‘Hayward’ Kiwifruit Responses to Ethylene in Controlled Atmosphere and Storage Performance in Modified Atmosphere Packaging

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Abstract

The relatively short storage life of some recently developed kiwifruit cultivars is the key limitation for sea freight. Modified atmosphere packaging (MAP) is a promising approach to maintain kiwifruit quality through the supply chain with high flexibility and relatively low cost, whereas there is a potential risk of ethylene accumulation in MAP that may accelerate kiwifruit softening. Meanwhile, controlled atmosphere (CA) can be used in research as a tool to investigate various atmospheric conditions. Hence, the objectives of this study were to investigate the influence of ethylene on kiwifruit quality in an optimal atmosphere, to evaluate the performance of kiwifruit in MAP during storage at low temperature and subsequent shelf-life at room temperature, and to decide whether an ethylene scavenger can reduce the damaging effects of ethylene in MAP.

Kiwifruit (*Actinidia chinensis* var. *deliciosa* 'Hayward') were stored in a flow-through system of air and optimal CA (5% CO_2 + 2% O_2) at 0 °C 95% RH for 13 weeks, and ethylene at the concentration of <1, 10, 100, 1000 $nL \cdot L^{-1}$ was added to the system after 3 weeks. The result has indicated that the responses of kiwifruit to ethylene were dose-dependent in both air and CA, but kiwifruit sensitivity to ethylene was lower in CA compared to that in air.

Kiwifruit was packed in commercial sourced MAP film and stored at 1 °C for 5 weeks before being repacked into a retail pack and kept at 20 °C for 10 days. The equilibrium gas compositions of 3-4% CO_2 + 12-15% O_2 and 12-16% O_2 + 7-10% CO_2 were created at 1 °C and 20 °C, respectively. No detectable ethylene was observed in MAP with sound fruit, but up to 5600 $nL \cdot L^{-1}$ ethylene was detected in MAP with rotten fruit. The quality assessment has shown that MAP retained kiwifruit firmness during coolstorage, but MAP at room temperature did not provide extra benefit. Applying potassium permanganate ($KMnO_4$) based ethylene scavenger sachet during the post-storage period did not improve kiwifruit quality.

This study has indicated that ethylene impact on kiwifruit quality is relatively low during short term storage in MAP, but assessment on longer storage is required in the future to determine the effect of MAP on maintaining kiwifruit quality at the post-storage stage.

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List of Abbreviations and Symbols

1-MCP	1-methyclycloprope ne
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	1-aminocyclopropane-1-carboxylic acid oxidase
ACS	1-aminocyclopropane-1-carboxylase synthase
ANOVA	analysis of variance
ATP	adenosine triphosphate
AVG	Aminoethoxyvinylglycine
C_2H_4	ethylene
CA	controlled atmosphere
CF	chlorophyll fluorescence
CI	chilling injury
CO_2	carbon dioxide
DCA	dynamic controlled atmosphere
DM	dry matter
ES	ethylene scavenger
FW	fresh weight
GC	gas chromatograph
GL	grower line
HDPE	high-density polyethene
ILOS	initial low oxygen stress
KMB	2-keto-4-methylthiobutyrate
$KMnO_4$	potassium permanganate
LDPE	low-density polyethene
LSD	least significant difference
LTB	low-temperature breakdown
MA	modified atmosphere
MAP	modified atmosphere packaging
MB	modular bulk
Met	methionine
ML	modular loose
MTA	5’-deoxy-5’methylthioadenosine
MTR	5’-methylthioribose

N_2	nitrogen
O_2	oxygen
O_3	ozone
OPP	oriented polypropylene
PE	polyethene
PG	polygalacturonase
PME	pectin methyl esterase
PP	polypropylene
PVC	polyvinyl chloride
RH	relative humidity
RLOS	repeated low oxygen stress
RQ	respiratory quotient
SAM	S-adenosylmethionine
SBD	storage breakdown disorder
SEM	standard error of means
SSC	soluble solid content
TA	titratable acid
TCA	tricarboxylic acid cycle
TCR	temperature-controlled room
TiO_2	titanium dioxide
ULO	ultra-low oxygen
WCI	white-core inclusions
WL	weight loss
β-GAL	beta-galactosidase

Chapter 1. Introduction

Kiwifruit is a cultivated plant in the genus *Actinidia*, with the origin and centre of diversity being China (Ferguson, 2004). The cultivation of *Actinidia* outside of China began in 1904 when Isabel Fraser brought seeds to New Zealand (Ferguson, 2011). The breeding of *Actinidia* occurred in New Zealand in the first half of the 20th century. The fruit was first commonly known as "Chinese gooseberry" because of the flavour similarity to European gooseberry. The name "kiwifruit" was invented in 1959, as part of a marketing strategy to associate the product with New Zealand when exporting to the United States (Ferguson, 2013). One of the very first kiwifruit cultivars, 'Hayward', was selected in 1930 and named after its selector Hayward Wright (Ferguson, 2011). 'Hayward' is still a major commercial cultivar worldwide because of its excellent performance in prolonged storage (Burdon & Lallu, 2011).

New Zealand is the leading kiwifruit exporter since the 1970s, and the third largest kiwifruit producer after China and Italy (Burdon & Lallu, 2011). Kiwifruit is a key horticultural product in New Zealand, and more than 90% of the kiwifruit produced in are for exporting (Aitken & Warrington, 2020). In the season of 2019/2020, a total of 552,800 tonnes of kiwifruit has been exported to more than 51 countries, representing 38% of the total horticultural export of New Zealand (Aitken & Warrington, 2020). The kiwifruit industry has seen a rapid growing both globally and in New Zealand. The global production of kiwifruit has increased from 2.7 million tonnes to 4 million tonnes from 2008 to 2018, and the production volume in New Zealand has increased by 200% over the last two decades (Aitken & Warrington, 2019; FAOSTAT, 2020).

The genetic diversity of the early kiwifruit cultivars was extremely low, with breeding programmes being entirely based on one male and two female plants from Isabel Fraser's seeds. Diversity of germplasm was not imported to New Zealand until 1975 when *Actinidia chinensis* joined the breeding of kiwifruit outside of China (Ferguson, 2007). New cultivars have been developed in New Zealand, China, Japan, and other countries over the last two decades. These new cultivars, such as 'SunGold' with yellow flesh, 'Cuixiang' with a red coloured centre, and 'Sanuki Gold' with large size fruit, have been popular in the market globally (Mworia *et al.*, 2011; Morton *et al.*, 2018; Jiao *et al.*, 2020). The production of 'SunGold' has increased from 27% to 53% of the kiwifruit production

amount in New Zealand from 2016 to 2019 (Aitken & Warrington, 2019). One of the key factors contributing to the rapid growth of the kiwifruit industry over the past decade is the success of new cultivars.

One of the recently released kiwifruit cultivars, 'Red19' (*A. chinensis* var. *chinensis*), has high market value due to the distinctive red-coloured flesh and attractive berry flavour (Aitken & Warrington, 2019). However, the storage life of 'Red19' is relatively short, which is a challenge for exporting by sea freight. Postharvest technology generally has five options to change in combination to attempt to extend storage life. Temperature control is considered the primary technology, followed by oxygen and carbon dioxide concentration manipulation to influence product respiration (Burdon, 2020). Ethylene management is particularly important given that kiwifruit is extremely sensitive (Arpaia *et al.*, 1987; Jabbar & East, 2016). Water vapour management is also required to be achieved to minimize weight loss and subsequent shrivel development (Burdon *et al.*, 2014b). For any storage solution, it is essential to understand the interaction of these postharvest control technologies in order to optimise quality maintenance. To extend the storage life of 'Red19', controlled atmosphere (CA) and modified atmosphere packaging (MAP) in conjunction with temperature-controlled storage may be promising.

This work was originally designed to determine the storability of 'Red19' in multiple CA conditions and MAP with currently available films. However, this part of the experiment was aborted due to the pandemic of COVID-19 and nationwide lockdown in New Zealand during the harvest season of 'Red19'. An alternate study of ethylene impact in CA and storage performance in MAP was carried out on the 'Hayward' cultivar to provide background knowledge in subsequent work on 'Red19'.

Atmosphere modification with reduced O_2 and elevated CO_2 can extend the storage life of kiwifruit (Burdon, 2020). Due to the high cost of CA facility, MAP is a cost-effective alternative for fruit storage. However, there is a risk of ethylene accumulation in MAP from damaged or rotten fruit, which accelerates kiwifruit softening (Masamichi & Yoshinori, 1990). It is essential to understand the impact of ethylene on fruit quality in modified atmosphere (MA) conditions. Although CA is not the optimal technology for long-term storage and transport, it can be used experimentally to simulate different conditions in the supply chain. On the other hand, it has been observed on some fruit that the benefit of CA and MAP on retaining fruit quality declined after fruit were removed

from storage (Ozturk *et al.*, 2019b). Whether applying MAP during the post-storage stage can maintain the quality of kiwifruit remains unknown. Additionally, it is also unclear if an ethylene scavenger can effectively reduce ethylene accumulation in MAP and preserve the freshness of kiwifruit.

Hence, the objectives of this study are:

a) to understand the impact of ethylene at various concentrations in CA.
b) to test the effect of commercial sourced MAP on kiwifruit quality during coolstorage and a subsequent period at room temperature (commonly called shelf-life).
c) to determine whether an ethylene absorbent sachet can reduce ethylene accumulation in MAP and maintain kiwifruit quality.

This thesis begins with background information about kiwifruit postharvest quality and physiology, and the impact of storage conditions including the impact of temperature, atmosphere modification, and ethylene. An assessment of ethylene effects in CA environments on kiwifruit quality is explored in Chapter 3. An assessment of kiwifruit storage performance in MAP including cool storage and shelf life is provided in Chapter 4. The scientific and industrial implications of the research and recommendations for future research are discussed in Chapter 5.

Chapter 2. Literature Review

2.1 Postharvest physiology and quality of kiwifruit

Kiwifruit is a climacteric fruit, which experiences a respiration peak and ethylene production peak during ripening (Burg & Burg, 1962). However, autocatalytic ethylene production does not start until late in kiwifruit ripening when the firmness is lower than 1 kg_f (Antunes *et al.*, 2000). This differs significantly from other climacteric fruit such as avocado and tomato, where ethylene production is more associated with the initiation of fruit softening (Burdon & Lallu, 2011).

The maturity of kiwifruit at harvest is one of the major indicators for storability and capacity to ripen (Burdon, 2015). Kiwifruit maturity has been defined by the industry using soluble solids content (SSC). The industry standard for 'Hayward' requires SSC at harvest to reach 6.2% or higher (Burdon *et al.*, 2016). Kiwifruit with lower SSC has poor performance in extended storage. An earlier study has shown that more mature kiwifruit harvested at 8.2% SSC remained three times firmer than less mature fruit (5.6% SSC) after 6 months of storage (Mitchell *et al.*, 1991).

The New Zealand kiwifruit industry recognises that another maturity indicator, dry matter (DM), may also have commercial value. DM is a measure of the dry weight to fresh weight ratio of the fruit. Major contributors to DM in fruit are cell walls, starch, sugar, and organic acids (Crisosto *et al.*, 2012). Fruit with higher DM at harvest therefore may have a higher starch content which acts as an energy reserve and means those fruit are more likely to survive long-term storage and still have superior flavour after storage, instead of low DM fruit that may have consumed proportionally more of their starch and sugar just to fuel respiration. It has been revealed that higher DM is associated with higher consumer acceptance (Hunter *et al.*, 2020). From the industry point of view, consumers have shown a high willingness of paying extra price for kiwifruit that began their storage life with high DM (Jaeger *et al.*, 2011; NZKGI, 2017b) and for this reason a premium price is available for fruit that meets a particular threshold.

2.1.1 Firmness of kiwifruit

Firmness is a key parameter determining the storage life of kiwifruit (Kim *et al.*, 2001). Rapid softening is the key issue for kiwifruit storage. Kiwifruit with low firmness are

more susceptible to physical injury and pathological infection (Mitchell, 1990). In addition, an over-soft texture is not acceptable to consumers. The firmness for the best eating quality is 0.5-1.0 kg_f for 'Hayward' (Stec *et al.*, 1989), while that for 'Hort16A' ranges from 0.6 to 0.8 kg_f (Burdon *et al.*, 2002a). Due to the variability in the kiwifruit population, the quality standard for kiwifruit exporting is not described by the mean firmness of the sampled population, while 'soft fractile' has been employed instead. The softest 3% fruit out of 300 fruit samples, which means the 9^{th} softest fruit has to be firmer than 1 kg_f (9.8 N) for 'Hayward' (Jabbar, 2014). Therefore, the storage life of kiwifruit is often terminated by either firmness falling below 1 kg_f, or disorders, including physiological disorders and pathological infection (Lallu, 1997).

Fruit texture change is associated with cell wall modifications (Wang *et al.*, 2021a). The plant cell wall is composed of complex polysaccharides such as cellulose, xyloglucans, heteroxylans, mannans, and pectic polysaccharides (Smith & Melton, 2012). In fruit tissue, cellulose exists as orderly microfibrils and is difficult to break down (Smith & Melton, 2012). A model of the primary cell wall has been established in fruit. A 3-D network is created with cellulose microfibrils which are cross-linked by xyloglucan (Figure 2.1). Attached to the cellulose microfibril network are polysaccharides, such as mannans, galactan and arabinan sidechains of pectin. Unesterified regions of a pectin molecule can be cross-linked with another pectin molecule via divalent charges on calcium ions, which created the "egg-box model" (Kirtil *et al.*, 2014). Fruit softening during ripening is attributed to structural changes of pectic polysaccharides in the cell wall, including middle lamella dissolution and primary cell wall disruption (Smith & Melton, 2012). Multiple cell wall-modifying enzymes, such as pectin methylesterase (PME) and polygalacturonase (PG), are involved in firmness decline (Brummell & Harpster, 2001). PME removes the methyl ester groups from the pectin polymer in cell wall, facilitating access of PG to its substrate. The subsequent activity of PG is responsible for middle lamella degradation, which reduces cell-to-cell adhesion (Shakya & Manju, 2018).

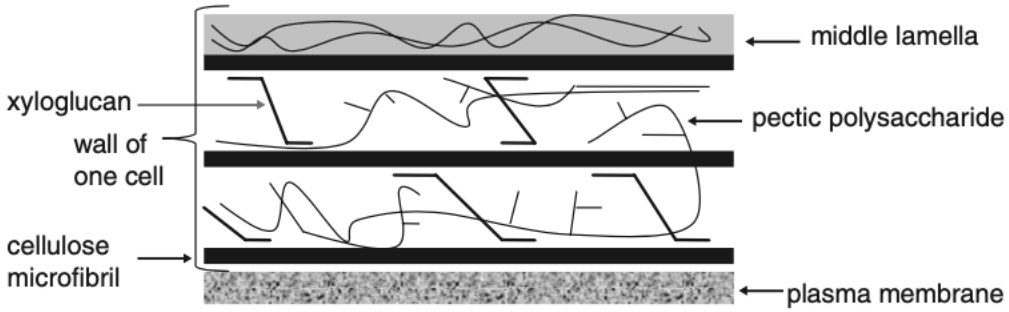

Figure 2.1 A model of the primary cell wall of fruit (Smith & Melton, 2012).

Softening of kiwifruit is characterized by a four-phase model (Figure 2.2) (Atkinson *et al.*, 2011). Fruit at harvest are firm and high in starch (phase 1). At this stage, fruit do not produce ethylene but are highly sensitive to exogenous ethylene (McAtee *et al.*, 2015). Phase 2 is a rapid softening period when soluble pectin in the cell wall depolymerizes. When the firmness decreases below 1 kg_f, phase 3 starts, i.e., endogenous ethylene production begins, and fruit start to enter the "eating window" for consumers. Fruit in phrase 4 are over-ripe with unpleasant flavour (Atkinson *et al.*, 2011).

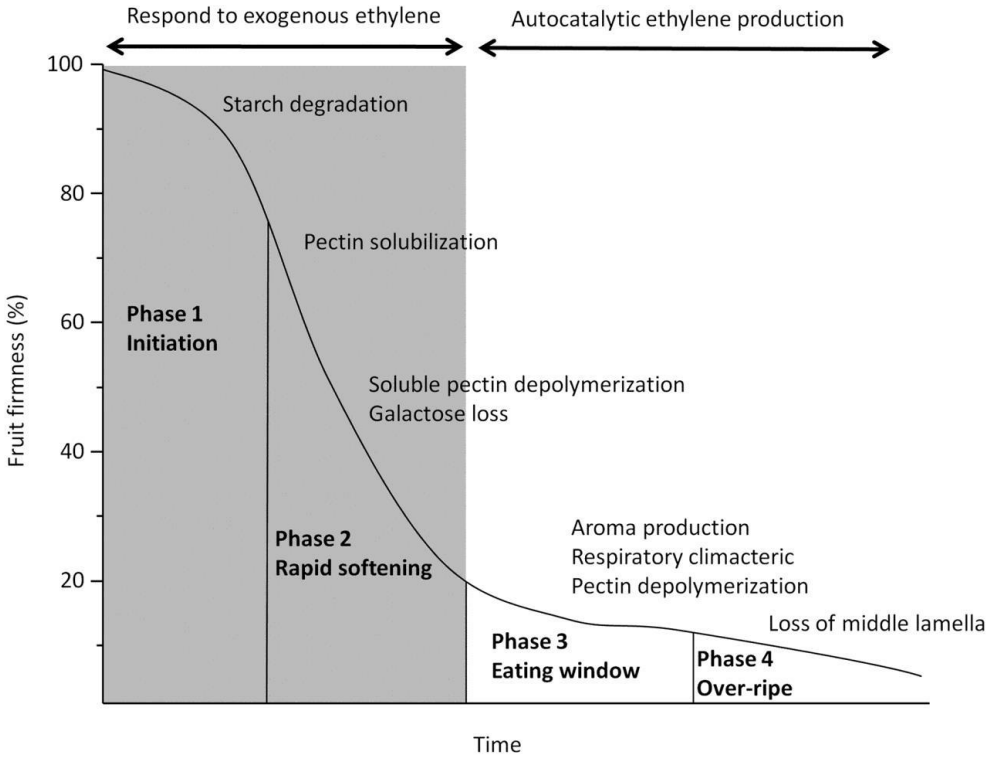

Figure 2.2 Key events in kiwifruit softening (Atkinson et al., 2011).

2.1.2 Taste of kiwifruit

Beyond texture, consumer acceptance of kiwifruit is determined by taste (Jaeger *et al.*, 2003). The SSC accumulation determines 'sweetness', while the 'sourness' is correlated to titratable acidity (TA) (Tandon *et al.*, 2003). Fruit with higher SSC are preferred by consumers. Ripe 'Hayward' kiwifruit with the minimum SSC of 14% are regarded as acceptable (Harker *et al.*, 2009). Sugar-acidity balance also impacts consumer acceptance. Consumers have higher acceptability to acidity when SSC increases (Rossiter *et al.*, 2000). Kiwifruits are generally harvested with low SSC and high TA. The SSC increases dramatically while TA experiences a slight decline during ripening, resulting in an SSC-TA ratio increase (Cha *et al.*, 2019; Shin *et al.*, 2020). The SSC of kiwifruit at eating ripeness is correlated with at-harvest DM. The kiwifruit with high at-harvest DM usually develops to high SSC after ripening, and high 'sweetness', which is preferred by the consumer. Hence, a premium price is generally paid to the growers for fruit with high DM (NZKGI, 2017b).

2.1.3 Water loss and shrivel of kiwifruit

Water loss during storage can lead to quality loss. Water loss is linked to weight loss and shrivel (Burdon & Clark, 2001) and saleable quantity (Saltveit, 2016). Severe water loss also affects fruit turgor, resulting in texture loss (Taglienti *et al.*, 2009). Water vapour diffusion from fruit to the environment is influenced by fruit characteristics and storage conditions. Surface structures, such as cuticles, epidermal cells, lenticels, and trichomes, are the morphological factors determined by the genetic background and preharvest conditions (Burdon & Clark, 2001; Burdon *et al.*, 2014b). Low relative humidity (RH), high temperature and high air circulation, as environmental factors, promote water loss (Yahia, 2011, p92). Postharvest technology, such as wrapping in films with low water vapour permeance, or surface coating, can suppress water loss (Mastromatteo *et al.*, 2011).

Shrivel symptoms are the visible signs of significant water loss (Gwanpua *et al.*, 2019). Shrivel in kiwifruit is induced by water loss reaching 4% to 6% of the at-harvest weight (Burdon *et al.*, 2015), which reduces the visual attraction of the fruit to the consumer. There is a variation of shrivel severity between cultivars. Shrivel is more often seen on the kiwifruit cultivars without substantial surface hair, such as 'Hort16A', 'Gold3' and 'Red19' (Burdon *et al.*, 2015).

2.1.4 Postharvest disorders

2.1.4.1 Chilling injury

The symptoms of chilling injury (CI) in kiwifruit are termed low-temperature breakdown (LTB) or storage breakdown disorder (SBD) (Lallu, 1995), which is described as a granular ring that forms in the outer pericarp and later develops to water-soaked tissue (Jabbar & East, 2016). CI is caused by low-temperature stress that leads to reactive oxygen species accumulation, membrane lipid peroxidation, cell membrane integrity loss, and cell death (Wang *et al.*, 2020a). The severity of CI is evaluated by visual assessment and rating with an index of 0-4 for kiwifruit, with 0 being sound fruit and 4 being severe CI (Wang *et al.*, 2020b).

The sensitivity to CI is determined by the genetic background and maturity of the commodity. 'Hayward' has a relatively high tolerance to low temperature, and can be stored at 0°C, while 'Hort16A' can only tolerate as low as 1 °C (Burdon & Lallu, 2011). The CI incidence of 80-100% has been observed on 'Hayward' kiwifruit with low maturity (at-harvest SSC < 5%) while that of more mature fruit (at-harvest SSC 6.5-8%) under the same storage condition was below 5%, suggesting immature kiwifruit is more susceptible to CI compared to mature fruit (Burdon *et al.*, 2016).

2.1.4.2 White-core inclusions

The symptom of white-core Inclusions (WCI) is white patches appearing in the core tissue of kiwifruit (Arpaia *et al.*, 1982). WCI was first observed in 'Hayward' kiwifruit with added C_2H_4 in CA storage in the 1980s. The incidence and severity of WCI are impacted by CO_2 concentration, C_2H_4 concentration and storage temperature (Arpaia *et al.*, 1986). It has been indicated that these white "inclusions" are clusters of starch. However, the exact mechanism of WCI formation remains unknown.

2.1.4.3 Hard core

Hard core is a physiological disorder of kiwifruit due to the softening difference between the flesh and core tissue. It has been demonstrated on 'Hayward' that the core softening pattern is close to linear, and the softening of the inner and outer pericarp is more exponential (Li *et al.*, 2017). The symptom of hard core can be described as core firmness that remains above 3.6 kg_f when flesh firmness falls below 1 kg_f, which leads to an unpleasant consumer experience (Zoffoli *et al.*, 2016).

Hard core emerges after long storage in CA storage or after 1-MCP treatment, the latter being associated with the core tissue losing the capacity to ripen (Peng *et al.*, 2019). The atmosphere in the storage environment after 1-MCP treatment impacts the occurrence of hard core. Zoffoli *et al.* (2016) found that a 1-MCP treatment followed by MAP storage did not induce hard core, while hard core was detected in kiwifruit treated with the same 1-MCP condition followed by air storage.

2.2 Storage condition effects on fruit quality

2.2.1 Ethylene

According to their respiration and ethylene metabolism, fruits can be classified into climacteric and non-climacteric. Traditionally, climacteric fruit experiences a climacteric increase of respiration and autocatalytic production of ethylene, and ethylene is the key signalling molecule regulating the downstream ripening processes in these fruit (Biale & Young, 1981). By contrast, the ripening of non-climacteric fruit is not controlled by ethylene and there is no climacteric peak of respiration and ethylene production in the ripening process (Pech *et al.*, 2012). However, this definition has been challenged by recent studies. Ethylene-dependent and independent events both exist in the ripening of climacteric fruit (Barry & Giovannoni, 2007). Meanwhile, ethylene-dependent events exhibit differential sensitivity to ethylene (Flores *et al.*, 2001). Kiwifruit, for instance, has been categorised as a climacteric fruit since the 1960s due to its high sensitivity to ethylene and climacteric peak during ripening (Burg & Burg, 1962), however, contemporary work has suggested that at an early ripening stage kiwifruit softening can occur in the absence of added ethylene and the peak of ethylene production rate does not appear until a late stage of ripening (McAtee *et al.*, 2015). Kiwifruit remains very sensitive to ethylene throughout ripening, so proper management of exogenous ethylene is critical for kiwifruit storage.

2.2.1.1 **Ethylene production and reception**

In climacteric fruit, ethylene regulation has been categorised into two systems. System 1 is characterised by a low level of ethylene production and ethylene production is auto-inhibited by itself, whereas in system 2, auto-catalytic ethylene is produced at the onset of climacteric (McMurchie *et al.*, 1972). The biological synthesis pathway of ethylene starts with the Yang cycle. Ethylene is produced from amino acid methionine (Met) via

S-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). Met is regenerated via 5'-deoxy-5'methylthioadenosine (MTA), 5'-methylthioribose (MTR), 5'-methylthioribose-1-phosphate (MTR-1-P) and 2-keto-4-methylthiobutyrate (KMB), from the recycled components of SAM hydrolysation (Yang & Hoffman, 1984). ACC is the precursor of ethylene. In the biosynthesis of ethylene, the key enzymes are 1-aminocyclopropane-1-carboxylase synthase (ACS) that catalyses SAM to ACC, and ACC oxidase (ACO) that catalyses ACC to ethylene (Grierson, 2018). Therefore, ethylene production of horticultural products can be regulated via manipulating the activity of ACS and ACO.

The regulation of plant growth and development by endogenous and exogenous ethylene is initialized by ethylene molecule binding to the receptors. Ethylene receptors are located in the endoplasmic reticulum and can be categorised into two subfamilies: ETR1-like subfamily (subfamily I) and ETR2-like subfamily (subfamily II) (Binder & Bleecker, 2002). The ethylene receptors negatively regulate ethylene signal transduction. With the absence of ethylene, the receptors interact with CTR1 and inhibit ethylene responses. With ethylene binding to the receptors, inhibition from CTR1 is released, and the cascading signalling processes, activating ethylene responses (Schaller & Kieber, 2002). Thus, ethylene action in high plants can be manipulated by interfering with the functioning of the ethylene receptor.

2.2.1.2 Ethylene response

Ethylene response has been characterized by "triple response" in germinating seedlings: elongation inhibition, hypocotyl swelling and apical hook formation (Guzmán & Ecker, 1990). The effects of ethylene on higher plants also include seed germination, plant growth, stress responses, flower senescence and fruit ripening (Ma & Dong, 2021). During fruit ripening, various biochemical and physiological changes may be induced by ethylene, such as cell wall breakdown, starch degradation, pigment accumulation and increase of respiration and ethylene production. Accordingly, fruit experience texture change, sugar accumulation, colour shifting as well as aroma development and acidity decrease (Acuna *et al.*, 2011; Cherian *et al.*, 2014; McAtee *et al.*, 2015; Saquet & Almeida, 2017; Shin *et al.*, 2020).

Softening is a key event in kiwifruit ripening induced by ethylene (Hertog *et al.*, 2016). Ethylene in the storage environment also increases the incidence of CI especially after

long exposure (Jabbar & East, 2016). Other responses of kiwifruit to ethylene include sugar accumulation, acidity decrease, aroma development, and respiration rate increase (Guenther *et al.*, 2015; Shin *et al.*, 2020). Kiwifruit is extremely sensitive to ethylene. An ethylene concentration as low as 10 $nL \cdot L^{-1}$ can subsequently induce the ripening of kiwifruit (Antunes, 2007; Pranamornkith *et al.*, 2012). The maturity at harvest affects the response of kiwifruit to ethylene, with immature kiwifruit having a lower response to ethylene treatment than mature kiwifruit (McAtee *et al.*, 2015). Meanwhile, it has been suggested that the role of ethylene in kiwifruit ripening is not just a trigger for initiation. On the contrary, it has been demonstrated that kiwifruit softened faster when exposed to higher concentrations for a longer period compared to lower concentrations and shorter duration, suggesting a dose effect in kiwifruit response to ethylene (Hertog *et al.*, 2016).

2.2.1.3 Ethylene management

Because of the effect of inducing fruit ripening, ethylene is sometimes utilized as a fruit conditioning agent to obtain desired quality in postharvest practice. For marketing purposes, a small proportion of kiwifruit is harvested before reaching the optimal maturity stage, which is known as "KiwiStart" in New Zealand (NZKGI, 2017a). However, early harvested kiwifruit may not spontaneously ripen within a short time. In addition, there exists large fruit-to-fruit variation in the softening process of early-season kiwifruit. To ensure uniform ripening and ready-to-eat condition when the fruit reaches the retail market, exogenous ethylene or warm conditions are required before or during shipping (Crisosto, 1999; Choi *et al.*, 2019). On the other hand, it has been demonstrated that the application of 1-MCP as a postharvest treatment to delay kiwifruit ripening may also induce hard core disorder, which can be alleviated by applying exogenous ethylene after storage (Zoffoli *et al.*, 2016). Due to the effect of accelerating fruit ripening and senescence, the dose of ethylene applied should be under strict regulation to obtain optimal fruit quality and avoiding over-ripening.

Apart from the exceptions listed above, minimising ethylene damage is key to prolonging the storage life of kiwifruit. Ethylene inhibitor, 1-methyclycloropene (1-MCP), can competitively bind to ethylene receptors and block the downstream signalling and gene expression, thus blocking the ethylene response pathway (Dias *et al.*, 2021). The effect of 1-MCP has been investigated on numerous fruit, such as apple, tomato, peach, and kiwifruit (Choi *et al.*, 2021; Mata *et al.*, 2021; Win *et al.*, 2021; Zhang *et al.*, 2021b).

However, there is a risk of applying 1-MCP to fresh products, as the binding to ethylene receptor is irreversible and treated fruit may not fully ripen (Chiriboga *et al.*, 2013). Application of 1-MCP on kiwifruit may induce hard core disorder (Zoffoli *et al.*, 2016), or failure in aroma development (Huan *et al.*, 2020). Meanwhile, 1-MCP cannot sufficiently delay the ripening of some kiwifruit cultivars with ethylene-independent ripening pathway, such as 'Sanuki Gold' (Mitalo *et al.*, 2019). Aminoethoxyvinylglycine (AVG) is another ethylene inhibitor that restrains ethylene biosynthesis by negatively regulating ACS activity (Muñoz-Robredo *et al.*, 2012). Preharvest and postharvest application of AVG have been investigated on fruit crops, such as plum, fig, and feijoa. It has been revealed that AVG application timing and concentration are critical for retaining fruit quality, and the effect of AVG also varies by species and cultivar (Rupavatharam *et al.*, 2016; Altuntas *et al.*, 2020; Qiao *et al.*, 2021). Studies on AVG application to kiwifruit have suggested that AVG does not have an impact on kiwifruit quality, in terms of firmness, SSC, and incidence of physical disorders (Manriquez *et al.*, 1999; Ozturk *et al.*, 2019b). Hence, neither 1-MCP nor AVG is recommended for extending kiwifruit storage.

Removing ethylene from the fruit storage environment is another strategy to minimize fruit spoilage caused by ethylene, which can be managed by high air circulation, applying ethylene removal agents, and separating ethylene producing products from ethylene sensitive commodities (Wei *et al.*, 2021). Air circulation rate normally depends on the operation in the storage, transport, or distribution facility, while applying ethylene removal agent and proper packaging can be managed at the product level. Ethylene absorbers, such as activated carbon and zeolite, have been utilized to reduce ethylene concentration within the package. However, they may become saturated in a short time and the efficiency reduces rapidly. Meanwhile, ethylene catalytic oxidants, such as ozone (O_3), titanium dioxide (TiO_2) and potassium permanganate ($KMnO_4$), have more stable performance. The most commonly used ethylene catalytic oxidant is $KMnO_4$, which is activated by moisture released by fresh products and chemically catalyses ethylene oxidation into water and CO_2 (Janjarasskul & Suppakul, 2018). The $KMnO_4$ product in form of a sachet can be applied in combination with packaging. However, the efficiency of $KMnO_4$ relies on several factors, such as storage condition, commodity cultivar and condition, and package properties (Álvarez-Hernández *et al.*, 2019a). The performance of $KMnO_4$ needs to be tested in the specific scenario before being applied to a particular

commodity and supply chain. On the other hand, packaging with low permeance to ethylene, as a physical barrier, can eliminate ethylene contamination from the environment and the adjacent commodity. Combining modified atmosphere packaging (MAP) with an ethylene scavenger can further repress ethylene damage on fresh products, as this should both minimise contact with exogenous ethylene and absorb ethylene produced by e.g., over-ripe or fungal-infected fruit.

2.2.2 Temperature

Temperature is a key factor that influences the storage life and quality of fresh products (Wills & Golding, 2016). Respiration rate and ethylene production are impacted by storage temperature. It has been revealed that high respiration rate and high ethylene production rate are associated with high storage temperature, while the low temperature can slow down the ripening process and delay deterioration (East *et al.*, 2009a; Asiche *et al.*, 2017; Shin *et al.*, 2018). Softening of kiwifruit can be significantly retared by 0 °C storage compared to 4-16 °C (Burdon *et al.*, 2017b). However, temperature below freezing point may cause freeze damage (Jha *et al.*, 2019), and CI may be induced by non-freezing low temperature (Jabbar & East, 2016; Gwanpua *et al.*, 2018; Suo *et al.*, 2018; Wang *et al.*, 2020b).

