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CRANFIELD HEALTH

Plant Science Laboratory

Ph.D THESIS

2010

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Academic Years 2006-2010

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EFFECT OF CONTROLLING ETHYLENE ON RIPENING OF AVOCADO CV. HASS FRUIT

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May 2010

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

ABSTRACT

Rigorous control of ethylene inside storage atmosphere is cardinal to maintain quality of climacteric fruit, including avocado cv. Hass. This can be achieved using the ethylene action inhibitor, 1-methylcyclopropene (1-MCP). The recent development of a novel palladium (Pd)-based ethylene scavenger, e+® Ethylene Remover, provides a new opportunity to delay avocado fruit ripening.

A new method was developed to sequentially extract and quantify both lipids and sugars from the same avocado mesocarp tissue sample. Extraction by homogenization with hexane yielded slightly less oil than the standard Soxhlet technique whilst the fatty acid profiles of the oil extracts were similar. Extraction of the resulting filter residue with methanol (62.5%, v/v) better recovered sucrose, perseitol and mannoheptulose as compared to ethanol (80%, v/v). The new method has a shorter extraction time, lower extraction temperature and requires less solvent.

Presence of e+® Ethylene Remover in storage atmosphere removed all ethylene and accordingly delayed the ripening of avocado cv. Hass stored at low temperature. 1-MCP also inhibited ripening, yet, unlike e+® Ethylene Remover it impaired subsequent ripening. It was possible to slow down the ripening rate after the climacteric has been induced by removing ethylene below 1 $\mu L\ L^{-1}$ in presence of e+® Ethylene Remover, and the scavenger was effective in combination with modified atmosphere packaging (MAP).

Blocking ethylene action or removing ethylene did very slightly affect the fatty acid composition of the mesocarp oil. Depending on the origin and maturity of the fruit, 1-MCP and e+® Ethylene Remover better maintained seven-carbon sugars concentrations in mesocarp. Results support the view that mannoheptulose and perseitol could be important features of the avocado ripening process but more research is necessary to elucidate their exact function.

Mesocarp abscisic acid (ABA) was quantified using a newly developed LC-ESI-MS/MS method. ABA increased as fruit ripened but appeared to be at least partly regulated by ethylene. Whether ABA influences the ethylene-associated ripening in avocado cv. Hass remains to be determined in future.

EXECUTIVE SUMMARY

Avocado (*Persea americana* Mill.) fruit often necessitate long distance transit under refrigeration to reach consumers overseas. Tight control of the ethylene levels or its effect inside the storage environment is cardinal to prevent ethylene-induced premature ripening. This can be achieved using 1-methylcyclopropene (1-MCP) but the ethylene binding inhibitor has been reported to have some problems on avocado fruit. Moreover, ethylene scavenging technologies are often ineffective under storage conditions (i.e. cold temperature, high %RH) and have not been sufficiently developed in recent years. The recent discovery of a new palladium (Pd)-based ethylene scavenger, e+® Ethylene Remover, provides a powerful tool to delay avocado fruit ripening and investigate the mechanisms of ripening and associated biochemical changes.

There is currently no convenient and fast method for extracting fatty acids and sugars from the same avocado mesocarp sample. A new method was developed that enabled, for the first time, sequential extraction and subsequent quantification of both lipids and sugars from the same avocado mesocarp tissue sample. Freeze-dried mesocarp of avocado cv. Hass fruit was extracted by homogenization with hexane, or using the standard Soxhlet technique for comparison, and the oil extracts quantified for fatty acid composition using an optimised GC method. Sugars were extracted from the resulting filter or thimble residue with methanol (62.5%, v/v), or ethanol (80%, v/v) for comparison. Average oil yield using the Soxhlet technique was significantly higher than that obtained by homogenization with hexane, although the differences remained slight, and fatty acid profiles of the oil extracts using both methods were very similar. Oil recovery improved with increasing ripeness of the fruit with minor differences observed in the fatty acid composition during postharvest ripening. After lipid removal, methanolic extraction was superior in recovering sucrose and perseitol as compared to 80% ethanol (v/v), whilst mannoheptulose recovery was less affected. The method presented herein has the benefits of shorter extraction time, lower extraction temperature and reduced amount of solvent.

There exist no comparative study on the effects of 1-MCP and an ethylene scavenger on physiology and biochemistry of avocado fruit. The effect of 1-MCP and e+®

Ethylene Remover on changes in firmness, colour, fatty acids and sugars content of imported avocado cv. Hass during storage at 12°C and 5°C with or without subsequent ripening at 20°C was reported. e+® Ethylene Remover effectively removed both exogenously applied and endogenously produced ethylene within storage atmospheres to levels below 1 and 0.1 μL L^{-1} at 12ºC (mid season fruit) and 5ºC (early and late season fruit), respectively. Where ethylene was removed below 0.1 μL L^{-1} a delay in ripening of avocado stored at 5°C was observed whilst removing ethylene below 1 μL L^{-1} did not prevent ripening. At 5°C, but not at 12°C, 1-MCP was more effective at inhibiting ripening, yet, unlike e+® Ethylene Remover it impaired subsequent ripening. Fatty acid profile of early season fruit was slightly, yet significantly, different according to treatments and storage time whereas that of mid and late season fruit remained unchanged. Substantial amounts of perseitol were found in all fruit. Mannoheptulose was only present in substantial amounts in mid and early season fruit whilst it was quasi-absent in late season fruit. A correlation between firmness and C7 sugars concentration in response to treatments was observed in early season fruit but this was not the case in mid and late season fruit, where mannoheptulose remained relatively low or almost absent, respectively. Results support the view that C7 sugars metabolism could be an important feature of the avocado fruit-ripening process but this necessitates more research.

Delaying the ripening process after the climacteric has been induced has received limited investigation, and rarely been attempted using an ethylene scavenger. A first experiment was conducted to test the efficacy of 1-MCP, e+® Ethylene Remover or combination of these at extending shelf life of avocado cv. Hass even though the climacteric had been induced. In a second experiment, fruit were stored inside modified atmosphere packaging (MAP) with or without e+® Ethylene remover after the climacteric was induced. Atmospheric gases, firmness, colour and sugars were measured at regular intervals. In the first experiment, removal of ethylene below 0.1 μL L^{-1} in presence of e+® Ethylene Remover, similarly to 1-MCP, delayed ripening of pre-climacteric avocado. Presence of the scavenger during/after climacteric induction reduced atmospheric ethylene level below 1 μL L^{-1} in both experiments and accordingly, fruit remained significantly less ripe vs. their respective controls. Additionally, fruit that were initially pre-treated with 1-

MCP in the first experiment remained generally harder and greener (albeit more heterogeneous) than fruit initially stored with e+® Ethylene Remover or controls. There was generally no treatment effect on sucrose, mannoheptulose or perseitol. This study has shown that delaying ripening once the climacteric has been initiated using a highly efficacious ethylene scavenger is possible. These findings are of commercial importance when, for example, cold chain abuse occurs. The mechanisms of MAP are also discussed.

Abscisic acid (ABA) has been associated with ethylene-mediated ripening in several fruit but little is known about the function of ABA in avocado. In order to investigate the function of abscisic acid in avocado cv. Hass ripening, changes in ethylene production, respiration rate, firmness, mesocarp C7 sugars and ABA content in response to 1-MCP (0.3 $\mu L\ L^{-1}$), e+® Ethylene Remover and the combination thereof were investigated. Presence of e+® Ethylene Remover significantly reduced atmospheric ethylene concentrations and, as such, fruit produced less ethylene, had a lower respiration rate and ripened more slowly vs. controls. Treatment with 1-MCP + e+® Ethylene Remover and, to a lesser extent 1-MCP alone, resulted in fruit with the lowest ethylene production, respiration rate and, consequently, improved maintenance of quality as compared with other treatments. The concentration of mannoheptulose decreased over time, whilst sucrose and perseitol content remained stable and there was no effect of treatment on concentration of sugars. Mesocarp ABA concentration, as determined by a newly developed LC-ESI-MS/MS method, increased as fruit ripened. The highest ABA concentrations were recorded in control fruit and the lowest in fruit treated with combined 1-MCP and e+® Ethylene Remover. Firmness was negatively correlated with ethylene production and ABA content with the relationship being described by an exponential decay. Results suggested that ABA may be partly mediated by ethylene since blocking ethylene, and to a larger extent blocking + removing ethylene resulted in lower ABA concentrations. Whether the physiological differences between treated and untreated fruit are related to differences in ABA contents is not clear. Indeed, whether ABA influences ripening (through promoting ethylene biosynthesis or stimulating tissue sensitivity to ethylene) needs to be determined in future research. The C7 sugars, in contrast, did not appear to be related to the ripening process and their function in avocado fruit still remains unclear.

ACKNOWLEDGEMENTS

I am heartily thankful to my supervisor, Dr. Leon A. Terry whose guidance, tireless support and encouragement throughout this project enabled me to develop a true understanding of the subject. I would also like to express my gratitude to Allen Hilton and Balwinder Cook, who have always made available their support in a number of ways, and to all staff at Cranfield University including Charles Marshall for statistical advice and Therese Groves for administrative tasks. I would particularly like to thank Dr. Gemma Chope for her precious help and collaboration during the project. Her knowledge of analytical phytochemistry and her competence in the horticultural domain has made possible several aspects of this project.

This studentship was funded by Johnson Matthey Plc. I would like to thank them for the financial support. This thesis would not have been possible without them and it is a pleasure to thank Dr. Andy J.W. Smith and Dr. Liz Rowsell for all their kindness and their help along the project. Thanks also are given to Anglo Platinum for financial support and Mack Multiples Division (M. W. Mack Ltd., Kent, UK) for kindly supplying me with avocado during the project.

This project was a rich experience shared with my colleagues from the Plant Science Laboratory team, who were a great support. And last but not least, I owe my deepest gratitude to my parents and family who encouraged me from the beginning until the end, and to Nacho who was always there for me.

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NOTATION & ABBREVIATION

<	less than
>	greater than
≥	less than or equal to
≤	greater than or equal to
%	per cent
=	equals
°C	degree Celsius
β	beta
α	gamma
µL	microlitre
µm	micrometer
1-MCP	1-methylcyclopropene
AAO	abscisic aldehyde dehydrogenase
ABA	abscisic acid
ACC	1-amino-cyclopropane-1-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
AOAC	Association of Analytical Communities
AVG	amino ethoxy vinyl glycine.
BOC	British Oxygen Company
bp	boiling point
CA	controlled atmosphere
ca.	approximately
Ca	calcium
CI	chilling injury
cm	centimetre

CO_2	carbon dioxide
cv.	cultivar
C7	seven carbon
DEFRA	Department for Environment Food and Rural Affairs
de novo	anew
d.f.	degrees of freedom
DM	dry matter
DRIFT S	diffuse reflectance infrared Fourier transform spectroscopy
DW	dry weight
ELSD	Evaporative Light Scattering Dectector
EPSRC	Engineering and Physical Sciences Research Council
EtOH	ethanol
et al.	and others
Exp.	experiment
FA	Fatty acid
FAO	Food and Agriculture Organisation of the United Nations
FID	Flame Ionisation Detection
FM	fresh matter
FW	fresh weight
g	gram
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
h	hours
HDL	high density lipoprotein
HPLC	High Performance Liquid Chromatography
H_2O	water
in vivo	inside a living organism
IS	internal standard
JM	Johnson Matthey
kg	kilogram

$KMnO_4$	potassium permanganate
kPa	kiloPascal
L	litre
LC-ESI-MS/MS	liquid chromatography electrospray ionization tandem mass spectrometry
LDL	low density lipoprotein
Ltd.	limited
LSD	least significant difference
m	metre
MACC	1-(malonylamino)- cyclopropane-l-carboxylic acid
MA	modified atmosphere
MAP	modified atmosphere packaging
MeOH	methanol
mg	milligram
min	minute
ml	millilitre
mm	millimetre
m/m	mass by mass
MRM	Multiple reaction monitoring
N_2	nitrogen
NBD	2,5-Norbornadiene
NDGA	nordihydroguaiaretic acid
ng	nanogram
NS	not significant
NSC	non-structural carbohydrate
O_2	oxygen
OECD	Organisation for Economic Co-operation and Development
P	probabiltity
Pd	Palladium
Pa	*Persea americana*

PA	phaseic acid
PG	Polygalacturonase
PME	pectin methyl esterase
RDC	Retail distribution center
RIA	Radio-immunoassay
RH	relative humidity
RNA	ribonucleic acid
rpm	revolutions per minute
SAM	S-adenosyl-L-methionine
SC-CO_2	supercritical carbon dioxide
S.E.	standard error
SPE	solid phase extraction
TCA	tricarboxylic acid
TEM	transmission electron microscopy
TiO_2	Titanium dioxide
UK	United Kingdom
UNECE	United Nations Economic Commission for Europe
US	United States
USA	United States of America
USDA	United States Department of Agriculture
UV	ultra violet
vs.	versus
viz.	namely
v/v	volume by volume
WRAP	Waste and Resources Action Programme
w/w	weight by weight
ZEP	zeaxanthin epoxydase

CHAPTER 1

Introduction

1.1 Project background

Avocado is a tropical/subtropical fruit which has gained considerable popularity all over the world. Beside its unique taste and flavour, the fruit is eaten for its health-promoting coumpounds, *viz.* unsaturated fats and uncommon carbohydrates. Avocados consumed in Europe, including UK, are often imported from distant production site and necessitate several weeks of transit under refrigerated storage. Presence of ethylene in the storage atmosphere is a major factor that can undermine avocado quality, and ethylene-induced premature ripening may generate significant economical losses. 1-methycyclopropene (1-MCP) is a synthetic chemical antagonist of ethylene that suppresses ripening and therefore prolongs storage life of many fruit, but may cause problems on avocado if used inappropriately (Jeong and Huber, 2004). Other methods of controlling ethylene effects exist, including ethylene scavengers, but these have not been fully exploited for avocado. In the past, work to improve postharvest techniques (other than 1-MCP) has remained limited with relatively little attention being paid to the mechanisms underlying avocado ripening.

A recent study by Terry *et al.* (2007a) has shown the efficacy of a newly developed palladium (Pd)-promoted material to remove ethylene below sub-physiologically active levels and hence maintain quality of climacteric fruit. The material, discovered and manufactured by Johnson Matthey (Johnson Matthey Plc., London, UK) consisted of a carefully selected zeolite impregnated with finely dispersed Pd particles (metal loading 2.5% (m/m)). Even when applied in low amounts, the scavenger removed ethylene below physiologically active levels at temperatures ranging between 5-16°C. Accordingly, a delay in the ethylene-induced ripening was observed for banana and avocado fruit (Terry *et al.*, 2007a). A subsequent paper by Smith *et al.* (2009) described the physico-chemical

properties of the material. This technology had not been applied to fresh produce before the study by Terry *et al.* (2007a) and was patented in 2009 [WO2007\052074]. The use of Pd in different forms and on different supports for ethylene removal from storage atmosphere containing horticultural commodities has been investigated before (Bailen *et al.,* 2006; Martinez-Romero *et al.,* 2009a, 2009b). However, the material used in these studies differed from the present one in that the Pd was impregnated into an active carbon support. The product was applied at 8ºC, 20ºC and in a heated device (>100ºC), hence acting as a catalyst. In contrast, the novel product described in this thesis consists of a specific combination of Pd with a carefully selected zeolite. Also, the present material does not necessitate application of heat and is effective when used at 5ºC (i.e. acting principally as a non-catalyst) (Terry *et al.,* 2007a, Smith *et al.*, 2009) or lower. Following the work by Terry *et al.* (2007a), a new formulation of the Pd-promoted material was produced by metal thrifting (from 2.5% to 1 % Pd (m/m)) and this formulation is currently registered as e+® Ethylene Remover (since 2009). This technique provides, beside an alternative or complementary to the use of 1-MCP, an opportunity for a better understanding of the mechanisms involved in avocado ripening. This project was funded by Johnson Matthey Plc and Anglo Platinum. New market opportunities for Pd are being sought, and the effectiveness of Pd-based materials at scavenging ethylene (see section 2.4.4, Chapter 2) renders them suitable for agricultural application, hence the present project.

1.2 Aim and objectives

1.2.1 Aim

The aim of this PhD project was to determine the effects of controlling ethylene using two different techniques, *viz.* 1-methylcyclopropene or an ethylene scavenger (e.g. blocking ethylene vs. removal), on physiological and biochemical changes occurring during storage and ripening of avocado cv. Hass. It was postulated that this research would result in a better understanding of the mechanisms of ripening and contribute to improved storage of avocado cv. Hass fruit.

1.2.2 Objectives

- To compare the effects of blocking ethylene action against ethylene removal, using 1-MCP or a novel Pd-promoted scavenger (e+® Ethylene Remover), respectively, on the physiological and biochemical (*viz.* fatty acids and sugars) attributes of avocado cv. Hass fruit during storage at different temperatures.
- To determine the effects of these two techniques on the ability of fruit to subsequently ripen under shelf life conditions.
- To determine the effects of 1-MCP and/or e+® Ethylene Remover on extending storage life of avocado fruit once the climacteric has been initiated.
- To determine the relationship between the seven-carbon (C7) carbohydrates present in avocado mesocarp and the ripening process, using 1-MCP and/or e+® Ethylene Remover to modulate ripening.
- To determine the relationship between ABA and avocado fruit ripening.

1.3 Thesis structure

The thesis is organised in nine chapters. Chapter 2 is a review of existing literature. First, it describes the current avocado fruit industry and the necessity for long periods of storage. The physiological and biochemical changes in avocado fruit associated with storage and ripening are described, with consideration of the health beneficial biochemical coumpounds and those likely to influence ripening. After this, the role of ethylene in avocado fruit ripening, ethylene biosynthesis and mechanisms of action are described, and the current strategies used to prevent detrimental effects of ethylene on stored crops, including avocado fruit, are outlined. There has been an increased interest in health-related properties of fruit and vegetables in the past years. Avocado fruit is a naturally rich dietary source of health-beneficial bioactive substances with reported medicinal effects toward many diseases, including monounsaturated fatty acid, which may prevent cardiovascular risk (Ledesma *et al.*, 1996), and C7 sugars with potential anti-cancer activity (Board *et al.*,

1995; Ishizu *et al.*, 2002) and insulin secretion inhibitory effects (Ferrer *et al.*, 1993). In addition, C7 may also have antioxidant activity (Bertling *et al.*, 2007).

Standard methods for extraction and quantification of fatty acids and sugars are tedious and time consuming. Extraction and quantification of both fatty acids and sugars from the same mesocarp sample has not been published before. Chapter 3 describes the development of a rapid method for extraction and quantification of both fatty acids and sugars from the same avocado mesocarp sample, thus enabling reliable analysis of a large number of samples. This new method was used for biochemical analysis of avocado mesocarp samples in subsequent experiments. Results from this work have been published as follows:

- 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*, 56, 7439–7445 (See Appendix B).
- A poster was presented at the Cranfield Health Postgraduate Conference (CHPC). *Cranfield Health Postgraduate Conference.* 17th September 2008, Cranfield University.

Part of this work has also been used in a subsequent publication:

- 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*, 57, 7039–7047.

A preliminary study by Terry *et al.* (2007a) demonstrated the ability of the newly developed Pd-promoted material to remove ethylene below sub-physiologically active levels hence delaying the ethylene-induced ripening of avocado cv. Hass fruit held at 12ºC. Ethylene removal vs. ethylene action blocking on avocado ripening has not been compared

previously. There is no published research on the effects of controlling ethylene on fatty acid and sugar composition in avocado. Chapter 4 describes three experiments which compared the effects of e+® Ethylene Remover and 1-MCP in controlling ethylene during storage at 12°C and 5°C of avocado cv. Hass, and subsequent shelf life ripening. Changes in physiological attributes, fatty acids and sugars in response to the treatments were considered. Special emphasize was given to C7 sugars status, based on the hypothesis that C7 sugars may be a factor involved with the control of the ripening process. Results from this work have been published as follow:

- Meyer, M.D. and Terry, L.A. (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,* 121, 1203–1210 (See Appendix B).
- Meyer, M.D. and Terry, L.A. (2010). Manipulating the ripening of imported avocado 'Hass' fruit during cold storage using e+® Ethylene Remover or 1-methylcyclopropene (1-MCP). *Acta Horticulturae*. (ISHS), 858, 295-300
- An oral presentation was given at the *10th Controlled and Modified Atmosphere Research Conference,* 4-7 April, 2009, Antalya, Turkey.
- An oral presentation was given at the *11th International Symposium on Plant Bioregulators in Fruit Production*, 20-24 September 2009, Bologna, Italy.

Most research on postharvest techniques to maintain fruit quality have focused on preventing the ethylene-induced climacteric event rather than reducing the ripening processes associated with ripening. Once the avocado has crossed over into the post-climacteric stage, shelf life is very short, and rapid perishability occurs in the retail and home environments. Successful storage of avocado relies on maintaining the cold chain (5-6°C) throughout storage; however cool chain abuse is not unusual, for instance when a power cut occur or where fruit consignments are transferred between different modes of transport. Chapter 5 tested whether it was possible to maintain avocado fruit quality even once the climacteric has been triggered. Therefore, the chapter describes the effect of e+®

Ethylene Remover and 1-MCP on quality attributes (firmness and colour) and sugars content of cold stored avocado cv. Hass subjected to a break in the cold chain (18°C for 24h) to induce ripening. The chapter also describes an experiment where e+® Ethylene Remover was combined with modified atmosphere packaging (MAP). This work is in preparation for publication.

Abscisic acid (ABA) metabolism in avocado fruit has not been well documented compared to other fruit, and its role remains unclear. Moreover, avocado is known to contain large amounts of ABA but methods to determine ABA concentration in mesocarp tissue are not recent and have not been optimised for avocado. Chapter 6 details the temporal changes in ABA and sugars concentration and other physiological aspects during storage at 12°C of avocado treated with e+® Ethylene Remover, 1-MCP or combination of both. In collaboration with Dr. Gemma Chope, an ABA extraction method was optimised and quantification was performed using a newly developed LC-ESI-MS/MS method. This work is in the process of being published.

Chapter 7 is a general discussion which integrates the findings from previous chapters and proposes recommendations for future research.

Chapter 8 is the literature cited and Chapter 9 presents appendeces (*viz.* Statistical tables and published articles). The results from this thesis resulted in a DEFRA FoodLINK project entitled 'AFM277 – Development of a prototype to reduce household waste' and an EPSRC (Engineering and Physical Sciences Research Council) Case studentship.

CHAPTER 2

Literature Review

2.1 The avocado *(Persea americana* Mill.)

2.1.1 Origins and geography of avocado

The commercial avocado tree (*Persea americana* Mill.) belongs to the large family Lauraceae and to the genus *Persea*. Other known members of the genus exists but have not been recognized as commercially important. The crop originated in a large geographic area extending from the eastern and central highlands of Mexico through Guatemala up to the Pacific coast of Central America (Smith, 1966). The crop was already semi-domesticated in both Mayan and Aztec civilizations, which supposedly selected for larger fruit size with improved eating quality (Smith, 1966, Storey *et al.,* 1986). Three distinct, ecologically separate races have been identified - Mexican, Guatemalan, and West Indian or Lowland- based on morphological differences and their respective ecological and climatic adaptations (Popenoe, 1920). The Mexican race is adapted to relatively elevated and cool habitats with a 6-8 months winter-spring dry period (Wolstenholme and Whiley, 1999). The Guatemalan race is native to tropical highlands, with year-round cool conditions, although it can also be found in warmer subtropical areas. The West Indian race is more adapted to hot and humid tropical, lowland climate with a short dry season. The West Indian and Guatemalan avocados have lower fat content than the Mexican type. Hybrids of these races represent the varieties dominating the international market. For instance, the cv. Hass, which is the most grown cultivar worldwide, is predominantly Guatemalan with some Mexican germplasm and cv. Fuerte, another economically important cultivar, is a Mexican x Guatemalan hybrid. Other commercial varieties include Ryan, Lula, Booth8, Walden, Pollock, Pinkerton, Bacon, Lamb Hass and Zuton.

From a botanical point of view, the avocado fruit is classified as a berry comprising a single seed (stone) and a pericarp. The pericarp is further divided into exocarp (skin),

mesocarp (flesh, edible portion) and the thin layer around the seed coat, the endocarp (Figure 2.1).

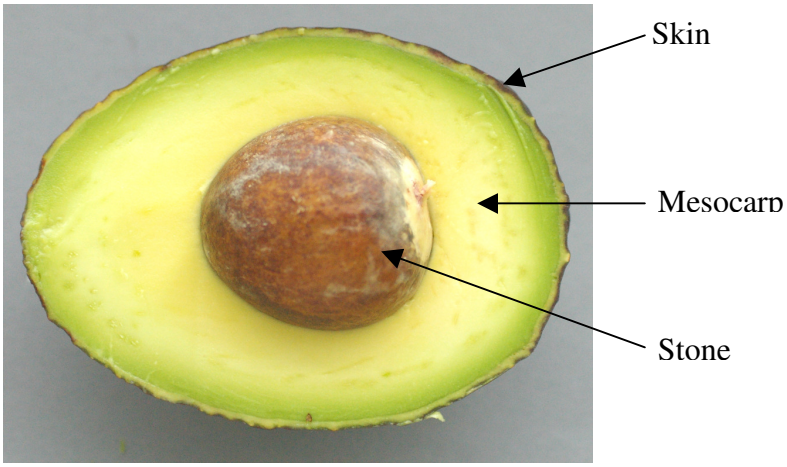

Figure 2.1. Avocado cv. Hass fruit. Source: Marjolaine Meyer

2.1.2 The cultivar Hass

The cv. Hass originates from a chance seedling of unknown parentage and was patented in April 1935 by R.G. Hass in California. It is the cultivar most grown in the last century. The fruit has an ovate shape (Figure 2.1) and weight varies between 140 – 400g. The skin is thick with a coarse corky texture, and forms a good protection from pests and disease. The fruit is green on the tree, but, unlike many other green-skin cultivars, the ripening process is accompanied by a distinct skin colour change from green to purplish-black when ripe. The purpling, to some extent, also masks minor imperfections and external damage. The cultivar is by far the preferred export cultivar due to its remarkable postharvest qualities and flavour. The fruit has a better storage capacity and internal quality than other cultivars and is appreciated by consumers for its superior taste.

2.1.3 Commercial importance of the avocado crop in the world

In the past decades, the trade of avocado fruit has considerably expanded with markets extended to North America and Europe, and volumes traded internationally have increased significantly. Avocado crop is nowadays cultivated in numerous countries at both tropical and subtropical latitudes, including Mexico, Chile, Indonesia, the Dominican Republic, Brazil, Peru, the United States, Israel, South Africa, Australia, New Zealand and Spain (Table 2.1) although Mexico remains, by far, the largest avocado producer in the world, while Chile has become the second largest producer. Producing countries can be distinguished between countries where the production was initially focused on the local market, e.g. Mexico and Peru, and countries where the avocado industry was originally developed for export, e.g. Israel, South Africa, Spain and Chile.

Table 2.1 Values in tonnes/year for main avocado producers and importers (from FAO Stat, 2007).

Country	Production (tonnes/year)	Country	Import (tonnes/year)
Mexico	1,142,892	U.S.A.	348,858
Chile	250,000	France	110,632
Indonesia	201,635	Netherlands	63,211
Colombia	193996	UK	44,526
Dominic Republic	183,468	Japan	26,511
U.S.A.	175,177	Canada	23,252
Spain	120,000		
Israel	85,913		
South Africa	65,203		

The United States, Europe, and to a much smaller extent Japan, are the main importers and are responsible for more than 90% of the world imports (Table 2.1). Avocado ranks as the 14th most commonly consumed raw fruit in the United State (US Food and Drug Administration, 2009).

With the globalization of avocado trade, the production of high quality fruit and efficient transport conditions has become a necessity in order to reduce waste and maintain fruit quality until it reaches the consumer.

2.1.4 Quality attributes of marketable avocado

The main objective of postharvest treatment of avocado is to preserve fruit quality until it reaches the consumers in markets which may be far away from growing regions. Avocado fruit quality relates to several factors which depend on the position of the recipient in the distribution chain. For the consumer, quality relates primarily to appearance (including firmness, colour, size, shape, and absence of defects), but also taste, flavour and nutritional value. The packinghouse handler and retailer, in contrast, may perceive quality as long term storage and shelf life capacities, uniformity of packaging, and absence of defects. Growers, on the other hand, rate quality as the overall freedom from defects and optimum size distribution (Arpaia *et al.,* 2004). The Organisation for Economic Co-operation and Development (OECD) has established an international standard (OECD, 2004), to be referred to for grading avocados in international trade under the OECD Scheme for the Application of International Standards for Fruit and Vegetables.

Whilst in most fruit, maturation is accompanied by physiological changes, in avocado fruit, maturation on the tree and the onset of ripening after harvest do not exhibit obvious external changes (Lewis, 1978). Therefore, postharvest ripening is a commercial pre-requisite and avocado is not acceptable for consumption until fully ripe. Normal avocado ripening with acceptable taste will only occur when fruit is harvested after a certain maturity level, as determined by a minimum dry matter content (Lee *et al.,* 1983; Ranney *et al.,* 1992) has been reached. The minimum dry matter content for cv. Hass avocado is 21% (United Nations Economic Commission for Europe (UNECE), 2009). Fruit

harvested before horticultural maturity may show irregular softening, a poor flavour and are most susceptible to decay.

Pre-harvest factors will not be considered in the present thesis, but it should be noted that pre-harvest environmental conditions and agricultural practice may strongly influence avocado postharvest quality. For instance mesocarp calcium concentration has been positively correlated with fruit quality and numbers of days to ripen and negatively correlated with disorders (Hofman *et al.,* 2002a). Chilling temperatures in the orchard have been shown to hasten postharvest ripening and detrimentally affect postharvest quality of avocado (Hershkovitz *et al.,* 2009). Water stress in the orchard may result in faster ripening and higher incidence of internal browning following postharvest cold storage (Bower and Cutting, 1988).

Avocado is one of the most rapidly ripening of all fruits and thus a highly perishable commodity. The principal biological factors leading to avocado fruit deterioration during storage are a high metabolic activity (respiration), storage disorders and pathogen attack. Efforts toward improving the postharvest management of avocados have been done in recent years, but this crop is among the most difficult commodities to manage in terms of postharvest quality maintenance (Hofman *et al.,* 2002b).

2.2 Physiological and biochemical changes in avocado fruit during ripening

2.2.1 Introduction

Fruit have four major physiological stages of development: cell division and growth, maturation, ripening and senescence. In avocado, similarly to most fleshy fruit, growth on the tree follows a single sigmoid curve with a lag period of *ca*. 10 weeks (commonly named phase I) followed by a growth phase of *ca*. 30 weeks (phase II) and a mature phase (phase III) characterized by slower growth rate (Valmayor, 1967). Like most other fruit, the initial fruit growth is characterized by rapid cell division. However cell division and enlargement in the mesocarp tissue is not limited to initial stage of growth but continues throughout fruit development and maturation on the tree, even when fruit has

reached maturity, albeit at a slower rate (Valmayor, 1967). Growth and maturation (collectively termed the development stage) always takes place when the fruit is still attached to the tree. As described in the previous section, maturation of avocado fruit is not accompanied by external changes, but the fruit is considered mature when it is capable of ripening.

Fruit ripening is the sum of a number of complex molecular, biochemical and physiological events resulting in a physiologically mature but inedible plant organ to become edible and desirable for a seed-dispersing animal (Giovannoni, 2001). Ripening corresponds to the completion of development and the start of senescence, and is normally non-reversible (Wills *et al.,* 1998). Typically, these changes include changes in respiration and ethylene production rates, modification of cell wall ultrastructure and texture, conversion of starch to sugars, change in organic acids, increased susceptibility to pathogen attack, alteration in pigment biosynthesis and accumulation, production of flavour and aromatic volatiles (Wills *et al.,* 1998; Giovannoni, 2001).

The avocado differs from most other fruits in that ripening does not take place on the tree, but will only do so after detachment from the tree (Blumenfeld *et al.,* 1986). The reason for this phenomenon is not yet known and early work by Tingwa and Young (1975) postulated that inhibitive components translocated from the tree to the fruit were responsible for the inhibition of the onset of ripening on the tree and shortly after harvest. The nature of the inhibitive substance(s), if any, has not been identified to date and hypothesis diverges with opinions. Recently, Hershkovitz *et al.* (2010), using the system of seeded and seedless avocado cv. Arad fruit, proposed a role for the seed in the regulation of avocado ripening, based on the observation that the expression of ethylene-related genes was differentially affected in seeded and seedless fruit (see section 2.3). Liu *et al.* (2002), on the other hand, proposed that C7 sugars may be inhibiting substances translocated from the tree to the fruit and control the onset of softening (see section 2.2.6).

2.2.2 Respiration and ethylene production

Respiration has been described as the oxidative breakdown of more complex materials present in cells (*viz.* starch, sugars and organic acids) into simpler molecules such as carbon dioxide and water, which generate heat, energy and molecules used by the cell for synthetic reactions (Wills *et al.,* 1998). The main substrates for respiration are primarily sugars, but also organic acids (Tucker and Grierson, 1987). The respiratory processes involved in the oxidation of sugars in fruits are mainly glycolysis, oxidative pentose pathway (OPP) and the tricarboxylic acid (TCA) pathway (Seymour and Tucker, 1993). The respiration rate is often used as an indicator of fruit metabolic activity and, consequently, of potential storability of the commodity. Ethylene production is integral to the ripening process of climacteric fruit and will be reviewed in more details in section 2.3.

The respiratory and ethylene biosynthesis behaviour of fruit has been used as the basis for their classification into two main categories: climacteric and non-climacteric fruits. Climacteric fruit, like tomato, cucurbits, avocado, banana, peaches, plums, and apples, differ from non-climacteric fruits, such as strawberry, grape, and citrus, in that they exhibit a marked increase in respiration and ethylene biosynthesis rates which coincides with ripening, and is known as the respiratory and ethylene climacteric (Lelièvre *et al.,* 1997). The climacteric event leads to rapid perishability of the fruit, whilst non-climacteric fruit complete ripening more slowly with separation of ripening events (Wills *et al.,* 1998). The respiration and ethylene production behaviour of the avocado follows a climacteric pattern with a sharp rise in respiration and ethylene production at the onset of ripening (Seymour and Tucker, 1993). The reason for a respiratory climacteric is not fully understood since non-climacteric fruit ripen without this physiological change (Giovannoni, 2001).

Whilst ethylene is generally not necessary for ripening of non-climacteric fruit (with some exceptions such as ethylene-induced mRNA and pigment production in the flavedo of orange; Alonso *et al.,* 1995), ethylene is essential for the coordination and completion of ripening in climacteric fruit. Climacteric and non-climacteric fruit are also distinguished from one another based on their response to exogenously applied ethylene. Ripening of

climacteric fruit can be induced by application of exogenous ethylene concentrations as low as 0.1- 1.0 μL L^{-1}, whilst applied ethylene does not normally cause a transient increase in the ethylene production of non-climacteric fruit (Wills *et al.,* 1998). It must be noted that whilst the effects that different ethylene concentrations have on several commodities is well documented (cf. Martinez-Romero *et al.*, 2007), there is a manifest lack of literature on the dose response (time x concentration) of commodities to ethylene.

The categorisation of climacteric and non-climacteric fruit types is often considered an over-simplification. Debate is still ongoing concerning classification of some fruits, such as strawberry and raspberry (Burdon and Sexton, 1990; Perkins-Veazie*,* 2000) where ethylene pattern and involvement in ripening is still not clear or kiwi fruits (Antunes *et al.,* 2000), where climacteric behaviour is temperature-dependent. The practical importance of such classification relies on the impact that exogenous ethylene has on the fruit, affecting postharvest handling and putting constraints on storage conditions of the commodity.

2.2.3 Firmness

Avocado fruit softens in 6-12 days at 20°C depending on physiological maturity at harvest (Eaks, 1980). Softening is the result of the activity of several cell wall degrading enzymes acting to solubilise and depolymerise cell wall polysaccharides (Seymour and Tucker, 1993). Early work has shown that cellulase (endo-β-1,4-glucanase), which targets the hemicellulosic matrix of the cell wall, plays an important role in the softening process (Pesis *et al.,* 1978, Awad and Young, 1979; Kanellis *et al.*, 1989, 1991; Jeong and Huber, 2004). Increase in the activity of cellulase (Pesis *et al.,* 1978; Feng *et al.,* 2000; Jeong and Huber, 2004) and in gene transcription for cellulase (Tucker and Laties, 1984) was stimulated by ethylene treatment whereas activity of the enzyme was significantly delayed or suppressed in avocado by 1-methylcyclopropene (1-MCP) treatment (Feng *et al.,* 2000; Jeong *et al.,* 2002; Jeong and Huber, 2004).

Cellulase activity precedes that of polygalacturonase (PG), an enzyme involved in depolymerisation of pectin (Awad and Young, 1979) and it has been proposed that cellulase disrupts the cell wall matrix, allowing the polygalacturans to access their pectic substrates

(Bower and Cutting, 1988). Therefore cellulase, which may be controlled at least partially by ethylene, appears to be more important in early softening with PG being more important in the later stages of softening (Bower and Cutting, 1988; Jeong *et al.,* 2002). Yet PG has little effect on fruit softening. It has been shown that in conditions of low oxygen atmosphere, gene transcription and activity of cellulase and PG were significantly reduced (Kanellis *et al.,* 1989, 1991). The role of another pectinase, pectin-methyl-esterase (PME), is believed to be methyl de-esterification of pectin, rendering it suitable for depolymerisation by PG (Awad and Young, 1979; Wakabayashi *et al.,* 2000).

Avocado softening, like for many other fruit, is therefore a complex phenomenon where not a single but a group of cell wall modifying proteins (enzymes) act in concert to bring about disassembly of both pectin and hemicellulose matrices (via solubilisation and depolymerisation of cell wall polysaccharides) leading to softening. Textural changes during ripeing of avocado fruit is also complicated by the high proportion of lipids in the mesocarp tissue.

2.2.4 Colour changes

The avocado cv. Hass fruit is characterized by a distinct change of colour from green to a purplish-black colour during ripening. Chlorophyll, the pigment responsible for the green colour, decreases as fruit ripen (Cox *et al.,* 2004) whilst anthocyanins, a type of flavonoid responsible for the red to purple colour in most fruit, increases in the peel of the fruit (Cox *et al.,* 2004; Ashton *et al.,* 2006). One anthocyanin in particular, cyanidin 3-*O*-glucoside, has been shown to account for the increase in total anthocyanin concentrations in the skin of avocado cv. Hass during ripening, resulting in the purpling development (Cox *et al.,* 2004; Ashton *et al.,* 2006). Concentration of cyanidin 3-*O*-glucoside in the fruit peel varies with ripening temperature (Cox *et al.,* 2004).

2.2.5 Lipids and fatty acids

Avocado is an oleaginous fruit with a lipid content up to 20 % fresh weight (Lewis, 1978) depending on cultivars. The lipid fraction is mainly monounsaturated, with oleic acid (C18:1) invariably being the most abundant fatty acid. The oil also contains, in decreasing order of abundance, the saturated palmitic acid (C16:0) and the unsaturated linoleic (C18:2), palmitoleic (C16:1) and linolenic (C18:3) acids. Trace amounts of stearic, myristic and arachidic acids have also been reported (Ahmed and Barmore, 1980; Ozdemir and Topuz, 2004; Vekiari *et al.,* 2004). Avocado ranks among the top natural dietary sources of food-derived monounsaturates and essential fatty acids, with amounts of unsaturated FA up to 5-fold that of saturated FA (Slater, 1975; Vekiari *et al.,* 2004). Consumption of monounsaturates have been reported to have potential cardiovascular benefits by acting on serum lipids (Ledesma *et al.,* 1996).

Although the oil/fatty acid composition remains generally consistent in avocado, with the predominance of oleic acid, the reported concentration of each fatty acid varies with cultivars (Ozdemir and Topuz, 2004; Vekiari *et al.,* 2004; Luza *et al.,* 1990). Fatty acid composition also varies with harvest time (Ozdemir and Topuz, 2004; Vekiari *et al.* 2004) but not always (Lu *et al.,* 2009). In particular, Ozdemir and Topuz (2004) reported a significant decrease in palmitic, palmitoleic, linoleic and linolenic acids and an increase in oleic acid between early and late season harvest of avocado cv. Hass. A recent detailed study has shown that the fatty acid profiles of avocado cv. Hass may also differ with different growing regions, hence with different agricultural practices and agro-environmental conditions, and varied in different parts of the fruit (Landahl *et al.,* 2009). This said, most studies have quoted FAs in a proportional fashion (% total fatty acid) rather than absolute concentration and therefore, there is a lack of quantifieable data on FA.

Changes in fatty acid profile during fruit growth and development has long been known, with a large and predominant increase in the oleic fraction (Kikuta and Erickson, 1968). However, fatty acid composition of the lipid fraction during postharvest storage and ripening is more recent and scarcely documented. Some changes in the proportion of some fatty acids have been reported during the fruit ripening process with an increase in

unsaturated and a decrease in saturated fatty acids (Ozdemir and Topuz, 2004). However, these changes remained very slight. Research with cvs. Fuerte and Hass avocados found that the fatty acid profiles remained constant during cold storage (De la Plaza *et al.,* 2003; Eaks, 1990; Luza *et al.,* 1990).

2.2.6 Non-Structural Carbohydrates (sugars)

Unlike most other fruit, the soluble seven carbon (C7) sugar D-mannoheptulose and its reduced form polyol, perseitol (Figure 2.2), are the major form of NSCs reserve in avocado cv. Hass. These rare sugars in nature are found only in a few plants but it has only been reported in large amounts in avocado (Nordal and Benson, 1954; Liu *et al.,* 1999a, b, 2002; Bertling and Bower, 2005; Landahl *et al.,* 2009). These soluble sugars have been measured in various tissues of the avocado tree such as leaves, shoots, trunk and roots, in equal or greater amounts to that of starch (Liu *et al.,* 1999a,b). In the fruit, these sugars are found in higher concentration than sucrose, glucose and fructose (Liu *et al.,* 1999b, 2002; Bertling and Bower, 2005). The mesocarp C7 sugar concentrations reported in the literature are variable, probably due to differences in origin, harvest dates and biological age of the fruit when measured which are factors of variations in levels of these compounds (Landahl *et al.,* 2009). Different method of extraction and quantification of NSCs may also have accounted for discrepancies in reported concentrations.

The concentration of the C7 sugars in fruit decreased as the season progressed (Liu *et al.*, 1999b). Research in California has reported *ca.* 30 mg g^{-1} dry matter (DM) of mannoheptulose and perseitol in the mesocarp of mature unripe avocado cv. Hass harvested mid-season (Liu *et al.,* 1999b). However, in another Californian study, Liu *et al.* (2002) recorded a 10-fold decrease in mannoheptulose in late season fruit. Experimental research has also provided evidence for a substantial decline in C7 coumpounds during cold storage and postharvest ripening (Liu *et al.,* 1999b, 2002; Landahl *et al.,* 2009). Most studies have used cv. Hass as material of investigation and did not specify the region of the mesocarp used when quantifying C7 sugars in the flesh. Landahl *et al.* (2009) examined the spatial distribution of non-structural carbohydrates within avocado cv. Hass and found that

perseitol concentrations were lower in the middle region. Mannoheptulose concentrations in the fruit tissue from stem to base end were highly heterogeneous, but there was a trend toward greater concentration in the apical region.

The mechanism for biosynthesis and metabolism of heptose sugars, as well as their function in avocado fruit remain, to date, largely unknown. The steps in the biochemical pathway for production of C7 sugars has not been elucidated (Liu *et al.,* 2002). Bean *et al.* (1962) suggested that avocado leaves synthesise mannoheptulose during periods of photosynthesis. However, it is not sure which Calvin cycle intermediates are the first products for the assembly of the mannoheptulose backbone or the localisation of the assembly in the leaf (Liu *et al.,* 2002).

Liu *et al.* (2002) established a positive correlation between softening initiation and decrease in C7 sugar content in the mesocarp. Based on an experiment where the fruit stalk was girdled, the authors observed that a significant reduction (below a threshold concentration of 20 mg g^{-1} DW) in mannoheptulose and perseitol seemed a physiological prerequisite for ripening to start, hence leading to the hypothesis that C7 substances may be the ripening inhibitor of fruit on the tree and shortly after harvest. In agreement with these results, Landahl *et al.* (2009) found greater C7 sugar concentrations in the apical region of the fruit, which appeared to be the firmest. Moreover, the phloem-mobile nature of these sugars makes them potential candidates as mobile inhibitors translocated from tree to fruit. Nevertheless, whether these sugars initiate and control the ripening process or whether the reduction in these carbohydrates is an artefact of fruit ripening still remains unknown. Cowan (2004) proposed various important potential functions for mannoheptulose activity, including protection from damage by reactive oxygen species (ROS) of certain key enzymes that are essential for fruit growth and development, role recently confirmed by Bertling *et al.* (2007). In addition, mannoheptulose and perseitol have been reported to have anti-cancer activity (Board *et al.*, 1995; Ishizu *et al.*, 2002) and mannoheptulose may have an inhibitory effect on insulin secretion (Ferrer *et al.*, 1993).

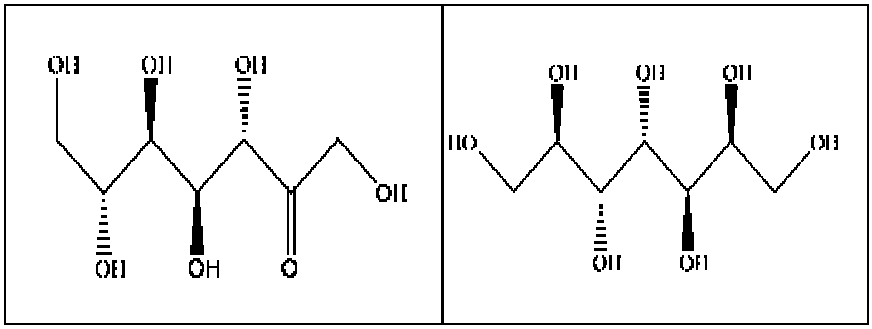

Figure 2.2. Structures of mannoheptulose (left) and perseitol (right)

2.2.7 Plant growth regulators

Among the several plant growth regulators existing in plants, ethylene has been by far the most studied in fruit (see section 2.3) and comparatively less work has been carried out on other phytohormones such as ABA, auxins, cytokinins and gibberellins. However, they are noteworthy as they regulate physiological processes at extremely low concentrations. Similarly to ethylene, ABA is generally (but not always) considered as a ripening promoter, while auxins, cytokinins and gibberellins, function as inhibitors of fruit ripening (Rhodes, 1981). Only the role of ABA will be outlined in this section.

Abscisic acid (ABA) is a carotenoid-derived phytohormone known to play a critical role in growth, development and responses to environment stress (e.g. cold, drought, and osmotic stress) of plants. ABA appears to play a key role in fruit ripening and the hormone has been identified as a promoting substance in avocado (Rhodes, 1981). The ABA biosynthesis pathway starts in chloroplasts and other plastids with the cleavage of a C40 carotenoid precursor, 9-cis-xanthophyll, to form xanthoxin. In the cytoplasm, xanthoxin is converted to ABA via ABA-aldehyde (Zeevaart, 1999). The cleavage of 9-cis-xanthophylls appeared to be the key regulatory step in the ABA biosynthetic pathway.

Endogenous ABA content increases during development and ripening of many climacteric fruit such as apple (Lara and Vendrell, 2000), tomato (Martinez-Madrid *et al.,* 1996), peach (Zhang *et al.,* 2009a) and in non-climacteric fruit such as grape (Zhang *et al.,* 2009a). In avocado, ABA levels during fruit development remained unchanged (Gazit and Blumenfeld, 1972) but concentrations increased considerably at the onset of ripening with a maximum peak occurring just after that of climacteric ethylene (Adato *et al.* 1976; Chernys and Zeevaart, 2000). In contrast, in peach, accumulation of endogenous ABA was maximal just prior to the ethylene peak (Zhang *et al.,* 2009a). Richings *et al.* (2000) found that phenotypically small avocado fruit had higher respiration rates and higher ABA content.

Application of exogenous ABA (usually by vacuum infiltration) has been shown to accelerate induction of ethylene biosynthesis and advance the onset of ripening in avocado (Blakey *et al.,* 2009); peaches (Zhang *et al.,* 2009a), apple (Lara and Vendrell, 2000), banana (Lohani *et al.,* 2004; Jiang *et al.,* 2000) and grape (Zhang et al; 2009a). In strawberry, exogenous ABA also induced ethylene production (Jiang and Joyce, 2003). The mechanisms by which ABA enhances fruit ripening are unknown and the role of the hormone in avocado fruit has not been elucidated. The function of ABA differs according to opinion: It was proposed that ABA stimulates ethylene biosynthesis (Riov *et al*., 1990) whereas others proposed that ABA increases sensitivity of tissues to ethylene (Lara and Vendrell, 2000). Zhang *et al*. (2009a) showed that following application of inhibitors of ABA synthesis (Fluridone and nordihydroguaiaretic acid (NDGA), ethylene production and ripening of peach fruit were suppressed. Whether ABA plays a role in ripening, through regulation of endogenous ethylene synthesis or enhancement of tissue responsiveness to ethylene, or not, remains unclear.

2.3 The role of ethylene in ripening of avocado fruit

Ethylene is a gaseous two-carbon hydrocarbon with a double bound which can diffuse into and out of plant tissues, from both biological and non-biological sources (Watkins, 2002). The phytohormone is involved in regulating many developmental

processes in plants, including fruit ripening, senescence, organ abscission, seed germination and stress responses, and responses to environmental variations (Abeles *et al.,* 1992). From an agricultural/horticultural perspective, the role of ethylene as a principal regulator of fruit ripening and its effects on colour, texture and flavour is the most important. Ethylene has been used historically to promote uniform ripening of climacteric fruits. However, the effects of ethylene can also be considered detrimental to fruit quality where ripening should be avoided, for instance when storage is required. Strategies for control of ethylene during storage, transport and handling operations have been widely developed for commercial purposes and will be outlined in this section.

The importance of ethylene in affecting ripening (or senescence) is reflected in the extensive literature on ethylene biosynthesis and perception, and its interaction with fruit ripening and quality (*cf.* Watkins, 2002). Especially, with the development of advanced techniques in genetic manipulation (i.e. transgenic plants with modified ethylene biosynthesis and perception genes) and the use of the binding inhibitor 1-MCP, significant progress has been made toward understanding the effects of ethylene on molecular regulation of a number of ripening parameters, although most molecular work has been carried out on tomato (Giovannoni, 2001).

