CRANFIELD UNIVERSITY

EPSRC CASE Studentship

FARDUSA ELMI

ELUCIDATING ETHYLENE-MEDIATED PHYSIOLOGY AND BIOCHEMISTRY IN SELECTED CLIMACTERIC AND NON-CLIMACTERIC FRUITS USING E+® ETHYLENE REMOVER

CRANFIELD HEALTH

Ph.D THESIS

Academic Year: 2009 - 2013

Supervisor: Prof Leon. A Terry

May 2013

CRANFIELD UNIVERSITY

CRANFIELD HEALTH
Plant Science Laboratory

Ph.D THESIS

Academic Year 2009 - 2013

FARDUSA ELMI

Elucidating ethylene-mediated physiology and biochemistry in selected climacteric and non-climacteric fruits using e+® Ethylene Remover

Supervisor: Prof Leon. A Terry
May 2013

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

ABSTRACT

The presence of ethylene in a storage environment can undermine both quality and postharvest life of many fruits, often generating significant waste and associated economic losses. A demand for discovering alternative technologies capable of scavenging ethylene has led to the development of a new material, e+® Ethylene Remover, which has significant ethylene adsorption capacity. The material has been shown to remove ethylene to below physiologically active levels during fruit storage at 0-20 ^{o}C and consequently extend postharvest life for a variety of fresh produce types. Different formats incorporating e+® Ethylene Remover have been developed. Successful application of e+® Ethylene Remover in laboratory settings has created opportunities to test new formats of the product. To this end, work was conducted herein to attest whether e+® Ethylene Remover, which has been shown to maintain avocado (*Persea americana* cv. Hass) firmness in recent laboratory trials, could result in a meaningful extension of storage life in a commercial setting. It was shown that e+® Ethylene Remover coated sheets were a highly efficacious format for suppressing ethylene and extending storage life of imported avocado and pluot plums in a series of commercial trials. Moreover, the potency of the e+® Ethylene Remover treatment in retarding ethylene induced ripening was significantly enhanced when avocados were treated during the early stages of ripening. After a storage period of up to 31 days (5-6 ^{o}C), avocado fruits which had received an early treatment at source and then treated again in the laboratory were significantly more firm and greener compared to fruits treated following 5 weeks of transit alone .

Strawberry is regarded as non-climacteric fruit; nevertheless, exogenous ethylene can negatively influence postharvest life. The low ethylene produced by non-climacteric fruits has been generally ignored and research reporting on the involvement of ethylene in these fruits is typically devoid in the literature. To this end, application of a highly sensitive laser based photoacoustic ethylene detector has revealed a possible role of ethylene in determining the postharvest life of strawberries. Moreover, fruit quality parameters including disease incidence, sugars, organic acids, phenolic compounds and plant phytohormones/metabolites were found to be profoundly affected by ethylene and

likewise the removal of ethylene. Storage of strawberries in the presence of e+® Ethylene Remover was associated with lower disease incidence, significantly less red fruits and higher ascorbic acid content. In contrast, ethylene and 1-methylcyclopropene (1-MCP) treatments resulted in the higher postharvest disease. Ethylene-treated fruits were associated with lower level of sucrose and higher simple sugars (fructose and glucose) suggesting a role of ethylene in promoting the rate of senescence and concomitant reduced postharvest quality of strawberries. Changes in ABA, ABA metabolites and auxins within different tissues of ripe strawberry during storage were investigated. ABA was more abundant in the flesh than in the achenes, while auxins were undetectable in the flesh tissue. Auxins indole-3-acetic acid (IAA) and the conjugated form indole-3-acetylaspartic acid (IAAsp) were detected in high concentrations in the achenes and were affected by ethylene and storage length.

ACKNOWLEDGEMENTS

I would like to first and foremost thank my supervisor Prof. Leon A. Terry. His encouragement, guidance and immense support were invaluable. Working with him has encouraged and inspired me greatly; it was an honour to have had the opportunity to work with him. Special thanks to Dr. Katherine Cools, for her immense patience; she has been the best mentor anyone could wish for. My thanks and appreciations are also extended to Dr. Marjolaine Meyer who assisted me during the start of my project. Furthermore, I would like to acknowledge with much appreciation Dr. Inmaculada Pradas for her help and collaboration during the last year of the project.

This studentship (case number: 09000987) was funded by Johnson Matthey Plc and Engineering and Physical Sciences Research Council (EPSRC). I would like to extend my gratitude to them for the financial support. In particular, thanks to Dr. Andy Smith, Dr. Liz Rowsell and Dr. Stephen Poulston from Johnson Matthey for their support throughout.

It would not have been possible to write my thesis without my friends and colleagues at Cranfield University, who were abundantly helpful.

Last but not least, I would like to express my deepest gratitude to my Mum Sofia Hussein and Dad Abdi Elmi for their endless love and understanding throughout my life. Special thanks to my Uncle Omar Elmi and Aunt Waris Abdirahman for their constant love and encouragement. I wish to also express my thanks to my beloved families; for believing in me throughout.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOTATION & ABBREVIATIONS

<	less than
>	greater than
%	percent
=	equals
µl	microliter
µmol	micromole
^{o}C	degree Celsius
7′-OH ABA	7′-hydroxy ABA
8′-OH ABA	8′-hydroxy ABA
1-MCP	1-methylcyclopropene
AAO	Abscisic-aldehyde oxidase
ABA	Abscisic acid
ABA-GE	ABA glucosyl ester
ACC	1-amino-cyclo-propane carboxylic acid
ACO	1-amino-cyclopropane-1-carboxylic oxidase
ACS	1-amino-cyclopropane-1-carboxylic acid synthase
ANOVA	Analysis of Variance
AOA	amino-oxyacetic acid
AVG	amino ethoxy vinyl glycine
BOC	British Oxygen Company
BF	buffer treated
C*	chroma
C_2H_4	ethylene
C	control
CA	controlled atmosphere
ca.	approximately

CAT	Catalyser
CI	chilling injuries
cm	centimetre
CO_2	carbon dioxide
C-3-G	cyanidin-3-glucoside
C-3-R	cyanidin-3-runtinoside
C6	six carbons
C7	seven carbons
CPPU	N-(2-chloro-4-pyridyl)-N'penylurea
CTR	constitutive triple response
CU	Cranfield University
cv.	cultivar
DAD	Diode array detector
d.f	degrees of freedom
DPA	dihydrophaseic acid
DW	dry weight
E+	e+® Ethylene Remover treated
EIN	ethylene insensitive
ELSD	Evaporative Light Scattering Detector
ERF	ethylene response factor
ERS	ethylene response sensor
ET	ethylene treated
EtOH	Ethanol
EPA	Environmental Protection Agency
et al.	and others
Exp	experiment
g	grams
GAs	Gibberellins
GC	Gas Chromatography

FAO	Food and Agriculture Organisation of the United Nations
FID	Flame Ionisation Detector
FW	fresh weight
H°	hue angle
h	hour
HPLC	High Performance Liquid Chromatography
H_2O	water
in vivo	inside a living organism
IAA	indole-3-acetic acid
IAAsp	indole-3-acetylaspartic acid
JM	Johnson Matthey Plc
kg	kilogram
kgf	kilogram force
$KMnO_4$	potassium permanganate
kPa	kilopascal
LDL	low-density lipoprotein
L*	lightness
l	litre
LSD	least significant difference
Ltd	limited company
M	molarity
MA	modified atmosphere
MAP	modified atmosphere packaging
MAPK	mitogen-activated protein kinase
MeOH	methanol
min	minutes
ml	millilitre
MET	methionine
MEP	methylerythritol 4-phosphate

MS/MS	tandem mass spectroscopy
MTA	5'-methylthioadenosine
mg	milligram
m/m	mass by mass
mm	millimeter
N	Newtons
NBD	2, 5 Norbornadiene
NCED	nine *cis*-epoxycarotenoid dioxygenase
ng	nanogram
NH_4OH	ammonium hydroxide
nl	nanolitre
NO	nitric oxide
O_2	oxygen
ORAC	oxygen radical absorbance capacity
P	probability
P-3-G	pelargonidin 3-glucoside
PA	phaseic acid
Plc	public limited company
Pd	palladium
PE	pectinesterase
PG	polygacturonase
PME	pectin methyl esterase
PDA	Potato Dextrose Agar
Q-3-G	Quercetin-3-Glucoside
Q-TOF-MS	Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometry
Rh	rhodium
RH	relative humidity
RNA	ribonucleic acid
rpm	revolutions per minute

Ru	ruthenium
s	seconds
SA	salicylic acid
S-AdoMet	*S*-adenosyl-L-methionine
SDR	short-chain dehydrogenase/reductase
TCC	Thermostatted column compartment
TEM	transmission electron microscopy
TSS	Total soluble solids
TR1	treatment 1
TR2	treatment 2
UK	United Kingdom
UN	untreated
UPLC	ultra-performance liquid chromatography
USA	United States of America
VC	valve controller
vs	versus
viz.	namely
v/v	volume by volume
WVP	Water vapour pressure
ZEP	zeaxanthin epoxidase

CHAPTER ONE

1 INTRODUCTION

1.1 Project Background:

Ethylene is a plant hormone which is intimately involved in almost every phase of plant development. Ethylene is able to evoke its effects at very low concentrations (Saltveit, 1999), where it can trigger the coordination of many hundreds of genes to affect a process. The response in relation to ethylene is highly dependent on the plant concerned. Fruits can be classified into two major groups according to their response to ethylene after harvest; either as climacteric or non-climacteric (Goldschmidt, 1997; Giovannoni, 2001). A period of rapid ripening termed as the 'climacteric' phase is thought to be unique for climacteric fruits. The climacteric phase is associated with a dramatic increase in respiration rate, as well as ethylene production during ripening. In climacteric fruits, ethylene is a key element in determining various quality parameters during ripening. Commercially, these fruits are usually harvested when unripe so that they can be manipulated to ripen according to supply chain and market requirements. The importance of ethylene in non-climacteric systems is less clear even though some such fruits and indeed vegetables may display a response to exogenous ethylene (Cools *et al.*, 2011). Consensus has typically disregarded the role of ethylene in regulating ripening or senescence in non-climacteric systems. Problems over understanding the role of ethylene in non-climacteric fruits may be associated with the inability to measure ethylene down to lower thresholds. Lately, more sensitive techniques which have high resolution and low threshold have been developed and used to measure ethylene production of non-climacteric fruits (Iannetta *et al.*, 2006). Most non-climacteric fruits mature while still attached to their parent plant and thus postharvest stages are described as maturation or senescence rather than ripening (Goldschmidt, 1997; Wills *et al.*, 2007).

Ethylene is responsible for accelerated postharvest ripening and thus, deterioration of climacteric fruits (Bapat *et al.*, 2010). The removal/and or inhibition of the effect of ethylene in a stored environment atmosphere is hence fundamental to maintaining

quality. The level of ethylene in the storage atmosphere of climacteric fruits is a major factor which can determine quality after harvest (Saltveit, 1999; Martinez-Romero *et al.*, 2007). However, although the effects of ethylene at different concentrations is well documented there is a little literature detailing the dose response (time x concentration) of ethylene for most fruits.

Reducing ethylene during postharvest stages can lead to an expected extension in shelf life and reduction in the deleterious effects associated with accelerated ripening. Techniques and procedures for retaining the postharvest freshness of climacteric produce that are currently in place are concerned with minimising ethylene exposure. Some strategies involve the use of low temperature storage, controlled atmosphere (CA) and appropriate ventilation (Thompson, 2003; Watkins, 2006). Respiration rate increases exponentially with temperature, thus the use of low temperature slows many metabolic processes. However, extended exposure to low temperature is limited for chilling sensitive fruits, while CA is often expensive and sometimes problematic to accurately maintain (Thompson, 2003). Other strategies include; adsorption on activated carbon (Martínez-Romero *et al.*, 2009a; Martínez-Romero *et al.*, 2009b), the use of potassium permanganate ($KMnO_4$) to oxidise the ethylene (Wills and Warton, 2004), ozone, ultraviolet irradiation or alternatively use of high temperature catalysts (Abeles *et al.*, 1992; Reid, 2004) and more recently the use of e+® Ethylene Remover (Terry *et al.*, 2007b; Meyer and Terry, 2010). The novel e+® Ethylene Remover differs from other ethylene adsorbing materials such as the activated carbon. The activated carbons (Martínez-Romero *et al.*, 2009a; Martínez-Romero *et al.*, 2009b) are impregnated on to carbon support where high temperature is required; thus they behave more like a true catalyst.

1-Methylcyclopropene (1-MCP) is an ethylene action inhibitor which it believed to bind to the ethylene receptor(s) to regulate tissue responses to ethylene. 1-MCP is the most commonly used ethylene inhibitor however it has been subject to scrutiny in its ability to extend the shelf-life (Watkins, 2001; Blankenship and Dole, 2003). 1-MCP is a competitive inhibitor of ethylene binding, thus it suppresses ripening and prolongs storage of many fruits. However, on many fresh produce 1-MCP can cause problems with the ripening process (Jeong and Huber, 2004; Meyer and Terry, 2010). The success

and efficacy of 1-MCP treatment is also dependent on a number of factors including genotype, horticultural maturity at harvest, temperature and duration of treatment as well as the ripening physiology of the fruit (Cin *et al.*, 2006).

A substantial amount of research has been devoted to developing methods in blocking ethylene related effects, while its removal from a storage environment or indeed within packaging has been less considered in the literature. $KMnO_4$ has been recognised as a ubiquitous method for removing exogenous ethylene from a defined atmosphere. However, in an environment of high relative humidity (RH) this ethylene scavenger has been reported to lose its efficaciousness (Terry *et al.*, 2007b). Similarly, Wills and Warton (2004) have reported that with increasing % RH the efficiency of $KMnO_4$ decreased substantially. They have also shown that ethylene adsorption at 90 % RH was 50 % less when compared to that at 70 % RH. In contrast, a Pd-impregnated zeolite, consisting of Pd-particles and a metal loading of 2.5 % Pd (m/m) was shown to have considerably high ethylene adsorption capacity (Terry *et al.*, 2007b). Terry *et al*. (2007b) has shown this material to remove ethylene to below physiologically active levels and to exhibit an adsorption capacity of 4162 µl g^{-1} at 20 ^{o}C and *ca.* 100 % RH. In comparison to the previously mentioned $KMnO_4$, this material was shown to have 60-fold higher ethylene adsorption capacity in conditions of high RH. In addition to this Smith *et al*. (2009) distinguished the physiological and chemical properties of the material. A new formulation of the Pd-impregnated material having only 1 % of Pd (m/m) was developed and reported to have beneficial effects in extending storage of avocado (cv. Hass) (Meyer and Terry, 2010). The material is now registered as e+® Ethylene Remover. It is hypothesised that given recent success of this treatment (Terry *et al.*, 2007b; Smith *et al.*, 2009; Meyer and Terry, 2010) both in its ethylene adsorption capacity and in preserving fruits quality (bananas cv. Cavendish and avocados cv. Hass) that there is great potential for improving and delaying the storage life of climacteric fruits. However, trials conducted previously have only looked at the use of treatment in a laboratory setting. Attesting the efficacy of the treatment in the real world supply chain is cardinal to determine its potential as a commercial product. The material (e+® Ethylene Remover) is now available in several formats. The current sheet format has a loading of 30 g of e+® Ethylene Remover coating per square meter, which equates to 38.6 % e+® Ethylene Remover.

Strawberries have been reported to produce ethylene and a concomitant rise in respiration rate at the red ripe stage (Iannetta *et al.*, 2006). Strawberries also deteriorate rapidly during storage due to their soft and perishable nature and this problem is further exacerbated due to disease caused by *Botrytis cinerea* Pers. Better understanding of ethylene involvement in non-climacteric fruits is necessary. Phytohormones including ABA and auxins are known to have a significant role in the development and ripening of fruits. However, whilst much of the focus of postharvest biology and technology has been focussed on understanding the role and effects of ethylene and measures to control its influence, little work has been conducted on the role of other phytohormones. Moreover, these phytohormones have been shown to be influenced by exogenous ethylene and it is likely that a cross talk between ethylene and other phytohormones exists.

1.2 Aim and objectives

1.2.1 Aim:

The aim of this project was to elucidate the effects of controlling ethylene using e+® Ethylene Remover on the postharvest physiological and biochemical changes of selected climacteric and non-climacteric fruits.

1.2.2 Objectives:

Several objectives were defined for this research including:

- To investigate the effects of e+® Ethylene Remover treatment on the physiological and biochemical changes occurring during storage and ripening of climacteric fruits (avocados cv. Hass and pluots cv. Flavor King).
- To explore the efficacy of a newly developed e+® Ethylene Remover coated sheet format in the real world supply chain.
- To study the efficacy of the e+® Ethylene Remover treatment once applied during the early stages of ripening (before transit) and whether this can optimise its usage.
- To determine the effects of exogenous ethylene on the physiology and biochemistry of strawberries during storage.

- To study the effects of postharvest treatments (1-MCP and e+® Ethylene Remover) on strawberry fruit quality (including sugars, organic acids, phenolics and phytohormones) during storage.
- To determine the relationship between exogenous ethylene and the concentrations of phytohormones in different strawberry tissues.

1.3 Thesis structure:

This thesis is separated into ten chapters. The first section is a review of the relevant literature on the biochemical and physiological changes that are associated with fruit ripening. This is covered mainly in Chapter 2 of the thesis where a great deal of emphasis is given to the role of ethylene in initiating and regulating fruit ripening. Also highlighted in Chapter 2 is the current literature on the postharvest technologies available for delaying the postharvest life of some fresh produces. Chapter 3 reports on two separate experiments, whereby avocados were treated with or without e+® Ethylene Remover at source in Chile (early season avocados) and Peru (late season avocados). Here, the effect of the initial e+® Ethylene Remover treatment at source and additional postharvest treatment after transit period (5 weeks) with or without e+® Ethylene Remover was investigated (Chilean Exp 3.1 and Peruvian Exp 3.2). In addition, the use of a newly developed e+® Ethylene Remover coated sheet format was explored. Results from Chapter 3 have been presented as follows:

- **Poster: F. Elmi, M. D. Meyer, L. A. Terry.** Extension of avocado storability using e+® Ethylene Remover coated sheets in sea containers. Johnson Matthey Academic Conference (JMAC11), 6 - 7th April 2011. Loughborough University, UK.
- **Oral presentation:** V Postharvest Unlimited, 23-26th May 2011. Leavenworth, WA, USA.
- **F. Elmi, M. D. Meyer, L. A. Terry** (2011), "Extension of Avocado Storability Using e+® Ethylene Remover Coated Sheets in Sea Containers". *Acta Horticulture*. 45, pp.325-330.

In the fourth chapter, the efficacy of e+® Ethylene Remover coated sheet on the postharvest quality of pluots from South Africa was studied, while the ethylene

evolution of treated and untreated fruits was monitored continuously throughout ripening. A newly developed laser based photoacoustic ethylene detector (ETD-300) was used to monitor ethylene production overtime. Some research has also hypothesised that infections caused by *B. cinerea* can be correlated with elevated ethylene. In Chapter 5, an experiment was conducted to investigate any relationship between infections from *B. cinerea* and the concentration of ethylene produced by the fruits during storage. This Chapter also reported on the effect of e+® Ethylene Remover on the biochemistry and physiology of strawberries infected *in planta* with or without the pathogen during green I stage. Results from these chapters have been presented as follows:

- **Poster: F. Elmi., K. Cools., L. A. Terry**. The role of ethylene and e+® Ethylene Remover on *Botrytis cinerea* infected and non-infected strawberry fruits (cv. Elsanta) during ripening. Postgraduate Cranfield Health Conference, 16th September 2011. Cranfield, UK.
- **Poster: F. Elmi, M. D. Meyer, L. A. Terry.** The use of e+® Ethylene Remover as a tool for preserving postharvest fruit quality. Johnson Matthey Academic Conference (JMAC12), 27-28th March 2012. Loughborough University, UK.
- **Oral presentation: F. Elmi., K. Cools., L. A. Terry**. The use of It'sFresh! Ethylene Remover Technology with e+® active as a practical means for preserving postharvest fruit quality. 7th International Postharvest Symposium (IPS 2012), 25–29th June 2012. Kuala Lumpur, Malaysia.
- **Oral presentation: F. Elmi., K. Cools., L. A. Terry**. The use of It'sfresh! Ethylene Remover technology with e+® active as a practical means for preserving postharvest fruit quality. Postgraduate Cranfield Health Conference, 19th September 2012. Cranfield, UK.

Chapter 6 details the role of ethylene in strawberries, whereby the influence of exogenous ethylene, ethylene inhibition and removal were investigated. Phytohormones including abscisic acid (ABA) and auxins have been shown to play a role in the development of strawberries. In Chapter 6 and 7 the influence of ethylene, 1-MCP and e+® Ethylene Remover on ABA, ABA metabolite and auxins concentrations using UPLC QToF-MS was determined. In Chapter 7 the changes in these phytohormones in both flesh (receptacle tissue only) and achene (seeds) of strawberries in response to the

treatments and storage length was investigated. Some of the results in these chapters have been presented as follows:

- **Oral presentation: F. Elmi., M. D. Meyer., K. Cools., L. A. Terry**. Reducing retail and household fresh produce waste using e+® ethylene remover. Johnson Matthey Academic Conference (JMAC13), 16-17th April 2013. Loughborough University, UK.

The eighth chapter discusses and concludes previous chapters and provides future recommendations for further research. Chapter 9 includes literature cited and Chapter 10 presents the Appendices. Appendix A contains mainly the statistical ANOVA tables from the Genstat statistics output. In Appendix B the ETD-300 ethylene detector is introduced and the mechanisms by which the measurements were determined are discussed. A preliminary experiment conducted on kiwi fruits (cv. Hayward) in The Netherlands using the Ethylene detector is reported. Appendix C shows some images of the analytical instruments used to extract important biochemical components. Appendix D details some knowledge transfer activities during this project including oral and poster presentations.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Fruit ripening

Fruits are commercially and nutritionally valuable due to their rich levels of nutrients including sugars, organic acids, vitamins and secondary metabolites. Botanically, fruits are a highly diverse species ranging from dry fruits such as nuts and grains to fleshy fruits that have highly complex flavours, textures, colours and aromas (Valero and Serrano, 2010). Fruit ripening is a combination of anabolic and catabolic processes, involving a series of molecular, biochemical as well as physiological changes. The biochemical transition leads to the synthesis of new pigments and flavour compounds. Consequently, ripening causes the mature plant organ to become much more appealing in terms of flavour and appearance to attract for seed dispersion. Fruit development is characterised into three main stages; growth, maturation and senescence. The timing of these stages will differ with each commodity or fruit. The growth stage is marked by significant and rapid cell division resulting in enlargement of tissues. Typical activities in the maturation stage will depend on the commodity; however, this stage refers to the processes that gives way to ripening and in some cases maturation may overlap with ripening. Senescence on the other hand, is a degenerative process which is accompanied by increasing anabolic (synthesis) and biochemical reactions that trigger catabolic activities. The increased anabolic and catabolic activities coincide with breakdown of macromolecular and sub-sequential death of tissues. Distinct biochemical and physiological changes will vary among different species however, main changes include altered sugar metabolism, loss of firmness, colour changes, production of aroma and increased susceptibility to pathogenic infections (Wills *et al.*, 2007; Valero and Serrano, 2010).

2.1.1 Climacteric vs. non-climacteric

The level of ethylene and carbon dioxide (CO_2) within a fruit is cardinal in determining the mechanism by which fruit ripen. Fresh produce are believed to exhibit either of two

major mechanisms of ripening; climacteric and non-climacteric. This categorisation of the fruits depends on whether the fruit displays an increase in respiration and ethylene production during the ripening period. Ethylene is highly important for the ripening events in climacteric fruits; both for coordination and completion (Giovannoni, 2001). These fruits can be ripened after harvest and will ripen most rapidly during postharvest storage. As such, climacteric fruits are often harvested in a mature state and are ripened postharvest. However, fruits harvested at an immature state can develop poor flavour and appearance once ripe. That said; over mature fruits once harvested have a limited shelf life and quality deteriorates more rapidly. Harvesting of fruits at the right stage of maturity is therefore highly important. The postharvest stages of non-climacteric fruits is not identified as ripening (Thompson, 2003).

Fruits such as tomato, avocado, banana, peaches, and apples present the characteristic traits of climacteric fruits, including rise in respiration and concurrent burst of ethylene production at the onset of ripening. In climacteric fruits, this surge of ethylene production is necessary for normal ripening to eventuate (Thompson, 2003; Valero and Serrano, 2010). In comparison, non-climacteric fruits demonstrate a progressive decrease in respiration and ethylene as the fruit develops from an immature state to full maturity (Seymour *et al.,* 1993; Burg, 2004). Fruits including sweet cherry, citrus fruits and pepper are classified as non-climacteric and show a gradual decrease in respiration and ethylene production during ripening (Goldschmidt, 1997; Lelièvre *et al.*, 1997; Barry and Giovannoni, 2007). The differences in this endogenous ethylene production between climacteric and non-climacteric fruits is seen to result from the existence of two ethylene biosynthetic pathways (system 1 and system 2), whereby climacteric fruits are thought to exhibit both. System 1, which is prominent in both climacteric and non-climacteric fruits, is associated with low ethylene production. System 2, also referred to as 'autocatalytic synthesis', is considered to be exclusive for climacteric fruits. The increase in ethylene observed in climacteric fruits during ripening is more significant to initiate the changes in the colour, aromas, texture, flavour and other biochemical and physiological changes. The low level of endogenous ethylene in non-climacteric fruits means their postharvest developments are limited, but are not insubstantial. Non-climacteric fruits show the ripening symptoms (increase in respiration & ethylene production) in response to exogenous ethylene, however once the ethylene treatment is

discontinued their respiration activities reverts to normal (Goldschmidt, 1997; Giovannoni, 2001). Exogenous ethylene dosage as low as 0.1-1.0 µl l^{-1} can be used to trigger ripening in climacteric fruits. Nonetheless, continuous ethylene throughout storage of onions and potatoes (non-climacteric vegetables) is an effective method of inhibiting sprout growth (Foukaraki *et al.*, 2010; Cools *et al.*, 2011).

A low level of ethylene, around 0.05 µl l^{-1}, is prominent in most plant tissues, but the peak level of ethylene will differ for different fruits (Wills *et al.*, 2007). Thus, the control of ethylene for non-climacteric and climacteric fruits will also differ. Research defining the regulatory mechanisms underlying the biochemical changes in non-climacteric fruits is lacking in the literature. Although non-climacteric fruits also synthesise ethylene in response to external stimul such as stress factors, physical injuries or pathogen infections, this ethylene is considered insignificant for their postharvest quality (Saltveit, 1999). The effect of ethylene on various commodities has been reported but the differences in the threshold level for individual fruits and the dose response (time x concentration) is often less reported.

Some debate is still centred on the role of ethylene in non-climacteric fruits. Chervin *et al.* (2004b) has documented that in grape fruit (non-climacteric) the ethylene biosynthesis is associated with an increase in berry diameter, decrease in berry acidity and anthocyanin accumulation. However, research has revealed that the response of non-climacteric fruits to ethylene is less consistent and uniform. There is still some debate with the categorisation of some fruits such as raspberry and strawberry as climacteric or non-climacteric (Burdon and Sexton, 1990; Iannetta *et al.*, 2006). Burdon and Sexton (1990) have shown red raspberry fruits *(Rubus idaeus* L. cv. Glen Clova) exhibit climacteric ethylene production. Ethylene production increased significantly as the fruit matured from the green stage to red ripe. Similarly, Iannetta *et al.* (2006) demonstrated similar findings with strawberry fruit. Ethylene production of strawberries in *planta* increased during development with a concurrent rise in respiration at red ripe stage. On the other hand, non-climacteric horticultural products including onions have consistently low ethylene production (<0.1 µl kg^{-1} h^{-1} at 0–5 ^{o}C) (Downes *et al.*, 2010) although these have been shown to respond to ethylene and 1-methylcyclopropene treatment (1-MCP), an ethylene action inhibitor (Cools *et al.*, 2011).

Since the role of ethylene is connected with the initiation and coordination of ripening in climacteric fruits, commercial strategies for horticultural products aim to minimise the exposure and production of ethylene during transportation and storage. Whilst it is imperative to minimise ethylene during ripening, harvest, storage and transport of the commodity, the control of temperature during handling as well as the atmosphere surrounding the commodity should also be considered.

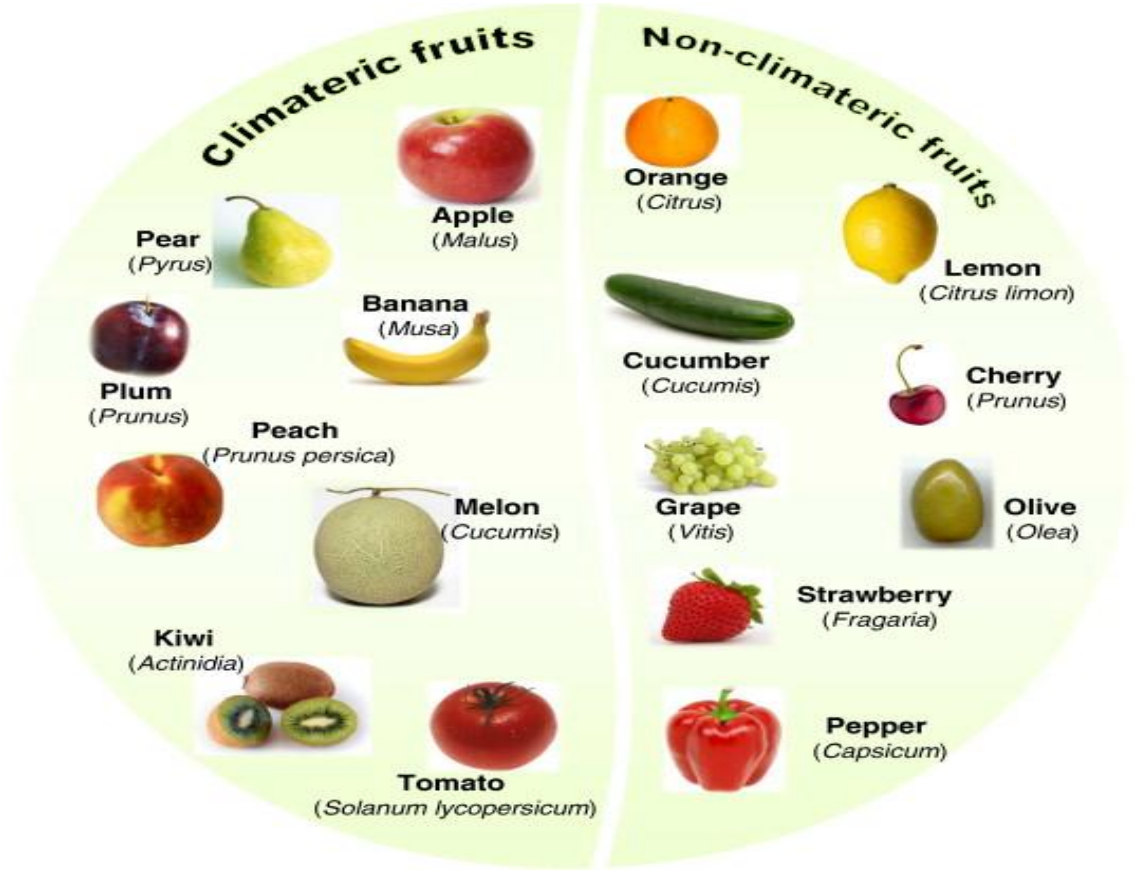


Figure 2.1. Some climacteric and non-climacteric fruits (Palma *et al.*, 2011).

2.2 Changes during ripening

Fruits may undergo distinct alteration in their chemical composition as well as appearance which directly correlates with ripening (Shewfelt, 2009)**.** The ripening process renders the fruits more attractive and palatable for consumption. The nature of ripening will vary depending on the fruit; however, the process usually features changes

in the appearance, texture, taste and flavour of the fruit. Fruit ripening is characterised by the loss of firmness due to changes in the ultra-structure of cell wall caused by degrading activities, changes in taste (hydrolysis of starch to sugars and a decline in organic acids), development of aroma (due to production of volatile compounds) and a decline in natural disease resistance. Along with these events there are concurrent changes in the type and numbers of metabolites present (Seymour *et al.,* 1993; Giovannoni, 2001).

In non-climacteric fruits the majority of the compositional changes take place during the course of development and maturation of the commodity while it is still attached to the plant. The burst of ethylene during the onset of ripening is responsible for the majority of the compositional changes occurring in climacteric fruits. These changes can continue to persist after harvest though it can be undesirable as fruits become increasingly susceptible to pathogens. Non-climacteric fruits such as citrus, grapes and strawberry do not seem to require ethylene for the ripening process. However, exogenous ethylene has been shown to influence their postharvest qualities (Valero and Serrano, 2010).

2.2.1 Colour development

Maturity of a commodity is accompanied by a colour change, in most fruits this is used as a maturity index. The change in colour will affect the peel and pulp tissues and is usually an important indicator of fruit eating quality. Accurate determination is carried out through the use of electronic and optic devices i.e., colorimeter which permits the determination of objective colour. There are three basic parameters used to describe colour: i) **hue (H^0)** reflects the distinct colour and corresponds to the dominant wavelength frequency; ii) **lightness (L*)** is a measure of the amount of light reflected and is an indicator of the brightness of the colour, and iii) **chroma (C*)** refers to the saturation of the colour. Hue is usually the most important parameter in the perception of fruit quality (Kays and Paull, 2004).

The external colour transformation of fruits is usually caused by decrease of chlorophyll pigments. Many climacteric fruits exhibit rapid loss of chlorophyll pigments during ripening. Similarly, non-climacteric fruits will show loss of green colour, for example

citrus fruit growing in temperate climates. Citrus peel respond to exogenous ethylene which is a phenomenon used for the degreening of citrus fruit. The decrease in the green colour is caused by the breakdown of the chlorophyll structure (Wills *et al.,* 2007). The disintegration of chlorophyll is associated with the synthesis and or the revealing of other pigments ranging from yellow to red. In many fruits these pigments are carotenoids composed of long unsaturated hydrocarbons often coupled to an oxygen atom. Carotenoids are usually synthesised during the developmental stage, however, these are masked by the chlorophyll pigments and only become visible once the chlorophyll degrades. In some fruits such as tomatoes, synthesis of lycopene, a carotenoid, occurs simultaneously with the loss of chlorophyll. The red, blue and purple colour of some fruits and vegetables are due to the presence of anthocyanins. Anthocyanins are water soluble pigments located in the vacuole and responsible for strong colours capable of masking the carotenoid and chlorophyll present. In avocados, the main anthocyanin responsible for the characteristic red to purple colour development is cyanidin-3-O-glucoside; on the other hand in apples this is believed to be cyanidin-3-galactoside (idaein) (Aaby *et al.*, 2005; Ashton *et al.*, 2006). Anthocyanin content generally increases during ripening in a range of climacteric and non-climacteric fruits, though variation exists in the total anthocyanin levels at commercial harvest between species and cultivars (Valero and Serrano, 2010). Fruit colour may result from a combination of several anthocyanins as well as the environmental condition such as the pH and other ions present in the vacuole (Paliyath *et al.,* 2008). Compositional changes and development of fruits characteristic colour is also associated with synthesis of other pigments including carotenes and xanthophylls (Seymour *et al.,* 1993).

2.2.2 Cell wall and texture

Changes in the texture of fruits and vegetables during ripening are mainly due to loss of tugor, starch degradation, but mostly due to the degradation of cell wall. Firmness is a primary feature that attributes to the quality of fruits and vegetables. Firmness is usually determined using destructive puncture test including handheld Effegi or with an instron machine. These techniques give an indication of firmness by providing the force required to penetrate a probe into the product at a specified distance (Shewfelt, 2009).

Ripening is associated with cell wall disassembly; however, this differs from that occurring during abscission. Whilst the latter involves reversible alteration of the walls and considerable biosynthesis, the former, involves irreversible but tightly regulated processes leading to reduced cell wall constituents. Both processes will show some features which are common to both (Rose and Bennett, 1999). In climacteric fruits over-ripening can develop within a few days. This will involve excessive softening and changes in taste, aroma and skin colour leading to fruit quality being lost (Bapat *et al.*, 2010). The ripening and softening of climacteric fruits are important features that contribute to perishability (Reid, 2004). Although this process is unavoidable, it can be delayed by several methods (Wills *et al.*, 2007), which are discussed in section 2.5. The loss of turgor occurs due to the breakdown of complex polymeric carbohydrates. The breakdown of hemicelluloses and pectin polymers leads to weakening of cell walls. Over 50 % of the total carbohydrates present in the cell wall of fruits is pectin and thus its degradation is associated with tissue softening (Kader, 2002). The increase in softening is due to cleavage of pectic chains by pectic enzymes which increases their solubility. Alteration and modification in the cell wall structure results from the release of these enzymes ithe three main pectic enzymes are pectinesterase, endopolygalacturonase and exopolygalacturonase. These are found in a wide variety of fruits (Huber, 1983; Huysamer *et al.*, 1997; Karakurt and Huber, 2004), but not all.

2.2.3 Non-structural carbohydrates

The taste of the fruit is heavily influenced by the quantity and type of sugars, phenolics as well as organic acids present. After water, the most abundant constituents in fruits and vegetables are the carbohydrates, accounting for 50-80 % of the total dry weight. Carbohydrates serve not only as an energy reserve, but additionally they make-up the structural framework of cells. The most prominent sugars in fruits and vegetables are the simple sugars; glucose and fructose. These sugars are the products of photosynthesis and impart important postharvest qualities. When one molecule of glucose and fructose combine, sucrose, a disaccharide sugar, is synthesised by sucrose synthase. The reaction is also reversible, where glucose and fructose are generated from sucrose; a reaction catalysed by sucrose invertase. Glucose, fructose and sucrose are water soluble and together they are responsible for the sweet taste of most fruits and vegetables. The

percentage of fructose, glucose and sucrose are important in the overall taste since fructose is the sweetest of the sugars 80 % sweeter than sucrose while glucose is 60 % sweeter than sucrose. In fruits such as avocados, the main carbohydrates are the seven carbon sugars mannoheptulose and perseitol. These sugars are found in only a few plants in nature, but large amounts have been reported in avocados (Liu *et al.*, 1999a; Landahl *et al.*, 2009). In avocados, glucose, fructose and sucrose are found though the concentrations are much less compared to the levels of mannoheptulose and perseitol (Meyer and Terry, 2010). However, the concentrations of sugars reported in fruits vary depending on their harvest date, origin and maturity (Landahl *et al.*, 2009). Liu *et al.* (2002) reported lower concentrations of C7 sugars (mannoheptulose and perseitol) in comparison to Landahl *et al.* (2009). Differences between soluble sugar results may be as a result of different extraction methods with each author using a different solvent to extract. Davis *et al.* (2007) compared methods of soluble sugar extractions and determined that methanol/water based solutions (O'Donoghue *et al.*, 2004) were more efficient at extracting monosaccharide sugars from onions than ethanol/water based mixtures. Their relative abundance will vary depending on the fresh produce, but will also vary depending on maturity. Fruits such as plum, nectarine and peach accumulate sucrose during ripening due to a rise in sucrose synthase activity. On the other hand, table grapes accumulate more glucose because sucrose is hydrolysed to glucose and fructose (Kays and Paull, 2004; Valero and Serrano, 2010).

2.2.4 Organic acids

Organic acids play a central role in the taste of many fruits and vegetables. Organic acids are also directly involved in the growth, maturation and senescence of fresh produce, and represent a source of stored energy that can be utilised during postharvest processes. These are small molecules and their acidic properties are due to the presences of carboxyl groups (COOH). The organic acids found in fresh produce will vary depending on the product and there are many types present in a single fresh produce however, malic, citric and tartaric acids are usually found in abundance in most fruits. Each acid represents a unique taste that contributes to the overall taste and flavour of the fruit. The concentrations of these acids will vary widely amongst different commodities, for example 70-75 mg of citric acid is found in 100 gfresh weight (FW) in lemon, in

bananas only 4 mg of malic acid is found in 100 gFW. However, the acidity in lemon is attributed to the high concentrations of citric acid, while increased acidity from malic acid would be undesirable in the taste of bananas (Kays and Paull, 2004). Malic acid is the major acid found in stone fruits and levels are particularly high during the growth period but decrease following maturation and ripening (Valero and Serrano, 2010).

Ascorbic acid, also known as vitamin C, is the second most abundant acid found in citrus fruit. Although, the ascorbic acid varies amongst fresh produce, fresh fruits and vegetables have the highest ascorbic acid. Along with citrus, strawberries also have a great reputation for their high ascorbic acid content (Pérez *et al.*, 1997). Concentrations of organic acids relating to different perspectives, such as the stage of fruit development and the storage condition have been investigated for various fresh produce (Alique and Oliveira, 1994; Holcroft and Kader, 1999; Suni *et al.*, 2000). The total organic acids tends to decrease during storage, however a number of other parameters need to be considered when determining the concentrations.

2.2.5 Phenolic compounds

Phenolic compounds are one of the main secondary metabolites in plants. These compounds are diverse in their structure and occurrence, though they are derived from the aromatic amino acids phenylalanine and tyrosine generated from the shikimate pathway (Tomás-Barberán *et al.*, 2001). Core structures from the shikimate pathway are used in the phenylpropanoid metabolism to generate a range of phenolic compounds. In the first step of the phenylpropanoid pathway, the aromatic amino acid phenylalanine is deaminated to *trans*-cinnamic acid by the enzyme phenylalanine ammonia-lyase (PAL) (Figure 2.2). This step is regarded as the main step in the biosynthesis of various phenylpropanoids. Subsequently, cinnamic acid is hydroxylated by cinnamic acid 4-hydroxylase to produce *p*-coumaric acid. In some species such as maize, this compound can also result from the deamination of tyrosine. The *p*-coumaric acid-derived secondary metabolites such as flavonoids, phenolic acids, coumarins, lignins and other phenolic compounds are characteristic for each plant species (Pereira *et al.*, 2009; Vogt, 2010).

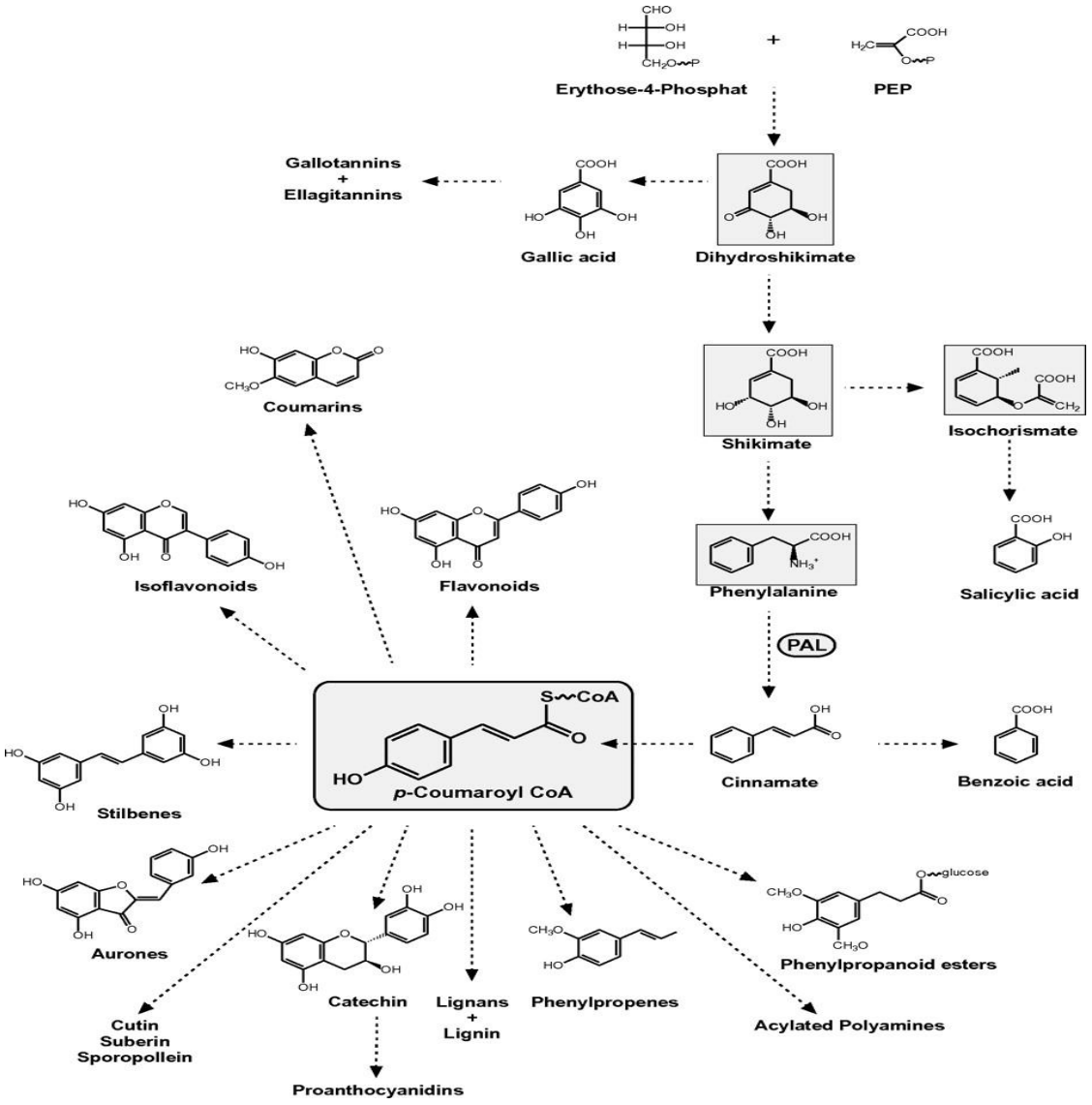


Figure 2.2. Diversification of phenylpropanoids based on the general phenylpropanoid pathway. The metabolites of the shikimate pathway and the central metabolite, 4-coumaroyl CoA, are shaded in grey. PAL (Phenylalanine ammonia lyase) is the enzyme that catalyses the formation of cinnamate (from Vogt, 2010).

The biological function of phenolic compounds includes attracting seed disseminators, protecting against UV damage, and contributing to the bitter and astringent taste in some products. These compounds are very beneficial once ingested, as they act as antioxidants along with ascorbic acid and other naturally occurring compounds (Tomás-Barberán *et al.*, 2001; Vicente *et al.*, 2009). There is an overwhelming research evidence confirming that diets rich in fruits and vegetables can protect against oxidative

damage from free radicals (molecules having unpaired electrons) (Wang *et al.*, 1996; Vicente *et al.*, 2009). Wang *et al.* (1996) compared the oxygen radical absorbance capacity (ORAC) of 12 different fruits and found that strawberry had the highest ORAC activity; plum and orange fruits also showed high ORAC. There is increasing evidence suggesting that antioxidants can reduce incidences of degenerative disease such as cardiovascular disease and certain cancers (Chen and Ho, 1997; Hollman and Katan, 1999). Berry fruits are particularly rich in these compounds and have received immense research interest (Kähkönen *et al.*, 1999; Kähkönen *et al.*, 2001; Lopes Da Silva *et al.*, 2007). There are large numbers of phenolic compounds present in plants; these can be further sub-divided into different classes such as phenolic acids, flavonoids and other compounds (Vicente *et al.*, 2009).

2.2.5.1 Phenolic acids

Phenolic acids are derived from either cinnamic acid or benzoic acid. Benzoic is formed by the cleavage of two carbons from the three carbon side chain in cinnamic acid (Figure 2.3). Derivatives of benzoic acid include *p*-hydrobenzoic acid, syringic acid and vanillic acid while *p*-coumaric, caffeic, sinapic and ferulic acid are derived from cinnamic acid (Natella *et al.*, 1999; Pereira *et al.*, 2009). In berries, the most abundant phenolic acid is caffeic acid. The antioxidant capacity of a phenolic compound is dependent on the number of hydroxyl groups present, where more hydroxyl groups mean increase in their antioxidant capacity.

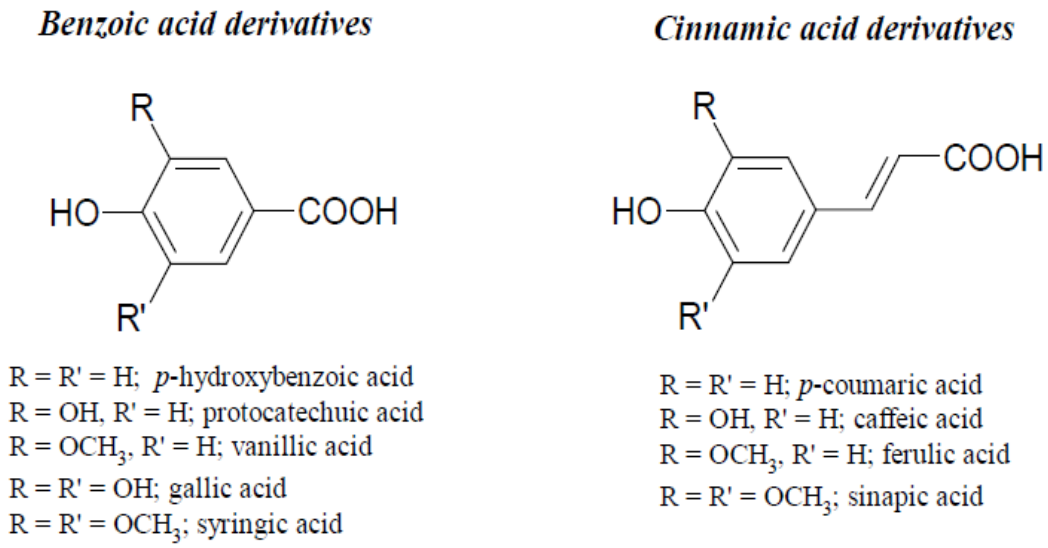


Figure 2.3. The chemical structures of phenolic acids, derivatives of cinnamic and benzoic acids (from Pereira *et al.*, 2009).

2.2.5.2 Flavonoids

Flavonoids are composed of two aromatic rings that are connected together by a 3C-oxygenated heterocycle. Flavonoids are one of the most abundant amongst the phenolic compounds. Flavonoids are usually bound to sugars as β-glycosides. It has been suggested that in the glycoside form they are non-absorbable; however, Hollman and Katan (1999) have shown that flavonoid found in onions (quercetin) was better adsorbed by humans in the glycoside form (52 %) than in the pure aglycone form (24 %). Flavonoids can be further divided into different sub-classes:

a) **Flavones and flavonols:** The flavonols have 3-hydroxypyran-4-one structure as their central ring, while flavones do not have a hydroxyl group on position 3. Flavones are the most abundant flavonoids in food. Blueberries are also rich source of flavonols and high concentrations are found in their skin since their synthesis is stimulated by light. Quercetin and Kaempferol are the main flavonols, while the most frequent flavones are luteolin and apigenin (Bravo, 1998; Vicente *et al.*, 2009).

b) **Flavanones and flavanols:** Flavanones have an aromatic ring lacking a double bond in position 2 and 3, while flavanols have no carbonyl group at position 4.

c) Anthocyanins: Anthocyanins are the pigments giving fruits their characteristic red/purple colour (see section 2.2.1). Anthocyanins are also important compounds that contribute to the antioxidant capacity of fruits and vegetables. There are six common anthocyanins found in fruits and vegetables; pelargonidin, cyanidin, delphynidin, peonidyn, petunydin and malvidin. The differences between them are the OH, H and OCH_3 functional groups attached to the aromatic ring. The hydroxyl group influences their antioxidant capacity. Anthocyanins are glycosides of anthocyanidins (Bravo, 1998; Vicente *et al.*, 2009). Hernanz *et al.* (2007) found 12 different phenolic compounds, including pelargonidin and cyanidin-3-O-glucoside which are the major anthocyanins present in strawberries**.**

There are a group of phenolics that are not synthesised via the phenylpropanoid pathway but instead directly from the shikimate pathway via the 5-dehydroshikimate precursor. Fruits of the Rosaceae family such as strawberries are known to be a rich source of phenylpropanoid derived phenolic compounds including anthocyanins, flavonols and phenolic acids, but in addition they contain significant amounts of ellagic acids and ellagitannins that are from the shikimate pathway (Hanhineva and Aharoni, 2010).

2.3 Plant growth regulators

Plant growth regulators are chemicals that are produced naturally by plants and can also be produced synthetically. Plant phytohormones include ethylene, abscisic acid (ABA), auxins, cytokinins, gibberellins, brassinosteroids and strigolactones. These hormones like ethylene play a significant role in many aspects of plant development. In particular, ABA has been widely studied and appears to influence numerous aspects of plant life cycle. Some studies have been conducted investigating the role of ABA on fruit ripening, but it is less considered when compared to ethylene. That said, ABA has also been associated with various developmental processes such as germination, dormancy, abscission and stomata regulation. Recent studies have elucidated that exposing strawberry plants to conditions such as drought, salinity and disease results in enhanced

levels of ABA (Xiong and Zhu, 2003; Terry *et al.*, 2007a). ABA treatment has been shown to enhance ethylene production in strawberries (Jiang *et al.*, 2000); however, the mechanism by which ABA increases ethylene biosynthesis has not been elucidated. Archbold and Dennis (1984) showed that ABA levels gradually decreased in strawberries during maturity. That said, the findings that ABA plays a role in strawberry fruit maturity are yet to be determined. In avocados, application of ABA was reported to accelerate ethylene biosynthesis leading to advanced ripening (Blakey and Bower, 2009). However, the mechanisms by which ABA advances fruit ripening are unknown and the role of this hormone in both climacteric and non-climacteric fruits requires more research. Moreover, although the role of ABA on the ripening of fruits has been investigated there is little information on the changes of this hormone during fruit senescence. The ABA biosynthetic pathway has been elucidated through the isolation of ABA deficient mutants.

The synthesis of ABA begins with the cleavage of C_{40} carotenoids from the methylerythritol 4-phosphate (MEP) pathway. The evidence that ABA is synthesised by carotenoids was revealed through molecular genetic analysis of auxotrophs (reviewed in Nambara and Marion-Poll, 2005). The synthesis starts in the plastid where a C_{40} carotenoid precursor undergoes hydroxylation. The next step is the epoxidation of the zeaxanthin to violaxanthin which occurs in the plastid. The conversion of zeaxanthin to violaxanthin is catalysed by zeaxanthin epoxidase (ZEP). Violaxanthin is converted to 9′-*cis*-neoxanthin; a 9-*cis*-epoxycarotenoid. The neoxanthin undergoes oxidative cleavage by 9-cis-epoxycarotenoid dioxygenase (NCED) to produce the C_{15} intermediate; C_{15} xanthoxin. The biologically active form of ABA is produced from the C_{15} xanthoxin through two enzymatic steps. The xanthoxin is firstly transported to the cytosol and is converted to ABA-aldehyde, a process catalysed by short-chain dehydrogenase/reductase (SDR). In the final stage of ABA biosynthesis ABA- aldehyde is oxidised by ABA-aldehyde oxidase (AAO) (Figure 2.4). The rate limiting step in the ABA biosynthesis pathway is the NCED step and has received particular interest (Seo and Koshiba, 2002; Nambara and Marion-Poll, 2003; Xiong and Zhu, 2003; Nambara and Marion-Poll, 2005). ABA catabolism occurs via oxidative and conjugative processes. ABA is oxidised through hydroxylation of the 8′-methyl group to produce 8′-hydroxy ABA (8′-OH ABA), which reversibly rearranges to a more stable catabolite

phaseic acid (PA). The PA is then reduced to give dihydrophaseic acid (DPA), an inactive ABA catabolite (Figure 2.4) (Nambara and Marion-Poll, 2003). Minor hydroxylation of the 7′-methyl group can occur to produce 7′-hydroxy ABA, a compound having similar hormonal activity as ABA. ABA and its catabolites can conjugate with glucose resulting in ABA glucosyl ester (ABA-GE) (Nambara and Marion-Poll, 2005; Zaharia *et al.*, 2005; Kepka *et al.*, 2011).

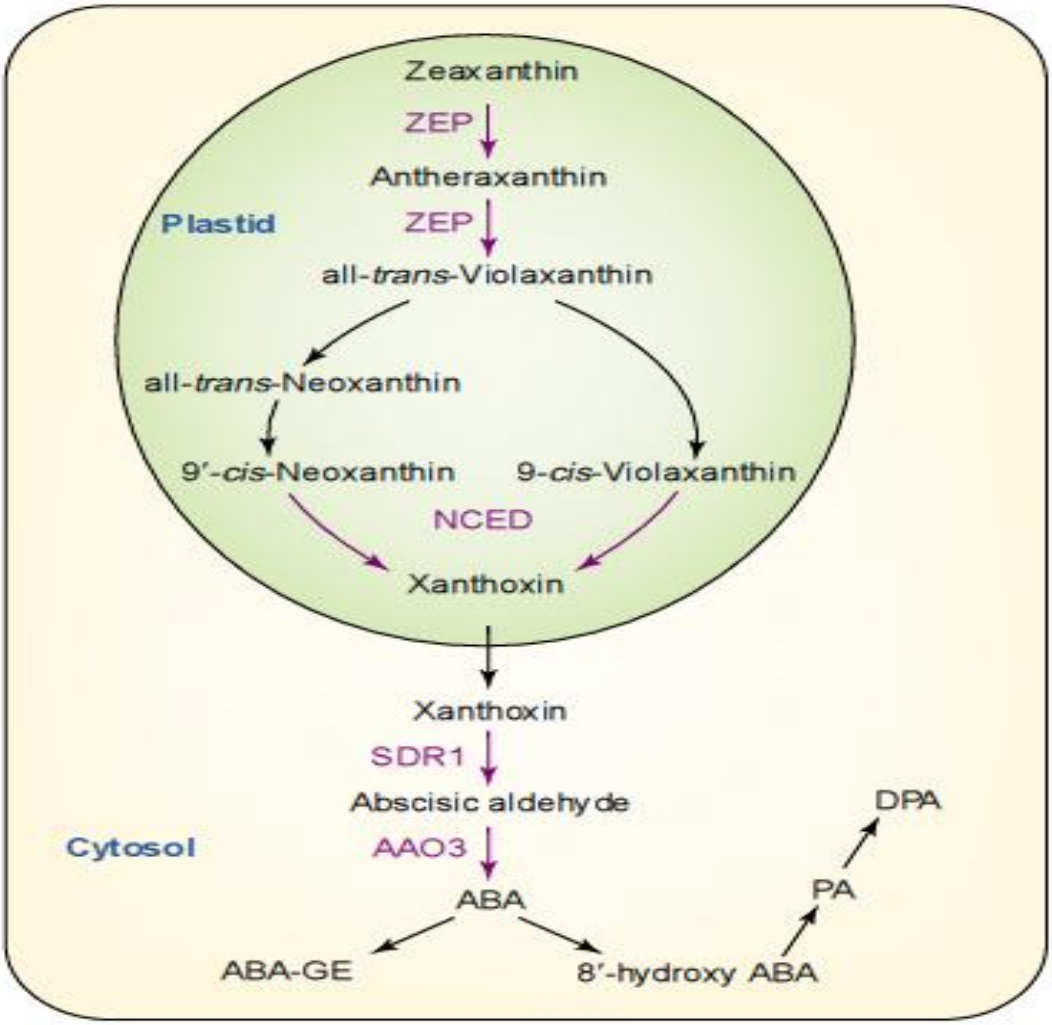


Figure 2.4. Abscisic acid (ABA) biosynthesis pathway and main catabolic route (from Nambara and Marion-Poll, 2003).

Auxins are necessary for the growth and expansion of the receptacles in strawberries. At early stages of fruit development auxins produced in the achenes stimulate fruit growth and development in strawberries (Aharoni *et al.*, 2002). The levels of auxins are highest in the receptacle and achenes just before the white stage and decline with maturity. Veluthambi and Poovaiah (1984) demonstrated that the application of auxins on strawberries at the middle green stage exhibited a delay on the firmness and colour development, hence the importance of auxins at the early stages of fruit development. Strawberry fruit ripening has been shown to be negatively correlated with auxins (Given *et al.*, 1988; Manning, 1994). Archbold and Dennis (1984) elucidated that auxins levels decline gradually with fruit maturity. In support of this Given *et al.* (1988) showed that reduced auxins levels triggers strawberry fruit ripening. However, there is much to be learnt about the mechanism in which auxins regulate strawberry ripening.

Gibberellins (GAs) are diterpenoid compounds; some of these compounds are plant growth regulators which are involved in processes such as germination, stem elongation and flowering in many species (García-Martínez *et al.*, 1997). Many different GAs have been identified however, of these, only a few are biologically active (Csukasi *et al.*, 2011). GAs are named in order of discovery from GA_1 through to GAn. GA_3 was the first GA to be structurally characterised. There are currently more than one hundred GAs identified in plants, fungi and bacteria. These bioactive compounds have been reported in strawberries (Villarreal *et al.*, 2009; Csukasi *et al.*, 2011). Csukasi *et al.* (2011) correlated the enlargement of the receptacles with external application of GAs. Serrani *et al.* (2007) investigated the involvement of GAs in tomato fruit and reported that inhibitors of GA biosynthesis resulted in decreased fruit development while the opposite was observed by GA_3 application. However, it is unclear how gibberellins promote fruit growth.

Like many other phytohormones, cytokinins have been identified as another component capable of promoting fruit development. Cell division and fruit growth were reported to be promoted by cytokinins. Lewis *et al.* (1996) linked fruit growth with the change in cytokinin content and proposed that increase in zeatin 10 days after anthesis correlated with fruit growth. Lewis also showed that treatment of kiwis with synthetic cytokinin (N-(2-chloro-4-pyridyl)-N'penylurea) (CPPU) enhanced fruit development. Synthetic

cytokinin CPPU has been shown to have cytokinin like activity as that of the true cytokinin zeatin (Curry and Greene, 1993). CPPU was used to influence the length to diameter ratio of apple hence a role of this hormone in fruit growth. In an earlier study on strawberry Kano *et al*. (1981) postulated that maturing of strawberries are accelerated by the rising of ABA and lowering of cytokinin.

2.4 Role of ethylene in ripening

2.4.1 Exogenous and endogenous ethylene

The simplest alkene ethylene is also a gaseous plant growth hormone, which diffuses into and out of plant tissues. This phytohormone influences the physiological processes of plants, including ripening and senescence. Ethylene is produced naturally by plant tissues as a product of plant metabolism and is therefore crucial in the initiation of ripening (Abeles *et al.,* 1992; Wang *et al.,* 2002). Exposure of ethylene can be via endogenous (i.e., biological) or exogenous (i.e., non-biological and biological) sources. Ethylene exposure or its application can have both advantageous and disadvantageous effects on plants (Table 2.1). Some beneficial effects of ethylene application listed include the promotion or acceleration of flowering in pineapple (*Ananas comosus* L.) as well as the initiation of ripening in tomato (*Solanum lycopersicum* L.). In fact ethylene treatment is commercially used for some fruits including banana and pear to promote uniform ripening. The effects, which are seen as deleterious, include the development of russet spotting in lettuce (*Lactuca sativa* L.) and excessive softening in some fruits (Saltveit, 1999; Wills *et al.,* 2007).