Low temperature and exposure duration are the two key factors impacting CI development. It has been observed on 'Hayward' that the CI symptom became visible after 125 days of storage at 0 °C and the incidence increased to as high as 50% after 200 days storage under the same conditions; while CI did not emerge until 175 days of storage at 2 °C and the incidence after 200 days at 2 °C was below 10% (Gwanpua *et al.*, 2018). To achieve a good effect on firmness retention as well as CI suppression, dual temperature storage is potentially helpful. It has been demonstrated that switching storage temperature from 0 °C to 2 °C between 50 and 125 days of storage can effectively reduce CI and fruit softening (Gwanpua *et al.*, 2018). However, elevated temperature in cool storage may lead to accelerated fruit ripening. It has been reported on 'Sanuki Gold' kiwifruit that ethylene-independent softening can be induced by 4 and 5 °C storage compared to 20 and 25 °C storage (Mworia *et al.*, 2012; Asiche *et al.*, 2018). In current industrial practice, kiwifruit are normally held at -1 °C to 1 °C during extended storage with variation among cultivars.

Apart from the storage temperature and duration, the cooling rate before storage also impacts fruit quality. Rapid cooling has led to higher CI incidence and postharvest rots compared to slow cooling (Manning *et al.*, 2016). It has been suggested that harvested kiwifruit rapidly cooled to 0 °C had lower firmness after 120 d of storage, while gradually cooling over 14 d led to high rot incidence (Zhao *et al.*, 2015). Low-temperature conditioning or step-down cooling, which refers to briefly holding fruit above chilling temperature before cool storage, can induce the cold resistance of kiwifruit thus reduce CI and retain higher firmness (Yang *et al.*, 2013).

In the real-life scenario, the fruit may be held at room temperature (20-25 °C) during transport and at retailer for several days before reaching consumers. The quality changes during this shelf life at room temperature also impact the consumer experience. The shelf-life performance of kiwifruit depends on fruit maturity and the previous cool storage (Tavarini *et al.*, 2008; Wang *et al.*, 2018). Extended cool storage generally shortens shelf-life. 'Jinmei' kiwifruit, for instance, can be held for up to 25 days at room temperature after harvest, while the shelf life after 3-4 months cold storage reduces to 7-10 days (Zhong *et al.*, 2018). Additionally, in the supply chain practice, other postharvest technologies can also be combined with temperature-controlled storage, such as ethylene management and atmosphere modification, to further preserve the quality of the fresh product (NZKGI, 2016).

2.2.3 Atmosphere modification

Modified atmosphere (MA) refers to technologies that apply atmospheres, in which the gas composition differs from air (78.08% N_2, 20.95% O_2, 0.93% argon, and 0.04% CO_2) to preserve fresh products such as fruit and vegetables (Dilley, 2006). Elevated carbon dioxide (CO_2) and reduced oxygen (O_2) are normally applied in MA (Brandenburg, 2020). Controlled atmosphere (CA) and modified atmosphere packaging (MAP) are the main technologies involved in MA storage of horticultural commodities.

Respiration is primarily altered in MA, as O_2 and CO_2 are the two key respiratory gas compounds. In the respiration process, carbohydrate is oxidized by O_2 into CO_2 and releases energy in the form of adenosine triphosphate (ATP), which supports the metabolism of living plant organs. During the initial stage of respiration, glycolysis, where glucose is converted to pyruvate, O_2 is not required. However, as the final electron acceptor, O_2 plays a vital role in the following tricarboxylic acid cycle (TCA cycle)

(Ponce-Valadez & Watkins, 2008). CO_2 as a product of TCA also regulates respiration by feedback inhibition (Saltveit, 2020). Reduction in respiration rate has been widely observed in fruit and vegetables stored in CA and MAP (East *et al.*, 2009b; Ghosh & Dash, 2020; Ho *et al.*, 2020b; Kanwal *et al.*, 2020). The respiration peak of blueberry stored in CA occurred earlier, but the respiration rate remained 60% lower after the climacteric peak compared to air storage (Falagán *et al.*, 2020). It has been reported that the respiration rate of 'Hayward' kiwifruit was reduced by up to 40% in MAP at 0 °C compared to kiwifruit in air (Ozturk *et al.*, 2019b). Reduced ATP supply generally results in slower metabolism and delay in fruit ripening.

On the other hand, anaerobic respiration may be initiated by low O_2 and high CO_2, which impacts the volatiles released by the fresh commodities. Without sufficient O_2 supply, pyruvate may enter fermentation and generate ethanol (Lal, 2018). The activity of TCA enzymes is suppressed by elevated CO_2 concentration, which also leads to fermentation (Ponce-Valadez & Watkins, 2008). In fresh commodities, ethanol produced by anaerobic respiration is toxic to plant tissue and may introduce off-flavours. It has been suggested that ethanol production was detected in mango stored in 5% O_2 with 25% CO_2 but was not observed in 10% CO_2 with the same O_2 concentration (Bender *et al.*, 2021). It has been reported that ethanol production of dragon fruit stored in 2% O_2 was reduced by the presence of 5% CO_2 (Ho *et al.*, 2021). These studies have revealed that the concentrations of both O_2 and CO_2 are critical for the storage atmosphere and that the optimal gas composition varies between products.

Besides, MA alters aroma evolution during cool storage. Aroma compounds play an essential role in fruit flavour, along with taste. Fruit are prone to aroma loss as a symptom of CI after long-term coolstorage (Nair *et al.*, 2002), while MA storage has the effect of reducing CI, thereby suppresses aroma loss. It has been demonstrated that peach stored in 3-5% O_2 and 3-5% CO_2 at 0 °C for 30 days produced higher levels of aroma compounds, including butyl acetate, ethyl acetate, and g-hexalactone, compared to air-stored peach fruit (Zhou *et al.*, 2018). Therefore, the influences of MA on fruit volatile are both reducing off-flavour caused by fermentation and suppressing aroma loss due to CI.

Soluble sugar is the substrate of respiration, and its metabolism is also affected by atmosphere modification (Brizzolara *et al.*, 2020). In freshly harvested kiwifruit, starch is the main carbohydrate storage form. During fruit ripening, starch is hydrolysed into

soluble sugar, which leads to the increase of sweetness (Zhang *et al.*, 2021a). Thus, the dynamic change of carbohydrates reflects the energy supply state as well as fruit quality. The effect of MA on delaying SSC increase has been observed on multiple products, which indicates the delay of starch degradation (Aglar *et al.*, 2017; Álvarez-Hernández *et al.*, 2020). A study on kiwifruit has revealed that CA (5% CO_2 + 2% O_2) supresses starch decrease by inhibiting five starch degradation genes (Hu *et al.*, 2016a). On the other hand, delaying the decline of SSC or maintaining SSC at a relatively higher level also suggested the sugar consumption for respiration has been reduced and the quality of the fresh product is retained during MA storage (Mworia *et al.*, 2011). Hence, delaying starch breakdown and slowing down sugar consumption by respiration are the key effects of MA on SSC evolution during fruit storage.

Besides sugar, acidity is another key component of fruit taste. As fruit ripens, TA decreases and SSC increases, leading to an increase in the SSC/TA ratio. Fruit with a relatively high SSC/TA ratio is generally preferred by consumers. It has been observed in various fruit, such as mango, apricot, and tomato, that MA delays TA decline (Álvarez-Hernández *et al.*, 2020; Cocetta *et al.*, 2020; Phakdee & Chaiprasart, 2020). With the delay of SSC increase, fruit stored in MA display slower development of SSC/TA ratio. However, the evolution of SSC and TA may not follow the same pace under the modified atmosphere compared to fruit stored in air, which potentially results in the taste alteration of ripe fruit (Álvarez-Hernández *et al.*, 2020; Phakdee & Chaiprasart, 2020). At the time of removal from storage, the TA of kiwifruit stored under MA with reduced O_2 and elevated CO_2 is higher than that stored in air, while the SSC/TA ratio is lower (Li *et al.*, 2015). However, the sensory quality assessment after a subsequent period at room temperature has shown less difference (Latocha *et al.*, 2014). The evolution of TA can provide information on the ripening process of MA stored fruit as a physiological parameter, but acidity as a part of taste and flavour quality needs to be considered at the ready-to-eat stage.

The skin or flesh colour change is impacted by MA for some fruit. As an indicator of ripening, the colour development of medlar was delayed by MAP, suggesting the pigment development was altered by the atmosphere (Ozturk *et al.*, 2019a). On the other hand, it has been reported that the senescent yellowing of dragon fruit was reduced by CA (Ho *et al.*, 2021), indicating pigment deterioration can also be impacted. For most kiwifruit

cultivars, the colour of fruit skin and flesh do not change during ripening. However, it has been observed that the flesh colour index decline of 'Sanuki Gold' kiwifruit was reduced by MA (Mworia *et al.*, 2011), which suggests MA has the effect of delaying pigment deterioration.

Fruit texture is affected by MA. The firmness of fruit reduces during ripening, as modification of cell wall pectin occurs. It has been demonstrated that CA retains the firmness by downregulating gene expression and suppressing the activity of pectin modifying enzymes such as polygalacturonase (PG), pectin methyl esterase (PME), and beta-galactosidase (β-GAL) (Gwanpua *et al.*, 2017). The effect of CA and MAP delaying kiwifruit softening has been well documented (Hertog *et al.*, 2004; Lallu *et al.*, 2005; Mworia *et al.*, 2011; Vieira *et al.*, 2012; Li *et al.*, 2015; Pegoraro *et al.*, 2016; Chang *et al.*, 2017; Li *et al.*, 2017; Sicari *et al.*, 2019). Meanwhile, the effect of MA on kiwifruit firmness displays various patterns between different tissues. The core tissue is more sensitive to MA compared to the outer pericarp. Hard core disorder may be induced by MA storage, where the core tissue remains firmer than the outer pericarp (Li *et al.*, 2017). The firmness of kiwifruit stored in MA was 30% higher than air-stored fruit after 30 days at 0 °C, but this difference gradually declined in the following 120 days of cold storage and firmness dropped dramatically after transfer to ambient condition (Ozturk *et al.*, 2019b). Apart from the influence of MA at the enzyme level, the softening of fruit, as a part of the ripening process, is also regulated by ethylene. The impact of MA on fruit texture also involves ethylene metabolism.

In ethylene biosynthesis, the conversion of ACC to ethylene by ACO is oxygen-dependent (Yang & Hoffman, 1984). The activities of ACO and ACS are also impacted by CO_2 (Mathooko, 1996a). Thus, MA with reduced O_2 and increased CO_2 can suppress ethylene production. It is suggested that the rapid softening after MA storage is the result of ACC accumulated during MA storage being catalysed by reactivated ACO in ambient conditions, which produces a large amount of ethylene and the accumulated ethylene, in turn, accelerates ripening (Chang *et al.*, 2017). This phenomenon is a potential limitation of MA application in the kiwifruit industry. On the other hand, it was suggested that CO_2 can reduce fruit sensitivity to ethylene by competitively binding to the ethylene receptor and blocking the downstream signalling (Burg & Burg, 1969). However, this was later challenged by Sisler (1979) that ethylene binding to receptor site was not competed by

CO_2. Although it has been suggested MA impacts ethylene action, the exact mechanism remains unclear (Domínguez *et al.*, 2016). Thus, applying MAP after CA storage to retain atmosphere modification may potentially reduce ethylene production, suppress ethylene responses and delay fruit softening. Meanwhile, combing ethylene scavenger with MAP may further reduce the post-storage surge in ethylene concentration around the fruit.

Modified atmosphere has been employed as an approach to reduce CI of fresh products in coolstorage (Kanwal *et al.*, 2020). It is recommended that similar storage life and fruit quality of 'Hort16A' kiwifruit can be achieved by storage under CA at elevated temperature (7 °C) compared to air storage at 1 °C, which reduces the risk of CI (Lallu *et al.*, 2011). Despite achieving the same fruit quality at elevated storage temperature, MA also alleviated the incidence and severity of CI at the temperature that induces CI of fruit stored in air. The concentration of CO_2 in the storage environment is critical for depressing the incidence of CI. It has been reported that CI of mango was inhibited by 10% CO_2 but induced by 25% CO_2 (Bender *et al.*, 2021). Meanwhile, for kiwifruit, up to 5% CO_2 is considered safe, but the effect of CO_2 at the concentration of 3% or lower on reducing CI is not sufficient (Jiao *et al.*, 2020).

Apart from over-ripening and senescence, postharvest rot caused by pathogens also impacts the storage life of fresh products. It has been illustrated that MA has the effect of suppressing rot development (Tahir *et al.*, 2009). It has been demonstrated on bell pepper that O_2 at the concentration of 2-5% reduced internal fruit rot by suppressing fungal growth (Frans *et al.*, 2021). On the other hand, elevated CO_2 contributes to resistance improvement, which reduces postharvest disease development (Gatto *et al.*, 2013). However, the effect of MA on postharvest rot development may be either positive or negative. applying CO_2 at the concentration of 10% and above to guava induces stylar end rot (Teixeira *et al.*, 2010), indicating excessive CO_2 may increase the damage of the postharvest disease. The concentrations of both O_2 and CO_2 are critical for postharvest disease control.

2.3 Factors impacting the effect of modified atmosphere

Apart from the concentration of O_2 and CO_2, the efficacy of MA on extending the storage life of fresh products is impacted by multiple factors, such as product cultivar, the physiological condition of the product, the timing of MA establishment, storage

temperature, and ethylene concentration (Saltveit, 2020); and all these factors may interact. Hence, the optimal condition for MA storage is product specific.

2.3.1 Respiratory gas concentration

The concentration of O_2 and CO_2 plays a central role in the effect of MA storage. Unsuitable MA conditions can lead to harmful effects. Exceedingly low O_2 concentration may lead to anaerobic respiration and the production of ethanol and acetaldehyde (Burdon *et al.*, 2007b). For most fruit and vegetables, when the concentration of O_2 drops below 1-3%, large amounts of CO_2, aldehydes and alcohols are produced as normal aerobic metabolism is replaced by anaerobic metabolism, which leads to off-flavours and off-odours (Watkins, 2020). Similarly, CO_2 at a higher level than the product threshold causes damage to fresh produce. The symptoms of CO_2 injury are internal browning, skin discolouration, flavour alteration, and postharvest rot development (Teixeira *et al.*, 2010; Jiao *et al.*, 2020; Ahn *et al.*, 2021; Du *et al.*, 2021). On the other hand, an atmosphere too close to the ambient cannot efficiently retain the quality of the commodity (Li *et al.*, 2017; Gudkovsky *et al.*, 2021). The optimal O_2 and CO_2 levels depend on the sensitivity and tolerance of the product to these respiratory gases, which depends on the genetic background.

2.3.2 Genetic background

The response of fresh produce varies between species. Cranberry can tolerate up to 30% CO_2 (Gunes *et al.*, 2002), while apple internal browning as the symptom of CO_2 injury can be induced by 5% CO_2 (Du *et al.*, 2021), and storing hardy kiwifruit in 15% CO_2 results in brown skin spots (Rebeaud *et al.*, 2018). Within the same species, different cultivars show different responses to atmosphere modification. A study on four blue honeysuckle fruit cultivars has demonstrated that under the same CA condition (20% CO_2 + 5% O_2), 'Vostorg' retained a higher firmness while 'Indigo Gem' experienced the highest fruit loss (Dziedzic *et al.*, 2020). It is the same case for kiwifruit that the optimal storage atmosphere for 'Hayward' is 5% CO_2 + 2% O_2 (Li *et al.*, 2017), while that for 'Hongyang' is 3% CO_2 + 2% O_2 (Li *et al.*, 2015), and the CA condition of 1.6% CO_2 + 2% O_2 has been recommended for 'Hort16A' (Lallu *et al.*, 2011).

2.3.3 Timing of establishing modified atmosphere

The timing of MA establishment affects the efficacy of MA. Generally, MA should be established immediately after harvest. It has been reported on 'Northern Spy' apple that CA applied from harvest is effective in retaining firmness, but this benefit is lost after a two-week delay before CA establishment (Deell & Lum, 2020). Similarly, kiwifruit packed into MAP after 30 days of cool storage received a reduced effect of delaying softening, while a delay of 60 days resulted in no benefit from MAP, and the most effective MAP was the one established immediately after harvest (Zoffoli *et al.*, 2006). This probably is due to the rapid softening of kiwifruit occurring within the 30-45 days after harvest, after which the fruit are already soft (Arpaia *et al.*, 1984). However, delaying MA establishment can alleviate some disorders. It has been reported that delayed CA can alleviate CO_2 injury and retain the freshness of multiple apple cultivars (Deell & Ehsani-Moghaddam, 2012; Harb *et al.*, 2013; Neuwald *et al.*, 2015), while for kiwifruit, delaying the establishment of CA for 30-45 days reduces stem end rot favoured by CA (Tonini *et al.*, 1999; Gregori *et al.*, 2002).

2.3.4 Temperature

Storage temperature, as a key factor impacting the quality of fresh produce, also interacts with the storage atmosphere. The primary effect of MA is on the respiration rate of fresh products. Elevated temperature enhances respiration while enriched CO_2 and reduced O_2 suppress respiration. To describe the respiration rate impacted by O_2, CO_2 and temperature, a number of models have been developed based on different products, such as blueberry, kiwifruit, banana, fig and mango (Cameron *et al.*, 1994; Hertog *et al.*, 2004; Ghosh & Dash, 2020; Ho *et al.*, 2020b). It has been illustrated that during MA storage, temperature affects the respiratory quotient (RQ), which refers to the ratio of CO_2 production to O_2 consumption (Bhande *et al.*, 2008). This result suggests that the point where respiration shifts from aerobic to anaerobic has been altered by temperature.

The effect of a certain O_2 and CO_2 concentration on the quality of fruit and vegetables can be altered by temperature. At 0 and 2 °C kiwifruit softening can be delayed for 45 days by both 2% O_2 and 5% CO_2, however, the same MA conditions were not as effective at 10 °C (Hertog *et al.*, 2004). On the other hand, the incidence and severity of physiological disorders are affected by the interaction between temperature and gas

composition. It has been demonstrated that electrolyte leakage, as a symptom of CO_2 injury on mango fruit, was induced by 25% CO_2 at 5 °C but not at 8 °C (Bender *et al.*, 2021).

Meanwhile, the impact of temperature on the effect of MAP is more complicated. As the atmosphere modification in MAP is driven by the respiration of the fresh product, the formation of gas composition is correlated with storage temperature. Temperature influences the fruit respiration rate and gas transmission rate of the packaging film, thus altering the gas composition in MAP. For a certain product in a certain packaging, elevating storage temperature results in higher CO_2 and lower O_2 concentration (Ho *et al.*, 2020b). Also, the time reaching equilibrium is affected by storage temperature. For example, kiwifruit packed in MAP reached gas equilibrium within 2 days at 20 °C, while it took 6 days at 10 °C (Kitsiou & Sfakiotakis, 2002). Apart from static storage temperature, temperature fluctuation also affects the gas composition in MAP headspace as well as the quality of the product. A study on MAP-stored kiwifruit has reported that temperature changes between 1 °C and 10 °C led to unstable atmospheres with 1% O_2 fluctuation and 2% CO_2 fluctuation, which results in 25% lower firmness than constant 5 °C storage (Ahmad *et al.*, 2002). Negative impacts of temperature fluctuation also include 50% higher WL due to RH change, and condensation also increases the risk of pathogen growth (Ahmad *et al.*, 2002). Thus, to ensure optimal storage life, MAP has to be used at the designed temperature with minimal fluctuation.

In the supply chain of horticultural commodities, MA is normally applied in combination with coolstorage (Brandenburg, 2020). At the late stage of the supply chain, when the product is transferred to room temperature for distribution and retail, the product is usually exposed to the regular atmosphere (air). The quality of MA-stored fresh products shows more significant changes at the post-storage stage, compared to air-stored products. Elevated temperature alters the effect of MA on fresh products. Respiration rate is accelerated at room temperature compared to coolstorage. The effect of MA storage at low temperature on reducing respiration rate and maintaining the firmness of kiwifruit declined within 5 days of post-storage shelf life (Ozturk *et al.*, 2019b). Rapid softening and CI symptoms generally occur in the shelf-life at room temperature after CA or MAP storage. Apple surface browning can be inhibited by CA during storage, but the symptom may appear in the cold chain after removal from CA (Poirier *et al.*, 2020). A similar

disorder has been observed on hardy kiwifruit during the post-storage shelf-life. Skin brown spots developed on hardy kiwifruit three days after removal from MAP storage (Rebeaud *et al.*, 2018). The effect of MA retaining kiwifruit firmness during cool storage may soon be lost once fruit are brought out of cool storage. A study has revealed that 'Hayward' kiwifruit stored in CA were 3.5 kg_f firmer than air-stored fruit after 90 days at 1 °C, however, the fruit firmness reached the same level after being transported at 10 °C for 20 days (Prencipe *et al.*, 2016).

It is clear that MA applied at low temperature can be very beneficial in delaying fruit softening, but this benefit may be quickly lost on returning the fruit to warm conditions without MA. Few studies have addressed the specific question of whether applying a new MA system to fruit removed from cold storage would allow the fruit to retain the firmness benefits they had received from MA during cold storage.

2.3.5 Ethylene

While reduced O_2 and elevated CO_2 delay fruit ripening, the ethylene existing in CA or MAP may reduce the beneficial effect of atmosphere modification on fruit quality and storability. Ethylene has the effect of enhancing the respiration of fruit and vegetable in air or MA. In the presence of 61.8 $\mu L \cdot L^{-1}$ ethylene, broccoli packed in MAP showed higher respiration rate and lower O_2 concentration in the headspace compared to that with 0.3 $\mu L \cdot L^{-1}$ ethylene, which led to a higher risk of fermentation (Esturk *et al.*, 2014). The benefit of MAP on retaining nutritional compounds is also impacted by ethylene. It has been illustrated that the contents of ascorbic acid and total phenols were lower in MAP-stored tomato in the presence of ethylene compared to that in ethylene-free MAP (Domínguez *et al.*, 2016). As for kiwifruit, ethylene interaction with modified atmosphere resulted in both offsetting the benefit of MA and inducing other physiological disorders. Ethylene at the concentration of as low as 50 $nL \cdot L^{-1}$ can reduce the benefit of CA on maintaining kiwifruit firmness (Arpaia *et al.*, 1982; McDonald & Harman, 1982). Meanwhile, the incidence of CI is higher in MA storage in the presence of ethylene, compared to ethylene-free MA storage (Pekmezci *et al.*, 2004). On the other hand, ethylene interacts with elevated CO_2 in CA and causes WCI, a physical disorder that does not occur in air storage (Arpaia *et al.*, 1982; Arpaia *et al.*, 1986).

Although ethylene alters kiwifruit metabolism in MA storage, the impact does not necessarily affect storability. Pegoraro *et al.* (2016) has reported that kiwifruit 'Tewi' stored in CA (3% O_2 + 5% CO_2) with ethylene management of either 1-MCP or $KMnO_4$ showed a higher firmness after short storage of two months compared to that in CA without ethylene scavenger, but CA effect overtook the impact of ethylene after extended storage of four months. This indicates the exposure duration affects the effect of ethylene and CA. On the other hand, a study conducted with kiwifruit exposed to ethylene at concentrations of 0.05, 0.1, 0.5, 1.0 and 5.0 $\mu L \cdot L^{-1}$ in CA has indicated that ethylene concentration influences fruit firmness (Arpaia *et al.*, 1986). However, the risk of ethylene damage in MAP under industrial conditions is unclear.

As kiwifruit produce little ethylene early in ripening but are highly sensitive to exogenous ethylene at that time, removal of environmental ethylene can reduce the risk of ethylene-induced fruit ripening and soften, thus extending the storage life of kiwifruit (Jabbar & East, 2016). Ethylene inhibitor or absorbent can be combined with MAP to minimize the influence of ethylene. 'Hayward' kiwifruit stored in MAP after treatment with 1-MCP retained higher firmness for up to 120 days compared to MAP or 1-MCP alone. However, 1-MCP also causes quality issues of kiwifruit. Hard core disorder has been observed as a harmful side effect of 1-MCP on kiwifruit (Zoffoli *et al.*, 2016). The effect of ethylene biosynthesis inhibitor, AVG, has also been studied in combination with MAP, but there was no additional impact on kiwifruit quality (Ozturk *et al.*, 2019b). On the other hand, an ethylene scavenger such as potassium permanganate ($KMnO_4$) can reduce the ethylene concentration in MAP. It has been suggested that ethylene scavengers in MAP can delay kiwifruit softening (Abe & Watada, 1991) and inhibit rot incidence (Pekmezci *et al.*, 2002). It seems ethylene scavenger is the optimal option for minimizing ethylene damage in MAP in the kiwifruit industry, however, the effectiveness under the conditions of the current supply chain remains unknown.

2.4 Modified Atmosphere Technologies

2.4.1 Controlled atmosphere (CA)

Conventional CA refers to placing fresh products in an enclosed system with reduced O_2 and elevated CO_2. The atmospheres in CA usually involve reduced O_2 below 5% and enriched CO_2 above 3%, and the gas composition in CA is monitored and controlled at a

specific, constant level (Dilley, 2006; Saltveit, 2020). The concept of CA can be dated back to ancient civilizations, but modern CA technology has only developed over recent decades (Falagán & Terry, 2018). A refrigeration system is often applied with CA to maintain the quality of fruit and vegetables during storage, transport and distribution (Watkins, 2020).

To maximize the benefit of CA, ultra-low oxygen (ULO) technology has been developed, which brings the O_2 level in the storage facility down to below 1% (Poirier *et al.*, 2020). In the pome fruit industry, ULO has been commonly applied. It has been reported that ULO significantly reduces superficial scald disorder of apple (Gudkovsky *et al.*, 2021). Additionally, combining ULO with temperature conditioning further improves the effect on disorder reduction (Zoffoli *et al.*, 2018). However, due to low oxygen stress, disorders such as internal breakdown and off-flavour may develop during ULO storage (Ntsoane *et al.*, 2019). To reduce physiological disorders caused by low O_2 damage, it is recommended to use a system that allows adjusting gas levels during the CA storage. Dynamic CA (DCA) was developed based on ULO but maintaining the O_2 level within the 'safe range' (Mditshwa *et al.*, 2018). However, the safe range varies in different stages of storage. Fruit respiration rate reduces during CA storage. Brief exposure to low O_2 reduces metabolism and induces the production of a relatively low level of ethanol, which is beneficial to some fruit and maintains better quality, but this is harmful during long term storage (Klein *et al.*, 2020; Weber *et al.*, 2020). Thus, it is more beneficial to manipulate O_2 concentration based on chlorophyll fluorescence (CF) or respiratory quotient (RQ) compared to static CA (Wright *et al.*, 2015). These technologies allow the fruit in storage to 'self-report' when they are experiencing low oxygen stress and the oxygen concentration can be temporarily raised, and then lowered again slowly as the fruit naturally show lower respiration rates during prolonged storage. The technology of applying temporary low O_2 at the beginning of CA storage is termed initial low oxygen stress (ILOS) (Van Der Merwe *et al.*, 2003). Similarly, pulling down oxygen levels multiple times during storage has been developed as repeated low oxygen stress (RLOS) (Torregrosa *et al.*, 2020).

Controlled atmosphere application in the kiwifruit industry

Currently, CA is used in the kiwifruit industry for extending packing windows. At the peak of the kiwifruit harvest season, when the harvested fruit volume exceeds the

packhouse processing capacity, kiwifruit is briefly stored under CA conditions and packed later in the season (NZKGI, 2016). It has not been applied for long term storage, because of the low cost-benefit efficiency and high risk to worker health and safety on a big operation scale. Furthermore, the optimal CA condition varies between different products, which makes it unsuitable for a large storage facility or distribution centres with multiple types of fruit and vegetables. Thus, an alternative technology that is low-cost, safe-to-operate and highly flexible would be beneficial for kiwifruit storage and transport.

2.4.2 Modified atmosphere packaging (MAP)

Compared to CA, MAP provides higher flexibility in practice during storage, transportation, and distribution of fresh produce, as the facility limitation is little more than for normal cool chains. From bulk packaging of pallet size to retail packages or individual produce coating, MAP can be designed to various forms that fit the required supply chain (Lee *et al.*, 1996). An internal atmosphere of elevated concentration of CO_2 and reduced O_2 can be created by MAP. The respiration rate of the fresh product is reduced in this way, which extends the storage life of the commodity (Fonseca *et al.*, 2002). Oxygen (O_2), CO_2 and N_2 are the main gases involved in MAP: a combination of reduced O_2, and enriched CO_2 is normally balanced with N_2 (Arvanitoyannis, 2012). The gas composition of MAP is created by the equilibrium between film permeance and the respiration rate of the given produce (Thompson, 2010a). The polymer film used can also reduce the diffusion of water vapour to the surrounding environment and maintain high relative humidity (RH) inside the package, hence reducing water loss of fresh product (Hu *et al.*, 2011; Ozturk *et al.*, 2019b).

The success of MAP relies on the interaction between the commodity, packaging, and the environment. Key factors that need to be considered in MAP design include product characteristics and mass, the target gas composition, storage temperature, and film permeability (Arvanitoyannis, 2012). Respiration of product creates the change in atmospheric conditions in passive modified atmosphere (MA). Consequently, a low respiration rate or small product mass delay the establishment of desired MA, which may lead to ineffectiveness (Pekmezci *et al.*, 2002). On the contrary, excessive respiration rate or large product mass may lead to gas compositions that are harmful to the product (Jiao *et al.*, 2020). As improper package design may result in ineffectiveness or negative impact on product quality (Fonseca *et al.*, 2000), MAP has to be designed based on each product.

Film permeability is determined by polymer properties with large variation between polymer types and is impacted by environmental factors such as temperature (Heilman *et al.*, 1956; Siracusa, 2012). Films commonly used for MAP are polyethene (PE), polyvinyl chloride (PVC), polypropylene (PP), with the thickness varying from 0.01 mm and 0.06 mm (Álvarez-Hernández *et al.*, 2020; Wang *et al.*, 2021b). However, some commonly used films such as oriented polypropylene (OPP) have low permeability to O_2 and CO_2, which limits their application in MAP. Micro-perforation technology has been employed to modify the permeance of MAP to meet the requirements for fresh products with a relatively high respiration rate (Ramos *et al.*, 2019).

Initial gas flush with N_2 or high CO_2 may alter fruit quality during MAP storage. It has been demonstrated that initial gas flush with 20% or 30% CO_2 improves strawberry firmness (Nakata & Izumi, 2020), whereas N_2 flushed active MAP reduces the skin browning of loquat fruit (Öz *et al.*, 2019). However, the effect of initial gas flush varies between cultivars and storage conditions. Red bell pepper in MAP flushed with 5% O_2 and 5% CO_2, resulting in an atmosphere of similar CO_2 level and higher O_2 concentration compared to MAP sealed in ambient atmosphere. Higher quality was achieved by the non-flushed MAP in this study (Cerit & Demirkol, 2020). Initial 6% CO_2 ended up with higher CO_2 level and lower O_2 level in MAP, and induced brown skin spot on hardy kiwifruit, compared to MAP begun in air (Rebeaud *et al.*, 2018). The optimal condition of the initial gas flush on kiwifruit has not been investigated.

Temperature influences the gas composition by altering product respiration (Ho *et al.*, 2020a) as well as modifying film permeability (Felder & Huvard, 1980). Generally, the storage temperature of MAP should be relatively stable to avoid changes in gas composition (Tano *et al.*, 2007; Pan *et al.*, 2019). Meanwhile, temperature also impacts the enzyme activities in multiple metabolic pathways of fruit and vegetable, which directly alters the quality and storability of fresh products. It has been demonstrated on king chilli that higher CO_2 and O_2 concentrations were achieved in MAP at 25 °C compared to that at 8 °C, whereas the longest storage life was obtained by MAP at 8 °C, but MAP at 25 °C still maintained higher quality compared to air-storage (Malakar *et al.*, 2020). Due to the dramatic respiration increase when the fresh product is moved from coolstorage to room temperature (20-25 °C), MAP applied during the cool storage is usually punctured once the product is moved to warmer temperatures to avoid

fermentation. It has been revealed on plum that the effect of MAP on alleviating CI was retained during the post-storage shelf-life, whilst the effect of maintaining flesh firmness soon declined after removal from storage (Wang *et al.*, 2021b). The post-storage quality decline has been observed on multiple crops, such as apple and kiwifruit (Ozturk *et al.*, 2019b; Poirier *et al.*, 2020). Applying MAP during the post-storage stage could be potentially beneficial, however, the effect of using MAP at room temperature has not been well studied.

As an enclosed system, there is a risk of ethylene accumulating in MAP, so that ethylene management is required for ethylene sensitive products stored in MAP. Studies on a number of fruit and vegetables have reported that pre-storage treatment with ethylene receptor inhibitor 1-MCP can reduce the impact of ethylene on fresh products during MAP storage (Li *et al.*, 2013; Özkaya *et al.*, 2016; Kanwal *et al.*, 2020; Zhao *et al.*, 2020). However, 1-MCP treatment is not always the optimal option. Aroma loss and hard core disorder have been reported on kiwifruit treated with 1-MCP (Burdon *et al.*, 2007c; Zoffoli *et al.*, 2016). Besides ethylene inhibitor, ethylene removal is another strategy to reduce ethylene impact in MAP. Ethylene scavenger, such as $KMnO_4$ based sachet, can sufficiently reduce ethylene concentration in small spaces like a sealed package i.e., MAP (Murmu & Mishra, 2018; Álvarez-Hernández *et al.*, 2019a; Álvarez-Hernández *et al.*, 2019b; Cerit & Demirkol, 2020). However, the effect of ethylene scavengers on product quality varies by cultivars and storage conditions. It has been demonstrated that applying $KMnO_4$ based ethylene scavenger to apricot in MAP can reduce ethylene concentration from 6.2 $nL \cdot L^{-1}$ to 0.3 $nL \cdot L^{-1}$, but the fruit quality was not impacted by the ethylene scavenger (Álvarez-Hernández *et al.*, 2020). Hence, the sufficiency of ethylene scavengers combined with MAP must be tested on the specified product.

