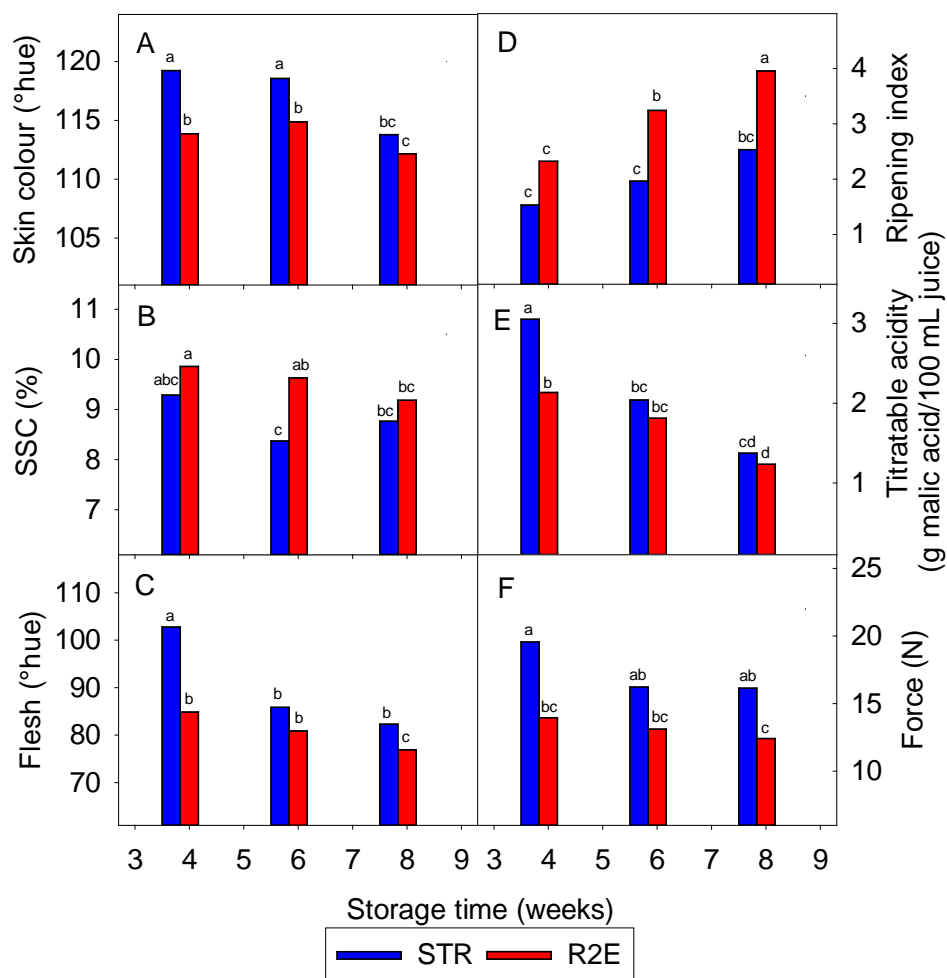


Fig. 6.7 Quality attributes of (A) compression firmness (B) SSC (C) flesh colour (D) internal ripening index (E) titratable acidity and (F) skin colour of 'Unique' feijoa graded as STR (>122 °hue) and R2E (<122 °hue). Fruit assessed after 4, 6 and 8 weeks of storage at 4 °C and a subsequent 4 d at 20 °C. Each data point represents at least 14 fruit. Same letters within a graph indicates means not significantly different ($p > 0.05$)

Success of segregation of feijoa was determined by comparing prediction of saleability with the internal ripening index after storage (Table 6.3). Fruit having a ripening index score of 2-3 were considered to be ripe and saleable while the remaining (<2 and >3) were considered unsalable. Over 91% of the fruit predicted as R2E and STR appear saleable after 4 weeks of storage (Table 6.3). The risk of unripe feijoa in R2E category (5.4%) was lower than STR

(18.7%). After 6 weeks of storage, success rate for saleability declined to 66.7% even when all the predicted STR were saleable (100%) due to a high proportion of over-ripe fruit in R2E (41.4%). Feijoa having skin colour above 122 °hue (STR) not only took longer time to ripen when compared to those having °hue below 122 (R2E) but also appeared saleable after 6 weeks of storage. After 8 weeks of storage, 80% of STR appear saleable while those of R2E declined to 31.6%. Skin colour (°hue) at the time of harvest was useful in segregating feijoa into longer storing STR (6 weeks) and those suitable for 4 weeks of storage (R2E).

Table 6.3 Resulting grading outcomes of feijoa segregated at-harvest by skin colour (R2E and STR) and storing at 4 °C. Saleable fruit determined by ripeness index and unsaleable feijoa include unripe and over ripe fruit

week	prediction	fruit number	Saleable	Un-saleable	success rate (%)	overall success (%)
4	R2E	56	53	3	94.6	91.7
	STR	16	13	3	81.3	
6	R2E	58	34	24	58.6	66.7
	STR	14	14	0	100	
8	R2E	57	18	39	31.6	41.7
	STR	15	12	3	80	

6.3.5 Reflectance spectra

A typical reflectance spectrum of feijoa skin at-harvest shows peaks at wavelengths of 550 and 720-740 nm, and a minimum at 680 nm (Fig. 6.8). On average, fruit from the earliest harvest (H₂) had lower reflectance at 550 nm wavelength (15.3%) than H₁ or H₀ (19.5-19.9%; Fig. 6.8A) indicating lower

absorption as maturity progressed. After *post hoc* segregation, the reflectance at 550 nm of STR (14.9%) was lower than R2E (19.9%; Fig. 6.8B). At-harvest skin °hue was found to correlate with reflectance at 550 nm ($r = -0.813$, $p < 0.0001$, data not shown).

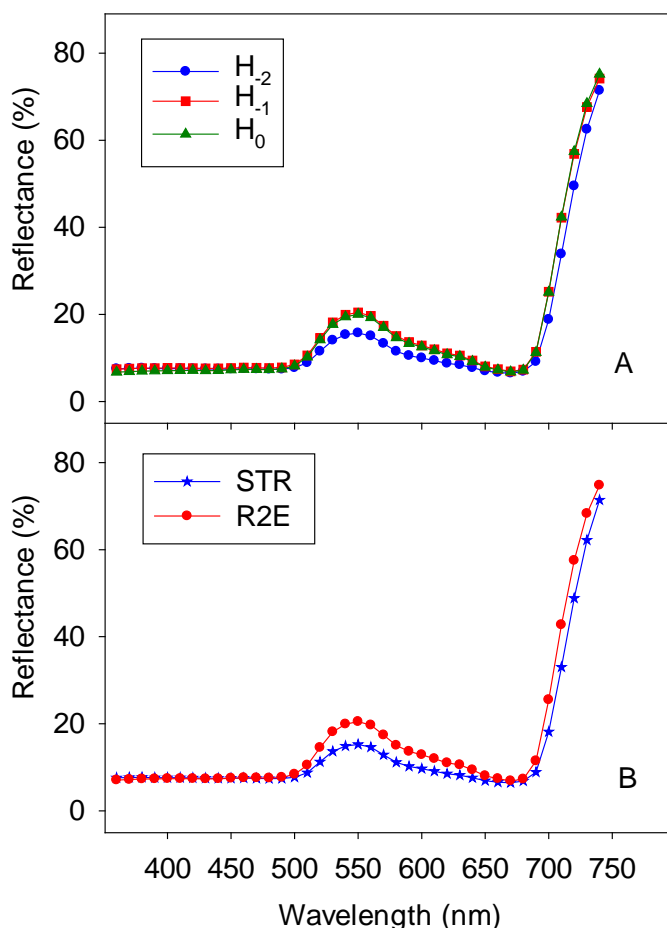


Fig. 6.8 Mean reflectance spectra of 'Unique' feijoa (A) harvested at different times (n of $H_{-2} = 52$, $H_{-1} = 115$ and $H_0 = 120$) and (B) Segregated as STR (> 122 °hue, $n = 58$) and R2E (< 122 °hue, $n = 229$). $HSD_{0.05}$ at 550 nm wave length in (A) = 1.3 and (B) = 0.9

Chappelle (1992) found that reflectance measurements are useful in estimating concentrations of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids). Our results mimic those found in soybean leaves where wavelengths 550 nm and 675 nm corresponded to the minimum and maximum

chlorophyll absorption (Chappelle et al., 1992). Merzlyak et al. (2003) and Wanitchang et al. (2011) found that changes in chlorophyll content contributed to differences in reflectance at 550 nm in apple and mango respectively. Cox et al. (2004) reported that the skin of a maturing avocado shows an initial degradation of chlorophyll followed by increase in anthocyanin concentration. Chlorophyll is transformed to pheophytin which is photosynthetically inactive during leaf senescence (Chappelle et al., 1992). Thomas and Gausman (1977) confirmed a better relationship between reflectance at 550 nm and chlorophyll concentration in comparison to its relationship with carotenoid concentration. Although 550 nm corresponds to green colour region of visible light, it was found to possess maximum absorption by anthocyanin (Merzlyak & Chivkunova, 2000). Increase in reflectance at 550 nm during progression of maturity in feijoa suggests chlorophyll degradation on fruit skin. There is paucity of research on pigment concentrations of feijoa. Reflectance at 550 nm may be useful to segregate feijoa and hence a robust commercial trial is recommended.