Table 2.1. Beneficial and detrimental effects of Ethylene on the quality of fresh fruits and vegetables (adapted from Saltveit, 1999).

Beneficial effects of ethylene
Promotes colour development in fruit.
Stimulates ripening of climacteric fruit.
Promotes de-greening of citrus.
Alters sex expression in the cucurbitaceae.
Promotes flowering in bromeliaceae (e.g. Pineapple).
Inhibition of sprouting in onions and potatoes
Detrimental effect of ethylene
Accelerates senescence.
Stimulates chlorophyll loss (e.g. yellowing).
Enhances excessive softening of fruits.
Promotes abscission of leaves and flowers.
Promotes discoloration (e.g. browning).
Hastens toughening of vegetables.

The production of endogenous ethylene is an important feature of climacteric fruits. The production as well as the response of different plants to ethylene will vary. Ethylene production, though highest during the ripening stage of development, is also enhanced in diseased or wounded tissues, and is seen to be more significant to affect nearby tissues, since ethylene exposure can occur not only from storage, transportation or atmospheric composition, but also by a neighbouring crop (Saltveit, 1999). A concentration level of 0.1 µl l^{-1} is stated as the threshold and low levels (nl-µl l^{-1}) are capable of exerting ethylene related responses (Kader, 1985; Wills *et al.*, 2001). Concentrations exceeding above the threshold (0.1 µl l^{-1}) can lead to significant losses due to induced ripening and resultant susceptibility to physical injuries, which in turn reduces storage and shelf-life. Research has demonstrated the relationship between storage life and exogenous ethylene concentration. Ethylene level exceeding 0.1-1 µl l^{-1} was capable of inducing significant losses and reducing storage and shelf life (Wills and Warton, 2004). The deleterious effects of the hormone are temperature dependent with sensitivity increasing with increased temperature (Wills *et al.*, 2001).

Vegetative tissues are less susceptible to stress induced ethylene and stress accounts for only a temporary increase in ethylene production, causing minimal effects. On the other hand, stress induced in climacteric fruits causes more pronounced and prolonged effects to the fruit tissues. For example, promoting ethylene production through chilling of pears (*Pyrus communis* L.) and wounding of figs is a commercial method used to trigger ripening (Saltveit, 1999). Although the ripening of non-climacteric fruits is thought to be ethylene independent, ethylene can have both negative and positive impact on these fruits and vegetables. Ethylene results in increased susceptibility to pathogen infections, physiological injuries, and senescence, leading to short postharvest life (Wills *et al.*, 2007). However, exogenous application of ethylene on onions (*Allium cepa* L.*)* during storage has been reported to suppress incidences of sprouting (Downes *et al.*, 2010). In accordance to this, similar finding were observed for potatoes whereby 4 µl l^{-1} of ethylene inhibited sprouting for 23-33 weeks of storage (Prange *et al.*, 2005). That said, some research has correlated the presence of ethylene with enhanced features related to ripening in some non-climacteric fruits including strawberries (Tian *et al.*, 2000) and grapes (Chervin *et al.*, 2004a). Studying the involvement of ethylene in the storage of non-climacteric fruits is important due to their high economic value. Recent literature has reported the impact of ethylene on the overall quality of strawberries. Below is a summary table showing research that has looked at the impact of ethylene on postharvest development of different strawberry cultivars (Table 2.2). As indicated in Table 2.2, strawberry fruit quality can be affected by ethylene during storage. It is therefore possible that non-climacteric fruits are not necessarily ethylene independent, but that there are common regulatory cascades operating in all fruits (White, 2002).

Table 2.2. Effect of ethylene on different strawberry cultivars (*Fragaria x ananassa* Duch).

Cultivar	Concentration (µl l^{-1})	Duration	Storage condition	Effects	Reference
Aiko	20	Shelf-life	CA. 21 d at 0.5 ˚C + 2 d at 20 ˚C *B.Cinerea* inoculation	▲Fruit decay, disease development, respiration rate ▼Firmness	El-Kazzaz *et al.* (1983)
G-3 / G-4	20	Shelf-life	21 d at 3.3 ˚C *B. cinerea* inoculation	▲Fruit decay, disease development ▼Firmness	
NS	10-100	12 h	-	▲Total RNA levels, poly(A) +RNA levels and RNase activity	Luo and Liu (1994a)
NS	10, 50 and 100	5 d	15 ˚C	▲Ripening, high molecular weight RNA and poly(A)+ RNA, RNase activity and in vitro protein synthesis activity	Luo and Liu (1994b)
Torrey/ Red Gaunlet	0.005, 0.01, 0.05, 0.1, 0.5 and 1	NS	0 and 20 ˚C	▲Storage life with low levels ▼Ethylene production	Wills and Kim (1995)
NS	0.005-10	NS	0 and 20 ˚C. Ventilated	▲Shelf-life linearly extended with low levels	Wills (1998)
Pajaro	0.5, 1, 5, 20, 50, 100	Continuous flow	20 ˚C	▲Colour development, softening, Respiration rate	Tian *et al.* (2000)
Chandler	100		3 d at 20 ˚C. 95 % RH	▲ACC oxidase activity, short ethylene production ▼ACC	Atta-Aly *et al.* (2000)
NS	0.01, 0.05, 0.1 and 1	Continuous flow	0 or 5 ˚C during shelf life	▲Postharvest decay, calyx browning in higher concentration.	Bower *et al.* (2003)
Kimberley	100	3 d	3 d at 5 ˚C in dark in sealed punnets; 10 d at 5 ˚C in vented punnets	▼loss of calyx and fruit colour	Terry *et al.* (2007b)
NS	1000	Shelf-life	4 d at 20 ˚C	▲AOX	Heredia and Cisneros-Zevallos (2009)
Camarosa	(ethephon) 0.048	5 min	2 d at 20 ˚C	▲Anthocyanin content▼FaXyl1 protein, mRNA levels	Bustamante *et al.* (2009)

Cultivar	Concentration (µl l^{-1})	Duration	Storage condition	Effects	Reference
NS	(ethephon) 1000 or 1500	-	2-6 ˚C	▼Grey mould	Al-Jamali and Al-Wardi (2010)
Toyonoka	(ethephon) 0.045	5 min	2 d at 22 ˚C	▲ anthocyanin content, PAL activity, total sugar,β-Gal activity▼β-(1,4)-glucan activity	Villarreal *et al.* (2010)

µl l-1 – microlitre per litre; NS - Not specified; h – hours; d – Days, ▲ - Increase; ▼- Decrease; PAL - Phenylalanine ammonia lyase activity; β-Gal - β-galactosidase; β-(1,4)-glucan endo-1,4-β-glucanase; AOX - Antioxidant capacity.

2.4.2 Ethylene Biosynthesis

Ethylene is involved in the coordination of many aspects of plants life cycle including growth, development and storage life. To determine the involvement of ethylene in plant functions, knowledge of how and when the hormone is produced, regulated and transduced is important. The discovery of the ethylene biosynthetic pathway (Yang and Hoffman, 1984) has allowed aspects of its regulation to be studied (reviewed in Kende, 1993). In the past three decades, there has been extensive research and hence considerable progress in understanding the ethylene biosynthetic pathway. The ethylene biosynthetic pathway is, as a result, well established. The production of ethylene via plant tissues is thought to exhibit a relatively simple biosynthetic pathway comprising of three consecutive steps (Figure 2.5). This biosynthetic pathway progresses from the conversion of the amino acid methionine to S-adenosyl methionine (S-AdoMet), catalysed by SAM synthase. The process then advances via an addition of a nucleotide base (adenine) to give 1-amino-cyclo-propane carboxylic acid (ACC) via ACC synthase (*S*-adenosyl-L-methionine methylthioadenosine-lyase) (ACS). The conversion of ACC to ethylene is catalysed by ACC oxidase (ACO) (Wang *et al.*, 2002; Barry and Giovannoni, 2007). When *S*-AdoMet is synthesised to ACC by ACC synthase, 5'-methylthioadenosine (MTA) is generated as a by-product. MTA is recycled back to methythio group for constant methionine concentration in the cells, hence continuous ethylene production. The production of MTA means continuous methionine production; thus this is not a limiting factor in ethylene synthesis (Wang *et al.*, 2002).

The ability of ACC to be converted to ethylene is enhanced with increasing ethylene concentration (from 0.1 to 100 µl l^{-1}, and duration (1 to 24 h)). In tomato, this capacity was removed with the use of an ethylene inhibitor, however exogenous application of ethylene meant that the increase in ethylene forming enzymes enhances the increase in ACC synthase and subsequent ethylene production (Liu *et al.*, 1985).This pathway is largely influenced not only by genetic factors but also environmental conditions, such as the temperature, level of oxygen and carbon dioxide. The synthesis of ACC requires oxygen. Gorny and Kader (1996) have shown that lowered O_2 and increased CO_2 in 'Golden Delicious' apples can lead to a suppression of ACC activity. The catalysis of ACS in the ethylene biosynthetic pathway is a rate limiting step, and thus more

investigation regarding the mechanisms involved has focused on this particular enzyme (Wang *et al.*, 2002). However, the extent of this mechanism whereby ethylene induces a phenotypic response is yet not fully explained.

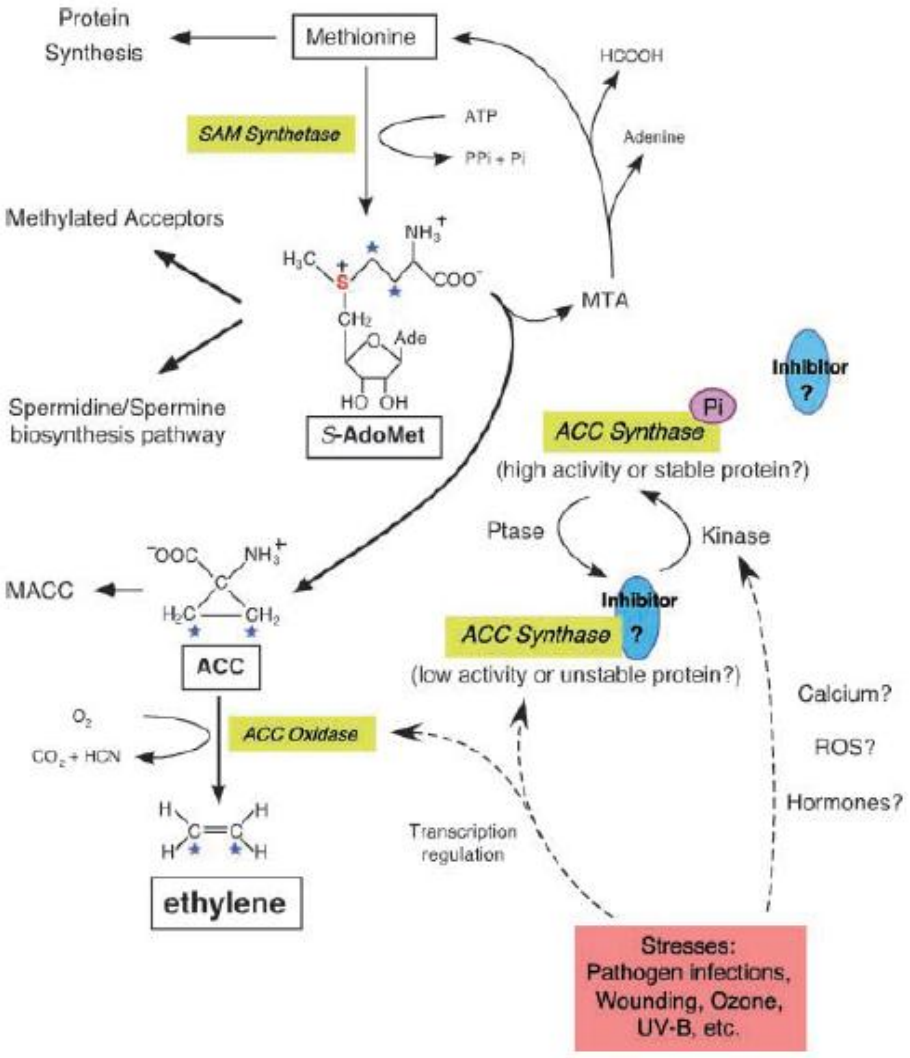


Figure 2.5. Ethylene biosynthesis and regulation (from Wang *et al.*, 2002).

Extensive research and the discovery of the ethylene biosynthetic pathway have led to emphasis in the role of regulatory enzymes ACS and ACC and the encoding genes (Pech *et al.*, 2008). There is also an ethylene independent ripening pathway. This independent process has been identified and associated with pulp colouration, increase in sugars, and loss of acidity (Abdi *et al.*, 1998; Alexander and Grierson, 2002; Pech *et*

al., 2008). Research has reported skin colour development to be an ethylene-independent phenomenon, but aroma production to be either ethylene-dependent or independent process, though this would depend on the cultivar (Alexander and Grierson, 2002; Pech *et al.*, 2008). Pech *et al.* (2008) has proposed that production of aroma volatiles, colour development and respiratory rise are ethylene dependent. However, the softening process constitutes of both, which suggest both processes ethylene dependent and independent co-exist during climacteric fruit ripening.

Ethylene biosynthesis in climacteric fruits is described as an autocatalytic process. There are two systems; in higher plants both systems operate (system 1 and system 2) refer to section 2.1.1. The response of climacteric fruits to ethylene analogue propylene gives rise to an autocatalytic synthesis of ethylene. Such a response is not apparent in non-climacteric fruit (McMurchie *et al.*, 1972). In response to exogenous ethylene, System 2 results in an autocatalytic synthesis of ethylene (Yang and Oetiker, 1995). However, even with the use of ethylene inhibitor, there was still ethylene (a quarter of the non-treated control fruits) detected, suggesting another mechanism of ethylene synthesis or the 1-methylcyclopropene (1-MCP) inhibitor did not exert complete inhibition (Yokotani *et al.*, 2004).

2.4.3 Ethylene Signalling Pathway

Much of the research identifying the signalling pathway for ethylene has used Arabidopsis as the model plant. This plant is still the model plant for many genetic and molecular analysis, by which the ETR1 (Ethylene receptor) was isolated (Cervantes, 2002). Receptors of ethylene are localised in the membrane of endoplasmic reticulum. These membrane proteins are similar to those present in bacteria, algae and plants. ETR1 was the first ethylene receptor described. Hua *et al.* (1998), isolated another ethylene receptor gene named *At-ERS1* (Ethylene sensor). These enzymes (At-ETR1 and At-ERS1) respond differently to the exogenous ethylene application. At-ETR1 response is ethylene independent during development and ripening. On the other hand, At-ERS1 is tissue specific, ethylene dependent and developmentally regulated (Vendrell *et al.*, 2003). Ethylene instigates its action by binding to the membrane localised receptors causing an alteration of the binding protein. A signal transduction pathway is triggered where the message ‘ethylene is bound to receptor’ is communicated through

numerous molecules; this triggers eventual expression of genes and subsequent physiological responses (Kays and Paull, 2004). Ethylene receptors consist of two proteins: a histidine kinase which acts as the sensor and in response to signals (e.g., environmental) autophosphorylates an internal histidine residue, and a regulator that activates the downstream component after receiving a phosphate from the histine residue on its aspartate residue (Wang *et al.*, 2002). Ethylene is considered to interact with group of five receptors (ETR1, ETR2, ERS1, ERS2 and EIN4) having similar structures in Arabidopsis.

These receptors can be divided into two subfamilies (type-I and type-II) depending on their structures. Type-I include ETR1 and ERS1, these have an amino-terminal, ethylene-binding domain (referred to as the sensor domain) as well as a carboxy-terminal histidine (His) kinase domain. On the other hand, type II receptors (ETR2, ERS2 and EIN4) contain a receiver domain that is similar to the bacterial response regulators at the C-terminus. The type-I receptors (ETR1 and ERS1) do not have a receiver domain and form heterodimers by binding to the receiver domain of other proteins (Chang *et al.*, 1993; Hua *et al.*, 1998; Guo and Ecker, 2004). Ethylene binding occurs on the ETR1 and ERS1 receptors, which have three membrane spanning regions at the N-terminus where ethylene binds. A single Cu (I) co-factor is believed to be required for ethylene binding and Cu is delivered to the receptors by transporter RAN. In the absences of ethylene a Raf-kinase, CTR1 is activated by ethylene receptors and in response CTR1 down regulates ethylene possibly through a MAP-kinase cascade. Binding of ethylene inactivates the receptors which results in deactivation, CTR1.EIN2 then functions as a positive regulator of ethylene pathway. EIN2 is similar in structure to the Nramp metal transporter proteins and the novel hydrophobic C-terminus as it contains the N-terminal hydrophobic domain. EIN3 is a nuclear-localised protein which acts downstream of EIN2. Three members are necessary for ethylene signalling: EIN3, EIN-like 1 (EIL1) and EIL2. Over expression of *EIN3* in an *ein2* mutant background reveals constitutive activation of ethylene response (Wang *et al.*, 2002). Homodimers of EIN3 and EIL1 are able to bind specific primary ethylene response elements found in the promoters of ERF1 (Solano *et al.*, 1998). ERF1 is part of a family of transcription factors that bind to the GCC-box found in many ethylene related genes (Guo and Ecker, 2004). Orthologues of these genes (*ETR1 and ERS1*) have been -isolated from peaches.

Rasori *et al.* (2002), has reported in peach *Pp-ETR1* gene to unlike *Pp-ERS1* be induced by ethylene, and inhibited by 1- MCP.

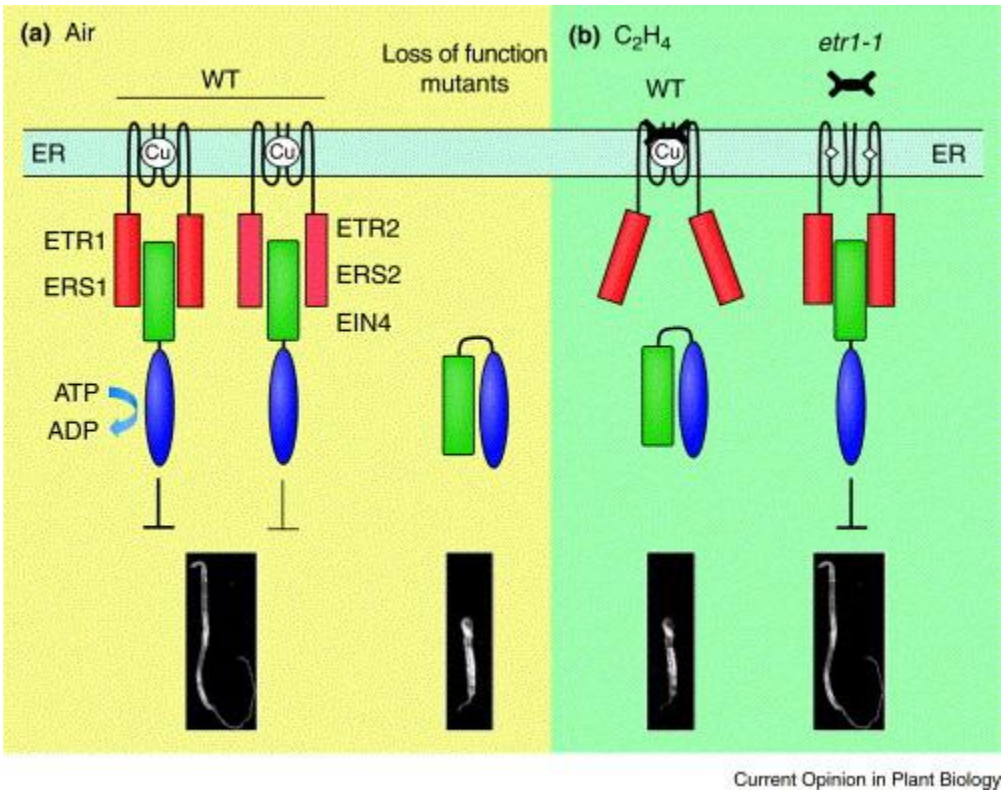


Figure 2.6. Ethylene perception and signalling pathway (from Guo and Ecker, 2004).

2.5 Control of ethylene production and action

Whilst direct exposure of fruits to ethylene is desirable in fruits such as bananas, and citrus to achieve more uniform ripening, in industry most strategies fundamentally aim to evade the effects associated with ethylene to achieve extended storage life. It is believed that ethylene levels higher than 0.1 µl l^{-1} can trigger the deleterious effects associated with ethylene. However, this depends on fruits, sensitivity to ethylene, and the duration of exposure. Controlling exposure of plants to ethylene and effects associated with the hormone may span from simple strategies, *viz.* segregating commodities from non-biological sources (internal combustion engine) and biological sources (e.g. other ripened, infected and injured commodities). Ethylene often

accumulates in the storage atmosphere of commodities during transportation, storage and handling of crops after harvest. Since ethylene can exert its effects at low concentrations (nl-µl l^{-1}) this concentration is believed to be dependent on the storage life whereby such concentration will reduce storage life and shelf-life (Wills and Warton, 2000).

2.5.1 Low temperature storage

The main purpose of low temperature storage is to reduce fruit metabolism and consequently delay the parameters associated with fruit ripening and quality degradation. The respiration rate of fruit is one of the main parameters defining the metabolic activity of the fruit and temperature is considered as the main variable affecting fruit respiration. In addition, reduction of fruit temperature storage reduces fruit ethylene production and sensitivity. This is particularly important in climacteric fruits where ethylene is cardinal for the ripening process. Low temperature storage reduces fruits, ethylene production and respiration which in turn slows processes related to fruit ripening and quality including colour and texture. It is therefore crucial that fresh produce are stored at low temperature immediately after harvest to maintain fruit quality due to reduced metabolic activity. However, among fruits there are many that are cold-sensitive and cold temperature may stimulate chilling injury (CI) disorder. The benefit of cold storage in plums is limited due to increased incidence of physiological disorders attributed to chilling injuries (CI) (Taylor *et al.*, 1993a; Candan *et al.*, 2008). Avocados demonstrate darkening of the mesocarp following period cold storage due to CI. Some literature has suggested that coupled with cold storage, exogenous ethylene can intensify the symptoms of chilling injury in avocados (Pesis *et al.*, 2002).

2.5.2 Reduction of endogenous ethylene production

Storage strategies such as modified atmosphere (MA) for delaying the ripening process and postharvest softening have been shown to be useful (Wright and Kader, 1997; Holcroft and Kader, 1999). MA is a preservation technique used for fresh and minimally processed food (Caleb *et al.*, 2012). The atmosphere around the commodity is usually controlled through packaging to prolong the state of the product and this atmosphere is thus different from that of air (78.08 % N_2, 20.95 % O_2 and 0.03 % CO_2), hence modified. In MA, fruits are packaged with sealed films that are selectively

permeable to specific gases. Modified atmosphere packaging (MAP) allow gas exchange to create a MA inside the packaging so that the interplay between the respiration of the fruits inside the packaging and the gas transfer through the packaging leads to an atmosphere that is higher in CO_2 and lower in O_2 (Thompson, 2003). MAP has been shown to reduce respiration, decay and ethylene sensitivity (Saltveit, 1997). Inhibition of banana fruit ripening when packed in polyethylene bags compared with non-packed fruits was demonstrated by Jiang *et al.* (1999). It is believed that the enhanced CO_2 inside MAP means that the ethylene receptors are saturated with CO_2 (Jiang *et al.*, 1999).

In controlled atmosphere (CA), atmosphere is predetermined and often involves reduction of O_2 and/or CO_2 concentrations. Zheng *et al.* (2005), have shown mangos treated with oxalic acid (5 mM dip for 10 min), while being stored in a CA (6 % CO_2 ± 2 %, 14 ± 1 %) to exhibit extended storage life and retarded decay. Kiwi storage life has been extended to 3-4 months with the use of CA of 5 kPa CO_2 and 2 kPa O_2 and ethylene free air providing CA is introduced within 1 week after harvest (Arpaia *et al.*, 1984). Retardation in flesh softening was also achieved with CO_2 levels between 4 to 10 kPa though levels higher than this can lead to storage defects. In addition, Burdon *et al.* (2008), demonstrated that avocado (cv. Hass) quality was better during CA storage. Similarly, MA can be used to reduce respiration, ethylene production and sensitivity to ethylene; and can therefore be successful in retaining the quality of fresh produce postharvest (Hoehn *et al.*, 2009). MA is used in combination with temperature, and % RH management. MA of 10 % O_2 and 15-20 % CO_2 for strawberries reduced respiration rate, retarded softening and other compositional changes associated with senescence (Ke *et al.*, 1991; Smith and Skog, 1992). Ventilation is also frequently used to control ethylene accumulation. Ventilation can be used in combination with appropriately designed containers to achieve effective air circulation. However, use of ventilation is not appropriate in enclosed environments such as CA or retail packaging.

2.5.3 Inhibition of ethylene biosynthesis and action

The synthesis of ethylene can be hindered through the use of inhibitors. Aminoethoxyvinylglycine (AVG) is an inhibitor of ethylene biosynthesis which works by inhibiting ACC synthase. AVG was found as an effective ethylene inhibitor

preharvest and postharvest. AVG also registered as Retain^{TM} has been widely studied for its use on apples preharvest. AVG was found as an effective method of reducing ethylene and protein synthesis of pre-climacteric mature tomato (Saltveit, 2005). Silver thiosulfate is another inhibitor of ACC synthesis. The preservative solution for cut flowers often consists of silver thiosulfate resulting in ethylene inhibition and subsequent longevity in vast life (Staby *et al.*, 1993).

Endogenous and exogenous ethylene can be reduced with the use of ethylene perception inhibitors. Cyclic olefin 2, 5-norbornadiene (NBD) is a competitive inhibitor of ethylene action preventing ethylene binding, hence response. Treatment of flowers with ethylene during anthesis in the presence of NBD (500 µl l^{-1}) resulted in higher ethylene needed for petal senescence, which means that NBD competes for ethylene binding sites in a competitive manner (Wang and Woodson, 1989). NBD has been shown to delay the ripening of apples (Blankenship and Sisler, 1989); however, continuous exposure of the material is required to maintain its efficaciousness. Hyodo and Fujinami (1989) have shown that NBD suppression can be overcome by continuous exposure to ethylene, hence the need for continuous treatment.

1-MCP is a commercially available ethylene inhibitor which is currently used for controlling ethylene production, ripening and senescence of mainly climacteric fruits and vegetative tissues (Watkins, 2006). 1-MCP has been shown to inhibit ethylene production at concentration of as low as 0.5 nl l^{-1}. This concentration of 1-MCP on bananas for 24 h exposure was found to inhibit ethylene production for a period of 12 days at 23 ^{o}C, after which the fruits were initiated with ethylene to ripen as normal (Sisler *et al.*, 2009). 1-MCP is believed to exert its effects by blocking the ethylene receptor and therefore inhibiting ethylene dependent responses (Sisler and Blankenship, 1996). According to Pathak *et al.* (2003), 1-MCP has also been reported to reduce the activities of ACS and ACO enzymes in banana fruit (Musa *Cavendishii* L. cv. Cavendish). In accordance, Ma *et al.* (2009), has shown 1-MCP to reduce gene expression of ACO and delayed the synthesis and concentration of ACC in broccoli (*Brussica oleracea* L.).

1-MCP was approved for extending the shelf life of ornamentals in 1999 by the Environmental Protection Agency (EPA). It was initially marketed as EthylBloc^{R} by

Floralife, Inc. (Walterboro, SC) and later developed by Agrofresh, Inc., under the trade name SmartFresh^{TM}. SmartFresh is now globally available for its use on horticultural products (Watkins, 2006). The discovery of this inhibitor of ethylene has driven an enormous amount of research into its effects on range of climacteric and non-climacteric fresh produce. The longevity of 1-MCP action on a fruits will depend on many factors such as species, concentration, duration, temperature and time of exposure as well as mode of ethylene biosynthesis induction (Jiang *et al.*, 2000; Sisler and Serek, 2006). 1-MCP effectiveness increases with concentration, however, this is up to a saturation point. The saturation point is dependent on the number of ethylene receptors available for binding. 1-MCP has greater affinity for binding than ethylene and it is effective at very low concentrations. Skog *et al.* (2001) reported the inhibitory effects of 1-MCP on ethylene of four different plum cultivars and showed that its effects were cultivar dependent. Salvador *et al.* (2003), demonstrated that 1-MCP treatment before storage on 'Santa Rosa' plums gave positive effects in comparison to those stored under cold storage. In the study, 1-MCP was successful in delaying processes such as softening, acidity loss, and colour evolution during cold storage and thus prolonged postharvest life. Although 1-MCP is commercially used for several commodities including apples, avocados and pears, treatment can incur some undesirable effects. 1-MCP has been found to be very selective in its effect on different apple cultivars (Watkins, 2006), while for peaches 1-MCP has limited effect (Liu *et al.*, 2005a). In avocados, 1-MCP treatment can interfere with fruit development after treatment ceases. A particular problem observed with avocados is uneven ripening or 'evergreen disorder' where fruits fail to ripen under ambient conditions after 1-MCP treatment (Watkins, 2006; Kruger and Lemmer, 2007; Meyer and Terry, 2010).

There is currently extensive literature focused on the effects of 1-MCP on climacteric fruits to delay ripening processes, but less on non-climacteric fruits. The effect of 1-MCP on non-climacteric fruits has received less consideration since ethylene is considered less important in determining fruit quality. However, there are some studies about 1-MCP application on non-climacteric fruits including citrus and strawberries (Valero and Serrano, 2010). A summary of the effect of 1-MCP on the physiological and biological changes in strawberry fruit after 1-MCP treatment is shown below (Table 2.3).

Table 2.3. Effect of 1-MCP on different strawberry cultivars (*Fragaria x ananassa* Duch).

Cultivar	Concentration (nl l^{-1})	Duration	Storage condition	Effects	Reference
-	500	-	5 or 20 ˚C	▲postharvest decay, quality	Ku *et al.* (1999)
Pajaro	2000	18 h	20 ˚C	▲postharvest decay, quality ▼respiration, loss in firmness, colour changes	Tian *et al*. 2000
Everest	100 and 250	2 h	3 d at 20 ˚C in dark at >95 RH %	▼loss of firmness and colour, ethylene production, postharvest decay (*Rhizopus stolonifer*)	Jiang *et al.* (2001)
	500 and 1000		as above	▲postharvest decay (R. stolonifer), PAL activity, anthocyanins ▼total phenolics	
NS	10, 50, 100 and 1000	24 h	0 or 5 ˚C during shelf life	▲Postharvest decay ▼ethylene production, calyx quality	Bower *et al.* (2003)
Camarossa / Seascape	1000	24 h	12 d at 5 ˚C	▲Respiration rate ▼Ethylene production; softening, TA, microbiological growth and deterioration when combined with $CaCl_2$+CA	Aguayo *et al.*(2006)
Jingnong 1st	500 and 1000	2 h	20 d at -1 ˚C Shelf life at 4 ˚C	▲Protein content, SOD, APX and CAT activity ▼rot rate	Shi *et al.* (2008)

Cultivar	Concentration (nl l^{-1})	Duration	Storage condition	Effects	Reference
Camarosa	1000	10 h	2 d at 20 ˚C	▲FaXyl1 protein ▼Anthocyanin accumulation	Bustamante *et al.* (2009)
Camarosa	250, 500, 750 and 1000	16 h	4 w at 1 ˚C in dark at >95RH %	▲ Store life, firmness colour, TSS. ▼ Fruit decay.	Modares *et al.* (2010)a
Toyonoka	1000	10 h	2 d at 22 ˚C	▲□-(1,4)-glucan activity ▼Anthocianin content, PAL activity, total sugar, □-Gal activity	Villarreal *et al.* (2010)

µl l^{-1} – microlitre per litre; NS - Not specified; h – hours; d – Days, ▲ - Increase; ▼- Decrease; PAL - Phenylalanine ammonialyase activity; β-Gal - β-galactosidase; β-(1,4)-glucan=endo-1,4-β-glucanase; SOD - Superoxide dismutase; CAT - Catalase activity; APX - Asorbate peroxidase; AOX - Antioxidant capacity.

2.5.4 Ethylene Removal

Methods described above are concerned with inhibiting ethylene biosynthesis or avoiding ethylene perception, however research rarely considers the removal of ethylene from the storage atmosphere. Potassium permanganate ($KMnO_4$) supported on activated alumina beads can be used to remove ethylene from the storage atmosphere. In the presence of $KMnO_4$ ethylene is oxidised to ethylene glycol or acetic acid and prolonged exposure causes further oxidation to CO_2 and H_2O. Research has shown $KMnO_4$ can retard the ripening of climacteric fruits by maintaining low ethylene levels in fruit environment. That said, the longevity of $KMnO_4$ was reduced in environments of high % RH, where ethylene usually accumulates during fruits storage (Wills and Warton, 2004; Terry *et al.*, 2007b; Wills *et al.*, 2007). This can be a great disadvantage since horticultural packaging and storage environments are under high RH usually over 90 %. Furthermore, authors above have also shown that the efficacy of $KMnO_4$ diminished continuously after 24 h, where within 14 days only 10 % of the ethylene administered was adsorbed. Wills and Warton (2004) have proposed large quantities of $KMnO_4$ adsorbent would be necessary inside large packing containers where there is high ethylene accumulation. Moreover, $KMnO_4$ is highly toxic and cannot be used in large quantities in contact with food products.

The use of activated carbon for gas adsorption started in the 1930s and its use for ethylene was reported in 1950s. The adsorption capacity of activated carbons depends on several factors such as the nature of the surface bound functional moiety and, the amount and arrangement of the pore structure (reviewed in Martinez-Romero *et al.*, 2007). Bailen *et al.* (2006) has reported the success of activated carbons in MAP as a means of preserving fruit postharvest quality (colour, weight loss and firmness). The efficacy of granular activated carbon was enhanced further when used with palladium as a catalyst; however the entirety of ethylene was not adsorbed.

The use of catalyst composed of pure metallic elements from the Platinum group metals (Ru, Rh, Pd) or oxides such as TiO_2 increases the rate of ethylene oxidation to CO_2 and oxygen. This has been shown to reduce ethylene in storage atmosphere of fresh produce. However, high temperatures (over 100 ^oC) are required to activate the reaction, which means high energy consumption. TiO_2 is a photocatalyst, which means it requires

UV light (artificial or natural) for the degradation of ethylene in fruits storage environment. Although TiO_2 offers advantages over other techniques since its cheap, photostable, clean and can be used at room temperature it requires permanent use of UV light which can be difficult inside fruit packaging (Martinez-Romero *et al.*, 2007).

2.5.5 E+® Ethylene Remover

Terry *et al.* (2007b) has shown the remarkable ethylene adsorption capacity of a newly developed Pd-promoted material. The Pd-promoted material adsorbed ethylene in humid condition until breakthrough occurred (Figure 2.7). The study showed Pd-material removed ethylene in cool-temperatures (5-16 ^{o}C) to sub-physiologically active levels and subsequent beneficial effects of delaying the climacteric ripening of bananas (cv. Cavandish) and avocados (cv. Hass). Bananas held in the presence of Pd-promoted material (0-50 g) showed reduction in respiration and colour development even when the ripening process was triggered with 100 $\mu l\ l^{-1}$ ethylene (Figure 2.8). Terry *et al.* (2007b) in another experiment compared the effects of Pd-material and $KMnO_4$ (Ethysorb ®) on avocados. Fruits held in the presence of Pd-promoted material were greener than untreated fruit or those treated with Ethysorb ®.

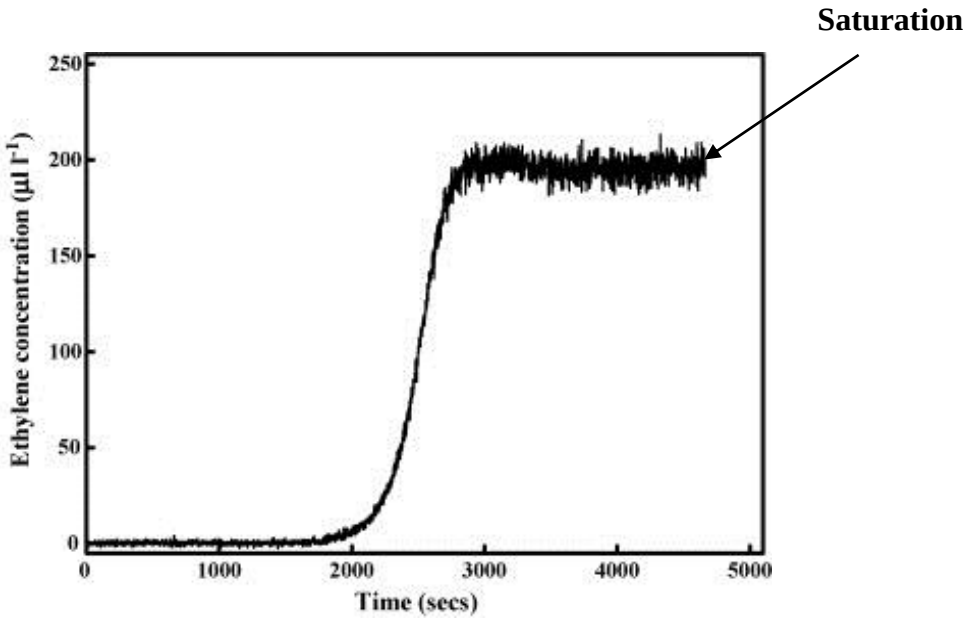


Figure 2.7. Ethylene breakthrough measurement on Pd-promoted material held in humid conditions (from Terry *et al.*, 2007b).

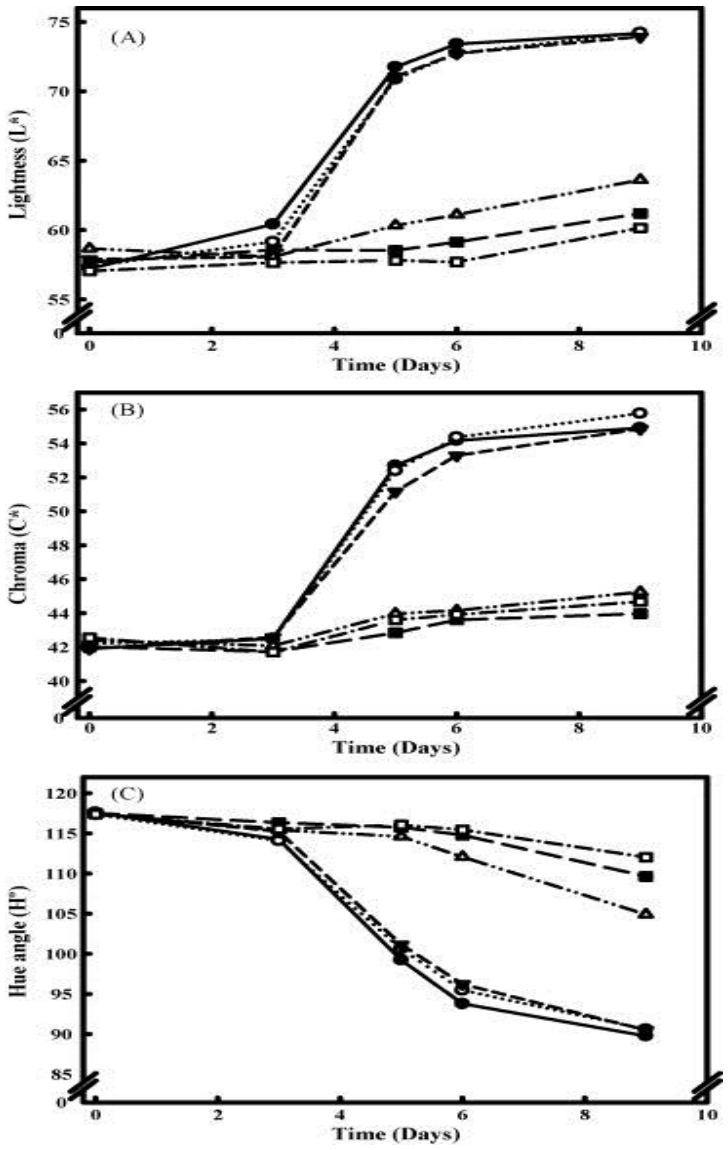


Figure 2.8. (A–C) Effect of Pd-promoted material (0, (●); 10, (○); 20, (▼); 30, (△); 40, (■); 50 g, (□)) on change in L*, C* and H° of pre-climacteric banana cv. Cavendish fruit held at 16 °C within 3 l sealed jars for 3 days which were initially treated with 100 µl l^{-1} ethylene. Fruit were removed and kept at 18 °C for 6 days. LSDs (P = 0.05) for A, B and C = 2.108, 1.188 and 1.547, respectively (from Terry *et al.*, 2007b).

The Pd-promoted material was developed by Johnson Matthey and reported to have significant ethylene adsorption after synthesising and testing a range of materials. The material was composed of Pd-impregnated zeolites producing finely dispersed Pd particles with metal loading of 2.5 % Pd (m/m). Active Pd-based material (0.1 g) was

held at room temperature in the presence of a gas mixture (ethylene 550 µl l^{-1}, 40 % (v/v) air balanced with argon). Within 2 h, the entirety of ethylene was removed and production of CO_2 and ethane gas was observed. Ethane was produced possibly by hydrogenation of the adsorbed ethylene (Figure 2.9) suggesting the Pb-material acts as an adsorbent rather than a catalyst. Images taken of the material with Transmission electron microscopy (TEM) reveal Pd particles (bright particles) are scattered over the support material and size of the particles are around 1.7 nm (Figure 2.10) (Smith *et al.*, 2009).

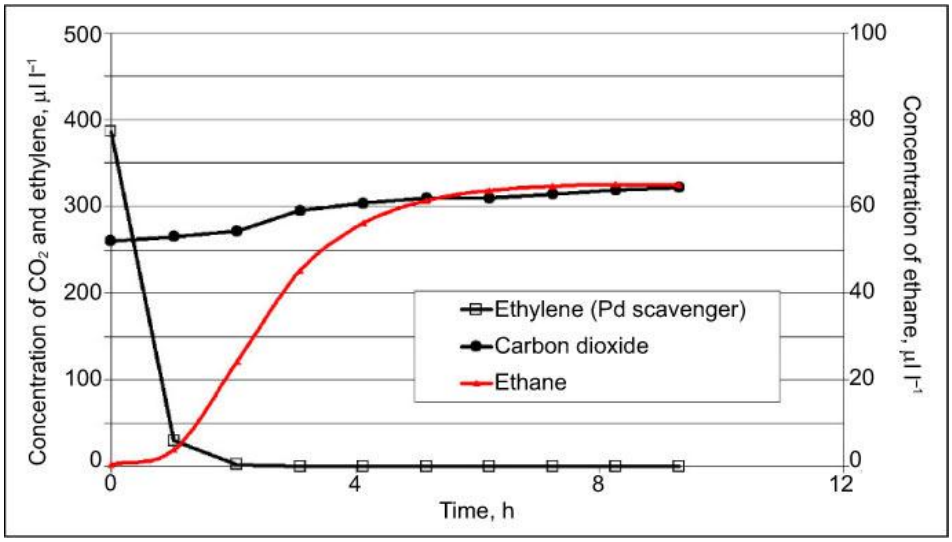


Figure 2.9. Gas concentrations in a batch reactor initially containing 550 µl l^{-1} ethylene, along with 0.1 g of the Pd-promoted ethylene scavenger (from Smith *et al.*, 2009).

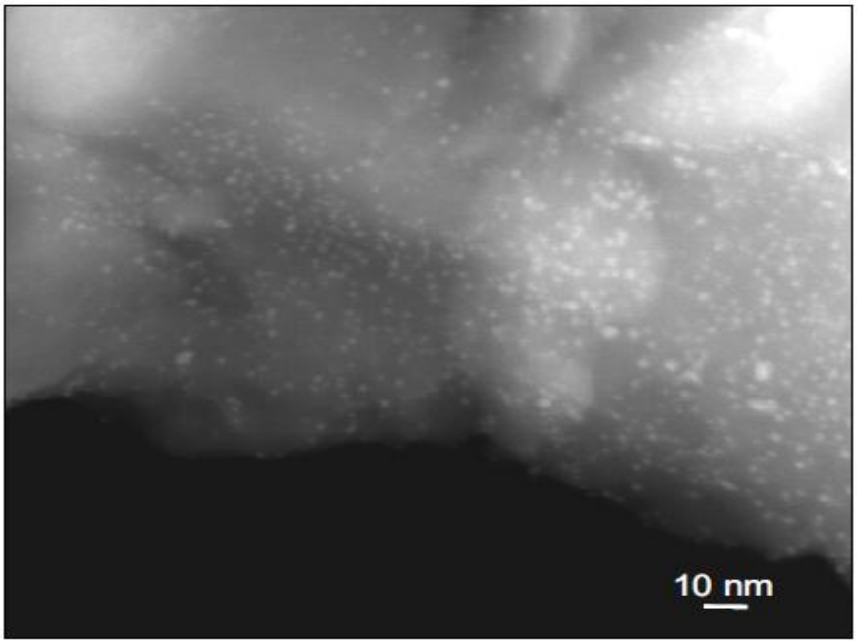


Figure 2.10. TEM image of the Pd-promoted zeolite material showing nanometre size palladium particles (bright areas) on the zeolite support (from Smith *et al.*, 2009).

Several potential Pd-ethylene bound species have been hypothesised following the exposure of the Pd-particles to ethylene (Figure 2.11). It is believed that the Pd-based material acts as an adsorber rather than as a catalyst. Further analysis of the material using Fourier transform spectroscopy (DRIFTS) showed that metal was required for the adsorption of ethylene and that an adsorption capacity of 45,600 μl g^{-1} was achieved when Pd was present.

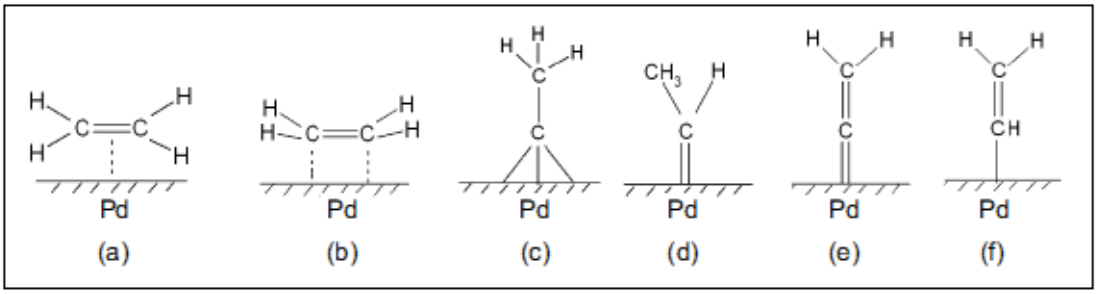


Figure 2.11. Potential adsorbed species following exposure of the Pd-containing scavenger to ethylene: (a) π-bond, (b) di-σ bonded, (c) ethylidyne, (d) ethylidene, (e) vinylidene and (f) vinyl (from Smith *et al.*, 2009).

Following on from these initial studies (Terry *et al.*, 2007b; Smith *et al.*, 2009), a new formulation of the Pd-promoted material was produced. This new formulation was registered as e+® Ethylene Remover. The chemical has the same properties of that used previously; however metal loading was 1 % Pd as opposed to 2.5 %. Meyer and Terry (2010) carried out a comparative study on the effects of 1-MCP (1.5 µl $l^{-1}$for 24 h) and e+® Ethylene Remover on avocados fruit (cv. Hass) stored at 5 ^{o}C and ripened at 20 ^{o}C. The e+® Ethylene Remover was effective in delaying ripening of fruits stored at 5 ^{o}C and fruits treated with the ethylene remover were significantly more green and firm in comparison to the untreated fruits. However, 1-MCP was more effective in inhibiting the ripening of avocados than e+® Ethylene Remover (Figure 2.12), but in contrast the subsequent ripening of 1-MCP treated fruits was impaired. This would be undesirable in the real world supply chain and would increase consumer's complaints due to inconsistency within fruit batch.

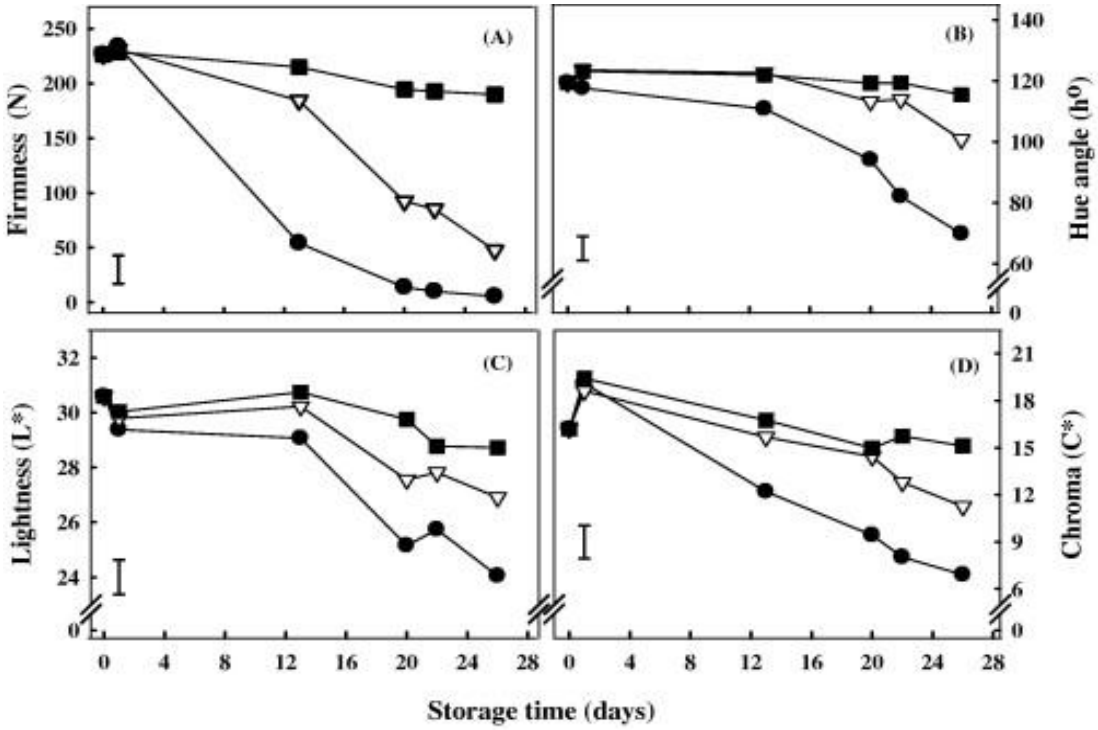


Figure 2.12. Effect of treatments (control, ●; e+® Ethylene Remover, ▽; 1-MCP, ■) on change in firmness (*N*), lightness (L^*), chroma (C^*) and hue angle ($H°$) of pre-climacteric avocado cv. Hass (late season, Exp 1) fruit held at 5 °C within 13 l boxes for 26 days in the presence of e+® Ethylene Remover (5 g) or initially pre-treated with 1-MCP (1.5 µl l^{-1} for 24 h). Values are means of ethylene-treated and ethylene non-treated fruits (from Meyer and Terry, 2010).

2.6 Conclusion

The role of ethylene in the ripening and postharvest quality of climacteric and non-climacteric fruits has been considered. This literature review has highlighted the advantages and significance in controlling ethylene in order to preserve the quality of climacteric fruits. Ethylene inhibition with the use of ethylene inhibitor 1-MCP has received enormous research interest. However, this inhibitor can incur some known problems of its own. There is therefore a great necessity for discovering and testing the efficacy of other technologies. The development of a new e+® Ethylene Remover, which has been shown to reduce ethylene in the storage atmosphere of the fruit through adsorption leading to subsequent delay in ripening was discussed. However, more research is necessary to better understand the efficacy of this treatment and discover

ways to optimise its usage for additional benefits. In recent times the role of ethylene in non-climacteric fruits such as strawberries and grapes has received some interest; that said, this requires more research and better understanding. Other phytohormones such as ABA and auxins also have a significant role in the ripening and development of fruits. However, there is lack of literature regarding the importance of these phytohormones in non-climacteric fruits. Investigating the role of ethylene and e+® Ethylene Remover on non-climacteric fruit development would also be of great relevance.

CHAPTER THREE

3 EXTENSION OF AVOCADO STORABILITY USING E+® ETHYLENE REMOVER IMPREGNATED SHEETS IN SEA CONTAINERS

3.1 Introduction

As a climacteric fruit, ethylene can be undesirable in avocado storage since it can cause reduced storage life and increased disease incidence (Pesis *et al.*, 2002; Maftoonazad and Ramaswamy, 2005). The ripening process of avocados is progressive where 5 to 7 days of shelf life is sufficient for complete ripeness following harvest depending on the physiological maturity (Awad and Young, 1979; Landahl *et al.*, 2009). Avocado fruit often require long distance transit under refrigeration to reach consumers overseas. Tight control over the ethylene levels within the storage environment is thus fundamental to prevent ethylene-induced premature ripening and decay leading to significant economic losses (Saltveit, 1999; Maftoonazad and Ramaswamy, 2005).

Research has demonstrated a direct relationship between softening of avocados and reduction in the content of C7 sugars. Authors (Liu *et al.*, 2002) proposed that these sugars may be involved in inhibiting fruit ripening when attached to the plant.

The material e+® Ethylene Remover was discovered to have significant ethylene adsorption capacity, adsorbing ethylene to sub-physiologically active levels as described in section 2.5.5. Subsequent studies have shown that the removal of ethylene from the storage environment of avocados (at low temperature and high relative humidity (% RH)) can offer great advantages. Storing avocado in the presence of e+® Ethylene Remover (metal loading 2.5% to 1% Pd (m/m)) was found to preserve postharvest qualities including firmness, heptose sugars and colour with fruits ripening normally when the treatment was discontinued (Terry *et al.*, 2007b; Smith *et al.*, 2009; Meyer and Terry, 2010). Previous studies which have reported the use e+® Ethylene Remover took place in laboratory settings and thus its efficacy in the real world supply chain is not

known. To this end, work was carried out to test the feasibility and effectiveness of e+® Ethylene Remover sheets in a real world supply chain scenario.

3.2 Materials and methods

3.2.1 Plant material and treatment

Two separate experiments were conducted herein (Exp 3.1 and Exp 3.2). In Exp 3.1, pre-climacteric avocado (*Persea americana.* cv. Hass) fruits [size code 18; 212-235 g], from commercial grower (Agricola Santa Sofia) in Talagante province in Chile were harvested (28th Jan 2010) and transported to the packing house (Soc. Agricola Campusano Ltda) within 24 h. Fruits were packed commercially in crates (4 kg) with or without e+® Ethylene Remover coated sheets (1 % Pd loading) (19 × 26.5 cm) on 4th February 2010. The e+® Ethylene Remover coated sheet was placed so that the coated side faced towards the bottom of the box and thus was not in direct contact with fruit. One pallet consisted of a mix of treated boxes (n=132, E+) and untreated boxes (n=132, BF) and a second pallet contained untreated boxes (n=264, UN) only. The untreated boxes (UN) acted as additional controls. Pallets were transported to the UK in a standard sea container at 5 °C and were held under standard controlled atmosphere (CA) (4 kPa O_2, 6 kPa CO_2). Upon arrival in the UK, following 5 weeks transit, fruit were transferred to Cranfield University within 24 h. Fruits (E+, BF and UN) were treated with or without e+® Ethylene Remover (5 g powder) and stored in 13 l boxes at 5 °C (refer to Figure 3.1for treatment combinations). Storage boxes were vented every day for 5 min to avoid CO_2 build up. In Exp 3.2, pre-climacteric late-season avocados (*Persea americana.* cv. Hass) [size 18] were harvested on the 27th May 2010. Fruits were obtained from a commercial orchard in Peru, and supplied by Worldwide Fruit Ltd, UK. Fruits were transported to the packing house within 24 h of harvest. Fruits were then commercially packed in crates of 4 kg, single layer and treated with or without e+® Ethylene Remover coated sheet (on 30th May 2010) as described above. Packed control and treated fruits were transported into the UK under refrigeration (5-6 ^oC) under CA (4 kPa O_2, 6 kPa CO_2) for approx. 5 week period. On arrival, fruits were sampled from two separate pallet containers. One pallet contained fruits without e+® Ethylene Remover (Control), while a second pallet contained fruits treated with e+® Ethylene Remover (E+ treated). Avocado boxes (n=24) were selected from each pallet

randomly. On arrival to Cranfield University, these boxes were stored at 5 °C overnight before commencing treatment.

3.2.2 Treatment of imported fruit at Cranfield University

On arrival at the laboratory (Exp 3.1), fruit from each of the treatments (n=72 per treatment) were randomly placed in 13 l plastic boxes (n=6 per treatment with 12 fruit per box) and treated with (n=3 boxes per treatment) or without (n=3 boxes per treatment) e+® Ethylene Remover, resulting in 6 treatments combinations (Figure 3.1). The e+® Ethylene Remover was achieved by placing 5 g of powdered e+® Ethylene Remover, divided into two petri dishes (Meyer and Terry, 2010). The e+® Ethylene Remover was not removed from the boxes for the duration of the experiment. Following treatment, boxes were stored for 31 days at 5 °C. The build-up of CO_2 in the storage boxes was avoided by venting the boxes manually on a daily basis.

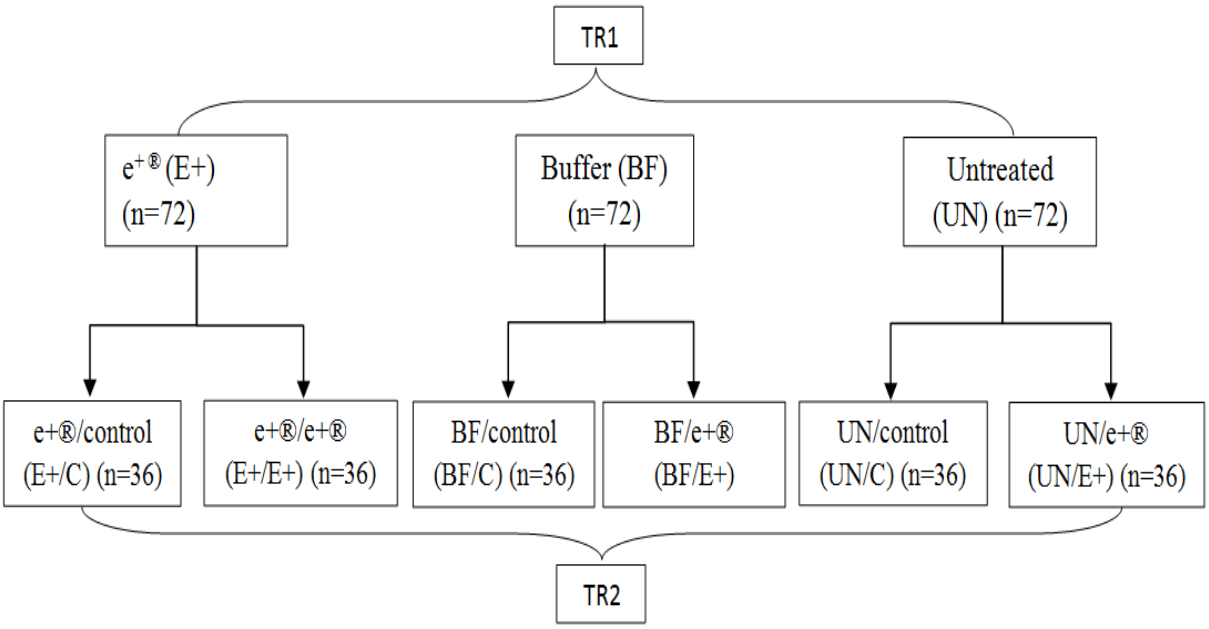


Figure 3.1. Experimental design for Exp 3.1, showing the three initial treatments (E+, BF, and UN) at source (TR1) and the subsequent treatments (TR2) after transit with (E+) or without (C) e+® Ethylene Remover to give six treatment combination (E+/C, E+/E+, BF/C, BF/E+, UN/C, UN/E+).

In Exp 3.2 there were two initial treatments; control and e+® Ethylene Remover treated fruits. The avocado boxes (n=24) selected randomly for each initial treatment were split in half (n=12 boxes). The e+® Ethylene Remover coated sheets inside half of the boxes (n=12) of avocados were removed, these were labelled as E+/C (E+ represented the initial treatment, C the postharvest treatment). The e+® Ethylene Remover coated sheets were placed inside the avocado boxes (n=12) of the control fruits and these were labelled as C/E+ (C being the initial treatment, E+ the postharvest treatment). There were four treatment combinations (Figure 3.2), with each treatment combination having 3 replicates and each replicate constituting 4 boxes stacked on top of each other. Within each replicate the two middle boxes were used for sampling. For Exp 3.2 the experimental setup was performed with the acknowledgement that in the commercial environment avocados are packed in their crates and stacked on top of each other. In acknowledging this, fruits were left in their original crates and not stored inside the plastic boxes (13 l) used in Exp 3.1.

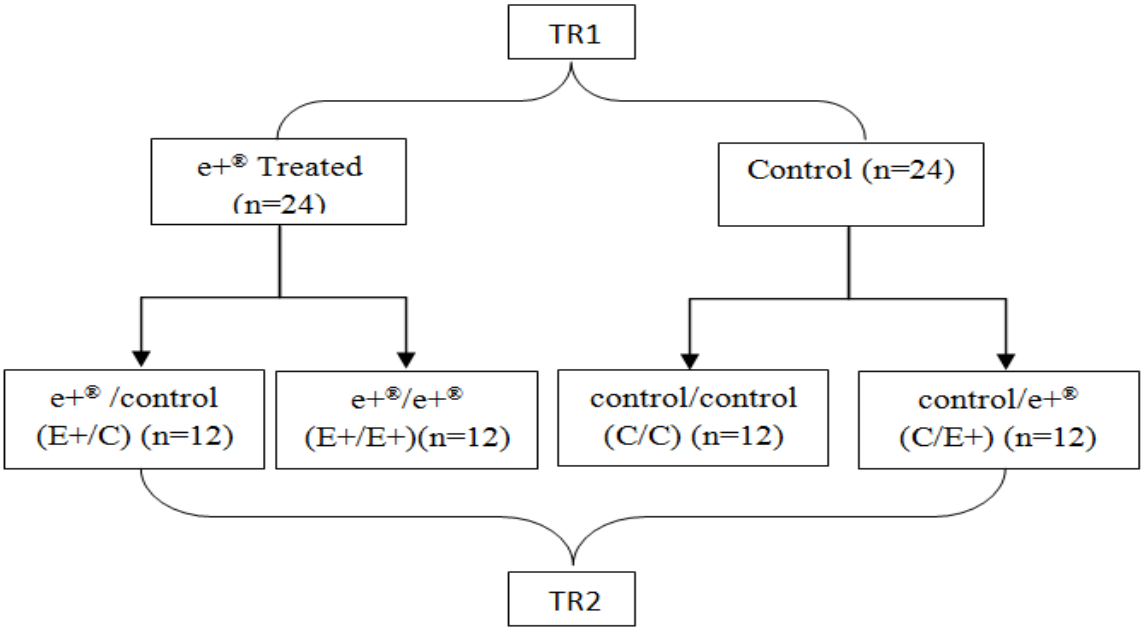


Figure 3.2. Experimental design for Exp 3.2: showing two initial treatments (TR1) with or without e+® Ethylene Remover sheets at source and treatment after transit (TR2) to give four treatment combinations (E/C, E+/E+, C/C, C/E+) with each treatment combination having three replicates.

