# Ethylene flux in postharvest kiwifruit systems

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# Abstract

Damaged or rotten kiwifruit or change of environmental conditions (temperature) through the supply chain may trigger premature ripening and softening of sound fruit as a result of the expected higher rates of ethylene production caused by these events. This thesis quantified some of the key factors which will govern ethylene composition within a commercial kiwifruit package (targeting 'Hayward' variety) as a preliminary step for constructing a predictive model that enable interpreting of ethylene from the sensor which could be used in detecting quality of kiwifruit within a package.

Ethylene production was found to be strongly associated with kiwifruit firmness and temperature of 'Hayward' kiwifruit. Maximal ethylene production (16,000 to 120,000 times that of minimal production) was observed when kiwifruit firmness reached less than 13 N suggesting that detection of ethylene concentration within a kiwifruit box should be able to be used to provide a reasonable estimate of the firmness of the fruit within the package. Lower rates of ethylene production were measured at 0 and 2 °C in comparison to previously reported data due to the advantage of using a newly developed high sensitivity ethylene detector, ETD-300 in present study. Ethylene production data obtained at a broad range of potential supply chain temperatures (0, 2, 5, 10 and 20 °C) concluded that at higher temperature (10 and 20 °C) initiation of an observable increase in ethylene production occurs at an earlier stage of firmness (10.5-13 N) while firmness of kiwifruit should reduce more (5.6-5.7 N) to observe this at lower temperature (0 and 2 °C). A simple mathematical model was developed which can be used to predict the ethylene production of 'Hayward' kiwifruit given a known fruit quality (firmness) and temperature condition.

Impact injured 'Hayward' kiwifruit produced high ethylene as a typical 'stress/wound' physiological response and results strongly indicated that temperature plays a significant role in controlling synthesis of wound ethylene by showing no effect at 0 °C and 2-3 times increase of ethylene production at 20 °C than at 5 °C. Two fold increase of rate of ethylene production was observed with different degree of impact damage (30, 60 and 120 cm drop heights) adding evidence to the effect of severity/degree of injury on increase of wound induced ethylene. Moreover, results

of two different maturity levels of kiwifruit demonstrated the further effect of firmness reduction of kiwifruit on increase of impact injury ethylene production.

A one to twenty times increase in ethylene evolution rate for 'Hayward' kiwifruit following subsequent transfer to a higher temperature from a lower temperature (0→2 °C, 2→5 °C, 5→10 °C, 10→20 °C) was demonstrated. Mathematical estimation of the desorbed ethylene at each transient increase of temperature using Henry's law revealed that there are other factors (via ethylene synthesis pathway) contribute to the escalation of ethylene evolution observed during and immediately subsequent to an increase in temperature other than contribution from the release of dissolved ethylene in the kiwifruit tissue based on Henry's law.

A six to eight times greater permeability of current commercial kiwifruit polyliner (HDPE) than what reported in literature for the similar type of film demonstrated the ethylene permeability differences that can be found as a result of the structure of the film (physical and chemical) as well as experimental conditions that are often not reported alongside the data presented. Permeability of the polyliner was found to be dependent on temperature as well as with concentration of ethylene. The model established to predict ethylene composition within different types of commercial packages available in the industry using log ethylene production rate ($fmol.kg^{-1}s^{-1}$) of kiwifruit and permeability of the polyliner ($mol.m.m^{-2}s^{-1}Pa^{-1}$) shows a 1.5 fold increase of log ethylene concentration (mPa) inside the kiwifruit package with the temperature decrease from 20 ºC to 0 ºC irrespective to the type of package.

Out of all the factors considered, the approximately 10,000–100,000 fold increase of ethylene production due to firmness change of kiwifruit dwarfs the 2-20 fold increase (due to injury or temporary temperature change) indicating that the ability to detect ethylene concentration inside a kiwifruit package could be applied in getting the information of the quality of the fruit (firmness) inside the package and hence identify the kiwifruit which require remedial action within the product stock.

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# Chapter 1

## PROJECT JUSTIFICATION

Kiwifruit which comes under the *Actinidiaceae* family, is considered as a major commercial crop which has a high potential for industrial exploitation throughout the world (Antunes, 2007) and also holds an important place in the New Zealand export industry (Martin, 2002). The green cultivar, 'Hayward' (*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson) and the gold cultivar, 'Hort16A' (*Actinidia chinensis* Planch.) are the two major commercial kiwifruit varieties currently produced in New Zealand (Mworia et al., 2010; Wang et al., 2011). Kiwifruit represents 28.7% of horticultural exports from New Zealand and has gained NZ$ 1,045 million, fob from kiwifruit exports in 2012 of which 'Hayward' (Green) represent the majority percentage. For the year 2011/2012, Zespri International sold 109 million trays of kiwifruit to more than 56 countries (Aitken et al., 2012). These statistics reflect that kiwifruit holds a good place in the international trade and mostly green cultivar 'Hayward' comes to the front out of all the varieties (such as Green, Gold3, Gold9) as the dominant variety of widely grown and relatively tolerant to the diseases such as Psa (Bacterial canker disease specific to kiwifruit caused by *Pseudomonas syringae* pv. *Actinidiae*). Ability to maintain reasonable shelf life of 6-10 months storage at 0 °C and 95% RH in air or CA conditions (5-8% $CO_2$ and 1-2% $O_2$) with ethylene concentration <30 $nL.L^{-1}$ enables exporters to transport 'Hayward' over long-distances with a good quality (Hewett et al., 1999). Considering the importance to the kiwifruit industry as described, the 'Hayward' variety of kiwifruit was selected for this research.

Factors such as temperature, damage, infection, exogenous ethylene concentration or exposure to ethylene inhibitors have been identified as factors influencing the level of ethylene production of kiwifruit. Biosynthesis of ethylene induced by these factors is generally called as stress induced ethylene production and can be divided into three categories: physical (wounding, high or low temperature), chemical (exposure to various chemicals) and biological (invasion by organisms such as fungi, bacteria, viruses) (Abeles et al., 1992). Kiwifruit is considered to be a unique climacteric fruit as its autocatalytic ethylene production occurs very late during ripening unless damaged or infected, whereas for classical climacteric fruit

ethylene production coincide with initiation and stimulation of ripening (Stavroulakis & Sfakiotakis, 1995; Antunes & Sfakiotakis, 1997; Antunes et al., 2000).

Firmness is considered as one of the most significant quality alterations consistently associated with ripening of fruit (Lelievre et al., 1997) which controls the consumer acceptability of the kiwifruit and is probably the best predictor of kiwifruit shelf life (Retamales & Campos, 1997; Kim et al., 1999). Fruit softening, even at low temperature storage such as 0 °C, is the main factor which contributes to the storage life limitation of kiwifruit and is a serious and costly problem for the New Zealand kiwifruit industry. Postharvest losses due to the rigors of transport and distribution of up to 5-10% have previously been reported (Hewett et al., 1999). Castillo et al., (1999); Kim et al., (1999); Menniti et al., (2005) and Antunes, (2007) have all reported that ethylene produced by kiwifruit [Healthy kiwifruit - 0.01 to 0.1 $pmol.kg^{-1}s^{-1}$ (Sfakiotakis et al., 1997; Castillo et al., 1999 and Pekmezci et al., 2004) and damaged or infected kiwifruit - 50 to 4000 $pmol.kg^{-1}s^{-1}$ (Niklis et al., 1997; Qadir et al., 1997; Sfakiotakis et al., 1997; Pekmezci et al., 2004)] is the potential promoter of kiwifruit softening and ethylene concentrations as low as 10 $nL.L^{-1}$ (produced by unripe sound kiwifruit) even at low temperatures (0 ºC) is sufficient for softening of kiwifruit (Jefffery & Banks, 1996). On the other hand, a number of reports describe kiwifruit producing detectable amounts of ethylene (600-1300 $pmol.kg^{-1}s^{-1}$) only after fruit have softened considerably [firmness below 13 N (Arapia et al., 1994); 10 N (Bonghi et al., 1996; Kim et al., 1999; Feng et al., 2003) or below 7 N (Ritenour et al., 1999)].

A vast variation of quality of fruit exists within stored stock. Ethylene production from a damaged or rotten fruit inside a package may trigger premature ripening or softening of the whole load of fruit during storage. The ability to check the quality of fruit within a package and to remove any damaged or rotten fruit from storage to avoid contamination of healthy fruit would be highly advantageous. As an answer to the problem, it has been identified that sensors could be used within the kiwifruit package as a tool to measure the current and future quality of the stock (East et al., 2011). Developing a sensor which could use measurement of ethylene concentration within an individual package to detect poor quality fruit (damaged or rotten) among a batch of kiwifruit and hence identify the need to take immediate action to remove poor quality fruit would provide financial benefit to the industry by reducing fruit wastage. However, to interpret the data obtained by the sensor it is

necessary to understand the relationships and interactions between the ethylene composition within the package with the status of the kiwifruit (firmness, injury), supply chain environment conditions (temperature) as well as package properties (permeability).

Cristescue et al., (2012) described the advantage of using the high sensitivity laser based technique applied in the ethylene detector (ETD-300, SensorSense, Nijmigen, The Netherlands) over other ethylene detection techniques. The minimum ethylene detection limit of ethylene detector (ETD-300) is 0.3 $nL.L^{-1}$ compared to the minimum detection limit of a traditional equipment like Gas Chromatography (GC) (5-10 $nL.L^{-1}$). Since ethylene production of sound kiwifruit is extremely low 0.01-0.1 $pmol.kg^{-1}s^{-1}$ (Sfakiotakis et al., 1997; Castillo et al., 1999; Pekmezci et al., 2004) especially at low temperature, there is a need to use a highly sensitive method to quantify ethylene production accurately. Even though there are several attempts recorded in the literature by Sfakiotakis et al. (1997); Castillo et al., (1999) and Pekmezci et al., (2004) based on quantifying ethylene production of kiwifruit under different conditions using low sensitive, time consuming, GC, there are no available data on attempts to use a highly sensitive ethylene detector (ETD-300).

This research project aims in providing some key quantification of flux of ethylene from kiwifruit and the associated packaging materials by using the ethylene detector (ETD-300) as an accurate method. The information obtained is necessary in enabling future construction of a predictive model of ethylene compositions within a commercial package that enables analysis of situation changes under different supply chain conditions. With the above identifications in mind the following specific objectives were set as research goals.

**Research Objectives**

a) Quantify rates of production of ethylene from 'Hayward' kiwifruit of different maturity stages (firmness) as a function of temperature and injury caused by impact damage.
b) Quantify rate and magnitude of desorption of ethylene from kiwifruit as caused by transient increased temperature within the supply chain.
c) Quantify transfer rates of ethylene through the polyliner used in the commercial kiwifruit package.

# Chapter 2

## LITERATURE REVIEW

### 2.1 Introduction

This chapter provides an insight into the past information on numerous factors affecting ethylene flux in the postharvest kiwifruit system at various stages of the supply chain. Information obtained was used as a basis for the development of the research strategy for this project and highlighted the significant issues which were taken into consideration throughout the research.

### 2.2 Kiwifruit

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson) which comes under the *Actinidiaceae* family, is an important commercial fruit in the world (Antunes, 2007). There are currently two major commercial kiwifruit varieties produced in New Zealand; the most widely grown 'Hayward' (*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson) and 'Hort16A' (*Actinidia chinensis* Planch.) (Patterson et al., 2003; Hunter et al., 2010; Mworia et al., 2010; Wang et al., 2011). According to the latest statistics, kiwifruit represents 28.7% of total horticultural exports from New Zealand and earned NZ$ 1,045 million, fob from kiwifruit exports in 2012. The variety 'Hayward' (Green) represent the majority percentage (66%) while remaining is from 'Hort16A' (Gold) (20%), and other cultivars (14%). For the year 2011/2012, Zespri™ sold 109 million trays of kiwifruit to more than 50 countries with Japan being the largest export market (Aitken et al., 2012). Nutritionally, kiwifruit have high levels of ascorbic acid or vitamin C (380 mg/100 g) which is twice that of orange (Crisosto & Kader, 1999) and is high in fiber content and folic acid concentration (Hewett et al., 1999). Apart from the nutritive value, customer's preferences are influenced by appearance, flavour and textural quality of the kiwifruit (Crisosto & Crisosto, 2001). The refreshing sweet/sour flavour in 'Hayward' attracts customers to have a mouth-watering, nutritious meal (Young et al., 1995; Martin, 2002).

#### 2.2.1 Morphology of kiwifruit

Kiwifruit is an elongated, oval shape fruit. The most important cultivar, 'Hayward' is typically 55-70 cm in length and 4-5 cm in width. When mature, weight of green

variety of kiwifruit is around 80-120 g and shape of the fruit gets flattened. The outer pericarp (flesh), the inner pericarp (seed area) and the columella (core) are the three main regions of the green-colored edible portion, with the columella being lighter green than the pericarp tissue (Hallet et al., 1992). The fruits fibrous, hard and inedible skin is initially green and becomes brown as the fruit ripens. The brown skin is relatively thin and includes a periderm (rather than an epidermis) and hypodermal cells (Crisosto & Kader, 1999). Kiwifruit is covered with multiseriate large and small hairs (mostly 2-3 mm long) called trichomes of which small hairs may be an arrested early stage of development of large hairs. Main function of this hair is to protect the fruit from excessive water loss by evaporation (Beever & Hopkirk, 1990). Gas exchange is mainly from openings where trichomes are removed. Stomata are not observed on the kiwifruit surface. Handling during harvesting and postharvest operations bend most of the small unicellular hairs on the surface of mature kiwifruit (Crisosto & Kader, 1999).

### 2.2.2 Horticultural maturity indices of kiwifruit

Kiwifruit are harvested at the pre climacteric stage with the aim to have a long storage and transport life. Presently fruit firmness, total soluble solids and days after full bloom to harvest are used as indices of maturity of kiwifruit. According to the New Zealand standard, a minimum brix value of 6.25–6.5% must be reached before harvesting kiwifruit. Flesh firmness equal to or higher than 60 N measured with the penetrometer (8 mm tip) is also used to identify the maturity level of kiwifruit (Crisosto & Kader, 1999). Kiwifruit harvested at later stage retain their flesh firmness during storage better than early harvested fruit and late harvested fruit usually have high SSC at harvest and at consumption (Arpaia et al., 1994). Internal ethylene concentration (IEC) cannot be used as a maturity index since it has been found that there was no remarkable change or increase in ethylene production during maturation (Sfakiotakis et al., 1997).

## 2.3 Supply chain

The variability in postharvest quality factors of fresh produce is as a result of pre and postharvest conditions and most importantly handling of fresh produce throughout the supply chain. Therefore, it is essential to demonstrate the best practices right through the supply chain to provide consumers with a highest quality fresh produce

(Martin, 2002). A significant amount of data is collected throughout the supply chain in order to fulfil this. For example, cold store temperature data is frequently monitored to ensure correct performance of the refrigeration and the quality attributes of fresh produce are recorded based on visual characteristics. Since the turn of the millennium, trays of kiwifruit produced have doubled but the number of pack-houses and cold stores has reduced by 43% and 22% respectively, reflecting the development of larger and more specialised facilities in the kiwifruit supply chain (Aitken et al., 2011).

### 2.3.1 Activities of the supply chain of kiwifruit

The New Zealand kiwifruit postharvest supply chain (Figure 2-1) consists of a number of activities such as harvest, packing, onshore cold storage, transporting and distribution. The harvesting season for kiwifruit is generally between the months of March and June (Gregori et al., 2002). Kiwifruit are picked manually by hand into picking bags once minimum maturity is reached (McDonald, 1990). However, in general all kiwifruit from a vineyard are harvested in a single pick as there are no visible unique features to help pickers identify immature from mature fruit and due to time and money wastage of using additional equipment to measure identified maturity indices of kiwifruit (Crisosto & Kader, 1999). Harvested fruit are placed in large wooden or plastic field bins and are transferred to the pack-house where the fruit is left in ambient air for 48-72 hours in a process known as 'curing' to allow picking wounds to heal and consequently reduce levels of postharvest rot (Gregori et al., 2002).

After curing, grading is conducted using kiwifruit graders. In New Zealand pack-houses, segregation of kiwifruit is conducted based on weight and size resulting in the rejection of damaged, misshapen or blemished fruit (approximately 10%) which is later used in fruit beverages or as stock feed (McDonald, 1990). However, development of new non-destructive technologies, such as Near Infrared (NIR) spectroscopy, density or impact force sensors may be useful to get more information on internal fruit quality attributes such as: dry matter, soluble solids and fruit firmness (Praat et al., 2003) to separate the sound kiwifruit. Adding to the new advancement in postharvest quality checking, this research is targeting quantifying data for constructing a model that would enable interpreting data from a sensor which is planning to be applied in the future to detect kiwifruit quality.

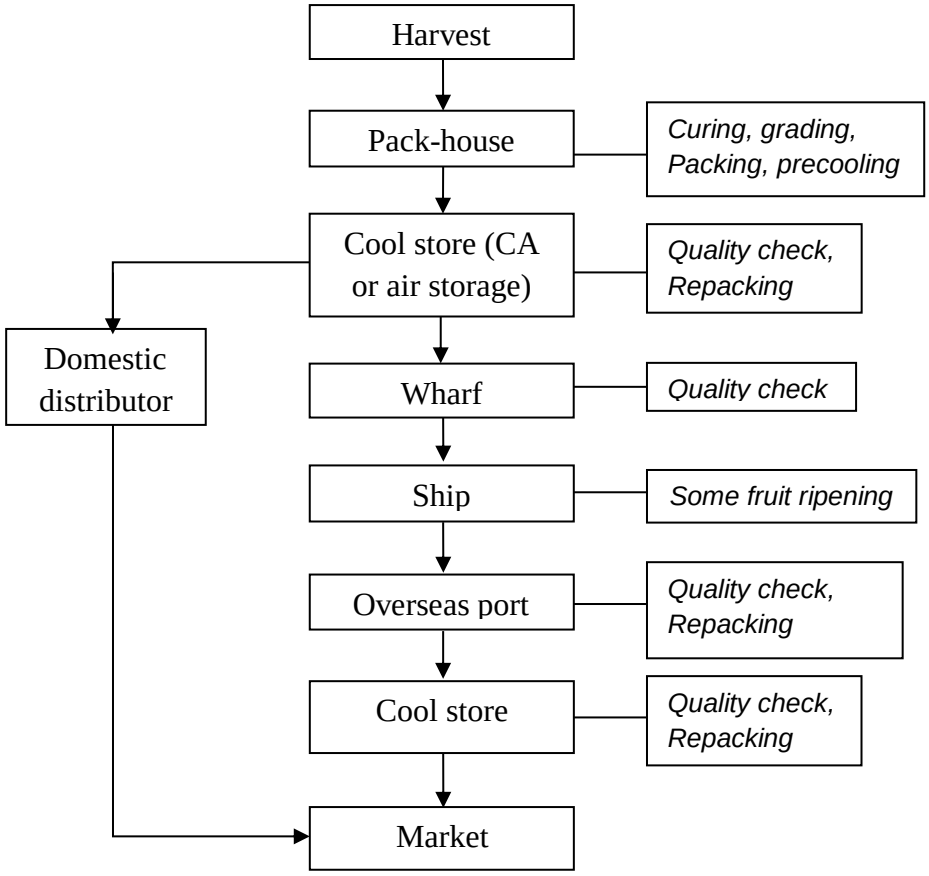


Figure 2-1: New Zealand kiwifruit supply chain and processes involved at each point

After sorting, fruit are packed into either single-layer trays that hold ≈3.6 kg or bulk packs that hold ≈10.6 kg. Both packs are lined with polyliners as a solution to water loss problems (Jeffery et al., 1991). Packs are then stacked onto pallets and identified by a unique number in order to facilitate traceability throughout the supply chain (Praat et al., 2003). Kiwifruit physiology, weight of the fruit, temperature, nature of the package such as polyliner characteristics (permeability, thickness, surface area etc.), free volume inside the package and environmental conditions all contribute to the resulting ethylene conditions developed inside the pack. In general, it is the balance between the total ethylene production of the kiwifruit inside the package and the rate of ethylene loss from the package that defines the resulting ethylene concentration (Ayhan, 2009). If there is a chance of ethylene accumulating in the packaging, stimulation of fruit ripening leading to fruit softening can be expected (Feng et al., 2003; Paz et al., 2005) as kiwifruit soften more rapidly when exposed to low concentrations of ethylene (10-30 $nL.L^{-1}$) for a few hours (McDonald & Harman, 1982; Jeffery & Banks, 1996). Potassium permanganate, which oxidizes or inactivates ethylene or dispersed minerals (such as zeolite, active carbon, pumice)

which absorb ethylene could be used to remove ethylene from the package (Ayhan, 2009).

Kiwifruit hold a good place in the international trade as exporters can rely on long-term storage of 6-10 months at 0 °C in air or controlled atmosphere (CA) for 'Hayward' (Hewett et al., 1999) and 12-16 weeks near 0 °C for 'Hort16A' (Patterson et al., 2003) to facilitate transport over long-distances (McDonald, 1983). Packs are precooled with the use of forced air coolers to reduce fruit temperature to about 2 °C in 8 hours before cold storage (0±0.5 °C) in an ethylene free environment (Fisk et al., 2008). According to Wiley et al., (1999) and Antunes & Sfakiotakis, (2002a) packaged fruit can be stored in an ethylene free environment for up to 4-6 months if forced air cooled. As ethylene during storage and storage temperature (Johnson et al., 1995) play a major role in promoting kiwifruit softening during storage, it is important to control both to maximize the postharvest storage life of kiwifruit. Kiwifruit should not be stored with commodities that are high ethylene producers such as avocados and apples even at temperatures below 4 °C (Shekarriz & Allen, 2008). Industry requires kiwifruit to be cold stored within 48 hours of harvest and fruit temperature should be below 0.5 °C within 10 days of storage (Lallu & Webb, 1997). Recommended relative humidity for kiwifruit storage is 90–98% (Mitchell, 1990). Regular quality checking and repacking of fruit is conducted when necessary to maintain a stock of kiwifruit with sound quality during storage (Mills, 2004). Scrubbing and ventilation are used to remove ethylene during storage (McDonald, 1990). Refrigerated vessels (reefers) are being used to export kiwifruit to overseas market under refrigeration (www.zespri.com). The 0±0.5 °C cool storage conditions are maintained during transport to supermarket shelves to facilitate kiwifruit to arrive to customers in optimal condition. Although storing at 0 °C can extend the storage life of kiwifruit, chilling injury can occur when they are stored at less than 1 °C (Wang et al., 2011) while freeze damage could occur when temperature declines to -1.5 °C. However, it is not recommended to lower kiwifruit storage temperature beyond 0 °C (Crisosto & Kader, 1999).

### 2.4 Ethylene

Ethylene is a simple, 2 carbon gaseous plant hormone, which has been involved in a number of plant growth and developmental processes including stimulating ripening of horticultural produce (Argueso et al., 2007) and senescence (Marques, 1998).

Additionally, ethylene can stimulate respiration; abscission and synthesis of ethylene itself and can profoundly affect quality factors of horticultural products such as texture, colour and flavour (Watkins, 2002). Various stimuli such as exposure to heat, oxidation, light or ionising radiation can result in ethylene production (Abeles et al., 1992) while decomposing fresh produce, burning coal or gas, internal combustion engines and garbage are all exogenous sources of ethylene (Wills et al., 2001).

### 2.4.1 Ethylene biosynthesis in kiwifruit

Adams & Yang, (1979) and Lursen et al., (1979) described the ethylene biosynthesis pathway of biological products after discovering 1-aminocyclopropane carboxylic acid (ACC) as an intermediate between methionine and ethylene. As Yang & Hoffman, (1984) elucidated, the pathway of ethylene biosynthesis is methionine, S-adenosylmethionine (SAM), ACC and ethylene (Figure 2-2). SAM synthase, ACC synthase (ACS) and ACC oxidase (ACO) are involved in catalysing the major steps of the ethylene biosynthesis pathway. Formation of ACC from SAM/AdoMet is mediated by ACC synthase (ACS) (Yu et al., 1979). ACS activity has been found to be unstable (Zarembinski & Theologis, 1994; Fluhr & Mattoo, 1996) and can be strongly inhibited by aminoethoxyvinyl glycine (AVG) (Owens et al., 1971). The conversion of ACC to ethylene is catalysed by ACC oxidase (ACO) and oxygen is required for this function. Like ACS, ACO activity is also unstable (Fluhr & Mattoo, 1996). Conversion of SAM/AdoMet to ACC is recognised as the rate limiting reaction and hence the main control site of ethylene synthesis (Yip et al., 1992). On the other hand, Larrigaudiere et al., (1997) demonstrated that changes in ethylene production depend on both the availability of ACC and activity of ACO.

Production of ethylene can be influenced by interactions with other metabolic pathways. The methionine from the Yang cycle feeds into ethylene biosynthesis (Miyazaki & Yang, 1987). ACS produces 5-methylthioadenosine (MTA) which is recycled to methionine and the rate of ethylene production could be limited with low methionine concentrations if this recycling does not happen (Wang et al., 1982). ACC could be coupled with malonate to form 1-(malonyl-amino) cyclopropane-1-carboxylic acid (M-ACC). M-ACC is well established as a primary conjugate form of ACC (Hoffman & Yang, 1983). In some systems M-ACC is the inactive end product of ACC not being oxidized to ethylene (Bouzayen et al., 1987). M-ACC acts

as a sink for ACC in the presence of high levels of ACC or when ACO is saturated. Accumulation of M-ACC could have an effect on auto-inhibition of ethylene production (Liu et al., 1985). Vangronsveld et al., (1988) observed a 40% reduction in ethylene production in parallel with 32% increase in M-ACC and 37% reduction in ACC in bean seedlings.

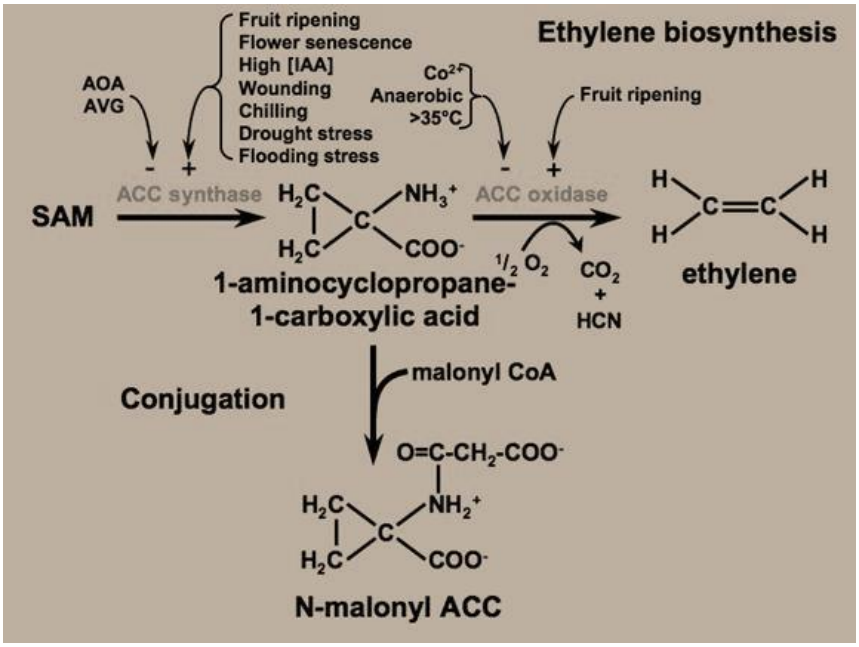


Figure 2-2: Schematic diagram representing pathway of ethylene biosynthesis (Source:Yang & Hoffman, 1984)

In considering the biosynthesis of ethylene during ripening, fruit can be classified as climacteric and non-climacteric. Fruit which undergo a significant increase in respiration associated with a large irreversible increase in ethylene production during ripening are known as climacteric fruit. Kiwifruit, apple, avocado and banana are all considered to be climacteric. In contrast, non-climacteric fruit like strawberry, orange and grape show little change in respiration and low levels of ethylene production during ripening (Wills et al., 2001). Kiwifruit is classified as a climacteric fruit since it has a rise in ethylene production as well as in respiration during ripening (Arpaia et al., 1994). Antunes et al., (2000) also confirmed kiwifruit (cv. Hayward) as climacteric since autocatalytic ethylene production occurred approximately 19 days after harvest at 20 °C. However, application of exogenous ethylene or propylene changed the climacteric pattern of 'Hayward' kiwifruit by demonstrating a burst of ethylene after a lag period of 68 to 79 h with higher

respiration occurring after 4-10 h (Antunes, 2007). This response differentiates kiwifruit from other climacteric fruit such as avocado and tomato in which ethylene production, respiration and ripening occur together upon exposing to exogenous ethylene (Abeles et al., 1992). Hence, kiwifruit is considered to be a unique climacteric fruit as it lacks the ability to produce ethylene through the autocatalytic pathway (unless damaged or infected) (Antunes, 2007).

Regulation of ethylene production has been described with a concept of two systems developed by McMurchie et al., (1972). System 1 is common to non-climacteric and pre-climacteric fruit and is associated with low basal ethylene production whereas autocatalytic ethylene burst in climacteric fruit along with ripening is referred to as system 2. Exogenous ethylene or propylene causes auto-inhibition of production of ethylene in system 1 and autocatalytic production in system 2 tissues (Sfakiotakis et al., 1997).

### 2.4.2. Factors influencing ethylene biosynthesis

Ethylene production rates of kiwifruit vary with storage condition and duration, cultivar difference, postharvest treatments as well as with numerous factors such as high temperature, fruit maturity, exogenous ethylene levels, damage or infection or ethylene inhibitors. In addition, considerable variation could be observed in individual fruit ethylene production within a batch of fruit kept under identical conditions (Feng et al., 2003). Work by Castillo et al., (1999) and Pekmezci et al., (2004) shows that healthy kiwifruit produce very low levels of ethylene at harvest (ranging between 0.01–0.1 $pmol.kg^{-1}s^{-1}$) and ethylene production significantly increases if fruit get damaged or infected. With increase of temperature (Sfakiotakis et al., 1997) and fruit maturity (Kim et al., 1999) ability of kiwifruit to produce ethylene becomes higher.

#### 2.4.2.1 Effect of fruit maturity/firmness on ethylene biosynthesis by kiwifruit

It is distinctive of kiwifruit to produce large amounts of ethylene only after fruit have softened (reached a certain maturity) considerably. 'Hayward' kiwifruit illustrate very low ethylene production until undergoing about 85% of their softening from harvest. Once reaching <10 N firmness, kiwifruit ethylene production is high at a rate between 0.4–0.7 $pmol.kg^{-1}s^{-1}$ (Kim et al., 1999). Similarly, this dramatic increase in ethylene production has been observed when kiwifruit firmness reached

<10 N (Jeffery et al., 1991), ≈ 50 N (Tonutti et al., 1993) and when <7 N (Ritenour et al., 1999). At the onset of ripening 'Hayward' kiwifruit ethylene production was 1.2 pmol.kg$^{-1}$s$^{-1}$ at 15 °C (Hyodo & Fukasawa, 1985) while ripened kiwifruit which have a firmness of <18 N produced ethylene at 619–1239 pmol.kg$^{-1}$s$^{-1}$ at 20 °C (Crisosto & Kader, 1999).

#### 2.4.2.2 Effect of temperature on ethylene biosynthesis by kiwifruit

Low temperature storage conditions enable maintenance of postharvest quality of perishables by inhibiting ethylene production (Wills et al., 1998). Sfakiotakis et al., (1989) found no significant ethylene production from kiwifruit stored at 0 °C and 10 °C while ripening and ethylene production occurred at 20 °C. Autocatalytic ethylene biosynthesis only occurred at temperatures within the range of 17-35 °C (Stavroulakis & Sfakiotakis, 1993) and 20-34 °C (Antunes & Sfakiotakis, 1997) in propylene treated kiwifruit stored at a range of temperatures from 0-35 °C while low ethylene production was observed at the temperature range of 11-14.5 °C (Antunes & Sfakiotakis, 1997). Crisosto & Kader, (1999) found mature but unripe kiwifruit producing less than 1.2 pmol.kg$^{-1}$s$^{-1}$ at 0 °C and 61.9 pmol.kg$^{-1}$s$^{-1}$ at 20 °C. $Q_{10}$ value of ethylene production between 20 and 40 °C was about 2 for 'Hayward' kiwifruit (Field, 1985).

In addition to inhibiting autocatalytic ethylene production it has been found that the onset of ethylene biosynthesis (and consequently ripening) is triggered in kiwifruit when exposed to refrigerated storage below 0 °C for a period of time. Autocatalytic ethylene production begins following removal to ambient temperatures (Manolopoulou & Papadopoulou, 1997). Antunes & Sfakiotakis, (2002a) found similar results in that kiwifruit started autocatalytic ethylene production within 24 h following removal to 20 °C after exposure to 0 °C for 12 days, while it took 19 days to start autocatalytic ethylene production for fruit kept continuously at 20 °C. A decrease in ethylene production following transfer of fruit from long duration cold storage to warm temperature showed that induction of ethylene production upon rewarming depended on the duration of cold storage (Manolopoulou & Papadopoulou, 1997; Antunes & Sfakiotakis, 2002a). Other fruit like European pear (Blankenship & Richardson, 1985) and 'Granny Smith' apple (Larrigaudiere & Vendrell, 1999) require long exposure to chilling temperature to induce ripening.

#### 2.4.2.3 Effect of damage on ethylene biosynthesis by kiwifruit

Injuries to fruit start while still on the plant, for instance when the fruit come into contact with other fruit or parts of the plant. Damage caused by postharvest activities start from harvest. The act of hand harvesting could cause injury to the fruit. After harvest fruit may pass through several containers and different modes of transport along the supply chain of which these changes could expose fruit to impact injury from falls from different heights as well as contact with other fruits. Bollen et al., (1995) categorized mechanical damage into two types: impacts during harvest, selection, handling and transport and compression loads during packing or storage. According to the comprehensive survey of data collection on incidence of mechanical injuries of fruit, impact force was recorded as the most common out of different types of injuries and it seems to be almost unavoidable in postharvest operations (Knee & Miller, 2002).

Even though fruit to fruit impact has been recorded as the most common source of damage in postharvest handling, most of the research has been concentrated on contact between fruit and a hard surface. Experiments done to determine the effect of impact injury on fruit could be followed in two ways: a fixed mass dropped into a fruit or the fruit itself dropped onto a surface. Impact force is constant throughout the experiment in the first method which is considered advantageous whereas the second method may be more representative of what happens to fruit after harvest (Knee & Miller, 2002). The degree of injury depends on the nature of the surface in which the fruit contacts and how contact is made (Knee & Miller, 2002). Round surfaces concentrate more energy than flat surfaces which have higher damage to lower energy levels. Mass of the fruit also could affect degree of damage by impact force (Maness et al., 1992; Knee & Miller, 2002).