6.3.6 Feijoa segregation

Grading feijoa at-harvest using skin °hue not only differentiated fruit maturity at-harvest but also created populations with different characteristics after storage. Individual feijoa having °hue > 122 (STR) had longer storability than those with less °hue (R2E). Segregation of feijoa using skin colour (°hue) at-harvest may enable grower/packhouse to separate fruit destined for local or export markets. Profitability in feijoa orchards may be able to be increased by segregating STR fruit and marketing them through sea-freight to distant places while directing R2E feijoa to local markets in New Zealand. However, the high TA and low SSC in STR fruit is likely to impact their taste profile (Fig. 6.1B,E).

Instrument techniques are needed to assist maturity assessment especially if earlier harvesting is to be adopted, but evaluation of acceptability of feijoa graded using skin °hue is critical before making a recommendation. Quality will be defined by the consumer and hence the goal to provide satisfaction to the buyers of a fresh product remains as important as extending the storage life (Shewfelt, 2014). In this work feijoa maturity was determined by colour, however using a single quality measure was found to be risky and hence multiple attributes are recommended for fresh products (Toivonen & Beveridge, 2005). Wiryawan (2005) suggested use of non-destructive firmness as a potential indicator of maturity at-harvest, there may be potential in combining skin colour along with firmness in future.

6.4 Conclusion

Feijoa maturity may be sorted using skin colour (°hue) at-harvest. Skin °hue at the time of harvest has moderate relationship with after storage quality attributes (firmness, SSC, ripening index and flesh colour). Fruit having skin °hue > 122 (STR) were less mature at harvest when compared to those with lower skin °hue < 122 (R2E). STR fruit took longer to ripen in storage and appeared saleable after 6 weeks of storage, while 42% of R2E fruit were unsalable after 6 weeks of storage. However low SSC and high TA in sorted fruit remains a potential problem in the STR population, which may impact the resulting taste profile and hence a sensory evaluation is needed before making a recommendation. Additionally, reflectance spectra at the wavelength of 550 nm suggest chlorophyll degradation during maturation of feijoa. There is a need to investigate if other cultivars of feijoa can be segregated using skin colour.

7 Overall discussion and recommendations

7.1 Summary of findings

Presently postharvest storage life of feijoa (*Acca sellowiana*) is typically 4 weeks at 4 °C with a further 5 d at 20 °C before losing their edibility. New Zealand produces commercial feijoa (≈ 500 T per annum) with export of the fresh fruit partially limited by the geographical isolation that requires 6 weeks to sea-freight in acceptable condition to the distant markets of Europe, Asia or North America (Al-Harthy, 2010). Earlier attempts to improve postharvest life of feijoa using postharvest temperature manipulations, calcium dips, hot-water treatments or by CA storage were unsuccessful. Harvest maturity influences storage life, therefore this study focussed on revisiting harvest timing of feijoa and investigated storage performance of earlier harvested fruit. Skin colour was used to identify harvest maturity of feijoa and reduce variability within a fruit batch. As feijoa demonstrate climacteric behaviour during ripening, reducing ethylene effects through use of 1-MCP or AVG to enable storage life extension was also investigated.

7.1.1 Storage life extension

7.1.1.1 *Controlled atmosphere storage*

Thorp and Bieleski (2002) found low O₂ along with no CO₂ best suitable for storage of 'Triumph' cultivar and East et al. (2009) tested a matrix of 16 atmospheres and found that low O₂ (1.15-3 kPa) and CO₂ (0-0.2 kPa) were

suitable for storage of 'Unique' variety with limited benefits on quality. East et al. (2009) found darkening of flesh at high O₂ atmospheres and increased surface injury at lower O₂ atmospheres. Al-Harthi et al. (2010a; 2010b) reported surface browning and over ripe fruit after 6 weeks of storage in CA (2 or 5 kPa O₂ with 0 or 3 kPa CO₂) in 'Unique' and 'Opal Star' feijoa.

Reduced oxygen atmospheres (at 2 kPa O₂ + 0 kPa C O₂) contributed to decline in respiration rates and improved some quality aspects (firmness, TA and flesh colour) in feijoa (Fig. 3.2, 3.3 and 3.4). However, after 6 weeks of storage in CA, feijoa suffers from a severe surface injury making CA technology inappropriate for extension of storage life (section 3.3.2). Moreover the quality benefits accrued may be minimal when compared to the higher costs incurred for commercial CA or MA application.

7.1.1.2 Harvest timing

Feijoa harvested at touch-picked maturity possess 4 weeks of storage at 4 °C (Klein & Thorp, 1987). Our results confirm that feijoa harvested at touch-picking maturity are suitable for immediate consumption as they are in advanced maturity stage (Section 4.4.1). Feijoa harvested 2 weeks earlier than touch-picked maturity were immature with higher firmness, skin °hue and TA (Table 4.1). Early harvested feijoa required longer time to ripen than those touch-picked. Early harvested feijoa were found to possess firmness and internal ripening suitable for edibility even after 6 weeks of storage at 4 °C and a subsequent 5 d at 20 °C (Figs 4.3, 4.6). Downs et al. (1988) found that feijoa harvested with higher force (similar to early harvested) had the same eating appeal after 4 weeks of storage as those that were touch picked. Early

harvested fruit had lower SSC and higher TA after storage which may influence their taste profile (Fig. 4.4; Fig. 6.3). However feijoa harvested 4 weeks before commercial maturity were unsuitable for sale or storage. Feijoa harvested at this time did not reach physiological maturity as fruit remained unripe even after 6 weeks of storage. Longer storage potential of early harvested feijoa (6 weeks) enables potential export to distant markets. As feijoa is relatively unknown around the world, there is potential to offer early harvested feijoa in markets where there is no expectation of taste unlike New Zealand consumers. A market survey on consumer's acceptance of early harvested feijoa in any marketplace is necessary.

Feijoa fruit grow rapidly in size during the final phase of maturation (Harman, 1987). Mean fresh weights of earlier harvests were lower than those at touch picking maturity (Table 4.1). Saleable feijoa (> 45 g) were drastically reduced when fruit were harvested earlier by 2 weeks (3.8 kg per tree) than at touch picking time (11.6 kg per tree; Table 5.2). This yield effect by harvest timing may have been exaggerated by the harvesting method adopted in this study. All the fruit from feijoa trees were removed irrespective of their size in a single day, however in a commercial orchard fruit are touch-picked based on their size and each tree is re-visited in a 2-3 week window (Thorp & Bielecki, 2002). In order to overcome reduced yields in early harvest, feijoa can be harvested by only picking fruit that are saleable (size > 45 g) and allowing smaller fruit to subsequently attain size on the tree in 2-3 week period.

Early harvested feijoa were stored in this study at a recommended temperature (4 °C) found suitable for fruit harvested at commercial touch-picking maturity (Klein & Thorp, 1987). However in tomato where ripening of

fruit is influenced by the stage of harvest maturity, change in fruit colour is affected by storage temperatures that are specific to the maturity stage (Takahashi et al., 2014). Effects of interaction between storage temperatures and maturities in feijoa are presently unknown and need to be explored in the future. It is possible that early harvested feijoa may benefit from a different storage regime than those recommended for touch-picked fruit.