After 14 and 30 days, 24 fruit subsamples per treatment (total n=96) were removed from cold storage. Half of the fruits (n=12 per treatment) were assessed for respiration rate, ethylene production, firmness and colour while the remaining fruits (n=12 per treatment) were allowed to ripen at 18 °C for an additional 5 days. On ripening C/E+ and E+/E+ fruits were placed on flat trays with e+® Ethylene Remover sheet (facing down) on the trays, while the control fruits were placed on trays having no treatment. After ripening ethylene production, respiration, colour and firmness measurements were performed. Additionally, baseline measurements were conducted on control and treated fruits (n=12 per treatment) after 24 h of cold storage (5 °C).

3.2.3 Physiological measurements and storage conditions

3.2.4 Carbon dioxide and ethylene production

Ethylene and CO_2 concentration in the storage atmosphere (13 l boxes) of the fruits were determined at regular intervals (over 31 days) during cold storage (5-6 ^oC, Exp 3.1). These were quantified with a GC model 8340 gas Chromatograph (Carlo Erba Instruments, Hert., UK) connected to a DP800 integrator (Thermoquest, Hert., UK) as described previously by Dauny *et al*. (2003) with minor modifications. This GC was fitted with flame ionisation detector (FID) for measuring ethylene and hot wire detector (HWD) for CO_2 quantification. For ethylene measurements oven and detector temperatures were set at 150 ^oC and 250 ^oC. A stainless steel 2 m long column packed with 80-100 mesh size Porapak P was used for ethylene quantification. The oven temperature was set at 80 °C and HWD at 120 ^oC for CO_2 measurements. A 2 m long x 4 mm column packed with Porapak Q mesh range 60-80 (Jones Chromatography, Mild Glamorgan, and UK) was used. Certified Standard from British Oxygen Company (BOC) as 10.6 µl l^{-1} ethylene in nitrogen was used for ethylene measurements while CO_2 quantification calibration was carried out against 10 % CO_2 (Certified standard of 10 % CO_2, 2 % O_2, 88 % N_2 from BOC).

The fruits were assessed for respiration and ethylene production rate (Exp 3.1 and 3.2). The CO_2 and ethylene emitted were measured by placing each subsample of three fruits for Exp 3.1 and two fruits for Exp 3.2 (each labelled) in a hermetically sealed 3 l glass jar with a rubber septum for 1 h (at 19 °C) (jars used are shown in Appendix C). After 1 h of incubation, the headspace gas within the jar was withdrawn using a gas syringe

(60 ml) and the ethylene and CO_2 concentration was quantified immediately using GC (as described above), respectively.

3.2.5 Colour measurements

The objective colour (lightness (L*), chroma (C*) and hue angle (H^o)) was measured as for each fruit, as previously described by Terry *et al*. (2007b). Measurements were obtained using a Minolta CR-400 colorimeter and DP-400 data processor (Minolta Co. Ltd., Japan) calibrated against a Minolta standard white tile CR-400 (Y = 93.5, x = 0.3114, y = 0.3190). The instrument was calibrated and three readings at the equidistant point at the equatorial regions were recorded on each fruit (n=9 per treatment in Exp 3.1and n=12 per treatment in Exp 3.2).

3.2.6 Fruit firmness evaluation

Flesh firmness was measured using a uniaxial testing machine (model 5542, Instron, Norwood, MA) programmed to Bluehill 2, version 2.11, Instron as described previously (Terry *et al.*, 2007b; Meyer and Terry, 2010). A flat cylinder probe (8 mm diameter) at a crosshead speed of 20 mm min^{-1} was used and the force (N) was recorded at maximum load. Fruit firmness was determined on a peeled surface. Two penetrations were taken on opposite sides of each fruit (n=9 in Exp 3.1 and n=12 in Exp 3.2 per treatment) at the equatorial region and the mean values of these penetrations taken. Fruits removed from cold storage were equilibrated to *ca*. 18 ^oC before the firmness could be determined (Meyer and Terry, 2010). Fruit firmness of approx. 200 N was measured on arrival (day 0) for both Exp 3.1 and 3.2. This method is depicted in Appendix C.

3.3 Biochemical measurements

3.3.1 Reagents, plant material and sample preparation

3.3.1.1 Reagents

All solvents used herein were of HPLC grade (hexane, methanol). To make the stock solution for preparation of standards the following were purchased from Sigma Aldrich (Dorset, UK): sucrose, glucose, fructose, mannoheptulose. Perseitol (D-glycero-D-galacto-heptitol) was obtained from Industrial Research Ltd (IRL-Fine Chemicals, New Zealand).

3.3.1.2 Plant material and sample preparation

Each avocado was cut in half vertically into two equal sections using a sharp knife. One half of the avocado was discarded. The skin of the remaining half of the avocado was removed and the flesh chopped into small chunks. The flesh (*ca*. 30 g) was snap-frozen in liquid nitrogen and held at -40 ^{o}C until required. The samples were freeze dried using an Alpha 1-4 Christ LDC-1 freeze-dryer and pump (Edwards Super Modulo, Sussex, UK). A finely disperse powder was obtained by grinding the sample with a mortar and pestle. Lipids were extracted using the method reported by Meyer *et al*. (2008). Briefly, lyophilized mesocarp tissue (1 g dry weight) was repeatedly homogenized with hexane. The mixture was filtered through a 5.5 cm diameter Fisherband QL 100 filter paper (Fisher Scientific, Leic., UK) under vacuum. The residue was washed with hexane and recovered from the filter paper. The oil fraction was discarded. Non-structural carbohydrates were extracted as previously reported (Davis *et al.*, 2007) with modifications. Freeze-dried avocado powder (150 mg) was combined with 3 ml of 62.5:37.5 HPLC grade methanol: water (v/v) and mixed well in a vial (7 ml polystyrene bijou vial). The vial containing the supernant was placed in a shaking water bath (to prevent layering) at 55 °C for 15 min, removed every 5 min and vortexed briefly. The sample was allowed to cool before being filtered through a 0.2 µm pore (Millipore Corp., MA) diameter filter unit and stored at -40 °C until required. Extracts were diluted 1:5 ml with water ready for immediate analysis (method depicted in Appendix C).

3.3.2 Quantification of non-structural carbohydrates (NSC)

Fructose, glucose, sucrose, mannoheptulose and perseitol were determined using HPLC system comprising a P580 pump, GINA 50 autosampler and ASI-100 Automated Sampling Injector (Dionex, CA, USA). The extract (20 µl) was injected into a Prevail Carbohydrate ES column (with 250 x 4.6 mm diameter, 5 µm particle size) (Alltech, UK; Part No. 35101) connected to a Prevail Carbohydrate ES guard cartridge (7.5 x 4.6 mm in diameter) (Alletech, UK; Part No. 96435). The mobile phase was HPLC graded water (A) and acetonitrile (B) filtered through a 0.4 µm filter and degassed by sparging with He for 15 min. There was a linear increase/decrease of solvent A. Solvent A was increased to 22 % at 25 min; 22:17 %, after 5 min at a flow rate of 0.5 ml min^{-1} and column temperature was held at 30 °C. Eluted carbohydrates

were monitored by an evaporative light scattering detector (ELSD 2420, Waters, MA, USA) connected to the Dionex system using a UCI-50 universal chromatography interface. Mixed standards of known concentration and composition were prepared. The presence and abundance of glucose, fructose, sucrose, mannoheptulose and perseitol in each sample were quantified by comparing the sample peak to that of the calibration standard (procedure shown in Appendix C).

3.4 Statistical analysis

All statistical analysis was performed using Genstat for Windows version 10 (VSN International Ltd., Herts., U.K.). Data obtained was subjected to analysis of variance (ANOVA), followed by a comparison of the means according to a Least significant difference (LSD) test at $P<0.05$.

3.5 Results and discussion

3.5.1 Quality attributes (physiological measurements)

Ethylene can accumulate in the storage environment accounting for premature ripening of climacteric fruits (Pesis *et al.*, 2002; Valero and Serrano, 2010). In this study (Exp 3.1 Chilean avocados cv. Hass) ethylene levels accumulated in the storage boxes every 24 h before being flushed daily. Reduction of exogenous ethylene in the storage atmosphere of avocado (cv. Hass; Exp 3.1) in response to e+® Ethylene Remover was observed in Table 3.1 and was consistent with that reported previously (Terry *et al.*, 2007b; Meyer and Terry, 2010).

Table 3.1. Ethylene concentration (μl l^{-1}) within 13 l sealed boxes (n=18) containing Chilean avocado cv. Hass (Exp 3.1) packed at source under different treatment (TR1): UN (untreated); E+ (e+® Ethylene Remover coated sheets); BF (buffer treatment). Fruit were held at 5 °C with or without e+® Ethylene Remover (5 g) (TR2). LSD (P<0.05) = 0.812.

TR1	**TR2**	**Ethylene concentration (μl l^{-1})**				
		Day 7	Day 10	Day 24	Day 28	Day 31
UN	Control	0.045^{a}	0.093^{ab}	2.560^{e}	2.414^{e}	3.008^{e}
	E+	0.006^{a}	0.005^{a}	0.071^{ab}	0.083^{ab}	0.123^{abcd}
E+	Control	0.036^{a}	0.017^{a}	0.061^{ab}	0.122^{abcd}	0.118^{abc}
	E+	0.055^{ab}	0.027^{a}	0.033^{a}	0.009^{a}	0.044^{a}
BF	Control	0.033^{a}	0.025^{a}	0.933^{d}	0.920^{c}	0.858^{bc}
	E+	0.003^{a}	0.004^{a}	0.025^{a}	0.358^{ab}	0.858^{bc}

Fruits were placed inside 13 l boxes on day 0 therefore no ethylene.

Pre-climacteric fruits which had received the initial e+® Ethylene Remover treatment at source had overall significantly reduced ethylene levels when stored at 5 °C for 31 days (refer to Appendix A, Table A.1 for the statistical data). The e+® Ethylene Remover treated fruits showed an average ethylene level of 0.05 μl l^{-1}. This concentration is below the threshold level (0.1 μl l^{-1}) for biological activity (Kader, 1985). Interestingly, fruits that were transported in the same pallet as the treated fruits (buffer (BF)) during transit, hence in closer proximity to the treated fruits, exhibited an average ethylene level of 0.3 μl l^{-1} in their storage boxes, which was significantly lower in contrast to the untreated fruits (0.8 μl l^{-1}). Exogenous ethylene stimulates the system 2 ethylene biosynthesis also known as the autocatalytic ethylene (Sisler and Serek, 2006; Barry and Giovannoni, 2007). Therefore, removing the exogenous ethylene will delay the autocatalytic ethylene production. During transit buffer fruits were in an environment where ethylene was adsorbed which possibly delayed the initiation of the autocatalytic ethylene production hence lower ethylene in the buffer fruit environment than the

untreated. The efficacy of e+® Ethylene Remover in reducing the exogenous ethylene within the storage atmosphere of the fruits was enhanced when fruits were treated at source. Fruits treated at source and later treated after transit (E+/E+ fruits) had reduced ethylene in their storage boxes after 31 days at 5 °C. In addition, untreated fruits treated after 5 weeks of transit with the e+® Ethylene Remover powder (UN/E+) exhibited lower ethylene levels compared to overall untreated fruits (UN/C), respectively (Table 3.1). Ethylene accumulated inside the untreated boxes resulting in an overall high ethylene production of these fruits during storage (Table 3.1). Results here verify previous findings regarding the effectiveness of e+® Ethylene Remover in its ethylene adsorption capacity in conditions of low temperature (5 °C) and high % RH (Terry *et al.*, 2007b; Meyer and Terry, 2010). In agreement with the observed ethylene reduction in the storage atmosphere of the treated fruits, the ethylene induced ripening was consequently retarded. Avocados like most climacteric fruits exhibit increase in respiration with maturity (Kader and Arpaia, 2002; Hershkovitz *et al.*, 2005). In Exp 3.1 avocados, respiration rate was significantly lower in response to the e+® Ethylene Remover applied after transit (Appendix A, Table A.6). Fruits treated after transit exhibited an average respiration rate of 155.4 ml kg^{-1} h^{-1} which was significantly lower compared to the corresponding control fruits (178.9 ml kg^{-1} h^{-1}). In accordance, in Exp 3.2 respiration rate increased significantly after 30 days (Appendix A, Table A.15) (at 5 ^{o}C) (Table 3.2). Treatment with e+® Ethylene Remover at source and or after 5 weeks of transit correlated with significantly reduced respiration. Following an incubation period (1 h) fruits treated at source exhibited significantly lower respiration rate (168.6 ml kg^{-1} h^{-1}) in contrast to the overall control fruits (185 ml Kg^{-1} h^{-1}) after 30 days under cold storage, respectively. In addition, treatment with e+® Ethylene Remover after transit also resulted in significantly reduced respiration rate. Following 30 days under 5 ^{o}C, fruits treated at source and after transit showed lowest respiration rate (183.2 ml Kg^{-1} h^{-1}), which was significant (P<0.05) in contrast to the fruits treated only at source (219.4 ml Kg^{-1} h^{-1}) and the control fruits (231.4 ml Kg^{-1} h^{-1}). Results discussed here are in accordance with previous findings using the ethylene remover treatment.

Table 3.2. Respiration rate (ml kg^{-1} h^{-1}) (Exp 3.2 late season Peruvian) of avocado cv. Hass (n=12 fruits) packed at source under different treatment (TR1): Control; E+ (e+® Ethylene Remover coated sheet). Fruit were held at 5 °C with or without e+® Ethylene Remover (coated sheets) (TR2). LSD: (P<0.05) =23.73.

Respiration rate (ml Kg^{-1} h^{-1})		**TR2**	
Day 5 °C	**TR1**	**Control**	**E+**
0	Control	125.9^{ab}	136.6^{abc}
14	Control	168.7^{de}	147.2^{bcd}
	E+	121.3^{a}	150.3^{cd}
30	Control	231.4^{h}	198.5^{fg}
	E+	219.4^{gh}	183.2^{ef}

Softening of avocados involves the evolution of ethylene (Jeong and Huber, 2004; Jeong and Huber, 2004; Maftoonazad and Ramaswamy, 2005). Consistent with this, softening was accelerated where high ethylene levels were observed in storage boxes (Figure 3.3). In agreement with the observed ethylene reduction in the storage atmosphere of the treated fruits, the ethylene induced ripening was consequently retarded as previously reported (Meyer and Terry, 2010). This is demonstrated by a significant reduction in softening of treated fruits during storage at 5 °C for 31 days (Appendix A, Table A.5a and 5b). Accordingly, the application of e+® Ethylene Remover sheets at source rather than after a transit period of up to 5 weeks when fruits are expected to be more mature, was found to enhance the efficacy of this treatment. Thus, fruits treated before and after transit (E+/E+ fruits) in Exp 3.1 showed significantly higher firmness in contrast to fruits treated only at source (E+/C) and those treated only after transit (UN/E) (Figure 3.3). These data demonstrate that e+® Ethylene Remover had a great efficacy on avocado quality, but the time of application must be chosen carefully for optimum efficacy. Higher firmness in fruits treated at source substantiates that ethylene was removed by the e+® Ethylene Remover coated sheets

during transit hence the benefits of this newly developed format. Interestingly, buffer fruits (fruits arriving in the same pallet as the treated fruits) were significantly firmer (Figure 3.3) in contrast to untreated fruits, which is in accordance with the results seen for ethylene levels in the storage boxes (Table 3.1).

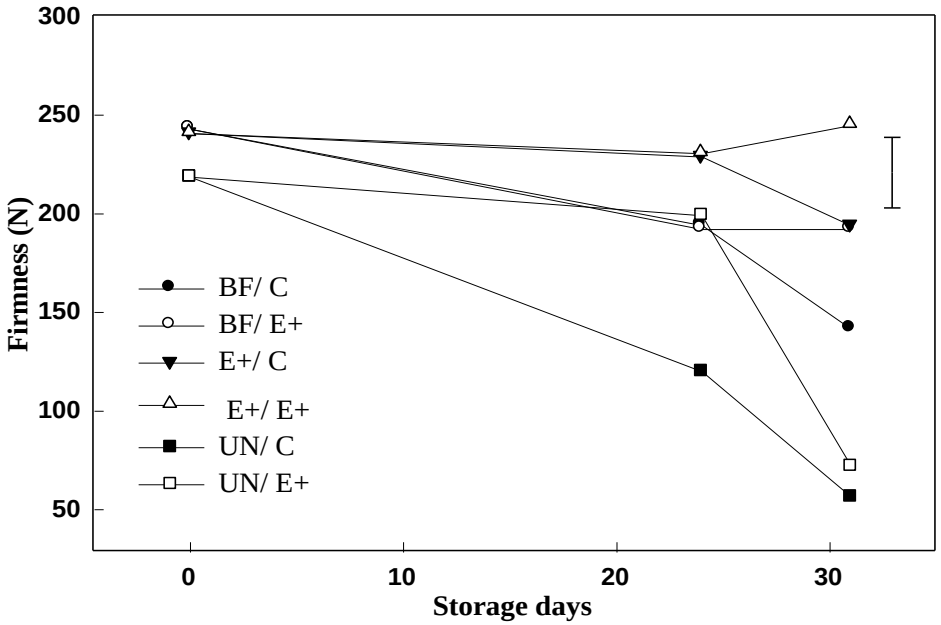


Figure 3.3. Effect of treatment at source : UN (untreated)); E+ (e+® Ethylene Remover coated sheet); BF (buffer treatment) and after transit with (E+) or without (C) e+® Ethylene Remover (5 g) giving six different treatment combination (BF/C, BF/E+, E+/C, E+/E+, UN/C, UN/E+) on the changes in firmness (N) of pre-climacteric avocado cv. Hass (early season Chilean Exp 3.1) fruits (n=9) stored at 5 °C within 13 l boxes for 31 days. LSD bar (P<0.05).

Similarly, in Exp 3.2 fruits treated at source and after transit with the e+® Ethylene Remover coated sheets exhibited significantly higher firmness compared to other fruits (Appendix A, Table A.11). Treated fruits were considerably more firm on arrival (152.3 N) in comparison to the control fruits (107.1 N), which confirms the effectiveness of the treatment at source. Coupled with this, fruits treated at source and after transit (E+/E+) were overall more firm (30 N) than those treated after transit

(18.8 N) after 30 days at 5 ^{o}C (Table 3.3, Figure 3.4). However, there was no significant interaction between treatment and storage with regards to firmness. Since there was an overall significant effect of treatment 1 (TR1) with e+® Ethylene Remover, results suggests that the e+® coated sheet was an effective method of delaying ripening during transit. However, once the fruits were allowed to ripen there were no significant differences in the firmness (Table 3.3, Table 3.4). In Exp 3.2, fruits were placed on trays with the coated sheets during ripening, however, differences between the treated and untreated fruits in shelf life condition were not evident. It is likely that at ambient condition the high rate of ethylene production and metabolic activity outstrips the ethylene adsorbed by the e+® Ethylene Remover. Equally, fruits in Exp 3.1 ripened heterogeneously once the treatment was discontinued and fruits were placed under shelf life condition (Table 3.4). This is especially important since 1MCP; the current commercial treatment for avocados, can cause uneven ripening within fruit batch (Woolf *et al.*, 2005; Meyer and Terry, 2010).

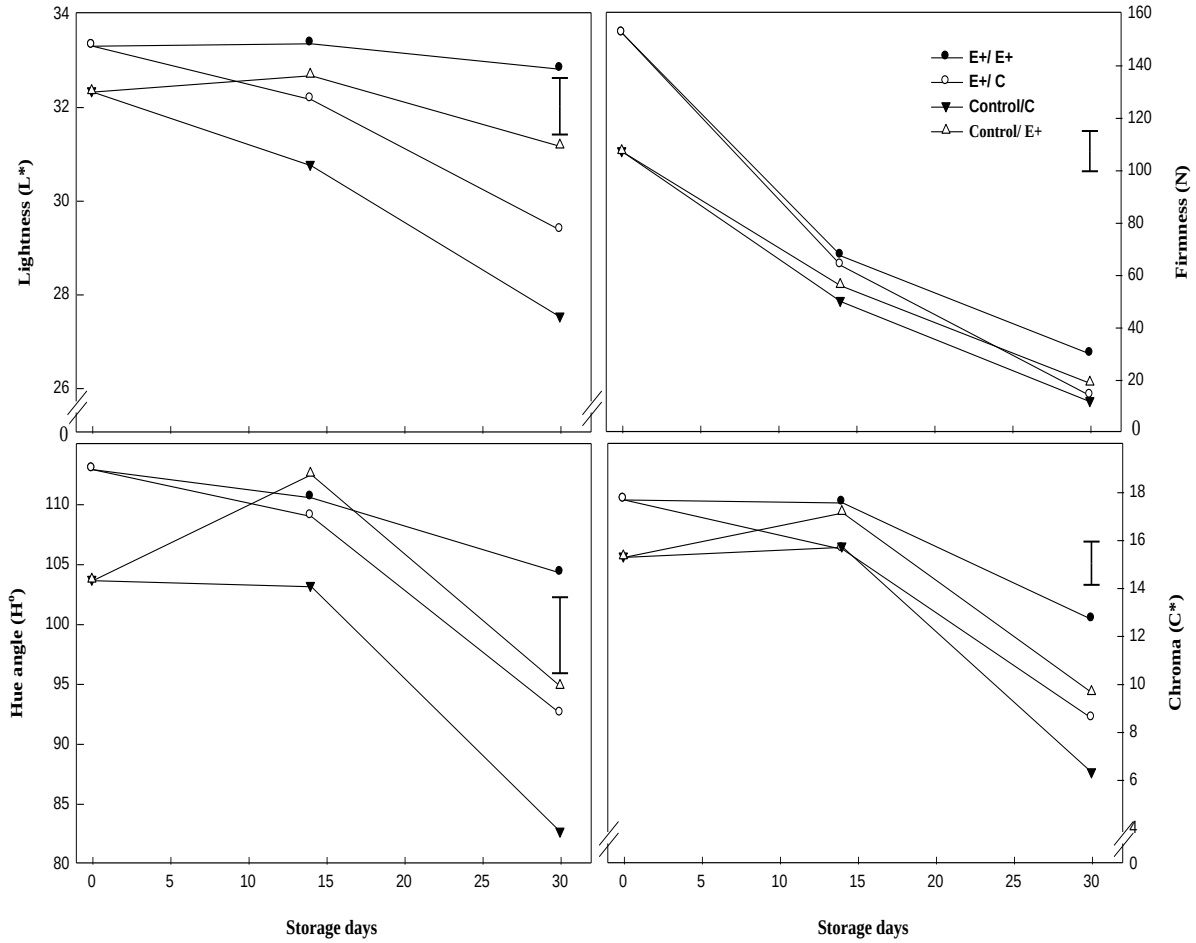


Figure 3.4. Effect of treatment at source (control; e+® Ethylene Remover (E+)) and after transit with (E+) or without (C) e+® Ethylene Remover sheets giving four different treatment combinations (E+/E+, E+/C, Control/C and Control/E+) on the changes in hue angle (H^o), lightness (L*) chroma (C*) and firmness (N) of pre-climacteric avocados cv. Hass (late season Peruvian Exp 3.2) fruits (n=12) held at 5 ^oC in open crates for 30 days.

Accordingly, the colour of avocado was significantly retained where ethylene levels had been reduced. Treatment with e+® Ethylene Remover caused a marked delay on the colour (Appendix A, Table A.4), which was complementary to the firmness data. Moreover, greener avocados with e+® Ethylene Remover treatment confirms ethylene role in chlorophyll catabolism (Hershkovitz *et al*., 2005). On arrival, fruits from all treatments showed similar colour values (L*, C* and H^o) (Table 3.3, Table 3.5) in both

experiments. In addition, in Exp 3.1 fruits which had received no treatment at source and after transit (UN/C) were significantly darker during storage at 5 ^{o}C and 19 ^{o}C and E+/E+ fruits were considerably greener in comparison to other treatments, this can be seen visually in Figure 3.5. Moreover, in Exp 3.2 following the ripening period, the colour (Appendix A, Table A.12) was better maintained in the treated fruits. Pictures (Figure 3.6) taken of fruits (Exp 3.2) taken after a ripening period of 5 days following day 0 of cold storage demonstrate the importance and the efficacy of treatment at source. That said, during ripening there were little differences in colour of the fruits which is consistent with firmness data where fruits exhibited normal ripening at ambient condition. That said, the external colour is not always consistent with the firmness of the fruits, which has been observed by others (Cox *et al.*, 2004). Moreover, following 31 days at 5 C, fruits which had received treatment at source (Exp 3.1) maintained greenness compared to the overall greenness of buffer and untreated fruits (Table 3.5, Figure 3.5). These results coincide with results from firmness and ethylene measurements. Firmness and colour of all fruits exhibited a decline during cold storage and transfer to ambient conditions, but more rapid softening and loss of colour was seen in the untreated fruits. Results are also in agreement with previous literature using e+® Ethylene Remover on avocados (Meyer and Terry, 2010) where e+® Ethylene Remover treatment retained greenness of avocados.

Table 3.3. Effects of treatment at source (TR1): UN (untreated)); E+ (e+® Ethylene Remover coated sheet); BF (buffer treatment) and treatment after transit (TR2) with (E+) or without (control) e+® Ethylene Remover (5 g) giving six different treatment combination (BF/Control, BF/E+, E+/Control, E+/E+, UN/Control, UN/E+) on the firmness (N) and hue angle (H^o) of pre-climacteric avocado cv. Hass (Exp 3.2 late season Peruvian) fruits (n=12) held at 5 ^oC.

			Firmness (N)		**Hue angle (H^o)**	
				TR2		
Day at 5 ^oC	**Days at 19 ^oC**	**TR1**	**Control**	**E+**	**Control**	**E+**
0	0	Control	209.6	234.0	124.48	124.82
	5	E+	4.6	70.6	82.87	101.07
14	0	Control	96.4	107.4	113.46	122.19
		E+	124.2	131.6	123.19	121.8
	5	Control	3.5	4.5	92.86	102.82
		E+	3.5	3.3	94.82	99.34
30	0	Control	14.6	22.5	88.15	102.25
		E+	16.6	49.3	98.15	108.27
	5	Control	8.9	15.1	77.12	87.35
		E+	11.3	10.8	86.94	100.31
LSD (*P<0.05*)			21.67		8.961	

* *No statistical differences in this data.*

Table 3.4. Effects of treatment at source (TR1): UN (untreated)); E+ (e+® Ethylene Remover coated sheet); BF (buffer treatment) and treatment after transit (TR2) with (E+) or without (control) e+® Ethylene Remover (5 g) giving six different treatment combination (BF/Control, BF/E+, E+/Control, E+/E+, UN/Control, UN/E+) on the firmness (N) of Chilean avocados cv. Hass (n=9 fruits, Exp 3.1). Firmness was determined on removal from cold storage (day 24 and 31) and following a ripening period of 4 days at 19 °C. LSD (P<0.05) = 31.41.

			Firmness (N)	
				TR2
Days at 5 °C	**Days at 19 °C**	**TR1**	**Control**	**E+**
24	0	UN	120.0^{d}	199.1^{e}
		E+	228.8^{f}	230.5^{f}
		Buffer	194.1^{e}	192.0^{e}
	4	UN	6.3^{a}	4.5^{a}
		E+	5.8^{a}	9.0^{a}
		Buffer	6.7^{a}	7.6^{a}
31	0	UN	56.4^{bc}	71.9^{c}
		E+	193.9^{e}	244.8^{f}
		Buffer	141.4^{d}	192.0^{e}
	4	UN	4.5^{a}	4.4^{a}
		E+	24.6^{a}	35.1^{ab}
		Buffer	7.2^{a}	10.1^{a}

Table 3.5. Effects of treatments at source (TR1): UN (untreated)); E+ (e+® Ethylene Remover coated sheet); BF (buffer treatment) and treatment after transit (TR2) with (E+) or without (control) e+® Ethylene Remover (5 g) giving six different treatment combination (BF/Control, BF/E+, E+/Control, E+/E+, UN/Control, UN/E+) on the hue angle (H^o) and lightness (L*) of Chilean avocados cv. Hass (n=9 fruits, Exp 3.1). Colour parameters were determined on removal from cold storage (day= 0) and after 4 days at 19 ^oC.

		Hue angle (H^o)		Lightness (L*)	
		TR2			
Days at 19 ^oC	**TR1**	**Control**	**E+**	**Control**	**E+**
0	UN	88.49^{d}	100.47^{e}	29.25^{c}	31.56^{d}
	E+	118.05^{fg}	121.45^{g}	32.07^{d}	33.13^{d}
	Buffer	101.40^{e}	110.89^{f}	31.51^{d}	31.85^{d}
4	UN	54.75^{ab}	46.14^{a}	27.34^{ab}	26.82^{a}
	E+	59.51^{b}	71.78^{c}	28.55^{bc}	28.25^{bc}
	Buffer	61.17^{b}	60.70^{b}	27.52^{ab}	28.35^{bc}
LSD (*P<0.05*)		9.496		1.379	

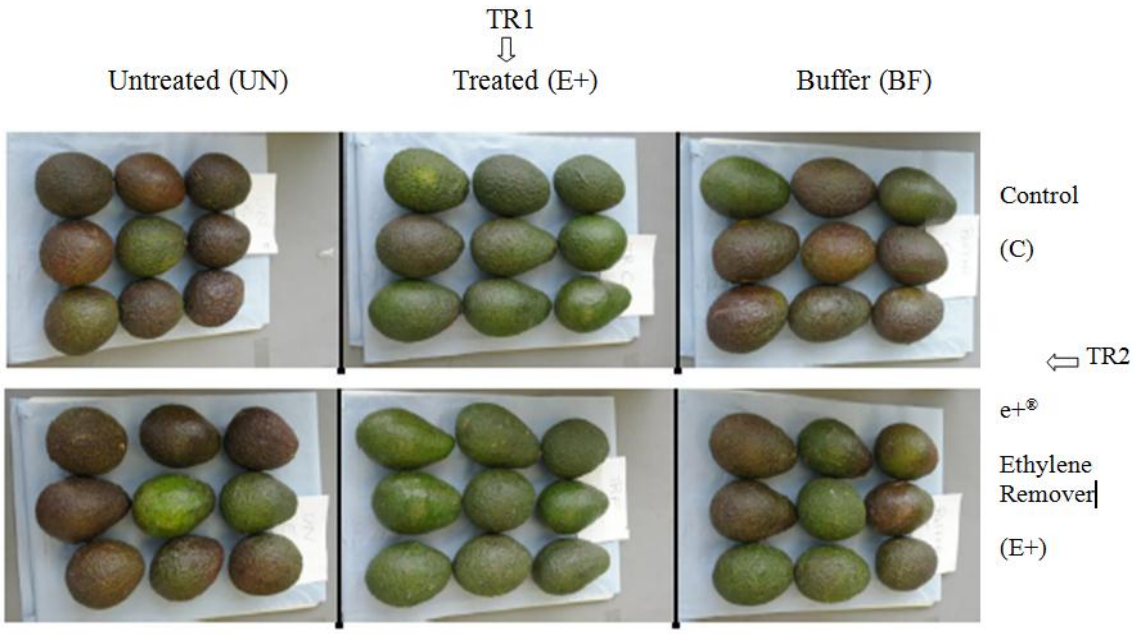
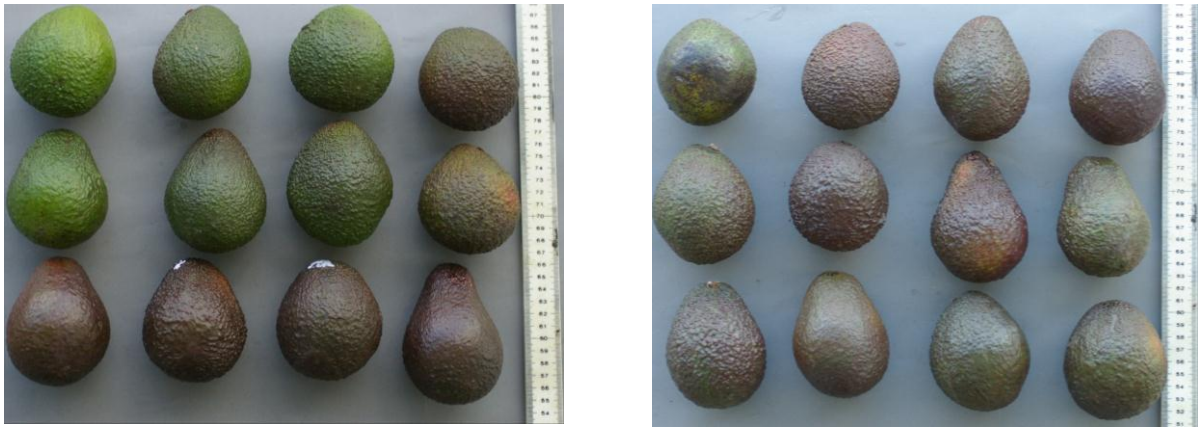


Figure 3.5. Pictures taken of fruits (n=9) treated at source (TR1): UN (untreated)); E+ (e+® Ethylene Remover coated sheet); BF (buffer treatment) and after transit (TR2) with (E+) or without (control) e+® Ethylene Remover (5 g). Pictures were taken after 31 days at cold storage (5 ^oC).



E+ **Control**

Figure 3.6. Pictures of avocados (Peruvian, Exp 3.2, n=12 fruits) treated with or without e+® Ethylene Remover coated sheets before transit. Pictures were taken after ripening period of 5 days following day 0 of cold storage.

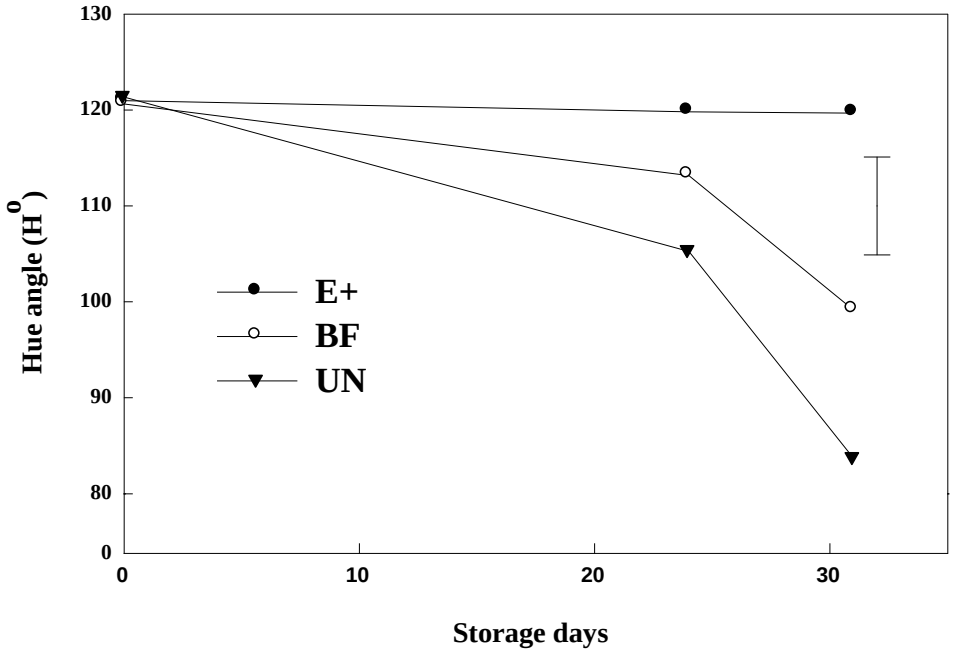


Figure 3.7. Effect of treatment at source: Buffer (BF), e+® Ethylene Remover coated sheets (E+) and Untreated (UN)) on the change in hue angle (H^o) of pre-climacteric avocado cv. Hass fruits (Exp 3.1) stored at 5 ^oC within 13 l boxes for 31 days. Values presented are means of subsample number of fruits (n=9). LSD bar (P<0.05).

3.5.2 Biochemical measurements

The flavour and nutritional benefits of avocados is highly attributed to the content of sugars and lipids in the mesocarp. As reported previously (Bertling and Bower, 2005; Landahl *et al.*, 2009; Meyer and Terry, 2010), mannoheptulose, perseitol and sucrose were found in substantial amounts in the mesocarp of avocados cv. Hass (Table 3.6). C6 sugars (glucose and fructose) were found to be present; however, these were not quantified due to low levels (Figure 3.8). The prominent sugars in avocados mannoheptulose and its alcohol form perseitol have received recent interest (Liu *et al.*, 1999a; Liu *et al.*, 2002) mainly because of their health promoting properties. The role of C7 sugars in avocados is an on-going research. A profound change in the C7 sugar concentration of avocados has been associated with maturity. Previous literature (Bergh, 1992; Landahl *et al.*, 2009; Meyer and Terry, 2010), has postulated that these carbohydrates may act as ripening inhibitors. Results herein show that mannoheptulose concentration was substantially higher in pre-climacteric avocados (early season avocados cv. Hass, Exp 3.1) on arrival (59.3 mg g^{-1} DW) than after 31 days at 5 ^{o}C (18.9 mg g^{-1} DW). Levels decreased considerably across all treatments (Table 3.6), for example UN/Control fruits after 24 days at 5 ^{o}C had a concentration of 27.8 mg g^{-1} of mannoheptulose on day 31, which decreased to 14.9 mg g^{-1} (Table 3.6). Likewise, perseitol content declined during storage nonetheless, concentration of this sugar was significantly affected by e+® Ethylene Remover treatment after transit (TR2) (Appendix A, Table A.9). Overall, fruits treated after transit exhibited significantly higher persitol content (37.0 mg g^{-1} DW) than those that were left untreated (29.5 mg g^{-1} DW). Similarly, Meyer and Terry (2010) also showed that avocados treated with e+® Ethylene Remover had higher persitol content vs. control. The decline in C7 sugars observed with storage and high persitol content detected in fruits treated with e+® Ethylene Remover after transit supports previous findings (Liu *et al.*, 1999b; Liu *et al.*, 2002; Bertling and Bower, 2005; Landahl *et al.*, 2009; Meyer and Terry, 2010) on the importance of C7 sugars in fruit quality. Sucrose concentration was substantially reduced during storage (Table 3.6, Table 3.7). An interaction between treatment 1 and outturn was observed (Appendix A, Table A.60) where significant differences in the data were mainly between outturns however, no particular pattern was apparent between treatments (Table 3.7). Fruit ripening is generally associated with sugar metabolism (Barry and

Giovannoni, 2007; Valero and Serrano, 2010) thus, decrease in sucrose may be attributed to the hydrolysis of sucrose to fructose and glucose during ripening; however these simple sugars were not quantified.

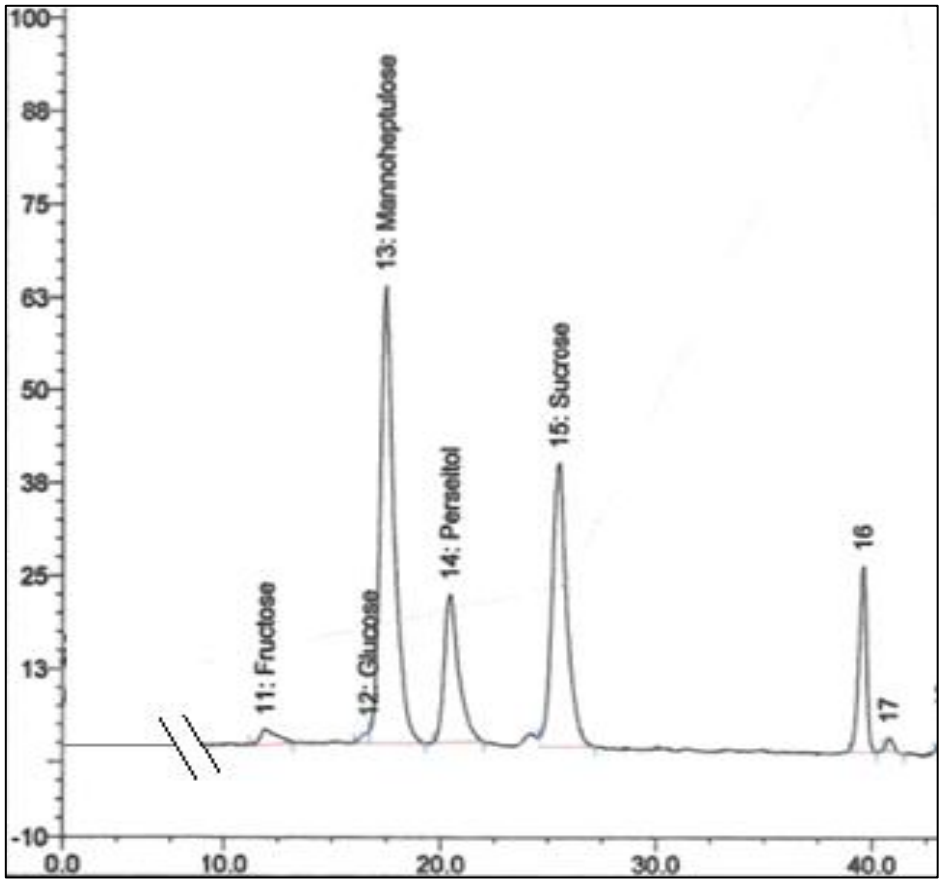


Figure 3.8. Typical HPLC chromatogram using prevail column connected to ELSD detector of the main sugars in avocados mesocarp cv. Hass (early season Chilean Exp 3.1).

Table 3.6. Effect of treatment at source: Buffer (BF), e+® Ethylene Remover sheet (E+) and Untreated (UN)) and treatment after transit (TR2) with (E+) or without (control) e+® Ethylene Remover (5 g) giving six different treatment combination (BF/Control, BF/E+, E+/Control, E+/E+, UN/Control, UN/E+) on the concentration of the main sugars in avocado cv. Hass (Exp 3.1) fruits (n=9) mesocarp stored for 31 days inside 13 l plastic boxes at 5 ^{o}C. Values expressed here are in mg g^{-1} in dry weight (DW). **No statistical differences in this data.*

Days at 5 °C	TR1	TR2	Sucrose	Mannoheptulose	Perseitol
0	UN	Arrival	42.88	62.1	71.3
	BF	Arrival	38.61	49.0	61.8
	E+	Arrival	38.03	67.0	77.6
24	UN	Control	21.88	27.8	32.7
		E+	22.87	25.3	44.7
	BF	Control	23.16	24.3	36.7
		E+	22.79	27.0	39.0
	E+	Control	28.65	30.1	24.2
		E+	22.34	36.4	42.5
30	UN	Control	25.31	14.9	21.3
		E+	30.56	19.9	31.4
	BF	Control	25.53	19.4	28.8
		E+	23.51	16.6	25.9
	E+	Control	28.60	19.1	33.3
		E+	29.69	23.3	38.3
LSD (*P<0.05*)			10.603	20.93	23.37

Table 3.7. Effect of treatment at source (TR1): Buffer (BF), e+® Ethylene Remover coated sheet (E+) and Untreated (UN)) on the concentration of sucrose in avocados cv. Hass (Exp 3.1) fruits mesocarp stored for up to 31 days (5 °C) and after subsequent ripening of 4 days (20 °C). Results are expressed in mg g-1 DW. LSD (P<0.05) = 7.498.

		Sucrose (mg g^{-1} DW)	TR1	
Days at 5 °C	**Days at 20 °C**	**UN**	**E+**	**Buffer**
0	0	39.56^{ef}	44.30^{ef}	38.64^{de}
	4	46.20^{f}	31.76^{cd}	38.58^{de}
24	0	17.42^{a}	27.87^{bc}	19.88^{a}
	4	27.32^{bc}	23.12^{ab}	26.07^{bc}
31	0	28.29^{bc}	28.63^{bc}	27.70^{bc}
	4	27.58^{bc}	29.67^{c}	21.34^{ab}

3.6 Conclusion

Results of the present study have substantiated previous findings using the e+® Ethylene Remover treatment in its ethylene removal capacity and preserving avocado quality. This is the first study to report on the efficacy of the newly developed e+® Ethylene Remover sheets. The e+® Ethylene Remover coated sheets efficacy was demonstrated in a 'real world' scenario and shown to delay softening and colour change in avocados 'Hass' fruit. Furthermore, results from two different trials have shown that the efficacy of the e+® Ethylene Remover can be enhanced once applied to avocados during the early stages of storage thus; the timing of the treatment is a crucial factor. This study substantiates previous findings on the role of C7 sugars in avocado ripening given a significant decrease was observed with storage. Perseitol was overall significantly higher in fruits treated with e+® Ethylene Remover after 5 weeks of transit, possibly relating firmer fruits.

CHAPTER FOUR

4 CONTROL OF ETHYLENE USING E+® ETHYLENE REMOVER DURING STORAGE OF PLUOTS (*PRUNUS SALICINA L. X PRUNUS ARMENIACA L.* CV. FLAVOR KING)

4.1 Introduction

Plum ripening is typical of climacteric fruit, however; some cultivars have been shown to display suppressed-climacteric behaviour, having reduced capacity to convert ACC to ethylene (Abdi *et al.*, 1997; Abdi *et al.*, 1998). Generally, control of plum ripening stems from the control of ethylene and storage at low temperature to decrease respiration and metabolic activity (Salvador *et al.*, 2003; Luo *et al.*, 2009; Singh *et al.*, 2009).

Most plums/pluots marketed in the UK are imported. During transit, fruits can accumulate ethylene in their surrounding atmosphere or ethylene may build up due to contamination from other sources.

Overcoming the effects of ethylene using 1-MCP has been studied numerously for plums (Abdi *et al.*, 1998; Menniti *et al.*, 2004; Luo *et al.*, 2009) and was found to be effective. Specifically, treatment of Japanese plum (cv. Tegan Blue) with 1-MCP correlated with firmer fruits (Khan *et al.*, 2009); however others (Abdi *et al.*, 1998; Martínez-Romero *et al.*, 2003) demonstrated that the responses to 1-MCP vary with different cultivars and conditions. Nevertheless, inhibiting or reducing ethylene biosynthesis may play a significant role in enhancing consumer acceptability and reducing waste through enhanced storage life. Therefore, there is a need to prevent ethylene exposure throughout the transportation and distribution of fruits.

Plums are not only valued by consumers for their characteristic flavour and texture, but also for their health promoting properties (Liu, 2003; Vicente *et al.*, 2011). Pluots are a hybrid between plums (*Prunus salicina* L.) and apricots (*Prunus armeniaca* L.). However, their flavour and texture is much like that of plums given this hybrid is 75 %

plum. Pluots (cv. Flavour King) are registered under the trader ‘Zaiger’s Genetics’. This hybrid (reddish purple skin and red flesh) comes in numerous varieties of which the most popular are the King Flavour and Dapple Dandy, which are improved varieties in terms of flavour and are produced from a popular plum variety Santa Rosa (Parsons, 2007).

The use of e+® Ethylene remover was associated with better quality fruits in Chapter 3 and in other studies (Terry *et al.*, 2007b; Meyer and Terry, 2010). The objectives of this work were: **(a)** to investigate the feasibility and effectiveness of e+® Ethylene Remover sheets on pluots in the real world supply chain **(b)** to explorethe effect of treatment on the physiology and biochemistry of pluot plums stored with or without the e+® Ethylene Remover in the real world supply chain. Ethylene production was measured using a highly sensitive ethylene detectorcapable of measuring ethylene at very low concentrations (0.3 nl l^{-1}) in real time. This was used to measure ethylene production continuously as it evolved from the fruit during the ripening period.

4.2 Materials and methods

4.2.1 Plant material

Pluots (*Prunus salicina* L. *x Prunus armenica* L. cv. ˈFlavor Kingˈ) were sourced from South Africa and supplied by Univeg UK. Fruits were harvested on 25th January and transported to the packing house, where they were commercially packed in crates (double layer) and treated with or without e+® Ethylene Remover sheets (19 cm x 12.5 cm) with 1 % Pd loading. The e+® Ethylene Remover sheets (5 sheets) were placed face up in each layer on top of the fruits (Figure 4.1). Fruits were held at 1-3 ^{o}C during transit and the temperature was raised towards the end of transit to 7 ^{o}C. However, the transit was delayed and fruits were received late. Following 4 week transit period, the fruits were received at the Plant Science Laboratory and stored at 2 ^{o}C in their original crates inside 320 l chambers.

e+® Ethylene Remover treatment (E+)

Control

Figure 4.1. Fruits packed in crates with (E+) or without (control) the e+® Ethylene Remover coated sheets (n=10) with five sheets in each layer (n=2).

4.2.2 Treatments and storage regime

For each treatment, there were 3 chambers and each chamber contained 2 crates, hence 6 crates were sampled per treatment. The chambers were not sealed; instead the lids were left slightly ajar. Fruits (control n=2 and E+ n=2) were removed from cold storage following 0, 7, 14 days at 2 ^{o}C. Fruits (n=2 per treatment) were weighed and each placed inside a 1 l glass jar at 18 ^{o}C, ready for ethylene measurements and thereafter colour determined. Fruits taken for the measurements were the same weight (82 ± 0.5 g) and size.

4.2.3 Ethylene production

Fruits receiving the e+® Ethylene Remover treatment were placed inside jars containing e+® Ethylene Remover powder (2.5 g). For each treatment, one jar was labelled 'Long (L)' and fruits in these jars were kept at ambient temperature to ripen for a period of (4-6 days). The other jars (n=1) per treatment were labelled 'Short (S)'. Fruits in these jars (S) were swapped every day for new fruits (which had been stored at 2 ^{o}C) after 24 h at 18 ^{o}C where ethylene production was measured. At each outturn (after 0, 7 and 14 days at 5 ^{o}C) the 6 jars available for ethylene measurements were divided as demonstrated in (Figure 4.2). Ethylene measurements were also done on two control jars; a sealed empty jar (Blank) and a jar containing fresh e+® Ethylene Remover powder (2.5 g) alone. The E+ fruits were kept in the presences of the e+® Ethylene

Remover material within the jars to observe how the material affects the ethylene production pattern over the course of ripening.

Real time ethylene production was monitored with a newly developed photoacoustic laser based ethylene detector (ETD-300; Sensor Sense B.V., Nijmegen, The Netherlands) incorporated with a gas handling system (refer to Appendix B for details of the instrument and instrumental set up). There are two types of gas measurements which can be performed using this machine: *Stop and Flow* and *Continuous flow*. In this trial, *Continuous flow* was performed to measure real time ethylene production. This guarantees continuous gas refreshment and stable conditions for the samples. There were a total of six jars connected to the ethylene detector each containing a single fruit and ethylene production of fruit (n=1) for each treatment (control and E+) were determined throughout ripening (4-6 days); this was done in two separate jars as described above and shown in (Figure 4.2). The jars were sealed with gas tight lids and rubber septum. Ethylene production was measured (18 ^{o}C) as it evolved from the fruit for a 10 min period. The jars were flushed at a continuous flow rate of 3 l h^{1} with cleaned compressed air (explained in detail in Appendix B) and a total pressure of 1 bar. This instrument has been described in detail by others (Cristescu *et al*., 2002). The experimental design lacked replicates due to the instrumental design and hence number of jars attached to the ETD-300 Ethylene detector, which was out of the operator control.

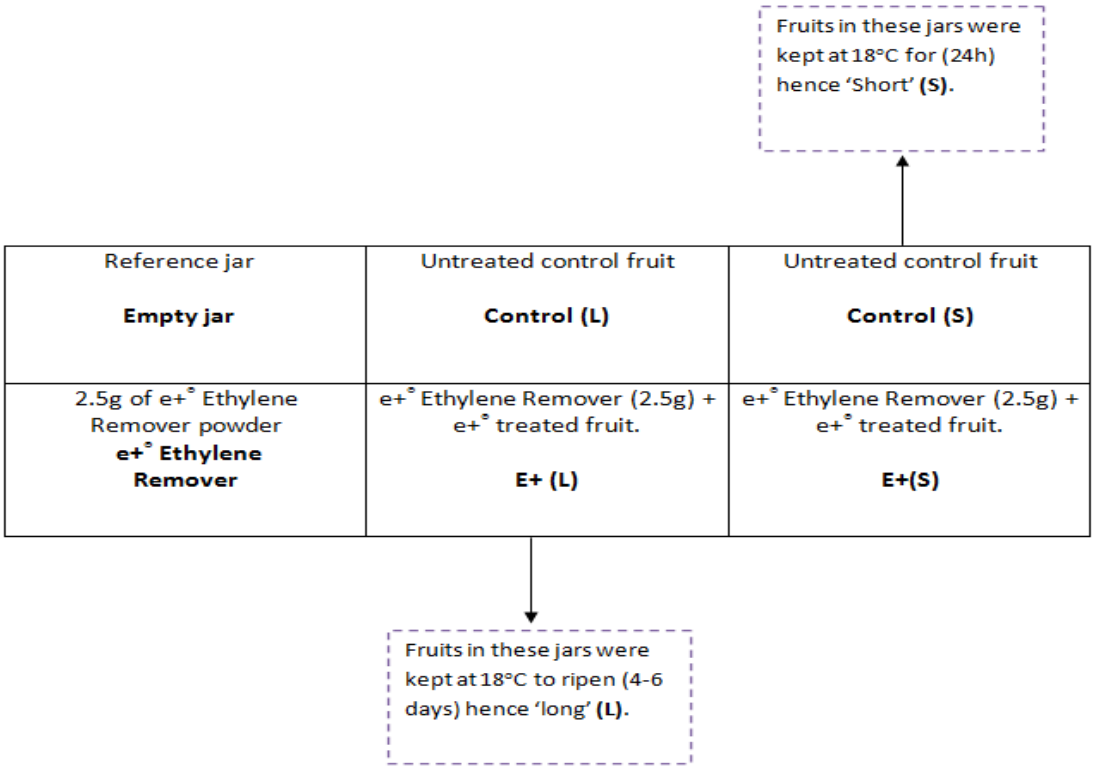


Figure 4.2. Experimental design showing the content of the six ethylene jars connected to the ETD-300 ethylene detector.

4.2.4 Colour measurements

The objective colour; lightness (L*), chroma (C*) and hue angle (H^o) were determined as described by others (Terry *et al.*, 2007b) and in Chapter 3 (section 3.2.5). Briefly, the mean of three readings around the equatorial regions was recorded for each fruit.

4.2.5 Firmness

Fruits (n=12) were measured for their firmness on arrival (day 0), thereafter fruits removed from the ethylene jars were taken for firmness measurement. Flesh firmness was measured as previously reported (Terry *et al.*, 2007b) and Chapter 3. Briefly, a uniaxial testing machine (model 5542, Instron, Norwood, MA) programmed to Bluehill 2, version 2.11, Instron with a curved 8 mm probe was used. The force required to puncture the tissue to a depth of 8 mm at a crosshead speed of 100 min^{-1} was recorded. Fruits were then snap frozen in liquid nitrogen for subsequent biochemical analysis.

4.3 Biochemical measurement

4.3.1 Reagents

The solvent used herein was HPLC grade (methanol). To make the stock solution for standard preparation the following compounds were purchased from Sigma Aldrich (Dorset, UK); sucrose, glucose, fructose, quercetin-3-glucoside, quercetin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-rutinoside and caffeic acid. For the determination of total antioxidant capacity, hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) and 2-2-Diphenyl-pikryl-hydrazyl (DPPH) used were purchased from Sigma Aldrich.

4.3.2 Extraction of individual anthocyanins, phenolic acids and flavonols

Anthocyanins, phenolic acid and flavonol compounds were extracted as previously reported (Terry *et al.*, 2007a). Briefly, 3 ml of MeOH: H_2O: HCl (70 %: 29.5 %: 0.5 % v/v) was added to freeze-dried pluot sample (150 mg) in a polystyrene bijou vial (7 ml). The sample was mixed thoroughly and placed immediately in a shaking water bath set at 35 ^oC for 1.5 h and agitated every 15 min. The sample was filtered and the extracts stored in a -40 ^oC freezer until required.

4.3.3 Quantification of individual anthocyanins, phenolic acids and flavonols

Phenolic acid (caffeic acid), anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) and flavonol (quercetin-3-rutinoside and quercetin-3-glucoside) were quantified using an Agilent 1200 series HPLC quaternary pump system equipped with an Agilent 1200 DE G1315D photodiode array with multiple wavelength detector (Alamar Gavidia *et al*., unpublished). Extracts were injected into a 250 x 4.6 mm, 5 µm Zorbax Eclipse XDB-C18 (Agilent) connected to an OPTI-GUARD 1 mm guard column (Crawford Scientific, UK). The mobile phase followed a quaternary gradient increase and decrease of solvent A (95 % water + 5 % methanol), solvent B (88 % water + 12 % methanol), solvent C (20 % water + 80 % methanol) and solvent D (100 % methanol). Formic acid (5 %) (v/v) was added to both HPLC grade water and methanol before preparing the following mobile phases. Mobile phase composition followed 100 % of solvent A and remained isocratic for 7 min., 7-9 min a linear gradient was applied to reach 50 % solvent B, 9-13 min isocratic 50 % solvent A and 50% solvent B,

13-20 min linear gradient to reach 100% B, 20-25 min isocratic 100 % B, 25-40 min linear gradient to reach 75 % B and 25 % C; 40-50 min, linear gradient to reach 72 % B and 28% C, 50-52 min linear gradient to reach 50 % B and 50 % C, 52-54 min linear gradient to reach 100 % D, 54-56 min isocratic at 100 % D. The flow rate was 1 ml min^{-1} and the column temperature was set at 40 ^{o}C. The anthocyanins (e.g. cyaniding-3-glucoside and rutinoside) were detected at 520 nm, phenolic acid (e.g. caffeic acid) at 280 nm, and flavonol (e.g. quercetin) at 355 nm. The concentration present in sample was determined by comparing sample peak area with that of authentic standard (0.01-0.2 mg ml^{-1}). Quercetin-3-glucoside and quercetin-3-rutinoside were found to elute at the same retention time, hence concentrations were reported as total quercetin.

4.3.4 Quantification of non-structural carbohydrates (NSC)

Sugars (fructose, glucose, and sucrose) were determined as described in Chapter 3 with some modifications. Briefly, the mobile phase was HPLC grade water (filtered through a 0.4 µm filter and degassed by sparging with He for 15 min). The flow rate was 0.5 ml min^{-1} and column temperature was set at 40 °C. Eluted carbohydrates were monitored by an evaporative light scattering detector (ELSD 2420, Waters, MA, USA) connected to the Dionex system using a UCI-50 universal chromatography interface.

4.3.5 Measurements of antioxidant capacity

The antioxidant capacity of the flesh and skin of pluot samples were measured as reported previously (Xu *et al.*, 2010). Extracts from the flesh of the pluot (100 µl) were diluted with 900 µl phosphate buffered saline (PBS) at pH 7.4, while the skin extract (50 µl) was diluted with 950 µl of the PBS buffer. A 100 µl aliquot of diluted sample was further added to a 3.9 ml methanolic solution of DPPH (0.0025 g^{-1}100 ml) in glass test tubes. The reaction mixture was kept in the dark to incubate for 60 min after which the samples were transferred to disposable cuvettes (1 cm × 1 cm × 4.5 cm) and the absorbance at 515 nm was recorded immediately using a Camspec M501 UV/Vis spectrophotometer (Camspec Ltd. Cambs., UK) where the concentration of the remaining radical activity of the DPPH (2-2-Diphenyl-pikryl-hydrazyl) was determined. The antiradical properties of the samples were calculated using the percentage inhibition of DPPH and known solution of Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic

acid (Trolox) purchased from Sigma Aldrich as a standard. The antiradical activities of the sample were expressed as Trolox equivalent antioxidant capacity (μmol Trolox g^{-1} DM) on their percentage inhibitions.

4.3.6 Statistical analysis

Statistical analysis was carried as described in Chapter 3 (section) however, given the limited number of jars connected to the ETD-300 Ethylene detector there were uneven replicates which was considered. For the long stored fruits n=1 fruit was taken per sampling week and for the short stored fruits n=1 fruits was taken daily for 4-6 days per sampling week. Thus, due to this imbalance and lack of replicates the anova only took into account the length of storage (Long and short) and treatments (E+ and control). Also a block structure was not used. Where the data was not normally distributed the logarithm was calculated.

4.4 Results and discussion

4.4.1 Ethylene production

Treatment of pre-climacteric avocados at source resulted in further reduction in ethylene during storage at 5 ^{o}C for 31 days (Chapter 3). Pluots (a plum hybrid) like avocados undergo long distant transit whereby ethylene can accumulate in the surrounding atmosphere. In the present study, fruits were treated with the e+® Ethylene Remover at source; in the packing house and treatment was continued throughout storage. Minimising the effect of ethylene via other treatments such as 1-MCP (Salvador *et al.*, 2003; Khan *et al.*, 2009; Luo *et al.*, 2009) and the use of nitric oxide (NO) as reported by Singh *et al*. (2009) has been shown to maintain fruit quality. However, this is the first study to report on the effect of e+® Ethylene Remover on pluot plums, which follows a different mode of action whereby ethylene is removed from the fruit environment.

Pluot fruits stored at 18 ^{o}C for 24 h (control (S) and (E+ (S)) exhibited a surge of ethylene production, which reached a maximum level and then decreased rapidly (Figure 4.3, Figure 4.4, Figure 4.5). Fruits stored with e+® Ethylene Remover treatment (2.5 g) (E+ (S)) displayed a similar ethylene production pattern as the controls; however, this peak in ethylene was reduced substantially. It is clear that when the fruits

were removed from cold storage and placed under ambient conditions (18 ^{o}C) they exhibit a dramatic rise in ethylene production. Measuring ethylene in real-time using the ETD-300 ethylene detector meant that the ethylene produced by the fruits could be monitored continuously throughout the ripening period, allowing this peak in ethylene production to be observed. Such observations are more difficult with conventional techniques such as gas chromatography given these are not in real time and thus trends overtime are difficult to observe. Concerning the ethylene produced by the fruits over the ripening period (4-6 days), fruits displayed a gradual increase in ethylene production (Figure 4.3, Figure 4.4, Figure 4.5) during the ripening period (control (L) and E+ (L)). Although the control fruits produced considerably higher ethylene in contrast to the E+ fruits both fruits presented similar ethylene pattern over the ripening (control (L) and E+ (L)) suggesting that e+® Ethylene Remover does not disrupt the normal ripening pattern. Moreover, ethylene was still detected for the E+ fruits however; it is probable that endogenous ethylene present in the tissues was sufficient to stimulate the autocatalytic ethylene production.