Mechanical injuries such as impacts, which are common during harvest and postharvest handling, can accelerate ethylene production (Pekmezci et al., 2004). Response of production of ethylene due to injuries has been described as ‘wound ethylene’ and that follows immediately after mechanical damage (between 2-3 hours) and lasts 2-10 hours (Bollen et al., 1995). As described by DeMartino et al., (2002) release of ethylene from the impact surface was significantly increased after 6 hours when compare with the production from the sound surface where ethylene production only increased after 12 hours and as discussed, time delay between release of ethylene from injured side and diffusion through the tissues and

stimulation of overall ethylene production including sound side must have caused this lag period. Sfakiotakis et al., (1997) reported ethylene production at 4300 $pmol.kg^{-1}s^{-1}$ from wounded (cut) kiwifruit stored at 5 °C while Mencarelli et al., (1996) reported doubling of ethylene production 14 hours after impact bruising of kiwifruit at 18 °C.

Low ethylene production is always observed at reduced temperature after impact injury (Mencarelli et al., 1996). Ethylene production was increased (5 times) at higher temperature (20 °C) than at lower temperature (2 °C) for peeled slices of kiwifruit (Agar et el., 1999). Apricots also revealed a reduction of ethylene production from fruit moved to low temperature (4 °C) after impact tests done at room temperature (18 °C) while ethylene production increased from 5 to11 $pmol.kg^{-1}s^{-1}$ in 30 hours when impact injury occurred at 4 °C and fruit were later moved to 18 °C (DeMartino et al., 2002).

Wound ethylene production increases with maturity (Kende & Boller, 1981). Acceleration of kiwifruit ethylene production from impact bruising occurred when fruit firmness was below 60 N suggesting fruit at, or recently after harvest (which typically have a firmness >60 N) are unlikely to produce ethylene due to injury (Arpaia et al., 1994). However, according to Crisosto et al., (1997) the maturity stage with a kiwifruit firmness of 18 N has been found as the minimum threshold limit to avoid physical damage and induction of wound ethylene production during standard postharvest handling. Ketsa & Koolpluksee, (1993) discussed that although climacteric fruit produce large amounts of ethylene during ripening, wounding at this ripening stage may not increase or may even decrease ethylene production.

Kiwifruit dropped from 60 and 120 cm heights increased ethylene production by 4.7 and 5.7 times respectively at 20 °C (Alves et al., 2010). Ethylene emission of 'Babygold' peaches was proportional to impact intensity (compression forces 30, 40 and 50 N) 3-5 hours after mechanical damage (Martìnez-Romero et al., 2000). Similarly, peeled kiwifruit showed 2-4 times higher ethylene production than unpeeled slices and ethylene production was 2 times higher in peeled fruit than whole fruit (Agar et el., 1999). Stress ethylene increases with the severity of injury until a point beyond which additional injury decreases ethylene production. Sub division of apples, sweet potatoes and tomato increases production of ethylene but decreased when tissue were subdivided further (Abeles et al., 1992).

#### 2.4.2.4 Effect of other factors on ethylene biosynthesis

Exogenous ethylene, infections, pre storage treatments as well as antagonists and inhibitors are identified as the other factors which can influence production of ethylene of kiwifruit after harvest. Exogenous ethylene can induce biosynthesis of ethylene in kiwifruit to reach the climacteric more rapidly at temperatures above 15 °C (Antunes, 2007).

Botrytis grey mold rot which is caused by *Botrytis cinerea* is the most important postharvest disease of kiwifruit (Antunes, 2007). According to Feng et al., (2003) fruit that have damaged cells from fungal rot produce higher ethylene to induce a wounding response. Ethylene production of kiwifruit incubated with *B. cinerea* was 10 $pmol.kg^{-1}s^{-1}$ when methionine was added as ethylene precursor and almost no ethylene was produced without the precursor (Qadir et al., 1997). Niklis et al., (1997) found ethylene production at a maximal rate of 2.5 $pmol.kg^{-1}s^{-1}$ 100 days after inoculation with *B. cinerea* at 0 °C. A similar ethylene production rate of infected fruit was found by Sfakiotakis et al., (1997) and production was generally correlated to the size of the infected area. Even though work has been done to demonstrate ethylene production of fruit that had been artificially inoculated with *B. cinerea*, there are no previous results regarding naturally inoculated fruit during the growing season or at harvest.

Table 2-1: Ethylene production values reported in literature for kiwifruit under different conditions.

| Condition of the kiwifruit | Ethylene production ($pmol.kg^{-1}s^{-1}$) | Source |
|---|---|---|
| Good fruit | 0.01-0.1 | Kim, 1999<br>Castillo et al., 1999 |
| Rotten fruit | 6.8 | Sfakiotakis et al., 1997<br>Nikilis et al., 1997 |
| Soft fruit at 20 °C | 123 | Hyodo & Fukasawa, 1985 |
| Mature but unripe at 0 °C | 1.2 | Crisosto & Kader, 1999 |
| Mature but unripe at 20 °C | 1.2–61.9 | |
| On set of ripening at >15 °C | 1.2 | Hyodo & Fukasawa, 1985 |
| 180 days stored fruit at 0 °C (firmness <10 N) | 0.4–0.7 | Kim, (1999) |
| 6 months stored sound fruit after transfer from 0 to 20 °C | 87 | Antunes & Sfakiotakis, 2002b |

| | | |
|---|---|---|
| Wounded fruit at 5 °C | 4300 | Sfakiotakis et al., 1997 |
| Fruit incubated with *B. cinerea* (Presence of methionine at 22 °C ) | 10 | Qadir et al., 1997 |
| Fruit incubated with *B. cinerea* (100 days after inoculation at 0 °C) | 2.5 | Niklis et al., 1997. |

#### 2.4.2.5 Internal ethylene concentration in kiwifruit

The concentration of ethylene in the fruit is referred to as the internal ethylene concentration (IEC). The IEC is arguably the actual concentration which affects fruit quality (Alonso & Stepanova, 2004) and also could be responsible for the variability of the composition of packaging atmosphere (Jeffery et al., 1991; Dadzie et al., 1996). Ethylene production by fruit, skin resistance to ethylene diffusion, resistance of packaging materials and environmental ethylene concentrations largely affect internal ethylene concentration of the fruit packed inside a container (Nicolaï et al., 2009). At steady state, endogenous ethylene production maintains the internal ethylene concentration of fruit at a higher concentration than the surrounding environment.

Even though use of IEC is advantageous in studying ethylene biosynthesis and softening during cold storage because of less variation and higher measurable concentrations (Castillo et al., 1999), availability of IEC data is rare (Sfakiotakis et al., 1989). Ethylene production data is primarily used to study physiological behavior of fruit. Knowledge of skin permeability of ethylene is necessary to estimate IEC from ethylene production data. However, there is very little information on skin resistance to ethylene for any fruit in comparison with the amount of data available for water, oxygen and carbon dioxide (Xu et al., 2001). Hyodo & Fukasawa, (1985) and Abeles et al., (1992) have observed a linear relationship between ethylene production and IEC, with IEC ($\mu L.L^{-1}$) values about 10 times higher than ethylene production values ($\mu L.kg.h^{-1}$). This generally results in 1 to 10 $\mu L.L^{-1}$ internal ethylene concentration being related to 1.2-12.4 $pmol.kg^{-1}s^{-1}$ of ethylene production (Abeles et al., 1992).

Some have argued that physiologically active ethylene is not what is present in the gaseous phase in intercellular spaces but what is dissolved in the solutes inside the tissue. Hence, understanding of the nature and physics of dissolved ethylene in fruit tissues takes an important place and estimation of the amount of ethylene

dissolved in liquid of any fruit tissue is commonly based on the analysis of ethylene in the gaseous phase above the solution (head space) (Bassi et al., 1981). Henry's law constant (HLC) represents the gas liquid equilibrium for any particular gas present in a dilute aqueous solution and could be given as the relationship of the partial pressure of the gas to the mole fraction of the gas in a solution at a given (temperature) condition. Every compound/liquid combination has a unique Henry's law constant (HLC) at a specific temperature and pressure (Altschuh et al., 1999). HLC increases with increasing temperature as solubility of ethylene in water decreases with increasing temperature at atmospheric pressure (Bradbury et al., 1952).

## 2.5 Kiwifruit quality attributes

Of the key factors which determine kiwifruit quality with regard to consumer acceptability (firmness, colour, soluble solid content and dry matter), firmness of kiwifruit has been considered as the key determinant of postharvest quality attributes which limit the storage life of kiwifruit (Papadopoulou & Manolopoulou, 1997; Kim et al., 2001; Patterson et al., 2003).

### 2.5.1 Firmness

Fruit softening occurs due to loss of cell wall integrity during ripening and it is not uniform for kiwifruit because of the distinctively different tissue types (section 2.2.1; Hallet et al., 1992). The softening curve for kiwifruit can be described with 3 distinctive phases in which the length varies and depends mainly on maturity at harvest and storage conditions (McDonald, 1990; Kim et al., 1999). Very little softening occurs in the first phase of softening curve which is a lag period. This phase has less influence in more mature fruit (Arpaia et al., 1987; MacRae et al., 1989). The second phase represents a period of rapid softening. This occurs after the lag period in early harvest fruit and immediately after harvest for late harvested fruit. Generally, kiwifruit stored at 0 °C in air soften from 70–80 N at harvest to 20–30 N within 8-12 weeks during the second phase (Hewett et al., 1999). The third slow phase of softening begins at about 20 N and continues until fruit become overripe with firmness less than 7 N. According to Hewett et al., (1999) there is evidence of another $4^{th}$ phase of sharp reduction in firmness at the completion of

softening where final tissue disintegration is taking place and the fruit becomes no longer edible.

Generally, kiwifruit are harvested at a flesh firmness of 69-98 N (McDonald, 1990). Kiwifruit firmness declines by about 94% and reaches around a range between 9-13 N when fruit is ready to eat (Harker & Hallet, 1994; Crisosto & Crisosto, 2001). At the ready to purchase stage firmness should be between 13-18 N (Natalia et al., 2010) whereas fruit firmness ranging from 8-10 N is considered as optimum for consumer acceptance (McDonald, 1990). It is best to transport kiwifruit with a firmness value of ≥22 N to avoid induction of ethylene production due to injury during handling and transportation (Mitchell, 1990). Kiwifruit are considered unacceptable for export when fruit firmness falls below 10 N (Hopkirk et al., 1996).

Premature softening has been identified as the key contributing factor to storage life limitation of kiwifruit (Castillo et al., 1999; Hewett et al., 1999). Ethylene produced during ripening (Menniti et al., 2005; Antunes, 2007) as well as storage temperature (Johnson et al., 1995) have been considered as potential promoters of kiwifruit softening. Irrespective of storage temperature (0 °C or 20 °C) fruit have accelerated softening when exposed to very low concentrations of ethylene such as 5-10 $nL.L^{-1}$ in storage (Shekarriz & Allen, 2008; Pranamornkith et al., 2012).

Firmness is commonly measured destructively using a penetrometer as the average peak force (N) required to puncture the skinned tissue to a depth of 8 mm at a known speed with a 7.9 mm round Effegi probe. Usually, two positions around the equator of the fruit at 90º separation are measured (Jeffery & Banks, 1994).

### 2.6 Correlation of ethylene with postharvest quality of kiwifruit

Knowledge of how ethylene levels correlate with fruit quality is essential in application of an ethylene sensor within fruit stock to observe current or predict future fruit quality, based on the measured ethylene concentration. However, even though fruit firmness is commonly used to describe kiwifruit quality, most research work has described a poor correlation between the internal ethylene concentration (IEC) or fruit ethylene production with firmness (Kim et al., 1999; Feng et al., 2003). Sfakiotakis et al., (1989) and Kim et al., (1999) had little success in relating ethylene production with fruit softening in cool storage and found ethylene concentrations are very low for healthy fruits stored in cool storage.

## 2.7 Ethylene movement within the package

Ethylene transmission through the kiwifruit package influences ethylene composition inside the package. The dynamics of reaching a steady state of ethylene composition inside the package depend on the ethylene influx and efflux, the dimensions of the package, amount of product packaged, ethylene production of the product and absorption of ethylene by the product. Solution–diffusion through permeable packaging material is the mechanism involved in exchanging ethylene between the package atmosphere and the environment surrounding the package in the absence of cracks, pinholes, or other flaws. This mechanism involves dissolution of permeate in the barrier at the higher concentration side, diffusion through the barrier driven by a concentration gradient and evaporation from the other surface (Gholizadeh et al., 2007).

### 2.7.1 Ethylene transmission through polymer films

Ethylene transmission rate or permeability through polymer materials is governed by factors which are controlled by polymer properties (intrinsic structure of the polymer: degree of crystallinity, density, crystallinity/amorphous phase ratio, chemical groups in the polymer, glass transition temperature and degree of cross linking, thermal and mechanical behaviour), permeate (ethylene) properties (solubility difference, size, shape and polarity of the penetrating molecule of the permeant) and degree of interaction between polymer and permeate (ethylene) and environmental conditions (Siracusa, 2012). Steady state gas permeability (P) through polymer films ($mol.s^{-1}m^{-1}Pa^{-1}$) can be described by a diffusion model applying Fick's first law and Henry's law as expressed in equation 2-1.

$$P = \frac{N_A \times l}{A \times (p_2 - p_1)} \qquad [2\text{-}1]$$

where $N_A$ is the steady-state flux of gas through the film ($mol.s^{-1}$), l is the film thickness (m), A is the surface area of the film ($m^2$) and $p_2$ and $p_1$ are the upstream (high) and downstream (low) partial pressures of gas (Pa) respectively (Lin & Freeman, 2004; Paz et al., 2005). Partial pressure gradient ($p_2$-$p_1$) can be replaced with the concentration gradient of the active compound established between the two surfaces of the film by using gas constant and absolute temperature (Lin & Freeman, 2004). The chemical structure of a polymer is considered as the predominant factor which influences permeability (Heilman et al., 1956). If there is no interaction

between the permeant and the film, the permeability values are usually considered to be concentration independent. However, this cannot apply when interactions are there as between water vapour and hydrophilic film (cellulose and EVOH) or between organic vapours and polyolefin films like LDPE and PP (Weerawate, 2008). Amorphous polymers have higher permeability than crystalline polymers. Permeability is inversely proportional to the volume fraction of the crystalline phase. With an increase in polymer crystallinity, permeability considerably decreases since the crystalline area is generally impermeable to the gas permeants (Wang et al., 1998; Siracusa, 2012). Permeability decreases significantly with the increase of density of the polymer structure (Heilman et al., 1956).

The 'isostatic method' is a common means in which to measure permeability. The setup for the 'isostatic method' in measuring permeability of a film consists of two similar permeation cells and the film which is mounted between each cell separating it in two halves. One side of the film is swept continuously with the test gas whilst other side is swept with an inert gas into which the test gas diffuses in order to keep a constant partial pressure gradient during the experimental period (Paz et al., 2005). The quantity of permeant gas passing through the film can be determined from the change in chemical composition of the carrier gas by monitoring the concentrations of the feeding gas and permeant streams as well as transmission rate when the system comes to equilibrium (Paz et al., 2005). In this method the total pressure on both sides of the film is assumed equal (Paz et al., 2005). One concern about this method is the fact that the stagnant air layer at the film surface which exists in real conditions, can be reduced because of the continuous gas flow (Charles et al., 2003).

When considering perforated packaging, like that used in the kiwifruit industry, there are two phenomena occurring: mass transfer through film and transfer through perforations **(**Pandey et al., 2012). Micro and macro perforations are the two main categories of classifications of gas diffusion through perforations of a package and some researchers employed macro perforation (Fishman et al., 1996; Paul & Clarke, 2002) while some use micro perforation (Hirata et al. 1996; Gonzalez et al., 2008) to establish models to describe the diffusion of gases through perforation of a package.

Polymer film permeability values reported in the literature (Table 2-2) demonstrate a large variation for the same type of film. This variation will have large effects when attempting to model ethylene concentration within a package.

Reported values of ethylene permeability values for synthetic films range from 2.68 x $10^{-16}$-8.49 x $10^{-15}$ mol.m.$m^{-2}s^{-1}Pa^{-1}$ which represents a 20 times difference between the slowest and fastest rates. Meanwhile edible wheat gluten film has been reported with the lowest permeability, being approximately 1000 times lower than synthetic films. Only a few works have dealt with the study of permeability of ethylene through synthetic films such as ethylene vinyl alcohol and polyvinyl chloride (Paz et al., 2005). Relatively higher permeation of ethylene can be observed when compared with $O_2$ and $CO_2$ in films like low-density polyethylene (LDPE) and poly[styrene-b-(ethylene-co-butylene)-bstyrene] (SEBS) as these films are hydrophobic polymers that possess strong interaction with the ethylene gas (Monprasit et al., 2011).

Paz et al., (2005) found that ethylene permeability can be affected by both temperature and relative humidity (RH). At high humidity, permeability of a film changes because of water absorbance or condensation of water on the film forming an extra barrier for diffusion through the film (Wang et al., 1998). Passing humidified stream gases through the measurement system allows measuring permeability at different relative humidity levels. Paz et al., (2005) study results revealed that ethylene permeability was very slight (≤3 amol.$m^{-1}s^{-1}Pa^{-1}$) within the temperature range of 3–5 °C and up to 60% RH and higher permeability value (≤ 3000 amol.$m^{-1}s^{-1}Pa^{-1}$) was observed at the temperature and relative humidity level between 3 °C (80% RH) and 45 °C (60% RH). For wheat gluten film ethylene permeability was low at 0% RH, indicating a very low solubility and diffusivity of ethylene in the wheat gluten protein network in the dry state. Although many fresh commodities are now packaged and stored for retail sale to the consumer between 2 and 15 °C it is difficult to obtain reliable permeability data between those temperatures and generally permeability tests are conducted at a temperature between 23 and 25 °C (Wang et al., 1998).

Table 2-2: Ethylene permeability values reported in literature for different types of polymer films

| Film Type | Sample thickness µm | Permeability value ($mol.m.m^{-2}s^{-1}Pa^{-1}$) | Reference | Temperature |
|---|---|---|---|---|
| HDPE | 40 | $2.68x10^{-16}$ | Wang et al., 1998 | 20 °C |
| LLDPE | 50 | $9.62x10^{-16}$ | Wang et al., 1998 | 20 °C |
| LDPE | 30 | $5.61x10^{-16}$ | Furuta et al., 1993 | 10 °C |
| | 30 & 60 | $9.41x10^{-16}$ | Furuta et al., 1993 | 20 °C |
| | 50 | $1.12x10^{-15}$ | Wang et al., 1998 | 20 °C |
| | 85 | $1.07x10^{-15}$ | Wang et al., 1998 | 20 °C |
| | 100 | $8.87x10^{-16}$ | Wang et al., 1998 | 20 °C |
| | 140 | $3.11x10^{-15}$ | Paz et al., 2005 | 23 °C |
| | 17 | $3.51x10^{-15}$ | Paz et al., 2005 | 23 °C |
| PVC | 13 | $6.05x10^{-16}$ | Savoie et al., 1993 | 3 °C |
| | 13 | $6.73x10^{-16}$ | Savoie et al., 1993 | 10 °C |
| | 13.5 | $2.59x10^{-15}$ | Piergiovanni et al.,1992 | 10 °C |
| | 13 | $1.14x10^{-15}$ | Savoie et al., 1993 | 17 °C |
| | 13 | $1.71x10^{-15}$ | Savoie et al., 1993 | 25 °C |
| | 13.5 | $5.79x10^{-15}$ | Piergiovanni et al.,1992 | 25 °C |
| | 13.5 | $8.49x10^{-15}$ | Piergiovanni et al.,1992 | 32 °C |
| EVA | 15 | $1.76x10^{-15}$ | Piergiovanni et al.,1992 | 10 °C |
| | 30 | $8.56x10^{-16}$ | Furuta et al., 1993 | 10 °C |
| | 30 | $1.68x10^{-15}$ | Furuta et al., 1993 | 20 °C |
| | 15 | $4.74x10^{-15}$ | Piergiovanni et al.,1992 | 25 °C |
| | 15 | $7.01x10^{-15}$ | Piergiovanni et al.,1992 | 32 °C |
| PEBA | 25 | $3.15x10^{-15}$ | Paz et al., 2005 | 25 °C |
| Wheat gluten | 80 | $1.90x10^{-16}$ | Paz et al., 2005 | 7 °C, 75% RH |
| | | $7.00x10^{-18}$ | Paz et al., 2005 | 9 °C, 33% RH |
| | 80 | $8.35x10^{-16}$ | Paz et al., 2005 | 20 °C, 85% RH |

## 2.8 Models of gas movement

Mathematical modelling is a suitable approach to predict the internal composition inside a package (Ho et al., 2008). Typically, models of gas partial pressures are developed from mass balance equations that describe permeation through the film and gas exchange by the packaged product (Cameron, 2001). Use of validated

models in evaluating the effect of different factors such as fruit quality and environmental conditions on composition of package gas will be convenient and reduce effort for experimental work. Empirical models or strongly simplified fundamental or kinetic models can be used to model the gas exchange of a package. Fick's first law has been used in the first model developed to determine the gas exchange in fruit and other bulky storage organs macroscopically (Nicolaï et al., 2009; Ho et al., 2008;2010).

Typically, it is necessary to make several assumptions when developing models dealing with flux within a package. One common assumption is to assume that there is perfect mixing of gas on each side of the film of the pack and that there is no boundary layer effects. Alternatively, Jeffery et al., (1991) assumed two dimensional ethylene diffusion within the tray (no vertical gradient) and also constant ethylene production from each individual fruit in developing a steady state model to predict ethylene concentration within a tray of kiwifruit. The entire surface area of the package is available for gas exchange is another common assumption. However, the surface area available for gas exchange is limited in practice because of the stacking of packages on each other or in boxes (Cameron, 2001). Uniform package gas composition and no effect of $CO_2$ on respiration were the assumptions made for a simple MAP model developed by Jurin & Karel, (1963) to predict steady state $O_2$ and $CO_2$ concentrations in an apple package.

Jeffery et al., (1991) found that maximum ethylene concentrations were in regions immediately adjacent to the climacteric fruit while zero ethylene concentration was observed along the fold of the polyliner when modelling ethylene flux in a kiwifruit package. This model could be used to develop improved packaging systems for kiwifruit storage and distribution. In another way, this model can be extended to determine non steady state changes such as those that occur when the storage atmosphere becomes polluted by external sources of ethylene and changes in the rates of ethylene production by the fruit over time.

A steady state model to illustrate external ethylene concentration (EEC) inside a kiwifruit package has been developed (East et al., 2011). The EEC inside the package ($\mu L.L^{-1}$) is the result of the difference between the total production of ethylene by the fruit ($mol.kg^{-1}s^{-1}$) and the rate of ethylene losses from the packaging. Ethylene loss from the packaging was considered to occur in three ways:1) diffusion through the polyliner ($mol.s^{-1}$), 2) losses via a small number of perforations within

the polyliner (mol.$s^{-1}$) and 3) losses through the fold of the unsealed polyliner (mol.$s^{-1}$) by considering the polyliner used within the corrugated cardboard box as the major barrier for ethylene diffusion from the packaging.

## 2.9 Conclusions and opportunities for research

During the supply chain of kiwifruit there are opportunities for exposure to different environmental conditions which induce deterioration of quality. Regular quality checking resulting in segregation of low quality fruit throughout the supply chain is essential as high ethylene produced by soft kiwifruit could cause rapid deterioration of the remaining fruit in the package. Therefore, identification and removal of poor quality fruit will maximize postharvest storage life of kiwifruit and increase profit by satisfying customers with high quality produce.

Other than using current techniques such as checking the visual quality, weight and size to detecting inferior quality kiwifruit within a batch, with the advancement of science there is a need to apply accurate and efficient kiwifruit segregation methods. Hence, as a step forward it has been identified that the use of sensors that enable analysis of situation changes under different supply chain conditions may be useful to detect poor quality fruit. To interpret the output from the sensor, a relationship between ethylene composition within the commercial kiwifruit package versus quality of kiwifruit is required. In general, composition of ethylene inside the package is the balance between the total ethylene production of the kiwifruit inside the package and the rate of ethylene loss from the package. Fruit maturity/firmness, fruit damage, fruit infection along with environmental conditions, storage conditions and duration, cultivar difference, postharvest treatments, exogenous ethylene levels and ethylene inhibitors have been identified as key factors affecting ethylene production of kiwifruit. Out of these,

- Fruit maturity/firmness
- Damage or injury to kiwifruit
- Environmental temperature variation

play a major role in controlling synthesis of ethylene from kiwifruit. Several attempts have been made to quantify ethylene production of kiwifruit in relation to these major factors by using traditional gas chromatography methods. This work aims to add to this body of knowledge by further establishing quantitative factors affecting ethylene production of ‘Hayward’ by utilizing a recently developed high

sensitivity ethylene detector, ETD-300 (SensorSense B.V., Nijmegen, The Netherlands). Additionally, due to variation in reported values for polymer film permeability, it is necessary to quantify accurate permeability values for the specific polyliner in the kiwifruit package as the barrier which impacts on the ethylene composition inside the package. Hence, to address the need identified in developing a proper correlation between ethylene composition inside the commercial kiwifruit pack with factors attributing, rates of production of ethylene from 'Hayward' kiwifruit of different maturity stages (firmness) as a function of temperature, and injury caused by impact damage along with quantification of transfer rates of ethylene through the specific polyliner used in the commercial kiwifruit package will be sought in this thesis. In addition, comparison of estimated and actual internal partial pressure of ethylene of kiwifruit for an increased temperature based on Henry's law constant (HLC) will be done to fill the information gap in clarifying the reasons behind the rapid ethylene evolution observed during a sudden increase in temperature within the supply chain of kiwifruit.

# Chapter 3

## METHODOLOGY

### 3.1 Plant material

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson) harvested from commercial orchards were provided by EastPack in the Bay of Plenty, New Zealand and transported to Massey University, Palmerston North by refrigerated truck after the commercial grading and packing. On arrival, fruit (100 g) were randomized in to plastic plix of commercial single layer corrugated fibreboard trays and surrounded by a polyethylene liner. Trays were immediately stored at 0 ºC with determination of ethylene production and firmness conducted at regular intervals during storage. Ethylene concentration inside the cold storage was maintained at a low level (<1-5 $nL.L^{-1}$, through regular monitoring) by utilising $KMnO_4$ (Purafil™, Doroville, Georgia, USA) ethylene scrubber granules inside the cold room and ensuring adequate room refresh.

### 3.2 Ethylene production measurement

A laser photo-acoustic ethylene detector, (ETD-300, SensorSense B.V., Nijmegen, The Netherlands) was used to determine ethylene production rate of kiwifruit exposed to different treatment conditions throughout the experimental work (Cristescu et al., 2012; Pranamornkith et al., 2012). A continuous flow measurement method was used to determine ethylene concentration of the head space of each jar at steady state and equated to fruit ethylene production. Dry air from the air compressor was delivered through the catalyzer to provide hydrocarbon and ethylene free air for the ETD-300 as an input into the controller. The valve control box controls the flow of the input supply into up to 6 channels and also the selection of which return flow is delivered to the ethylene detector. Mass flow is controlled as an output from the control box with the return gas flow rate being measured as a control measure of the seal of the closed loop that includes the sampling jars. The selected flow from the valve controller passes through scrubbers to eliminate $CO_2$ and water prior to being delivered to the detector (Figure 3-1).

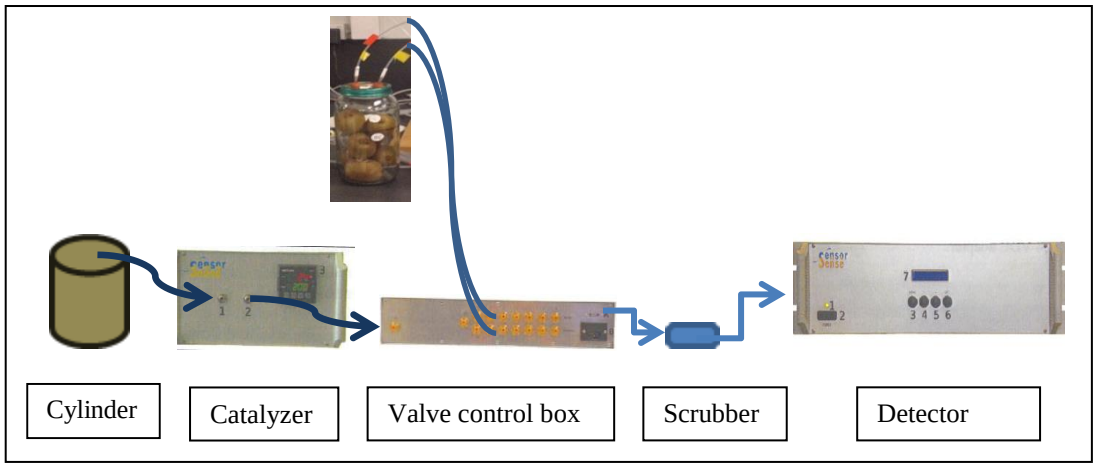


Figure 3-1: Experiment set up of for ethylene determination using an ethylene detector (ETD-300, SensorSense B.V., Nijmegen, The Netherlands).

As the main characteristic of the continuous flow measurement, air flows through all the selected channels throughout the experiment resulting in constant gas refreshment and establishment of a steady state gas conditions for the samples. This method is also advantageous in that it avoids accumulation of carbon dioxide which can both influence and inhibit measurements of ethylene production when using a static measurement method.

Measurement of the ethylene concentration in each of the sample jars was set to ten minute time intervals. Out of 6 channels, 5 channels were used for determining ethylene production of kiwifruit inside jars while the other channel was used as a zero reference point. As a result, it takes 1 h to cycle through measurements of each channel. Measurements were continued until concentration of ethylene of each jar (consecutive cycles) attained a maximum constant level, ensuring steady state establishment. The number of 10 minute cycles (time for one consecutive cycle) or total time to obtain the equilibrium level depended on the ethylene production of kiwifruit as well as the flow rate of the ethylene detector (Figure 3-2). Generally, 4 h (4 cycles/each cuvette) was required to reach equilibrium state with the selected flow rate of 1 $L.h^{-1}$ when ethylene production of kiwifruit were low (<0.01 $pmol.kg^{-1}s^{-1}$) while it took only 1-2 h (1-2 cycles/ cuvette) with the selected flow rate of 5 $L.h^{-1}$ when fruit produced high ethylene (>100 $pmol.kg^{-1}s^{-1}$). Irrespective of fruit ethylene production, each measurement was continued for 5 h to ensure accurate and reliable data.

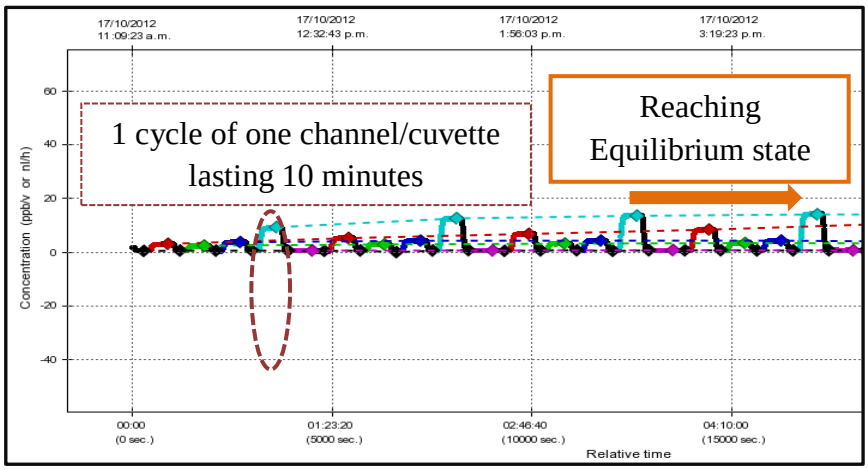


Figure 3-2: Example of data plot obtained from ETD-300, representing ethylene concentration ($nL.L^{-1}$) versus time (seconds) and describing cycles from each channel and stage of equilibrium state. Each colour represents the measured ethylene concentration of each channel.

Kiwifruit were placed inside sealed glass jars (Figure 3-3) fitted with two rubber septum to connect tubes to and from the valve controller box. Total fruit weight inside each jar (1 replicate) was measured using a three decimal place digital balance (PG503-S, Mettler Toledo, Switzerland) before each measurement. Glass jar size and the number of fruit inside the jar varied with the condition/maturity of the kiwifruit.

Figure 3-3: Sealed glass jars (with kiwifruit) fitted with two rubber septum to connect tubes to and from the valve controller box of the ethylene detector used in determination of ethylene production.

According to the equation 3-1 calculation of rate of ethylene production of kiwifruit utilizing data obtained by ethylene detector depends on the gas flow rate ($F$) and the total weight ($W$) of the fruit used. In order to detect small values of ethylene produced by fruit (early maturity) it was necessary to use the minimum flow rate of the detector (1 $L.h^{-1}$) as well as maximum number of fruit could put in a suitable sized jar. Thus, ethylene production of kiwifruit at early stages of maturity (>15 N) was obtained by placing seven fruit in 1.75 L sealed glass jars (Figure 3-3) since fruit at this stage produced very low ethylene. Number of fruit and the size of the jar had

to be reduced with the increase of ethylene production rate (or maturity) of kiwifruit. Hence, when fruit firmness reduced to <15 N, three fruit were placed in 750 mL sealed glass jars and finally when fruit firmness was <10 N one kiwifruit was used in a 500 mL jar.