Fruit trees undergo different phenological stages during their annual growth cycle from bud dormancy up to fruit ripening (Salazar et al., 2006). Temperature fluctuations in the environment influenced fruit set and development in tomato (Adams et al., 2001). Time taken to mature and ripen was found to depend upon the time of flowering and the span of fruit development period (FDP) and in turn regulated by the temperature ranges specific to crops on field. The summation of heat units as growing degree days (GDD) is used to predict anthesis, fruit set and harvest time in peach (Marra et al., 2001). Parra-Conronado et al. (2014) estimated thermal time of Colombian feijoa as 2651 GDD and suggested that harvest date may be predicted. Future work on modelling phenological stages of New Zealand feijoa and predict harvest time may increase our knowledge on assessing maturity. However changes to grading standard of feijoa may be required before adopting changes to harvest timing by the industry in New Zealand.

7.1.1.3 Postharvest 1-MCP treatment

Postharvest treatment of feijoa with ethylene action inhibitor (1-MCP) had no impact either on fruit physiology (respiration and ethylene production rates) or on delaying ripening of touch-picked feijoa (section 4.3.2). An unpublished report by White and Woolf also noted that 1-MCP was not effective in delaying

ripening in 'Apollo' feijoa (Schotsmans et al., 2011). Similarly Velho et al. (2008) reported that commercially harvested 'Brazilian' feijoa were unaffected by 1-MCP treatments. Only Amarante et al. (2008) found 1-MCP applied at 500 or 1500 nL L⁻¹ for 8 h retained firmness and skin colour °hue after 30 d storage (4 °C) in 'Brazil (242)' feijoa. Thorp and Bielecki (2002) reported that all the modern cultivars in New Zealand have come from a single population of Uruguay type feijoa. It is possible that the 1-MCP effects found by Amarante et al. (2008) may be due to cultivar differences as reported in apple (Watkins & Nock, 2005).

Efficacy of 1-MCP depends upon maturity stage at the time of harvest in apple (Bulens et al., 2012) and pear (Chiriboga et al., 2013). However feijoa harvested earlier than touch-picked maturity showed few responses to 1-MCP. In addition, when observed, the effects of 1-MCP were modest when compared to the influence of harvest maturity on after storage quality.

1-MCP interactions based on concentration x temperature determines the duration of application (Blankenship & Dole, 2003). 1-MCP can be effective at concentrations ranging from 2.5 to 1000 nL L⁻¹. In this study feijoa were exposed to a high dosage of 1000 nL L⁻¹ for 12 h at 20 °C. Binding of 1-MCP to receptors is better at higher temperatures (Sisler & Serek, 1997). In tomato, ripening delays were directly related to very high concentration of 1-MCP at 5-20 µL L⁻¹ (Wills & Ku, 2002) while effects of 1-MCP at higher concentration at 1.5 µL L⁻¹ were found to be useful for Brazilian feijoa (Amarante et al., 2008). Higher concentrations of 1-MCP on other varieties of New Zealand feijoa may still need to be explored in the future.

Time taken from harvest to treatment with 1-MCP is important in fruit crops (Watkins, 2006). In apple the recommended time of application is within 7 d from harvest but Watkins and Nock (2005) found that minimizing the delay of 1-MCP treatment from harvest increased quality after long term storage. In this study 1-MCP was applied within 26 h after harvesting feijoa. It is possible that feijoa may respond differently if 1-MCP application was applied immediately after harvest.

1-MCP competes with ethylene and blocks the receptors in fruit tissue (Watkins, 2006). Internal concentration of ethylene in fruit was found to modulate efficacy of 1-MCP in tomato (Zhang et al., 2009), pear (Macnish et al., 2012) and apple (Jung & Watkins, 2014). The limited response to 1-MCP application in feijoa suggests that the fruit may have saturated concentration of endogenous ethylene at the time of harvest. Hypobaric pressure conditions were found to increase efficacy of 1-MCP in tomato (Dong et al., 2013) and avocado (Zhang et al., 2011) as this treatment results in a decrease in internal ethylene concentration (Zhang et al., 2010). It may be possible that feijoa exposed to hypobaric conditions and later treated with 1-MCP can result in a ripening delay and extend their storage life.

Effect of 1-MCP can be transient as tissue recovers sensitivity to ethylene in apple (McArtney et al., 2009) and pear (Ekman et al., 2004) by regeneration of new receptors. This was overcome by multiple application of 1-MCP (Lu et al., 2013). It is possible that feijoa generated new ethylene receptors and multiple 1-MCP treatments may be effective in delaying ripening. Treating 'Unique' feijoa with 1-MCP pre-storage is not suitable for storage life extension, however other cultivars may respond differently.

7.1.1.4 Preharvest AVG

Preharvest AVG treatment suppressed ethylene production and reduced fruit drop at commercial touch-picked maturity (Table 5.2). AVG treatments were effective when applied at 4 weeks but not at 2 weeks before commercial harvest. AVG treatment effected maturity by retaining firmness and reducing flesh colour (°hue) when compared to untreated fruit at touch-picking maturity (Table 5.3). Nevertheless storage life was not extended by AVG treatment as quality (firmness, SSC, TA or flesh colour) was not altered during storage (Fig.5.4). This work is the first published research of using preharvest AVG on feijoa.

Preharvest AVG has the potential to increase profitability of feijoa orchards in New Zealand. Feijoa yields (fruit number) appear higher in AVG treated trees (Table 5.3). This study found that feijoa yields may increase by 18.6% (number of fruit) if fruit drop is reduced. While harvesting was performed on a single day in this study, AVG effects on feijoa yields may be higher in a commercial scenario where saleable fruit (>45 g) are touch picked in a 2-3 week window. Mean fresh weights were not affected by AVG treatment in this study (Table 5.3). Green (2006) suggests that apple fruit weight can be increased using AVG treatment as the maturity delay enables fruit to remain longer on the plant and accumulate size. If feijoa harvesting was delayed then increase in average fruit weight may be possible.

Preharvest AVG at 4 weeks before touch picking time retained firmness at the time of harvest but flesh colour (°hue) reduced (Table 5.3). Ethylene production rates were suppressed during storage (Fig. 5.2) but there was no

impact on quality during storage (Fig. 5.4). Similar effects were found in peach and nectarine (Bregoli et al., 2006). In this study AVG was applied at similar dose as recommended for apple and stone fruit (Retain™, 125 mg L⁻¹ at 0.83 g L⁻¹ water). Effects of AVG in apple in reducing internal ethylene concentration increased linearly with higher concentration from 75 to 225 mg L⁻¹ (Schupp & Greene, 2004). Similar effects may be possible in feijoa with increased dose of AVG. Hence a robust commercial trial is needed to understand the effects of higher concentrations of AVG in delaying harvest time and storability of feijoa before making a recommendation to the industry.

7.2 Maturity grading of feijoa

Feijoa are harvested by a touch-picking method that provides ready to eat fruit for immediate consumption in local markets rather than to withstand a period of storage (Fig. 4.5 and Fig. 5.5). The results of this study confirm earlier findings of Al-Harthy (2010) who found that maturity of fruit harvested by touch-picking included a mix of immature, mature and over-mature feijoa (Fig. 4.1). In this study feijoa harvested early possessed longer storage potential but these fruit do not meet the present grade standards used by the industry. Harvesting early can result in amplifying uneven maturity within fruit batches (Fig. 4.5). If feijoa are harvested early, then touch picking of the fruit will not be needed by the industry and individual fruit segregation by skin colour at the time of harvest may be useful to divert fruit to appropriate markets based on their storage potential.

Grading feijoa maturity at harvest was attempted with limited success in the past using fruit density (Clark et al., 2005), acoustic impulse response (Gaddam et al., 2005) and non-destructive firmness (Wiryawan et al., 2005). Al-Harthy

(2010) found that non-destructive compression firmness and acoustic firmness were unable to distinguish maturity of feijoa. In this study skin colour ($^{\circ}$ hue) declined as maturity progressed (Table 4.1 and Table 5.3). It was hypothesised that skin $^{\circ}$ hue can be used to predict feijoa maturity at harvest and segregate fruit for storage potential.

In this study harvesting was performed at 4, 2, 1 and at touch picked maturity across 3 seasons (section 4.2.1, section 5.2.1 and section 6.2.1). Details on how 122 $^{\circ}$ hue threshold was chosen to segregate feijoa is detailed in section 6.3.3. A skin colour > 122 $^{\circ}$ hue (categorised as STR) resulted in maintaining quality with extended storage life (up to 6 weeks) when compared to those < 122 $^{\circ}$ hue (R2E; Fig. 7.1). Volumes of STR were higher in early harvested feijoa when compared to latter harvests. Likewise R2E fruit were in higher proportion as feijoa approached touch picked maturity. However STR fruit were found to be low in SSC and high in TA (Fig. 6.7). Feijoa harvested 4 weeks earlier than touch-picking maturity included fruit in $^{\circ}$ hue ranges between 122-130 $^{\circ}$ hue (Fig. 7.1), however these feijoa did not ripen even after 6 weeks of storage (Fig. 4.5). This indicates that skin colour ($^{\circ}$ hue) was useful in discriminating feijoa within batches only when fruit were physiologically mature.