Following 14 days of storage (at 5 ^{o}C) the fruits stored for a short period (24 h) (control (S) and (E+ (S)) at 18 ^{o}C exhibited a gradual increase in ethylene after each 24 h for a period of 4 days where maximum level was observed. In accordance, the control (L) and E+ (L) fruits showed similar behaviour in the ethylene production during the ripening period (4 days). Similarly, Valero *et al*. (2004) showed that plums (*Prunus domestica* L., cv. 'President') exhibited climacteric ethylene production after 2 weeks of cold storage and 7 days at 20 ^{o}C. High ethylene produced by the control fruits infers that these fruits senesce more rapidly. Results herein are in accordance with results reported in Chapter 3 and in literature (Terry *et al.*, 2007b; Meyer and Terry, 2010) where the efficacy of e+® Ethylene Remover in reducing exogenous ethylene was demonstrated. Nonetheless, fruits were received late due to delayed transit to the UK and prior to the delay the ripening was triggered by a slight increase in temperature (7 ^{o}C). Triggering the ripening too early would have meant that the fruits were more mature than anticipated, influencing the data obtained.

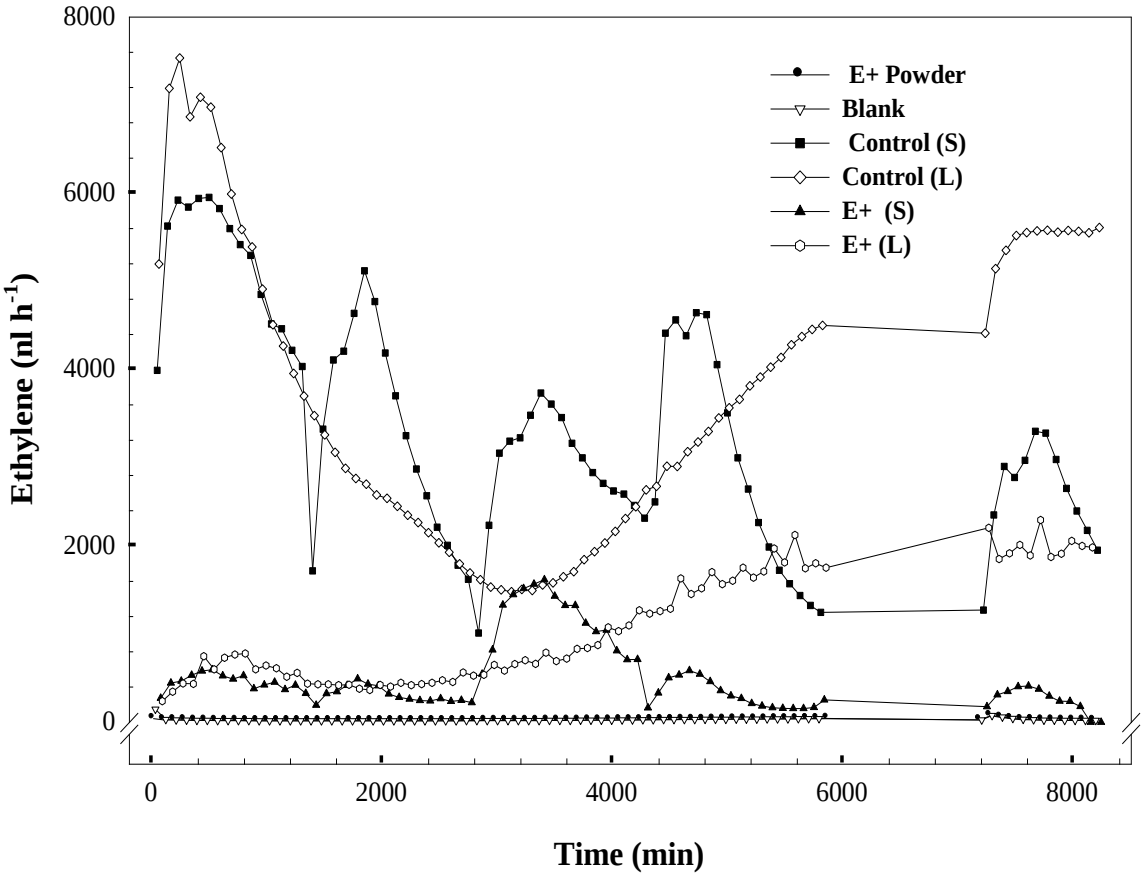


Figure 4.3. Ethylene production (nl h^{-1}) of pluots (cv. Flavor King) during ripening at 18 ^{o}C, on day 0 of storage (arrival). Long (L) stored fruits (n=1 fruit per treatment) were stored at 18 ^{o}C for the ripening period of 5 days. Short (S) stored fruits (n=5) were stored at 18 ^{o}C for 24 h. Ethylene production was measured using ETD-300 detector over 5 days.

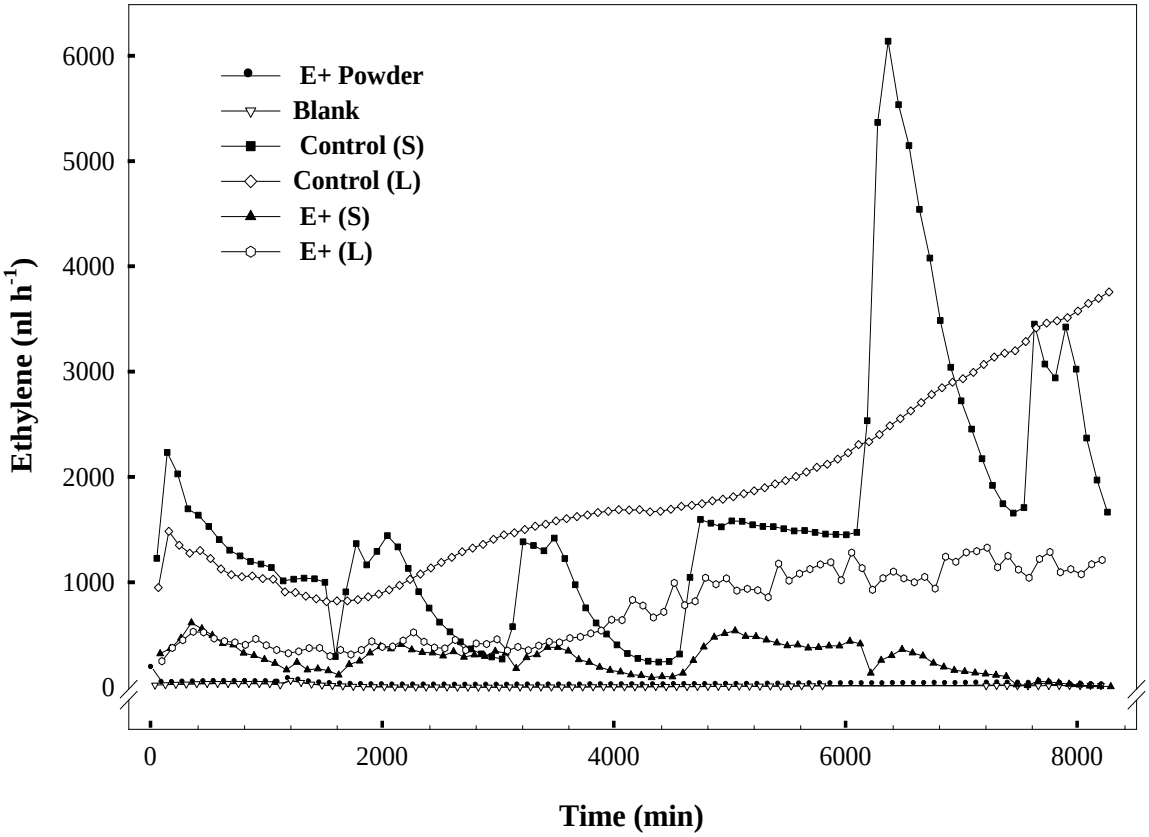


Figure 4.4. Ethylene production (nl h^{-1}) of pluots (cv. Flavor King) during ripening at 18 ^{o}C, after fruits were removed from cold storage following 7 days at 2 ^{o}C. Long (L) stored fruits (n=1) were stored at 18 ^{o}C for the ripening period of 6 days. Short (S) stored fruits (n=6) were stored at 18 ^{o}C for 24 h. Ethylene production was measured using ETD-300 detector over 6 days.

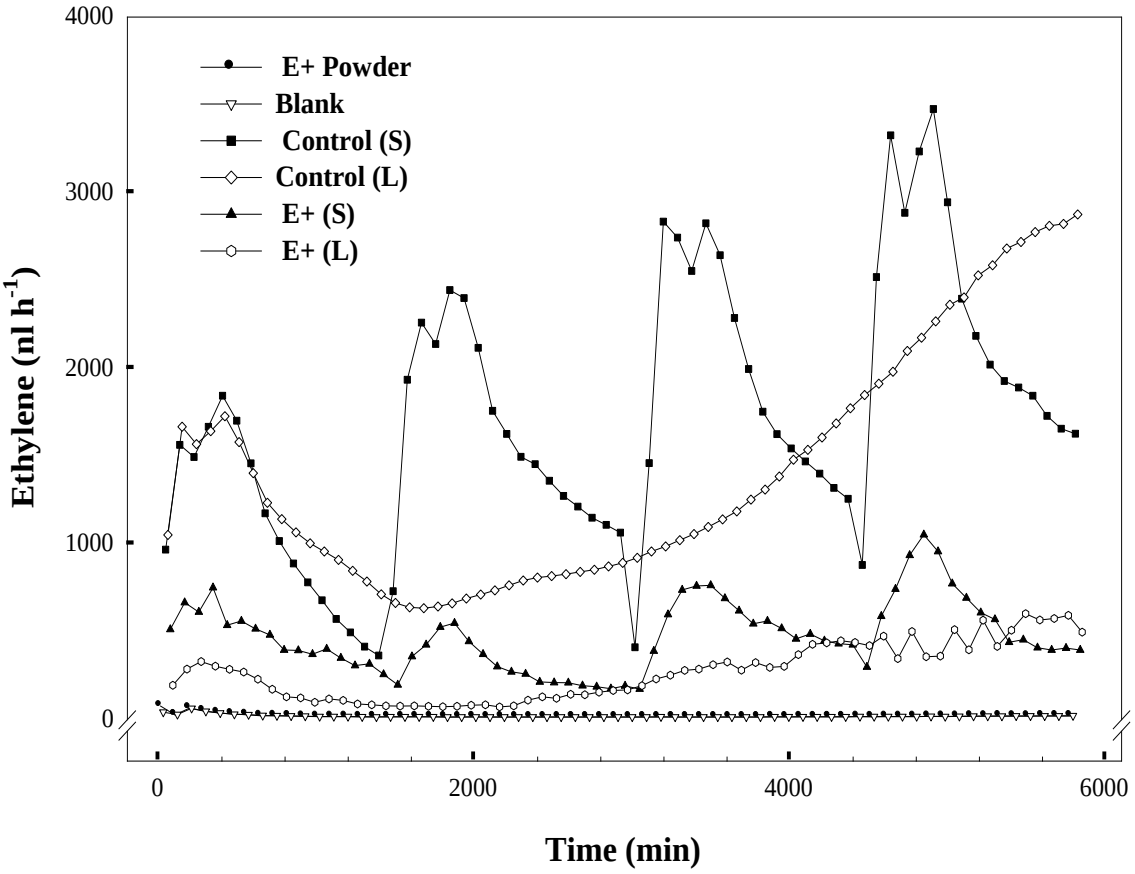


Figure 4.5. Ethylene production (nl h^{-1}) of pluots (cv. Flavor King) during ripening at 18 ^{o}C, after fruits were removed from cold storage following 14 days at 2 ^{o}C. Long (L) stored fruits (n=1) were stored at 18 ^{o}C for the ripening period of 4 days. Short (S) stored fruits (n=4) were stored at 18 ^{o}C for 24 h. Ethylene production was measured using ETD-300 detector over 4 days.

4.4.2 Flesh firmness and colour

Treatment with the e+® Ethylene Remover sheets helped to delay the onset of ripening and senescence, corroborating with previous results (Chapter 3). There was a significant effect of storage length on the colour of pluots (Appendix A, Table A.30) as defined by hue angle (H^{o}). Fruits ripened (4-6 days) at 18 ^{o}C were overall significantly darker, hence lower hue angle (12.58) when compared to fruits stored at 18 ^{o}C for a 24 h period

(17.35). Although fruits treated with e+® Ethylene Remover were overall less dark (17.58) than their corresponding controls (15.54), there was no significant effect of treatment on the colour of pluots. Plums and pluots of different cultivars will vary significantly in their colour, however this change in colour has been observed for various plum cultivars (Abdi *et al.*, 1998; Skog *et al.*, 2001; Khan *et al.*, 2009). Other modes of controlling ethylene such as the use of the ethylene inhibitor have been shown to influence the colour of plums (Khan *et al.*, 2009). That said, differences or lack of differences in colour of fruits from the two treatments may be influenced by the position of the fruit on the canopy. Taylor *et al*. (1993b) showed that the position of plums within the canopy affected fruit quality characteristics including fruit colour.

Fruit texture is the most important feature affecting postharvest life and commercial value. Fruit firmness also diminished significantly during storage, the decrease in firmness being significantly higher in control than E+ fruits (Appendix, Table A.29). As demonstrated in Figure 4.6, a strong effect of e+® Ethylene Remover in delaying the firmness loss of pluots was observed. Results indicate that treated fruits were overall significantly firmer in comparison to the overall control fruits. On arrival (day 0) fruits (n=12 per treatment) were measured for their firmness as a baseline measurement and the average firmness for the control fruits was 14.12 N (1.44 kgf), however for the E+ fruits this was 29.68 N (3.02 kgf). Fruits were also supplied to Marks and Spencer (M&S) and according to M&S specification the lowest firmness required is 17.65 N (1.8 kgF). This suggests that fruits were quite advanced when received, which may have influenced the overall quality parameters. However, initial measurements (day 0) shows that treatment with e+® Ethylene Remover sheets at source had beneficial effects on firmness of pluots. Fruits treated at source with the e+® Ethylene Remover coated sheets were considerably firmer than the control fruits (day 0), which suggests an effect of treatment at source. This supports the necessity in controlling ethylene during transportation, since control fruits here and those in Exp 3.2 (Chapter 3) were notably softer on arrival compared to the E+ fruits. In spite of this, fruits were delayed during transit, which had an overall impact on the quality hence the measure of the overall effect of treatment.

Previous studies (Terry *et al.*, 2007a; Meyer and Terry, 2010) have shown that e+® Ethylene Remover treatment does not completely inhibit fruit ripening, but rather delays rate of ripening. In accordance, to that observed in Chapter 3, results demonstrate that fruits treated at source with e+® Ethylene Remover sheets maintained better firmness and colour. Menniti *et al*. (2004) showed that after 30 days of storage the differences between 1-MCP treated and control fruits of (cv. Fortune) fruits became smaller and after 7 days of shelf life the hue angle reached similar levels. Pluots were overall of better quality then the control fruits however, the efficacy of the treatment declined as fruits became older. Overall the firmness results suggest that the reduction of ethylene (E+ fruits) suppressed the ethylene response and signalling (Kevany *et al.*, 2007) since the enzymes involved in fruit softening are mainly regulated by ethylene (Wills *et al.*, 2007), leading to significantly higher firmness (refer to appendix A; Table A. 29). That said, other factors such as relative humidity may have influenced the overall efficacy of the e+® Ethylene Remover. Terry *et al*. (2007b) showed that the material exhibited its highest ethylene adsorption capacity 45,600 µl g^{-1}under dry conditions. The e+® Ethylene Remover coated sheets were slightly moist after the end of the trial; hence ethylene adsorption capacity of the sheets may have been affected.

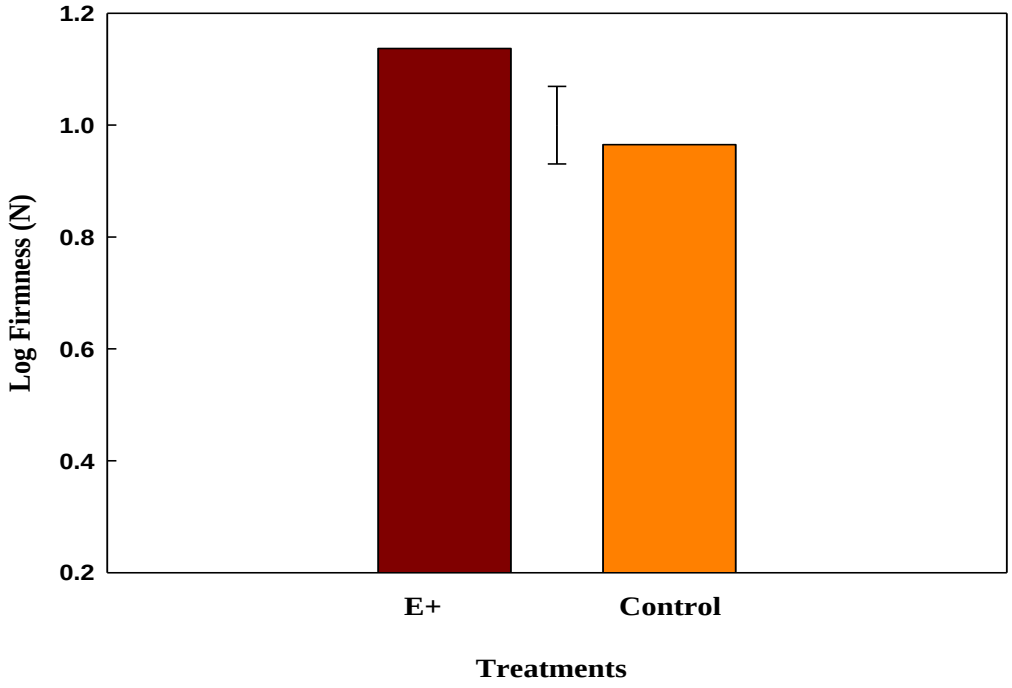


Figure 4.6. Effects of treatments on the overall firmness of pluots treated at source with (E+) or without (Control) e+® Ethylene Remover coated sheets and later treated with (E+) or without (Control) e+® Ethylene Remover (2.5 g) for a period of 1-6 days (stored at 18 °C). LSD bar (*P<0.05*). Firmness data was converted to log (10) to normalise distribution.

4.4.3 Sugars, phenolic compounds and total antioxidant capacity in pluots

The main sugars contributing to the taste of pluots are sucrose, glucose and fructose. These sugars were quantified to observe any effect of treatment, yet, as shown in Table 4.1, treatment with e+® Ethylene Remover had no impact on the sugar content of pluots (Appendix A, Table A.31, A.32, A.33) . Similarly, studies have also shown that controlling ethylene through inhibition via 1-MCP does not necessarily affect soluble solid content (Dong *et al.*, 2002; Menniti *et al.*, 2004). However, the soluble solid content does not necessarily mirror the content of individual sugars. However, although, some studies have correlated sugar accumulation with the ripening of plums (Abdi *et al.*, 1997), in this study it is likely that since the fruits were quite mature on arrival as noted from the firmness measurements of the control fruits that the sugars may have accumulated before being received.

Table 4.1. Effects of treatments on the concentration of the main sugars in pluot fruits stored for long (up 6 days) (n=3 fruits) and short (24 h) (n= 15 fruits) period at 18 ^{o}C inside 1 l glass jars with or without e+® Ethylene Remover (2.5 g): values are expressed here are in mg g^{-1} DW.

Length	**Glucose**		**Fructose**		**Sucrose**	
	Control	E+	Control	E+	Control	E+
Long	188.5	181.0	196.4	193.3	230.8	243.1
Short	193.3	213.8	198.6	211.7	240.5	235.4
LSD(*P<0.05*)	44.29		47.06		66.93	

**No statistical differences in this data.*

The reports of the health benefits of phytochemicals have encouraged research to investigate factors that influence their concentration. Some phenolic compounds are also associated with the astringency of fruit (Vizzotto *et al.*, 2007), which decreases with ripening; for this reason it would be expected that these compounds decrease with ripening. Phenolic compounds in plums have also been studied due to their health benefits, since consumption of foods rich in antioxidant compounds have been considered as a way to prevent diseases such as cancer (Noratto *et al.*, 2009; Vicente *et al.*, 2011). The phenolic compounds in plums are likely to have an impact on the quality of the fruit. In this study, caffeic acid, quercetin derivatives, cyanidin-3-glucoside and cyanidin-3-rutinoside were quantified since these phenolics have been identified by others in various plum cultivars (Fang *et al.*, 2002; Kim *et al.*, 2003). Caffeic acid is the most abundant phenolic acid in most fruits (Vicente *et al.*, 2011). Results show that caffeic acid was significantly higher in E+ fruits that were ripened at 18 ^{o}C (4-6 days, Table 4.2) than the control fruits (Appendix A, Table A.37). Reduced phenolic acids including caffeic acid have been associated with ripening (Tomás-Barberán *et al.*, 2001). Equally, the changes in the types and concentrations of phenolics have been shown to differ between cultivars of apricots (Kan and Bostan, 2010) and plums (Tomás-Barberán *et al.*, 2001; Chun and Kim, 2004).

Anthocyanins are the main pigments responsible for the colour of fruits and colour is an important indicator of maturity and quality in many fruits (Gao and Mazza, 1995). Anthocyanins; cyanidin-3-glucoside (C-3-G) and cyanidin-3-rutinoside (C-3-R) have been reported as the main constituents for the colour development in the red plum cultivars (Tomás-Barberán *et al.*, 2001; Fang *et al.*, 2002; Kim *et al.*, 2003). Results show that cyanidin-3-glucoside, the prominent anthocyanin, increased in the long stored e+® Ethylene Remover treated fruits than the short stored fruits, but similar observations were not observed in control fruits (Table 4.2). That said, cyanidin-3-glucoside was significantly higher in the E+ fruits (stored for 4-6 days at 18 ^{o}C (Long)) than the control fruits (Appendix A, Table A.35), which does not correlate with the colour data, where control fruits were generally darker than the E+. Nonetheless, anthocyanins are mainly localised in the skin cells (Tomás-Barberán *et al.*, 2001), thus the anthocyanins extracted from the flesh of the pluots may not reflect the overall concentration. That said environmental condition such as pH can influence the overall colour and concentration of anthocyanins (Młodzińska, 2009; Valero and Serrano, 2010). In terms of the other phenolic compounds measured (quercetin and cyanidin-3-rutinoside) no significant effect of treatment or storage was observed (Table 4.2).

Table 4.2. Effects of treatments on the concentration of phenolic compounds in pluot fruits stored for long (up to 6 days) (n=3 fruits) and short (24 h) (n= 15 fruits) period at 18 ^{o}C inside 1 l glass jars with or without e+® Ethylene Remover (2.5 g): values are expressed here are in mg g^{-1} DW.

Length	**Quercetin**		**Caffeic acid**		**C-3-G**		**C-3-R**	
	Control	E+	Control	E+	Control	E+	Control	E+
Long	0.937^{a}	0.918^{a}	0.157^{a}	0.394^{c}	0.757^{a}	1.627^{b}	0.326^{a}	0.337^{a}
Short	0.917^{a}	0.898^{a}	0.307^{bc}	0.246^{ab}	0.902^{a}	0.669^{a}	0.279^{a}	0.239^{a}
LSD (*P<0.05*)	0.2245		0.1126		0.3038		0.1166	

C-3-G (cyanidin-3-glucoside), C-3-R (cyanidin-3-rutinoside)

The antioxidant capacity of fruits may be from a range of compounds including vitamins, β-carotene and phenolic compounds. The high antioxidant capacity of the pluots is attributed to high phenolic compounds (Wang *et al.*, 1996). Kevers *et al*. (2007) showed that storage does not negatively affect the antioxidant capacity however; others have shown the antioxidant activity can be affected by ripening (Yoo *et al.*, 2004; Khan *et al.*, 2009). The antioxidant capacity was considerably higher in the skin possibly due to high content of phenolics in the skin than in the flesh as demonstrated by Tomás-Barberán *et al*. (2001). That said, no effect of treatment (Appendix A, Table A.38, A.39) was observed on the antioxidant capacity of the pluots and in addition there was no storage effect (Table 4.3).

Table 4.3. Effects of treatments on the total antioxidant capacity of pluot fruits stored for long (up to 6 days) (n=3 fruits) and short (24 h) (n= 15 fruits) period at 18 ^{o}C inside 1 l glass jars with or without e+® Ethylene Remover (2.5 g): values are expressed here are in µmol Trolox g^{-1} DW.

Length	**Total antioxidant capacity** (µmol Trolox g^{-1} DW)		
	Treatment	**Flesh**	**Skin**
Long	Control	168.8	432
	E+	211.4	421
Short	Control	168.6	341
	E+	189.0	388
LSD (*P<0.05*)		40.29	88.6

**No statistical differences in this data.*

4.5 Conclusion

The results of this study consolidates the benefits of the newly developed e+® Ethylene Remover coated sheets. Here pluots imported from South Africa with e+® Ethylene Remover sheets at source were of better quality on arrival and at the end of storage these fruits exhibited significantly higher firmness and were generally less dark than the untreated fruits. In addition, the use of a highly sensitive ETD-300 Ethylene detector meant the ethylene production of pluots stored with e+® Ethylene Remover and the

untreated fruits could be monitored continuously over a short and long periods under ambient condition (18 ^{o}C). Where e+® Ethylene Remover treatment was concerned considerably lower ethylene was observed, however, the ethylene production pattern of the treated and the untreated fruits displayed the same pattern. While the sugar content were not affected by treatment, caffeic acid was more abundant in the E+ fruits during ripening, that said, other phenolic compounds measured and the total antioxidant capacity of pluots were not influenced by the e+® Ethylene Remover treatment. However, the quality of the fruits both physiologically and biochemically may have been influenced by the state of the fruits on arrival.

CHAPTER FIVE

5 THE ROLE OF ETHYLENE AND E+® ETHYLENE REMOVER ON STORED STRAWBERRY FRUIT (*FRAGARIA* X *ANANASSA*) QUALITY

5.1 Introduction

Strawberries are highly perishable and their delicate nature makes them especially prone to postharvest diseases during storage, accounting for significant losses (Ayala-Zavala *et al.*, 2004; Babalar *et al.*, 2007; Giné Bordonaba and Terry, 2011). Several studies have considered the effect of ethylene on the flavour, texture and colour development of strawberries refer to Table 2.2. Some research has suggested that the deterioration of strawberries can be exacerbated by the presence of ethylene during storage (Jiang *et al.*, 2001; Bower *et al.*, 2003) hence increasing losses. Wills and Kim (1995) reported an ethylene concentration of 0.36 µl l^{-1} in strawberry punnets; however, a concentration of 0.1 µl l^{-1} was reported to reduce storage life of strawberries.

In addition to the increased and rapid softening of strawberries during postharvest storage, significant postharvest losses occur due to grey mould caused by the *Botrytis cinerea* (*B. cinerea*). Moreover, removal of ethylene from the storage environment of strawberries (cv. Torrey) using potassium permanganate was reported to hinder the development of grey mould (*B. cinerea*) at 20 ^{o}C (Wills and Kim, 1995). That said, there is no clear underlying evidence correlating ethylene concentration with rot development. For instance, it was shown that treatment of strawberries (cv. Brighton) with ethylene inhibitors; norbordiene and aminoethyoxyvinylglycine (AVG) had no impact on fruit ripening (Given *et al.*, 1988). However, studies have shown that the ethylene binding inhibitor, 1-MCP, can maintain strawberry quality postharvest (Tian *et al.*, 2000; Jiang *et al.*, 2001; Bower *et al.*, 2003). Recently, Trainotti *et al.* (2005), identified ethylene receptors in strawberries and have argued increased synthesis of receptors were concomitant with the increased synthesis of ethylene. However, more research is necessary to elucidate the role of the plant hormone in the process of infection and storage life of strawberries.

Strawberries are greatly appreciated for their taste properties and significant nutritional benefits. Reduced levels of anthocyanins and phenolic content were reported following 1-MCP treatment (Jiang *et al.*, 2001). Therefore ethylene also seems to play a key role in some aspects of strawberry fruit quality. Nevertheless, this is an area that requires more research to better understand the impact and effect of ethylene in non-climacteric fruits.

The advantages of e+® Ethylene Remover in reducing the ripening rate of climacteric fruits such as avocado and/or pluots has been reported in literature (Terry *et al.*, 2007b; Meyer and Terry, 2010) and previous chapters (3 and 4). This study was conducted to investigate the role of ethylene in the postharvest life of strawberries infected with or without *B. cinerea* at green stage. Impact of treatments on the physiology and biochemistry of strawberries during postharvest was also investigated.

5.2 Materials and methods

5.2.1 Isolation of the *B. cinerea*

A single spore isolate of *B. cinerea* (Strain BC 143) purchased from Plant Research International, Wageningen (Netherlands) was cultured in a 9 cm petri dish (Fisher Scientific, Leic., UK) on potato dextrose agar (PDA; 39 g PDA l^{-1} distilled water) (Sigma Aldrich., UK) for 14 days at 22 °C. Streptomycin (1.0 mg ml^{-1}) was added to the autoclaved PDA to inhibit the potential growth of bacteria (Terry *et al.*, 2007a). A conidial suspension was prepared by transferring the fungus from the cultured plates through gentle sweeping of the plate surface using a glass rod and then suspended in distilled water. The concentration of conidia was determined using a haemocytometer/cell counter and a light microscope. The concentration 2×10^5 conidia ml^{-1} was prepared in sterilized water supplemented with 0.03 % Tween 20 to ensure uniform distribution of the conidia on the fruit surface.

5.2.2 Plant material and experimental design

Three separate experiments were conducted. In the first of the three experiments maiden year A+ grade strawberry (cv. Elsanta) plants were purchased from R. W. Walpole (Norfolk, UK) (n=72, Exp 5.1) and grown in a glasshouse (at Cranfield University) during 2007 (April-June) in 1 l capacity pots containing compost. The treated and

untreated plants were kept separate in the glasshouse to avoid infection of non-infected plants. In Exp 5.2 and 5.3 strawberry fruits (cv. Jubilee) were purchased from a local grower (H & H Duncalfe, Cambs., UK) and transported to the laboratory within 2 h. In Exp 5.2 and 5.3 there was no treatment involving the pathogen; however fruits (cv. Jubilee) were picked on two different dates. Exp 5.2 fruits were harvested on 23rd July 2011, while fruits in Exp 5.3 were harvested on the 18th Aug 2011 (Table 5.1).

Table 5.1. Details of the experiments conducted herein.

Experiment	Cultivar	Plant supplier/ origin	Growing condition	Harvest date
5.1	Elsanta	R.W. Walpole Strawberry plants Ltd. (Norfolk, UK)	Plants were irrigated with *ca.* 200 ml day^{-1}.	11/06/2011
5.2	Jubilee	H & H Duncalfe (Cambs., UK)	Standard commercial growing conditions	23/07/ 2011
5.3	Jubilee	H & H Duncalfe (Cambs., UK)	Standard commercial growing conditions	18/08/ 2011.

5.2.3 Fruit sampling (cv. Elsanta Exp 5.1)

Strawberry plants (Exp 5.1; cv. Elsanta n=72) were subjected to two initial treatments in the glasshouse once the majority of the primary fruits from the primary truss were at green stage I (*ca*. 7 days after anthesis). *B. cinerea* conidial solution (2 x 10^5 pores ml^{-1}) was sprayed (*ca.* 10 ml) on plants (n=36 infected (I)) while the remaining plants (n=36 non-infected (NI)) were sprayed with sterile distilled water containing 0.03 % Tween 20. The tertiary fruits (n=96 per treatment) on the primary truss were harvested at optimum ripeness (red stage). Fruits of similar size and weight (8 ± 0.5 g) (I and NI) were immediately stored with or without e+® Ethylene Remover (2.5 g powder) and stored in 13 l plastic boxes (at 5 ^{o}C). A 20 channel gas blender (series 850) and channel selector was used to achieve controlled continuous air change every 5 min within the boxes. Refer to Figure 5.1 for details of the treatments. Following a subsequent storage

period (0, 2, 4, 7 days) fruits were removed from cold storage and the weight, objective colour and ethylene production determined.

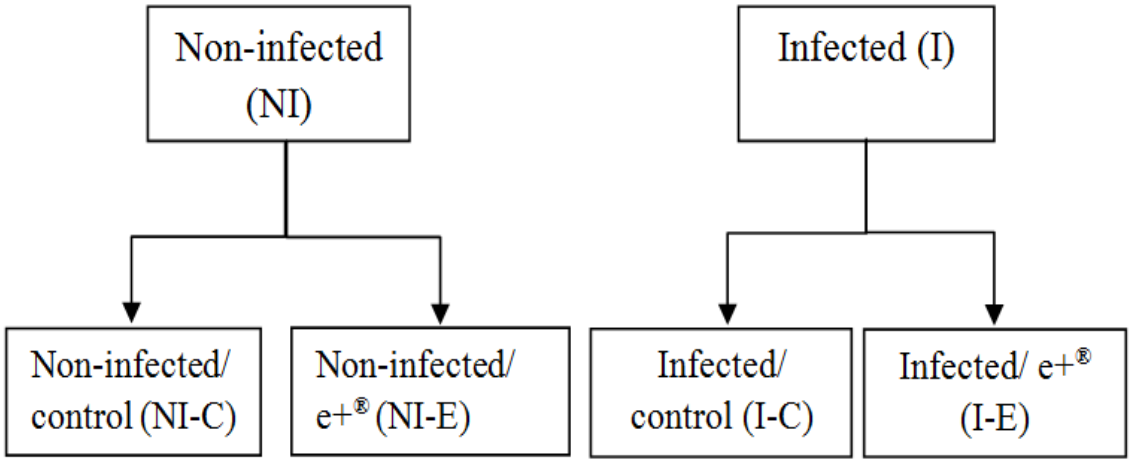


Figure 5.1. Experimental design (Exp 5.1) showing fruits infected with (I, n=96) or without (NI, n=96) *B. cinerea* during preharvest and later treated with (E+) or without (control (C)) e+® Ethylene Remover (2.5 g powder) during postharvest to give four treatment combinations. Fruits (n=12) were separated into blocks (n=3) with each block containing n=4 fruits and taken for measurements on 0, 2, 4 and 7 days following storage at 5 ^{o}C.

5.2.4 Fruit sampling (cv. Jubilee, Exp 5.2 and 5.3)

On arrival (Exp 5.2) fruits (n=120) were placed inside 13 l plastic boxes with (E+, n=60) or without (C, n=60) e+® Ethylene Remover (2.5 g) and stored at 5 ^{o}C. Following storage period (0, 1, 2, 4 and 7 days) fruit weight, colour and ethylene production were measured. A subsample number of fruits (n=24) were taken for immediate ethylene measurements (day 0). The e+® Ethylene Remover was repeated in another trial (0, 1, 2, 4 and 7 days) on cv. Jubilee (Exp 5.3) fruits purchased from the same grower and measurements conducted as described above.

5.2.5 Ethylene determination

The ethylene production was monitored using the laser based ethylene detector ETD-300 as described in Chapter 4 with minor modifications. In Exp 5.1 fruits of the

same initial treatment (n=24) (Infected (I) or non-infected (NI)) treated with (n=12) or without (n=12) e+® Ethylene Remover were separated into the 1 l jars (6 jars, 3 for each treatment) (n=4 fruits in each jar). Fruits treated with e+® Ethylene Remover were placed inside jars containing fresh e+® Ethylene Remover (2.5 g powder in petri dish). The jars were flushed at a continuous flow rate of 3 l h^1 with cleaned air (Appendix B) and a total pressure of 1 bar. Real time ethylene production was monitored continuously every 10 min (for 6 h) at 18 ^oC. This system has been described in more detail elsewhere (Cristescu *et al.*, 2002; Salman *et al.*, 2009). Fruits were removed from the jars and then snap frozen in liquid nitrogen ready for biochemical analysis.

In Exp 5.2 and 5.3 the e+® Ethylene Remover treated fruits (n=12) were placed inside the jars (n=3 jars, 4 fruits in each) containing e+® Ethylene Remover (2.5 g), while the control fruits (n=12) were separated into the remaining jars (n=3 jars, 4 fruits in each jar) containing no e+® Ethylene Remover powder. Fruits of similar size and weight (8.5 ± 0.5 g) were used within and between treatments. After 24 h at 18 ^oC fruits were snap frozen in liquid nitrogen.

5.3 Colour measurements

Colour measurements on all fruits were obtained as described in previous chapters (3 and 4) using the colorimeter. The average of three readings at 3 equidistant points around the equatorial axis of the fruits was recorded.

5.3.1 Biochemical analysis

5.3.1.1 Extraction and quantification of sugars

Sugars were extracted in 62.5 % (v/v) methanol as previously reported (Terry *et al.*, 2007a). Sugars in strawberries (Exp 5.1) were quantified as described in Chapter 3 with some modifications. Briefly, the diluted strawberry extract (20 µl) and sugar standards were injected into a Rezex RCM monosaccharide Ca^+ (8 %) column of 300 mm × 7.8 mm diameter (Phenomenex, Torrance, CA; Part No. 00H-01030-K0) with a Carbo-Ca^{2+} security guard cartridge of 4 mm × 3 mm diameter (Phenomenex, CA). The temperature of column was held at 75 ^oC. The mobile phase was HPLC graded water at a flow of 0.6 ml min^{-1}. The abundance of sugars (fructose, glucose and sucrose) was calculated against authentic standard ranging from 0.05 -2.5 mg ml^{-1}.

The sugar extracts from Exp 5.3 were analysed using an Agilent 1200 series HPLC binary pump system (purchased from Agilent, Berks., UK), coupled with an Agilent refractive index detector (RID) G1362A. Sugar extracts were diluted (1:10) before being injected (20 µl) into the Rezex RCM monosaccharide column used above. Here, column temperature was set at 80 ^{o}C using a thermostat G1316A. The mobile phase was HPLC grade water and flow rate was 0.6 ml min^{-1}. The temperature of the detector was set at 30 ^{o}C while the autosampler was set at 4 ^{o}C with the use of Agilent cooled autosampler G1330B.

5.3.1.2 Extraction and quantification of organic acids

Strawberry organic acid extract were prepared as previously described (Giné Bordonaba and Terry, 2008). Briefly, freeze dried strawberry powder (75 mg) was dissolved in HPLC grade water (1.5 ml). Extracted samples were kept at room temperature (25 ^{o}C) for 10 min and then filtered using a 0.2 µm syringe filter. Standards consisting of selected organic acids were used and malic, L-ascorbic and citric acid content present in samples were quantified. Extracts (20 µl) were injected into an Alltech Prevail Organic Acid column 250 mm×4.6 mm diameter, with particle size of 5 µm (Alltech, CA; Part no. 88645), attached to an Alltech Prevail Organic Acid guard column, 7.5 mm×4.6 mm diameter (Alltech, CA; Part no.96429) connected to an evaporative light scattering detector (ELSD 2420, Waters, MA, USA). The mobile phase consisted of analytical grade KH_2PO_4 (25 mM) (BDH, Dorset, UK) in HPLC grade water. The mobile phase flow rate was 1.5 ml min^{-1} (isocratic) for 10 min. The temperature of the column was set at 35 ^{o}C. The organic acid content in the extracts were detected at 210 nm, using a UVD 170S/340S (Dionex, CA) connected to the Dionex system described above. The presence and abundance of the selected organic acid were determined by comparing peak area of the sample against that of the standard (0.05-2.5 mg ml^{-1}).

5.3.2 Determination of anthocyanins and antioxidant capacity of strawberry

5.3.2.1 Extraction of antioxidants compounds

Extraction of antioxidants for anthocyanin quantification and total antioxidant capacity was conducted as previously described (Terry *et al.*, 2007a) from strawberries (sample from Exp 5.1 and 5.3) by adding 1.5 ml of MeOH: H_2O: HCl (70 %: 29.5 %: 0.5 % v/v/v) solvent to 75 mg of freeze-dried sample in a polystyrene

bijou vial (7 ml). The sample was mixed thoroughly and placed immediately in a shaking water bath set at 35 ^{o}C for 1.5 h; sample was mixed every 15 min. The sample was filtered as described above and the extracts stored in a -40 ^{o}C freezer until required.

5.3.2.2 Measurements of antioxidant capacity

The antioxidant capacity of the strawberry samples (Exp 5.1) were measured as reported previously (Xu *et al.*, 2010) and in Chapter 4. The antiradical activities of the sample were expressed as Trolox equivalent antioxidant capacity (μM TE g^{-1}DW) on their percentage inhibitions.

5.3.2.3 Quantification of individual anthocyanins

Individual anthocyanins were determined for samples from Exp 5.1 and 5.3 as described previously (Aaby *et al.*, 2007; Terry *et al.*, 2007a). Briefly, anthocyanins were determined using Agilent 1200 series HPLC system (Agilent, Berks., UK). The HPLC system was equipped with an Agilent 1200 DE G1329A/G1315D photodiode array with multiple wavelength detector. Chromatographic separation was performed on 150 x 4.6 mm, 5 μm Zorbax Eclipse XDB-C18 (Agilent) connected to an OPTI-GUARD 1 mm guard column (Crawford Scientific). The mobile phase consisted of degassed and filtered HPLC grade acetonitrile, 1 % phosphoric acid and 10 % acetic acid (A) (purchased from Fischer Scientific, Leics., UK) and HPLC grade water (B). The composition of the mobile phase followed a linear gradient starting from 2 to 20 % of solvent A in 25 min and then 20 to 40 % of solvent A in 1 min at a flow rate of 1 ml min^{-1}, while the column temperature was set at 40 ^{o}C. Anthocyanins cyanidin-3-glucoside and pelargonidin-3-glucoside (purchased from Extrasyntheses, Lyon, France) were observed at 520 nm and their presence and abundance determined by comparing the peak areas with external standards.

5.4 Results and Discussion

5.4.1 Ethylene production of strawberries

Studies have shown that deterioration of strawberries can be exacerbated by the presence of ethylene during storage, hence increasing potential waste (El-Kazzaz *et al.*, 1983; Wills and Kim, 1995; Bower *et al.*, 2003). Grey mould is the most widespread disease affecting strawberries. The host usually gains entry to the flower at early stage

of development and remains quiescent until later during storage when conditions are more favourable (El-Kazzaz *et al.*, 1983; Fallik *et al.*, 1993). Results of the present study (Exp 5.1) demonstrated that fruits infected (I) with *B. cinerea* (Exp 5.1) produced significantly higher ethylene than non-infected fruits (Appendix A, Table A.41) as presented in Table 5.2. The elevated ethylene observed in infected fruits could be mediated by processes that encourage fruit senescence and may result from the response of the host to the stress caused by *B. cinerea* infection. Nonetheless, literature has also shown that the *B. cinerea* itself produces ethylene (Cristescu *et al.*, 2002), thus it is difficult to exclude the contribution of fungal-derived ethylene.

Table 5.2. Concentration of ethylene from fruits treated during preharvest infected (I) or without (NI) *B. cinerea* and during postharvest with (E+) or without (Control) e+® Ethylene Remover. The average ethylene production of strawberries is given (cv. Elsanta, Exp 5.1, n=12) stored at 5 °C for a total of 7 days, ethylene levels measured at 18 °C using ETD-300 Ethylene Sensor.

Treatment 1	**Treatment 2**	**Ethylene concentration (nl h^{-1})**			
	Days at 5 °C	**0**	**2**	**4**	**7**
I	**Control**	26.703^{j}	19.442^{b}	27.198^{j}	27.253^{j}
	E+	25.666^{gh}	18.029^{a}	24.998^{gh}	23.485^{de}
NI	**Control**	24.668^{gh}	23.581^{de}	24.427^{fg}	25.172^{hi}
	E+	23.959^{ef}	22.051^{c}	23.134^{d}	23.853^{ef}

NI (non-infected); I (infected). LSD (P<0.05) = 0.8275

The presence of e+® Ethylene Remover resulted in significantly reduced ethylene levels (Appendix A, Table A.41, A.52, A.54) in all three trials (Table 5.2, Table 5.3, Figure 5.2). In Exp 5.1, the non-infected fruits treated with the ethylene remover overall exhibited significantly lower ethylene in contrast to the other treatments (Table 5.2). Furthermore, enhanced ethylene observed in the infected fruits was reduced significantly by e+® Ethylene Remover. In Exp 5.3, significant difference in the

ethylene production of the control and E+ fruits was only apparent after day 4 of storage (Table 5.3). It is likely that after day 4 of storage control fruits exhibited enhanced ethylene due to increased rates of senescence. Additionally, if ethylene contributes to the natural disease resistance of the fruits then there may be a potential effect of e+® Ethylene Remover on the growth of the pathogen. Wills and Kims (1995) reported that strawberries treated with the ethylene scrubber $KMnO_4$ were found to be less susceptible to grey mould caused *B. cinerea,* since reducing ethylene below 0.005 µl l^{-1} at 0 ^{o}C significantly delayed the rate of deterioration. The e+® Ethylene Remover also adsorbs exogenous ethylene however, research on the effect of e+® Ethylene Remover on disease incidence in fruits has not been investigated.

Table 5.3. Concentration of ethylene from strawberry fruits (cv. Jubilee n=12, Exp 5.3) stored at 5 ^{o}C for a total of 7 days, ethylene levels measured at 18 ^{o}C using ETD-300 Ethylene Sensor. LSD (P<0.05) = 6.009.

	Ethylene concentration (nl h^{-1})	
Days	**Control**	**E+**
0	38.68^{cd}	34.65^{bc}
1	30.09^{ab}	29.52^{ab}
2	32.12^{ab}	32.17^{ab}
4	46.87^{e}	27.98^{a}
7	83.86^{f}	43.93^{de}

Strawberry fruit is considered non-climacteric due to its low ethylene production; nonetheless it is likely these low levels are essential in determining fruit quality parameters such as colour as observed herein. Moreover, the present study has identified an ethylene production pattern similar to climacteric fruits albeit at a much lower level. The ethylene production of strawberry fruit (Exp 5.2, cv. Jubilee) increased significantly after harvest peaking after 4 days of cold storage (5 °C) followed by a rapid decrease to

its lowest level (after 7 days at 5 ^{o}C) (Figure 5.2). These results support the study by Ianneta *et al*. (2006), where strawberries were shown to undergo a respiratory increase and concomitant ethylene production during ripening *in planta* and in detached red ripe fruits. Intriguingly, studies have found commonality between climacteric and non-climacteric ripening. Trainotti *et al*. (2005) observed enhanced expression of *FaACO1* as well as increased ethylene production similar to climacteric fruits; however, this study was conducted on strawberries during preharvest. Ianneta *et al*. (2006) determined ethylene production using the real time ETD-300 ethylene detector also used herein. It is likely that the use of less sensitive conventional techniques in the past did not enable these findings. Treatment with e+® Ethylene Remover treatment resulted in significantly reduced ethylene (Figure 5.2), however, ethylene was still detected for the E+ fruits, which was probably caused by a diffusion of ethylene from the internal structure through a concentration gradient resulting in the presence of some ethylene in the exogenous environment. Having said this, ethylene production of the treated fruits followed a similar pattern as the control fruits though the peak in the ethylene was less apparent and levels were significantly lower. Similar findings were also observed in pluots (Chapter 4); this suggests that fruits exhibit normal development but rate of the ethylene dependent processes are reduced under low ethylene environment.

Aharoni *et al*. (2002) reported increased expression of ACC oxidase genes in strawberries at red stage. Nevertheless, many processes associated with strawberry maturity have been shown to be regulated by the presence or absence of the achenes (Nitsch, 1950; Given *et al.*, 1988; Manning, 1994). Thus, it is likely that different areas of the fruit (achenes and receptacles) may exhibit different ripening mechanisms. In support of this notion, Ianneta *et al*. (2006) analysed the ethylene production of dissected fruit. It was demonstrated that the ethylene produced by the achenes at red stage accounted for 50% of the ethylene produced by the fruit, even though they make up a small fraction of the weight. That said, work by Ianneta *et al*. (2006) did not follow the fruit ethylene production during postharvest. Moreover, other plant phytohormones such as auxins have been shown to play a significant role in strawberry fruit development. Auxins have been found to be abundant in the achenes and were shown to inhibit strawberry ripening (Symons *et al.*, 2012). It is likely that exogenous ethylene would influence auxins and other phytohormones however, the mechanism by which

ethylene influences strawberry fruit quality requires better understanding and warrants further research.

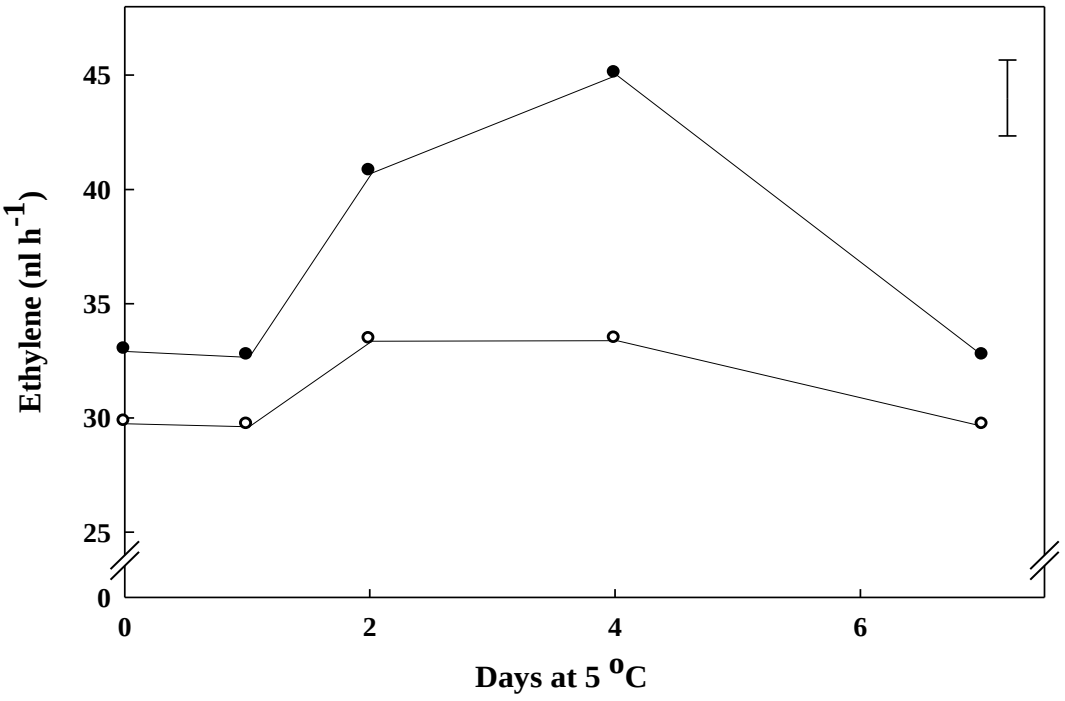


Figure 5.2. Ethylene production (nl h^{-1}) of strawberry fruits (cv. Jubilee n=12, Exp 5.2) following storage at 5 °C in 13 l boxes with (○, E+) or without (●, control) e+® Ethylene Remover powder (2.5 g). Ethylene production was measured at 18 °C inside 1 l capacity jars (n=3 per treatment) connected to ETD-300. LSD par (P<0.05).

5.4.2 Colour and anthocyanins

Removal of ethylene significantly maintained the colour of strawberries (Appendix A, Table A.40, A.53, A.55) in all three trials (cvs. Elsanta and Jubilee) (Table 5.4, Table 5.5) which suggests an involvement of ethylene in modulating fruit quality after harvest. Results are consistent with previous studies (Terry *et al.*, 2007b; Meyer and Terry, 2010) and results in Chapter 3 where e+® Ethylene Remover was found to maintain the colour of avocados. That said, avocado is a climacteric fruit where ethylene is known to

regulate fruit quality parameters during postharvest (Wills *et al.*, 2007). In addition, exogenous ethylene induced colour in other strawberry cultivars (Wills and Kim, 1995; Tian *et al.*, 2000; Villarreal *et al.*, 2010). Moreover, others have shown that ethylene inhibition using ethylene inhibitor 1-MCP and ethylene removal with potassium permanganate ($KMnO_4$) maintained the colour of strawberries (Wills and Kim, 1995; Jiang *et al.*, 2001). Strawberry postharvest life is short and colour development during postharvest is associated with senescence such that ethylene may have a role in this process. However, more research is required on the role of ethylene in strawberry fruit quality since some studies have shown no effect of exogenous ethylene on strawberries, while others have suggested positive effects (Table 2.5).

Low ethylene in the fruit environment correlated with fruits that were significantly less red thus suggesting a relationship between colour development and ethylene in strawberries. Literature has also demonstrated that ethylene treatment enhanced fruit physiological and biochemical characteristics. Villarreal *et al*. (2010) has shown that strawberry fruit treated with ethephon, an ethylene releasing chemical, showed a reduced chlorophyll level during white stage and accumulated more anthocyanins. Nevertheless, inhibition of ethylene using higher concentration of 1-MCP has been shown to negatively impact on strawberries. Jiang *et al*. (2001) showed that disease was accelerated in fruits treated with high concentrations of 1-MCP (500 and 1000 n l^{-1}). This suggests that, ethylene maybe necessary for normal fruit development and complete inhibition of ethylene may result in undesirable effects.

Table 5.4. Effect of treatment on the colour of infected (I) and non-infected (NI) strawberry fruits (cv. Elsanta n=12, Exp 5.1) stored for 7 days at 5 °C with or without e+® Ethylene Remover (2.5 g powder).

Treatment 1	**Treatment 2**	**Hue angle (H^o)**			
	Days at 5 °C	**0**	**2**	**4**	**7**
I	**Control**	33.31^{cdef}	31.35^{bc}	33.36^{d}	29.94^{ab}
	E+	33.66^{def}	33.54^{def}	32.34^{bcd}	32.55^{bcd}
NI	**Control**	33.74^{def}	32.56^{cd}	28.52^{a}	30.58^{b}
	E+	34.33^{ef}	34.80^{f}	33.25^{cdef}	33.69^{def}

NI (non-infected); I (infected). LSD (P<0.05) = 1.990

Table 5.5. Effect of treatment with (E+) or without (Control) e+® Ethylene Remover (2.5 g) on the hue angle of strawberry fruits (cv. Jubilee n=60, Exp 5.2 and 5.3) stored for 7 days at 5 °C.

	Hue angle (H^o)		
	Control	**E+**	**LSD (P<0.05)**
Exp 5.2	34.77^{a}	35.70^{b}	0.862
Exp 5.3	34.28^{a}	35.19^{b}	0.827

Anthocyanins are the pigments responsible for the red colour in strawberries. The most prominent anthocyanins in strawberries are pelargonidin and cyanidin mainly in their glucoside form (Lopes Da Silva *et al.*, 2002). Three main peaks were identified using the HPLC chromatogram and labelled using retention times of the authentic standards. The first two peaks were identified as cyanidin-3-glucoside and pelargonidin-3-glucoside. Cyanidin-3-glucoside was not calculated due to low levels as seen in the chromatogram (Figure 5.3); minor amounts of cyanidin-3-glucoside were

also identified by Aaby *et al*. (2005) in strawberries (cv. Totem). The third peak eluted after pelargonidin-3-glucoside, was recognised as a pelargonidin derivative as identified by others (Gil *et al.*, 1997; Hernanz *et al.*, 2007; Terry *et al.*, 2007a). Similar concentrations of perlargonidin-3-glucoside and pelargonidin derivative were detected in both experiments (Exp 5.2 and Exp 5.3) which were in line with others (Terry *et al.*, 2007a).

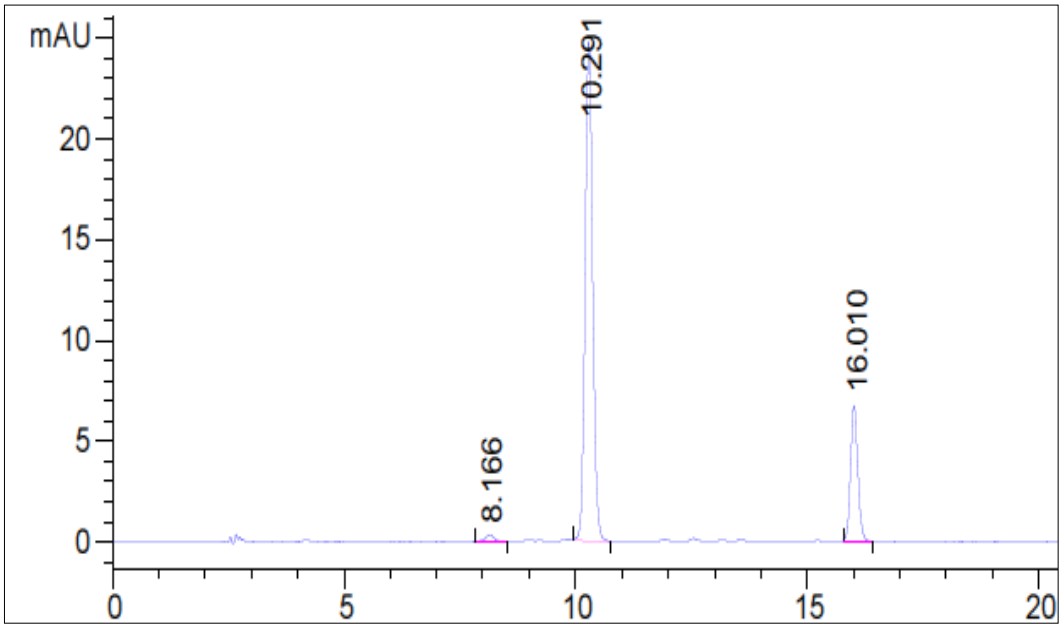


Figure 5.3. HPLC anthocyanin profile of strawberry extracts (cv. Elsanta, Exp 5.1). Three peaks corresponding to cyanidin-3-gluoside (at 8.166 min), pelargonidin-3-glucoside (10.291 min) and pelargonidin derivative (at 16.010 min).

Anthocyanins are the pigments responsible for the red colour of strawberries however; anthocyanin concentrations were not consistent with the colour measurements. Preharvest treatment with *B. cinerea* resulted in significant redder fruits after 7 days at 5 ^{o}C (Table 5.4) and the use of e+$^{®}$ Ethylene Remover retarded the change in colour (Table 5.4, Table 5.5). This said, similar results were not observed in the anthocyanin concentrations (Appendix A, Table A.61, A.62) (**Error! Reference source not found.**, Table 5.7). However, it is possible that factors affecting anthocyanin colouration including light, temperature, various stresses, may have contributed to the differences

between anthocyanins and the colour data (Kalt *et al.*, 1999). Moreover, the analysis of extracted pigments does not always correlate with visual appearance. For example, the anthocyanins are the pigments responsible for the purple colour in blueberries however; these pigments appear red in colour when extracted, having minimal resemblance to their actual purple colouration (Boulton, 2001). The colour of anthocyanins is highly affected by the intracellular environment in particular pH (Holcroft and Kader, 1999). Gil and Kader (1997) illustrated that CO_2 had little impact on the content of anthocyanins of external tissues, but caused a decrease in anthocyanin levels of the internal tissues. The concentration and colour intensity of anthocyanins can be affected by the organic acid concentration and hence pH, but also by numerous other factors including temperature, light and extraction solvents. Although in Exp 5.1 there was a significant decrease in organic acids over time (Table 5.8), during storage as seen by others, (Cordenunsi *et al.*, 2005; Koyuncu and Dilmacunal, 2010) no treatment effect was apparent (Appendix A, Table A.42, A.43, A.44, A.45). In Exp 5.3, the levels fluctuated, but there was no significant increase or decrease or an overall effect of treatment (Appendix A, Table A.56, A.57, A.63) during storage of strawberries (*No *statistical differences in this data.*

Table 5.9). In both experiments (Exp 5.1 and 5.3) the citric acid was the main organic acid found in agreement with that reported previously (Olsson *et al.*, 2004; Terry *et al.*, 2007a). Nevertheless, organic acids will have an impact on the pH of the environment and hence may affect the perceived colour of the anthocyanins.

Table 5.6. Effects of treatment with (I) or without (NI) *B.cinerea* (Treatment 1) on the concentration of the anthocyanins in strawberry fruits (cv. Elsanta n=12, Exp 5.1) stored for 7 days stored (at 5 ^{o}C) with (E+) or without (Control) e+® Ethylene Remover (2.5 g) (Treatment 2): values are expressed here are in mg g^{-1} in DW. NI (non-infected); I (infected).

		Pelargonidin-3- glucoside		**Pelargonidin derivative**	
		Treatment 2			
Days at 5 ^{o}C	**Treatment 1**	**Control**	**E+**	**Control**	**E+**
0	I	0.585a	0.684	2.159	2.181
	NI	0.851	0.723	2.706	2.412
2	I	0.660	0.463	2.005	1.608
	NI	0.697	0.522	2.234	1.696
4	I	0.522	0.650	1.793	2.188
	NI	0.676	0.545	2.290	2.223
7	I	0.650	0.540	2.583	2.124
0	NI	0.568	0.686	2.501	2.199
LSD (*P*<0.05)		0.2089		0.7975	

**No statistical differences in this data.*

Table 5.7. Concentration of the anthocyanins in strawberry fruits (cv. Jubilee n=12, Exp 5.2) stored for 7 days stored (at 5 ^{o}C) with (E+) or without (Control) the presence of e+® Ethylene Remover (2.5 g): values are expressed here are in mg g^{-1} DW.

	Pelargonidin-3-glucoside		**Pelargonidin derivative**	
	Treatments			
Days at 5 ^{o}C	**Control**	**E+**	**Control**	**E+**
0	2.54	2.08	0.228	0.207
1	2.15	1.81	0.173	0.173
2	2.99	1.84	0.251	0.197
4	2.22	2.62	0.221	0.248
7	2.37	2.10	0.244	0.217
LSD (*P*<0.05)	1.391		0.1312	

**No statistical differences in this data.*

Table 5.8. Concentration of the main organic acids in strawberries (cv. Elsanta n=12, Exp 5.1) infected with (I) or without (NI) *B. cinerea* (Treatment 1) and stored with (E+) or without (control) e+® Ethylene Remover (2.5 g) for 7 days at 5 °C (Treatment 2): values are expressed here are in mg g^{-1} DW.

		ASCORBIC ACID		CITRIC ACID		MALIC ACID		OXALIC ACID	
		Treatment 2							
Days at 5 °C	**Treatment 1**	**Control**	**E+**	**Control**	**E+**	**Control**	**E+**	**Control**	**E+**
0	I	4.93	5.53	57.2	63.1	6.44	10.86	6.78	15.62
	NI	6.61	7.16	76.1	75.4	5.20	7.43	10.24	11.61
2	I	3.47	3.63	54.9	50.0	6.95	6.01	8.61	6.93
	NI	4.60	3.23	58.9	48.2	8.21	6.65	10.70	10.58
4	I	3.32	4.12	30.4	35.5	4.84	4.96	4.71	7.14
	NI	3.01	4.64	28.2	40.2	5.00	5.71	5.59	6.45
7	I	3.32	2.95	34.4	28.0	4.81	4.25	4.30	5.38
	NI	2.62	2.46	25.4	27.2	3.25	4.48	6.44	6.61
LSD (*P<0.05*)		2.323		25.23		3.380		4.920	

**No statistical differences in this data.*

Table 5.9. Effects of treatments on the concentration of the main organic acids in strawberry fruits (cv. Jubilee n=12, Exp 5.3) stored for 7 days stored (at 5 ^{o}C) in the presence of e+$^{®}$ Ethylene Remover (2.5 g): values are expressed here are in mg g^{-1} DW.