2.3.1 Ethylene biosynthesis

In higher vascular plants, ethylene is produced via a relatively simple biosynthetic pathway (reviewed in Yang and Hoffman, 1984). The first stage in ethylene biosynthesis is the conversion of the amino acid methionine to S-adenosylmethionine (SAM) by methionine adenosyltransferase. S-adenosylmethionine is then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase, which is pyridoxal phosphate dependent. This reaction is the rate-limiting step in ethylene production. The synthesis of ACC requires oxygen, and because the enzyme is membrane-bound, the maintenance of membrane integrity. The final reaction is the conversion of ACC to ethylene by ACC oxidase (ACO) (Figure 2.3).

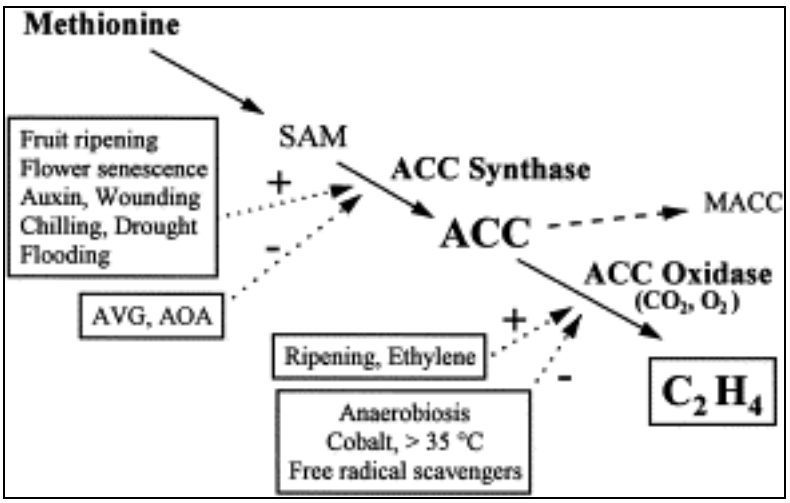

Figure 2.3: Biosynthesis of ethylene in higher vascular plants. Some of the intrinsic and extrinsic factors that promote (+) or inhibit (–) ethylene (C_2H_4) synthesis in higher vascular plants (from Saltveit, 1999). S-adenosylmethionine (SAM), 1-aminocyclopropane-1-carboxylic acid (ACC), 1-(malonylamino)- cyclopropane-l-carboxylic acid (MACC), amino ethoxy vinyl glycine,(AVG), amino-oxyacetic acid (AOA).

Ethylene may exert either positive or negative feedback regulation of its own biosynthesis and two different patterns (systems) of ethylene production have been defined, based on response to exogenous ethylene treatment (Lelièvre *et al.,* 1997). System 1 is common to non-climacteric tissues and preclimacteric fruit where the rate of ethylene production is low and is suppressed by exogenous ethylene. System 2 is the autocatalytic ethylene-production system active during the climacteric fruit ripening, and the rate of ethylene synthesis is significantly increased and further enhanced by exogenous ethylene. Ethylene production in plant tissue is primarily modulated by the level of activity of ACS and ACO (Bleecker and Kende, 2000) and autoinhibition of ethylene production in system 1 is attributed to suppression of activity of ACO and/or ACS (Riov and Yang, 1982; Atta-Aly *et al.,* 2000). ACS and ACO are encoded by multigene families which are differentially regulated during ripening (Cara and Giovannoni, 2008).

In preclimacteric avocado, autoinhibition of ethylene production has been demonstrated (Zauberman and Fuchs, 1973). Trace amounts of ACC and detectable expression but very low activity of ACS and ACO were measured in preclimacteric

avocado fruit at harvest, and their activity increased markedly with the onset of the climacteric (Sitrit *et al.,* 1986; Owino *et al.,* 2002; Hershkovitz *et al.,* 2010). Specifically, ACC content and ACS activity reached a maximum shortly before the climacteric peak, whilst ACO activity increased markedly only at the upsurge of climacteric ethylene production (Owino *et al.,* 2002). Hershkovitz *et al.* (2010) additionally showed that seedless avocado cv. Arad ripened earlier than seeded ones, and these fruit already had higher transcript levels of *PaACO* and *PaACS*1 at harvest. Exogenous pulses of ethylene or propylene to fruit within 24h of harvest caused an increase in ACO activity but not in the low, basal level of ACS activity and fruit did not ripen (Starrett and Laties, 1991). In that sense, the inability of avocado to produce ethylene as long as they are attached to the tree has been attributed to repression of ACS activity, as the limiting factor (Blumenfeld *et al.,* 1986; Sitrit *et al.,* 1986, Hershkovitz *et al.,* 2010).

The transition from system 1 to system 2 is the result of increased sensitivity of tissue to ethylene as fruit mature and is presumably mediated by differential regulation of multigene families of ACO and ACS (Cara and Giovannoni, 2008).

2.3.2 Ethylene binding, perception and response during ripening

Three steps are generally recognised in response to ethylene, which ultimately results in the phenotypic changes: 1) the perception of the hormone, 2) the transduction of the ethylene signal through regulation of gene expression and 3) the expression of genes and synthesis of proteins that are sensitive to the received signal (Cara and Giovannoni, 2001).

Ethylene is perceived by a family of receptors that are homologous to bacterial two component regulators. They are integral membrane proteins associated to the endoplasmic reticulum (Chen *et al.,* 2002) and exhibit protein kinase activities (Moussatche and Klee, 2004). The receptors are disulphide-linked dimmers and ethylene binds to a metal in the receptor (Burg and Burg, 1967), which is believed to be copper. The exact structure of the binding site is still not known. However, it was proposed that the mechanism of ethylene action was similar to the *trans* effect observed in inorganic chemistry (Sisler, 1977) which

involves substitution of the ligand in *trans* position upon ethylene binding. A rearrangement of ligands may then result in a conformational change in receptor (or an interaction with another component) and somehow induce an ethylene response through transduction of a signal downstream (Sisler, 1977).

Receptors normally act as negative regulators of the ethylene response pathway (Tieman *et al.,* 2000). In absence of ethylene, the receptor actively suppresses ethylene response and upon ethylene binding, this suppression is removed and a response then occurs. In agreement with this, experiments have shown that reduction in receptor content increased ethylene sensitivity (Tieman *et al.,* 2000) and vice versa (Ciardi *et al.,* 2000). Recent work on tomato has further demonstrated that receptor levels during fruit development may determine the timing of ripening: levels of receptor proteins were highest in immature tomato and decreased significantly at the onset of ripening, facilitating ethylene-mediated ripening (Kevany *et al.,* 2007). These authors also hypothesised that a conformational change in receptors due to ethylene binding, as mentioned above, render receptors susceptible to degradation, in turn modulating ethylene sensitivity. Nevertheless, how ethylene receptor signals downstream components remains unclear. Moreover, the above proposed mechanisms are based on work on tomato, yet it has not been proved that such systems operates in avocado fruit.

Receptors are encoded by a multigene family that includes some genes that are up-regulated during the onset of ripening (Hershkovitz *et al.,* 2010). The ethylene response gene (*ETR1*) was the first characterised in *Arabidopsis* and comprises a number of homologues characterised in both *Arabidopsis* and tomato (5 and 6 receptors isoforms, respectively), which have been classified in two sub-families according to predicted peptide structure (Cara and Giovannoni, 2008). In tomato, *LeETR*4 and *LeETR*6 (subfamily II receptors), are important negative regulators of ethylene response (Kevany *et al.,* 2007). CTR1, a key negative regulator of ethylene response, acts immediately downstream of the receptor (Kieber *et al.*, 1993). A multigene family of functional CTR1 genes is present in tomato and its members are differentially regulated by ethylene during fruit development (Adams-Phillips *et al.,* 2004).

Increases in ethylene response genes during ethylene production has been reported in many fruit including avocado *PaERS*1 (Owino *et al.,* 2002), *PaETR* and *PaERS*1 (Hershkovitz *et al.,* 2009). In avocado cv. Arad, Hershkovitz *et al.* (2010) found that the ethylene response genes exhibited low transcripts levels at harvest and only *PaETR* transcript accumulated significantly in parallel with ethylene production. In contrast, in seedless cv. Arad fruit, *PaERS*1 and *PaCTR*1 transcript levels were much higher than in seeded ones. The authors suggested a role for the seed in inhibiting the induction of ethylene response genes, therefore modulating ripening (Hershkovitz *et al.,* 2010). A cross talk mechanism from seed to mescocarp was not postulated.

Chilling stress of avocado cv. Arad in the orchard stimulated expression of *PaACS*1, *PaACS*2 and *PaACO*, ethylene and CO_2 production in fruit while still attached to the tree resulting in accelerated softening after harvest (Hershkovitz *et al.,* 2009). Cold storage stimulated expression of genes for ethylene biosynthesis and ethylene action compared with their levels at ambient temperature (Hershkovitz *et al.,* 2010). Similarly, low temperatures storage also stimulated expression of ACO and ACS genes in apples and pears (Tian *et al.,* 2002; El-Sharkawy *et al.,* 2003).

2.4 Strategies to prevent detrimental effects of ethylene on stored crops

Ethylene has been used historically to ripen fruit such as citrus, bananas and avocados in a uniform and predictable fashion. However, in the fruit industry, most strategies rely on avoiding ethylene effects in order to prolong storage life. A number of simple and basic strategies may be used to avoid deleterious ethylene effects, *viz.* keeping commodities away from both non-biological source (e.g. internal combustion engines) and biological sources (e.g. ripening, diseased and injured commodities). However, ethylene commonly accumulates during transport, handling and storage of harvested crops. Although no standard for detrimental effects of ethylene has been set due to important differences in ethylene sensitivity between commodities (Wills *et al.,* 2001), although it is known that ethylene is biologically active at extremely low (nL-μL L^{-1}) concentrations. Indeed, a relationship between storage life and ethylene concentrations in the atmosphere has been

demonstrated whereby ethylene levels higher than 0.10 μL L^{-1} would induce significant quality loss (Wills and Warton, 2000), with reduction in storage life and shelf life. Accelerated ripening/senescence depends on factors such as tissue sensitivity to ethylene, duration of exposure, ethylene concentration, atmospheric composition and temperature (Saltveit, 1999). In particular, deleterious effects of the hormone are temperature-dependent, with sensitivity to ethylene increasing as temperature increases in the range 0-20°C (Wills *et al.,* 2001). As mentioned earlier in this Chapter, the effect of ethylene dose (time x concentration) is not well documented, and this constitutes a gap in the scientific knowledge on mechanisms of ethylene action. It has been advised that ethylene should be kept at concentrations below 0.015 μL L^{-1} in storage areas (Wills *et al.,* 2001). However, this is not always achievable and other techniques, based on inhibition of ethylene production or more importantly action inhibition have been found useful. This section will review existing tools aiming at controlling ethylene.

2.4.1 Ventilation, storage temperature, controlled atmospheres

Ventilation is the easiest technique to overcome the ethylene effect. Ventilation however, is not applicable in sealed atmosphere (CA, some packaging types) and generates important loss of energy by subsequent necessity of cooling down the cold room. Low temperature storage is the primary means of reducing metabolic activity and increasing postharvest life of commodities (Watkins, 2002). However, most tropical fruit are prone to physiological disorders and chilling injury (CI) when exposed to low temperature (depending on commodities). Avocados are chilling sensitive and recommended storage temperature for cv. Hass is 5-7°C (Zamorano *et al.,* 1994). It has been suggested that postharvest hot water treatment prior to placement in cold storage could render fruit more resistant to chilling injury (Hofman *et al.,* 2002c).

Controlled atmosphere (CA) storage can extend storage life by reducing O_2 and/or increasing CO_2 concentrations. CA recommendation for storage of avocado fruit is 2-5% O_2 and 3-10% CO_2 (Kader, 2002). Burdon *et al.* (2008) showed that quality of avocado cv. Hass was better maintained during CA storage than air storage. Trials in South Africa have

shown that CA storage decreased considerably the risk of chilling injury (Truter and Eksteen, 1987).

2.4.2 Inhibitors of ethylene biosynthesis

Ethylene biosynthesis can be blocked using chemical compounds that act by repressing ACS and ACO or diversion of SAM through treatment with polyamines (cf. Martinez-Romero *et al.,* 2007). Aminoethoxyvinyl glycine (AVG), marketed as ReTain™, has been the most studied and is widely used to block ethylene synthesis of apples in the field. AVG acts by suppressing ACS activity. A potent ACO inhibitor is silver, formulated as silver thiosulfate, which is used as an active component in preservative mixtures for cut flowers (Staby *et al.,* 1993). This said, inhibitors of ethylene biosynthesis do not give long-lasting protection to the fruit from exogenous ethylene.

2.4.3 Inhibitor of ethylene perception

Inhibitors of ethylene perception have the advantage of protecting the tissue from both endogenous and exogenous ethylene, which is convenient for use on agricultural products. The compound 2,5-norbornadiene (NBD) counteracts with the ethylene receptor in a competitive manner hence preventing ethylene binding and action. NBD has been shown to be effective at delaying ripening of apples (Blankenship and Sisler, 1989). However, continuous exposure and application at high concentration are required for lasting efficacy. Moreover, its strong and repellent odour renders it unsuitable for agricultural purposes.

Cyclopropenes have been shown to be good antagonists of the ethylene response (Sisler *et al.,* 1996). They compete with ethylene prior to binding, but do not appear to be a competitor of the hormone once they are bound to the receptor (Dupille and Sisler, 1995). 1-methylcyclopropene (1-MCP) has been by far the most extensively studied ethylene treatment (*cf.* Blankenship and Dole, 2003; Watkins, 2006, 2008) due to its efficacy on

various crops. It is more stable than cyclopropene (CP) and 1000 times more active than 3,3-dimethylcyclopropene (3,3-DMCP; Sisler and Serek, 1997). 1-MCP exerts its action through interacting with the ethylene receptor and competing for binding sites (Blankenship and Dole, 2003). Whereas ethylene diffuses rapidly from the binding site after ethylene treatment, the antagonist in contrast remains bound for a longer period, hence preventing ethylene from binding and forming an active complex (Watkins, 2002). The beneficial effect of 1-MCP at maintaining postharvest quality has been reported for a wide array of climacteric (but also non-climacteric) commodities, including apples, tomatoes, plums, and avocado (cf. Watkins, 2006 for a detailed review). Other fruits for which 1-MCP has been shown to prevent ethylene action include apricot, banana, custard apple, mango, papaya and strawberry (Blankenship and Dole, 2003).

1-MCP is structurally related to ethylene, is effective at very low (nL L^{-1}) concentration, is odourless and non-toxic to humans and the environment (USA Environmental Protection Agency, 2002) and leaves little residue on commodities. 1-MCP was first developed commercially by the American company FloraLife Inc. and registered in 1999 as EthylBloc® for use on ornamentals. The gaseous compound was manufactured as a powder with 1-MCP active mixed within γ-cyclodextrin, which releases the gas when dissolved in water or in a solvent. Currently, 1-MCP is registered as Smartfresh® (AgroFresh Inc., a subsidiary of Rohm and Haas, Spring House, PA) and used commercially as postharvest treatment for various fruit in 6 EU countries and 15 countries outside Europe including US and Canada (Martinez-Romero *et al.,* 2007). Registered crops varies with countries but generally include apple, apricot, avocado, kiwifruit, mango, melon, nectarine, papaya, peach, pear, pepper, persimmon, pineapple, plantain, plum, squash, tomatoes and tulip bulbs (Watkins, 2002). Besides representing a much-appreciated commercial product, the ethylene action inhibitor constitutes a powerful tool to investigate ethylene involvement in ripening and senescence of horticultural commodities.

The extent and longevity of 1-MCP action is a function of time and concentration of the treatment, and depends on numerous factors such as species, cultivar, tissue and mode of ethylene biosynthesis induction (Watkins, 2002). In avocado, the ethylene antagonist effectively delayed or suppressed the respiration and ethylene production rates, softening,

colour change and activity of cell wall degrading enzymes (Feng *et al.,* 2000; Hofman *et al.,* 2001; Jeong *et al.,* 2002; Jeong and Huber, 2004; Adkins *et al.,* 2005; Hershkovitz *et al.,* 2005, Woolf *et al.,* 2005). 1-MCP has also been shown to down-regulate transcript levels of ethylene biosynthesis (*PaACS*1, *PaACS*2, *PaACO*) and ethylene response (*PaETR*, *PaERS*1*, PaCTR*1) genes (Owino *et al.,* 2002; Hershkovitz *et al.,* 2010). However, if results with 1-MCP have shown large effects on delaying ripening of many fruits and vegetables, it has been shown to have limited success in some fruits, such as peaches (Liu *et al.* 2005), and apricots (Dong *et al.* 2002; Lippert and Blanke 2004). Additionally, research has recently shown that 1-MCP also binds to non-target analytes present in storage facilities (*viz.* wet cardboard and wood material; Vallejo and Beaudry, 2006) and lipids in avocado (Dauny *et al.,* 2003). In avocado particularly, 1-MCP may cause problems of heterogeneous ripening within a fruit batch, uneven ripening within the fruit or cause "evergreen" disorders, when fruit will not ripen under shelf life conditions (Kruger and Lemmer, 2007; Ochoa- Ascencio *et al.*, 2008), thus generating considerable logistical issues.

2.4.4 Removal of ethylene

In recent years, research has focused mainly on the control of ethylene action by using 1-MCP rather than on effective removal of ethylene inside storage atmospheres. However, ethylene removal is often desirable in situations where ethylene accumulates along the food chain. This can be achieved using potassium permanganate ($KMnO_4$)-supported on activated alumina spheres (Ethysorb®), which oxidises ethylene to form CO_2 and H_2O. However, this scavenger may have limited long-term efficacy in environments of high relative humidity and where high ethylene accumulation occurs, large quantities of the adsorbent would be required rendering its use questionable (Wills and Warton, 2004). Moreover, it is not suitable in contact with food due to its high toxicity.

Ethylene can be adsorbed using activated carbon (Bailen *et al.,* 2006) and zeolites (Martinez-Romero *et al.,* 2007). The ability of activated carbon to adsorb depends on both a large surface area and the pore volume. A study on the use of activated carbon inside

packaging containing tomatoes showed that the totality of ethylene was not adsorbed. (Bailen *et al.,* 2006). Catalytic degradation (Conte *et al.,* 1992; Maneerat *et al.,* 2003, Bailen *et al.,* 2006; Martinez-Romero *et al.,* 2009a,b) entails the use of a catalyst (usually Platinum Group metals or titanium (TiO_2)) fixed on a support (usually activated carbon) to increase the rate of the chemical reaction and have been shown to reduce ethylene levels in storage environments containing fresh produce. However these techniques frequently require the system to be working at high temperature (100-250°C) to be effective, hence consuming energy. Another means of removing ethylene is by photo catalysis, which is based on light-activated catalyits. In this technique, the catalyst (usually TiO_2) is activated by natural or an artificial source of UV light (Martinez-Romero *et al.,* 2007). Titanium is relatively inexpensive, photostable and clean, and ethylene can be removed at room temperature. However, there is a need for permanent UV light.

In a previous study, Terry *et al.* (2007a) have demonstrated that a newly developed palladium (Pd)-promoted material was capable of removing ethylene at cool temperature (5-16°C) to sub-physiologically active levels and, accordingly, to effectively delay the climacteric-induced ripening of banana and avocado fruit. When pre-climacteric banana fruit were held in the presence of the Pd-promoted material (0-50 mg) for 3 days at 16°C, CO_2 production was reduced and control of colour change from green to yellow was observed (Figure 2.4 and 2.5).

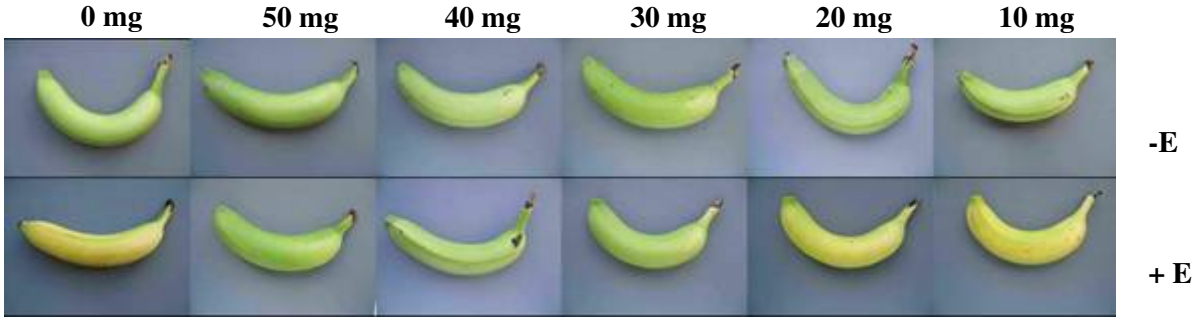

Figure 2.4. Colour of 5-day-old banana cv. Cavendish fruit previously held for 3 days at 16°C in 3 L sealed jars containing Pd-promoted material (0-50 mg) and previously treated with (+E) or without (-E) 100 µL L^{-1} ethylene when at pre-climacteric stage (i.e. green) at day 0 (Terry *et al.*, 2007a; Smith *et al.*, 2009).

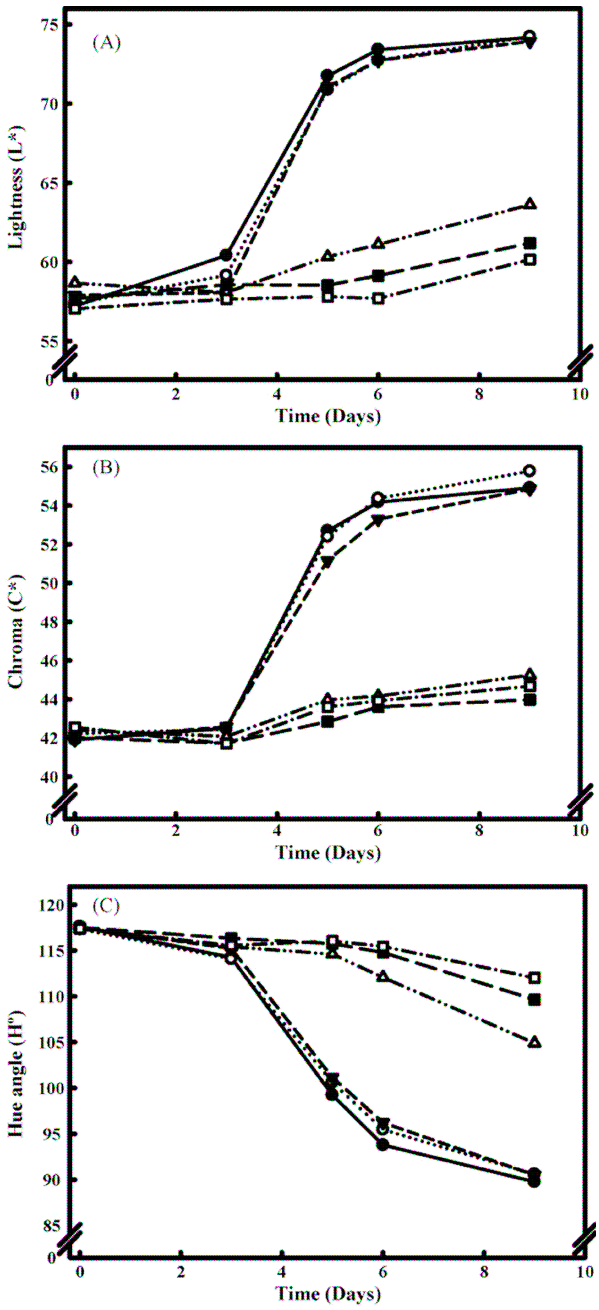

Fig. 2.5. (A–C) Effect of Pd-promoted material (0, (●); 10, (○); 20, (▼); 30, (Δ); 40, (■); 50, (□)) on change in L*, C* and H◦ of pre-climacteric banana cv. Cavendish fruit held at 16°C within 3 L sealed jars for 3 days which were initially treated with 100 µL L^{-1} ethylene. Fruit were removed and kept at 18°C for 6 days. LSDs (P = 0.05) for A, B and C= 2.108, 1.188 and 1.547, respectively (Terry *et al.*, 2007a).

In another experiment, avocado cv. Hass were enclosed with Pd-promoted material (0-1000 mg) for 3 days at 12°C, and then held 7 days in open air at 12°C. Presence of Pd-promoted material removed ethylene to sub-physiologically active levels and, accordingly, visual colour change of avocado cv. Hass fruit was affected (Figure 2.6). After 7 days, fruit held in the presence of 100 or 1000 mg Pd-promoted material for 3 days were generally greener than control fruit or fruit treated with Ethysorb® (Figure 2.6).

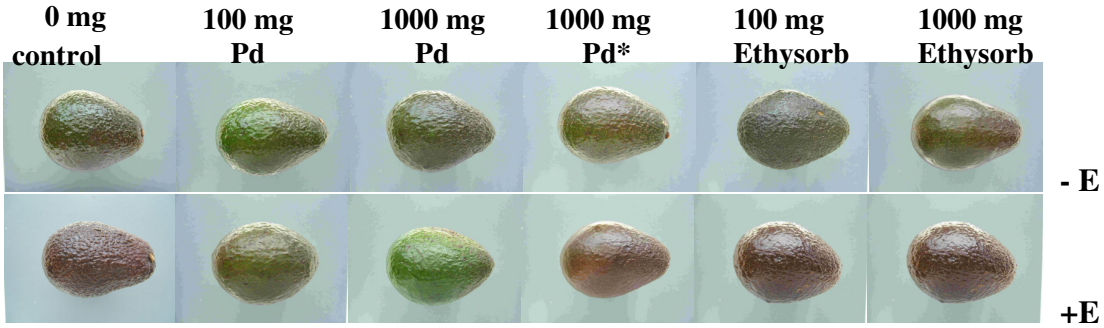

Figure 2.6. Colour of 7-day-old avocado cv. Hass fruit previously held for 3 days at 12°C in 3 L sealed jars containing Pd-promoted material (0, 100, 1000 mg) and Ethysorb (100, 1000 mg) and previously treated with (+E) or without (-E) 100 µL L^{-1} ethylene when at pre-climacteric stage (i.e. green) at day 0. * = JM Pd-promoted material (1000 mg) was put in jars after day 1 after fruit had been treated with or without 100 µL L^{-1} ethylene (Terry *et al.*, 2007a; Smith *et al.*, 2009).

The material, manufactured by Johnson Matthey Plc., consisted of a zeolite impregnated with fine Pd particles (metal loading 2.5% Pd (m/m)). Transmission electron microscopy (TEM) analysis (Figure 2.7) indicated that the Pd particles (bright particles) were well dispersed over the zeolite, which contributed to the efficacy of the scavenger (Smith *et al.*, 2009).

Initial tests were conducted to determine the ethylene adsorption capacity of the material using a synthetic gas stream. Ethylene adsorption capacity measurements were carried out at room temperature (21ºC) in a plug flow reactor using 0.1 g of active Pd-based material inside a gas composition of 200 µL L^{-1} ethylene, 10% (v/v) oxygen balanced with

helium, at a flow rate of 50 mL min^{-1}, with and without *ca.* 100% relative humidity (RH). Reactor outlet gas concentrations were analysed by mass spectrometry (Smith *et al.,* 2009). Results showed a considerable ethylene absorption capacity of typically 45,600 μL g^{-1} under low %RH and 4162 μL g^{-1} under *ca.* 100% RH.

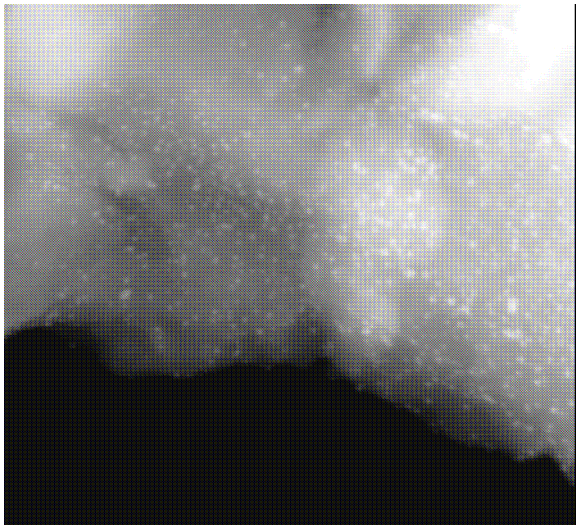

Figure 2.7. Transmission electron microscopy (TEM) image of the Pd-promoted zeolite material showing nanometre size palladium particles (bright areas) on the zeolite support (from Smith *et al.*, 2009).

In another experiment, 0.1 g active Pd-based material was held in the presence of a gas mixture (550 μL L^{-1} ethylene, 40% (v/v) air balanced with argon) at room temperature in a sealed, unstirred batch reactor (0.86 L). The totality of ethylene was removed within 2 hours (Figure 2.8). A slight production of ethane and CO_2 arose from the catalytic oxidation of some ethylene over the Pd-promoted material. However, these products represented a minor fraction of the total carbon balance, and the majority of the ethylene was adsorbed on the Pd-promoted material (Terry *et al.,* 2007a; Smith *et al.,* 2009).

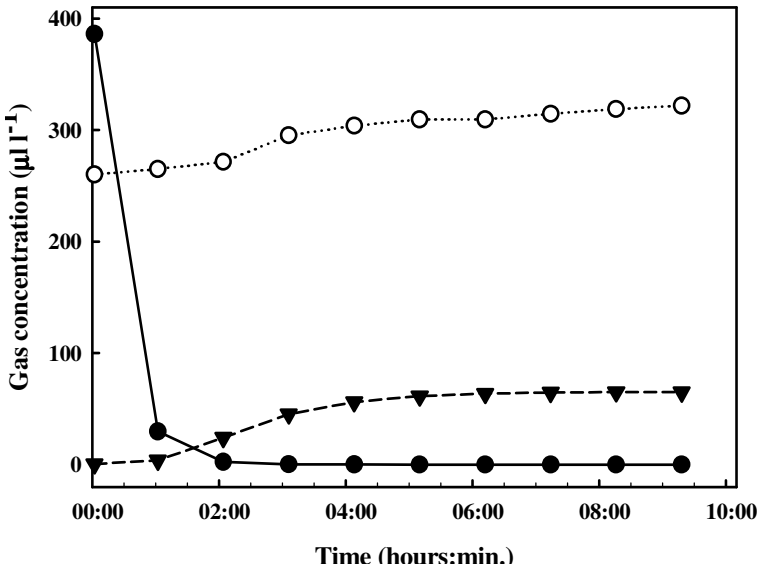

Figure 2.8. Gas concentrations (ethylene, ●; CO_2, ○; ethane, ▼) in a batch reactor in presence of 0.1 g of the Pd-promoted ethylene scavenger. The reactor initially containing 550 µL L^{-1} ethylene. Some ethylene has been removed by the scavenger prior to first measurement point (from Terry *et al.*, 2007a; Smith *et al.,* 2009).

The Pd-based material is acting largely as an adsorber rather than as a catalyst. Further characterisation of the ethylene–metal interaction using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) analysis indicated that presence of the metal played a significant role in the retention of the ethylene and that Pd was required to achieve the 45,600 µL g^{-1} adsorption capacity observed under dry conditions (Smith *et al.,* 2009).

Although zeolites are commonly considered as an adsorbent, the key to the Pd-promoted material is the interaction of a specific platinum group metal with a carefully selected zeolite support to remove significant amounts of ethylene at low and room temperature. Over 100 samples were screened to investigate the best metal and support combination leading to a patent application [WO2007\052074] on this novel material. This technology had never been applied to fresh produce before the study by Terry *et al.* (2007a). Whilst previous work (Bailen *et al.,* 2006; Martinez-Romero *et al.,* 2009a, 2009b) has used a Pd-based ethylene scavenger at 8ºC, 20ºC and in a heated device (>100ºC), hence acting as a catalyst, the present material does not necessitate application of heat and

is effective when used at 5ºC (i.e. acting principally as a non-catalyst) (Terry *et al.,* 2007a). Moreover, in prior studies (Bailen *et al.,* 2006; Martinez-Romero *et al.,* 2009a, 2009b), Pd was supported on granular activated carbon rather than on a powdered zeolite as herein.

Following the study by Terry *et al.* (2007a), a new formulation of the Pd-promoted material was produced by metal thrifting and this formulation is now registered as e+® Ethylene Remover since 2009. e+® Ethylene Remover has the same properties as the Pd-promoted material used in previous studies (Terry *et al.*, 2007a; Smith *et al.*, 2009) but with a metal loading of 1 % Pd (m/m).

2.5 Conclusions

The role and importance of ethylene, as well as its mechanisms of action, and the biochemical and physical changes that occur in avocado fruit during storage and ripening have been considered. The hormone ethylene and its mode of action on fruit ripening have been extensively studied whereas less is known about ABA, which appears to be an important feature of fruit ripening. Most research concerned with extending avocado storage life has concentrated on the use of 1-MCP. In contrast, and in spite of the known problems encountered with the use of 1-MCP on certain fruit including avocado, efforts toward developing new and more efficacious methods to control ethylene in avocado storage has remained practically non-existent. The novel Pd-promoted ethylene scavenger, e+® Ethylene Remover, may provide an alternative (or may complement) to 1-MCP for maintaining quality of avocado and possibly bring new insights to ripening mechanisms.

CHAPTER 3

Development of a rapid method for extraction and quantification of fatty acids and sugars from avocado mesocarp sample

3.1 Introduction

Avocado fruit is valued for the high lipid content in mesocarp tissue, which can vary from between 15 and 30% (on a fresh weight basis) depending on cultivar and seasonality (Lewis, 1978; Lee, *et al.,* 1983). Avocado fruit is considered to be one of the most important natural sources of monounsaturated food-derived lipids and essential fatty acids such as linoleic and linolenic acid (Jakab *et al.,* 2002). The avocado mesocarp is an excellent source of monounsaturated fatty acids (FA) and research has shown that a high avocado enriched diet may contribute to decrease risks of cardiovascular disease, possibly by lowering total and low density lipoproteins (LDL) cholesterol levels whilst increasing high density lipoproteins (HDL) (Ledesma *et al.,* 1996; Carranza-Madrigal *et al.,* 1997). Oil accumulates during fruit growth and maturity on the tree, with a large increase in oleic acid. Once fruit has been harvested, oil no longer accumulates and it has been shown that there is little change in fatty acid profile during postharvest ripening (Ozdemir and Topuz, 2004).

Soluble sugar content in avocado mesocarp tissue is dominated by the seven carbon (C7) sugar, D-mannoheptulose, and the corresponding sugar alcohol, perseitol (Liu *et al.,* 1999b, 2002; Bertling and Bower, 2005), whilst sucrose, glucose, fructose are present in lower concentrations. Sugars play an essential role in avocado fruit growth and development but are also considered important respiratory substrates during fruit ripening (Liu *et al.,* 2002). It has also been suggested that the C7 sugars could be involved in the ripening inhibition of the fruit while still attached to the tree and shortly after harvest (Liu *et al.,* 2002). Mannoheptulose has been linked to improved health and recent research has reported that it may inhibit insulin secretion and have anticancer activity (Ferrer *et al.,* 1993; Board *et al.,* 1995; Xu *et al.,* 1995).

The standard method for determining lipid content in various foodstuffs is the Soxhlet technique, which commonly uses conventional solvents such as hexane with a boiling point (bp) of 66-69ºC or petroleum ether (bp 40-60ºC). Accordingly, the Soxhlet method has been used extensively to extract oil from avocado mesocarp (Lewis *et al.,* 1978; Lee *et al.,* 1983; Werman and Neeman, 1987; Ortiz-Moreno *et al.,* 2003; Ozdemir and Topuz, 2004; Mostert *et al.,* 2007). However, this technique has the disadvantage of being time-consuming and requires operation at relatively high temperature. Alternative lipid extraction techniques such as homogenization with a solvent (e.g. petroleum ether) or supercritical carbon dioxide (SC-CO_2) have been compared to Soxhlet extractions in avocado fruit (Lewis *et al.,* 1978; Mostert *et al.,* 2007). In contrast to lipid analysis in avocado, there is a paucity of published methods describing the extraction and quantification of soluble sugars from avocado mesocarp tissue. Most protocols rely on the use of 80% ethanol (v/v; Liu *et al.,* 1999b, 2002; Bertling and Bower, 2005) as the extracting solvent. Crucially, lipids are not recovered during this process and to the best of our knowledge, no method has yet been reported whereby both lipids and sugars have been extracted from the same mesocarp sample.

The aim of this work was, therefore, to develop a simple and rapid procedure to sequentially extract and quantify both neutral lipids and sugars from the same mesocarp sample of avocado fruit at three different ripening stages, and compare this to previously published methods that have quantified lipids or sugars separately.

3.2 Materials and method

3.2.1 Reagents, plant material and sample preparation.

All chemicals used were of analytical grade. Hexane, methanol and ethanol were purchased from Fisher Scientific Chemicals (Leics., UK). Methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, methyl linolenate, sucrose, D-glucose, D-fructose and D-mannoheptulose standards were purchased from Sigma (Dorset, UK).

Perseitol (D-glycero-D-galacto-heptitol) was obtained from Industrial Research Ltd. (IRL - Fine Chemicals, New Zealand).

Early season avocado (*Persea americana* Mill.) cv. Hass fruit (n = 72), originating from Malaga (Spain), were harvested on the 25th January 2007 and supplied by Mack Multiples Division (M. W. Mack Ltd., Kent, U.K.). Fruit were stored overnight at 12°C. Fruit were not pre-treated with 1-MCP. On arrival at the laboratory, fruit were 4 days-old after harvest and were considered to be pre-climacteric. Fruit were held in 3 L jars at 12°C for 3 days then removed to avoid CO_2 poisoning (Terry *et al.,* 2007a). On days 3, 5 and 9, a fruit sub-sample (n = 18) was removed, and lightness (L*), chroma (colour saturation; C*) and hue angle (Hº) were measured as described in section 4.2.6 of Chapter 4. Firmness was measured using an Instron Universal Testing Machine (Model 1122, Bucks., UK) fitted with an 8 mm diameter flat probe as described in section 4.2.6 of Chapter 4. Three levels of ripeness, defined by firmness range (*viz.* under ripe (>50 N), medium ripe (50-15 N) and eat-ripe (<5 N)), were selected, comprising three fruits per maturity level and used for further lipid and soluble sugars analysis. Each fruit was a replicate and extracted for lipid and soluble sugars in triplicate.

Fruit were cut in half vertically into two equal sections. The stone and peel of one half were removed manually and slices were sequentially cut starting from the apex towards the basal end of the fruit. These slices were then quickly chopped into small chunks, mixed and pooled to ensure randomization between tissues originating from the apical and basal end of the fruit. Approximately 30 g of pooled sample was immediately snap-frozen in liquid nitrogen and held at -40°C before being freeze-dried in a Christ ALPHA-RVC freeze-drier with cooling-trap ALPHA 1-4 (Christ, Osterode, Germany) for 7 days. Dry weight (DW) was determined and samples returned to -40°C prior to analysis.

3.2.2 Lipid extraction.

Lyophilized mesocarp tissue (1 g, *ca.* 3.7 g fresh weight (FW)) was ground to a powder using a pestle and mortar, and homogenized with hexane (30 mL) for 30s using an Ultra-Turrax T25 homogeniser (Janken & Kunkel Ika-Labortechnik, Staufen, Germany).

The mixture was allowed to stand at room temperature for 1 min before filtering under vacuum, using a Buchner flask and funnel, through a 5.5 cm diameter Fisherbrand QL 100 filter paper (Fisher Scientific, Leics., UK). The powdered residue was recovered from the filter paper and washed again with 20 mL of fresh hexane. The mixture was again allowed to stand at room temperature for another 1 min before being filtered as before. Additional hexane (10 mL) was used to rinse the beaker and funnel. All lipid-containing filtrates were combined (60 mL) and the solvent removed using a rotary evaporator (Buchi Rotovapor, Büchi Labortechnik AG, Flawil, Switzerland) under vacuum at 40°C. The recovered oil was weighed and stored under nitrogen in capped amber glass vials at -40°C until lipid analysis. The filter residue was allowed to stand for approx. 2 h at room temperature until no more hexane was present. The residue was weighed and stored in vials at -40°C for subsequent extraction and analysis of non-structural carbohydrates.

The Soxhlet technique was used for validation of the method described above and carried out according to AOAC 963.15 (AOAC, 1995) with modifications. The thimble containing the same ground freeze-dried mesocarp sample (1 g, *ca*. 3.7 g FW) was placed in the Soxhlet device and 150 mL of hexane placed in the round flask with few defatted antibumping granules (Fisher Scientific, Leics., UK). The sample was refluxed for approx. 1 h, with the heat adjusted so that the extractor siphoned eight times (approx. 70ºC). The flask was removed and the solvent evaporated on a rotor evaporator as previously described. The recovered oil was weighed and stored as before. The thimble residue was allowed to stand at room temperature until no more hexane was present and the residue then stored as described earlier.

3.2.3 Sugar extraction

Extracts for soluble sugars were prepared from the residue obtained following either hexane homogenization or Soxhlet extractions, using either methanol or ethanol (following homogenization only) as solvents. Powdered residue (150 mg) was combined with 3 mL of 62.5% aqueous methanol (v/v) (Terry *et al.,* 2007b) or 3 mL of ethanol 80% (v/v) (Liu *et al.,* 1999b) and mixed well. Vials (7 mL polystyrene bijou vials; Sterilin, Staffs., UK)

containing the slurry were placed in a shaking water bath at 55ºC for 15 min, removed briefly and vortexed (Vortex Genie 2, Scientific Industries, NY) for 20s every 5 min. The samples were then filtered through syringe filters (0.2 µm pore diameter; Millipore Corp., MA) and stored at -40ºC until needed. Extracts were diluted 1:10 with water (HPLC grade) immediately before analysis.

3.2.4 Fatty acid identification and quantification

Fatty acid methyl esters (FAMEs) were produced according to the method prescribed by the IOOC (International Olive Oil Council, 2001) with modifications. Briefly, 0.2 ml of methanolic KOH (2 N) was added to 0.1 g avocado oil extract in 2 mL hexane. Hexane was chosen as the preferred solvent due to improved peak resolution. The mixture was shaken vigorously for 30 s and left to stratify until the upper layer became clear. The hexane layer containing the methyl esters was decanted and kept for no more than 12 h at 5ºC until needed. This solution was diluted 1:100 (v/v) with fresh hexane immediately before injection into an Agilent 6890N GC (Agilent Technologies, Cheshire, UK) equipped with a G1540N flame ionisation detector (FID) and a 7683B autosampler. The identification and quantification of selected compounds was performed on a CP-Sil 88 fused silica capillary column (30 m x 0.25 mm i.d., 0.2 µm film thickness; Varian, CA). Column temperature was programmed at 55°C for 3 min, and then raised to 175°C at 13°C/min intervals followed by an isothermal period of 1 min and increased again to a final temperature of 220°C at 8 °C/min. The carrier gas was He at a constant flow rate of 1.6 mL min^{-1}. The injector and detector temperatures were set at 220 and 250°C, respectively. The presence and abundance of fatty acids was calculated by comparison of peak area with standards (methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, methyl linolenate).

3.2.5 Sugar identification and quantification

Concentrations of fructose, glucose, sucrose, mannoheptulose and perseitol were determined using a HPLC system comprising a P580 pump, Dionex STH column thermostat and GINA 50 autosampler (Dionex, CA) based on that described previously (Terry *et al.,* 2007b). The diluted avocado extract (20 µL) or standard sugar solution was injected into a Rezex RCM monosaccharide Ca^{+} (8%) size exclusion column of 300 mm x 7.8 mm diameter, 8 µm particle size (Phenomenex, Torrance, CA; part no.00H-0130-K0) with a Carbo-Ca^{+} security guard cartridge of 4 mm x 3 mm diameter (Phenomenex, CA). The mobile phase was HPLC grade water at a flow rate of 0.6 mL min^{-1}. Column temperature was held at 75ºC using Dionex STH column thermostat. Eluted soluble sugars were monitored using an evaporative light scattering detector (ELSD 2420, Waters, MA) (Terry *et al.,* 2007b) connected to the Dionex system using UCI-50 universal chromatography interface. The presence and abundance of the selected sugars were automatically calculated by comparison of peak area with peak area of known standards using Chromeleon version 4.6 software (Dionex).

3.2.6 Statistical analysis

All statistical analyses were carried out using Genstat for Windows vers. 10 (VSN International Ltd., Herts., UK). Data were subjected to analysis of variance (ANOVA). Least significant difference values (LSD; $P = 0.05$) were calculated for mean separation using critical values of t for two-tailed tests. Tests for correlations between mean values for sugars concentrations were made using Pearson's product moment correlation. Correlations are presented with the Pearson's correlation coefficient (r) and P value based on a two-tailed test. Unless otherwise stated significant differences were $P < 0.05$. Means with different letters in tables are significantly different from one another ($P < 0.05$).

3.3 Results

3.3.1 Physical measurements

During 9 days of storage at 12ºC, there was a decrease in fresh weight, firmness, L* C* values and H° and an increase in dry matter content (**Table 3.1**). Fresh weight, L*, C* and Hº were all significantly lower at eat-ripe stage as compared with medium and under ripe stage. Eat-ripe and medium ripe fruits were significantly less firm as compared with under ripe fruits. Concomitant to changes in colour, weight and firmness, dry matter content increased (but not significantly) as ripening advanced. (**Table 3.1**).

Table 3.1. Effect of ripening stage (*viz.* under ripe (UR), medium ripe (MR) and eat-ripe (ER)) and storage time on fresh weight (FW), dry matter (DM) content (% FW), firmness (N), lightness (L*), chroma (C*) and hue angle (Hº) of avocado cv. Hass fruit stored for 9 days at 12ºC.

Ripening stage	Storage days	FW (g)	DM (% FW)	Firmness (N)	L*	C*	Hº
UR	2	181.74[a]	25.63[a]	74.67[a]	33.86[a]	17.33[a]	123.58[a]
MR	5	179.50[a]	26.71[a]	25.00[b]	30.46[ab]	12.90[a]	114.80[a]
ER	9	166.33[b]	27.09[a]	3.83[b]	26.76[b]	6.45[b]	48.11[b]

[ab] different letters indicate significant differences ($P < 0.05$).

3.3.2 Oil yield and fatty acid identification and quantification

The Soxhlet extraction technique resulted in a significantly higher quantity of oil extracted from avocado mesocarp tissue (0.61 g oil g^{-1} total mesocarp tissue DW, 16% FW) as compared with the homogenization extraction technique (0.54 g oil g^{-1} DW, 14% FW), respectively. The gas chromatography method developed and presented in this study successfully identified and quantified fatty acids in avocado oil extracts. A final runtime of <20 min. was required to elute all fatty acids present (Figure 3.1).

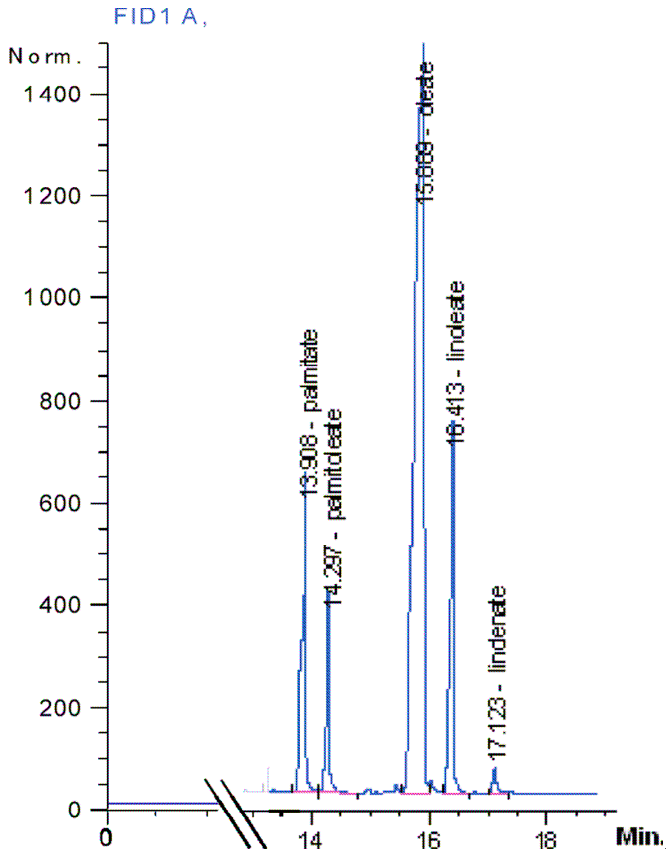

Figure 3.1: Typical GC-FID chromatogram of the main fatty acids present in oil extracted from avocado cv. Hass fruit mesocarp tissue.

In all oil samples, oleic acid was predominant constituting 56.93% of total fatty acids. In descending order of abundance, other fatty acids quantified were palmitic (20.92%), linoleic (12.16 %), palmitoleic (8.88 %) and linolenic acids (1.12%). The fatty acid profiles (% total fatty acids) of the oils extracted by homogenization and by Soxhlet were very similar with no significant differences found between extraction methods for palmitic, palmitoleic, oleic and linoleic acids (Table 3.2). However, the proportion of the polyunsaturated fatty acid linolenic acid was significantly, yet slightly, higher following homogenization with hexane (1.14%) in comparison to the Soxhlet technique (1.10%).

The effect of ripening stage on oil yield, oil fatty acid composition and concentrations was investigated for samples extracted by homogenization. Oil recovery was improved as fruit ripened, with values significantly higher at eat-ripe stage (0.61 g g^{-1} DW) as compared with medium and under ripe stages (0.53 g g^{-1} DW and 0.48 g g^{-1} DW, respectively). Additionally, ripening stage had a main effect on the fatty acid profile, whereby the dominant fatty acid, oleic acid, was significantly lower at medium ripe stage (54.90%) as compared with under ripe and eat- ripe stages (57.72 and 58.25%, respectively). On the other hand, the second most abundant fatty acid, palmitic acid, was significantly higher at medium ripe (21.83%) and eat-ripe stages (20.89%) vs. under ripe stage (19.84%). Per DW, concentrations of all fatty acids increased concomitantly to progressing ripeness. Specifically, palmitic, palmitoleic and linoleic acid amounts were significantly greater at medium and eat-ripe stages as compared with under ripe stages, whilst significantly greater concentrations of oleic acid were found at eat-ripe stage vs. under and medium ripe stages (Table 3.2).

Table 3.2. Effect of extraction methods on the concentration of fatty acids (FA) in avocado cv. Hass fruit mesocarp at under ripe (UR), medium ripe (MR) and eat-ripe (ER) stages, expressed as % total (FA), per dry weight (DW) and per g oil.