Ethylene production of kiwifruit ($\mu L.kg^{-1}h^{-1}$) was calculated by applying ethylene concentration value ($nL.L^{-1}$) and the flow rate (($L.h^{-1}$) obtained from the detector and the total weight of fruit (kg) used in the experiment for each measurement (Equation 3-1). Equation 3-2 was used to convert ethylene production of kiwifruit in $\mu L.kg^{-1}h^{-1}$ to $mol.kg^{-1}s^{-1}$ by applying the specific temperature value in kelvin (K) (Banks et al., 1995).

$$EP = \left[\frac{(C/10^9).F}{W}\right] \text{ x } 10^{-6} \qquad [3\text{-}1]$$

Where,

*EP* – Ethylene production of kiwifruit ($\mu L.kg^{-1}h^{-1}$)

*C* - Measured ethylene concentration ($nL.L^{-1}$)

*F* - Flow rate ($L.h^{-1}$)

*W* - Fresh total weight of kiwifruit per jar (kg)

$$EP \ (mol.kg^{-1}s^{-1}) = EP \ (\mu L.kg^{-1}h^{-1}) \left[\frac{\left(3.341 \text{ x } 10^{-14}\right).P}{T}\right] \qquad [3\text{-}2]$$

Where,

P - Standard atmospheric pressure (101,325 Pa)

*T* - Temperature (K)

### 3.3 Determination of firmness

Firmness of fruit from each treatment was evaluated after equilibration to 20 °C using a QALink Penetrometer (Willowbank, Electronics Ltd., Napier, New Zealand) interfaced to a computer (Pranamornkith et al., 2012). The penetrometer was fitted with the standard 7.9 mm round Effegi probe. Fruit skin at two locations (90º apart) around the equator of the fruit were removed (depth 2 mm) using a fruit slicer before each measurement. The average peak force (N) required to puncture the skinned tissue to a depth of 8 mm at a speed of 20 $mm.s^{-1}$ was recorded.

# Chapter 4

## RATE OF ETHYLENE PRODUCTION OF 'HAYWARD' KIWIFRUIT AS A FUNCTION OF FIRMNESS AND TEMPERATURE

### 4.1 Introduction

Temperature is a key factor which governs ethylene biosynthesis and ultimately affects fruit quality (Johnson et al., 1995). Hence, low temperature is used throughout the supply chain to provide opportunities to transport fresh produce to long distance markets and to store produce for long periods (Ritenour et al., 1999).

Kiwifruit are picked at mature but firm and unripe stage, and within 24-72 hours fruit are graded, packed and then forced air cooled at 2 °C before storing at 0 °C cold storage for up to 4-6 months. Industry requirement is to have kiwifruit cool stored within 48 hours of harvest and fruit temperature should be below 0.5 °C within 10 days of storing in the cold storage (Lallu & Webb, 1997). Refrigerated vessels (reefers) are being used to export kiwifruit to overseas market under refrigeration (www.zespri.com).

However, despite efforts to maintain optimal storage conditions at each stage of the supply chain there are some problems arising in maintaining constant suitable temperature continuously (Zhao et al., 2013). Delays between transportation of kiwifruit from field to pack-house, pre-cooler and cold storage facility, time during pack-house operations such as sorting, grading and packing and specially transport from cold storage to end market are potential occasions where maintaining the perfect temperature throughout the supply chain is affected. For example to reduce cost and time, the New Zealand kiwifruit industry does not actively control temperature between on-shore storage facilities and the port. Commercial monitoring of cool chain temperature has identified frequent breaks in temperature control (from 2-8 °C) at this time which may have an effect on fruit quality over the time period in shipping and marketing. Further, there are possibilities of kiwifruit being exposed to higher temperatures (>20 °C) through underdeveloped cool chain infrastructure when the export market for kiwifruit is expanded to South East Asia and the Indian subcontinent (Zhao et al., 2013).

Kiwifruit has been classified as a climacteric fruit since it has a rise in ethylene production accompanied by rise in respiration during ripening (Arpaia et al., 1994).

Regulation of ethylene production has been described by Yang & Hoffman, (1984) and Oetiker et al., (1997) with a concept of two systems developed by McMurchie et al., (1972). System 1 is associated with low basal level production of ethylene prior to fruit ripening and autocatalytic burst of ethylene occurs along with ripening refers to system 2. However, 'Hayward' kiwifruit variety has been considered as a unique climacteric fruit since that they lack the ability to produce ethylene through the autocatalytic pathway until fruit has ripened to a certain stage (fully ripen) of which it differs from other climacteric fruit where ethylene production and ripening are corresponding (Stavroulakis & Sfakiotakis, 1995; Antunes & Sfakiotakis, 1997 ).

Moreover as Stavroulakis & Sfakiotakis, (1995) noted 'Hayward' kiwifruit lack the ability to produce ethylene through the autocatalytic pathway at temperatures below a critical range of 11-14.5 °C unless damaged or infected. Autocatalytic of ethylene production was only reported to occur in the temperature range of 20-34 °C (Antunes & Sfakiotakis, 1997) and from 17-35 °C (Stavroulakis & Sfakiotakis, 1993).

Firmness is the critically important physical component considered as the key determinant of postharvest kiwifruit quality (Retamales & Campos, 1997; Kim et al., 1999). It is considered as one of the most significant quality alterations consistently associated with ripening of fruit (Lelievre et al., 1997) which controls the consumer acceptability of the kiwifruit and probably the best predictor of kiwifruit shelf life (Bonghi et al., 1996).

Postharvest ripening of kiwifruit has been described in relation to four distinct phases in the softening curve of which the length varies and depends mainly on maturity at harvest and storage atmosphere (Lallu et al., 1989; MacRae et al., 1989; 1990; McDonald, 1990; Kim et al., 1999). First phase has very little softening and cannot identify in more mature fruit. The second phase which has a rapid softening occurs after the lag period (first phase) in early harvest fruit and immediately after harvest for late harvest fruits. Generally, kiwifruit stored at 0 °C in air, soften from 70–80 N at harvest to 20–30 N within 8-12 weeks during the second phase (Hewett et al., 1999) and produce very low ethylene production varying between 0.5-0.7 $pmol.kg^{-1}s^{-1}$(Kim et al., 1999). The third phase begins at a firmness of around 10 N and is marked by onset of autocatalytic ethylene production and slow rate of softening (Bonghi et al., 1996; Kim et al., 1999) which continues until fruit become overripe with firmness less than 7 N (Hallett et al., 1992; Bonghi et al., 1996). There

is evidence of another phase (phase 4) of sharp reduction in firmness at the very end where final tissue disintegration is taking place and the fruit is no longer edible (Hewett et al., 1999).

There are several examples of kiwifruit producing large amounts of ethylene (ethylene climacteric) only after fruit have softened considerably (Beever & Hopkirk, 1990). According to Tonutti et al., (1993) ethylene production was undetectable until kiwifruit reached about 50 N firmness and Kim et al., (1999) illustrated that ethylene production remained very low (0.1-1.2 $pmol.kg^{-1}s^{-1}$) until kiwifruit underwent about 85% of their softening from harvest (90 N firmness) and increased only when fruit softened to 10 N at 20 ºC. Similarly, a dramatical increase of ethylene production was observed only when the fruit firmness dropped below 7 N at 20 ºC (Ritenour et al., 1999), below 10 N (Bonghi et al., 1996; Kim et al., 1999; Feng et al., 2003) or below 13 N (Arapia et al., 1994).

While there is sufficient evidence correlating ethylene production of 'Hayward' kiwifruit variety as a function of fruit quality (firmness) in the literature, most data is collected at either 0 or 20 ºC temperature. No attention has been given to finding a precise relationship between ethylene production and fruit firmness at a broad range of potential supply chain temperatures. Determination of the relationship of ethylene production for a range of temperatures and quality (firmness) will assist the kiwifruit industry in predicting the magnitude of ethylene in the commercial packaging environment throughout the supply chain.

Moreover, some reports (Stavroulakis & Sfakiotakis, 1995 and Antunes & Sfakiotakis, 1997) include observations of no ethylene production, especially at low temperatures (<10 ºC). It is possible that limitations in measuring very low levels of ethylene produced by Gas Chromatogarphy (GC) could have contributed to these no ethylene production measurements. As reported, minimum ethylene detection limit of the conventional method (GC) is 5-10 $nL.L^{-1}$ (Cristescue et al., 2012). The advantage of using high sensitivity laser based technique applied in the ethylene detector (ETD-300, SensorSense, Nijmigen, The Netherlands) has been described in Cristescue et al., (2012).

As reported, minimum ethylene detection limit of the ETD-300 is 0.3 $nL.L^{-1}$ which is equivalent to 0.005 $pmol.kg^{-1}s^{-1}$ ethylene production rate comparing to 0.08 $pmol.kg^{-1}s^{-1}$ ethylene production rate for 5-10 $nL.L^{-1}$ minimum ethylene detection limit in GC [ethylene production rates at each ethylene concentration ($nL.L^{-1}$) were

calculated for a flow rate of 1 $L.h^{-1}$ of ETD-300 and for a total weight of seven kiwifruit (~ 700 g) as referring to equation 3-1]. This shows the benefit of usage of ethylene detector (ETD-300) in detecting ethylene production rates that are un-measurable through more conventional methods [Gas Chromatography (GC)] utilized in most of the previous literature.

Hence, the objective of this research is to establish the relationship between production of ethylene of 'Hayward' kiwifruit at different quality stages (firmness) whilst at different temperature by utilizing the new advancement in ethylene analysis, the ETD-300 (SensorSense, Nijmigen, The Netherlands). As a result, the data collected in this work aims to explore the relationships established with more precision and at production rates that were previously un-measurable through traditional method (GC) because of the advantage of high sensitivity of the ethylene detector. In addition, the data produced is mathematically modelled, enabling prediction of ethylene production of 'Hayward' kiwifruit given knowledge of fruit firmness and temperature.

Outcomes of this work will be helpful in interpreting ethylene concentration measurements (interpreted by sensors) as indicators of kiwifruit quality, enabling detection of kiwifruit quality within the supply chain and potential decisions throughout the kiwifruit supply chain to be made.

## 4.2 Materials and methods

### 4.2.1 Plant material

Kiwifruit ('Hayward') were obtained and prepared for the experiment as explained in the section 3.1.

### 4.2.2 Temperature

Different temperatures for measuring ethylene production were selected (0, 2, 5, 10 and 20 ºC) to enable construction of a temperature dependent model applicable to different environmental exposure throughout the activities of the supply chain. Kiwifruit were taken out from cold storage (0 ºC) and put into glass jars after weighing using a digital balance (PG503-S, Mettler Toledo, Switzerland) [Size of the jar and number of kiwifruit used in each jar varied with the firmness of the fruit as explained in section 3.2]. Jars with kiwifruit were kept inside a temperature

controlled incubator (MIR153, SANYO, Tokyo, Japan) to equilibrate under required temperatures (0, 2, 5, 10 and 20 ºC) for 24 hours.

### 4.2.3 Determination of ethylene production by kiwifruit

Ethylene production rate of kiwifruit at different temperatures (0, 2, 5, 10 and 20 °C) were determined using the ethylene detector (ETD-300, SensorSense B.V., Nijmegen, The Netherlands) by following the method described in section 3.2. Measurements of ethylene production of different fruit removed from cool storage (0 °C) and equilibrated under each specific temperature were conducted with 3 replicates (single jar with either seven, three or one fruit depending on the rate of ethylene production of kiwifruit - section 3.2 represented 1 replicate) for each specific temperature at regular intervals throughout the storage period of six months.

### 4.2.4 Determination of firmness of kiwifruit

At the end of each measurement of ethylene production, firmness of each kiwifruit was evaluated destructively after equilibration to 20 °C using a QALink Penetrometer (Willowbank, Electronics Ltd., Napier, New Zealand) following the method explained in section 3.3.

### 4.2.5 Modelling of data

A simple mathematical model was developed to enable prediction of the ethylene production of kiwifruit as a function of firmness and temperature. An empirical model (Equation 4-1) of logistic form was applied to the data obtained at each temperature to predict the relationship between ethylene production and firmness for each temperature (0, 2, 5, 10 and 20 °C). $F_0$ and μ value (Equation 4-1) were obtained using the solver command/function in Microsoft Excel (Microsoft Excel solver) by minimising the value of the average of standard error of ethylene production [(predicted – actual)$^2$] for each temperature.

$$Log\ EP = \mathrm{EP_{Max}} - \left(\frac{\mathrm{EP\ Max.} - \mathrm{EP\ Min.}}{1+\exp.^{-\mu\,(F-\mathrm{F_0})}}\right) \quad [4\text{-}1]$$

Where,

*Log EP* = Log ethylene production at specified firmness value (mol.kg$^{-1}$s$^{-1}$)

EP $_{Max.}$ = Maximum ethylene production obtained (mol.kg$^{-1}$s$^{-1}$)

EP $_{Min.}$ = Minimum ethylene production obtained (mol.kg$^{-1}$s$^{-1}$)

*F* = Specified firmness value (N)

$F_0$ = Minimum firmness value (N)

μ = Ethylene production rate constant (s$^{-1}$)

To develop a combine model to explain the temperature effect, a secondary joint model (Equation 4-2) was developed by estimating new values of $F_0$ (= 0.12 T + 5.02) and μ (= 0.64 s$^{-1}$).

$$Log\ EP = \mathrm{EP\ _{Max}} - \left[\frac{\mathrm{EP\ Max.} - \mathrm{EP\ Min.}}{1+\exp.^{-0.64\,[F-(0.12\,T+5.02)]}}\right] \quad [4\text{-}2]$$

Where,

*T* = Temperature (°C)

### 4.2.6 Calculation of temperature co-efficient ($Q_{10}$)

Equation 4-3 was used for calculation of $Q_{10}$ values for change of rate of ethylene production of kiwifruit at temperature change between 0 and 10 ºC and 10 and 20 ºC.

$$\mathrm{Q_{10}} = \frac{R_2}{R_1}^{\left[\frac{10}{(T_2 - T_1)}\right]} \quad [4\text{-}3]$$

Where,

$R_2$ = Ethylene production rate at $T_2$ temperature (mol.kg$^{-1}$s$^{-1}$)

$R_1$ = Ethylene production rate at $T_1$ temperature (mol.kg$^{-1}$s$^{-1}$)

$T_2$ = Higher temperature (ºC)

$T_1$ = Lower temperature (ºC)

### 4.3 Results

Figure 4-1 illustrates the relationship between ethylene production and firmness (51.6-1.4 N) of kiwifruit obtained at a temperature range of 0, 2, 5, 10 and 20 °C. Ethylene production was very low, but detectable: 0.008-0.011 pmol.kg$^{-1}$s$^{-1}$ (8-11 fmol.kg$^{-1}$s$^{-1}$) at 0 and 2 °C, at early stage of measurements when the fruit firmness was in a range of 51-44 N. The concentration of ethylene detected through the ethylene detector was 0.5-0.8 nL.L$^{-1}$ for the ethylene production value of 0.008-0.011 pmol.kg$^{-1}$s$^{-1}$ [for a flow rate of 1 L.h$^{-1}$ of ETD-300 and for a total weight of seven kiwifruit (~700 g) as referring to equation 3-1]. The range of ethylene concentrations measurable in the ethylene detector (ETD-300) is 0.3-5000 nL.L$^{-1}$. The minimum detection limit observed in the experiment was 0.3 nL.L$^{-1}$, which was equivalent to 0.005 pmol.kg$^{-1}$s$^{-1}$ ethylene production rate [for a flow rate of 1 L.h$^{-1}$ of ETD-300 and for a total weight of seven kiwifruit (~700 g) as referring to equation 3-1]. Ethylene production ranging from 0.012-0.016 pmol.kg$^{-1}$s$^{-1}$ (12-16 fmolkg$^{-1}$s$^{-1}$) were observed for kiwifruit with similar firmness value (51-44 N) at 20 °C. Data are not available for 5 °C, 10 °C for 51-44 N firmness value range.

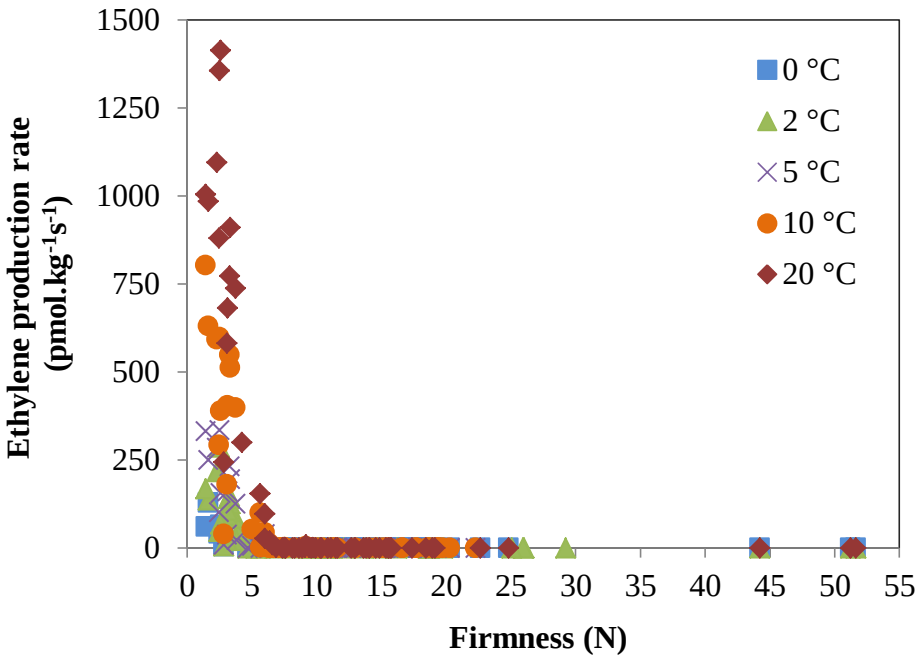


Figure 4-1: Ethylene production of 'Hayward' kiwifruit (pmol.kg$^{-1}$s$^{-1}$) as a function of firmness value (N) and temperature (ºC) for the firmness range from 51.6 to 1.4 N. (Each data point represents ethylene production obtained from a single jar with either seven, three or one fruit depending on the rate of ethylene production of kiwifruit - section 3.2).

Log ethylene production values of kiwifruit for the firmness range from 29.3-1.4 N (excluding data of 51-44 N) are presented in figure 4-2 for clear observations of data of the later stage of the experiment. Ethylene production of kiwifruit with the firmness range of 24-18 N (~after 2 weeks of storage at 0 ºC) was still low (0.008-0.011 pmol.kg$^{-1}$s$^{-1}$) at 0 and 2 ºC (Figure 4-2). However, ethylene production observed at 5 and 10 ºC were approximately twice that of 0 and 2 ºC. At 20 ºC ethylene production was approximately 3 times that at low temperature (0 and 2 ºC) for fruit with similar firmness: 24-18 N.

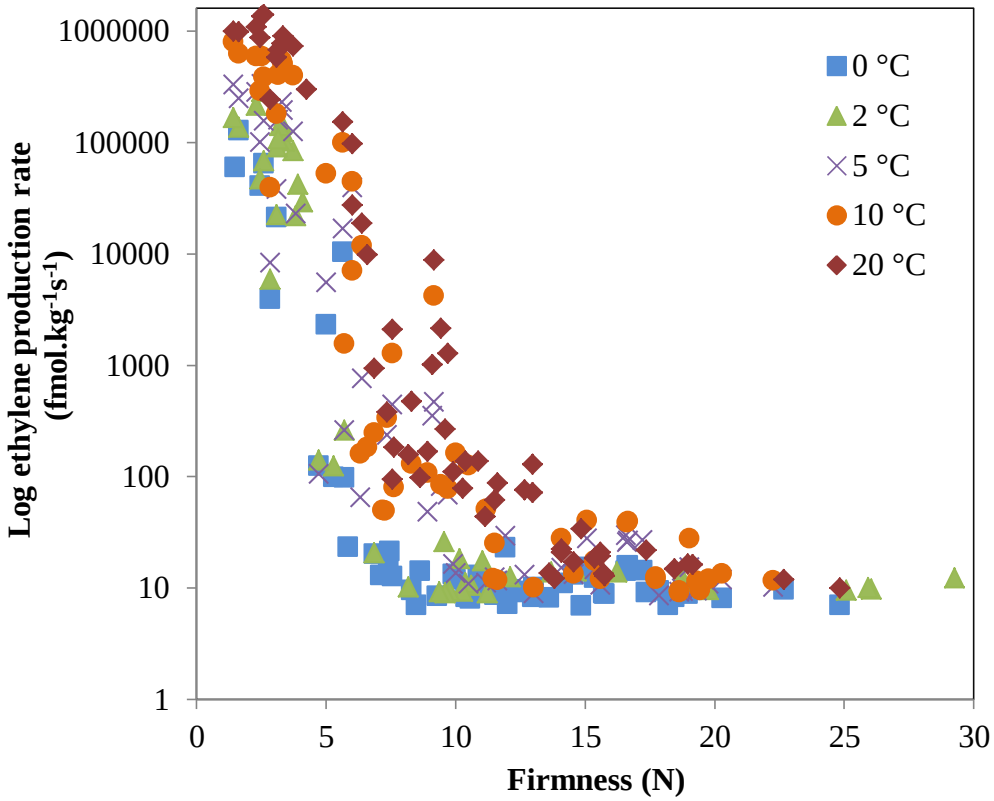


Figure 4-2: Log ethylene production of 'Hayward' kiwifruit (fmol.kg$^{-1}$s$^{-1}$) as a function of firmness value (N) and temperature (ºC) for the firmness range from 29.3 to 1.4 N. (Each data point represents ethylene production obtained from a single jar with either seven, three or one fruit depending on the rate of ethylene production of kiwifruit - section 3.2).

At the time fruit firmness was below 15 N (after four months of storage at 0 ºC), production of ethylene was slightly higher than when kiwifruit firmness was above 15 N at lower temperatures (<10 ºC). Ethylene production of kiwifruit with a firmness <15 N was 2-3 fold higher at 0, 2 and 5 ºC when compare to ethylene production obtained at >15 N. In comparison, at 10 and 20 ºC ethylene production

values of kiwifruit with a firmness <15 N increased by 5 and 10 fold respectively in comparison to fruit with a firmness >15 N (Figure 4-2).

After reaching a certain firmness (<13 N) a very distinct (~10,000–100,000 times) increase in ethylene production was observed in kiwifruit at all temperatures (Figure 4-2). However, firmness level where increase of production of ethylene was observed varied with temperature (Table 4-1). As detected, spike of ethylene production in ten to hundred thousand fold occurred at early stage of firmness for higher temperatures (20 ºC; 13 N) and with the reduction of temperature, point of firmness also got lowered (0 ºC; 5.6 N). Further, it was observed that firmness value where substantial ethylene production was observed at 0 ºC (5.6 N) and 2 ºC (5.7 N) was well away from the value of ready to eat stage of kiwifruit (<8 N). Up to 16,000, 30,000, 33,000, 70,000, 120,000 fold burst of ethylene production was detected at 0, 2, 5, 10 and 20 ºC respectively at each specific firmness level (Table 4-1).

Table 4-1: Different points of firmness value of which substantial increase of ethylene production was observed for 'Hayward' kiwifruit at different temperatures.

| Temperature | Increase of ethylene production | Firmness value of which increase of ethylene production occurred |
|---|---|---|
| 0 ºC | 16,000 fold | 5.6 N |
| 2 ºC | 30,000 fold | 5.7 N |
| 5 ºC | 33,000 fold | 9.7 N |
| 10 ºC | 70,000 fold | 10.5 N |
| 20 ºC | 120,000 fold | 13.0 N |

Substantial differences in ethylene production as a result of temperature were detected following the ethylene burst. Values of ethylene production were nearly 1.7, 2, 4.7 and 8.3 times higher at 2 ºC, 5 ºC, 10 ºC and 20 ºC respectively than at 0 ºC. Further, it could be noted that kiwifruit at the stage of burst of ethylene production produced 16,000 times more ethylene even at 0 ºC against early stage fruit (>15 N).

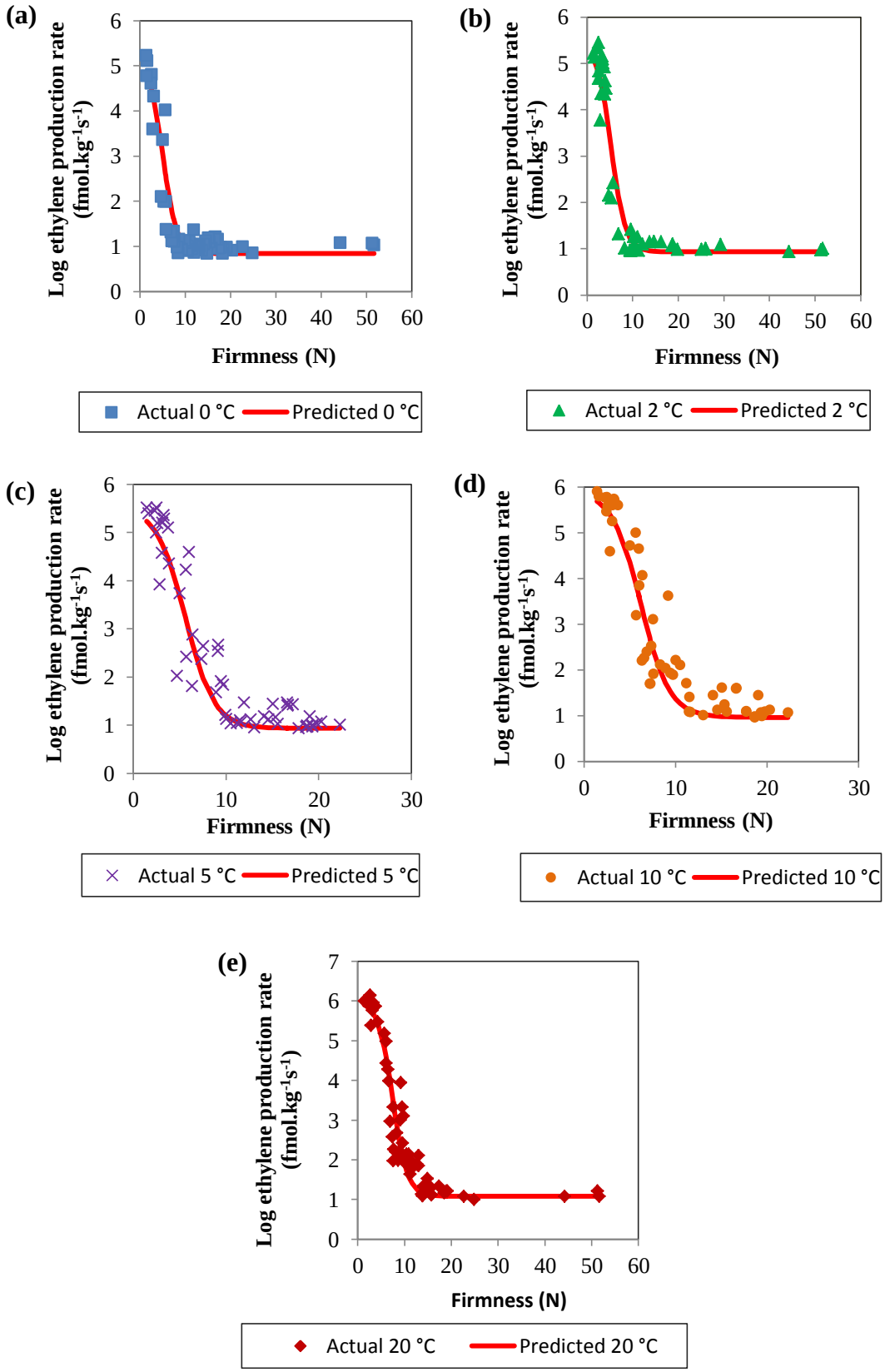


Figure 4-3 (a-e): Mathematical models illustrating the relationship between log ethylene production of 'Hayward' kiwifruit (fmol.kg$^{-1}$s$^{-1}$) as a function of fruit quality/firmness (N) at each specific temperature (°C).

Ethylene production peaked to a value of 172 pmol.kg$^{-1}$s$^{-1}$ (172,000 fmol.kg$^{-1}$s$^{-1}$) for fruit at 0 ºC whereas it was 286 pmol.kg$^{-1}$s$^{-1}$ (286,000 fmol.kg$^{-1}$s$^{-1}$) for fruit at 2 ºC. Kiwifruit at 5 ºC produced high ethylene at a rate of 335 pmol.kg$^{-1}$s$^{-1}$ (335,000 fmol.kg$^{-1}$s$^{-1}$) and it was 803 pmol.kg$^{-1}$s$^{-1}$ (803,000 fmol.kg$^{-1}$s$^{-11}$) and 1413 pmol.kg$^{-1}$s$^{-1}$ (1413,000 fmol.kg$^{-1}$s$^{-1}$) for fruit at 10 and 20 ºC respectively.

Obtained experimental data at each specific temperature was well fitted with the logistic equation explained in the section 4-2-5 (Equation 4-1) by minimising the value of the average of standard error of ethylene production up to 0.1-0.3 using the Microsoft Excel solver (Figure 4-3). The developed model to explain the combine temperature effect on the relationship between log ethylene production of 'Hayward' kiwifruit (fmol.kg$^{-1}$s$^{-1}$) and the firmness value (N) is presented in Figure 4-4. The result model describes the ethylene production of sound kiwifruit as a function of fruit quality (firmness) and temperature.

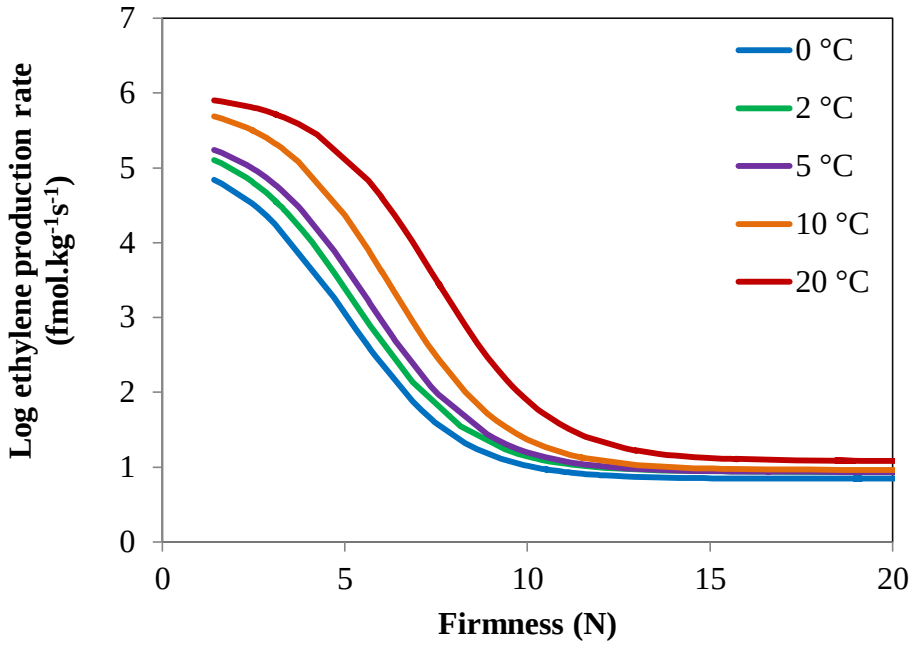


Figure 4-4: Mathematical model illustrating the relationship between log ethylene production of 'Hayward' kiwifruit (fmol.kg$^{-1}$s$^{-1}$) as a function of fruit quality/firmness (N) and temperature (°C).

Equation 4-4 was obtained through the mathematical model (Figure 4-4) developed in predicting log ethylene production of sound kiwifruit as a function of fruit quality (firmness) and temperature.

$$\text{Log EP} = 6.0 - \left[\frac{(6.0-0.8)}{1+\exp^{(-0.64\,[F-(0.12\,T+5.02)]}}\right] \qquad [4\text{-}4]$$

Where,

T = Temperature (°C)

F = Specified firmness value (N)

As described in section 4-2-6, $Q_{10}$ (temperature coefficient) values calculated for rate of ethylene production was 4.7 between 0 and 10 ºC and 1.2 for between 10 and 20 ºC temperature change.

### 4.4 Discussion

Low ethylene production (less than 1.2 pmol.kg$^{-1}$s$^{-1}$) obtained in this study for kiwifruit with high firmness (51-44 N) has been found to occur in a number of previous studies. Kim, (1999) found ethylene production was in a range of 0.25-1.2 pmol.kg$^{-1}$s$^{-1}$ for kiwifruit stored at 0 °C when fruit firmness was around 50-40 N. Ethylene production was reported as less than 1.2 pmol.kg$^{-1}$s$^{-1}$ at 0 °C for mature but unripe fruit by Crisosto & Kader, (1999). However, results obtained for rate of ethylene production of kiwifruit with high firmness (51-44 N) in the present investigation are very low (0.008-0.011 pmol.kg$^{-1}$s$^{-1}$) compared with reported values.

When firmness of kiwifruit reduced further from 90 to 10-12 N, ethylene production still remained low between 0.1-1.2 pmol.kg$^{-1}$s$^{-1}$ even at 20 ºC according to the findings of Kim et al., (1999). In the same way Crisosto & Kader, (1999) also mentioned that mature but unripe kiwifruit produced low ethylene ranging from 1.2-6.2 pmol.kg$^{-1}$s$^{-1}$ at 20 ºC but there was no statement about the exact firmness level of the kiwifruit measured. Going in parallel, kiwifruit of the current research also maintained low ethylene production (~0.03 pmol.kg$^{-1}$s$^{-1}$) even at 20 ºC when fruit firmness reduced to a value of 24-18 N from 51-44 N.

Very low ethylene production values detected in the present work (0.008-0.03 pmol.kg$^{-1}$s$^{-1}$) comparing with the earlier reported values (0.25-6.2 pmol.kg$^{-1}$s$^{-1}$) in Kim, (1999) and Crisosto & Kader, (1999) is reasonably explained by the high sensitivity of the ethylene detector (ETD-300) used for the current work. This high sensitivity method gives the benefit of detecting production rates of ethylene that are un-measurable through conventional methods [Gas Chromatograph (GC)] utilized in most of the previous literature. As reported, minimum ethylene detection limit of the conventional method (GC) is 5-10 nL.L$^{-1}$ (Cristescue et al., 2012) which is

equivalent to 0.08 pmol.kg$^{-1}$s$^{-1}$ ethylene production rate [for a flow rate of 1 L.h$^{-1}$ of ETD-300 and for a total weight of seven kiwifruit (~700 g) as referring to equation 3-1]. In comparison, minimum detectable ethylene production rate for ETD-300 is 0.005 pmol.kg$^{-1}$s$^{-1}$ [for a flow rate of 1 L.h$^{-1}$ of ETD-300 and for a total weight of seven kiwifruit (~700 g) as referring to equation 3-1] for the minimum detection limit of 0.3 nL.L$^{-1}$. Hence, this explains the limitation in measuring very low ethylene production values such as 0.008 pmol.kg$^{-1}$s$^{-1}$ in employing traditional methods like GC and the ability of usage of ETD-300 for determination of similar ethylene production rate (0.008 pmol.kg$^{-1}$s$^{-1}$).