	ASCORBIC ACID		**CITRIC ACID**		**MALIC ACID**		**OXALIC ACID**	
				Treatment				
Days at 5 ^{o}C	**Control**	**E+**	**Control**	**E+**	**Control**	**E+**	**Control**	**E+**
0	4.97	5.96	65.8	71.9	7.34	11.87	12.87	17.02
1	4.01	5.09	62.5	68.4	15.66	17.34	12.24	11.31
2	5.73	5.98	95.6	111.2	12.32	14.62	16.72	17.71
4	5.00	5.95	89.7	100.9	5.43	4.26	10.40	12.97
7	6.17	5.18	114.1	89.5	6.81	8.33	14.03	12.38
LSD (*P<0.05*)	1.936		36.31		5.827		7.746	

**No statistical differences in this data.*

5.4.3 Sugars and antioxidant capacity

Sugar accumulation in climacteric fruits has been widely studied (Giovannoni, 2001; Pech *et al.*, 2008) however; this process is less reported for non-climacteric fruits since these fruits are harvested at optimum ripeness fruits. Strawberries have been shown to have higher concentration of total sugars when treated with ethephon (Villarreal *et al.*, 2010) and when fully ripe (Cordenunsi *et al.*, 2005; Kafkas *et al.*, 2007; Basson *et al.*, 2010). Infected fruits (Exp 5.1, cv. Elsanta) accumulated significantly higher monosaccharide sugars in comparison to the non-infected (Appendix A, Table A.49, A.50). This overall higher concentration of monosaccharaides in infected fruits maybe due to the hydrolysis of sucrose as a result of higher ethylene production possibly caused by stress response of host to the *B. cinerea* infection. Non-infected fruits overall showed higher sucrose content (81.9 mg g^{-1} DW) compared to the infected (71.7 mg g^{-1} DW) which means the catabolism rate of sucrose to simple sugars was lower in these fruits. High concentrations of sucrose were also measured in E+ fruits (Exp 5.1 and 5.3). For example the overall sucrose content for the E+ fruits was 83 mg g^{-1} DW, which was significantly higher compared to the control fruits (70 mg g^{-1} DW) in Exp 5.1 (Appendix A, Table A.51). This suggests that controlling ethylene with the e+® Ethylene Remover may impact on the process of sugar hydrolysis and therefore suppressed senescence, which coincides with the colour data.

Principal component analysis (PCA) was used to demonstrate the source of variation on the sugars, ethylene, hue angle and total antioxidant capacity data of fruits treated with or without *B. cinerea* during preharvest (Exp 5.1) (Figure 5.4). The first principal component (PC1) was calculated to explain 33 % of the variance, whereas the second principal component (PC2) explained 21.5 % of the variance. Two main clusters were observed and separated by PC2. The infected fruits (I) were clustered together, while the non-infected fruits were clustered separately (Figure 5.4). The variables antioxidant capacity and ethylene were negatively correlated with sucrose and hue angle. Sucrose and colour (hue angle) were positively correlated while the ethylene and antioxidant capacity were also positively correlated.

Overall the infected fruits showed an antioxidant capacity of 168.5 µmol while in the non-infected fruits this was 147.8 µmol. Phenolic compounds are believed to be the

most important antioxidants contributing to the overall antioxidant capacity of fruits (Valero and Serrano, 2010). Moreover, the phenylpropanoid metabolism was found to be activated by exogenous ethylene in strawberries (Cisneros-Zevallos, 2003) suggesting that the high ethylene in the infected fruits triggered this process resulting in an overall high antioxidant capacity.

In Exp 5.3, significantly higher sucrose was apparent in the E+ fruits (Appendix A, Table A.60). PCA considered hue angle, ethylene and sugars data. PC1 explained 41 % of the variance and PC2 24 %. The E+ and control fruits were clustered separately along PC2. As observed in Exp 5.1 the ethylene and sucrose were also negatively correlated (Exp 5.3). Sucrose, hue angle and ethylene were responsible for the separation of the samples along PC2 (Figure 5.5). Results suggest that ethylene is associated with senescence in strawberries and enhanced ethylene accelerates processes such as sugar degradation which are associated with senescence.

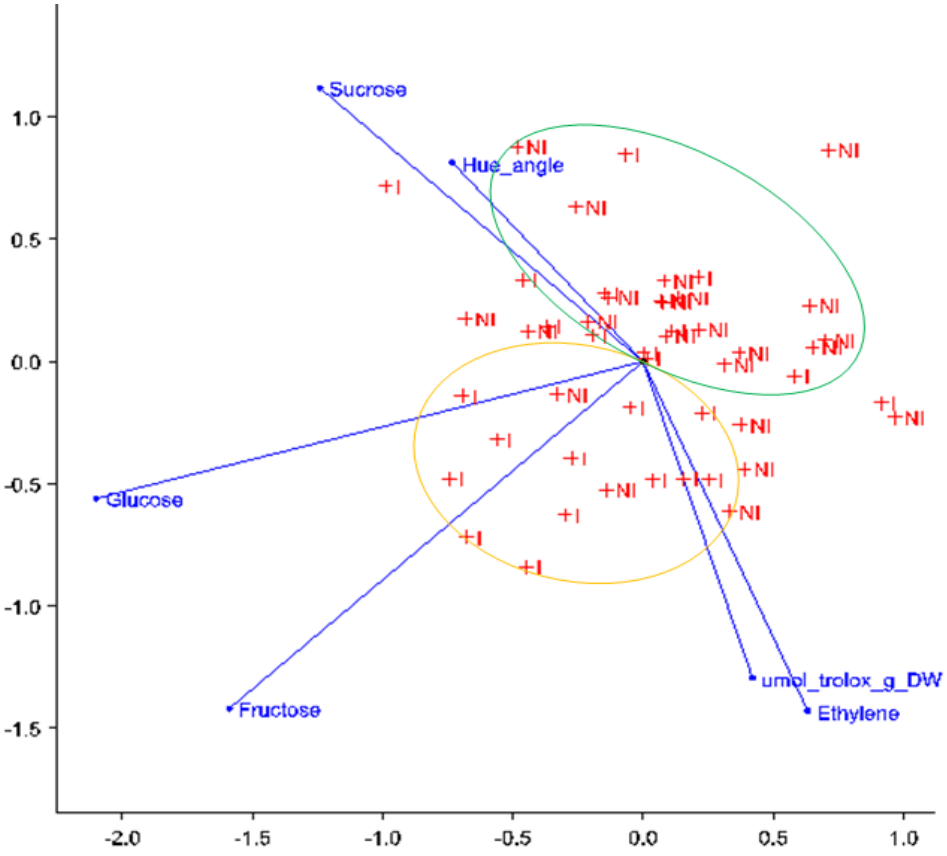


Figure 5.4. Principal component analysis (PCA) analysis of glucose, fructose, sucrose, hue angle, total antioxidant capacity (µmol trolox g^{-1} DW) and ethylene concentrations in infected (I ——) and non-infected (NI ——) fruits. X axis = PC1 (33 %) and Y axis = PC1 (21.5 %).

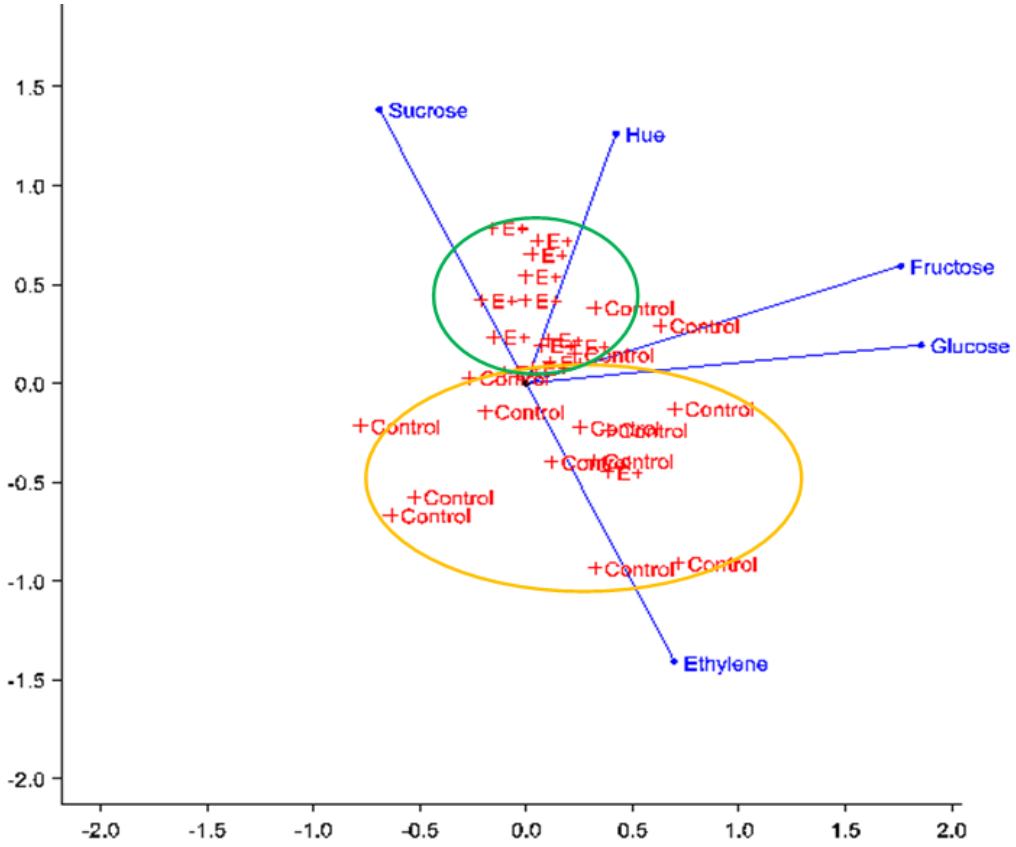


Figure 5.5. Principal component analysis (PCA) analysis of glucose, fructose, sucrose, hue angle, and ethylene concentrations of strawberries (cv. Jubilee, Exp 5.3) treated with (——) or without (——) e+® Ethylene Remover (2.5 g). X axis = PC1 (24 %) and Y axis = PC1 (41 %).

5.5 Conclusions

In conclusion the results here demonstrate a possible relationship between strawberry postharvest quality and ethylene in the fruit environment. Here, the use of e+® Ethylene Remover decreased ethylene in the fruit environment which was associated with reduced postharvest colour development of strawberries (cvs. Elsanta and Jubilee). Infection of strawberries with *B. cinerea* was associated with enhanced ethylene production, while the use of e+® Ethylene Remover reduced the ethylene of infected fruits and maintained the colour development (cv. Elsanta). This study is the first to look at the effect of e+® Ethylene Remover on the quality of fruits infected with *B. cinerea*. Infection of fruits with *B. cinerea* resulted in the accumulation of simple

sugars, while sucrose was enhanced in non-infected fruits, possibly attributed to enhanced rate of sucrose catabolism in the infected fruits. In agreement, fruits treated with e+® Ethylene Remover were also reported to have significantly higher sucrose content in two separate experiments (cvs. Elsanta and Jubilee). Phytohormones auxins and abscisic acid (ABA) have been shown to regulate many aspects relating to strawberry fruit ripening and development. Cross talk between ABA and ethylene in strawberry has also been suggested but there is little research elucidating the role of specific phytohormones after harvest.

CHAPTER SIX

6 DISSECTING THE ROLE OF ETHYLENE IN DETERMINING POSTHARVEST STRAWBERRY FRUIT QUALITY

6.1 Introduction

Plant phytohormones play an important role in non-climacteric fruit development (Chen *et al.*, 2011; Symons *et al.*, 2012). Auxin and abscisic acid are recognised to have a significant role in the development and ripening of strawberries (Nitsch, 1955; Given *et al.*, 1988; Terry *et al.*, 2007a). ABA has been shown to promote the pre-harvest ripening of strawberry (Jia *et al.*, 2011) and ABA applications have been shown to accelerate fruit colour and softening of strawberry while stimulating ethylene production (Jiang and Joyce, 2003). In addition, others have proposed that ABA is upregulated when fruits are exposed to abiotic and biotic stress (Terry *et al.*, 2007a). Auxins have been associated with the inhibition of receptacle ripening (Given *et al.*, 1988; Manning, 1994) and the application of synthetic auxins delayed strawberry ripening (Given *et al.*, 1988). Given all this evidence that phytohormone flux is important in strawberry fruit ripening (Archbold and Dennis, 1984; Jia *et al.*, 2011; Symons *et al.*, 2012), it is surprising that little work has evaluated the role of ethylene and other phytohormones during postharvest, especially given the senescent processes and water loss observed after harvest. This study therefore reports on the effect of ethylene and ethylene inhibition and removal on the physiology and biochemistry (during postharvest of strawberries) including taste and health related compounds and phytohormones. Moreover, real time ethylene measurements were conducted using a highly sensitive ethylene detector to detect very low levels. Respiration rate was also measured in real-time during storage with a highly sensitive technique.

6.2 Materials and methods

6.2.1 Plant material and treatment structure

Strawberry fruits (cv. Sonata, n=252) were purchased from a local grower (H & H Duncalfe in Cambs., UK) during August 2012. Fruits were grown under standard commercial growing conditions and harvested on 6th August. Fruits were received in the Plant Science Laboratory

within 2 h of harvest. On arrival, fruits of similar size and weight were selected, placed in plastic punnets and subjected to four different treatments. In each punnet there were n=12 fruits and for each treatment there were n=5 punnets (n=60 fruits per treatment). Punnets containing the fruits were stored within a 320 l rigid polypropylene fumigation chamber (n=4 in total). Fruits were subjected to the following treatments: 1-MCP (1 µl l^{-1}), Ethylene (50 µl l^{-1}), e+® Ethylene Remover (E+) (2.5 g powder), and control chamber containing no treatment control. Fruits were kept inside the chambers for a 24 h period. The e+® treatment remained in the punnets throughout storage.

6.2.2 Application of 1-MCP, ethylene and e+® Ethylene Remover

A 1-MCP (1 µl l^{-1} concentration) evolving solution was prepared according to Chope *et al.* (2007). Briefly 1.80 g of SmartFresh (Rohm and Haas, PA, USA) was placed inside a 50 ml conical flask, and sealed with parafilm (Fisher Scientific Bed, UK) then warm (50 ºC) distilled water (20 ml) was injected into the flask through the seal. The flask was immediately opened and placed in the chamber with the fruits inside. The chamber was closed with a moat of water providing an air tight seal. All chambers were kept sealed for 24 h at 5 ºC. Ethylene treatment (50 µl l^{-1}) was achieved by injecting 16.25 ml ethylene (100 % ethylene; SIP Analytical Ltd., Kent, UK) into the sealed chamber through a tapped tube (polyvinyl chloride) followed by repeated withdrawal-injection to homogenise the ethylene into the chamber. The e+® Ethylene Remover treatment was achieved by weighing e+® Ethylene Remover powder (2.5 g) into a glass petri dish, which was then placed inside each punnet (n=5 in total). In addition there was a control chamber where fruits received no treatment.

6.2.3 1-MCP and ethylene quantification

The concentration of 1-MCP (1 µl l^{-1}) and ethylene (50 µl l^{-1}) were quantified using flame ionisation gas chromatography (GC model 8340, EL980 FID and DP800 integrator, Carlo Erba Instrument, Herts, UK) as described previously (Chope *et al.*, 2007; Terry *et al.*, 2007b). After 2 h 1.103 µl l^{-1} of 1-MCP and 55 µl l^{-1} of ethylene were quantified inside the chamber.

6.2.4 Fruit sampling

After 24 h fruit punnets were removed from chambers. For each treatment n=1 punnet was assessed immediately (day 1), meanwhile the remaining punnets were each placed inside labelled 13 l plastic boxes at 5 ^{o}C, which were flushed constantly (7 l min^{-1}) using a blower manifold (custom built and supplied by Air Equipment, Beds., UK). This ensured complete

air exchange within the boxes and to prevent the accumulation of carbon dioxide (CO_2) and ethylene. Strawberries were analysed for CO_2, ethylene, colour and disease incidence during cold storage (0, 1, 2, 4, 6, and 10 days at 5 ^oC) and snap frozen for biochemical analysis. Fruits assessed on day 0 before treatment were used for baseline measurements.

6.2.5 Ethylene production rate

Real time ethylene production was monitored continuously (for 3 h) with a newly developed laser based photoacoustic ethylene detector (ETD-300) as described in Chapter 5 with minor modifications. Briefly, three jars were used for each treatment at each given time. Thus, only two of the four treatments could be assessed at the same time. Strawberry fruits (n=4) were placed inside glass jars (0.5 l) connected to the ethylene detector. Final values were adjusted for fruit weight to determine the ethylene rate in nl kg^{-1} h^{-1}.

6.2.6 Respiration Measurements

Respiration rate was assessed (at 5 ^oC) after 0, 1, 2, 4, 6 and 10 days of cold storage. Gas measurements were taken directly from the 13 l plastic boxes (which was flushed during sampling) and were analysed using a Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, NV, USA), as previously described (Collings *et al.*, 2012). Briefly, the instrument was calibrated with 10.06 % CO_2 and 1.99 % O_2 (10 % CO_2, 2 % O_2, 88 % N_2; certified standard from BOC, Surrey, UK). Sequencing was controlled by a MUX flow multiplexer (hardware version 4, firmware version 1.05) and air was subsampled at 1.5 l min^{-1} from each box via a 'pull mode' set up using an SS4-subsampler (firmware version 2.0). The water vapour pressure (WVP) was also determined with a RH-300 WVP detector (Sable Systems International, NV, USA). Sampled air was then passed through a water scrubber (Drierite) and analysed with a CA-10 carbon dioxide detector (firmware version 1.05) and a FC-10 oxygen detector (firmware version 3.0), to determine CO_2 (%) and O_2 (%), respectively. Additionally, flow rate (FR, ml min^{-1}) and barometric pressure (BP, kPa) was also recorded using the SS4-subsampler and CA-10 carbon dioxide detector, respectively. Data was analysed and interpreted by the instrument software (ExpeData Release 1.3.8, Version: PRO, Sable Systems International, NV, USA).

6.2.7 Colour measurements

The objective colour (lightness; L*), chroma (colour saturation; C*), hue angle (H^o) was determined for each fruit as described in Chapter 3 and previously reported by Terry *et al*. (2007b) using colorimeter.

6.2.8 Disease incidence

The total number of diseased berries was recorded for each treatment and sampling date for the percentage of diseased berries at each storage day per treatment.

6.3 Biochemistry

6.3.1 Sugars extraction and quantification

Sugars were extracted as previously reported (Terry *et al.*, 2007a). Sugars were measured as described in Chapter 5.

6.3.2 Organic acid extraction and quantification

Freeze-dried strawberry residue (150 mg) was extracted with 3 % metaphosphoric acid in water (v/v) (6 ml). The samples were homogenized and vortexed for 30 seconds and kept on ice for 5 min. The samples were vortexed again and filtered through 0.2 µm pore-size filter. The extracts were immediately measured by HPLC-DAD.

Organic acids were measured as described in Chapter 5 and by others (Giné Bordonaba and Terry, 2008) with some modifications. Briefly, organic were measured using an Agilent 1200 series HPLC system (Agilent, Berks., UK) equipped with an Agilent 1200 DE G1364C/G1315D photodiode array with multiple wavelength detector. The mobile phase consisted of 0.2 % metaphosphoric acid in water adjusted to pH 2.5 using metaphosphoric acid. The flow rate was 1 ml min^{-1} (isocratic) and the sample injection volume was 20 µl. The column temperature was set at 35 ^oC. Peaks were recorded at 210 nm for oxalic, citric and malic acids and 245 nm for ascorbic acid. The organic acids were identified and quantified using an external calibration curve prepared with authentic standards and the concentrations expressed as mg g^{-1} of DW.

6.3.3 Phenolic compounds extraction and quantification

Phenolic compounds were extracted as previously described (Terry *et al.*, 2007a). Phenolic compounds were measured using the HPLC above. Chromatographic separation was

performed on a 4.6 x 150 mm, 5 µm Zorbax Eclipse XDB-C18 column (Agilent) connected to an OPTI-GUARD 1 mm guard column (Crawford Scientific). The mobile phase consisted of 1 % phosphoric acid: 10 % acetic acid in water (A) and acetonitrile (B). The mobile phase followed a linear gradient from 2 to 20 % of solvent B over 25 min, and from 20 to 98 % B in 5 min and held for 2 min. The flow rate was 1 ml min^{-1}, the injection volume 20 µl and the column temperature set at 40 °C. All peaks were observed in the range 200-700 nm. The phenolic compounds were characterized by their UV-Vis spectra and retention time relative to external standards. Flavan-3-ol (catechin and epicatechin) were quantified with catechin standards at 280 nm, phenolic acid (chlorogenic acid) at 332 nm, flavonol (quercitin-3-glucoside), ellagic acid at 355 nm and anthocyanins (cyanidin-3-glucoside and pelargonidin-3-glucoside) at 520 nm. Concentration of each phenolic compound was calculated using authentic standards. The results were expressed as µg g^{-1} of DW.

6.3.4 Extraction and quantification of strawberry phytohormones

6.3.4.1 Extraction of strawberry phytohormones

Phytohormones were extracted following the procedure outlined by Ordaz Ortiz *et al*. (2012). Briefly freeze-dried samples (*ca*.150 mg) were placed in 15 ml centrifuge tubes (Fisher Scientific., UK) and 5 ml of methanol/water/formic acid (72: 20: 5 v/v) was directly added to the samples. Thereafter, 50 µl of internal standard containing d_4-ABA, d_5-ABA-GE, d_3-PA, d_4-7'-OH-ABA, d_3-DPA, d_2-GA_1 and d_2-GA_4 (400 ng ml^{-1} concentration) was also added to the sample mixture. The mixture was vortexed thoroughly and left for 12 h (at -20 ^oC) to be cold-extracted. The extracted samples were vortexed and then centrifuged for 15 min at 1960 g (4500 rpm) at 4 ^oC. The supernatant was collected (extraction1) and the pellet was re-extracted with 2 ml of the extraction solvent (above) for 30 min. The extract was again vortexed and centrifuged for 15 min at 1960 g. The supernatant was removed (extraction 2) and combined with the first supernatant into one fraction (extraction 1 + 2). Samples were filtered using a 0.2 µm filters. A Sep-Pak Plus C18 cartridge 6cc/500 mg, 37-55 µm (Waters Associates, Milford, MA) was conditioned with 5 ml of 100 % methanol and equilibrated with 5 ml of formic acid 1 M. The supernatant were loaded onto the C18-cartidge and kept. Methanol in the collected sample was evaporated by applying a flow of N_2 gas, until a volume of 1.5-2 ml was achieved. Centrifuge tubes containing the samples were immersed in liquid nitrogen to ensure samples were fully frozen. Immediately the frozen supernatant was freeze dried in the dark overnight. The freeze dried samples were then reconstituted with 1 ml of formic acid (1 M). Oasis MCX 6cc/150 mg, 60 µm cartridge (Waters Associates, Milford,

MA) were conditioned with 5 ml of 100 % methanol and equilibrated with 5 ml of formic acid (1 M). The supernatant was then loaded onto the Oasis MCX cartridges and the hormones were eluted with 100 % methanol (2 ml) and 2 ml of 0.35 M NH_4OH in 60 % methanol. The methanol in the samples was again evaporated with a flow of N_2 until a sample volume reached 1.5-2 ml. Samples were freeze dried overnight. Samples were reconstituted with 400 µl of mobile phase A (0.1 % formic acid in water). A 50 µl of external standard (d_6-ABA, 400 ng ml^{-1} solution) was added to 1 out of 10 samples. Samples containing the external standard were reconstituted in 350 µl of mobile phase.

6.3.4.2 LCMS quantification

Phytohormones were determined according to Ordaz Ortiz *et al*. (2012) with modifications. Shortly, samples (5 µl) were injected into an Agilent 1290 Infinity UPLC system. A Zorbax Eclipse Plus RRHD C18 column (2.1 x 50 mm, 1.8 µm) was used to obtain chromatographic separation: an Agilent 1290 Infinity Thermostatted column compartment (TCC) was operated at 30 °C. Compounds were separated at a flow rate of 0.6 ml min^{-1} using a linear gradient of solvent A (water + 0.1 % formic acid) and solvent B (acetonitrile + 0.1 % formic acid) programmed as following: 0 min, 4 % B + 96 % A; 7.0 min, 26 % B + 74 % A; 10 min, 40 % B + 60 % A; 10.60 min 100 % B and hold for 1 min; followed by 1.35 min for re-equilibration with 4 % B + 96 % A.

An Agilent 6540 Ultra High Definition Accurate Mass Q-TOF LC-MS System was used to quantify phytohormones. All the samples were analysed using a Dual ESI Agilent Jet Stream source in negative and positive mode. The following settings were applied for both modes: nebulizer gas temperature (N_2) 325 °C, at a flow of 8 l min^{-1}, sheath gas temperature (N_2) 350 °C at a flow rate of 1 l min^{-1}. Full scan data was acquired in the range of 100-1000 m z^{-1}, at an acquisition rate of 3 spectra s^{-1}.The LC-MS system and data acquisition were controlled by Agilent MassHunter Data Acquisition software B.04.00, data analysis was processed using Agilent MassHunter Quantitative Analysis software B.05.00. Endogenous phytohormones concentration was calculated using internal standard calibration curve prepared at the following concentrations 5, 10, 25, 50, 75, 100, 150, 300 ng ml^{-1}. Quantitation was performed automatically by the software taking into account the area underneath each standard compound peak divided by the area integrated by its corresponding deuterated internal standard (relative response); calibration curve were plotted using relative response against known concentration level.

6.4 Statistical analysis

Statistical analysis was performed using Genstat as described in Chapter 3. Data were subjected to analysis of variance (ANOVA), followed by a comparison of the means according to a Least significant difference (LSD) test at $P<0.05$.

6.5 Results and discussion

6.5.1 Respiration, ethylene and colour measurements

This is the first piece of work which has measured the temporal change in ethylene production and respiration rate during strawberry postharvest. To this end, ethylene production was monitored in real time using a highly sensitive (0.3 nl l^{-1}) laser based ethylene detector with high time resolution (5 s) while respiration was measured in real time with a Sable Respirometry System having high resolution (1 µl l^{-1}).

The ethylene production of strawberries (cv. Sonata) exhibited a significant increase throughout storage (Appendix A, Table A.65). Ethylene production rate increased from 90.8 nl kg^{-1} h^{-1} on day 0 to 240.5 nl kg^{-1} h^{-1} on day 10. Moreover, a significant increase in respiration was observed for ethylene-treated fruits in contrast to the other treatments (Table 6.1). Although there was no treatment effect on the ethylene production of strawberries (Appendix A, Table A.64,), fruits stored under low ethylene environment overall had the lowest ethylene level (196.70 nl kg^{-1} h^{-1}), while ethylene treated fruit produced the highest (210 nl kg^{-1} h^{-1}). Iannetta *et al*. (2006) demonstrated enhanced ethylene production at red ripe stage and a concomitant respiratory increase in strawberries. Iannetta *et al*. (2006) demonstrated increase in the ethylene and respiration *in planta* and on detached fruits suggesting that the former was due to ripening and the latter to senescence. Additionally, this study also employed the same highly sensitive ethylene detector used herein, which no doubt contributed to the results obtained. In comparison, conventional techniques for measuring ethylene and respiration such as gas chromatography are incapable of measuring in real time and are much less sensitive (*ca.* 1 µl l^{-1}) making it difficult to observe trends overtime. Fruits stored in a low ethylene environment (E+) had a significantly higher hue angle (Appendix A, Table A.66), thus less red after 10 days at 5 ^{o}C (Table 6.1) which is consistent with results in Chapter 5.

During storage fruits exhibited significant water loss (Appendix A, Table A.67), however, this was accelerated by ethylene treatment (Figure 6.1). Overall fruits treated with ethylene

exhibited significantly higher water loss in comparison to the other treatments which could be attributed to high rates of senescence.

Table 6.1. The effect of treatments on the hue angle (H°), respiration rate (ml kg^{-1} h^{-1}), ethylene production (nl kg^{-1} h^{-1}) and % water loss of strawberries (cv. Sonata, n=60) during 10 days storage at 5 °C.

	Control	**1-MCP**	**E+**	**Ethylene**	LSD (P<0.05)
H° angle	35.50[a]	35.33[a]	37.26[b]	35.77[a]	1.039
Respiration rate	67.10[ab]	78.10[b]	52.40[a]	110.2[c]	17.44
Ethylene	204.50[a]	209.40[a]	196.70[a]	210.20[a]	37.06

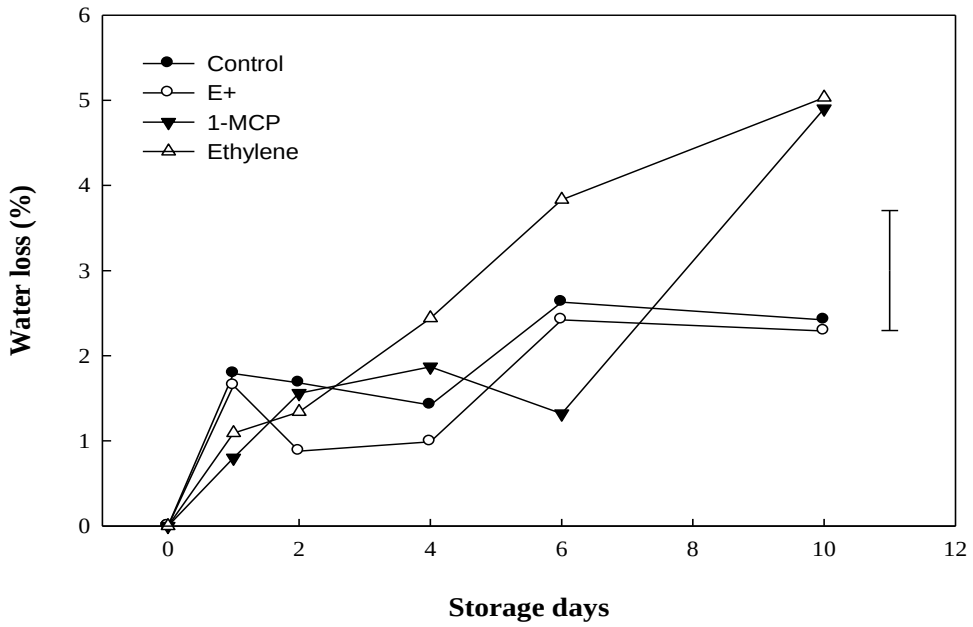


Figure 6.1. The effect of treatments on the % water loss of strawberries (cv. Sonata, n=12) stored at 5 °C for 10 days. LSD (P<0.05).

6.5.2 Disease incidence

The most prominent disease affecting strawberry fruit quality during postharvest is grey mould caused by *B. cinerea* (El-Kazzaz *et al.*, 1983; Fallik *et al.*, 1993). During postharvest there is a natural decline in the disease resistance in fruits whereby the growth of pathogens including *B. cinerea* is enhanced once conditions are more favourable. In recent literature, the deterioration of strawberries has been shown to be stimulated by the presence of ethylene during storage, hence increasing potential waste (Wills and Kim, 1995; Bower *et al.*, 2003). The percentage of diseased berries caused by *B. cinerea* found in each treatment during 10 days at 5 ^{o}C was recorded (Table 6.2). Treatments with ethylene and 1-MCP were associated with high percentage of diseased berries. Fruits treated with ethylene for 24 h showed signs of disease after 48 h of storage. However, in the 1-MCP treatment diseased berries were observed from day 6 of storage. This is in accordance with literature where ethylene was shown to accelerate deterioration of strawberries (Wills and Kim, 1995; Bower *et al.*, 2003). Nevertheless, an increased decay of strawberries treated with high 1-MCP concentrations (up to 0.5 µl l^{-1}) has been observed previously (Ku *et al.*, 1999; Jiang *et al.*, 2001). Bower *et al*. (2003) suggested that blocking ethylene perception may negatively affect disease resistance in strawberries. Ku *et al*. (1999) suggested that 1-MCP may interfere with the natural defence system that is responsible for resistance. In accordance, inhibition of ethylene with the 1-MCP correlated with high disease incidence, while fruits stored under low ethylene environment (E+) had the lowest percentage of diseased fruits (Table 6.2) suggesting that 1-MCP may be affecting processes related to defence against disease. Application of exogenous ethylene accelerated disease whereas the presences of e+® Ethylene Remover delayed this process suggesting a role of ethylene in the growth of *B. cinerea* in strawberries.

Table 6.2. The effect of treatments on the percentage (%) of diseased strawberries (cv. Sonata, n=12) during storage at 5 ^{o}C.

	Control	**Ethylene**	**1-MCP**	**E+**
Day 1	0	0	0	0
Day 2	0	1.6	0	0
Day 4	0	1.6	0	0
Day 6	0	1.6	5.0	0
Day 10	13.3	13.3	11.6	3.3

6.6 Biochemistry

The proportion of sugars and acids in strawberry is an important indicator of fruit taste and quality (Kafkas *et al.*, 2007; Giné Bordonaba and Terry, 2009). Some recent literature has considered the role of ethylene in the development, ripening and postharvest life of non-climacteric fruits including strawberries (Atta-Aly *et al.*, 2000; Bower *et al.*, 2003; Villarreal *et al.*, 2010). As found by others (Cordenunsi *et al.*, 2003; Olsson *et al.*, 2004; Kafkas *et al.*, 2007) glucose and fructose were the dominant sugars measured, but a considerable portion of sucrose was also detected (Figure 6.2). Sucrose was the dominant sugar at the start; however sucrose content decreased greatly following storage where levels reduced from 298.8 mg g^{-1} on day 0 to 149.8 mg g^{-1} after day 10. Similarly, others have demonstrated such trend in sucrose during storage (Cordenunsi *et al.*, 2005; Kafkas *et al.*, 2007), which was attributed to enhanced invertase activity whereby sucrose is broken down to other carbohydrates. In addition, sucrose catabolism is an indicator of senescence affecting the natural disease resistance of the fruit. Moreover, the decline of sucrose was mirrored in the increase of simple sugars (glucose and fructose) during storage (Figure 6.2).

As demonstrated in Appendix A, (Table A.68), sucrose content was significantly affected by treatments and storage. Application of ethylene resulted in the lowest sucrose content which was significant in contrast to the E+ and control fruits, but not significant when compared to those treated with 1-MCP. The simple sugars (glucose and fructose) were significantly higher in fruits treated with ethylene indicating effect of ethylene in the concentration of sugars in

strawberries (Appendix A, Table A. 69, A.70). Similarly; Villarreal *et al.* (2010) reported an increase in sugar concentrations in strawberries at white stage following ethylene treatment. The effect of ethylene on the concentration of sucrose and the simple sugars could be explained by the increase in senescence and respiration rate during storage.

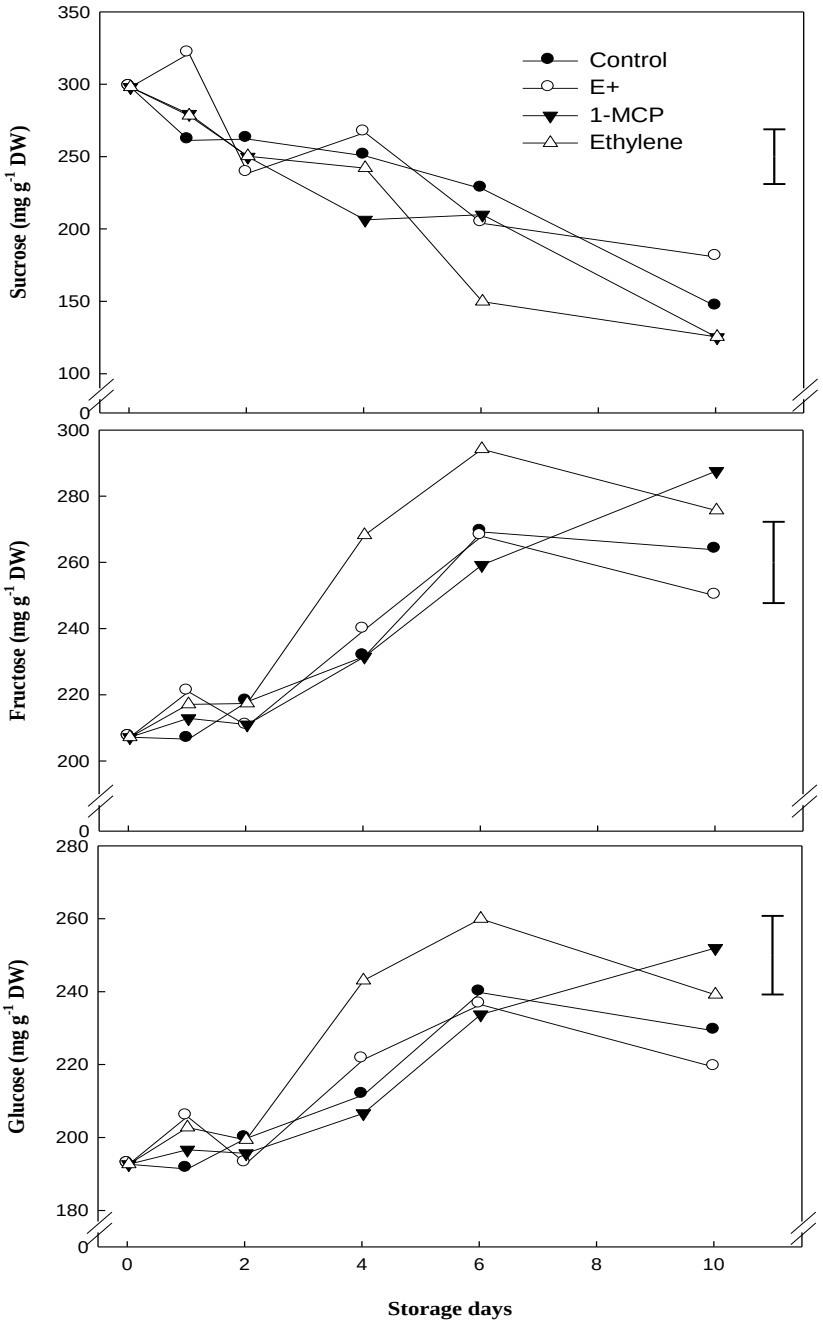


Figure 6.2. The effect of treatments on the sugar concentration in strawberries (cv. Sonata, n=12) stored at 5 ^{o}C for 10 days. LSD (P<0.05).

Strawberries are valued for their high content of ascorbic acid. Ascorbic and citric acid are the most important organic acids in strawberries (Cordenunsi *et al.*, 2003; Olsson *et al.*, 2004; Cordenunsi *et al.*, 2005; Terry *et al.*, 2007a). Citric acid decreased significantly with storage (Figure 6.3). All organic acids measured decreased significantly after storage (Appendix A, Table A.71-A.74) which is consistent with that reported by others (Moing *et al.*, 2001). The decrease in ascorbic acid was most prominent in fruits treated with ethylene which reflects in the overall quality of the ethylene treated fruits (Figure 6.3). In contrast, significantly higher ascorbic acid (Appendix A, Table A.71) was observed in the fruits stored under low ethylene environment (E+) than those treated with ethylene (Figure 6.3). Similar trends were also observed in the malic acid content whereby ethylene treated fruits exhibited considerably lower levels.

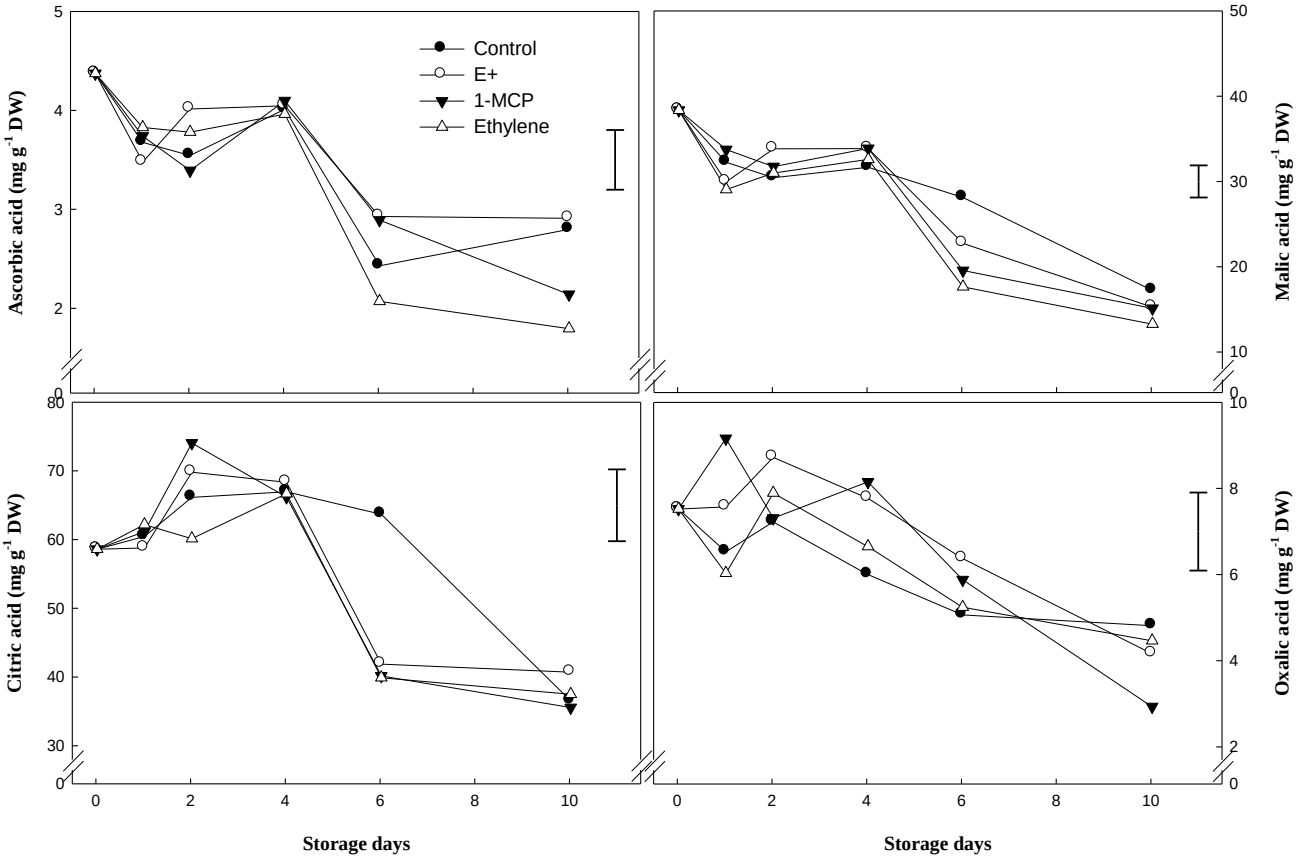


Figure 6.3. The effect of treatments on the concentration of organic acids in strawberries (cv. Sonata, n=12) stored at 5 ^{o}C for 10 days. Values are expressed in mg g^{-1} DW.

Three anthocyanins pelargonidin-3-glucoside, pelargonidin-3-glucoside derivative and cyanidin-3-glucoside were detected. The concentration of anthocyanins increased during storage as reported previously (Gil *et al.*, 1997; Cordenunsi *et al.*, 2005). All phenolic compounds measured except quercetin-3-glucoside exhibited a significant increase during storage (Appendix A, Table A.75-A.82). Epicatechin and chlorogenic acid content was overall significantly higher in fruits treated with ethylene (Appendix A, Table A.79, A.80). Furthermore, fruits stored under low ethylene environment exhibited significantly lower epicatechin content with respect to control and ethylene treatments (Table 6.3). This increase in phenolic compounds in the ethylene treated fruits could be due to activation of phenylpropanoid metabolism, which has been shown to be induced by exogenous application of ethylene in strawberries (Cisneros-Zevallos, 2003).

Table 6.3. The effect of treatments on the concentration of individual phenolic compounds (μg g^{-1} DW) measured in strawberries (cv. Sonata, n=60) stored at 5 ^{o}C for 10 days.

	Control	**E+**	**1-MCP**	**Ethylene**	LSD (P<0.05)
Pelargonidin-3-glucoside	1280^{a}	1278^{a}	1330^{a}	1288^{a}	120.6
Pelargonidin-3-gluc.deriv.	372.3^{a}	360.8^{a}	385.2^{a}	348.5^{a}	39.16
Cyanidin-3-glucoside	59.4^{a}	54.5^{a}	60.3^{a}	58.3^{a}	11.45
Catechin	477^{a}	519^{a}	490^{a}	543^{a}	81.2
Epicatechin	795^{b}	666^{a}	739^{ab}	933^{c}	111.3
Chlorogenic acid	362.6^{ab}	345.7^{a}	382.2^{bc}	404.6^{c}	35.93
Ellagic acid	38.8^{a}	44.8^{a}	47.3^{a}	42.8^{a}	7.53
Quercetin-3-glucoside	273^{a}	268^{a}	259^{a}	228^{a}	80.6

6.6.1 Phytohormones content

Abscisic acid (ABA) and its metabolites (7'-OH abscisic acid (7'-OH ABA), abscisic acid glucosyl ester (ABAGE), and auxins, indole-3-acetic acid (IAA) and indole-3-acetylaspartic

acid (IAAsp) were detected in strawberry. However, the auxins were below the quantification limit and were not considered in this study.

Endogenous ABA content is determined by a dynamic balance between biosynthesis and catabolism rates (Sun *et al.*, 2010). ABA can be degraded through the hydroxylation of 7', 8' or 9' methyl group, or stored in a conjugated form as ABA-glucosyl ester (ABAGE) (Kepka *et al.*, 2011). In this study ABA levels increased nearly 2 fold after 10 days at 5 ^{o}C. Throughout storage this significant increase in ABA (Appendix A, Table A.83) was observed for all treatments (Figure 6.4). Recent reports have also provided evidence to suggest a role of ABA in strawberry fruit ripening and an upregulation of ABA was observed in fruits at the onset of preharvest ripening (Archbold, 1988; Chen *et al.*, 2011; Jia *et al.*, 2011). That said, there is little literature on the change of this hormone during the storage of strawberries. However, in other non-climacteric fruits such as grapes and cherries ABA increased and the accumulation of ABA to a higher level was associated with berry senescence (Blanusa *et al.*, 2006; Sun *et al.*, 2010). Furthermore, ABA synthesis has been shown to be induced by drought and salt stress. Terry *et al*. (2007a) demonstrated that the stress caused by deficit irrigation was associated with increased ABA concentration during postharvest. On the other hand, others have suggested that sucrose may serve as a signal molecule for ABA biosynthesis possibly having an important role in strawberry ripening (Jia *et al.*, 2011; Jia *et al.*, 2013). Here, ethylene treated fruits exhibited higher rates of water loss due to enhanced senescence. Moreover, these fruits showed higher ABA concentration, which was possibly caused by stress due to high water loss. However, results here did not demonstrate a correlation between ABA and sucrose levels during storage. Nonetheless, after postharvest increase in ABA was possibly caused by stress from senescence suggesting that the interplay between sucrose and ABA is not present during postharvest. In addition, ABA metabolites 7'-OH ABA and ABAGE followed the same trend as that of ABA with levels increasing steadily during storage (Figure 6.4). The increase in ABA metabolites could be explained since ABA may be stored as ABAGE (Sun *et al.*, 2010) or catabolized to 7'-OH ABA. High concentration of ABA was also observed in 1-MCP treated fruits which was possibly due to senescence since high number diseased berries were recorded in this treatment. However, no treatment effect was observed in the content of ABA metabolites (Appendix A, Table A.84, A.85) (Figure 6.4).

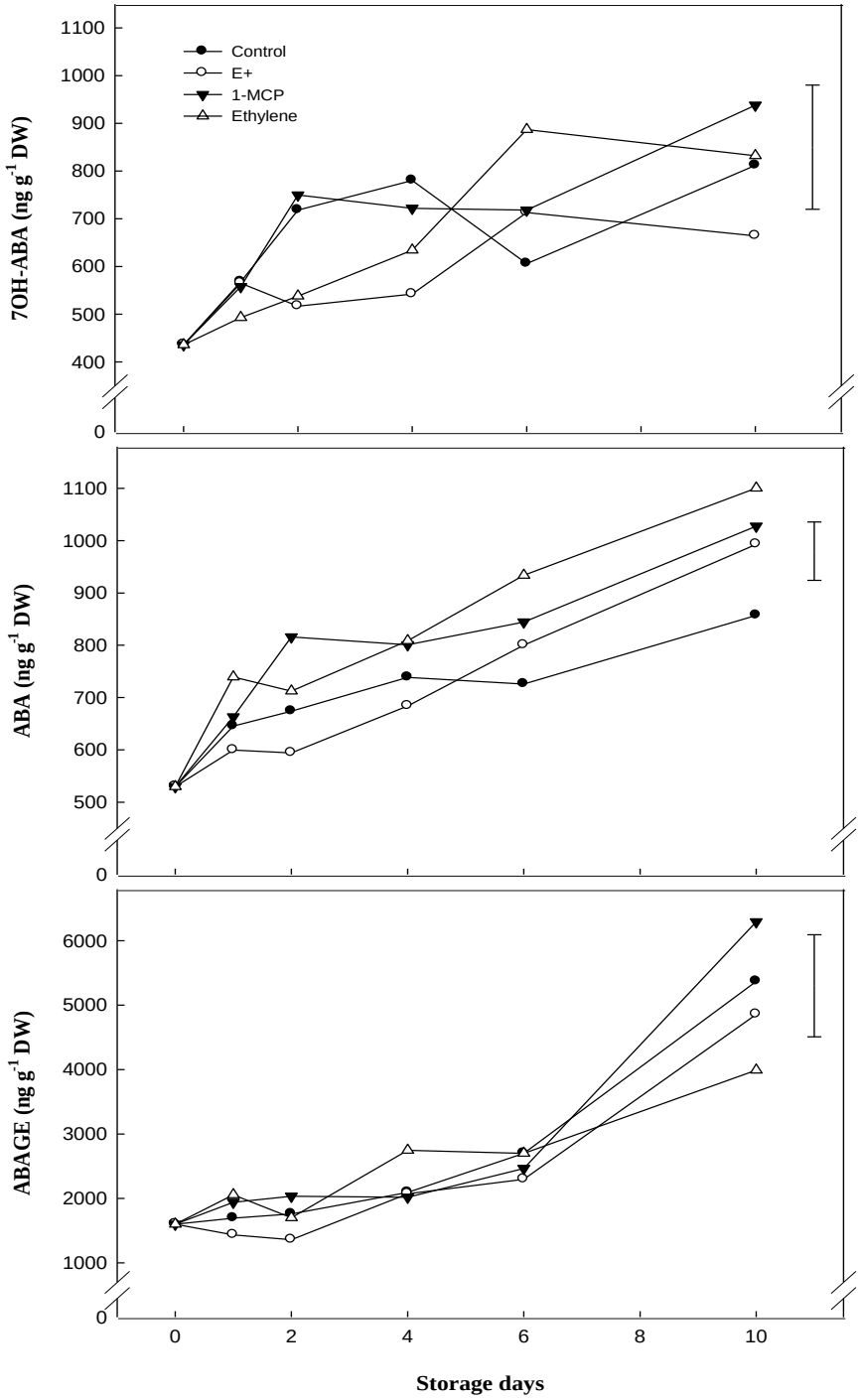


Figure 6.4. The effect of treatments on ABA and ABA metabolites (ABAGE and 7'-OH ABA) content in strawberries (cv. Sonata, n=12) stored at 5 ^{o}C for 10 days. LSD (P<0.05).

6.7 Conclusion

The study suggests a role of ethylene in the postharvest quality of strawberries. During storage strawberries demonstrated enhanced, water loss, disease incidences, accumulation of simple sugars, decrease in organic acids and enhanced synthesis of ABA. That said, ethylene treatment stimulated these processes. In conclusion, the postharvest application of ethylene regulates many aspects of strawberry fruit quality. Results suggest interplay between ethylene and ABA where ABA synthesis was increased following ethylene treatment. However, strawberries are classified as non-climacteric but in this study a significant role of ethylene in the storage of strawberries is demonstrated.

CHAPTER SEVEN

7 ACHENES AND RECEPTACLE PHYTOHORMONES PROFILE OF STRAWBERRIES TREATED WITH ETHYLENE, 1-MCP AND E+® ETHYLENE REMOVER DURING POSTHARVEST

7.1 Introduction

Auxins are believed to be synthesised in achenes and are often found conjugated to various amino acids and peptides. This plant hormone is involved in regulating numerous aspects of plant growth and development (González-Lamothe *et al.*, 2012). In strawberries auxins have been shown to be negatively correlated with fruit ripening (Given *et al.*, 1988; Manning, 1994). Archbold and Dennis (1984) elucidated that auxin levels decline gradually with fruit maturity, while Given *et al*. (1988) showed that the application of synthetic auxins inhibited fruit ripening. Abscisic acid (ABA) has been previously reported to play an important role in the regulation of strawberry ripening (Chai *et al.*, 2011; Jia *et al.*, 2011). Changes in the ABA concentrations during strawberry development have been reported (Archbold and Dennis, 1984; Symons *et al.*, 2012). ABA has been shown to accumulate during the late stages of development and ripening (Chai *et al.*, 2011; Jia *et al.*, 2011; Symons *et al.*, 2012).

The changes in the phytohormones profile at different stages of strawberry fruit development have been previously reported (Chai *et al.*, 2011; Jia *et al.*, 2011; Symons *et al.*, 2012), however this has not been characterised during postharvest. The purpose of this study was to investigate the changes in the phytohormones in the achenes and receptacles of strawberries after harvest. The aim was to determine the role of ethylene in determining postharvest strawberry fruit quality and cross talk with other phytohormones.

7.2 Material and methods

7.2.1 Achenes, flesh and whole

Samples from Chapter 6 were used for this study. Fruits (n=12 per treatment) from each storage day (0, 1, 2, 4, 6, 10) were frozen in liquid nitrogen and freeze dried. Fruits were separated into 3 blocks with each block containing n=4 fruits. The fruits (n=4) from each block were pulled together to give 3 replicates per treatment, per day. The achenes were separated manually from the freeze-dried strawberries using a pair of tweezers and then ground with a mortar and pestle separately.

7.2.2 Extraction and quantification of strawberry phytohormones

Phytohormones were extracted from the different strawberry tissues following the procedure outlined by Ordaz Ortiz *et al*. (2012) and described in Chapter 6.

7.2.3 LCMS quantification

Phytohormones were determined according to Ordaz Ortiz *et al*. (2012) with minor modifications as described in Chapter 6. Endogenous concentration was calculated against using authentic standards at the following concentration 5, 10, 25, 50, 75, 100, 150, 300 ng ml^{-1}.

7.3 Statistical analysis

Statistical analysis was performed using Genstat as described in Chapter 3.

7.4 Results and discussion

7.4.1 Phytohormones

In this study the levels of various forms of two different phytohormones (abscisic acid (ABA), 7'-OH abscisic acid (7'-OHABA), abscisic acid glucosyl ester (ABAGE), indole-3-acetic acid (IAA), indole-3-acetylaspartic acid (IAAsp) were detected and measured in strawberry tissues and the response to different ethylene treatments studied.

7.4.1.1 ABA and ABA metabolites

Studies concerning the role of ABA have focused on the whole fruit however; there is little consideration in the content of ABA within different tissues. Significant difference

between ABA concentration in the flesh and achene of strawberries was observed (Appendix A, Table A.95) as demonstrated in Figure 7.1. The content of ABA in the flesh was over 4 fold higher than in the achenes. Symons *et al*. (2012) studied ABA concentration in the different tissues at white stage of development and demonstrated that ABA concentration in achenes was twice that in the flesh. However, ABA has been reported to have a role in fruit ripening thus it is possible that the ripening process may have resulted in enhanced ABA in the receptacle tissues (Jiang and Joyce, 2003; Symons *et al.*, 2012). Moreover, it is possible that ABA content may have increased due to senescence as previously reported in grapes (Sun *et al.*, 2010).

1-MCP and ethylene treatment resulted in overall the highest ABA content in the flesh tissue. However, ABA was significantly higher in the ethylene treatment for the achenes (Figure 7.1). Similarly; Sun *et al*. (2010) observed a significant increase in the ABA levels in the pulp of grapes treated with ethephon during storage. Generally in both tissues ABA content increased significantly with storage. During storage strawberries experienced significant water loss due to senescence. Moreover, ABA synthesis was found to be induced by stress (Terry *et al.*, 2007a). Similarly, Sun *et al*. (2010) observed that the gene (*VvNCED1*) encoding the enzyme responsible for the ABA biosynthesis was upregulated due to stress caused by water loss in grapes. Although an increase with ripening has been reported for the whole fruit (Chai *et al.*, 2011; Jia *et al.*, 2011; Symons *et al.*, 2012) there is no literature on the change of this hormone in the separate tissues during storage. There are several different ABA metabolites which can be produced following the oxidation of ABA for example 7'OH-ABA (Kepka *et al.*, 2011). ABA metabolite 7'OH-ABA was detected in all tissues in similar concentrations (Figure 7.2). ABAGE metabolite is produced after conjugation of ABA with glucose ester acting as an inactive storage form of ABA and a reservoir of ABA. There was no significant difference on the concentration of ABAGE in the different tissues studied (Appendix A, Table A.97). Consistent with ABA, 7'OH-ABA also increased with storage which was significant (Appendix A, Table A.96). In the flesh tissue no significant differences were observed in ABA metabolites (7'-OHABA and ABAGE) between treatments (Appendix A, Table A.91, A.92). In addition, dehydration was found to induce the expression of the main enzyme (8'-ABA hydroxylase) responsible for ABA catabolism (Roychoudhury *et al*., 2013). In the achenes significantly higher

ABA metabolites were observed for the 1MCP treatment (Figure 7.2). In strawberries 1-MCP concentration of up to 0.5 µl l^{-1} has been shown to enhance the rate of senescence and therefore water loss. It is probable that the high concentration of 1-MCP (1 µl l^{-1}) used in this study resulted in stress thus increased ABA metabolites.

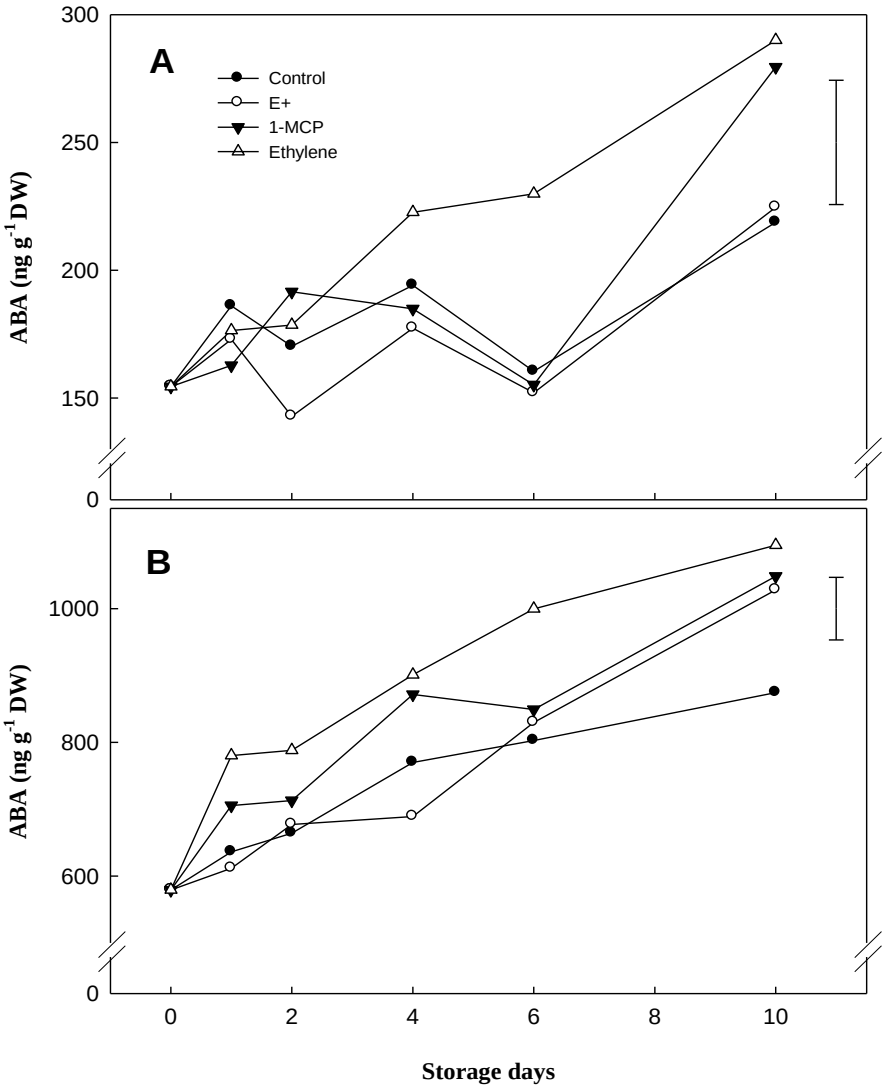


Figure 7.1. The effect of treatments on the content of ABA in the achenes (**A**) and flesh (**B**) of strawberries (cv. Sonata, n=12) stored at 5 °C for 10 days. LSD (P<0.05).