Modified atmosphere packaging studies on kiwifruit

MAP has not been commonly applied in the kiwifruit industry. However, the effect of MAP on kiwifruit quality has been studied on several cultivars, including old cultivars like ‘Hayward’ and ‘Bruno’, as well as new cultivars, such as ‘Cuixiang’ (*A. deliciosa*), ‘Sanuki Gold’ (*A. chinensis*) and ‘Wanjin’ (Table 2.1). The majority of studies have been conducted on ‘Hayward’, as it has been the main cultivar for decades. However, other cultivars with different genetic backgrounds may respond differently from ‘Hayward’.

Table 2.1 The conditions of MAP applied on kiwifruit in previous studies.

Cultivar	Film	Thickness (mm)	O_2 (%)	CO_2 (%)	C_2H_4 ($\mu L \cdot L^{-1}$)	C_2H_4 management	Temperature (°C)	Storage (day)	Shelf-life (day)	Reference
'Bruno'	PE	0.02	20-21	0.5-1.0		$KMnO_4$	-1	120	7	(Ben-Arie & Sonego, 1985)
		0.04	16-18	2-3						
		0.05	12-15	4-5						
A. chinensis	PE	0.04	12-15	0.5-1			2	120		(Lee *et al.*, 1992)
		0.06	2-10	2-3						
		0.08	1-10	4-5						
		0.1	1-10	7-8						
'Hayward'	LDPE	0.06	2-4	11-13			0	180		(Manolopoulou *et al.*, 1995)
	MDPE	0.075	16-18	4-5						
'Hayward'	stretch film	0.016	13-15.8	1.7-2.3	0.07		10	77		(Kitsiou & Sfakiotakis, 2002)
		0.016	8.96-11	2.3-3.5	0.19		20		21	
'Hayward'	PE	0.02	7-18	1-8		$KMnO_4$	0	180		(Pekmezci *et al.*, 2002)
'Hayward'	FF Kiwi®		8.6-16.9	4.1-9.9			0	60	14	(Zoffoli *et al.*, 2006)
'Hayward'	LDPE	0.013				$KMnO_4$	0	200		(Bal & Celik, 2010)
						salicylic acid				
'Lushanxiang'	PE	0.03	14-16	2-4		1-MCP	20	35		(Li *et al.*, 2011)
	PVC	0.03	10-14	3-5						
	PVC	0.05	2-5	4-8						
'Qinmei'	LDPE	0.05			15	nano-silver, nano-TiO_2	4	42		(Hu *et al.*, 2011)
					40					
'Sanuki Gold'	PE	0.05	2-10	3-5		1-MCP	4	120		(Mworia *et al.*, 2011)
'Zhonghua'	PE	0.03	14-16	5-6			0	120		(Gao *et al.*, 2014)
	PVC	0.03	16-18	3-4						

Table 2.1 (continued)

Cultivar	Film	Thickness (mm)	O_2 (%)	CO_2 (%)	C_2H_4 ($\mu L \cdot L^{-1}$)	C_2H_4 management	Temperature (°C)	Storage (day)	Shelf-life (day)	Reference
'Hayward'	Stepac®		17.5-19.5	1.5-3			0	112		(García, 2015)
	San Jorge®		10.5-15	5-9						
	Lifespan®		13-15	3.5-4.5						
	Viewfresh®		14-18	3.5-5						
	Paclife®		15-17.5	3-4						
	Viewfresh®		14.5-17	3.5-4.5						
'Hayward'	LDPE	0.06	10-15	5-8		1-MCP	0	120	20	(Zoffoli *et al.*, 2016)
A. chinensis	LDPE	0.04	10.8-11.5	7.8-8.9		1-MCP	20		28	(Qian *et al.*, 2018)
'Hayward'	BOPP		8	14			0.5	140		(Sicari *et al.*, 2019)
	PP		6	15						
'Hayward'	Xtend®		13.0	8.7		AVG	0	180	5	(Ozturk *et al.*, 2019b)
'Cuixiang'	LDPE	0.02	17.1–17.5	2.5–3.2			0	91		(Jiao *et al.*, 2020)
		0.03	14.0–14.8	4.2–4.8						
		0.05	10.1–10.6	6.4–7.0						
'Wanjin'	PE	0.01	16.6-17.5	2.0-2.6			1	90		(Wang *et al.*, 2021c)
		0.03	15.0-16.2	3.2-3.7						
		0.05	13.4-14.5	4.4-5.5						

The atmosphere created in the MAP headspace is the key factor impacting kiwifruit quality and storage life in MAP. In previous studies, the concentrations of O_2 varied from 1% to 20%, and that of CO_2 ranged between 1% and 10% (Lee *et al.*, 1992; Zoffoli *et al.*, 2006; García, 2015). With no initial gas flush being investigated, the ratio of O_2 to CO_2 ranged between 1:1 and 4:1, due to the transmission rate of polymer films (Beaudry, 1999). The gas transmission rate of O_2 and CO_2 depend on film type and thickness. The most frequently used film types are polyethene (PE), low-density polyethene (LDPE), and PVC, with film thickness ranges between 0.01 mm and 0.08 mm (Table 2.1). Generally, PE, LDPE or medium density polyethene (MDPE) films with the thickness of 0.06 mm and above are not suitable for kiwifruit, as the permeance is too low, and anaerobic respiration may be induced (Lee *et al.*, 1992; Manolopoulou *et al.*, 1995; Zoffoli *et al.*, 2016). Meanwhile, several commercial films with unknown material types and thickness have been tested, the outcomes of which were not always satisfactory. For example, the film of San Jorge® applied on 'Hayward' has induced fruit rot during the storage at 0 °C (García, 2015).

The primary effect of MAP on kiwifruit is reducing respiration rate (Wang *et al.*, 2021c), thereby slowing down the ripening process. As softening is a key issue during kiwifruit storage, MAP is mainly used for maintaining kiwifruit firmness. Another contribution of MAP to kiwifruit quality is suppressing CI (Jiao *et al.*, 2020). However, negative impacts may occur if the atmosphere created by MAP is undesirable. Fermentation and ethanol production may happen in MAP, which creates off-flavours in kiwifruit and leads to value loss (Lee *et al.*, 1992; Jiao *et al.*, 2020). Fruit rot can be enhanced by MAP in coolstore when the CO_2 level is beyond the safe level of the kiwifruit cultivar (García, 2015; Wang *et al.*, 2021c). With the presence of ethylene, the relatively high level of CO_2 in MAP may also induce WCI on kiwifruit (Lee *et al.*, 1992; Manolopoulou *et al.*, 1995).

The beneficial atmospheres during storage also depended on the temperature. The acceptable gas composition for kiwifruit in MAP storage at low temperature (below 4 °C) are 2-18% O_2 and 2-7% CO_2 (Pekmezci *et al.*, 2002; Mworia *et al.*, 2011; Gao *et al.*, 2014). However, the effectiveness of gas composition varies between kiwifruit cultivars. The gas composition of 17.1-17.5% O_2 and 2.5-3.2% CO_2 was ineffective on 'Cuixiang' kiwifruit at 0 °C (Jiao *et al.*, 2020). On the other hand, lower O_2 or higher CO_2 concentration may result in fermentation. Ethanol production and off-flavours have been detected when O_2 was below 2% or CO_2 was above 7% (Lee *et al.*, 1992; Jiao *et al.*, 2020),

indicating respiration has shifted from aerobic to anaerobic at these points. Meanwhile, the beneficial atmospheres at room temperature differs from that at low temperature. At 20 °C, the gas composition of 9-11% O_2 and 2-10% CO_2 were effective and safe for kiwifruit (Kitsiou & Sfakiotakis, 2002; Qian *et al.*, 2018), suggesting kiwifruit has lower sensitivity and higher tolerance to low O_2 and high CO_2 at room temperature compared to low temperature.

Due to the influence of temperature on respiration and fruit sensitivity to reduced O_2 and elevated CO_2, different films were applied during coolstore and room temperature to create desired atmospheres (Table 2.1). In industrial practice, kiwifruit are usually kept at low temperatures for long storage and may be transferred to uncontrolled temperatures at the end of the supply chain for distribution and retailing. The temperature change during this shift potentially impacts fruit quality. To monitor the quality change during this temperature shift, a shelf-life assessment at 20 °C following coolstorage has been used in some of the experiments. As the MAP film applied during coolstorage cannot maintain the desired atmosphere once it is placed at elevated temperature, the packages were cut open upon removal from low temperature, and the fruit were exposed to air during the shelf-life assessment (Ben-Arie & Sonego, 1985; Zoffoli *et al.*, 2006; Ozturk *et al.*, 2019b). The results of these studies have commonly shown a rapid quality decline during the post-storage shelf-life. Meanwhile, beneficial effects on kiwifruit have been obtained by applying MAP at 20 °C (Kitsiou & Sfakiotakis, 2002; Li *et al.*, 2011; Qian *et al.*, 2018), which suggests it is possible to maintain kiwifruit quality at room temperature by MAP. However, the previous studies on MAP applied at room temperature only included 20 °C during the whole assessment, and no previous coolstore was involved. Thereby, it is unknown whether repacking kiwifruit into a different MAP film upon removal from coolstore can alleviate the rapid quality decline during the post-storage shelf-life.

With the low permeability of PE and PVC films that ranges from 2.68×10^{-16} to 8.49×10^{-15} mol.m.m^{-2}s^{-1}Pa^{-1} (Samarakoon, 2013), there is a high chance that ethylene produced by kiwifruit accumulates in MAP, especially after extended storage and at room temperature. In fact, ethylene accumulation up to 40 µL·L^{-1} has been detected in MAP after 42 days of storage at 4 °C (Hu *et al.*, 2011). On the other hand, it has been revealed that the atmosphere of reduced O_2 and elevated CO_2 can suppress the impact of ethylene on kiwifruit softening; but ethylene interacting with CO_2 also leads to WCI disorder

(Arpaia *et al.*, 1982). Hence, it is critical to evaluate the ethylene level that is likely to accumulate in MAP and to investigate the impact of ethylene at these levels on kiwifruit stored under MA.

Ethylene management approaches have been tested in combination with MAP. Ethylene receptor inhibitor, 1-MCP has been widely applied with MAP in the horticultural industry. The effects of delaying fruit ripening and reducing ethylene production have been observed on 1-MCP treated kiwifruit (Mworia *et al.*, 2011; Qian *et al.*, 2018). However, hard core disorder and failure to ripen were also observed on 1-MCP treated kiwifruit (Zoffoli *et al.*, 2016), suggesting 1-MCP is not an optimal method to reduce ethylene damage in MAP for kiwifruit. Meanwhile, ethylene biosynthesis inhibitor AVG did not sufficiently reduce the impact of ethylene on kiwifruit in MAP (Ozturk *et al.*, 2019b). Apart from ethylene inhibitors, the effect of ethylene scavengers in MAP has also been investigated. As an enclosed package, it is possible to reduce the ethylene concentration by applying an ethylene absorbing or catalysing agent. It has been reported that adding nano-TiO_2 and nano-silver into MAP film can reduce the ethylene level from 40 $\mu L \cdot L^{-1}$ to 15 $\mu L \cdot L^{-1}$ (Hu *et al.*, 2011), however, 15 $\mu L \cdot L^{-1}$ is still a very high ethylene concentration and is capable to induce kiwifruit softening (Jabbar & East, 2016). The effect of another commonly used ethylene scavenger, $KMnO_4$, has also been studied in MAP. Ethylene concentration in MAP has been reduced by $KMnO_4$ sachet and fruit ripening has been delayed at 0 and -1 ℃ (Ben-Arie & Sonego, 1985; Pekmezci *et al.*, 2002; Bal & Celik, 2010). However, the effect of $KMnO_4$ on reducing ethylene concentration and maintaining kiwifruit quality in MAP at room temperature has not been revealed.

2.5 Conclusions and Objectives

The relatively short storage life of some new kiwifruit cultivars has created challenges for international trading. To extend the storage life of kiwifruit, atmosphere modification is a potential approach. Due to the high facility requirement and high cost, CA is not ideal for long term storage and not suitable for transport. Meanwhile, MAP has high flexibility and is easy to apply, which is a promising alternative to CA. However, as an enclosed system, ethylene accumulation is a potential risk for kiwifruit storage in MAP. On the other hand, rapid post-storage quality decline has been observed in MAP stored kiwifruit. It is unknown whether this can be reduced or delayed by applying MAP during shelf-life.

Hence, the objectives of this study are: to evaluate the influence of ethylene at various levels in MA conditions; to test the effect of commercial sourced MAP on kiwifruit quality during coolstorage and the subsequent shelf-life; and to determine whether $KMnO_4$-based ethylene absorbent sachet can reduce ethylene damage in MAP and extend kiwifruit shelf-life.

Chapter 3. Kiwifruit responses to ethylene in controlled atmosphere

3.1 Introduction

Kiwifruit (*Actinidia deliciosa* and *A. chinensis*) is a key export horticultural product for New Zealand. More than 90% of New Zealand grown kiwifruit are exported (Burdon & Lallu, 2011). The kiwifruit industry has been growing fast and various new cultivars have been generated from the genus *Actinidia* over the recent years, such as 'Cuixiang', 'Hongyang' and 'Sanuki Gold' (Mworia *et al.*, 2010; Ma *et al.*, 2014; Jiao *et al.*, 2020). However, 'Hayward' is still the main cultivar because of the excellent postharvest performance for up to six months of storage at 0 °C (Harman, 1981). The storage life of most new cultivars is relatively short compared to 'Hayward'. One option for extending storage life and providing greater flexibility in supply chains is the use of controlled atmosphere storage (Mworia *et al.*, 2011; Li *et al.*, 2015; Burdon, 2020).

Controlled atmosphere (CA) is the technology that applies atmosphere differing from air (79% N_2, 21% O_2, and 0.05% CO_2) in an enclosed space to preserve the quality of fresh produce (Dilley, 2006). The gas compositions applied in CA are usually oxygen (O_2) below 5% and carbon dioxide (CO_2) above 3% (Saltveit, 2020). Reduced O_2 and enriched CO_2 can extend the storage life of fresh products by slowing down respiration (East *et al.*, 2009b), reducing C_2H_4 production (Yang & Hoffman, 1984), suppressing C_2H_4 sensitivity (Burg & Burg, 1969), and alleviating physiological disorders (Burdon *et al.*, 2008). Optimal CA (2% O_2 + 5% CO_2) (McDonald & Harman, 1982) has been commercially applied in kiwifruit industry to expand the packing window and reduce packhouse pressure (Burdon & Lallu, 2011). Due to the relatively high cost of CA facilities, CA is not commonly used for long term storage in the kiwifruit industry. Instead, modified atmosphere packaging (MAP) has shown a higher potential for maintaining kiwifruit quality during extended storage at a relatively low cost. Although CA technology is not employed in long-term storage for kiwifruit, the closely monitored and highly regulated gas condition in CA makes it an ideal tool to study fruit responses in various gas mixes, which produces information for MAP development.

Ethylene (C_2H_4) is a gaseous plant hormone that plays a significant role in fruit ripening (Burg & Burg, 1962). It can be produced by ripe fruit and postharvest pathogens (Tonutti

et al., 1993; Qadir *et al.*, 1997), and accelerate kiwifruit softening (Redgwell *et al.*, 1990). Kiwifruit is extremely sensitive to C_2H_4 so that as little as 0.01 $\mu L \cdot L^{-1}$ C_2H_4 can stimulate kiwifruit softening when the fruit is stored in air (Arpaia *et al.*, 1987). C_2H_4 contamination in the CA system can negatively impact fruit quality and storage life (Arpaia *et al.*, 1982). The effect of C_2H_4 on kiwifruit ripening appears to be an acceleration rather than initiation of ripening (Beever, 1990). Higher C_2H_4 concentrations can further accelerate kiwifruit softening and induce chilling injury (CI) in air (Jabbar & East, 2016). It has been reported that 50 $\mu L \cdot L^{-1}$ C_2H_4 in CA (2% O_2 + 5% CO_2) storage can accelerate kiwifruit softening and induce white core inclusions (WCI) disorder (Arpaia *et al.*, 1986). However, the effects of C_2H_4 concentrations from 0.01 $\mu L \cdot L^{-1}$ to 1 $\mu L \cdot L^{-1}$ have not been compared between air and CA environments in previous studies.

For recently developed cultivars, especially those with relatively short storage life, there is a need to determine CA conditions suitable for extending the storage life and meeting the requirements for long-distance shipping. As this study was undertaken during the COVID-19 pandemic, an initial plan to work on new varieties was not possible due to enforced lockdown restrictions coinciding with harvest timing. As a result, when access to the lab returned, the focus of the work moved to increasing our understanding of CA and ethylene effects on 'Hayward' fruit, which are harvested later in the season.

In commercial practice, kiwifruit is normally harvested mature but unripe (Burdon & Lallu, 2011). After passing a sorting line, only the sound fruit are moved into long-term storage or shipped to overseas markets. At this stage, fruit firmness is relatively high, and the ethylene production of kiwifruit is merely detectable. A large amount of ethylene is only produced by kiwifruit when flesh firmness is below 1 kg_f (Antunes & Sfakiotakis, 2002) or rot develops (Qadir *et al.*, 1997). Hence, ethylene is not likely to accumulate in the kiwifruit storage environment until a few weeks after harvest.

This study aimed to quantitatively determine the responses of 'Hayward' kiwifruit to delayed exposure to C_2H_4 under both air and CA condition within the exporting timeframe. The result of this study will inform C_2H_4 management in commercial practice.

3.2 Materials & methods

3.2.1 Fruit Material

Kiwifruit (*A. deliciosa* cv. 'Hayward') was sourced from three growers, creating grower lines (GLs) as replicates. The fruit were couriered to Massey University, Palmerston North, after commercial grading and packing in modular bulk (MB) packs. Fruit size of 'Count 36' (95-108 g) were used in this study. Ninety fruit from each GL were randomly taken out of the MBs upon arrival for initial quality assessment (described in 3.2.6 Fruit quality assessment), including dry matter (DM), firmness, and soluble solid content (SSC) (Table 3.1). Twelve mesh bags each containing 15 fruit from the same GL were filled and randomly placed in a 60 L barrel upon fruit arrival, with a separate barrel for each GL in the same treatment conditions.

Table 3.1 Initial quality of 'Harward' kiwifruit from three grower lines.

Grower	Firmness (kg_f)	SSC (%)	DM (%)
GL1	5.12 ± 0.15	14.03 ± 0.22	18.75 ± 0.13
GL2	6.82 ± 0.11	12.12 ± 0.19	18.80 ± 0.10
GL3	5.68 ± 0.12	12.57 ± 0.19	17.52 ± 0.10

*Data represents mean ± SEM, n=90.

3.2.2 Controlled atmosphere and ethylene treatment

Air and optimum CA (5% CO_2 + 2% O_2, balanced by N_2) were used as gas treatments for the first 3-week storage, as identified in a previous study (Pranamornkith *et al.*, 2012). Ethylene concentrations at 10, 100 and 1000 $nL \cdot L^{-1}$ were added to a flow-through system (section 3.2.3 Flow-through system) after 3 weeks of storage in CA or air, which created 8 treatments:

1. Air (21% O_2, <0.1% CO_2)
2. Air + 10 $nL \cdot L^{-1}$ C_2H_4
3. Air + 100 $nL \cdot L^{-1}$ C_2H_4
4. Air + 1000 $nL \cdot L^{-1}$ C_2H_4
5. CA (2% O_2, 5% CO_2)
6. CA + 10 $nL \cdot L^{-1}$ C_2H_4
7. CA + 100 $nL \cdot L^{-1}$ C_2H_4

8. CA + 1000 $nL \cdot L^{-1}$ C_2H_4

3.2.3 Flow-through system

A flow-through system was set up in a temperature-controlled room (TCR). Compressed air, compressed N_2 and CO_2 (BOC Gas, Palmerston North), were mixed with a set of needle valves to create optimum CA (5% CO_2 + 2% O_2, balanced with N_2). Ethylene from cylinders containing 9.6 ± 0.4 $\mu L \cdot L^{-1}$ C_2H_4 in air (BOC Gas, Palmerston North) was mixed with compressed air and CA using needle valves to create 100 and 1000 $\mu L \cdot L^{-1}$ C_2H_4 treatment. Likewise, 92 ± 4 $\mu L \cdot L^{-1}$ C_2H_4 in air (BOC Gas, Palmerston North) was added to the flow-through system using mass flow controllers (GSC-A9TA-BB21, Vögtlin Instruments GmbH, Switzerland) for 10 $nL \cdot L^{-1}$ C_2H_4 treatments. The pressure at the point of CA mixing, mixer input and mixer output were maintained at 15, 10 and 5 psi respectively, using diaphragm regulators (R57M-2W, Master Pneumatic, USA). To limit CO_2 concentration change inside the barrels (<0.2%), the flow rate was set at 300 mL/min based on calculations of kiwifruit respiration rate (East *et al.*, 2009a). The desired gas composition and flow rate were achieved by adjusting the needle valves, mixer and manifold, simultaneously monitoring manifold outflow using a multi-gas sampling data logger (CM-1000, CO_2 Meter, USA), an ethylene analyser (MacView®, EMS, The Netherlands) and a flow meter (G6691A, Agilent, USA).

Each of the gas mixes was split by a manifold to create identical lines for all 3 replicates. To maintain 95% relative humidity (RH), the gas mix was bubbled through a 1 L gastight jar containing 500 mL 21.1% glycerol solution before entering the barrel. Twenty-four sealable plastic barrels with a volume of 60 L were used as fruit containers. The barrels were fixed on two racks in a temperature-controlled room (TCR) and labelled with grower and treatment details. After passing through the barrel, outflow gas was released to an outdoor space through the TCR ventilation system. All the elements in the flow-through system were connected by 6 mm (outside diameter, OD) nylon tubes (Ledalon, Leda, New Zealand) with an inside diameter of 4 mm. The system passed a pressurised leak-checking before the experiment. Storage temperature in the TCR was set at 0 °C, and a short defrost cycle was run twice daily.

3.2.4 System Monitoring

The concentrations of CO_2 and O_2 were monitored weekly by connecting the flow meter and the gas logger to the barrel outflow starting from week 1, while C_2H_4 concentration

was monitored weekly by connecting the ethylene analyser to the barrel outflow from Week 3. Gas monitoring was conducted 16 h after barrel re-tightening at each fruit sampling time to allow the internal atmosphere to be re-established. The level of glycerol solution in each jar was checked weekly. Water was refilled in the glycerol jar with a syringe whenever the solution level was clearly lower than the initial level mark.

3.2.5 Fruit Sampling

Fruit were sampled at 0, 3, 5, 7, 9, 11 and 13 weeks after harvest for quality assessment (section 3.2.6 Fruit quality assessment), including firmness, SSC, and disorder assessing. In addition, core firmness was measured at Week 13 to determine whether hard core disorder occurs when flesh firmness is below 1 kg_f (Jeffery *et al.*, 2012). Thirty fruit (2 bags) were taken out from each barrel in the afternoon and arranged in a tray for each assessment.

3.2.6 Fruit quality assessment

Quality assessment was performed the following morning (16 h after removal from cool storage) to allow fruit internal temperature to recover to the same as room temperature (20 °C), as fruit temperature impacts firmness (Jeffery & Banks, 1994). The fruit stored at room temperature were assessed at the same temperature.

3.2.6.1 Dry matter

Dry matter (DM) was measured at harvest. Flesh (2 mm) was sliced from the equator of each fruit and placed in a petri dish, then dried using a food dehydrator (ULTRA FD1000, Ezidri, New Zealand) at 65°C for 24 h. The fresh weight (FW) and dry weight (DW) of the flesh slice were determined using an electronic balance (TW423L, Shimadzu, Japan).

Dry matter (%DM) =

$$\frac{DW}{FW} \times 100\%$$

Where:

DW = the weight of fruit slice after dehydrating (g),

FW = the weight of fruit slice before dehydrating (g).

3.2.6.2 Firmness

Fruit firmness was measured using a penetrometer (Willowbank Electronics Ltd., New Zealand), with a 7.9 mm diameter probe inserted to a depth of 8 mm at the speed of 8 $mm \cdot s^{-1}$. Two points (90° apart) at the fruit equator were measured on each fruit. At each of the measuring points, skin of 1 mm depth was removed with a slicer before penetration. The peak force was recorded. The average of the two measurements was recorded as the firmness of the measured fruit.

3.2.6.3 Core Firmness

Approximately 5 mm flesh from the flat side of fruit was removed before penetration. Core firmness was measured at the centre of the cut surface using a penetrometer (Willowbank Electronics Ltd., New Zealand) with a 6 mm probe at the depth of 25 mm. The peak force was recorded.

3.2.6.4 Disorder assessment

Chilling injury

Chilling injury (CI) was assessed from the cut at 1.5 mm from the blossom end and the equator (Figure 3.1-A and B).

Scuffing

The incidence of scuffing was visually assessed on the skin surface before any other assessment. The fruit with scuffing was marked as “1”, and fruit without scuffing was marked as “0” (Figure 3.1-C).

White core inclusions

White-core inclusions (WCI) were assessed from the cut surface at the fruit equator. Fruit with WCI was marked “1”, otherwise was marked “0” (Figure 3.1-D and E).

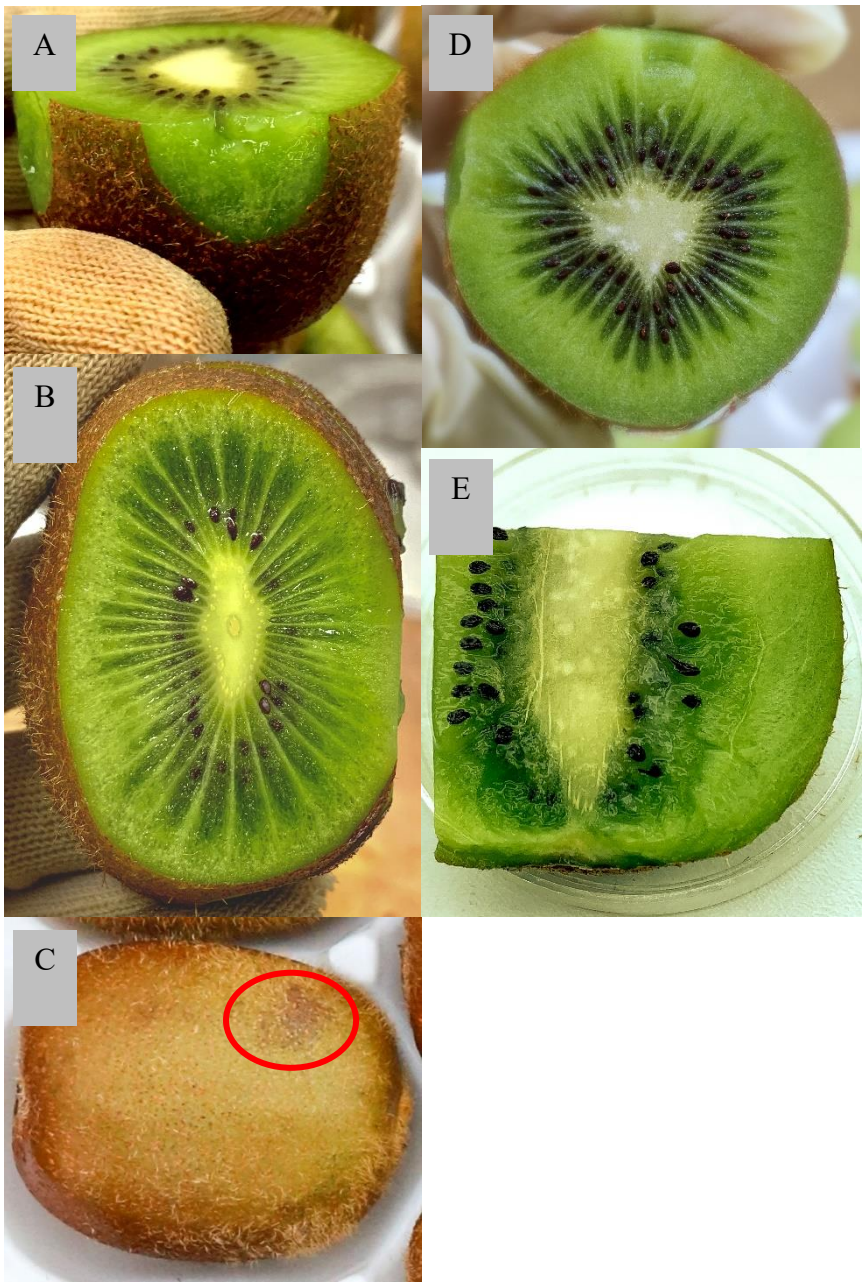

Figure 3.1 Disorder symptoms in 'Hayward' kiwifruit. A and B: chilling injury (CI). C: scuffing. D and E: white-core inclusions (WCI).

3.2.6.5 Soluble solids content

Soluble solids content (SSC) was determined by squeezing out approximately 0.3 mL of juice from the cut surface of the blossom half of the fruit and measuring it with a refractometer (PR-32α, Atago, Japan). The refractometer was returned to zero with reverse osmosis (RO) water before the first measurement and after finishing each tray of fruit.

3.2.7 Statistical analysis

Data has been described by means and standard error of means (SEM). Statistical separation of treatment means was by analysis of variance (ANOVA). Multiple comparisons used Fisher's protected least significant difference (LSD) test at P=0.05. Analysis was conducted using R version 4.0.3 (R Core Team, Vienna, Austria). Graphs were created using the package ggplot2.

3.3 Results and discussion

3.3.1 Firmness

3.3.1.1 Firmness change in initial storage

The fruit were stored in air and CA for three weeks before ethylene was added to the system. The mean initial firmness for all GLs was 5.9 kg_f at harvest. Data for individual GLs will be discussed later in this chapter. Rapid softening occurred in both air and CA storage during the first 3 weeks, before C_2H_4 treatment started. There was no significant difference in terms of firmness between air and CA stored fruit after the first 3 weeks, however firmer fruit appeared in CA at Wk 5 compared to air (Figure 3.2), suggesting low O_2 (2%) and high CO_2 (5%) condition did not affect the loss of firmness in the initial three weeks of storage.

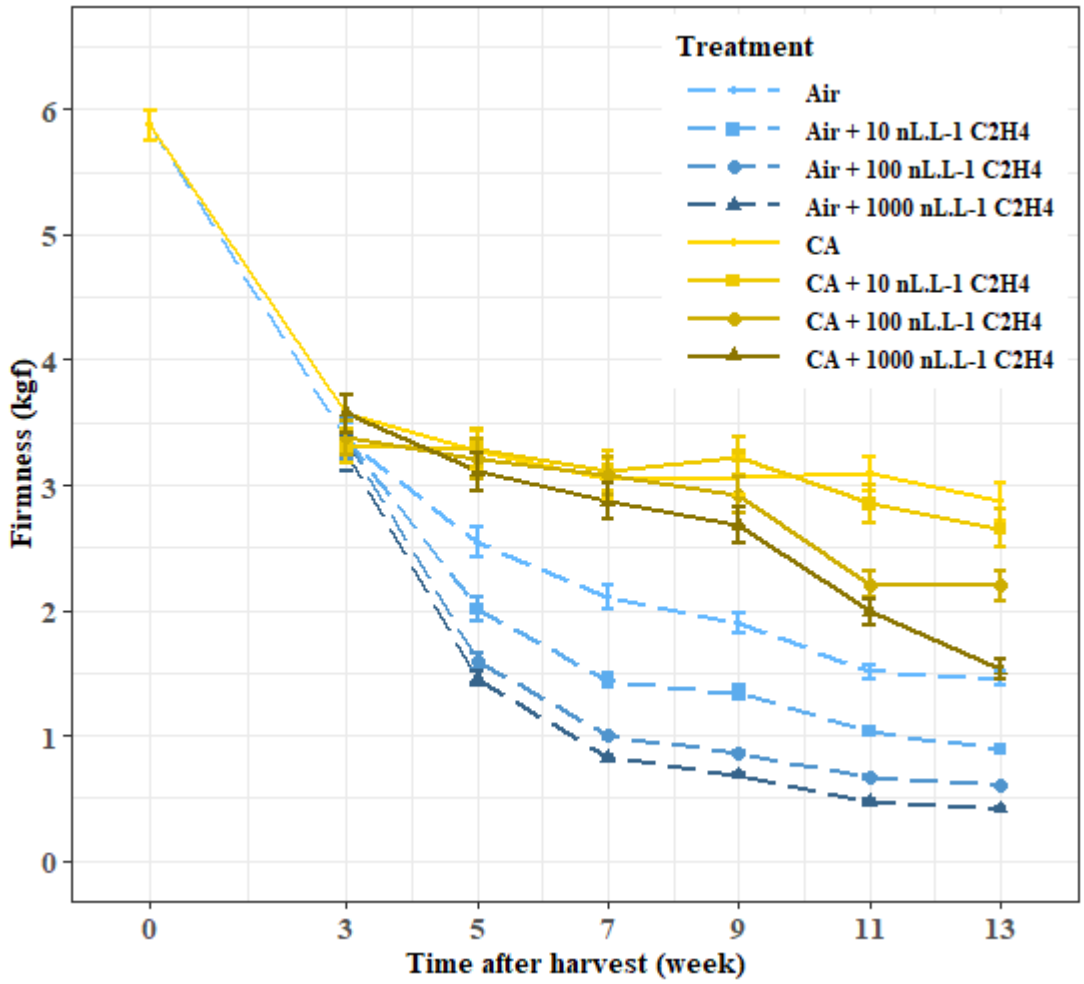

Figure 3.2 Firmness of 'Hayward' kiwifruit stored in air and optimal CA (2% O_2 + 5% CO_2) at 0 ºC 95% RH for 13 weeks with additional C_2H_4 at the concentration of 0, 10, 100 and 1000 nL·L^{-1} from Week 3 to Week 13. Each data point represents mean ± SEM, n = 90.