When compared with the results reported previously, the maximum ethylene production obtained at 20 °C in this experiment when fruit firmness reduced below 18 N is not dissimilar to what has been reported previously. Crisosto & Kader, (1999) reported kiwifruit with a firmness of less than 18 N produced 620–1239 pmol.kg$^{-1}$s$^{-1}$ at 20 °C. Ethylene production value of kiwifruit in the present research reached a maximum level of 1400 pmol.kg$^{-1}$s$^{-1}$ at 20 °C when the firmness of the fruit was below 18 N. However, Hyodo & Fukasawa, (1985) reported the maximum ethylene production obtained from 'Hayward' kiwifruit at 21 ºC as 744 pmol.kg$^{-1}$s$^{-1}$, only two third's of what was measured in the present work. Ethylene production of 'Hayward' kiwifruit was 298 pmol.kg$^{-1}$s$^{-1}$ at 20 °C as reported by Kim, (1999), almost 4 times less than what was observed in current research. Unknown exact firmness values of the kiwifruit used in past studies may have given this ethylene production gap at similar temperature (20 °C).

Perkins-Veazie et al., (1999) described ethylene production for non-climacteric fruit like strawberry as low and constant between 0.3-6.2 pmol.kg$^{-1}$s$^{-1}$ during ripening and that has been described as associated with low ACC (1-amynocyclopropane-1-carboxylic acid) concentration between 1.19 and 1.95 nmol.g$^{-1}$ and ACO (ACC oxidase) activity varying between 0.72-2.01 nL.g$^{-1}$h$^{-1}$. Similarly, Kim, (1999) stated that as a climacteric fruit, low ethylene production of kiwifruit at 0 °C was associated with low ACC concentration, varying between 0.15 and 0.5 nmol.g$^{-1}$ and ACO activity varying between 0.01-0.66 nL.g$^{-1}$h$^{-1}$ when firmness ranged from 70-20 N. Other climacteric fruit including avocado, banana and tomato have also shown low ACC concentration and low ACO activity at the pre climacteric stage.

While attributing a reason to the low ethylene production of kiwifruit associated with high firmness in the current work was not the focus of this study, future research in obtaining change of intermediates and enzymes related to ethylene synthesis pathway (ACC and ACO) as a result of temperature change and firmness change in parallel on determination of ethylene production of kiwifruit would add more value to the relationship established in this work.

In previous studies firmness of kiwifruit has been reported to decrease with storage time (MacRae et al., 1989; 1990; Kim et al., 1999). Loss of firmness occurs through three phases, the first phase being considered as a lag period which consists of a very small softening. This initial phase is not observed in more mature fruit. The kiwifruit received for this experiment also didn't have a slow softening phase as they were relatively over mature at the time of the receipt (51-44 N firmness). The lag phase is followed by a second phase of rapid decline in firmness and a third phase of slow softening. The rapid firmness reduction was observed in the kiwifruit used for this study within the first month of storage at 0 ºC.

According to Crisosto & Kader, (1999) the conversion of starch to soluble sugars corresponds to the drop in flesh firmness roughly and research work demonstrate that first few weeks of storage are the critical stage for flesh softening where conversion of starch to soluble sugars increases rapidly. Approximately one-third to one-half of the remaining flesh firmness can be lost even when fruit are held at 0 °C for a period of one month storage. Several cell wall hydrolases including β galactosidase (β GAL), pectin methylesterase (PME) and xyloglucan endotransglycosylase (XET) induce fruit softening (MacRae & Redgwell, 1992). As stated by MacRae & Redgwell, (1992) probably low ethylene produced at 0 °C (<1 $pmol.kg^{-1}s^{-1}$) is enough to activate these cell wall hydrolases. In supported of this, Tonutti et al., (1993) and Arpaia et al., (1994) found that low concentrations of ethylene produced (below 2.5 $pmol.kg^{-1}s^{-1}$) could have an impact on accelerating kiwifruit softening. Kiwifruit used in the present experiment lost half of fruit firmness (from 51-44 N to 24-18 N) within two weeks of storage at 0 ºC and continued to soften reaching around 18-13 N (losing approximately two third of firmness) within one month of storage at 0 ºC. Considering the statements by MacRae & Redgwell, (1992); Tonutti et al., (1993) and Arpaia et al., (1994) it could be discussed that very low ethylene production observed from kiwifruit of early stage

of storage (51-44 N to 24-18 N) of present trial even at 0 ºC may have an effect to this rapid firmness reduction.

Past research documenting ethylene production of kiwifruit as a function of firmness is focused only for either 0 °C (Kim, 1999; Crisosto & Kader, 1999) or 20 °C (Hyodo & Fukasawa, 1985; Kim, 1999; Crisosto & Kader, 1999). The current work dedicated time to collecting ethylene production at more temperatures between 0 and 20 °C namely: 2, 5 and 10 °C allowing development of a model that describes kiwifruit ethylene production as a function of both firmness and temperature. To develop and to give benefits of these findings to the industry it is important to extend this research further to other commercial varieties of kiwifruit available in New Zealand.

Kiwifruit has been classified as a climacteric fruit since it has a rise in ethylene production in parallel with rise in respiration during ripening (Arpaia et al., 1994). However, there is some conflicting evidence on this climacteric ethylene production of kiwifruit. Stavroulakis & Sfakiotakis, (1995) noted that 'Hayward' kiwifruit is considered to be a unique climacteric fruit since they lack the ability to produce ethylene through the autocatalytic pathway at temperatures below a critical range of 11-14.5 °C unless damaged or infected. Autocatalysis of ethylene production was only reported to occur in the temperature range of 20-34 °C (Antunes & Sfakiotakis, 1997) and from 17-35 °C (Stavroulakis & Sfakiotakis, 1993). Interestingly, kiwifruit not treated with propylene did not produce ethylene even at temperatures above 15 °C (Antunes & Sfakiotakis, 1997). However, absence of autocatalytic ethylene production at temperature below a critical range of 11-14.5 °C is a debatable point when considering the research results of the present work since autocatalytic ethylene production was observed even at temperature less than 10 °C for 'Hayward' kiwifruit. As explained earlier in section 4-1 as well as beginning of this section 4-4, it could suggest that limitations in measuring very low ethylene produced by kiwifruit at temperature below 11-14.5 °C using conventional method (GC) may be the reason behind the comments made by Stavroulakis & Sfakiotakis, (1993); Stavroulakis & Sfakiotakis, (1995) and Antunes & Sfakiotakis, (1997).

Earlier Stavroulakis & Sfakiotakis, (1993) attributed limited ACC production due to decreased activity of ACS rather than decreased ACO activity to low ethylene production at temperatures below 11-14.8 °C and later Antunes & Sfakiotakis, (1997) attributed inhibition of ethylene production below 10 °C to low activities of

both ACS and ACO. Hence, this statement also raises the need of quantifying intermediates (ACC) and enzymes (ACO and ACS) attached to ethylene synthesis pathway in parallel with ethylene production determination as a function of firmness and temperature for further explanation of the results obtained in the present work at low temperature (below 15 °C).

In agreeing with the explanations presented in number of previous studies on phenomenon of substantial increase of ethylene production at a certain firmness level of fruit, similar tendency was observed in results of present work. As evidences to demonstrate that it is distinctive to kiwifruit to produce large amount of ethylene only after fruit have softened considerably, results of Kim et al., (1999) revealed that even at 20 ºC ethylene production remained low when kiwifruit softened from 90 N to 10-12 N and only increased when kiwifruit underwent about 85% of their softening from harvest or when fruit softened to below 10 N. According to Bonghi et al., (1996) autocatalytic ethylene production was a late event occurring where it was observed when kiwifruit had softened to 10 N at 20 ºC coinciding with the stage where activity of cell wall degrading enzymes: β-GAL and PG markedly increased. Going in parallel, Feng et al., (2003) also reported that kiwifruit itself did not produce ethylene until it softens to flesh firmness less than 10 N at 20 ºC. Contrastingly, dramatically increase of ethylene production at 20 ºC was only observed by Ritenour et al., (1999) when firmness dropped below 7 N with no statement about the temperature while Arpaia et al., (1994) stated that kiwifruit climacteric occurred when fruit softened below 13 N. The current research results obtained at 20 ºC agrees with the information from Arpaia et al., (1994) whereas deviates from Bonghi et al., (1996); Kim et al., (1999) and Feng et al., (2003) research results since substantial increase in ethylene production at 20 ºC was observed when fruit firmness was at 13 N. Considering Ritenour et al., (1999) results of 7 N point of firmness, current research results noted that increase of ethylene at later firmness values like less than 7 N was observed at lower temperatures (0 and 2 ºC). Nevertheless, more detailed work by correlating the fruit maturity at harvest with the relationship of ethylene production of kiwifruit between fruit firmness and temperature is required to elucidate the variation exist in point of firmness of which ethylene spike occur at different temperature.

Most of the past information relating kiwifruit firmness to the observed ethylene production increase has occurred at 20 °C. Hence, the current work conducted at different temperatures, including that of commercial applicability adds significantly to the understanding of the ethylene production behaviour of 'Hayward' kiwifruit. Results of this work have indicated that with the reduction of temperature, point of firmness of which substantial increase of ethylene production occurring gets lowered. Further, results leads us to suggest that firmness value where burst of ethylene production was observed at 0 ºC and 2 ºC (5.6 N and 5.7 N) was well away from the value of ready to eat stage of kiwifruit (8 N). This may remind and would be an important information to the industry as kiwifruit can be stored and kept at lower temperature levels (0 ºC and 2 ºC) safely before ripening and state of which customers prefer.

### 4.5 Conclusion

Very low ethylene production values observed from the kiwifruit of high firmness (51-18 N) in the present investigation comparing with reported results of ethylene production for similar stage kiwifruit revealed the sensitivity of the equipment, ethylene detector (ETD-300) used in the current study on determining ethylene production. The current work dedicated on collecting ethylene production at a wider temperature range between 0 and 20 °C (2, 5 and 10 °C) allowed observation of the variation of firmness of kiwifruit where substantial increase of ethylene production (autocatalytic ethylene production) occurred at different temperature. As observed with the reduction of temperature, point of firmness of which increase of ethylene production occurring got lowered and this information would give benefits to the industry in concerning the duration of storage of kiwifruit according to the temperature the fruit exposed. A mathematical model was established that could be used in predicting ethylene production of sound kiwifruit as a function of fruit quality (firmness) and temperature. Future research on similar kind of model development targeting other kiwifruit varieties available in New Zealand would help the kiwifruit industry when concern reducing postharvest losses of kiwifruit occurring through the supply chain and ultimately to increase profits.

# Chapter 5

## RATE OF ETHYLENE PRODUCTION OF 'HAYWARD' KIWIFRUIT CAUSED BY IMPACT DAMAGE

### 5.1 Introduction

Mechanical injury is a key cause of significant postharvest loss of perishables (FAO, 1989) that occur during postharvest operations. Starting from dropping fruit into the picking bag during harvest, kiwifruit has the potential to be exposed to various types of mechanical injuries through different phases of the supply chain. Namely, compression, impact, abrasion, puncture and vibration injuries could occur to fruit during loading, unloading, transport, sorting, grading and packing operations (Bollen et al., 1995). Out of these, impact injury is more related to harvest and handling and provides an important issue within the kiwifruit supply chain. Generally, fruit may be exposed to impact damage through falling of fruit from different heights (dropping fruit to the collector at harvest, loading and unloading) as well as due to contact with surrounding fruit (packing). Preliminary observations have noticed that impact injury is the most frequent injury happening to randomly packed kiwifruit (Mencarelli et al., 1996). Mostly, pallet reconstruction takes place a number of times through the kiwifruit supply chain leading to opportunities to exposing fruit to short term impact injuries.

Information related to the increase of ethylene of fresh produce due to injury comes from ancient history. Scraping of sycamore fig (*Ficus sycomorus*) with iron claws was practiced to ripen fruit in early Egyptian civilization (Abeles et al., 1992). Scientific research over the past four decades has identified that induction of ripening of an injured fruit occurs due to an increase of ethylene as a physiological response to mechanical wounding (Yu & Yang, 1980; Yang & Hoffman, 1984; Abeles et al., 1992; DeMartino et al., 2002). Ethylene produced due to injury is referred to as "stress or wound ethylene" and this follows with other responses including destruction of cells leading to de-compartmentalization of enzymes and substrates, decrease of firmness, increase of respiration, accumulation of secondary metabolites and increase of soluble solid concentration as indicators of induction of ripening due to mechanical injury (Yang & Pratt, 1978; Miller et al., 1987). Detection of wound ethylene is considered as an important index of revealing any mechanical damage to

fruit since most mechanical injuries, especially as impact injuries on kiwifruit are undetectable externally due to invisibility of symptoms of bruises (Bollen et al., 1995; Mencarelli et al., 1996).

Recently, ethylene production of 'Bruno' kiwifruit was found to increase by 2.5 and 5 times for fruit dropped at 60 and 120 cm respectively when compared with control (without drop) at 20 °C (Alves et al., 2010). A two fold increase of ethylene evolution in comparison to the control was found for dropped 'Hayward' kiwifruit from 30 cm at 18 °C (Mencarelli et al., 1996). After 14 and 21 h of impact injury, rate of production of ethylene of 'Hayward' kiwifruit had increased by 3 and 5 times than what observed at 7 h of injury at 18 °C. This information provides an insight into the existence of damaged kiwifruit and suggests that damaged fruit may have a significant influence on the ethylene accumulation within the kiwifruit pack.

Current knowledge determining ethylene production of kiwifruit exposed to impact injuries is concentrated to mostly high temperatures of 18 °C (Mencarelli et al., 1996; DeMartino et al., 2002) and 20 °C (Alves et al., 2010) apart from a few pieces of research done at 4 °C (Mencarelli et al., 1996; DeMartino et al., 2002). As a potential key factor contributing to the ethylene concentration within kiwifruit packaging systems, this research aimed to determine effect of impact injury on ethylene production of kiwifruit for across a range of temperatures (0, 5 and 20 °C). The results will aid future construction of a predictive model and the interpretation of ethylene sensors implemented in kiwifruit packaging systems for the purpose of reducing postharvest losses throughout the kiwifruit supply chain.

## 5.2 Materials & methods

### 5.2.1 Plant Material

Kiwifruit without any visual damage, rot development or misshapen character were selected for the trials and the experimental work was performed at two stages with two different maturities of kiwifruit. Kiwifruit stored for 2 months at 0 ºC and with an average firmness of 20 N were used for the experiment done as first stage (stage 1), while the experiment done as second stage (stage 2) was conducted with kiwifruit stored for nearly 10 months at 0 ºC which had a firmness value of 12-15 N.

Mass of the fruit has the greatest effect to the energy absorbed by injured fruit (Knee & Miller, 2002) as well as the bruise volume (Brusewitz & Barstch, 1991; Maness et al., 1992). Thus, to prevent the influence of fruit mass variation on stress

ethylene produced due to injury, kiwifruit of a similar weight (100±5 g) were selected after measuring weight electronically using a three decimal place digital balance (PG503-S, Mettler Toledo, Switzerland).

### 5.2.2 Method of impact injury

Knee & Miller, (2002) identified two means of determining effect of impact force on fruit namely a fixed mass dropped onto a fruit or the fruit itself dropped onto a surface. Even though there is an advantage of having a constant impact force throughout the experiment in the first method, second method has been introduced as more representative of what occurs to fruit during postharvest handling and storage and is the most common method used in the investigations on effect of impact force on fruit. Hence, fruit drop onto a surface was selected as the method to determine ethylene production of 'Hayward' kiwifruit induced by impact injury for the present research.

The degree of injury to the fruit is dependent on the impact surface of the fruit as well as the nature of the surface which the fruit falls on (Knee & Miller, 2002). Round surfaces concentrate more energy than flat surfaces. In order to attempt to consistently apply impact, kiwifruit were dropped from a controlled environment. Initially, kiwifruit were held with a clamp fixed to a clamp stand and fruit were released to the dropping surface by orienting with the longitudinal axis parallel to the impact surface to make sure that the flat surface of the fruit always made contact with the dropped surface at each time of dropping (Figure 5-1). The drop surface was constructed of a high density fiberboard/hard board (5 mm thickness). A bottomless cardboard box was used to create a barrier around the drop location to limit further contact damage to the kiwifruit after the initial impact.

Figure 5-1: Method used to drop kiwifruit from different heights

### 5.2.3 Temperature

The experimental work was conducted at three different temperatures: 0, 5 and 20 °C. Kiwifruit were acclimatized at each temperature for 24 h before the impact test and were dropped from three different heights (30 cm, 60 cm and 120 cm) under each temperature conditions inside a refrigerated storage room. A temperature controlled incubator (MIR153, SANYO, Tokyo, Japan) was used to control temperature during ethylene production determination at the 3 temperature levels: 0, 5 and 20 °C. Fruit acclimatized to the same temperature but without any exposure to damage were used as the control.

### 5.2.4 Determination of ethylene production of kiwifruit

Following the condition of variation of glass jar size and the number of fruit inside the jar with the maturity/firmness of the kiwifruit as explained in section 3.2, seven kiwifruit in 1.75 L glass jars and three fruit in 750 mL glass jars were used for determination of ethylene production, for the stage 1 and stage 2 respectively with each jar considered as one replicate. Ethylene production of fruit exposed to three different impact forces were carried out using the ethylene detector, (ETD-300, SensorSense B.V., Nijmegen, The Netherlands) by following the method described in section 3.2. Detection of ethylene was initiated as soon as possible after exposing the fruit to different degrees of impact injury (within 1-2 minutes) and continued for nearly 80 h for all observations. Determination of ethylene production of fruit exposed to three different degrees of damage (drop heights) at one temperature was conducted at one time so that there was only one measure for each temperature treatment (out of six channels available in ETD-300, four channels were used for each treatment: 30, 60, 120 cm drop heights and control). Separate measurements were done for each temperature.

### 5.2.5 Determination of firmness of kiwifruit

At the end of quantification of ethylene production, determination of firmness of control (sound kiwifruit) and injured kiwifruit (of the whole fruit) at each selected temperature was conducted after re-equilibration to 20 °C following the technique described in section 3.3.

### 5.2.6 Data analysis

All the data of firmness of kiwifruit were subjected to analysis of variance (ANOVA) with Minitab Version 15 (Minitab Inc., State College, PA, USA). Means were compared by a Tukey's test at a significant level of 0.05.

## 5.3 Results

### 5.3.1 Effect of intensities of impact injury on ethylene production at different temperatures

As expected, an increase of ethylene production was observed for 'Hayward' kiwifruit exposed to different intensities of injury at 5 and 20 °C (Figure 5-2B and C). However, fruit injured at 0 °C displayed no ethylene production increase due to injury (Figure 5-2A) in comparison to the control. Similar trend of ethylene production with time was observed in injured fruit as well as control (sound kiwifruit) at 0 °C.

The different degree of impact force caused a rise in ethylene production after a lag period for kiwifruit injured at 5 °C (Figure 5-2B). Ethylene production remained unchanged around a value range of 0.2-0.25 pmol.kg$^{-1}$s$^{-1}$ till the 20$^{th}$ h from impact damage for all treatments. After 20$^{th}$ h of injury, a similar pattern of rapid increase in ethylene production was observed till 35$^{th}$ h at 5 °C following a state of equilibrium at peak ethylene production for another 5 h (35-40 h after injury) for all treatments despite of intensity of damage. Even though the pattern was similar, rate of ethylene production values at peak varied with the intensity of damage (drop height). Maximum ethylene production rates obtained from kiwifruit exposed to 30, 60 and 120 cm drop heights at the peak level of 5 °C were 0.55, 0.79 and 0.87 pmol.kg$^{-1}$s$^{-1}$ respectively. Even though it followed the same pattern of ethylene evolution with time as injured, control (sound fruit) produced ethylene at a lower rate of 0.46 pmol.kg$^{-1}$s$^{-1}$ at the peak level. Kiwifruit exposed to 30, 60 and 120 cm drop heights produced 1.2, 1.7 and 1.9 times greater ethylene production than control (sound fruit) at the peak level of 5 °C. The peak was followed by a fall in ethylene production rate (after around 40$^{th}$ h) reaching an equilibrium state 70 h after injury of which values were same for each treatment including the control (approximately 0.3 pmol.kg$^{-1}$s$^{-1}$). With the initiation of increase in ethylene production, control (sound fruit) always had the lowest ethylene production in comparison with all three drop heights (Figure 5-2B).

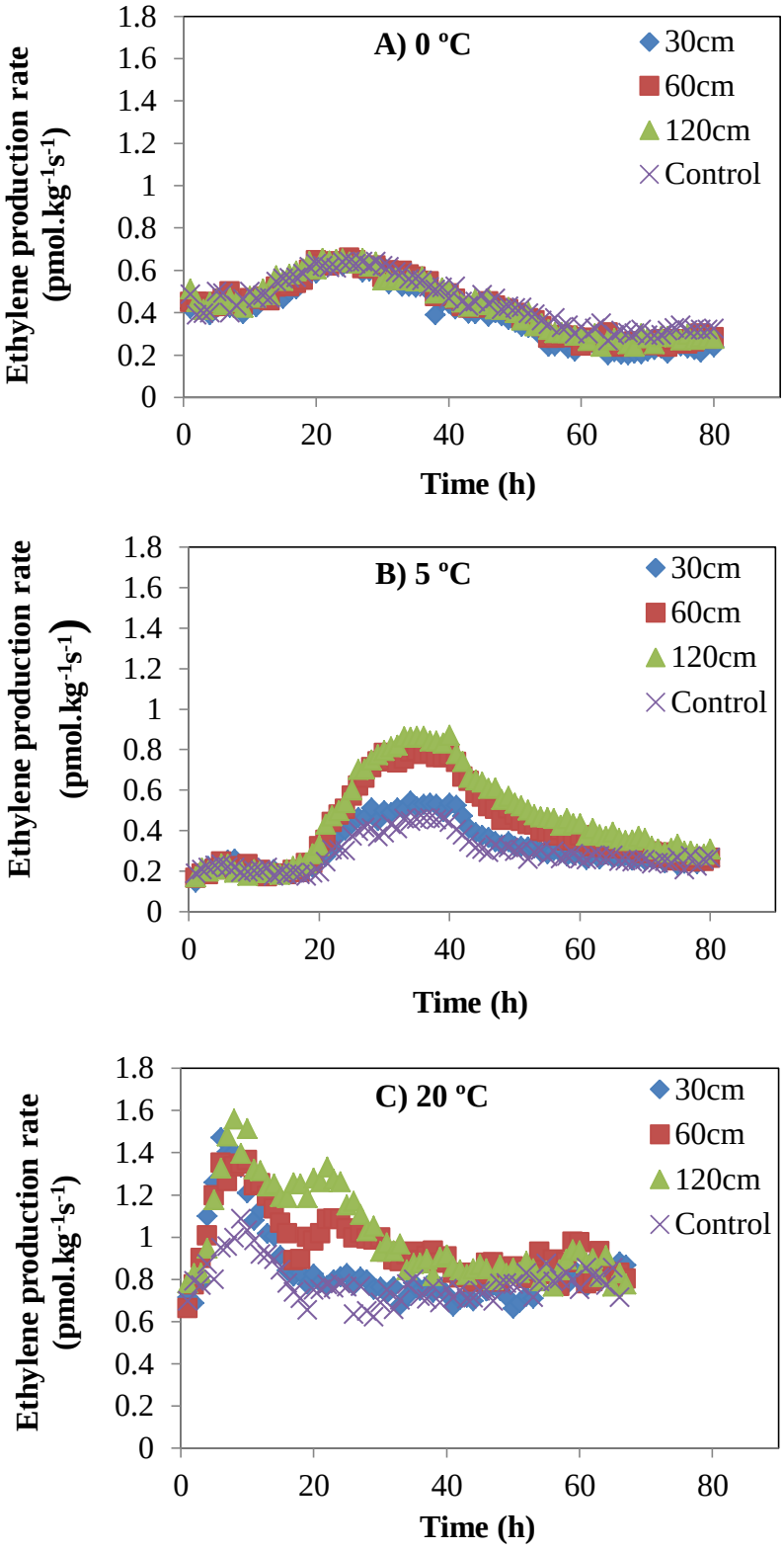


Figure 5-2: Rate of ethylene production of kiwifruit dropped from 30, 60 & 120 cm heights (to inflict with different degrees of impact injury) and control (sound kiwifruit) with time obtained at 0 °C (5-2A), 5 °C (5-2B) and 20 °C (5-2C).

A different trend in the change of ethylene production rate with time was observed for kiwifruit exposed to impact injury at 20 °C (Figure 5-2C). A pattern featuring an initial burst was observed for all treatments without the lag phase observed at 5 °C. A two fold increase in ethylene production rate was observed at this initial burst comparing to basal rate (0.7 pmol.kg$^{-1}$s$^{-1}$). Ethylene production at 20 °C continued to increase during the first 10 h and then declined in the next 10 h subsequently accompanying a second peak lower than first peak around 22 h after injury. Noticeable variation between ethylene production rates due to intensity of damage (except 30 cm drop height) was observed at this second peak. An approximate 44% and 72% increase in comparison to the control (sound fruit) was found in kiwifruit exposed to 60 and 120 cm drop heights respectively at the stage of the second peak (22 h after exposing to damage). The second peak was followed with a gradual decline to a steady state after 30 h of injury and was subsequently observed for all treatments including control (sound fruit) producing a rate of 0.7-0.8 pmol.kg$^{-1}$s$^{-1}$ ethylene at this equilibrium stage (Figure 5-2C).

### 5.3.2 Effect of temperature on wound induced ethylene production at different intensities of impact injury

When comparing results obtained at each temperature (0, 5 and 20 °C) increase in rate of ethylene production was observed with increase of temperature for each intensity of impact injury: 30, 60 and 120 cm drop height (Figure 5-3). Maximum ethylene production of fruit exposed to 30 cm drop height at 20 °C was 2.8 times higher than at 5 °C (Figure 5-3A). Similarly, maximum ethylene production was 1.8 and 2 times higher at 20 °C than at 5 °C when consider 60 cm (Figure 5-3B) and 120 cm (Figure 5-3C) drop heights correspondingly. Temperature effect between 0 and 5 °C was observed with 1.3 times increase of ethylene production at the peak for both drop heights of 60 and 120 cm (Figure 5-3B & C) while temperature effect between 0 and 5 °C was not clearly observable at 30 cm drop height (Figure 5-3A). At the same time Figure 5-3 illustrates that maximum rate of ethylene evolution was observed within 10 h of exposing to damage at 20 °C whereas it took 20-35 h to reach maximum after 20 h lag phase at 5 °C. Firmness variation between the three fruit used in each jar of each treatment might have caused the slightly higher ethylene production observed at the beginning for 0 °C (0.4 pmol.kg$^{-1}$s$^{-1}$) fruit than 5 °C (0.2 pmol.kg$^{-1}$s$^{-1}$).

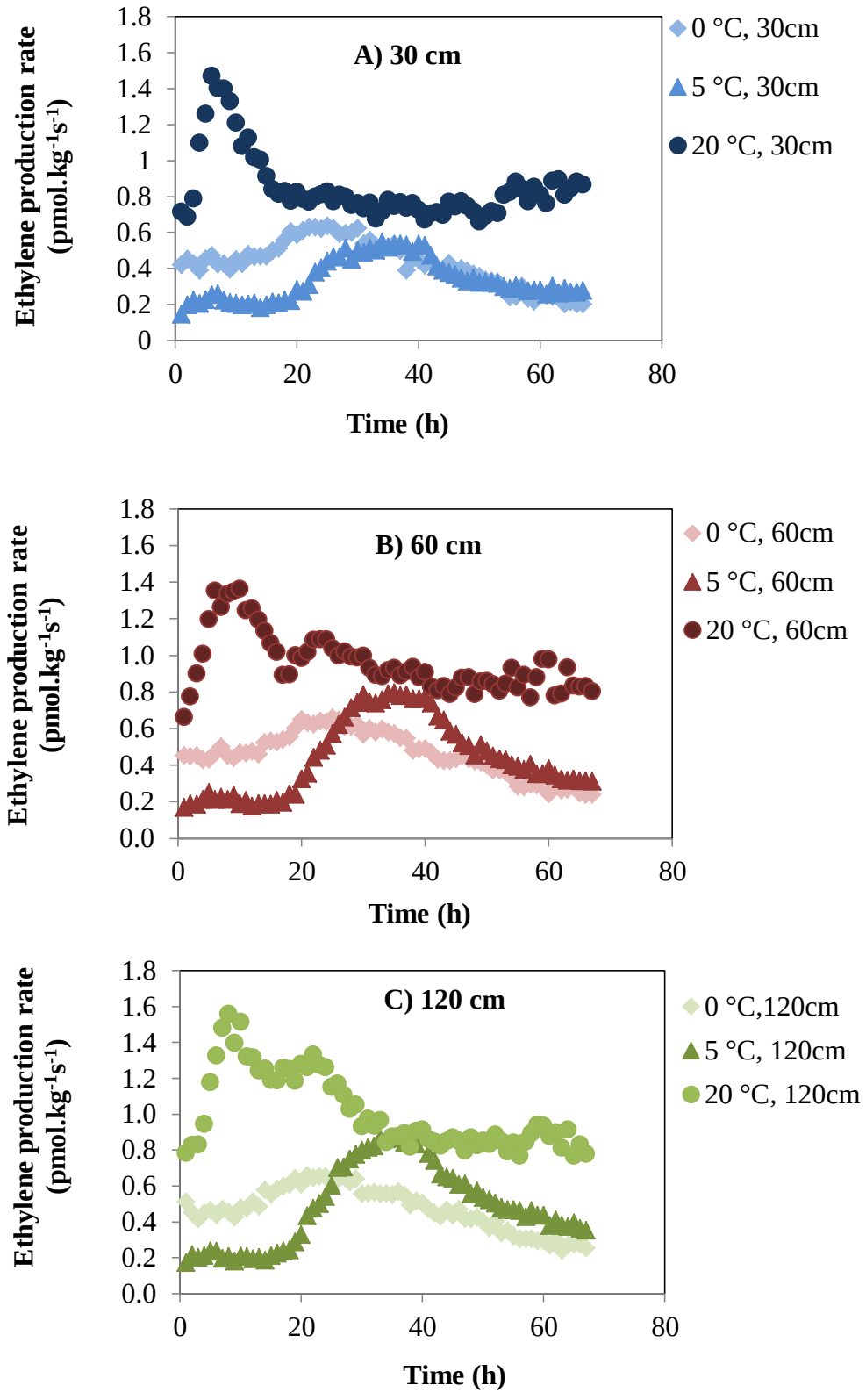


Figure 5-3: Rate of ethylene production of kiwifruit obtained at 0, 5 and 20 °C subjected to different intensities of impact damage (30, 60 and 120 cm drop heights).

### 5.3.3 Effect of fruit maturity (firmness) on ethylene production at different intensities of impact injury

Figure 5-4 compares the results obtained at 20 ºC from the experiment conducted in the stage 1 where firmness of kiwifruit was approximately 20 N (after two months of cold storage at (0 ºC) and results of stage 2 (Figure 5-2C) conducted for kiwifruit of a firmness value of 12-15 N (nearly 10 months after cold storage).

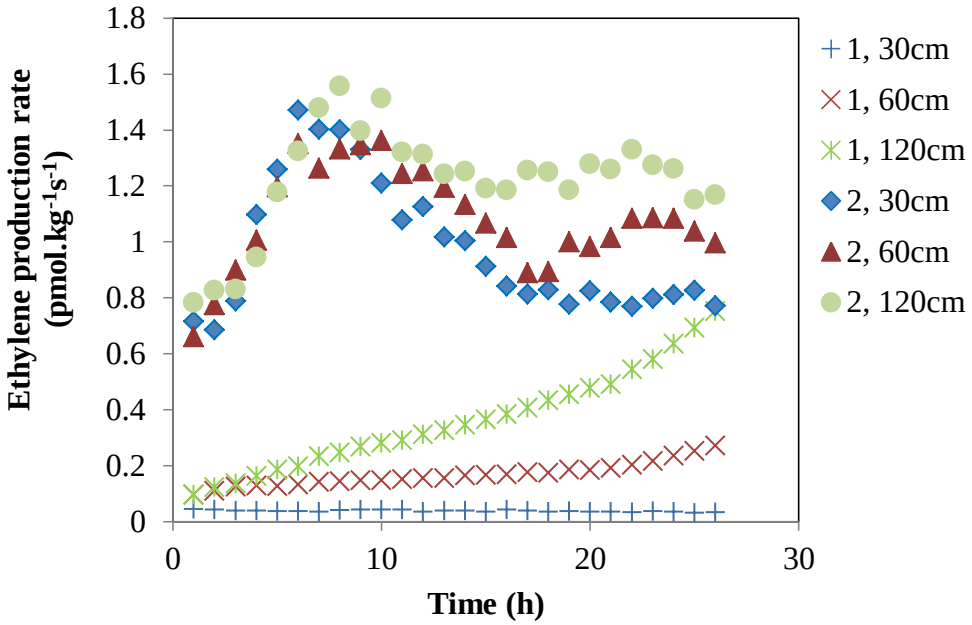


Figure 5-4: Rate of ethylene production of kiwifruit of stage 1 (fruit firmness; 20 N) and stage 2 (fruit Firmness; 12-15 N) subjected to three intensities of impact damage (30, 60 and 120 cm drop heights) at 20 °C (1 represents stage 1 and 2 represents stage 2).

As expected, rate of ethylene production values were higher (2 to 4 times) for kiwifruit of stage 2 than fruit from stage 1 because of the firmness difference of the kiwifruit (see chapter 4). Kiwifruit of first stage (20 N) exposed to 60 and 120 cm drop heights produced ethylene till a maximum rate of 0.27 and 0.75 pmol.kg$^{-1}$s$^{-1}$ respectively within 26 h observation time and it was a 7 and 20 times increase than 30 cm dropped fruit. In comparison, even though production rates of stage 2 fruit (12-15 N) were higher than stage 1 (1.1 and 1.3 pmol.kg$^{-1}$s$^{-1}$) at 22 h, increase was only 1.4 and 1.8 time for 60 and 120 cm drop heights in comparison to the 30 cm dropped fruit. The data for stage 2 fruit suggests the further effect of impact injury on ethylene production and firmness reduction of kiwifruit, as the most damaged most rapidly increase their ethylene production which may be symptomatic of the rapid softening caused by undetected increases in ethylene caused by the damage.