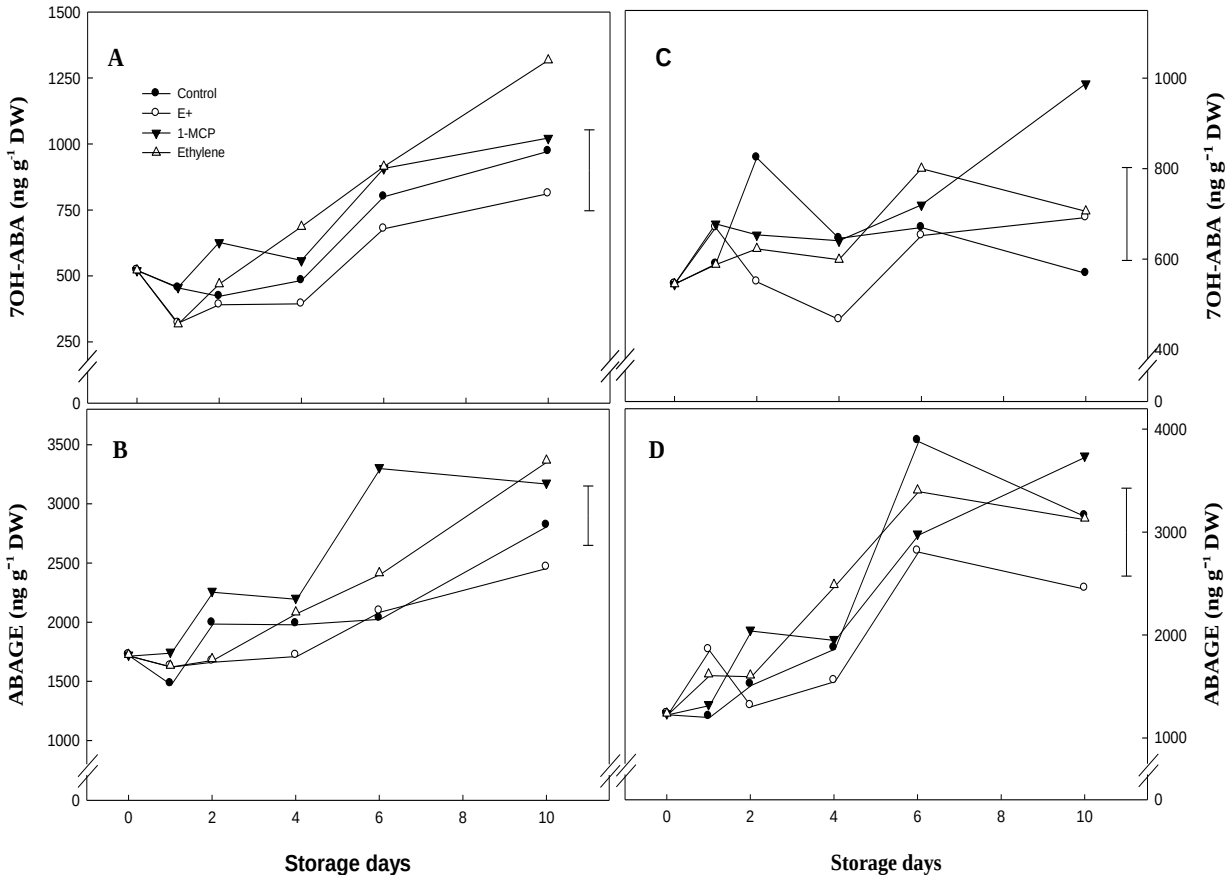


Figure 7.2. The effect of treatments on the content of ABA metabolites in the achenes (**A** and **B**) and flesh (**C** and **D**) tissue of strawberries (cv. Sonata, n=12) stored at 5 °C for 10 days. LSD (P<0.05).

ABA represented 20-25 % of the total ABA and ABA metabolite measured in the flesh; however in the achenes this hormone represented only 6 %. This coupled with the fact that higher concentration of ABA was detected in the flesh (4 fold) than in the achenes suggests that the process of ABA catabolism was enhanced more in the achenes during postharvest. The fact that the proportion of ABA catabolites was considerably higher in the achenes suggests there was higher rate of ABA catabolism in tissue. Moreover, the achenes were more responsive to the treatments and ethylene and 1-MCP resulted in higher content of ABA metabolites (Figure 7.2). Ianneta *et al*. (2006) also demonstrated differences in the behaviour of the achenes and flesh whereby achenes were shown to

exhibit climacteric like activity. This coupled with the results herein suggest that the achenes behave differently to the flesh tissue, which could be explained by the fact that the achenes are the true fruit.

7.4.1.2 IAA and IAAsp

The auxins, IAA (445 ng g^{-1} DW) and the conjugated form IAAsp (2170 ng g^{-1} DW) were detected in the achenes. However in the flesh tissue these compounds were below the quantification limit. Symons *et al*. (2012) found IAA was present in achenes (65 ng g^{-1} FW), however the level of IAA in the flesh was below 1 ng g^{-1} FW. These results are consistent with results demonstrated herein and with previous evidence that the auxins are synthesised in the achenes (Nitsch, 1950; Nitsch, 1955; Given *et al.*, 1988). Bombarely *et al.* (2010) also reported that auxins were more abundant in the achenes acting as key regulator of growth and ripening. It is widely accepted that decrease in auxins triggers the ripening process of strawberries. Moreover, the application of the synthetic auxin 1-naphthalenacetic acid (NAA) has been shown to prevent ripening (Given *et al.*, 1988; Manning, 1994).

During storage the IAA content in achene tissue decreased while the IAA-amide conjugates (IAAsp) increased. IAAsp the conjugated form of IAA is believed to result from the catabolism of IAA. The concentration of IAA-amide conjugates was in accordance with that reported in Archbold and Dennis (1984) for ripe strawberries. Previous studies on climacteric (tomatoes) and non-climacteric fruits (grapes and strawberries) reported high levels of IAA-amide conjugates during ripening while the levels of free IAA were maintained low (Archbold and Dennis, 1984; Böttcher *et al.*, 2010). Böttcher *et al*. (2010) proposed that the IAAsp may be involved in IAA degradation. The conjugation of IAA to amino acids is catalysed by auxin-inducible GH3 protein (Staswick *et al.*, 2005). The activity of GH3 protein has been associated with fruit ripening in pepper, a non-climacteric fruit (Liu *et al.*, 2005b). Moreover, it was reported that GH3 gene was induced by ethylene which could explain the significantly high level of IAAsp in the ethylene treated fruits (Appendix A, Table A.89) as depicted in Figure 7.3. Lower level of IAA was observed in the achenes of fruits treated with ethylene (Figure 7.3) although this was not significant (Appendix A, Table A.90). Moreover, the IAAsp was significantly higher in the ethylene treatment

suggesting that accelerated rates of IAA catabolism enhanced the concentration of the conjugated form (IAAsp). Auxins negatively regulate strawberry fruit ripening and senescence (Given *et al.*, 1988; Civello *et al.*, 1999). Nearly all the genes associated with strawberry fruit ripening and softening are upregulated by dcline in auxins (Civello *et al.*, 1999). Here, ethylene was shown to increase the catabolism of the main auxin in strawberry IAA. IAA was considerably lower in the ethylene treated fruits suggesting that ethylene may regulate strawberry quality by affecting auxins. Moreover, phytohormones in the achenes were overall more responsive to the ethylene treatment than in the flesh suggesting that this tissue may exhibit climacteric like behaviour.

However, achenes only constitute about 1 % of the total fresh weight of the fruit (7 % of the total dry weight). Therefore the proportion of these phytohormones detected in the achenes is not representative of that present in the whole fruit. Moreover, previous studies that measured phytohormones concentration in the whole fruit reported the content of these compounds for the flesh.

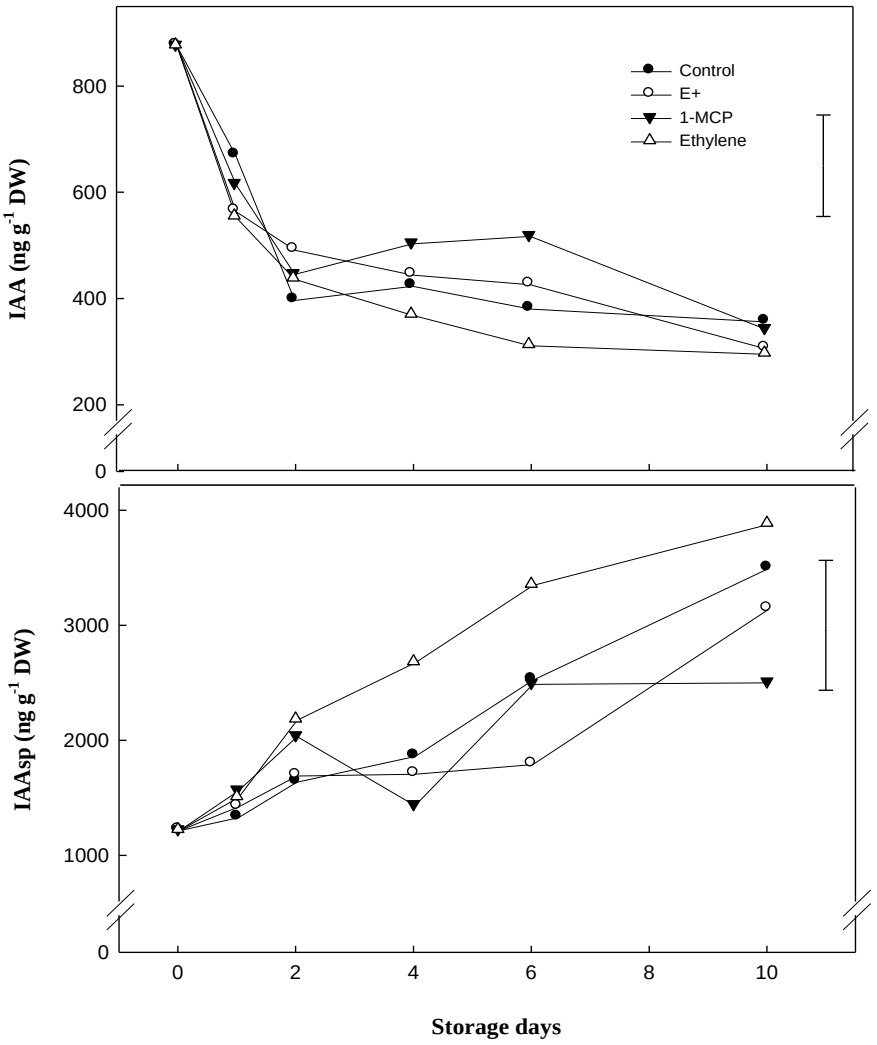


Figure 7.3. The effect of treatments on the content of auxins (IAA and IAAsp) in the achenes tissue of strawberries (cv. Sonata, n=12) stored at 5 °C for 10 days. LSD (P<0.05).

7.5 Conclusion

Achenes and flesh showed different phytohormone profiles after harvest. ABA concentration was more abundant in the flesh while auxins were presented only in the achenes tissue. It is probable that ABA is more significant in the flesh during postharvest of strawberries while the auxins are more essential in the achenes. The decrease of IAA correlated with the increase of IAAsp, this suggests that during postharvest IAA is catabolised and conjugated with amino acids. ABA concentration in

the achenes and the flesh was affected by exogenous ethylene. In contrast, control of ethylene through inhibition and removal did not have the opposite effect. Ethylene treatment caused an accelerated rate of IAA catabolism resulting in significantly higher IAAsp.

CHAPTER EIGHT

8 GENERAL DISCUSSION & CONCLUSIONS

8.1 Discussion

8.1.1 Introduction

The concentration of ethylene in the storage atmosphere of climacteric fruits can dictate their overall quality and shelf life. Where ethylene concentration is above the physiological active level there is a greater risk of induced premature ripening resulting in subsequent economic losses due to reduced postharvest life and accelerated senescence. Ethylene can accumulate at any point in the supply chain (e.g. transportation, storage and handling) and a concentration of 0.1 µl l^{-1} has been quoted as the threshold above which fruits become physiologically more mature (Saltveit, 1999). However, there is a lack of research on the dose response to ethylene (time × concentration). Although significant research has focused on the exposure of fruits to absolute ethylene concentration the influence of time is rarely considered. Therefore, the interactions between ethylene concentration and exposure time as well as the threshold dose would help to improve the postharvest handling of many commodities.

8.1.2 Limitations of existing techniques

The ethylene binding inhibitor, 1-methylcyclopropene (1-MCP), has been demonstrated as an effective tool for overcoming the effects associated with ethylene (reviewed in Chapter 2). 1-MCP has been shown to delay ripening of many perishable crops as expressed in colour, firmness retention, suppression of ethylene production and respiration (Golding *et al.*, 1998; Fan and Mattheis, 2001; Adkins *et al.*, 2005; Fu *et al.*, 2007). For all these benefits, a growing body of literature has shown 1-MCP to present some problems of its own for some fresh produce. Research has associated the use of 1-MCP with uneven ripening in avocados (Kruger and Lemmer, 2007); hence alternative treatments for regulating the ripening of avocados are desirable. In addition, the longevity and action of 1-MCP is dependent on numerous factors such as species, cultivar and mode of ethylene biosynthesis (Watkins, 2006). Other technologies

available such as $KMnO_4$ have been reviewed in Chapter 2, however $KMnO_4$ ethylene scrubber has been found to be unsuitable for the typical storage environment used for fruits (high relative humidity (RH)) and low temperature) and has been found to lose efficacy under such conditions (Wills and Warton, 2004).

8.1.3 Development of e+® Ethylene Remover

The need for alternative technologies to control ethylene in fruit environment has led to the invention and patent of e+® Ethylene Remover. The material, which is made from a unique combination of a selected platinum (Pt) group metal *viz*. Palladium and specifically selected zeolite support, has been discovered to have significant ethylene adsorption capacity under low and room temperature. The e+® Ethylene Remover was first tested on avocados by Terry *et al.* (2007b), who showed that the treatment maintained fruit quality. Subsequent studies by Smith *et al*. (2009) provided greater information about the physio-chemical properties of the ethylene remover itself. The e+® Ethylene Remover is unique compared to other Pt group metal-based ethylene scavengers that have been tested previously since these acted as catalysts and the active was supported on activated carbon which required elevated temperatures for adsorption to occur (Bailén *et al.*, 2006; Martínez-Romero *et al.*, 2009a; Martínez-Romero *et al.*, 2009b). Initial testing by Smith *et al*. (2009) revealed the significant ethylene adsorption capacity of the e+® Ethylene Remover using a synthetic gas mixture of 200 µl l^{-1} ethylene and 10% oxygen balanced with helium, at a flow rate of 50 ml min^{-1} under dry and humid conditions (100 %). Typically the material adsorbed all measureable ethylene and was found to have a considerable ethylene adsorption capacity in humid (4162 µl g^{-1}) and dry condition (45,600 µl g^{-1}). Moreover, e+® Ethylene Remover is effective at lower temperature and has been demonstrated to remove ethylene to below physiologically active levels during fruit storage at 5-20 ^{o}C (Terry *et al.*, 2007b; Smith *et al.*, 2009; Meyer and Terry, 2010). Meyer and Terry (2010), demonstrated that this material (5 g) removed ethylene to below the threshold (0.1 µl l^{-1}), which effectively maintained the postharvest quality of pre-climacteric avocados stored at 5 ^{o}C. Nevertheless, the discovery of this material offers considerable implications for the fruit industry given its efficacy at low temperature and high RH. Earlier studies on e+® Ethylene Remover (Terry *et al.*, 2007b; Meyer and Terry, 2010) were conducted using

the powdered material, however, commercial application of the material requires a compatible format. The need for effective and compatible format has motivated the development of an e+® Ethylene Remover coated sheet. This newly developed format is a more convenient method for the application of this treatment in the real world supply chain as it can be incorporated inside fruit packaging and containers.

8.2 Classification of climacteric and non-climacteric fruits

Fruit species are classified as either climacteric or non-climacteric based on the presence of a climacteric rise in respiration and ethylene production during the onset of ripening. However, detailed studies have revealed that this climacteric peak which is considered unique for climacteric fruits exist in some non-climacteric fruits (Trainotti *et al.*, 2005; Iannetta *et al.*, 2006; Paul *et al.*, 2012). In addition, the ripening behaviour of non-climacteric fruits has been shown to resemble that of climacteric fruits. Moreover, ethylene dependent and independent pathways have been shown to coexist in both climacteric and non-climacteric fruits suggesting that classification of fruits based on the climacteric rise is an over simplification (Chervin *et al.*, 2004b; Iannetta *et al.*, 2006; Paul *et al.*, 2012).

8.2.1 Climacteric Fruits

8.2.1.1 Avocado

As a climacteric fruit, avocado exhibits a surge of ethylene production and respiratory increase during ripening (Feng *et al.*, 2000; Hershkovitz *et al.*, 2005). Avocados are also subtropical fruits that are highly valued for their unique taste and health promoting benefits however; avocados consumed in the UK are usually imported from abroad. During transportation, ethylene can accumulate and impact on the overall quality of avocados.

In previous studies the e+® Ethylene Remover was applied on avocados after transit (Terry *et al.*, 2007b; Meyer and Terry, 2010), which is often after several weeks of harvest in which fruits are more advanced. Furthermore, research on the feasibility and effectiveness of postharvest treatments in the commercial world is rarely reported in literature. The aim of the experiments reported in Chapter 3 was to attest the feasibility of a newly developed e+® Ethylene Remover coated sheet format in the real world

supply chain. In addition, the aim was to investigate the efficacy of the material once it is applied at the early stages of storage. The sheet (19 × 26.5 cm) was coated with 38.6 % e+® Ethylene Remover and 1 % Pd loading. Significant differences in response to the treatment at source and during later postharvest treatment were observed. Application of e+® Ethylene Remover at the point of packing resulted in a significantly lower ethylene in the storage atmosphere than the untreated fruits. Consequently, the potency of the e+® Ethylene Remover in retarding ethylene induced ripening was significantly enhanced with the earlier treatment. After storage period of 31 days (5-6 ^{o}C), fruits which had received this early treatment during transit and then treated in the laboratory were significantly more firm compared to fruits treated following 5 weeks of transit. Results in Exp 3.1 are also consistent with previous findings (Terry *et al.*, 2007b; Meyer and Terry, 2010), whereby ethylene was reduced to below 0.1 µl l^{-1} during storage at 5 ^{o}C. In both experiments (Exp 3.1 and 3.2), avocados that were packed with the treatment (E+/E+) were overall firmer and more green compared to the other treatments. It is evident from Exp 3.1 that ethylene was adsorbed during transit, since the buffer fruits which were fruits packed in the same pallet as the treated fruits were overall significantly firmer and green when compared to the overall untreated fruits. Treatment with e+® Ethylene Remover has so far been conducted under enclosed or confined environment, since scrubbers need to be close to the fruits to adsorb the exogenous ethylene (Terry *et al.*, 2007b; Martínez-Romero *et al.*, 2009b; Meyer and Terry, 2010). In Exp 3.2, where fruits were not stored in the confined 13 l plastic boxes, the overall effect of treatment was not as pronounced as that observed in Exp 3.1 suggesting that e+® Ethylene Remover may be most efficacious in confined environment. That said, a number of factors such as origin and the use of e+® Ethylene Remover coated sheet on arrival instead of the powdered material, may have contributed to the differences between the results of the two trials (Exp 3.1 and 3.2). However, results from both trials show that employing e+® Ethylene Remover treatment in the 'real world' supply chain, is feasible and effective since this was associated with considerable benefits on the quality of avocados during storage. These findings are of commercial importance in the fresh produce industry. E+® Ethylene Remover could serve as a potential strategy to control and modulate avocado fruit quality during storage and transportation. This material can also serve as an alternative treatment to the current

commercial treatment on avocados (1-MCP), which causes uneven ripening. Besides serving as a potential commercial product the e+® Ethylene Remover constitutes a tool to study the role of ethylene in ripening and senescence of horticultural commodities.

Avocados are plentiful in C7 sugars, which are especially recognised to have anti-cancer activity (Ishizu *et al.*, 2002). Liu *et al.* (2002), suggested a correlation between the induction of ripening and decrease in C7 sugars; mannoheptulose and perseitol. Moreover, Landahl *et al*. (2009) found that firmer regions of the avocado accumulated more C7 sugars. The data presented for sugar concentration of the avocados (Exp 3.1) do provide evidence that C7 sugars decrease during storage (Bertling *et al.*, 2007; Landahl *et al.*, 2009; Meyer and Terry, 2010). Although, mannoheptulose was not affected by the treatment high content of perseitol sugar was observed in fruits treated with e+® Ethylene Remover after transit. In accordance, Meyer and Terry (2010), showed that C7 sugars (mannoheptulose and perseitol) were more abundant in 1-MCP and e+® Ethylene Remover treated fruits than soft control fruits. Nonetheless, sugars in avocados do vary with origin, season and maturity (Liu *et al.*, 1999b; Landahl *et al.*, 2009) all of which may have impacted on results obtained.

8.2.1.2 Pluot plums

Pluots like avocados are identified as climacteric fruits. Plums and their hybrids have short postharvest life and can quickly descend from ideal ripeness to over maturity (Crisosto *et al.*, 1995). Flavour King is a hybrid of plums (Santa Rosa) and apricots. Santa Rosa plums (dark red skin and amber flesh) became more unpopular as they rarely grow into greater sizes. However, Santa Rosa has been used to create crosses such as pluots where plum characteristics dominate (Parsons, 2007). In Chapter 4, pluots were sourced from South Africa and held with or without the e+® Ethylene Remover coated sheets. During transit pluots were subjected to a brief increase of temperature to 7 ^{o}C, to achieve uniform ripening during postharvest; a common method used by the South African industry. However, prior to the temperature treatment, the transport of fruit to the UK was delayed, which meant that fruits were at an advanced stage of ripening once received. Nevertheless, fruits treated at source with the e+® Ethylene Remover sheets were not only more firm than the control fruits on arrival, but their firmness met the Marks & Spencer (M&S) specification in contrast to the control

fruits. Firmness of the control fruits on arrival was below the minimum required to be received by M&S suggesting that ethylene was adsorbed during transit.

Real-time ethylene production was monitored using a laser-based ethylene detector (ETD-300). In contrast, conventional techniques such as gas chromatography (GC) involve the collection of gas samples from hermetically sealed containers to give data only at a particular time point, which means that temporal fluctuation are not considered. In addition, it can be argued that ethylene builds up in the hermetically sealed containers and can thus result in increased ethylene production in a closed system, which is different from the ETD-300 since this is connected to a flow through system. Monitoring the ethylene production of control and E+ fruits during the course of the ripening period enabled the differences in the ethylene production pattern of the fruits to be observed. Fruits treated with the e+® Ethylene Remover coated sheets at source were placed inside jars containing the e+® Ethylene Remover powder. Indeed, the treated fruits produced considerably lower ethylene, but both fruits exhibited similar ethylene production pattern, suggesting that E+ fruits undergo normal ripening patterns. This is in contrast to the 1-MCP treatment which has been shown to impact on the normal ripening process in fruits such as plums, pears and avocados (Abdi *et al.*, 1998; Dauny *et al.*, 2003; Ekman *et al.*, 2004). Consistent with Chapter 3, fruits sourced with the e+® Ethylene Remover treatment were overall significantly more firm than the control fruits. Concerning the biochemistry of the pluots little differences were observed between the treatments however, it is possible that the biochemistry may not have been affected as expected since the fruits were received in an advanced state.

8.2.2 Non-climacteric

8.2.2.1 Strawberries

Strawberries are classified as non-climacteric fruits, however, an increase in ethylene production and respiration during red ripe stage has been observed (Iannetta *et al.*, 2006). Furthermore, Trainotti *et al*. (2005) identified ethylene receptors in strawberry and demonstrated increased expression in the gene encoding for the ethylene receptor during development and in response to exogenous ethylene. The postharvest life of strawberries is short involving undesirable changes in colour, texture and water loss, which occurs as a consequence of the natural process of senescence. Besides the natural

process of senescence major cause of strawberry postharvest losses occurs due to mould growth (Cordenunsi *et al.*, 2003). The main pathogen responsible for postharvest decay of strawberries is *B. cinerea*. Infection is often initiated at the stem end of the fruit leading to contamination of the flower. The mould lies dormant until the fruit reach maturation, when sugars accumulate; natural disease resistance declines and conditions become more favourable (Terry and Joyce, 2004). Literature has postulated that growth of this pathogen can be enhanced by exogenous ethylene (Wills and Kim, 1995). Moreover, Wills and Kim (1995), showed that reducing ethylene in the strawberry fruit environment using $KMnO_4$ ethylene scrubber was associated with better quality fruits and reduced disease incidence. In Chapter 5, treatment of strawberry (cv. Elsanta) plants with *B. cinerea* resulted in fruits producing significantly higher ethylene and was more red fruits than the non-infected. This confirmed that disease associated with *B. cinerea* may be ethylene dependent. Similarly, Babalar *et al*. (2007) showed that exogenous salicylic acid (SA) application decreased fungal decay on strawberries by inhibiting the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene, thereby decreasing ACC oxidase was down-regulated in expression by SA. However, in Chapter 5 it was shown that dormant disease in strawberry fruit caused enhanced ethylene production and colour change. In addition, infection was also associated with sugar accumulation possibly resulting from accelerated rates of senescence which could be exacerbated by the high endogenous ethylene production. Presence of e+® Ethylene Remover in fruit storage environment not only reduced the ethylene production of the fruits, but infected fruits treated with the ethylene remover were of better quality in terms of colour when compared to the corresponding control.

The role of ethylene during the postharvest quality of non-climacteric fruits has been considered less in postharvest biology. Moreover, results (Chapter 5) demonstrated an increase in ethylene production during storage as the fruits became more advanced. In Exp 5.2, fruits exhibited similar ethylene production pattern as climacteric fruits. That said, the classification of fruits to either climacteric or non-climacteric might be an over simplification. Literature has demonstrated that the ripening and postharvest life of some non-climacteric fruits resembles that of climacteric fruits. Chervin *et al*. (2004) showed that ethylene influences several aspects of grape fruit ripening and a significant increase in ethylene was observed at the onset of ripening. Atta-Aly *et al*. (2000)

suggested that ethylene exhibits a negative feedback mechanism (ethylene does not induce its own synthesis) in non-climacteric fruits, while a positive feedback mechanism underlies the ethylene production of climacteric fruits (ethylene induces its own synthesis). In Chapter 5, removal of ethylene from the strawberry storage environment was associated with significantly lower ethylene. In Chapter 6 strawberries treated with exogenous ethylene did not produce the highest ethylene yet the ethylene treatment enhanced fruit respiration suggesting that exogenous ethylene stimulated ethylene production rather than inhibiting its own synthesis. However, in Ianneta *et al*. (2006), it was also demonstrated that the achenes produced over 50 % of the ethylene suggesting that different tissues may exhibit different ripening patterns. It may be possible to propose that the achenes (true fruits) are climacteric in that they produce most of the ethylene even though these only represent a fraction of the fruit weight and that the receptacle (flesh tissue) is truly non climacteric.

Although exogenous ethylene has been shown to negatively influence strawberry postharvest life, the inhibition of ethylene has been shown to have both negative and positive effects (Tian *et al.*, 2000; Jiang *et al.*, 2001; Villarreal *et al.*, 2010). Moreover, in Chapter 6 sugars, organic acids, phenolic compounds were all found to be negatively influenced by ethylene treatment. Similarly, Villareal *et al*. (2010), showed that ethephon induced accumulation of sugars and anthocyanins, while the opposite was observed for the 1-MCP treated fruits. However, others (Jiang *et al.*, 2000; Tian *et al.*, 2000; Bower *et al.*, 2003) have shown that 1-MCP can have negative effects and this was associated with enhanced disease incidence in strawberries. That said, it is likely that 1-MCP impacts on the natural resistance of the fruit since controlling ethylene via the use of e+® Ethylene Remover did not have the same effect (Chapter 6). However, some literature (Ku *et al.*, 1999; Jiang *et al.*, 2001) has associated high concentration of 1-MCP with the accelerated loss of quality. In Chapter 6, 1-MCP and ethylene treatments were found to both accelerate the rate of senescence. Moreover, e+® Ethylene Remover was shown to have the opposite effect which could be attributed to the low ethylene environment and low respiration rate of these fruits. While results in Chapter 5 and 6 have highlighted the importance of ethylene in determining the postharvest life of strawberries, the majority of the studies concerning the role of ethylene in strawberry fruit quality have focused on fruits at different developmental stages and there is less

consideration as to how ethylene can influence postharvest life. Here, ethylene was shown to cause strawberries to deteriorate more rapidly and quality parameters associated with the overall fruit taste were also negatively influenced.

The involvement of phytohormones in the development and storage of fruits is not fully understood. Although there is some evidence suggesting the involvement of phytohormones including ABA and auxins in strawberry fruit development (Archbold and Dennis, 1984; Chen *et al.*, 2011; Jia *et al.*, 2011) there is little information regarding the role of these phytohormones during postharvest and the cross talk with ethylene. ABA has been regarded as an important phytohormone regulating grape fruit ripening also a non-climacteric fruit (Davies *et al.*, 1997), while upregulation of ABA was also observed during the ripening of strawberries (Chai *et al.*, 2011; Jia *et al.*, 2011). In Chapter 6, significant increase of ABA was observed during the storage of strawberries. It is likely that increase of ABA observed during storage could be attributed to senescence or indeed water loss. Similarly, in other non-climacteric such as grapes and cherries, ABA content was found to increase during senescence. In addition, Terry *et al*. (2007a) showed that stress from deficit irrigation of strawberries was associated with a significant increase in ABA. Moreover, Jiang *et al*. (2003) demonstrated that exogenous ABA stimulated ethylene production of strawberries during storage. In Chapter 6, ABA was also found to increase significantly in response to ethylene and 1-MCP treatment suggesting that this may be the result of enhanced senescence in these fruits. Ethylene treatment resulted in significant water loss due to accelerated rate of senescence. It is likely that stress from the water loss accelerated ABA accumulation in these fruits. In addition, ethephon or ethylene treatment favoured the accumulation of ABA in grapes (Sun *et al.*, 2010) and mandarins (Lafuente *et al.*, 1997), which suggests interplay between ethylene and ABA.

Botanically, strawberries are acknowledged as a false fruit and considered as a swollen receptacle. The true fruits are the seeds (achenes) which are embedded in the epidermis of the receptacle through vascular connections. It was demonstrated by Ianneta *et al*. (2006) that the achenes are responsible for the majority of ethylene produced by strawberries. Achenes are also regarded as the main site for the synthesis of auxins (Nitsch, 1950; Given *et al.*, 1988; Symons *et al.*, 2012). Auxins have been shown to

negatively regulate strawberry fruit ripening. Moreover, the removal of achenes triggered the ripening of strawberries while exogenous application was found to inhibit it (Given *et al.*, 1988). In Chapter 7, the role of ABA and auxins in different strawberry tissues was investigated. Endogenous ABA concentration is determined by a balance between biosynthesis and catabolism of this phytohormone. Moreover, ABA can be conjugated with glucose, and the most widespread is the ABA-glucosyl ester (ABA-GE). The hydroxylated catabolite 7'OH-ABA and the conjugated form ABA-GE were detected in strawberries (Chapter 6 and 7). Although ABA was significantly higher in the flesh tissue, the ABA metabolites were enhanced in the achenes suggesting a higher rate of ABA catabolism in this tissue. These ABA metabolites were found to increase significantly following ethylene and 1-MCP treatment in the achenes and thus were more responsive to the treatments. In accordance, there is research evidence that the conjugated ABA accumulates in the vacuoles during stress and senescence (Nambara and Marion-Poll, 2005). In addition, auxins were abundant in the achenes as previously reported (Nitsch, 1950; Given *et al.*, 1988). However, levels of auxins; indole-3-acetic acid (IAA) and indole-3-acetylaspartic acid (IAAsp) were below the quantification limit in the flesh tissue. IAA is thought to promote receptacle enlargement and regulate strawberry ripening (Nitsch, 1950; Archbold and Dennis, 1984; Given *et al.*, 1988). A decline of IAA is believed to trigger the ripening and senescence of strawberries (Given *et al.*, 1988; Civello *et al.*, 1999). Consistent with this, in Chapter 7, IAA exhibited a significant decrease and lower concentration was observed in the ethylene treated fruits, which could be attributed to the enhanced senescence in these fruits. IAAsp is thought to occur from the catabolism of IAA, which is then conjugated to amino acid (aspartic acid). IAAsp not only increased with storage but it was significantly higher in the ethylene treated fruits. This increase not only confirms that IAA was considerably lower in these fruits due to accelerated rate of senescence but that the ethylene may regulate strawberry fruit quality by affecting the auxins.

8.3 Recommendation for future work

The ethylene adsorption capacity of the material under different conditions (temperature and % RH) has been determined however, more trials are necessary to observe the optimum conditions at which this treatment is made most efficacious.

- The performance of e+® Ethylene Remover in combination with modified atmosphere (MA) and controlled atmosphere (CA) should be considered. Deducing any additional benefits presented by the e+® Ethylene Remover treatment in combination with CA or MA will mean new directions in terms of what is attainable with this treatment.
- Moreover, quantitative analysis to determine the amount of the e+® Ethylene Remover required to achieve optimum efficacy is necessary to determine the desired amounts or loading for individual fresh produce.
- To get a better understanding of the mechanism in which e+® Ethylene Remover effects fruit ripening would be useful to carry out molecular analysis to determine the genes involved in the e+® Ethylene Remover mediated response.
- There is little literature on the dosage of ethylene (time × concentration). Although ethylene concentration is important, the timing of the exposure is just as important. Therefore more research on the interaction between ethylene concentration and exposure time on fruit physiology as well as defining the threshold is required.
- Although strawberries are classified as non-climacteric fruit a significant role of ethylene in storage was demonstrated in Chapters 5-7. That said, more research is required regarding the mechanism by which ethylene controls strawberry fruit quality.

8.4 Project conclusion

The projects objectives are described in Chapter 1 section 1.2.2. The overall conclusions of the project are briefly summarised below.

- To investigate the effects of e+® Ethylene Remover treatment on the physiological and biochemical changes occurring during storage and ripening of climacteric fruits (avocados cv. Hass and pluots cv. Flavor King). Avocados and pluots treated with the e+® Ethylene Remover showed better quality parameters during postharvest storage.
- To explore the efficacy of a newly developed e+® Ethylene Remover coated sheet format in the real world supply chain. A newly developed format was

shown to be feasible and effective during transit of avocados and pluots. Fruits receiving the treatment were of better qualities in terms of firmness and colour.

- To study the efficacy of the e+® Ethylene Remover treatment once applied during the early stages of ripening (before transit) and whether this can optimise its usage. Avocados fruits receiving the treatment at source were of better quality than those treated after 5 weeks of transit.

- To determine the effects of exogenous ethylene on the physiology and biochemistry of strawberries during storage. Strawberry fruit quality was negatively influenced by exogenous ethylene. Exogenous ethylene was associated with disease incidences, accumulation of simple sugars, decrease in organic acids and enhanced synthesis of ABA.

- To study the effects of postharvest treatments (1-MCP and e+® Ethylene Remover) on strawberry fruit quality (including sugars, organic acids, phenolics and phytohormones) during postharvest. Strawberries treated 1-MCP showed higher incidence of disease and enhanced ABA concentration. Storage of strawberries in the presences of e+® Ethylene Remover delayed the development of rot caused by *B.cinerea*. Strawberries treated with the ethylene remover were also significantly less red and exhibited lower ethylene and respiration rate compared to the corresponding control fruits.

- To determine the relationship between exogenous ethylene and the concentrations of phytohormones in different strawberry tissues. ABA was significantly higher in the flesh tissue than the achenes while auxins were only detected in the achenes. Both tissues exhibited a significant increase in ABA concentration in response to exogenous ethylene treatment. Reduced content of IAA was observed with the ethylene treatment while the catabolite (IAAsp) was significantly increased.

CHAPTER NINE

9 LITERATURE CITED

Aaby, K., Ekeberg, D. and Skrede, G. (2007), "Characterization of phenolic compounds in strawberry (*Fragaria× ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity", *Journal of Agricultural and Food Chemistry*, vol. 55, no. 11, pp. 4395-4406.

Aaby, K., Skrede, G. and Wrolstad, R. E. (2005), "Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria x ananassa*)", *Journal of Agricultural and Food Chemistry*, vol. 53, no. 10, pp. 4032-4040.

Abdi, N., McGlasson, W. B., Holford, P., Williams, M. and Mizrahi, Y. (1998), "Responses of climacteric and suppressed-climacteric plums to treatment with propylene and 1-methylcyclopropene", *Postharvest Biology and Technology*, vol. 14, no. 1, pp. 29-39.

Abdi, N., Holford, P., McGlasson, W. and Mizrahi, Y. (1997), "Ripening behaviour and responses to propylene in four cultivars of Japanese type plums", *Postharvest Biology and Technology*, vol. 12, no. 1, pp. 21-34.

Abeles, F. B., Morgan, P. W. and Saltveit, M. E. (1992), *Ethylene in Plant biology*, 2nd ed, Academic Press, San Diego, California.

Adkins, M. F., Hofman, P. J., Stubbings, B. A. and Macnish, A. J. (2005), "Manipulating avocado fruit ripening with 1-methylcyclopropene", *Postharvest Biology and Technology*, vol. 35, no. 1, pp. 33-42.

Aguayo, E., Jansasithorn, R. and Kader, A. (2006), "Combined effects of 1-methylcyclopropene, calcium chloride dip, and/or atmospheric modification on quality changes in fresh-cut strawberries", *Postharvest Biology and Technology*, vol. 40, no. 3, pp. 269-278.

Aharoni, A., Keizer, L. C. P., Van den Broeck, H. C., Blanco-Portales, R., Muñoz-Blanco, J., Bois, G., Smit, P., De Vos, R. C. H. and O'Connell, A. P. (2002), "Novel insight into vascular, stress, and auxin-dependent and -independent gene

expression programs in strawberry, a non-climacteric fruit", *Plant Physiology,* vol. 129, no. 3, pp. 1019-1031.

Alexander, L. and Grierson, D. (2002), "Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening", *Journal of Experimental Botany,* vol. 53, no. 377, pp. 2039-2055.

Alique, R. and Oliveira, G. S. (1994), "Changes in sugars and organic acids in cherimoya (Annona cherimola Mill.) fruit under controlled atmosphere storage", *Journal of Agricultural and Food Chemistry,* vol. 42, no. 3, pp. 799-803.

Al-Jamali, A. F. and Al-Wardi, S. S. (2010), "Croplife-B and ethephon: Candidates for use in strawberry sustainable organic horticulture", *International Journal of food, Agriculture and environment,* vol. 8, no. 3-4, pp. 683-688.

Antunes, M. and Sfakiotakis, E. (2002), "Ethylene biosynthesis and ripening behaviour of 'Hayward'kiwifruit subjected to some controlled atmospheres", *Postharvest Biology and Technology,* vol. 26, no. 2, pp. 167-179.

Archbold, D. D. and Dennis, F. G. (1984), "Quantification of free ABA and free and conjugated IAA in strawberry achene and receptacle tissue during fruit development.", *Journal of the American Society for Horticultural Science,* vol. 109, no. 3, pp. 330-335.

Archbold, D. D. (1988), "Abscisic acid facilitates sucrose import by strawberry fruit explants and cortex disks in vitro", *HortScience,* vol. 23, pp. 880-881.

Arpaia, M., Mitchell, F., Mayer, G. and Kader, A. (1984), "Effects of delays in establishing controlled atmospheres on kiwifruit softening during and following storage", *J.Amer.Soc.Hort.Sci,* vol. 109, no. 6, pp. 768-770.

Ashton, O. B. O., Wong, M., McGhie, T. K., Vather, R., Wang, Y., Requejo-Jackman, C., Ramankutty, P. and Woolf, A. B. (2006), "Pigments in avocado tissue and oil", *Journal of Agricultural and Food Chemistry,* vol. 54, no. 26, pp. 10151-10158.

Atta-Aly, M. A., Brecht, J. K. and Huber, D. J. (2000), "Ethylene feedback mechanisms in tomato and strawberry fruit tissues in relation to fruit ripening and climacteric patterns", *Postharvest Biology and Technology,* vol. 20, no. 2, pp. 151-162.

Awad, M. and Young, R. (1979), "Postharvest variation in cellulose, polygalacturonase and pectinmethylesterase in avocado fruit in relation to respiration and ethylene production", *Plant Physiol,* vol. 64, pp. 306-308.

Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y. and González-Aguilar, G. A. (2004), "Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit", *LWT-Food Science and Technology,* vol. 37, no. 7, pp. 687-695.

Babalar, M., Asghari, M., Talaei, A. and Khosroshahi, A. (2007), "Effect of pre-and postharvest salicylic acid treatment on ethylene production, fungal decay and overall quality of Selva strawberry fruit", *Food Chemistry,* vol. 105, no. 2, pp. 449-453.

Bailén, G., Guillén, F., Castillo, S., Serrano, M., Valero, D. and Martínez-Romero, D. (2006), "Use of activated carbon inside modified atmosphere packages to maintain tomato fruit quality during cold storage", *Journal of Agricultural and Food Chemistry,* vol. 54, no. 6, pp. 2229-2235.

Bapat, V. A., Trivedi, P. K., Ghosh, A., Sane, V. A., Ganapathi, T. R. and Nath, P. (2010), "Ripening of fleshy fruit: molecular insight and the role of ethylene", *Biotechnology Advances,* vol. 28, no. 1, pp. 94-107.

Barry, C. S. and Giovannoni, J. J. (2007), "Ethylene and fruit ripening", *Journal of Plant Growth Regulation,* vol. 26, no. 2, pp. 143-159.

Basson, C., Groenewald, J., Kossmann, J., Cronjé, C. and Bauer, R. (2010), "Sugar and acid-related quality attributes and enzyme activities in strawberry fruits: Invertase is the main sucrose hydrolysing enzyme", *Food Chemistry,* vol. 121, no. 4, pp. 1156-1162.

Bergh, B. (1992), "The avocado and human nutrition. I. Some human health aspects of the avocado", *Proc. of Second World Avocado Congress,* pp. 25.

Bertling, I. and Bower, J. P. (2005), "Sugars as energy sources–is there a link to avocado fruit quality", *South African Avocado Growers' Association Yearbook,* vol. 28, pp. 24-27.

Bertling, I., Tesfay, S. and Bower, J. (2007), "Antioxidants in 'Hass' avocado", *South African Avocado Growers' Association Yearbook,* vol. 30, pp. 17-19.

Blakey, R. and Bower, J. (2009), "The importance of maintaining the cold chain for avocado ripening quality", *South African Avocado Growers' Association Yearbook,* vol. 32, pp. 48-52.

Blankenship, S. M. and Dole, J. M. (2003), "1-Methylcyclopropene: a review", *Postharvest Biology and Technology,* vol. 28, no. 1, pp. 1-25.

Blanusa, T., Else, M., Davies, W. and Atkinson, C. (2006), "Regulation of sweet cherry fruit abscission: The role of photo-assimilation, sugars and abscisic acid", *Journal of Horticultural Science & Biotechnology,* vol. 81, no. 4, pp. 613-620.

Bombarely, A., Merchante, C., Csukasi, F., Cruz-Rus, E., Caballero, J., Medina-Escobar, N., Blanco-Portales, R., Botella, M., Muñoz-Blanco, J. and Sánchez-Sevilla, J. (2010), "Generation and analysis of ESTs from strawberry (Fragaria xananassa) fruits and evaluation of their utility in genetic and molecular studies", *BMC genomics,* vol. 11, no. 1, pp. 503.

Böttcher, C., Keyzers, R. A., Boss, P. K. and Davies, C. (2010), "Sequestration of auxin by the indole-3-acetic acid-amido synthetase GH3-1 in grape berry (Vitis vinifera L.) and the proposed role of auxin conjugation during ripening", *Journal of Experimental Botany,* vol. 61, no. 13, pp. 3615-3625.

Boulton, R. (2001), "The copigmentation of anthocyanins and its role in the color of red wine: A critical review", *American Journal of Enology and Viticulture,* vol. 52, no. 2, pp. 67-87.

Bower, J., Biasi, W. and Mitcham, E. (2003), "Effects of ethylene and 1-MCP on the quality and storage life of strawberries", *Postharvest Biology and Technology,* vol. 28, no. 3, pp. 417-423.

Bravo, L. (1998), "Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance", *Nutrition Reviews,* vol. 56, no. 11, pp. 317-333.

Burdon, J., Lallu, N., Haynes, G., McDermott, K. and Billing, D. (2008), "The effect of delays in establishment of a static or dynamic controlled atmosphere on the quality of 'Hass' avocado fruit", *Postharvest Biology and Technology,* vol. 49, no. 1, pp. 61-68.

Burdon, J. and Sexton, R. (1990), "The role of ethylene in the shedding of red raspberry fruit", *Annals of Botany,* vol. 66, no. 1, pp. 111-120.

Burg, S. P. (2004), "Ethylene", in *Postharvest physiology and hypobaric storage of fresh produce*, CABI, Oxfordshire, pp. 132-232.

Bustamante, C. A., Civello, P. M. and Martínez, G. A. (2009), "Cloning of the promoter region of β-xylosidase (FaXyl1) gene and effect of plant growth regulators on the expression of FaXyl1 in strawberry fruit", *Plant Science*, vol. 177, no. 1, pp. 49-56.

Caleb, O. J., Opara, U. L. and Witthuhn, C. R. (2012), "Modified atmosphere packaging of pomegranate fruit and arils: a review", *Food and Bioprocess Technology*, vol. 5, no. 1, pp. 15-30.

Candan, A. P., Graell, J. and Larrigaudière, C. (2008), "Roles of climacteric ethylene in the development of chilling injury in plums", *Postharvest Biology and Technology*, vol. 47, no. 1, pp. 107-112.

Cervantes, E. (2002), "Ethylene: new interactions, still ripening", *Trends in Plant Science*, vol. 7, no. 8, pp. 334-335.

Chai, Y., Jia, H., Li, C., Dong, Q. and Shen, Y. (2011), "FaPYR1 is involved in strawberry fruit ripening", *Journal of Experimental Botany*, vol. 62, no. 14, pp. 5079-5089.

Chang, C., Kwok, S. F., Bleecker, A. B. and Meyerowitz, E. M. (1993), "Arabidopsis ethylene-response gene ETR1: similarity of product to two-component regulators.", *Science (New York, NY)*, vol. 262, no. 5133, pp. 539.

Chen, J., Liu, D., Jiang, Y., Zhao, M., Shan, W., Kuang, J. and Lu, W. (2011), "Molecular characterization of a strawberry FaASR gene in relation to fruit ripening", *PloS one*, vol. 6, no. 9, pp. e24649.

Chen, J. H. and Ho, C. T. (1997), "Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds", *Journal of Agricultural and Food Chemistry*, vol. 45, no. 7, pp. 2374-2378.

Chervin, C., El-Kereamy, A., Roustan, J. -., Latché, A., Lamon, J. and Bouzayen, M. (2004a), "Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit", *Plant Science*, vol. 167, no. 6, pp. 1301-1305.

Chervin, C., El-Kereamy, A., Roustan, J. -., Latché, A., Lamon, J. and Bouzayen, M. (2004b), "Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit", *Plant Science*, vol. 167, no. 6, pp. 1301-1305.

Chope, G. A., Terry, L. A. and White, P. J. (2007), "The effect of 1-methylcyclopropene (1-MCP) on the physical and biochemical characteristics of onion cv. SS1 bulbs during storage", *Postharvest Biology and Technology*, vol. 44, no. 2, pp. 131-140.

Chun, O. K. and Kim, D. (2004), "Consideration on equivalent chemicals in total phenolic assay of chlorogenic acid-rich plums", *Food Research International*, vol. 37, no. 4, pp. 337-342.

Cin, D. V., Rizzini, F. M., Botton, A. and Tonutti, P. (2006), "The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit", *Postharvest Biology and Technology*, vol. 42, no. 2, pp. 125-133.

Cisneros-Zevallos, L. (2003), "The Use of Controlled Postharvest Abiotic Stresses as a Tool for Enhancing the Nutraceutical Content and Adding-Value of Fresh Fruits and Vegetables", *Journal of Food Science*, vol. 68, no. 5, pp. 1560-1565.

Civello, P. M., Powell, A. L., Sabehat, A. and Bennett, A. B. (1999), "An expansin gene expressed in ripening strawberry fruit", *Plant Physiology*, vol. 121, no. 4, pp. 1273-1279.

Collings, E., Garcia Cas, J. A., Ordaz Ortiz, J. J. and Terry, L. A. (2012), " A new real-time automated method for measuring in situ respiration rates of fresh produce ", *7th International Postharvest Symposium 2012 (IPS2012)*, 25-29th May, Kuala Lumpur, Malaysia, .

Cools, K., Chope, G. A., Hammond, J. P., Thompson, A. J. and Terry, L. A. (2011), "Ethylene and 1-methylcyclopropene differentially regulate gene expression during onion sprout suppression", *Plant Physiology*, vol. 156, no. 3, pp. 1639-1652.

Cordenunsi, B. R., Genovese, M. I., Oliveira do Nascimento, J. R., Aymoto Hassimotto, N. M., José dos Santos, R. and Lajolo, F. M. (2005), "Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars", *Food Chemistry*, vol. 91, no. 1, pp. 113-121.

Cordenunsi, B. R., Nascimento, J. R. O. and Lajolo, F. M. (2003), "Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage", *Food Chemistry*, vol. 83, no. 2, pp. 167-173.

Cox, K. A., McGhie, T. K., White, A. and Woolf, A. B. (2004), "Skin colour and pigment changes during ripening of 'Hass' avocado fruit", *Postharvest Biology and Technology,* vol. 31, no. 3, pp. 287-294.

Crisosto, C. H., Mitchell, F. G. and Johnson, S. (1995), "Factors in fresh market stone fruit quality", *Postharvest News and information,* vol. 6, no. 2, pp. 17-21.

Cristescu, S. M., De Martinis, D., te Lintel Hekkert, S., Parker, D. H. and Harren, F. J. M. (2002), "Ethylene production by Botrytis cinerea in vitro and in tomatoes", *Applied and Environmental Microbiology,* vol. 68, no. 11, pp. 5342-5350.

Csukasi, F., Osorio, S., Gutierrez, J. R., Kitamura, J., Giavalisco, P., Nakajima, M., Fernie, A. R., Rathjen, J. P., Botella, M. A. and Valpuesta, V. (2011), "Gibberellin biosynthesis and signalling during development of the strawberry receptacle", *New Phytologist,* vol. 191, no. 2, pp. 376-390.

Curry, E. A. and Greene, D. W. (1993), "CPPU influences fruit quality, fruit set, return bloom, and preharvest drop of apples", *HortScience,* vol. 28, no. 2, pp. 115-119.

Dauny, P. T., Joyce, D. C. and Gamby, C. (2003), "1-Methylcyclopropene influx and efflux in 'Cox'apple and 'Hass' avocado fruit", *Postharvest Biology and Technology,* vol. 29, no. 1, pp. 101-105.

Davies, C., Boss, P. K. and Robinson, S. P. (1997), "Treatment of grape berries, a nonclimacteric fruit with a synthetic auxin, retards ripening and alters the expression of developmentally regulated genes", *Plant Physiology,* vol. 115, no. 3, pp. 1155-1161.

Davis, F., Terry, L. A., Chope, G. A. and Faul, C. F. J. (2007), "Effect of extraction procedure on measured sugar concentrations in onion (Allium cepa L.) bulbs", *Journal of Agricultural and Food Chemistry,* vol. 55, no. 11, pp. 4299-4306.

Dong, L., Lurie, S. and Zhou, H. (2002), "Effect of 1-methylcyclopropene on ripening of 'Canino'apricots and 'Royal Zee'plums", *Postharvest Biology and Technology,* vol. 24, no. 2, pp. 135-145.

Downes, K., Chope, G. A. and Terry, L. A. (2010), "Postharvest application of ethylene and 1-methylcyclopropene either before or after curing affects onion (*Allium cepa*) bulb quality during long term cold storage", *Postharvest Biology and Technology,* vol. 55, no. 1, pp. 36-44.

Ekman, J., Clayton, M., Biasi, W. and Mitcham, E. (2004), "Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett'pears", *Postharvest Biology and Technology,* vol. 31, no. 2, pp. 127-136.

El-Kazzaz, M., Sommer, N. and Fortlage, R. (1983), "Effect of different atmospheres on postharvest decay and quality of fresh strawberries.", *Phytopathology,* vol. 73, no. 2, pp. 282-285.

Fallik, E., Klein, J., Grinberg, S., Lomaniec, E., Lurie, S. and Lalazar, A. (1993), "Effect of postharvest heat treatment of tomatoes on fruit ripening and decay caused by *Botrytis cinerea*", *Plant Disease,* vol. 77, no. 10, pp. 985-988.

Fan, X. and Mattheis, J. P. (2001), "1-Methylcyclopropene and storage temperature influence responses of 'Gala'apple fruit to gamma irradiation", *Postharvest Biology and Technology,* vol. 23, no. 2, pp. 143-151.

Fang, N., Yu, S. and Prior, R. L. (2002), "LC/MS/MS characterization of phenolic constituents in dried plums", *Journal of Agricultural and Food Chemistry,* vol. 50, no. 12, pp. 3579-3585.

Feng, X., Apelbaum, A., Sisler, E. C. and Goren, R. (2000), "Control of ethylene responses in avocado fruit with 1-methylcyclopropene", *Postharvest Biology and Technology,* vol. 20, no. 2, pp. 143-150.

Foukaraki, S. G., Chope, G. A. and Terry, L. A. (2010), "Ethylene Exposure after Dormancy Break Is as Effective as Continuous Ethylene to Control Sprout Growth in Some UK-Grown Potato Cultivars", *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on* 934, pp. 1175.

Fu, L., Cao, J., Li, Q., Lin, L. and Jiang, W. (2007), "Effect of 1-methylcyclopropene on fruit quality and physiological disorders in Yali pear (Pyrus bretschneideri Rehd.) during storage", *Food Science and Technology International,* vol. 13, no. 1, pp. 49-54.

Gao, L. and Mazza, G. (1995), "Characterization, quantitation, and distribution of anthocyanins and colorless phenolics in sweet cherries", *Journal of Agricultural and Food Chemistry,* vol. 43, no. 2, pp. 343-346.

García-Martínez, J. L., López-Diaz, I., Sánchez-Beltrán, M. J., Phillips, A. L., Ward, D. A., Gaskin, P. and Hedden, P. (1997), "Isolation and transcript analysis of

gibberellin 20-oxidase genes in pea and bean in relation to fruit development", *Plant Molecular Biology,* vol. 33, no. 6, pp. 1073-1084.

Gil, M. I., Holcroft, D. M. and Kader, A. A. (1997), "Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments", *Journal of Agricultural and Food Chemistry,* vol. 45, no. 5, pp. 1662-1667.

Giné Bordonaba, J. and Terry, L. A. (2008), "Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars", *Journal of Agricultural and Food Chemistry,* vol. 56, no. 16, pp. 7422-7430.

Giné Bordonaba, J. and Terry, L. A. (2011), "Strawberry", in Terry, L. (ed.) *Health-promoting properties of fruits and vegetables,* CABI Publishing, King's Lynn, UK, pp. 291-320.

Giné Bordonaba, J. and Terry, L. A. (2009), "Development of a glucose biosensor for rapid assessment of strawberry quality: relationship between biosensor response and fruit composition", *Journal of Agricultural and Food Chemistry,* vol. 57, no. 18, pp. 8220-8226.

Giovannoni, J. (2001), "Molecular biology of fruit maturation and ripening", *Annual review of Plant biology,* vol. 52, no. 1, pp. 725-749.

Given, N., Venis, M. and Gierson, D. (1988), "Hormonal regulation of ripening in the strawberry, a non-climacteric fruit", *Planta,* vol. 174, no. 3, pp. 402-406.

Golding, J., Shearer, D., Wyllie, S. and McGlasson, W. (1998), "Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit", *Postharvest Biology and Technology,* vol. 14, no. 1, pp. 87-98.

Goldschmidt, E. E. (1997), "Ripening of citrus and other non-climacteric fruits: A role for ethylene", *VIII International Symposium on Plant Bioregulation in Fruit Production 463,* pp. 335.

González-Lamothe, R., El Oirdi, M., Brisson, N. and Bouarab, K. (2012), "The Conjugated Auxin Indole-3-Acetic Acid–Aspartic Acid Promotes Plant Disease Development", *The Plant Cell Online,* vol. 24, no. 2, pp. 762-777.

Gorny, J. and Kader, A. (1996), "Fresh-cut fruit products", *Fresh-cut Products: Maintaining Quality and Safety,* , pp. 1-14.

Guo, H. and Ecker, J. R. (2004), "The ethylene signaling pathway: new insights", *Current opinion in Plant biology,* vol. 7, no. 1, pp. 40-49.

Hanhineva, K. and Aharoni, A. (2010), "Metabolomics in fruit development", in Jain, S. M. and Barr, D. S. (eds.) *Molecular Techniques In Crop Improvements,* 2nd ed, Springer, Dordrecht, pp. 675-694.

Heredia, J. B. and Cisneros-Zevallos, L. (2009), "The effects of exogenous ethylene and methyl jasmonate on the accumulation of phenolic antioxidants in selected whole and wounded fresh produce", *Food Chemistry,* vol. 115, no. 4, pp. 1500-1508.

Hernanz, D., Recamales, A. F., Meléndez-Martínez, A. J., González-Miret, M. L. and Heredia, F. J. (2007), "Assessment of the differences in the phenolic composition of five strawberry cultivars (*Fragaria x ananassa* Duch.) grown in two different soilless systems", *Journal of Agricultural and Food Chemistry,* vol. 55, no. 5, pp. 1846-1852.

Hershkovitz, V., Saguy, S. I. and Pesis, E. (2005), "Postharvest application of 1-MCP to improve the quality of various avocado cultivars", *Postharvest Biology and Technology,* vol. 37, no. 3, pp. 252-264.

Hoehn, E., Prange, K. R. and Vigneault, C. (2009), "Storage Technology and Applications ", in Yahia, E. M. (ed.) *Modified and controlled atmospheres for the storage, transportation, and packaging of horticultural commodities,* CRC, Boca Raton, FL., pp. 17-37.

Holcroft, D. M. and Kader, A. A. (1999), "Controlled atmosphere-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit", *Postharvest Biology and Technology,* vol. 17, no. 1, pp. 19-32.

Hollman, P. C. H. and Katan, M. B. (1999), "Dietary flavonoids: intake, health effects and bioavailability", *Food and Chemical Toxicology,* vol. 37, no. 9-10, pp. 937-942.

Hua, J., Sakai, H., Nourizadeh, S., Chen, Q. G., Bleecker, A. B., Ecker, J. R. and Meyerowitz, E. M. (1998), "EIN4 and ERS2 are members of the putative ethylene receptor gene family in Arabidopsis", *The Plant Cell Online,* vol. 10, no. 8, pp. 1321-1332.

Huber, D. (1983), "Polyuronide degradation and hemicellulose modifications in ripening tomato fruit", *Journal-American Society for Horticultural Science,* vol. 108.

Huysamer, M., Greve, L. C. and Labavitch, J. M. (1997), "Cell Wall Metabolism in Ripening Fruit (IX. Synthesis of Pectic and Hemicellulosic Cell Wall Polymers in the Outer Pericarp of Mature Green Tomatoes (cv XMT-22)", *Plant Physiology,* vol. 114, no. 4, pp. 1523-1531.

Hyodo, H. and Fujinami, H. (1989), "The Effects of 2, 5-Norbornadiene on the Induction of the Activity of 1-Aminocyclopropane-l-carboxylate Synthase and of Phenylalanine Ammonia-lyase in Wounded Mesocarp Tissue of Cucurbita maxima", *Plant and Cell Physiology,* vol. 30, no. 6, pp. 857-860.

Iannetta, P. P. M., Laarhoven, L. J., Medina-Escobar, N., James, E. K., McManus, M. T., Davies, H. V. and Harren, F. J. M. (2006), "Ethylene and carbon dioxide production by developing strawberries show a correlative pattern that is indicative of ripening climacteric fruit", *Physiologia Plantarum,* vol. 127, no. 2, pp. 247-259.

Ishizu, T., Winarno, H., Tsujino, E., Morita, T. and Shibuya, H. (2002), "Indonesian medicinal plants. XXIV. Stereochemical structure of perseitol·K+ complex isolated from the leaves of Scurrula fusca (Loranthaceae)", *Chemical and Pharmaceutical Bulletin,* vol. 50, no. 4, pp. 489-492.

Jeong, J. and Huber, D. J. (2004), "Suppression of avocado (*Persea americana Mill*.) fruit softening and changes in cell wall matrix polysaccharides and enzyme activities: Differential responses to 1-MCP and delayed ethylene application", *Journal of the American Society for Horticultural Science,* vol. 129, no. 5, pp. 752-759.

Jia, H., Chai, Y., Li, C., Lu, D., Luo, J., Qin, L. and Shen, Y. (2011), "Abscisic acid plays an important role in the regulation of strawberry fruit ripening", *Plant Physiology,* vol. 157, no. 1, pp. 188-199.

Jia, H., Li, C., Chai, Y., Xing, Y. and Shen, Y. (2013), "Sucrose promotes strawberry fruit ripening by stimulation of abscisic acid biosynthesis", *Pak.J.Bot,* vol. 45, no. 1, pp. 169-175.

Jiang, Y. and Joyce, D. C. (2003), "ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit", *Plant Growth Regulation,* vol. 39, no. 2, pp. 171-174.

Jiang, Y., Joyce, D. C. and Macnish, A. J. (1999), "Extension of the shelf life of banana fruit by 1-methylcyclopropene in combination with polyethylene bags", *Postharvest Biology and Technology,* vol. 16, no. 2, pp. 187-193.

Jiang, Y., Joyce, D. C. and Macnish, A. J. (2000), "Effect of abscisic acid on banana fruit ripening in relation to the role of ethylene", *Journal of Plant Growth Regulation,* vol. 19, no. 1, pp. 106-111.

Jiang, Y., Joyce, D. C. and Terry, L. A. (2001), "1-Methylcyclopropene treatment affects strawberry fruit decay", *Postharvest Biology and Technology,* vol. 23, no. 3, pp. 227-232.

Kader, A. A. (1985), "Ethylene-induced senescence and physiological disorders in harvested horticultural crops", *HortScience,* vol. 20, no. 1, pp. 54-57.

Kader, A. A. (2002), *Postharvest technology of horticultural crops,* Agriculture & Natural Resources.

Kader, A. A. and Arpaia, L. M. (2002), "Postharvest handling systems subtropical fruits", in Kader, A. A. (ed.) *Postharvest technology of horticultural crops,* 3rd ed, Agriculture & Natural Resources, California, pp. 375-397.

Kafkas, E., Koşar, M., Paydaş, S., Kafkas, S. and Başer, K. (2007), "Quality characteristics of strawberry genotypes at different maturation stages", *Food Chemistry,* vol. 100, no. 3, pp. 1229-1236.

Kähkönen, M. P., Hopia, A. I. and Heinonen, M. (2001), "Berry phenolics and their antioxidant activity", *Journal of Agricultural and Food Chemistry,* vol. 49, no. 8, pp. 4076-4082.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S. and Heinonen, M. (1999), "Antioxidant activity of plant extracts containing phenolic compounds", *Journal of Agricultural and Food Chemistry,* vol. 47, no. 10, pp. 3954-3962.

Kalt, W., Forney, C. F., Martin, A. and Prior, R. L. (1999), "Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits", *Journal of Agricultural and Food Chemistry,* vol. 47, no. 11, pp. 4638-4644.

Kan, T. and Bostan, S. Z. (2010), "Changes of Contents of Polyphenols and Vitamin a of Organic and Conventional Fresh and Dried Apricot Cultivars (Prunus

armeniaca L.)", *World Journal of Agricultural Sciences,* vol. 6, no. 2, pp. 120-126.

Kano, Y. and Asahira, T. (1981), "Roles of cytokinin and abscisic acid in the maturing of strawberry fruits.", *Journal of the Japanese Society for Horticultural Science,* vol. 50, pp. 31-36.

Karakurt, Y. and Huber, D. J. (2004), "Ethylene-induced gene expression, enzyme activities, and water soaking in immature and ripe watermelon (Citrullus lanatus) fruit", *Journal of Plant Physiology,* vol. 161, no. 4, pp. 381-388.