Generally, it is recommended that CA should be established within a short time after fruit harvest, as delayed CA for 30 days reduces the benefit of retaining kiwifruit firmness while a 60-day delay leads to complete loss of CA benefit (Zoffoli *et al.*, 2006). In the present study, the steady-state was established within 24 h in the flow-through system, which was immediate enough to obtain a beneficial effect. However, the fact that fruit firmness did not differentiate within the first three weeks may be the result of short exposure to CA. The satisfactory effect of CA or MA on kiwifruit are normally observed after at least 30 days of CA or MA exposure (Harman & McDonald, 1989a; Hertog *et al.*, 2004; Fisk *et al.*, 2008). The firmness change during the fourth week may be critical for a statistical difference to be detected. One exception was that Cornacchia *et al.* (2008) reported a higher kiwifruit firmness was obtained as early as two weeks after CA storage started, however, the fruit was stored at 0 °C for five weeks before entering CA storage,

which means the impact of CA on fruit firmness appeared after seven weeks of cool storage but reacted within two weeks. Thereby, the effect of CA on delaying kiwifruit softening may be affected by not only the CA exposure duration but also the total storage time after harvest, which also leads to the phase of kiwifruit softening. The storage duration is not the only factor that changes over time. The rapid softening phase occurs during the first 30 to 45 days (Arpaia *et al.*, 1984), after which the softening slows down. It is likely that kiwifruit does not respond to CA during the rapid softening phase, while the effect of CA on maintaining kiwifruit firmness takes place at the slow softening phase or establishing a different flat level where the slow softening phase settles. The mechanisms of kiwifruit responding to CA at different softening phases are yet unclear.

3.3.1.2 Ethylene effect on firmness in air

Fruit firmness was 3.3-3.6 kg_f at Wk 3 before the C_2H_4 treatment started (Figure 3.2). Differences in firmness started to emerge from Week 5, the first assessment after C_2H_4 treatment had begun. At Wk 5, the firmness of fruit stored in air with no additional C_2H_4 was 2.5 kg_f, followed by Air + 10 $nL \cdot L^{-1}$ C_2H_4 (2.0 kg_f), Air + 100 $nL \cdot L^{-1}$ C_2H_4 (1.6 kg_f), and Air + 1000 $nL \cdot L^{-1}$ C_2H_4 (1.5 kg_f). During C_2H_4 exposure in air, low firmness was associated with high C_2H_4 concentration and vice versa. All the air treatments followed a similar softening pattern during C_2H_4 exposure: rapid softening before Wk 5, softening slowed down between Wk5 and Wk 7 and entered a plateau at the end of the experiment. The fruit stored in air with 100 $nL \cdot L^{-1}$ and 1000 $nL \cdot L^{-1}$ C_2H_4 fell below 1 kg_f at Wk 7. Fruit exposed to10 $nL \cdot L^{-1}$ C_2H_4 did not reach the firmness of 1 kg_f until Wk 11, while those exposed to 0 $nL \cdot L^{-1}$ C_2H_4 remained above 1 kg_f till the end of the experiment. The difference between 100 and 1000 $nL \cdot L^{-1}$ was much smaller than that between the other treatments, indicating the response of kiwifruit firmness to C_2H_4 was close to saturated at 1000 $nL \cdot L^{-1}$. At the end of the experiment (Wk 13), fruit stored in air with no additional C_2H_4, compared to the other treatments in air, retained the highest firmness (1.5 kg_f), followed by Air + 10 $nL \cdot L^{-1}$ C_2H_4 (0.9 kg_f), Air + 100 $nL \cdot L^{-1}$ C_2H_4 (0.6 kg_f), and Air + 1000 $nL \cdot L^{-1}$ C_2H_4 (0.4 kg_f).It is suggested by this study that kiwifruit softening in cool storage under commercial conditions (0 °C, 95% RH) is C_2H_4 concentration dependent. Previous studies on 'Hayward' with the same C_2H_4 concentration gradient have illustrated similar results: as low as10 $nL \cdot L^{-1}$ C_2H_4 can induce kiwifruit softening in air; effect of C_2H_4 at the concentration of 100 $nL \cdot L^{-1}$ and 1000 $nL \cdot L^{-1}$ on kiwifruit softening were very similar; rapid softening occurred at the early stage of C_2H_4 exposure; fruit stored in 100

$nL \cdot L^{-1}$ and 1000 $nL \cdot L^{-1}$ C_2H_4 started to soften below 1 kg_f after 4 weeks of C_2H_4 treatment and fruit stored in air with no additional C_2H_4 stayed above 1 kg_f after 10 weeks of C_2H_4 treatment (Jabbar & East, 2016). The minimal concentration of C_2H_4 inducing kiwifruit softening (10 $nL \cdot L^{-1}$) has been well documented (Arpaia *et al.*, 1987; Mitchell, 1990). The existence of the saturation concentration for kiwifruit softening response to ethylene has also been investigated for kiwifruit recently. Hertog *et al.* (2016) has reported that 200 $\mu L \cdot L^{-1}$ C_2H_4 did not further accelerate kiwifruit softening compared to 10 $\mu L \cdot L^{-1}$, indicating that kiwifruit softening response to C_2H_4 was fully saturated below 10 $\mu L \cdot L^{-1}$ at 0 °C. The saturating concentration may be less than this as has been illustrated by both this study and previous work (Jabbar & East, 2016) where the effect of 1000 $nL \cdot L^{-1}$ C_2H_4 on inducing kiwifruit softening at 0 °C was very similar to that of 100 $nL \cdot L^{-1}$, suggesting that the saturation concentration may be within this range.

The three-week delay before C_2H_4 exposure provided more potential for capturing the difference between different C_2H_4 concentrations compared to the previously applied ten-week delay (Jabbar & East, 2016). Other research with the same C_2H_4 concentrations and three-week delay of C_2H_4 exposure resulted in 'Hort16A' experiencing more rapid softening during the initial cool storage (compared to 'Hayward' in the current study) resulting in a lower firmness when C_2H_4 treatment started, and hence providing less potential for the fruit to respond to various C_2H_4 levels (Pranamornkith *et al.*, 2012). The 'Hort16A' experiment also indicated that the effect of 1000 $nL \cdot L^{-1}$ C_2H_4 on fruit softening was substantially greater than 10 $nL \cdot L^{-1}$ and 100 $nL \cdot L^{-1}$, (Pranamornkith *et al.*, 2012) which differs from the current study, suggesting that C_2H_4 concentration impacts on kiwifruit softening vary between cultivars. Therefore, C_2H_4 effects on air-stored kiwifruit softening is dose-dependent, with a minimum level of 10 $nL \cdot L^{-1}$ and saturating concentration near 1000 $nL \cdot L^{-1}$; and the response of kiwifruit to C_2H_4 is very rapid but varies between cultivars.

3.3.1.3 Ethylene effect on firmness in controlled atmosphere

The firmness of CA-stored fruit was the same as air-stored fruit (3.3-3.6 kg_f) at Week 3 before C_2H_4 was introduced to the system. CA-stored fruit showed higher firmness compared to air-stored fruit from Week 5 to Week 13, regardless of C_2H_4 concentration. Fruit stored in CA + 0 $nL \cdot L^{-1}$ C_2H_4 and CA + 10 $nL \cdot L^{-1}$ C_2H_4 maintained relatively high firmness until the end of Week 13 (2.7-2.9 kg_f), and there was no significant difference

($p>0.05$) between the firmness of these two C_2H_4 concentrations during the 10-week C_2H_4 exposure. Fruit in CA + 100 $nL \cdot L^{-1}$ C_2H_4 retained the same firmness as fruit in the lower ethylene concentrations until Week 9 and accelerated fruit softening appeared in CA + 100 $nL \cdot L^{-1}$ C_2H_4 from Week 11. Enhanced fruit softening in CA + 1000 $nL \cdot L^{-1}$ C_2H_4 started earlier (from Week 9) compared to 100 $nL \cdot L^{-1}$ C_2H_4 in CA. At the end of the experiment, fruit firmness in CA + 100 $nL \cdot L^{-1}$ C_2H_4 (2.2 kg_f) and CA + 1000 $nL \cdot L^{-1}$ C_2H_4 (1.5 kg_f) did not fall below 1 kg_f, and firmness was higher than that in air treatments regardless of C_2H_4 concentration, with one exception: CA + 1000 $nL \cdot L^{-1}$ C_2H_4 reached the same firmness as air with no additional C_2H_4, i.e., 1.5 kg_f (Figure 3.2).

In comparison to air storage, the C_2H_4 effect on fruit firmness was altered by CA (Figure 3.2). Firstly, the lowest C_2H_4 concentration inducing softening was elevated from 10 $nL \cdot L^{-1}$ in air to a higher level between 10 and 100 $nL \cdot L^{-1}$ in CA, as 10 $nL \cdot L^{-1}$ in CA did not induce any further softening compared to no additional C_2H_4 in CA. Secondly, C_2H_4 accelerated softening was delayed from 2 weeks of C_2H_4 exposure in air to 6 weeks in CA + 1000 $nL \cdot L^{-1}$ C_2H_4 and 8 weeks in CA + 100 $nL \cdot L^{-1}$ C_2H_4. Thirdly, the severity of C_2H_4 induced softening was suppressed by CA. At the end of the 10-week C_2H_4 exposure (Week 13), fruit firmness reduction induced by 10, 100 and 1000 $nL \cdot L^{-1}$ C_2H_4 in air were 40%, 60% and 73% of 0 $nL \cdot L^{-1}$ C_2H_4 respectively, while that in CA were 0%, 24% and 48% respectively. Additionally, in absence of C_2H_4, the firmness of fruit stored in CA was 93% higher than that in air. Finally, the firmness of CA-stored fruit did not fall below 1 kg_f irrespective of C_2H_4 concentration during the experiment, while firmness of fruit exposed to 1000, 100 and 10 $nL \cdot L^{-1}$ C_2H_4 reached 1 kg_f after 4, 4 and 10 weeks respectively.

The negative effect of C_2H_4 in CA on kiwifruit firmness has been reported in previous studies. Arpaia *et al.* (1982) found that C_2H_4 at the concentration of 50 $nL \cdot L^{-1}$ enhanced kiwifruit softening in optimal CA (Arpaia *et al.*, 1982). In conjunction with the present study, the minimum C_2H_4 concentration to accelerate kiwifruit softening in CA may be between 10 $nL \cdot L^{-1}$ and 50 $nL \cdot L^{-1}$. Whereas C_2H_4 concentrations as high as 5000 $nL \cdot L^{-1}$ in CA are required to induce kiwifruit softening to the same level as C_2H_4-free air after 12 weeks storage (Arpaia *et al.*, 1986).

Apart from C_2H_4 concentration, exposure duration is another factor that impacts kiwifruit firmness in CA. Arpaia *et al.* (1986) found that the firmness of the kiwifruit exposed to

500 $nL \cdot L^{-1}$ C_2H_4 for 2 or 4 weeks was higher than that continuously exposed for 21 weeks, and the accelerated softening in 50-100 $nL \cdot L^{-1}$ and 500-5000 $nL \cdot L^{-1}$ C_2H_4 in CA was detectable after 16 weeks and 6 weeks respectively, while enhanced softening in CA started after 6 weeks and 8 weeks in C_2H_4 exposure at 1000 $nL \cdot L^{-1}$ and 100 $nL \cdot L^{-1}$ in the present study. These effects of concentration and exposure duration of C_2H_4 of kiwifruit softening indicate dose dependency. However, the difference of softening rate and absolute firmness between the present work and previous research (Zoffoli *et al.*, 2006) have suggested the initial firmness at the beginning of C_2H_4 exposure or other pre-harvest factors also impacts kiwifruit softening in CA.

3.3.1.4 Initial fruit condition effect on firmness

Fruit from the 3 different Grower Lines (GLs) behaved differently during storage (Figure 3.3). The softening rate of the 3 GLs differed during the initial 3 weeks. The at-harvest firmness of GL1, GL2 and GL3 were 5.1 kg_f, 6.8 kg_f and 5.7 kg_f, respectively, while those at Wk 3 were 4.2 kg_f, 3.7 kg_f and 2.3 kg_f respectively. The at-harvest firmness of GL1 was the lowest (5.1 kg_f), but the firmness of GL1 at Week 3 was the highest (4.2 kg_f), whereas the softening rate of GL3 was the most dramatic and firmness of GL3 was the lowest at Week 3 (2.3 kg_f). At the end of Week 13, the firmness of air-stored fruit were quite similar for each C_2H_4 concentration among the GLs, but that of CA-stored fruit differed among GLs. The firmness of fruit stored in CA with various levels of C_2H_4 from GL3 were lower than that of the other 2 GLs. This result indicates that the benefit of CA, irrelative of the C_2H_4 level, is affected by grower line differences.

To ensure consistent high fruit quality at the end of storage, a maturity assessment, such as non-destructive firmness or dry matter content determination, is recommended (Thompson, 2010b). However, apart from the at-harvest fruit condition, the variance of storage performance among GLs is also associated with multiple pre-harvest factors, such as soil nutrition, weather condition and orchard management (Gerasopoulos & Drogoudi, 2005). Thus, a model of 'softening rate prediction' based on pre-harvest and at-harvest data is helpful for segregating fruit for short-term and long-term storage. However, such a model has not yet been developed.

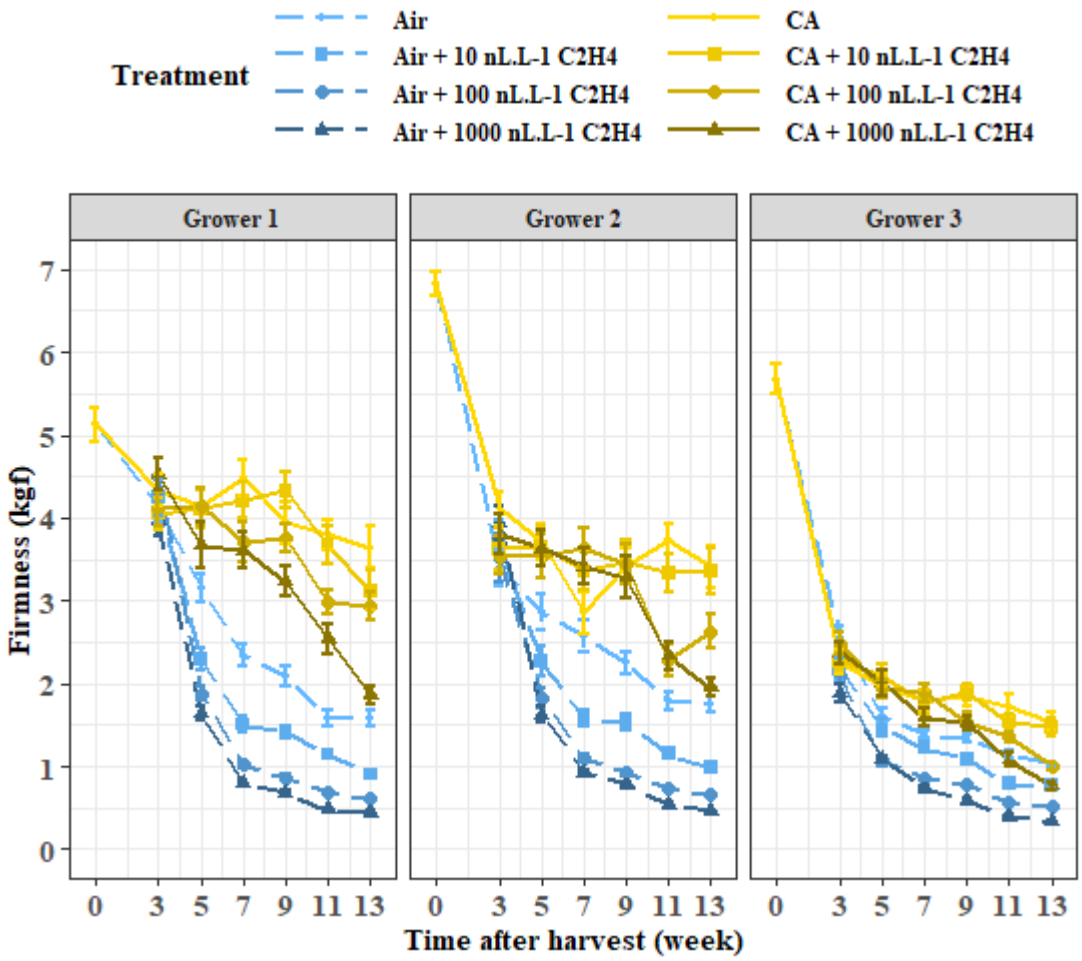

Figure 3.3 Firmness of 'Hayward' kiwifruit from 3 grower lines in response to optimal CA (2% O_2 + 5% CO_2) and C_2H_4 at concentrations of 0, 10, 100 and 1000 nL·L^{-1}, under 0 ºC 95% RH storage for 13 weeks. Each data point represents mean ± SEM, n = 30.

3.3.1.5 Softening rate

As destructive methods were employed in this study and different batches of fruit were examined at each assessment, the fruit softening rate could not be calculated simply by following the firmness change of each individual fruit. The "softening rate" was determined by population firmness changes between two sampling times. The highest softening rate for both air and CA occurred in the first 3 weeks of storage before C_2H_4 was introduced (Figure 3.4). During the C_2H_4 treatment, air-stored fruit experienced relatively rapid softening for the whole time. Whilst softening of CA-stored fruit was quite slow that no significant softening occurred between contiguous fruit sampling times, apart from significant ($p<0.01$) softening of fruit in CA + 100 nL·L^{-1} C_2H_4 between Week 9 and Week 11, and in CA + 1000 nL·L^{-1} C_2H_4 between Week 9 and Week 13.

It has been claimed that C_2H_4 can reduce the fruit-to-fruit difference of kiwifruit firmness (Lallu *et al.*, 1989). The data of air-stored fruit agreed with this statement. The range of fruit firmness in Air + 10, 100 and 1000 $nL \cdot L^{-1}$ C_2H_4 reduced over time, and much faster compared to that in air without additional C_2H_4. However, the range of population firmness in CA-stored fruit did not change except for CA + 1000 $nL \cdot L^{-1}$ C_2H_4 at the last 2 weeks that decreased slightly (Figure 3.4), suggesting that the fruit-to-fruit variation was not impacted by C_2H_4 in CA. On the other hand, the decreased firmness range has demonstrated the existence of a "bottom line": the potential for softening became very limited when the firmness of a kiwifruit population was near or below 1 kg_f; there seems to be an 'asymptote' for kiwifruit, i.e., the fruit doesn't soften below about 0.4 kg_f; and only the fruit with higher firmness can potentially soften. Thus, the C_2H_4 effect of reducing fruit-to-fruit variation may be caused by inducing more rapid softening of fruit with higher firmness compared to fruit with lower firmness.

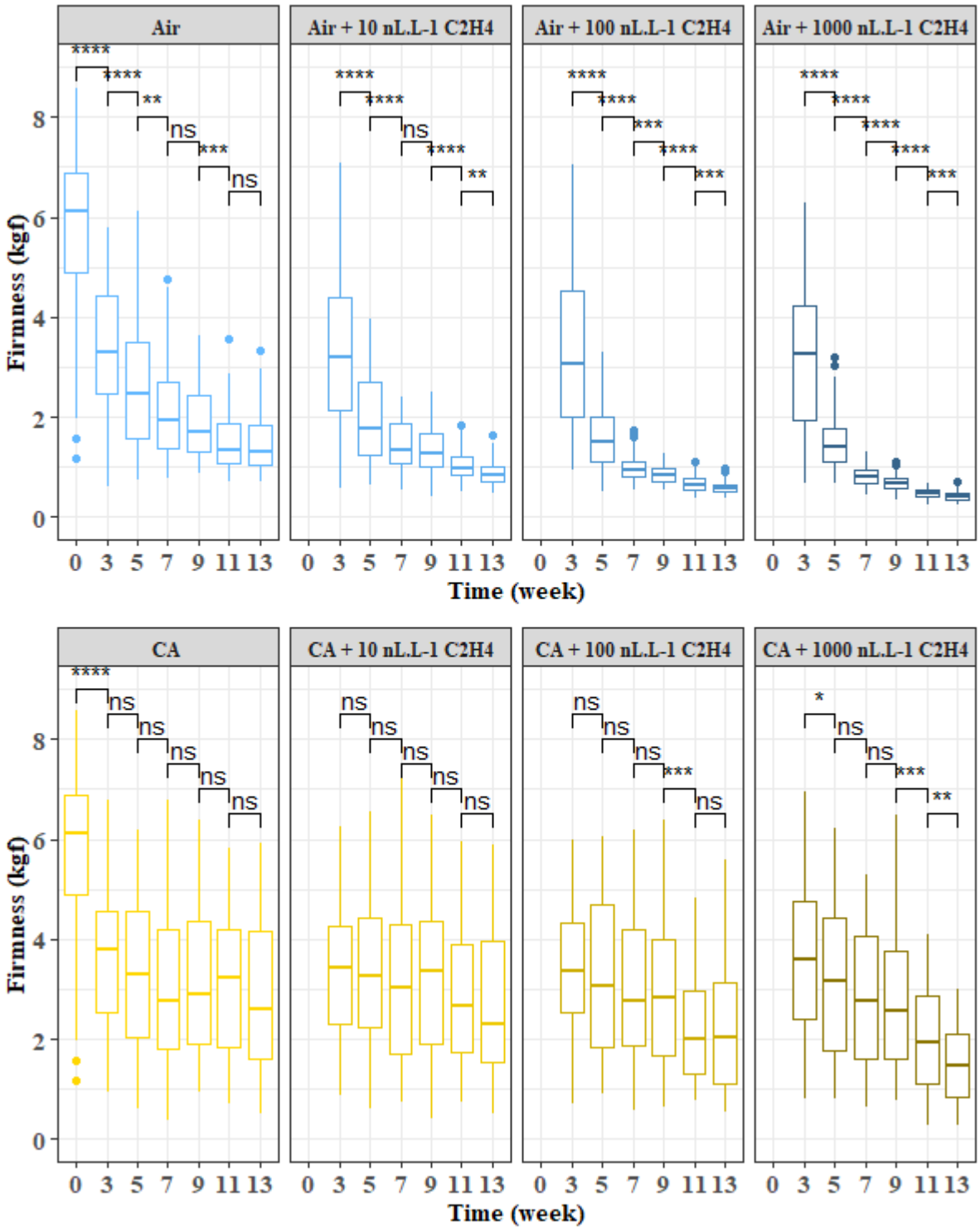

Figure 3.4 ANOVA of firmness changes over 13-week storage of 'Hayward' kiwifruit in responses to optimal CA (2% O_2 + 5% CO_2) and C_2H_4 at the concentration of 0, 10, 100 and 1000 nL·L^{-1}, under 0 ºC storage. Significant codes: '**' 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. n=90.**

3.3.2 Soluble solid content

3.3.2.1 Storage atmosphere effect on soluble solid content

The fruit mean soluble solid content (SSC) at harvest was 12.9%. After 3 weeks of storage, SSC of fruit in air increased to 13.0-13.4%, while that in CA remained at a low level (12.6-13.0%). The increase of SSC in air-stored fruit was rapid before Week 7 and slowed down in the following 6 weeks; while the SSC increase of CA-stored fruit was only rapid between Week 3 and Week 5, and relatively constant growth was maintained until Week 13. At the end of storage at Week 13, SSC of air-stored fruit was 15.3-15.7%, whereas that of CA-stored fruit was 14.8-15.1%. During the 13 weeks of storage, the SSC of fruit in CA were lower compared to that in air, irrespective of C_2H_4 concentration (Figure 3.5).

SSC is commonly used as a maturity index in the kiwifruit industry (Burdon *et al.*, 2016). Fruit SSC of 6.2% has been used as the minimum requirement, as kiwifruit with at-harvest SSC below 6.2% generally have poor performance in extended storage (Mitchell *et al.*, 1991). In the present study, 12.9% SSC indicated relatively high maturity at harvest.

On the other hand, SSC also impacts consumer acceptance (Rossiter *et al.*, 2000). Fruit with higher SSC are preferred by the consumer, and ripe 'Hayward' kiwifruit with ≥ 14% SSC are regarded as acceptable (Harker *et al.*, 2009). Even though CA stored fruit did not fully ripen by Week 13, SSC at Week 13 was above 14% for both air and CA stored fruit. But longer storage time is required to determine the SSC of CA stored fruit at the eating stage for this experiment.

SSC of kiwifruit normally increases during the early stage of storage (Burdon *et al.*, 2014a; Burdon *et al.*, 2016; Lin *et al.*, 2020), but a reduction of SSC at the end of storage has been observed (Pranamornkith *et al.*, 2012; Narae *et al.*, 2019). Meanwhile, as the increase of SSC is because of starch conversion to soluble sugar, the final SSC is generally defined by at-harvest DM (Crisosto *et al.*, 2012). Lower SSC has been reported in CA-stored kiwifruit compared to air-stored fruit, however, the lower SSC was also accompanied by higher firmness (Cornacchia *et al.*, 2008; Xia *et al.*, 2016). It is suggested that the CA effect on SSC evolution is delaying the increase of SSC by delaying fruit ripening, rather than reducing SSC at eating ripeness.

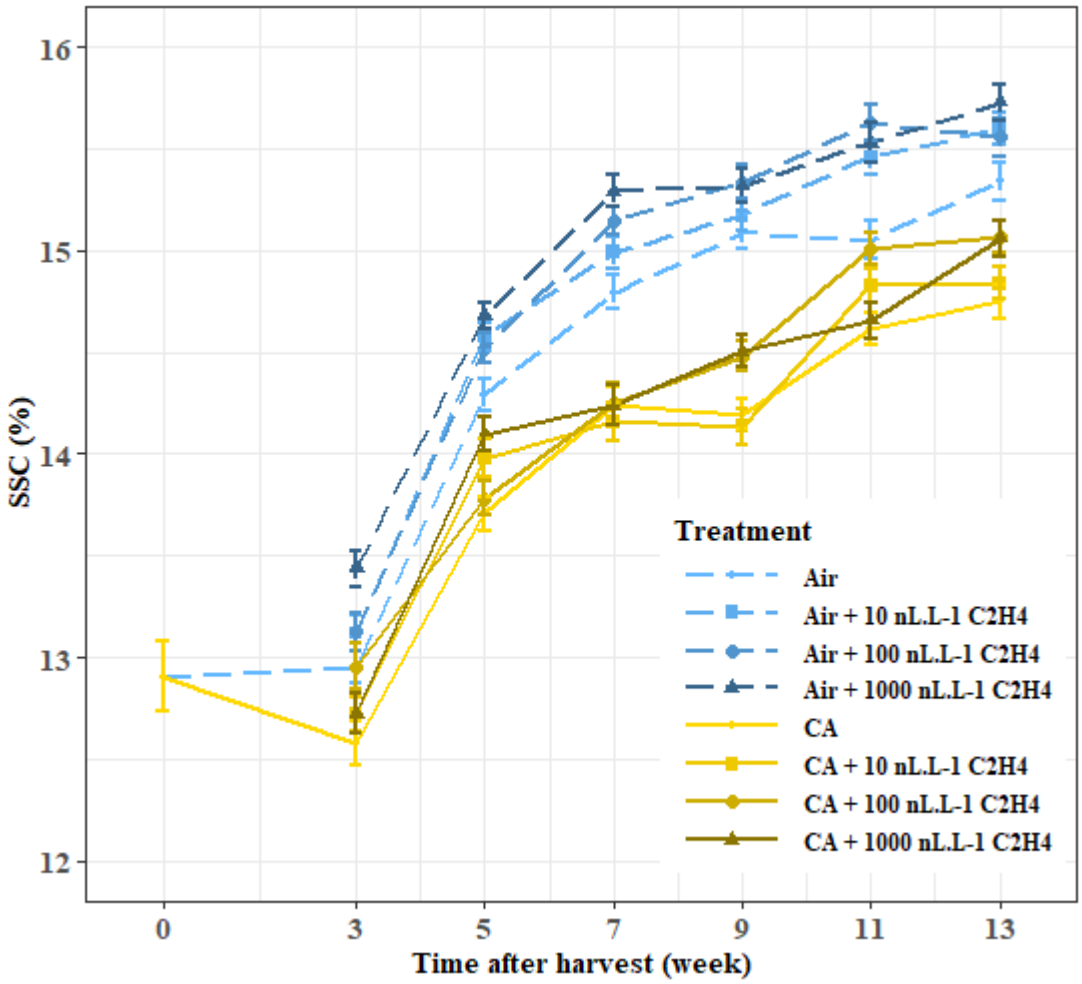

Figure 3.5 Soluble solid content (SSC) changes over 13-week storage of 'Hayward' kiwifruit in responses to optimal CA (2% O_2 + 5% CO_2) and C_2H_4 at the concentration of 0, 10, 100 and 1000 nL·L^{-1}, under 0 ºC 95% RH storage. Each data point represents mean ± SEM, n = 90.

3.3.2.2 Ethylene effect on soluble solid content

In air stored fruit, fruit SSC was increased by C_2H_4, and the impact was concentration dependent. SSC of fruit exposed to air with C_2H_4 was higher than that in ethylene-free air. SSC of fruit in Air + 10 nL·L^{-1} C_2H_4 was lower than that in Air + 100 and 1000 nL·L^{-1} C_2H_4. There was no significant difference ($p>0.05$) between SSC of air stored fruit exposed to 100 nL·L^{-1} C_2H_4 and that in 1000 nL·L^{-1} C_2H_4. Conversely, SSC of CA stored fruit was not affected by C_2H_4 exposure (Figure 3.5).

The effect of C_2H_4 on SSC has been observed during kiwifruit storage previously (Ritenour *et al.*, 1999; Kim *et al.*, 2009). Increased SSC was associated with lower firmness, indicating C_2H_4 impact on kiwifruit SSC is due to accelerating fruit ripening, instead of solely increasing SSC level (Ritenour *et al.*, 1999; Kim *et al.*, 2009).

Concentration dependency has not been observed in a previous study. C_2H_4 at the concentration of 1000 $\mu L \cdot L^{-1}$ did not further enhance kiwifruit SSC increase compared to 100 $\mu L \cdot L^{-1}$ (Kim *et al.*, 2009). The saturating level of C_2H_4 has been narrowed to below 100 $nL \cdot L^{-1}$ by the current study. On the other hand, the C_2H_4 impact on SSC in CA storage has not been reported previously.

3.3.2.3 Initial fruit condition effect on SSC

The at-harvest SSC of the fruit of GL1 (14.1%) was significantly higher than that of GL2 (12.1%) and GL3 (12.6%), while fruit SSC of GL1 became the lowest at Wk 3, and fruit SSC of GL3 maintained the highest from Wk 3 to Wk 13 (Figure 3.6). According to the firmness evolution, the firmness of GL1 experienced the lowest softening rate among the three grower lines during the initial three weeks (Figure 3.2). The SSC reduction at Wk 3 suggests this unexpected high SSC of GL1 at week 0 might be caused by sampling error.

It is suggested that mature fruit can develop higher SSC after storage compared to immature fruit (Burdon, 2015; Narae *et al.*, 2019). Thus, the fruit of GL3 was potentially more mature compared to fruit from GL1 and GL2. However, the lower at-harvest DM also suggested that GL3 was less mature compared to GL1 and GL2 (Table 3.1). Since SSC increase during the post-harvest stage is caused by starch degradation, the high SSC of GL3 with low DM suggested a relatively large proportion of starch had already converted to soluble sugar at Wk 0. The conflict of maturity estimations is probably due to fruit from GL3 was less mature but at a more advanced ripening stage.

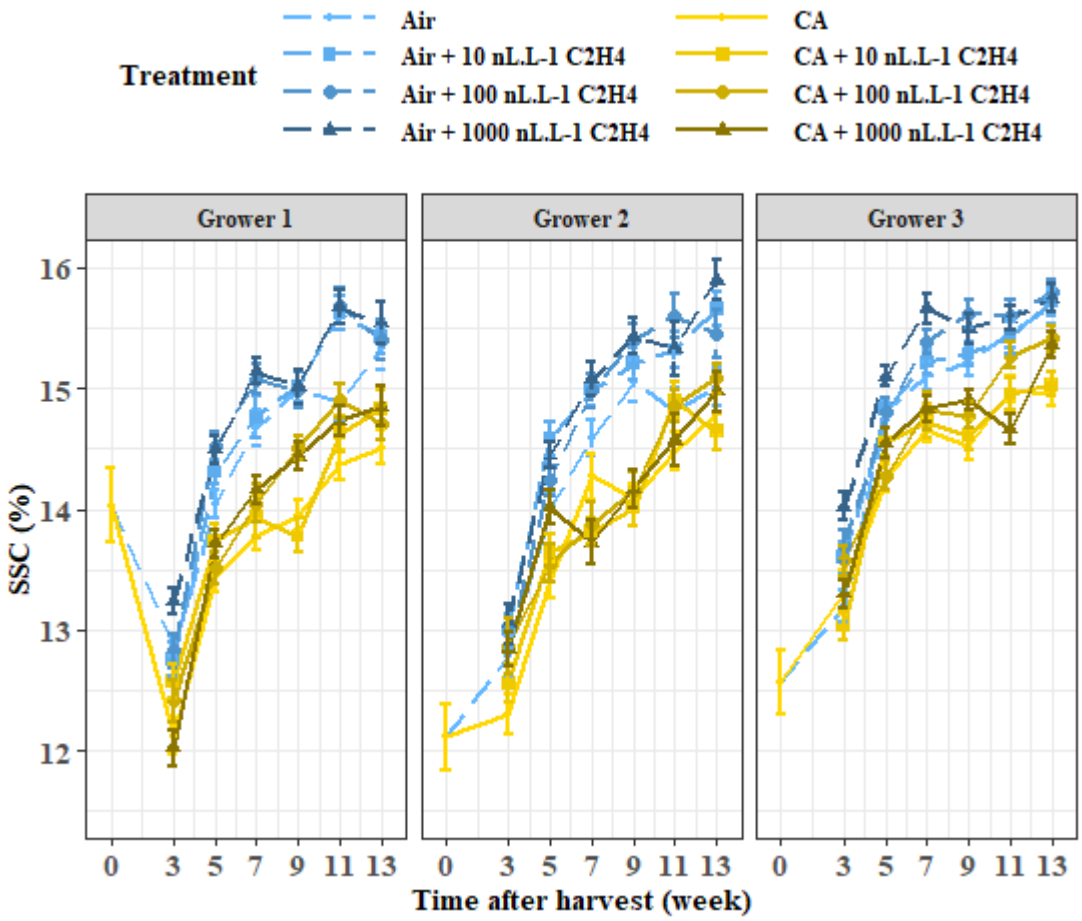

Figure 3.6 SSC of 'Hayward' kiwifruit from 3 grower lines responding to optimal CA (2% O_2 + 5% CO_2) and C_2H_4, at 0 ºC for 13 weeks. Each data points represents mean ± SEM, n = 30.