Figure 5-4 clearly illustrates a substantial difference in pattern of ethylene production, with a gradual inclination of ethylene production rate with time for stage 1 fruit (20 N), and a rapid inclination with stage 2 fruit (12-15 N) by adding evidence to the effect of firmness reduction on impact injury induced ethylene production of kiwifruit.

### 5.3.4 Firmness difference of kiwifruit exposed to different degrees of impact injury at different temperatures.

A significant difference in firmness was observed between the control (sound kiwifruit) and the fruit exposed to different degrees of impact injury at 20 °C for stage 1 fruit (20 N) and at 5 and 20 °C for stage 2 (12-15 N) kiwifruit (Table 5-1). However, when considering stage 1 fruit (20 N), at 20 °C there was no significant firmness difference between 30 and 60 cm drop heights even though there was a significant firmness difference between 120 cm dropped fruit with other drop heights. Stage 2 kiwifruit (12-15 N) showed a significant firmness difference between each drop height when the temperature was at 5 and 20 °C. At 0 °C, there was no significant difference of firmness between control and the 30 cm drop height as well as with 60 and 120 cm drop heights for stage 2 kiwifruit (12-15 N).

Table 5-1: Firmness (N) of kiwifruit of stage 1 and stage 2 after exposure to different degrees of impact injury (dropping heights; 30, 60 and 120 cm) at 0, 5 and 20 °C with values of control (sound fruit). Each value represents the average firmness value of six kiwifruit. Values with different letters show significant difference ($P<0.05$) from each treatment (degree of impact injury) at 0, 5 and 20 °C separately as determined by Tukey's test. (Stage 1 refers to experiment done with 20 N fruit and stage 2 refers to experiment done with 12-15 N fruit).

| | **Stage 1** | **Stage 2** | | |
|---|---|---|---|---|
| | **20 °C** | **0 °C** | **5 °C** | **20 °C** |
| **Control (Sound fruit)** | $19.9^{a}$ | $12.0^{a}$ | $12.9^{a}$ | $14.9^{a}$ |
| **30 cm drop height** | $16.8^{b}$ | $11.8^{a}$ | $10.8^{b}$ | $8.1^{b}$ |
| **60 cm drop height** | $17.6^{b}$ | $7.6^{b}$ | $8.8^{c}$ | $7.6^{c}$ |
| **120 cm drop height** | $15.2^{c}$ | $7.2^{b}$ | $7.3^{d}$ | $6.8^{d}$ |

Comparing with the firmness value of the control (sound fruit), percentage loss of firmness after exposing fruit to impact injury was increased with the exposed temperature. Highest percentage loss of firmness was observed at 20 ºC while lowest was observed at 0 ºC for each drop height (Table 5-2). On the other hand, stage 2 kiwifruit (12-15 N) showed higher firmness loss percentage compared with stage 1 fruit (20 N) for same high temperature (20 ºC). The increase of intensity of impact force (drop height) resulted in increased firmness loss percentage at each temperature.

Table 5-2: Percentage loss of firmness of kiwifruit of both stages (stage 1 and stage 2) exposed to different degrees of impact damage (30, 60 and 120 cm) at 0 °C, 5 °C and 20 °C compared with the firmness values of the control (sound fruit) obtained at each treatment (Stage 1 refers to experiment done with 20 N fruit and stage 2 refers to experiment done with 12-15 N fruit)

| **Drop height** | **Percentage loss of firmness** | | | |
|---|---|---|---|---|
| | **Stage 1** | **Stage 2** | | |
| | **20 °C** | **0 °C** | **5 °C** | **20 °C** |
| **30 cm** | 16% | 1.7% | 16% | 46% |
| **60 cm** | 12% | 37% | 32% | 49% |
| **120 cm** | 24% | 40% | 43% | 54% |

## 5.4 Discussion

Previous to this study no work has been conducted to investigate the effect of degree of impact injury on ethylene production of 'Hayward' kiwifruit. Work by Mencarelli et al., (1996) for 'Hayward' kiwifruit focused on impact injury related to a 30 cm drop height only, while Alves et al., (2010) found the effect of impact injury on ripening for 'Bruno' kiwifruit. Hence, present ethylene production results obtained for different intensities of impact injury on 'Hayward' kiwifruit will be useful to fill that gap in the scientific research.

### 5.4.1 Effect of temperature on wound induced ethylene production at different intensities of impact injury.

Miller et al., (1987); Mencarelli et al., (1996); DeMartino et al., (2002) all speculated that temperature plays an important role in controlling induction of ripening through

regulating ethylene production as a result of mechanical injury. No effect of degree of damage was observed on ethylene production of injured kiwifruit at 0 °C in this study, suggesting that temperature plays a role in retarding wound induced ethylene production. In the same way, Mencarelli et al., (1996) observed a reduction of ethylene production after impact damage of kiwifruit when temperature was reduced to 4 °C. DeMartino et al., (2002) also found that bruised apricot had an ethylene production similar to sound apricots kept at 4 °C. Despite the method of injury being more severe, physical tissue damage by slicing or peeling of kiwifruit also has shown an unchanged low ethylene production for 3 days at 2 °C (Agar et el., 1999).

An increase in rate of ethylene production was observed with increase of temperature for each intensity of injury. Maximum ethylene production of kiwifruit exposed to a 30 cm drop height at 20 °C was 2.8 times higher than at 5 °C (Figure 5-3A) while it was 1.8 and 2 times higher at 20 °C than at 5 °C when dropped 60 cm (Figure 5-3B) and 120 cm (Figure 5-3C) respectively. Similarly, Agar et al., (1999) found a 5 times increase in ethylene production at 20 °C than at 2 °C for peeled kiwifruit slices. However, a temperature effect between 0 and 5 °C was observed only for drop heights of 60 and 120 cm in the present work where increase of ethylene production was 1.3 times at the peak (Figure 5-3B and C).

According to Mencarelli et al., (1996), production of ethylene of bruised 'Hayward' kiwifruit by impact force of 30 cm drop to a smooth solid steel surface was increased by three times that of initial after 14 h and increased by 5 times after 21 h at 18 °C. In comparison, current impact test for 30 cm drop at 20 °C resulted in 2.2 times increase of ethylene production than initial after 10 h followed by a drop to an equilibrium level equal to basal rate of ethylene production. In the current work at 20 °C a burst of ethylene production was observed instantaneously after injury and continued to increase within first 10 h for all treatments. Similar instantaneous rises in ethylene production after injury have been described previously by Kende & Boller, (1981) for sliced ripe tomato which lasted for 2-10 h at room temperature. Similarly, rise in ethylene production was observed from injured apricots stored at 18 °C after being dropped from 30 cm height at 4 °C (DeMartino et al., 2002).

Pattern of ethylene production obtained at 20 °C in present work are consistent with that of McGlasson & Pratt, (1963) for cantaloupe fruit where ethylene production attained a maximum 48 h after injury and then declined to a minimum after 144 h (6 days) followed by another gradual increase. Ethylene production

results obtained in current work at 20 °C continued to increase during the first 10 h to a maximum and then declined in next 10 h subsequently accompanying another gradual increase (second peak) around 22 h after injury for all intensities of damage (30, 60 and 120 cm drop heights). Research results of McGlasson, (1969) revealed that ethylene synthesis from sliced green banana increased from 0.6 to 5 pmol.kg$^{-1}$s$^{-1}$ after 1 h of cutting at 20 °C. As described in the same research of McGlasson, (1969), production was further increased up to 12 pmol.kg$^{-1}$s$^{-1}$ by 10$^{th}$ hour and then declined to 5 pmol.kg$^{-1}$s$^{-1}$ by 20$^{th}$ hour. Ethylene production of intact fruit was 0.2 pmol.kg$^{-1}$s$^{-1}$. Following the pattern observed in McGlasson, (1969) for banana, ethylene production of impact bruised kiwifruit of present work increased from 0.7 to 1.65 pmol.kg$^{-1}$s$^{-1}$ within first 10 h after injury for all degree of damages at 20 °C followed by a declination to a second peak of having 0.8, 1.1 and 1.3 pmol.kg$^{-1}$s$^{-1}$ for 30, 60 and 120 cm drop heights respectively after 22 h of injury. This second inclination was accompanied with an ethylene production drop to 0.7-0.8 pmol.kg$^{-1}$s$^{-1}$ by 30$^{th}$ h and remained at equilibrium till end of the experiment (67 h) going parallel with the pattern of ethylene production observed in sliced potato of McGlasson, (1969) research.

At 5 °C, an ethylene production response to damage was observed only after a delay of 20 h (1200 minutes) after exposing to injury. Similar occurrences have been observed in several other studies on different fruit varieties and are often referred to as the lag phase. A delay of 16 h was observed before an increased in ethylene production for squash after wounding sliced layer of mesocarp tissue (Kato et al., 2000). However, duration of lag phase was mostly less than 5 h for some other injured plant segments such as 3-5 h for compressed peach (Martìnez-Romero et al., 2000) and 1.5 h for cutting of Japanese morning glory (*Pharbitis nil*) stem tissue in to segments (Prasad & Cline, 1987). Only 90 minute lag period was required for sliced tomato (Abeles et al., 1992).

Even though time duration is much lower, more examples of occurrence of lag phase are listed in Table 5-3. Comparing with present work, difference between the type of plant part exposed to injury as well as the type of injury must have caused the lower duration lag phase in examples listed in Table 5-3.

Table 5-3: Maximum and basal values of wound induced ethylene production rates of selected species of plants with time taken for lag phase and to reach peak level.

| Plant part | Time (Minutes) | | Maximum rate ($pmol.kg^{-1}s^{-1}$) | Basal rate ($pmol.kg^{-1}s^{-1}$) |
|---|---|---|---|---|
| | Lag | Peak | | |
| Pea, Subapical *(Pisum sativum),* | 26 | 55 | 131 | 32 |
| Cucumber, Cotyledon (*Cucumis sativus)* | 24 | 57 | 48 | 6 |
| Tomato, Apical *(Lycopersicon esculentum)* | 18 | 39 | 65 | 30 |
| Maize, Coleoptile *(Zea may)* | 20 | 58 | 740 | 32 |
| Wheat, Coleoptile *(Triticum aestivum)* | 18 | 53 | 382 | 233 |

Source: Abeles et al., (1992)

Hyodo et al., (1989) and Mencarelli et al., (1996) equate the observed lag time after injury before increased ethylene production to the requirement for ethylene produced at the wound site to translocate or diffuse through inter cellular spaces away from the site of injury and stimulate dramatic global ethylene burst. Moreover, Knee & Miller, (2002) indicated that wound ethylene must be produced in neighboring cells of the area of damage because enzymes relate to ethylene synthesis could be inactivated by cell breakage. Hence, time for lag phase could be interpreted as time takes for ethylene production increase at the site of damage or adjoining area and then to permeate through the fruit to ultimately result in whole fruit (or global) increase in ethylene production after some time. Nevertheless, Dunlap & Robacker, (1994) noted that it is one of the intermediates of the ethylene synthesis pathway: 1-aminocyclopropane carboxylic acid (ACC) rather than ethylene which translocate through cells and accumulate in tissue cells further away from injury and induce wound ethylene production.

For both 5 °C and 20 °C, an equilibrium state was attained after the rate of ethylene production declined from a peak (after around 70 h for 5 °C and 30 h for 20 °C). Kende & Boller, (1981) observed a similar result with wound ethylene synthesis ceasing or descending 18-20 h after excision of tomato and settling to equilibrium at room temperature. Various reasons have been considered to explain the decline to a point of equilibrium. Anaerobic conditions can occur inside interior cells of injured tissue that may result in the inhibition of oxidation of ACC to ethylene (Adams & Yang, 1979). Watada et al., (1990) postulated that the cell de-

compartmentalisation that occurs with tissue disruption due to injury leads to intermixing of enzyme and substrates and may release acids and hydrolysing enzymes which affect rate of ethylene production. One or all of the reasons stated might have an effect to the cessation of ethylene production and reaching an equilibrium state at later stage of which values became similar despite of the degree of damage of the current study.

### 5.4.2 Effect of intensity of impact injury on ethylene production

As observed in the present work, a substantial body of evidence is available that demonstrates an increase of stress ethylene with severity of the injury irrespective of the type of stress (Abeles et al., 1992). In the current research kiwifruit exposed to 30, 60 and 120 cm drop heights produced 16, 74 and 88% greater ethylene production than control (sound kiwifruit) at the peak level of 5 °C. At 20 °C, 44 and 72% higher production of ethylene was observed for 60 and 120 cm drop heights respectively than control (sound fruit) after 22 h of injury (No variation from the control was observed for 30 cm drop height at 20 °C). Research results of Alves et al., (2010) demonstrated 2.5 and 5 times increase of ethylene emission rates when dropped 'Bruno' kiwifruit from 60 and 120 cm heights respectively at 20 °C (Alves et al., 2010). Ethylene production rate of 'Babygold' peaches was proportional to applied compression forces even at 2 °C (Martìnez-Romero et al., 2000) while a significant correlation between production of ethylene and degree of vibration was observed for fig fruit (Mao et al., 1995). Ethylene production of sweet potato roots was in proportion to the logarithm of the cut area (Imaseki et al., 1968) while cutting bean in to segments induced ethylene production in proportion to the cut surface area (Jackson & Osborne, 1970). Similarly, study results of Agar et al., (1999) revealed 2-4 times higher ethylene production in peeled kiwifruit slices than unpeeled slices and it was 2 times higher in peeled fruit than whole fruit. Cutting of Japanese morning glory (*Pharbitis nil*) stem tissue in to segments increased ethylene production into three fold (Prasad & Cline, 1987). All of these evidences demonstrate the influence of intensity of damage to wound ethylene biosynthesis as found with the different degree of impact injury on 'Hayward' kiwifruit in the current research.

### 5.4.3 Effect of fruit maturity (firmness) on ethylene production at different intensities of impact injury.

Ethylene production response differences were observed from fruit of two different stages of maturity (firmness) in this work. Although Menesatti & Paglia, (2001) also acknowledged the strong effect of degree of ripening to the physical response due to impact injury, debatable comments were found in literature to oppose what was found in the present work. According to the work of Abeles et al., (1992), cut pre-climacteric apple produced higher ethylene than climacteric apple. DeMartino et al., (2002) described an effect of cellular turgor on fruit sensitivity and leading of low turgor in ripen fruit to less sensitivity to damage and less induction of wound ethylene production. Moreover, cells in ripe fruit have more tendencies to slide against each other to reduce the mechanical stress without breaking cells which gives benefit of low emission of ethylene in ripe fruit than unripe fruit (Knee & Miller, 2002). However, as noted in Kende & Boller, (1981) even though variation of production of ethylene was observed with the ripening level of damage fruits, clear pattern of increase or decrease of ACC content with progressive ripening has not been identified yet. Hence, considering the comment from Kende & Boller, (1981) further research is required to clarify the effect of maturity of fruit on damage induced ethylene production.

### 5.4.4 Ethylene production of injured fruit

According to Hoffman & Yang, (1982), unripe cantaloupe fruit after excision had produced 600 times more ethylene from 1.2 to 744 $pmol.kg^{-1}s^{-1}$ after 24 hours at 25 °C. Dropping freshly harvested red snow and northern spy apples have caused increase of ethylene formation by 3-20 fold after 24 hour of bruising (Lougheed & Franklin, 1974). Wounding increases stress ethylene production by 10 fold in spinach leaves (Wilson & Lucas, 1988) and 14 fold for pea leaves (Walters & Osborne, 1979). All of these give evidence for extent of influence of any type of injury on induction of high ethylene production and current experiment done with kiwifruit exposed to impact injury also revealed around 2-5 fold increase of ethylene at 5 and 20 °C presenting a greater influence of availability of injured fruit on change in ethylene composition inside a package.

Yang & Pratt (1978); Wang & Adams, (1980) and Yu & Yang, (1980) commented that biosynthetic pathway for ethylene in wounded tissue of a fruit is

same as the pathway of ethylene synthesis. Given the ethylene production pathway identified and described by Adams & Yang, (1979) (Methionine→SAM→ACC→ethylene: detailed description in Chapter 2), high ethylene emission due to injury could be attributable to increase of ACC by activation of ACC synthase (ACS) and subsequent conversion to ethylene (Miller et al., 1987). Parallel studies conducted over the last three decades have demonstrated that production of wound ethylene in fruit is controlled by a coordinated expression of both ACS and ACO genes (Katz et al., 2004). Observations with wounded melon (Dunlap & Robucker, 1994) and dissected mesocarp tissue of pre-climacteric melon (Shiomi et al., 1999) revealed a dramatic production of ethylene coincided and paralleled by increase of ACC levels, ACS and ACO activity. Confirming earlier results of Kende & Boller, (1981) for wounded tomato, Shiomi et al., (1998) also found a good correlation between activity of ACS and rate of wound ethylene production in sliced mature cucumber. Study on finding the effect of combinations of wounding and applications of the translational inhibitor cycloheximide (CHX), the ethylene action inhibitor 1-methylcyclopropene (MCP) also revealed that increase of ACC and ACO was in parallel with production of ethylene for injured pea (Mathooko et al., (2001). Similar pattern occurred for wounded avacado (Owino et al., 2002), squash (Kato et al., 2000), tomato (Yu & Yang, 1980; Kende & Boller, 1981), winter squash (Hyodo et al., 1989), citrus (Shimokawa, 1983) and cantaloupe (Hoffman & Yang, 1982). Decline of ACS in wounded green tomato after treating with translational inhibitor cycloheximide (CHX) confirmed the comment of increase of ACC and ACO in parallel with production of ethylene of injured fruit (Abeles et al., 1992). Yet, considering all these findings, future research targeting determination of intermediates and enzymes attached to ethylene synthesis pathway in parallel with current work should be planned for further interpretation of these findings relating to wound induced ethylene production of 'Hayward' kiwifruit.

Interestingly, not all research investigating effect of damage on ethylene production of fresh produce reports increased production of ethylene. Damaged mango fruit did not show significant increase in ethylene probably due to the presence of phenolic compounds released from the cell wall that could compete with biosynthesis of ethylene (Ketsa & Koolpluksee, 1993). Similarly, high impact and abrasion forces did not produce high ethylene in papaya (Quintana & Paul, 1993) and in water fern (*Regnellidium diphyllum*) (Walters & Osborne, 1979). Even though

there was visible damage in immature cucumber due to bruising, ethylene production did not increase over 48 h period (Miller et al., 1987). Moreover, production of ethylene decreased by 90% after sugar beet leaf was pressed between plastic piston and board (Elstner & Konze, 1976). However, there are no records available of kiwifruit not producing ethylene as an effect of impact injury and present work also confirmed the response of impact injury on ethylene production.

### 5.5 Conclusion

The outcome of this research work supported the theory of acceleration of rate of 'stress/wound' ethylene production of fresh produce as a response to mechanical injury caused by impact damage during handling and storage. Results strongly indicated that temperature plays a significant role in controlling synthesis of wound ethylene and confirmed a beneficial effect at low temperature (0 °C) storage. An increase in rate of ethylene production was observed with increase of temperature for each intensities of injury. A 2-3 times increase of ethylene production was observed at 20 °C than at 5 °C. Further, it could be confirmed that ethylene production of 'Hayward' kiwifruit is proportional to the degree of intensity of impact injury. Research results of two different maturity levels of kiwifruit demonstrated the further effect of firmness reduction of kiwifruit on impact injury induced ethylene production as kiwifruit of 12-15 N firmness produced 2 to 4 times higher ethylene than 20 N fruit. Future research targeting determination of wound induced ethylene production of different maturity levels of kiwifruit (including less matured fruit with a firmness of >20 N) for broad range of temperatures (2, 10 and >20 °C) than what was used in the present research as well as for different degrees of impact injury (> 120 cm drop heights) representing existing variables throughout kiwifruit supply chain would give benefits as essential realistic inputs for the future constructive model. Along with that quantification and studying of the behavioral pattern of intermediates (ACC) and enzymes (ACS and ACO) attached to ethylene synthesis pathway, in parallel with determination of impact injury, induced ethylene production should be planned for further interpretation of the results obtaining at different temperatures under different degrees of impact injury.

# Chapter 6

## DESORPTION OF ETHYLENE FROM KIWIFRUIT CAUSED BY INCREASED TEMPERATURE WITHIN THE SUPPLY CHAIN

### 6.1 Introduction

Low temperature is used throughout the supply chain to enable transport of fresh produce to long distance markets and to store produce for a long period (Arpaia et al., 1994; Ritenour et al., 1999). Kiwifruit harvested for export markets are stored in cool storage (with a recommended range of 0–2 °C; Kader, 2002) for up to 4-6 months. However, irrespective to the effort to maintain optimal storage conditions at each stage of the supply chain there are instances when greater than optimal temperatures occur within modern commercial supply chains (Zhao et al., 2013). Specifically, recent research by Zespri International has identified several points within the supply chain where temperature variation occurs. Kiwifruit are often exposed to breaks in temperature control during transport between coolstore (0–2 °C) and port (8 °C peak), while high temperature exposure (20 °C) at the end of the supply chain in the market also could exist (Zhao et al., 2013). There are further possibilities of kiwifruit being exposed to higher temperatures (>20 °C) through underdeveloped cool chain infrastructure when Zespri International expands the export market of kiwifruit to South East Asia and the Indian subcontinent (Zhao et al., 2013). Use of non-refrigerated trucks between coolstore and port could expose kiwifruit to 12-24 h temperature breaks while reaching fruit to low facilitated markets without low temperature display cabinets could increase the severity and time duration of high temperature exposure especially when containers of kiwifruit are transshipped to developing countries like India. All of these scenarios draw attention towards the possibilities of sudden exposure of kiwifruit to increased temperature from lower temperature during supply chain activities as a reality.

Revealing of kiwifruit to increased temperature following removal from low temperature has been shown to cause increase in ethylene evolution (Hyodo et al., 1987; Kim, 1999; Antunes & Sfakiotakis, 2002a; Antunes & Sfakiotakis, 2002b). Increase of ethylene evolution from kiwifruit during transient increase in temperature could critically contribute to the change in composition of ethylene inside the commercial kiwifruit package when sudden temperature increases occur within the supply chain. This increase in ethylene concentration may affect fruit quality by

inducing ripening and fruit softening as kiwifruit respond to very low concentrations of ethylene such as 5-10 $nL.L^{-1}$ (Mitchell, 1990; Retamales & Campos, 1997; Castillo et al., 1999; Kim et al., 1999; Antunes, 2007).

Considerable work has been conducted to determine the effect of increasing temperature on production of ethylene from a fruit. Antunes & Sfakiotakis, (2002b) demonstrated for 'Hayward' kiwifruit that ethylene production was negligible at 0 °C and reached a maximum of 86 $pmol.kg^{-1}s^{-1}$ when transferred to 20 °C. 'Hayward' kiwifruit in the study of Antunes & Sfakiotakis, (2002a) started autocatalytic ethylene production within 24 hours following transfer to 20 °C from 0 °C. Kim et al., (1999) also demonstrated that 'Hayward' kiwifruit stored for more than 14 days at 0 ºC prior to removal to 20 ºC produced more ethylene sooner at 20 ºC than fruit kept at 0 ºC for 7 days. Hyodo & Fukasawa, (1985); Manolopoulou & Papadopoulou, (1997) also investigated variation in ethylene production of 'Hayward' kiwifruit following transfer of fruit from 1 °C to 21 °C. All of these published work with other research (Wang & Adams, 1980; Hyodo et al., 1987) on effect of transient increase in temperature on production of ethylene of fruit has been confined only between refrigerated storage (0 °C) and ambient temperature (20 °C) and at the same time all works account for the increase in ethylene production caused by increased temperature to be due to increases in ethylene synthesis of the kiwifruit.

As described in Apel & Patterson, (1983) physiologically active ethylene is not what is present in the gas phase (within the intercellular spaces) but what is dissolved in solutes inside the tissue. The solubility of any gas dissolved in a liquid is dependent on temperature, the partial pressure of the gas over the liquid, the nature of the solvent and the nature of the gas. An increase of temperature or decrease of partial pressure of the gas over the liquid causes dissolved gas to desorb. Given that rapid evolution of ethylene from kiwifruit is observed due to an increase in temperature, this increase in observed ethylene production could be contributed to from release of ethylene dissolved in tissue liquid. An approximation of the dissolved ethylene in fruit tissue as a function of temperature is essential in clarifying the reasons behind the rapid ethylene evolution observed during an increase in temperature.

Estimation of the amount of ethylene dissolved in liquid of any fruit tissue is commonly based on the analysis of ethylene in the gaseous phase above the solution (head space) (Bassi et al., 1981). Henry's law constant (HLC) represents the gas liquid equilibrium for any particular gas present in a dilute aqueous solution and could be given as the relationship of the partial pressure of the gas to the mole fraction of the gas in a solution at a given (temperature) condition. Hence, the HLC values obtained for ethylene and the temperature dependency could be used in estimating internal partial pressure of ethylene in the gaseous phase related to specific solubility change of gas in the liquid phase of the kiwifruit tissue for a specific temperature increase. Every compound/liquid combination has a unique Henry's law constant (HLC) at a specific temperature and pressure (Altschuh et al., 1999). Frolich et al., (1931) found that gas solubility tended to follow HLC over a wide range of pressure according to the extent to which the considered gas obeys the ideal gas law. Further, validity of Henry's law is relying upon the negative interaction of gas with the solvent without forming a compound. With evidence ethylene obeys following criteria, HLC could be applied in determining dissolved ethylene in kiwifruit tissue.

Apel & Patterson, (1983) obtained a mean HLC value for ethylene in water at 20 °C as $7.99 \times 10^6$. A similar value ($7.74 \times 10^6$) calculated by Winkler, (1928) provided confidence in the Apel & Patterson, (1983) value. Henry's law constant expressed for apple juice was higher than that for water due to the presence of other solutes (Apel & Patterson, 1983). The HLC was estimated as $1.215 \times 10^7$ for post-climacteric 'Starkrimson' apple and was found to be not substantially different from 'Rome' and 'Shotwell' cultivars. HLC was however lower at 4 °C (approximately 2.5 times) than at 20 °C indicating increase of the HLC with increase of temperature (Apel & Patterson, 1983). As HLC is temperature dependent, the van't Hoff equation can be used to calculate HLC across a range of temperatures when a HLC at a specific temperature and the enthalpy of the reaction are known (Pinar, 2004).

The aim of this research was to quantify the amount of ethylene released from 'Hayward' kiwifruit for a range of transient temperature increase (0→2 °C, 2→5 °C, 5→10 °C, 10→20 °C) and to find out the contribution of ethylene desorption from dissolved ethylene in kiwifruit tissue to the increase rate of ethylene evolution of kiwifruit observed when exposed to an increase in temperature based on Henry's law constant (HLC).

## 6.2 Materials and Methods

### 6.2.1 Determination of ethylene evolution rate

Kiwifruit ('Hayward', *Actinidia deliciosa*) stored for nearly four months at cold temperature (0 ºC) were used for the experiment. Firmness values of the kiwifruit used for the trial varied in a range of 18-2.3 N (as firmness of fruit changed with the time taken to conduct totally 8 trials within 2.5 months). Kiwifruit without any visual defects were carefully selected and weighed to three decimal places using a digital balance (PG503-S, Mettler Toledo, Switzerland). Evolution of ethylene of kiwifruit during and subsequent to increased temperature were determined using the ethylene detector (ETD-300, SensorSense, Nijmegen, The Netherlands) by following the method described in section 3.2. Jars used in measuring ethylene production were kept inside a temperature controlled incubator (MIR153, SANYO, Tokyo, Japan) to maintain required temperatures of the experiment during measurement of ethylene production. Size of the jar and number of kiwifruit used in each trial varied with the firmness of the kiwifruit as explained in section 3.2. The experiment was conducted with six replicates starting at 0 °C and then increasing the temperature to 2 °C, 5 °C, 10 °C and finally 20 °C respectively.

### 6.2.2 Determination of internal temperature of kiwifruit

Determination of ethylene evolution at each temperature was continued until the ethylene concentration reached an equilibrium stage. In parallel, internal temperature of kiwifruit was recorded throughout the experiment by employing squirrel temperature data logger with thermocouples (SQREM1200, Eltek, Cambridge, United Kingdom). Four readings of internal temperature were measured and averaged using four thermocouples inserted into four fruit kept inside a dummy glass jar placed with other replicates (Figure 6.1). A total of 8 experiments were conducted as several trials for further investigation and clarification of the results obtained.

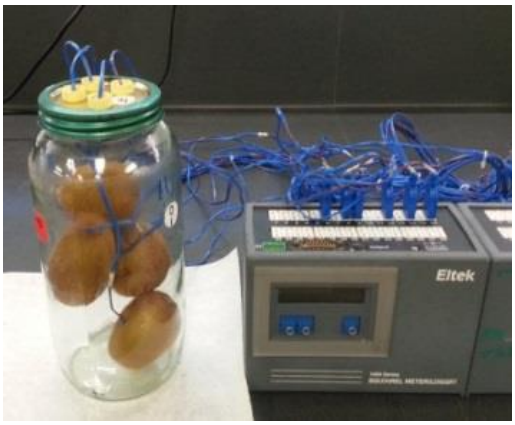


Figure 6-1: Method established to measure internal temperature of kiwifruit by using a Squirrel temperature data logger with thermocouples (SQREM1200, Eltek, Cambridge, United Kingdom).

### 6.2.3 Determination of firmness

Firmness of kiwifruit at the end of each trial was conducted after re-equilibration to 20 °C by following the technique described in section 3.3.

### 6.2.4 Determination of contribution of release of dissolved ethylene in kiwifruit tissue to the increased evolution rate of ethylene observed at each temperature increase

Contribution from the release of dissolved ethylene in kiwifruit tissue to the evolution rate of ethylene observed at each temperature increase was determined by comparing (difference) the actual internal partial pressure of ethylene (Actual $P_{inHT}$) and estimated internal partial pressure of ethylene (Estimated $P_{inHT}$) at higher temperature. To obtain Actual $P_{inHT}$ and Estimated $P_{inHT}$ for comparison initially, Henry's law constant (HLC) at each specific temperature was estimated (Table 6-1) as explained in section 6.2.4.1. Secondly, the actual internal partial pressure of ethylene at each specific low and high temperature (Actual $P_{inLT}$ and Actual $P_{inHT}$) were calculated based on experimentally obtained ethylene evolution rates at each specific temperature (Sections 6.2.4.2 and 6.2.4.3). Thirdly, estimated internal partial pressure of ethylene at higher temperature (Estimated $P_{inHT}$) was calculated (Section 6.2.4.4) based on calculated actual internal partial pressure of ethylene at each specific low temperature (actual $P_{inLT}$) and Henry's law constants at each specific low and high temperature (Table 6-1).

#### 6.2.4.1 Estimation of Henry's law constant (HLC) at different temperature

Henry's law constant (HLC) for ethylene was estimated (Table 6-1) for different temperatures (0, 2, 5 and 10 °C) by applying the HLC value of ethylene in water at 20 °C extracted from Apel & Patterson, (1983) to the van't Hoff equation (Pinar, 2004) described in Equation 6-1.

$$\ln \frac{\mathrm{HLC_{T1}}}{HLC_{T2}} = \mathrm{C}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \qquad [6\text{-}1]$$

Where,

$\mathrm{HLC_{T1}}$ = Henry's law constant at $T_1$ temperature (°K)

(HLC of ethylene in water at 293 °K (20 °C) =$7.99 \times 10^6$)
[Apel & Patterson, (1983)]

$HLC_{T2}$ = Henry's law constant at $T_2$ temperature (°K)

$$C = \frac{\Delta\mathrm{H\ (Standard\ enthalpy\ change\ of\ vaporization)[\ J.mol^{-1}]}}{\mathrm{R\ (Universal\ gas\ constant)[J.K^{-1}mol^{-1}]}}$$

($\Delta$H = 13,500 J.mol$^{-1}$) [Dean, 1999]

(R = 8.314 J.K$^{-1}$ mol$^{-1}$)

$C$ = 1624 K

Table 6-1: Henry's law constant (HLC) for ethylene in water calculated for each specific temperature (0 °C, 2 °C, 5 °C and 10 °C) using information extracted from Apel & Patterson, (1983).

| Temperature | HLC of ethylene in water |
|---|---|
| 0 °C | 5323831 |
| 2 °C | 5559179 |
| 5 °C | 5924932 |
| 10 °C | 6569023 |
| 20 °C | 7990000 |

Henry's law constant (HLC) represents the relationship between the partial pressure of a gas (Pa or mol.kg$^{-1}$) in the gaseous phase to the mole fraction of the gas in a solution/liquid phase (mol.L$^{-1}$) at a given temperature. Decrease of solubility of the gas in the liquid phase with the increase of temperature causes increase of partial pressure of gas in the gaseous phase due to desorption of gas from liquid phase to gaseous phase leading to increase of HLC value with the increase of temperature as shown in Table 6-1.

#### 6.2.4.2 Calculation of internal partial pressure for observed ethylene evolution rate at lower temperature ($P_{inLT}$)

Ethylene produced by kiwifruit is lost through the skin into the surrounding environment and this can be expressed using Fick's law of diffusion:

$$EL = \mathrm{A.P}\ (P_{in} - \mathrm{P_{ex}}) \qquad [6\text{-}2]$$

Where,

$EL$ = Ethylene loss rate ($mol.s^{-1}$)

A = Surface area of kiwifruit (0.01 $m^2$) [Buxton, 2005]

P = Skin permeance of the kiwifruit (2 x $10^{-10}$ $mol.m^{-2}s^{-1}Pa^{-1}$) [East et al., 2011]

$P_{in}$ = Internal partial pressure of ethylene (Pa)

$P_{ex}$ = External partial pressure of ethylene (Pa) [Assumed as 0]

At steady state the rate of ethylene produced is balanced by the rate lost through the skin and it could be identified as ethylene evolution rate of kiwifruit.

$$\mathrm{m}.EE = \mathrm{A.P}\ (P_{in} - \mathrm{P_{ex}}) \qquad [6\text{-}3]$$

Where,

$EE$ = Ethylene evolution rate ($mol.kg^{-1}s^{-1}$)

m = Mass of the kiwifruit (kg) [0.09 kg]

Rearranging Equation 6-3 to make the internal partial pressure of ethylene ($P_{in}$) the subject and assuming $P_{ex}$ = 0 the equation results in,

$$P_{in} = \frac{EE.\mathrm{m}}{\mathrm{A.P}} \qquad [6\text{-}4]$$

Hence, $P_{in}$ at lower temperature ($P_{inLT}$) could be obtained from the ethylene evolution rate obtained at lower temperature ($EE_{LT}$).

$$P_{inLT} = \frac{EE_{LT}.m}{A.P} \qquad [6\text{-}5]$$

Where,

$EE_{LT}$ = Ethylene evolution rate at lower temperature ($mol.kg^{-1}s^{-1}$)

#### 6.2.4.3 Calculation of actual internal partial pressure for observed ethylene evolution rate at higher temperature (actual $P_{inHT}$)

With the increase of temperature actual internal pressure of ethylene at higher temperature (Actual $P_{inHT}$) could be estimated from the ethylene evolution rate at high temperature ($EE_{HT}$).

$$\text{Actual } P_{inHT} = \frac{EE_{HT}.m}{A.P} \qquad [6\text{-}6]$$

Where,

$EE_{HT}$ = Ethylene evolution rate at higher temperature ($mol.kg^{-1}s^{-1}$)

#### 6.2.4.4 Calculation of estimated or new internal partial pressure at increased temperature (estimated $P_{inHT}$)

Following the theory of decrease of solubility of gas in the liquid phase of the fruit with increase of temperature, a temporary increase in $P_{in}$ at high temperature could be expected because of the decrease in ethylene solubility. Figure 6-2 describes the relationship between partial pressure of ethylene gas (Pa) and mole fraction of ethylene gas in the liquid ($mol.L^{-1}$) based on Henry's law constant [Henry's law constant (HLC) = Partial pressure of ethylene in gas phase (Pa)/Mole fraction of ethylene in liquid phase ($mol.L^{-1}$)] for ethylene in water at different temperature (Table 6-1) and the phenomenon behind the obtaining of a new $P_{in}$ at higher temperature.