	Ripening st	Palmitic acid		Palmitoleic acid		Oleic acid		Linoleic acid		Linolenic acid	
		Homog.*	Soxhlet**	Homog.	Soxhlet	Homog.	Soxhlet	Homog.	Soxhlet	Homog.	Soxhlet
% total FA	UR	19.84^{aB}	19.87^{a}	8.82^{aAB}	8.82^{a}	57.72^{aA}	57.76^{a}	12.44^{aA}	12.43^{a}	1.19^{aA}	1.11^{a}
	MR	21.83^{aA}	22.06^{a}	9.50^{aA}	9.44^{a}	54.90^{aB}	54.70^{a}	12.71^{aA}	12.64^{a}	1.16^{aA}	1.16^{a}
	ER	20.89^{aA}	21.01^{a}	8.34^{aB}	8.38^{a}	58.25^{aA}	58.26^{a}	11.45^{aA}	11.31^{a}	1.07^{aA}	1.03^{a}
	Mean	20.85^{x}	20.98^{x}	8.89^{x}	8.88^{x}	56.95^{x}	56.91^{x}	12.20^{x}	12.13^{x}	1.14^{x}	1.10^{y}
mg g^{-1} oil	UR	29.05^{aC}	29.19^{a}	12.93^{aB}	12.95^{a}	84.53^{aA}	84.93^{a}	18.35^{aA}	18.17^{a}	1.75^{aA}	1.62^{a}
	MR	34.70^{aA}	30.04^{b}	15.37^{aA}	12.82^{b}	87.34^{aA}	74.48^{b}	20.32^{aA}	17.13^{b}	1.86^{aA}	1.58^{a}
	ER	31.93^{aB}	29.98^{a}	12.76^{aB}	11.96^{a}	88.95^{aA}	83.14^{b}	17.50^{aA}	16.13^{b}	1.63^{aA}	1.47^{a}
	Mean	31.89^{x}	29.73^{y}	13.69^{x}	12.58^{y}	86.94^{x}	80.85^{y}	18.72^{x}	17.14^{y}	1.75^{x}	1.56^{y}
mg g^{-1} DW	UR	13.74^{aB}	16.43^{a}	6.17^{aB}	7.29^{a}	40.62^{aB}	47.95^{a}	8.77^{aB}	10.30^{a}	0.84^{aA}	0.92^{a}
	MR	18.35^{aA}	18.04^{a}	8.09^{aA}	7.65^{a}	46.14^{aB}	44.87^{a}	10.70^{aA}	10.35^{a}	0.98^{aA}	0.95^{a}
	ER	19.40^{aA}	20.09^{a}	7.78^{aA}	8.03^{a}	54.07^{aA}	55.45^{a}	10.64^{aA}	10.78^{a}	0.99^{aA}	0.98^{a}
	Mean	17.16^{x}	18.19^{x}	7.35^{x}	7.65^{x}	46.95^{x}	49.43^{x}	10.03^{x}	10.47^{x}	0.94^{x}	0.95^{x}

* Homogenization with hexane. ** Soxhlet extraction with hexane. ^{a,b} different letters within the same ripening stage between the methods are significantly different ($P < 0.05$). LSD used for comparing means within same levels of ripeness. ^{x,y} different letters between the methods mean are significantly different ($P < 0.05$). ^{A,B} different letters within the column 'Homog.' between the ripening stage are significantly different ($P < 0.05$). LSD used for comparing means within the method of homogenization with hexane only.

3.3.3 Soluble sugars

Soluble sugars were extracted from the filter residue recovered after lipid extraction. For the extraction and quantification of sugars, the method described in this work was successfully adapted and slightly modified from previously reported methods applied to other fruit (Terry *et al.,* 2007b). Mannoheptulose, perseitol, and sucrose were the main sugars identified in all samples (Figure 3.2). Fructose and glucose were detected, but their presence was at or near the detection limit, and thus they were not considered (Figure 3.2).

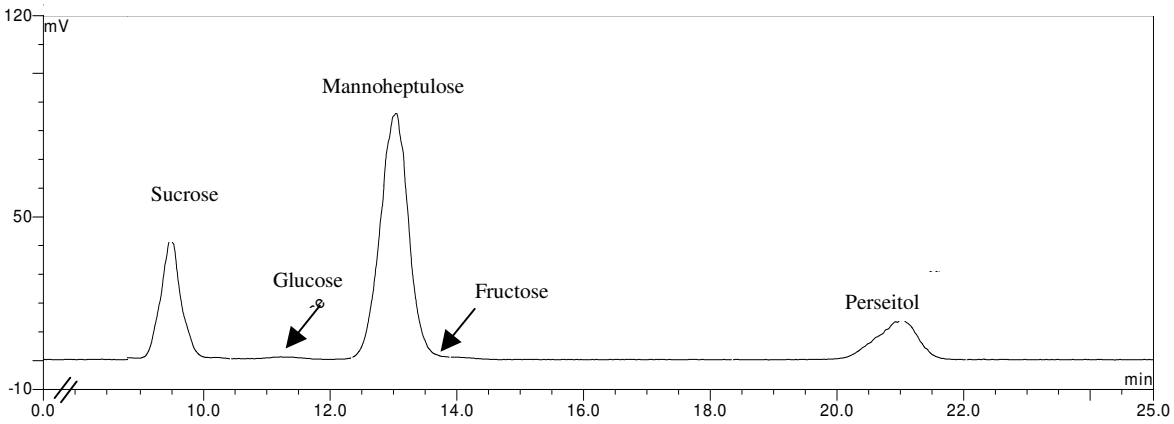

Figure 3.2. Typical HPLC-ELSD chromatogram of main sugars extracted from avocado cv. Hass fruit mesocarp tissue after lipid removal.

Sugars were differentially affected by the extraction method (Table 3.3). Specifically, sucrose and perseitol concentrations (residue and DW basis) were both significantly lower in ethanol extracts than in methanol extracts following either homogenization or Soxhlet extraction (Table 3.3). In contrast, the use of either methanol or ethanol as extraction solvent had no effect on mannoheptulose for residues obtained from homogenization with hexane. This said, sugar extractions from Soxhlet residue consistently resulted in the highest concentrations of sucrose, mannoheptulose and perseitol (Table 3.3). When the effect of ripening stage on sugar concentrations was investigated for samples

extracted by homogenization with hexane followed by methanolic extraction, mannoheptulose and perseitol (residue weight, DW and FW) were generally lowest at eat-ripe stage. In particular, greater amounts of mannoheptulose, on a residue and DW basis, were extracted from under ripe and medium ripe fruit vs. eat-ripe fruit, whereas perseitol concentration was significantly higher at under ripe vs. medium and eat-ripe stages. In contrast, little difference between ripeness was seen for sucrose concentrations on a residue basis (Table 3.3). Again, there was a significant interaction between extraction method and ripening stage for all sugars, whereby significantly lower amounts of sucrose, mannoheptulose and perseitol (residue basis) were obtained in ethanol extracts vs. methanol extracts at the under ripe stage. At the medium ripe stage, sucrose concentration was also significantly lower following ethanol extraction as compared with methanol, while no differences at medium and eat-ripe stages were found between methods for mannoheptulose and perseitol. Per DW, significant differences between the methods for sucrose and perseitol were found at under ripe stage, with greater concentrations in methanol extracts as compared with ethanol extracts, whereas no significant differences were found between methods for mannoheptulose (Table 3.3).

Table 3.3. Effect of extraction methods on the concentrations of sugars in avocado cv. Hass fruit mesocarp at under ripe (UR), medium ripe (MR) and eat-ripe (ER) stages, expressed per residue weight and per dry weight.

	Ripening stage	Sucrose			Mannoheptulose			Perseitol		
		Homog. + MeOH*	Soxhlet + MeOH**	Homog.+ EtOH***	Homog. + MeOH*	Soxhlet + MeOH**	Homog.+ EtOH***	Homog. + MeOH*	Soxhlet + MeOH**	Homog.+ EtOH***
mg g^{-1}residue	UR	41.20^{aA}	38.99^{b}	30.94^{c}	57.49^{aA}	59.62^{a}	53.75^{b}	58.98^{bA}	66.95^{a}	53.13^{c}
	MR	29.63^{bB}	32.69^{a}	26.59^{c}	80.52^{bA}	94.12^{a}	81.86^{b}	44.77^{bB}	51.83^{a}	43.73^{b}
	ER	36.41^{bAB}	44.03^{a}	34.64^{b}	32.12^{bB}	35.83^{a}	32.57^{b}	40.66^{bB}	43.59^{a}	39.33^{b}
	Mean	35.75^{y}	38.57^{x}	30.72z	56.71^{y}	63.19^{x}	56.06^{y}	48.14^{y}	54.13^{x}	45.40z
mg g^{-1} DW	UR	19.74^{aA}	17.72^{b}	14.56^{c}	27.82^{aA}	27.51^{a}	25.85^{a}	28.14^{bA}	30.62^{a}	25.16^{c}
	MR	14.01^{abB}	14.95^{a}	12.37^{b}	37.96^{aA}	42.64^{a}	38.32^{a}	21.03^{bB}	23.47^{a}	20.44^{b}
	ER	13.70^{bB}	15.91^{a}	13.17^{b}	12.14^{aB}	12.83^{a}	12.26^{a}	15.54^{aC}	15.66^{a}	14.93^{a}
	Mean	15.81^{x}	16.19^{x}	13.37^{y}	25.97^{y}	27.66^{x}	25.48^{y}	21.57^{y}	23.25^{x}	20.18z

* Homogenization with hexane followed by methanolic extraction. ** Soxhlet extraction with hexane followed by methanolic extraction. *** Homogenization with hexane followed by ethanolic extraction. ^{a,b,c} different letters within the same ripening stage between the methods are significantly different ($P < 0.05$). LSD used for comparing means within same levels of ripeness. ^{x,y,z} different letters between the methods are significantly different ($P < 0.05$).^{A,B} different letters within the column 'Homog. + MeOH' between ripening stages are significantly different ($P < 0.05$). LSD used for comparing means within the method of homogenization with hexane followed by methanolic extraction only.

Pearson's product moment correlations were drawn between the three extraction method for sugars, *viz.* methanol extraction following either Soxhlet or homogenization and ethanol extraction following homogenization. There was generally a good correlation (r = 0.8 - 0.9) between the methanol and ethanol extractions following homogenization, for all sugars and all ripening stages. However, the correlation between the methanol extractions following homogenization or Soxhlet was much poorer ($P > 0.05$) for sucrose (0.48) and for perseitol (0.47) at eat-ripe stage.

3.4 Discussion

Oil content and dry matter of avocado fruit is associated with avocado fruit horticultural maturity and therefore has often been used as a basis for determining harvesting time. Fatty acid composition defines oil quality. Therefore, it is important to have an appropriate method that adequately recovers lipids from avocado fruits. Oil yield from avocado mesocarp is variable and differs according to genotype, harvesting time and postharvest ripening (Lewis, 1978; Ozdemir and Topuz, 2004). Nevertheless, the results of the present study (0.54-0.61 g oil g^{-1} DW; 0.14-0.16 g oil g^{-1} FW) are in general agreement with that reported in earlier studies, where values of 56-58 % DW were found for avocado cv. Hass fruit (Ozdemir and Topuz, 2004) and *ca*. 54% DW were found for unknown cultivars extracted with hexane (Ortiz Moreno *et al.,* 2003). Higher values of 74-75% DW (*ca*. 23.5% FW; Lewis *et al.,* 1978) and *ca*. 70% have been reported for avocado cv. Fuerte (Mostert *et al.,* 2007).

The fatty acid profile from all tested samples (Table 3.2) was as expected for avocado and consistent with that reported in the literature for avocado fruit in general (Werman and Neeman, 1987; Ortiz Moreno *et al.,* 2003; Ozdemir and Topuz, 2004; Haiyan *et al.,* 2007*).* The predominance of the monounsaturated fatty acid, oleic acid (from 55-58%), has been reported previously (Werman and Neeman, 1987; Ortiz Moreno *et al.,* 2003*;* Ozdemir and Topuz, 2004; Pacetti *et al.,* 2007). Palmitic acid, the major saturated fatty acid, was the second most abundant fatty acid (20–22%). Linolenic acid was very

scarce in the neutral lipids of the mesocarp (less than 1.2%), but in agreement with others (Pacetti *et al.,* 2007) who found less than 1.1% linolenic acid.

3.4.1 Effect of method on oil yield

Oil extraction from avocado mesocarp with petroleum using the Soxhlet technique has previously been compared to sample homogenization with petroleum ether (Lewis *et al.,* 1978) and similarities in oil recovery were found for both methods (74-75% DW; *ca*. 23.5% FW). More recently, the efficacy of extracting avocado oil using hexane or supercritical carbon dioxide (SC-CO_2) was investigated (Mostert, *et al.,* 2007); better results were found with the former, possibly because hexane is less selective during extraction and better permeates whole plant material leading to more exhaustive extraction. In the present study, the differences in the oil yielded by hexane extraction using either the conventional Soxhlet technique or homogenization, although statistically different, remained very slight (Table 3.2). Any differences are probably due to a longer extraction time (i.e. eight time siphoning; approx. 1.5 h) at higher operating temperature (approx. 70ºC) when using the Soxhlet system. Yet, the new method described here only requires homogenization of the sample with hexane at ambient temperature and, once samples have been freeze-dried, the method takes ca. 10 min per sample as opposed to 1-6 h for Soxhlet extraction. Considerably less solvent (60 mL per sample) is required compared to Soxhlet extraction (150 mL). It must be noted, however, that a neutral solvent, such as hexane, will only tend to recover non-polar lipids (triglycerides), and that a more polar solvent is usually required to extract more polar lipids, namely glycolipids and phospholipids.

It has been reported that oil content in avocado fruit does not increase after harvest (Lee *et al.,* 1983). Greater recovery in oil yield during ripening of avocado fruit observed herein has been reported elsewhere (Ozdemir and Topuz, 2004; Mostert *et al.,* 2007*)*. Changes in the mesocarp at the ultrastructural level typically occur during fruit ripening and have previously been associated with the activity of the cell-wall degrading enzymes cellulase and polygalacturonase (Zauberman and Schiffmann-Nadel, 1972; Awad and Lewis, 1980; Reymond and Phaff, 1985). It has been hypothesised that this structural

degradation possibly causes oil to be liberated from cellular bodies, specifically triacylglycerols from parenchyma cells, making it more available for extraction (Platt and Thomson, 1992). This said, the idioblastic oil cells, which have a different composition and function than parenchyma cells, are less sensitive to the activity of these enzymes and remain intact during ripening (Platt and Thomson, 1992).

3.4.2 Effect of extraction method on fatty acid composition

There was little change in the fatty acid profile during postharvest ripening (Table 3.2), as already reported previously for avocado cv. Hass fruit (Ozdemir and Topuz, 2004). Crucially, this study showed that the fatty acid profile of oil obtained following hexane extraction with either Soxhlet or homogenization were not different, suggesting that high temperature (approx. 70ºC) lipid extraction may not be detrimental to fatty acids. Most studies which have analyzed avocado oil have not quantified fatty acids on either a fresh or dry weight basis or per gram oil recovered, but have rather stated the relative proportion of each fatty acid (Werman and Neeman, 1987; Ortiz Moreno, 2003*;* Ozdemir and Topuz, 2004; Haiyan *et al.,* 2007; Pacetti *et al.,* 2007). This study presents concentrations of fatty acids and results showed that the oil extracted by homogenization generally contained higher concentrations of fatty acids than the oil extracted by the Soxhlet method (Table 3.2). A possible explanation may be that Soxhlet extracted in a more exhaustive manner than homogenization with hexane; therefore recovering more non-target compounds other than triglycerides such as gums, waxes, and non-saponifiable material (*viz.* sterols, pigments and hydrocarbons), resulting in a higher overall oil value. Although the fatty acid profile (% total fatty acid) did not change as fruit ripened, there was an overall increase in fatty acid concentrations (DW and FW basis) as fruit ripen, and this was most probably caused by the apparent increase in oil content associated with ripening.

3.4.3 Effect of extraction method on soluble sugars

The principal sugars present in mesocarp tissue were mannoheptulose, perseitol and sucrose (Table 3.3), as previously reported (Biale and Young, 1971; Liu *et al.,* 1999b, 2002; Bertling and Bower, 2005). Mannoheptulose and sucrose concentrations found in the present study were higher than previously reported (Liu *et al.,* 2002) whilst perseitol concentrations were in agreement with others (Liu *et al.,* 1999b, 2002). Differences in the harvest season and fruit origin could have accounted for these discrepancies as it has been shown that non-structural carbohydrates, especially the seven carbon (C7) sugars, tend to decline throughout the season (Liu *et al.,* 1999b). Other work (Vekiari *et al.,* 2004) that used a crude method to quantify total sugars and therefore did not discriminate between individual sugars also found a seasonal decline in the total sugar content of avocado cvs. Fuerte, Ettinger and Hass fruit. Nevertheless, and in accordance with that previously found (Liu *et al.,* 1999b, 2002), all sugars studied herein exhibited a decreasing trend during fruit ripening. Comparison of the efficacy of methanol (62.5%) and ethanol (80%) as extraction solvents has been reported for sucrose in onion (Davis *et al.,* 2007) but not for sugars in avocado fruit. In the present study, the efficacy of these solvents was compared on residues obtained from homogenization with hexane. Results showed that methanolic extraction was *ca.* 1.2-fold more efficacious (on a residue basis) in extracting sucrose and *ca*. 1.1-fold better for perseitol than 80% ethanol (v/v; Liu *et al.,* 1999b, 2002), while mannoheptulose concentration was not affected by the solvent used. It is known that sucrose is nearly 3 times more soluble in a water/methanol mixture than in a water/ethanol mixture (Peres and Macedo, 1997; Macedo and Peres, 2001) and this could explain the higher concentrations of sucrose in methanol-based extraction (Davis *et al.,* 2007). Furthermore, methanol (62.5%, v/v), being a more polar solvent mixture than ethanol, could simply have wetted the powdered sample more efficaciously than ethanol (Davis *et al.,* 2007). Additionally, it was noted that lyophilized under-ripe samples, when ground with mortar, resulted in coarser particles than eat-ripe or medium ripe samples. The difference in the physical nature of these powders may have accounted for discrepancies in solvent efficiency at under ripe stage. Moreover, sugar analysis of residue samples derived from Soxhlet

extraction showed that some chromatograms had poorer peak separation (especially for sucrose; data not shown) as compared with the excellent peak separation obtained from residue following homogenization with hexane (Figure 3.2). This suggests that an alteration of some sugars might have occurred when samples were subjected to longer extraction at higher temperature when using the Soxhlet technique. Additionally, the nature of the solvent used for sugar extraction (*viz.* ethanol vs. methanol) could have affected recovery of sugars. For example, the use of proton-donating alcohols will tend to cause inversion of sucrose to a limited extent. In this context, spiking of the samples by adding external sugar standards to the freeze-dried sample prior to lipid extraction, or to the filter residues prior to sugar extraction, would provide additional information on target analyte recoveries, facilitating the discrimination between the methods investigated.

To summarize, sequential extraction of lipids and sugars from the same mesocarp sample can be achieved by recovering and extracting filter residues with methanol following homogenization of freeze-dried avocado mesocarp tissue with hexane. The brevity of this method and its relative simplicity make it especially suitable for extraction of large numbers of samples, without altering the fatty acid profile of the avocado oil. Removal of lipids before sugar analysis also has the advantage of extending HPLC column and guard column working life. The present study also demonstrated that the extraction efficiency for sucrose and perseitol is affected by the solvent used, with 62.5% (v/v) methanol being more effective than 80% (v/v) ethanol.

CHAPTER 4

Fatty acid and sugar composition of avocado cv. Hass in response to treatment with e+® Ethylene Remover or 1-methylcyclopropene to extend storage life.

4.1 Introduction

Avocado (*Persea americana* Mill.) fruit is an oleaginous fruit increasingly consumed, not only for its flavour, but also for its high nutritional value and reported health- benefits, including anti-cancer activity (Ding *et al.*, 2007). As reported in Chapter 3, the avocado mesocarp is an excellent source of monounsaturated fatty acids (FA) with supposed cholesterol-lowering effect (Ledesma *et al.,* 1996), and heptose sugars, mannoheptulose and perseitol, which have been associated with anti-cancer activity (Board *et al.*, 1995; Ishizu *et al.,* 2002) and inhibition of insulin secretion (Ferrer *et al.,* 1993). Whilst the properties of avocado and its fatty acid composition have been studied extensively, the biosynthesis and exact function of C7 sugars in avocado remains unclear, despite these particular sugars being the predominant form of non-structural carbohydrates in avocado cv. Hass (Liu *et al.*, 1999a). Liu *et al.,* (1999b) and others (Liu *et al.,* 2002) have suggested a possible association between C7 sugar metabolism and fruit ripening, possibly by acting as a factor of ripening inhibition.

Avocado fruit can be stored for up to 6 weeks under refrigeration (5–6°C), for instance when long distance shipment is required, and presence or effect of ethylene in the storage environment should be tightly controlled for optimum quality maintenance and storage life extension. This can be achieved using the inhibitor of ethylene action 1-MCP or ethylene scavengers, which have been reviewed in Chapter 2, section 2.4.3 and 2.4.4. The recent development of e+® Ethylene remover (Chapter 2, section 2.4.4) provides a new opportunity to control ethylene, as a potential alternative or addition to 1-MCP.

There are, to date, no published data on the effect of 1-MCP on fatty acids (FA) and sugars in avocado fruit. Literature on the use of ethylene scavengers is very scarce for avocado fruit, probably due to a lack of commercially available ethylene scavengers

that are sufficiently effective at removing ethylene for extended periods of time and under conditions of low temperature and high RH% (e.g. holding chambers, packaging). Only one study has reported fatty acid distribution of avocado stored in the presence of a $KMnO_4$-based ethylene absorber (De La Plaza *et al.,* 2003). There are also no comparative studies of the effects of ethylene removal vs. ethylene action inhibition on physical and biochemical attributes of avocado fruit. In this context, the aim of this work was to study the influence of delaying fruit ripening, using either 1-MCP or e+® Ethylene Remover, on physical attributes of quality (namely colour, firmness) and major health-associated biochemical components (namely FA and sugars) of imported avocado cv. Hass fruit during cool and cold storage and subsequent shelf-life ripening. The study focuses on changes in fatty acid composition and C7 sugar content in relation to the fruit-ripening process, using e+® Ethylene Remover or 1-MCP as tools to modulate ethylene-induced ripening and maintain fruit quality. It is anticipated that results arising from this study would help to clarify the role of C7 sugars in avocado fruit and their possible contribution to regulating fruit ripening.

4.2 Materials and methods

4.2.1 Plant material

Three experiments were conducted on pre-climacteric avocado cv. Hass fruit (size code 22) originating from a commercial farm in Malaga, Spain. Fruit were harvested on the 13th March 2007, 15th May 2007 and 21st January 2008 and used for Exp. 1 (mid-season n = 270), Exp. 2 (late season n = 270) and Exp. 3 (early season n = 324), respectively. Fruit were supplied by Mack Multiples Division (M. W. Mack Ltd., Kent, U.K.) and were transported under refrigeration to the UK within 6 days of harvest. Fruit were not pre-treated with 1-MCP. Fruit were kept overnight at 12°C (Exp. 1) or 5ºC (Exp. 2 and 3) before commencing treatments.

4.2.2 Treatments

Fruit (Exp. 1 and 2 n = 15; Exp. 3 n = 12) were placed into 13 L hermetically sealed polypropylene boxes (approx. 32 cm x 14.5 cm x 28 cm; Exp. 1 and 2 n = 18; Exp. 2 n = 27) and treated with e+® Ethylene Remover or 1-MCP (1.5 µL L^{-1}, 24h). e+® Ethylene Remover treatment was achieved by adding 5 g of powdered material (metal loading of 1% Pd (m/m)) placed in two Petri dishes within boxes. The amount of e+® Ethylene Remover used was determined by preliminary trials. The material was not removed for the duration of the storage trial. 1-MCP was obtained by releasing the gas from a commercial powdered formulation (a.i. 1-MCP 0.14% (m/m); SmartFresh; AgroFresh, Rohm and Haas Inc., Italy). A stock gas (200 µL L^{-1}) was prepared by dissolving 1.5 g SmartFresh powder with 50 mL distilled water at 50°C in a 3 L sealed jar. To achieve the desired concentration, appropriate levels of headspace were removed from the prepared concentrated stock and injected into experimental containers using a syringe. The boxes were kept sealed for 24 h at 12ºC (Exp. 1) or 5ºC (Exp. 2 and 3). Untreated fruit acted as controls and were held in the same conditions as treated fruit. After 24h (= day 0), boxes (Exp. 1 and Exp. 2 only) were treated with or without 100 µL L^{-1} ethylene for an additional 24h, resulting in 6 treatment combinations. Ethylene treatment (100 L L^{-1}) was administered by flushing ethylene (100 µL L^{-1} ethylene balanced in N_2; British Oxygen Company (BOC) Gases, Surrey, UK) directly into the boxes via a tapped tube until the desired concentration was reached (approx. 5 minutes at 15 L min^{-1}). After 24h, boxes were vented for 30 min to remove excess ethylene. Boxes were not pre-treated with ethylene in Exp. 3.

4.2.3 1-MCP and ethylene quantification

The concentration of 1-MCP and ethylene were quantified using a GC model 8340 gas chromatograph (Carlo Erba Instruments, Herts., UK) fitted with an EL980 flame ionisation detector (FID) and DP800 integrator (Thermoquest, Herts., UK) as previously described (Dauny *et al.,* 2003; Terry *et al.,* 2007a) with modifications. Oven and detector temperature were operated at 100°C and 250°C, respectively, for 1-MCP quantification and 150°C and 250°C, respectively, for ethylene quantification.

Calibration was carried out against 0.9 μL L^{-1} isobutylene (1 μL L^{-1} isobutylene in nitrogen; Certified Standard from BOC) as 1-MCP standard and 10.6 μL L^{-1} ethylene (10 μL L^{-1} ethylene in nitrogen; Certified Standard from BOC) as ethylene standard.

4.2.4 Storage conditions and sampling regime

Following treatments, boxes were stored in the dark at 12°C (Exp. 1 for 15 days) or 5ºC (Exp. 2 for 26 days and Exp. 3 for 21 days) and *ca.* 98% RH. For the duration of the storage trial, CO_2 poisoning was avoided by venting the boxes manually on a daily basis (Exp. 1) or flushing boxes with air for 9 min each day using a gas-mixing blender (Signal Series 850; Signal Instrument Co., Surrey, UK) (Exp. 2 and 3). CO_2 level within boxes was monitored at regular intervals using the same GC system as before with hot wire detection (Terry *et al.,* 2007a). The hot wire detector was set at 120°C and the oven at 80°C. Quantification of CO_2 was performed on a 2 m long x 4 mm column packed with 60-80 mesh size Porapak Q (Jones Chromatography, Mid Glamorgan, UK). Calibration was carried out against 10% CO2 (10% CO2, 2% O2, 88% N2; Certified Standard from BOC).

Fruit sub-samples were removed before commencing treatments (baseline n = 15). Samples were then taken after 1 (corresponding to 24 h after ethylene treatment; 48h after 1-MCP or e+® Ethylene Remover), 6, 8, 13 and 15 days from the 12ºC storage treatment (Exp. 1) and after 1 (corresponding to 24 h after ethylene treatment; 48h after 1-MCP or e+® Ethylene Remover application), 13, 20, 22 and 26 from the 5ºC storage treatment (Exp. 2) for physical assessment and then prepared for subsequent biochemical analysis. Each treatment had 9 replicates and 1 fruit constituted one replicate. For Exp. 3, fruit subsamples (n = 27 per treatment) were removed after 0 (corresponding to 24h after 1-MCP or e+® Ethylene Remover application), 7, 14 and 21 days at 5ºC and placed on open plastic trays for ripening at 20ºC. Fruit (n = 9 per treatment) were assessed after 0 (direct from cold storage), 3 and 6 days shelf life at 20ºC, which gave a total of 324 analyzed fruit.

4.2.5 Measurement of respiration and ethylene production rate

The respiration and ethylene production rates were measured for Exp. 1 only and were assessed as the rate of CO_2 and ethylene emission by fruit under standard air condition at 20°C. At each sampling interval, fruit (n = 9 per treatment) were removed from storage boxes and placed by group of 3 into 3 L jars (n = 18) fitted with air tight lids. Jars were kept sealed for 2 h at 20ºC. After this incubation period, headspace gas samples were removed with repeated full withdrawal-injection displacements of a 60 ml plastic syringe. Ethylene and CO_2 were immediately quantified by gas chromatography with FID and hot wire detector (HWD), respectively, as described in sections 4.2.3 and 4.2.4.

4.2.6 Firmness and colour measurement

Objective colour, as determined by lightness (L*), chroma (colour saturation; C*), and hue angle (Hº), was measured using a Minolta CR-400 colourimeter with an 8 mm light aperture and DP-400 data processor (Minolta Co. Ltd., Japan). The instrument was calibrated against a Minolta standard white tile CR-400. At each sampling interval, the mean of three readings taken at equidistant point around the equatorial axis was recorded on each fruit (n = 9 per treatment).

Firmness was determined after fruit internal core temperature had equilibrated to *ca.* 18ºC. For Exp. 1, firmness was determined using an Instron Universal Testing Machine (Model 1122, Bucks., UK) fitted with an 8 mm diameter flat probe (Terry *et al.,* 2007a). The probe was driven with a crosshead speed of 20 mm min^{-1} and the force was recorded at bioyield. Results were taken from the mean of two penetrations on opposite sides of whole fruits supported in a sand bath, where small pieces of skin had been removed. For Exp. 2 and 3, firmness measurement was performed on an Instron Uniaxial Testing Machine (model 5542, MA) equipped with calibrated 500 N load cell, again fitted with an 8 mm diameter flat probe. The machine was programmed (Bluehill 2, version 2.11, Instron) with the crosshead speed set at 20 mm min^{-1}. The force (N) at bioyield was recorded. Firmness of avocado fruit was measured before commencing

treatments and average baseline firmness were 117 N, 227 N and 252 N for Exp. 1, 2 and 3, respectively.

4.2.7 Biochemical analysis: fatty acids and sugars extraction and quantification

After firmness measurement, fruit samples were prepared for subsequent fatty acids and sugar extraction, carried out as described in Chapter 3 (section 3.2.1 to 3.2.3). Fatty acids were analyzed as fatty acids methyl esters (FAMEs) and quantified by GC-FID as described in section 3.2.4. The fatty acid profile was calculated as percentage of total of the five detected FAMEs, after comparison of peak areas of samples and peak areas of standards of known composition. Sugars were identification and quantified by HPLC equipped with an evaporative light scattering detector (ELSD) with parameters set as described in section 3.2.5 of Chapter 3. Each sugar was quantified by comparing sample peak areas to mixed standards of known composition and concentration. The minimum detection limit for the sugars was 20 mg g^{-1} powder residue (approx. 0.7% substance dry mass; 0.2% substance fresh weight (FW)). Results below the detection limit were set at zero which caused an unavoidable underestimation of the mean value of sugar content. For Exp. 3, sugar content in avocado extracts was determined as before with slight modification since analysis was performed on an Agilent 1200 series HPLC binary pump system (Agilent, Berks., UK), equipped with an Agilent refractive index detector (RID) G1362A and cooled autosampler set at 4°C. The presence and abundance of fructose, glucose, sucrose, mannoheptulose and perseitol were automatically calculated by comparing sample peak area to standards of known concentration using ChemStation Rev. B.02.01.

4.2.8 Statistical analysis

Analysis of variance (ANOVA) was carried out according to Chapter 3. Tests for correlations between mean values for colour data (L*, C* and Hº) and firmness were made using Spearman's Rank correlation. Correlations are presented with the Spearman Correlation Coefficient (r) and P value on a two-tailed test. Unless otherwise stated

significant differences were $P < 0.05$. Means with different letters in tables are significantly different from one another ($P < 0.05$).

4.3 Results and discussion

4.3.1 Quality attributes (physical variables)

Ethylene-induced accelerated ripening accounts for a large proportion of the postharvest losses of perishable crops. Ethylene commonly accumulates during postharvest handling, transportation and storage of climacteric commodities. A concentration of ethylene in air of 0.1 μL L^{-1} is often quoted as the threshold level, above which fruit becomes evidently more physiologically active (Kader, 1985, Wills and Warton, 2000). In the present study, ethylene accumulated daily (boxes opened every day) inside boxes containing control and 1-MCP-treated fruit accumulated daily (boxes flushed every 24 h) to concentrations above 0.1 μL L^{-1} (Exp. 1, Table 4.1; Exp 2, Table 4.2 and Exp. 3, data not shown). During storage at 12ºC, the overall mean ethylene concentration measured inside 1-MCP-treated boxes was significantly higher (22.47 μL L^{-1}) as compared with that measured in controls (13.78 μL L^{-1}).

Table 4.1: Ethylene concentration (μL L^{-1}) within 13 L boxes containing avocado cv. Hass fruit (mid season, Exp.1) stored at 12°C and held in presence of e+® Ethylene Remover (e+® ER, 5 g) or initially pre-treated with 1-MCP (1.5 μL L^{-1}). Boxes were vented every day from day 1. Boxes were treated with or without 100 μL L^{-1} ethylene (24h) on day 0.

	Ethylene treatment	0 μL L^{-1}			100 μL L^{-1}		
Day	Treatment	Control	e+® ER	1-MCP	control	e+® ER	1-MCP
0*		10.34	0.15	17.53	22.01	0.15	9.75
1**		4.29	0.36	7.33	69.82	0.28	80.04
2		17.84	0.22	24.06	22.12	0.18	20.26
6		11.75	0.51	42.77	22.84	0.52	21.40
8		7.82	0.36	18.60	7.21	0.20	9.79
13		13.07	0.40	26.07	16.36	0.33	32.58
15		6.64	0.29	13.71	12.12	0.85	15.43

*Values after 24h after 1-MCP treatment or e+ Ethylene Remover application, before ethylene treatment ** Values 24h after ethylene application. LSD used for comparison within the interaction day x treatment x ethylene treatment (day 2-15 only) = 9.900

Table 4.2: Ethylene concentration (μL L^{-1}) within 13 L sealed boxes containing avocado cv. Hass fruit (late season, Exp. 2) stored at 5°C and held in presence of e+® Ethylene Remover (5 g) or initially pre-treated with 1-MCP (1.5 μL L^{-1}). Boxes were flushed with air for 9 min every 24h from day 1. Boxes were treated with or (without) 100 μL L^{-1} ethylene on day 0.

Treatment	Ethylene concentration (μL L^{-1})											
	Day 0		Day 1		Day 7		Day 13		Day 20		Day 26	
Control	96.50	(0.00)	65.83	(0.28)	4.64	(1.25)	1.63	(2.29)	1.15	(1.27)	0.70	(0.52)
e+® Ethylene Remover	82.17	(0.00)	0.03	(0.00)	0.02	(0.01)	0.05	(0.06)	0.05	(0.03)	0.02	(0.01)
1-MCP	94.98	(0.00)	73.58	(0.17)	0.66	(0.57)	0.78	(1.76)	1.11	(2.77)	0.58	(0.49)

LSD (P<0.05) = 1.177**LSD calculated for values of day 7, 13, 20 and 26 only

However, presence of e+® Ethylene Remover reduced significantly both exogenous and endogenously produced (fruit-derived) ethylene (Exp. 1, Table 4.1; Exp 2, Table 4.2 and Exp. 3, data not shown). Approx. 22% (Exp. 1) and 15% (Exp. 2) of exogenously administered ethylene were scavenged within seconds. However, whilst ethylene levels were only reduced below 1 μL L^{-1} during 15 days storage at 12°C, concentrations were further reduced below 0.1 μL L^{-1} during storage at 5°C (Exp. 2 and Exp. 3), and thus was in agreement with Terry *et al.* (2007a).

Accordingly, where ethylene level had been reduced below 0.1 μL L^{-1} in the presence of e+® Ethylene Remover, the ethylene- induced ripening was delayed, as demonstrated by significant improvement in firmness and colour retention during 26 days (Exp. 2, Fig. 4.1) and 21 days of storage at 5°C (Exp. 3, Table 4.3). After 26 days of storage, late season fruit treated with e+® Ethylene Remover were still firmer (47.5 N) as compared to controls (5.5 N; Fig. 4.1A). In early season fruit (Exp. 3) a difference between e+® Ethylene Remover -treated and control fruit for firmness was observed after day 14 day and for peel after day 21 (Table 4.3). Prevention of ethylene action using 1-MCP strongly influenced ethylene-induced ripening of avocado fruit stored for 26 days and 21 days at 5°C, resulting in highest firmness and colour values vs. other treatments (Fig. 4.1 and Table 4.3), and thus was in agreement with other studies reporting the effects of the ethylene antagonist on avocado cv. Hass (Adkins *et al.*, 2005; Feng *et al.,* 2000; Jeong and Huber, 2004; Hershkovitz *et al.,* 2005; Woolf *et al.,* 2005; Hershkovitz *et al.,* 2010). Whilst there was a tendency toward lower firmness and H° with increasing storage duration in both control and e+® Ethylene Remover -treated fruit, 1-MCP application suppressed softening and colour change of avocado fruit over time at 5°C (Table 4.3 and Fig. 4.1).

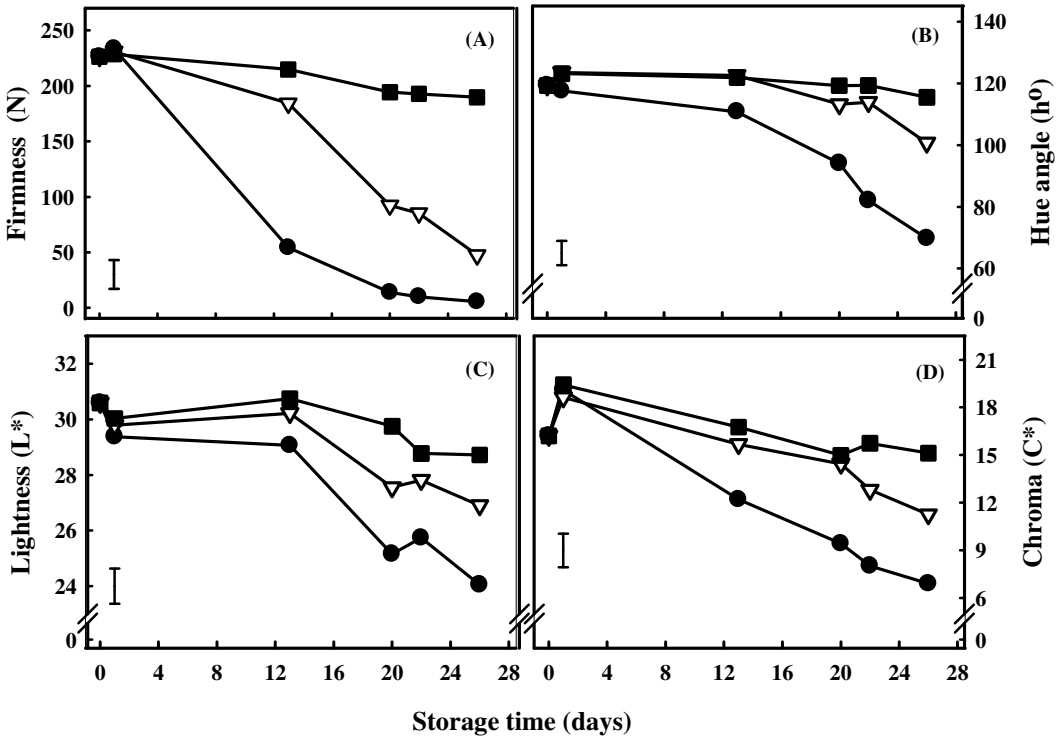

Figure 4.1: Effect of treatments (control, •; e+® Ethylene Remover, ∇; 1-MCP, ■) on change in firmness (N), lightness (L*), chroma (C*) and hue angle (Hº) of pre-climacteric avocado cv. Hass (late season, Exp. 2) fruit held at 5ºC within 13L boxes for 26 days in presence of e+® Ethylene Remover (5 g) or initially pre-treated with 1-MCP (1.5 µL L^{-1} for 24h). Values are mean of ethylene-treated and ethylene non-treated fruits.

Upon removal from cold storage and transfer to shelf life conditions at 20°C, firmness and greenness of all fruit exhibited a decline (Exp.3, Table 4.3). However, these changes were more pronounced for control and e+® Ethylene Remover -treated fruit than for 1-MCP-treated fruit: avocado previously stored in presence of e+® Ethylene Remover resumed normal ripening (i.e. softening and peel purpling) and were fully ripe (firmness<10 N) within 3 days, similarly to controls.

Table 4.3: Effect of treatments on firmness (N) and hue angle (Hº) of avocado cv. Hass (early season, Exp. 3) fruit held under shelf life conditions at 20ºC. Firmness and hue angle were determined after 0 (direct from cold storage), 3 and 6 days shelf life. Fruit were previously stored for 0, 7, 14 or 21 days at 5ºC within 13L boxes in presence of e+® Ethylene Remover (5 g) or initially pre-treated with 1-MCP (1.5 µL L^{-1}, 24h at 5ºC).

Days at 5ºC	Days at 20ºC	Firmness (N)			Hue angle (Hº)		
		Control	e+® Ethylene Remover	1-MCP	control	e+® Ethylene Remover	1-MCP
0	0	240.6±3.9	246.4±5.3	240.6±6.0	125.87±0.3	125.93±0.5	125.49±0.6
	3	5.9±0.6	9.6±2.0	155.7±16.9	113.71±1.7	116.13±3.4	123.72±1.3
	6	3.2±1.0	5.8±2.7	124.3±18.3	63.04±10.3	73.89±11.5	120.51±2.1
7	0	217.6±12.9	240.0±7.5	238.7±8.0	126.13±0.4	125.18±0.5	125.99±0.3
	3	3.7±0.3	6.5±2.4	118.6±22.4	75.20±7.0	99.42±5.1	121.58±2.4
	6	2.2±0.1	2.1±0.2	84.9±20.0	42.06±5.3	44.50±8.0	92.06±12.1
14	0	105.5±27.1	169.3±31.9	234.2±13.4	122.21±0.8	123.84±0.9	123.67±1.0
	3	2.8±0.2	2.8±0.2	123.5±20.7	57.19±5.0	60.57±6.0	121.65±1.1
	6	2.4±0.2	2.3±0.2	102.7±18.1	35.15±3.5	32.22±2.2	96.55±11.1
21	0	38.8±16.8	54.3±25.9	163.2±26.5	108.13±4.1	113.87±4.2	122.39±1.7
	3	3.3±0.4	3.1±0.2	131.2±30.1	64.91±6.3	63.17±6.1	108.47±9.2
	6	2.6±0.2	2.1±0.1	87.8±26.1	54.51±2.8	43.34±8.5	67.05±14.7

Results represent mean values of 9 replicates ± SE.

Avocados treated with 1-MCP did not fully ripen during shelf life and, after 6 days at 20°C, fruit were 38- and 32-fold more firm than were controls and e+® Ethylene Remover -treated fruit, respectively (Exp. 3, Table 4.3). Also, considerable heterogeneity in softening of fruit treated with 1-MCP was observed, as demonstrated by the standard error (SE) after 3 and 6 days shelf life. In particular, this SE was proportionally higher with longer cold storage period (Table 4.3). Uneven ripening (fruit-to-fruit and batch-to-batch variation) as a result of 1-MCP treatment is not unusual in avocado cv. Hass (Kruger and Lemmer, 2007; Ochoa-Ascencio *et al.,* 2009). It is acknowledged that concentrations and time of 1-MCP application in the present study (1.5 μL L^{-1} for 24h) may have been inappropriate since 300 nL L^{-1} for 16h is now recommended (G. Regiroli, AgroFresh Inc., pers. comm.) and therefore could explain why the strong and persistent ripening inhibition was observed. This said, 1-MCP is usually applied at production site rather than after transit when fruit are biologically older, and therefore a larger concentration was chosen to ensure efficacy of the inhibitor. Also, administering a specific concentration can be challenging within a large storage volume especially considering that desired 1-MCP levels can be altered by the environment and may not be uniform when reliant, in part, on diffusion.

In contrast, where ethylene was only reduced below 1 μL L^{-1} in presence of the scavenger (Exp. 1), no delay in ethylene-induced ripening was observed. It has been advised to maintain ethylene levels below 0.1 μL L^{-1} in storage atmospheres since concentrations above this level may induce important quality loss (Wills and Warton, 2000). Hence, it is probable that the ethylene concentration present in the surrounding environment was high enough to stimulate an ethylene response in these fruit. Ethylene production (Exp. 1, Table 4.4) and respiration rate (data not shown) by controls and fruit treated with e+® Ethylene Remover with or without exposure to ethylene were not different during 15 days storage. Ethylene production increased over storage time to reach a maximum after 8 days, followed by a decreased in ethylene production (Table 4.4), whilst respiration rate increased steadily over storage time and was highest toward the end of the storage period (data not shown).

Table 4.4. Effect of treatment on ethylene production rate (μL kg^{-1} h^{-1}) of mid-season avocado cv. Hass (Exp. 1) stored at 12ºC in presence of e+® Ethylene Remover (e+® ER, 5 g) or initially pre-treated with 1-MCP (1.5 μL L^{-1} for 24h). Fruit were treated with or without 100 μL L^{-1} ethylene for 24h on day 0. Values are mean of 3 replicates and 3 fruits per jar constituted one replicate. Ethylene production rate was measured after 2h at 20°C.

	0 μL L^{-1} ethylene			100 μL L^{-1} ethylene		
Day	control	e+® ER	1-MCP	control	e+® ER	1-MCP
1	8.22^{ef}	3.62^{f}	5.94^{ef}	9.43^{def}	6.88^{ef}	7.01^{e}
6	6.18^{ef}	10.43^{def}	22.33^{b}	11.25^{cde}	10.80^{de}	10.59^{de}
8	15.61^{bcd}	18.40^{b}	19.41^{b}	18.04^{bc}	29.86^{a}	10.12^{def}
13	6.43^{ef}	6.14^{ef}	6.98^{ef}	6.01^{ef}	6.16^{ef}	6.86^{ef}
15	4.70^{ef}	5.17^{ef}	5.20^{ef}	4.53^{ef}	5.44^{ef}	5.31^{ef}

^{abcdef} different letters within the interaction day x treatment x ethylene treatment indicate differences (P<0.05)

Accordingly, fruit softened and changed colour similarly to controls (Exp.1, Table 4.5 and Figure 4.2). Although application of 100 μL L^{-1} ethylene hastened firmness loss of controls on day 1 storage (P>0.05), the effect was not very large. On the other hand, fruit treated with 1-MCP (0 μL L^{-1} ethylene) at 12°C exhibited a maximum ethylene concentration two days earlier, on day 6 storage, whilst fruit treated with 1-MCP and 100 μL L^{-1} ethylene did not exhibited a peak and ethylene production remained relatively stable over storage time (Table 4.4). Both fruit exhibited significantly higher respiration rate than other treatments toward the end of storage period (data not shown). Since 1-MCP treatment did not suppress ethylene biosynthesis, the softening of fruit treated with 1-MCP (0 μL L^{-1} ethylene) did not differ significantly from that of controls, and firmness and colour decreased rapidly until fully ripe (firmness 5-10 N) already after day 6 (Exp. 1, Table 4.5). In contrast, fruit treated with 1-MCP and 100 μL L^{-1} ethylene softened at a lower rate than all other treatments and were the firmest (P<0.05) fruit (Table 4.5). These fruit were eventually ripe as storage trial terminated. Colour development from green (H° ~ 121.19, baseline) to reddish/black was less pronounced for 1-MCP- treated fruit (H° ~70.79) vs. control (H°

~ 61.76) and e+® Ethylene Remover -treated fruit (H° ~ 64.46). The overall effect of 1-MCP treatment was largely due to the delay in colour change of fruit treated with 1-MCP and exogenous ethylene vs. other treatments (Exp. 1, Figure 4.2).

Table 4.5. Effect of treatment on firmness (N) of mid-season avocado cv. Hass (Exp. 1) stored at 12ºC within 13L boxes for 15 days in presence of e+® Ethylene Remover (e+® ER, 5 g) or initially pre-treated with 1-MCP (1.5 µL L^{-1} for 24h). Boxes were treated with or without 100 µL L^{-1} ethylene for 24h on day 0.

Day	0 µL L^{-1} ethylene			100 µL L^{-1} ethylene		
	control	e+® ER	1-MCP	control	e+® ER	1-MCP
1	48.98	35.43	53.70	29.91	45.58	81.06
6	5.74	6.81	6.91	4.72	10.61	21.89
8	4.28	3.81	6.59	2.80	4.63	15.23
13	2.89	3.05	6.59	3.70	3.61	19.13
15	2.51	3.04	4.09	2.14	3.02	4.47
Mean	12.88^{bc}	10.43^{bc}	15.58^{b}	8.66^{c}	13.49^{bc}	28.36^{a}

^{abc} different letters indicate differences between treatment means (P<0.05)

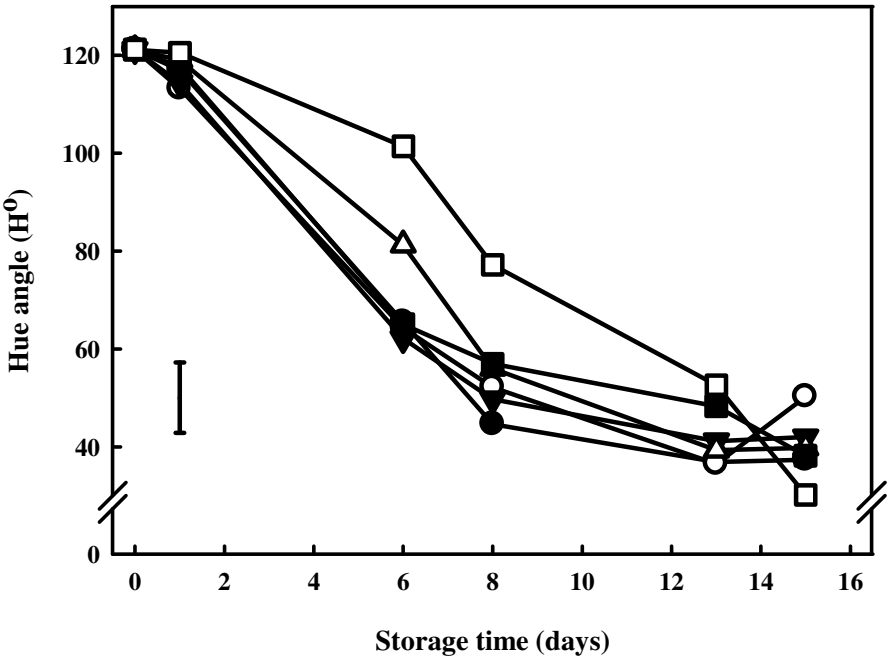

Figure 4.2. Effect of treatment (control-0 µL L^{-1} ethylene, •; control-100 µL L^{-1} ethylene,○; e+® Ethylene Remover -0 µL L^{-1} ethylene,▼; e+® Ethylene Remover -100 µL L^{-1} ethylene, Δ; MCP-0 µL L^{-1} ethylene, ■; MCP-100 µL L^{-1} ethylene, □) on hue angle (Hº) of mid-season avocado cv. Hass (Exp. 1) stored at 12ºC within 13L boxes for 15 days in presence of e+® Ethylene Remover (5 g) or initially pre-treated with 1-MCP (1.5 µL L^{-1} for 24h). Boxes were treated with or without 100 µL L^{-1} ethylene for 24h on day 0.