Feijoa having < 122 $^{\circ}$ hue (R2E) were mature at-harvest and possessed lower storage (4 weeks) when compared to fruit > 122 $^{\circ}$ hue (STR; Fig. 6.7). R2E can be directed towards local markets for immediate consumption while STR feijoa that are less-mature at-harvest with longer storage potential (6 weeks) can be exported to distant markets using sea-freight. However the resulting low SSC and high TA that develops in STR fruit is likely to impact the

taste profiles of feijoa (Jayasena & Cameron, 2008). Hence before this strategy can be adopted an examination of consumer's taste acceptance is necessary.

Only 20.8% of fruit were found to be segregated for long storage using skin °hue in feijoa indicating that the benefits of grading may be uneconomical for orchards having low production volumes (Table 6.3). It is possible that the harvest method used for conducting this work influenced the proportion of long stored fruit within the population. If size of feijoa (> 45 g) was used as a criteria for picking the fruit as followed in commercial touch picking then it may be possible to have higher STR within a batch. Additional research is required by extending this work to other cultivars of feijoa before making a recommendation.

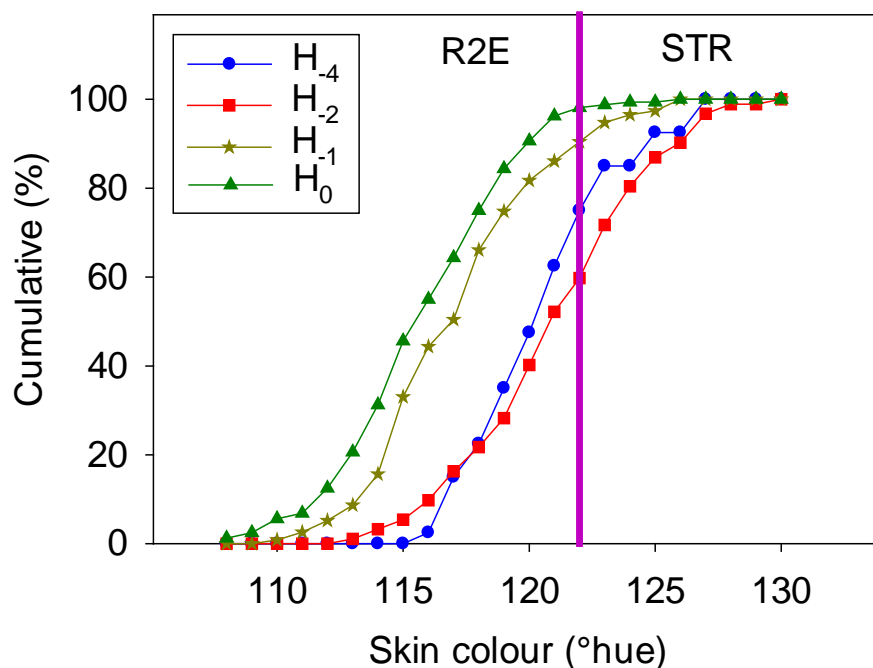


Fig. 7.1 Distribution of feijoa population harvested at 4 different times; H₋₄, H₋₂, H₋₁ and H₀ were harvested at 4, 2, 1 week before and at touch-picked maturity respectively. Vertical bar on 122 °hue represents the threshold for segregation of long storing feijoa (STR) from ready-to-eat (R2E) fruit. Seasonal data of section 4.2, 5.2 and 6.2 included in this graph

Visible light spectroscopy (wavelengths 360-740 nm) was also investigated to discern feijoa maturity at harvest. Reflectance at 550 nm was found to differentiate maturity at the time of harvest. Reflectance of STR (14.9%) feijoa was lower than R2E (19.9%) fruit (Fig 6.7). Chappelle (1992) found that reflectance at 550 nm for soybean leaves was influenced by chlorophyll content. Merzlyak et al. (2003) found that changes in reflectance at 550 nm was due to differences in chlorophyll content in green skinned apple. In both cases (Chappelle et al., 1992; Merzlyak et al., 2003) higher reflectance at 550 nm was related to lower chlorophyll content. With the progression of maturity chlorophyll degradation on feijoa skin may be responsible for the resulting changes in skin colour (°hue). Dark green coloured skin became lighter as feijoa matured which may have resulted in an increase in reflectance at 550 nm in R2E fruit. Reflectance measurements at 550 nm may decode feijoa maturity at-harvest and have potential for grading fruit at the time of harvest. Skin colour as a non-destructive predictor of maturity at-harvest may add value in re-designing grade standards for the feijoa industry in the future. There is a need for further trials on other varieties of feijoa to see if skin colour can segregate fruit within a batch of feijoa.

7.2.1 X-ray computed tomography (CT)

A cross section of feijoa fruit reveals an internal fleshy pericarp enclosing a jellied section of 4 to 6 locules with seed pulp (Al-Harthy, 2010; Thorp & Bieleski, 2002). X-ray CT can be used for inspecting fruit and vegetables quality to identify internal defects (Lammertyn et al., 2003). Potential use of X-ray CT for monitoring maturity differences was reported in tomato (Brecht et al., 1991), mango and peaches (Barcelon et al., 1999a, 1999b). Magwaza & Opara (2014)

found x-ray CT technique useful in quantifying volume of edible fruit portion in pomegranate.

7.2.1.1 Methods

Feijoa were harvested from a commercial farm at Matamata, New Zealand in April, 2011. Feijoa of three grades namely touch picked, harvested with greater force than touch-picking (considered immature) and those collected from ground (considered over mature fruit). Fruit were transported to postharvest laboratory in a corrugated fiber board with plix.

Fruit were scanned using a CT scanner (Philips Medical Systems, Cleveland OH, USA). Scanned images were processed using MERGE™ Healthcare software to calculate CT number (Hounsfield unit, HU) and Matlab™ software used a threshold algorithm to distinguish feijoa fruit.

7.2.1.2 Results

Major differences in feijoa were distinguished based on changes to CT number (HU) as region with fleshy pericarp were lower when compared to the locular region (Fig.7.2). In the pericarp tissue there was a dramatic reduction in mean CT number of a mature feijoa (≈ -39.4 HU) when compared with an immature (≈ 21.9 HU) fruit while there were no noticeable differences in the locules.

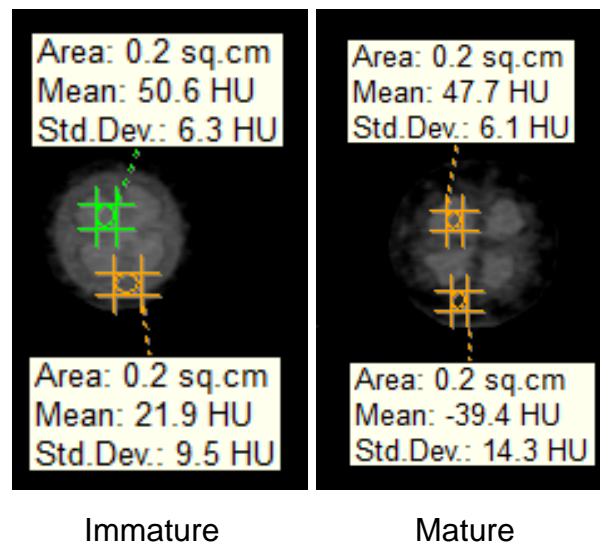


Fig. 7.2 X-ray CT scan image of feijoa (transverse slice) using Philips six slice helical CT scanner (Philips Medical Systems, Cleveland OH, USA) and processed using MERGE™ Healthcare software showing differences in CT number (HU) between locule and pericarp regions of an immature and mature feijoa

It was possible to observe and report two distinct regions within the pericarp of a mature feijoa fruit. The outer pericarp and inner pericarp of feijoa can be delineated with further image processing using Matlab™ software (Fig. 7.3). Matlab™ software distinguished feijoa into three conspicuous regions with > 19 HU representing locular gel. Inner pericarp ranged between > -47 and < 19 HU while outer pericarp was at > -145 and < 47 HU. During maturation of feijoa the fleshy locular region becomes clear (jelly), however X-ray CT appears to show potential for identifying maturity of feijoa in region of the pericarp as mean CT number declined (Fig. 7.2). It is postulated that the CT number declines during maturation of feijoa. Future studies using X-ray CT may be helpful in determining maturity of feijoa and aid in re-defining grading standards of the industry.