New $P_{in}$ at high temperature (Estimated $P_{inHT}$) can be calculated as,

$$\text{Estimated } P_{inHT} = P_{inLT} \cdot \left(\frac{HLC_{HT}}{HLC_{LT}}\right) \qquad [6\text{-}7]$$

Where,

Estimated $P_{inHT}$ = Estimated Internal partial pressure of ethylene at higher temperature (Pa)

$P_{inLT}$ = Internal partial pressure of ethylene at lower temperature (Pa)

$HLC_{HT}$ = Henry's law constant at higher temperature

$HLC_{LT}$ = Henry's law constant at lower temperature

In comparison of Actual $P_{inHT}$ from the experimental ethylene evolution data and Estimated $P_{inHT}$ from the Henry's law calculation, two scenarios that could occur are,

(I) Actual $P_{inHT}$ > Estimated $P_{inHT}$: If rate of ethylene evolution change with immediate and subsequent increased temperature is due to effect of desorption of ethylene with change in solubility as well as due to some other factors which cause increase in ethylene evolution.

(II) Actual $P_{inHT}$ = Estimated $P_{inHT}$: If rate of ethylene evolution change with immediate and subsequent increased temperature is solely due to desorption of ethylene with change in solubility.

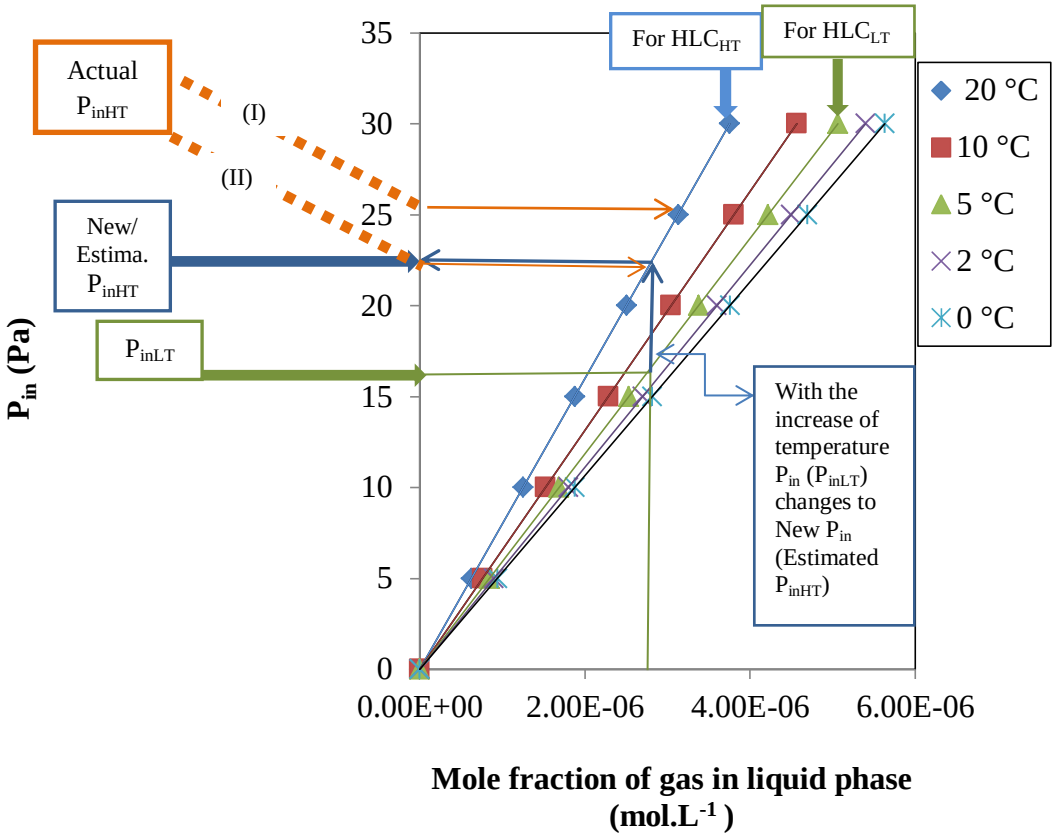


Figure 6-2: Relationship between partial pressure of ethylene gas (Pa) and mole fraction of ethylene gas in the liquid (mol.$L^{-1}$) according to the Henry's law constants for ethylene in water at different temperatures (0, 2, 5, 10 and 20 °C).

## 6.3 Results

### 6.3.1 Observed ethylene evolution rate

Figure 6-3A to 6-3C illustrates the ethylene evolution rate of 'Hayward' kiwifruit as a response to increased temperature from 0→5 °C, 5→10 °C and 10→20 °C obtained at three different trials. Firmness of kiwifruit of each trial was different (Table 6-2) due to variation of the date of experiment conducted (length of storage at 0 °C) between each trial. These firmness differences result in a 3 log range of ethylene production (0.01-10 $pmol.kg^{-1}s^{-1}$) within the data set (Figure 6-3).

Firmness of kiwifruit used in the first trial (Figure 6-3A) was 14.6 N and 15.3 N for replicate 1 (R1) and replicate 2 (R2) respectively. Even though this trial did not demonstrate significant fluctuations in ethylene evolution rate at the temperature change of 0→5 °C, there was 1.2 to 1.7 fold increase of ethylene evolution rate at 10 °C than what observed at 5 °C (Figure 6-3A). A small single point spike (3 to 4 fold increase) in ethylene evolution rate was found after 9 h of temperature change from 10→20 °C. This sudden peak was followed with a rapid decline of ethylene evolution rate to the basal rate.

Figure 6-3B [firmness of kiwifruit: 9.1 N (R1)] and 6-3C [firmness of kiwifruit: 6.9 N (R1) and 5.7 N (R2)] results demonstrated a clear escalation in ethylene evolution rate for every temperature increase (0→5 °C, 5→10 °C and 10→20 °C). As found with first trial (Figure 6-3A), a small single point spike in ethylene evolution rate was found after 9 h of temperature change from 10→20 °C in Figure 6-3B also. However, increase was only 1.9 fold in Figure 6-3B compare to 3-4 fold increase observed in Figure 6-3A at the temperature change of 10→20 °C. Increase in ethylene evolution rate was 1.7, 1.8 fold at the temperature change from 0→5 °C, 5→10 °C respectively for the second trial (Figure 6-3B).

Ethylene evolution rate of kiwifruit in the third trial (Figure 6-3C) increased by approximately 1-3, 6-12 and 2-3 times with the increase of temperature from 0→5 °C, 5→10 °C and 10→20 °C respectively. Higher increase in ethylene evolution rate was observed at the temperature change of 5→10 °C where a 12 fold increase was detected in R2 and a 6 fold increase in R1 of which firmness of fruit was higher in R1 (6.9 N) than in R2 (5.7 N). A spike in ethylene evolution rate was found after 5-6 h of temperature change from 10→20 °C in Figure 6-3C.

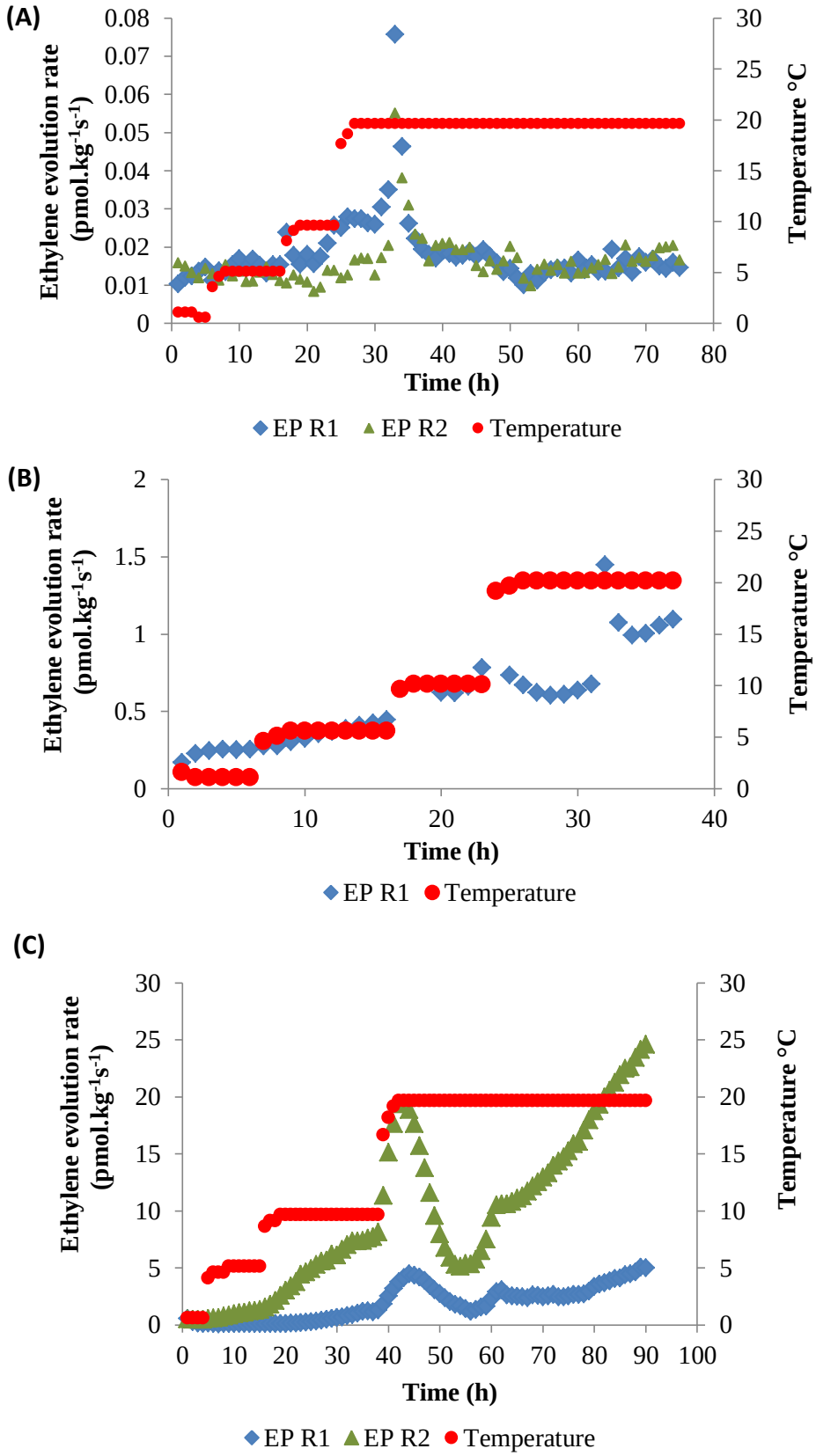


Figure 6-3: (A-C): Pattern of ethylene evolution rate of 'Hayward' kiwifruit as a response to increased temperature from 0⟶5 °C, 5⟶10 °C and 10⟶20 °C. [Firmness of kiwifruit - 6-3A:14.6 N (R1) and 15.3 N (R2), 6-3B:9.1 N and 6-3C:6.9 N (R1) and 5.7 N (R2)].

The ninety hour experiment duration in Figure 6-3C allowed observation of a burst of ethylene evolution followed by a decline and then another rapid increase with time at 20 °C. Even though the pattern of ethylene evolution rate with change of temperature was similar between R1 and R2 of the third trial (Figure 6-3C), magnitude of ethylene evolution rate at each transient temperature increase was different for R1 and R2 as with the observed firmness difference between R1 (6.9 N) and R2 (5.7 N). R2 of Figure 6-3C with low firmness value (5.7 N) demonstrated higher ethylene evolution rate (overall 4-12 times) than R1 with high firmness (6.9 N). Highest proportional difference (12 fold) between ethylene evolution rate of R2 (1.3 $pmol.kg^{-1}s^{-1}$) and R1 (0.1 $pmol.kg^{-1}s^{-1}$) was observed at the temperature change of 0→5 °C and ethylene evolution rate was 4 fold higher in R2 (18 $pmol.kg^{-1}s^{-1}$) than R1 (4.4 $pmol.kg^{-1}s^{-1}$) at the ethylene spike observed after 5-6 h of temperature change from 10→20 °C. At $90^{th}$ hour the difference between ethylene evolution rate was 5 times higher in R2 (23 $pmol.kg^{-1}s^{-1}$) than R1 (4.9 $pmol.kg^{-1}s^{-1}$).

Figure 6-4 represents a different set of results for ethylene evolution rate of kiwifruit with a firmness of 13.3 N (R1), 18.1 N (R2) and 17.5 N (R3) where temperature has increased from 2→5 °C and 5→20 °C. Similar to the trend observed in Figure 6-3C at 20 °C, a spike in ethylene evolution rate followed by a gradual declination was observed after 19 h of changing from 5→20 °C. A 14, 6 and 7 times increase in ethylene evolution rate was observed in replicate 1, 2 and 3 respectively when temperature changed from 5→20 °C (Figure 6-4). This increase in ethylene evolution rate at the temperature change from 5→20 °C (Figure 6-4) was much higher than compared to what observed in Figure 6-3B (2 fold) and 6-3C (3-4 fold) at the temperature change from 10→20 °C. However, a longer time duration (19 h) after the temperature change from 5→20 °C was taken to reach the peak in comparison to that observed when the change in temperature is from 10→20 °C (5-9 h, Figure 6-3 B and C). As observed in earlier trials (Figure 6-3C) 2 times higher increase in ethylene evolution rate was observed in R1 than R2 and R3 of which fruit firmness of R1 was lower (13.3 N) than other 2 replicates (18.1 and 17.5 N in R2 and R3 respectively).

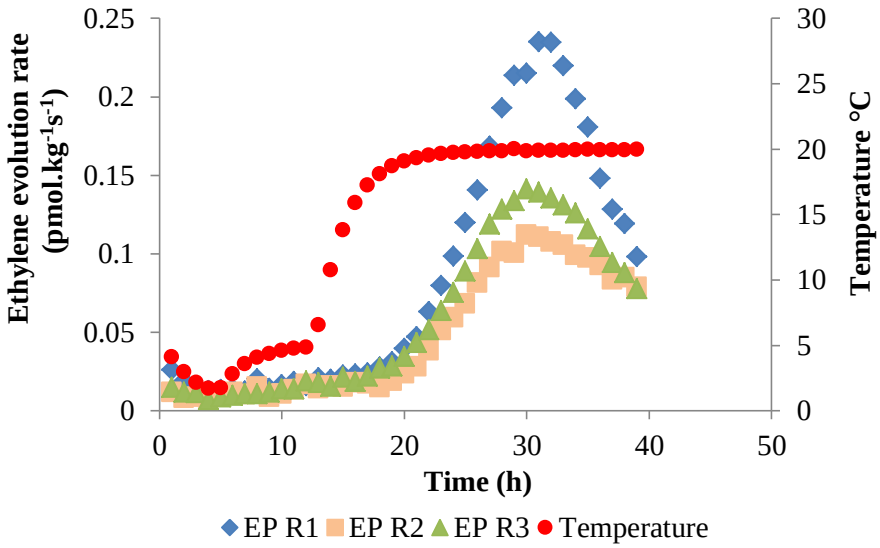


Figure 6-4: Pattern of ethylene evolution rate of 'Hayward' kiwifruit as a response to increased temperature from 2→5 °C and 5→20 °C. [Firmness of kiwifruit - 13.3 N (R1), 18.1 N (R2) and 17.5 N (R3)].

Figure 6-5A (firmness of R1:8.3 N) and 6-5B (firmness of R1:2.6 N, R2:3.1 N and R3:2.5 N) illustrates the ethylene evolution rate of kiwifruit with temperature change from 0→2 °C, 2→5 °C, 5→10 °C and 10→20 °C while Figure 6-6 represents the ethylene evolution rate of kiwifruit [firmness 3.7 N (R1), 2.3 N (R2), 3.1 N (R3), 2.5 N (R4), 3.3 N (R5) and 3.3 N (R6)] when change of temperature from 2→5 °C, 5→10 °C and 10→20 °C (excluding 0→2 °C). Figure 6-7 [firmness of kiwifruit 6.0 N (R1) and 6.6 N (R2)] only represents the temperature change from 10→20 °C.

Generally, a 2-3 fold increase of ethylene evolution rate was observed with the temperature change from 2→5 °C as well as from 5→10 °C in Figure 6-5 and 6-6 (Table 6-3). Ethylene evolution rate increased by around 2 times with a temperature change from 10→20 °C in Figure 6-5 and 6-6 whereas increase was 3 fold in R1 and 20 fold in R2 in Figure 6-7 at the temperature change from 10→20 °C (Table 6-3). Similar pattern of rate of change in ethylene evolution rate (spike followed by a decline and then increment) as found in earlier trials (Figure 6-3B and 6-3C) were again observed in Figure 6-5A, 6-5B, 6-6 and 6-7 at the time of change in temperature from 10→20 °C. Despite the similar pattern in the change in ethylene evolution after a change of temperature to 20 °C from either 5 °C (Figure 6-4) or 10 °C in all the trials conducted, the time taken to reach the peak rate at ethylene burst differed (Table 6-4). With temperature change from 10→20 °C, a second increase of

rate of ethylene evolution was observed after a decline following the initial ethylene spike (Figure 6-3C and Figure 6-5 to 6-7). In other trials the restriction of the observation time to less than 20 h at 20 °C may have resulted in this second ethylene rise not being observed. This rapid inclination of ethylene evolution began to be observed after around 10 to 12 h of decline after the initial ethylene burst.

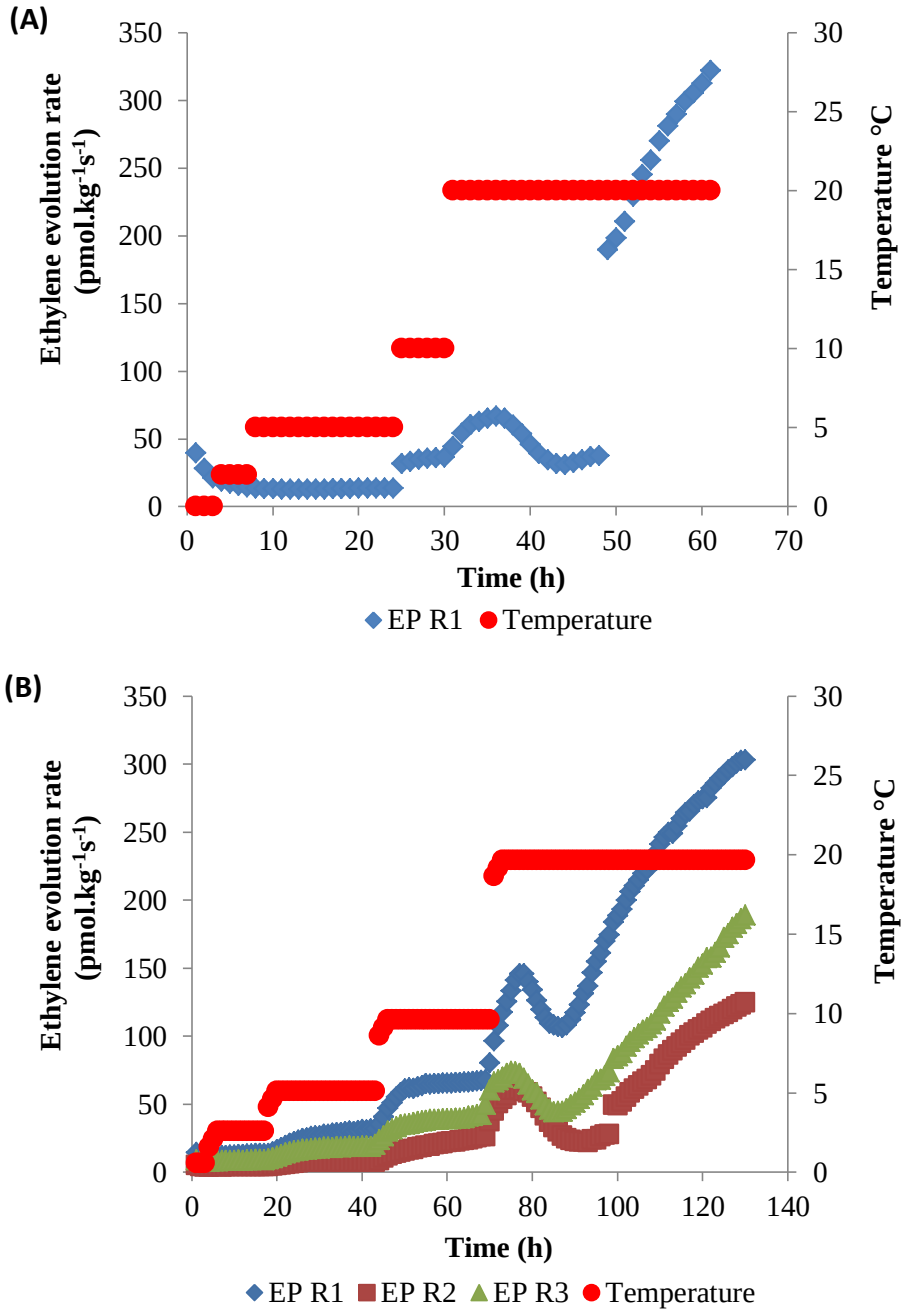


Figure 6-5: Pattern of ethylene evolution rate of 'Hayward' kiwifruit as a response to increased temperature from 0⟶2 °C, 2⟶5 °C, 5⟶10 °C and 10⟶20°C. [Firmness of kiwifruit - 6-5A:8.3 N (R1) and 6-5B:2.6 N (R1), 3.1 N (R2) and 2.5 N (R3)].

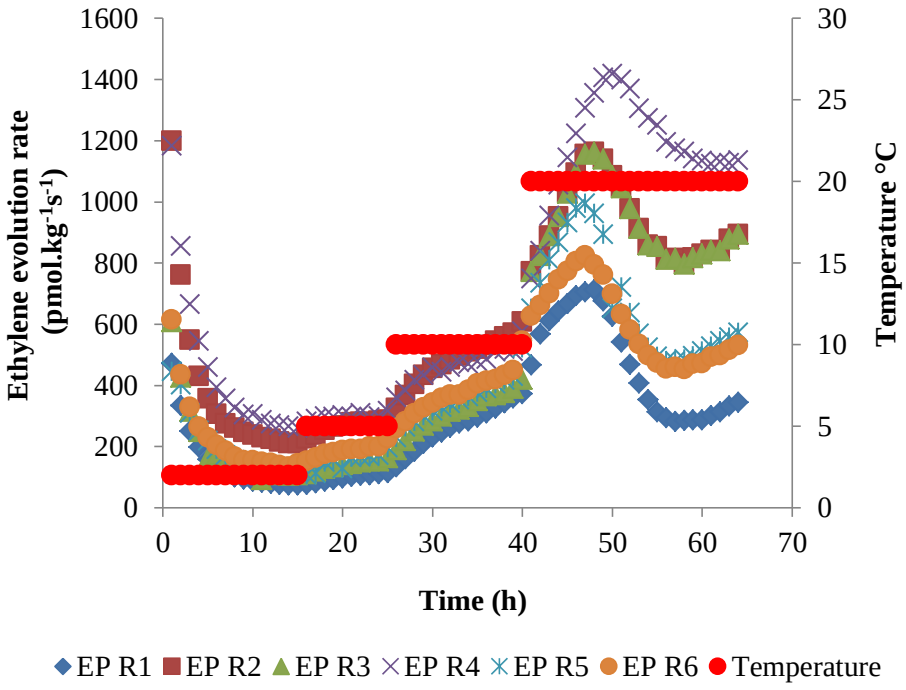


Figure 6-6: Pattern of ethylene evolution rate of 'Hayward' kiwifruit as a response to increased temperature from 2⟶5 °C, 5⟶10 °C and 10⟶20 °C. [Firmness of kiwifruit - 3.7 N (R1), 2.3 N (R2), 3.1 N (R3) and 2.5 N (R4), 3.3 N (R5) and 3.3 N (R6)].

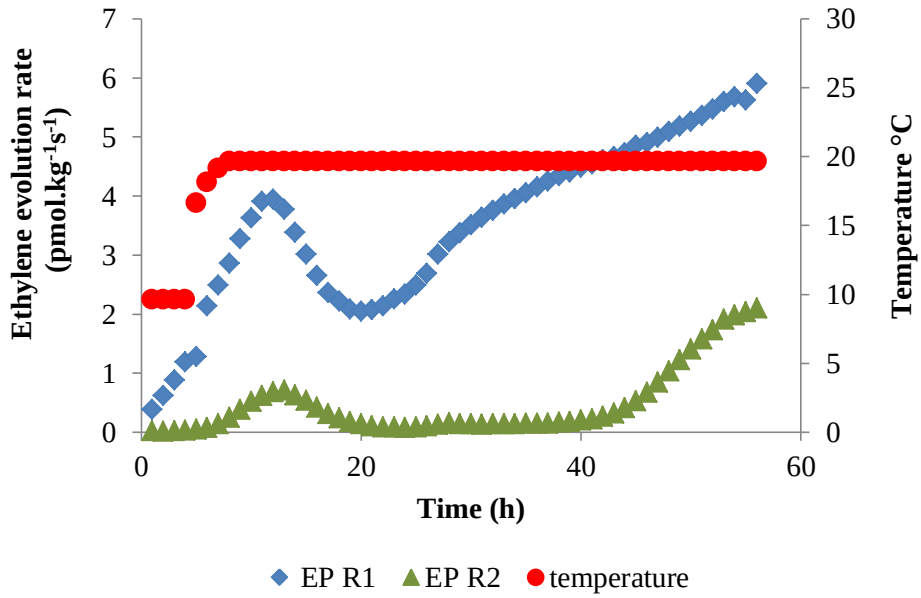


Figure 6-7: Pattern of ethylene evolution rate of 'Hayward' kiwifruit as a response to increased temperature from 10⟶20°C. [Firmness of kiwifruit - 6.0 N (R1) and 6.6 N (R2)].

As presented in Table 6-2, rate of ethylene evolution differed with each trial where the highest rate was observed in the trials representing Figure 6-5B and 6-6 of which firmness of kiwifruit was lowest (ranging from 2.3-3.7 N) compare with the other trials. Ethylene evolution rate was increased up to 1400 pmol.kg$^{-1}$s$^{-1}$ at the temperature change of 10→20 °C in Figure 6-6 (Replicate 4). Even at 0 °C ethylene evolution rate of kiwifruit was within a range of 4-13 pmol.kg$^{-1}$s$^{-1}$ (Figure 6-5B and 6-6). On the other hand very low ethylene production ranging from 0.011 to 0.2 pmol.kg$^{-1}$s$^{-1}$ was observed from kiwifruit representing figure 6-3A and 6-4 where firmness of kiwifruit was higher (18.1-13.3 N) than in figure 6-5B and 6-6 (ranging from 2.3-3.7 N).

Table 6-2: Observed ethylene evolution rate (pmol.kg$^{-1}$s$^{-1}$) and firmness (N) of kiwifruit obtained at each temperature (0 °C, 2 °C, 5 °C, 10 °C and 20 °C) in relation to the figure presented and the replicate.

| **Figure** | **Replicate** | **Firmness (N)** | **Observed ethylene evolution rate (pmol.kg$^{-1}$s$^{-1}$)** | | | | |
|---|---|---|---|---|---|---|---|
| | | | **0 °C** | | **5 °C** | **10 °C** | **20 °C** |
| 6.3A | R1 | 14.6 | 0.15 | | 0.015 | 0.026 | 0.076 |
| | R2 | 15.3 | 0.14 | | 0.011 | 0.14 | 0.055 |
| | | | **0 °C** | | **5 °C** | **10 °C** | **20 °C** |
| 6.3B | R1 | 9.1 | 0.3 | | 0.4 | 0.8 | 1.4 |
| 6.3C | R1 | 6.9 | 0.1 | | 0.1 | 1.3 | 4.4 |
| | R2 | 5.7 | 0.5 | | 1.3 | 7.7 | 18.5 |
| | | | | **2 °C** | **5 °C** | | **20 °C** |
| 6.4 | R1 | 13.3 | | 0.01 | 0.02 | | 0.2 |
| | R2 | 18.1 | | 0.01 | 0.01 | | 0.1 |
| | R3 | 17.5 | | 0.01 | 0.01 | | 0.1 |
| | | | **0 °C** | **2 °C** | **5 °C** | **10 °C** | **20 °C** |
| 6.5A | R1 | 8.3 | 21.1 | 14.5 | 13.6 | 36.4 | 66.7 |
| 6.5B | R1 | 2.6 | 13.0 | 13.7 | 31.8 | 80.7 | 146.0 |
| | R2 | 3.1 | 4.3 | 4.4 | 7.7 | 37.4 | 61.1 |
| | R3 | 2.5 | 8.1 | 9.4 | 20.6 | 60.5 | 74.4 |
| | | | | **2 °C** | **5 °C** | **10 °C** | **20 °C** |
| 6.6 | R1 | 3.7 | | 74.5 | 116.1 | 372.9 | 713.7 |
| | R2 | 2.3 | | 212.2 | 287.2 | 608.1 | 1163.2 |
| | R3 | 3.1 | | 107.7 | 164.5 | 420.5 | 747.5 |
| | R4 | 2.5 | | 262.4 | 318.3 | 578.2 | 1419.5 |
| | R5 | 3.3 | | 126.2 | 196.6 | 523.1 | 980.4 |
| | R6 | 3.3 | | 145.8 | 226.8 | 545.7 | 824.8 |
| | | | | | | **10 °C** | **20 °C** |
| 6.7 | R1 | 6.0 | | | | 1.2 | 3.9 |
| | R2 | 6.6 | | | | 0.03 | 0.7 |

Table 6-3 gathers the data of magnitude of increase in observed ethylene evolution rate at every temperature increase (0→2 °C, 2→5 °C, 5→10 °C and 10→20 °C) and firmness value while Table 6-4 represents the time taken to reach the peak ethylene evolution rate at temperature change of 10→20 °C (5→20 °C in Figure 6-4) along with firmness value in relation to the figure presented and the replicate.

Table 6-3: Magnitude (fold) of increase in ethylene evolution rate at every temperature increase and firmness value (N) in relation to the figure presented and the replicate

| **Figure** | **Replicate** | **Firmness (N)** | **Magnitude of increase in ethylene evolution rate at each temperature increase** | | | |
|---|---|---|---|---|---|---|
| | | | **0→5 °C** | | **5→10 °C** | **10→20 °C** |
| 6.3A | R1 | 14.6 | - | | 1.7 | 3 |
| | R2 | 15.3 | - | | 1.2 | 4 |
| | | | | | | |
| | | | **0→5 °C** | | **5→10 °C** | **10→20 °C** |
| 6.3B | R1 | 9.1 | 1.7 | | 1.8 | 1.9 |
| | | | | | | |
| 6.3C | R1 | 6.9 | 1 | | 12 | 3 |
| | R2 | 5.7 | 3 | | 6 | 2 |
| | | | | | | |
| | | | | **2→5 °C** | | **5→20 °C** |
| 6.4 | R1 | 13.3 | | 1 | | 14 |
| | R2 | 18.1 | | 2 | | 6 |
| | R3 | 17.5 | | 2 | | 7 |
| | | | | | | |
| | | | **0→2 °C** | **2→5 °C** | **5→10 °C** | **10→20 °C** |
| 6.5A | R1 | 8.3 | 0.7 | 0.9 | 3 | 2 |
| 6.5B | R1 | 2.6 | 1 | 2 | 3 | 2 |
| | R2 | 3.1 | 1 | 2 | 5 | 2 |
| | R3 | 2.5 | 1 | 2 | 3 | 1 |
| | | | | | | |
| | | | | **2→5 °C** | **5→10 °C** | **10→20 °C** |
| 6.6 | R1 | 3.7 | | 2 | 3 | 2 |
| | R2 | 2.3 | | 1 | 2 | 2 |
| | R3 | 3.1 | | 2 | 3 | 2 |
| | R4 | 2.5 | | 1 | 2 | 2 |
| | R5 | 3.3 | | 2 | 3 | 2 |
| | R6 | 3.3 | | 2 | 2 | 2 |
| | | | | | | |
| | | | | | | **10→20 °C** |
| 6.7 | R1 | 6.0 | | | | 3 |
| | R2 | 6.6 | | | | 20 |

Table 6-4: Time (h) taken to reach the peak ethylene evolution rate at ethylene burst after temperature change 10→20 °C (5→20 °C in Figure 6-4) and firmness of kiwifruit (N) used in the trials in relation to the figure presented and the replicate.

| Figure | Replicate | Time taken to obtain peak ethylene evolution rate (h) | Firmness (N) |
|---|---|---|---|
| 6.3A | R1 | 9 | 14.6 |
| | R2 | 9 | 15.3 |
| | | | |
| 6.3B | R1 | 9 | 9.1 |
| | | | |
| 6.3C | R1 | 6 | 6.9 |
| | R2 | 5 | 5.7 |
| | | | |
| 6.4 | R1 | 19 | 13.3 |
| | R2 | 19 | 18.1 |
| | R3 | 19 | 17.5 |
| | | | |
| 6.5A | R1 | 6 | 8.3 |
| | | | |
| 6.5B | R1 | 8 | 2.6 |
| | R2 | 7 | 3.1 |
| | R3 | 5 | 2.5 |
| | | | |
| 6.6 | R1 | 8 | 3.7 |
| | R2 | 8 | 2.3 |
| | R3 | 8 | 3.1 |
| | R4 | 10 | 2.5 |
| | R5 | 7 | 3.3 |
| | R6 | 7 | 3.3 |
| | | | |
| 6.7 | R1 | 7 | 6.0 |
| | R2 | 8 | 6.6 |

### 6.3.2 Comparison of actual $P_{inHT}$ and estimated $P_{inHT}$ for each temperature increase

As described in section 6.2.4, actual $P_{inHT}$ and estimated $P_{inHT}$ values were obtained and compared by calculating percentage difference between actual $P_{inHT}$ and estimated $P_{inHT}$ (Pa) [% difference = (actual $P_{inHT}$ - estimated $P_{inHT}$)/(actual $P_{inHT}$)] for each temperature increase in order to determine the contribution of release of dissolved ethylene of kiwifruit tissue to the ethylene evolution rate increase with transient increase of temperature.