1-MCP is believed to exert its effect through interacting with receptors and competing with ethylene for binding sites (Sisler and Serek, 1997). By occupying the ethylene-binding site in an apparent irreversible manner, the inhibitor prevents ethylene from binding and inducing signal transduction and translation downstream, therefore inhibiting ripening (Blankenship and Dole, 2003). Treated fruits will remain insensitive to ethylene for a certain period (depending on commodity, cultivar, tissue and maturity), after which the effect disappears. Although the mechanism(s) of recovery from 1-MCP action is not yet known, it has often been invoked that the effect eventually ceases due to dissociation of 1-MCP from the binding site, metabolism of the receptor-protein complex and/or because new receptors are formed (Sisler *et al.,* 1996; Golding *et al.,*

1998, Jeong *et al.,* 2002; Sisler and Serek, 2003). In the present study, the ability of 1-MCP to delay ripening at 12ºC (Exp.1) was not comparable to other studies where locally produced fruit were treated with the inhibitor within 24 h of harvest (Feng *et al.,* 2000; Adkins *et al.,* 2005, Hofman *et al.,* 2001; Jeong *et al.,* 2002; Jeong and Huber, 2004; Hershkovitz *et al.,* 2005; Hershkovitz *et al.,* 2010). 1-MCP did not block ethylene production, but rather attenuated production of ethylene as compared with other treatments (Exp.1, Table 4.4), and fruit softened over the course of storage. It has been shown that avocado fruit becomes gradually less responsive to 1-MCP once the climacteric ethylene production has been initiated (Adkins *et al.,* 2005) as seen for banana (Golding *et al.,* 1998; Jiang *et al.,* 1999a). In particular, the capacity of 1-MCP to interrupt softening of avocado cv. Hass was negated within two days of exposure to 100 μL L^{-1} ethylene for 24h (Adkins *et al.,* 2005). Recent research on tomato found that the level of internal ethylene strongly influenced 1-MCP efficacy to delay ripening (Zhang *et al.,* 2009b). In Exp.1, fruit may have been too mature already at the time of treatment, although initial ethylene production by fruit at treatment time was not measured. Assuming fruit were at the onset of ripening when treated, the skin of avocado fruit could have acted as a barrier to gas flow, increasing the internal ethylene concentration. In such a scenario, minimizing detrimental effects of ethylene may have represented a greater challenge for e+® Ethylene Remover.

In Exp.1, it was expected that ethylene-treated fruit which had previously been exposed to 1-MCP would soften at the same rate, if not faster, than those treated with 1-MCP only. Exogenous application of ethylene has been shown to hasten the ripening of avocado fruit (Feng *et al.,* 2000, Adkins *et al.,* 2005). In tomato, Kevany *et al.* (2007) recently provided evidence for the receptors being rapidly degraded in the presence of ethylene. In the present study, ethylene treatment however maintained firmness of 1-MCP-treated avocado fruit better than 1-MCP treatment alone (Exp.1) and as such this paradox remains unexplained. Evidence has shown that pre-treatment with 1-MCP renders avocado fruit insensitive to subsequent exposure to ethylene for a set period of time (Feng *et al.,* 2000; Jeong and Huber, 2004; Adkins *et al.,* 2005). Yet, there exist no scientific evidence for new receptors being produced or old receptors being reactivated (Sisler and Serek, 2003), although both hypothesis may explain that 1-MCP action is not infinite (Jeong *et al.,* 2002). Additionally, it is known that 1-MCP can bind to non-

specific tissue such as oil in avocado mesocarp (Dauny *et al.,* 2003). In such scenarios, the rate at which 1-MCP residues are subsequently desorbed and released into the atmosphere, and whether they bind to newly formed ethylene binding site is unknown, but would potentially explain re-sensitization of tissue to 1-MCP. Besides the hypothesised influences of internal ethylene concentration on 1-MCP responsiveness, it has been suggested that the lack of tissue sensitivity to 1-MCP may in part reflect processes that occur independently of ethylene or necessitate ethylene only for initiation (Golding *et al.,* 1998). In that sense, the importance of abscicic acid (ABA) in ripening of climacteric fruit has already been highlighted (Zhang *et al.,* 2009a) and will be developed further in Chapter 6.

4.3.2 Oil content and fatty acid composition

Oil content, together with dry matter, is often associated with avocado horticultural maturity and used as a basis for determining harvesting time. The values found in the present study were in the normal range expected for avocado cv. Hass (Chapter 3; Ozdemir and Topuz, 2004) and averaged 0.62 g g^{-1} DW (19.1% FW) for mid-season fruit (Exp. 1), 0.59 g g^{-1} DW (18.97% FW) for late season fruit (Exp. 2) and 0.57 g g^{-1} DW (15.33% FW) for early season fruit (Exp. 3). A slight, yet significant increase in oil yield during storage at 5°C, 12°C and shelf life period was found (data not shown) which has previously been reported in ripening avocado (Chapter 3; Ozdemir and Topuz, 2004; Mostert *et al.,* 2007). Moreover, where ripening had been delayed in response to 1-MCP or e+® Ethylene Remover treatment, slightly less (significant in Exp. 2; non-significant in Exp. 3) oil was extracted as compared with controls (data not shown). Such phenomenon of increased oil yield with increasing ripening has been shown in Chapter 3 and can be explained by changes in tissue texture through action of cell wall hydrolases (Chapter 3; Platt and Thomson, 1992).

The fatty acid profiles found in this study were consistent with those published for avocado cv. Hass (Eaks, 1990; Ozdemir and Topuz, 2004; Vekiari *et al.,* 2004) with oleic acid consistently representing the main fraction, followed in decreasing order of abundance by palmitic, linoleic, palmitoleic and linolenic acids (Exp. 1 and 2, data not shown and Exp. 3, Table 4.6). In mid and late season fruit (Exp. 1 and 2), the fatty acid

composition remained relatively unchanged over storage time at 12°C or 5°C, similarly to that observed by others for cold- and cool-stored avocado cv. Hass (Eaks, 1990; Chapter 3), and was not affected by treatments applied. However, in early season fruit (Exp. 3), the proportion of some fatty acids showed significant changes during 21 days storage at 5°C, with a decrease in palmitic and palmitoleic acids and an increase in linolenic acid (Table 4.6). There was a treatment effect on palmitic acid only, since 1-MCP resulted in higher palmitic acid content (21.8%) vs. e+® Ethylene Remover - treated fruit (20.5%, $P < 0.05$) and controls (21.0%, $P > 0.05$) over 21 days of storage

During subsequent ripening at 20°C, a decrease in palmitic acid and increase in polyunsaturated fatty acids was observed, whilst monounsaturated fatty acids remained relatively unchanged (Table 4.6). Again, overall palmitic acid content was significantly higher for 1-MCP-treated fruit (21.5%) as compared with controls (20.5%) and e+® Ethylene Remover (20.4%) over 3 days of ripening. The temporal decrease in saturated fatty acids and increase in polyunsaturated fatty acids found herein has previously been reported by Ozdemir and Topuz (2004) during ripening of avocado cv. Hass held at 18–22°C. However, although these changes in oil fatty acid composition were statistically different they were very small numerically and not considered nutritionally important (Ozdemir and Topuz, 2004). Despite ripening being delayed by e+® Ethylene Remover, presence of the scavenger did not affect the fatty acids profile, and the proportion of each FA was not different from that of controls. De La Plaza *et al.* (2003) previously reported that reducing ethylene level within storage atmosphere by addition of a $KMnO_4$-based ethylene absorber resulted in a relatively steady fatty acid composition in avocado fruit throughout a storage period at 20°C or at 4°C.

Fatty acid composition is the characteristic feature which defines oil quality. It is known that major changes in the fatty acid profile occur during fruit growth and development, with a marked increase in the oleic fraction. However, few studies have looked at changes in fatty acid profile during postharvest fruit life and authors found relatively little change in the oil composition during postharvest ripening (Eaks, 1990; Ozdemir and Topuz, 2004). The present data suggest that fatty acids are probably not related to the ripening event, since no considerable changes in response to treatments were found. Discrepancies between experiments for oil content and fatty acid composition probably arise from the different harvest dates (*viz.* early, mid and late

season), which is expected with increasing maturity (Ozdemir and Topuz, 2004; Vekiari *et al.,* 2004).

Table 4.6: Fatty acid composition (% total fatty acids) in avocado cv. Hass (early season, Exp. 3) mesocarp during 3 days ripening at 20°C. Fruit were previously stored for 21 days at 5ºC within 13L boxes in presence of e+® Ethylene Remover (5 g) or initially pre-treated with 1-MCP (1.5 µL L^{-1}).

Fatty acids (%)	Storage days	Shelf life days at 20°C	
		0	3
Palmitic	0	21.00^{AB}	20.61
	7	21.81^{A}	20.70
	21	20.50^{B}	20.20
	mean	21.10^{X}	20.50^{Y}
Palmitoleic	0	9.77^{Aa}	8.82^{b}
	7	9.42^{Aab}	9.10^{abc}
	21	8.69^{Bc}	9.02^{bc}
	mean	9.29^{NS}	8.98^{NS}
Oleic	0	55.02	55.54
	7	55.15	55.41
	21	56.06	56.12
	mean	55.41^{NS}	55.69^{NS}
linoleic	0	12.96	13.57
	7	12.46	13.32
	21	13.21	13.12
	mean	12.88^{X}	13.34^{Y}
linolenic	0	1.25^{B}	1.46
	7	1.16^{B}	1.47
	21	1.54^{A}	1.54
	mean	1.32^{NS}	1.49^{NS}

^{AB} different letters within same column (shelf life 0) indicate significant differences between cold storage days ($P<0.05$), LSD used for comparison of values directly out of cold storage. ^{XY}different letters within same row indicate significant differences between shelf life days ($P<0.05$). ^{abc} different letters within the interaction "storage days x shelf life days" indicate significant differences ($P<0.05$). ^{NS} not significant ($P>0.05$).

4.3.3 Sugars

Sucrose, mannoheptulose and the alcohol perseitol were the main soluble components present in avocado mesocarp tissue (Tables 4.7-4.10), as previously reported (Bertling and Bower, 2005; Cowan, 2004; Liu *et al.,* 1999b, 2002; Chapter 3). Fructose and glucose were detected, but were at or below the limit of quantification in almost all samples and thus they were not considered. Despite the importance of reserve carbohydrates as an energy source for the respiratory processes during fruit storage and ripening (Kozlowski, 1992), little is known about the nature of carbon substrates which support the respiratory process in avocado fruit. Moreover, the exact function of heptose sugars in avocado fruit is not completely understood and the number of related research papers published in the past decade remain limited (Bertling and Bower, 2005; Cowan, 2004; Liu *et al.,* 1999a, 1999b, 2002).

In the present study, substantial amounts of mannoheptulose was found in mid-season fruit (Exp. 1, Table 4.7) with concentrations being 5.1-fold lesser after 15 days vs. 1 day at 12ºC. Greatest amounts were measured in early season fruit, with a decline in concentrations (reduction by half) over 21 days of storage (Exp. 3, Table 4.9). In contrast, very low amounts of mannoheptulose were found in late season fruit stored at 5°C (on average 2.92 mg g^{-1} residue; 1.11 mg g^{-1} DW) with most values below quantification, regardless of treatments or storage time (Exp. 2, Table 4.8). Large quantities of perseitol were consistently detected in all samples (Tables 4.7-4.9) with lower concentrations measured toward the end of the cold storage period (approximately 1.5–2.6 fold less than at the beginning of storage).

Table 4.7. Effect of treatments on the concentration of main sugars in avocado cv. Hass (mid-season, Exp.1) fruit mesocarp stored for 15 days at 12ºC within 13L boxes in presence of e+® Ethylene Remover (5 g) or initially pre-treated with 1-MCP (1.5 µL L^{-1}, 24h at 12ºC); values are expressed per residue (dry weight after lipid removal), per dry weight and per fresh weight. Values are mean of ethylene treated and non-treated fruit.

	Treatment	sucrose		mannoheptulose		perseitol	
		Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
mg g^{-1} residue	Control	40.18^{bc}	39.13^{c}	39.43^{a}	9.55^{a}	43.62^{a}	17.71^{a}
	e+® Ethylene Remover	48.71^{ab}	37.67^{c}	50.53^{a}	9.22^{a}	48.32^{a}	17.84^{a}
	1-MCP	52.58^{a}	34.70^{c}	59.99^{a}	10.55^{a}	50.11^{a}	18.17^{a}
	Column mean	47.16^{x}	37.17^{y}	49.98^{x}	9.77^{y}	47.35^{x}	17.91^{y}
mg g^{-1} DW	Control	16.23^{a}	12.68^{a}	16.78^{a}	3.10^{a}	18.01^{a}	5.96^{a}
	e e+® Ethylene Remover	19.77^{a}	12.71^{a}	21.13^{a}	2.90^{a}	19.83^{a}	5.96^{a}
	1-MCP	21.40^{a}	11.36^{a}	24.24^{a}	3.56^{a}	19.97^{a}	6.04^{a}
	Column mean	19.14^{x}	12.25^{y}	20.72^{x}	3.19^{y}	19.27^{x}	5.99^{y}
mg g^{-1} FW	Control	5.05^{a}	3.62^{a}	4.99^{a}	0.94^{a}	5.55^{a}	1.58^{a}
	e+® Ethylene Remover	6.07^{a}	3.63^{a}	6.43^{a}	0.84^{a}	6.08^{a}	1.68^{a}
	1-MCP	6.55^{a}	3.44^{a}	7.53^{a}	1.02^{a}	6.23^{a}	1.79^{a}
	Column mean	5.89^{x}	3.56^{y}	6.32^{x}	0.93^{y}	5.95^{x}	1.69^{y}

^{abc} different letters indicate significant differences (P<0.05) within the interaction day x treatment. ^{xy} different letters between different storage days (column mean) indicate significant difference (*P*<0.05).

Table 4.8: Effect of treatments on the concentration of main sugars in avocado cv. Hass (late season, Exp. 2) fruit mesocarp stored for 26 days at 5ºC within 13L boxes in presence of e+® Ethylene Remover (e+® ER, 5 g) or initially pre-treated with 1-MCP (1.5 µL L^{-1}); values are expressed per residue (dry weight after lipid removal), per dry weight and per fresh weight. Values are mean of ethylene treated and non-treated fruit.

	Treatment	sucrose			mannoheptulose			perseitol		
		Day 1	Day 13	Day 26	Day 1	Day 13	Day 26	Day 1	Day 13	Day 26
mg g^{-1} residue	Control	17.45^{de}	33.86^{b}	51.76^{a}	2.20^{a}	1.59^{a}	6.40^{a}	52.65^{a}	33.90^{bc}	22.23^{de}
	e+® ER	11.87^{ef}	22.26^{cd}	30.70^{bc}	5.84^{a}	3.59^{a}	1.09^{a}	55.72^{a}	37.63^{b}	17.79^{e}
	1-MCP	4.47^{f}	30.06^{bc}	9.80^{ef}	4.27^{a}	0.00^{a}	1.33^{a}	50.10^{a}	39.14^{b}	28.45^{cd}
	Mean	11.26^{y}	28.72^{x}	30.75^{x}	4.10^{x}	1.73^{x}	2.94^{x}	52.82^{x}	36.89^{y}	22.83^{z}
mg g^{-1} DW	Control	7.16^{de}	12.85^{b}	18.24^{a}	0.76^{a}	0.57^{a}	2.39^{a}	21.30^{a}	12.87^{a}	7.99^{a}
	e+® ER	4.75^{ef}	9.17^{cd}	11.72^{bc}	2.24^{a}	1.48^{a}	0.42^{a}	22.20^{a}	15.62^{a}	6.94^{a}
	1-MCP	1.98^{f}	12.36^{bc}	3.67^{ef}	1.59^{a}	0.00^{a}	0.51^{a}	21.08^{a}	16.52^{a}	11.12^{a}
	Mean	4.63^{y}	11.46^{x}	11.21^{x}	1.53^{x}	0.68^{x}	1.11^{x}	21.53^{x}	15.00^{y}	8.68^{z}
mg g^{-1} FW	Control	2.27^{de}	4.20^{b}	5.64^{a}	0.24^{a}	0.19^{a}	0.73	6.84^{a}	4.23^{a}	2.47^{a}
	e+® ER	1.50^{ef}	2.87^{cd}	3.54^{bc}	0.66^{a}	0.45^{a}	0.11^{a}	7.12^{a}	4.96^{a}	2.10^{a}
	1-MCP	0.59^{f}	3.81^{bc}	1.19^{f}	0.51^{a}	0.00^{a}	0.16^{a}	6.66^{a}	5.13^{a}	3.67^{a}
	Mean	1.45^{y}	3.63^{x}	3.46^{x}	0.47^{x}	0.21^{x}	0.33^{x}	6.87^{x}	4.77^{y}	2.74^{z}

^{abcdef} different letters within the interaction "treatments x days" indicate significant difference (P<0.05). ^{xyz} different letters between different storage days indicate significant difference (P<0.05).

Table 4.9: Effect of treatments on the concentration of main sugars in avocado cv. Hass (early season, Exp. 3) fruit mesocarp stored for 21 days at 5ºC within 13L boxes in presence of e+® Ethylene Remover (e+® ER, 5 g) or initially pre-treated with 1-MCP (1.5 µL L^{-1}); values are expressed per residue (dry weight after lipid removal), per dry weight and per fresh weight.

	Treatment	sucrose			mannoheptulose			perseitol		
		Day 0	Day 7	Day 21	Day 0	Day 7	Day 21	Day 0	Day 7	Day 21
mg g^{-1} residue	Control	27.95[a]	20.61[a]	30.84[a]	77.10[a]	56.18[a]	33.03[a]	74.01[a]	46.47[a]	41.48[a]
	e+® ER	25.48[a]	28.41[a]	38.04[a]	82.14[a]	70.08[a]	49.72[a]	77.45[a]	52.61[a]	57.70[a]
	1-MCP	27.86[a]	20.68[a]	26.77[a]	97.76[a]	89.42[a]	51.70[a]	65.11[a]	53.89[a]	50.19[a]
	Mean	27.09[xy]	23.23[y]	31.88[x]	85.67[x]	71.89[x]	44.82[y]	72.19[x]	50.99[y]	49.79[y]
mg g^{-1} DW	Control	11.41[a]	8.50[a]	13.01[a]	31.09[a]	23.17[a]	14.02[a]	30.25[a]	19.36[a]	17.73[a]
	e+® ER	10.84[a]	11.95[a]	16.74[a]	34.83[a]	29.42[a]	22.24[a]	32.62[a]	22.20[a]	25.45[a]
	1-MCP	11.17[a]	8.66[a]	11.42[a]	39.24[a]	37.05[a]	22.40[a]	26.05[a]	22.15[a]	21.24[a]
	Mean	11.14[y]	9.70[y]	13.72[x]	35.05[x]	29.88[x]	19.55[y]	29.64[x]	21.24[y]	21.47[y]
mg g^{-1} FW	Control	3.08[a]	2.34[a]	3.24[a]	8.60[a]	6.40[a]	3.56[a]	8.24[a]	5.30[a]	4.50[a]
	e+® ER	2.98[a]	3.23[a]	3.96[a]	9.42[a]	7.79[a]	5.31[a]	8.96[a]	5.92[a]	6.05[a]
	1-MCP	3.12[a]	2.46[a]	2.96[a]	11.08[a]	10.71[a]	5.74[a]	7.37[a]	6.42[a]	5.54[a]
	Mean	3.06[xy]	2.68[y]	3.39[x]	9.70[x]	8.30[x]	4.87[y]	8.19[x]	5.88[y]	5.36[y]

[a] values with same letter within the interaction 'treatments x days' are not significantly different ($P>0.05$). [xyz] different letters between different storage days indicate significant difference ($P<0.05$).

Table 4.10: Effect of treatments on the concentration of main sugars in avocado cv. Hass (early season, Exp. 2) fruit mesocarp sampled after 0 and 3 days at 20ºC following cold storage at 5ºC. Fruit were stored for 0, 7 or 21 days at 5ºC within 13L boxes in presence of e+® Ethylene Remover (e+® ER, 5 g) or initially pre-treated with 1-MCP (1.5 µL L^{-1}); values are expressed per residue (dry weight after lipid removal), per dry weight and per fresh weight

	Treatment	sucrose		mannoheptulose		perseitol	
		Day 0	Day 3	Day 0	Day 3	Day 0	Day 3
mg g^{-1} residue	Control	26.46^{bc}	35.24^{a}	55.44^{a}	29.43^{a}	53.99^{a}	37.98^{a}
	e+® ER	30.66^{ab}	31.58^{a}	67.35^{a}	27.38^{a}	62.60^{a}	40.40^{a}
	1-MCP	25.08^{cd}	20.25^{d}	79.49^{a}	40.62^{a}	56.27^{a}	44.40^{a}
	Mean	27.40^{x}	29.03^{x}	67.43^{x}	32.48^{y}	57.62^{x}	40.89^{y}
mg g^{-1} DW	Control	10.97^{b}	13.93^{a}	22.76^{a}	11.71^{a}	22.45^{b}	15.01^{d}
	e+® ER	13.19^{a}	12.08^{ab}	28.84^{a}	10.47^{a}	26.77^{a}	15.45^{cd}
	1-MCP	10.41^{b}	8.13^{c}	32.84^{a}	16.86^{a}	23.09^{b}	18.04^{c}
	Mean	11.52^{x}	11.38^{x}	28.15^{x}	13.01^{y}	24.10^{x}	16.17^{y}
mg g^{-1} FW	Control	2.89^{bc}	3.62^{a}	6.19^{a}	3.09^{a}	6.01^{b}	3.94^{d}
	e+® ER	3.39^{ab}	3.19^{abc}	7.52^{a}	2.76^{a}	6.99^{a}	4.06^{d}
	1-MCP	2.85^{c}	2.24^{d}	9.16^{a}	4.54^{a}	6.43^{ab}	4.94^{c}
	Mean	3.04^{x}	3.02^{x}	7.62^{x}	3.46^{y}	6.48^{x}	4.31^{y}

^{abcd} different letters within the interaction "treatments x days" indicate significant difference (P<0.05). ^{xy} different letters between different shelf life days (column mean) indicate significant difference (P<0.05).

Further decline in C7 sugars was observed as fruit ripened at 20°C (Table 4.10) with mannoheptulose decreasing by half and perseitol by 1.4-fold within 3 days shelf life. This decrease in C7 sugar content during low temperature storage and during ripening of avocado has already been reported by others (Liu *et al.,* 1999b, 2002; Bertling and Bower, 2005) and in Chapter 3, and supports the hypothesis that avocado fruit may have an enzymatic mechanism to metabolize C7 sugars (Liu *et al.,* 1999b).

It has long been known that avocado fruit do not ripen on the tree but will only do so after detachment from the tree, possibly due to the presence of a ripening inhibitor transported from tree to fruit. The nature of this ripening inhibitor remains, as yet, unclear. Liu *et al.* (2002) hypothesised that C7 sugars may be partly responsible for ripening inhibition, based on ripening data and given the phloem mobility nature of these sugars. These authors used a different method for sugars extraction than herein (*viz*. extraction with 80% ethanol (v/v) rather than with methanol-based solvent). There is some evidence in this study for an association between mannoheptulose and perseitol metabolism and the ripening process (Tables 4.7-4.10). Specifically, where ripening of early season fruit stored at 5°C (Exp. 3) was delayed in response to e+® Ethylene Remover and 1-MCP treatment, better maintenance of mannoheptulose and perseitol during 21 days was found (Table 4.9). In particular, 1-MCP-treated fruit, which were also the firmest fruit, had, on average, more mannoheptulose (79.6 mg g^{-1} residue; 32.9 mg g^{-1} DW) vs. the more ripe e+® Ethylene Remover - treated fruit (67.3 mg g^{-1} residue; 28.8 mg g^{-1} DW; $P > 0.05$) and significantly more than controls (55.4 mg g^{-1} residue; 22.8 mg g^{-1} DW) over 21 days of storage. Perseitol concentrations of cold stored fruits declined more slowly in 1-MCP-treated fruit vs. other treatments (Exp. 2, Table 4.8) and were significantly higher in e+® Ethylene Remover - treated fruit vs. controls during cold storage (Exp. 3, Table 4.9). As fruit ripened at 20°C, a decline in mannoheptulose and perseitol concentrations from 28.2 to 13.0 mg g^{-1} DW and 24.3 to 16.2 mg g^{-1} DW, respectively (Table 4.10) occurred, concomitant with the rapid softening and colour changes observed. Again, more mannoheptulose ($P < 0.05$) and perseitol ($P > 0.05$) were found in the firmer 1-MCP-treated fruit vs. controls over 3 days of shelf life. Spearman's Rank Correlations were drawn between physical variables (firmness, L*, C* and H°) and each of the main sugars (Exp. 2 and 3). In accordance with

data presented herein, mannoheptulose in early season fruit (Exp. 3) exhibited a significant ($P < 0.001$) and good correlation with firmness (r = 0.70) and H° (r = 0.64), but a weaker correlation with L* (r = 0.40) and C* (r = 0.49). Perseitol, in contrast, was more poorly correlated with firmness (r = 0.57), C* (r = 0.43) and H° (r = 0.49). On the other hand, in late season fruit stored at 5°C (Exp. 2), fruit from both e+® Ethylene Remover and 1-MCP treatments took longer to ripen than did controls and this in spite of quasi-absence of mannoheptulose present in the mesocarp tissue (Table 4.8). This explains the very poor correlation ($P > 0.1$) found between mannoheptulose and each of the physical markers of fruit quality (r = 0.03–0.11), whilst there was a significant ($P < 0.001$) and better (r = 0.62–0.67) correlation between perseitol and firmness, C* and H°. This substantiates that if C7 sugars, and in particular mannoheptulose, participate in the regulation of the fruit-ripening process, these particular sugars may not be the only ripening inhibitor and other factors (possibly modulated by the action of ethylene), could be involved in the control of the fruit-ripening process.

Sucrose showed dissimilar patterns of change during storage at 12°C and 5°C (Tables 4.7-4.9): concentrations decreased significantly during storage at 12ºC (Exp. 1, Table 4.7) and the opposite trend was seen in late season fruit stored at 5ºC (Exp. 2, Table 4.8). In particular, sucrose levels in fruit were 1.3-fold lower at day 15 as compared with day 1 storage at 12ºC whereas at the end of storage period at 5ºC concentration was almost triple that at day 0. This decrease in sucrose at 12°C was largely due the decrease in 1-MCP and e+® Ethylene Remover fruit as concentration found in control fruit remained relatively stable over time (Table 4.7). Conversely, in early season fruit (Exp. 3, Table 4.9), sucrose declined during the first days of storage at 5°C but concentrations measured at day 21 were not different from those measured at day 0. Control fruits had, overall, significantly more sucrose vs. e+® Ethylene Remover -treated fruit and both fruit had significantly more sucrose than had 1-MCP-treated fruit (Exp. 2, Table 4.8). During shelf life at 20°C, the overall mean sucrose concentration was significantly greater in fast-ripening ripening controls and e+® Ethylene Remover-treated fruit vs. 1-MCP (Exp. 3, Table 4.10). Although sucrose has been regarded as less important than C7 carbohydrates in the carbon balance and has not been recognised as an indicator of postharvest quality in avocado fruit (Bertling

and Bower, 2005), previous work (Liu *et al.,* 1999b) suggested that this stored sugar could also contribute to the carbon energy source used by the respiratory process. There was no evidence in the present study to support this role, since sucrose concentrations increased during ripening at 20°C (Table 4.10), contrary to that previously observed (Liu *et al.,* 1999b). The fact that fruit were sourced from a different country of origin, at different harvest dates and assessed at different physiological age from that in other studies (Liu *et al.,* 1999a, 1999b) could have accounted for discrepancies in results. Nevertheless, clear differences were observed between 1-MCP-treated fruit and both e+® Ethylene Remover - treated and control fruit (Tables 4.8 and 4.10) indicating that indeed the metabolism of sucrose might be important.

4.4 Conclusion

This study is the first piece of research that reports on fatty acid and sugar changes in imported avocado fruit in response to 1-MCP and an ethylene scavenger. It is also the first direct comparison between ethylene blocking vs. ethylene removal effects on both physical and biochemical changes in avocado fruit. Results showed that passively removing ethylene below sub-physiologically active level using e+® Ethylene Remover has the ability to delay ripening of avocado cv. Hass fruit at low temperature, similarly to 1-MCP and consistent with the involvement of ethylene in softening process (Lelievre *et al.,* 1997). However, ripening resumed normally under shelf life conditions substantiating that, unlike after 1-MCP, tissues regain full sensitivity to ethylene when required. Corroborating this, no delay in ripening was observed where ethylene was reduced only below 1 uL L^{-1}. In Exp. 1, 1-MCP and e+® Ethylene Remover were tested under challenging conditions since i) fruit were stored at 12°C, which is higher than the recommended 5-6°C for avocado storage ii) treatments were applied on imported fruit after the transit period, whilst most studies have applied 1-MCP straight after harvest.

The fatty acid composition was generally not markedly affected by delayed ripening. This work constituted a good system in which to study the role of C7 sugars during avocado fruit ripening. Results have shown that C7 sugars are important

biochemical compounds in avocado cv. Hass fruit and, since changes in their content generally mirrored that of changes in ripening, C7 sugar metabolism may be an important feature of the fruit-ripening process. Clearly, in unripe fruit, C7 sugars were the major form of soluble sugars and concentrations declined substantially as ripening advanced. However, lack of evidence from some results (i.e. late season fruit) substantiate that more research is necessary to elucidate the function of the particular sugars in the fruit-ripening process. In the future, the different levels at which the e+® Ethylene Remover and 1-MCP exert their action (viz. ethylene removal vs. blocking) represents an opportunity to further understand the mechanisms by which fruits modulate various responses to ethylene, in particular ethylene signalling and receptor function.

CHAPTER 5

Delaying ripening is possible even when the climacteric has been induced

5.1 Introduction

Avocado is a highly perishable commodity and one of the most rapidly softening of all fruit. It was shown previously (Chapter 4) that 1-MCP and e+® Ethylene Remover were effective at delaying the ethylene-induced softening and colour change of imported unripe avocado cv. Hass stored for 26 days at 5°C. Whilst fruit that had previously been stored in the presence of e+® Ethylene Remover ripened normally under shelf life conditions, those treated with 1-MCP softened and changed colour more unevenly.

Most reviews addressing the effects of techniques to maintain avocado quality, including 1-MCP, have been attempted on unripe pre-climacteric fruit as the material of investigation and have focused on preventing the onset of ripening rather than slowing down the process after the climacteric has been initiated. Convention states that once the avocado has crossed over into the post-climacteric stage, ripening is irreversible and accompanied by a large production of ethylene resulting in a shortened shelf life. Adkins *et al.* (2005) showed that ripening of avocado cv. Hass could be delayed when 1-MCP was applied within 2 days following ethylene treatment (100 μL L^{-1}) to initiate the climacteric; the fruit became unresponsive to 1-MCP action when the inhibitor was applied at the start of softening, suggesting that ethylene-induced ripening could not be reversed after a certain time following ethylene perception. Terry *et al.* (2007a) demonstrated that application of the Pd-promoted ethylene scavenger within 1 day of ethylene treatment (100 μL L^{-1}) could still delay softening of avocado fruit held at 12°C.

Commercially, there are many instances when cold-stored fruit may be exposed to warmer temperatures. The effects can be viewed as positive or negative, according to the commercial purpose. For instance in the packhouse facility, fruit may be warmed up (18-21°C) for 1-3 days, before being gradually cooled down to 5°C until packing and

distribution (A. Shaw, Mack Multiples, pers. com.). A stepped temperature programme is a commonly used ripening regime that has the advantage of reducing chilling disorders and better controls the ripening rate. In that sense, Hofman *et al.* (2002c) showed that hot water treatment of about 41 °C for 25–30 min, or 42 °C for 25 min enhanced avocado external and internal fruit quality following cold disinfestation of cv. Hass avocado. The disadvantage is that such a process is energy-consuming due to large differences between temperatures. On the other hand, undesirable interruption in the cold chain (or cold chain abuse) is not unusual, especially when long distance transport to reach far away markets is required, and will result in faster ripening and quality loss (Blakey and Bower, 2009). Cool chain abuse is more important when product is transfered from different modes of transport. For example, fruit imported into Europe necessitate transportation by truck from the orchard to the port, shipment for several weeks and eventually transport from the port to the retail distribution centre (RDC) and retailer in Europe by truck. This puts high logistical constraints on the supply chain and it is near inevitable that cold chain abuse will occur, either during shipment or due to delays at customs. As a consequence, temperature abuse occurring between orchard and retailers often impact on produce quality and therefore on consumer acceptability for the commodity. Any technique aimed at minimizing detrimental effects of cool chain abuse on quality of fresh commodities would be useful. In addition, poor temperature management may also occur in the lower segment of the supply chain, i.e. during transport from the distribution center to the store, inside the retail (store) display, and at home (Nunes *et al.*, 2009), further affecting sensory quality and reducing shelf life of the produce, and ultimately increasing wastes. Wastes further down the supply chain leads to not only lost profits, but also accrure all the imbedded costs of logistics and increase cumulative greenhouse gas emissions, which in a current context of climate change cannot be neglected. Generation of wastes is of growing concern and there would be advantages of reducing them.

Therefore, the objective of the present study was to determine whether the efficacy of 1-MCP and e+® Ethylene Remover at maintaining firmness and greeness of cold stored (5°C) avocado cv. Hass persist upon an intervening exposure to warmer temperature (18°C for 24h) to simulate cold chain abuse and induce the climacteric. Additionally, in another

experiment, e+® Ethylene Remover was tested in combination with modified atmosphere packaging (MAP).

5.2 Material and methods

5.2.1 Plant materials

Two experiments were conducted. For Exp 1., late season pre-climacteric avocado cv. Hass fruit [size code 22] were sourced from a commercial farm in Malaga, Spain and supplied by Mack Multiples Division (M. W. Mack Ltd., Kent, U.K.). Fruit were harvested on 2nd May 2008 and transported into UK under refrigeration (5-6°C). Fruit were received at the Plant Science Laboratory on 12th May 2008 and were unripe upon arrival, as confirmed by initial firmness and colour. Fruit were not pre-treated with 1-MCP. Fruit were kept overnight at 5ºC before commencing treatments. In Exp. 2, early season avocado cv. Hass fruit [size code 16] were sourced from a commercial farm in Melipilla, Chile, and supplied by Mack Multiples Division (M. W. Mack Ltd., Kent, U.K.). Fruit were harvested on the 4th September 2008 and shipped into UK under standard commercial conditions at 5°C for approx. 5 weeks. Fruit were received at the Plant Science Laboratory on 10th October 2008 and fruit were unripe upon arrival, as confirmed by initial firmness and colour. Again, fruit were not pre-treated with 1-MCP. Fruit were kept for one day and one night at 5ºC before commencing treatments.

5.2.2 Treatments and storage regimes

5.2.2.1 Experiment 1

Fruit (n = 252) were randomly placed into transparent 13 L hermetically sealed polypropylene boxes (approx. 32 cm x 14.5 cm x 28 cm; n = 18 with 14 fruit per box) and treated with e+® Ethylene Remover (5 g; n = 6 boxes) or 1-MCP (1.5 µL L^{-1}, 24h at 5°C; n = 6 boxes) as described in Chapter 4. Untreated fruit (n = 6 boxes) acted as controls and

were otherwise held in the same conditions as treated fruit. Following treatments, boxes containing the fruit were then stored in the dark at 5°C and *ca.* 98% RH for 7 days (storage S1).

After 0 or 7 days storage, a subsample of fruit from all treatments (n = 42 per treatment) were removed from cold storage and firmness and colour measurements were taken on some fruit (n = 6 per treatment; n = 3 treatments) directly ('before break'). The remaining fruit (n = 36 per treatment) were placed in new boxes (n = 4 boxes per treatment, 9 fruit per box) of the same dimensions as before. To half of the fruit (n = 2 boxes per treatment) was added fresh e+® Ethylene Remover (4 g per box), resulting in 6 treatment combinations (cf. Figure 5.1). Boxes were then held at 18°C for *ca.* 24h to simulate cold chain abuse and triggered to ripen ('temperature break'). After 24h, boxes were vented and transferred back to 5°C for an additional 4 or 7 days of storage at 5°C (post-break cold storage, S2) followed by two days of shelf life in open air at 20°C. CO_2 poisoning during storage in boxes was avoided by manually venting boxes each day.

Individual firmness, and colour measurements were taken on fruit subsamples after 4 and 7 days cold storage S2 (after break; n = 6 treatments combinations) and after an additional 2 days shelf life. Firmness and colour were measured as described in Chapter 4. Samples derived from day 0 and 7 were prepared for subsequent sugar analysis, as described in Chapter 3. Each treatment at each sampling had 6 fruit and each fruit constituted a replicate. Firmness and colour measurements were taken on additional fruit at arrival at Plant Science Laboratory (baseline n = 15).

5.2.2.2 Experiment 2

Fruit in single layer cardboard trays were held for 24h or 48h (n = 81 each period) at 18°C to simulate cold chain abuse and trigger the climacteric. After 24h or 48h, fruit were removed and placed individually in polypropylene plastic punnets (140 mm×115 mm; Nicholas Ltd., Derbys., UK). Punnetted fruit were separated into 3 groups of 27 fruit. Two groups of punnetted fruit were individually placed in NatureFlex™ NVS films of 20 x 28 cm dimension and 30 μm thickness (Innovia Films Ltd., Cumbria, UK) and sealed using a

hand-operated heat sealer (Hulme Martin Ltd., Surrey, UK). The films were not perforated and had a permeability of 360 g of water vapour m^{-2} 24 h^{-1} (test at 38°C, 90% RH) and 3 cc of O_2 m^{-2} 24 h^{-1} bar^{-1} (test at 23°C, 0% RH). The CO_2 permeability for this film was not known but tests carried out on a film similar to NVS 30 µm gave a figure of 42 cc of CO_2 m^{-2} 24 h^{-1} (test condition 23°C and 0.5% RH) (C. McKeown, Innovia Films Ltd., pers. com.) To one packed group was added e+® Ethylene Remover by placing 0.5 g of the powdered material nested in a polystyrene disposable weighing boat (55 x 30 mm diamond shape, Fisher Scientific, Leics., UK) inside each packaging just before sealing (MAP/ e+® Ethylene Remover). To the other packed group was added nothing (MAP only). The last group remained unwrapped (control). All fruit were then stored at 5°C in darkness for 7 days. The film was selected on the basis of its high moisture permeability providing a drier environment for e+® Ethylene Remover.

After 3, 7 and 9 days storage, individual bags (n = 9 per treatment) were sampled for atmospheric gase measurements and fruit physical assessment. For measurement of CO_2 and ethylene concentrations inside bags, a headspace gas sample was removed using a syringe fitted with a hypodermic needle through a small septum (9 mm diameter) taped to the bag. Ethylene and CO_2 present in the headspace were quantified by gas chromatography with FID and hot wire detector (HWD), respectively, as described in Chapter 4. Firmness and colour were determined on individual fruit (n = 9 per treatment per sampling) according to Chapter 4. Additional fruit were measured for firmness and colour on arrival at Plant Science Laboratory (baseline, n = 9) and just after 24h or 48h triggering at 18°C, before packing (n = 9 each period). Sugars were measured in fruit sampled at arrival (baseline), after 24h temperature break, and after 3 and 7 days subsequent storage at 5°C following 24h break, using the method described in Chapter 3.

5.2.3 Statistical analysis

Analysis of variance (ANOVA) was carried out as detailed in Chapter 4. Firmness data for Exp. 1 was log transformed to fit the requirements for analysis of variance. Data presented are log and back transformed values that represent biological data.

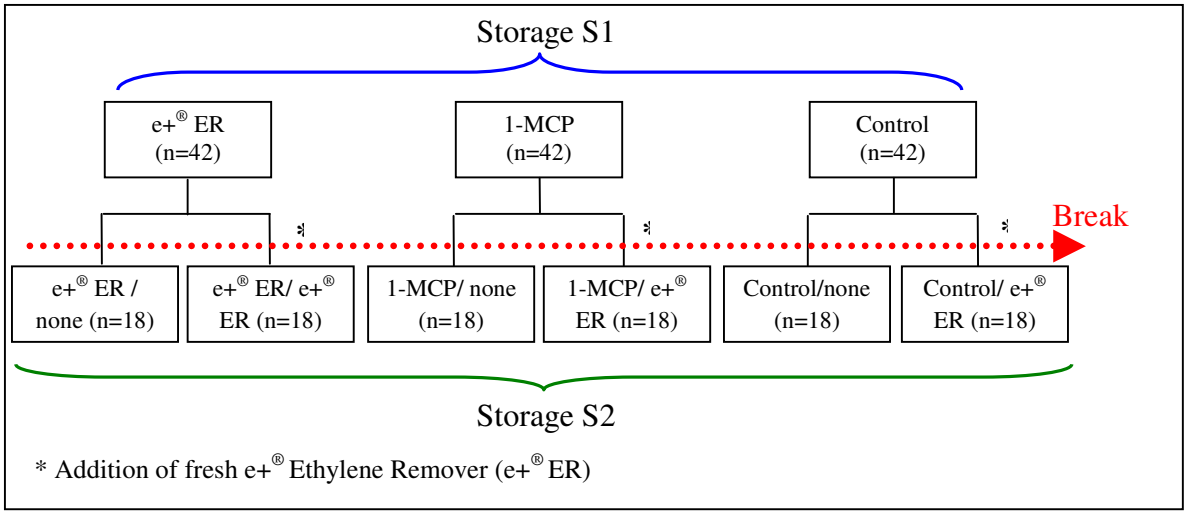

Figure 5.1. Experimental design of Exp. 1: at each sampling period in S1 (0 or 7 days), fruit subsample (n = 42 per treatment) were removed and treated with or without fresh e+® Ethylene Remover before being exposed to 18°C for 24h (temperature break) and resuming storage at 5°C (S2) for 4 (n = 6 per treatment) or 7 (n = 6 per treatment) days. Following 7 days storage S2, remaining fruit (n = 6 per treatment) were held for an additional 2 days shelf life in open air at 20°C.

5.3 Results

5.3.1 Ethylene levels inside storage atmospheres

In Exp. 1, during initial storage (S1) of preclimacteric fruit at 5ºC, ethylene level inside control boxes was 0.44 µL L^{-1} after 2 days and 0.84 µL L^{-1} after 7 days storage. Boxes pre-treated with 1-MCP contained more ethylene than controls, at concentrations of 1.46 µL L^{-1} and 0.88 µL L^{-1} after 2 and 7 days storage, respectively. Presence of e+® Ethylene Remover however reduced ethylene concentrations to 0.02 µL L^{-1} during 7 days. After the temperature break, the ethylene concentration inside boxes increased with levels highest at day 1 of post-break storage (S2), after which levels decreased over time (Figure 5.2.). However, addition of fresh e+® Ethylene Remover prior to transfer at 18°C maintained ethylene concentrations between 0.049 and 0.430 µL L^{-1} over storage time. It

was observed that boxes containing 1-MCP-treated fruit had a lower ethylene concentration as compared to fruit initially treated with e+® Ethylene Remover or non-treated in S1, especially after 0 days storage S1 (Figure 5.2.).

In Exp. 2, ethylene inside non-treated packaging accumulated during 7 days storage at 5°C whereas presence of e+® Ethylene Remover reduced levels to below 1 μL L^{-1} (Table 5.1). CO_2 levels were relatively elevated but did not differ significantly between treatments. Measurement of a headspace gas sample withdrawn inside the cold room were additionally analysed and showed no detectable ethylene.

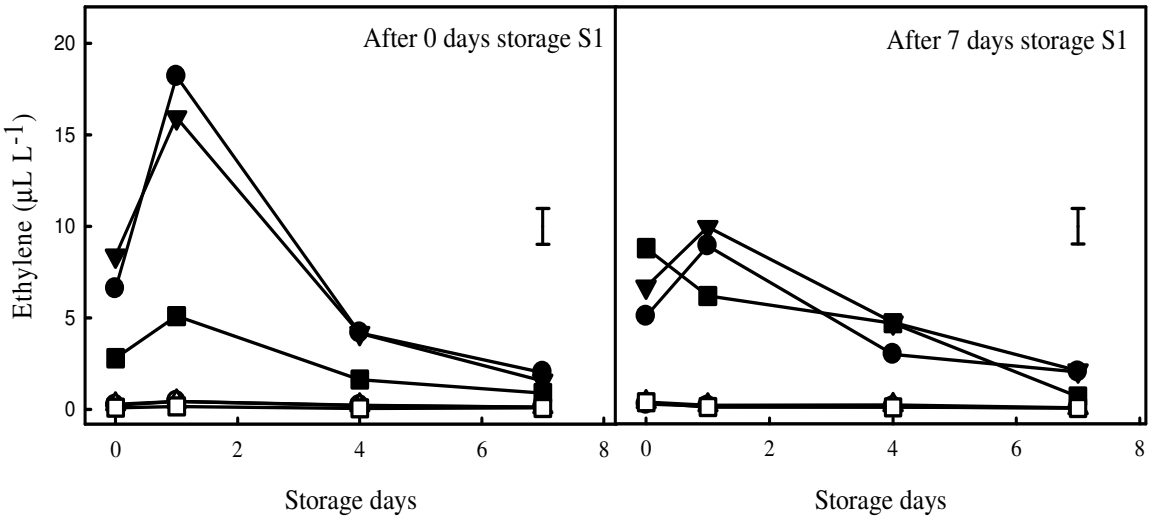

Figure 5.2. Ethylene concentrations (μL L^{-1}) within boxes (boxes opened every day) containing avocado cv. Hass during 7 days storage at 5°C (S2) and held in presence (open symbol) or absence (close symbol) of e+® Ethylene Remover. Fruit were initially treated with 1-MCP (square symbol), e+® Ethylene Remover (triangle symbol) or non-treated (control; round symbol) and stored for 0 or 7 days at 5°C (S1) before temperature break (18°C for 24h). Values on day 0 are concentrations just after break, before resuming cold storage. Values on day 1 are concentrations 1 day after resuming cold storage following break.

Table 5.1. Ethylene (μL L^{-1}) and CO_2 (%) concentrations inside packaging containing individual avocado cv. Hass fruit without (MAP) or with e+® Ethylene Remover (MAP/e+® ER) during storage at 5°C. Fruit were initially held for 24h or 48h at 18°C on day 0 (temperature break). Values are mean of 9 replicates.

Break	Storage day	Ethylene (μL L^{-1})		CO_2 (%)	
		MAP	MAP/e+® ER	MAP	MAP/e+® ER
24h	1	23.0	0.6	4.74	3.56
	3	23.5	0.5	4.86	4.42
	7	12.1	0.4	5.32	4.81
	Mean	19.6[a]	0.5[b]	4.97[a]	4.26[a]
48h	1	45.6	0.5	7.67	8.86
	3	35.3	0.2	10.90	11.58
	7	24.8	0.3	8.98	10.24
	Mean	35.2[a]	0.3[b]	9.18[a]	10.23[a]

[a,b] different letters within each row indicate significant differences ($P<0.05$) between treatments.

5.3.2 Fruit firmness and colour

5.3.2.1 Experiment 1

Flesh softening and skin colour change was measured after 0 or 7 days of cold storage before temperature break (S1), after 4 and 7 days of cold storage after temperature break (S2) and after 2 additional days under shelf life conditions in open air.

During 7 days of storage S1, the control fruit softened with a 2.6-fold reduction in firmness between day 0 and 7 storage whereas treatment with e+® Ethylene Remover or 1-MCP resulted in significantly better firmness maintenance over the same period (Table 5.2, column 'before break'). For all fruit, temperature break induced a significant decrease in firmness, from an average of 166.4 N before break to 48.2 N and 32.4 N after 4 and 7 days

storage S2, respectively (Table 5.2). In particular, firmness of control fruit declined by 75% of its initial value within 4 days following break and this decrease was more pronounced for control fruit that had previously been cold stored for 7 days in S1, with a decrease of 87% within 4 days post-break storage (Table 5.2). Whilst this decline occurred for all treatments, all fruit held in presence of fresh e+® Ethylene Remover in S2 were firmer (on average 45.3 N) vs. untreated fruit (35.3 N) (Table 5.2). This said, the standard error for fruit treated with e+® Ethylene Remover in S2 was proportionally higher than for non-treated fruit, especially after 0 days S1. In addition, there was a treatment in S1 main effect, whereby fruit initially stored with e+® Ethylene Remover were overall (S1 and S2 combined) significantly less soft (average 59.8 N) than controls (45.6 N) whilst both fruit were significantly softer than 1-MCP-treated fruit (91.1 N). The standard error for 1-MCP-treated fruit was generally higher than that of control (1-2.6 fold) or e+® Ethylene Remover- treaed fruit (0.7-3 fold) (Table 5.2), indicating more heterogeneity in ripening.

Table 5.2. Firmness of avocado cv. Hass fruit after 0 and 7 days cold storage S1 ('before break') and after 4 and 7 days storage S2 following temperature break. Fruit were initially treated with e+® Ethylene Remover (e+® ER), 1-MCP or non-treated (control) and cold stored for 0 or 7 days at 5°C (S1) before temperature break. Fruit were then stored without (none) or with e+® Ethylene Remover (e+® ER) for 4 or 7 days at 5°C (S2). Values are mean of 6 replicates ± SE. Values in brackets are log transformed data.