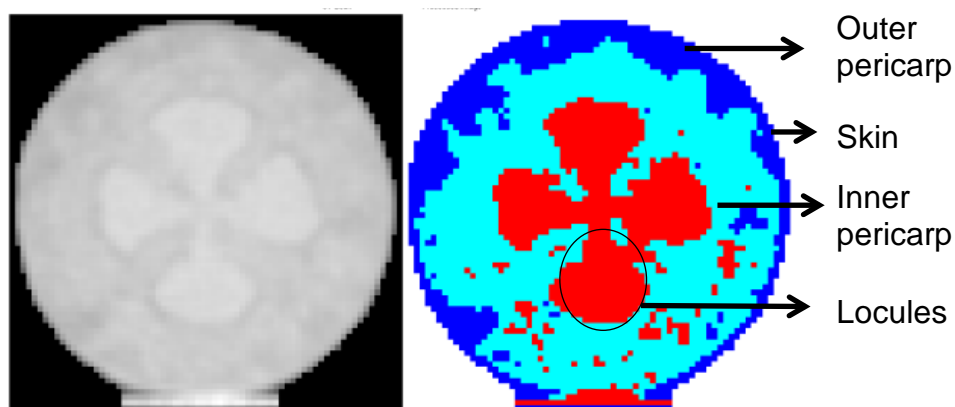


Fig. 7.3 X-ray CT scan image of a mature feijoa (transverse slice on left) processed using Matlab™ software (on right) that discriminated fruit regions into locular jelly (red), inner pericarp (light blue) and outer pericarp (dark blue)

7.3 Ethylene in feijoa

The gaseous plant hormone ethylene regulates ripening of climacteric fruit. Feijoa's climacteric behaviour was earlier demonstrated by Biale et al. (1954) and Reid (1975). Controlling the start and the rate of ripening in fruit is a major goal for postharvest researchers as this enables extension of storage life and spoilage reduction (Bleecker & Kende, 2000). Controlling biosynthesis or perception of ethylene is useful in providing valuable insight into the ripening processes of fruit and vegetables (Watkins, 2006). In our study feijoa's dependency on ethylene to ripen was investigated with the application of preharvest biosynthesis inhibitor AVG or by applying 1-MCP prior to storage or by applying ethylene during storage.

7.3.1 Biosynthesis of ethylene

In this study ethylene production was undetectable ($< 0.08 \text{ pmol kg}^{-1} \text{ s}^{-1}$) in feijoa harvested 4 or 2 weeks earlier than commercial touch-picked maturity

(Fig. 4.2). At the time of 'touch-picked' harvest ethylene production was < 1 pmol kg⁻¹ s⁻¹. These low rates of ethylene production suggest that fruit were at a pre-climacteric stage (system 1) at the time of harvest. During storage increase in ethylene production was observed in both early harvested and touch-picked feijoa reaching up to 220 pmol kg⁻¹ s⁻¹ at 20 °C (Fig. 5.2). Ethylene production rates declined after 8 weeks storage indicating that feijoa entered a post-climacteric stage.

Ethylene biosynthesis is controlled by the rate limiting action of ACS enzyme which converts SAM into ACC (Kende, 1989). Low rates of ethylene production at harvest with increasing rates during postharvest ripening indicate the likelihood of a rise in the activity of ACS enzyme. During tomato ripening ACS enzyme activity is controlled by 9 *LEACS* genes (Lin et al., 2009). It is possible that multiple genes of ACS enzyme are involved in the regulation of ethylene biosynthesis during ripening of feijoa.

Ethylene production within a fruit can vary greatly in different tissues as evident from studies on tomato where pericarp produced higher amounts when compared to the central region (locules with septa and seed) indicating a tissue specific ethylene regulation (Van de Poel et al., 2014). Ethylene is produced as a result of internal development factors as well as external factors (Yang & Hoffman, 1984). An external factor that induces ethylene production is water stress caused by detachment from the tree (Apelbaum & Yang, 1981). Barry & Giovannoni (2007) suggested that initial water loss near the abscission zone of detached fruit triggers ethylene synthesis that can influence ripening in a climacteric fruit. This mechanism was demonstrated after harvest in persimmon where ethylene was produced in the calyx located near the abscission zone (Nakano et al., 2003). Feijoa has a persistent calyx at the distal end of the fruit

opposite to the abscission zone. It still remains entirely possible that ethylene production induced by stress may be responsible for initiation of system 2 production postharvest. However, contrastingly feijoa tissue was insensitive to postharvest application of exogenous ethylene treatment (Fig. 4.3) and hence stress may not be responsible for transition into system 2 ethylene.

Ethylene production was reduced when fruit were treated by preharvest AVG while 1-MCP and exogenous ethylene had minimal effect on production rates during storage (Fig. 7.4). Feijoa displays a fairly typical ethylene climacteric during its postharvest life. While a climacteric rise is witnessed in postharvest ripening of feijoa, its purpose remains unclear and it may be possible that ethylene synthesised during climacteric is required for the production of volatiles during fruit ripening (Al-Harthy, 2010).

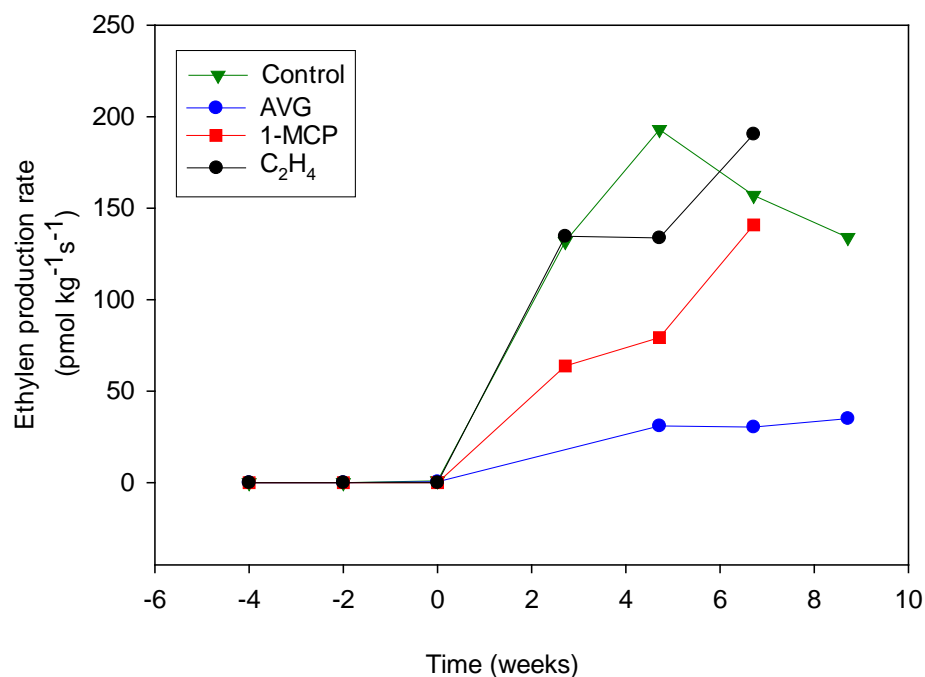


Fig. 7.4 Effect of 1-MCP, AVG and exogenous ethylene treatments on ethylene production of feijoa. Y-axis are mean values at 20 °C, data from Fig. 4.3 and Fig. 5.2

7.3.2 Ethylene during fruit maturation

Feijoa size increases during the final phase of fruit development in the last 4 weeks prior to natural fall (Harman, 1987). Our data confirmed this increase in mean fresh weights during the final stage of feijoa maturation (Table 4.1; Table 5.3). Feijoa flesh changes during maturation as the locules become clear and softening occurs on the tree (Fig. 4.1).