Kays, S. J. and Paull, R. E. (2004), *Postharvest biology,* Exon Press, Athens, Greece.

Ke, D., Goldstein, L., O'MAHONY, M. and Kader, A. A. (1991), "Effects of Short-term Exposure to Low O2 and High CO2 Atmospheres on Quality Attributes of Strawberries", *Journal of Food Science,* vol. 56, no. 1, pp. 50-54.

Kende, H. (1993), "Ethylene biosynthesis", *Annual review of plant biology,* vol. 44, no. 1, pp. 283-307.

Kepka, M., Benson, C. L., Gonugunta, V. K., Nelson, K. M., Christmann, A., Grill, E. and Abrams, S. R. (2011), "Action of natural abscisic acid precursors and catabolites on abscisic acid receptor complexes", *Plant Physiology,* vol. 157, no. 4, pp. 2108-2119.

Kevany, B. M., Tieman, D. M., Taylor, M. G., Cin, V. D. and Klee, H. J. (2007), "Ethylene receptor degradation controls the timing of ripening in tomato fruit", *The Plant Journal,* vol. 51, no. 3, pp. 458-467.

Kevers, C., Falkowski, M., Tabart, J., Defraigne, J. O., Dommes, J. and Pincemail, J. (2007), "Evolution of antioxidant capacity during storage of selected fruits and vegetables", *Journal of Agricultural and Food Chemistry,* vol. 55, no. 21, pp. 8596-8603.

Khan, A. S., Singh, Z. and Swinny, E. E. (2009), "Postharvest application of 1-Methylcyclopropene modulates fruit ripening, storage life and quality of 'Tegan Blue'Japanese plum kept in ambient and cold storage", *International Journal of Food Science & Technology,* vol. 44, no. 6, pp. 1272-1280.

Kim, D. O., Chun, O. K., Kim, Y. J., Moon, H. Y. and Lee, C. Y. (2003), "Quantification of polyphenolics and their antioxidant capacity in fresh plums", *Journal of Agricultural and Food Chemistry,* vol. 51, no. 22, pp. 6509-6515.

Koyuncu, M. A. and Dilmacunal, T. (2010), "Determination of vitamin C and organic acid changes in strawberry by HPLC during cold storage", *Not.Bot.Hort.Agrobot.Cluj*, vol. 38, no. 3, pp. 95-98.

Kruger, F. and Lemmer, D. (2007), "Commercial ripening trials with South African avocados in the United Kingdom", *Proceedings VI World Avocado Congress*, Vol. 30, 12 – 16 Nov, Viña Del Mar, Chile, South African Avocado Growers Association Yearbook, South Africa, pp. 12-15.

Ku, V. V. V., Wills, R. B. H. and Ben-Yehoshua, S. (1999), "1-Methylcyclopropene can differentially affect the postharvest life of strawberries exposed to ethylene", *HortScience*, vol. 34, no. 1, pp. 119-120.

Lafuente, M., Martínez-Téllez, M. and Zacarias, L. (1997), "Abscisic Acid in the Response of 'Fortune'Mandarins to Chilling. Effect of Maturity and High-Temperature Conditioning", *Journal of the Science of Food and Agriculture*, vol. 73, no. 4, pp. 494-502.

Landahl, S., Meyer, M. D. and Terry, L. A. (2009), "Spatial and temporal analysis of textural and biochemical changes of imported avocado cv. hass during fruit ripening", *Journal of Agricultural and Food Chemistry*, vol. 57, no. 15, pp. 7039-7047.

Lelièvre, J. M., Latche, A., Jones, B., Bouzayen, M. and Pech, J. C. (1997), "Ethylene and fruit ripening", *Physiologia Plantarum*, vol. 101, no. 4, pp. 727-739.

Lewis, D. H., Burge, G. K., Schmierer, D. M. and Jameson, P. E. (1996), "Cytokinins and fruit development in the kiwifruit (Actinidia deliciosa). I. Changes during fruit development", *Physiologia Plantarum*, vol. 98, no. 1, pp. 179-186.

Liu, H., Jiang, W., Zhou, L., Wang, B. and Luo, Y. (2005a), "The effects of 1-methylcyclopropene on peach fruit (Prunus persica L. cv. Jiubao) ripening and disease resistance", *International Journal of Food Science & Technology*, vol. 40, no. 1, pp. 1-7.

Liu, K., Kang, B., Jiang, H., Moore, S. L., Li, H., Watkins, C. B., Setter, T. L. and Jahn, M. M. (2005b), "A GH3-like gene, CcGH3, isolated from Capsicum chinense L. fruit is regulated by auxin and ethylene*", *Plant Molecular Biology*, vol. 58, no. 4, pp. 447-464.

Liu, R. H. (2003), "Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals", *The American Journal of Clinical Nutrition,* vol. 78, no. 3, pp. 517S-520S.

Liu, X., Robinson, P. W., Madore, M. A., Witney, G. W. and Arpaia, M. L. (1999a), "Hass' avocado carbohydrate fluctuations. I. Growth and phenology", *Journal of the American Society for Horticultural Science,* vol. 124, no. 6, pp. 671-675.

Liu, X., Robinson, P. W., Madore, M. A., Witney, G. W. and Arpaia, M. L. (1999b), "Hass' avocado carbohydrate fluctuations. II. Fruit growth and ripening", *Journal of the American Society for Horticultural Science,* vol. 124, no. 6, pp. 676-681.

Liu, X., Sievert, J., Arpaia, M. L. and Madore, M. A. (2002), "Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado", *Journal of the American Society for Horticultural Science,* vol. 127, no. 1, pp. 108-114.

Liu, Y., Hoffman, N. E. and Yang, S. F. (1985), "Promotion by ethylene of the capability to convert 1-aminocyclopropane-1-carboxylic acid to ethylene in preclimacteric tomato and cantaloupe fruits", *Plant Physiology,* vol. 77, no. 2, pp. 407-411.

Lopes Da Silva, F., de Pascual-Teresa, S., Rivas-Gonzalo, J. and Santos-Buelga, C. (2002), "Identification of anthocyanin pigments in strawberry (cv Camarosa) by LC using DAD and ESI-MS detection", *European Food Research and Technology,* vol. 214, no. 3, pp. 248-253.

Lopes Da Silva, F., Escribano-Bailón, M. T., Pérez Alonso, J. J., Rivas-Gonzalo, J. C. and Santos-Buelga, C. (2007), "Anthocyanin pigments in strawberry", *LWT-Food Science and Technology,* vol. 40, no. 2, pp. 374-382.

Luo, Y. and Liu, X. (1994a), "Effects of ethylene on RNA metabolism in strawberry fruit after harvest", *Journal of Horticultural Science,* vol. 69. pp. 137-139.

Luo, Y. and Liu, X. (1994b), "Effects of ethylene on the RNA metabolism and protein synthesis activity in postharvest strawberry fruit", *Acta Phytophysiologica Sinica,* vol. 20, pp. 235-239.

Luo, Z., Xie, J., Xu, T. and Zhang, L. (2009), "Delay ripening of 'Qingnai'plum (*Prunus salicina* Lindl) with 1-methylcyclopropene", *Plant Science,* vol. 177, no. 6, pp. 705-709.

Ma, G., Wang, R., Wang, C. R., Kato, M., Yamawaki, K., Qin, F. and Xu, H. L. (2009), "Effect of 1-methylcyclopropene on expression of genes for ethylene biosynthesis enzymes and ethylene receptors in post-harvest broccoli", *Plant Growth Regulation,* vol. 57, no. 3, pp. 223-232.

Maftoonazad, N. and Ramaswamy, H. (2005), "Postharvest shelf-life extension of avocados using methyl cellulose-based coating", *LWT-Food Science and Technology,* vol. 38, no. 6, pp. 617-624.

Manning, K. (1994), "Changes in gene expression during strawberry fruit ripening and their regulation by auxin", *Planta,* vol. 194, no. 1, pp. 62-68.

Martinez-Romero, D., Bailen, G., Serrano, M., Guillen, F., Valverde, J. M., Zapata, P., Castillo, S. and Valero, D. (2007), "Tools to maintain postharvest fruit and vegetable quality through the inhibition of ethylene action: a review", *Critical reviews in Food Science and Nutrition,* vol. 47, no. 6, pp. 543-560.

Martínez-Romero, D., Guillén, F., Castillo, S., Zapata, P. J., Serrano, M. and Valero, D. (2009a), "Development of a carbon-heat hybrid ethylene scrubber for fresh horticultural produce storage purposes", *Postharvest Biology and Technology,* vol. 51, no. 2, pp. 200-205.

Martínez-Romero, D., Guillén, F., Castillo, S., Zapata, P. J., Valero, D. and Serrano, M. (2009b), "Effect of ethylene concentration on quality parameters of fresh tomatoes stored using a carbon-heat hybrid ethylene scrubber", *Postharvest Biology and Technology,* vol. 51, no. 2, pp. 206-211.

Martínez-Romero, D., Dupille, E., Guillén, F., Valverde, J. M., Serrano, M. and Valero, D. (2003), "1-Methylcyclopropene increases storability and shelf life in climacteric and nonclimacteric plums", *Journal of Agricultural and Food Chemistry,* vol. 51, no. 16, pp. 4680-4686.

McMurchie, E., McGlasson, W. and Eaks, I. (1972), "Treatment of fruit with propylene gives information about the biogenesis of ethylene", .

Menniti, A., Gregori, R. and Donati, I. (2004), "1-Methylcyclopropene retards postharvest softening of plums", *Postharvest Biology and Technology,* vol. 31, no. 3, pp. 269-275.

Meyer, M. D., Landahl, S., Donetti, M. and Terry, L. (2011), "Avocado", in Terry, L. (ed.) *Health-promoting properties of fruits and vegetables,* CABI Publishing, King's Lynn, UK, pp. 27-50.

Meyer, M. D. and Terry, L. A. (2008), "Development of a rapid method for the sequential extraction and subsequent quantification of fatty acids and sugars from avocado mesocarp tissue", *Journal of Agricultural and Food Chemistry,* vol. 56, no. 16, pp. 7439-7445.

Meyer, M. and Terry, L. (2010), "Fatty acid and sugar composition of avocado, cv. Hass, in response to treatment with an ethylene scavenger or 1-methylcyclopropene to extend storage life", *Food Chemistry,* vol. 121, no. 4, pp. 1203-1210.

Młodzińska, E. (2009), "Survey of plant pigments: molecular and environmental determinants of plant colors", *Acta Biologica Cracoviensia Series Botanica,* vol. 51, no. 1, pp. 7-16.

Modares, B., Ramin, A. and Ghobadi, C. (2010), "1-Methylcyclopropene Treatment Enhanced Strawberry Fruit Quality during Storage", *Acta Horticulturae,* vol. 876, pp. 255-258.

Moing, A., Renaud, C., Gaudillère, M., Raymond, P., Roudeillac, P. and Denoyes-Rothan, B. (2001), "Biochemical changes during fruit development of four strawberry cultivars", *Journal of the American Society for Horticultural Science,* vol. 126, no. 4, pp. 394-403.

Montalvo, E., García, H. S., Tovar, B. and Mata, M. (2007), "Application of exogenous ethylene on postharvest ripening of refrigerated ‘Ataulfo’mangoes", *LWT-Food Science and Technology,* vol. 40, no. 8, pp. 1466-1472.

Nambara, E. and Marion-Poll, A. (2003), "ABA action and interactions in seeds", *Trends in plant science,* vol. 8, no. 5, pp. 213-217.

Nambara, E. and Marion-Poll, A. (2005), "Abscisic acid biosynthesis and catabolism", *Annu.Rev.Plant Biol.*, vol. 56, pp. 165-185.

Natella, F., Nardini, M., Di Felice, M. and Scaccini, C. (1999), "Benzoic and cinnamic acid derivatives as antioxidants: structure-activity relation", *Journal of Agricultural and Food Chemistry,* vol. 47, no. 4, pp. 1453-1459.

Nitsch, J. (1950), "Growth and morphogenesis of the strawberry as related to auxin", *American Journal of Botany,* , pp. 211-215.

Nitsch, J. (1955), "Free Auxins and Free Tryptophane in the Strawberry.", *Plant Physiology,* vol. 30, no. 1, pp. 33-39.

Noratto, G., Porter, W., Byrne, D. and Cisneros-Zevallos, L. (2009), "Identifying peach and plum polyphenols with chemopreventive potential against estrogen-independent breast cancer cells", *Journal of Agricultural and Food Chemistry,* vol. 57, no. 12, pp. 5219-5226.

O'Donoghue, E. M., Somerfield, S. D., Shaw, M., Bendall, M., Hedderly, D., Eason, J. and Sims, I. (2004), "Evaluation of carbohydrates in Pukekohe Longkeeper and Grano cultivars of Allium cepa", *Journal of Agricultural and Food Chemistry,* vol. 52, no. 17, pp. 5383-5390.

Olsson, M. E., Ekvall, J., Gustavsson, K., Nilsson, J., Pillai, D., Sjöholm, I., Svensson, U., Åkesson, B. and Nyman, M. G. L. (2004), "Antioxidants, low molecular weight carbohydrates, and total antioxidant capacity in strawberries (*Fragaria* × *ananassa*): effects of cultivar, ripening, and storage", *Journal of Agricultural and Food Chemistry,* vol. 52, no. 9, pp. 2490-2498.

Ordaz Ortiz, J. J., Foukaraki, S. G. and Terry, L. A. (2012), "A new liquid chromatography tandem ultra high definition accurate mass spectrometry method for the simultaneous quantitation of nine plant hormones in fruits and vegetables", *7th InternationalPostharvest Symposium 2012(IPS2012),* 25-29th May, Kuala Lumpur, Malaysia .

Paliyath, G., Murr, D. P., Handa, A. K. and Lurie, S. (2008), *Postharvest biology and technology of fruits, vegetables, and flowers,* Wiley-Blackwell Ames, Singapore.

Palma, J. M., Corpas, F. J. and del Río, L. A. (2011), "Proteomics as an approach to the understanding of the molecular physiology of fruit development and ripening", *Journal of proteomics,* vol. 74, no. 8, pp. 1230-1243.

Palou, L., Crisosto, C. H., Garner, D. and Basinal, L. M. (2003), "Effect of continuous exposure to exogenous ethylene during cold storage on postharvest decay development and quality attributes of stone fruits and table grapes", *Postharvest Biology and Technology,* vol. 27, no. 3, pp. 243-254.

Parsons, R. (2007), "How to Pick a Peach: The Search for Flavor from Farm to Table", in *Plums*, Houghton Mifflin Harcourt, New York, USA, pp. 208-220.

Pathak, N., Asif, M. H., Dhawan, P., Srivastava, M. K. and Nath, P. (2003), "Expression and activities of ethylene biosynthesis enzymes during ripening of banana fruits and effect of 1-MCP treatment", *Plant Growth Regulation*, vol. 40, no. 1, pp. 11-19.

Paul, V., Pandey, R. and Srivastava, G. C. (2012), "The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene—An overview", *Journal of Food Science and Technology*, vol. 49, no. 1, pp. 1-21.

Pech, J. C., Bouzayen, M. and Latché, A. (2008), "Climacteric fruit ripening: ethylene-dependent and independent regulation of ripening pathways in melon fruit", *Plant Science*, vol. 175, no. 1, pp. 114-120.

Pereira, D. M., Valentão, P., Pereira, J. A. and Andrade, P. B. (2009), "Phenolics: from chemistry to biology", *Molecules*, vol. 14, no. 6, pp. 2202-2211.

Pérez, A. G., Olías, R., Espada, J., Olías, J. M. and Sanz, C. (1997), "Rapid determination of sugars, nonvolatile acids, and ascorbic acid in strawberry and other fruits", *Journal of Agricultural and Food Chemistry*, vol. 45, no. 9, pp. 3545-3549.

Perkins-Veazie, P. M., Huber, D. J. and Brecht, J. K. (1996), "In vitro growth and ripening of strawberry fruit in the presence of ACC, STS or propylene", *Annals of applied biology*, vol. 128, no. 1, pp. 105-116.

Pesis, E., Ackerman, M., Ben-Arie, R., Feygenberg, O., Feng, X., Apelbaum, A., Goren, R. and Prusky, D. (2002), "Ethylene involvement in chilling injury symptoms of avocado during cold storage", *Postharvest Biology and Technology*, vol. 24, no. 2, pp. 171-181.

Pesis, E., Fuchs, Y. and Zauberman, G. (1978), "Cellulase activity and fruit softening in avocado", *Plant Physiology*, vol. 61, no. 3, pp. 416-419.

Prange, R. K., Daniels-Lake, B. J., Jeong, J. C. and Binns, M. (2005), "Effects of ethylene and 1-methylcyclopropene on potato tuber sprout control and fry color", *American Journal of Potato Research*, vol. 82, no. 2, pp. 123-128.

Rasori, A., Ruperti, B., Bonghi, C., Tonutti, P. and Ramina, A. (2002), "Characterization of two putative ethylene receptor genes expressed during peach fruit development and abscission", *Journal of Experimental Botany,* vol. 53, no. 379, pp. 2333-2339.

Reid, M. S. (2004), "Ethylene in postharvest technology", in Kader, A. A. (ed.) *Postharvest technology of horticultural crops,* 3rd ed, University of California, Division of Agriculture and Natural Resources, California, pp. 149-162.

Ritenour, M. A., Crisosto, C. H., Garner, D. T., Cheng, G. W. and Zoffoli, J. P. (1999), "Temperature, length of cold storage and maturity influence the ripening rate of ethylene-preconditioned kiwifruit", *Postharvest Biology and Technology,* vol. 15, no. 2, pp. 107-115.

Rose, J. K. C. and Bennett, A. B. (1999), "Cooperative disassembly of the cellulose–xyloglucan network of plant cell walls: parallels between cell expansion and fruit ripening", *Trends in plant science,* vol. 4, no. 5, pp. 176-183.

Roychoudhury, A., Paul, S. and Basu, S. (2013), "Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress", *Plant Cell Reports*, vol. 32, no. 7, pp.1-22.

Salman, A., Filgueiras, H., Cristescu, S., Lopez-Lauri, F., Harren, F. and Sallanon, H. (2009), "Inhibition of wound-induced ethylene does not prevent red discoloration in fresh-cut endive (Cichorium intybus L.)", *European Food Research and Technology,* vol. 228, no. 4, pp. 651-657.

Saltveit, M. E. (1997), "A summary of CA and MA requirements and recommendations for harvested vegetables. In: Seventh International Controlled Atmosphere Research Conference.", *Proceedings of the 7th international controlled atmosphere research conference,* Vol. 4, California, Vegetables and Ornamentals, Univ. Calif., Davis, pp. 98.

Saltveit, M. E. (1999), "Effect of ethylene on quality of fresh fruits and vegetables", *Postharvest Biology and Technology,* vol. 15, no. 3, pp. 279-292.

Saltveit, M. E. (2005), "Aminoethoxyvinylglycine (AVG) reduces ethylene and protein biosynthesis in excised discs of mature-green tomato pericarp tissue", *Postharvest Biology and Technology,* vol. 35, no. 2, pp. 183-190.

Salvador, A., Cuquerella, J. and Martínez-Jávega, J. (2003), "1-MCP Treatment Prolongs Postharvest Life of'Santa Rosa'Plums", *Journal of Food Science*, vol. 68, no. 4, pp. 1504-1510.

Seo, M. and Koshiba, T. (2002), "Complex regulation of ABA biosynthesis in plants", *Trends in Plant Science*, vol. 7, no. 1, pp. 41-48.

Serrani, J. C., Sanjuán, R., Ruiz-Rivero, O., Fos, M. and García-Martínez, J. L. (2007), "Gibberellin regulation of fruit set and growth in tomato", *Plant Physiology*, vol. 145, no. 1, pp. 246-257.

Seymour, G. B., Taylor, J. and Tucker, G. A. (1993), *Biochemistry of fruit ripening*, Chapman & Hall, London.

Shewfelt, L. R. (2009), "Measuring Quality and Maturity", in Shewfelt. L. R. and Florkowski, W. J., Shewfelt. L. R., Brueckner. B., Prussia. E. Stanley. (eds.) *Postharvest handling: A systems approach*, 2nd ed, Academic Press, New York, pp. 462-476.

Shi, L., Shen, L., Yu, M., Ouyang, L., Fan, B. and Sheng, J. (2008), "Effects of 1-Methylcyclopropene Treatment on the Shelf-Life Quality of Strawberry cv. Jingnong 1st", *Acta Horticulturae*, , no. 768, pp. 311.

Singh, S., Singh, Z. and Swinny, E. (2009), "Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina Lindell*)", *Postharvest Biology and Technology*, vol. 53, no. 3, pp. 101-108.

Sisler, E. C., Goren, R., Apelbaum, A. and Serek, M. (2009), "The effect of dialkylamine compounds and related derivatives of 1-methylcyclopropene in counteracting ethylene responses in banana fruit", *Postharvest Biology and Technology*, vol. 51, no. 1, pp. 43-48.

Sisler, E. and Blankenship, S. (1996), "Methods of counteracting an ethylene response in plants", *US Patent*, vol. 5, no. 518, pp. 988.

Sisler, E. and Serek, M. (2006), "Inhibitors of ethylene responses in plants at the receptor level: recent developments", *Physiologia Plantarum*, vol. 100, no. 3, pp. 577-582.

Skog, L., Schaefer, B. and Smith, P. (2001), "1-Methylcyclopropene preserves the firmness of plums during postharvest storage and ripening", *Acta Horticulturae*, vol. 533, pp. 171-172.

Smith, A. W. J., Poulston, S., Rowsell, L., Terry, L. A. and Anderson, J. A. (2009), "A new palladium-based ethylene scavenger to control ethylene-induced ripening of climacteric fruit", *Platinum Metals Review*, vol. 53, no. 3, pp. 112-122.

Smith, R. and Skog, L. (1992), "Postharvest carbon dioxide treatment enhances firmness of several cultivars of strawberry", *HortScience*, vol. 27, no. 5, pp. 420-421.

Solano, R., Stepanova, A., Chao, Q. and Ecker, J. R. (1998), "Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1", *Genes & development*, vol. 12, no. 23, pp. 3703-3714.

Soliva-Fortuny, R. C. and Martín-Belloso, O. (2003), "New advances in extending the shelf-life of fresh-cut fruits: a review", *Trends in Food Science & Technology*, vol. 14, no. 9, pp. 341-353.

Staby, G. L., Basel, R. M., Reid, M. S. and Dodge, L. L. (1993), "Efficacies of commercial anti-ethylene products for fresh cut flowers", *HortTechnology*, vol. 3, no. 2, pp. 199-202.

Staswick, P. E., Serban, B., Rowe, M., Tiryaki, I., Maldonado, M. T., Maldonado, M. C. and Suza, W. (2005), "Characterization of an Arabidopsis enzyme family that conjugates amino acids to indole-3-acetic acid", *The Plant Cell Online*, vol. 17, no. 2, pp. 616-627.

Sun, L., Zhang, M., Ren, J., Qi, J., Zhang, G. and Leng, P. (2010), "Reciprocity between abscisic acid and ethylene at the onset of berry ripening and after harvest", *BMC Plant Biology*, vol. 10, no. 1, pp. 257-268.

Suni, M., Nyman, M., Eriksson, N. A., Björk, L. and Björck, I. (2000), "Carbohydrate composition and content of organic acids in fresh and stored apples", *Journal of the Science of Food and Agriculture*, vol. 80, no. 10, pp. 1538-1544.

Symons, G., Chua, Y., Ross, J., Quittenden, L., Davies, N. and Reid, J. (2012), "Hormonal changes during non-climacteric ripening in strawberry", *Journal of Experimental Botany*, vol. 63, no. 13, pp. 4741-4750.

Taylor, M. E., Jacobs, G., Rabe, E. and Dodd, M. C. (1993a), "Physiological factors associated with over-ripeness, internal breakdown and gel breakdown in plums stored at two temperatures", *Journal of Horticultural Science*, vol. 68, pp. 825-830.

Taylor, M., Rabe, E., Dodd, M. and Jacobs, G. (1993b), "Influence of sampling date and position in the tree on mineral nutrients, maturity and gel breakdown in cold stored 'Songold'plums", *Scientia Horticulturae*, vol. 54, no. 2, pp. 131-141.

Terry, L. A., Chope, G. A. and Giné Bordonaba, J. (2007a), "Effect of water deficit irrigation and inoculation with Botrytis cinerea on strawberry (*Fragaria x ananassa*) fruit quality", *Journal of Agricultural and Food Chemistry*, vol. 55, no. 26, pp. 10812-10819.

Terry, L. A., Ilkenhans, T., Poulston, S., Rowsell, L. and Smith, A. W. J. (2007b), "Development of new palladium-promoted ethylene scavenger", *Postharvest Biology and Technology*, vol. 45, no. 2, pp. 214-220.

Terry, L. A. and Joyce, D. C. (2004), "Elicitors of induced disease resistance in postharvest horticultural crops: a brief review", *Postharvest Biology and Technology*, vol. 32, no. 1, pp. 1-13.

Thompson, A. K. (2003), *Postharvest Technology of Fruit and Vegetable*, 2nd ed, Blackwell Science., Oxford.

Tian, M., Prakash, S., Elgar, H., Young, H., Burmeister, D. and Ross, G. (2000), "Responses of strawberry fruit to 1-methylcyclopropene (1-MCP) and ethylene", *Plant Growth Regulation*, vol. 32, no. 1, pp. 83-90.

Tomás-Barberán, F. A., Gil, M. I., Cremin, P., Waterhouse, A. L., Hess-Pierce, B. and Kader, A. A. (2001), "HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums", *Journal of Agricultural and Food Chemistry*, vol. 49, no. 10, pp. 4748-4760.

Trainotti, L., Pavanello, A. and Casadoro, G. (2005), "Different ethylene receptors show an increased expression during the ripening of strawberries: does such an

increment imply a role for ethylene in the ripening of these non-climacteric fruits?", *Journal of Experimental Botany*, vol. 56, no. 418, pp. 2037-2046.

Valero, D. and Serrano, M. (2010), *Postharvest biology and technology for preserving fruit quality*, CRC Press.

Valero, D., Martınez-Romero, D., Valverde, J., Guillén, F., Castillo, S. and Serrano, M. (2004), "Could the 1-MCP treatment effectiveness in plum be affected by packaging?", *Postharvest Biology and Technology*, vol. 34, no. 3, pp. 295-303.

Veluthambi, K. and Poovaiah, B. (1984), "Auxin-regulated polypeptide changes at different stages of strawberry fruit development", *Plant Physiology*, vol. 75, no. 2, pp. 349-353.

Vendrell, M., Klee, H., Pech, C. J. and Romojaro, F. (2003), *Biology and biotechnology of the plant hormone ethylene III*, Ios Press Inc, Amsterdam.

Vicente, A. R., Manganaris, G. A., Cisneros-Zevallos, L. and Crisosto, C. H. (2011), "Prunus", in Terry, L. A. (ed.) *Health-promoting properties of fruits and vegetables*, CABI Publishing, King's Lynn, UK, pp. 238-259.

Vicente, A. R., Manganaris, G. A., Sozzi, G. O. and Crisosto, C. H. (2009), "Nutritional quality of fruits and vegetables", in Florkowski, W. J., Shewfelt, L. R., Brueckner, B., et al (eds.) *Postharvest Handling A System Approach*, 2nd ed, Academic Press., New York, pp. 58-93.

Villarreal, N. M., Bustamante, C. A., Civello, P. M. and Martínez, G. A. (2010), "Effect of ethylene and 1-MCP treatments on strawberry fruit ripening", *Journal of the Science of Food and Agriculture*, vol. 90, no. 4, pp. 683-689.

Villarreal, N. M., Martínez, G. A. and Civello, P. M. (2009), "Influence of plant growth regulators on polygalacturonase expression in strawberry fruit", *Plant Science*, vol. 176, no. 6, pp. 749-757.

Vizzotto, M., Cisneros-Zevallos, L., Byrne, D. H., Ramming, D. W. and Okie, W. (2007), "Large variation found in the phytochemical and antioxidant activity of peach and plum germplasm", *Journal of the American Society for Horticultural Science*, vol. 132, no. 3, pp. 334-340.

Vogt, T. (2010), "Phenylpropanoid biosynthesis", *Molecular Plant*, vol. 3, no. 1, pp. 2-20.

Wang, H., Cao, G. and Ronald, L. (1996), "Total antioxidant capacity of fruits", *Journal of Agricultural and Food Chemistry,* vol. 44, no. 3, pp. 701-705.

Wang, H. and Woodson, W. R. (1989), "Reversible inhibition of ethylene action and interruption of petal senescence in carnation flowers by norbornadiene", *Plant Physiology,* vol. 89, no. 2, pp. 434-438.

Wang, K. L. C., Li, H. and Ecker, J. R. (2002), "Ethylene biosynthesis and signaling networks", *The Plant Cell Online,* vol. 14, no. suppl 1, pp. S131-S151.

Watkins, C. B. (2006), "The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables", *Biotechnology Advances,* vol. 24, no. 4, pp. 389-409.

White, P. J. (2002), "Recent advances in fruit development and ripening: an overview", *Journal of Experimental Botany,* vol. 53, no. 377, pp. 1995-2000.

Wills, R. B. H. (1998), "Enhancement of senescence in non-climacteric fruit and vegetables by low ethylene levels", *Acta Horticulturae,* vol. 464, pp. 159-164.

Wills, R. B. H. and Kim, G. H. (1995), "Effect of ethylene on postharvest life of strawberries", *Postharvest Biology and Technology,* vol. 6, no. 3-4, pp. 249-255.

Wills, R. B. H., McGlasson, W. B., Graham, D. and Joyce, D. C. (2007), *Postharvest. An introduction to the physiology and handling of fruit and vegetables*. 5th ed, CAB International, Wallingford, UK.

Wills, R. B. H. and Warton, M. A. (2000), "A new rating scale for ethylene action on postharvest fruit and vegetables", *Improving Postharvest Technologies of Fruits, Vegetables and Ornamentals, Institute International of Refrigeration,* Murcia, Spain, , pp. 43-47.

Wills, R. B. H. and Warton, M. A. (2004), "Efficacy of potassium permanganate impregnated into alumina beads to reduce atmospheric ethylene", *Journal of the American Society for Horticultural Science,* vol. 129, no. 3, pp. 433-438.

Wills, R. B. H., Warton, M. A., Mussa, D. and Chew, L. P. (2001), "Ripening of climacteric fruits initiated at low ethylene levels", *Animal Production Science,* vol. 41, no. 1, pp. 89-92.

Woolf, A. B., Requejo-Tapia, C., Cox, K. A., Jackman, R. C., Gunson, A., Arpaia, M. L. and White, A. (2005), "1-MCP reduces physiological storage disorders of 'Hass' avocados", *Postharvest Biology and Technology,* vol. 35, no. 1, pp. 43-60.

Wright, K. P. and Kader, A. A. (1997), "Effect of controlled-atmosphere storage on the quality and carotenoid content of sliced persimmons and peaches", *Postharvest Biology and Technology*, vol. 10, no. 1, pp. 89-97.

Xiong, L. and Zhu, J. K. (2003), "Regulation of abscisic acid biosynthesis", *Plant Physiology*, vol. 133, no. 1, pp. 29-36.

Xu, C., Zhang, Y., Cao, L. and Lu, J. (2010), "Phenolic compounds and antioxidant properties of different grape cultivars grown in China", *Food Chemistry*, vol. 119, no. 4, pp. 1557-1565.

Yang, S. F. and Hoffman, N. E. (1984), "Ethylene biosynthesis and its regulation in higher plants", *Annual Review of Plant Physiology*, vol. 35, no. 1, pp. 155-189.

Yang, S. and Oetiker, J. (1995), "The role of ethylene in fruit ripening", *Acta Horticulturae*, vol. 398, pp. 167-178.

Yokotani, N., Tamura, S., Nakano, R., Inaba, A., McGlasson, W. B. and Kubo, Y. (2004), "Comparison of ethylene- and wound-induced responses in fruit of wild-type, rin and nor tomatoes", *Postharvest Biology and Technology*, vol. 32, no. 3, pp. 247-252.

Yoo, K. M., Lee, K. W., Park, J. B., Lee, H. J. and Hwang, I. K. (2004), "Variation in major antioxidants and total antioxidant activity of Yuzu (Citrus junos Sieb ex Tanaka) during maturation and between cultivars", *Journal of Agricultural and Food Chemistry*, vol. 52, no. 19, pp. 5907-5913.

Zaharia, L. I., Walker-Simmon, M. K., Rodríguez, C. N. and Abrams, S. R. (2005), "Chemistry of abscisic acid, abscisic acid catabolites and analogs", *Journal of Plant Growth Regulation*, vol. 24, no. 4, pp. 274-284.

Zheng, X., Tian, S., Xu, Y. and Li, B. (2005), "Effects of exogenous oxalic acid on ripening and decay incidence in mango fruit during storage at controlled atmosphere", *Journal of Fruit Science*, vol. 22, no. 4, pp. 351-355.

CHAPTER TEN

10 APPENDICES

Appendix A

A.1 Statistical tables

A.1.1 ANOVA tables for Chapter 3

A. 1. Statistical output for the effect of treatment 1 and treatment 2 on the concentration of ethylene in storage boxes at 5 ^{o}C of avocados (cv. Hass Chilean early season avocados).

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
TR1	2		9.6424	4.8212	19.66	<.001
TR2	1		10.7079	10.7079	43.67	<.001
storage day	4		8.5271	2.1318	8.69	<.001
TR1.TR2	2		9.3324	4.6662	19.03	<.001
TR1.storage_day	8		6.7069	0.8384	3.42	0.003
TR2.storage_day	4		6.7811	1.6953	6.91	<.001
TR1.TR2.storage_day	8		5.8482	0.7310	2.98	0.009
Residual	48	(12)	11.7683	0.2452		
Total	77	(12)	67.3553			

A. 2. Lightness (L*) of cv. Hass avocados (early season Chilean Exp 3.1).

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		61.806	61.806	9.89	0.002
Baseline.OT	1		42.399	42.399	6.78	0.010
Baseline.T1	4		91.093	22.773	3.64	0.008
Baseline.T2	1		41.356	41.356	6.62	0.011
Baseline.OT.T1	2		39.952	19.976	3.20	0.044
Baseline.OT.T2	1		6.284	6.284	1.01	0.318
Baseline.T1.T2	2		17.979	8.990	1.44	0.241
Baseline.OT.T1.T2	2		21.655	10.827	1.73	0.181
Residual	119	(1)	743.896	6.251		
Total	133	(1)	1066.119			

A. 3. Chroma of (C*) avocados cv. Hass (Exp 3.1).

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		210.72	210.72	20.49	<.001
Baseline.OT	1		158.23	158.23	15.39	<.001
Baseline.T1	4		184.61	46.15	4.49	0.002
Baseline.T2	1		134.12	134.12	13.04	<.001
Baseline.OT.T1	2		13.29	6.64	0.65	0.526
Baseline.OT.T2	1		1.45	1.45	0.14	0.708
Baseline.T1.T2	2		2.51	1.25	0.12	0.885
Baseline.OT.T1.T2	2		8.81	4.41	0.43	0.652
Residual	118	(2)	1213.25	10.28		
Total	132	(2)	1907.08			

A. 4. Hue angle (hue) of Chilean avocados (Exp 3.1).

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		4348.1	4348.1	36.32	<.001
Baseline.OT	1		3837.6	3837.6	32.06	<.001
Baseline.T1	4		11517.4	2879.3	24.05	<.001
Baseline.T2	1		1855.5	1855.5	15.50	<.001
Baseline.OT.T1	2		2126.2	1063.1	8.88	<.001
Baseline.OT.T2	1		12.2	12.2	0.10	0.750
Baseline.T1.T2	2		350.8	175.4	1.47	0.235
Baseline.OT.T1.T2	2		364.6	182.3	1.52	0.222
Residual	119	(1)	14246.1	119.7		
Total	133	(1)	38643.5			

A. 5a. Firmness (Exp 3.1) with baseline.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Baseline	1	83001.	83001.	56.90	<.001
Baseline.T1	4	234958.	58740.	40.27	<.001
Baseline.T2	1	28784.	28784.	19.73	<.001
Baseline.OT	1	52326.	52326.	35.87	<.001
Baseline.T1.T2	2	2925.	1463.	1.00	0.370
Baseline.T1.OT	2	36833.	18417.	12.62	<.001
Baseline.T2.OT	1	1100.	1100.	0.75	0.387
Baseline.T1.T2.OT	2	19702.	9851.	6.75	0.002
Residual	120	175049.	1459.		
Total	134	634679.			

A.5b. Firmness (Exp 3.1) with shelf life

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
OT	1		17836.	17836.	15.63	<.001
SL	1		1410345.	1410345.	1236.00	<.001
T1	2		144414.	72207.	63.28	<.001
T2	1		16773.	16773.	14.70	<.001
OT.SL	1		36080.	36080.	31.62	<.001
OT.T1	2		27370.	13685.	11.99	<.001
SL.T1	2		91034.	45517.	39.89	<.001
OT.T2	1		911.	911.	0.80	0.373
SL.T2	1		12193.	12193.	10.69	0.001
T1.T2	2		941.	471.	0.41	0.663
OT.SL.T1	2		12428.	6214.	5.45	0.005
OT.SL.T2	1		280.	280.	0.25	0.621
OT.T1.T2	2		10346.	5173.	4.53	0.012
SL.T1.T2	2		2263.	1132.	0.99	0.373
OT.SL.T1.T2	2		9402.	4701.	4.12	0.018
Residual	187	(5)	213378.	1141.		
Total	210	(5)	1973173.			

A. 6. Respiration rate (Exp 3.1)

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
OT	1		59797.	59797.	52.93	<.001
SL	1		282240.	282240.	249.81	<.001
T1	2		3512.	1756.	1.55	0.222
T2	1		9817.	9817.	8.69	0.005
OT.SL	1		1385.	1385.	1.23	0.274
OT.T1	2		3638.	1819.	1.61	0.211
SL.T1	2		53926.	26963.	23.86	<.001
OT.T2	1		723.	723.	0.64	0.428
SL.T2	1		822.	822.	0.73	0.398
T1.T2	2		862.	431.	0.38	0.685
OT.SL.T1	2		6245.	3122.	2.76	0.074
OT.SL.T2	1		5022.	5022.	4.44	0.040
OT.T1.T2	2		3527.	1763.	1.56	0.221
SL.T1.T2	2		2162.	1081.	0.96	0.392
OT.SL.T1.T2	2		1876.	938.	0.83	0.442
Residual	46	(2)	51972.	1130.		
Total	69	(2)	472161.			

A. 7. Ethylene production rate (Exp 3.1).

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
OT	1		2038.6	2038.6	3.89	0.055
SL	1		31460.8	31460.8	59.99	<.001
T1	2		3337.2	1668.6	3.18	0.051
T2	1		605.9	605.9	1.16	0.288
OT.SL	1		5781.7	5781.7	11.02	0.002
OT.T1	2		627.7	313.9	0.60	0.554
SL.T1	2		5165.7	2582.8	4.92	0.012
OT.T2	1		188.7	188.7	0.36	0.552
SL.T2	1		128.8	128.8	0.25	0.623
T1.T2	2		1811.8	905.9	1.73	0.189
OT.SL.T1	2		581.7	290.8	0.55	0.578
OT.SL.T2	1		73.0	73.0	0.14	0.711
OT.T1.T2	2		813.0	406.5	0.78	0.467
SL.T1.T2	2		1795.9	898.0	1.71	0.192
OT.SL.T1.T2	2		725.8	362.9	0.69	0.506
Residual	45	(3)	23600.4	524.5		
Total	68	(3)	77268.9			

A. 8. D-Mannoheptulose per g^{-1} DW (Exp 3.1).

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		18308.5	18308.5	85.80	<.001
Baseline.Outturn	1		1672.0	1672.0	7.84	0.007
Baseline.Shelflife	2		11122.9	5561.5	26.06	<.001
Baseline.TR1	4		1485.5	371.4	1.74	0.155
Baseline.TR2	1		83.7	83.7	0.39	0.534
Baseline.Outturn.Shelflife	1		182.8	182.8	0.86	0.359
Baseline.Outturn.TR1	2		60.6	30.3	0.14	0.868
Baseline.Shelflife.TR1	4		327.4	81.8	0.38	0.819
Baseline.Outturn.TR2	1		0.0	0.0	0.00	1.000
Baseline.Shelflife.TR2	1		30.4	30.4	0.14	0.707
Baseline.TR1.TR2	2		90.9	45.4	0.21	0.809
Baseline.Outturn.Shelflife.TR1	2		431.4	215.7	1.01	0.371
Baseline.Outturn.Shelflife.TR2	1		309.1	309.1	1.45	0.234
Baseline.Outturn.TR1.TR2	2		136.6	68.3	0.32	0.728
Baseline.Outturn.TR1.TR2	2		136.6	68.3	0.32	0.728
Baseline.Shelflife.TR1.TR2	2		286.8	143.4	0.67	0.515
Baseline.Outturn.Shelflife.TR1.TR2	2		275.2	137.6	0.64	0.529
Residual	53	(7)	11309.5	213.4		
Total	82	(7)	41544.0			

A. 9. Perseitol per g^{-1} DW (Exp 3.1).

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		19735.5	19735.5	96.62	<.001
Baseline.Outturn	1		832.0	832.0	4.07	0.048
Baseline.Shelflife	2		4757.4	2378.7	11.65	<.001
Baseline.TR1	4		819.7	204.9	1.00	0.413
Baseline.TR2	1		1011.9	1011.9	4.95	0.030
Baseline.Outturn.Shelflife	1		58.5	58.5	0.29	0.594
Baseline.Outturn.TR1	2		783.6	391.8	1.92	0.156
Baseline.Shelflife.TR1	4		369.8	92.5	0.45	0.770
Baseline.Outturn.TR2	1		206.0	206.0	1.01	0.320
Baseline.Shelflife.TR2	1		182.5	182.5	0.89	0.348
Baseline.TR1.TR2	2		544.8	272.4	1.33	0.272
Baseline.Outturn.Shelflife.TR1	2		891.1	445.5	2.18	0.122
Baseline.Outturn.Shelflife.TR2	1		658.2	658.2	3.22	0.078
Baseline.Outturn.TR1.TR2	2		102.6	51.3	0.25	0.779
Baseline.Shelflife.TR1.TR2	2		218.3	109.1	0.53	0.589
Baseline.Outturn.Shelflife.TR1.TR2	2		70.1	35.1	0.17	0.843
Residual	57	(3)	11642.9	204.3		
Total	86	(3)	42446.8			

A. 10. Sucrose per g^{-1} DW (Exp 3.1).

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		2999.05	2999.05	71.36	<.001
Baseline.Outturn	1		231.74	231.74	5.51	0.022
Baseline.Shelflife	2		31.79	15.90	0.38	0.687
Baseline.TR1	4		239.58	59.89	1.43	0.238
Baseline.TR2	1		0.94	0.94	0.02	0.882
Baseline.Outturn.Shelflife	1		150.72	150.72	3.59	0.063
Baseline.Outturn.TR1	2		48.35	24.18	0.58	0.566
Baseline.Shelflife.TR1	4		417.30	104.33	2.48	0.054
Baseline.Outturn.TR2	1		50.22	50.22	1.20	0.279
Baseline.Shelflife.TR2	1		62.24	62.24	1.48	0.229
Baseline.TR1.TR2	2		107.02	53.51	1.27	0.288
Baseline.Outturn.Shelflife.TR1	2		304.64	152.32	3.62	0.033
Baseline.Outturn.Shelflife.TR2	1		5.27	5.27	0.13	0.725
Baseline.Outturn.TR1.TR2	2		63.26	31.63	0.75	0.476
Baseline.Shelflife.TR1.TR2	2		19.12	9.56	0.23	0.797
Baseline.Outturn.Shelflife.TR1.TR2	2		65.36	32.68	0.78	0.464
Residual	56	(4)	2353.40	42.03		
Total	85	(4)	6804.81			

Table A. 11-A28. Effect of treatment on the firmness, L*, C*, H^o, Respiration and Ethylene (Exp 3.2 on Chapter3).

A. 11. Firmness

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		316089.7	316089.7	435.94	<.001
Baseline.Outturn	1		79438.8	79438.8	109.56	<.001
Baseline.Shelflife	2		595956.8	297978.4	410.96	<.001
Baseline.T1	2		28980.2	14490.1	19.98	<.001
Baseline.T2	1		3203.0	3203.0	4.42	0.037
Baseline.Outturn.Shelflife	1		112777.3	112777.3	155.54	<.001
Baseline.Outturn.T1	1		423.1	423.1	0.58	0.446
Baseline.Shelflife.T1	2		10456.4	5228.2	7.21	<.001
Baseline.Outturn.T2	1		547.7	547.7	0.76	0.386
Baseline.Shelflife.T2	1		2068.7	2068.7	2.85	0.093
Baseline.T1.T2	1		135.9	135.9	0.19	0.666
Baseline.Outturn.Shelflife.T1	1		380.0	380.0	0.52	0.470
Baseline.Outturn.Shelflife.T2	1		229.5	229.5	0.32	0.574
Baseline.Outturn.T1.T2	1		390.5	390.5	0.54	0.464
Baseline.Shelflife.T1.T2	1		640.7	640.7	0.88	0.348
Baseline.Outturn.Shelflife.T1.T2	1		867.3	867.3	1.20	0.275
Residual	213	(7)	154440.2	725.1		
Total	232	(7)	1284617.2			

A. 12. H^o

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		1946.2	1946.2	15.69	<.001
Baseline.Outturn	1		11155.9	11155.9	89.95	<.001
Baseline.Shelflife	2		26669.7	13334.8	107.51	<.001
Baseline.T1	2		2662.5	1331.2	10.73	<.001
Baseline.T2	1		3639.0	3639.0	29.34	<.001
Baseline.Outturn.Shelflife	1		1568.2	1568.2	12.64	<.001
Baseline.Outturn.T1	1		717.6	717.6	5.79	0.017
Baseline.Shelflife.T1	2		969.0	484.5	3.91	0.022
Baseline.Outturn.T2	1		506.7	506.7	4.08	0.044
Baseline.Shelflife.T2	1		31.7	31.7	0.26	0.614
Baseline.T1.T2	1		201.1	201.1	1.62	0.204
Baseline.Outturn.Shelflife.T1	1		233.0	233.0	1.88	0.172
Baseline.Outturn.Shelflife.T2	1		45.0	45.0	0.36	0.548
Baseline.Outturn.T1.T2	1		162.2	162.2	1.31	0.254
Baseline.Shelflife.T1.T2	1		104.2	104.2	0.84	0.360
Baseline.Outturn.Shelflife.T1.T2	1		4.5	4.5	0.04	0.848
Residual	219	(1)	27162.6	124.0		
Total	238	(1)	77532.1			

A.13. Lightness (L*)

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		96.427	96.427	21.44	<.001
Baseline.Outturn	1		196.219	196.219	43.63	<.001
Baseline.Shelflife	2		290.348	145.174	32.28	<.001
Baseline.T1	2		106.237	53.118	11.81	<.001
Baseline.T2	1		312.305	312.305	69.45	<.001
Baseline.Outturn.Shelflife	1		173.940	173.940	38.68	<.001
Baseline.Outturn.T1	1		6.080	6.080	1.35	0.246
Baseline.Shelflife.T1	2		39.131	19.566	4.35	0.014
Baseline.Outturn.T2	1		47.519	47.519	10.57	0.001
Baseline.Shelflife.T2	1		39.929	39.929	8.88	0.003
Baseline.T1.T2	1		2.684	2.684	0.60	0.441
Baseline.Outturn.Shelflife.T1	1		0.555	0.555	0.12	0.726
Baseline.Outturn.Shelflife.T2	1		8.000	8.000	1.78	0.184
Baseline.Outturn.T1.T2	1		0.818	0.818	0.18	0.670
Baseline.Shelflife.T1.T2	1		0.209	0.209	0.05	0.830
Baseline.Outturn.Shelflife.T1.T2	1		0.184	0.184	0.04	0.840
Residual	218	(2)	980.331	4.497		
Total	237	(2)	2291.614			

A.14. Chroma (C*)

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		494.68	494.68	49.36	<.001
Baseline.Outturn	1		2500.03	2500.03	249.45	<.001
Baseline.Shelflife	2		2351.71	1175.86	117.33	<.001
Baseline.T1	2		164.33	82.16	8.20	<.001
Baseline.T2	1		352.75	352.75	35.20	<.001
Baseline.Outturn.Shelflife	1		878.92	878.92	87.70	<.001
Baseline.Outturn.T1	1		75.44	75.44	7.53	0.007
Baseline.Shelflife.T1	2		160.24	80.12	7.99	<.001
Baseline.Outturn.T2	1		50.46	50.46	5.03	0.026
Baseline.Shelflife.T2	1		0.24	0.24	0.02	0.877
Baseline.T1.T2	1		5.14	5.14	0.51	0.475
Baseline.Outturn.Shelflife.T1	1		8.78	8.78	0.88	0.350
Baseline.Outturn.Shelflife.T2	1		24.11	24.11	2.41	0.122
Baseline.Outturn.T1.T2	1		0.23	0.23	0.02	0.880
Baseline.Shelflife.T1.T2	1		3.49	3.49	0.35	0.556
Baseline.Outturn.Shelflife.T1.T2	1		3.57	3.57	0.36	0.551
Residual	219	(1)	2194.86	10.02		
Total	238	(1)	9236.09			

A.15. Respiration rate

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Baseline	1		41109.6	41109.6	47.97	<.001
Baseline.OT	1		90028.8	90028.8	105.06	<.001
Baseline.SL	2		123192.0	61596.0	71.88	<.001
Baseline.T1	2		8383.7	4191.8	4.89	0.010
Baseline.T2	1		5706.8	5706.8	6.66	0.011
Baseline.OT.SL	1		75250.0	75250.0	87.82	<.001
Baseline.OT.T1	1		430.6	430.6	0.50	0.480
Baseline.SL.T1	2		3622.0	1811.0	2.11	0.126
Baseline.OT.T2	1		8825.3	8825.3	10.30	0.002
Baseline.SL.T2	1		45.8	45.8	0.05	0.818
Baseline.T1.T2	1		3340.1	3340.1	3.90	0.051
Baseline.OT.SL.T1	1		5316.4	5316.4	6.20	0.014
Baseline.OT.SL.T2	1		4731.8	4731.8	5.52	0.021
Baseline.OT.T1.T2	1		4365.9	4365.9	5.09	0.026
Baseline.SL.T1.T2	1		335.5	335.5	0.39	0.533
Baseline.OT.SL.T1.T2	1		159.8	159.8	0.19	0.667
Residual	95	(5)	81406.9	856.9		
Total	114	(5)	438441.1			

A.1.2 APPENDIX A: ANOVA tables for Chapter 4

Table A.29–A.39. Effect of treatment on the firmness, hue angle (H^o), fructose, glucose, sucrose, quercetin, cyanidin-3-glucoside, cyanidin-3-rutinoside, caffeic acid, antioxidant capacity (flesh), antioxidant capacity (skin).

A. 29. Firmness

Source of variation	d.f	s.s	m.s	v.r	F pr.
Length	1	0.52077	0.52077	12.50	0.001
Treatment	1	0.26520	0.26520	6.36	0.017
Length.Treatment	1	0.00078	0.00078	0.02	0.892
Residual	32	1.33370	0.04168		
Total	35	2.12045			

A. 30. Hue (H^o)

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		114.14	114.14	5.24	0.029
Treatment	1		37.33	37.33	1.71	0.201
Length.Treatment	1		6.48	6.48	0.30	0.590
Residual	30	(2)	653.74	21.79		
Total	33	(2)	778.29			

A.31. Fructose

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		532.	532.	0.40	0.531
Treatment	1		974.	974.	0.74	0.397
Length.Treatment	1		326.	326.	0.25	0.623
Residual	28	(4)	36942.	1319.		
Total	31	(4)	38514.			

A.32. Glucose

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		1764.	1764.	1.51	0.229
Treatment	1		2250.	2250.	1.93	0.176
Length.Treatment	1		983.	983.	0.84	0.367
Residual	28	(4)	32727.	1169.		
Total	31	(4)	36941.			

A.33. Sucrose

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		391.	391.	0.12	0.731
Treatment	1		2083.	2083.	0.64	0.429
Length.Treatment	1		1357.	1357.	0.42	0.523
Residual	30	(2)	97405.	3247.		
Total	33	(2)	101019.			

A.34. Quercetin

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		0.0020	0.0020	0.02	0.893
Treatment	1		0.0031	0.0031	0.03	0.868
Length.Treatment	1		0.0000	0.0000	0.00	0.999
Residual	29	(3)	3.1452	0.1085		
Total	32	(3)	3.1500			

A.35. Cyanidin-3-glucoside

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		0.8258	0.8258	4.16	0.051
Treatment	1		0.0221	0.0221	0.11	0.741
Length.Treatment	1		1.5215	1.5215	7.66	0.010
Residual	29	(3)	5.7582	0.1986		
Total	32	(3)	7.4813			

A.36. Cyanidin-3-rutinoside

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		0.02633	0.02633	0.90	0.351
Treatment	1		0.00917	0.00917	0.31	0.580
Length.Treatment	1		0.00336	0.00336	0.11	0.737
Residual	30	(2)	0.87938	0.02931		
Total	33	(2)	0.91711			

A.37. Caffeic acid

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		0.00126	0.00126	0.06	0.816
Treatment	1		0.01318	0.01318	0.58	0.452
Length.Treatment	1		0.13620	0.13620	6.00	0.021
Residual	29	(3)	0.65871	0.02271		
Total	32	(3)	0.80247			

A.38.Antioxidant capacity (flesh)

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		3.1	3.1	0.00	0.955
Treatment	1		2584.5	2584.5	2.65	0.115
Length.Treatment	1		5.4	5.4	0.01	0.941
Residual	27	(5)	26293.2	973.8		
Total	30	(5)	28550.9			

A.39. Antioxidant capacity (skin)

Source of variation	d.f	m.v	s.s	m.s	v.r	F pr.
Length	1		19209.	19209.	4.08	0.052
Treatment	1		12595.	12595.	2.68	0.112
Length.Treatment	1		4235.	4235.	0.90	0.350
Residual	30	(2)	141202.	4707.		
Total	33	(2)	175906.			

A.1.3 APPENDIX A: ANOVA tables for Chapter 5

Table A. 40-A. 63. Effect of treatment on the colour (hue angle) ethylene, organic acids, sugars and anthocyanins of strawberries (cv. Elsanta and Jubilee). Outturn represents storage days 0, 2, 4 and 7 for Exp 5.1 and 0, 1, 2, 4 and 7 (at 5 ^{o}C) for Exp 5.2 and 5.3.

A.40. Hue angle (H^{o}) Exp 5.1

Source of variation	d.f	s.s	m.s	v.r	F pr.
Outturn	3	35.034	11.678	8.08	<.001
Treatment_1	1	0.366	0.366	0.25	0.619
Treatment_2	1	41.305	41.305	28.57	<.001
Outturn.Treatment_1	3	19.049	6.350	4.39	0.011
Outturn.Treatment_2	3	9.207	3.069	2.12	0.118
Treatment_1.Treatment_2	1	7.993	7.993	5.53	0.025
Outturn.Treatment_1.Treatment_2	3	16.999	5.666	3.92	0.018
Residual	30	43.371	1.446		
Total	47	175.774			

A.41. Ethylene (Exp 5.1)

Source of variation	d.f	s.s	m.s	v.r	F pr.
Outturn	3	500.2102	166.7367	224.49	<.001
Treatment_1	1	1.4086	1.4086	1.90	0.178
Treatment_2	1	93.9316	93.9316	126.47	<.001
Outturn.Treatment_1	3	227.9212	75.9737	102.29	<.001
Outturn.Treatment_2	3	12.9181	4.3060	5.80	0.003
Treatment_1.Treatment_2	1	8.6093	8.6093	11.59	0.002
Outturn.Treatment_1.Treatment_2	3	8.6894	2.8965	3.90	0.018
Residual	32	23.7678	0.7427	4.25	
Time	2	1.7915	0.8957	5.12	0.013
Time.Outturn	6	8.6077	1.4346	8.20	<.001
Time.Treatment_1	2	5.4731	2.7365	15.64	<.001
Time.Treatment_2	2	2.2873	1.1437	6.54	0.005
Time.Outturn.Treatment_1	6	7.0642	1.1774	6.73	<.001
Time.Outturn.Treatment_2	6	2.8642	0.4774	2.73	0.029
Time.Treatment_1.Treatment_2	2	3.3278	1.6639	9.51	<.001
Time.Outturn.Treatment_1.Treatment_2	6	4.8995	0.8166	4.67	0.001
Residual	64	11.1973	0.1750		
Total	143	924.9687			

A.42. Malic acid (Exp 5.1)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	3		85.482	28.494	6.92	0.001
Treatment_1	1		1.919	1.919	0.47	0.500
Treatment_2	1		5.959	5.959	1.45	0.238
Outturn.Treatment_1	3		19.008	6.336	1.54	0.224
Outturn.Treatment_2	3		32.678	10.893	2.64	0.067
Treatment_1.Treatment_2	1		0.032	0.032	0.01	0.930
Outturn.Treatment_1.Treatment_2	3		6.479	2.160	0.52	0.669
Residual	31	(1)	127.730	4.120		
Total	46	(1)	271.911			

A.43. Citric acid (Exp 5.1)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	3		11784.0	3928.0	17.12	<.001
Treatment_1	1		126.5	126.5	0.55	0.463
Treatment_2	1		0.7	0.7	0.00	0.955
Outturn.Treatment_1	3		682.0	227.3	0.99	0.410
Outturn.Treatment_2	3		438.9	146.3	0.64	0.597
Treatment_1.Treatment_2	1		1.3	1.3	0.01	0.940
Outturn.Treatment_1.Treatment_2	3		141.3	47.1	0.21	0.892
Residual	31	(1)	7114.7	229.5		
Total	46	(1)	19864.1			

A.44. Ascorbic acid (Exp 5.1)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	3		67.885	22.628	11.63	<.001
Treatment_1	1		1.764	1.764	0.91	0.348
Treatment_2	1		0.624	0.624	0.32	0.575
Outturn.Treatment_1	3		7.945	2.648	1.36	0.273
Outturn.Treatment_2	3		6.071	2.024	1.04	0.389
Treatment_1.Treatment_2	1		0.055	0.055	0.03	0.867
Outturn.Treatment_1.Treatment_2	3		2.269	0.756	0.39	0.762
Residual	31	(1)	60.316	1.946		
Total	46	(1)	144.705			

A.45. Oxalic acid (Exp 5.1)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	3		243.637	81.212	9.30	<.001
Treatment_1	1		14.341	14.341	1.64	0.209
Treatment_2	1		31.423	31.423	3.60	0.067
Outturn.Treatment_1	3		19.094	6.365	0.73	0.542
Outturn.Treatment_2	3		58.479	19.493	2.23	0.104
Treatment_1.Treatment_2	1		13.223	13.223	1.51	0.228
Outturn.Treatment_1.Treatment_2	3		32.935	10.978	1.26	0.306
Residual	31	(1)	270.606	8.729		
Total	46	(1)	681.323			

A.48. Antioxidant capacity (Trolox) (Exp 5.1)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	3		2251.7	750.6	0.77	0.520
Treatment_1	1		5182.5	5182.5	5.32	0.029
Treatment_2	1		1301.9	1301.9	1.34	0.258
Outturn.Treatment_1	3		11059.7	3686.6	3.79	0.022
Outturn.Treatment_2	3		830.0	276.7	0.28	0.836
Treatment_1.Treatment_2	1		3.5	3.5	0.00	0.952
Outturn.Treatment_1.Treatment_2	3		288.5	96.2	0.10	0.960
Residual	27	(3)	26285.9	973.6		
Total	44	(3)	47526.5			

A.49. Fructose (Exp 5.1)

Source of variation	d.f	s.s	m.s	v.r	F pr.
Outturn	3	404.4	134.8	0.32	0.811
Treatment_1	1	2721.5	2721.5	6.47	0.016
Treatment_2	1	1113.5	1113.5	2.65	0.114
Outturn.Treatment_1	3	2830.3	943.4	2.24	0.104
Outturn.Treatment_2	3	29.2	9.7	0.02	0.995
Treatment_1.Treatment_2	1	1370.1	1370.1	3.26	0.081
Outturn.Treatment_1.Treatment_2	3	2343.9	781.3	1.86	0.158
Residual	30	12626.0	420.9		
Total	47	23527.9			

A.50. Glucose (Exp 5.1)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	3		2746.3	915.4	3.52	0.027
Treatment_1	1		1490.4	1490.4	5.73	0.023
Treatment_2	1		654.8	654.8	2.52	0.124
Outturn.Treatment_1	3		626.1	208.7	0.80	0.503
Outturn.Treatment_2	3		2414.7	804.9	3.09	0.042
Treatment_1.Treatment_2	1		12.1	12.1	0.05	0.831
Outturn.Treatment_1.Treatment_2	3		650.6	216.9	0.83	0.486
Residual	29	(1)	7546.7	260.2		
Total	46	(1)	16456.1			

A.51. Sucrose (Exp 5.1)

Source of variation	d.f	s.s	m.s	v.r	F pr.
Outturn	3	16815.	5605.	5.42	0.004
Treatment_1	1	2785.	2785.	2.69	0.111
Treatment_2	1	4320.	4320.	4.18	0.050
Outturn.Treatment_1	3	9735.	3245.	3.14	0.040
Outturn.Treatment_2	3	5467.	1822.	1.76	0.176
Treatment_1.Treatment_2	1	1448.	1448.	1.40	0.246
Outturn.Treatment_1.Treatment_2	3	2212.	737.	0.71	0.552
Residual	30	31018.	1034.		
Total	47	74643.			

A.52. Ethylene (Exp 5.2)

Source of variation	d.f	s.s	m.s	v.r	F pr.
Outturn	4	3940.923	985.231	23.56	<.001
Treatment	1	2625.917	2625.917	62.79	<.001
Outturn.Treatment	4	961.423	240.356	5.75	0.003
Residual	20	836.398	41.820	17.83	
Time	10	722.479	72.248	30.81	<.001
Time.Outturn	40	1551.671	38.792	16.54	<.001
Time.Treatment	10	98.840	9.884	4.21	0.043
Time.Outturn.Treatment	40	479.381	11.985	5.11	0.002
Residual	200	468.997	2.345		
Total	329	11686.029			

A.53. Hue angle (H^o) Exp 5.2

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	4		80.989	20.247	3.57	0.009
Treatment	1		25.755	25.755	4.54	0.035
Outturn.Treatment	4		5.676	1.419	0.25	0.909
Residual	106	(2)	600.857	5.668	3.57	
Total	117	(2)	755.619			

A.54. Ethylene (Exp 5.3)

Source of variation	d.f	s.s	m.s	v.r	F pr.
Outturn	4	54322.38	13580.59	90.92	<.001
Treatment	1	14447.94	14447.94	96.72	<.001
Outturn.Treatment	4	20974.18	5243.54	35.10	<.001
Residual	20	2987.44	149.37	7.77	
Time	11	6171.47	561.04	29.18	<.001
Time.Outturn	44	6074.94	138.07	7.18	<.001
Time.Treatment	11	2248.02	204.37	10.63	0.001
Time.Outturn.Treatment	44	2953.62	67.13	3.49	0.011
Residual	220	4230.49	19.23		
Total	359	114410.48			

A.55. Hue angle (H^o) Exp 5.3.