Storage day S1	Treatment S1	Storage day S2	Treatment S2		
			before break[1]	none	e+® ER
0	Control	before break[1]	201.2±7.48^{A}		
		4		48.9±10.65	54.3±11.64
		7		13.8±1.07	28.6±7.06
	e+® ER	before break	195.5±6.52^{A}		
		4		39.1±6.57	77.2±20.56
		7		29.3±9.25	42.3±17.83
	1-MCP	before break	197.7±16.80^{A}		
		4		117.5±21.77	88.2±27.03
		7		70.1±14.02	104.3±29.32
7	Control	before break	75.1±17.00^{B}		
		4		9.7±2.39	10.8±2.60
		7		7.9±1.53	6.0±0.62
	e+® ER	before break	163.1±34.00^{A}		
		4		12.9±4.69	19.3±6.85
		7		5.9±0.69	12.8±2.54
	1-MCP	before break	165.5±13.39^{A}		
		4		35.2±19.36	64.7±31.10
		7		33.1±11.67	35.1±9.39
		mean	166.4 (5.005)^{X}	35.3 (3.030)^{Yx}	45.3 (3.253)^{Yy}

[1] Values at removal from initial cold storage S1, before break.^{A,B} different letters within same column indicate significant differences between treatments ($P<0.05$), LSD used for comparison of treatments before break only. ^{X,Y} different letters within same row indicate significant differences between means ($P<0.05$), LSD used for comparison of 'before break' with treatments in S2 (log transformed value). ^{xy} different letters within same row indicate significant differences between means ($P<0.05$), LSD used for comparison between treatments in S2 only (log transformed data)

Similarly to softening, a decline in L*, C* and H° (Table 5.3) was observed following the break. However, and as observed for firmness, there was a treatment main effect for S1 in that fruit which had initially been stored with e+® Ethylene Remover were overall greener (average S1 and S2, Hº~106.02) as compared with controls (Hº~100.48) and both treatments were less green than 1-MCP-treated avocado (Hº~110.03). On the other hand, L* and C* values were highest ($P<0.05$) in both 1-MCP-treated and e+® Ethylene Remover -treated fruit than in controls (data not shown). There was also a main effect of treatment applied in S2 on H°, as fruit treated with fresh e+® Ethylene Remover in S2 exhibited a higher value (average Hº~104.16) vs. untreated fruit (Hº~99.86) (Table 5.3). In particular, controls held in presence of e+® Ethylene Remover were significantly greener after 0 days storage S1 and 7 days post-break storage than untreated controls, and the same trend was seen after 7 days storage S1 and 4 days storage S2 (Table 5.3).

Following an additional 2 days at 20°C in open air, fruit previously treated with e+® Ethylene Remover in S1 and controls were fully ripe (firmness < 5N and H°~63). In contrast, fruit initially treated with 1-MCP were significantly less soft (24.4 N) and more green (H°~78.3), and exhibited proportionally more heterogeneity in ripening as demonstrated by higher standard errors (Table 5.4).

Table 5.3. Hue angle (H°) of avocado cv. Hass fruit after 0 and 7 days cold storage S1 ('before break') and after 4 and 7 days storage S2 following temperature break. Fruit were initially treated with e+® Ethylene Remover (e+® ER), 1-MCP or non-treated (control) and cold stored for 0 or 7 days at 5°C (S1) before temperature break. Fruit were then stored without (none) or with e+® Ethylene Remover (e+® ER) for 4 or 7 days at 5°C (S2). Values are mean of 6 replicates ± SE.

Storage day S1	Treatment S1	Storage day S2	Treatment S2		
			before break[1]	none	e+® ER
0	Control	before break[1]	123.23^{Aa}		
		4		119.06^{abcd}	112.74^{abcdef}
		7		87.77^{klmn}	102.27^{fghij}
	e+® ER	before break	118.89^{Aabcd}		
		4		117.86^{abcd}	115.14^{abcdef}
		7		107.21^{defghi}	103.37^{efghij}
	1-MCP	before break	120.83^{Aab}		
		4		118.63^{abcd}	112.67^{abcdef}
		7		110.66^{bcdefg}	114.91^{abcde}
7	Control	before break	$111.88^{Aabcdefg}$		
		4		82.52^{mn}	95.28^{ijk}
		7		87.28^{klmn}	82.80^{lmn}
	e+® ER	before break	120.72^{Aabc}		
		4		96.72^{hijk}	107.20^{defghi}
		7		78.31^{n}	94.76^{jkl}
	1-MCP	before break	121.54^{Aab}		
		4		98.44^{hijk}	108.73^{cdefgh}
		7		93.84^{jklm}	100.06^{ghij}
		mean	119.51^{X}	99.86^{Yy}	104.16^{Yx}

[1] Values at removal from initial cold storage S1, before temperature break. [A]same letter within column indicate no significant differences between treatments (P>0.05), LSD used for comparison of treatments before break only. [a-n] different letters indicate significant differences (P<0.05), LSD used for comparison within the interaction 'storage S1 x treatment S1 x storage S2 x treatment S2'. [X,Y] different letters within row indicate significant differences between means (P<0.05), LSD used for comparison of 'before break' with treatments in S2. [x,y] different letters within row indicate significant differences between means (P<0.05), LSD used for comparison between treatments in S2 only.

Table 5.4. Firmness and hue angle (H°) of avocado cv. Hass fruit held for 2 days at 20°C after 7 days at 5°C (S2) without (none) or with e+® Ethylene Remover (e+® ER). Fruit were initially treated with e+® Ethylene Remover (e+® ER), 1-MCP or non-treated (control) and cold stored at 5°C (S1) before temperature break (18°C for 24h). Data shown are average of 0 and 7 days storage S1. Values are mean of 12 replicates ± SE.

	Firmness (N)			Hue angle (H°)		
	Treatment S1					
Treatment S2	control	e+® ER	1-MCP	control	e+® ER	1-MCP
None	3.1± 0.17	3.2± 0.11	20.3± 4.75	58.2± 4.97	64.5± 3.78	77.2± 5.78
e+® ER	4.2± 0.34	3.2± 0.23	28.5± 7.76	66.0± 3.63	64.7± 4.85	79.4± 7.01
Mean	3.6^{y}	3.2^{y}	24.4^{x}	62.1^{y}	64.6^{y}	78.3^{x}

^{x,y} different letters indicate significant differences between treatment means ($P<0.05$).

5.3.2.2 Experiment 2

On arrival at the laboratory, fruit were unripe as confirmed by firmness (195.3 N) and greenness (H°~121) values. Following the temperature break, firmness declined significantly to 136.6 N and 46.6 N after 24h and 48h at 18°C, respectively (Table 5.5). During subsequent storage at 5°C, fruit further softened in a significant fashion (Table 5.5). However, whilst this decrease occurred in all fruit, avocado held with e+® Ethylene Remover remained significantly firmer over 7 days storage as compared with fruit packed without the scavenger or unwrapped controls (Table 5.5).

Table 5.5. Firmness and hue angle of avocado cv. Hass packed individually without (MAP) or with e+® Ethylene Remover (MAP/e+® ER) or unpacked (control) and stored at 5°C. Fruit were initially held for 24h or 48h at 18°C (temperature break). * Day 0 corresponds to value just after 24h or 48h triggering, before treatments.

		Firmness (N)				Hue angle (H°)			
Temperature break	Storage day	Before treatment	Control	MAP	MAP/e+® ER	Before treatment	Control	MAP	MAP/e+® ER
24h	0*	136.6				121.91^{a}			
	3		49.2	56.7	70.6		119.69^{abc}	120.70^{abc}	121.64^{a}
	7		31.4	28.4	58.3		118.60^{abc}	118.71^{abc}	121.02^{ab}
48h	0	46.6				119.02^{abc}			
	3		12.9	19.3	33.8		113.80^{d}	117.86^{abcd}	118.41^{abc}
	7		6.0	7.0	8.0		97.36^{e}	116.71^{bcd}	116.57^{cd}
	mean	91.6^{X}	24.9^{Yy}	27.8^{Yy}	42.7^{Yx}	120.46^{X}	112.36^{Yy}	118.49^{Xx}	119.41^{Xx}

$^{a-e}$ different letters indicate significant differences (P<0.05). ^{X,Y} different letters within row indicate significant differences between means (P<0.05), LSD used for comparison of 'before treatment' with treatments. ^{x,y} different letters within row indicate significant differences between means (P<0.05), LSD used for comparison between treatments only.

On the other hand, skin colour did not change after 24h triggering at 18°C and subsequent storage, whereas after 48h triggering, unwrapped controls were the only fruit to present a significant decrease in hue angle after 3 and 7 days at 5°C, and thus were less green ($P<0.05$) than other treatments (Table 5.5). L* and C* were also affected by treatments whereby fruit held with e+® Ethylene Remover had overall higher L* (34.82) and C* (20.15) values than untreated packed fruit (33.63 and 17.22, respectively) or unwrapped controls (32.89 and 15.58, respectively).

5.3.3 Effect of treatments on sugar content

5.3.3.1 Experiment 1

Sugar content was measured after 0 and 7 days of cold storage S1, after 7 days of cold storage after temperature break (S2) and after 2 days of shelf life. Fruit treated with 1-MCP were not analysed for sugar content. The main sugars detected were mannoheptulose, perseitol and sucrose (Table 5.6 and 5.7). Glucose and fructose were detected in very low quantity and were not considered in this study. During 7 days of cold storage S1, mannoheptulose and sucrose content remained constant at a concentration of 21.13 mg g^{-1} residue (7.35 mg g^{-1} DW) and 25.26 mg g^{-1} residue (8.86 mg g^{-1} DW) respectively, and were not affected by treatments. This said, differences were observed between treatments for sucrose, whereby concentrations declined in controls but increased in e+® Ethylene Remover -treated fruit, although in a non-significant fashion. Similarly, perseitol content remained unchanged over the same period, although a considerably higher (more than double) concentration was measured in mesocarp tissue (49.60 mg g^{-1} residue; 17.42 mg g^{-1} DW), independent of treatments applied.

Following the temperature break, concentration of mannoheptulose and perseitol contents decreased significantly with lower concentrations measured after 7 day storage S2 than before temperature break (Table 5.6), except for perseitol content following 7 days storage S1, where content remained unchanged before and after break. For perseitol, concentrations further declined after an additional 2 days shelf life whilst for

mannoheptulose, the same trend was observed in fruit initially stored 0 day S1 only (Table 5.6). There was no treatment effect on C7 sugars content.

Changes in sucrose differed from that of C7 sugars since amounts increased considerably after temperature break, from 25.26 mg g^{-1} residue (8.86 mg g^{-1} DW) before break to 52.97 mg g^{-1} (16.75 mg g^{-1} DW) after break (average 7 days S2 and 2 days shelf life), and this decline was seen in all treatments (Table 5.7). There was generally no effect of treatment on sucrose content, except for fruit initially stored 7 days with e+® Ethylene Remover, which after 7 days S2 had significantly higher sucrose content when held without (67.89 mg g^{-1} residue) than with (41.84 mg g^{-1} residue) e+® Ethylene Remover, or than controls without e+® Ethylene Remover (36.98 mg g^{-1} residue; Table 5.7).

Table 5.6. Concentrations of main sugars in mesocarp of avocado cv. Hass fruit immediately after 0 and 7 cold storage S1 ('before break'), after 7 days storage S2 following temperature break and after an additional 2 days shelf life at 20°C ('shelf life'). Values are mean of treatment in S1 (e+® Ethylene Remover and non-treated) and treatment in S2 (with or without e+® Ethylene Remover). Before break: n = 12; day 7 and shelf life: n=24.

		mannoheptulose		perseitol	
Storage day S1	Storage day S2	mg g^{-1} residue	mg g^{-1} DW	mg g^{-1} residue	mg g^{-1} DW
0	before break[1]	21.09^{X}	7.17^{X}	50.20^{X}	17.30^{X}
	day 7	16.73^{Ya}	5.62^{Ya}	49.51^{Xa}	16.66^{Xa}
	shelf life	13.09^{Zb}	3.92^{Zb}	32.04^{Zc}	9.59^{Zc}
7	before break	21.17^{X}	7.52^{X}	48.99^{X}	17.54^{X}
	day 7	12.31^{Zb}	3.95^{Zb}	38.76^{Yb}	12.60^{Yb}
	shelf life	13.10^{Zb}	4.11^{Zb}	33.03^{YZc}	10.30^{YZc}

^{X,Y,Z} different letters indicate significant differences (P<0.05), LSD used for comparison of 'before break' with all other times. ^{a,b,c} different letters indicate significant differences (P<0.05), LSD used for comparison of all times except 'before break'

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Table 5.7. Sucrose concentration in avocado cv. Hass mesocarp tissue after 0 and 7 days cold storage S1 ('before break'), after 7 days storage S2 following temperature break and after an additional 2 days shelf life at 20°C ('shelf life'). Fruit were initially treated with e+® Ethylene Remover (e+® ER), or non-treated (control) and cold stored for 0 or 7 days at 5°C (S1) before temperature break. Fruit were then stored without (none) or with e+® Ethylene Remover (e+® ER) for 7 days at 5°C (S2). Values are mean of 6 replicates ± SE.

Storage day S1	Treatment S1	Storage S2	Sucrose (mg g^{-1} residue)			mg g^{-1} DW		
			Before	none	e+® ER	Before	none	e+® ER
0	control	before break[1]	27.13^{de}			9.16^{def}		
		day 7		49.37^{abcd}	49.97^{abc}		16.05^{abcd}	16.38^{abcd}
		shelf life		53.83^{abc}	51.52^{abc}		16.34^{abcd}	15.21^{abcd}
	e+® ER	before break[1]	19.52^{e}			6.66^{f}		
		day 7		53.04^{abc}	49.77^{abc}		18.26^{abc}	16.91^{abc}
		shelf life		68.96^{ab}	47.06^{bcd}		20.83^{ab}	14.02^{bcde}
7	control	before break[1]	19.41^{e}			6.98^{ef}		
		day 7		36.98^{cde}	57.21^{abc}		14.12^{bcde}	18.38^{abc}
		shelf life		70.43^{a}	51.60^{abc}		21.36^{a}	17.15^{abc}
	e+® ER	before break[1]	34.97^{cde}			12.62^{cdef}		
		day 7		67.89^{ab}	41.84^{cd}		19.75^{abc}	13.41^{cdef}
		shelf life		50.72^{abc}	47.39^{bcd}		15.57^{abcd}	14.32^{abcd}

[a-f] different letters indicate significant differences (P<0.05), LSD used for comparison within the interaction 'storage S1 x treatment S1 x storage S2 x treatment S2'.

5.3.3.2 Experiment 2

Sucrose, mannoheptulose and perseitol were the main sugars measured. Mannoheptulose content measured at each sampling stage was not significantly different (Figure 5.3.) and was not affected by treatments applied. Perseitol, on the other hand, did not differ between baseline concentration, following 24h at 18°C and subsequent storage at 5°C, but was significantly higher after 3 days than after 7 days at 5°C, independent of treatments applied. Sucrose levels were not different between baseline value, after 24h at 18°C and 3 days at 5°C, but concentration found after 7 days at 5°C was significantly higher from that after 24h at 18°C or after 3 days at 5°C. This said, this difference remained slight (Figure 5.3.).

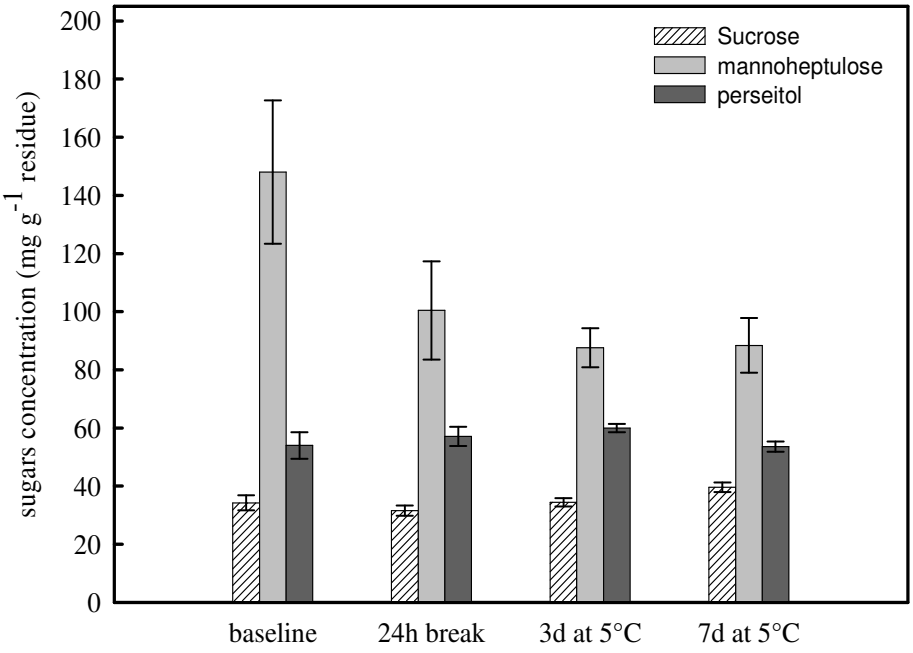

Figure 5.3. Concentrations of main sugars in avocado cv. Hass mesocarp at arrival (baseline), after 24h break at 18°C and after 3 and 7 days storage at 5°C after break. Data shown on day 3 and 7 at 5°C are means of treatments. Baseline: n = 9; 24h break: n = 9; 3 d and 7 d at 5°C: n = 27. Bar shows the standard error.

5.4 Discussion

Although it has been ascertained that the cold chain should be maintaining at all time during postharvest handling of avocado, temperature abuse in the 'real world' often occurs, especially when many intermediates are involved as fruit progresses from orchard to consumer (Dodd *et al.*, 2007). Interrupting the cold chain during postharvest handling is detrimental to avocado fruit quality (Undurraga *et al.*, 2007; Blakey and Bower, 2009). Undurraga *et al.*, (2007) found that interrupting the cold chain toward the end of the storage period resulted in the fruit softening and changing colour before end of storage. The effect of breaking the chain at various points during cold storage of avocado has been studied (Blakey and Bower, 2009) and these authors found that fruit stored at 5.5°C and subjected to a cold break had poor quality upon ripening (below 30% sound fruit). Despite the inevitable risk of temperature abuse, hence premature ripening, along the cold storage chain, little work has been conducted aimed at minimizing the ripening rate after climacteric induction. Moreover, whilst some research has looked at the effect of 1-MCP when applied following ethylene treatment, no study has looked at the detailed effects of ethylene removal after climacteric induction.

In this Chapter, e+® Ethylene Remover was used as a means of quality preservation during postharvest storage of preclimacteric fruit, but also during the climacteric event and subsequent cold storage. In Exp. 1, addition of the scavenger reduced the ethylene levels below 0.1 $\mu L\ L^{-1}$ during the preclimacteric storage phase, and accordingly a delay in softening and colour change were observed (Table 5.2), corroborating previous results (Chapter 4) and consistent with the role of negative regulators played by receptors in the absence of ethylene. Similarly, 1-MCP effectively blocked ethylene binding resulting in suppressed softening and colour change over storage time (Table 5.2-5.3), in agreement with Chapter 4 and published reports on the effects of 1-MCP for avocado cv. Hass (*cf.* Watkins, 2006). In contrast, untreated fruit showed substantial softening during cold storage at 5°C, which has already been reported elsewhere (Blakey and Bower, 2009; Chapter 4). It has been shown that ethylene synthesis and response genes are induced during cold storage of avocado

(Hershkovitz *et al.*, 2010), and may probably be the reason why untreated fruit were still sensitive to ethylene and ripened during initial storage at 5°C (S1).

Following exposure to ambient temperature, the climacteric event was triggered as demonstrated by the extensive and rapid softening (Exp. 1, Table 5.2; Exp. 2, Table 5.5) and colour change (Exp. 1, Table 5.3 and Table 5.5) of untreated fruit. In Exp. 2, control fruit remained green or changed very little in colour during ripening; a similar phenomenon was reported for fruit from the same origin harvested that same year (A. Shaw, pers. com.) Although ethylene production rate on single fruit was not measured in this work, it is clear that, upon transfer to ambient temperature, fruit produced considerably more ethylene as demonstrated by ethylene levels measured at day 1 inside boxes or bags (Exp. 1, Figure 5.2.; Exp. 2, Table 5.1), suggesting that fruit must have entered the phase of ethylene autocatalytic production. Antunes and Sfakiotakis (2002) also showed that kiwifruit started autocatalytic ethylene production 24h after they were removed from 12 days of low temperature storage. However, in the present study, where ethylene was removed below 1 μL L^{-1} in presence of e+® Ethylene Remover, firmness and colour changes evolved more slowly and extension of the storage period was observed (Exp. 1, Table 5.2 and 5.3; Exp. 2, Table 5.5). A possible explanation would be that fruit may measure the ethylene level to which it is exposed and modulate its response accordingly. It has been shown that the more ethylene to which an immature fruit is exposed, the earlier it ripens (Yang, 1987). Exposure of mature but unripe avocado fruit to exogenous ethylene shortly after harvest augmented the expression of *PaACO* and ACO activity, but ACS activity remained suppressed (Owino *et al.*, 2002). Unlike these studies, we did not use exogenous ethylene to trigger the ripening but rather an intervening exposure to ambient temperature. However, reducing ethylene levels below 1 μL L^{-1} in the atmosphere could have exerted a reductive effect on ethylene ACS transcript and activity, hence attenuating ethylene biosynthesis and in turn minimizing ripening process. Moreover, in Exp. 1, fruit treated with e+® Ethylene Remover in S1 and in S2 had higher firmness (77.2 N), although not significantly, than either double controls (48.9 N), non-treated in S1 but treated in S2 (54.3 N) or treated in S1 but not in S2 (39.1 N; Table 5.1) after 4 days post-break storage. This suggests a cumulative beneficial effect of removing ethylene, maybe by slowing down expression of ethylene-related genes first during preclimacteric stage and then further during post-

climacteric phase. This said, ethylene-related genes or ethylene production by fruit were not measured in the present study. Another explanation for the observed effects (Exp. 1, Table 5.2 and 5.3; Exp. 2, Table 5.5) is that low atmospheric ethylene levels (below 1 μL L^{-1}) may have been sufficiently high for some small ethylene perception and binding, resulting in receptors continuing to exert a negative feedback regulation as a 'counterintuitive' behaviour, as suggested in tomato (Kevany *et al.*, 2007), since receptors are negative regulators. Yet the concentration may not have been high enough to deplete receptors to the point where a constitutive response, leading to rapid ripening, is induced and which may occur when sufficiently enough receptors have been degraded via ethylene binding (Kevany *et al.*, 2007). The mechanism by which fruits sense cumulative ethylene response remains unclear, but Kevany *et al.*, (2007) recently suggested that, in tomato, the timing of the onset of ripening is controlled by the level of ethylene receptors. Changes in receptors abundance occurred through protein turnover (Kevany *et al.*, 2007). Whether a similar principle operates in avocado has not been shown. Also, it must be noted that fruit used in this Chapter were from different origin with different transit time and thus this may account for any differences between experiments. Nevertheless, these results are in agreement with findings by Adkins *et al.* (2005) that it was possible to suppress the ripening process (using 1-MCP treatment in their study) within 2 days of ethylene treatment, after which fruit became recalcitrant to the 1-MCP. Similarly, Owino *et al.* (2002) showed that 1-MCP applied to avocado at the onset of the climacteric rise still completely inhibited ACS activity whereas it temporarily delayed ACO activity and there was no rise in ethylene production. Temperature break also triggered the ripening of 1-MCP-treated fruit but the rate of softening diverged significantly from that of other e+® Ethylene Remover–treated fruit and controls, with higher firmness throughout the storage period (Exp. 1, Table 5.2 and 5.3). 1-MCP is known to suppress expression and activity of ethylene-related genes (Owino *et al.*, 2002, Hershkovitz *et al.*, 2010). As the 1-MCP-mediated suppression ceases, it may take longer for 1-MCP-treated tissue to regain sensitivity and respond to ethylene. On the other hand, since 1-MCP preferably binds to lipids in the mesocarp (Dauny *et al.*, 2003), it is also possible that 1-MCP was gradually released from the lipids in the mesocarp and binded to newly formed receptors, if any (Jiang *et al.*, 1999b), hence further exerting an effect.

In modified atmospheres (MA) or controlled atmospheres (CA), gas concentrations surrounding the commodity are manipulated to create an atmospheric composition different from that of air (78.08% N_2, 20.95% O_2 and 0.03% CO_2). CA is active and the gas composition is dynamically controlled whilst standard MA is passive and reliant on the product, the temperature (and possibly other environmental cues) and the nature and gas permeability of the film. Both postharvest techniques can supplement low temperature storage of fruit and can retard ripening (or senescence) through lower respiration and ethylene production rates, softening and compositional changes. Also, carbon dioxide is a competitive inhibitor of ethylene action (Faubion *et al.*, 1992). CA is sometimes (but not systematically) used commercially on avocado when long distance shipping is required and recommended MA or CA conditions for this fruit range between 2-5% O_2 and 3-10% CO_2 within the temperature range 5-13°C (Kader, 2002). In avocado, CA has been reviewed but most of the work was done in relation to physiological and pathological storage disorders and the need to reduce these (Burdon *et al.*, 2008). CA conditions of 5 kPa O_2 and CO_2 was shown to increase the number of days to ripen (Burdon *et al.*, 2008).

MA packaging are created when fruit is sealed in films with specific permeability to gases (Meir *et al.*, 1997). Accordingly, as the fruit respires, O_2 level decreases whereas CO_2 concentration increases in the bag, thus passively creating an atmosphere. Such atmospheric conditions reduce the respiration rate of fruit and delay the ethylene climacteric (Kanellis *et al.,* 1989; Kader, 2002). Although laboratory-based research on application of MA to avocado exists, there is no large commercial-scale use of MAP with avocado. Good quality retention for up to 7 weeks was achieved when sealed fruit were stored at temperature ranging 7-14°C and the O_2 and CO_2 concentrations inside bags varied between 2-6 and 3-7%, respectively (Scott and Chaplin, 1978). Sealing avocado cv. Hass individually in polyethylene films reduced texture and weight losses during 4 weeks at 5°C (Gonzales *et al.*, 1990). Meir *et al.* (1997) found that fruit (3.2 kg) sealed in polyethylene bags at 5°C with a gas composition of 4% O_2 and 5% CO_2 had reduced weight loss, softening and chilling injury. Inhibition of softening in low atmospheric O_2 (2.5-5.5%) has been attributed to diminished activity and protein accumulation of cellulase and polygalacturonase, presumably through a diminution of the biological activity of ethylene under low O_2

conditions. (Kanellis *et al.*, 1989, 1991). Therefore, MA may affect ripening through inhibition of ethylene action,

In the present work, O_2 concentrations were not measured but CO_2 inside MA packaging was not different between fruit treated with and without e+® Ethylene Remover and concentration were 4-5% (24h triggering) and 10% (48h triggering) (Table 5.1), and hence were within ranges previously reported (Meir *et al.*, 1997, Pesis *et al.*, 2002). There was no beneficial effect of MAP alone on fruit firmness as compared with air controls, and fruit softened rapidly (Table 5.5) which is contrary to what has been observed previously (Meir *et al.*, 1995, 1997; Hertog *et al.*, 2003). Whilst high atmospheric CO_2 concentrations have been frequently associated with reduced ripening, the balance between O_2 and CO_2 in the environment, however, is probably more important than the effect of the concentration of a single gas *per se.* The interaction between different O_2 (not measured herein) and CO_2 levels, and their effects on fruit (particularly on CO_2 injury) is rather conflicting in the literature (Hertog *et al.*, 2003; Meir *et al.*, 1995). Meir *et al.* (1995) reported that treating Israeli avocado cv. Hass at 5°C with 8% CO_2 and any of 3 or 21% O_2 was more effective than treating fruit with 3% CO_2 and either O_2 levels. Specifically the combination of 3% O_2 and 8% CO_2 gave the best firmness retention and almost no chilling injury (mesocarp discoloration) upon ripening, in agreement with Hertog *et al.* (2003) that increasing CO_2 and lowering O_2 improve firmness and colour retention. On the other hand, Hertog *et al.* (2003) found that treatment of New-Zealand-grown avocado cv. Hass with 2 kPa O_2 and 0 kPa CO_2 gave the best result since under these conditions the change in colour, weight loss and softening were minimised (as was the case with low O_2 and high CO_2) but no CO_2 injury occurred under these conditions. Discrepancies in results between these two studies could be due to fruit being from different origins (i.e. Israel vs. New-Zealand). It must be noted that in most studies, fruit are placed under MA conditions shortly after harvest, whilst in the present work fruit have been treated and packed after 5 weeks of transit, which may have had an impact on fruit response to treatment. Notwithstanding, even though these two studies only assessed fruits upon removal from cool CA storage at 20°C rather than along CA storage, the results clearly indicated that the effect of O_2 and CO_2 is rather synergistic and complex. Thus, having measured only CO_2 in the present study may be insufficient to explain any MAP effect in the present work.

Presence of e+® Ethylene Remover inside MAP retarded softening compared with MAP alone or air controls (Table 5.5), which could be partly due to elimination of autocatalytic ethylene production, as suggested earlier (Pesis *et al.*, 2002). Since CO_2 concentrations were not different between untreated MAP and MAP/e+® Ethylene Remover, the beneficial effects of MAP with e+® Ethylene Remover on fruit texture may be attributed to addition of the scavenger rather than to a single CO_2 effect. It could also possibly be, considering the importance of O_2 mentioned above, due to different O_2 levels inside packaging in response to e+® Ethylene Remover, although this is not proven herein. It must be reminded that the oxidative reaction of ethylene on e+® Ethylene Remover generates some CO_2 (section 2.4.4, Chapter 2). It is therefore possible that fruit respiration may have been lower in presence of e+® Ethylene Remover (individual fruit respiration not measured) but CO_2 generated by the oxidative reaction of ethylene may have accounted, together with respiration CO_2, for the total CO_2 measured inside MAP.

Several studies have reported on the importance of maintaining low levels of ethylene in CA or MAP. Faubion *et al.* (1992) found that continuous introduction of 1 or 10 μL L^{-1} ethylene inside a 2% O_2 + 2.5% CO_2 atmosphere containing avocado resulted in significant decrease in firmness relative to normal CA after 9 weeks and 6 weeks storage, respectively. High ethylene levels (10 μL L^{-1}) also reduce the quality of fruit stored under CA (Hatton and Reeder, 1972; Faubion *et al.*, 1992). Pesis *et al.* (2002) found that even lower concentration (4 μL L^{-1}) continuously applied during 5°C storage induced severe browning of cv. Fuerte after 3 weeks. Application of ethylene scavengers inside MAP has been experimentally tested on tomato (Bailen *et al.*, 2006) and avocado cv. Hass (Pesis *et al.*, 2002). For tomato, the scavenger was a granular-activated carbon impregnated with Pd, but was different from e+® Ethylene Remover (see section 2.4.4., Chapter 2). Unlike herein (Table 5.1), the ethylene inside tomato-containing bags was not scavenged below sub-physiologically active levels and remained at a concentration of *ca.* 8 μL L^{-1}, although colour change, softening and weight loss of tomato were still reduced in presence of the scavenger (Bailen *et al.*, 2006). It must be reminded that avocado and tomato are different fruit, yet the presence of their inferior Pd-based scavenger inside MAP increased O_2 and decreased CO_2 vs. MAP alone (Bailen *et al.*, 2006), in contrast with present results where no differences

between treatments on CO_2 were observed (Table 5.1). The films used in both studies were not similar: Bailen *et al*. (2006) used 20 μm thick non-perforated oriented polypropylene film with high permeability to O_2 and CO_2 whilst in the study herein, bags were thicker (30 μm) and had lower permeability to O_2 and CO_2 (section 5.2.2.2). Similarly, Pesis *et al.* (2002) published that addition of $KmNO_4$-based ethylene absorbent sachets (Ethysorb®, see section 2.4.4., Chapter 2) to micro perforated polyethylene bags (40 μm, permeability not precised) containing avocado cv. Hass stored at 5°C reduced ethylene and CO_2 concentrations whilst increased O_2 levels in bags relative to MAP only. Improved quality (less decay and mesocarp discoloration) of fruit upon ripening at 20°C was observed when fruit were previously held with Ethysorb®, in agreement with benefits of removing ethylene by an absorbent to prevent chilling injury in CA (Hatton and Reeder, 1972). This said, if the ethylene level was reduced in presence of the scavenger, the exact concentration achieved was not precised in their study, and also fruit were a different cv. (*viz.* cv. Ettinger) than that herein. Notwithstanding these discrepancies, the present findings have clearly shown that e+® Ethylene Remover was able to reduce ethylene below sub-physiologically active levels inside MAP and accordingly delay ripening of avocado fruit (Table 5.5). However, the interaction of low ethylene level and MAP effect is less clear and more trials with more control over both CO_2 and O_2 would be required. It must be noted that the film in the present study was selected on the basis of high moisture permeability, thus creating a drier environment to the scavenger (which is not the case when using sealed boxes), but may not have been optimal for avocado storage. Also the low CO_2 and especially O_2 permeabilities of the film may have led to anaerobic respiration, although no off-flavour (indicating formation of alcohol) was detected at that stage. Anaerobic respiration and CO_2 injuries may arise in conditions of extremely low O_2 (0 %) and high CO_2 (10-15% in presence of low % O_2) concentrations (Hertog *et al.*, 2003).

The major sugars reported in both experiments were the C7 sugars, mannoheptulose and perseitol, and sucrose and thus is in agreement with previous reports (Liu *et al.*, 1999b, 2002; Bertling and Bower, 2005; Chapter 3 and Chapter 4). In Exp. 1, mannoheptulose and perseitol concentrations were much lower and similar, respectively, than those measured in early season (mannoheptulose: 85.67 mg g^{-1} residue; perseitol: 72.19 mg g^{-1} residue) and mid season (mannoheptulose: 49.98 mg g^{-1}

residue; perseitol: 47.35 mg g^{-1} residue) avocado cv. Hass originating from Spain (Chapter 4). On the other hand, mannoheptulose content was higher than that detected in very late season from same origin (mannoheptulose: 4.10 mg g^{-1} residue; perseitol: 52.82 mg g^{-1} residue) (Chapter 4), consistent with the data of Liu *et al.* (1999b) that mannoheptulose declines as the season progress. Mannoheptulose and perseitol concentration in early season Chilean fruits (Exp. 2) were high and in the same range as that found in mid-season Chilean fruit (Landahl *et al.,* 2009) or in early season Spanish fruit (Chapter 4) using the same method for extraction and quantification of sugars than in this Chapter. In Exp.1, mannoheptulose and perseitol remained unchanged during the initial 7 days pre-climacteric storage, and there was no difference in concentrations between treatments despite variations observed in fruit softening behaviour. This is contrary to that observed in Chapter 4, whereby firmer fruit, as treated with 1-MCP and e+® Ethylene Remover, maintained better levels of C7 coumpounds vs. controls. On the other hand, in very late season fruit (Chapter 4), mannoheptulose levels did not differ between treatments even though some fruit ripened faster than other, as seen herein.

A decline in both mannoheptulose and perseitol occurred as fruit ripened extensively following temperature break in Exp. 1 (Table 5.6) whilst only perseitol declined slightly (yet significantly) in Exp. 2 (Figure 5.3.). In both experiments, there was no treatment effect on the C7 sugars concentrations. It has been suggested that C7 sugars may act as ripening inhibitors (Liu *et al.*, 2002) and this has been reviewed in Chapter 2 and discussed in Chapter 4. The present experiments do not provide support for a role of mannoheptulose or perseitol in ripening and the role of C7 sugars remains, as such, still unclear.

The role of the six-carbon sugars, sucrose, in avocado fruit is equivocal and whilst this sugar has been deemed as less important than C7 substances in the carbon balance (Bertling *et al.*, 2005), Liu *et al.* (1999b) suggested that sucrose may contribute to the energy source utilized during the climacteric process since a reduction in sucrose occurred as fruit softened (Liu *et al.*, 1999b). There is no evidence in the present study for sucrose being metabolised as fruit ripened and the pattern of change in sucrose was not consitent. In Exp. 1, concentrations detected in controls during initial storage decreased (although non-significantly) as fruit ripened from 200 N to 75 N after 7 days at 5°C, whilst level tended to increase in non-softening e+® Ethylene Remover- treated

fruit (Table 5.7). On the other hand, after temperature break, sucrose levels increased significantly in all fruit concomitant with softening, with no distinguishable pattern between treatments. Similarly in Exp. 2, sucrose increased significantly between 3 and 7 days post-break storage (Figure 5.3.). Accumulation of sucrose during fruit ripening at 20°C has been observed before in early season fruit (Chapter 4) and therefore the role of sucrose as part of the carbon energy source is questionable.

5.5 Conclusions

This work has shown that removing ethylene in an atmosphere was effective at delaying ripening even after the climacteric has been induced, corroborating previous work (Terry *et al.,* 2007a). The storage life extension was not comparable to that achieved using e+® Ethylene Remover or 1-MCP on pre-climacteric fruit, but has practical significance when fruit requires long distance transport with frequent "change of hand" and could limit excessive softening of fruit in the retail and home environments. An analysis at the molecular changes would contribute to a better understanding of the mechanism involved. As seen in previous experiments (Chapter 4), fruit ripened quickly and normally once removed from presence of the scavenger under shelf life conditions, whilst fruit pre-treated with 1-MCP were less ripe at that stage (Exp. 1). This study has also shown that e+® Ethylene Remover is compatible with MAP and such findings could provide an opportunity for new formatting and commercial development of the scavenger in synergy with new packaging design. It must be noted that e+® Ethylene Remover is a passive system. The role of sugars has been discussed and it is suggested that more research is necessary to elucidate the role of C7 sugars. In particular, only two trials were conducted herein but a greater quantity of data from more fruit from with different origins, different transit time and stored at different temperatures should be analysed in a systematic manner. C7 sugars may have a role in certain circumstances and their function may be affected by preharvest factors and the transit time.

CHAPTER 6

Investigation of the role of endogenous abscisic acid in ripening of imported avocado cv. Hass.

6.1 Introduction

It is widely accepted that increase in the rate of ethylene production occurs before most of the compositional changes related to ripening occur, and that ethylene affects the expression of many genes involved in fruit ripening, including avocado (Owino *et al.*, 2002; Hershkovitz *et al.*, 2010; Cara and Giovannoni, 2008). The importance of ethylene in the ripening of avocado has been extensively studied using the ethylene inhibitor (*cf.* Owino *et al.*, 2002; Watkins, 2006; Hershkovitz *et al.*, 2010; section 2.4.3. of Chapter 2). Ethylene biosynthesis is regulated by 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS) and ACC oxidase (ACO) and, in avocado, activity of these enzymes increase with the onset of climacteric rise and at the upsurge of ethylene production during ripening, respectively (see section 2.3 of Chapter 2; Owino *et al.,* 2002; Hershkovitz *et al.*, 2010).

Considerably less is known about the function of the phytohormone abscisic acid (ABA) in avocado ripening. ABA is generally recognized as a ripening promoter and is synthesised from the carotenoid, xanthoxin, which is converted to ABA via ABA-aldehyde (Chernys and Zeevart, 2000, section 2.2.7 Chapter 2). The concentration of the hormone in avocado mesocarp tissue typically increases during maturity and ripening of many fruits, and in avocado this increase closely follows the rise in ethylene biosynthesis, with a peak occurring just after the peak in ethylene production (Adato *et al.*, 1976; Cutting *et al.,* 1986; Chernys and Zeevaart, 2000). Richings *et al.* (2000) found that phenotypically small fruit, cv. Hass, had higher respiration rates, higher ABA and lower indolacetic acid contents than fruit of larger size. Furthermore, a single application of exogenous ABA (48 µg/fruit, as 1.5 mL of a 32 µg/mL solution) by infiltration to pre-climacteric avocado has been shown to advance climacteric ethylene biosynthesis and respiration peak, causing more rapid ripening (Blakey *et al.*, 2009). The authors also used a single application of 1.5 mL water as a control. Application of

exogenous ABA also accelerated induction of ethylene biosynthesis and advanced the onset of ripening in peaches (Zhang *et al.,* 2009a), apple (Lara and Vendrell, 2000), banana (Lohani *et al.,* 2004; Jiang *et al.,* 2000) and grape (Zhang *et al.*, 2009a). In contrast, in onion, a non-climacteric commodity, ABA content decreased exponentially following harvest, as did the storage potential of the cvs. studied (*viz.* cv SS1, Renate and Ailsa Craig; Chope *et al.*, 2006). Although ABA appears to be an important feature of fruit ripening, the exact role of this hormone in the avocado ripening process has not been established.It has been postulated in the literature that ABA may have an indirect action by increasing tissue sensitivity to ethylene (Bower and Cutting, 1988) and stimulate ACS accumulation and activity, as proposed for apples (Lara and Vendrell, 2000), peaches and grapes (Zhang *et al.*, 2009a). Zhang et al (2009a), in particular, showed in peaches that application of inhibitors of ABA synthesis (Fluridone and nordihydroguaiaretic acid (NDGA)) suppressed ethylene production and ripening of fruit. Whether ABA plays a role in ripening, possibly through regulation of endogenous ethylene synthesis or enhancement of tissue responsiveness to ethylene, or whether its metabolism is an effect rather than a cause of normal ripening remains unclear and has not been investigated for avocado fruit.

Mature avocado fruits do not ripen whilst still attached to the tree but only do so after harvest. Additionally, the fruits do not respond to exogenously applied ethylene immediately after harvest (Starrett and Laties, 1991; Hershkovitz *et al.* 2010). The nature of the factor(s) of ripening inhibition on the tree and shortly after picking is not yet known. It has been suggested that inhibition of ethylene production on the tree and after harvest could be due to repressed ACS activity (Sitrit *et al*., 1986; Hershkovitz *et al.*, 2010). Recently, Hershkovitz *et al.* (2010), using the system of seeded and seedless avocado cv. Arad, proposed a role for the seed in inhibiting the induction of ethylene response and delaying climacteric in mature seeded fruit. Others have hypothesised that the seven-carbon (C7) sugar mannoheptulose and its corresponding alcohol, perseitol, may act as ripening inhibitors (Liu *et al.*, 2002; section 2.2.6 Chapter 2). Supporting evidence was provided by Landahl *et al.* (2009) since greater concentrations of mannoheptulose were measured in the apical part of the fruit, where mesocarp tissues were firmest. However, the absolute concentrations of C7 sugars may vary greatly according to harvest season and storage, with very low amounts of mannoheptulose

present in late harvested fruit (Liu *et al.*, 1999b; section 2.2.6 Chapter 2, Chapter 4 and 5) and the function, if any, of C7 sugars has not yet been elucidated.

It has been shown in Chapter 4 and 5 that e+® Ethylene Remover was effective at delaying ripening of avocado cv. Hass during pre-climacteric storage at 5°C and following induction of the climacteric. 1-MCP, also strongly inhibited ripening under the same conditions (Chapter 4 and 5), even though its mechanism of action differs from that of e+® Ethylene Remover since it binds to the receptor rather than removes atmospheric ethylene (section 2.4.3., Chapter 2). A combination of these two approaches has been previously investigated in Chapter 5 but only following climacteric induction and not on pre-climacteric fruit. It is presumed that 1-MCP-treated fruit overcome inhibition by synthesising new receptors (Jiang *et al.*, 1999b) and may regain sensitivity to ethylene during storage, which would explain some fruit ripening during transit (Kruger and Lemmer, 2007). Hence, it is expected that ethylene removal could further extend postharvest life of 1-MCP-treated fruit. Moreover, it is postulated that the different way fruit respond to 1-MCP and e+® Ethylene Remover, as investigated in Chapter 4 and 5, constitutes a useful system in which to investigate ABA. As far as is known, there is no published study on ABA metabolism in avocado fruit in response to 1-MCP or an ethylene scavenger.

In order to verify these hypotheses, the present study addressed the effect of 1-MCP (0.3 $\mu L\ L^{-1}$), e+® Ethylene Remover and the combination thereof on ripening of imported avocado cv. Hass fruit stored for 7 days at 12°C. The temporal change in ethylene production, respiration, C7 sugars and ABA concentration, as measured using a newly developed LC-ESI-MS/MS method, in mesocarp tissue were assessed and discussed in relation to differences in firmness and colour. The great majority of research on avocado has been conducted on home-grown fruit with shelf life simulation carried out shortly after harvested. However, there is a lack of research on postharvest quality of imported fruit. Europe, including UK, is a major importer of avocado from overseas and fruit are typically stored for lengthy periods under refrigeration before being ripened and consumed. Such conditions are likely to affect fruit quality at arrival, and uneven ripening within consignments of imported avocado fruit is not unusual. Therefore, the purpose of this work was also to detail the biochemical and physiological changes in imported avocado fruit in an attempt to identify a biochemical marker, i.e.

ABA or C7 sugars, which may explain heterogeneity in ripening and could be used as a potential indicator of fruit storability.

6.2 Materials and methods

6.2.1 Plant material

Mid-season pre-climacteric avocado cv. Hass fruit (size code 18) were sourced from a commercial farm in White River (Mpumalanga, South Africa) and were supplied by Mack Multiples Division (M. W. Mack Ltd., Kent, U.K.). Avocados were harvested on 4-8th June 2009 and shipped to the UK under refrigeration (*ca.* 5°C). Fruit were containerised by the grower, which ensured that the cool chain was maintained from harvest until arrival in the UK and was a reflection of 'real world' commercial practice. The cold stores and containers in South African were set at *ca*. 5°C. Fruit arrived at the Plant Science Laboratory on 13th July 2009 and were hence 35-39 days old, which is not unusual for fruit imported into UK. Fruit were unripe upon arrival as confirmed by initial colour, firmness and ethylene production measurements and fruit were not pre-treated with 1-MCP.

6.2.2 Treatments, storage conditions and sampling regime

All treatments were carried out at 12°C. Equal batches of fruit (n = 42) were placed into water-sealed air tight polypropylene chambers (88 cm × 59 cm × 59 cm) which housed a 8 cm × 8 cm electric fan (Nidec Beta SL, Nidec, Japan). Fruit were treated with e+® Ethylene Remover (n = 42), 1-MCP (0.3 μL L^{-1} for 16 h; n = 84) or un-treated (n = 42). Treatment with e+® Ethylene Remover was achieved by placing 32 g of powdered e+® Ethylene Remover (metal loading of 1% Pd (m/m)) in Petri dishes (n = 8) within the box. 1-MCP treatment was achieved as described in Chapter 4, with modification in that the final concentration obtained inside boxes was 0.3 μL L^{-1} instead of 1.5 μL L^{-1}. Following 1-MCP application, all boxes were kept sealed for 16 h (G. Regiroli, pers.com.). Fans placed within the boxes ensured homogenous distribution of

the 1-MCP gas within chambers. Untreated fruit acted as controls and were held in the same conditions as treated fruit.

After 16 h, 1-MCP-treated fruit (n = 84) were further divided into two batches. To one batch was added 4 g of fresh e+® Ethylene Remover (1-MCP/ e+® Ethylene Remover; n = 42) as described above and to the other batch was added nothing (1-MCP (first treatment); n = 42). In order to verify the effects of delaying application of e+® Ethylene Remover by 16 h, some additional fruit (n = 42) were held as controls in a chamber for 16 h after which the ethylene scrubber was applied (delayed e+® Ethylene Remover; n = 42) (Figure 6.1). Following treatments fruit were stored for 7 days at 12°C.

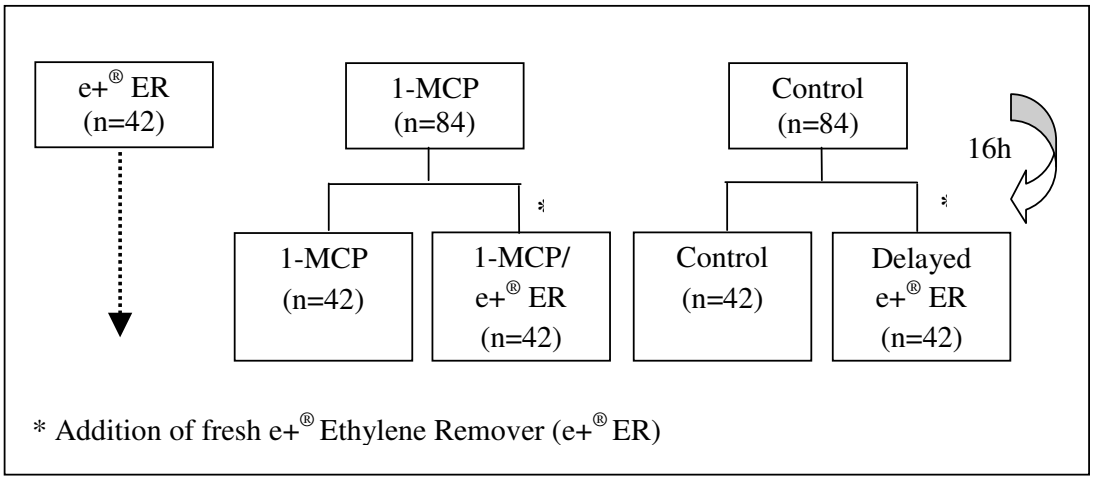

Figure 6.1. Experimental design

Samples (n = 6 per treatment) were taken at day 1 (24 h after treatments were completed), 2, 3, 4, 5 and 7 for respiration, ethylene production, firmness and colour assessment. Respiration and ethylene production rate were measured as in Exp.1 of Chapter 4, with slight modification in that fruit (*n* =6 per treatment per sampling) were placed individually in 3 L jars for 2 h at 12ºC (storage temperature; *n* =3 fruit) or 20ºC (standard temperature; *n* = 3 fruit). Firmness and colour were measured as before (Chapter 4). Samples derived from days 1, 3 and 5 were prepared and analysed for sugars (n = 6 per treatment) and ABA (n = 3 per treatment) concentration according to Chapter 3 and section 6.2.below, respectively. It must be noted that in this experiment, samples were freeze-dried in the dark. For the duration of the storage trial, CO_2

poisoning was avoided by opening the boxes every 24 h, coinciding with sampling time. A subsample of fruit (n = 6, baseline) was assessed upon arrival at the laboratory for firmness, colour, ethylene production, sugars and ABA content. In summary, the trial had 5 treatments, 6 sampling times and 6 fruit per replicate, which gave a total of 186 fruit analyzed.