Ethylene production was undetectable ($< 0.08 \text{ pmol kg}^{-1} \text{ s}^{-1}$) at the time of application of AVG (4 weeks before commercial harvest time) and very low ($< 1 \text{ pmol kg}^{-1} \text{ s}^{-1}$) at commercial harvest time indicating a pre-climacteric stage (Fig.5.2). Preharvest AVG treatment applied 4 weeks prior to commercial harvest time retained fruit firmness with lower flesh colour ($^{\circ}\text{hue}$) at touch-picking maturity (Table 5.3). Maturity delays observed at the time of commercial harvest is likely due to the suppression of ethylene synthesis by preharvest AVG sprays. Hence, it would seem that low basal levels (system I) of ethylene promoted softening of feijoa before harvest, similar to apple where pre-climacteric ethylene plays an active role in controlling maturation on the tree (Varanasi et al., 2013). The mechanism of softening on the tree may be due to expansion of cells as the fruit increases size as suggested for maturing apple (Johnston et al., 2002). However, feijoa fruit size was not influenced by AVG in this study (Table 5.3). Greene (2006) suggested that AVG can indirectly effect size by allowing fruit to remain longer on the tree, however this effect was not tested in this study as harvesting was performed at commercial touch-picking maturity.

Preharvest AVG application in feijoa resulted in reducing fruit drop at commercial touch-picking maturity (Table 5.2). Ethylene accelerates the

process of abscission (González-Carranza et al., 1998) and results of this study confirm the active role of ethylene in forming the abscission zone of feijoa during fruit development. Abscission zone formation is complex with both ethylene-dependent and independent regulation (Binder & Patterson, 2009). For example, Meir et al. (2006) found that formation of the abscission zone in leaves of *Mirabilis jalapa* was regulated by auxins (indole-3-acetic acid, IAA) which also affected sensitivity to ethylene. The interaction of other plant hormones with ethylene during feijoa abscission is possible.

7.3.3 Postharvest 1-MCP responses

Feijoa harvested at touch-picking maturity did not respond to 1-MCP treatments as fruit physiology (respiration and ethylene production rates) and quality were not altered after storage (section 3.4.2 and section 4.4.2). High internal ethylene concentrations reduce the efficacy of 1-MCP treatment in tomato (Zhang et al., 2009), apple (Jung & Watkins, 2014) and pears (Macnish et al., 2012). Internal ethylene concentrations were not monitored for feijoa in this work and since endogenous ethylene competes with 1-MCP (Serek et al., 2006), it appears that the binding sites in feijoa may be saturated by endogenous ethylene at commercial touch-picking maturity and hence did not respond to 1-MCP treatments.

It is assumed that 1-MCP binds irreversibly to ethylene receptors and prevent responses of ethylene (Sisler & Serek, 1997; Watkins, 2006). The mode of ethylene action has been elucidated from studies using model plants *Arabidopsis thaliana* and tomato (*Lycopersicon esculentum*). Binder and Bleecker (2003) proposed an “inverse antagonistic model” where ethylene receptors negatively regulated ethylene responses. In this model, ethylene

binds to the receptor and results in inactivation of the receptor (Hirayama et al., 1999). Induction of ethylene responses is due to the inactivation of multiple ethylene receptors (Hua & Meyerowitz, 1998). This model predicts that the magnitude and sensitivity of ethylene responses increases with a decrease in the number of receptors (Tieman et al., 2000).

Ethylene responses can occur when 0.1% of receptors are bound to ethylene (Schaller & Bleecker, 1995). Receptor clustering has been proposed by Binder (2008) to explain the possible interactions between different receptors leading to a response by a plant even at low occupancy of ethylene on receptors. This may result in an amplified response as plant senses even at low concentrations (5 nmol) of ethylene (Bray et al., 1998). Binder (2008) updated the ethylene receptor signalling model by including an intermediate state after ethylene binds to the receptor in order to explain the complex responses of the plant at saturating levels of ethylene. At this stage the ethylene receptor is not immediately inactivated in the presence of ethylene. If this is true and to date no information is available on ethylene receptors in feijoa, hence further work is required to quantify ethylene receptors of feijoa during postharvest ripening.

The number of receptors that may be blocked by 1-MCP is specific to fruit species and hence different concentration recommendations are available (Blankenship & Dole, 2003; Watkins, 2006). In this study 1-MCP was applied at concentrations (1000 nL L^{-1}) that evoked responses in closely related guava (Myrtaceae family), however it may be possible that feijoa responds differently to a higher dose of 1-MCP.

Effects of 1-MCP can be transitory as tissue can regain their sensitivity to ethylene after some time as observed in tomato (Tassoni et al., 2006). A

hypothesis for the recovery from inhibition of perception by 1-MCP is due to the generation of new ethylene receptors is supported by studies conducted in tomato (Tassoni et al., 2006). Hence in some fruits multiple 1-MCP treatments performed better than a single treatment in delaying ripening as witnessed in pear (Ekman et al., 2004) and apple (Lu et al., 2013). Commercial application now allows for multiple applications (Nock & Watkins, 2013). Apple exposed to multiple applications within 7 d after harvest had the most beneficial effect for long term storage (Lu et al., 2013). It is possible that new ethylene receptors were regenerated in feijoa and these new binding sites limited the effects of pre-storage 1-MCP application. If this hypothesis is true then multiple 1-MCP applications may have a positive effect in blocking new ethylene binding sites and result in delayed ripening in feijoa. Alternatively, Huber et al. (2010) suggested that metabolism of 1-MCP directly influences tissue's sensitivity to applied 1-MCP. This raises the question that in addition to ethylene receptors other processes like degradation and sorption of 1-MCP are important to understand plant sensitivity to 1-MCP.

Maturity stage at the time of harvest influences efficacy of postharvest 1-MCP treatment in many fruit (Blankenship & Dole, 2003). There are diverse responses of climacteric fruit to the application of 1-MCP after initiation of ripening. For example, only 2 days after the initiation of ripening avocado becomes insensitive to 1-MCP (Adkins et al., 2005) while tomato (Ergun et al., 2006), papaya (Manenoi et al., 2007) and pear (Hiwasa et al., 2003) are sensitive even in advanced stages of ripening. During commercial harvest, high variability in the internal ethylene concentration was noticed depending upon the maturity stage of apple (Watkins & Nock, 2012). Maturity effects may

appear to be inter-related in combination with factors like internal ethylene concentration and gene expression of cultivars in apple (Jung & Watkins, 2014). In this study effects of 1-MCP was minimal irrespective of harvest maturity in 'Unique' feijoa and there are no reports on maturity effects on other varieties.

7.3.4 Exogenous ethylene on feijoa

In this study feijoa harvested early (2 weeks before touch-picking maturity) appeared visually immature with undetectable ethylene production ($< 0.08 \text{ pmol kg}^{-1} \text{ s}^{-1}$) at the time of harvest (pre-climacteric). However during storage there was an increase in ethylene production rates reaching up to $220 \text{ pmol kg}^{-1} \text{ s}^{-1}$ (climacteric system 2). Addition of ethylene to climacteric fruit usually triggers advancement of ripening (McMurchie et al., 1972). However exposure of feijoa (of different maturities) to high concentrations of ethylene ($1000 \text{ }\mu\text{L L}^{-1}$) at $4 \text{ }^{\circ}\text{C}$ was observed to have no effect on ethylene production rates or quality (section 4.4.4) after storage (Al-Harthy, 2010; Velho et al., 2008). Only Akerman et al. (1993) reported that feijoa exposed to exogenous ethylene resulted in increasing ethylene production and a decline in TA during storage. Feijoa responses reported in this study contrasts the expectation of exogenous ethylene or propylene during the natural pre-climacteric phase inducing a rise in the production of endogenous ethylene that is associated with climacteric fruit systems (McMurchie et al., 1972). Questions remain on the purpose of climacteric rise in ethylene production during ripening of feijoa. In this study quality attributes of feijoa firmness, TA, SSC, ripening index, colour of skin and flesh remained unaffected after storage by exogenous ethylene. It may be possible that ethylene is required for stimulation of aroma volatiles development in feijoa ripening (Al-Harthy, 2010).

It was suggested that once ripening is initiated in some climacteric fruit then their endogenous ethylene levels increase and reach saturation, making the fruit insensitive to further exposure to ethylene (Saltveit, 1999). In apple Johnston et al. (2009) found that ripening attributes became insensitive after tissue reached saturation and further exposure to higher concentrations of ethylene had no effect. Likewise ripening events in feijoa may be dependent upon ethylene for initiation but may have low sensitivity to ethylene with progression of time. The hypothesis that feijoa tissue in 'Unique' cultivar may be saturated with endogenous ethylene explains the results of this work as fruit were unresponsive to 1-MCP and insensitive to exogenous ethylene.

In banana, Golding et al. (1999) suggested that some processes during fruit development after initiation by ethylene can become independent of ethylene. Pech et al. (2008) demonstrated that ripening of climacteric melons was regulated by both ethylene-dependent and independent pathways. It is possible that postharvest ripening in climacteric feijoa may be substantially regulated by ethylene-independent pathways.