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	4		72.530	18.132	3.47	0.010
Treatment	1		24.917	24.917	4.77	0.031
Outturn.Treatment	4		44.006	11.002	2.11	0.085
Residual	106	(2)	553.506	5.222		
Total	117	(2)	706.051			

A.56. Ascorbic acid (Exp 5.3)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	4		6.091	1.523	1.21	0.344
Treatment	1		1.558	1.558	1.23	0.282
Outturn.Treatment	4		4.559	1.140	0.90	0.484
Residual	17	(1)	21.467	1.263		
Total	28	(1)	31.588			

A.57. Citric acid (Exp 5.3)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	4		8121.1	2030.3	4.57	0.011
Treatment	1		60.2	60.2	0.14	0.717
Outturn.Treatment	4		1506.4	376.6	0.85	0.514
Residual	17	(1)	7552.5	444.3		
Total	28	(1)	18783.3			

A.58. Fructose (Exp 5.3)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	4		4898.	1224.	0.56	0.697
Treatment	1		98.	98.	0.04	0.835
Outturn.Treatment	4		3458.	864.	0.39	0.811
Residual	17	(1)	37409.	2201.		
Total	28	(1)	48146.			

A.59. Glucose (Exp 5.3)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	4		12715.	3179.	2.32	0.101
Treatment	1		978.	978.	0.71	0.410
Outturn.Treatment	4		9815.	2454.	1.79	0.179
Residual	16	(2)	21887.	1368.		
Total	27	(2)	39274.			

A.60. Sucrose (Exp 5.3)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	4		8935.	2234.	0.86	0.507
Treatment	1		16576.	16576.	6.42	0.023
Outturn.Treatment	4		11706.	2927.	1.13	0.378
Residual	15	(3)	38747.	2583.		
Total	26	(3)	71615.			

A.61. Pelargonidin_Derivative (Exp 5.3)

Source of variation	d.f	s.s	m.s	v.r	F pr.
Outturn	4	0.014737	0.003684	0.63	0.647
Treatment	1	0.001680	0.001680	0.29	0.598
Outturn.Treatment	4	0.005654	0.001413	0.24	0.911
Residual	18	0.105226	0.005846		
Total	29	0.135130			

A.62. Pelargonidin_Glucoside (Exp 5.3)

Source of variation	d.f	s.s	m.s	v.r	F pr.
Outturn	4	0.7813	0.1953	0.30	0.876
Treatment	1	1.0010	1.0010	1.52	0.233
Outturn.Treatment	4	1.8551	0.4638	0.71	0.599
Residual	18	11.8356	0.6575		
Total	29	15.7559			

A.63. Oxalic acid (Exp 5.3)

Source of variation	d.f		s.s	m.s	v.r	F pr.
Outturn	4		131.20	32.80	1.62	0.214
Treatment	1		7.91	7.91	0.39	0.540
Outturn.Treatment	4		34.82	8.70	0.43	0.785
Residual	17	(1)	343.75	20.22		
Total	28	(1)	555.52			

A.1.4 APPENDIX A: ANOVA tables for Chapter 6

Table A. 64-85. Effect of treatment on the physiology (respiration, colour (H^o), ethylene, disease incidence, water loss) and biochemistry (sugars organic acids, phenolics and phytohormones) of strawberries (cv. Sonata). Outturn represents storage days 0, 1, 2, 4, 6 and 10 (at 5 ^oC).

A.64. Respiration rate

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		16.4	16.4	0.03	0.865
Baseline.Storage_days	4		4995.9	1249.0	2.23	0.082
Baseline.Treatment	3		27121.4	9040.5	16.16	<.001
Baseline.Storage_days.Treatment	12		12031.4	1002.6	1.79	0.082
Residual	41	(1)	22935.5	559.4		
Total	61	(1)	66575.3			

A.65. Ethylene production

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		37381.	37381.	14.80	<.001
Baseline.storage_days	4		171537.	42884.	16.98	<.001
Baseline.treatment	3		1730.	577.	0.23	0.876
Baseline.storage_days.treatment	12		66738.	5562.	2.20	0.030
Residual	41	(1)	103537.	2525.		
Total	61	(1)	364081.			

A.66. Hue angle (H^o)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		13.766	13.766	1.65	0.200
Baseline.Storage_days	4		244.303	61.076	7.31	<.001
Baseline.Treatment	3		140.509	46.836	5.61	<.001
Baseline.Storage_days.Treatment	12		105.670	8.806	1.05	0.400
Residual	229	(1)	1912.021	8.349		
Total	249	(1)	2413.187			

A.67. % Water loss

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		12.8101	12.8101	17.63	<.001
Baseline.storage_days	4		47.3961	11.8490	16.30	<.001
Baseline.treatment	3		9.5668	3.1889	4.39	0.009
Baseline.storage_days.treatment	12		27.0446	2.2537	3.10	0.003
Residual	41	(1)	29.7974	0.7268		
Total	61	(1)	118.1812			

A.68. Sucrose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		15722.7	15722.7	29.93	<.001
Baseline.Outturn	4		141021.6	35255.4	67.12	<.001
Baseline.Treatment	3		10124.8	3374.9	6.42	0.001
Baseline.Outturn.Treatment	12		18649.8	1554.2	2.96	0.005
Residual	41	(1)	21536.6	525.3		
Total	61	(1)	201983.6			

A.69. Fructose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		3581.0	3581.0	16.16	<.001
Baseline.Outturn	4		38541.7	9635.4	43.48	<.001
Baseline.Treatment	3		2895.0	965.0	4.35	0.009
Baseline.Outturn.Treatment	12		4701.7	391.8	1.77	0.087
Residual	41	(1)	9085.8	221.6		
Total	61	(1)	58265.0			

A.70. Glucose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		1953.2	1953.2	11.42	0.002
Baseline.Outturn	4		20286.7	5071.7	29.66	<.001
Baseline.Treatment	3		2067.7	689.2	4.03	0.013
Baseline.Outturn.Treatment	12		3767.1	313.9	1.84	0.074
Residual	41	(1)	7011.6	171.0		
Total	61	(1)	34764.5			

A.71. Ascorbic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		3.4399	3.4399	25.69	<.001
Baseline.Outturn	4		25.5565	6.3891	47.71	<.001
Baseline.Treatment	3		1.1438	0.3813	2.85	0.050
Baseline.Outturn.Treatment	12		3.8115	0.3176	2.37	0.021
Residual	38	(4)	5.0891	0.1339		
Total	58	(4)	38.4201			

A.72. Citric acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		21.83	21.83	0.55	0.463
Baseline.Outturn	4		8489.99	2122.50	53.52	<.001
Baseline.Treatment	3		228.84	76.28	1.92	0.145
Baseline.Outturn.Treatment	12		1357.44	113.12	2.85	0.008
Residual	33	(9)	1308.79	39.66		
Total	53	(9)	10258.82			

A.73. Malic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		391.789	391.789	74.28	<.001
Baseline.Outturn	4		2876.522	719.130	136.35	<.001
Baseline.Treatment	3		85.710	28.570	5.42	0.003
Baseline.Outturn.Treatment	12		198.263	16.522	3.13	0.003
Residual	41	(1)	216.246	5.274		
Total	61	(1)	3760.878			

A.74. Oxalic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		3.611	3.611	3.01	0.091
Baseline.Outturn	4		110.878	27.719	23.09	<.001
Baseline.Treatment	3		10.458	3.486	2.90	0.048
Baseline.Outturn.Treatment	12		29.139	2.428	2.02	0.051
Residual	36	(6)	43.225	1.201		
Total	56	(6)	185.513			

A.75. Cyanidin-3-glucoside

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	123.2	123.2	0.51	0.479
Baseline.Outturn	4	317.2	79.3	0.33	0.857
Baseline.Treatment	3	292.3	97.4	0.40	0.751
Baseline.Outturn.Treatment	12	1884.6	157.0	0.65	0.786
Residual	42	10133.1	241.3		
Total	62	12750.3			

A.76. Pelargonidin derivative

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		184.	184.	0.07	0.800
Baseline.Outturn	4		24169.	6042.	2.14	0.093
Baseline.Treatment	3		11100.	3700.	1.31	0.283
Baseline.Outturn.Treatment	12		29005.	2417.	0.86	0.594
Residual	41	(1)	115613.	2820.		
Total	61	(1)	180012.			

A.77. Pelargonidin-3-glucoside

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		52418.	52418.	1.96	0.169
Baseline.Outturn	4		653096.	163274.	6.11	<.001
Baseline.Treatment	3		26412.	8804.	0.33	0.804
Baseline.Outturn.Treatment	12		321521.	26793.	1.00	0.464
Residual	41	(1)	1096278.	26738.		
Total	61	(1)	2132816.			

A.78. Catechin

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		30062.	30062.	2.48	0.123
Baseline.Outturn	4		162879.	40720.	3.36	0.018
Baseline.Treatment	3		38924.	12975.	1.07	0.372
Baseline.Outturn.Treatment	12		245856.	20488.	1.69	0.105
Residual	41	(1)	496852.	12118.		
Total	61	(1)	964875.			

A.79. Epicatechin

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		650322.	650322.	28.53	<.001
Baseline.Outturn	4		3205045.	801261.	35.15	<.001
Baseline.Treatment	3		574630.	191543.	8.40	<.001
Baseline.Outturn.Treatment	12		497956.	41496.	1.82	0.077
Residual	41	(1)	934489.	22792.		
Total	61	(1)	5652661.			

A.80. Chlorogenic acid

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		8403.	8403.	3.54	0.067
Baseline.Outturn	4		29316.	7329.	3.09	0.026
Baseline.Treatment	3		29034.	9678.	4.08	0.013
Baseline.Outturn.Treatment	12		8681.	723.	0.30	0.985
Residual	41	(1)	97325.	2374.		
Total	61	(1)	170049.			

A.81. Ellagic acid

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		163.9	163.9	1.57	0.217
Baseline.Outturn	4		3298.3	824.6	7.91	<.001
Baseline.Treatment	3		570.5	190.2	1.83	0.158
Baseline.Outturn.Treatment	12		1314.9	109.6	1.05	0.423
Residual	41	(1)	4271.9	104.2		
Total	61	(1)	9566.7			

A.82. Quercetin-3-glucoside

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		1974.	1974.	0.17	0.687
Baseline.Outturn	4		32267.	8067.	0.68	0.613
Baseline.Treatment	3		17835.	5945.	0.50	0.686
Baseline.Outturn.Treatment	12		35543.	2962.	0.25	0.994
Residual	41	(1)	489966.	11950.		
Total	61	(1)	576948.			

A.83. Abscisic acid (ABA)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	190928.	190928.	41.40	<.001
Baseline.outturn	4	826052.	206513.	44.78	<.001
Baseline.treatment	3	199397.	66466.	14.41	<.001
Baseline.outturn.treatment	12	99680.	8307.	1.80	0.080
Residual	42	193680.	4611.		
Total	62	1509737.			

A.84. 7OH-ABA

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		167020.	167020.	6.72	0.013
Baseline.outturn	4		483884.	120971.	4.87	0.003
Baseline.treatment	3		149323.	49774.	2.00	0.129
Baseline.outturn.treatment	12		325325.	27110.	1.09	0.393
Residual	41	(1)	1019106.	24856.		
Total	61	(1)	2131405.			

A.85. ABA-GE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	3317829.	3317829.	3.59	0.065
Baseline.outturn	4	95283412.	23820853.	25.75	<.001
Baseline.treatment	3	2286698.	762233.	0.82	0.488
Baseline.outturn.treatment	12	8838131.	736511.	0.80	0.652
Residual	42	38850983.	925023.		
Total	62	148577052.			

A.1.5 APPENDIX A: ANOVA tables for Chapter 7

Table A. 86-93. Effect of treatment on the phytohormones (ABA, ABA metabolites and auxins) content within different strawberries (cv. Sonata) tissues. Outturn represents storage days 0, 1, 2, 4, 6 and 10 (at 5 ^{o}C).

A.86. 7OH-ABA (Achenes)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		57664.	57664.	1.68	0.203
Baseline.outturn	4		4069287.	1017322.	29.72	<.001
Baseline.treatment	3		573506.	191169.	5.58	0.003
Baseline.outturn.treatment	12		355838.	29653.	0.87	0.586
Residual	35	(7)	1198057.	34230.		
Total	55	(7)	5584262.			

A.87. ABA-GE (Achenes)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		619962.	619962.	6.67	0.014
Baseline.outturn	4		13222705.	3305676.	35.55	<.001
Baseline.treatment	3		3229464.	1076488.	11.58	<.001
Baseline.outturn.treatment	12		2500588.	208382.	2.24	0.029
Residual	39	(3)	3626896.	92997.		
Total	59	(3)	20371797.			

A.88. ABA (Achenes)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		4379.6	4379.6	5.06	0.030
Baseline.outturn	4		57693.8	14423.5	16.67	<.001
Baseline.treatment	3		16764.5	5588.2	6.46	0.001
Baseline.outturn.treatment	12		16040.7	1336.7	1.54	0.151
Residual	38	(4)	32881.6	865.3		
Total	58	(4)	115175.2			

A.89. IAAsp (Achenes)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		2529188.	2529188.	5.51	0.025
Baseline.outturn	4		17702729.	4425682.	9.65	<.001
Baseline.treatment	3		6225236.	2075079.	4.52	0.010
Baseline.outturn.treatment	12		4640138.	386678.	0.84	0.609
Residual	31	(11)	14223870.	458835.		
Total	51	(11)	39323951.			

A.90. IAA (Achenes)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		536068.	536068.	40.37	<.001
Baseline.outturn	4		480876.	120219.	9.05	<.001
Baseline.treatment	3		64404.	21468.	1.62	0.204
Baseline.outturn.treatment	12		78182.	6515.	0.49	0.906
Residual	34	(8)	451503.	13280.		
Total	54	(8)	1530432.			

A.91. 7OH-ABA (Flesh)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		42019.	42019.	2.72	0.107
Baseline.outturn	4		174368.	43592.	2.83	0.038
Baseline.treatment	3		128556.	42852.	2.78	0.054
Baseline.outturn.treatment	12		399733.	33311.	2.16	0.035
Residual	39	(3)	601437.	15421.		
Total	59	(3)	1302706.			

A.92. ABA-GE (Flesh)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		3220393.	3220393.	12.02	0.001
Baseline.outturn	4		33916906.	8479227.	31.66	<.001
Baseline.treatment	3		1878848.	626283.	2.34	0.088
Baseline.outturn.treatment	12		5649728.	470811.	1.76	0.090
Residual	40	(2)	10714184.	267855.		
Total	60	(2)	54016399.			

A.93. ABA (Flesh)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		161528.	161528.	49.93	<.001
Baseline.outturn	4		839874.	209968.	64.91	<.001
Baseline.treatment	3		249655.	83218.	25.73	<.001
Baseline.outturn.treatment	12		67293.	5608.	1.73	0.096
Residual	40	(2)	129394.	3235.		
Total	60	(2)	1360105.			

Table A. 94-96. The phytohormones (ABA and ABA metabolites) content within different strawberries (cv. Sonata) tissues. Outturn represents storage days 0, 1, 2, 4, 6 and 10 (at 5 ^{o}C).

A. 95. ABA (tissues)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		272942.	272942.	45.35	<.001
Baseline.outturn	4		1333409.	333352.	55.39	<.001
Baseline.tissue	4		15092698.	3773174.	626.98	<.001
Baseline.outturn.tissue	8		349235.	43654.	7.25	<.001
Residual	166	(5)	998983.	6018.		
Total	183	(5)	17339968.			

A.96. 7OH-ABA

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		241277.	241277.	8.12	0.005
Baseline.outturn	4		2936624.	734156.	24.70	<.001
Baseline.tissue	4		26896.	6724.	0.23	0.923
Baseline.outturn.tissue	8		1488362.	186045.	6.26	<.001
Residual	161	(10)	4786073.	29727.		
Total	178	(10)	9162558.			

A. 97. ABA-GE (tissues)

Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.
Baseline	1		6404580.	6404580.	13.91	<.001
Baseline.outturn	4		107686485.	26921621.	58.47	<.001
Baseline.tissue	4		8682494.	2170624.	4.71	0.001
Baseline.outturn.tissue	8		34373729.	4296716.	9.33	<.001
Residual	166	(5)	76430037.	460422.		
Total	183	(5)	231683302.			

Appendix B

B.1 Measuring ethylene production using laser-based ethylene detector

B.2 Introduction

It has been recognised that induced ethylene can trigger climacteric phase in fleshy fruits. Although climacteric fruits demonstrate some diversity in their ripening mechanism, the ripening in climacteric fruits is usually associated with colour change, change in sugar metabolism, softening of the flesh and changes in the texture and aroma (Barry and Giovannoni, 2007). Infected and wounded fruits produce more ethylene at higher rates, which will lead to accelerated ripening and senescence. Soft or decayed fruits will in turn affect softening of nearby fruits (Saltveit, 1999). Single softened or decayed kiwi fruit in a container can induce softening of surrounding fruits (Kader, 2002). This has been correlated with increased activity of cellulase (endo-β-1, 4-glucanase), which targets the hemi cellulosic matrix of the cell wall. Increased activity of the cellulase and gene transpiration for cellulase can be stimulated by ethylene treatment (Pesis *et al.*, 1978; Feng *et al.*, 2000). Studies have associated the stage of maturity with fruits susceptibility to wounds (Soliva-Fortuny and Martín-Belloso, 2003). Kiwi fruits are highly perishable commodity and soften rapidly during postharvest making them even more susceptible to physical injuries. Kiwi fruits that soften below 6 kilogram-force show physiological responses and elevated ethylene production, which continues to persist over 2 weeks after injury. For this reason kiwi fruits are harvested before firmness reaches below this level (Kader, 2002). In the fruit industry the control of ethylene in storage atmosphere is crucial in order to prevent the deleterious effects of ethylene. The detrimental effects of this hormone are temperature dependent, with sensitivity increasing with increasing temperature within 0-20 ^{o}C (Wills *et al.*, 2001).

Most studies investigating the effects of storage treatments on the level of endogenous and exogenous ethylene production have reported ethylene concentration using injection techniques such as gas chromatography (GC). Recent studies have reported the use of a recently developed laser based photoacoustic ethylene detector having high sensitivity and resolution time to measure on-line ethylene evolution (Cristescu *et al.*, 2002;

Iannetta *et al.*, 2006). This laser based photoacoustic ethylene detector enables the determination of ethylene produced in real time with nl h^{-1} detection limit. The main objective here was to demonstrate the advantage of this on-line measuring technique and hence preliminary experiments were conducted to demonstrate this. Aim was to investigate the effects wound on the ethylene production of kiwi fruits stored at different temperatures using laser based photoacoustic ethylene detector.

B.3 Materials and methods

Experiment here was conducted at Radboud University, Nijmegen Netherlands in collaboration with the Life Science gas facility.

B.3.1 Plant material

Kiwi fruits (n=12 cv. Hayward) originally from Chile packed in plastic punnets (n=2) were purchased from local supermarket in Nijmegen. Three experiments were conducted herein, whereby kiwis were stored and or wounded and effect of this on ethylene production was measured.

B.3.2 Experimental conditions

In the first of the three experiments (Exp A.1) kiwi fruits (n=5) of similar size and weight were selected and placed inside 1 l jars (n=1 fruit in each jar) with a gas tight lid and a rubber septum (Appendix C). To look for any relationship in the ethylene produced and state of the fruit it was decided to wound some fruits (n=3) by dropping them from shoulder level to the ground. Fruits were placed inside jars and stored at 4 ^{o}C and ethylene production was measured continuously. In the second experiment (Exp A.2) kiwi fruits of similar size and weight previously stored at 4 ^{o}C were later stored at 18 ^{o}C and ethylene production measured using the ethylene detector for a period of 24 h. Once again in this trial fruits were either wounded (n=3) by dropping fruits to the ground or left as control (*n*=2). In the third experiment (Exp A.3) kiwi fruits (*n*=5) stored at room temperature for 24 h were then wounded (bruised) (*n*=3), cut (*n*=2) or left as control (*n*=1) and ethylene concentration was determined for each fruit at 18 ^{o}C.

B.3.3 Laser-based ethylene detector

Ethylene evolution was monitored via a laser-based ethylene detector (ETD-300, incorporated with a gas handling system) in real-time (image shown in Appendix C). The experimental setup for the ethylene detector is demonstrated below (Figure B.1). There are two types of gas measurements which can be performed on this machine: *Stop and Flow and Continuous flow*. In these trials the continuous measurements were performed. The valve controller (VC) has 6 channels which are designed to measure from 6 containers alternately (containing biological sample) at any one time (jars shown on Appendix C). The gas flow into the detector is controlled with the use of three-way valves directing the gas of a selected jar into the photoacoustic detector (on position) and that of the remaining jars out into the laboratory (of position) (Figure B.2). The switching between jars gives rise to a delayed period before which a complete refill of gas is attained in the photoacoustic cell. The flow was set by the analyst, while the ethylene laser-based detector and the valve controller were operated automatically via sensor-sense software. Moreover, the machine is capable of taking measurements continuously up to a period of several weeks. The experimental setup also includes a platinum containing catalyser (platinum on Al_2O_3) operating at 400 ^oC minimum; the purpose of this is to eliminate potential interfering gases.

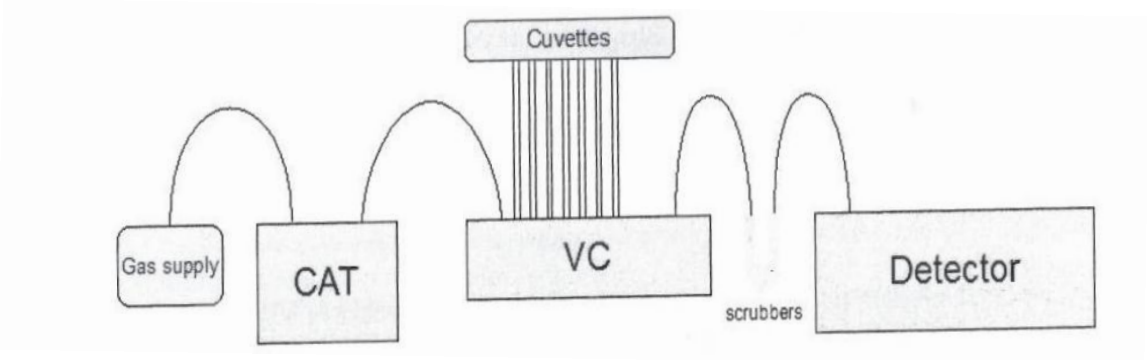


Figure B.1. Typical valve controller experiment setup.

To remove any CO_2 and water from the external atmosphere or that produced from the biological sample inside the jars a scrubber containing sodalime (granules) and $CaCl_2$ (granules) was placed before the detector hence gas from the VC passes through the scrubber to avoid any interferences. An empty jar was used as the reference, hence the

ethylene concentrations inside this jar was subtracted from the calculated ethylene measured for each sample.

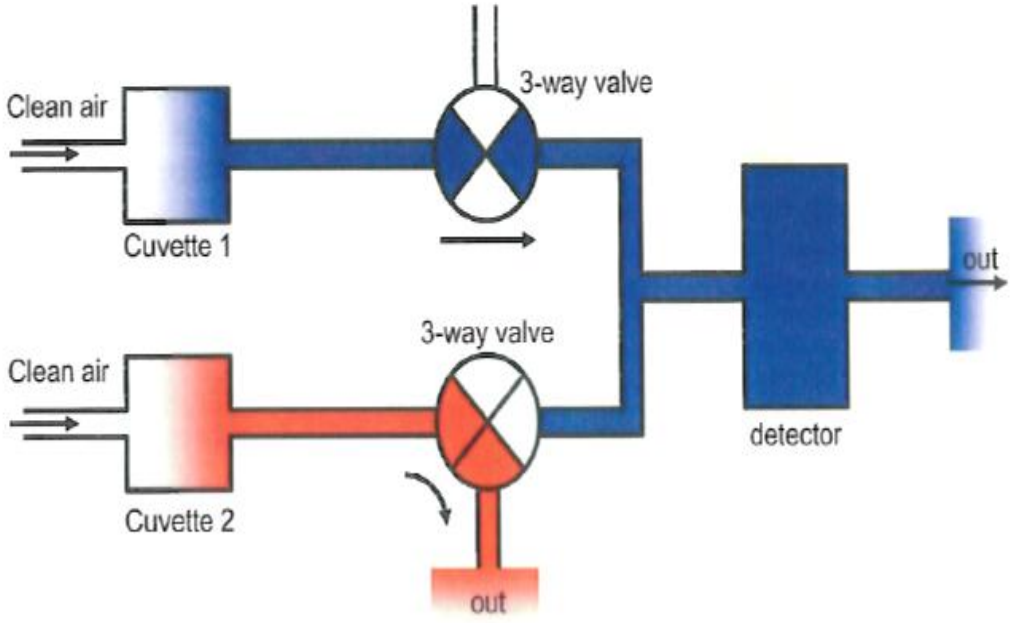


Figure B.2. Continuous flow setup

Here the gas from selected jar (blue) is directed to the detector. However for the jar which is not selected (i.e., jar 2 red) the gas flow is blocked from the detector and instead goes straight out of the VC box. Once the VC box has switched to a new jar the measured concentration will gradually reach the concentration of this jar and once this is reached the signal is stabilised. The stabilised period is important for determining the correct concentration of ethylene. Figure B.2 shows the period of transition from one jar to the next and the stable period whereby the ethylene will be averaged for each jar.

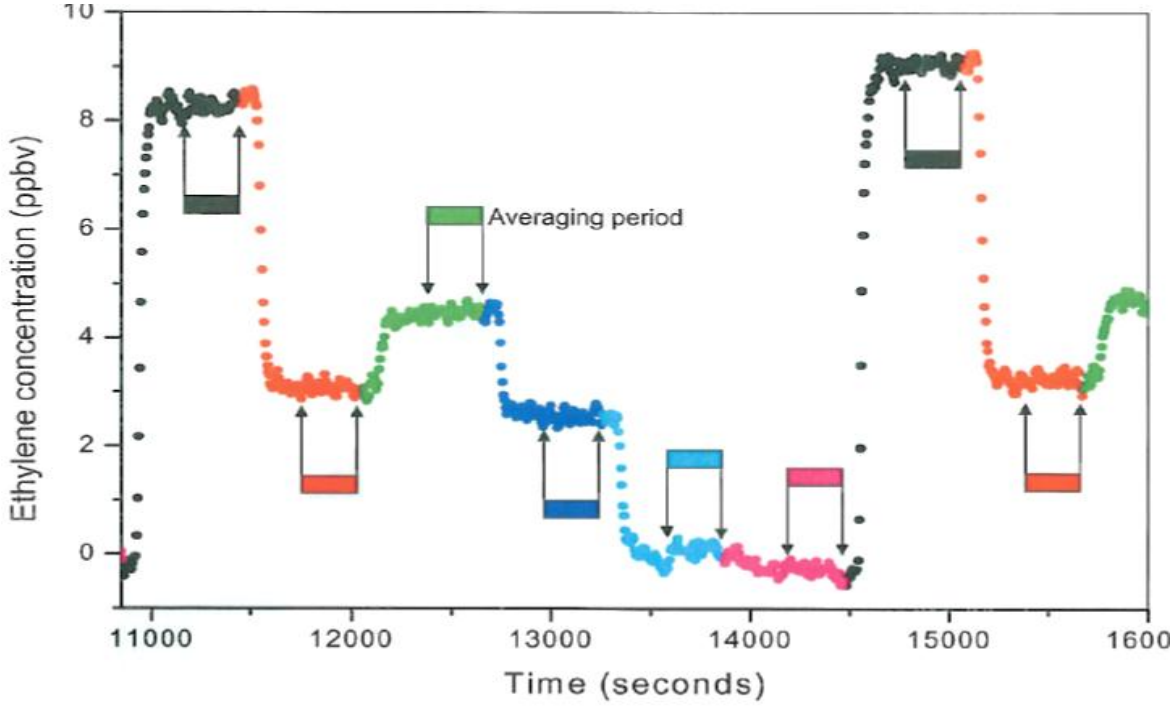


Figure B.3. Typical continuous flow measurements with the appropriate averaging period.

The time it takes until the concentration is stabilised will be selected (i.e., every 7 min) and the program will average the level within each jar for this period for each measurement. The point of this is to avoid selecting from the transition period and selecting from a period where results look consistent. The time it takes until the concentration is stabilised is dependent on the flow rate and the volume of the gas line (Figure B.3).

Wounded or control kiwi fruits were placed in 1 l volume jars, these jars were closed and continuously flashed with 1 l h^{-1} constant flow of air. In the trials conducted herein one jar was left empty in all cases. The ethylene production was measured in each experiment by the ETD-300 detector for 10 min for Exp B.1 and B.2 and 15 min in Exp B.3.

B.4 Results

B.4.1 Ethylene production of wounded kiwis (stored at 4 ^{o}C)

The ethylene production presented herein (Figure B.4) shows there was no relationship between ethylene production and wounded kiwis in this trial. However, measurements were conducted for each jar for a period of 10 min and at low temperature (4 ^{o}C) it is likely that the ethylene production took longer. That said, it is possible also that ethylene production from the kiwis at this temperature was lower hence it would have been desirable to have run this experiment overnight.

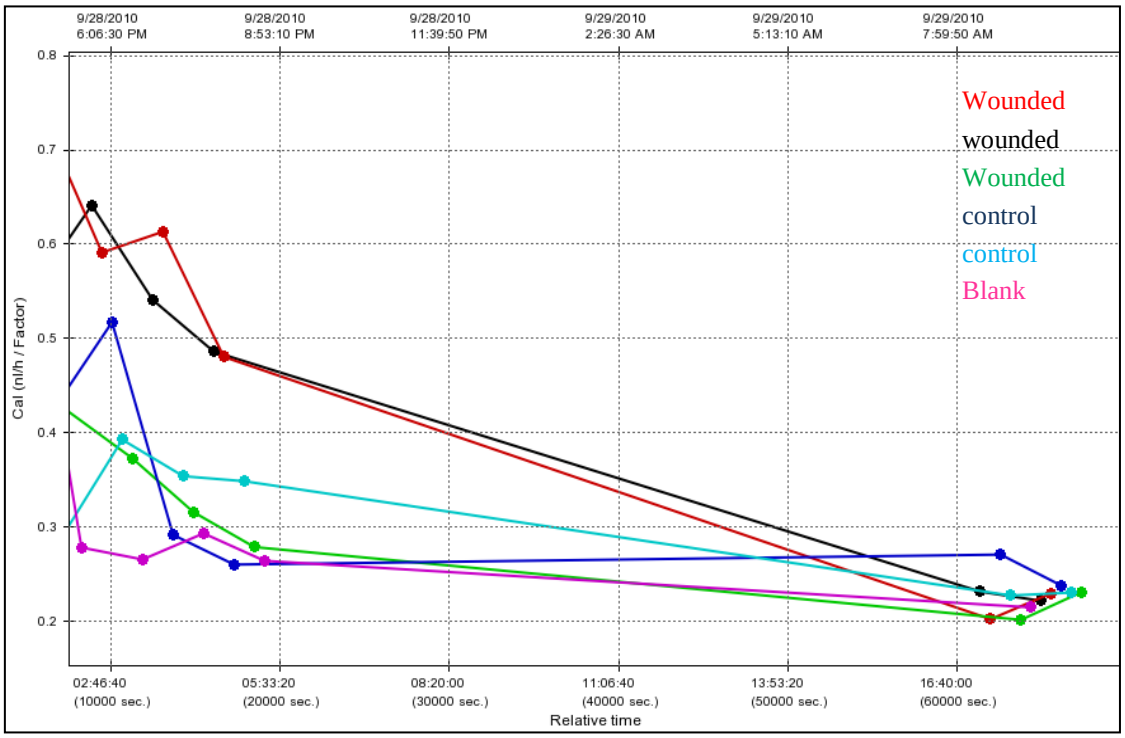


Figure B.4. Calculated ethylene (nl h^{-1}) for each jar containing wounded or control fruit (Exp B.1).

The results for Exp B.2 (Figure B.5) are when the ethylene concentration in the reference jar was subtracted from the calculated ethylene in the other jars, hence results demonstrated below (Figure B.5) are the final corrected ethylene values in nl h^{-1} for each fruit. Results here (Exp B.2, Figure B.6) indicate that wounded kiwis stored at 18 ^{o}C not only exhibited a rapid increase in ethylene production until a threshold was reached after which it decreased, but also it is evident that wounded kiwis produced higher ethylene throughout the 24 h period.

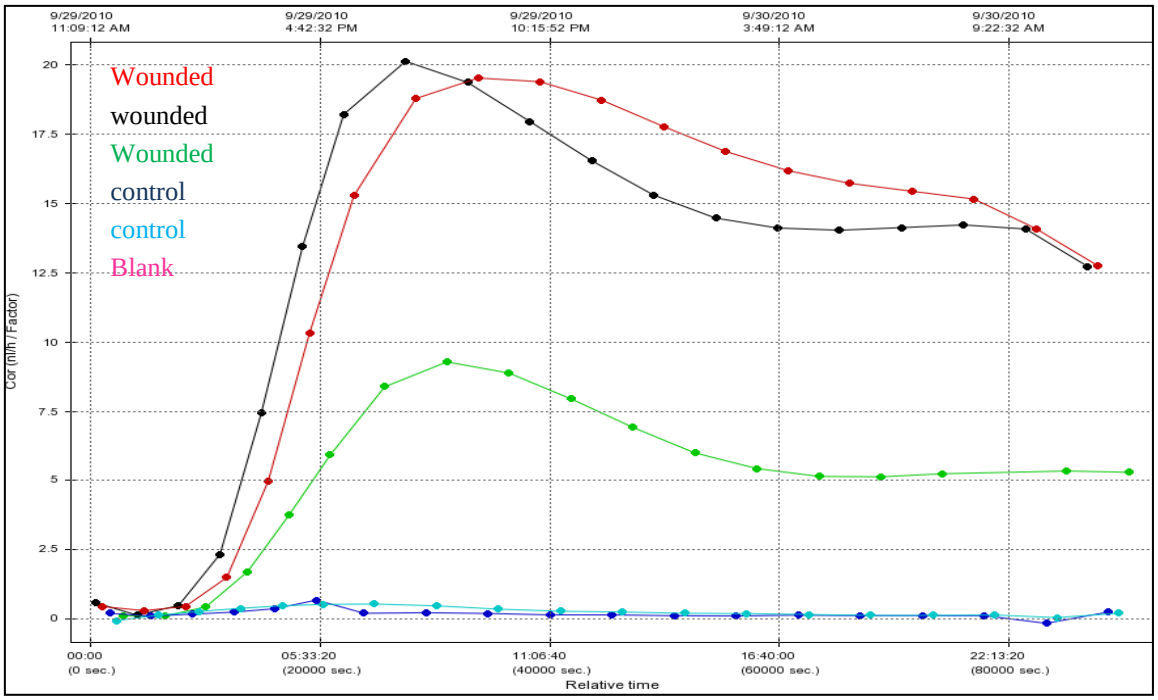


Figure B.5. Output for corrected ethylene concentration in Exp B.2 for wounded and control kiwis stored at 18 ^{o}C for 24 h.

In Exp B.3 kiwi fruits previously stored at room temperature for 24 h were subjected to different wounds (cut or bruised) and ethylene evolution determined (at 18 ^{o}C). Here results (Figure B.6) are similar as above (Exp B.2), whereby wounded fruits once again showed higher ethylene production rate over time in comparison to control. Concerning different wounds (cut and bruised fruits); higher ethylene concentration was observed during the start in both cases although towards the end, fruits which were cut rather than bruised demonstrated an increase in ethylene. In contrast bruised fruits after a threshold showed a decrease in ethylene production rate. On the other hand, ethylene in the control fruit remained very steady throughout the 24 h period. The corrected values where the ethylene in the reference jar is subtracted are shown in (graph B).

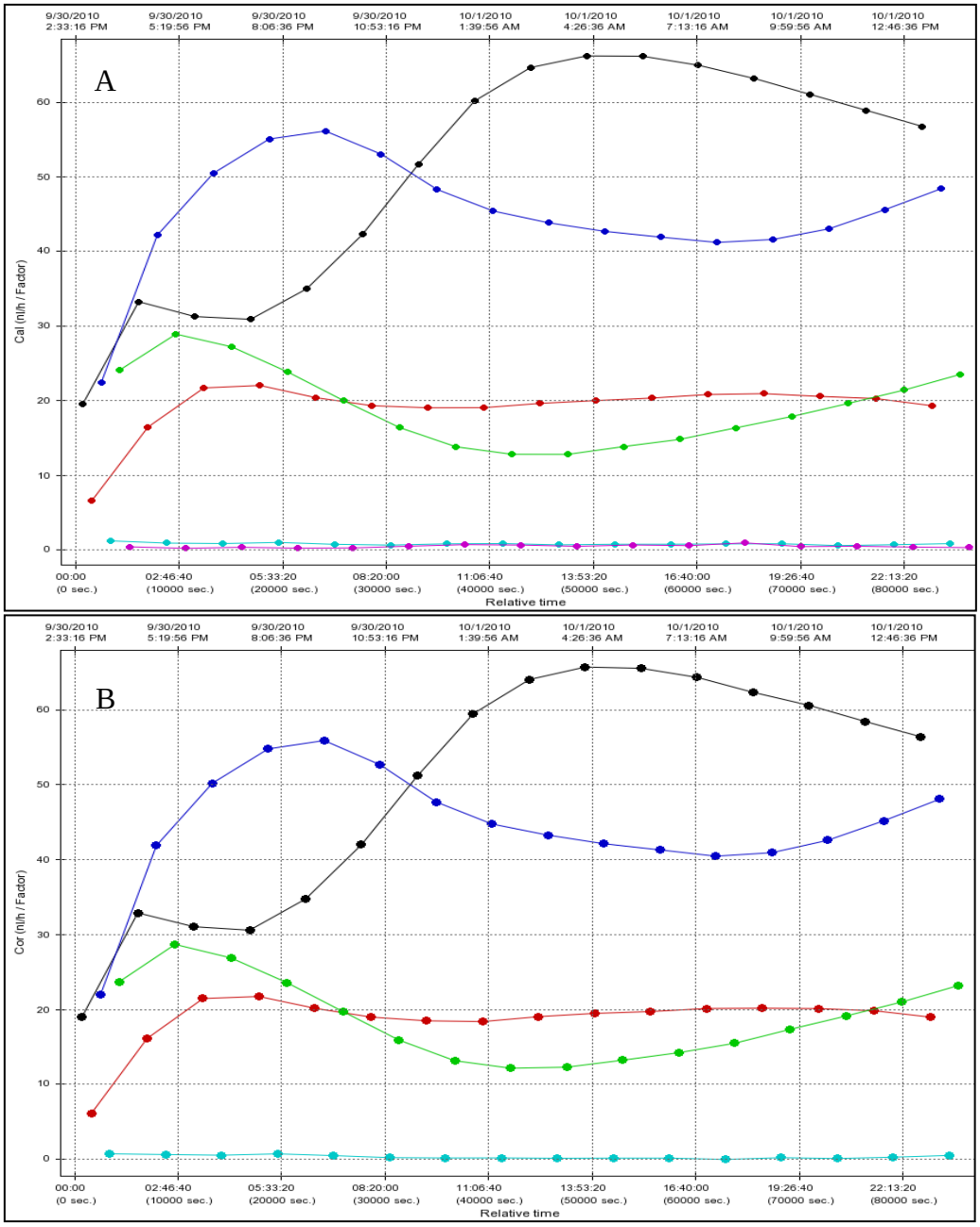


Figure B.6. Output from ethylene detector for the calculated (graph A) and corrected (graph B) ethylene concentration of kiwi fruits stored at 18 ^{o}C (Exp B.3).

B.5 Discussion

Ethylene in the atmosphere of the fruit will influence the quality and developmental processes therefore measure of ethylene in their storage atmosphere is important.

Economic losses can occur when ethylene is induced via wounding or when fruits are exposed to high concentrations; this can result in induced premature ripening (Wills *et al.,* 2007).

Determining ethylene concentrations is imperative in studies concerning fruit development and physiology as these processes are greatly influenced by this phytohormone. Ethylene in response to storage conditions and influence of ethylene in storage atmosphere of different fresh produces is consistently studied (Wills and Warton, 2000; Palou *et al.*, 2003; Montalvo *et al.*, 2007). Techniques capable of determining the rate of ethylene production with time are less available and most conventional methods rely on injection for example gas chromatography (GC). However, with the use of GC and other injection techniques, there is risk of missing important trends in the gas evolution with time. This on-line ethylene detector overcomes this disadvantage in that ethylene evolution of sample throughout a desired period (i.e., 24 h) is attainable. This instrument is capable of performing measurement on six separate samples. After each measurement the jar container is refreshed with fresh gas before being analysed again. Recent literature has highlighted the advantages of the on-line measurement of ethylene using the laser based photoacoustic ethylene detector. In Cristescu *et al*. (2002), ethylene production was clearly demonstrated via a line graph whereby the influence of fungus (160 µl of a conidial) suspension at various concentrations of conidia; 1.5×10^8, 2×10^7, 2×10^5 conidia ml^{-1} when plated on PDA having 25 mM L-methionine. Here the trend of ethylene production over a period 84 h was depicted, and the influences of the concentration of conidia were also simultaneously demonstrated. Demonstrating such trend is the advantage of this on-line monitoring ethylene detector. Recent studies, Salman *et al*. (2009) have similarly confirmed the sensitivity and advantages presented by this technique in monitoring ethylene with time hence demonstrating crucial patterns.

The efficacy of laser based photoacoustic detector was tested with kiwis purchased from a local supermarket in Nijmegen. It is widely believed that climacteric fruits including kiwis respond to physical damage via increase in their ethylene production. This is believed to result from the ethylene induced and combination of other hormones such as abscisic acid (ABA) which in turn impact cell wall hydrolases enzymes (Seymour *et al.*,

1993). The kiwis purchased were subject to stress through bruising and cuts and the ethylene produced overtime was measured.

The effects of ethylene are temperatures-dependent, whereby sensitivity to ethylene increases with temperature increase in the range 0-20 ^{o}C (Wills *et al.*, 2001). Low ethylene production of wounded and control fruits stored at 4 ^{o}C is presented (Figure B.5). During the start of the measurement ethylene concentration was seen to be at its highest with concentrations of 0.6-0.7 nl h^{-1} for the wounded fruits; however this decreased rapidly overtime possibly because as fruits spend more time in the cold temperature this ethylene emission decreased or was suppressed. The control fruits (*n*=2), wounded (*n*=1) and the blank jar show a similar trend over time where ethylene concentration was highest at the start and then decreased rapidly overtime. The ethylene in the reference jar is also slightly higher than ethylene in the control fruit jar (Figure B.5); however measurements may have been different if this trial proceeded for longer. A repeat of this experiment when fruits were at ambient temperatures (18 ^{o}C) shows more direct effect of wounding. Results imply that the effect of wounding or bruising to the fruit in triggering evolution of ethylene is more pronounced at this temperature and is conspicuous from the results (refer Figure B.6 and B.7). This is in accordance with literature (Ritenour *et al.*, 1999; Antunes and Sfakiotakis, 2002), where an increase in respiration and biochemical alteration as a result of higher temperature storage has been identified with kiwis. In both experiments results (Figure B.5 and B.6) show fruits which were subject physical stress (wounding or cut) showed an elevated ethylene in comparison to control fruits having no wounds. It is clearly demonstrated (Figure B.6) that the fruits (n=3) which were wounded produced considerably higher ethylene when compared control fruits (n=2), which on the other produced consistently low level. When comparing if different wounds affected the overall ethylene produced (Figure B.6) results do not demonstrate any relationship between the concentration of ethylene produced and the type of wound. All fruits (n=4) which were physically wounded through bruising or cut exhibited a substantially higher ethylene levels in comparison to the control fruit which produced consistently low ethylene over approximately 24 h period.

Appendix C

C.1 Appendix C: Pictures

Figure C.1. Pictures of the quantification of CO_2 and ethylene, objective colour using colorimeter, determination of firmness using instron and sample preparation for subsequent biochemical analysis.

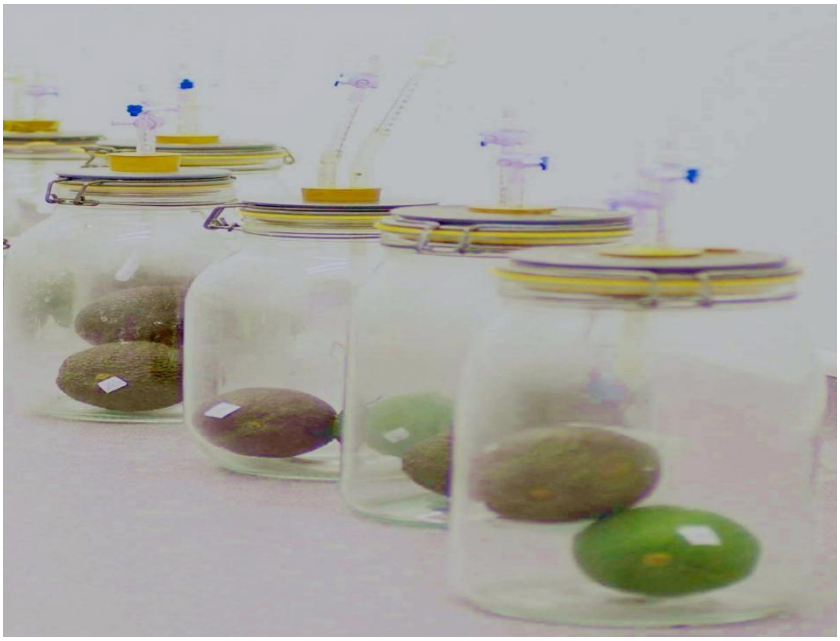


Figure C.2. Incubation jars (3 l) for measuring ethylene and CO_2 .

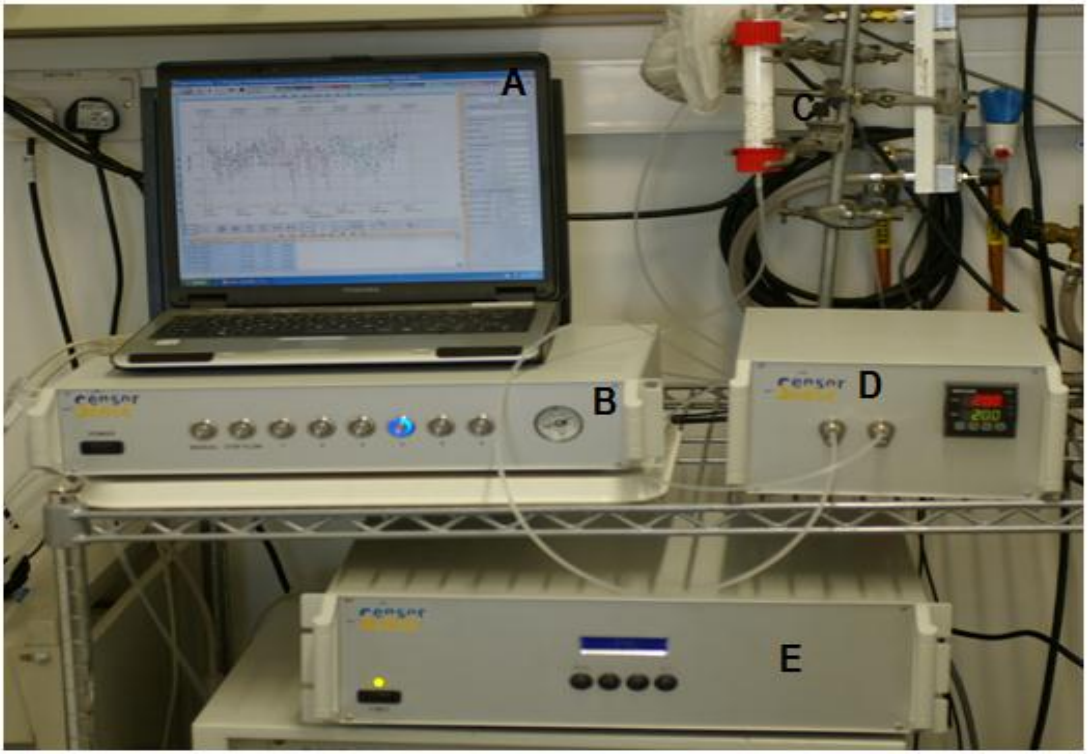


Figure C.3. The laser based photoacoustic ethylene detector (ETD-3000). Output (A), valve controller (B), scrubber (C), catalyser (D) and ethylene detector (E).

Figure C.4. Six 1 l jars connected to the valve control in which air inside the jars is carried to the detector.

Figure C.5. Picture of the Sable system set up. Connected to 3 l jars (A), MUX Flow multiplexer (B), SS4 subsampler (C), RH-300 water vapour detector (D), CA-10 CO_2 detector (E), FC-10 O_2 detector (F), UI-2 universal interface (G) and computer (H).

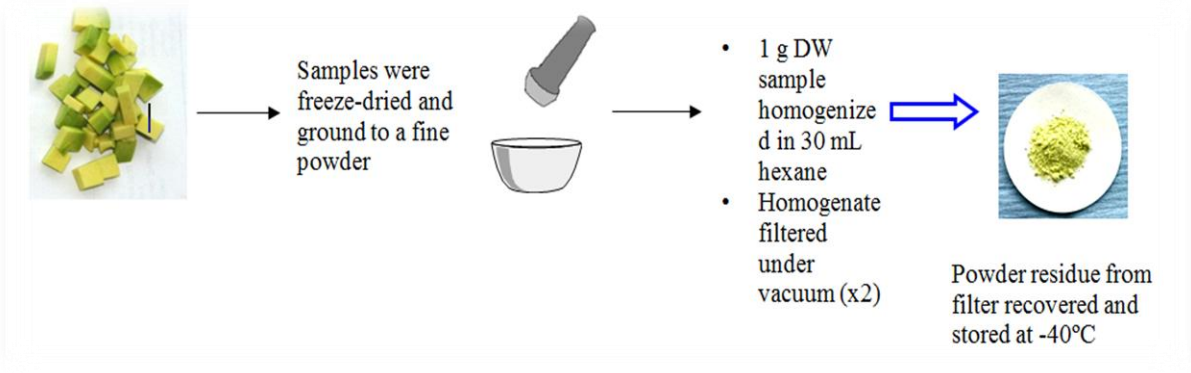


Figure C.6. Sample preparation for biochemical analysis and oil extraction.

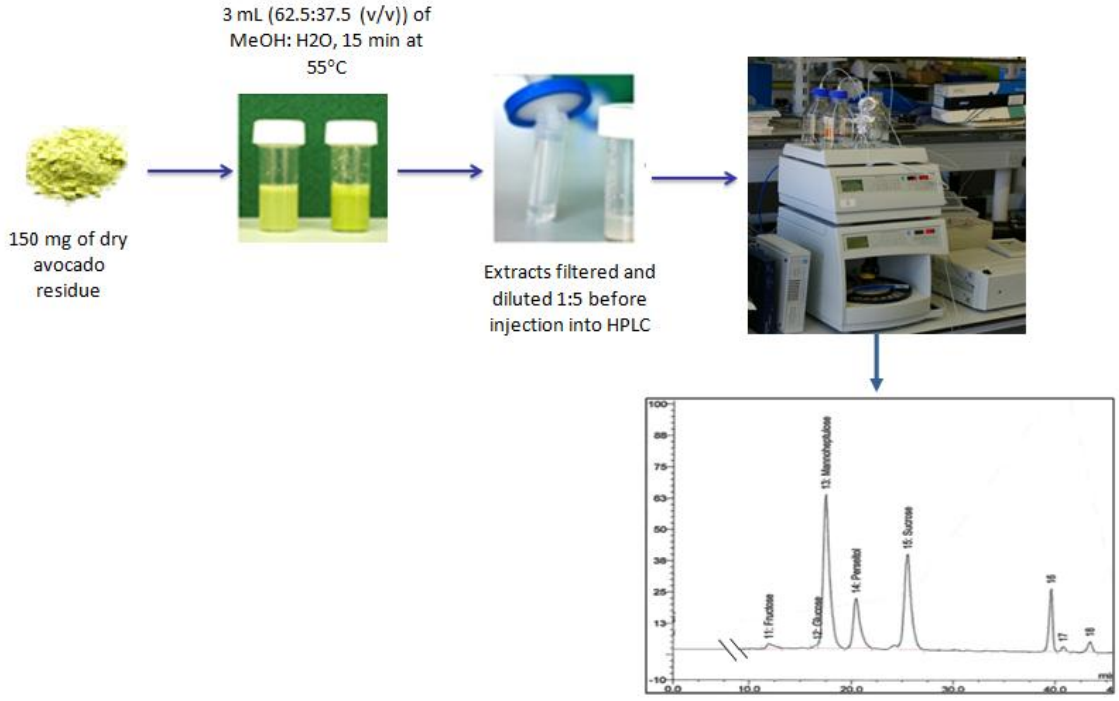


Figure C.7. Sugar extraction and quantification.

Appendix D

D.1 Conference proceedings and publications

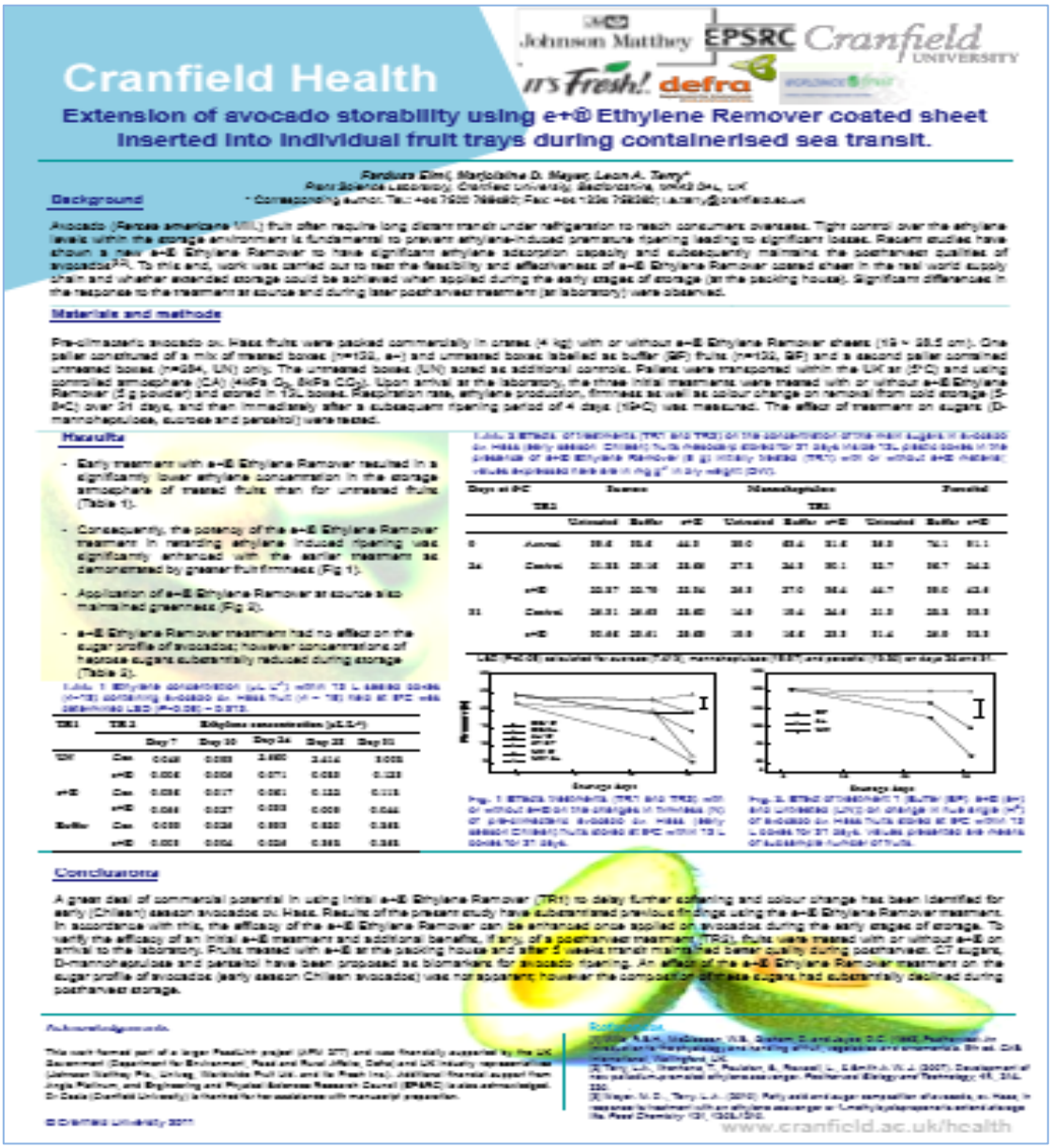
- **Oral presentation: F. Elmi., M. D. Meyer., L. A. Terry**. Extension of avocado storability using e+® Ethylene Remover coated sheets in sea containers. IV Postharvest Unlimited, 23-26th May 2011. Leavenworth, WA, USA.

- **Oral presentation: F. Elmi., K. Cools., L. A. Terry**. The use of It'sFresh! Ethylene Remover Technology with e+® active as a practical means for preserving postharvest fruit quality. 7th International Postharvest Symposium (IPS 2012), 25 – 29th June 2012. Kuala Lumpur, Malaysia.

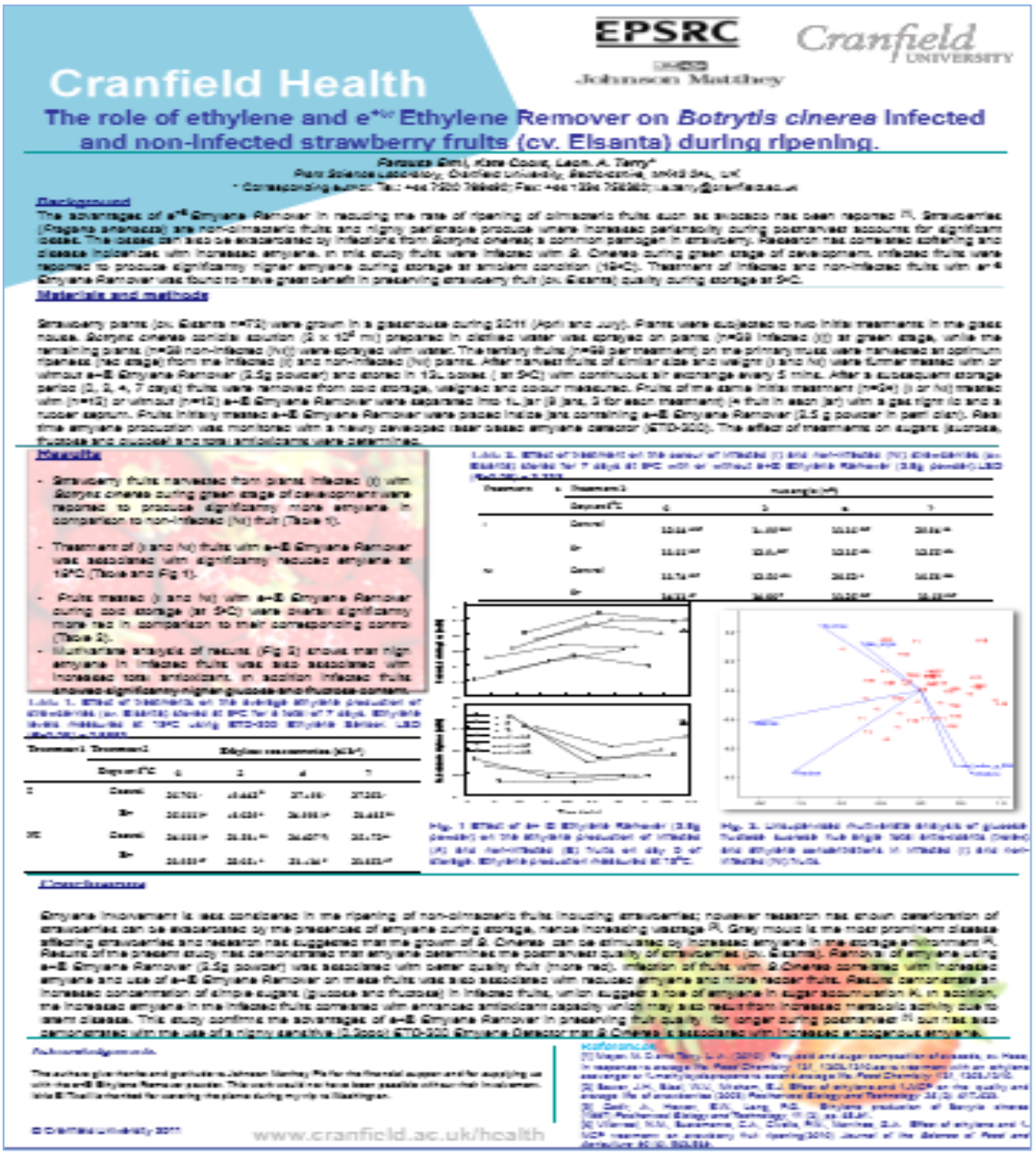
- **Oral presentation: F. Elmi., K. Cools., L. A. Terry**. The use of It'sfresh! Ethylene Remover technology with e+® active as a practical means for preserving postharvest fruit quality. Postgraduate Cranfield Health Conference, 19th September 2012. Cranfield, UK.

- **Oral presentation: F. Elmi., M. D. Meyer., K. Cools., L. A. Terry**. Reducing retail and household fresh produce waste using e+® ethylene remover. Johnson Matthey Academic Conference (JMAC13), 16 - 17th April 2013. Loughborough University, UK.

Poster: F. Elmi., M. D. Meyer., L. A. Terry. Extension of avocado storability using e+® Ethylene Remover coated sheets in sea containers. Cranfield University & Colworth: Unilever regional partners working together for global success. 23rd June 2011. Bedford, UK.



Poster: F. Elmi., K. Cools., L. A. Terry. The role of ethylene and e$^{+®}$ Ethylene Remover on *Botrytis cinerea* infected and non-infected strawberry fruits (cv. Elsanta) during ripening. Postgraduate Cranfield Health Conference. 16th September 2011. Cranfield, UK.



Poster: F. Elmi, M. D. Meyer, L. A. Terry. The use of e+® Ethylene Remover as a tool for preserving postharvest fruit quality. Johnson Matthey Academic Conference (JMAC12), 27-28th March 2012. Loughborough University, UK.