3.3.2.4 SSC-firmness correlation

It was difficult to analyze the different effects of storage conditions, as SSC evolution during storage was relatively subtle. However, the correlation between SSC and firmness revealed more details than SSC alone. Firmness and SSC were negatively correlated in the present study (Figure 3.7). SSC-firmness correlation of fruit in CA storage and fruit in ethylene-free air were linear, while that of fruit stored in air with additional C_2H_4 were non-linear: i.e., there was a greater increase in SSC during the latter phases of fruit softening in air than in CA. For the fruit in air + C_2H_4 + 100 and 1000 $nL \cdot L^{-1}$ C_2H_4, rapid SSC increase occurred when firmness was between 0.5 kg_f and 2 kg_f; while the rapid increase of SSC in the fruit stored in Air + 10 $nL \cdot L^{-1}$ C_2H_4 occurred when firmness was between 1 kg_f and 5 kg_f. The SSC level of fruit in CA and ethylene-free air was higher than fruit in air with additional C_2H_4 when firmness was above 1.5 kg_f, except for Air +

10 $nL \cdot L^{-1}$ C_2H_4 above 2.5 kg_f. It is suggested that C_2H_4 had a greater impact on the softening of kiwifruit stored in air compared to that on SSC increase, and the effect of CA on retaining kiwifruit firmness was greater than that on delaying SSC development.

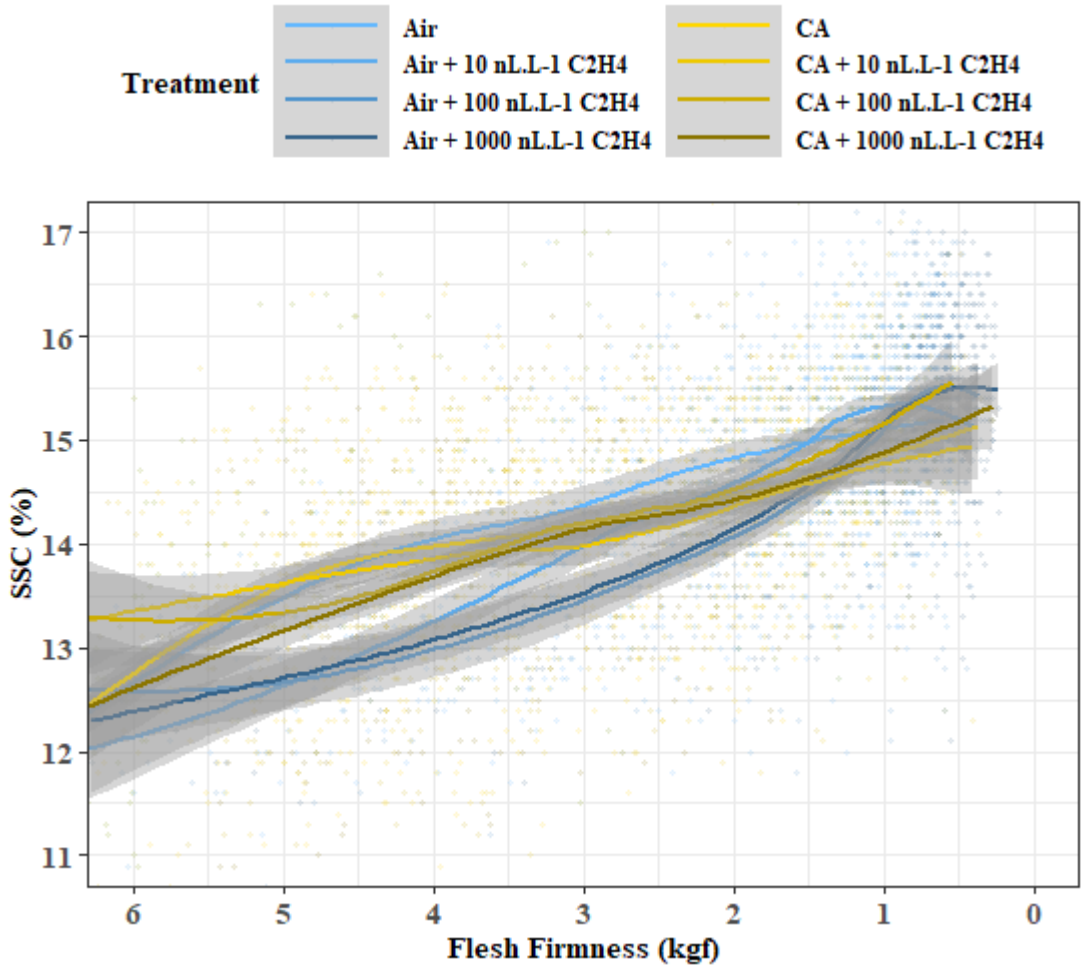

Figure 3.7 Correlation between firmness and soluble solid content (SSC) of 'Hayward' kiwifruit stored in air and CA with 0, 10, 100 and 1000 $nL \cdot L^{-1}$ C_2H_4 at 0 ºC 95% RH for 13 weeks. The shade shows SEM.

SSC increase during postharvest storage of kiwifruit is due to starch conversion to sugar (MacRae *et al.*, 1992). The activity of starch degradation enzymes, such as α-amylase, are responsible for SSC evolution (Wegrzyn & MacRae, 1995). Whereas softening during kiwifruit ripening is mainly caused by cell wall changes (Brett & Waldron, 1990), which involves the activity of pectin degradation enzymes, such as polygalacturonase (PG) and pectinesterase (PE) (Soda *et al.*, 1986). The desynchrony of fruit softening and SSC increase suggested that cell wall metabolism is more sensitive to ethylene compared to starch degradation, while the impact of CA is greater on carbohydrate evolution than that on cell wall modification.

3.3.3 Disorders

3.3.3.1 Chilling injury

The symptom of chilling injury (CI) on kiwifruit is also termed low-temperature breakdown (LTB) or storage breakdown disorder (SBD) (Jabbar & East, 2016). The symptoms of CI normally start from graininess in the outer pericarp and develop into tissue water-soaking (Burdon & Lallu, 2011). CI is caused by low temperature-induced membrane lipid peroxidation (Sheng *et al.*, 2016). Increased permeability leads to cell compartmentalisation loss and ion leakage (Saltveit, 2002). The tolerance to low temperature is influenced by antioxidant enzyme activity and antioxidants, as a part of the defence system (Yang *et al.*, 2013).

Figure 3.8 The graininess symptom of chilling injury (CI) on 'Hayward' kiwifruit.

In the present study, noticeable CI symptoms were only observed on 1 fruit, with graininess appearing in the flesh tissue close to the skin (Figure 3.8). However, severe CI was observed in the spare fruit from the same batch stored at 0 °C in air for a further 2-month time (data not shown). This is probably because the current storage duration (13 weeks) at low temperature was too short to induce CI. Storage temperature and duration play a key role in inducing CI (Burdon *et al.*, 2014b; Gwanpua *et al.*, 2018). Lallu (1997)

found that increasing the storage temperature from -0.5 ºC to 1 ºC reduced the incidence of CI by over 31%. Gerasopoulos andDrogoudi (2005) reported that CI incidence in 'Hayward' kiwifruit stored at -0.5 ºC for 16 weeks was 13%, while the incidence of CI increased to 27% after 24 weeks of storage. More recently, the work of Gwanpua *et al.* (2018) has suggested that CI of kiwifruit occurs after 120 days of storage at 0 °C, and the incidence increases with the time in coolstorage. In the present study, 13-week storage at 0 ºC was much shorter than the durations of cool storage in those earlier studies, thus severe CI was not observed during the experiment but detected in the extended storage time.

The effect of C_2H_4 and CA on CI was not observed within this time frame of the current study. However, the effect of C_2H_4 on inducing CI has been detected in previous research as early as 6 weeks in 0 ºC storage, and the CI incidence in 1000 $nL \cdot L^{-1}$ C_2H_4 was markedly higher than 100 and 10 $nL \cdot L^{-1}$ C_2H_4 (Jabbar & East, 2016). As CI is peroxidation damage (Sheng *et al.*, 2016), reducing O_2 concentration can suppress CI (Burdon *et al.*, 2008).

The induction of CI is largely related to the cooling process. Kiwifruit is more susceptible to CI when it is cooled rapidly (Lallu & Webb, 1997). It has been demonstrated that rapid cooling to 0 °C accelerates 'Hayward' softening during the coolstorage, while gradual cooling to the same temperature can reduce the softening rate (Zhao, 2017). The fruit used in this work was transferred by a non-refrigerated truck at approximately 10°C for approximately 6 h, after which the fruit were cooled to 0°C from 10°C in 24 hours. This cooling process was relatively slow and similar to step-down cooling (Lallu, 1997), which potentially reduces CI.

The incidence of CI is also related to fruit maturity. Mature fruit are generally less prone to CI than less mature fruit (Sfakiotakis *et al.*, 2005; Zhao, 2017). Research on 'Tomua' kiwifruit revealed an 80% CI on the fruit with at-harvest SSC of 6%, while the incidence of CI on the fruit with 12% SSC in the same experiment was below 5% (Burdon *et al.*, 2007a). In this study, the fruit was harvested with SSC > 12%, much higher than the minimum requirement in the industry for kiwifruit harvesting (> 6.2%). The high SSC of fruit material in this study may be another key reason of low CI incidence.

3.3.3.2 White-core inclusions

White-core inclusions (WCI) is a disorder of white patches appearing in the core tissue of kiwifruit (Arpaia *et al.*, 1982). WCI was observed in the present study (Figure 3.9). Iodine-starch test was performed to indicate starch distribution with a few drops of iodide-potassium iodide solution (0.5% iodine and 2% potassium iodide dissolved in water) being applied to the cut surface, and the colour change being assessed in a few minutes. It has been revealed by the iodine-starch test that the white patches are groups of cells packed with clusters of starch grains, as the patches are too large to be starch grains in a single cell (Figure 3.9-C). The symptom of WCI first started from the outer regions of core tissue, then developed to the whole core area (Figure 3.9-D, E). The distribution of WCI symptoms did not differ along the fruit longitude in the current observation. There was no such symptom in the flesh tissue.

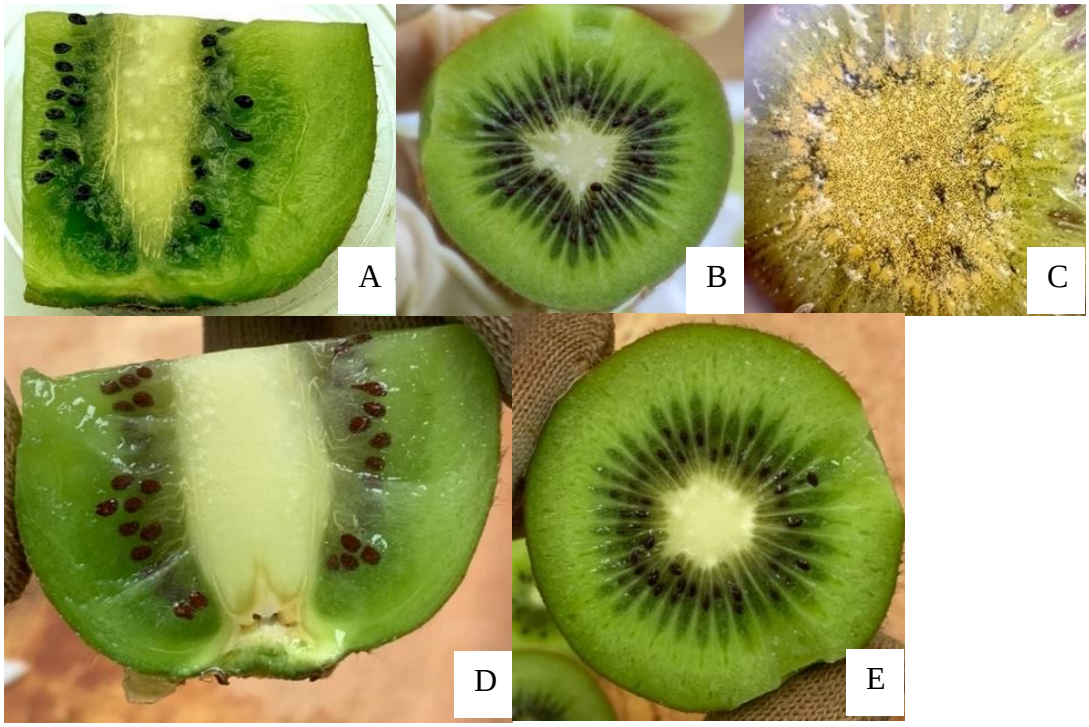

Figure 3.9 Symptom of white-core inclusions (WCI) in 'Hayward' kiwifruit. A: late stage of WCI (longitudinal section); B: late stage of WCI (transection); C: WCI dyed with potassium iodide solution; D: the early stage of WCI (longitudinal section); E: the early stage of WCI (transection).

The presence of WCI was observed in CA stored fruit with 100 and 1000 $nL \cdot L^{-1}$ C_2H_4 at Wk 11 and 13 (Figure 3.10), while there was no WCI at lower ethylene concentrations or within the first 9 weeks of storage. No WCI was observed in fruit stored in air. WCI incidence in CA + 1000 $nL \cdot L^{-1}$ C_2H_4 was higher than that in CA +100 $nL \cdot L^{-1}$ C_2H_4. WCI

incidence increased in both 100 and 1000 nL·L^{-1} C_2H_4 from Week 11 to Week 13. The incidence of WCI was lower in GL3 compared to GL1 and GL2.

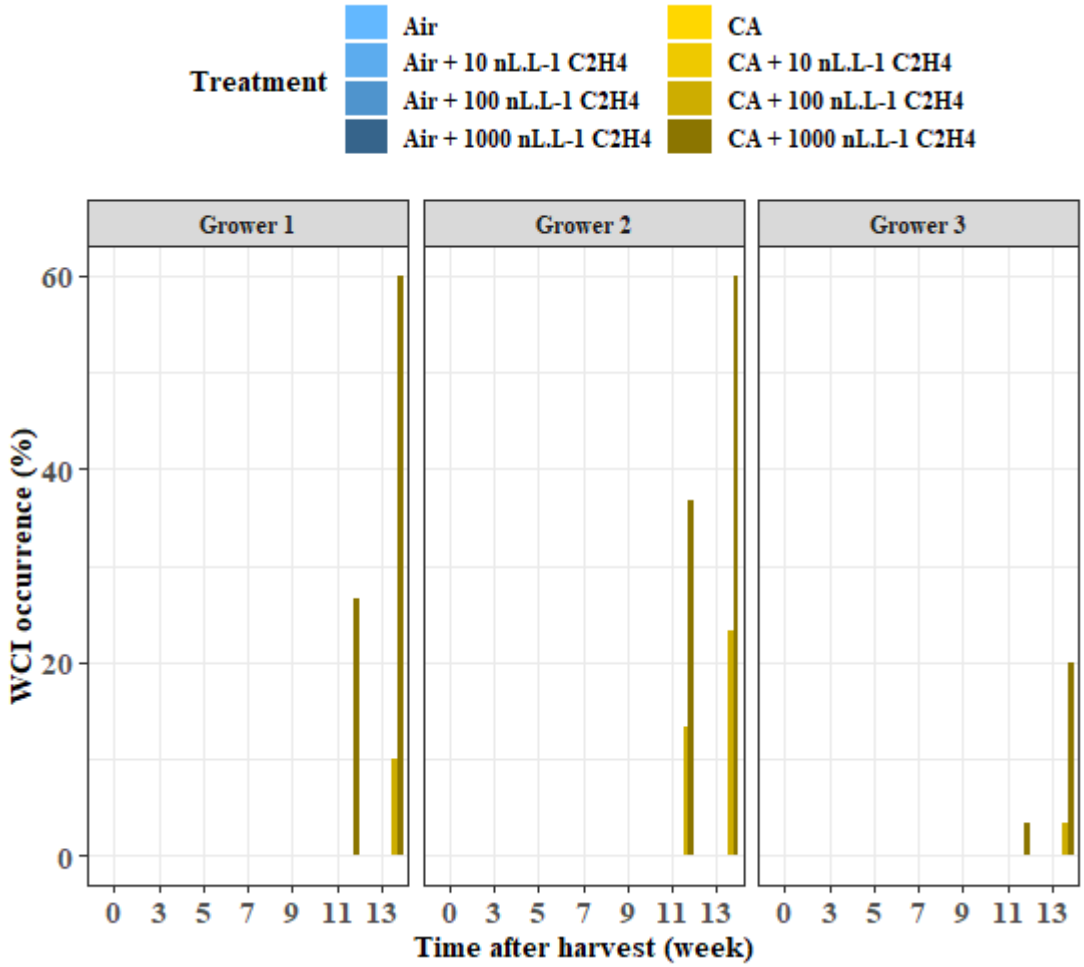

Figure 3.10 Occurrence of white core inclusions (WCI) in 'Hayward' kiwifruit of 3 grower lines stored in air and optimal CA (2% O_2 + 5% CO_2) and C_2H_4 at the concentration of 0, 10, 100 and 1000 nL·L^{-1}, under 0 °C 95% RD storage for 13 weeks after harvest. N=30.

It is suggested WCI is caused by starch degradation interruption during kiwifruit ripening, which is the result of C_2H_4 being present in CA storage with elevated CO_2 (Arpaia *et al.*, 1986). Crisosto (1997) previously observed WCI during the storage of kiwifruit under in 3-7% CO_2 with 50 nL·L^{-1} C_2H_4 within 3 weeks of storage, while WCI was not observed in the CA storage of 4-14% CO_2 with C_2H_4 < 30 nL·L^{-1} up to 24 weeks (Harman & McDonald, 1989b). In the current study, WCI was observed in 5% CO_2 with 100 nL·L^{-1} and 1000 nL·L^{-1} C_2H_4 after 8 weeks of C_2H_4 exposure. It would seem that occurrence of WCI requires the interaction of both relatively high C_2H_4 and elevated CO_2.

Arpaia *et al*., (1986) found that storage temperature affects WCI incidence. WCI occurrence and severity was significantly lower at 5 and 10 °C compared to 0 and 2.5 °C under the same CO_2 level and C_2H_4 concentration. Arpaia *et al*., (1986) also suggested that WCI can develop at any stage of kiwifruit storage, and fruit cannot recover from WCI in the following cool storage or shelf life once it has developed. However, the WCI level was lower in a 10-week delay of C_2H_4 exposure in CA compared to that in C_2H_4 presenting at the beginning of CA storage (Arpaia *et al.*, 1986). In the current research, the WCI incidence of GL3 fruit was lower than that of GL1 and GL2 (Figure 3.10). Given that the at-harvest DM of GL3 was lower than that of GL1 and GL2 (Table 3.1), and that the SSC of GL3 was higher than GL1 and GL2 at Wk3 when ethylene was introduced to the system (Figure 3.6), it is indicated that the starch retention in GL3 was the lowest among the three GLs during ethylene treatment. Hence, it is suggested that the starch content at the beginning of C_2H_4-CO_2 exposure influences WCI occurrence. Kiwifruit with higher starch content are more susceptible to WCI compared to that with less starch when exposed to C_2H_4 in CA. This is probably because that the WCI symptoms are retention of starch in patches, not the synthesis of new starch in those patches so that WCI can only happen when there is sufficient starch in the first place. However, quantitative data of starch content is needed to confirm this hypothesis.

Since WCI is caused by starch degradation interruption, this disorder potentially impacts SSC. However, the SSC of the fruit with WCI symptoms did not show any difference from the rest of the fruit in this study. This is probably because WCI symptoms only affect the core tissue, while SSC measurement was based on the juice from the outer pericarp. Thereby, it also indicates that the starch breakdown in the pericarp tissue was not negatively impacted by the C_2H_4-CO_2. However, the reason for this tissue variation remains unclear.

In the present study, WCI was quantified by occurrence, without indication of severity. Even though the occurrence of WCI was as high as 60% at Wk 13, some of the WCI symptoms were quite mild and barely noticeable. In the previous study, a scoring system of 0-3 was applied to evaluate the WCI severity of none, slight, moderate and severe (Arpaia *et al.*, 1986). However, this scoring system is quite subjective and no score chart has been published. The person-to-person variance may impact the severity scoring result. Thus, an objective method of evaluating WCI severity needs to be developed in the future, should this become an industrially important issue.

WCI is not frequently reported in the kiwifruit industry, nor in peer-reviewed scientific literature, indicating a low chance of kiwifruit being exposed to the high CO_2 high C_2H_4 conditions that seem to be required to induce WCI. However, the situation may change in the future, as such a micro-environment may be created for the storage of new cultivars. And it raises the caution for future research that WCI should be taken into consideration, in terms of method of evaluation, physiological mechanism, and actions to prevent or reduce the occurrence.

3.3.3.3 Scuffing

Scuffing is kiwifruit skin damage generally caused by poor fruit handlings, such as fruit-to-fruit or fruit-to-container contact during picking, transferring, or grading (Burdon & Lallu, 2011). Scuffing was detected in this study with the symptom of brown patches on the fruit skin (Figure 3.11). Brown patches were of various sizes and shapes. There was no obvious pattern of scuffing distribution on the fruit surface. Scuffing was seen at various locations on the fruit. One or more patches were observed on the scuffed fruit. The severity was not recorded in this study as there was no established index to evaluate the severity of kiwifruit scuffing. Instead, the incidence was recorded to quantify scuffing in this study. However, scuffing in this work is likely to be more severe than that in industrial practice, as the fruit were packed into mesh bags with rough surfaces, and there was more movement and rubbing during fruit handling in this experimental system.

Figure 3.11 'Hayward' kiwifruit with (left) and without (right) scuffing symptom.

Scuffing incidence was discovered mainly on the air stored fruit but was rarely observed in CA stored fruit. The scuffing symptom was not observed in the first 7 weeks of storage. The first occurrence was in air stored fruit with 100 and 1000 $nL \cdot L^{-1}$ C_2H_4 at Wk 9. In the

following two assessments, scuffing was also found in air + 0 $nL \cdot L^{-1}$ C_2H_4, and the occurrence rate increased compared to Wk 9. The occurrence at Wk 11 and Wk 13 were quite similar, between 20% and 30%. And there was no significant difference in the incidence among C_2H_4 levels (Figure 3.12).

In the scuffed area, layers of cells near the fruit surface are dead and compressed from the physical injury, however, the appearance does not change immediately (Hallett & Sutherland, 2005). It normally takes a few days for scuffing symptoms of 'Hort16A' to become obvious after skin damage (Burdon & Lallu, 2011). For the current study on 'Hayward' the scuffing appearance was only occurred after much longer (9 weeks in coolstore). According to the experiment operation, there was no physical movement on the fruit after loading to the barrels. The scuffing assessment was processed right after fruit being removed from the barrels so that the observed scuffing must have been the result of pre-storage handling (at harvest and/or during transit). Whereas the impact of post-storage handling on scuffing should be assessed after a few days at 20 °C. The physical damage to skin cells may have occurred before storage. Therefore, the 9-week delay of scuffing appearance was not due to handling the fruit, but a delayed physiological reaction.

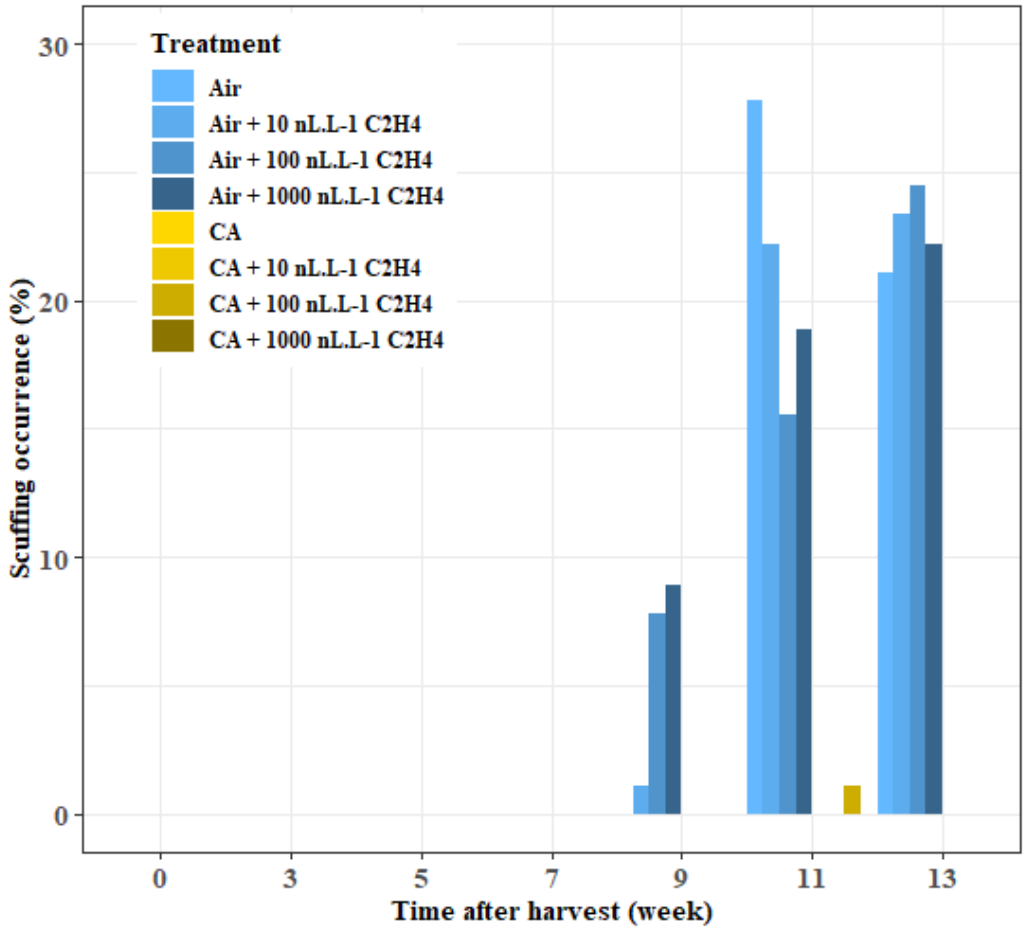

Figure 3.12 Scuffing occurrence in 'Hayward' kiwifruit stored in air and optimal CA (2% O_2 + 5% CO_2) and C_2H_4 at the concentration of 0, 10, 100 and 1000 nL·L^{-1}, under 0 ºC storage for 13 weeks after harvest. N = 90.

Scuffing symptoms are thought to be generated by phenolic compounds released from damaged cells and enzymatically oxidized to produce quinones (Bai *et al.*, 2006). Under optimal CA (5 % CO_2 + 2 % O_2), reduced O_2 suppresses the oxidation, thus reduces skin surface browning. However, it is unknown whether this effect can be extended after removal from CA. There exists the possibility that the oxidation process restarts when the fruit is exposed to 21 % O_2 in the atmosphere, as the cell damage already occurred before storage. Further study on scuffing is required to investigate the shelf-life behaviour following CA storage.

The skin structure of 'Hayward' (*A. deliciosa*) is thick and complex, while the skin of some other kiwifruit species, such as *A. chinensis* and *A. arguta*, is thinner and more fragile (Hallett & Sutherland, 2005). This structural difference potentially determines

fruit sensitivity to scuffing. Scuffing may be more severe on the kiwifruit cultivars with hairless, thinner skin, such as ‘Gold3’ and ‘Red19’. At this point, reducing scuffing by CA storage may be more beneficial to these cultivars than to ‘Hayward’.

3.3.3.4 Core firmness

The symptom of hard core disorder can be described as the firmness of core tissue remaining above 3 kg_f when the flesh firmness is lower than 1 kg_f, resulting in unpleasant texture when consumer bites or scoop into the fruit (Jeffery *et al.*, 2012). It has been observed in kiwifruit stored under CA with the CO_2 concentration of 8% or higher (Harman & McDonald, 1989a), as well as kiwifruit treated with 1-MCP (Zoffoli *et al.*, 2016). Hard core occurs because the firmness evolution of core tissue follows a different pattern from that of the outer pericarp (flesh) during storage (Li *et al.*, 2017), and the difference becomes noticeable when kiwifruit is stored under undesirable conditions (Gwanpua *et al.*, 2019).

In the current study, core firmness was assessed in Wk 13 along with flesh firmness to determine the occurrence of hard core. A linear model has been generated between the core firmness and flesh firmness of each treatment (Figure 3.13). Most of the fruit did not have the issue of HC. Only 5 data points out of a total of 720 fruit fell into this field (flesh firmness < 1 kg_f and core firmness > 3 kg_f). Two (2) of these 5 fruit were stored under CA + 0 $nL \cdot L^{-1}$ C_2H_4 and the other 3 fruit were stored under CA + 10 $nL \cdot L^{-1}$ C_2H_4. The core firmness of the rest of the fruit fell in the same area.

The current data has also indicated that the C_2H_4 effect on core firmness differed from flesh tissue. At the same flesh firmness, higher ethylene levels resulted in lower core firmness in CA. However, this relationship was not observed in air stored fruit, as most of the fruit treated with high ethylene were already very soft at Wk13. However, It has been reported in a previous study that ethylene has a greater effect on core tissue softening than on flesh softening in air storage of kiwifruit (Zoffoli *et al.*, 2016). Thus, it is indicated that core tissue is more sensitive to C_2H_4 induced softening compared to outer pericarp in both air and CA.

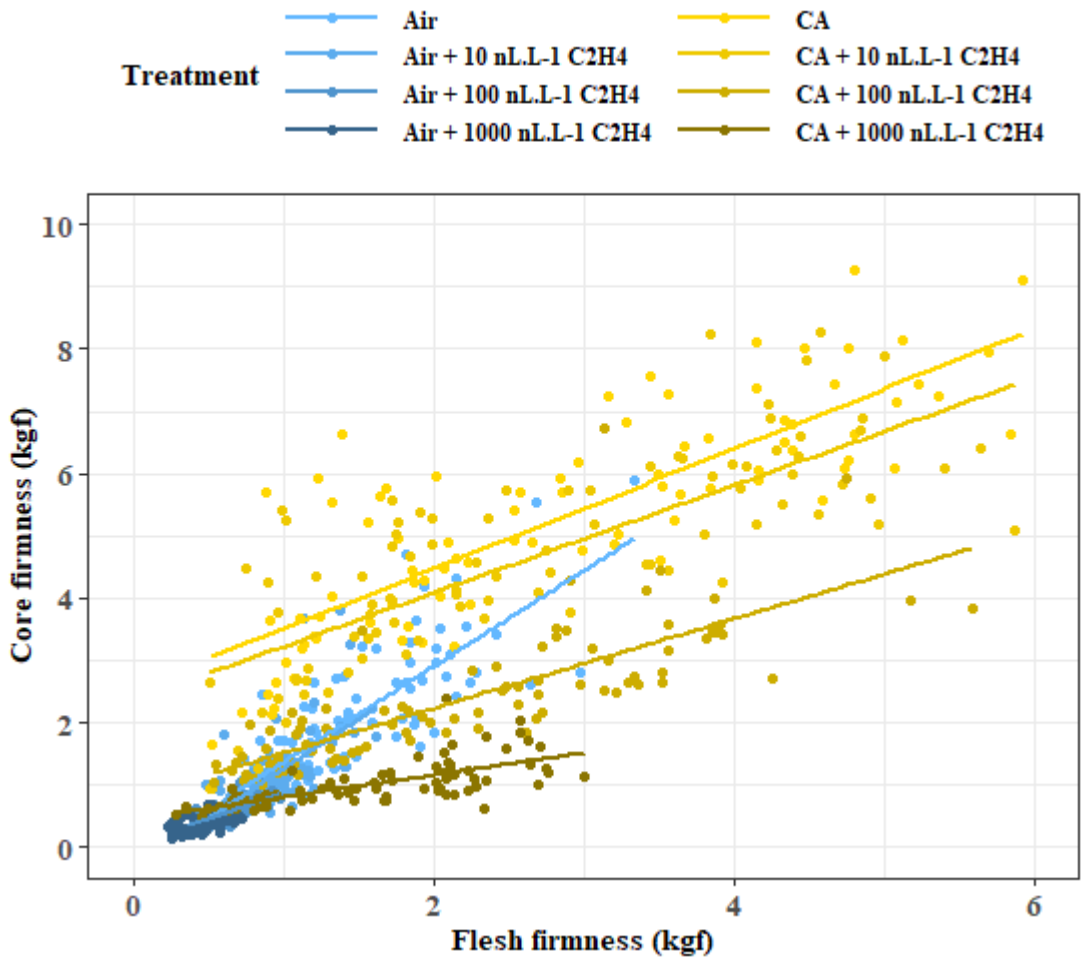

Figure 3.13 Correlation between core firmness and flesh firmness of 'Hayward' kiwifruit after 13 weeks of storage in air and optimal CA (2% O_2 + 5% CO_2) and C_2H_4 at the concentration of 0, 10, 100 and 1000 nL·L^{-1} at 0 ºC.