6.2.3 ABA extraction and quantification

The defatted residue powder (50 mg) obtained after lipid extraction (see Chapter 3) was extracted overnight in 5 mL of a solution of 80:19:1 (v/v/v) of acetone:water:acetic acid at 4°C in the dark on a suspension mixer. Extracted samples were vortexed and then centrifuged at 3000 rpm for 10 min. The supernatant was collected and the residue pellets were re-extracted with 1 mL fresh extraction solvent. The second extract was centrifuged and supernatants were combined. After dilution (1:25) of the extract with fresh solvent, 20 ng of the deuterated internal standard (IS; d_4-ABA, National Research Council of Canada, Saskatchewan, Canada) was added from a concentrated stock solution. The dilution factor and amount of IS required for accurate results were determined by preliminary experiments. The solution was then filtered through a syringe filter (0.2 µm pore diameter; Millipore Corp., MA). The solvent was evaporated to dryness under vacuum at 4°C in the dark. Dried extracts were re-suspended in 3 mL HPLC-grade water and purified by loading extracts onto a Sep-Pak C18 SPE cartridge (Waters, Herts., UK) under gentle vacuum to remove more polar coumpounds. The eluted fraction was taken to dryness under vacuum at 4°C and kept at -40°C until further analysis. Lyophilised samples were re-suspended in 500 µL methanol prior to injection.

Samples were analysed using a Waters Alliance 2795 HPLC coupled to a Micromass Quattro quadrupole tandem mass spectrometer (Waters, MA, USA) with an electrospray ion source. Both the HPLC and the mass spectrometer were operated by MassLynx v4.0 SP3 software (Waters). Samples (10 µL) were separated on a Zorbax Eclipse XDB-C18 analytical column (3.5 µm, 2.1 x 100 mm, Agilent, CA, USA) with 1 mm C18 guard column (Optiguard, Optimize Technologies, OR, USA) maintained at 25˚C. The mobile phase consisted of HPLC-grade methanol (A), water (B) and 5% acetic acid (C). The gradient involved an increase/decrease in solvent A; 10-60%, 15

min; 60-99.2%, 15 min; 99.2-10%, 2 min; 10%, 3 min, at a constant proportion of solvent C (0.8%) at a flow rate of 0.2 mL min^{-1}. Mass spectrometry was carried out using multiple reaction monitoring in negative ionisation mode, with a capillary potential of 2.75 kV, a source temperature of 120˚C, a desolvation temperature of 350˚C; cone gas and desolvation gas flow rates of 50 and 950 L h^{-1}, respectively, and a collision gas (Ar) pressure of 5 x 10^{-3} mbar. The multiple reaction monitoring (MRM) transitions were 263>153 for ABA and 267>156 for d_4-ABA, with a cone voltage of 30 and 25 V, respectively, and a collision energy of 9 eV for both compounds. The retention time was 18.00 min for ABA and also for the IS d_4-ABA.

Calibration curves were prepared using a range of standard solutions containing an increasing amount of ABA with a constant amount of d_4-ABA. The area beneath the MRM product ion peak was determined for the analyte and IS, and the response calculated according to the formula: Response = analyte product ion peak area x ([IS]/IS product ion peak area), where the [IS] is the known concentration of IS added. Concentration of ABA in samples was quantified in relation to the internal standard using the calibration curves generated.

6.2.4 Statistical analysis

Analysis of variance (ANOVA) and tests for correlations between mean values for physical parameters (Hº and firmness), ethylene production, respiration rate, sugars and ABA using Pearson's Product Moment correlation were performed according to Chapter 3. Since respiration rate was affected by temperature, statistical analysis of this parameter was performed at 12°C and 20°C separately. Firmness data was log transformed to fit the requirements for analysis of variance. Data presented are back transformed values that represent biological data. Non-linear regression against an exponential standard curve was used to model the change in firmness against ABA concentration and ethylene production.

6.3 Results

6.3.1 Ethylene levels within storage chambers

Concentrations of ethylene within control boxes ranged between 0.64-2.9 μL L^{-1} over 7 days of storage (Figure 6.2). Ethylene concentrations of 0.04-2.60 μL L^{-1} were measured in boxes containing 1-MCP-treated fruit. However, addition of the ethylene scavenger reduced ethylene concentrations to below 0.1 μL L^{-1} for the first 2 days, after which concentrations were between 0.1-0.2 μL L^{-1}. The combination of 1-MCP and e+® Ethylene Remover treatments had the lowest ethylene concentrations (below 0.1 μL L^{-1}) for the duration of the storage trial.

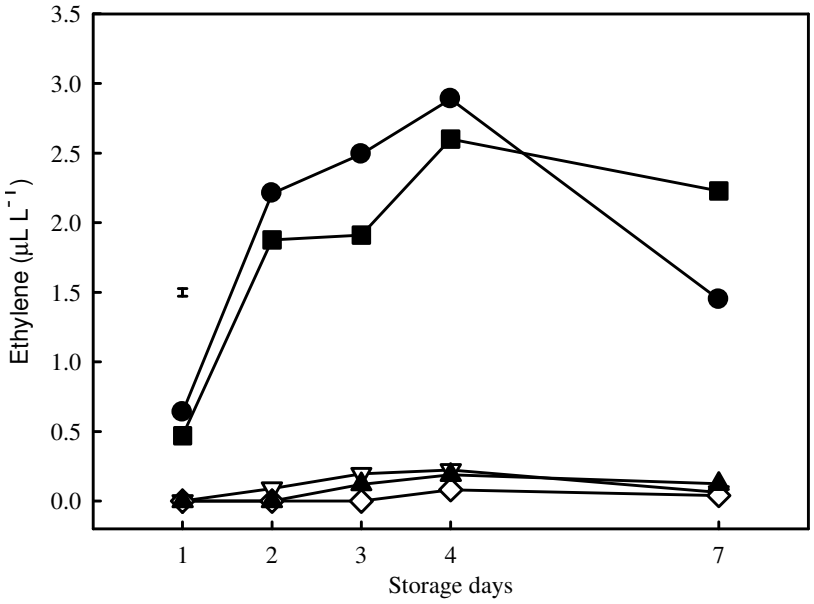

Figure 6.2: Effect of treatments (e+® Ethylene Remover,∇; 1-MCP, ■; MCP/ e+® Ethylene Remover, ◊; delayed e+® Ethylene Remover, ▲; controls, ●) on ethylene concentrations inside experimental chambers containing avocado cv. Hass stored at 12ºC. Boxes were opened at regular intervals. Bar represents LSD (P<0.05).

6.3.2 Respiration rate and ethylene production

Respiration and ethylene production rates were measured at 12ºC (storage temperature, n = 3) or 20ºC (standard temperature, n = 3). There was a significant difference between respiration rates at different temperatures. At 12°C, the overall respiration rate increased significantly between days 1 and 2, and concentration reached a maximum on day 4 (Table 6.1). All fruit exhibited a maximum respiration rate between days 4-5, except for fruit treated with delayed application of e+® Ethylene

Remover which showed a maximum rate at day 7. The overall mean was significantly higher in controls vs. e+® Ethylene Remover -, 1-MCP- and 1-MCP/ e+® Ethylene Remover -treated fruit whilst delayed e+® Ethylene Remover fruit was not different from any other treatment (Table 6.1). On the other hand, when the respiration rate was measured at 20°C, there was no effect of storage time or treatments, and the overall respiration rates increased from 43.4 mL kg^{-1} h^{-1} (day 1) to a maximum value of 58.4 mL kg^{-1} h^{-1} at day 5. The standard error for 1-MCP-treated fruit on day 3 and 7 (at 20°C only) was proportionally greater than for any other treatments (Table 6.1), indicating more heterogeneity in respiration rate for these fruit.

The ethylene production of fruit upon arrival was 0.96 µL kg^{-1} h^{-1} (baseline) indicating that fruit were pre-climacteric prior to treatments. There was no significant effect of temperature on ethylene production. The concentration rose significantly during storage from 1.5 µL kg^{-1} h^{-1} on day 1 to a maximum of 20 µL L^{-1} on day 7. Ethylene production was significantly affected by treatments, whereby overall mean ethylene production by 1-MCP and 1-MCP/e+® Ethylene Remover - treated fruit was significantly lower than controls (Table 6.2). Ethylene production by fruit treated with e+® Ethylene Remover (delayed or not) was not different from that of control or 1-MCP-treated fruit. As for respiration, the standard error for 1-MCP-treated fruit on day 7 was greater than for any other treatments (Table 6.2).

6.3.3 Firmness and colour

The firmness of all avocado fruit decreased significantly over time, from an initial value of 196.0 N upon arrival (baseline) to an average value of 8.5 N after 7 days at 12ºC, indicating full ripeness. Significant differences between treatments were observed; controls generally softened faster than treated fruit and were ripe (<20 N) after 3 days storage (Table 6.3). Treatment with e+® Ethylene Remover and delayed e+® Ethylene Remover significantly reduced softening over the first 3 days, after which fruit softened similarly to controls. Treatment with 1-MCP and 1-MCP/ e+® Ethylene Remover resulted in the best maintenance of firmness over storage time and fruit only attained ripe stage (<20N) after 7 days. Specifically, these fruit were less soft than controls for the entire storage duration, whilst they were firmer than e+® Ethylene Remover -treated fruit only after day 4 (Table 6.3).

Table 6.1: Effects of treatments on respiration rate (mL kg^{-1} h^{-1}) of avocado cv. Hass stored for 7 days at 12°C and treated with 1-MCP, e+® Ethylene Remover (e+® ER), delayed application of e+® Ethylene Remover (delayed e+® ER)or 1-MCP/ e+® Ethylene Remover (1-MCP/ e+® ER) together. Respiration rate was measured at 12°C or 20°C. Results represent mean values of 3 replicates ± SE.

Temperature	Storage (days)	Control	e+® ER	Delayed e+® ER	1-MCP	1-MCP/ e+® ER	Mean
12°C	1	40.7 ± 0.51	34.9 ± 1.88	27.7 ± 4.29	29.1 ± 2.87	31.1 ± 5.56	32.7[b]
	2	51.9 ± 3.09	50.0 ± 9.34	43.9 ± 5.91	38.9 ± 0.90	34.5 ± 2.36	43.8a
	3	53.4 ± 0.85	42.6 ± 8.20	43.9 ± 2.63	41.0 ± 3.69	41.2 ± 2.82	44.4[a]
	4	59.3 ± 5.45	49.0 ± 3.47	50.4 ± 4.36	48.1 ± 5.14	40.4 ± 3.58	49.4[a]
	5	49.8 ± 3.59	40.2 ± 4.18	49.1 ± 12.48	42.9 ± 17.78	47.9 ± 18.12	46.0[a]
	7	58.5 ± 12.73	46.4 ± 8.93	51. 9 ± 8.22	37.3 ± 6.74	28.4 ± 1.36	44.5[a]
	Mean	52.3[a]	43.9[b]	44.5[ab]	39.6[b]	37.3[b]	
20°C	1	47.8 ± 4.04	46.1 ± 17.86	34.0 ± 1.93	51.7 ± 13.72	37.3 ± 4.61	43.4[a]
	2	45.5 ± 2.74	43.0 ± 3.17	45.9 ± 7.49	36.9 ± 3.35	45.0 ± 6.61	43.3[a]
	3	61.7 ± 2.84	54.5 ± 6.49	47.7 ± 3.83	66.1 ± 25.94	50.4 ± 7.00	56.1[a]
	4	62.9 ± 2.68	54.1 ± 2.05	56.1 ± 1.32	38.2 ± 3.01	41.9 ± 0.41	50.7[a]
	5	82.4 ± 19.56	57.3 ± 4.71	52.2 ±2.46	49.0 ± 12.12	32.0 ± 2.51	58.4[a]
	7	44.9 ± 2.54	49.8 ± 3.48	48.4 ± 10.54	54.8 ± 24.05	51.5 ± 18.89	49.9[a]
	Mean	57.5[a]	54.0[a]	47.4[a]	49.5[a]	43.0[a]	

[ab] different letters indicate significant difference between means (P<0.05)

Table 6.2: Effects of treatments on ethylene production (μL kg^{-1} h^{-1}) of avocado cv. Hass stored for 7 days at 12°C and treated with 1-MCP, e+® Ethylene Remover, delayed application of e+® Ethylene Remover or 1-MCP/ e+® Ethylene Remover together. Values are average of respiration at 12°C (*n* =3 replicates) and 20°C (*n* = 3 replicates) ± SE.

Storage (days)	Control	e+® Ethylene Remover	Delayed e+® Ethylene Remover	1-MCP	1-MCP/e+® Ethylene Remover	Mean
1	3.3 ± 1.27	0.7 ± 0.41	0.8 ± 0.32	0.9 ± 0.57	1.8 ± 0.73	1.5^{c}
2	7.5 ± 1.01	5.6 ± 1.43	5.0 ± 1.45	4.3 ± 0.81	1.5 ± 0.85	4.8^{c}
3	17.8 ± 1.99	9.6 ± 4.00	14.2 ± 1.91	5.8 ± 1.25	5.2 ± 0.74	10.5^{b}
4	14.0 ± 3.09	15.9 ± 3.00	16.0 ± 3.13	5.6 ± 1.44	8.3 ± 1.93	12.0^{b}
5	18.6 ± 2.65	15.8 ± 2.94	14.2 ± 2.23	4.0 ± 0.92	8.2 ± 3.57	12.1^{b}
7	20.0 ± 1.47	19.5 ± 1.85	18.3 ± 2.68	29.2 ± 13.51	12.8 ± 1.32	20.0^{a}
Mean	13.5^{a}	11.2^{ab}	11.4^{ab}	8.3^{bc}	6.3^{c}	

^{abc} different letters within the same row or the same column indicate significant difference between means (P<0.05).

Table 6.3. Effects of treatments on firmness of avocado cv. Hass stored for 7 days at 12°C and treated with 1-MCP, e+® Ethylene Remover, delayed e+® Ethylene Remover or 1-MCP/ e+® Ethylene Remover together. Values in brackets are log transformed means.

Storage (days)	Control	e+® Ethylene Remover	Delayed e+® Ethylene Remover	1-MCP	1-MCP/ e+® Ethylene Remover	Mean
1	86.4 (4.260^{y})	146.7 (4.936^{x})	148.8 (4.969^{x})	172.5 (5.095^{x})	171.9 (5.050^{x})	145.2^{A} (4.862^{a})
2	46.0 (3.669^{y})	66.7 (4.036^{xy})	74.2 (4.102^{xy})	62.6 (4.053^{xy})	113.0 (4.563^{x})	72.5^{B} (4.085^{b})
3	11.4 (2.316^{y})	43.8 (3.338^{x})	27.8 (3.142^{x})	35.8 (3.533^{x})	37.6 (3.491^{x})	31.3^{C} (3.164^{c})
4	18.8 (2.793^{yz})	15.9 (2.604^{z})	13.1 (2.438^{z})	33.3 (3.437^{x})	38.4 (3.325^{xy})	23.9^{CD} (2.919^{c})
5	7.9 (1.910^{y})	7.6 (1.993^{y})	8.0 (2.023^{y})	20.8 (2.992^{x})	26.9 (3.105^{x})	14.2^{D} (2.405^{d})
7	6.7 (1.697^{yz})	3.7 (1.284^{z})	3.1 (1.130^{z})	10.9 (2.279^{xy})	17.9 (2.806^{x})	8.5^{D} (1.839^{e})
Mean	29.6^{C} (2.774^{b})	47.4^{B} (3.032^{b})	45.8^{B} (2.967^{b})	56.0^{AB} (3.565^{a})	67.6^{A} (3.723^{a})	

^{xyz} different letters within the same row (day) indicate significant difference between treatments (P<0.05); LSD used for log transformed data. ^{abcde} different letters indicate significant difference between means of treatments or storage days (P<0.05); LSD used for log transformed data. ^{ABCD} different letters indicate significant difference between means of treatments or storage days (P<0.05); LSD used for back transformed data.

Lightness (L*) and chroma (intensity, C*) of the peel decreased significantly over time but were not affected by the treatment applied (data not shown). Similarly, hue angle (Hº) decreased over time, from 118.22 (at arrival) to 65.5 after 7 days, indicating the change in skin colour from green to purplish-brown (Table 6.4). Generally, and mirroring softening trend, controls changed more in colour than treated fruit over 4 days storage (Table 6.4). Fruit treated with e+® Ethylene Remover and delayed e+® Ethylene Remover maintained better greenness over the first 4 days, after which they changed colour comparably to controls. There was a main effect of treatment on Hº, since fruit treated with 1-MCP and 1-MCP/ e+® Ethylene Remover were on average greener (H°~100.50 and 101.98, respectively) as compared with controls (H°~93.0), whilst fruit treated with e+® Ethylene Remover (H°~96.28) and delayed e+® Ethylene Remover (H°~96.69) were not statistically different from either 1-MCP-treated or control fruit (Table 6.4).

Table 6.4. Effects of treatments on hue angle (H°) of avocado cv. Hass stored for 7 days at 12°C and treated with 1-MCP, e+® Ethylene Remover (e+® ER), delayed application of e+® Ethylene Remover (delayed e+® ER) or 1-MCP/ e+® Ethylene Remover together. (1-MCP/ e+® ER).

Days	Control	e+® ER	Delayed e+® ER	1-MCP	1-MCP/ e+® ER	**Mean**
1	113.51	119.14	121.38	120.46	119.96	118.89^{A}
2	106.27	109.78	111.58	113.51	118.23	111.87^{B}
3	97.67	106.53	105.88	105.44	109.79	105.06^{C}
4	94.22	93.35	94.47	102.38	97.63	96.41^{D}
5	80.70	90.21	83.44	92.93	94.72	88.40^{E}
7	65.61	58.66	63.43	68.25	71.54	65.50^{F}
Mean	93.00^{C}	96.28^{BC}	96.69^{BC}	100.50^{AB}	101.98^{A}	

^{ABCDEF} different letters within the same row or same column indicate significant difference between means (P<0.05)

6.3.4 Sugars

Sucrose and, the heptose sugars, mannoheptulose and perseitol, were the main sugars measured in all samples and were present at concentrations of 41.0 mg g^{-1} residue (15.4 mg g^{-1} DM, 92.4 mg g^{-1} residue (35.6 mg g^{-1} DM) and 50.5 mg g^{-1} residue (18.7 mg g^{-1} DM), respectively, before storage. Sugars varied differently with storage time, whereby mannoheptulose decreased significantly over time from 107.2 mg g^{-1} residue (43.8 mg g^{-1} DM) at day 1 storage to 75.9 mg g^{-1} residue (28.4 mg g^{-1} DM) after 5 days whilst perseitol and sucrose content remained constant over time, at an overall concentration of 55.4 mg g^{-1} residue (21.09 mg g^{-1} DM) and 41.0 mg g^{-1} residue (15.62 mg g^{-1} DM), respectively. Treatment had no effect on sugar concentrations in the fruit mesocarp. Glucose and fructose were detected but concentrations were at quantification limit and hence these sugars were not considered in this study.

6.3.5 Abscisic acid

The initial ABA content prior to application of the different treatments was 1081 ng g^{-1} powder (454 ng g^{-1} dry matter (DM); 124.6 ng g^{-1} fresh matter (FM)). The ABA concentration increased significantly during 7 days storage for all treatments (Table 6.5). There was a treatment main effect as controls contained overall significantly more ABA (2480 ng g^{-1} residue) than treated fruit. Also, fruit treated with e+® Ethylene Remover-treated had overall more ABA (1929 ng g^{-1}) than those treated with 1-MCP-treated (1584 ng g^{-1}; $P>0.05$) and 1-MCP/ e+® Ethylene Remover -treated fruit (1443 ng g^{-1}; $P<0.05$). There was also a significant interaction between storage time and treatments (Table 6.5) whereby the concentration in 1-MCP-treated fruit at day 1 was significantly lower than that present in controls and e+® Ethylene Remover-treated fruit. After 3 days, controls contained more ABA vs. all treated fruit yet after 5 days, both controls and e+® Ethylene Remover -treated fruit contained more ABA than fruit from 1-MCP and 1-MCP/ e+® Ethylene Remover treatments. A difference between 1-MCP and 1-MCP/ e+® Ethylene Remover -treated fruit was observed at day 3.

Table 6.5: Effects of treatments on ABA concentration in mesocarp of avocado cv. Hass stored at 12°C and treated with 1-MCP, e+® Ethylene Remover (e+® ER) or 1-MCP/e+® Ethylene Remover (1-MCP/ e+® ER). Values are expressed per residue mass (dry weight after lipid removal), per dry mass and per fresh mass. Values are mean values of 3 replicates.

	Storage (day)	Control	e+® ER	1-MCP	1-MCP/ e+® ER	Mean
ng g^{-1} residue	1	1429.8^{ef}	1413.4^{ef}	586.2^{g}	951.9^{fg}	1095.3^{C}
	3	2564.6^{bc}	1321.4^{ef}	1794.7^{de}	1033.8^{fg}	1678.6^{B}
	5	3446.4^{a}	3053.2^{ab}	2370.8^{cd}	2343.4^{cd}	2803.5^{A}
ng g^{-1} DM	1	571.2	554.5	242.9	385.7	438.6^{C}
	3	923.8	466.8	678.5	407.3	619.1^{B}
	5	1277.6	1169.2	902.6	873.0	1055.6^{A}
ng g^{-1} FM	1	166.3	155.9	70.6	112.8	126.4^{C}
	3	272.5	138.1	203.2	111.6	181.3^{B}
	5	371.0	333.8	275.7	273.5	313.5^{A}

^{abcdefg} different letters within the interaction "storage day x treatment" indicate significant difference $P<0.05$. ^{ABC} different letters indicate significant difference between storage days ($P<0.05$).

There was generally a significant and good correlation between firmness and Hº (r = 0.72) indicating coordination in the ripening event. There was also a negative correlation between the ethylene production, and firmness and greenness (r = -0.59 and r = -0.70, respectively). However, firmness, Hº and ethylene production were weakly correlated with respiration rate and sugars content. The ABA concentration exhibited a good yet negative correlation with firmness (r = -0.69), Hº (r = -0.82) and positive correlation with ethylene production (r = 0.56). In contrast, ABA was poorly correlated with sugars and respiration rate.

In order to further describe the relationship between ethylene production (x) and softening (y), non-linear regression was applied to the data. The relationship was well described by an exponential decay curve $y = a \exp(-bx)$, with a coefficient of determination of R= 0.87. The same equation was found to describe the relationship between ABA (x) and softening (y) with a coefficient of determination of R= 0.78 (Table 6.6).

Table 6.6: Values of constant for the exponential decay curve $y = a \exp(-bx)$ fit to the decline in firmness (y) of avocado fruit stored at 12°C.

	a	b	R	R^2
Firmness vs. ethylene	188.2051	0.4435	0.87	0.76
Firmness vs. ABA	348.9954	0.0011	0.78	0.61

Where a reflects the estimated maximum firmness (initial firmness), b is a function of the initial rate of decline, R^2 is the coefficient of determination for the fitted curve.

6.4 Discussion

Rigorous control of ethylene concentration or inhibiting its action is fundamental to maintaining postharvest quality of horticultural climacteric commodities. For avocado, this can be achieved by using 1-MCP (see Watkins, 2006) or ethylene scavengers (section 2.4.4., Chapter 2; Terry *et al.*, 2007a, Chapter 4 and 5). Previous work has shown that e+® Ethylene Remover had the ability to reduce ethylene levels below physiologically active concentrations and accordingly maintain better

quality of imported avocado cv. Hass stored at 5°C and after climacteric induction (Chapter 4 and 5). However, research on the effect of ethylene removal on fruit ripening remains limited. Moreover, most studies on avocado have quantified ethylene production, respiration and characteristics associated with quality in fruit ripened or treated shortly after harvest. In contrast, details of postharvest physiological and biochemical changes on imported avocado are almost non existent. A large proportion of avocado fruit consumed in Europe are imported from overseas, necessitating long distance shipment under refrigeration before fruit are then ripened. Although fruit are shipped at a pre-climacteric stage, it is likely that biochemical changes occur during transit time, affecting subsequent ripening. Therefore, using imported fruit with varied biological age in the present study reflects commercial practice and thus the 'real world'. In the present study, e+® Ethylene Remover, alone or in combination with 1-MCP, effectively removed ethylene from the storage atmosphere (Figure 6.2) and, accordingly, had an effect on delaying fruit ripening (Table 6.3-6.4). The role of ABA and C7 sugars, which are present in avocado and have been considered as candidates for ripening regulation, was addressed in this study.

The typical climacteric pattern of ethylene production was not observed herein during storage of avocado (Table 6.1), and the maximum ethylene production remained relatively low compared with that reported in other works where fruit were ripened around 20°C (Adato *et al.*, 1976). This could be explained by the lower temperature used, which probably accounted for differences in ethylene production behaviour, as reported before (Zamorano *et al.,* 1994; Perez *et al.*, 2004). The fact that fruit have been stored for a long period and were aged may also have altered fruit capacity to produce ethylene, as seen elsewhere (Donetti and Terry, unpublished). On the other hand, the respiration rates (Table 6.2) were in the range of those documented by others (Adato and Gazit, 1977; Perez *et al.*, 2004).

Differences between treatments for both ethylene and respiration rate were observed and suggest that treatments had an effect on physiological activity. Where ethylene was reduced below 0.1 $\mu L\ L^{-1}$ in the presence of the scavenger, fruit had lower respiration rate (significant) and ethylene production (non-significant), accompanied by improved firmness maintenance vs. controls over 3 days (Table 6.3) and overall higher greenness (Table 6.4). After 3 days of storage, fruit held in the presence of the

scavenger ripened similarly to controls. As expected, treatment with 1-MCP/ e+® Ethylene Remover, and to a lesser extend 1-MCP alone, consistently resulted in the lowest ethylene production and respiration rates (Table 6.1-6.2) and, accordingly, delayed ripening (Table 6.3-6.4). 1-MCP treatment did not completely inhibit ethylene biosynthesis, as demonstrated by ethylene levels within boxes containing 1-MCP-treated fruit (Figure 6.2) and ethylene production by these fruit (Table 6.2). This substantiates the notion that ethylene production resumed in spite of 1-MCP treatment (albeit at a lower rate than that of control fruit), and is in accordance with the start of softening already after 2 days storage in these fruit (Table 6.3). It is acknowledge that 1-MCP is usually applied directly after harvest and not after transit. Although ripening proceeded in both 1-MCP- and 1-MCP/ e+® Ethylene Remover -treated fruit, presence of the scavenger generally led to lower ethylene production and higher firmness and H° compared than 1-MCP alone (Table 6.3-6.4). It is believed that the ethylene antagonist binds irreversibly to the ethylene receptors and that plants presumably overcome inhibition by producing new receptors (Sisler *et al.*, 1996, Jiang *et al.*, 1999b). Ethylene (either exogenously applied or endogenously produced) may then activate the newly formed receptors and induce a ripening response. In accordance with this, ethylene removal further repressed ethylene production, softening and colour change of ripening 1-MCP-treated fruit (Table 6.2-6.4), and demonstrates the possible benefits of removing ethylene in the storage atmosphere following 1-MCP treatment.

Heptose sugars were the predominant non-structural carbohydrates in all samples, in agreement with prior studies (Liu *et al.*, 1999b, 2002; Landahl *et al.*, 2009; Chapter 3-5). The C7 sugars have been shown to decline during the season, during storage at 5°C, 12°C and as fruit ripened at 20°C or following climacteric (Liu *et al.*, 1999b; Chapter 3-5). It was earlier postulated that C7 sugars metabolism may contribute to controlling the ripening process, since a drop in their concentration appeared to be a physiological pre-requisite for softening to occur (Liu *et al.*, 2002). In Chapter 4 (Exp.1), it was observed that firmer fruit, as treated with 1-MCP or e+® Ethylene Remover, contained more mannoheptulose and perseitol than untreated fruit. However, the Chapter observed that metabolism of C7 sugars was not systematically related to the ripening process since late season fruit softened in spite of very low levels of mannoheptulose in mesocarp tissues (Exp. 2, Chapter 4).

In this study, mannoheptulose concentrations followed a predictable decrease during ripening but this decrease was only 1.5-fold between day 1 and day 5, hence the change remained smaller than the decrease reported in previous studies (Liu *et al.*, 1999b; Chapter 3-5). Also, perseitol remained unchanged during ripening and none of the heptose sugars were affected by 1-MCP or e+® Ethylene Remover. The present experimental conditions were different from that in other studies: namely, with fruit from different origins, with different harvest season and of varied biological age, as well as a different ripening regime, which might account for discrepancies in findings. All nutrients required for the high energy-demanding respiration and ethylene production are likely to come from carbohydrates reserves within the fruit, which in avocado are constituted mainly by mannoheptulose and perseitol. It is expected that differences in respiration rate according to treatments would have affected carbohydrate utilization, and hence C7 sugars content. However, similar concentrations were found for all treatments. There is still uncertainty as to the role of C7 sugar metabolism in avocado fruit and more systematic research would be required to elucidate their function. Recently, Hershkovitz *et al.* (2010) hypothesised that the seed is likely to play a role in delaying fruit ripening process of avocado cv. Arad, but still there is not a consensus of opinion.

Endogenous plant hormones are known to play a vital role in fruit growth and development, and changes in their ratios occur during ripening. Avocado contains high amounts of ABA and the importance of the hormone for avocado fruit quality has been highlighted (Cutting *et al.*, 1986). In this study, ABA was successfully extracted and quantified using a newly developed LC-ESI-MS/MS method and concentrations were in the same range as that measured in South African fruit by radioimmunoassay (Cutting *et al.*, 1986). LC-ESI-MS/MS is a highly selective and sensitive technique and the preferred tool for plant hormones analysis since a wide range of phytohormones with different chemical properties can be simultaneously analysed with high accuracy (Chiwocha *et al.*, 2003). The ABA content measured was initially high before storage commenced, and large amounts of ABA have been reported in unripe, freshly harvested fruit (Adato *et al.*, 1976, Truter *et al.*, 1992). Fruit were harvested during the mid-season and it is known that ABA content in avocado flesh increases as the season progress (Cutting *et al.*, 1986). The observed rise in ABA concentrations as fruit

softened has been previously reported (Milborrow and Robinson, 1973; Adato *et al.,* 1976, Cutting *et al.*, 1986, Chernys and Zeevart, 2000). Concentrations were significantly affected by treatment since the highest ABA concentrations were recorded in control fruit and the lowest in 1-MCP/ e+® Ethylene Remover -treated fruit (Table 6.5). Indeed, trends in ABA content appeared to mirror that of respiration, ethylene production and softening of the fruit. Confirming these findings, the ABA concentration was positively correlated with ethylene production (although not with respiration) and negatively correlated with firmness. It should be noted that in the present study, only the free form of ABA was quantified and activity of ABA extract was not measured. ABA action not only depends on its concentration but is also related to the activity of signal receptors of ABA (Zhang *et al.*, 2009a). Nevertheless, results substantiate that the physiological differences between 1-MCP-treated and untreated fruit may be, in part, related to changes in ABA metabolism. The nature of the interaction between ethylene and ABA is, however, far from understood.

The role of ABA in fruit ripening is equivocal and differing opinions exist. It has been suggested that ABA stimulates ethylene biosynthesis, by enhancing 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis (Riov *et al.*, 1990; Goren *et al.*, 1993). ABA may also cause alteration of tissue sensitivity to ethylene (Rhodes, 1981). In their study on 'Granny Smith' apples, Lara and Vendrell (2000) suggested that an increase in endogenous ABA concentration may precede the increase in ethylene responsiveness, although it was not clarified how this ABA increase promoted sensitivity to ethylene. The authors concluded that ABA could have a role in the onset of the climacteric process in 'Granny Smith' apples. Recently, treating peaches with inhibitors of ABA synthesis (Fluridone and nordihydroguaiaretic acid (NDGA)), resulted in suppressed ethylene production and prevented fruit ripening, suggesting that ABA plays a significant role in the induction of ethylene synthesis and ripening (Zhang *et al.*, 2009a). This was supported by the findings that an increase in ABA levels to a certain concentration could stimulate transformation of ACC into ethylene (Lara and Vendrell, 2000; Zhang *et al.*, 2009a). Taken altogether, this suggests that ABA may play an indirect role by facilitating the initiation and progress of the ethylene-mediated ripening, with higher importance before the onset of ripening (Jiang *et al.*, 2000; Lara

and Vendrell, 2000), but could as well have a more direct role on ethylene biosynthesis, and particularly stimulation of ethylene-related genes.

The present study found that treatment with 1-MCP/ e+® Ethylene Remover, 1-MCP, and to a lesser extend e+® Ethylene Remover, resulted in lower ABA content, reduced ethylene production and, consequently, delayed ripening (Table 6.5). This suggests that ABA metabolism may be to some degree influenced by ethylene. Also, this would explain the lowest ABA and ethylene production and highest quality maintenance found in fruit treated with 1-MCP/ e+® Ethylene Remover as compared with 1-MCP only (Table 6.5), since further perception of ethylene had been suppressed by addition of e+® Ethylene Remover. This is in agreement with Jiang *et al.* (2000) who showed in banana that exogenous ABA, alone or in combination with ethylene, enhanced flesh softening whilst this effect was partially inhibited by pre-treatment with 1-MCP. Similarly, Lohani *et al.* (2004) found that 1-MCP treatment suppressed the otherwise promoting effect of exogenous ABA on the activity of softening hydrolases in ripening banana. However, none of these studies measured endogenous ABA concentrations. Conversely, it is also possible that reduction of ABA (by an unknown factor or via ethylene action) could have in turn affected ethylene biosynthesis and/or ripening. However, whether ABA induces fruit ripening and, if yes, whether this is achieved via a direct action on ethylene biosynthesis in avocado, or indirect effect by enhancing tissue responsiveness remains unknown. Zhang *et al.* (2009a) showed that softening of peaches treated with both ABA and 1-MCP was inhibited in response to 1-MCP whilst endogenous ABA was still being synthesised in response to exogenous ABA. In other words, ABA was suggested to act upstream of ethylene perception/action but ethylene appeared to have a more important role in the later stages of ripening. There is a general consensus that ABA and ethylene may act synergistically, with different importance according to the developmental stage of the fruit (Zhang *et al.,* 2009a).

The purpose of the present study was to investigate the biochemical and physiological profile of imported avocado cv. Hass and assess whether ABA plays a role in the regulation of ripening. Using 1-MCP and/or e+® Ethylene Remover to modulate ethylene-response pathways, differences in endogenous ABA content and in fruit physiology were observed, substantiating that ABA may be partially associated

with ethylene. However, whether ABA directly induces ethylene synthesis in imported avocado fruit, or alters the sensitivity of tissue to ethylene remains to be clarified. An investigation of ABA at the genetic level would be useful for adequate understanding of the interaction between ABA and ethylene in avocado ripening. On the other hand, C7 sugars did not appeared to be related to the ripening process in this specific study and their function in avocado fruit still remains unclear.

CHAPTER 7

General discussion and conclusions

7.1 Discussion

Avocado fruit consumed in UK are imported from distant growing sites and thus necessitate medium to long term-storage. Presence of ethylene in the storage atmosphere is a major factor that can undermine avocado quality, and ethylene-induced premature ripening may generate significant economical losses. 1-MCP represents an effective way of delaying ripening and improves quality of stored avocado. Beneficial effects of 1-MCP on avocado fruit have been reviewed (section 2.4.3 of Chapter 2). However, 1-MCP is likely to bind to non-target analytes present in the storage room but also lipids present in avocado mesocarp (Dauny *et al.*, 2003; Vallejo and Beaudry, 2006) and problems of uneven ripening, both during storage and under shelf life, have been reported (Kruger and Lemmer, 2007; Ochoa- Ascencio *et al.*, 2008), which constitutes a logistical problem. This has highlighted the need to find alternatives to 1-MCP for mediation of ethylene induced ripening in avocado.

Ethylene scavenging technologies (e.g. catalytic degradation, activated carbon, potassium permanganate ($KMnO_4$)-promoted materials) can reduce ethylene levels in stored environments and therefore help maintain postharvest quality of climacteric fruits (section 2.4.4, Chapter 2). However, these scavengers often do not remove ethylene below sub physiologically active levels and their efficacy at removing ethylene for extended periods diminishes in environments with high relative humidity (RH%) and often at low temperature. High temperature catalytic degradation is more efficient than absorbers but requires operation at high temperature (>200°C) which consumes energy. Progress in developing new and more efficacious scavenging material has been scant in the past few years. The novel ethylene scavenger e+® Ethylene Remover (section 2.4.4, Chapter 2) provides a new opportunity to control ethylene, as a potential alternative or complement to 1-MCP, and was investigated in this project. The novelty of the scrubber lies in the combination of a specific platinum group metal with a specifically selected zeolite support, which has been shown to remove significant amounts of ethylene at low

and room temperature. Pd-based ethylene scavengers have already been previously studied (Bailen *et al.,* 2006; Martinez-Romero *et al.,* 2009a, 2009b) but in these cases the catalyst was supported on activated carbon and used at temperatures higher than 5°C (8ºC and 20ºC) or was heated (>100ºC) in a device, hence acting as a catalyst. In the present work and in contrast, e+® Ethylene Remover does not necessitate application of heat and is effective when used at 5ºC (i.e. acting principally as a non-catalyst (Terry *et al.*, 2007a; Smith *et al.*, 2009). This said, the disadvantage of e+® Ethylene Remover, as for most scavengers, is the necessity for it to be continuously in the presence of the commodity, which is not the case with 1-MCP (or for only a very short period, e.g. 24h). Efficacy of e+® Ethylene Remover relies on an enclosed or near enclosed environment, although this may not be an issue anymore since modern commerce frequently use palletisation and compartmentalisation through containerisation and packaging.

This project was also innovative in that both physiological behaviour and biochemical profiles of fruit imported from three of the main exporters to Europe (*viz.* South Africa, Chile and Spain), all with different transport duration, was reported in detail. Most studies have investigated home-grown avocado fruit (i.e. in country of origin), and storage or shelf life have been simulated within 24h of harvest. No study has however detailed physiology and, especially, biochemistry of imported fruit. Quality of fruit upon arrival from transit is cardinal as this is where the margins are made. Also, it is crucial for exporting countries to understand how their products perform in the markets to which they are sold. The work has therefore concentrated on the end user, i.e. the consumer. The consumer is the person paying the highest price for the product, and its perception of the fruit is paramount and one of the most important factors in postharvest management.

Avocado is rich in monounsaturated fatty acids, which in the literature have been associated with decreased risks of cardiovascular disease (Ledesma *et al.,* 1996). Avocado also contains non-negligible amounts of C7 sugar, mannoheptulose, and its corresponding sugar alcohol, perseitol. Mannoheptulose (Board *et al.*, 1995) and perseitol (Ishizu *et al.,* 2002) may have anti-cancer activity, and mannoheptulose has additionally been associated with an insulin secretion inhibitory effect (Ferrer *et al.,* 1993). On the negative aspect, if there is an increasing interest in avocado consumption

due to its health benefits, the caloric value associated with its high fat content may possibly be a factor of reluctance to consumption.Yet, little is known about the biochemical changes occurring in avocado fruit during ripening or in response to postharvest conditions/treatment, and biochemical profiling is lacking for avocado fruit, especially after long transit period. It was postulated that understanding biochemical changes in response to treatments with 1-MCP and/or e+® Ethylene Remover may provide important clues to the mechanisms underlying avocado ripening, and ultimately contribute to improve postharvest management to maintain quality and nutritional value. Changes in fatty acids (FA) (Chapter 4) and sugars (Chapter 4, 5 and 6) in response to ethylene control were systematically analysed in response to treatments.

Since there is currently no convenient and fast method for extracting fatty acids and sugars from the same avocado mesocarp sample, a new method has been developed in Chapter 3 which enabled sequential extraction and analysis of FA and sugars from the same mesocarp sample. To date, studies have quantified FA and sugars on separate avocado tissue samples. The Soxhlet method is the standard method used for lipid extraction, but is relatively tedious and it is not known whether operating lipid extraction at high temperature would affect the fatty acids profile of the oil. In the novel method described in Chapter 3, lipids were extracted with hexane at ambient temperature and the amount of oil yielded using this technique was generally comparable to that obtained with Soxhlet. Terry (2002) previously used a similar technique of sequential extraction to remove lipids from freeze-dried strawberry samples. It was demonstrated in Chapter 3 (Table 3.2) that the FA was not affected by the extraction temperature. The parameters set for the GC method enabled the elution of all fatty acids in a final runtime of <20 min (Figure 3.1), which is shorter than other GC runtimes reported in the literature (Haiyan *et al.,* 2007). In the methods reported in the literature for sugar extraction, the extraction solvent is invariably 80% (v/v) ethanol (Liu et al, 1999b; Cowan, 2004; Bertling and Bower, 2005). The aim of Chapter 3 was hence to compare the efficacy of ethanol and methanol at extracting sugars in tissues of different ripeness, since previous work on onion has shown a better sugar recovery using methanol-based vs. ethanol-based solvent (Davies *et al.*, 2007). Since a defatted residue was obtained after lipid extraction (by Soxhlet or homogenisation), the analysis of sugars was performed on the residue powder rather than dry tissue sample before

lipid removal. This is novel since sugars are generally extracted from lyophilized but not defatted tissue samples (Liu et al, 1999b; Cowan, 2004; Bertling and Bower, 2005). Presence of lipids in the sample may render the sugars extraction process less efficient: although lipids (i.e. triglycerides) are neutral coumpounds, hence less prone to extraction by a polar solvent (*viz*. ethanol and methanol), it is possible that some polar compounds from the lipid fraction may have been extracted, hence reducing the purity of the sugar extract to be analysed. Results in this Chapter (Table 3.3) have shown that sucrose, mannoheptulose and perseitol were better extracted by methanol than by ethanol in under-ripe fruit. In mid-ripe fruit, only sucrose was present in higher concentration in methanol vs. ethanol extract. Better recovery of sucrose with methanol-based vs. ethanol-based solvent has been shown before (Peres and Macedo, 1997; Macedo and Peres, 2001, Davies *et al.*, 2007) and could be due to methanol (62.5%, v/v) being a more polar solvent mixture than 80% ethanol (v/v). However, as far as is known, no study has compared the efficacy of different solvent on extraction exists for mannoheptulose or perseitol. Besides the chemical nature of the solvents, it is possible that the varying particle size of the ground tissue sample (i.e. coarser in under-ripe tissue) may also have affected the efficacy of the different solvent (Davis *et al.,* 2007), which would explain the significant difference between solvents in under-ripe tissue only. Overall, the novel technique can be used as an alternative to standard methods reported in literature. The method is reliable and relatively fast, and has the advantage of using less solvent (*viz.* hexane) than the standard methods. Results from Chapter 3 have been published in Journal of Agricultural and Food Chemistry (Meyer and Terry, 2008).

In Chapter 4, removal of ethylene below 0.1 μL L^{-1} using e+® Ethylene Remover effectively delayed the ripening of pre-climacteric fruit stored at 5°C, consistent with the role of ethylene in fruit ripening (section 2.3, Chapter 2). In the absence of ethylene, the receptors function as negative regulators, suppressing ethylene response and signalling (Kevany *et al.*, 2007). Also, cellulase and polygalacturonase, the main enzymes involved in fruit softening, are largely regulated by ethylene and triggered by the climacteric rise (Starrett and Laties, 1993). Similarly, during storage of pre-climacteric avocado imported from South Africa and stored at 12°C (Chapter 6), ethylene was reduced in presence of the scavenger below 0.1 μL L^{-1} for the first 2 days,

after which concentrations increased between 0.1-0.2 μL L^{-1} (Figure 6.1). Accordingly, fruit remained firmer and greener than controls over 3 and 4 days, respectively, after which fruit ripened similarly to controls (Table 6.3-6.4). The difference in persistence of e+® Ethylene Remover efficacy at removing ethylene below 0.1 μL L^{-1}, and therefore extend storage life, observed between Chapter 4 (Exp. 2, Figure 4.1; Exp.3, Table 4.3) and Chapter 6 (Table 6.3) could have been due to (1) different storage temperature (*viz.* 5°C vs. 12°C), consistent with effects of cooling avocado on metabolism and ethylene production rate (Perez *et al.*, 2004), (2) different biological age when treated because of different transit time (*viz. ca.* 6 days vs. *ca*. 30 days) and (3) different origin, i.e. differences in rootstock and environmental conditions. On the other hand, in Exp. 1 of Chapter 4, where ethylene was removed below 1 μL L^{-1} in presence of e+® Ethylene Remover at 12°C, fruit ripened similarly to controls (Table 4.5 and Figure 4.2), showing that either the threshold ethylene level or ethylene dose was not achieved.

Ethylene has biological activity at very low concentrations (nL-μL L^{-1}concentrations; Saltveit, 1999) but the ethylene concentration required to affect fruit physiology is uncertain. Threshold, half-maximal and saturating concentrations for ethylene-induced response in vegetative tissue have been identified as 0.01, 0.1 and 10 μL L^{-1} respectively (Abeles *et al.*, 1992), but these concentrations sound too convenient. In general, an ethylene concentration of 0.1 μL L^{-1} in the atmosphere is frequently quoted as the threshold level above which senescence of mature fruit is promoted (fruit becomes physiologically active) (Kader, 1985) and can induce important quality loss (Wills and Warton, 2000). However, the degree of effects from ethylene depends on, not only exposure concentration, but also on a number of other factors such as species, cultivar, tissues sensitivity to ethylene, duration of exposure and storage temperature (Saltveit, 1999), rendering any establishment of a universal threshold for the detrimental effects of ethylene difficult (Wills *et al.*, 2001). Therefore, the rapid ripening of avocado stored at 12°C (Exp.1, Chapter 4, Table 4.5 and Figure 4.2) may have been due to a combination of factors such as ethylene concentration, non-cold storage temperature and advanced maturity of tissues when treated, rather than because of just the concentration being higher than 0.1 μL L^{-1}. Additionally, Zauberman and Fuchs (1973) reported that only continuously applied ethylene to avocado at low temperature caused accelerated softening whereas exposure for 24h was not sufficient. Similarly, Pesis *et al.*

(2002) demonstrated that softening of avocado cv. Hass at 5°C was negligibly hastened by 100 μL L^{-1} ethylene (concentration far above the saturating level) for 24h before storage, and similar results were seen herein (Figure 4.1; Exp. 2, Chapter 4). This suggests a dose (time x concentration) effect of ethylene rather than just concentration *per se*. Despite the likely importance of ethylene exposure time at a given concentration, the literature on a dose effect of ethylene is scant.

Pre-treatment with 1-MCP resulted in stronger ripening inhibition during storage at 5°C (Chapter 4, Figure 4.1, Table 4.3) and during storage at 12°C (Chapter 6, Table 6.3) than with e+® Ethylene Remover. 1-MCP may offer better protection against exogenous ethylene effects than ethylene removal since it acts at the receptor level, whereas efficacy of ethylene scavenger is reliant on sufficient ethylene removal. This said, 1-MCP did not completely inhibit ethylene production, and fruit still produced endogenous ethylene which accumulated in the storage environment (Chapter 4, Table 4.1 and 4.2 and Chapter 6, Figure 6.1). Thus, when 1-MCP effect ceases (presumably because new receptors are produced; Blankenship and Dole, 2003) this endogenously produced ethylene may bind to newly available receptors and induce a ripening response (Jiang *et al.*, 1999b). Therefore, it is logical that ethylene removal further repressed ethylene production, softening and colour change of ripening 1-MCP-treated fruit (Chapter 6, Table 6.3-6.4). This has commercial significance when large batches of 1-MCP-treated avocado are stored, since e+® Ethylene Remover may protect non-ripening 1-MCP-treated fruit from the ethylene produced by those already ripening. Part of this work (Exp. 2 and Exp. 3, Chapter 4) has been published in Food Chemistry (Meyer and Terry, 2010).

Most research has focused on preventing the ethylene-induced climacteric (Chapter 4 and 6). Attempts to minimize softening and colour change of ripening fruit after climacteric induction were made in Chapter 5 (Exp. 1 and Exp. 2). In Exp. 2, e+® Ethylene Remover was applied in combination with MAP (rather than in boxes), with the aim to test the scavenger in a more commercial format. Results showed that lowering atmospheric ethylene below 1 μL L^{-1} significantly slowed down softening and degreening of avocado after the onset of ripening (Exp. 1, Table 5.2 and 5.3; Exp. 2, Table 5.5). Owino *et al.* (2002) showed that 1-MCP applied to avocado even at the onset of climacteric rise still completely inhibited ACS activity and temporarily delayed

ACO activity, hence no ethylene was produced. Similarly, it is not impossible that reducing ethylene concentration below 1 μL L^{-1} may have exerted a reductive effect on ethylene ACS transcript and activity, hence attenuating ethylene biosynthesis and in turn minimizing the ripening process. It was also suggested that an ethylene concentration in air below 1 μL L^{-1} may have been insufficient for depletion of receptors to the point where a constitutive ethylene response, and subsequent ripening, would be triggered. In that sense, Kevany *et al.*, (2007) proposed a model where the timing of the onset of ripening in tomato is controlled by the level of ethylene receptors. Whether such mechanisms can be extrapolated to avocado remain unknown, but this hypothesis could be verified through measuring ethylene biosynthesis genes and receptor proteins in conjunction with e+® Ethylene Remover.

Modified atmospheres have the benefits of reducing the respiration rate of fruit and delaying the ethylene climacteric (Kanellis *et al.*, 1989), hence suppressing softening and compositional changes. The optimal gas composition required to delay ripening depends on a number of variables such as cultivar, temperature and tissue maturity (Hertog *et al.*, 2003). Also, although much effort has been done in an attempt to define optimum MA conditions for a range of fresh food commodities, the underlying mechanisms for the action of MA are still poorly understood (Hertog *et al.*, 2003), as is the nature of the interaction, if any, between ethylene and CO_2 under MA conditions. MAP has been investigated experimentally but has not yet been adopted as a routine technique for extending storage life of avocado. Nevertheless, research has shown beneficial effects of MAP on quality retention of avocado fruit at temperatures between 5-14°C (Scott and Chaplin, 1978; Meir *et al.*, 1997).