7.3.5 Conceptual model of ethylene's role in feijoa

Combining the results of the effects that AVG, 1-MCP and exogenous ethylene had on feijoa, a conceptual model is outlined to encapsulate the role of ethylene during maturation and postharvest ripening (Fig. 7.5). Responses of cv. 'Unique' observed in this study displayed peculiarities contrasting classical climacteric fruit ripening. There was no detectable ethylene during the last 4 weeks prior to commercial harvest time but low rates of ethylene production were observed at touch picking maturity ($< 1 \text{ pmol kg}^{-1}\text{s}^{-1}$). These low levels of ethylene influenced fruit development during the final stages of feijoa maturation

on the tree. Ripening events like abscission zone formation, softening and flesh colour were influenced by AVG treatments demonstrating the critical role played by ethylene during feijoa maturation. Ethylene production rates increased during storage and AVG suppressed ethylene production rates without altering quality during postharvest storage. 1-MCP treatments had limited effect on the physiology (respiration and ethylene biosynthesis) and quality of postharvest feijoa irrespective of maturity stage of fruit. Postharvest quality of firmness, SSC, TA and colour of skin and flesh of feijoa appear to exhibit ethylene-independent ripening. Feijoa were insensitive to the application of exogenous ethylene. The climacteric rise in ethylene production may be influencing the production of volatiles in feijoa (Al-Harthy, 2010).

A schematic chart that attempts to summarise the role played by ethylene prior to harvest and during postharvest storage of feijoa is provided (Fig. 7.5). This conceptual model has been proposed after compiling the current findings of this study along with the existing literature available on feijoa by Al-Harthy, (Al-Harthy, 2010), Velho et al., (Velho et al., 2008), Thorp & Bieleski (Thorp & Bieleski, 2002) and Reid (1975). Amarante et al. (2008) was not included in this model primarily due to the contrasting results. The highlights of this model are

- Ethylene production (system 1) is observed up to the time of commercial harvest of feijoa. Feijoa turns to autocatalytic (system 2) ethylene production during postharvest life.
- Firmness, TA and skin °hue decline with the progression of maturity in feijoa.
- Ethylene suppression at preharvest by AVG reduces fruit abscission and delayed maturity at commercial harvest time.

- Touch-picked feijoa cannot withstand 6 weeks of storage while those harvested 4 weeks earlier are not physiologically mature.
- Feijoa harvested 2 weeks before touch-picked maturity have ripening delays and appear storable for 6 weeks (high TA and low SSC may change their taste profile).
- Blocking ethylene responses of postharvest feijoa using 1-MCP was not effective in substantially delaying ripening and storage quality.
- Feijoa are insensitive to exogenous ethylene both at the time of maturation and during ripening stages.

No enzymatic or molecular studies have been undertaken on feijoa (fruit development & ripening) and hence it was not possible to identify specific mechanisms in this model. In climacteric fruit like pears (Hiwasa et al., 2003); banana (Trivedi & Nath, 2004) and kiwifruit (Boquete et al., 2004) ethylene not only initiates ripening but also controls the progression of ripening events. However in feijoa ethylene initiated ripening has been demonstrated while fruit is attached to the tree, however postharvest ripening events are found to be substantially regulated by ethylene-independent processes. Ethylene's role in feijoa ripening is an interesting case study of unconventional behaviour observed in natural biological systems.

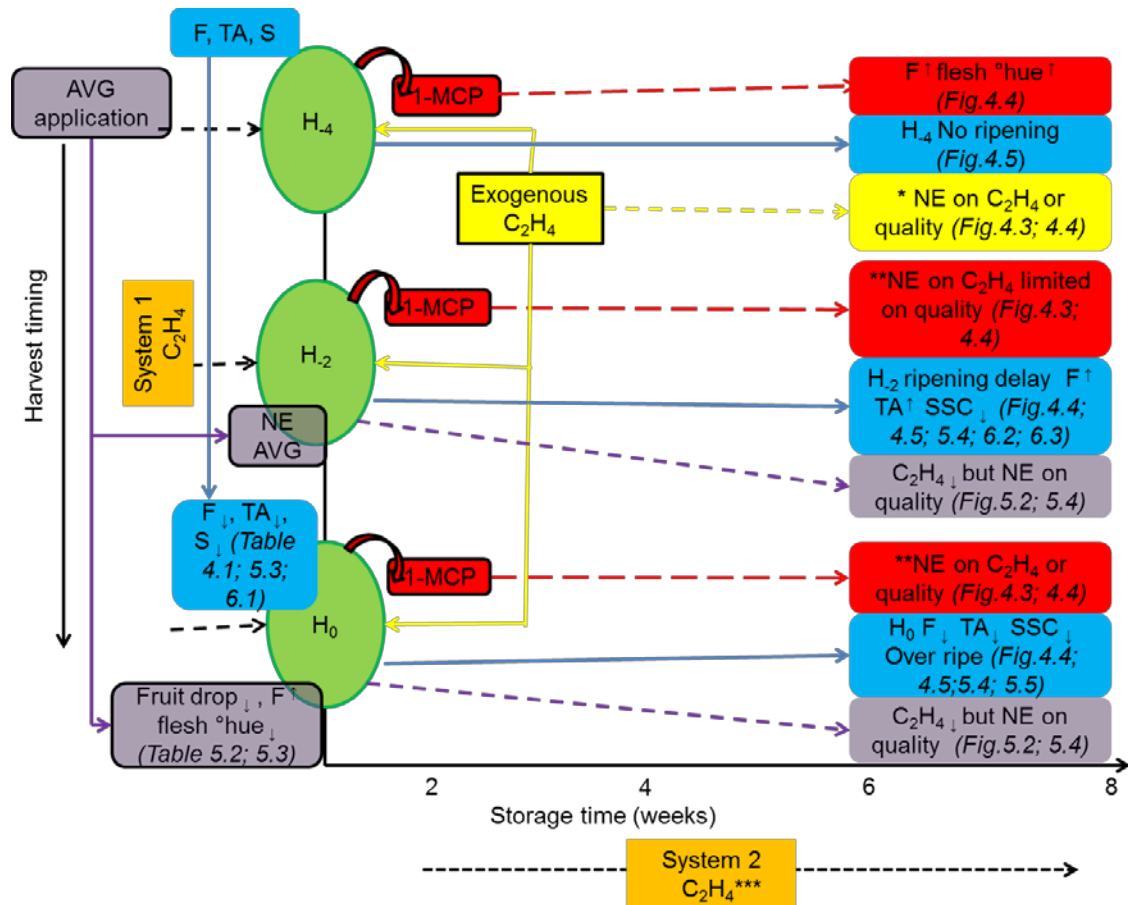


Fig. 7.5 Schematic chart showing ethylene interactions during maturation and ripening of feijoa. H_{-4} , H_{-2} and H_0 are harvests conducted at 4, 2 week before and at touch-picked maturity. Symbols indicate firmness (F), titratable acidity (TA), skin °hue (S), soluble solids content (SSC), reduced (\downarrow), higher (\uparrow) no effect (NE), * (Al-Harthy, 2010 & Velho et al., 2008), ** (Velho et al., 2008) and * (Al-Harthy, 2010 & Reid, 1975). Colour red indicates 1-MCP treatment, violet for AVG, yellow for exogenous C_2H_4 and blue for no manipulation**

7.4 Unexplored opportunities

7.4.1 Role of polyamines

Ethylene and polyamines have an antagonistic effect during fruit development as polyamines compete for the common substrate (SAM) that is involved in the production of ethylene (Torrighiani et al., 2004). Exogenous

polyamines were found to delay ethylene production and delay ripening in guava (Mondal et al., 2008), plums (Serrano et al., 2003) and peaches (Liu et al., 2006). Interaction between polyamines and ethylene during feijoa ripening is presently not known and future research may improve our understanding of postharvest fruit responses. However use of polyamines to manipulate ethylene may not yield storage life enhancement in feijoa as postharvest ripening appears to be independent of ethylene.