It has been reported that core firmness behaves differently under different temperatures. In the 20 ºC shelf life following 0 ºC cool store, core firmness declines rapidly (Jeffery *et al.*, 2012). However, in the current study, core firmness was assessed within 24 h after fruit removal from coolstore, while shelf-life was not included. A further softening could possibly occur afterwards. It seems extremely unlikely to be a problem, but it is suggested that a 5 to 10 days shelf-life should be included in future studies to determine the effect of CA with ethylene on kiwifruit core firmness and the occurrence of hard core.

3.4 Conclusion

Responses of 'Hayward' kiwifruit to C_2H_4 at the concentrations of 0, 10, 100 and 1000 nL·L^{-1} have been examined in both air and optimal CA (2% O_2 + 5% C O_2) during storage of 13 weeks under 0 °C 95% RH.

- CA retained higher firmness of kiwifruit than air in extended storage but did not affect softening in the initial three weeks of storage. C_2H_4 can induce fruit softening in air storage, and the C_2H_4 effect on firmness was dose-dependent, with the response saturated between 100 and 1000 $nL \cdot L^{-1}$. CA can reduce kiwifruit response to C_2H_4 by increasing the minimum C_2H_4 concentration that accelerates softening, delaying C_2H_4 triggered softening, and reducing the severity of C_2H_4 induced softening. However, the benefit of CA is determined by the initial fruit firmness before C_2H_4 exposure. CA can also slow down the softening rate of fruit exposed to C_2H_4.
- CA can delay kiwifruit SSC development and offset the C_2H_4 effect on accelerating SSC increase, but preharvest factors such as maturity can impact the effect of CA on SSC. Cell wall degradation and starch breakdown were impacted by CA and C_2H_4 differently during kiwifruit ripening.
- Chilling injury was not induced up to 13 weeks of storage.
- White core inclusions disorder can be induced by 100 $nL \cdot L^{-1}$ or higher concentration of C_2H_4 in CA after 11 weeks of storage. WCI incidence is dose-dependent, and also impacted by maturity.
- Visible scuffing can be inhibited by CA.
- Hard core disorder was not observed in this study.

A low level of C_2H_4 ($\leq$ 10 $nL \cdot L^{-1}$) present in CA storage cannot alter kiwifruit quality, however, excessive softening and WCI disorder may negatively impact kiwifruit quality when exposed to a high concentration of C_2H_4 in CA for extended storage time. Meanwhile, storage time longer than 13 weeks and the following shelf life are required in the future to determine the performance of kiwifruit in CA with C_2H_4 at an advanced ripening stage.

Chapter 4. Responses of 'Hayward' kiwifruit to modified atmosphere packaging

4.1 Introduction

Kiwifruit (*Actinidia deliciosa* and *A. chinensis*) has been the leading export horticultural product of New Zealand, and the export value was over 2300 million NZD in 2019 (Aitken & Warrington, 2019). Although 'Hayward' has been the main cultivar since the 1970s (Okuse & Ryugo, 1981), cultivars with different characteristics have become popular, especially in the foreign markets, i.e. Asia and Europe. The export quantity for the gold-flesh kiwifruit has increased by 250% since the first release in 2005 (Aitken & Warrington, 2019). New cultivars are merging recently, such as 'Red19' with red-coloured flesh (Aitken & Warrington, 2020). However, the storage life of these new cultivars is not as long as 'Hayward' (Kim *et al.*, 2009; Li *et al.*, 2015).

Long-distance transport is a challenge for some new cultivars with relatively short shelf life. It takes 4 to 6 weeks for New Zealand products to reach the offshore market by sea freight. Regular practice in the supply chain does not provide sufficient maintenance of fruit quality (Suo *et al.*, 2018). Postharvest technology, such as controlled atmosphere (CA) can potentially improve the shelf life of kiwifruit (Burdon *et al.*, 2002b), however, the facility requirement of CA limits its application in transport.

Modified atmosphere packaging (MAP) can be used to create an internal atmosphere of elevated concentration of CO_2 and reduced O_2, in this way reducing the respiration rate of the fresh product, thus extending the storage life of the commodity (Fonseca *et al.*, 2002). The gas composition of MAP is created by the equilibrium between the film permeance and the respiration rate of the given produce (Thompson, 2010a). The film used can also reduce the diffusion of water vapour to the surrounding environment and maintain high relative humidity (RH) inside the package, hence reducing water loss of fresh product (Hu *et al.*, 2011; Ozturk *et al.*, 2019b).

Kiwifruit, as a climacteric fruit (Pratt & Reid, 1974), is sensitive to ethylene (C_2H_4) (Wills *et al.*, 2001). As low as 10 $nL \cdot L^{-1}$ C_2H_4 can induce kiwifruit softening (Antunes, 2007). But unlike most climacteric fruit, 'Hayward' kiwifruit does not produce a large amount of C_2H_4 until the fruit firmness is close or just above 1 kg_f (Sfakiotakis *et al.*, 1989). However, in some fruit, the softening might be accelerated due to physical injury (e.g.

wounding, impact injury, abrasion, etc.) or pathogen infection, resulting in increased C_2H_4 production (Masamichi & Yoshinori, 1990). As MAP is an enclosed system, there is a potential risk of C_2H_4 accumulation inside the packaging. Ethylene scavenger (ES) may reduce the C_2H_4 concentration in MAP (Park *et al.*, 2016), but the effect has not been well studied in kiwifruit. Whilst the MAP effect on kiwifruit quality has been studied under low temperature (0-4 °C) conditions (Öztürk & Ağlar, 2019; Sicari *et al.*, 2019; Jiao *et al.*, 2020) the responses of kiwifruit to MAP at room temperature (20 °C) are unclear. This study aims to understand kiwifruit responses to MAP in both cool storage and subsequent shelf life, and also to investigate the impact of ethylene scrubbing in MAP.

4.2 Material and methods

4.2.1 Fruit

Kiwifruit (*A. deliciosa* cv. 'Hayward') was sourced from three grower lines (GLs). The fruit were packed in modular bulk (MB) cardboard boxes after commercial grading and couriered to Massey University, Palmerston North. 'Count 36' (95-108 g) fruit were used in this study. Ninety fruit from each GL were randomly selected upon arrival for the initial quality assessment (as described in section 2.4), including dry matter (DM), firmness, soluble solid content (SSC) and titratable acidity (TA) (Table 4.1).

Table 4.1 Initial quality of 'Hayward' kiwifruit from three grower lines.

Grower	Firmness (kg_f)	SSC (%)	DM (%)	TA (%)
GL1	5.62 ± 0.12	11.20 ± 0.12	18.36 ± 0.09	1.59 ± 0.01
GL2	5.66 ± 0.12	13.08 ± 0.14	19.77 ± 0.11	1.77 ± 0.04
GL3	6.06 ± 0.11	12.85 ± 0.15	19.86 ± 0.10	1.66 ± 0.01

*Data represents mean ± SEM, n=90.

Weight loss

Weight loss (WL) was measured for all the fruit packs after cool storage and after shelf life. An industrial scale (24000D-SCS, Precisa, Switzerland) was used for coolstore packs (50 fruit per bag), and a balance (TW423L, Shimadzu, Japan) was used for shelf packs (8 fruit per bag). Initial weight (W_0) was measured right after package sealing on the packing

day. The final weight (W_1) was measured before the package was opened on the day of quality assessment after storage.

Weight loss (%) =

$$\frac{W_1 - W_0}{W_0} \times 100\%$$

Where:

W_0 = Initial bag weight at packing (g),

W_1 = Final bag weight after storage (g).

4.2.2 MAP treatments

4.2.2.1 Cool storage

From each GL 50 fruit were randomly packed into one modular loose (ML) cardboard box at the start of the trials. Ten of the MLs were lined with polyethene film and were not sealed (Air), while the other 10 MLs were lined with an MAP film (Xtend® 885-KW45/L, StePac, Israel) (Figure 4.1) and sealed with a rubber band (MA). A total of 60 MLs was created with 3 GLs (Figure 4.1).

Sample ports were attached to 3 packages of each treatment for gas sampling. An iButton® temperature/humidity logger (DS1923, Maxim Integrated, USA) was placed in each of the three sampling packs to monitor temperature and RH. The MLs were randomly stacked in a refrigerated shipping container and stored at 1 °C, 60-70% RH for 35 d. A gas sample was taken from the sample port on a daily basis using a 1 mL syringe. The concentration of O_2 and CO_2 of each sample was measured using an O_2/CO_2 analyser. Three fruit from each ML (a total of 30 fruit per GL per treatment) were randomly sampled after 35 d of cool storage and placed into a tray. Quality assessments, including WL, firmness, TA, SSC and disorder assessment (details as in section 2.4) were carried out after placing the fruit for 16 h at 20 °C, 60-70% RH.

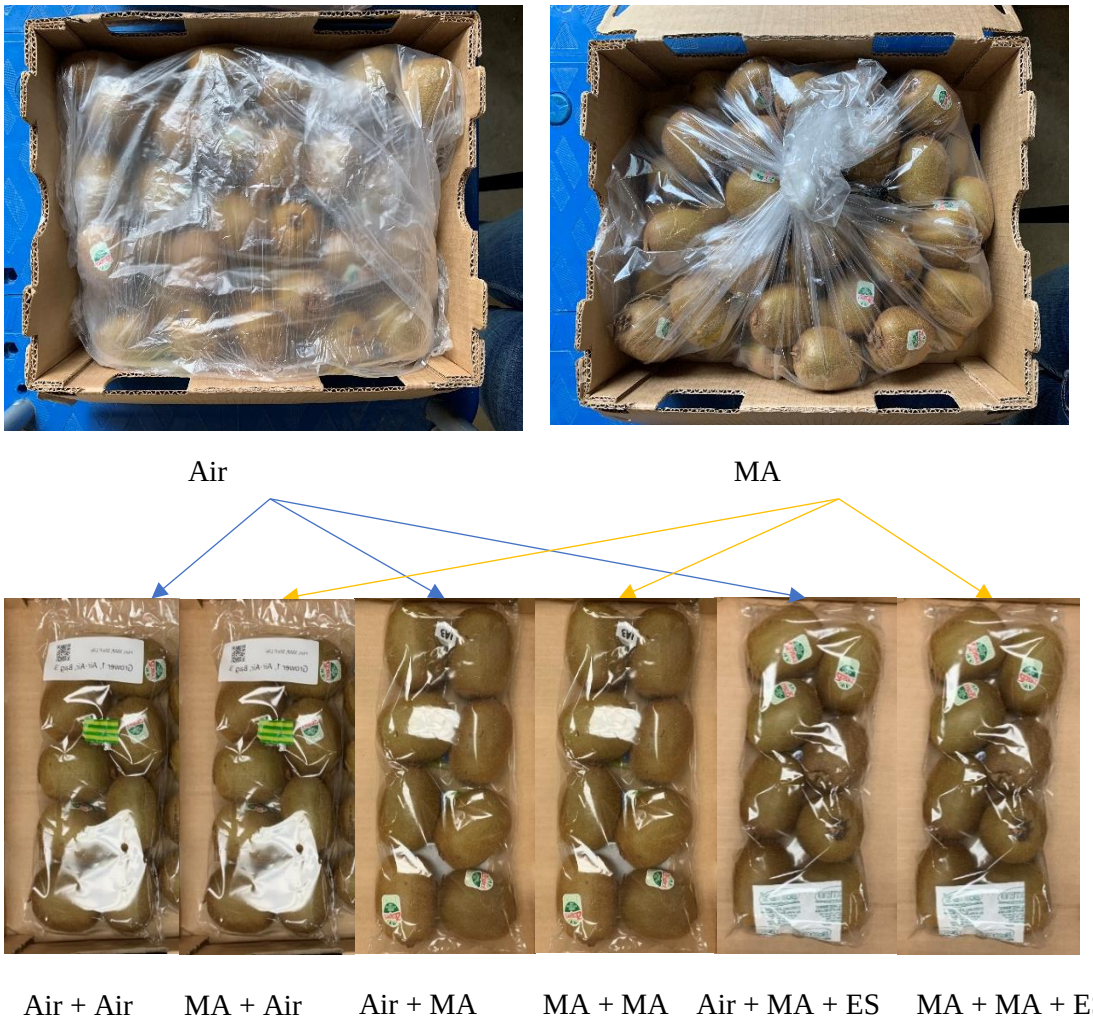

Figure 4.1 Treatments for 'Hayward' kiwifruit in cool storage and shelf life. 50 fruit were packed in a modular loose (ML) box lined with either polyethene film (Air) or MAP film (MA). 10 Air and 10 MA boxes were created from each grower line (GL). A total of 60 MLs were stored at 1 °C for 35 days. After removal from cool storage (1 °C, 60-70% RH), fruit from the same GL were repacked into shelf-life packs. 8 fruit were randomly picked from the 10 MLs of the same cool storage treatment and packed into a shelf-life pack. Following cool storage treatments, 6 shelf-life treatments were generated. Air + Air: cool-stored in air followed by shelf life in perforated bag; MA + Air: cool-stored in MAP followed by shelf life in perforated bag; Air + MA: cool-stored in air followed by shelf life in non-perforated bag; MA + MA: cool-stored in MAP followed by shelf life in non-perforated bag; Air + MA+ ES: cool-stored in air followed by shelf life in the non-perforated bag with ethylene scrubbing sachet; MA + MA +ES: cool-stored in MAP followed by shelf life in the non-perforated bag with ethylene scrubbing sachet. 15 packs of each shelf-life treatment were generated from each GL. A total of 270 shelf-life packs were stored at 20 °C, 60-70% RH for up to 10 days.

4.2.2.2 Shelf life

Upon removal from 1 ºC storage, eight fruit per treatment per GL were randomly picked from the 10 MLs and packed into a shelf-life bag (Xtend® 885-KW55/L, StePac, Israel). 15 shelf-life bags were perforated with 8 holes with 8 mm diameter (Air), 15 shelf-life bags were non-perforated (MA), and another 15 non-perforated shelf-life bags were packed with potassium permanganate ($KMnO_4$) ethylene scrubbing (ES) sachets (MA + ES). Six treatments were created upon removal of fruit from air/MA cold storage:

1. Air + Air
2. Air + MA
3. Air + MA + ES
4. MA + Air
5. MA + MA
6. MA + MA + ES

A total of 270 shelf-life packs were produced with fruit from all 3 GLs (Figure 4.1). The shelf-life packs were sealed with a heat sealer. The shelf-life bags were randomly organized in MB boxes and stored at 20 °C, 60-70% RH for up to 10 days. Internal gas composition was monitored daily by sampling 1 ml of gas with a syringe and analysed with an O_2/CO_2 analyser. C_2H_4 concentration was measured using a gas chromatograph (GC-2014 Gas Chromatograph, Shimadzu, Japan) at the end of shelf life. 5 shelf-life packs (8 fruit per pack) of fruit from each treatment were sampled after storage at 20 ºC for 3, 6 and 10 days for quality assessment (described in section 2.4), including WL, firmness, TA, SSC, disorder assessment.

4.2.2.3 Measurement of film properties

The bags used in the cool storage and shelf-life assessment were commercially supplied with no property data provided by the supplier. The thickness was measured using a digital calliper (IP54, Shahe, China). The permeability of film was determined by a steel permeability cell (ID = 0.1 m) (Figure 4.2). The measuring methods were adapted from Samarakoon (2013). The film was placed between the two chambers of the permeability cell. N_2 at the flow rate of 2 ml $\cdot min^{-1}$ was supplied to the top chamber, and pure CO_2 or air with 21% O_2 was supplied to the bottom chamber at the same flow rate to determine the film permeability of O_2 and CO_2, respectively. The environment temperature was

20 °C. The gas composition of the inlet and outlet of both the top and bottom chamber was measured daily for 3 weeks using an O_2/CO_2 analyser.

Figure 4.2 Permeability cell (ID = 0.1 m) used in film permeability measurement.

Permeability at steady state was calculated using Equation 4.1.

$$P = \frac{Q_L(C_{LO} - C_{LI})\,L}{A(C_{HO} - C_{LO})RT} \qquad \textbf{Equation 4.1}$$

Where,

P = Film permeability ($mol \cdot m \cdot m^{-2}s^{-1}Pa^{-1}$)

Q_L = Gas flow rate through low concentration side (m^3s^{-1})

C_{LO} = Outlet gas concentration from low concentration side ($\mu L \cdot L^{-1}$)

C_{LI} = Inlet gas concentration from low concentration side ($\mu L \cdot L^{-1}$)

L = Thickness of film (m)

A = Area of film (m^2)

C_{HO} = Outlet gas concentration from high concentration side ($\mu L \cdot L^{-1}$)

R = Universal gas constant (8.314 $J \cdot K^{-1}mol^{-1}$)

T = Absolute temperature (K)

4.3 Results and discussion

4.3.1 Film properties

The film properties are demonstrated in Table 4.2.

Table 4.2 Properties of MAP film applied in cool storage and shelf life.

Film	PO_2 ($mol.m.m^{-2}s^{-1}Pa^{-1}$)	PCO_2 ($mol.m.m^{-2}s^{-1}Pa^{-1}$)	Thickness (mm)
Cool storage	9.78×10^{-17}	2.23×10^{-17}	0.025
shelf-life	1.55×10^{-16}	2.97×10^{-17}	0.036

4.3.2 Gas composition in headspace of packaging

4.3.2.1 Oxygen and carbon dioxide concentration in cool-storage packs

The gas composition inside the packaging was tracked from the time of bag-sealing throughout the whole cool storage. The O_2 and CO_2 concentration in control (Air) remained the same as ambient (21% O_2 and 0.05% CO_2) throughout the 35 days of storage. The gas composition in MA at packing (Day 0) was the same as ambient (21% O_2 and 0.05% CO_2). After a rapid change, the internal atmosphere of MA reached 15% O_2 and 3% CO_2 on Day 2 (Figure 4.3). O_2 concentration kept declining to 12% until day 7, and slowly increased to 15% at the end of cool storage. Whereas CO_2 concentration was relatively stable between 3% and 4% after Day 2. The modified atmosphere established rapidly and remained relatively stable (12-15% O_2, and 3-4% CO_2) for the cool storage duration.

The concentrations of O_2 and CO_2 are the key factors impacting fruit quality in MAP in controlled temperature storage. The optimum atmosphere for 'Hayward' kiwifruit in cool storage is 2% O_2 + 5% CO_2 (Harman & McDonald, 1989b). However, this atmosphere is rarely achieved by MAP. The internal atmosphere of 5-8% CO_2 and 10-15% O_2 has previously been reported in MAP applications for 'Hayward' kiwifruit stored at 0 ºC with a positive impact on fruit quality (Zoffoli *et al.*, 2006; Zoffoli *et al.*, 2016). On the other hand, O_2 and CO_2 concentrations close to the ambient cannot be expected to make a large impact on fruit quality. In a study of MAP application, 17.1-17.5% O_2 with 2.5-3.2% CO_2

has been created by using 20 µm low-density polyethene (LDPE) film but did not significantly alter the quality of kiwifruit (Jiao *et al.*, 2020). The O_2 concentration (12-15%) in the current study was within the previous range, whereas the CO_2 concentration of 3-4% was lower compared to the previous studies. It has been suggested that modifying the initial gas composition in MAP to create an active MAP, instead of using air as the starting point (passive MAP), alters the equilibrium in the headspace and reduces the product respiration and further extends the storage life (Belay *et al.*, 2019). Initial gas flushing with N_2 or CO_2 may be a potential approach to achieve a better gas composition.

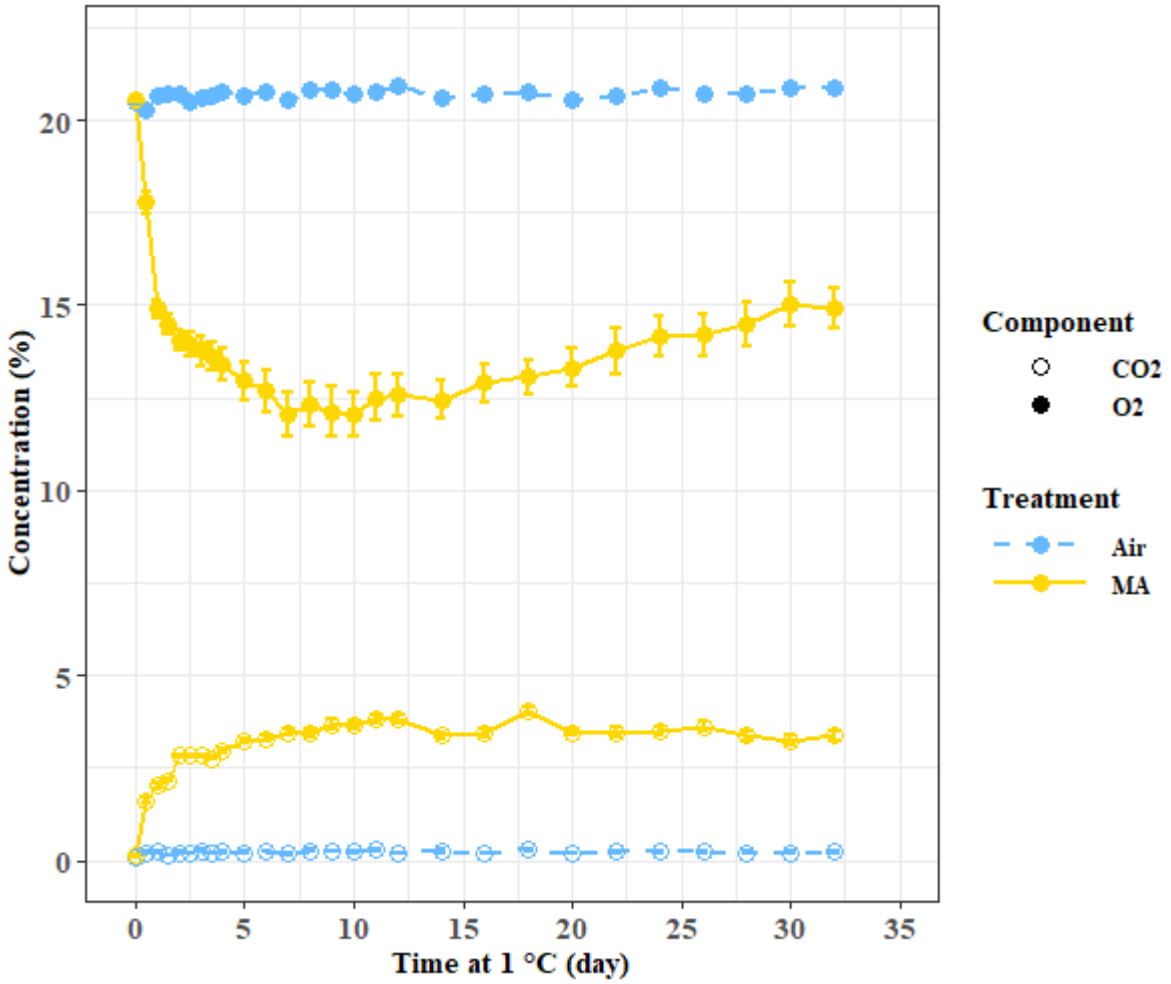

Figure 4.3 CO_2 and O_2 concentration in modified atmosphere (MA) packaging and polyethylene (Air) with 50 'Hayward' kiwifruit stored at 1 ºC, 60-70 % RH for 35 days. Each data points represents mean ± SEM, n = 9.

Although both O_2 and CO_2 concentration impact fruit quality, CO_2 concentration has a greater influence on fruit quality compared to O_2 (Zoffoli *et al.*, 2006). It has been indicated that CO_2 at a concentration higher than 2% can reduce the sensitivity of plant tissue to C_2H_4 (Kader & Saltveit, 2002). However, excessively high CO_2 has a negative

impact on kiwifruit, but the tolerance to high CO_2 varies between cultivars. CO_2 concentration higher than 8% can induce CO_2 injury on 'Hayward' (Burdon, 2020), while 'Cuixiang' kiwifruit stored under 6.4%–7.0% CO_2 produced off-smell and bitter taste (Jiao *et al.*, 2020). At the concentration of 3-4%, the CO_2 level in the current study was within the safe range for 'Hayward'. Indeed, there was no unpleasant odour. The details of MAP effect on fruit quality will be discussed later in this chapter.

4.3.2.2 Oxygen and carbon dioxide concentration in shelf-life packs

Kiwifruit were repacked into shelf-life bags within 2 h after removal from cool storage and stored at 20 °C for 10 days. The O_2 concentration in perforated bags (Air) was close to ambient (21%) for the whole 10-day shelf life. The CO_2 concentration in perforated bags (Air) was higher than ambient, ranged from 0.5% to 1%. The perforated unsealed package allowed the free gas exchange without a 'strict' barrier. However, the CO_2 level was elevated at room temperature, indicating the respiration rate was high enough to generate 1% CO_2 before the CO_2 diffused to the environment. This CO_2 accumulation also suggests that there is also a possibility of ethylene accumulating in the same packaging if the production rate is higher than the diffusion rate.

Steady-state was created in non-perforated bags (MA) after 24 h in shelf-life assessment. O_2 concentration in MA was between 12% and 15% on Day 1. It stabilized between 15% and 16% after Day 3. The CO_2 concentration in MA reached 7.5% on Day 1. The peak was at 8% to 10% for different treatments on Day 2, after which the CO_2 concentration experienced a reduction to 7.5% in the following day and entered a slow decrease to 7% in the remaining 7 days. The gas composition in MA at a steady-state was 12-16% O_2 and 7-10% CO_2 (Figure 4.4).

MAP application in shelf life has rarely been reported for kiwifruit or other climacteric fruit but has been studied in the shelf life of non-climacteric fruit, such as strawberry and dragon fruit (Wu *et al.*, 2019; Ho *et al.*, 2020a). Established MA at 20-25 °C varies from 15% O_2 and 5% CO_2, 17% O_2 and 2% CO_2 (Wu *et al.*, 2019) to 0% O_2 and 21% CO_2 (Ho *et al.*, 2020a). The O_2 and CO_2 concentration in the current study was similar to that in the MAP of strawberry (Wu *et al.*, 2019), indicating kiwifruit in the 10 days of shelf life did not experience a respiration peak. Although there is a CO_2 concentration peak at the early stage of this 10 days of shelf life, the fruit remained firmer than 1 kg_f, indicating the climacteric peak did not start during this period.

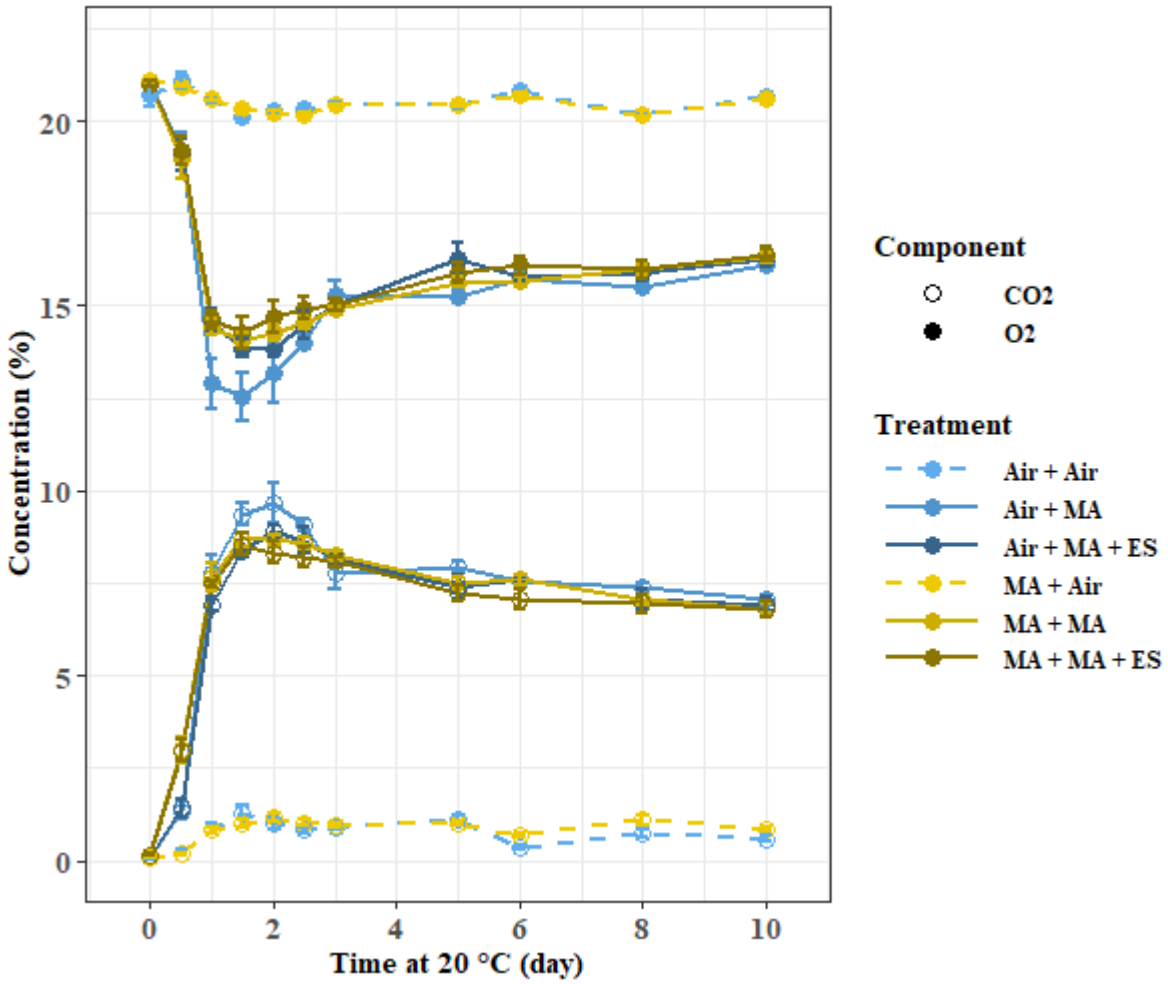

Figure 4.4 O_2 and CO_2 concentration in shelf-life packs stored at 20 °C, 60-70% RH for up to 10 days. Air + Air: cool-stored in air followed by shelf life in perforated bag; MA + Air: cool-stored in MAP followed by shelf life in perforated bag; Air + MA: cool-stored in air followed by shelf life in non-perforated bag; MA + MA: cool-stored in MAP followed by shelf life in non-perforated bag; Air + MA + ES: cool-stored in air followed by shelf life in the non-perforated bag with ethylene scrubbing sachet; MA + MA + ES: cool-stored in MAP followed by shelf life in the non-perforated bag with ethylene scrubbing sachet. Each data point represents mean ± SEM, n = 3.

4.3.2.3 Ethylene concentration in shelf packs

Ethylene concentration inside packages was measured on Day 7 of shelf life. There was a large variance of ethylene concentration between packages from undetectable (below 30 $nL \cdot L^{-1}$) to 5600 $nL \cdot L^{-1}$. It was found that, in every case, the packages with high ethylene were associated with one or more rotten fruit. In the packs without rotten fruit, the ethylene level was merely detectable by the GC with the minimum detection concentration of 30 $nL \cdot L^{-1}$. For the shelf life packs with rotten fruit, the ethylene level ranged from 100 $nL \cdot L^{-1}$ to 5600 $nL \cdot L^{-1}$ (Figure 4.5). The high ethylene concentrations (>200 $nL \cdot L^{-1}$) appeared in the packs following cool storage in air, while that of MA + Air

and MA + MA was below 200 $nL \cdot L^{-1}$, and no rotten fruit was found in MA + MA + ES. High levels of ethylene were observed in a perforated 'control' bag, suggesting there is the possibility of ethylene accumulation when the ethylene production rate is high, and the perforation did not reduce the concern of ethylene accumulation.