It has been ascertained through experimental data that ethylene inside MA should be kept at low levels for optimal storage and reduced disorders of avocado (Hatton and Reeder, 1972; Faubion *et al.*, 1992; Pesis *et al.*, 2002). Application of an ethylene scavenger inside MAP has been shown to reduce colour change, softening and weight loss of tomato fruit (Bailen *et al.*, 2006). The bags were made of 20 μm thick non-perforated oriented polypropylene film with high permeability to O_2 and CO_2. Pesis *et al.* (2002) reported that addition of $KMnO_4$-based ethylene absorbent sachets to microperforated polyethylene bags (40 μm, permeability not given) containing avocado cv. Hass stored at 5°C improved quality (less decay and mesocarp discoloration) of fruit

upon ripening at 20°C. Results in Chapter 5 (Exp. 2) showed that although CO_2 level inside MA packaging (Table 5.1) was in agreement with others (Meir *et al.*, 1997, Pesis *et al.*, 2002), there was no beneficial effect of MAP alone on fruit softening (Table 5.5) and thus is contrary to what has been observed previously (Meir *et al.*, 1997; Hertog *et al.*, 2003). Presence of e+® Ethylene Remover inside MAP, on the other hand, retarded softening as compared with MAP alone or air controls (Table 5.5), and since CO_2 concentrations were not different between untreated MAP and MAP/e+® Ethylene Remover, the beneficial effects of MAP with e+® Ethylene Remover on fruit texture could be attributed to the addition of the scavenger rather than to a single CO_2 effect, but also to a low O_2 level. Preventing ethylene perception may result in elimination of autocatalytic ethylene production, as suggested earlier (Pesis *et al.*, 2002), possibly through ACS transcript and activity suppression, as aforementioned in this Chapter. O_2 concentration was not measured, but it is likely that it must have been low since the film permeability to O_2 was very low. Kanellis *et al.* (1989, 1991) have provided useful information concerning the mode of action of low oxygen on fruit ripening through investigating the biochemical and molecular aspects of low oxygen action on fruit ripening. The authors found that low atmospheric O_2 (2.5-5.5%) suppressed the activity and protein accumulation of the softening-related hydrolytic enzymes, cellulase and polygalacturonase. The authors partly attributed such suppression to a diminution of the biological activity of ethylene under low O_2 conditions (Kanellis *et al.*, 1989, 1991). Therefore, MA may indirectly affect ripening through not only inhibition of ethylene biosynthesis but also inhibition of ethylene action and additional application of e+® Ethylene Remover may have further enhanced this effect.

Chapter 5 has demonstrated that it is possible to delay ripening of avocado even after climacteric has been induced, using a powerful ethylene scrubber. Also, e+® Ethylene Remover has the potential to be used in combination with MAP, but more work is needed to design the appropriate packaging material alongside the e+® Ethylene Remover. Whilst these findings have significant importance for waste reduction through prolonging fruit shelf life, it must be notified that there is debate between the use of packaging and preventing waste. As mentioned in the introduction of Chapter 5, wastes toward the end of supply chain results in profit loss, and increase the imbedded costs of logistics. However, the use of packaging is an important source of waste, and should be

reduced where appropriate, alongside better design of packaging to allow recycling (Waste and Resources Action Programme (WRAP), 2009). Also, under the Producer Responsibility Obligations (Packaging Waste) Regulations (2007), businesses are constrained to recover and recycle a certain proportion of the packaging waste they generate.

The fatty acid composition of oil is an indicator of its quality and has been used as a basis for determining harvesting time. The concentration of each fatty acid may vary with cultivars (Ozdemir and Topuz, 2004; Vekiari *et al.,* 2004; Luza *et al.,* 1990), harvest time (Ozdemir and Topuz, 2004; Vekiari *et al.* 2004) although not always (Lu *et al.,* 2009), ripening stage (Ozdemir and Topuz, 2004), different growing regions and different parts of the fruit (Landahl *et al.,* 2009). Research with cvs. Fuerte and Hass avocado also found that the fatty acid profile remained stable during cold storage (De la Plaza *et al.,* 2003; Eaks, 1990; Luza *et al.,* 1990). However, no study has looked at fatty acid profile in avocado following 1-MCP treatment and only one study has looked at fatty acid composition in presence or absence of an ethylene scavenger (De la Plaza *et al.*, 2003), with no difference observed in the composition of fatty acids treated with or without the scavenger.

In Chapter 4, the fatty acid profiles found in each experiment were consistent with those published for avocado cv. Hass (Eaks, 1990; Ozdemir and Topuz, 2004; Vekiari *et al.,* 2004) (data not shown and Exp.3, Table 4.6). The fatty acid profiles measured for each experiment were different, with a progressive decrease in palmitic and palmitoleic acid and an increase in oleic acid as the season progressed (Chapter 7, Figure 7.1) which is expected for avocado cv. Hass (Ozdemir and Topuz, 2004; Vekiari *et al.* 2004). Only fruit harvested early season (Exp. 3, Table 4.6) presented changes in the proportion of some fatty acids during 21 days storage at 5°C, with a significant decrease in palmitic and palmitoleic acids and an increase in linolenic acid (Table 4.6). 1-MCP resulted in higher palmitic acid content (21.8%) vs. e+® Ethylene Remover - treated fruit (20.5%, $P < 0.05$) and controls (21.0%, $P > 0.05$) over the same period (Table 4.6). During 3 days ripening at 20°C after storage, palmitic acid decreased from 21.1% to 20.5%, with overall more palmitic acid content found in 1-MCP-treated fruit as compared with controls and e+® Ethylene Remover. On the other hand, the polyunsaturated fatty acids linoleic and linolenic acid increased during 3 days at 20°C

from 12.9% to 13.3%, and from 1.3% to 1.5%, respectively, independent of treatments (Table 4.6) and consistent with data from Ozdemir and Topuz (2004) during ripening of avocado cv. Hass held at 18– 22°C. These changes in oil fatty acid composition were however very small numerically. Despite ripening being delayed by e+® Ethylene Remover (Exp 2 and 3, Chapter 4), presence of the scavenger did not affect the fatty acids profile, and the proportion of each FA was not different from that of controls, as found by De La Plaza *et al.* (2003). The present data suggest that fatty acids are probably not related to the ripening event, since no significant changes in response to treatments were found. However, present findings also suggest that fruit harvested earlier are more affected by postharvest conditions than fruit harvested later in the season.

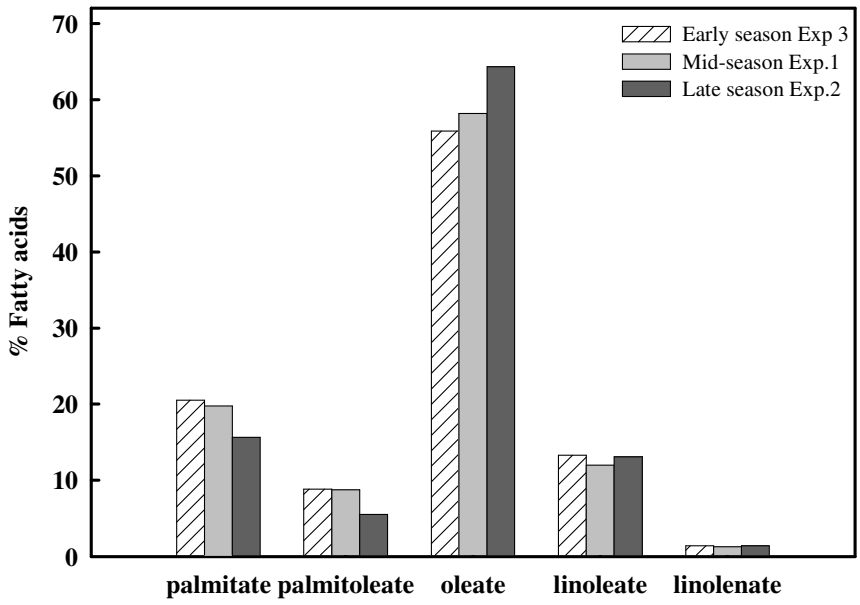

Figure 7.1. Fatty acids profile (% fatty acids) in avocado cv. Hass fruit originating from Spain and harvested at different periods in the year (*viz.* early, mid and late season). Only values of untreated (control) fruit from each experiment (Exp.1, 2, 3 of Chapter 4) are included.

Avocado is unique in its sugar composition, since the soluble seven carbon (C7) sugar D-mannoheptulose and its reduced form polyol, perseitol, are the major form of carbohydrate reserve in the fruit, and present in higher concentration than sucrose, glucose or fructose (Liu *et al.,* 1999b, 2002; Bertling and Bower, 2005; Chapter 4, Table 4.7-4.10; Chapter 5, Table 4.6-4.7 and Figure 5.3; Chapter 6, section 6.3.4). Reported C7 sugar concentrations in avocado mesocarp varies according to publications and it is likely that different harvest dates (Liu *et al.*, 1999b), origin and biological age of the fruit (Landahl *et al.,* 2009) impact on amounts present in the mesocarp tissue. Most studies have measured sugar concentration in Californian-derived (Liu *et al.*, 1999b, 2002) or South-African-derived (Bertling and Bower, 2005; Bertling *et al.*, 2007) fruit shortly after harvest, with or without storage. In contrast, the present work has quantified sugar content in imported fruit from different origins and harvest date, and with different transit time and storage conditions (Figure 7.2).

It is demonstrated herein that sugar content in the mesocarp vary greatly with harvest date. In particular, mannoheptulose decreased progressively, albeit sharply, during the season with much lower concentration in very late and, to a lower extend, late harvested fruit than in fruit harvested earlier. Mannoheptulose was also affected greatly by the growing origin, whereby, Spanish fruit contained less mannoheptulose than South African or Chilean-derived fruit from the same season. Perseitol seemed more constant and less affected by origins or season (Figure 7.2), although early season Spanish fruit contains more perseitol than both mid and late season fruit. Sucrose was also detected in substantial amounts in all fruit, and tended to increase toward the middle of the season and decrease again to the lowest concentration at the end of the season. The observed seasonal decrease in C7 sugars has already been reported earlier (Liu *et al.*, 1999b), corroborating the present findings.

It has been demonstrated in Californian fruit harvested in March (considered early to middle season) that mannoheptulose, and to a lower extent perseitol, decreased during low temperature storage and subsequent ripening (Liu *et al.*, 1999b). In the present study, different trends were observed in response to storage time or treatments applied (Table 7.1 below). Mannoheptulose decreased during storage at 5°C (Exp 3, Chapter 4, Table 4.9) and as fruit ripened at 12°C (Exp.1 Chapter 3, Table 4.7), 20°C, (Exp 3, Chapter 4, Table 4.10) or at 5°C following climacteric induction (Exp 1 and 2,

Chapter 5, Table 5.6 and Figure 5.3). In contrast, mannoheptulose did not decrease in pre-climacteric late season Spanish fruit stored at 5°C (Exp. 2, Chapter 4, Table 4.8; Exp. 1, Chapter 5, Table 5.6), and this maybe because concentrations were relatively low and fruit were kept at cold temperature, hence with a reduced metabolic activity. Mannoheptulose was found in very high quantity in fruit imported from Chile and South Africa compared with Spanish fruit (Figure 7.2), but did not decrease during storage at 12°C (Chapter 6) or 5°C following climacteric induction (Exp. 2 Chapter 5, Table 5.6), and these findings remain unexplained. Whether there is a transit time effect or not remains unknown. On the other hand, perseitol declined for almost all origins and season during storage at 5°C and ripening (Exp. 1, 2 and 3, Chapter 4, Table 4.7-4.10; Exp. 1 and 2, Chapter 5, Table 5.6 and Figure 5.3; Chapter 6; Table 7.1). The sugar alcohol did not decrease in pre-climacteric Spanish fruit stored at 5°C (Exp. 1, Chapter 5, Table 5.6) or in South African fruit held at 12°C (Chapter 6). This demonstrates that C7 sugars metabolism is reliant on factors such as origin and biological age of the fruit, and maybe transports conditions.

In another study where fruit were girdled on their stalk, Liu *et al.* (2002) reported a clear correlation between induction of ripening and a drop in mannoheptulose and perseitol levels below a threshold concentration of 20 mg g^{-1} DW. The authors suggested that the ripening process is associated with metabolism of C7 sugars and that these substances may possibly control the ripening process. Supporting evidence was found by Landahl *et al.* (2009), whereby C7 sugars accumulated more in the apical than in the basal region of the fruit, which were also shown to be the firmest. It has long been known that avocado ripening does not take place on the tree, but only after detachment from the tree. The reason for this phenomenon is not clear but early work by Tingwa and Young (1975) already postulated that inhibitory substances translocated from the tree to the fruit must be responsible for the inhibition of the onset of ripening on the tree and shortly after harvest. Following harvest, the inhibitor may be deactivated during the preclimacteric period and endogenously produced ethylene can initiate ripening (Tingwa and Young, 1975). Similarly, Blumenfeld *et al.* (1986) hypothesized that low basal ACS activity which is recognized as the limiting factor to on-tree ethylene production and ripening, could increase once the ripening inhibiting factor has been removed. In that sense, Hershkovitz *et al.* (2010) also recently found very low levels of

ACS and ACO activity at harvest which increased during ripening, and since ethylene biosynthesis genes appeared to be differentially expressed between seeded and seedless fruit (cv. Arad), the authors proposed the seed to function as a ripening regulator of the onset of ripening. It must be highlighted that the work from these authors was conducted on cv. Arad, a less well known variety than cv. Hass, and hence their results might not be transferable to cv. Hass. Additionally, the authors have not postulated how seed cross talks to the mesocarp. Differential effects observed between seeded and non-seeded fruit does not necessarily mean that the seed has a function in ripening, and these discrepancies could, as well, be due to the position of the flower on the tree, or indeed that the development of the seed is a key factor rather than the impact of the seed on ripening *per se*. Nevertheless, whether C7 sugars are the on-tree ripening inhibitor regulating through its own metabolism the initiation of ripening, or whether the reduction in these carbohydrates is an artefact of fruit ripening remains unknown. In the present work, both supporting and non-supporting data are provided (Table 7.1). In early season fruit (Exp. 3, Chapter 4, Table 4.9), mannoheptulose and perseitol were present in significantly higher concentration in firmest e+® Ethylene Remover and 1-MCP-treated fruits than in softening controls. In late season fruit, perseitol was more abundant in 1-MCP-treated fruit than in controls ($P>0.05$) and e+® Ethylene Remover-treated fruit ($P<0.05$) (Exp.2, Chapter 4, Table 4.8). Additionally, fruit treated with 1-MCP and ethylene softened slower and contained significantly more perseitol than fruit treated with 1-MCP alone (Exp. 1, Chapter 4, Table 4.7). In contrast, in other experiments, there were no differences in mannoheptulose (Exp. 2, Chapter 4, Table 4.8; Chapter 5, Table 5.6 and Figure 5.3; Chapter 6) or perseitol (Chapter 5, Table 5.6 and Figure 5.3; Chapter 6) between treatments, in spite of fruit ripening at different rates in response to 1-MCP or e+® Ethylene Remover (Table 7.1). The lack of evidence for a regulating role of C7 sugars in parts of the work substantiates that more research is necessary to elucidate the function of the particular sugars in the fruit-ripening process.

Sucrose also showed dissimilar patterns of change during ripening (Table 7.1) and no clear and consistent trend could be seen. This sugar generally increased or remained constant in controls but decreased or remained constant in treated fruits (Table 7.1). Also, it was noticed that fruit treated with e+® Ethylene Remover or untreated fruit contained more sucrose than 1-MCP treated fruit (Exp.2 Chapter 4, Table 4.8; Exp.3,

Chapter 4, Table 4.9). In many fruit species, sucrose is recognised as being the main translocated carbohydrate from sources to sinks. However, sucrose has been very little studied in avocado fruit, probably because C7 sugars appear to be the major form of carbohydrate of translocated photosynthate. Sucrose has been deemed as less important than C7 carbohydrates in the carbon balance of avocado fruit and has not been recognised as an indicator of postharvest quality (Bertling and Bower, 2005). In one study, Liu *et al.* (1999b) observed a decline in sucrose during cold storage and ripening and thus suggested that this stored sugar may contribute to the carbon energy source utilized by the respiratory process. Sucrose may also contribute to changes in osmotic potential. Yet, in a following study, sucrose increased concomitant with firmness loss (Liu *et al.*, 2002). There was no evidence in the present study to support this role, since sucrose concentrations increased during ripening at 20°C (Chapter 4, Table 4.10) and during post-climacteric storage (Exp. 1 and 2, Chapter 5, Table 5.7, Figure 5.3). Nevertheless, clear differences were observed between 1-MCP-treated fruit and both e+® Ethylene Remover -treated and control fruit (Exp. 1 , 2 and 3, Chapter 4) indicating that indeed the metabolism of sucrose might be important. Parts of the present results have been published in Food Chemistry (Meyer and Terry, 2010).

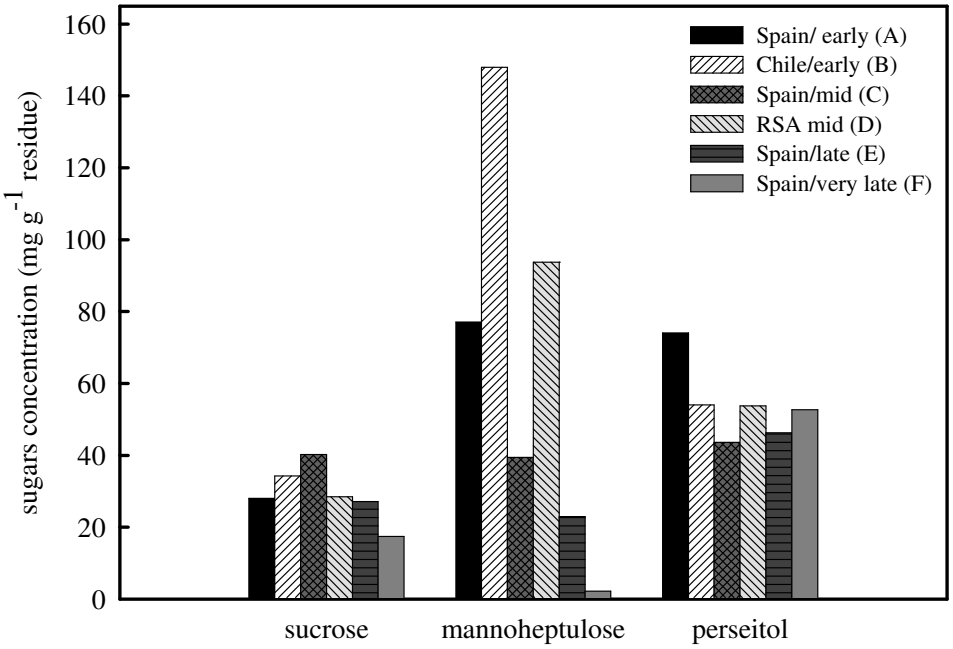

Figure 7.2. Effect of seasons (*viz.* early, mid, late, very late) on sugar content of avocado cv. Hass from different origin (*viz.* Spain, Chile, South Africa (RSA)). Fruit were used in Exp.3 Chapter 4 (A); Exp.2 Chapter 5 (B); Exp.1 Chapter 4 (C); Chapter 6 (D); Exp.1 Chapter 5 (E); Exp.2 Chapter 4 (F). Harvest dates were as follow: (A) 21st January 2008; (B) 4th September 2008; (C) 13th March 2007; (D) 4-8th June 2009; (E) 2nd May 2008; (F) 15th May 2007. Values are from untreated (controls) fruit only and correspond to the measurements taken at the earliest sampling date of each experiment (day 0 or 1 storage).

Table 7.1. Summary of trends in sucrose, mannoheptulose (Manno.) and perseitol concentrations in avocado cv. Hass fruit for each experiment. Treatment abbreviations are: 1-MCP ('MCP'), e+® Ethylene Remover ('e+'), controls ('con') and ethylene (100 µL L^{-1}; 'Et')

Experiment	Origin/season	Temp/sto-rage time	Time effect			Treatment effect		
			Sucrose	Manno.	perseitol	Sucrose	Manno.	perseitol
Exp. 1 Chapter 4	Spain/mid	12°C,/15d	con ↔ e+ ↓ MCP ↓	↓	↓	MCP/Et>MCP	No effect	MCP/Et>MCP
Exp. 2 Chapter 4	Spain/late	5°C/26d	con ↑ e+ ↑ MCP ↑↓	↔	↓	con>e+>MCP	No effect	MCP≥con≥e+ (day 26 only)
Exp. 3 Chapter 4	Spain/early	5°C/21d	↔	↓	↓	No effect	MCP≥e+≥con	e+≥MCP≥con
		20°C/3d	con ↑ e+ ↔ MCP ↔	↓	↓	e+≥con >MCP	MCP>e+≥con	e+≥MCP≥con
Exp. 1 Chapter 5	Spain/ late	5°C/7d	↔	↔	↔	No effect	No effect	No effect
		18-5°C/7d	↑	↓	↓	none > e+ (7d S1+7d S2)	No effect	No effect
		20°C/2d	↔* ↓**	↓* ↔ **	↓	No effect	No effect	No effect
Exp. 2 Chapter 5	Chile/ early	18-5°C/7d	↑	↔	↓	No effect	No effect	No effect
Chapter 6	RSA/ mid	12°C/5d	↓	↔	↔	No effect	No effect	No effect

↔ unchanged (P>0.05), ↑ increase (P<0.05), ↓ (P<0.05), * previously stored 0 days S1, ** previously stored 7 days S1, ≥ superior (P>0.05), >superior (P<0.05),

The phytohormone ABA was investigated in an attempt to identify a marker of storage potential. In avocado flesh ABA increases during maturity and ripening and has been recognized as a major factor in avocado fruit quality in relation to internal browning (Cutting *et al.*, 1986; Cutting and Bower, 1987). There is some evidence in the literature for an association between ABA and the ripening process in several other fruits. For instance, the maximum ABA content in tomato tissues was considerably higher in the fast ripening than in the slow ripening cultivar (Martinez-Madrid *et al.*, 1996). In peach, ABA content, together with ripening, was stimulated or delayed by exogenous ABA or Fluridone (an inhibitor of ABA synthesis), respectively. As yet, limited research has been directed in recent years to determine the relationship between endogenous ABA and ripening in avocado. Nevertheless, based on evidence in other fruits and the occurrence in avocado of increased ABA content at the climacteric (Chernys and Zeevart, 2000), it is likely that ABA may be intimately associated with the ripening process, and thus storage potential, in avocado.

To test this hypothesis, novel work to investigate the changes in mesocarp ABA concentration in response to 1-MCP and/or e+® Ethylene Remover was undertaken (Chapter 6). In this present experiment, ABA was extracted using acidified acetone as solvent (Zeevart *et al.*, 1989, section 6.2.5, Chapter 6). Most studies on ABA have used either fresh or lyophilised avocado tissues, but not defatted residue. A small preliminary trial was conducted to compare the newly optimised ABA extraction method (section 6.2.5, Chapter 6) to a technique published by Cutting *et al.* (1986). The principle of extraction for the comparative method was essentially the same as that described in section 6.2.5 (Chapter 6), with differences being that fresh frozen tissue rather than defatted residue powder was used, the extraction solvent was 90% methanol acidified with 1% acetic acid (v/v/v) rather than 80% acetone acidified with 1% acetic acid (v/v/v), and an additional step of partition with hexane to remove lipids was added after centrifugation. Both extracts were purified in the same way and were analysed by LC-ESI-MS/MS under same conditions. Sample size consisted of two fruits (the same fruits for both method, but mesocarp tissue prepared as fresh frozen or defatted residue), with each fruit a replicate. ABA mesocarp was extracted in triplicate (n = 6 extracts per method) and each extract was purified in duplicate (total n =

12 samples analysed per method). Results showed that ABA recovery was much higher (*ca*. 7-fold) using the acetone-based extraction on defatted residue (optimised method) than the methanol-based solvent on fresh frozen samples (data not presented). In order to compare the effect of solvent, a few additional defatted mesocarp samples were also extracted with either 90% acidified methanol or 80% acidified acetone (n = 3 samples analysed per method) and it was found that ABA recovery was better, albeit not largely, using the acetone-based solvent than the methanol-based one (data not provided). Most studies have profiled and quantified ABA in avocado extracts by radioimmunoassay (Cutting *et al.*, 1986; Cutting and Bower, 1987) or GC-MS (Zeevart *et al.*, 1989). However, radioimmunoassay is time consuming and difficult to perform. GC-MS has the disadvantage of requiring derivatisation of the compound prior to analysis and operating the GC column at high temperature may cause thermal degradation of labile target analytes (Chiwocha *et al.*, 2003). LC-ESI-MS/MS is a highly sensitive and selective technique and is particularly useful since it allows for simultaneous profiling and quantification of a wide range of plant hormone and their metabolites. The advantage of such analytical tool is that (1) it can be set to take into account the chemical properties of each of the analytes under investigation (2) phytohormones can be analysed using positive- or negative-ion electrospray, according to their chemical properties, in a single run mode (ABA is run in negative mode) and (3) since coumpounds are firstly separated by HPLC, application of high temperature is not necessary (Chiwocha *et al.*, 2003). Therefore, using this advanced technique of analysis provided a good degree of confidence in profiling and quantifying avocado endogenous ABA.

Results in Chapter 6 showed that quantified endogenous ABA concentrations were significantly affected by treatment (Table 6.4) and appeared to mirror that of respiration, ethylene production and softening of the fruit. These results suggested that ABA is at least partially mediated by ethylene, as seen in banana where 1-MCP suppressed the ripening promoting effect of ABA (Jiang *et al.*, 2000). It was postulated that its accumulation may conversely regulate ethylene biosynthesis and response. However, whilst it has been shown in this study that ethylene perception affects ABA content, whether ABA enhanced fruit ripening and, if yes, whether ABA has a direct action on ethylene biosynthesis in avocado

or indirect effect by enhancing tissue responsiveness is not known and the interaction between ethylene and ABA in avocado needs clarification. It is likely that ABA and ethylene act synergistically, but that ABA act upstream of ethylene perception/action whilst ethylene play a more important role in the later stages of ripening (Lara and Vendrell, 2000; Zhang *et al.,* 2009a). Considering this hypothesis, it would have been useful in the present research to measure ABA evolution, together with ethylene production, starting from harvest onwards. It is also worthwhile mentioning that although ABA concentrations generally increase in ripening fruit, the endogenous ABA level in plant tissue is driven by its biosynthesis and by its catabolism (Cutlers and Krochko, 1999). ABA synthesis requires the oxidative cleavage of carotenoids such as neoxanthin and violaxanthin to yield *cis*-xanthoxin, a precursor of ABA (section 2.2.7 of Chapter 2). The main ABA breakdown pathway occurs via hydroxylation at the 8´ position by the enzyme ABA 8´-hydroxylase, which produces 8'-hydroxy-ABA which is unstable and readily cyclises to phaseic acid (PA). Finally, PA can be converted to dihydrophaseic acid by PA reductase (Cutler and Krochko, 1999, Nambara and Marion-Poll, 2005). Phaseic acid has negligible hormonal activity in most assays, whereas 8´-hydroxy-ABA still has some hormonal action. Despite ABA levels increasing during maturation and ripening of several fruits, changes in amount of coumpounds which are involved in ABA biosynthetic and metabolic pathway are not well known, and may be important to clarify the regulatory role of ABA in fruit. Ethylene may control ABA levels through regulation of the conversion rate of xanthoxin to ABA but *in vivo* factors such as transport and degradation of ABA are equally important in regulating ABA levels (Zeevart, 1999). Changes in ABA metabolic activity and resulting metabolites during avocado ripening and in response to 1-MCP or e+ ® Ethylene Remover treatments should also be considered in the future.

7.2 Recommendation for future experimental work

Much effort has been done in recent years to understand the mechanisms of ethylene action and its implication in various ripening events, notably at the molecular level. In avocado fruit, ethylene plays a crucial role in the ripening event. Notwithstanding this, little

is known about the factor(s) involved in the initiation of ripening of avocado fruit. As mentioned throughout this work, avocado is peculiar in that ripening will not occur on the tree but will only do so after picking. Whether the onset of ripening is regulated by ethylene solely, by inhibitive substances, or by an interaction between these remains unknown. Various hypotheses have been proposed such as a role for the seed or for C7 sugars at inhibiting ripening (see section 2.2.1 of Chapter 2), but none have been confirmed. The importance of sugars, in particular C7 coumpounds in avocado fruit and their possible participation in ripening regulation has been outlined in only two pieces of research (Liu *et al.*, 2002; Bertling and Bower, 2005). These reports have not investigated the interaction, if any, between sugars content and ethylene. It is likely that ripening is a complex system, in which ethylene plays an importantrole. The discovery of e+® Ethylene Remover as a powerful tool to remove ethylene below sub physiologically active levels provides an opportunity to better understand the mechanisms of ethylene response and action, and its interaction with other substances potentially involved in ripening such as C7 sugars and ABA.

In the present work, e+® Ethylene Remover was applied on imported fruit after transit. In order to gain more control over the ripening process, research in the future should consider applying e+® Ethylene Remover at different stages of avocado life, starting straight from harvest until advanced ripeness. By sequentially removing ethylene and measuring the physiological and biochemical response of fruit to presence/absence of the hormone, it is expected to have a more complete picture of the respective roles of the factors involved in avocado ripening, but also their relative importance at different time along the avocado life (i.e. from harvest to full ripeness). It is possible that the importance of C7 sugars in avocado ripening depends on the maturity stage and therefore such an approach would contribute to understanding the role of C7 sugars in avocado, particularly regarding their hypothesised inhibitive properties or their possible utilization as a carbon source sustaining energy during the climacteric event.

Since the onset and the progress of ripening involves the expression of a wide set of genes, it would be beneficial to investigate the genes involved in e+® Ethylene Remover-mediated response, *viz.* ethylene-related genes, but also genes encoding for cell wall

degrading enzymes, anthocyanins accumulation in the peel or sugar metabolizing enzyme. This would contribute to elucidate the genetic mechanisms underlying the physiological and biochemical processes and, ultimately, identify molecular markers of ripening suppression in conjunction with physical and biochemical traits. The e+® Ethylene Remover also constitutes a useful tool to characterise ethylene-dependant and ethylene independent pathways, which until now have been unravelled using either 1-MCP or transgenic plants. The different levels at which the e+® Ethylene Remover and 1-MCP exert their action (*viz.* ethylene removal vs. blocking) represents an additional opportunity to further apprehend the mechanisms by which fruits modulate various responses to ethylene, in particular ethylene signalling and receptor function.

The present chapter has highlighted the significant lack of research on the dose response to ethylene (time x concentration). Besides the absolute ethylene concentration to which a fruit is exposed, the time of exposure is likely to influence the ethylene effects on physiological processes. This is particularly relevant if we take into account that receptors act as negative regulators and reduction in their level may control the onset of ripening (at least in tomato, Kevany *et al.*, 2007). Therefore, in the future, a more systematic research on the interaction between ethylene concentration and exposure time on fruit physiology, as well as defining threshold doses above which detrimental effects occur, would be much needed and help improve the postharvest handling of many commodities. Such research would necessitate a technique, in which atmospheric ethylene levels can be modulated without interfering with receptor integrity, and e+® Ethylene Remover represents a potential candidate for such approach since, unlike 1-MCP, the scavenger does not interact with the receptor.

ABA has clearly been identified as being associated with avocado ripening process (Chapters 6). Endogenous ABA content also appeared to be at least partially affected by ethylene. This said, ABA has been measured herein in one experiment only and following a long transit time. The mechanisms controlling endogenous ABA levels in the avocado mesocarp during the different physiological stages should be investigated in more detail. From the literature, it is likely that the role of ABA is not continuous and will vary during fruit development and ripening, as will its interaction with ethylene. ABA may have more

significance before the climacteric but less effect than ethylene in later stages of ripening (Lara and Vendrell, 2000, Zhang *et al.*, 2009a). Therefore, a more systematic quantification of ABA in response to ethylene removal from harvest until later stages of ripening may help elucidate the interaction and relative role of ABA and ethylene during postharvest life of avocado. In order to understand the molecular mechanisms of ABA, both the genes and enzymes involved in ABA biosynthesis/metabolism and those that require ABA for expression/action must be studied in more detail and in a temporal fashion (i.e. before harvest, at harvest, during the pre-climacteric and post-climacteric stages). Agricultural benefits could be achieved through further understanding the function of ABA in the regulation of ripening-related gene expression.

Lastly, the discovery of e+® Ethylene Remover has significant implications for the fruit industry. This powerful ethylene scavenger is efficacious at low temperature and in conditions of high relative humidity. In addition, when the scavenger is removed fruit will ripen when required and in a controlled manner, which is not always the case when using 1-MCP. However, any future commercial application of e+® Ethylene Remover requires finding an adequate format in which to use the scavenger, and which is compatible with current commercial practice. Additional research will also be needed to determine the optimum timing of application, temperature of application and amount of the material necessary to exert a desirable effect.

7.3 Project conclusions

The project objectives were listed in Chapter 1, Section 1.2.2. In summary, the overall conclusions of the project in terms of the objectives are as follow:

- A new method was developed that enabled the sequential extraction and subsequent quantification of both fatty acids and sugars from the same mesocarp sample. The method has the benefits of shorter extraction time, lower extraction temperature and is suitable for the analysis of a large number of samples.

- By using a powerful ethylene scavenger (e+® Ethylene Remover) it was possible to remove the totality of ethylene and therefore delay the ripening of avocado fruit for up to 26 days at 5°C, similarly to 1-MCP, albeit to a lower extend. Blocking ethylene action or removing ethylene did not, or very slightly, affect the fatty acid composition of the mesocarp oil. Depending on the origin and maturity of the fruit, 1-MCP and e+® Ethylene Remover better maintained C7 sugars concentrations in fruit mesocarp.

- Removing ethylene did not impair subsequent ripening under shelf life conditions at 20°C, whilst blocking the receptors using 1-MCP resulted in uneven ripening.

- It was possible to slow down the ripening rate once the climacteric had been initiated by removing ethylene below 1 $\mu L\ L^{-1}$ in presence of e+® Ethylene Remover.

- The role of ripening inhibitors earlier proposed for C7 sugars was not always verified in this project. The lack of evidence for a regulating role of C7 sugars in some experiments substantiates that more research is necessary to elucidate the function of these sugars in the fruit-ripening process.

- ABA has been identified as being intimately associated with the ripening process. ABA also appeared to be, at least to some degree, mediated by ethylene. It remains to be determined whether ABA has a direct action on ethylene biosynthesis in avocado or indirect through enhancing tissue responsiveness, a role likely to be important before the climacteric.

CHAPTER 8

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CHAPTER 9

APPENDICES

9.1 APPENDIX A: Statistical tables

9.1.1 ANOVA tables for Chapter 3

Table A.1-A.6. Effect of ripening stage (Maturity) on FW, DM content, firmness, L*, C* and Hº of avocado cv. Hass during storage (section 3.3.1, Table 3.1)

Table A.1. FW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	416.01	208.00	8.69	0.017
Residual	6	143.58	23.93		
Total	8	559.59			

Table A.2. DM content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	3.411	1.706	0.83	0.481
Residual	6	12.360	2.060		
Total	8	15.771			

Table A.3. Firmness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	7932.2	3966.1	18.54	0.003
Residual	6	1283.5	213.9		
Total	8	9215.7			

Table A.4. L*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	75.588	37.794	8.64	0.017
Residual	6	26.239	4.373		
Total	8	101.827			

Table A.5. C*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	179.551	89.775	17.63	0.003
Residual	6	30.553	5.092		
Total	8	210.104			

Table A.5. H°

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	10218.94	5109.47	60.60	<.001
Residual	6	505.89	84.31		
Total	8	10724.83			

Table A.7-A.8. Effect of extraction method and ripening stage (Maturity) on oil yield (g g^{-1} DW and % FW) of avocado cv. Hass mesocarp (section 3.3.2)

Table A.7. Oil (g g^{-1} DW)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum						
Maturity	2		0.096420	0.048210	2.78	0.140
Residual	6		0.104002	0.017334	4.50	
Sample.*Units* stratum						
Method	1		0.078891	0.078891	20.46	<.001
Maturity.Method	2		0.005448	0.002724	0.71	0.500
Residual	38	(4)	0.146499	0.003855		
Total	49	(4)	0.389612			

Table A.8. Oil (% FW)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum						
Ripening stage	2		110.382	55.191	2.57	0.156
Residual	6		128.990	21.498	7.96	
Sample.*Units* stratum						
Method	1		54.577	54.577	20.21	<.001
Maturity.Method	2		2.780	1.390	0.51	0.602
Residual	38	(4)	102.599	2.700		
Total	49	(4)	359.204			

Table A.9.-A.25. Effect of extraction method and ripening stage (Maturity) on palmitic, palmitoleic, oleic, linoleic and linolenic acid content (% total FA; mg g^{-1} oil, mg g^{-1} DW), on total FA content (mg g^{-1} oil) and on total unsaturated fatty acid content (mg g^{-1} oil) extracted from avocado cv. Hass mesocarp (section 3.3.2, Table 3.2).

Table A.9. Palmitic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	39.45087	19.72543	2.87	0.133
Residual	6	41.22318	6.87053	88.24	
Sample.*Units* stratum					
Method	1	0.21951	0.21951	2.82	0.101
Maturity.Method	2	0.08897	0.04448	0.57	0.569
Residual	42	3.27035	0.07787		
Total	53	84.25287			

Table A.10. Palmitoleic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	11.06204	5.53102	1.18	0.370
Residual	6	28.13073	4.68846	116.48	
Sample.*Units* stratum					
Method	1	0.00044	0.00044	0.01	0.917
Maturity.Method	2	0.02409	0.01205	0.30	0.743
Residual	42	1.69054	0.04025		
Total	53	40.90785			

Table A.11. Oleic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	125.0525	62.5262	4.28	0.070
Residual	6	87.7021	14.6170	87.01	
Sample.*Units* stratum					
Method	1	0.0281	0.0281	0.17	0.685
Maturity.Method	2	0.1685	0.0842	0.50	0.609
Residual	42	7.0553	0.1680		
Total	53	220.0064			

Table A.12. Linoleic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	17.02884	8.51442	0.76	0.507
Residual	6	66.98752	11.16459	559.04	
Sample.*Units* stratum					
Method	1	0.07449	0.07449	3.73	0.060
Maturity.Method	2	0.04527	0.02264	1.13	0.332
Residual	42	0.83878	0.01997		
Total	53	84.97490			

Table A.13. Linolenic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	0.133526	0.066763	0.61	0.576
Residual	6	0.660721	0.110120	45.89	
Sample.*Units* stratum					
Method	1	0.020530	0.020530	8.55	0.006
Maturity.Method	2	0.013869	0.006934	2.89	0.067
Residual	42	0.100795	0.002400		
Total	53	0.929441			

Table A.14. Palmitic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	95.427	47.713	4.57	0.062
Residual	6	62.688	10.448	2.15	
Sample.*Units* stratum					
Method	1	62.888	62.888	12.93	<.001
Maturity.Method	2	52.193	26.097	5.37	0.008
Residual	42	204.266	4.863		
Total	53	477.461			

Table A.15. Palmitoleic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	27.929	13.964	1.28	0.345
Residual	6	65.598	10.933	7.69	
Sample.*Units* stratum					
Method	1	16.582	16.582	11.67	0.001
Maturity.Method	2	15.483	7.741	5.45	0.008
Residual	42	59.691	1.421		
Total	53	185.282			

Table A.16. Oleic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	255.90	127.95	2.89	0.132
Residual	6	265.70	44.28	1.24	
Sample.*Units* stratum					
Method	1	501.21	501.21	14.06	<.001
Maturity.Method	2	396.71	198.35	5.57	0.007
Residual	42	1496.91	35.64		
Total	53	2916.44			

Table A.17. Linoleic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	35.635	17.817	0.66	0.551
Residual	6	161.962	26.994	15.63	
Sample.*Units* stratum					
Method	1	33.675	33.675	19.50	<.001
Maturity.Method	2	20.760	10.380	6.01	0.005
Residual	42	72.529	1.727		
Total	53	324.560			

Table A.18. Linolenic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	0.27544	0.13772	0.52	0.619
Residual	6	1.58750	0.26458	14.10	
Sample.*Units* stratum					
Method	1	0.50533	0.50533	26.94	<.001
Maturity.Method	2	0.0599	0.02997	1.60	0.214
Residual	42	0.78789	0.01876		
Total	53	3.21610			

Table A.19. Palmitic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	202.641	101.320	8.53	0.018
Residual	6	71.245	11.874	2.21	
Sample.*Units* stratum					
Method	1	14.170	14.170	2.64	0.113
Maturity.Method	2	21.003	10.502	1.96	0.155
Residual	38 (4)	204.087	5.371		
Total	49 (4)	474.257			

Table A.20. Palmitoleic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	16.0892	8.0446	1.49	0.299
Residual	6	32.4736	5.4123	5.77	
Sample.*Units* stratum					
Method	1	1.2672	1.2672	1.35	0.252
Maturity.Method	2	5.5084	2.7542	2.94	0.065
Residual	38 (4)	35.6271	0.9376		
Total	49 (4)	86.1067			

Table A.21. Oleic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	1181.93	590.96	3.97	0.080
Residual	6	893.03	148.84	4.04	
Sample.*Units* stratum					
Method	1	82.99	82.99	2.25	0.142
Maturity.Method	2	174.60	87.30	2.37	0.107
Residual	38 (4)	1398.94	36.81		
Total	49 (4)	3493.20			

Table A.22. Linoleic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	14.409	7.204	0.61	0.574
Residual	6	70.846	11.808	5.89	
Sample.*Units* stratum					
Method	1	2.594	2.594	1.29	0.263
Maturity.Method	2	8.610	4.305	2.15	0.131
Residual	38 (4)	76.200	2.005		
Total	49 (4)	170.061			

Table A.23. Linolenic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	0.10722	0.05361	0.53	0.616
Residual	6	0.61234	0.10206	5.35	
Sample.*Units* stratum					
Method	1	0.00219	0.00219	0.11	0.737
Maturity.Method	2	0.03360	0.01680	0.88	0.423
Residual	38 (4)	0.72542	0.01909		
Total	49 (4)	1.46906			

Table A.24. Total FA (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	12.9	6.4	0.63	0.566
Residual	6	61.6	10.3	0.09	
Sample.*Units* stratum					
Method	1	1673.1	1673.1	15.04	<.001
Maturity.Method	2	1282.8	641.4	5.77	0.006
Residual	42	4672.5	111.2		
Total	53	7702.9			

Table A.25. Total unsaturated FA (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	42.170	21.085	0.65	0.554
Residual	6	194.041	32.340	15.69	
Sample.*Units* stratum					
Method	1	42.430	42.430	20.59	<.001
Maturity.Method	2	23.005	11.503	5.58	0.007
Residual	42	86.551	2.061		
Total	53	388.198			

Table A.26- A.40. Effect of ripening Stage (Maturity) on palmitic, palmitoleic, oleic, linoleic and linolenic acid content analysed within the method of homogeneisation only.

Table A.26. Palmitic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	17.902	8.951	8.94	0.001
Residual	24	24.040	1.002		
Total	26	41.942			

Table A.27. Palmitoleic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	6.0592	3.0296	4.49	0.022
Residual	24	16.1918	0.6747		
Total	26	22.2510			

Table A.28. Oleic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	58.157	29.078	14.64	<.001
Residual	24	47.658	1.986		
Total	26	105.815			

Table A.29. Linoleic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	7.890	3.945	2.89	0.075
Residual	24	32.818	1.367		
Total	26	40.708			

Table A.30. Linolenic acid (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	0.06805	0.03403	2.24	0.128
Residual	24	0.36421	0.01518		
Total	26	0.43226			

Table A.31. Palmitic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	143.613	71.807	15.35	<.001
Residual	24	112.285	4.679		
Total	26	255.898			

Table A.32. Palmitoleic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	38.205	19.102	5.22	0.013
Residual	24	87.873	3.661		
Total	26	126.078			

Table A.33. Oleic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	90.35	45.17	1.70	0.205
Residual	24	639.20	26.63		
Total	26	729.55			

Table A.34. Linoleic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	37.707	18.854	2.49	0.104
Residual	24	181.464	7.561		
Total	26	219.171			

Table A.35. Linolenic acid (mg g^{-1} oil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	0.23139	0.11569	1.55	0.233
Residual	24	1.79080	0.07462		
Total	26	2.02219			

Table A.36. Palmitic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	159.240	79.620	15.46	<.001
Residual	23 (1)	118.435	5.149		
Total	25 (1)	265.813			

Table A.37. Palmitoleic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	19.886	9.943	5.53	0.011
Residual	23 (1)	41.371	1.799		
Total	25 (1)	59.760			

Table A.38. Oleic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	837.33	418.67	8.84	0.001
Residual	23 (1)	1089.34	47.36		
Total	25 (1)	1884.06			

Table A.39. Linoleic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	26.825	13.412	4.41	0.024
Residual	23 (1)	70.000	3.043		
Total	25 (1)	94.763			

Table A.40. Linolenic acid (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	0.16277	0.08138	2.56	0.099
Residual	23 (1)	0.73128	0.03179		
Total	25 (1)	0.88160			

Table A.41.-A.49. Effect of method and ripening stage (Maturity) on mannoheptulose, perseitol and sucrose (mg g^{-1} residue, mg g^{-1} DW and mg g^{-1} FW) (section 3.3.3, Table 3.3).

Table A.41. Mannoheptulose (mg g^{-1} residue)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	36617.744	18308.872	2.83	0.136
Residual	6	38777.822	6462.970	835.43	
Sample.*Units* stratum					
Method	2	839.756	419.878	54.28	<.001
Maturity.Method	4	404.577	101.144	13.07	<.001
Residual	66	510.582	7.736		
Total	80	77150.480			

Table A.42. Mannoheptulose (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	10032.044	5016.022	3.08	0.120
Residual	6	9779.604	1629.934	209.79	
Sample.*Units* stratum					
Method	2	70.738	35.369	4.55	0.014
Maturity.Method	4	74.084	18.521	2.38	0.061
Residual	59 (7)	458.384	7.769		
Total	73 (7)	19215.084			

Table A.43. Mannoheptulose (mg g^{-1} FW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	703.7609	351.8805	3.47	0.099
Residual	6	607.5937	101.2656	188.98	
Sample.*Units* stratum					
Method	2	4.7930	2.3965	4.47	0.016
Maturity.Method	4	4.9319	1.2330	2.30	0.069
Residual	59 (7)	31.6151	0.5358		
Total	73 (7)	1258.2169			

Table A.44. Perseitol (mg g^{-1} residue)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	4860.164	2430.082	2.03	0.212
Residual	6	7179.999	1196.667	300.11	
Sample.*Units* stratum					
Method	2	1075.341	537.670	134.84	<.001
Maturity.Method	4	225.878	56.469	14.16	<.001
Residual	66	263.169	3.987		
Total	80	13604.551			

Table A.45. Perseitol (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	2142.200	1071.100	2.58	0.156
Residual	6	2495.539	415.923	90.71	
Sample.*Units* stratum					
Method	2	128.141	64.071	13.97	<.001
Maturity.Method	4	55.918	13.979	3.05	0.024
Residual	59 (7)	270.532	4.585		
Total	73 (7)	4857.291			

Table A.46. Perseitol (mg g^{-1} FW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	116.5805	58.2902	2.64	0.151
Residual	6	132.6769	22.1128	72.93	
Sample.*Units* stratum					
Method	2	8.4228	4.2114	13.89	<.001
Maturity.Method	4	3.3690	0.8423	2.78	0.035
Residual	59 (7)	17.8890	0.3032		
Total	73 (7)	265.2591			

Table A.47. Sucrose (mg g^{-1} residue)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	1193.787	596.893	1.09	0.394
Residual	6	3277.754	546.292	86.61	
Sample.*Units* stratum					
Method	2	853.614	426.807	67.67	<.001
Maturity.Method	4	287.523	71.881	11.40	<.001
Residual	66	416.288	6.307		
Total	80	6028.965			

Table A.48. Sucrose (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	201.610	100.805	0.55	0.603
Residual	6	1096.018	182.670	54.68	
Sample.*Units* stratum					
Method	2	127.135	63.567	19.03	<.001
Maturity.Method	4	64.318	16.080	4.81	0.002
Residual	59 (7)	197.119	3.341		
Total	73 (7)	1571.965			

Table A.49. Sucrose (mg g^{-1} FW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Sample stratum					
Maturity	2	8.4363	4.2182	0.43	0.671
Residual	6	59.2862	9.8810	43.63	
Sample.*Units* stratum					
Method	2	8.4657	4.2329	18.69	<.001
Maturity.Method	4	4.3538	1.0885	4.81	0.002
Residual	59 (7)	13.3624	0.2265		
Total	73 (7)	88.1150			

Table A.50.-A.58. Effect of ripening stage (Maturity) on mannoheptulose, perseitol and sucrose content (mg g^{-1} residue, mg g^{-1} DW and mg g^{-1} FW) when analysed within the method'homogenization+ MeOH' only (section 3.3.3, Table 3.3).

Table A.50. Mannoheptulose (mg g^{-1} residue)

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	10552.9	5276.5	8.88	0.001
Residual	24(3)	14256.7	5.94.0		
Total	26 (3)	24809.6			

Table A.51.Mannoheptulose (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	3172.5	1586.3	10.09	<.001
Residual	21 (3)	3300.0	157.1		
Total	23 (3)	6120.0			

Table A.52. Mannoheptulose (mg g^{-1} FW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	211.385	105.692	10.65	<.001
Residual	21 (3)	208.318	9.920		
Total	23 (3)	396.215			

Table A.53. Perseitol (mg g^{-1} residue)

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	1664.3	832.1	7.72	0.003
Residual	24(3)	2586.1	107.8		
Total	26 (3)	4250.3			

Table A.54. Perseitol (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	961.03	480.52	16.16	<.001
Residual	21 (3)	624.50	29.74		
Total	23 (3)	1478.75			

Table A.55. Perseitol (mg g^{-1} FW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	51.491	25.745	16.01	<.001
Residual	21 (3)	33.780	1.609		
Total	23 (3)	79.549			

Table A.56. sucrose (mg g^{-1} residue)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity	2	608.22	304.11	3.94	0.033
Residual	24	1851.49	77.15		
Total	26	2459.71			

Table A.57. Sucrose (mg g^{-1} DW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	266.76	133.38	5.95	0.009
Residual	21 (3)	471.01	22.43		
Total	23 (3)	708.13			

Table A.58. Sucrose (mg g^{-1} FW)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Maturity	2	13.500	6.750	5.30	0.014
Residual	21 (3)	26.753	1.274		
Total	23 (3)	38.753			

9.1.2 ANOVA tables for Chapter 4

Table A.59. Effect of storage day, ethylene treatment and treatments on atmospheric ethylene inside boxes (Exp. 1 section 4.3.1, Table 4.1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	1259.43	314.86	8.57	<.001
Ethylene	1	0.36	0.36	0.01	0.921
Treatment	2	7423.38	3711.69	101.01	<.001
Day.Ethylene	4	175.97	43.99	1.20	0.321
Day.Treatment	8	1024.26	128.03	3.48	0.002
Ethylene Treatment	2	364.90	182.45	4.97	0.010
Day.Ethy.Treatment	8	624.55	78.07	2.12	0.047
Residual	60	2204.76	36.75		
Total	89	13077.60			

Table A.60. Effect of storage day, ethylene treatment and treatments on atmospheric ethylene inside boxes (Exp. 2 section 4.3.1, Table 4.2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	7.3653	2.4551	4.78	0.005
Ethylene	1	0.0155	0.0155	0.03	0.863
Treatment	2	33.4775	16.7387	32.57	<.001
Day.Ethylene	3	9.0403	3.0134	5.86	0.002
Day.Treatment	6	18.6241	3.1040	6.04	<.001
Ethylene Treatment	2	5.2134	2.6067	5.07	0.010
Day.Ethy.Treatment	6	9.3841	1.5640	3.04	0.013
Residual	48	24.6654	0.5139		
Total	71	107.7855			

Table A.61. Effect of storage day and treatments on atmospheric ethylene inside boxes (Exp. 3, section 4.3.1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	9.7128	3.2376	14.77	<.001
Treatment	2	23.2870	11.6435	53.12	<.001
Day.Treatment	6	18.3635	3.0606	13.96	<.001
Residual	96	21.0419	0.2192		
Total	107	72.4053			

Table A.62.-A.65. Effect of storage day, ethylene treatment and treatments on firmness, L,* C* and H° (Exp. 2 section 4.3.1, Figure 4.1)

Table A.62. Firmness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	814625.	203656.	130.66	<.001
Ethylene	1	244.	244.	0.16	0.693
Treatment	2	891670.	445835.	286.04	<.001
Day.Ethylene	4	9663.	2416.	1.55	0.189
Day.Treatment	8	304996.	38125.	24.46	<.001
Ethylene Treatment	2	3772.	1886.	1.21	0.300
Day.Ethy.Treatment	8	14520.	1815.	1.16	0.321
Residual	240	374080.	1559.		
Total	269	2413570.			