7.4.2 Other pre or postharvest treatments

Postharvest nitric oxide fumigation at $10 \mu\text{L L}^{-1}$ delayed ripening of Japanese plums (Singh et al., 2009). Possible mode of action of nitric oxide is not fully known but a recent study by Hong et al. (2014) found that nitric oxide suppressed expression of ACO and ERS1 genes leading to decrease in ethylene production and delayed ripening during storage of mango. Methyl jasmonate is found to effect ripening without requiring ethylene action (Fan & Mattheis, 1999). Preharvest application of methyl jasmonate improved storage quality of plums by reducing ethylene production and respiration rates and increasing the activity of total phenolics and antioxidant enzymes (Zapata et al., 2014). Preharvest application of nickel extended postharvest storage life in persimmon (Zheng et al., 2006). Nickel, cobalt and silicon were found to lower ethylene production and increase vase life in carnations (Jamali & Rahemi, 2011). Feijoa is a less researched crop that is yet to be explored by a range of pre and postharvest treatments that influence in storage life extension.

7.4.3 Other non-destructive grading

Time-reflectance spectroscopy (TRS) is a novel technique that is found to be reliable in predicting maturity of peaches (Shinya et al., 2013) and nectarines (Eccher Zerbini et al., 2009). TRS instruments detect the distribution of time-of-flight of photons from a fixed distance (Torricelli et al., 2008). Near infra-red (NIR) radiation using wavelengths in the region of 760-2500 nm was found to predict maturity at harvest in fruit crops (Burdon et al., 2014a). Chlorophyll pigment analysis using DA meter by monitoring absorbance spectrum at 670 and 720 nm is commercially available for apple industry to determine harvest maturity (DeLong et al., 2014). Calculating normalised difference vegetation index (NDVI) using reflectance measurements in the visible region (670 nm) and NIR (780 nm) was also useful to measure chlorophyll content in apple (Kuckenberg et al., 2008). In our study reflectance at 550 nm was found to show potential in segregating feijoa maturity at harvest. Using other spectral reflectance measuring instruments may be useful in predicting feijoa

Nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) techniques are used extensively in laboratories to detect fruit quality (Butz et al., 2005). These techniques were useful to monitor ripening stages of tomato (Musse et al., 2009). Al-Harthy (2010) found reasonable relationship between spin-spin relaxation time and compression firmness of feijoa and suggested that MRI may be useful in determining maturity of feijoa. In this study X ray CT was useful to identify maturity differences in feijoa. These non-destructive techniques may be explored for segregating at-harvest feijoa.

7.4.4 Ripening index

Presently a feijoa internal visual maturity scale developed by Plant & Food Research, New Zealand in 2004 is used to grade fruit at the time of harvest (Schotsmans et al., 2011). This index has been based on the locular gel clearing and browning of internal flesh of feijoa. In this study ripening index after storage was based upon this at-harvest maturity scale. In the present scale fruit ranked as '1' are immature at the time of harvest and were considered not to ripen, however this study demonstrates ripening of these feijoa after 6 weeks of storage. Similarly feijoa flesh and locule turning brown are ranked '4' and were considered over mature at the time of harvest (suitable only for processing industry). However after a period in storage fruit ranked as '4' appear saleable and may be suitable for consumption. Hence a ripening index based on visual, non-destructive and sensory attributes may be developed for feijoa in the future. This will contribute towards consistence in quality which is desired by customers of fresh products. Having well understood standards of feijoa by all the market players is necessary for growth of the industry.

7.4.5 Other cultivars of feijoa

Effects of ethylene action inhibitor (1-MCP) were found to be cultivar dependent in apple (Watkins et al., 2000) and likewise AVG effects was variable between varieties of same fruit species (Byers, 1997a). Responses of 'Unique' feijoa to ethylene manipulations may be entirely because of cultivar specific factors. Presently more than 23 commercial varieties of feijoa are available for plantation in New Zealand alone (Anon, 2014a). 'Unique' cultivar appears to be no longer popular in new orchard plantings due to its fruit size as 'Kakariki',

'Wiki Tu', 'Anatoki' and 'Kaiteri' produce bigger fruit (Frans de Jong, personal communication, 29th December, 2014). Hence future investigations on other popular cultivars of feijoa are recommended for extending storage life of feijoa.

7.4.6 Genetically modified feijoa

Pech et al. (2012) suggests that ripening processes can be altered in some fruit only when there is complete inhibition (>99%) of ethylene biosynthesis. This physiological state has been witnessed in genetically modified (anti-senescence) cultivars of apple, tomato and cantaloupe melons. Development of modified cultivars of apple by silencing ACS enzyme displayed extended storage life (Dandekar et al., 2004), similar applications can be useful for development of a new feijoa cultivar in the future. Espley et al. (2013) genetically engineered an increase in anthocyanin pigment in white fleshed apple cultivar by turning it into a red fleshed fruit. Future studies through genetic engineering may be useful for introducing an anthocyanin producing gene which triggers skin colour change at the onset of ripening in feijoa. However there may be opposition to the consumption of genetically modified products from a number of consumers.

7.5 Thesis conclusion

Feijoa storage life was extended from 4 weeks (present) at 4 °C up to 6 weeks by harvesting fruit early (2 weeks from commercial touch-picked maturity). There is potential to export early harvested feijoa to distant places in Europe and North America using sea-freight in acceptable condition. However, low SSC and high TA may affect their taste necessitating consumer's taste acceptance study before making a recommendation. Harvesting feijoa early can

rule out the use of touch-picking method that is presently employed by the industry in New Zealand. This may impact the grading standards used by the market players. There is a need to undertake identification of a ripening index while redefining the grading standard of feijoa in New Zealand.

Skin colour (°hue) and reflectance (550 nm wavelength) demonstrated potential for use as a non-destructively segregation method for individual feijoa within a batch. Feijoa graded by skin colour can be useful in diverting long storage fruit (having °hue >122) to export markets and target the local markets with ready-to-eat fruit (<122 °hue).

Blocking postharvest ethylene action using 1-MCP was ineffective in extending storage life of feijoa harvested at different maturities. Preharvest application of ethylene inhibitor AVG at 4 weeks before commercial maturity was found to reduce fruit drop and delay maturity of feijoa at the time of harvest. There is potential for commercial application of preharvest AVG sprays to increase profitability of feijoa orchards. A conceptual model summarising the role of ethylene during maturation and postharvest storage has been prepared. This study showcases unusual behaviour in climacteric feijoa where ethylene plays an active role during fruit maturation but postharvest ripening was found to be substantially driven by ethylene-independent pathways. This model may be helpful to design future strategies for storage life extension in feijoa.

7.6 Recommendations

7.6.1 Sensory analysis

Feijoa harvested 2 weeks earlier than touch-picking maturity appear visibly acceptable after 6 weeks of storage at 4 °C and a subsequent 5 d at 20

°C, however °Brix/acid ratios may affect their taste profile. Hence a sensory analysis of consumer's acceptance of taste is recommended in a relatively new marketplace like Singapore, Hong Kong or Japan where feijoa is relatively unknown. The outcome of this investigation may have profound impact on export revenues for feijoa industry in New Zealand.

7.6.2 Commercial trial of preharvest AVG

Preharvest AVG sprays resulted in reduction of fruit drop and delayed maturity in feijoa. Results from this study suggest potential use of preharvest AVG to increase profitability of feijoa orchards. A robust commercial trial is recommended for use of preharvest AVG on other cultivars of feijoa.

7.6.3 Multiple 1-MCP treatments

It is possible that the new receptors generated by some plant tissues were blocked by repeated 1-MCP treatments. Multiple doses of 1-MCP treatment was found to be useful in apple (Lu et al., 2013; Mir & Beaudry, 2001) and pear (Ekman et al., 2004). It will be interesting to investigate if repeated dosage of 1-MCP can delay ripening and extend storage life of feijoa.

7.6.4 Developing maturity and ripening index of feijoa

The outcome of this research with harvesting early feijoa necessitates changes to the existing grading standards employed by the industry in New Zealand. Touch picking of feijoa may be replaced in the grading standard and a separate ripening index should be designed for feijoa. Developing indices involves all the stakeholders of the industry (researcher, grower, packhouse,

exporter and consumer) in New Zealand. This is pertinent for the future expansion of feijoa industry in New Zealand.

7.6.5 Studies on other cultivars

In many climacteric fruit crops reducing ethylene effected responses depended upon cultivar traits. 'Unique' cultivar of feijoa is becoming less popular among recently established orchards in New Zealand. It is entirely possible that other upcoming varieties of feijoa (Kakariki, Wiki Tu, Anatoki and Kaiteri) may respond with extended storage life.

7.6.6 Non-destructive techniques

In our work we found X-ray CT to have the potential in determining maturity of feijoa. We have not explored NDVI measurements or DA meter for estimating chlorophyll content of feijoa skin during fruit development. These methods may be worthwhile to explore in assisting the determination of harvest maturity in feijoa.

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