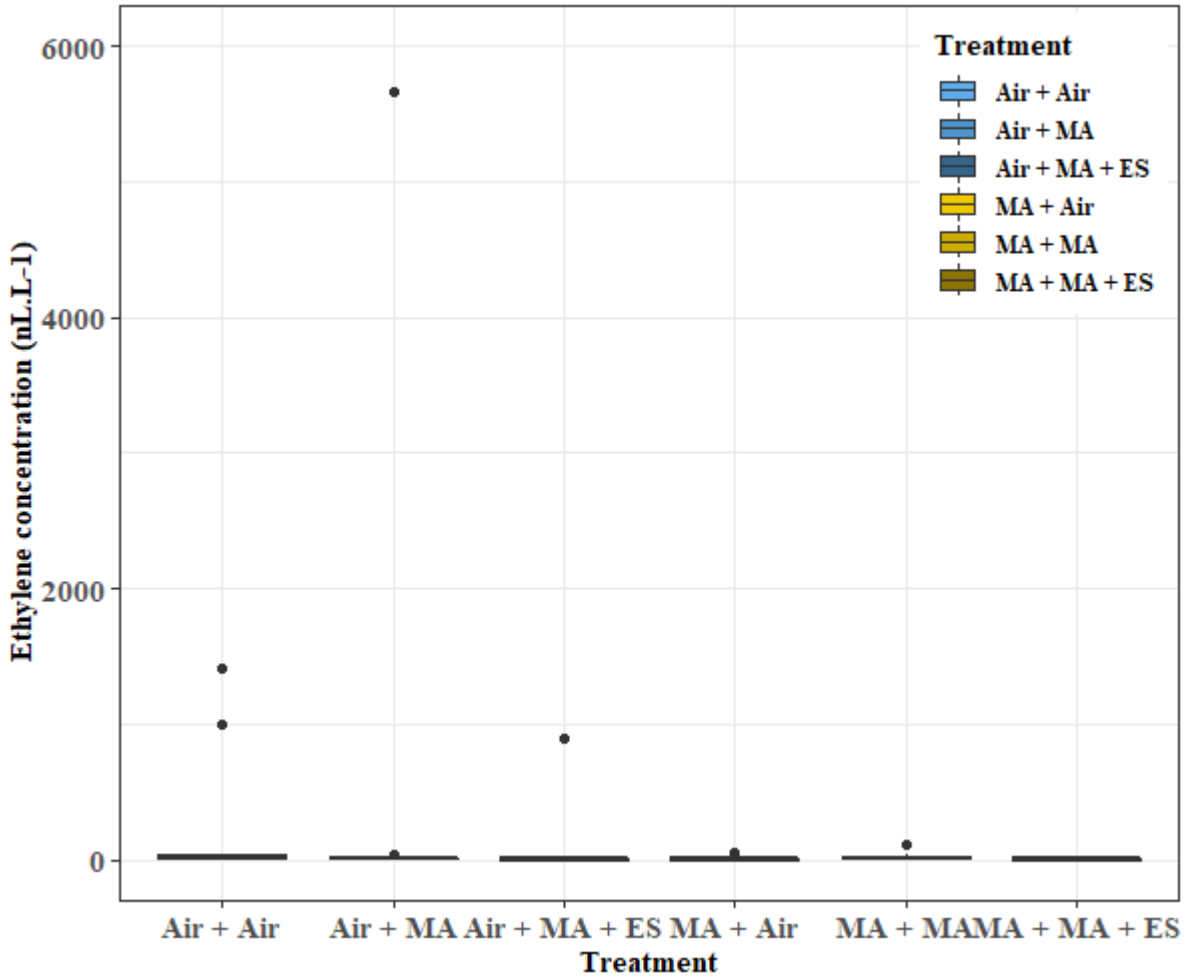

Figure 4.5 Ethylene concentration in shelf-life packs of 'Hayward' kiwifruit stored at 20 ºC, 60-70% RH after 7 days. Air + Air: cool-stored in air followed by shelf life in perforated bag; MA + Air: cool-stored in MAP followed by shelf life in perforated bag; Air + MA: cool-stored in air followed by shelf life in non-perforated bag; MA + MA: cool-stored in MAP followed by shelf life in non-perforated bag; Air + MA + ES: cool-stored in air followed by shelf life in the non-perforated bag with ethylene scrubbing sachet; MA + MA + ES: cool-stored in MAP followed by shelf life in the non-perforated bag with ethylene scrubbing sachet.

The large variation of ethylene concentration in MAP at 20 °C has been reported, and it is due to pack-to-pack variation rather than the treatment effect (D'Aquino *et al.*, 2016). The current result agrees with this statement, as high ethylene concentration was only observed in 6 shelf-life packs with rotten fruit, and the difference between treatments was not statistically significant. Meanwhile, it has been reported that MAP can reduce the

ethylene production rate of kiwifruit by up to 43% and delay the peak of C_2H_4 production by 14 days (Jiao *et al.*, 2020). A similar result has not been found in the current study, as most of the fruit did not reach firmness below 1 kg_f, when detectable ethylene is produced. Longer storage time is needed to observe the ethylene production peak. Although it has been observed that the ethylene production rate can be reduced by MAP (Phakdee & Chaiprasart, 2020), this benefit was not observed in the current study. The benefit of MAP in shelf life with firm kiwifruit was not significant.

Generally, rot is more common in air stored kiwifruit compared to MA stored kiwifruit, unless the CO_2 level exceeds 7-8% which favours some fungal infection (Wang *et al.*, 2021c). In this study, given the rot incidence was very low, it is too early to conclude MAP impact on rot incidence. Future work is required to classify the effect of MAP on kiwifruit, with a longer room temperature storage and softer fruit.

The effect of ethylene absorbent on fruit in MAP has been studied on multiple fruit. Studies on fruit, such as apple, peach and tomato, have shown ES significantly reduced the ethylene level in the headspace of MAP and maintained the fruit quality (Wei *et al.*, 2021). However, this is not necessarily the case. A study on apricot has demonstrated the $KMnO_4$ based ES reduced ethylene level in MAP from 6 $nL \cdot L^{-1}$ to below 1 $nL \cdot L^{-1}$, but the fruit data were not significantly altered, as 6 $nL \cdot L^{-1}$ was so low that could not impact the quality of apricot (Álvarez-Hernández et al., 2020). In the current study, neither ethylene concentration nor kiwifruit quality was altered by ES due to the fruit not starting to produce ethylene by the end of the experiment. On the other hand, ethylene at the concentration above 100 $nL \cdot L^{-1}$ was occasionally found in this experiment, and always associated with rotten fruit, and in one bag with a rotten fruit and ES, which suggests ES did not sufficiently reduce ethylene damage from rotting fruit. Longer shelf-life duration will be required in the future study to determine the effect of ES on the ethylene concentration in MAP headspace of undamaged ripening fruit and on kiwifruit quality.

Apart from the ethylene produced by the fruit in the package, the ethylene in the storage environment also affects the fruit quality. In some distribution centres and retail stores, kiwifruit are held in the same area as some fruit producing a large amount of ethylene like apple. With unsealed packaging, ethylene contamination has a relatively high possibility to impact kiwifruit quality, while MAP as an enclosed system is capable to eliminate environmental contamination.

4.3.3 Weight Loss

Weight loss (WL) in cool storage and shelf life were calculated based on the initial weight at harvest (Day 0). WL by the end of cool storage was 0.1% in MA, and 0.25% in Air. WL in shelf life was more drastic compared to that in cool storage. Cool stored in MA followed by the non-perforated bag in shelf life (MA + MA and MA + MA + ES) had the lowest WL (0.37%). Cool stored in Air followed by the non-perforated bag in shelf life (Air + MA and Air + MA +ES) had slightly higher WL (0.49-0.51%). WL in perforated bags in shelf life (Air + Air and MA + Air) was the highest (1.11-1.22%) after 10 days of storage at 20 ºC (Figure 4.6). WL in non-perforated bags was impacted by the previous cool storage treatments, while the WL in the shelf-life of perforated bags was not impacted by the previous cool storage treatments. Ethylene scrubbing sachet did not affect WL. Temperature and perforation are the two main factors influencing WL in MAP.

Weight loss of fresh products is mainly caused by water loss (Lufu *et al.*, 2019). Generally, water loss in fresh products can induce physical and physiological changes, as well as direct economic loss i.e., reduction of saleable weight (Saltveit, 2016). It has been demonstrated that water loss increases respiration rate and cell wall enzyme activity and accelerates kiwifruit softening (Taglienti *et al.*, 2009). Shrivel has become an important factor impacting the storage life of 'Gold3' kiwifruit, and obvious symptoms of shrivel start to emerge when WL is above 1% (Burdon *et al.*, 2014b). Managing weight loss could potentially improve the quality and extend the storage life of kiwifruit.

In the present study, MAP significantly reduced WL at both 1 °C and 20 °C. Similar results of MAP reducing weight loss have been well documented for kiwifruit (Hu *et al.*, 2011; Ozturk *et al.*, 2019b) and other fruit, such as sweet cherry (Aglar *et al.*, 2017) and plum (Erkan & Eski, 2012). These results indicate that MAP is a promising approach for maintaining the fresh weight of the product during cool storage and subsequent shelf life.

MAP reduces WL both physically and physiologically. MAP, being an enclosed system, physically reduces the water vapour diffusion from the package to the outer environment, thus maintains the RH in the package at a high level (near 100%) (Saltveit, 2016). In return, water vapour diffusion from the fruit surface to the package is suppressed. On the other hand, water loss can be accelerated by physiological changes of the fruit surface during ripening (Celano *et al.*, 2009). MAP delays fruit ripening, which indirectly reduces WL.

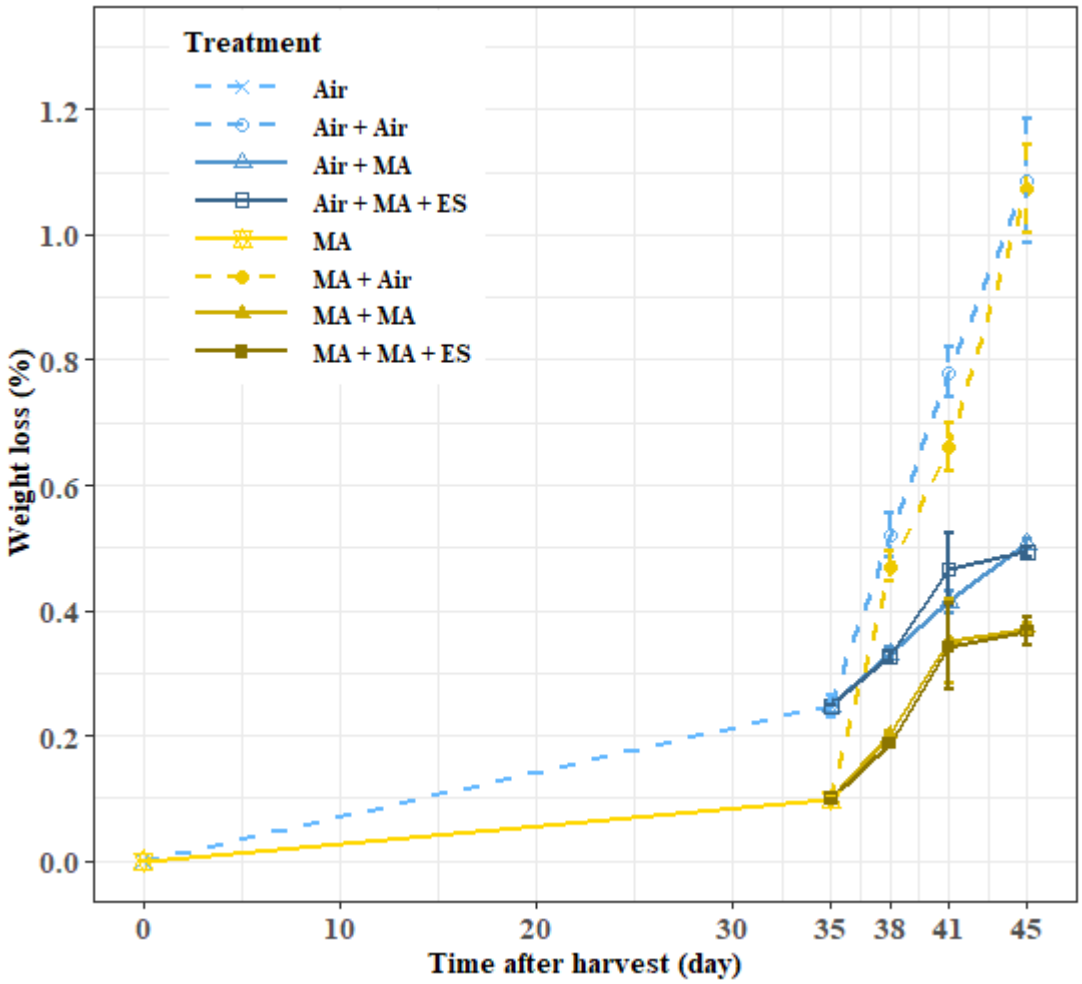

Figure 4.6 Weight loss of 'Hayward' kiwifruit stored in modified atmosphere packaging at 1 °C, 60-70% RH for 35 days followed by 10 days shelf life at 20 °C, 60-70% RH. Air + Air: cool-stored in air followed by shelf life in perforated bag; MA + Air: cool-stored in MAP followed by shelf life in perforated bag; Air + MA: cool-stored in air followed by shelf life in non-perforated bag; MA + MA: cool-stored in MAP followed by shelf life in non-perforated bag; Air + MA + ES: cool-stored in air followed by shelf life in the non-perforated bag with ethylene scrubbing sachet; MA + MA +ES: cool-stored in MAP followed by shelf life in the non-perforated bag with ethylene scrubbing sachet. Each data points represents mean ± SEM, n = 15.

4.3.4 Firmness

The at-harvest firmness of fruit was 5.8 kg_f. After 35 days of cool storage at 1 ºC, significant differences ($p<0.001$) emerged between MA and Air stored fruit. The firmness of fruit in MA was 2.9 kg_f, while that of Air stored fruit was 2.0 kg_f on Day 35. In the subsequent shelf life, the bag perforation or ethylene scrubbing sachet did not create significant differences. Following cool storage in Air, the firmness at Day 3, 6 and 10 of shelf life was 1.7-1.8 kg_f, 1.6-1.7 kg_f and 1.5-1.6 kg_f, respectively. Following cool storage in MA, the firmness at Day 3, 6 and 10 of shelf life was 2.6-2.7 kg_f, 2.3-2.5 kg_f and 2.1-

2.3 kg_f, respectively. At the end of the 10-day shelf life, fruit firmness was dependent on cool storage treatments (Figure 4.7).

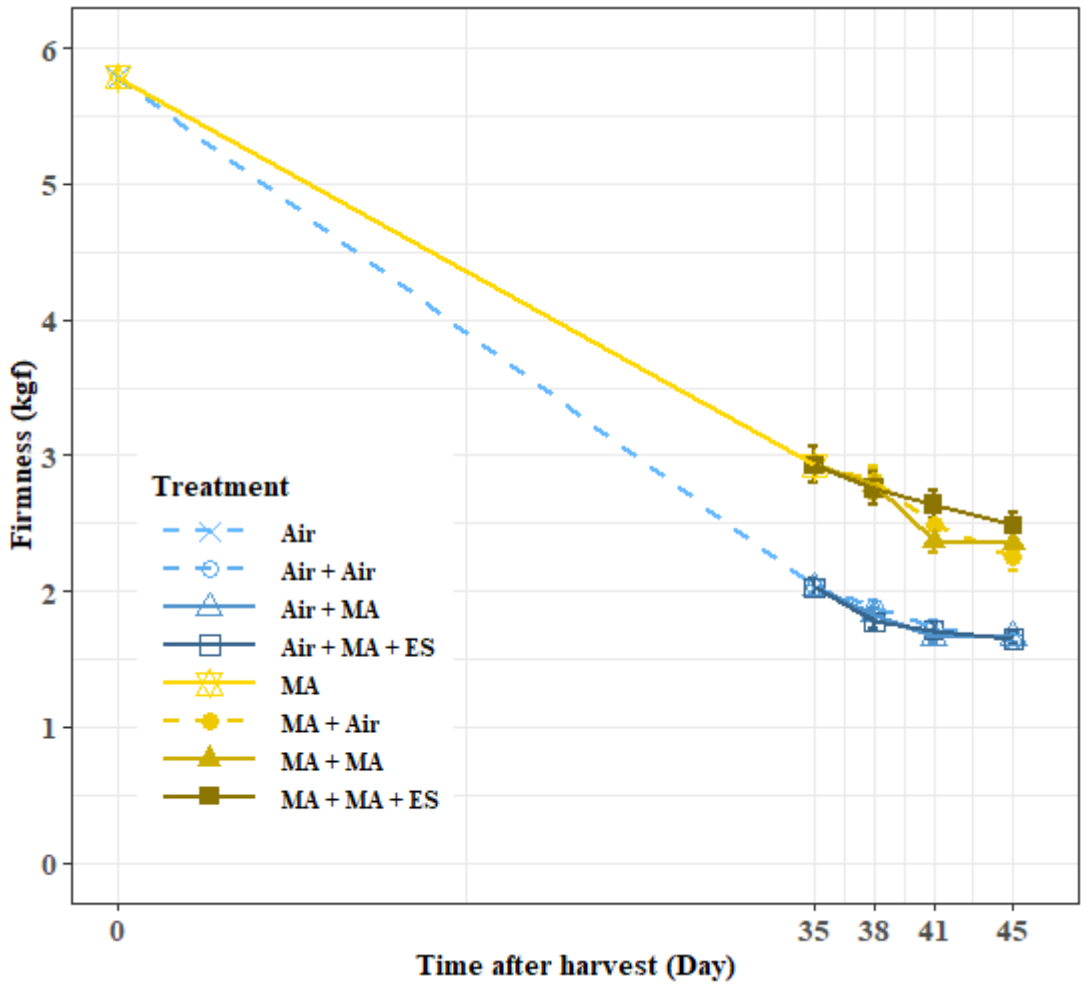

Figure 4.7 Firmness of 'Hayward' kiwifruit stored in polyethene film (Air) and modified atmosphere bag (MA) at 1 ºC for 35 days followed by shelf life at 20 ºC for 10 days. Air + Air: cool-stored in air followed by shelf life in perforated bag; MA + Air: cool-stored in MAP followed by shelf life in perforated bag; Air + MA: cool-stored in air followed by shelf life in non-perforated bag; MA + MA: cool-stored in MAP followed by shelf life in non-perforated bag; Air + MA + ES: cool-stored in air followed by shelf life in the non-perforated bag with ethylene scrubbing sachet; MA + MA + ES: cool-stored in MAP followed by shelf life in the non-perforated bag with ethylene scrubbing sachet. Each data point represents mean ± SEM, n = 120.

MAP significantly maintained higher firmness in cool storage, but there was no significant difference between perforated and non-perforated bags in the following shelf life for up to 10 days. Fruit firmness for all the treatments was above 1 kg_f, indicating there was still reasonable storage potential after 10 days of shelf life. On the other hand, there was no rapid softening either in cool storage or during shelf life, suggesting the current MAP did not negatively impact fruit firmness (fermentation or CO_2 injury).

Longer shelf life is needed to determine the effect of MAP and ethylene-scrubbing agents on kiwifruit quality.

The firmness of kiwifruit stored in MAP relies on the atmosphere created in MAP. In the present study, fruit stored in MAP (2-4% CO_2, and 12-15% O_2) were 44.3% firmer than control after cool storage. Another study conducted with 'Hayward' kiwifruit has seen a 54% higher firmness in MAP with 5-8% CO_2 and 10-15% O_2 compared to control after 60 days of cool storage (Zoffoli *et al.*, 2016). An atmosphere of 3% CO_2 and 17% O_2 created by MAP has been reported to have little effect on the firmness of 'Cuixiang' kiwifruit, but an atmosphere of 5% CO_2 and 15% O_2 from the same experiment significantly improved the firmness (Jiao *et al.*, 2020). It has been suggested that compared with low O_2 concentration, high CO_2 concentration has a greater influence on retaining kiwifruit firmness (Stec *et al.*, 1989). CO_2 at a concentration of higher than 5% can effectively suppress respiration (Mastromatteo *et al.*, 2011), reduce intercellular pH (Lange & Kader, 1997), inhibit ACO activity (Mathooko, 1996a) and competitively bind to C_2H_4 receptors (Mathooko, 1996b).

WL in some occasions impacts kiwifruit firmness. High WL results in non-cell-wall related softening, as turgor reduces. In this study, the high WL in the perforated bag did not impact firmness, therefore, the small difference of WL probably did not contribute to the softening during coolstorage. Thus, the difference of firmness during storage was more of the result of gas composition rather than the water loss.

Apart from the gas composition, a higher temperature can impair the effect of MAP on retaining fruit firmness. The atmosphere created by MA in shelf-life was 7-10% CO_2 and 12-15% O_2 in the current study. The CO_2 concentration was higher than that in cool storage (2-4%), while the O_2 concentration was similar to that in cool storage (12-15%). But the MAP at room temperature did not alter the firmness during the 10 days shelf life (Figure 4.7). MAP application at room temperature has not been well studied on climacteric fruit like kiwifruit, whereas studies on blueberry (Randolph *et al.*, 1992) and avocado (Burdon *et al.*, 2017a) have shown the benefits of MA are reduced by elevated storage temperature. Meanwhile, research on non-climacteric fruit, such as strawberry (Wu *et al.*, 2019), dragon fruit (Ho *et al.*, 2020a) and grapes (Xu *et al.*, 2013) has shown MAP effect on maintaining fruit quality at 20-25 °C. In the shelf life following MAP in cool storage of kiwifruit, the package is normally opened or removed, and the same film

of MAP in cool storage is not continuously used in the following shelf life (Zoffoli *et al.*, 2016; Ozturk *et al.*, 2019b), due to the high respiration rate of fresh product at room temperature which can alter the internal gas composition. Thus, a repacking into another film with higher CO_2 and O_2 permeance is essential, as an alternative to removing the packaging and exposing the commodity to ambient. The current study provided information on MAP of climacteric fruit at room temperature, but further details are to be revealed in the future.

The timing of MAP establishment also impacts the effect. In the current study, the stable state of gas composition was established within 2 days in cool storage and maintained a higher firmness than control (Figure 4.7). In another study, a 3-day delayed MAP has retained flesh firmness significantly (Zoffoli *et al.*, 2016). However, MA of 8% CO_2 and 7% O_2 delayed for 28 days has not provided any benefit on 'Hayward' kiwifruit storage (Pekmezci *et al.*, 2002). In this study, non-perforated bags following air storage did not retain fruit firmness. This result is in line with the previous studies that establishing after 5 weeks of delay was not effective in retaining firmness. Effective MA should be established within a few days in storage to maintain fruit quality and delayed MA loses the benefit.

4.3.5 Soluble solid content

Soluble solid content (SSC) has been used as an at-harvest maturity index to predict storage performance (Burdon & Lallu, 2011). Immature kiwifruit may fail to ripen and is more susceptible to chilling injury (Burdon *et al.*, 2007a). The standard of minimum at-harvest SSC for the New Zealand kiwifruit industry is 6.2% (Harman, 1981). The at-harvest SSC of kiwifruit in this study was 12.38%, demonstrating this batch of fruit were at a suitable maturity.

The SSC of fruit stored in both MA and Air increased to 15.42-15.53% after 35 days of cool storage at 1 ºC. There was no significant difference ($p=0.442$) in the cool storage between MA and Air. However, differences appeared in the following shelf life. In the following shelf life at 20 ºC, the SSC of most treatments, apart from Air + Air, experienced an increase from 15.42-15.53% on Day 35 to 15.49-15.88% on Day 41, and reached the peak on Day 41, after which SSC decreased to 15.01-15.67% on Day 45; while the SSC of Air + Air reached the peak (15.73%) earlier than the other treatments on Day 38 and decreased afterwards. The time difference of SSC peak appearing

suggested MAP delays the reduction of SSC. The SSC peak of Air + MA and Air + MA + ES were the highest (15.85% and 15.88% respectively); Air + Air, MA + MA and MA + MA + ES were lower (15.73%, 15.70% and 15.57% respectively); and MA + Air was the lowest (15.46%). At the end of shelf life, SSC of Air + MA (15.67%) and Air + MA + ES (15.61%) were the highest, MA + MA (15.36%) and MA + MA + ES (15.39%) were lower and Air + Air (15.27%) and MA + Air (15.01%) were the lowest. The peak and end value of SSC suggested coolstorage in MA suppresses SSC increase, but shelf-life in MA (non-perforated) retains a higher SSC. However, the difference of SSC in shelf life between treatments and between assessing days were quite small (ranged from 15.01% to 15.88%) (Figure 4.8). The statistical difference may not fully reveal the effect of MAP on SSC. Meanwhile, the fruit did not fully ripen on Day 10 of shelf life. Longer storage time is required to reveal the impact of MAP on the SSC of kiwifruit, especially in shelf life.

SSC impacts consumer acceptance of kiwifruit. It is recommended that SSC should be above 14% at eating-firmness (0.4-1 kg_f for 'Hayward') (Harker *et al.*, 2009). The SSC value during the shelf-life of the current study was above 15%, which was higher than the standard of 14%. MAP did not alter the normal sugar accumulation associated with good flavour. the flesh firmness was above 1 kg_f, suggesting the fruit was not fully ripened at the end of the 10-day shelf life, therefore the SSC at eating-firmness was unknown. Thus, longer storage time is needed to determine the impact of MAP and ES on consumer acceptance.

SSC generally shows a continuous increase during the storage of kiwifruit. The increase of SSC in 'Hayward' kiwifruit stored in MAP at 0 °C for 60-120 days has been delayed but the SSC value of 14.2 - 14.8% has been reached when the firmness was below 13 N (Zoffoli *et al.*, 2016). Similarly, SSC increase of 'Sanuki Gold' stored at 4 °C has been delayed by MAP for up to 2 months, but the SSC reached the same level at the end of storage (Mworia *et al.*, 2011), indicating MAP delays kiwifruit ripening but with the capacity of the fruit to ripen not being impacted. In the current study, SSC after cool storage (at Day 35) was not altered by MAP, which may be related to the short storage time (35 days in cool storage). In addition, the fruit did not reach the eating firmness within the 45 days of storage, and the MAP impact on the SSC at eating firmness could not be determined by the current data.

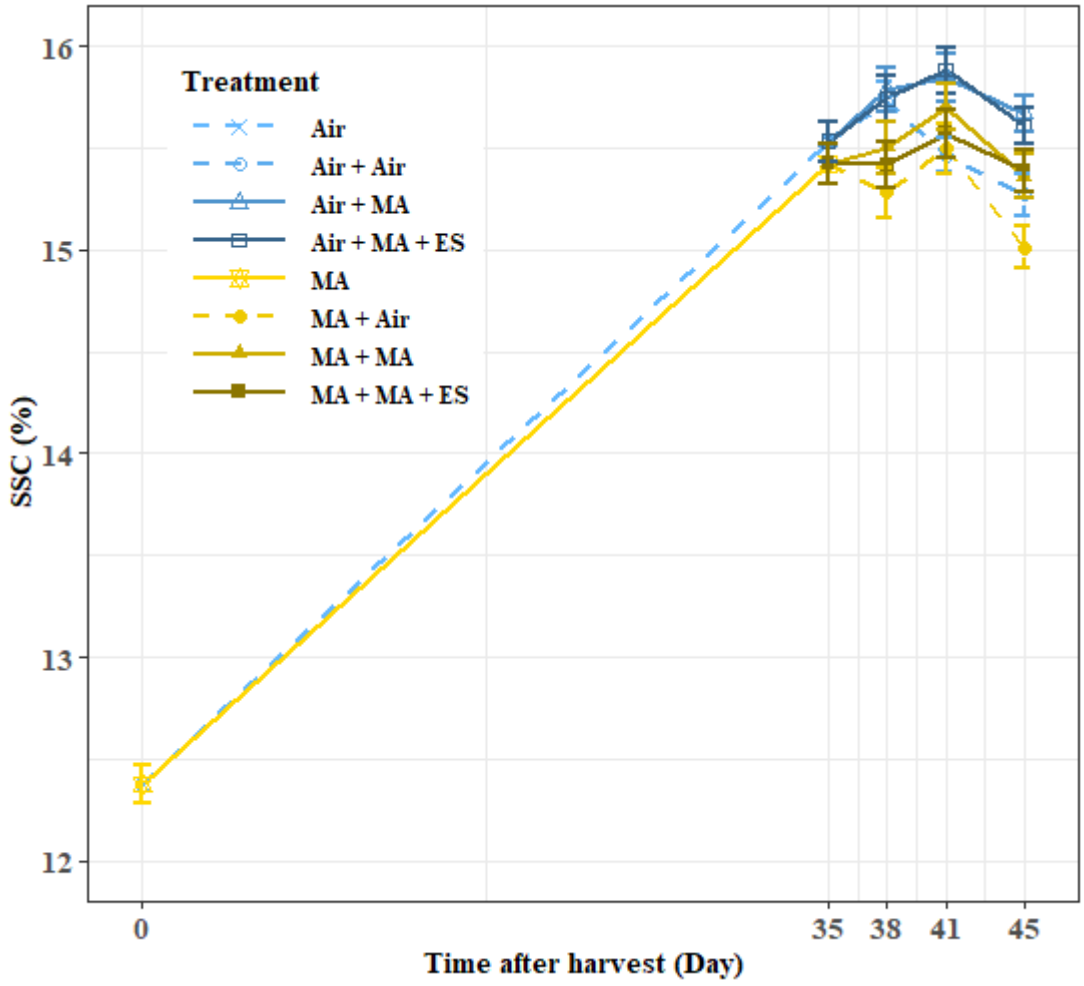

Figure 4.8 Soluble solid content (SSC) of 'Hayward' kiwifruit stored in polyethene film (Air) and modified atmosphere bag (MA) at 1 ºC for 35 days followed by shelf life at 20 ºC for 10 days. Air + Air: cool-stored in air followed by shelf life in perforated bag; MA + Air: cool-stored in MAP followed by shelf life in perforated bag; Air + MA: cool-stored in air followed by shelf life in non-perforated bag; MA + MA: cool-stored in MAP followed by shelf life in non-perforated bag; Air + MA + ES: cool-stored in air followed by shelf life in the non-perforated bag with ethylene scrubbing sachet; MA + MA +ES: cool-stored in MAP followed by shelf life in the non-perforated bag with ethylene scrubbing sachet. Each data point represents mean ± SEM, n = 120.

On the other hand, SSC decline during post-MAP shelf-life has been reported in other fresh products, such as tomato (Majidi *et al.*, 2014) and nectarine (Özkaya *et al.*, 2016). In the current study, the SSC peak value was delayed by MAP but reached a higher level compared to the control. A similar result has been reported on tomatoes that the peak value of SSC is not altered but the time of SSC peak emerging is delayed and the loss of SSC after the peak is reduced by MAP (Majidi *et al.*, 2014). It is suggested that MAP delays SSC increase in cool storage, while MAP reduces SSC loss in shelf life.

4.3.6 Titratable acidity

Titratable acidity (TA) at harvest was 1.64%. After cool storage, the TA of MA stored fruit (1.57%) was significantly ($p<0.001$) lower than those stored in Air (1.64%). After 10 days of shelf life, TA was reduced in all treatments. The TA of MA + MA (1.46%) and MA + MA + ES (1.48%) were the lowest on Day 45. There was no significant difference between Air + Air, MA + Air, Air + MA and Air + MA + ES at the end of shelf life (Figure 4.9).

It has been claimed that MAP impact on TA of kiwifruit is greater at an early stage of storage (Marsh *et al.*, 2004). MAP reducing TA at 0 °C has been reported on 'Hayward' in the first 60 days of storage, but the difference between MAP and control reduced after 90 days of storage (Zoffoli *et al.*, 2016). A similar result has been reported in a previous study that TA of kiwifruit stored in MAP for 30 days in cool storage is approximately 6% higher than that of control, while the difference declines to approximately 3% after 5 days of shelf life (Ozturk *et al.*, 2019b). However, MAP delaying TA reduction has been reported on 'Sanuki Gold' kiwifruit (Mworia *et al.*, 2011). The contrast may be caused by cultivar variation. The result of the present study was in line with the previous study on 'Hayward'. TA in the current study was significantly decreased by MAP in the 35 days of cool storage, but the MAP effect reduced in the following 10 days of shelf life. At the end of storage, only fruit stored in MAP for both cool storage and shelf life reached a lower TA level, while TA of fruit stored out of MA in either of the two stages remained at higher level. Longer storage at 20 °C is required to determine the influence of MAP on TA of kiwifruit at eating-firmness.

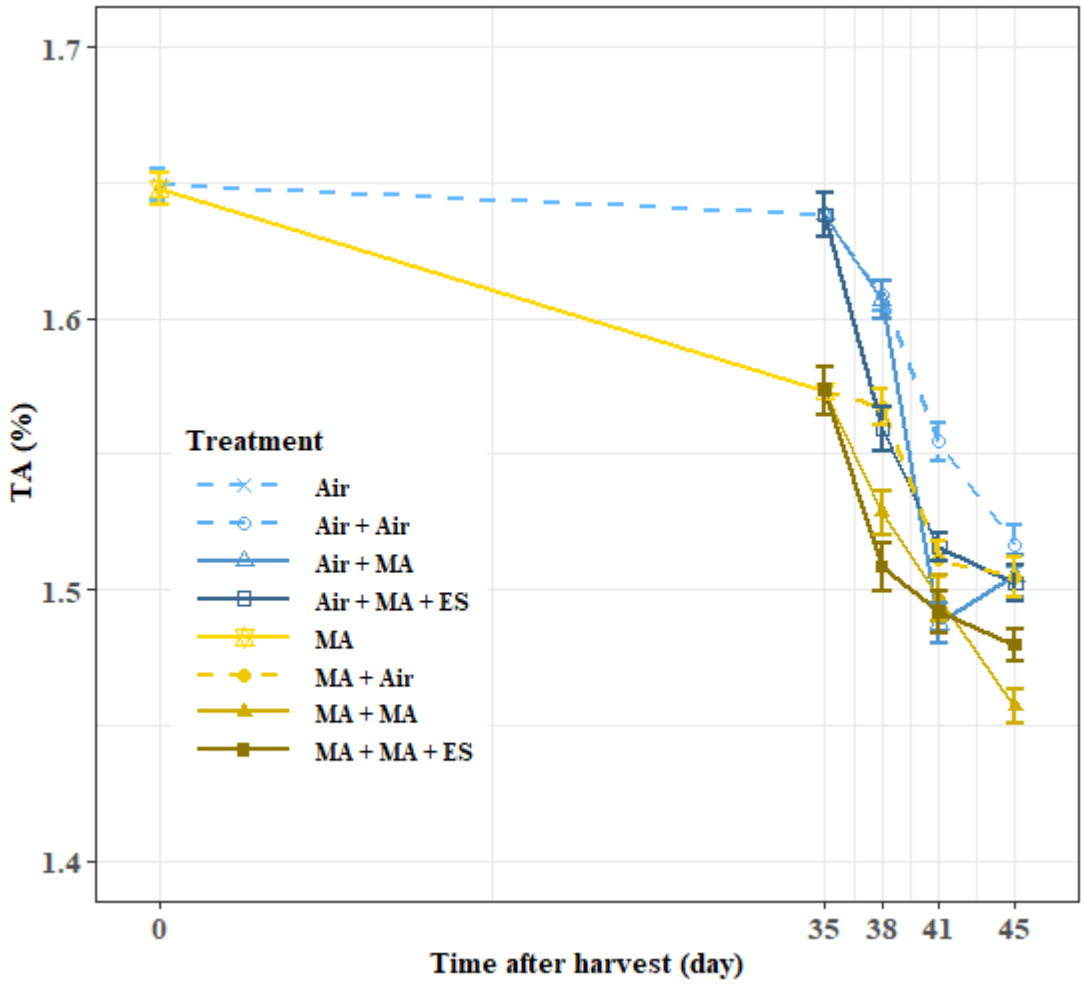

Figure 4.9 Titratable acidity (TA) of 'Hayward' kiwifruit stored in polyethene film (Air) and modified atmosphere bag (MA) at 1 ºC for 35 days followed by shelf-life at 20 ºC for 10 days. Air + Air: cool-stored in air followed by shelf life in perforated bag; MA + Air: cool-stored in MAP followed by shelf life in perforated bag; Air + MA: cool-stored in air followed by shelf life in non-perforated bag; MA + MA: cool-stored in MAP followed by shelf life in non-perforated bag; Air + MA + ES: cool-stored in air followed by shelf life in the non-perforated bag with ethylene scrubbing sachet; MA + MA +ES: cool-stored in MAP followed by shelf life in the non-perforated bag with ethylene scrubbing sachet. Each data point represents mean ± SEM, n = 30.