Table 6-5: Actual $P_{inHT}$ and estimated $P_{inHT}$ and the % difference between Actual $P_{inHT}$ and Estimated $P_{inHT}$ at each temperature increase (0⟶2 °C, 2⟶5 °C, 5⟶10 °C and 10⟶20 °C) in relation to figure presented and the replicate.

| **Fig.** | **Rep.** | **Firmnes. (N)** | **Actual $P_{inHT}$ & Estimated $P_{inHT}$ values and the % difference between Actual $P_{inHT}$ & Estimated $P_{inHT}$ values of ethylene at each increased temperature** | | | | |
|---|---|---|---|---|---|---|---|
| | | | | **0⟶5 °C** | | **5⟶10 °C** | **10⟶20 °C** |
| 6.3A | R1 | 14.6 | Actual | 0.00070 | | 0.00120 | 0.0035 |
| | | | Estimated | 0.00077 | | 0.00003 | 0.0014 |
| | | | ***% Differ.*** | ***-6%*** | | ***97%*** | ***59%*** |
| | R2 | 15.3 | Actual | 0.00050 | | 0.00070 | 0.0026 |
| | | | Estimated | 0.00075 | | 0.00003 | 0.0007 |
| | | | ***% Differ.*** | ***42%*** | | ***95%*** | ***69%*** |
| | | | | **0⟶5 °C** | | **5⟶10 °C** | **10⟶20 °C** |
| 6.3B | R1 | 9.1 | Actual | 0.021 | | 0.037 | 0.068 |
| | | | Estimated | 0.016 | | 0.023 | 0.044 |
| | | | ***% Differ.*** | ***25%*** | | ***37%*** | ***34%*** |
| 6.3C | R1 | 6.9 | Actual | 0.0047 | | 0.0590 | 0.204 |
| | | | Estimated | 0.0052 | | 0.0053 | 0.072 |
| | | | ***% Differ.*** | ***-9%*** | | ***91%*** | ***65%*** |
| | R2 | 5.7 | Actual | 0.059 | | 0.361 | 0.865 |
| | | | Estimated | 0.024 | | 0.066 | 0.440 |
| | | | ***% Differ.*** | ***59%*** | | ***82%*** | ***49%*** |
| | | | | | **2⟶5 °C** | | **5⟶20 °C** |
| 6.4 | R1 | 13.3 | Actual | | 0.0007 | | 0.011 |
| | | | Estimated | | 0.0006 | | 0.001 |
| | | | ***% Differ.*** | | ***10%*** | | ***91%*** |
| | R2 | 18.1 | Actual | | 0.0008 | | 0.0052 |
| | | | Estimated | | 0.0005 | | 0.0011 |
| | | | ***% Differ.*** | | ***43%*** | | ***79%*** |
| | R3 | 17.5 | Actual | | 0.0009 | | 0.0066 |
| | | | Estimated | | 0.0004 | | 0.0012 |
| | | | ***% Differ.*** | | ***50%*** | | ***82%*** |
| | | | | **0⟶2 °C** | **2⟶5 °C** | **5⟶10 °C** | **10⟶20 °C** |
| 6.5A | R1 | 8.3 | Actual | 0.67 | 0.64 | 1.70 | 3.11 |
| | | | Estimated | 1.03 | 0.72 | 0.71 | 2.07 |
| | | | ***% Differ.*** | ***-53%*** | ***-13%*** | ***58%*** | ***34%*** |
| 6.5B | R1 | 2.6 | Actual | 0.64 | 1.49 | 3.76 | 6.81 |
| | | | Estimated | 0.63 | 0.68 | 1.65 | 4.58 |
| | | | ***% Differ.*** | ***1%*** | ***54%*** | ***56%*** | ***33%*** |
| | R2 | 3.1 | Actual | 0.21 | 0.36 | 1.75 | 2.85 |
| | | | Estimated | 0.21 | 0.22 | 0.39 | 2.13 |
| | | | ***% Differ.*** | ***-1%*** | ***39%*** | ***77%*** | ***25%*** |
| | R3 | 2.5 | Actual | 0.44 | 0.96 | 2.82 | 3.47 |
| | | | Estimated | 0.39 | 0.47 | 1.07 | 3.43 |
| | | | ***% Differ.*** | ***9%*** | ***52%*** | ***62%*** | ***1%*** |
| | | | | | **2⟶5 °C** | **5⟶10 °C** | **10⟶20 °C** |
| 6.6 | R1 | 3.7 | Actual | | 5.42 | 17.40 | 33.29 |
| | | | Estimated | | 3.71 | 6.00 | 21.16 |
| | | | ***% Differ.*** | | ***32%*** | ***65%*** | ***36%*** |
| | R2 | 2.3 | Actual | | 13.40 | 28.37 | 54.26 |
| | | | Estimated | | 10.55 | 14.85 | 34.50 |
| | | | ***% Differ.*** | | ***21%*** | ***48%*** | ***36%*** |
| | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | R3 | 3.1 | Actual | | 7.68 | 19.61 | 35.11 |
| | | | Estimated | | 5.36 | 8.51 | 23.86 |
| | | | ***% Differ.*** | | ***30%*** | ***57%*** | ***32%*** |
| | R4 | 2.5 | Actual | | 14.85 | 26.97 | 66.22 |
| | | | Estimated | | 13.06 | 16.46 | 32.81 |
| | | | ***% Differ.*** | | ***12%*** | ***39%*** | ***50%*** |
| | R5 | 3.3 | Actual | | 9.17 | 24.40 | 46.42 |
| | | | Estimated | | 6.28 | 10.17 | 29.68 |
| | | | ***% Differ.*** | | ***32%*** | ***58%*** | ***36%*** |
| | R6 | 3.3 | Actual | | 10.58 | 25.46 | 38.48 |
| | | | Estimated | | 7.25 | 11.73 | 30.97 |
| | | | ***% Differ.*** | | ***31%*** | ***54%*** | ***20%*** |
| | | | | | | | **10→20 °C** |
| 6.7 | R1 | 6.0 | Actual | | | | 0.18 |
| | | | Estimated | | | | 0.07 |
| | | | ***% Differ.*** | | | | ***63%*** |
| | R2 | 6.6 | Actual | | | | 0.030 |
| | | | Estimated | | | | 0.002 |
| | | | ***% Differ.*** | | | | ***94%*** |

Overall, there was a positive difference between actual $P_{inHT}$ and estimated $P_{inHT}$ where actual $P_{inHT}$ was always greater than estimated $P_{inHT}$ following the scenario I explained in the section 6.2.4.4 (except for few cases at temperature change of 0→5 °C or 0→2 °C). Percentage difference between actual $P_{inHT}$ and estimated $P_{inHT}$ generally ranged from 30% to 97% specially at the temperature change of 10→20 °C in all the trials.

## 6.4 Discussion

According to Abeles et al., (1992) ethylene production from a plant tissue has been associated with many types of stresses including high temperature stress where exposure of fruit to increased temperature when transferred from lower (chilling temperature) to higher (warm/ambient temperature) causes escalation in evolution of ethylene. This research work done for 'Hayward' kiwifruit also demonstrates increase in ethylene evolution rate following sudden transfer to a higher temperature from a lower temperature as previously reported by Hyodo et al., (1987); Kim, (1999); Antunes & Sfakiotakis, (2002a); Antunes & Sfakiotakis, (2002b). As demonstrated in Antunes & Sfakiotakis, (2002a) kiwifruit started autocatalytic ethylene production within 24 hours following transfer to 20 °C from 0 °C for 12 days following harvest while Antunes & Sfakiotakis, (2002b) also revealed that ethylene production of 'Hayward' kiwifruit was negligible at 0 °C and reached a maximum of 90 $pmol.kg^{-1}s^{-1}$ when transfer to 20 °C. 'Hayward' kiwifruit of Kim et al., (1999) study produced more ethylene soon after removal to 20 ºC from 14 days at

0 ºC than fruit kept at 0 ºC for 0 or 7 days. However, current study aimed at a range of transient temperature increase (0→2 °C, 2→5 °C, 5→10 °C, 10→20 °C) allowed detailed description of ethylene evolution rate at increased temperature rather than work concentrated only for temperature change from 0→20 °C as documented in literature.

Ethylene evolution rate of fruit in the present investigation varied between each trial regardless of the temperature increase. This variation was contributed to by the differences in firmness of the fruit used (Table 6-2). Kiwifruit of Figure 6-3A and 6-4 had higher firmness (18.1-13.3 N) than other trials resulting in the evolution of very low ethylene. On the other hand, the highest rate of ethylene evolution was observed in kiwifruit used in Figure 6-5B and 6-6 which had low firmness ranging from 2.3 to 3.7 N. These results agree with the dramatic ethylene production rate and firmness relationship observed in chapter 4.

The solubility of any gas in a liquid is dependent on temperature along with the partial pressure of the gas over the liquid, and the nature of the solvent. With an increase of temperature, dissolved gas in a tissue liquid will desorp (Apel & Patterson, 1983). Hence, desorption of dissolved ethylene in the kiwifruit tissue with the temperature increase could contribute to the increase in ethylene evolution of kiwifruit observed with increased temperature. In this work, mathematical estimation of the desorbed ethylene was conducted to clarify the contribution of the desorption phenomenon on the escalation of ethylene evolution rate of kiwifruit caused by increased temperature. Two scenarios were identified could occur at the comparison of actual $P_{inHT}$ and mathematically estimated $P_{inHT}$ namely actual $P_{inHT}$ > estimated $P_{inHT}$ or actual $P_{inHT}$ = estimated $P_{inHT}$ (Section 6.2.4.4). Scenario I (actual $P_{inHT}$ > estimated $P_{inHT}$) observed in most of the results (Table 6-5) suggests that observed ethylene evolution rate of kiwifruit at each increased temperature was not solely contributed from the release of dissolved ethylene in the kiwifruit tissue based on Henry's law and there are some other factors that contribute to the escalation of ethylene evolution observed during and immediately subsequent to an increase in temperature.

The overestimation (actual $P_{inHT}$ < estimated $P_{inHT}$ or negative percentage difference values) observed in few instances (Table 6-5) specially at low temperature change (0→2 °C or 0→5 °C) could be discussed as due to inadequate time duration

given (need more time to attain equilibrium state due to low ethylene evolution rate at low temperature) in the experimental trials specially at low temperature.

An attempt was done to clarify the presence of the ethylene peak at the time of temperature change from 10→20 °C or even 5→20 °C (Figure 6-4). Ethylene evolution rate was calculated using Equation 6-7 for estimated internal partial pressure of ethylene at increased temperature (Estimated $P_{inHT}$) and was compared with the ethylene evolution rate calculated using the area under the peak at increased temperature (20 °C) of the graph. However, ethylene evolution rate calculated for the area under the peak is smaller than the estimated ethylene evolution rate at increased temperature (20 °C). This allows us to consider that the peak observed at increased temperature (20 °C) may be from induction of increase of ethylene production of kiwifruit due to high temperature stress via ethylene synthesis pathway or due to both the effect of release of dissolved ethylene with increased temperature (Henry's law effect) and effect of temperature on biological ethylene synthesis pathway. Generally, values of 30-97% difference obtained between actual $P_{inHT}$ and estimated $P_{inHT}$ (Table 6-5) and the time gap between the temperature change from 10→20 °C or 5→20 °C and the observation of the peak (Table 6-4) also reminds us that the existence of peak is not solely due to the sudden release of dissolved ethylene.

Description of the ethylene synthesis pathway was initiated by Adams & Yang, (1979) and Lursen et al., (1979). As Yang & Hoffman, (1984) elucidated major stages in the pathway of ethylene biosynthesis is methionine→S-adenosylmethionine (SAM)→1-aminocyclopropane carboxylic acid (ACC)→ethylene. ACC synthase (ACS) and ACC oxidase (ACO) are involved in catalysing the formation of ACC and ethylene (Abeles et al., 1992). Generally conversion of SAM/AdoMet to ACC facilitated by ACS has been identified as the rate limiting reaction of the ethylene synthesis pathway (Abeles et al., 1992). As Kim et al., (1999) demonstrated, ACC concentration and ACO activity of 'Hayward' kiwifruit remained low throughout storage at 0 ºC and generally increased after removal to 20 ºC at which time the fruit produced more ethylene sooner than fruit kept at 0 ºC for 0 or 7 days. The expected increase of ACC and ACO activity due to increase of temperature in Kim et al., (1999) was likely to have contributed to the escalation of ethylene observed when kiwifruit are transferred to a higher temperature. Activity of ACS remained low in pears stored at cold temperature and ACC levels increased upon removal to 20 °C

due to increased ACS activity (Knee, 1984). Correlation between burst of ethylene synthesis on transfer of 'Granny Smith' apple and pear fruit from chilled temperature to warmer temperature and increase in ACC concentration and ACO activity was demonstrated by Larrigaudiere et al., (1997). Hence, it could be suggested that contribution to the increase of ethylene evolution rate of 'Hayward' kiwifruit through ethylene synthesis pathway may be due to increase of ACC, ACS or ACO activity due to high temperature stress to the biological pathway.

As described, ethylene desorption pattern of present investigation at 20 °C illustrated a fall in the rate of ethylene evolution followed by rapid increment after a peak at the beginning agreeing with previous observations of Riov & Yang, (1982) for citrus; Antunes et al., (2000) for 'Hayward' kiwifruit; Atta-Aly et al., (2000) for tomato and strawberry and East et al., (2007) for 'Cripps Pink' apple. Adding to the past evidence, the consistency of this pattern in the observed data in all the trials conducted provides confidence that this is a true effect of an increase in temperature on ethylene evolution.

Yu et al., (1979) has demonstrated an accumulation of endogenous levels of ACC in auxin-treated mung bean and apple tissue as a response to high temperature and as explained in Riov & Yang, (1982) a decline of ethylene evolution could be due to reduction of ACC. Moreover, production of ethylene could be influenced or controlled by interactions between other metabolic pathways connected with the pathway of ethylene biosynthesis. The methionine or Yang cycle is one important interconnecting link to the pathway of ethylene biosynthesis (Miyazaki & Yang, 1987). As Wang et al., (1982) briefed, ACS produces 5-methylthioadenosine (MTA) which is recycled to methionine. If decline of ethylene evolution could be due to reduction of ACS activity as a result of existence of high temperature stress, low activity of ACS could also limit methionine recycling which could ultimately effect ethylene production. Further, ACC could be coupled with malonate or glutathione to form 1-(malonyl-amino) cyclopropane-1-carboxylic acid (M-ACC) and 1-(γ-L-(glutamylamino) cyclopropane-1-carboxylic acid (G-ACC) respectively (Pieser & Yang, 1998). Conjugation of ACC into M-ACC which is catalyzed by ACC malonyltransferase enzyme causes a rapid decline of ethylene production (Yang et al., 1990). M-ACC is considered as an inactive end product which is not oxidized to ethylene (Bouzayen et al., 1987). Presence of malonyltransferase may behave as an alternative way of conversion of high concentration of ACC into an inactive product

(Hoffman et al., 1983). In another way M-ACC is acting as a sink for ACC in the presence of high levels of ACC or when ACO activity is saturated. Vangronsveld et al., (1988) observed a 40% reduction in ethylene production in parallel with 32% increase in MACC and 37% reduction in ACC in bean seedlings. Likewise decrease in ethylene evolution observed at 20 °C in the current study could have been associated with either due to accumulation or reduction of ACO activity due to high temperature or due to formation into M-ACC and G-ACC or due to low activity of ACS. However, rapid ethylene evolution following this short fall of ethylene evolution at 20 °C suggest that whatever the reason behind, the effect is not lasting for long time duration. Hence, further investigation is needed to clarify this obtained pattern at 20 °C.

Applying chilling to trigger ethylene production at a subsequent increased temperature has been applied in industry to induce ripening of European pear (Blankenship & Richardson, 1985), kiwifruit (Sfakiotakis et al., 1997) and some cultivars of apples (Knee et al., 1983). Wang & Adams, (1982) proposed that the chilling stress activates a signal for increased activity of enzymes involved in ethylene synthesis. Kim et al., (1999) demonstrated that a period of 52-64 days at 0 ºC was required to activate both ACS and ACO activity that resulted in an increase in ethylene production at 20 ºC. In contrast, Sfakiotakis et al., (1997) demonstrated only 12 days at 0 ºC was enough to induce biosynthesis of ethylene at 20 ºC. In comparison, pear varieties need extensive exposure of cold treatment (12 weeks of 0 °C) for ripening while 'Granny Smith' apple needs only a few days for the same effect (Larrigaudiere & Vendrell, 1999). Research results of present experiment also revealed that even exposing to temperatures above 0 ºC (2, 5 or 10 ºC) for a small time duration (10 to 20 h) could stimulate ethylene production of kiwifruit as results indicated a minimum 2 to maximum 14 times increase in ethylene evolution rate as a result of expose to higher temperature. Discussion of contribution of ethylene production of kiwifruit via ethylene synthesis pathway to the increase of ethylene evolution rate at increased temperature (through evaluation of under estimated internal partial pressure values obtained at higher temperature based on HLC) further clarifies the statement of stimulation of ethylene production at increased temperature after short exposure to low temperature (0, 2, 5 and 10 ºC). However, most importantly it is important to consider the detrimental effect to the quality of the kiwifruit because of triggering of ethylene production at sudden exposure to

temperature (which is a reality through supply chain) as that leads to high postharvest loss as well as profit loss.

Previous research on ethylene production responses of apples cultivars suggests that 'Hayward' kiwifruit may not represent other cultivars of kiwifruit. Larrigaudiere et al., (1997) found that 'Royal Gala' and 'Starking Delicious' apples produced the same amount of ethylene at 20 °C irrespective of previous chilling while chilled 'Granny Smith' produced three times higher ethylene than non-chilled fruit after transfer to 20 °C. Hence, further research is required to understand the ethylene production response of other cultivars of kiwifruit available in New Zealand to transient temperature increase as it is one of the important point to consider in reducing the postharvest loss of kiwifruit through the supply chain.

## 6.5 Conclusion

One to twenty times increase of ethylene evolution of 'Hayward' kiwifruit was observed upon sudden transfer to higher temperature from a lower temperature for a range of temperatures (0→2 °C, 2→5 °C 5→10 °C and 10→20 °C). Mathematical estimation of internal partial pressure of ethylene for an increased temperature (Estimated $P_{inHT}$) based on Henry's law constant (HLC) and comparison with the actual internal partial pressure of ethylene for an increased temperature (Actual $P_{inHT}$) revealed that both the effect of desorption of dissolved ethylene in kiwifruit tissue following HLC as well as increase of enzymatic activity of ethylene synthesis pathway cause increase in ethylene evolution rate of kiwifruit to sudden temperature increase within the supply chain of kiwifruit. As found with the results in chapter 4 the present study confirmed the effect of change in firmness value of kiwifruit to the rate of ethylene evolution with the increase of temperature by triggering higher ethylene evolution rate by kiwifruit with low firmness.

# Chapter 7

## ETHYLENE TRANSMISSION THROUGH THE COMMERCIAL KIWIFRUIT POLYLINER

### 7.1 Introduction

Commonly, kiwifruit are packed in a corrugated fibreboard package with an internal polyliner as a barrier to water loss. This polyliner could also provide a major barrier to ethylene transmission to and from the kiwifruit package and subsequently influences the ethylene concentration in the environment immediately surrounding the fruit. Subsequently, the ethylene transmission property of the polyliner affects the extent of accumulation of ethylene in the package and hence the kiwifruit postharvest storage life.

The development of a model for predicting ethylene composition within a commercial package will require accurate knowledge of packaging film ethylene permeability. While permeability data for water, $O_2$ and $CO_2$ have been published in the literature for a wide range of polymers (synthetic and edible), little information is available for ethylene (Table 2-2). The limited information on the transmission of ethylene through commonly used polymer materials has previously been obtained by using conventional Gas Chromatograph (GC) method. Ethylene detection limit of these conventional methods is 5-10 $nL.L^{-1}$ while 0.3 $nL.L^{-1}$ is achievable with the advanced ethylene detector which applies laser based technique (Cristescue et al., 2012). The high sensitivity of the ethylene detector provides a benefit in detecting previously un-measurable values through conventional methods.

While there is some data available for permeability of ethylene for various types of polymer films, obtaining a value for the commercial kiwifruit polyliner is essential as physical and chemical structure of each film can vary while the composition of the film can also be altered by the addition of other functional additives such as fillers, pigments or plasticisers when manufacturing (Siracusa, 2012). Thus, as one of the essential pieces of information which will enable future construction of a predictive model of ethylene compositions within a commercial package, this investigation was focused on accurate quantification of ethylene transmission value for the specific polyliner by using an advanced ethylene detection technique.

## 7.2 Materials & method

The methods used were adopted from Wang et al., (1998) and Paz et al., (2005). Experimental data was collected using a stainless steel circular permeability cell (Figure 7-1) with two chambers (ID = 0.18 m). HDPE polyliner (10 μm) was placed between the two chambers and sealed by means of a screw press. Butyl rubber O-rings were placed between poly-film and each chamber to ensure a tight seal.

Figure 7-1: Stainless steel permeability cell (ID = 0.18m) with two chambers

Initially (prior to starting the experimental work), a leakage test of the permeability cell was conducted by flushing $CO_2$ (0.5% in air) to the permeability cell and sealing. Carbon dioxide concentration drop inside the permeable cell was investigated by sampling three gas samples of 1 mL from the permeability cell initially and after a known time interval (Weerawate, 2008). Remaining $CO_2$ concentration was determined with a $CO_2$ analyser equipped with a miniature infrared $CO_2$ transducer (Analytical Development Company, Hoddesdon, UK) at 20 °C and integrator (HP 3396A, Hewlett Packard, CA, USA) to record output signals.

For the ethylene permeability measurement, different concentrations of ethylene gas (10 and 100 $\mu L.L^{-1}$ in air) were flushed through the top chamber whilst the bottom chamber was supplied with ethylene free air by passing dry air through the catalyzer of the ethylene detector (ETD-300, SensorSense, Nijmegen, The Netherlands) both with a flow rate of 80 $mL.min^{-1}$ (Figure 7-2)

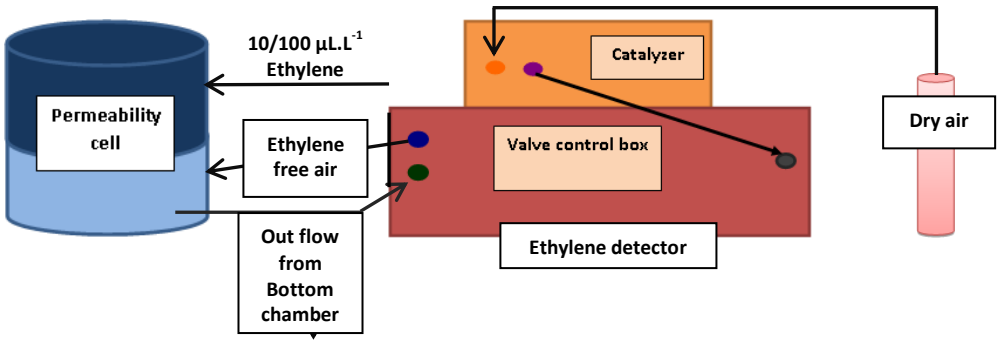


Figure 7-2: Experimental set up of for determination of ethylene transmission of polyliner using ethylene detector (ETD-300, SensorSense B.V., Nijmegen, The Netherlands)

Continuous gas flow through both top and bottom chambers were provided to establish a constant partial pressure gradient in the system (Figure 7-3). Out flow ethylene concentration in the bottom chamber (low ethylene side) was measured until the system attained steady state by directing the out flow through the ethylene detector (Figure 7-2). The experiment was conducted at two temperatures: 0 ºC and 20 ºC by keeping the permeability cell inside a temperature controlled incubator (MIR153, SANYO, Tokyo, Japan).

At steady state, permeability was calculated using the equation (Equation 7-1) derived from Fick's first law of diffusion (Appendix I).

$$P = \frac{Q_L(C_{LO} - C_{LI})\mathrm{L_{Film}}}{\mathrm{A_{Film}}(C_{HO} - C_{LO})\mathrm{R}T} \qquad [7\text{-}1]$$

Where,

$P$ = Effective film permeability (mol.m.m$^{-2}$s$^{-1}$Pa$^{-1}$)

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

$C_{LO}$ = Outlet ethylene concentration from low ethylene side (µL.L$^{-1}$)

$C_{LI}$ = Inlet ethylene concentration from low ethylene side (µL.L$^{-1}$)

$\mathrm{L_{Film}}$ = Thickness of film (m)

$\mathrm{A_{Film}}$ = Area of film (m$^2$)

$C_{HO}$ = Outlet ethylene concentration from high ethylene side (µL.L$^{-1}$)

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

$T$ = Absolute temperature (K)

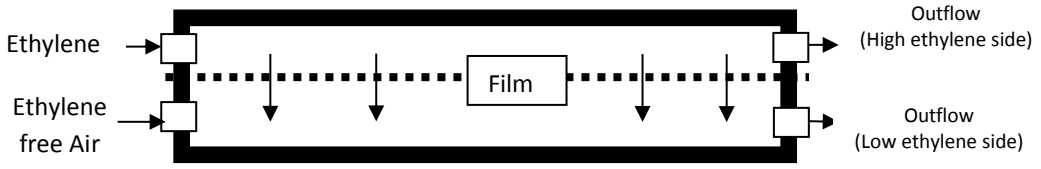


Figure 7-3: Schematic diagram to show air flow inside the permeability cell

## 7.3 Results

Table 7-1 presents the permeability values obtained for the HDPE polyliner used in commercial kiwifruit package. Outlet ethylene concentration from low ethylene side or lower chamber at 20 °C was detected at a steady state of 1.07 $\mu L.L^{-1}$ and 0.05 $\mu L.L^{-1}$ for 100 $\mu L.L^{-1}$ and 10 $\mu L.L^{-1}$ inlet ethylene concentrations respectively. It took around 12 and 25 h to reach this equilibrium stage respectively. At 0 °C concentration of ethylene detected from low ethylene side after nearly 50 h was 0.35 $\mu L.L^{-1}$ for 100 $\mu L.L^{-1}$ inlet concentration.

Adapting these concentrations obtained through the experiment, 1.65 x $10^{-15}$ $mol.m.m^{-2}s^{-1}Pa^{-1}$ permeability value was calculated for the polyliner at 20 °C for 10 $\mu L.L^{-1}$ inlet concentration and 2.19 x $10^{-15}$ $mol.m.m^{-2}s^{-1}Pa^{-1}$ for 100 $\mu L.L^{-1}$. This was approximately 6 and 8 times higher than the previously reported value 0.27 x $10^{-15}$ $mol.m.m^{-2}s^{-1}$ $Pa^{-1}$ (Wang et al., 1998) for the same type of material (HDPE) with a thickness of 40 μm at the same temperature (20 °C).

Table 7-1: Ethylene permeability values of HDPE polyliner used in the commercial kiwifruit package obtained for two different concentrations of ethylene at two different temperatures.

| **Inlet ethylene concentration ($\mu L.L^{-1}$)** | **Temperature (ºC)** | **Permeability value ($mol.m.m^{-2}s^{-1}Pa^{-1}$)** |
|---|---|---|
| 10 | 20 | 1.65 x $10^{-15}$ |
| 100 | 20 | 2.19 x $10^{-15}$ |
| 100 | 0 | 0.76 x $10^{-15}$ |

Permeability was nearly 30% higher with 100 $\mu L.L^{-1}$ inlet concentration when compare to 10 $\mu L.L^{-1}$ concentration obtained at 20 °C. Furthermore, nearly three times increase in ethylene permeability at 20 °C (2.19 x $10^{-15}$ $mol.m.m^{-2}s^{-1}$ $Pa^{-1}$) was

observed in comparison to 0 °C permeability value ($0.76 \times 10^{-15}$ $mol.m.m^{-2}s^{-1}$ $Pa^{-1}$) obtained for similar inlet concentration 100 $\mu L.L^{-1}$. Calculated $Q_{10}$ (temperature co-efficient) value (applying permeability values obtained at 0 and 20 °C for 100 $\mu L.L^{-1}$ inlet concentration) for the specific polyliner is 1.7.

## 7.4 Discussion

Previously reported data (Table 2-2) demonstrated ethylene permeability differences between film types as well as for the similar type of polymer film (e.g. HDPE). Differences in measured permeability may be a result of difference in various factors found within the structure of the film as well as experimental conditions that are often not reported alongside the data presented. Barrier properties of packing material depend on intrinsic structure (chemical and physical) of the polymer including degree of crystallinity, density, crystallinity/amorphous phase ratio, orientation, chemical groups in the polymer, glass transition temperature, degree of cross linking, thermal and mechanical behaviour (Heilman et al., 1956; Siracusa, 2012).

Many researchers have investigated the influence of chemical structure and morphological properties on polymer permeability and reported correlations between properties like density, crystalinity and molecular orientation with the permeability behaviour of any polymer (Siracusa, 2012). Kofinas et al., (1994) and Wang et al., (1998) reported that amorphous polymers have higher permeability than crystalline polymers. With the increase of polymer crystallinity, permeability considerably decreases since the crystalline area is generally impermeable to the gas permeant confining gas permeation of semi crystalline polymer only to the amorphous region. Pauly, (1999) found a 38 and 19.3% permeability reduction with increasing of crystalinity of 60% and 81% respectively for HDPE, in comparison to 90% crystaline HDPE when $O_2$ is used as the permeant. Kofinas et al., (1994) also observed a permeability decrease with the increase in crystallinity for polyethylene/poly(ethylene-propylene) semicrystalline diblock copolymers to several gases such as He, $CO_2$, $CH_4$, $O_2$ and $N_2$. These studies suggest that despite data for HDPE existing, the crystallinity/amorphous ratio of the polyliner has the potential to cause the 6-8 times difference in permeability to ethylene between this experiment and that reported by Wang et al., (1998) and also emphasises the need to measure

each manufactured film in order to gain accurate data to facilitate accurate gas transfer modelling.

Mrkić et al., (2007) showed that not only physiochemical characteristics but also temperature associated stress can have an effect on gas transport through films. The increase in film permeability with increased temperature is mostly due to greater motion of polymer segments and energy level increase in the permeate molecule (Siracusa, 2012). Additionally, free volume in the polymer can increased with high temperature due to creation of larger gaps in the polymer matrix at high temperature. Permeability of ethylene through edible wheat gluten film at 9 ºC (33% RH) was as low as 0.007 x $10^{-15}$ mol.m.$m^{-2}s^{-1}Pa^{-1}$ while at 20 ºC (85% RH) it was 0.8 x $10^{-15}$ mol.m.$m^{-2}s^{-1}Pa^{-1}$ (Paz et al., 2005). Savoie et al., (1993) found permeability of PVC to ethylene was 1.8 and 2.8 times higher at 17 and 20 ºC respectively in comparison to the permeability observed at 3 ºC. HDPE in present research showed 2.2 fold increase at 20 ºC than at 0 ºC. Therefore, it could be suggest while increase in permeability can be expected as a consequence of increase in temperature, the magnitude of temperature dependency on permeability of ethylene may differ substantially depending on the type of film.

The observed increase in permeability with increase of temperature ($Q_{10}$=1.7) agrees with past research. Previously, Piergiovanni et al., (1992) and Savoie et al., (1993) found that for every 10 ºC increase in temperature the ethylene permeability of PVC increased 1.7 times ($Q_{10}$=1.7) while Furuta et al., (1993) found a $Q_{10}$ of 1.7 for LDPE and 2 for EVA. $Q_{10}$ of EVA is 1.9 as found by Piergiovanni et al., (1992).

Standard test conditions to determine film permeability are usually between 23 and 25 °C. However, fresh produce including kiwifruit are stored at cool storage temperature (Wang et al., 1998). Therefore, it is important to obtain gas transmission data under low temperature conditions. Thus, although creating additional experimental complexity, the present attempt to obtain an ethylene permeability value at 0 °C is very relevant to the kiwifruit industry. Further research is needed to be done at a range of low temperature values (0-15 °C) and for a range of ethylene concentration driving forces.

Permeability values are usually considered to be concentration independent if there is no interaction between the permeant and the film (Lin & Freeman, 2004; Weerawate, 2008). However, this does not apply when interactions between the

permeant and the film occur, such as what occurs between water vapour and hydrophilic film (cellulose and EVOH) or between organic vapours such as ethylene and polyolefin films like LDPE, HDPE and PP. Weerawate, (2008) observed effective film permeability to increase exponentially with increasing concentrations. A 2.5-4 fold increase in hexanal permeability was observed in all film types (LDPE, PP and Tyvek) with a 2.5 time increase in hexanal vapour concentration at 10 ºC. Meanwhile a 24 and 3 fold increase of permeability for LDPE and PP respectively was observed for 24 times hexanal concentration increase at 20 °C (Weerawate, 2008). In comparison, a 1.3 fold increase in ethylene permeability at 20 °C through HDPE was observed with a 10 times ethylene concentration increase in this current work.

While Wang et al., (1998) found permeability of a film to decrease at high humidity due to absorbed or condensed water forming an extra diffusion barrier, Paz et al., (2005) found that high relative humidity caused a higher rate of flow of gases through a wheat gluten film because of structural modifications with water uptake of the film. These results of Wang et al., (1998) and Paz et al., (2005) demonstrate the potential for high dependency of ethylene permeability on the relative humidity in addition to temperature, in agreement with past evidence for oxygen and carbon dioxide permeation through hydrophilic films. Considering this potential for an RH effect on the kiwifruit HDPE polyliner, in the future, further research should be targeted by controlling RH, as in commercial use high RH conditions are likely to prevail, at least inside the polyliner, given that this is its primary function, yet this initial study used dry air for the ease of experiment set-up and ethylene measurement. Determination of the relationship between water content in the polyliner and the permeability at different temperature and relative humidity levels will be useful in determining the ethylene composition inside a commercial kiwifruit package exposed to variable environmental conditions through different stages of the supply chain.

To the best of knowledge there have been no efforts to study film behaviour or specifically permeability of a film under realistic conditions when a significant portion of the film is in contact with the fruit. Under real situations, absorption of vapour and liquid from the product to the film can cause an increase of polymer plasticization, resulting in a decrease in permeability due to alteration of polymer performances (Siracusa, 2012).

Further, as discussed in Siracusa, (2012) handling and packaging procedures for kiwifruit may have an effect on the polyliner barrier properties due to creation of cracks and folds. Hence, it would be important to set a goal in the future to study the permeability of the film under real situations by following a suitable method. Additionally, findings would provide more understanding on interactions between the kiwifruit and the polymer film if there are any and the factors influencing the material permeability under real conditions.

## 7.5 Conclusion

Reported values in the past for ethylene permeability through synthetic films range from 0.27 x $10^{-15}$ - 8.49 x $10^{-15}$ mol.m.m$^{-2}$s$^{-1}$Pa$^{-1}$ within a temperature range of 3-23 ºC which represents a 20 times difference between the slowest and fastest rates (Table 2-2). Meanwhile edible and biodegradable wheat gluten film has been reported with the lowest permeability, being approximately 1000 times lower than synthetic films. Permeability values acquired in the present experiment: 0.76 x $10^{-15}$ - 2.19 x $10^{-15}$ mol.m.m$^{-2}$s$^{-1}$Pa$^{-1}$ for 0 and 20 ºC are consistent with the values found in literature. This data will be important for any future prediction of the ethylene accumulation within a commercial kiwifruit package, as the polyliner film with the package represents the major barrier to ethylene diffusion from the package. Further research on permeability evaluation of kiwifruit HDPE polyliner under realistic conditions (for a range of low and high temperature values) would provide more accurate quantification and understanding on interactions between the kiwifruit and the polymer film.

# Chapter 8

## DISCUSSION AND CONCLUSIONS

### 8.1 Introduction

The kiwifruit industry will benefit from reduction of postharvest losses. This potentially could be achieved through application of a suitable sensor which could be used to detect poor quality fruit (rotten or damaged) as they advance along the different stages of the supply chain. A potential target for a sensor may be ethylene concentration within an individual commercial kiwifruit package. To interpret the data obtained by the sensor it is necessary to understand the relationships and interactions between the ethylene concentration within the package with the status of the kiwifruit (firmness, injury), supply chain environment conditions (temperature) as well as package properties (permeability).

This thesis aimed to quantify some of the key factors which govern ethylene composition within the commercial kiwifruit package: ethylene production of kiwifruit as a function of fruit maturity/firmness, temperature and degree of injury, ethylene desorption from kiwifruit with transient increase of temperature as well as transfer of ethylene across the packaging material. The 'Hayward' kiwifruit cultivar was selected for the study as the most widely grown cultivar. Furthermore, an attempt was made to develop simple mathematical models to illustrate a relationship between ethylene composition within the package and obtained experimental data as a preliminary step towards constructing a predictive model which could be used in analyzing situation changes under different supply chain conditions.

### 8.2 Review of results

Results in quantifying rate of ethylene production as a function of firmness and temperature (Chapter 4) clearly indicated a strong relationship between ethylene production with temperature and firmness of 'Hayward' kiwifruit (Figure 4-2). Much lower rates of ethylene production (0.008-0.011 $pmol.kg^{-1}s^{-1}$) were detected at 0 and 2 °C in the current work in comparison with previously reported ethylene production data [0.25-1.2 $pmol.kg^{-1}s^{-1}$ in Kim et al., (1999) and <1.2 $pmol.kg^{-1}s^{-1}$ in Crisosto & Kader, (1999)] at similar low temperature (0 °C) for same ('Hayward') variety of kiwifruit, demonstrating the advantage of using a high sensitivity of ethylene detector, ETD-300 in the present study. Moreover, statements made by

Stavroulakis & Sfakiotakis, (1995) describing lack of production of ethylene of 'Hayward' kiwifruit through the autocatalytic pathway at temperatures below a critical range of 11-14.5 °C unless damaged or infected and occurring only in the temperature range of 20-34 °C (Antunes & Sfakiotakis, 1997) or at 17-35 °C (Stavroulakis & Sfakiotakis, 1993) suggests the limitations in measuring very low levels of ethylene (0.008-0.011 $pmol.kg^{-1}s^{-1}$) produced by kiwifruit at low temperature (<11-14.5 °C) by usage of conventional methods such as gas chromatography (GC) in prior work.

As observed in earlier work (Arapia et al., 1994; Bonghi et al., 1996; Kim et al., 1999 and Feng et al., 2003), a dramatic increase in ethylene production was observed as the fruit softened below 13 N at 20 °C. Maximal production of ethylene occurs in very soft fruit, which produce 16,000-120,000 times more ethylene than fruit of >13 N even at low temperature (0 °C). Commercially, storage occurs at 0 °C and hence an approximate 100,000 times difference in ethylene production is possible between a very soft fruit and a sound fruit in these conditions. Since there are 100 boxes of (on average) 100 kiwifruit in each box (modular bulk pack) per pallet, this result indicates that if there was a single fruit of 1 N within a pallet of >20 N fruit, that one 1 N fruit would produce as much ethylene as the remainder of the entire pallet combined. The fact that there is such a dynamic range of ethylene production in the 13 N to 1 N firmness range would suggest that detection of ethylene concentration within a kiwifruit box should be able to be used to provide a reasonable estimate of the firmness of the fruit within the box. This idea is further investigated later in this chapter (section 8.3).

Ethylene production data obtained at a broad range of potential supply chain temperatures (0, 2, 5, 10 and 20 °C) rather than previous research evidence obtained only either at 0 °C (Kim, 1999; Crisosto & Kader, 1999) or 20 °C (Hyodo & Fukasawa, 1985; Kim, 1999; Crisosto & Kader, 1999) concluded that at higher temperatures (10 and 20 °C) initiation of an observable increase in ethylene production occurs at an earlier stage of firmness (10.5-13 N) while firmness of kiwifruit should reduce more (5.6-5.7 N) to observe this at lower temperatures (0 and 2 °C). Further, substantial differences in ethylene production as a result of temperature variation were detected following the ethylene burst at kiwifruit firmness of <13 N.

At 20 °C, the stage of firmness of kiwifruit (13 N) of present work which spike in ethylene production was detected is similar to what demonstrated in Arpaia et al., (1994) of which kiwifruit of less than 13 N firmness initiated high production of ethylene. Agreeing with the previous comments by MacRae & Redgwell, (1992), Tonutti et al., (1993) and Arpaia et al., (1994), it could be suggested that very low ethylene production (<2.5 $pmol.kg^{-1}s^{-1}$ in past literature and 0.008-0.03 $pmol.kg^{-1}s^{-1}$ in present work) detected from kiwifruit with firmness around 51-18 N even at 0 °C might have an effect to the rapid firmness reduction observed in kiwifruit at early stage of storage. Adapting the data described in chapter 4, a simple mathematical model was developed (Figure 4-4, Equation 4-4). This could be used to predict the ethylene production of 'Hayward' kiwifruit given a known fruit quality (firmness) and temperature condition.

As previously identified in earlier research for kiwifruit as well as other fruit (Yu & Yang, 1980; Yang & Hoffman, 1984; Wilson & Lucas, 1988; Abeles et al., 1992; Mencarelli et al., 1996; DeMartino et al., 2002; Alves et al., 2010), impact damage produced high ethylene as a typical 'stress/wound' physiological response (Chapter 5). Results of the current research strongly indicate that temperature plays a significant role in controlling synthesis of wound ethylene (Section 5.3.2 and Figure 5-3) as speculated by Miller et al., (1987); Mencarelli et al., (1996) and DeMartino et al., (2002). No effect was observed on impact damage induced ethylene production at 0 °C in present work agreeing with the results of Mencarelli et al., (1996) with impact injured 'Hayward' kiwifruit at 4 °C; Agar et el., (1999) with peeled kiwifruit at 2 °C and DeMartino et al., (2002) with bruised apricot at 4 °C. This result confirms the beneficial effect of low temperature (especially 0 °C) on impact injury induced ethylene production.

Following the results of Agar et al., (1999) where peeled kiwifruit produced 5 times the ethylene at 20 °C than at 2 °C, an increase in rate of wound ethylene production was observed with increase of temperature in present research producing 2-3 times increase of ethylene production at 20 °C than at 5 °C while increase was 1.3 times when compare with what observed at 5 °C than at 0 °C in impact injured kiwifruit exposed to different drop heights.

Instantaneous rise of ethylene production observed after injury at 20 °C (Figure 5-2C) agrees with the similar instantaneous rises of ethylene production detected in sliced ripe tomato (Kende & Boller, 1981) at room temperature and in injured

apricots stored at 18 °C after being dropped from 30 cm height at 4 °C (DeMartino et al., 2002). The ethylene production pattern of injured kiwifruit observed at 20 °C (instantaneous rise during the first 10 h and then declination in the next 10 h subsequently accompanying a second peak lower than first peak around 22 h followed with a gradual decline to a steady state after 30 h of injury) had previously been demonstrated by McGlasson & Pratt, (1963) for sliced cantaloupe, green banana and potato.

A 1.2, 1.7 and 1.9 times greater ethylene produced by kiwifruit exposed to 30, 60 and 120 cm drop heights respectively than control (sound fruit) at 5 °C and 0.8 and 1.8 times higher ethylene produced by kiwifruit exposed to 60 and 120 cm drop heights respectively at 20 °C strengthens the past evidence available on effect of severity/degree of injury on wound/stress induced ethylene (Imaseki et al., 1968; Jackson & Osborne, 1970; Prasad & Cline, 1987; Mao et al., 1995; Agar et el., 1999; Martìnez-Romero et al., 2000; Alves et al., 2010) where Alves et al., (2010) demonstrated a 2.5 and 5 times increase of ethylene emission rates when 'Bruno' kiwifruit were dropped from 60 and 120 cm. Similarly, Agar et el., (1999) observed a 2-4 times higher ethylene production in peeled kiwifruit slices in comparison to unpeeled slices and 2 times higher in peeled fruit than whole fruit.

Results from two different maturity levels of kiwifruit demonstrated the further effect of firmness of kiwifruit on impact injury induced ethylene production as 12-15 N impact injured kiwifruit produced 2 to 4 times higher ethylene than 20 N fruit. In parallel with increase in ethylene production with increase of temperature as well as degree of damage, percentage loss of firmness after exposing fruit to impact injury was increased with the exposed temperature as well as with the increase of intensity of impact force (drop height). At the same time low firmness impact injured kiwifruit (12-15 N) showed higher firmness loss percentage than high firmness fruit (20 N) for same high temperature (20 ºC).

This study demonstrated a 1 to 20 times increase in ethylene evolution rate for kiwifruit following subsequent transfer to a higher temperature from a lower temperature as previously described by Hyodo et al., (1987); Kim, (1999); Antunes & Sfakiotakis, (2002a) and Antunes & Sfakiotakis, (2002b). The current study (Chapter 6) also aimed at describing the ethylene evolution effects of a range of transient temperature increases (0→2 °C, 2→5 °C, 5→10 °C, 10→20 °C) rather than a 0→20 °C temperature change that features in past literature.

Mathematical estimation of the desorbed ethylene at each transient increase of temperature using Henry's law revealed lower estimated values of ethylene desorption than actual ethylene evolution observed. This under estimation of the observed data by the mathematical estimation indicates there are other factors contribute to the escalation of ethylene evolution observed during and immediately subsequent to an increase in temperature other than contribution from the release of dissolved ethylene in the kiwifruit tissue based on Henry's law. An upsurge of ethylene production due to increase of intermediary, ACC (Kim et al., 1999) or enzymes associated with the ethylene biosynthetic pathway, ACC synthase or ACC oxidase (Knee, 1987; Larrigaudiere et al., 1997; Kim et al., 1999) with increased temperature may have contributed to increased ethylene evolution with transient increase of temperature other than contribution from desorbed ethylene.

Ethylene permeability differences found in reported data (Table 2-2) between film types and similar films (HDPE) are likely to be due to the structure of the film (physical and chemical) and the experimental conditions that are often not reported alongside the data presented. The present research work (Chapter 7) assessed the permeability of the commercial kiwifruit polyliner (HDPE) and found values of 0.76 x $10^{-15}$ mol.m.m$^{-2}$s$^{-1}$Pa$^{-1}$ at 0 ºC and 2.19 x $10^{-15}$ mol.m.m$^{-2}$s$^{-1}$Pa$^{-1}$ at 20 ºC. The influence of the intrinsic structure (physical and chemical) of a film including degree of crystallinity, density, crystallinity/amorphous phase ratio, orientation, chemical groups in the polymer, glass transition temperature, degree of cross linking, thermal and mechanical behaviour may have caused the 6-8 times greater permeability (Kofinas et al., 1994; Wang et al., 1998; Pauly 1999; Siracusa, 2012) of current commercial kiwifruit polyliner (HDPE) in comparison to that reported by Wang et al., (1998) for HDPE film (2.68 x $10^{-16}$ mol.m.m$^{-2}$s$^{-1}$Pa$^{-1}$) at 20 ºC.

Permeability was found to be dependent on temperature showing a 1.7 times increase of permeability for 10 ºC increase in temperature agreeing with past research which demonstrated $Q_{10}$ of 1.7 for PVC (Piergiovanni et al., 1992; Savoie et al., 1993), $Q_{10}$ of 1.7 for LDPE (Furuta et al., 1993) and $Q_{10}$ of 2 (Furuta et al., 1993) and 1.9 (Piergiovanni et al., 1992) for EVA.

The effect of increasing concentration of permeate (ethylene) on permeability observed in present work with 1.3 fold increase in permeability of HDPE with 10 times increase in ethylene concentration at 20 ºC has previously been demonstrated

in Weerawate, (2008) by showing a 2.5-4 fold increase in hexanal permeability with a 2.5 times increase in hexanal vapour concentration at 10 ºC for LDPE, PP and Tyvek and a 24 and 3 fold increase of hexanal permeability for LDPE and PP respectively with 24 times hexanal concentration increase at 20 °C.

From the different parameters identified as influencing the magnitude and the variability of ethylene movement and accumulation within the kiwifruit package, this thesis focused on quantifying ethylene production of kiwifruit as a function of fruit maturity (firmness), temperature and degree of injury, ethylene desorption from kiwifruit with transient increase of temperature as well as transfer of ethylene across the packaging material. One of the identified factors which govern the ethylene composition of the package: production of ethylene by rotten fruit infected by *Botrytis cinerea* was not conducted in the present work. However, past literature reveals that ethylene production of rotten kiwifruit ranges from 2.5 to 9.9 $pmol.kg^{-1}s^{-1}$ [2.5 $pmol.kg^{-1}s^{-1}$ (Sfakiotakis et al., 1997), 3.7 $pmol.kg^{-1}s^{-1}$ (Niklis et al., 1997), 9.9 $pmol.kg^{-1}s^{-1}$ (Qadir et al., 1997)]. Comparing with the value of ethylene production of a sound fruit (0.008-0.011 $pmol.kg^{-1}s^{-1}$), increase in ethylene production due to rot is approximately 250-1000 fold. In this study, ethylene production of impact injured kiwifruit was in a range of 0.2 to 1.6 $pmol.kg^{-1}s^{-1}$ leading to an effect of 2-3 fold increase of ethylene production due to impact damage. Whereas it was 1-20 fold increase of ethylene production due to instantaneous increase in temperature as detected in current study.

Hence, considering the effect of all the factors which govern ethylene concentration within the commercial kiwifruit package, firmness change of 'Hayward' kiwifruit (Figure 4-2) has the most impact. Over the range of potential fruit firmness, ethylene production ranges from 10,000 – 100,000 fold, dwarfing the 2-20 fold increase (due to injury, temperature) and 250-1000 increase (due to rot) of ethylene production with change of other factors. So, it could be concluded that the ability to detect ethylene concentration inside a kiwifruit package could be applied in getting the information of the quality of the fruit (firmness) inside the package and to identify the kiwifruit which require remedial action within the stock.

## 8.3 First draft mathematical model to illustrate the relationship between ethylene concentration within the package and production of ethylene of 'Hayward' kiwifruit

Polyliner permeability values obtained at 0 ºC and 20 ºC (Table 7-1) and predicted log ethylene production rates of kiwifruit obtained at 0 ºC and 20 ºC as a function of fruit quality/firmness (Figure 4-4) were used as inputs to a model to established the log ethylene concentration (mPa) within different types of commercial packages available in the industry as a function of log ethylene production of kiwifruit (fmol.kg$^{-1}$s$^{-1}$) [Figure 8-1] and as a function of kiwifruit firmness (N) [Figure 8-2].

The following assumptions were made when developing the model presented in Figure 8-1 and 8-2.

1. At steady state, ethylene production of kiwifruit inside the pack = Ethylene loss through the polyliner of the package.

   By assuming,

   1.1 No ethylene adsorption to the kiwifruit or to the package.

   1.2 No ethylene transmission through the holes and the gap between the fold.

2. Ethylene concentration outside the package as zero.
3. Ethylene loss through the polyliner is uniform throughout the total area available.

According to the Fick's law of diffusion,

$$\text{Ethylene loss through polyliner} = \mathrm{P} \times A \times (E_{in} - \mathrm{E_{out}})/\mathrm{x} \qquad [8\text{-}1]$$

Where,

P – Permeability of polyliner (mol.m.m$^{-2}$s$^{-1}$Pa$^{-1}$)

$A$ – Total area of the film (m$^2$)

x – Thickness of the polyliner (m)

$E_{in}$ - Ethylene inside the package (Pa)

$E_{out}$ - Ethylene outside the package (Pa)

At steady state ethylene production of kiwifruit inside the package is balanced by rate it is lost through the polyliner (Assumption 1).

$$m.\ EP = \mathrm{P} \times A \times (E_{in} - \mathrm{E_{out}})/\mathrm{x} \qquad [8\text{-}2]$$

Where,

*EP*- Ethylene production rate of kiwifruit inside the package (mol.kg$^{-1}$s$^{-1}$)

*m*- Mass of kiwifruit inside the package (kg)

Rearranging Equation 8-2 to make the ethylene inside the package ($E_{in}$) the subject and assuming ethylene outside the package ($E_{out}$) = 0 results in

$$\mathrm{E_{in}} = \frac{m \times \mathrm{x}}{\mathrm{P} \times A}\ \mathrm{EP} \qquad [8\text{-}3]$$

Four types of kiwifruit packages namely, modular bulk pack for green and gold variety and single layer pack for green and gold variety were considered in establishing the model presented in Figure 8-1 and 8-2. Total weight of each pack (loaded with fruits) and total area of the polyliner of each pack used in the model are listed in the Table 8-1.

Table 8-1: Total weight (kg) and total area (m$^2$) of the polyliner of four types of commercial packs available in the kiwifruit industry considered in establishing the model

| **Type of pack** | **Weight (kg)** | **Area available (m$^2$)** |
|---|---|---|
| Modular bulk pack (green) | 10.23 | 0.50 |
| Modular bulk pack (gold) | 5.92 | 0.42 |
| Single layer pack (green) | 3.68 | 0.37 |
| Single layer pack (gold) | 3.41 | 0.38 |

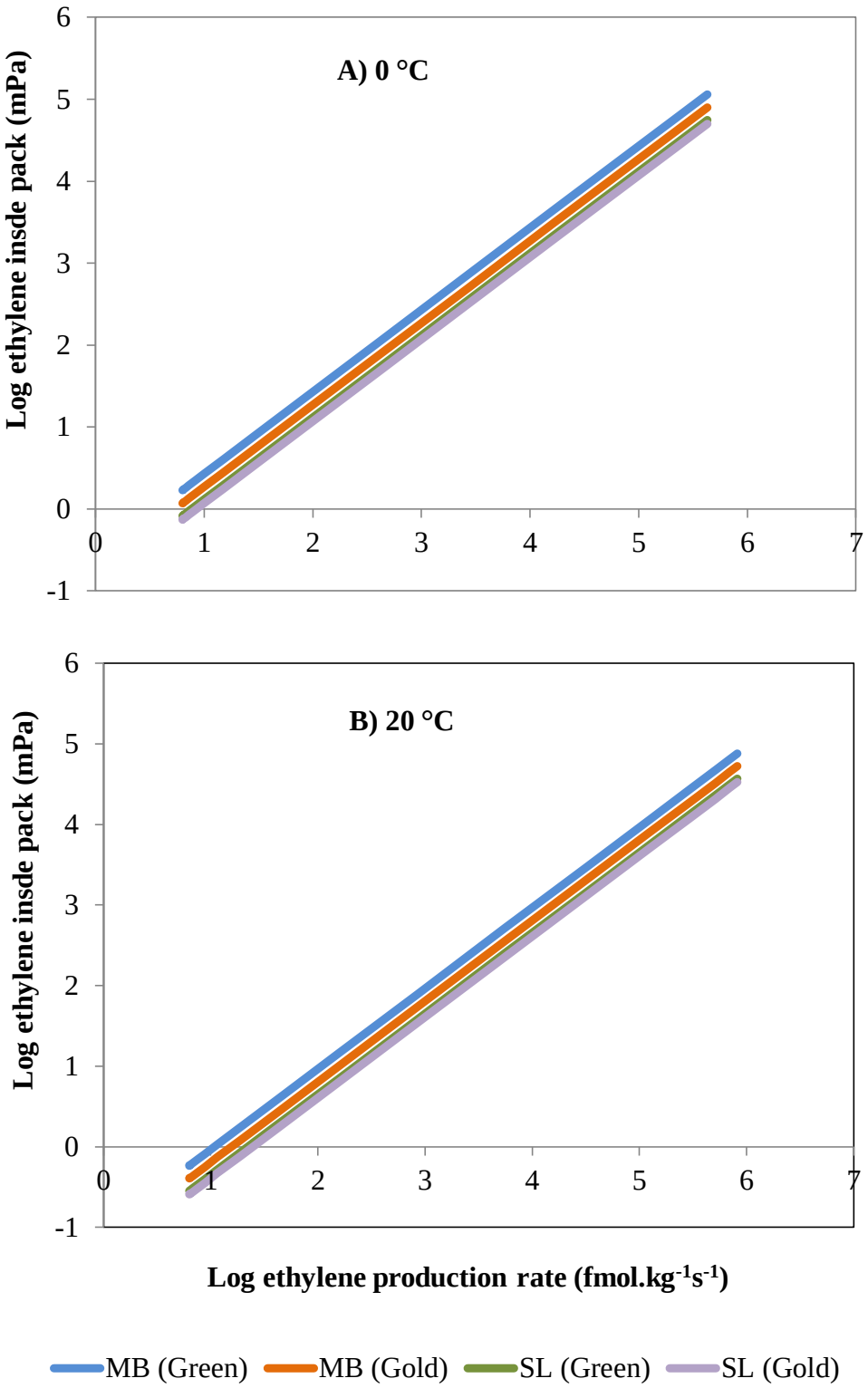


Figure 8-1: Models established to predict log ethylene composition (mPa) inside different packages available in the kiwifruit industry as a function of log ethylene production rate (fmol.kg$^{-1}$s$^{-1}$) of kiwifruit at 0 °C (Figure 8-1A) and 20 °C (Figure 8-1B). [MB (Green) - Modular bulk pack for Green variety, MB (Gold) - Modular bulk pack for Gold variety, SL (Green) - Single layer pack for Green variety, SL (Gold) - Single layer pack for Gold variety].

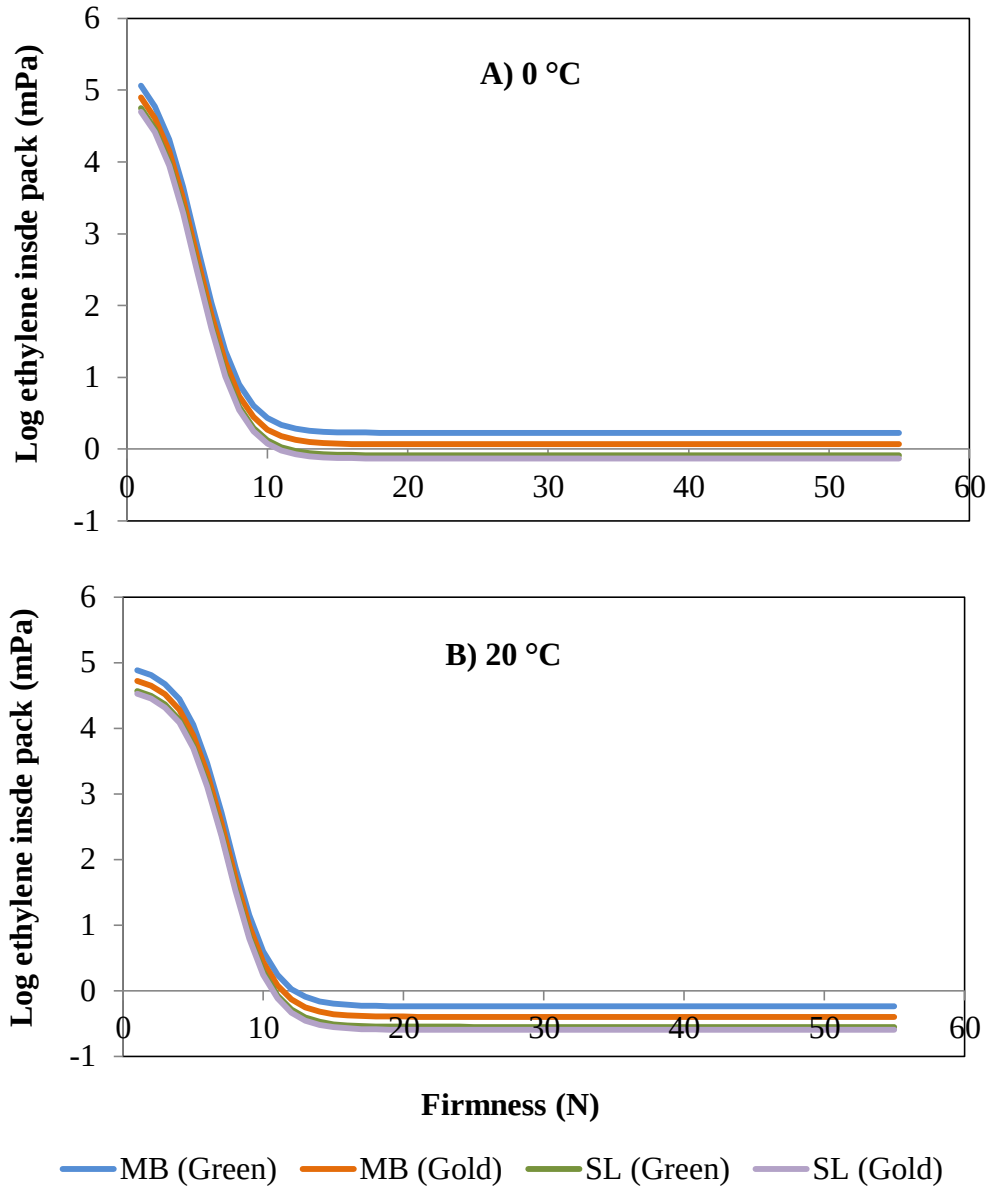


Figure 8-2: Models established to predict log ethylene composition (mPa) inside different packages available in the kiwifruit industry as a function of firmness (N) of kiwifruit at 0 °C (Figure 8-2A) and 20 °C (Figure 8-2B). [MB (Green) - Modular bulk pack for Green variety, MB (Gold) - Modular bulk pack for Gold variety, SL (Green) - Single layer pack for Green variety, SL (Gold) - Single layer pack for Gold variety].

When consider the models established to predict log ethylene composition (mPa) within different types of commercial packages available in the industry as a function of log ethylene production ($fmol.kg^{-1}s^{-1}$) of kiwifruit (Figure 8-1) and firmness (N) of kiwifruit (Figure 8-2) a 1.5 fold increase of ethylene composition (mPa) inside the package could be observed with the temperature decrease from 20 ºC to 0 ºC irrespective to the type of package due to permeability reduction of the polyliner at

lower temperatures even though the rate of ethylene production of kiwifruit is lower at 0 ºC than at 20 ºC. More clear interpretation could be observed with the Figure 8-2 (model established to predict ethylene composition within different types of commercial packages available in the industry as a function of firmness of kiwifruit) at the time of substantial increase of ethylene production was observed when kiwifruit firmness reached below 13 N. Higher permeability at high temperature is favourable to reduce any accumulation of ethylene generated inside the package which may lead to reduction of detrimental effects from ethylene as a result of the physiological effects on kiwifruit. Out of all types of packages considered, modular bulk pack for green kiwifruit [MB (Green)] accumulates more ethylene inside the package at both temperatures due to the lower surface area of the polyliner to fruit volume ratio.

### 8.4 Suggestions for further research

This work has highlighted several areas which require further research. Application of the results obtained in this work to other cultivars of kiwifruit in New Zealand is difficult due to different physiological nature of each cultivar. This work represents the most detailed investigation on ethylene production of 'Hayward', whereas other varieties have not been studied to a similar depth. Thus, it is important to consider in conducting similar work for different varieties of kiwifruit in the future to find out the varietal difference on production of ethylene as a function of firmness, temperature and impact injury.

Further, establishing the relationship between ethylene production and fruit firmness at higher temperatures (>20 °C) will provide benefit to the industry since there is a possibility of kiwifruit being exposed to more extreme environmental conditions (20-30 °C) through underdeveloped cool chain infrastructure when expanding the export market of kiwifruit to South East Asia and the Indian subcontinent. While there is information available for upper temperature limit (>38 °C) where ethylene production get inhibited for 'Hayward' kiwifruit variety (Antunes & Sfakiotakis, 1997) updating the data available considering all the current varieties available in the market will be an advantage for the industry. Moreover, finding the upper temperature limit of kiwifruit of which resumption of ethylene production occurs when again exposed to low temperature would give more benefits to the industry when consider the tropical underdeveloped supply chains.

Determination of wound induced ethylene production for a broader range of temperatures than what was used in the present research as well as for different degrees of impact injury (>120 cm drop heights) representing existing variables throughout kiwifruit supply chain would give benefits as essential realistic inputs for the future constructive model. Further, future research targeting determination of intermediates (ACC) and enzymes (ACO and ACS) attached to ethylene synthesis pathway in parallel with determination of ethylene production as a function of temperature, fruit quality and impact injury would benefit in understanding and further interpretation of the mechanism behind ethylene production variation due to various factors considered as key contributors to the change in ethylene composition within the package.

To the best of knowledge there have been no efforts to study film behaviour or specifically permeability of a film under real conditions when in contact with the fruit except studies directed with simulated conditions. Further, handling and packaging procedures of kiwifruit pack may have an effect to the polyliner barrier properties. Additional findings would provide more understanding on interactions between the kiwifruit and the polymer film if there are any and the factors influencing the material permeability under real conditions. Considering the potential for a relative humidity (RH) effect on the permeability of kiwifruit HDPE polyliner, further research should be targeted by controlling relative humidity (RH) as in commercial use high RH conditions are likely to prevail. Determination of the relationship between water content in the polyliner and the permeability at different temperature and relative humidity levels will be useful in determining the ethylene composition inside a commercial kiwifruit package exposed to variable environmental conditions through different stages of the supply chain.

Quantification of other factors which contribute to the ethylene composition inside the kiwifruit pack: ethylene production by rotten kiwifruit, ethylene adsorption by the package and the kiwifruit itself, ethylene transfer through the fold and holes present in the pack should be done to fill the gap in obtaining data for the future constructive model which has been planned as the base of the application of the sensor in the kiwifruit industry. Moreover, as quality attributes which affect ethylene production, quantification of other volatiles (ethanol, methanol, ethyl butonoate) as a function of same factors considered in this study may be beneficial in establishing the future model.

# Chapter 9

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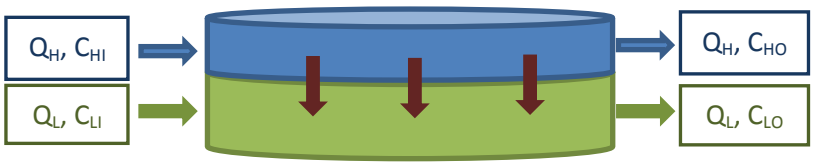
# APPENDIX I

**Derivation of permeability equation (Equation 7-1)**




Where,

$C_{HI}$ = Inlet ethylene concentration from high ethylene side ($\mu L.L^{-1}$)
$C_{HO}$ = Outlet ethylene concentration from high ethylene side ($\mu L.L^{-1}$)
$C_{LI}$ = Inlet ethylene concentration for low ethylene side ($\mu L.L^{-1}$)
$C_{LO}$ = Outlet ethylene concentration from low ethylene side ($\mu L.L^{-1}$)
$Q_H$ = Ethylene flow rate of high ethylene side ($m^3s^{-1}$)
$Q_L$ = Ethylene flow rate of low ethylene side ($m^3s^{-1}$)
$P$ = Effective film permeability ($mol.m.m^{-2}\,s^{-1}Pa^{-1}$)
$L_{Film}$ = Thickness of film (m)
$A_{Film}$ = Area of film ($m^2$)
R = Universal gas constant ($8.314\ J.K^{-1}mol^{-1}$)
$T$ = Absolute temperature (K)

Ethylene balance on high side

Rate of ethylene flow in to chamber = Rate of ethylene flow out from chamber + Rate of ethylene transfer through film

$$Q_H \cdot C_{HI} = Q_H \cdot C_{HO} + \frac{P.\ A\,(C_{HO} - C_{LO})\ R.T}{L}$$

Ethylene balance on low side

Rate of ethylene flow in to chamber + Rate of ethylene transfer through film = Rate of ethylene flow out from chamber

$$Q_L \cdot C_{LI} + \frac{P.\ A\,(C_{HO} - C_{LO})\ R.T}{L} = Q_L \cdot C_{LO}$$

$$Q_L \cdot C_{LO} - Q_L \cdot C_{LI} = \frac{P.\ A\,(C_{HO} - C_{LO})R.T}{L}$$

$$Q_L\,(C_{LO} - C_{LI}) = \frac{P.\ A\,(C_{HO} - C_{LO})R.T}{L}$$

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