Fruit with both low acidity and low sugar is likely to produce a 'blander' flavour which is not preferable by consumers. However, again, this result is based on unripe fruit. The actual flavour of ripe fruit has not been assessed in this study. As dramatic physiological changes occur during a later stage of ripening when flesh firmness reduces to below 1 kg_f and ethylene production rises, it is possible that sugar and acidity contents change by then.

4.3.7 Rot and disorders

Rotten fruit were observed in 6 shelf-life packs on the 10th day of 20 °C storage (Figure 4.10). C_2H_4 at a concentration higher than 100 $nL \cdot L^{-1}$ was detected in the packs with one or more rotten fruit. The rest of the fruit in the same packs with the rotten fruit were markedly softer compared to the other packs in the same treatment (data not shown). There was no statistical difference of rot incidence between treatments. No other physiological disorder was observed in the cool storage or shelf life of this experiment.

Figure 4.10 Rotten kiwifruit after storage for 35 days at 1 ºC and 10 days at 20 ºC.

The rot of kiwifruit is caused by pathogens, such as *Botrytis cinerea*, which enter fruit through the picking wound at the stem end or wound from physical damage on the skin (Bautista-Baños *et al.*, 1997). The high RH and relatively high temperature (20 °C) create an optimal environment for pathogen growth (Bautista-Baños *et al.*, 1997). A large amount of C_2H_4 can be produced by the infected fruit as well as the pathogen (Qadir *et al.*, 1997). Slow diffusion of C_2H_4 through the film can potentially enhance the C_2H_4 accumulation in the package and induce rapid softening of other fruit in the same pack (Antunes & Sfakiotakis, 2002). However, packing fruit in the small unit of shelf-life pack can limit the C_2H_4 in the pack, which protects the surrounding packs of fruit from C_2H_4 contamination. At this point, MAP can potentially reduce fruit spoilage especially in a large distribution centre or during long storage.

4.4 Conclusion

The gas composition in MAP headspace was steady during cool storage and shelf life. An internal atmosphere of 3-4% CO_2 and 12-15% O_2 was established in MAP during 35 days of storage at 1 °C. The atmosphere generated in the MAP shelf pack at 20 °C was 12-16% O_2 and 7-10% CO_2. There was no detectable C_2H_4 accumulation in shelf packs, except for a few packs with rotten fruit. The effect of MAP and ethylene scrubbing sachet include:

- MAP can effectively reduce weight loss in both cool storage and shelf life.
- MAP in cool storage retained higher firmness, but MAP and MAP with ethylene scrubbing sachet did not alter flesh firmness in shelf life.
- MAP can slow down SSC increase, delay the peak of SSC and suppress the subsequent reduction of SSC. However, this difference was only statistical, but this difference does not necessarily impact fruit quality.
- MAP can reduce TA at the early stage of storage, but the difference of TA between MAP and control declined at the end of shelf life.
- Rotten fruit was observed at the end of shelf life associated with high C_2H_4 concentration and soft fruit in the same packs, which may be caused by physical injury, relatively high temperature and high humidity. Ethylene scrubbing sachet did not reduce C_2H_4 concentration in MAP, nor alter the fruit quality during 10 days of shelf life.

However, the fruit did not reach the end of storage life within the current timeframe. A longer storage time is required to determine the effect of MAP and ethylene absorbent on the eating quality of kiwifruit.

Chapter 5. General Discussion and Conclusion

5.1 Introduction

Applying MAP during coolstorage or after storage, at elevated temperatures, are approaches to extend kiwifruit storage life for international trading and year-round supplying. There exists a risk of ethylene accumulation in MAP due to physical injury or fruit rot. As kiwifruit is highly sensitive to ethylene, it is critical to understand the impact of ethylene on fruit quality in MAP storage. In addition, rapid softening of fruit previously stored in CA or MAP during the post-storage stage has been previously reported and MAP storage at warm temperatures may be particularly beneficial in preventing this. The objective of this study was to understand the influence of ethylene at different concentrations on kiwifruit stored under an optimal controlled atmosphere, and the effect of MAP applied on kiwifruit during coolstorage or the following shelf-life, and also the impact of ethylene scavenger in MAP. This chapter is going to cover the key findings, the industry implications and suggestions for future study will be discussed.

5.2 The impact of ethylene in modified atmosphere

5.2.1 Dose effect of ethylene on kiwifruit

Ethylene effect on kiwifruit softening in air during cold storage

The effect of ethylene on kiwifruit softening in air storage is affected by ethylene concentration and exposure duration. Excessive fruit softening was induced by ethylene at the concentration of as low as 10 $nL \cdot L^{-1}$, and higher ethylene levels further accelerated softening. The softening was accelerated as soon as the fruit were exposed to ethylene (Figure 3.2). It has been well documented that the role of ethylene in accelerating kiwifruit ripening is proportional to the applied dose, rather than ethylene serving as a trigger of rapid ripening (Arpaia *et al.*, 1986; Hertog *et al.*, 2016; Jabbar & East, 2016). Previous research indicated that ethylene at the concentration of 10-1000 $nL \cdot L^{-1}$ promoted kiwifruit softening during cool storage in air for ‘Hayward’ and ‘Hort16A’. Longer exposure duration and higher ethylene concentration led to more severe softening (Pranamornkith *et al.*, 2012; Jabbar & East, 2016). A similar result has been obtained in the present study, but a saturation point has been discovered. The softening of fruit exposed to 1000 $nL \cdot L^{-1}$ ethylene was slightly greater than that exposed to 100 $nL \cdot L^{-1}$

ethylene during the ten-week ethylene treatment, suggesting fruit response to ethylene might be approaching saturation at that dose. Higher ethylene concentrations were assessed than in previous research. 'Hayward' kiwifruit stored in 200 $\mu L \cdot L^{-1}$ ethylene showed no further softening compared to that in 10 $\mu L \cdot L^{-1}$ ethylene (Hertog *et al.*, 2016). Thus, in air storage, this thesis has demonstrated that the minimum concentration of ethylene that induces 'Hayward' kiwifruit softening is 10 $nL \cdot L^{-1}$, while the response saturating point is close to 1000 $nL \cdot L^{-1}$. Between 10 and 1000 $nL \cdot L^{-1}$, the effect of ethylene on 'Hayward' softening is dose-dependent.

Ethylene effect on kiwifruit softening in controlled atmosphere at low temperature

The effect of ethylene on kiwifruit stored under optimal CA (2% O_2 and 5% CO_2) has shown a different pattern. The excessive softening did not appear instantly after ethylene was added to the treatments. Fruit exposed to 1000 $nL \cdot L^{-1}$ ethylene first showed accelerated softening after 6 weeks of ethylene treatment, and fruit exposed to lower levels of ethylene showed advanced softening even later. Ethylene level of 10 $nL \cdot L^{-1}$ did not induce additional kiwifruit softening during 10 weeks of exposure (Figure 3.2). This result indicates that the effect of ethylene on kiwifruit softening was suppressed by CA. Compared to air storage, CA-stored kiwifruit showed reduced sensitivity, delayed softening and the degree of softening was not as great as air-stored kiwifruit. The dose-effect of ethylene exists in CA storage as well, but the minimum ethylene concentration that induces 'Hayward' softening was between 10 and 100 $nL \cdot L^{-1}$, i.e., higher than that in air. Meanwhile, the saturation point was not observed in this study so is presumably above 1000 $nL \cdot L^{-1}$ in CA. Previous studies on the effect of ethylene on the softening of CA-stored kiwifruit were done in the 1980s (Arpaia *et al.*, 1982; Arpaia *et al.*, 1985, 1986). The minimum ethylene concentration that accelerated 'Hayward' kiwifruit softening was 50 $nL \cdot L^{-1}$. Combined with the current study, CA reduces the sensitivity of 'Hayward' from 10 $nL \cdot L^{-1}$ in air to somewhere between 10 and 50 $nL \cdot L^{-1}$ in CA. Meanwhile, the softening of kiwifruit in 5000 $nL \cdot L^{-1}$ ethylene was similar to that in 1000 $nL \cdot L^{-1}$ ethylene, suggesting the saturation point was close to 1000 $nL \cdot L^{-1}$ in CA. In between 50 and 1000 $nL \cdot L^{-1}$, the ethylene effect of inducing kiwifruit softening in CA is also dose-dependent.

It is worth noticing that even with the saturation level of 5000 $nL \cdot L^{-1}$ ethylene, CA stored kiwifruit was still at the same firmness as that stored in ethylene-free air (Arpaia *et al.*, 1986). A similar result has been observed in the present work that the firmness of kiwifruit

exposed to 1000 $nL \cdot L^{-1}$ ethylene in CA for 10 weeks only just reached the same level of that in ethylene-free air. These data have suggested the effect of optimal CA on kiwifruit firmness is greater than that of ethylene, even when ethylene concentration reaches the saturation point. At this point, ethylene contamination is not a concern for kiwifruit stored in CA, as ethylene cannot enhance softening beyond air-storage level even in the worst scenario.

However, the firmness was assessed within 24h of removal from CA storage at 0 °C, with no additional room temperature assessment. It is possible that rapid softening occurs after a few days of shelf-life assessment (Ozturk *et al.*, 2019b). Thus, a post-storage shelf-life assessment is required to determine the quality of CA stored kiwifruit with ethylene exposure. If rapid softening occurs within a short time after removal from storage, ethylene management is still essential to obtain desired fruit quality. To investigate the post-storage quality issue, a ten-day shelf-life has been included in the MAP experiment in the current study, but not in the ethylene dose experiment. It is worthwhile for future work to assess the quality evolution during the shelf-life after kiwifruit exposure to ethylene in CA storage at low temperatures.

In the meantime, in the practice of the current supply chain, it takes four to six weeks to deliver kiwifruit to key overseas markets by sea freight. The current result has shown that the firmness of kiwifruit stored in CA was not dramatically impacted by ethylene during six weeks exposure at 0 °C, suggesting ethylene is not an issue for fruit that are directly shipped after harvest. However, as the impact of ethylene on kiwifruit firmness is dose-dependent, longer exposure to ethylene can lead to a more severe influence. In fact, kiwifruit can be stored up to six months from production in both the north and south sphere to ensure year-round supply. The impact of ethylene in CA or MA for such a long time has not been revealed.

On the other hand, the gas composition of 2% O_2 and 5% CO_2 in this study is the optimal CA condition, but this atmosphere has not been successfully created by current MAP films. The O_2 concentration in MAP is likely to be between 10% and 18% for kiwifruit, while that of CO_2 ranges from 1.5% to 9% (Gao *et al.*, 2014; García, 2015; Zoffoli *et al.*, 2016; Jiao *et al.*, 2020; Wang *et al.*, 2021c). The impact of ethylene in these non-optimal atmospheres may differ from the current result, which requires further investigation as well.

Ethylene effect on kiwifruit disorder in controlled atmosphere

Apart from accelerating softening of CA-stored kiwifruit, ethylene also causes a physiological disorder termed white-core inclusions (WCI), which is due to starch degradation interrupted by CO_2 and ethylene interaction (Arpaia *et al.*, 1982). WCI was observed in the present work, after 8 weeks of exposure to ethylene at the concentration of 0.1 $\mu L \cdot L^{-1}$ or higher, with the highest incidence being 60% in case of fruit stored with 1 $\mu L \cdot L^{-1}$ ethylene (Figure 3.10). However, in the previous work (Arpaia *et al.*, 1986), WCI emerged from 4 weeks at 0.05 $\mu L \cdot L^{-1}$ ethylene exposure and the incidence reached 100% from 8 weeks of storage. The lower WCI incidence and later occurrence in the present study may be related to higher at-harvest maturity of the fruit, which was 12.9% SSC in the current work compared to 8% in the study of Arpaia *et al.* (1986). The lower WCI incidence in mature kiwifruit may suggest that kiwifruit at more advanced maturity are more sensitive to ethylene. However, the lower WCI incidence may also be the result of most starch has already converted to sugar before ethylene exposure, On the other hand, in the work of Arpaia *et al.* (1986), the assessment was carried out after 7 days of shelf-life in air at 20 °C, while fruit was assessed within 24 h after removal from storage the present work, with no subsequent shelf-life. Hence, it is possible that WCI symptom progresses during the post-storage shelf-life. Since the mechanism of the formation of WCI is unclear, future work conducted with multiple fruit maturities and the observation of symptom development during shelf-life are required; and the influence of CA and ethylene on starch enzyme activity is to be discovered in the future.

5.2.2 Mechanisms of O_2, CO_2 and ethylene interaction on kiwifruit ripening

The two key events involved in kiwifruit ripening are fruit softening caused by pectin modification in the cell wall and SSC increase from starch degradation (Oscar *et al.*, 2019; Lin *et al.*, 2020). Firmness is a critical quality parameter to determine storability, whilst SSC affects fruit taste. In the present study, the evolution of firmness and SSC have been monitored throughout the storage period. Both results from CA and MAP stored fruit showed that the response of SSC to multiple treatments differed from that of firmness, suggesting starch breakdown and cell wall metabolism are influenced by different mechanisms.

The firmness of kiwifruit is sensitive to both ethylene and respiratory gases. There is clearly a dose-effect of ethylene on kiwifruit softening, and the softening process of

kiwifruit responds to atmosphere modification (Figure 3.2). Cell wall degradation genes in kiwifruit are highly regulated by ethylene signalling (Lin *et al.*, 2020). It is very likely that CA and MAP delay kiwifruit softening via ethylene perception and biosynthesis. On the other hand, the change of SSC is sensitive to atmosphere modification but not as sensitive to ethylene treatments (Figure 3.5), indicating the existence of ethylene-independent responses of carbohydrate metabolism in kiwifruit.

Kiwifruit ripening is a complex of multiple physiological changes, including respiration rise, ethylene production increase, firmness decrease, soluble sugar accumulation, acidity decline, aroma development, etc. Ripening, as a whole process, is impacted by ethylene metabolism. Ethylene reception begins with ethylene molecule bind to ethylene receptor, which releases the negative regulation and triggers downstream responses (McManus, 2012). After ethylene-dependent ripening genes are activated, ripening processes are accelerated, including autocatalytic ethylene production, cell wall modification and starch degradation (Pech *et al.*, 2012). However, ethylene is not the sole factor that regulates ripening. Ethylene-independent ripening has been reported in several kiwifruit cultivars, such as 'Rainbow Red' and 'Sanuki Gold' (Mworia *et al.*, 2012; Mitalo *et al.*, 2018). It has been suggested that the effect of MA on delaying fruit ripening is due to O_2 and CO_2 interfering with ethylene receptors as well as enzymes in ethylene biosynthesis pathway (Burg & Burg, 1967; Rothan & Nicolas, 1994). However, recent studies have revealed that the regulation of O_2 and CO_2 on some ripening events do not impact ethylene metabolism (Park *et al.*, 2018). It has been illustrated on tomatoes that some genes involved in starch metabolism are CO_2 responsive (Park *et al.*, 2021), and CA-regulated starch degradation genes have been discovered in kiwifruit (Hu *et al.*, 2016b). Hence, it is indicated that ethylene, O_2 and CO_2 impact kiwifruit ripening via different pathways. Ethylene accelerates kiwifruit ripening by triggering the whole ripening process, whereas O_2 and CO_2 affect kiwifruit ripening via both ethylene-dependent and ethylene-independent pathways. Firmness decline during kiwifruit ripening is ethylene dependent, while ethylene-independent regulation functions in starch metabolism.

Other than regulating gene expression via both ethylene-dependent and ethylene-independent pathways, O_2 and CO_2 also impact kiwifruit ripening by directly interfering with enzyme activities. Reduced O_2 and elevated CO_2 suppress respiration and reduce ATP supply, which slows down processes that require ATP, such as glucan-water dikinase activity in starch degradation (Bornke & Sonnewald, 2011). High CO_2 can also

reduce cellular pH, which favours the enzyme activity that requires low pH but restricts the enzymes active in higher pH, such as α-amylase activity in starch conversion to soluble sugars (Wegrzyn & MacRae, 1995). On the other hand, oxidising reactions, for instance, lipid peroxidation in fruit ripening (Huang *et al.*, 2019), require the participation of O_2, which can be slowed down when the O_2 level is low. Since O_2 and CO_2 affect kiwifruit ripening at multiple points, which involve cell wall changes, carbohydrate metabolism, and membrane breakdown, the effect on these processes could be different from different O_2-CO_2 concentrations and the interaction between respiratory gas compositions and ethylene concentration or temperature. It has been observed in the current study that applying MAP during post-storage shelf-life did not alter the firmness of kiwifruit, but slightly delayed SSC decrease. As the taste of the fruit is highly correlated with SSC, firm fruit with higher SSC are preferred by consumers. Even though firmness reduction is a key issue impacting kiwifruit storage life, different parameters of fruit quality, such as SSC evolution, should be considered when determining the MAP effect on fruit quality instead of firmness only.

5.3 The effect of MAP on kiwifruit quality

5.3.1 Mechanism of MAP retaining firmness

Firmness is a key parameter determining the storage life of kiwifruit (Goldberg *et al.*, 2019). Fruit texture changes during ripening involve multiple processes, such as turgor pressure loss, starch breakdown, membrane composition change and cell wall modification (Hallett *et al.*, 1995; Burdon & Clark, 2001; Schroder & Atkinson, 2006; Shakya & Manju, 2018). Water loss and cell wall degradation are the two main factors leading to kiwifruit softening (Burdon & Clark, 2001; Fullerton, 2015). Theoretically, MAP contributes to both reducing water loss and delaying cell wall degradation, but which is the key contribution of MAP to firmness retaining?

This question can be answered by comparing the firmness change to WL during both coolstorage and shelf-life. In the current MAP study, fruit stored in MAP during coolstorage were 1 kg_f firmer than that in control, while there was no firmness difference between perforated and non-perforated packaging during shelf-life assessment (Figure 4.7). On the other hand, the WL of fruit in MAP was just 0.15% lower than control during coolstorage, but the WL difference between perforated and non-perforated packs during

shelf-life assessment was 0.6% to 0.7% (Figure 4.6). Thereby, MAP retaining kiwifruit firmness was mainly due to slowing down cell wall degradation rather than reducing turgor pressure loss. The key contribution of MAP to retaining kiwifruit quality is delaying ripening by reduced O_2 and elevated CO_2, while reducing water loss is just an additional benefit. However, reducing WL during storage can potentially lead to a higher saleable weight which is a commercial benefit.

5.3.2 MAP effect on kiwifruit quality during coolstorage and shelf-life

The main effect of MAP on kiwifruit is delaying cell wall degradation and retaining firmness. In the current work, this benefit emerged during the five weeks of storage at 1 °C immediately started after harvest while the fruit firmness was not altered by bag perforation during subsequent storage at 20 °C. Neither additional impact nor loss of beneficial effect was observed during shelf-life assessment (Figure 4.7). It has been revealed that MA should be established within a short time after harvest, otherwise the benefit of retaining kiwifruit firmness reduces. The rapid softening of kiwifruit occurs during the first 30-45 days after harvest (Arpaia *et al.*, 1984). In the current work, MAP was established within one week of harvest for the coolstorage, which provided a beneficial effect on delaying softening. Whereas, the shelf-life assessment started after five weeks of storage when fruit were relatively soft and had passed the rapid softening stage, which leads to no additional effect on firmness maintenance. However, all the fruit experienced slow softening during the 10 days of shelf-life assessment at 20 °C and did not reach the threshold of 1 kg_f when a large amount of ethylene was likely to be produced (Antunes, 2007). It is possible that the 10 days at 20 °C was not long enough for advanced softening to happen, thus no difference between the treatments was observed. It is possible that the firmness of fruit in perforated and non-perforated bags start to differ after a longer shelf-life duration. Hence, a longer shelf-life assessment is required for future research to investigate the effect of MAP at room temperature on softer fruit.

The increase of SSC is a result of starch conversion (MacRae *et al.*, 1992). The delay of SSC increase indicates MAP suppressing starch metabolism. The SSC value depends on the balance between starch degradation and energy consumption. The optimal quality refers to high SSC, which means the stage after starch is converted to sugar and before sugar is consumed for energy. SSC decline in shelf life refers to high respiration which consumes energy. This is more likely to happen in shelf life than in cool storage due to the higher temperature.

Temperature is another key factor that changed during repacking. It is suggested that at a temperature higher than 10 °C the effect of reduced O_2 and elevated CO_2 is very limited (Hertog *et al.*, 2004). Elevating the storage temperature from 1 °C to 20 °C can enhance enzyme activities that were suppressed by MA at low temperatures. This could be another reason for no difference of firmness being observed between fruit stored in perforated and non-perforated bags. However, the value of SSC decreased in fruit stored in perforated bags at the late stage of shelf-life assessment, regardless of the previous treatment during coolstorage (Figure 4.8). This could be explained by respiration enhanced by elevated temperature in air, and MAP significantly suppresses the respiration of kiwifruit, as sugar is the direct carbohydrate source for respiration (Wang *et al.*, 2021c). In this case, MAP has the potential benefit of delaying SSC decrease by reducing respiration rate, but the difference was only statistically significant and unlikely to influence taste. The interaction between temperature and atmosphere could be examined by monitoring enzyme activities during temperature shifts and gas composition change in the future.

Although applying MAP at room temperature did not provide extra benefit, no negative effect was observed after storage. Rapid softening and quality decline has been reported for apple and kiwifruit after removal from CA or MAP storage (Ozturk *et al.*, 2019b; Poirier *et al.*, 2020). It is suggested that the phenomenon of post-storage quality decline is caused by elevated temperature reactivating the enzymes that were previously inhibited by low temperature, and the accumulated intermediate substrates were catalysed rapidly. If this is the case, applying MAP at room temperature after CA or MAP storage could potentially alleviate this post-storage quality decline. However, rapid softening or other quality drop was not observed in the present study, perhaps because of the relatively short storage period. Thus, the effect of MAP on post-storage quality maintenance has not been examined. It is likely that the accumulation of intermediate substrates was low during the five-week coolstore. A longer storage duration would be helpful to investigate the effect of MAP on the post-storage quality of kiwifruit in the future.

5.4 Risk assessment of ethylene in MAP

Apart from the ethylene produced by the adjacent kiwifruit, ethylene contamination from other fruit in the distribution facility or from fossil fuel combustion during transport is also risky for kiwifruit. The sealed MAP has the advantage to isolate kiwifruit from this

part of ethylene in the environment compared to other types of packaging. Thus, ethylene risk to MAP-packed kiwifruit mainly comes from the packed fruit themselves.

MAP is an enclosed system that restricts gas transmission through the film. Permeability of polymer film used in MAP to ethylene is between 2.68×10^{-16} and 3.51×10^{-15} $mol \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$ (Wang *et al.*, 1998; Paz *et al.*, 2005). On the other hand, the ethylene production rate of kiwifruit firmer than 1 kg_f is below 0.1 $\mu L \cdot kg^{-1} \cdot h^{-1}$ (Kim *et al.*, 1999), while the ethylene production of soft kiwifruit (< 1 kg_f) can reach up to 1.2 $\mu L \cdot kg^{-1} \cdot h^{-1}$ at 0 °C (Yang *et al.*, 2013) and 15 $\mu L \cdot kg^{-1} \cdot h^{-1}$ at 20 °C (Antunes & Sfakiotakis, 2002). Taking the packaging used in the shelf-life assessment of the current MAP experiment, for example, fruit mass of 800 g per pack, the ethylene production of one soft fruit with seven firm fruit (100 g per fruit) is 0.19 $\mu L \cdot h^{-1}$ at 20 °C. With the surface area of 0.09 m^2, thickness of 0.036 mm, the ethylene diffusion rate of the packaging will be 7.48×10^{-17} to 9.79×10^{-16} $L \cdot h^{-1} \cdot Pa^{-1}$. The ethylene concentration in the headspace of 1 L will reach 10 $nL \cdot L^{-1}$ within 20 h. Thus, there is a potential risk for ethylene accumulation in MAP for kiwifruit, especially at the late stage of storage.

In the current study, ethylene concentration in shelf-life packs after 7 days of storage at 20 °C was undetectable for packages with sound fruit (Figure 4.5), indicating no risky ethylene accumulation was occurring. In conjunction with the firmness data (> 1.5 kg_f for all treatments) (Figure 4.7), it suggested that the current films and practices were safe for kiwifruit storage within the 10-day timeframe. Meanwhile, it has been observed in this study that the ethylene levels in the packages with rotten fruit reached between 1000 and 6000 $nL \cdot L^{-1}$, and the rest of the fruit in the same pack were soft (Figure 4.5), which suggests ethylene produced by rotten fruit causes ethylene build-up in MAP that was not prevented by a sachet of ethylene scavenger. The impact of ethylene on fruit quality in these packs was not inhibited by MAP.

Due to the high possibility of ethylene accumulation and the existence of an ethylene effect on kiwifruit softening in MAP, ethylene management is essential for applying MAP on kiwifruit during long storage. Removing soft, damaged, and rotting fruit during pre-packing quality check could delay and reduce ethylene production in the MAP, and ethylene scavenging could potentially contribute to lower ethylene accumulation in MAP.

5.5 Ethylene scavenger

Ethylene-removal agents have been developed to address the ethylene accumulating issue in fruit packaging. Oxidation-based materials, such as potassium permanganate ($KMnO_4$), sodium permanganate ($NaMnO_4$) and titanium dioxide (TiO_2), can catalyse ethylene to water and CO_2; whereas absorption-based material, such as active charcoal, zeolites, and clays, reduce ethylene by absorption (Awalgaonkar *et al.*, 2020). The ethylene scavenger applied in the current study was $KMnO_4$ in the form of a sachet inserted within MAP prior to sealing.

In the present study, there was no significant difference in ethylene concentration between packs of fruit with and without ethylene scavenging sachet (Figure 4.5), perhaps because the fruit firmness was relatively high (> 1 kg_f) and did not reach the point where larger amounts of ethylene are produced. In the meantime, ethylene at the concentration of 960 $nL \cdot L^{-1}$ was detected in one pack with ethylene scavenger which contained one rotten fruit (Figure 4.5), indicating the scavenger could not prevent ethylene accumulation in the presence of a single rotten fruit. In addition, it has been reported that $KMnO_4$ sachet applying in MAP with blueberry and apricot sufficiently reduced ethylene concentration in the headspace from 50-60 $nL \cdot L^{-1}$ to below 5 $nL \cdot L^{-1}$, however, the fruit quality in both of these experiments was not impacted (Álvarez-Hernández *et al.*, 2019b; Álvarez-Hernández *et al.*, 2020). In the situations listed above, the ethylene scavenger did not alter fruit quality due to: a) ethylene production was low and there was no ethylene accumulation in MAP; b) ethylene concentration was too high that exceeded the capacity of scavenger, or ethylene production by rotten fruit was faster than $KMnO_4$ catalyzing; c) the ethylene concentration was within the capacity of scavenger but was lower than the threshold of triggering fruit ripening. Hence, it is suggested that ethylene scavenger is effective on reducing ethylene impact on fruit quality only under the circumstances that: a) the fruit has begun to produce ethylene and b) that the ethylene production is within the capacity of the scavenger, and c) the potential accumulation is higher than the minimum level that accelerates fruit ripening.

The capacity of ethylene scavengers is limited. It has been demonstrated that the effective weight of $KMnO_4$ applied in the fresh product was generally 4-6 % of the product weight (Awalgaonkar *et al.*, 2020), however, the effectiveness declined rapidly during the storage due to the increased difficulty of ethylene diffusion to the internal part of the

sachet before $KMnO_4$ was fully reacted (Keller *et al.*, 2013). A supporting agent with micropores, such as zeolite and silica, has been employed to improve the surface area of ethylene oxidation catalyzers and improve the performance of ethylene removal (Wei *et al.*, 2021). However, the sachet used in the present study was a commercial product that contains both KMnO4 and supporting agent, but the $KMnO_4$ near the sachet surface was still rapidly consumed at the beginning of ethylene rise, while a large amount of ethylene produced by rotten fruit did not effectively react with $KMnO_4$ in the centre of the sachet (Álvarez-Hernández *et al.*, 2019a), which leads to the subsequent fruit softening and ethylene production. Thus, the $KMnO_4$ sachet as ethylene removal approach cannot always effectively reduce ethylene level in the MAP with rotten fruit, and other ethylene removal methods may be required to address the issue.

5.6 Cultivar variation

The storability of fruit is highly impacted by genetic background. The effect of cultivar diversity on MAP storage has been demonstrated on tomato, peach and nectarine (Akbudak & Eris, 2004; D'Aquino *et al.*, 2016). As for kiwifruit, with 'Hayward' being the first commercialized and still the major cultivar worldwide, most studies have been based on it. However, some recently developed cultivars have shown a diversity of requirements for storage conditions. For instance, the optimal gas composition in CA storage is 5% CO_2 + 2% O_2 for 'Hayward', but that for 'Hongyang' and 'Tewi' are 2% O_2 +3% CO_2 and 3% O_2 +5% CO_2, respectively (Li *et al.*, 2015; Pegoraro *et al.*, 2016). As for ripening induction, 'Garmrok' has displayed higher sensitivity to exogenous ethylene compared to 'Hayward' (Shin *et al.*, 2020), and 'Sanuki Gold' has shown a low temperature modulated ripening pathway (Mworia *et al.*, 2012). With the recent development of new cultivars, such as 'Gold3' (Morton *et al.*, 2018), 'Red9' (Henwood *et al.*, 2018), 'Hongyang' (Ma *et al.*, 2014), 'Cuixiang' (Jiao *et al.*, 2020) and kiwiberries (Latocha *et al.*, 2014), the responses of these cultivars to different storage conditions are unknown. Further study is required on each of the new cultivars. The initial purpose of this study was to prolong the storage life of 'Red19', while the current work was conducted on 'Hayward' due to the COVID pandemic. As the genetic background and physiological metabolism of these cultivars are quite different, the results of this study cannot be directly applied to 'Red19'. Hence, validation is needed on 'Red19' in the future.

5.7 Conclusion

The relatively short storage life of new kiwifruit cultivars has created a challenge for international trading. Applying MAP with reduced O_2 and elevated CO_2 during low-temperature storage and the subsequent room temperature shelf-life can retain fruit freshness and extend the storage life. However, ethylene accumulation in the enclosed packaging is a potential risk that induces kiwifruit storage. Hence, it is critical to understand the interactions between ethylene, O_2, CO_2, and the storage temperature, and to estimate the risk of ethylene under modified atmosphere.

It is indicated by the present study that the effect of ethylene on kiwifruit quality is dose-dependent in both air and CA storage. The damage of ethylene can be reduced and delayed by CA. The impact of ethylene is greater on firmness compared to that on SSC, suggesting cell wall metabolism is ethylene dependent, while starch metabolism is regulated by both ethylene dependent and independent pathways. The mechanism of the atmosphere regulating carbohydrate metabolism remains unclear. Future work on ripening-related gene expression and enzyme activities impacted by O_2 and CO_2 is required.

Fruit quality assessment after short-term storage has suggested that ethylene accumulation in CA and MAP is not an issue for the current sea freight timeframe but could be a potential risk for long term storage due to the excessive softening and the occurrence of WCI disorder. Thus, ethylene management is required for extended storage.

Storing kiwifruit in MAP at low temperature can delay fruit softening while applying MAP at room temperature following short-term storage has neither negative effect nor additional benefit. However, rapid quality decline usually occurs after kiwifruit being removed from long-term storage and is remains unknown whether repacking kiwifruit into MAP improves this fruit ‘crash’. Hence, a longer storage period at low temperature is required in the future to determine the effect of MAP on kiwifruit quality during the post-storage period.

There was no detectable ethylene accumulation in the tested MAP films with firm kiwifruit, but high levels of ethylene have been observed in the packs with the presence of rotten fruit. Ethylene scavenger cannot improve fruit quality of firm kiwifruit due to extremely low ethylene production, but it also did not inhibit fruit softening in the

presence of a single rotten fruit as ethylene produced by rotten kiwifruit exceeded the capacity of ethylene scavenging sachet to lower the free ethylene concentration in the sachet to below 10 $nL \cdot L^{-1}$. It is recommended that a post-storage assessment would be required to ensure that. If MAP were applied (using bags optimised for warm temperature handling) after storage, all soft, damaged, or infected fruit should be first removed. A hand-held non-destructive device could be tested for this purpose.

This work was originally designed for 'Red19' to investigate the optimal O_2 and CO_2 concentration for storage and to assess the effect of MAP during coolstorage and post-storage period. However, due to the pandemic of COVID-19, the fruit season of 'Red19' was missed. Hence, the research content for this past season was modified and conducted on 'Hayward'. Meanwhile, the work on 'Red19' is proposed to form a subsequent study.

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