Table A.63. L*

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	4	508.110	127.027	34.24	<.001
Ethylene	1	3.013	3.013	0.81	0.368
Treatment	2	391.523	195.762	52.76	<.001
Day.Ethylene	4	43.498	10.875	2.93	0.022
Day.Treatment	8	115.732	14.466	3.90	<.001
Ethylene Treatment	2	27.519	13.759	3.71	0.026
Day.Ethy.Treatment	8	13.222	1.653	0.45	0.893
Residual	239 (1)	886.740	3.710		
Total	268 (1)	1987.444			

Table A.64. C*

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	4	2115.87	528.97	51.35	<.001
Ethylene	1	3.07	3.07	0.30	0.586
Treatment	2	1289.89	644.94	62.61	<.001
Day.Ethylene	4	38.72	9.68	0.94	0.442
Day.Treatment	8	413.44	51.68	5.02	<.001
Ethylene Treatment	2	27.69	13.85	1.34	0.263
Day.Ethy.Treatment	8	63.45	7.93	0.77	0.630
Residual	239 (1)	2461.95	10.30		
Total	268 (1)	6391.17			

Table A.65. H°

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	4	23878.6	5969.7	42.90	<.001
Ethylene	1	92.2	92.2	0.66	0.417
Treatment	2	31313.7	15656.9	112.51	<.001
Day.Ethylene	4	28.1	7.0	0.05	0.995
Day.Treatment	8	10976.8	1372.1	9.86	<.001
Ethylene Treatment	2	724.0	362.0	2.60	0.076
Day.Ethy.Treatment	8	244.3	30.5	0.22	0.987
Residual	239 (1)	33259.3	139.2		
Total	268 (1)	100357.5			

Table A.66.-A.69. Effect of cold storage days, shelf life days and treatments on firmness, L,* C* and H° (Exp. 3 section 4.3.1, Table 4.3). Baseline separates values in cold storage from values in shelf life.

Table A.66. Firmness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	1436425.	1436425.	733.43	<.001
Baseline.Storage	6	429457.	71576.	36.55	<.001
Baseline.Shelf_life	1	7823.	7823.	3.99	0.047
Baseline.Treatment	4	691234.	172808.	88.23	<.001
Baseline.Storage.Shelf_life	3	461.	154.	0.08	0.972
Baseline.Storage.Treatment	12	82821.	6902.	3.52	<.001
Baseline.Shelf_life.Treatment	2	11135.	5568.	2.84	0.060
Baseline.Storage.Shelf_life.Treatment	6	770.	128.	0.07	0.999
Residual	288	564052.	1959.		
Total	323	3224178.			

Table A.67. L*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	1247.057	1247.057	290.15	<.001
Baseline.Storage	6	409.354	68.226	15.87	<.001
Baseline.Shelf_life	1	387.260	387.260	90.10	<.001
Baseline.Treatment	4	633.078	158.269	36.82	<.001
Baseline.Storage.Shelf_life	3	3.855	1.285	0.30	0.826
Baseline.Storage.Treatment	12	96.803	8.067	1.88	0.037
Baseline.Shelf_life.Treatment	2	5.042	2.521	0.59	0.557
Baseline.Storage.Shelf_life.Treatment	6	38.008	6.335	1.47	0.187
Residual	288	1237.825	4.298		
Total	323	4058.282			

Table A.68. C*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	4464.34	4464.34	269.69	<.001
Baseline.Storage	6	927.11	154.52	9.33	<.001
Baseline.Shelf_life	1	508.42	508.42	30.71	<.001
Baseline.Treatment	4	2006.63	501.66	30.31	<.001
Baseline.Storage.Shelf_life	3	43.85	14.62	0.88	0.450
Baseline.Storage.Treatment	12	194.22	16.18	0.98	0.470
Baseline.Shelf_life.Treatment	2	32.36	16.18	0.98	0.378
Baseline.Storage.Shelf_life.Treatment	6	218.19	36.36	2.20	0.043
Residual	288	4767.41	16.55		
Total	323	13162.52			

Table A.69. H°

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	136976.7	136976.7	427.70	<.001
Baseline.Storage	6	45726.7	7621.1	23.80	<.001
Baseline.Shelf_life	1	48830.1	48830.1	152.47	<.001
Baseline.Treatment	4	83395.2	20848.8	65.10	<.001
Baseline.Storage.Shelf_life	3	2017.0	672.3	2.10	0.100
Baseline.Storage.Treatment	12	10811.0	900.9	2.81	0.001
Baseline.Shelf_life.Treatment	2	1222.7	611.4	1.91	0.150
Baseline.Storage.Shelf_life.Treatment	6	8612.5	1435.4	4.48	<.001
Residual	288	92235.9	320.3		
Total	323	429827.8			

Table A.70.-A.75. Effect of storage day, ethylene treatment and treatments on ethylene production rate, respiration rate, firmness, L,* C* and H° (Exp. 1 section 4.3.1, Table 4.4, Table 4.5, Figure 4.2).

Table A.70. Ethylene production rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	2233.64	558.41	32.26	<.001
Ethylene	1	1.25	1.25	0.07	0.789
Treatment	2	25.33	12.67	0.73	0.485
Day.Ethylene	4	44.71	11.18	0.65	0.632
Day.Treatment	8	504.45	63.06	3.64	0.002
Ethylene Treatment	2	209.00	104.50	6.04	0.004
Day.Ethy.Treatment	8	346.30	43.29	2.50	0.021
Residual	60	1038.57	17.31		
Total	89	4403.26			

Table A.71. Respiration rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	535357.	133839.	25.93	<.001
Ethylene	1	2565.	2565.	0.50	0.484
Treatment	2	43420.	21710.	4.21	0.020
Day.Ethylene	4	9624.	2406.	0.47	0.760
Day.Treatment	8	103920.	12990.	2.52	0.020
Ethylene Treatment	2	5809.	2905.	0.56	0.573
Day.Ethy.Treatment	8	24840.	3105.	0.60	0.773
Residual	60	309639.	5161.		
Total	89	1035175.			

Table A.72. Firmness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	80057.5	20014.4	87.97	<.001
Ethylene	1	1011.7	1011.7	4.45	0.036
Treatment	2	6808.2	3404.1	14.96	<.001
Day.Ethylene	4	343.3	85.8	0.38	0.825
Day.Treatment	8	4723.4	590.4	2.60	0.010
Ethylene Treatment	2	3275.5	1637.7	7.20	<.001
Day.Ethy.Treatment	8	2968.2	371.0	1.63	0.117
Residual	240	54604.4	227.5		
Total	269	153792.1			

Table A.73. L*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	1830.980	457.745	136.43	<.001
Ethylene	1	22.199	22.199	6.62	0.011
Treatment	2	20.340	10.170	3.03	0.050
Day.Ethylene	4	3.675	0.919	0.27	0.895
Day.Treatment	8	11.662	1.458	0.43	0.900
Ethylene Treatment	2	0.779	0.390	0.12	0.890
Day.Ethy.Treatment	8	88.968	11.121	3.31	0.001
Residual	60	805.222	3.355		
Total	89	2783.825			

Table A.74. C*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	3534.014	883.504	154.34	<.001
Ethylene	1	35.244	35.244	6.16	0.014
Treatment	2	63.795	31.898	5.57	0.004
Day.Ethylene	4	64.739	16.185	2.83	0.026
Day.Treatment	8	49.176	6.147	1.07	0.382
Ethylene Treatment	2	25.323	12.661	2.21	0.112
Day.Ethy.Treatment	8	162.841	20.355	3.56	<.001
Residual	60	1373.883	5.725		
Total	89	5309.017			

Table A. 75. H°

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	216093.3	54023.3	224.27	<.001
Ethylene	1	2810.1	2810.1	11.67	<.001
Treatment	2	3861.0	1930.5	8.01	<.001
Day.Ethylene	4	3386.4	846.6	3.51	0.008
Day.Treatment	8	5512.9	689.1	2.86	0.005
Ethylene Treatment	2	799.1	399.6	1.66	0.193
Day.Ethy.Treatment	8	4230.6	528.8	2.20	0.028
Residual	60	57813.6	240.9		
Total	89	294507.0			

Table A.76.-A.77. Effect of storage time, ethylene treatment and treatment on oil content (g g^{-1} DW; % FW) (Exp.1, section 4.3.2).

Table A.76. Oil (g g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	1	0.147294	0.147294	93.97	<.001
Ethylene	1	0.000887	0.000887	0.57	0.454
Treatment	2	0.001948	0.000974	0.62	0.539
Day.Ethylene	1	0.000611	0.000611	0.39	0.534
Day.Treatment	2	0.000343	0.000172	0.11	0.896
Ethylene Treatment	2	0.002031	0.001015	0.65	0.526
Day.Ethy.Treatment	2	0.004370	0.002185	1.39	0.253
Residual	93 (3)	0.145772	0.001567		
Total	104 (3)	0.299930			

Table A.77. Oil (%FW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	1	44.413	44.413	5.16	0.025
Ethylene	1	10.673	10.673	1.24	0.268
Treatment	2	15.171	7.586	0.88	0.418
Day.Ethylene	1	4.934	4.934	0.57	0.451
Day.Treatment	2	12.025	6.012	0.70	0.500
Ethylene Treatment	2	27.549	13.775	1.60	0.208
Day.Ethy.Treatment	2	14.725	7.362	0.85	0.429
Residual	93 (3)	800.983	8.613		
Total	104 (3)	928.816			

Table A.78-A.79. Effect of storage time, ethylene treatment and treatment on oil content (g g^{-1} DW; % FW) (Exp.2, section 4.3.2).

Table A.78. Oil (g g^{-1} DW)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2		0.020657	0.010329	9.28	<.001
Ethylene	1		0.000295	0.000295	0.27	0.607
Treatment	2		0.021195	0.010597	9.52	<.001
Day.Ethylene	2		0.000360	0.000180	0.16	0.851
Day.Treatment	4		0.005196	0.001299	1.17	0.328
Ethylene.Treatment	2		0.003140	0.001570	1.41	0.247
Day.Ethylene.Treatment	4		0.011185	0.002796	2.51	0.044
Residual	142	(2)	0.158024	0.001113		
Total	159	(2)	0.219678			

Table A.79. Oil (% FW)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2		7.679	3.840	0.90	0.408
Ethylene	1		0.423	0.423	0.10	0.753
Treatment	2		28.086	14.043	3.30	0.040
Day.Ethylene	2		0.311	0.155	0.04	0.964
Day.Treatment	4		41.985	10.496	2.47	0.047
Ethylene.Treatment	2		8.751	4.375	1.03	0.360
Day.Ethylene.Treatment	4		42.057	10.514	2.47	0.047
Residual	142	(2)	603.615	4.251		
Total	159	(2)	731.355			

Table A.80-A.81. Effect of cold storage time and treatment on oil content (mg g^{-1} DW; % FW) (Exp.3, section 4.3.2).

Table A.80. Oil (g g^{-1} DW)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2		0.003719	0.001860	1.33	0.271
Treatment	2		0.001498	0.000749	0.54	0.587
Day. Treatment	4		0.002037	0.000509	0.36	0.833
Residual	70	(2)	0.097713	0.001396		
Total	78	(2)	0.104842			

Table A.81. Oil (% FW)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2		51.644	25.822	4.89	0.010
Treatment	2		23.198	11.599	2.20	0.119
Day.Treatment	4		3.117	0.779	0.15	0.963
Residual	69	(3)	364.379	5.281		
Total	77	(3)	440.491			

Table A.82-A.83. Exp.3. Effect of cold storage time (Cold), subsequent shelf life (SL) days and treatments on oil content (mg g^{-1} DW; % FW) (Exp.3, section 4.3.2).

Table A.82. Oil (g g^{-1} DW)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2		0.010962	0.005481	3.97	0.021
SL	1		0.031841	0.031841	23.04	<.001
Treatment	2		0.002303	0.001151	0.83	0.437
Cold.SL	2		0.001060	0.000530	0.38	0.682
Cold.Treatment	4		0.005006	0.001252	0.91	0.463
SL.Treatment	2		0.003136	0.001568	1.13	0.324
Cold.SL.Treatment	4		0.001166	0.000291	0.21	0.932
Residual	141	(3)	0.194889	0.001382		
Total	158	(3)	0.250165			

Table A.83. Oil (% FW)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2		44.631	22.315	3.93	0.022
SL	1		22.191	22.191	3.91	0.050
Treatment	2		16.132	8.066	1.42	0.245
Cold.SL	2		15.508	7.754	1.37	0.259
Cold.Treatment	4		6.775	1.694	0.30	0.879
SL.Treatment	2		8.645	4.323	0.76	0.469
Cold.SL.Treatment	4		2.142	0.536	0.09	0.984
Residual	139	(5)	789.440	5.679		
Total	156	(5)	903.713			

Table A.85-A.89. Effect of storage day, ethylene treatment and treatments on palmitic, palmitoleic, oleic, linoleic and linolenic content (% FA) (Exp. 1 section 4.3.2).

Table A.85. Palmitic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	11.029	11.029	2.42	0.123
Ethylene	1	2.164	2.164	0.48	0.492
Treatment	2	0.243	0.121	0.03	0.974
Day.Ethylene	1	6.938	6.938	1.52	0.220
Day.Treatment	2	18.218	9.109	2.00	0.141
Ethylene Treatment	2	1.166	0.583	0.13	0.880
Day.Ethy.Treatment	2	8.003	4.002	0.88	0.418
Residual	93 (3)	423.223	4.551		
Total	104 (3)	470.266			

Table A.86. Palmitoleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	0.601	0.601	0.34	0.561
Ethylene	1	3.405	3.405	1.93	0.168
Treatment	2	2.038	1.019	0.58	0.563
Day.Ethylene	1	0.901	0.901	0.51	0.477
Day.Treatment	2	2.045	1.023	0.58	0.562
Ethylene Treatment	2	1.187	0.594	0.34	0.715
Day.Ethy.Treatment	2	6.575	3.287	1.86	0.161
Residual	93 (3)	164.090	1.764		
Total	104 (3)	180.324			

Table A.87. Oleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	14.930	14.930	1.58	0.212
Ethylene	1	10.849	10.849	1.15	0.286
Treatment	2	7.161	3.580	0.38	0.685
Day.Ethylene	1	15.193	15.193	1.61	0.208
Day.Treatment	2	28.442	14.221	1.51	0.227
Ethylene Treatment	2	7.228	3.614	0.38	0.683
Day.Ethy.Treatment	2	12.916	6.458	0.68	0.507
Residual	93 (3)	877.639	9.437		
Total	104 (3)	973.257			

Table A.88. Linoleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	0.050	0.050	0.05	0.830
Ethylene	1	0.008	0.008	0.01	0.931
Treatment	2	2.059	1.030	0.95	0.390
Day.Ethylene	1	0.188	0.188	0.17	0.678
Day.Treatment	2	0.231	0.115	0.11	0.899
Ethylene Treatment	2	0.111	0.056	0.05	0.950
Day.Ethy.Treatment	2	2.818	1.409	1.30	0.277
Residual	93 (3)	100.719	1.083		
Total	104 (3)	105.995			

Tabe A.89. Linolenic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	0.20843	0.20843	5.46	0.022
Ethylene	1	0.00459	0.00459	0.12	0.730
Treatment	2	0.06043	0.03021	0.79	0.456
Day.Ethylene	1	0.01414	0.01414	0.37	0.544
Day.Treatment	2	0.00598	0.00299	0.08	0.925
Ethylene Treatment	2	0.05041	0.02521	0.66	0.519
Day.Ethy.Treatment	2	0.04202	0.02101	0.55	0.579
Residual	93 (3)	3.55134	0.03819		
Total	104 (3)	3.91776			

Table A.90.-A.94. Effect of storage day, ethylene treatment and treatments on palmitic, palmitoleic, oleic, linoleic and linolenic content (% FA) (Exp.2 section 4.3.2, Exp.2).

Table A.90. Palmitic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	2.399	1.199	0.40	0.672
Ethylene	1	0.012	0.012	0.00	0.949
Treatment	2	2.725	1.363	0.45	0.636
Day.Ethylene	2	13.043	6.522	2.17	0.118
Day.Treatment	4	11.659	2.915	0.97	0.426
Ethylene Treatment	2	2.221	1.111	0.37	0.692
Day.Ethy.Treatment	4	38.753	9.688	3.22	0.014
Residual	143 (1)	429.607	3.004		
Total	160 (1)	499.621			

Table A.91. Palmitoleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	0.4484	0.2242	0.26	0.773
Ethylene	1	1.2039	1.2039	1.39	0.241
Treatment	2	3.1517	1.5758	1.81	0.167
Day.Ethylene	2	2.1713	1.0857	1.25	0.289
Day.Treatment	4	3.3606	0.8401	0.97	0.427
Ethylene Treatment	2	0.0047	0.0024	0.00	0.997
Day.Ethy.Treatment	4	5.7262	1.4316	1.65	0.165
Residual	143 (1)	124.1586	0.8682		
Total	160 (1)	139.5558			

Table A.92. Oleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	13.405	6.702	1.33	0.269
Ethylene	1	5.091	5.091	1.01	0.317
Treatment	2	23.450	11.725	2.32	0.102
Day.Ethylene	2	18.591	9.296	1.84	0.163
Day.Treatment	4	3.887	0.972	0.19	0.942
Ethylene Treatment	2	16.475	8.237	1.63	0.200
Day.Ethy.Treatment	4	41.916	10.479	2.07	0.087
Residual	143 (1)	722.872	5.055		
Total	160 (1)	844.766			

Table A.93. Linoleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	12.515	6.257	3.03	0.052
Ethylene	1	1.358	1.358	0.66	0.419
Treatment	2	4.341	2.170	1.05	0.353
Day.Ethylene	2	0.132	0.066	0.03	0.969
Day.Treatment	4	15.902	3.975	1.92	0.110
Ethylene Treatment	2	5.962	2.981	1.44	0.240
Day.Ethy.Treatment	4	9.834	2.459	1.19	0.318
Residual	143 (1)	295.643	2.067		
Total	160 (1)	345.339			

Table A.94. Linolenic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	0.84877	0.42439	7.13	0.001
Ethylene	1	0.01115	0.01115	0.19	0.666
Treatment	2	0.10362	0.05181	0.87	0.421
Day.Ethylene	2	0.16737	0.08368	1.41	0.249
Day.Treatment	4	0.32059	0.08015	1.35	0.256
Ethylene Treatment	2	0.02533	0.01267	0.21	0.809
Day.Ethy.Treatment	4	0.44932	0.11233	1.89	0.116
Residual	143 (1)	8.51629	0.05955		
Total	160 (1)	10.41553			

Table A.95.-A.99. Effect of cold storage days (storage) and treatment on palmitic, palmitoleic, oleic, linoleic, linolenic acid (% total FA) (Exp. 3 section 4.3.2, Table 4.6.)

Table A.95. palmitic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage day	2	23.516	11.758	4.28	0.018
Treatment	2	23.109	11.555	4.21	0.019
Storage day.Treatment	4	28.971	7.243	2.64	0.041
Residual	69 (3)	189.489	2.746		
Total	77 (3)	262.418			

Table A.96. palmitoleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage day	2	16.397	8.198	4.79	0.011
Treatment	2	4.563	2.282	1.33	0.270
Storage day.Treatment	4	5.109	1.277	0.75	0.563
Residual	69 (3)	118.009	1.710		
Total	77 (3)	143.951			

Table A.97. Oleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage	2	17.512	8.756	2.10	0.130
Treatment	2	14.784	7.392	1.77	0.177
Storage.Treatment	4	38.364	9.591	2.30	0.067
Residual	69 (3)	287.582	4.168		
Total	77 (3)	357.854			

Table A.98. Linoleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage	2	7.841	3.921	2.11	0.129
Treatment	2	7.862	3.931	2.12	0.128
Storage.Treatment	4	4.257	1.064	0.57	0.683
Residual	69 (3)	128.100	1.857		
Total	77 (3)	147.221			

Table A.99. Linolenic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage	2	2.04896	1.02448	11.06	<.001
Treatment	2	0.53592	0.26796	2.89	0.062
Storage.Treatment	4	0.39501	0.09875	1.07	0.380
Residual	69 (3)	6.39415	0.09267		
Total	77 (3)	9.26830			

Table A.100-A.104. Effect of cold storage days (Cold), shelf life days (SL) and treatment on palmitic, palmitoleic, oleic, linoleic, linolenic acid (%) (Exp. 3 section 4.3.2, Table 4.6.).

Table A.100. palmitic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	21.645	10.822	3.80	0.025
SL	1	14.593	14.593	5.12	0.025
Treatment	2	36.055	18.027	6.33	0.002
Cold.SL	2	5.456	2.728	0.96	0.386
Cold.Treatment	4	36.887	9.222	3.24	0.014
SL.Treatment	2	4.869	2.434	0.85	0.428
Cold.SL.Treatment	4	6.141	1.535	0.54	0.707
Residual	140 (4)	398.830	2.849		
Total	157 (4)	518.180			

Table A.101. Palmitoleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	6.498	3.249	1.97	0.143
SL	1	3.903	3.903	2.37	0.126
Treatment	2	6.467	3.234	1.96	0.144
Cold.SL	2	10.995	5.498	3.34	0.038
Cold.Treatment	4	2.104	0.526	0.32	0.865
SL.Treatment	2	9.524	4.762	2.89	0.059
Cold.SL.Treatment	4	10.302	2.576	1.56	0.187
Residual	140 (4)	230.648	1.647		
Total	157 (4)	279.569			

Table A.102. Oleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	23.598	11.799	2.50	0.086
SL	1	3.060	3.060	0.65	0.422
Treatment	2	23.569	11.784	2.49	0.086
Cold.SL	2	1.475	0.738	0.16	0.856
Cold.Treatment	4	37.173	9.293	1.97	0.103
SL.Treatment	2	18.421	9.211	1.95	0.146
Cold.SL.Treatment	4	10.407	2.602	0.55	0.699
Residual	140 (4)	661.863	4.728		
Total	157 (4)	778.807			

Table A.103. Linoleic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	4.018	2.009	0.95	0.390
SL	1	8.621	8.621	4.07	0.046
Treatment	2	6.401	3.201	1.51	0.224
Cold.SL	2	6.615	3.308	1.56	0.214
Cold.Treatment	4	1.698	0.425	0.20	0.938
SL.Treatment	2	2.892	1.446	0.68	0.507
Cold.SL.Treatment	4	6.487	1.622	0.77	0.549
Residual	140 (4)	296.640	2.119		
Total	157 (4)	331.587			

Table A.104. Linolenic acid

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	1.4993	0.7496	5.70	0.004
SL	1	1.2301	1.2301	9.35	0.003
Treatment	2	0.4827	0.2414	1.84	0.163
Cold.SL	2	0.6503	0.3252	2.47	0.088
Cold.Treatment	4	0.2200	0.0550	0.42	0.795
SL.Treatment	2	0.1532	0.0766	0.58	0.560
Cold.SL.Treatment	4	0.2824	0.0706	0.54	0.709
Residual	140 (4)	18.4116	0.1315		
Total	157 (4)	22.6813			

Table A.105-A.113. Effect of storage day, ethylene treatment and treatments on sucrose, mannoheptulose and perseitol (mg g^{-1} resdiue, mg g^{-1} DW and mg g^{-1} FW) (Exp. 1 section 4.3.3, Table 4.7)

Table A.105. Sucrose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	2695.2	2695.2	15.72	<.001
Ethylene	1	415.7	415.7	2.42	0.123
Treatment	2	343.9	171.9	1.00	0.371
Day.Ethylene	1	263.8	263.8	1.54	0.218
Day.Treatment	2	1289.7	644.9	3.76	0.027
Ethylene Treatment	2	2670.5	1335.2	7.79	<.001
Day.Ethy.Treatment	2	5.6	2.8	0.02	0.984
Residual	93 (3)	15947.8	171.5		
Total	104 (3)	22709.5			

Table A.106. Sucrose per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	1280.33	1280.33	36.50	<.001
Ethylene	1	58.33	58.33	1.66	0.200
Treatment	2	83.13	41.56	1.18	0.310
Day.Ethylene	1	60.57	60.57	1.73	0.192
Day.Treatment	2	189.88	94.94	2.71	0.072
Ethylene Treatment	2	445.52	222.76	6.35	0.003
Day.Ethy.Treatment	2	2.27	1.13	0.03	0.968
Residual	93 (3)	3262.39	35.08		
Total	104 (3)	5165.91			

Table A.107. Sucrose per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	146.104	146.104	66.34	<.001
Ethylene	1	8.019	8.019	3.64	0.059
Treatment	2	8.589	4.294	1.95	0.148
Day.Ethylene	1	6.962	6.962	3.16	0.079
Day.Treatment	2	12.804	6.402	2.91	0.060
Ethylene Treatment	2	36.012	18.006	8.18	<.001
Day.Ethy.Treatment	2	1.014	0.507	0.23	0.795
Residual	92 (4)	202.622	2.202		
Total	103 (4)	397.718			

Table A.108. Mannoheptulose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	43653.9	43653.9	72.06	<.001
Ethylene	1	34.9	34.9	0.06	0.811
Treatment	2	2090.0	1045.0	1.72	0.184
Day.Ethylene	1	64.1	64.1	0.11	0.746
Day.Treatment	2	1738.1	869.1	1.43	0.243
Ethylene Treatment	2	2181.0	1090.5	1.80	0.171
Day.Ethy.Treatment	2	404.9	202.4	0.33	0.717
Residual	93 (3)	56340.4	605.8		
Total	104 (3)	103223.7			

Table A.109. Mannoheptulose per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	8297.6	8297.6	74.29	<.001
Ethylene	1	3.3	3.3	0.03	0.863
Treatment	2	282.1	141.1	1.26	0.288
Day.Ethylene	1	6.1	6.1	0.05	0.816
Day.Treatment	2	226.6	113.3	1.01	0.367
Ethylene Treatment	2	364.7	182.4	1.63	0.201
Day.Ethy.Treatment	2	82.9	41.4	0.37	0.691
Residual	93 (3)	10386.8	111.7		
Total	104 (3)	19087.6			

Table A.110. Mannoheptulose per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	783.403	783.403	85.07	<.001
Ethylene	1	0.736	0.736	0.08	0.778
Treatment	2	31.009	15.505	1.68	0.191
Day.Ethylene	1	0.476	0.476	0.05	0.821
Day.Treatment	2	28.068	14.034	1.52	0.223
Ethylene Treatment	2	30.394	15.197	1.65	0.198
Day.Ethy.Treatment	2	9.880	4.940	0.54	0.587
Residual	92 (4)	847.175	9.208		
Total	103 (4)	1666.748			

Table A.111. Perseitol per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	23403.1	23403.1	127.04	<.001
Ethylene	1	7.7	7.7	0.04	0.838
Treatment	2	227.9	114.0	0.62	0.541
Day.Ethylene	1	185.1	185.1	1.00	0.319
Day.Treatment	2	177.6	88.8	0.48	0.619
Ethylene Treatment	2	1413.1	706.5	3.84	0.025
Day.Ethy.Treatment	2	894.5	447.3	2.43	0.094
Residual	93 (3)	17132.4	184.2		
Total	104 (3)	42594.0			

Table A.112. Perseitol per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	4763.61	4763.61	148.60	<.001
Ethylene	1	5.34	5.34	0.17	0.684
Treatment	2	22.51	11.25	0.35	0.705
Day.Ethylene	1	35.11	35.11	1.10	0.298
Day.Treatment	2	20.78	10.39	0.32	0.724
Ethylene Treatment	2	201.22	100.61	3.14	0.048
Day.Ethy.Treatment	2	96.77	48.39	1.51	0.226
Residual	93 (3)	2981.20	32.06		
Total	104 (3)	7959.17			

Table A.113. Perseitol per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	1	491.814	491.814	197.50	<.001
Ethylene	1	0.466	0.466	0.19	0.666
Treatment	2	3.725	1.863	0.75	0.476
Day.Ethylene	1	3.769	3.769	1.51	0.222
Day.Treatment	2	1.191	0.595	0.24	0.788
Ethylene Treatment	2	17.498	8.749	3.51	0.034
Day.Ethy.Treatment	2	7.790	3.895	1.56	0.215
Residual	92 (4)	229.101	2.490		
Total	103 (4)	729.200			

Table A.114-A.122. Effect of storage day, ethylene treatment and treatments on sucrose, mannoheptulose and perseitol (per residue, per DW and per FW) (Exp. 2 section 4.3.3, Table 4.8)

Table A.114. Sucrose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	12396.9	6198.5	34.33	<.001
Ethylene	1	163.1	163.1	0.90	0.343
Treatment	2	10670.0	5335.0	29.55	<.001
Day.Ethylene	2	238.0	119.0	0.66	0.519
Day.Treatment	4	7967.9	1992.0	11.03	<.001
Ethylene Treatment	2	45.4	22.7	0.13	0.882
Day.Ethy.Treatment	4	1125.2	281.3	1.56	0.189
Residual	142 (2)	25637.6	180.5		
Total	159 (2)	58015.2			

Table A.115. Sucrose per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	1618.62	809.31	28.80	<.001
Ethylene	1	18.04	18.04	0.64	0.424
Treatment	2	1254.74	627.37	22.33	<.001
Day.Ethylene	2	44.35	22.17	0.79	0.456
Day.Treatment	4	1050.03	262.51	9.34	<.001
Ethylene Treatment	2	9.01	4.51	0.16	0.852
Day.Ethy.Treatment	4	160.30	40.07	1.43	0.228
Residual	142 (2)	3990.06	28.10		
Total	159 (2)	8112.32			

Table A. 116. Sucrose per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	158.445	79.222	30.56	<.001
Ethylene	1	1.401	1.401	0.54	0.463
Treatment	2	131.137	65.569	25.30	<.001
Day.Ethylene	2	4.580	2.290	0.88	0.416
Day.Treatment	4	89.654	22.413	8.65	<.001
Ethylene Treatment	2	1.288	0.644	0.25	0.780
Day.Ethy.Treatment	4	17.092	4.273	1.65	0.165
Residual	142 (2)	368.073	2.592		
Total	159 (2)	768.589			

Table A.117. mannoheptulose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	152.38	76.19	1.12	0.330
Ethylene	1	0.61	0.61	0.01	0.925
Treatment	2	90.84	45.42	0.67	0.515
Day.Ethylene	2	75.92	37.96	0.56	0.574
Day.Treatment	4	468.56	117.14	1.72	0.149
Ethylene Treatment	2	115.03	57.52	0.84	0.432
Day.Ethy.Treatment	4	369.93	92.48	1.36	0.252
Residual	142 (2)	9675.10	68.13		
Total	159 (2)	10901.19			

Table A. 118. Mannoheptulose per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	19.49	9.75	0.96	0.385
Ethylene	1	1.17	1.17	0.12	0.735
Treatment	2	13.98	6.99	0.69	0.503
Day.Ethylene	2	9.02	4.51	0.45	0.641
Day.Treatment	4	70.38	17.59	1.74	0.145
Ethylene Treatment	2	12.30	6.15	0.61	0.546
Day.Ethy.Treatment	4	45.45	11.36	1.12	0.349
Residual	142 (2)	1438.57	10.13		
Total	159 (2)	1603.84			

Table A.119. Mannoheptulose per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	1.7704	0.8852	0.93	0.396
Ethylene	1	0.0980	0.0980	0.10	0.748
Treatment	2	1.0734	0.5367	0.57	0.570
Day.Ethylene	2	1.0344	0.5172	0.54	0.581
Day.Treatment	4	6.6443	1.6611	1.75	0.142
Ethylene Treatment	2	1.2985	0.6492	0.68	0.506
Day.Ethy.Treatment	4	4.2680	1.0670	1.12	0.348
Residual	142 (2)	134.8450	0.9496		
Total	159 (2)	150.4729			

Table A. 120. Perseitol per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	24327.3	12163.7	96.51	<.001
Ethylene	1	14.7	14.7	0.12	0.733
Treatment	2	255.4	127.7	1.01	0.366
Day.Ethylene	2	86.2	43.1	0.34	0.711
Day.Treatment	4	1324.9	331.2	2.63	0.037
Ethylene Treatment	2	21.6	10.8	0.09	0.918
Day.Ethy.Treatment	4	127.2	31.8	0.25	0.908
Residual	142 (2)	17897.0	126.0		
Total	159 (2)	43487.2			

Table A. 121. perseitol per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	4456.47	2228.23	92.16	<.001
Ethylene	1	0.02	0.02	0.00	0.979
Treatment	2	130.71	65.36	2.70	0.070
Day.Ethylene	2	11.44	5.72	0.24	0.790
Day.Treatment	4	181.93	45.48	1.88	0.117
Ethylene Treatment	2	15.81	7.91	0.33	0.722
Day.Ethy.Treatment	4	97.55	24.39	1.01	0.405
Residual	142 (2)	3433.29	24.18		
Total	159 (2)	8247.63			

Table A. 122. perseitol per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	459.904	229.952	97.22	<.001
Ethylene	1	0.010	0.010	0.00	0.950
Treatment	2	11.409	5.705	2.41	0.093
Day.Ethylene	2	1.646	0.823	0.35	0.707
Day.Treatment	4	22.688	5.672	2.40	0.053
Ethylene Treatment	2	0.335	0.168	0.07	0.932
Day.Ethy.Treatment	4	5.788	1.447	0.61	0.655
Residual	142 (2)	335.859	2.365		
Total	159 (2)	827.675			

Table A.123-A.131. Exp. 3. Effect of cold storage day and treatments on sucrose, mannoheptulose and perseitol (per residue, per DW and per FW) (section 4.3.3, Exp. 3, Table 4.9)

Table A.123. Sucrose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	1023.05	511.52	6.13	0.004
Treatment	2	455.50	227.75	2.73	0.072
Day.Treatment	4	534.37	133.59	1.60	0.184
Residual	70 (2)	5839.00	83.41		
Total	78 (2)	7803.74			

Table A.124. Sucrose per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	225.77	112.89	6.80	0.002
Treatment	2	116.25	58.13	3.50	0.035
Day.Treatment	4	88.93	22.23	1.34	0.264
Residual	70 (2)	1161.45	16.59		
Total	78 (2)	1583.66			

Table A.125. Sucrose per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	6.9643	3.4822	3.92	0.024
Treatment	2	5.0813	2.5407	2.86	0.064
Day.Treatment	4	4.0653	1.0163	1.15	0.343
Residual	69 (3)	61.2269	0.8873		
Total	77 (3)	76.9409			

Table A.126. Mannoheptulose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	23331.8	11665.9	17.40	<.001
Treatment	2	7923.1	3961.6	5.91	0.004
Day.Treatment	4	1108.0	277.0	0.41	0.799
Residual	70 (2)	46944.2	670.6		
Total	78 (2)	78806.5			

Table A.127. Mannoheptulose per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	3364.4	1682.2	12.33	<.001
Treatment	2	1410.4	705.2	5.17	0.008
Day.Treatment	4	179.8	44.9	0.33	0.857
Residual	70 (2)	9551.6	136.5		
Total	78 (2)	14423.5			

Table A. 128. Mannoheptulose per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	333.447	166.723	18.26	<.001
Treatment	2	121.213	60.607	6.64	0.002
Day.Treatment	4	18.537	4.634	0.51	0.730
Residual	69 (3)	630.077	9.132		
Total	77 (3)	1090.140			

Table A. 129. perseitol per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	8585.3	4292.6	29.06	<.001
Treatment	2	1100.5	550.3	3.72	0.029
Day.Treatment	4	1087.1	271.8	1.84	0.131
Residual	70 (2)	10340.8	147.7		
Total	78 (2)	21073.0			

Table A. 130. perseitol per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	1235.82	617.91	17.16	<.001
Treatment	2	300.96	150.48	4.18	0.019
Day.Treatment	4	215.73	53.93	1.50	0.212
Residual	70 (2)	2520.30	36.00		
Total	78 (2)	4265.00			

Table A. 131. perseitol per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	122.599	61.299	30.86	<.001
Treatment	2	13.050	6.525	3.28	0.043
Day.Treatment	4	14.988	3.747	1.89	0.123
Residual	69 (3)	137.080	1.987		
Total	77 (3)	281.160			

Table A.132-A.140. Effect of cold storage day (Cold), shelf life day (SL) and treatments on sucrose, mannoheptulose and perseitol (per residue, mg g^{-1} per DW and mg g^{-1} per FW) (Exp. 3 section 4.3.3, Table 4.10)

Table A. 132. Sucrose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	161.98	80.99	1.00	0.370
SL	1	112.05	112.05	1.38	0.241
Treatment	2	2498.90	1249.45	15.43	<.001
Cold.SL	2	2215.90	1107.95	13.68	<.001
Cold.Treatment	4	310.79	77.70	0.96	0.432
SL.Treatment	2	1266.81	633.40	7.82	<.001
Cold.SL.Treatment	4	587.83	146.96	1.82	0.129
Residual	137 (7)	11091.71	80.96		
Total	154 (7)	17884.40			

Table A. 133. Sucrose per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	24.89	12.45	0.81	0.448
SL	1	0.66	0.66	0.04	0.836
Treatment	2	386.34	193.17	12.54	<.001
Cold.SL	2	364.22	182.11	11.82	<.001
Cold.Treatment	4	62.97	15.74	1.02	0.398
SL.Treatment	2	205.44	102.72	6.67	0.002
Cold.SL.Treatment	4	91.04	22.76	1.48	0.212
Residual	137 (7)	2110.98	15.41		
Total	154 (7)	3196.25			

Table A. 134. Sucrose per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	0.8451	0.4225	0.48	0.618
SL	1	0.0214	0.0214	0.02	0.876
Treatment	2	19.3205	9.6603	11.03	<.001
Cold.SL	2	17.8377	8.9189	10.18	<.001
Cold.Treatment	4	3.2965	0.8241	0.94	0.442
SL.Treatment	2	12.9792	6.4896	7.41	<.001
Cold.SL.Treatment	4	6.4416	1.6104	1.84	0.125
Residual	135 (9)	118.2615	0.8760		
Total	152 (9)	175.7269			

Table A. 135. Mannoheptulose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	15416.5	7708.2	14.98	<.001
SL	1	49808.8	49808.8	96.77	<.001
Treatment	2	9008.9	4504.5	8.75	<.001
Cold.SL	2	8831.3	4415.7	8.58	<.001
Cold.Treatment	4	892.4	223.1	0.43	0.784
SL.Treatment	2	1577.4	788.7	1.53	0.220
Cold.SL.Treatment	4	965.6	241.4	0.47	0.758
Residual	137 (7)	70518.5	514.7		
Total	154 (7)	153057.0			

Table A. 136. Mannoheptulose per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	2264.1	1132.1	10.74	<.001
SL	1	9345.0	9345.0	88.67	<.001
Treatment	2	1653.5	826.8	7.84	<.001
Cold.SL	2	1214.9	607.5	5.76	0.004
Cold.Treatment	4	141.2	35.3	0.33	0.854
SL.Treatment	2	361.3	180.7	1.71	0.184
Cold.SL.Treatment	4	151.1	37.8	0.36	0.838
Residual	137 (7)	14438.9	105.4		
Total	154 (7)	28855.8			

Table A. 137. Mannoheptulose per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	209.043	104.521	15.20	<.001
SL	1	703.807	703.807	102.36	<.001
Treatment	2	146.440	73.220	10.65	<.001
Cold.SL	2	130.907	65.453	9.52	<.001
Cold.Treatment	4	15.780	3.945	0.57	0.682
SL.Treatment	2	22.262	11.131	1.62	0.202
Cold.SL.Treatment	4	12.512	3.128	0.45	0.769
Residual	135 (9)	928.249	6.876		
Total	152 (9)	2089.013			

Table A. 138. perseitol per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	5305.7	2652.9	18.36	<.001
SL	1	11434.1	11434.1	79.13	<.001
Treatment	2	945.1	472.6	3.27	0.041
Cold.SL	2	3598.6	1799.3	12.45	<.001
Cold.Treatment	4	421.4	105.3	0.73	0.574
SL.Treatment	2	736.6	368.3	2.55	0.082
Cold.SL.Treatment	4	1143.6	285.9	1.98	0.101
Residual	137 (7)	19796.1	144.5		
Total	154 (7)	42906.4			

Table A. 139. perseitol per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	880.40	440.20	14.08	<.001
SL	1	2575.80	2575.80	82.39	<.001
Treatment	2	176.50	88.25	2.82	0.063
Cold.SL	2	424.16	212.08	6.78	0.002
Cold.Treatment	4	80.40	20.10	0.64	0.633
SL.Treatment	2	275.84	137.92	4.41	0.014
Cold.SL.Treatment	4	224.66	56.17	1.80	0.133
Residual	137 (7)	4282.93	31.26		
Total	154 (7)	8815.97			

Table A. 140. perseitol per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Cold	2	80.442	40.221	23.17	<.001
SL	1	190.244	190.244	109.61	<.001
Treatment	2	14.993	7.496	4.32	0.015
Cold.SL	2	45.272	22.636	13.04	<.001
Cold.Treatment	4	3.850	0.962	0.55	0.696
SL.Treatment	2	14.197	7.099	4.09	0.019
Cold.SL.Treatment	4	17.461	4.365	2.52	0.044
Residual	135 (9)	234.303	1.736		
Total	152 (9)	576.994			

9.1.3 ANOVA tables for Chapter 5

Table A.141. Effect of storage time (S1) and treatment in S1 (T1) on atmospheric ethylene inside boxes. (Exp. 1 section 5.3.1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
S1	2	43.225	21.613	13.26	<.001
T1	2	48.917	24.459	15.01	<.001
S1. T1	4	26.579	6.645	4.08	0.007
Residual	44	71.714	1.630		
Total	53	200.422			

Table A.142. Effect of storage time S1 (S1), treatment in S1 (T1), storage time S2 (S2) and treatment in S2 (T2) on atmospheric ethylene inside boxes. (Exp. 1 section 5.3.1, Figure 5.2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
S1	1	3.1701	3.1701	3.34	0.074
T1	2	40.2814	20.1407	21.24	<.001
S2	3	290.2847	96.7616	102.04	<.001
T2	1	701.2177	01.2177	739.44	<.001
S1.T1	2	34.6283	17.3142	18.26	<.001
S1.S2	3	35.4447	11.8149	12.46	<.001
T1.S2	6	49.2615	8.2103	8.66	<.001
S1.T2	1	2.8971	2.8971	3.06	0.087
T1.T2	2	34.7243	17.3621	18.31	<.001
S2. T2	3	269.9262	89.9754	94.88	<.001
S1.T1.S2	6	20.1265	3.3544	3.54	0.006
S1.T1.T2	2	30.6910	15.3455	16.18	<.001
S1.S2.T2	3	30.1028	10.0343	10.58	<.001
T1.S2.T2	6	45.9593	7.6599	8.08	<.001
S1.T1.S2. T2	6	18.8426	3.1404	3.31	0.008
Residual	48	45.5187	0.9483		
Total	95	653.0768			

Table A.143.-A.146. Effect of storage time and treatment on atmospheric ethylene (μL L^{-1}) and CO_2 (%) inside MAP following 24h or 48h trigger (Exp. 2 section 5.3.1, Table 5.1)

Table A. 143. Ethylene (24h trigger)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage	2	382.78	191.39	3.00	0.059
Treatment	1	4919.53	4919.53	77.15	<.001
Storage.treatment	2	364.86	182.43	2.86	0.067
Residual	48	3060.59	63.76		
TStorageal	53	8727.75			

Table A. 144. Ethylene (48h trigger)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage	2	990.8	495.4	1.75	0.185
Treatment	1	16468.1	16468.1	58.07	<.001
Storage.treatment	2	960.1	480.0	1.69	0.195
Residual	47 (1)	13327.7	283.6		
TStorageal	52 (1)	30957.7			

Table A. 145. CO_2 (24h trigger)

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Storage	2	7.467	3.734	1.60	0.212
treatment	1	6.773	6.773	2.91	0.095
Storage.treatment	2	1.544	0.772	0.33	0.719
Residual	47 (1)	109.357	2.327		
TStorageal	52 (1)	125.103			

Table A. 146. CO_2 (48h trigger)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage	2	80.04	40.02	2.89	0.065
treatment	1	14.73	14.73	1.07	0.307
Storage.treatment	2	0.92	0.46	0.03	0.967
Residual	48	663.68	13.83		
TStorageal	53	759.36			

Table A.147.-A.151. Effect of storage time S1 (S1), treatment in S1 (T1), storage time S2 (S2) and treatment in S2 (T2) on firmness, log_transformed firmness, L*, C* and H° of avocado (Exp.1, section 5.3.2.1, Table 5.2-5.3).*baseline separate values in S1 from values in S2.

Table A.147. Firmness

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
T1	2	65104.	32552.	22.94	<.001
S1	1	84718.	84718.	59.70	<.001
Baseline*	1	457784.	457784.	322.61	<.001
T1.S1	2	2059.	1029.	0.73	0.486
T1.baseline	2	7906.	3953.	2.79	0.065
S1.baseline	1	4591.	4591.	3.24	0.074
baseline.T2	1	3620.	3620.	2.55	0.112
baseline.S2	1	8905.	8905.	6.28	0.013
T1.S1.baseline	2	19646.	9823.	6.92	0.001
T1.baseline.T2	2	783.	391.	0.28	0.759
S1.baseline.T2	1	263.	263.	0.19	0.667
T1.baseline.S2	2	30.	15.	0.01	0.989
S1. baseline.S2	1	1802.	1802.	1.27	0.262
baseline.T2.S2	1	81.	81.	0.06	0.812
T1.S1. baseline.T2	2	1672.	836.	0.59	0.556
T1.S1. baseline.S2	2	1128.	564.	0.40	0.673
T1. baseline.T2.S2	2	1384.	692.	0.49	0.615
S1. baseline.T2.S2	1	1507.	1507.	1.06	0.304
T1.S1. baseline.T2.S2	2	5315.	2657.	1.87	0.157
Residual	149 (1)	211428.	1419.		
Total	178 (1)	878668.			

Table A.148. log_transformed firmness

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
T1	2	32.8623	6.4311	37.39	<.001
S1	1	49.8492	9.8492	113.44	<.001
Baseline*	1	99.9657	9.9657	227.49	<.001
T1.S1	2	1.4247	0.7123	1.62	0.201
T1.baseline	2	3.8592	1.9296	4.39	0.014
S1.baseline	1	2.8113	2.8113	6.40	0.012
baseline.T2	1	1.7825	1.7825	4.06	0.046
baseline.S2	1	6.3090	6.3090	14.36	<.001
T1.S1.baseline	2	0.6455	0.3228	0.73	0.481
T1.baseline.T2	2	0.7427	0.3714	0.85	0.432
S1.baseline.T2	1	0.0588	0.0588	0.13	0.715
T1.baseline.S2	2	1.6758	0.8379	1.91	0.152
S1. baseline.S2	1	0.7779	0.7779	1.77	0.185
baseline.T2.S2	1	0.0184	0.0184	0.04	0.838
T1.S1. baseline.T2	2	0.9070	0.4535	1.03	0.359
T1.S1. baseline.S2	2	0.3365	0.1683	0.38	0.683
T1. baseline.T2.S2	2	0.0179	0.0089	0.02	0.980
S1. baseline.T2.S2	1	0.4619	0.4619	1.05	0.307
T1.S1. baseline.T2.S2	2	1.7118	0.8559	1.95	0.146
Residual	149 (1)	65.4751	0.4394		
Total	178 (1)	271.6412			

Table A.149. L*

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
T1	2	49.398	24.699	6.27	0.002
S1	1	212.039	212.039	53.82	<.001
Baseline*	1	410.062	410.062	104.08	<.001
T1.S1	2	31.959	15.979	4.06	0.019
T1.baseline	2	9.567	4.783	1.21	0.300
S1.baseline	1	55.758	55.758	14.15	<.001
baseline.T2	1	9.670	9.670	2.45	0.119
baseline.S2	1	486.732	486.732	123.54	<.001
T1.S1.baseline	2	7.463	3.731	0.95	0.390
T1.baseline.T2	2	1.529	0.764	0.19	0.824
S1.baseline.T2	1	1.823	1.823	0.46	0.497
T1.baseline.S2	2	14.176	7.088	1.80	0.169
S1. baseline.S2	1	21.821	21.821	5.54	0.020
baseline.T2.S2	1	10.750	10.750	2.73	0.101
T1.S1. baseline.T2	2	13.191	6.596	1.67	0.191
T1.S1. baseline.S2	2	4.280	2.140	0.54	0.582
T1. baseline.T2.S2	2	10.787	5.394	1.37	0.258
S1. baseline.T2.S2	1	6.009	6.009	1.53	0.219
T1.S1. baseline.T2.S2	2	32.560	16.280	4.13	0.018
Residual	149 (1)	587.034	3.940		
Total	178 (1)	1964.805			

Table A.150. C*

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
T1	2	247.918	123.959	12.43	<.001
S1	1	272.519	272.519	27.33	<.001
Baseline*	1	475.345	475.345	47.68	<.001
T1.S1	2	42.289	21.144	2.12	0.124
T1.baseline	2	39.666	19.833	1.99	0.140
S1.baseline	1	33.921	33.921	3.40	0.067
baseline.T2	1	1.886	1.886	0.19	0.664
baseline.S2	1	6.167	6.167	0.62	0.433
T1.S1.baseline	2	32.485	16.243	1.63	0.200
T1.baseline.T2	2	7.069	3.534	0.35	0.702
S1.baseline.T2	1	34.672	34.672	3.48	0.064
T1.baseline.S2	2	45.942	22.971	2.30	0.103
S1. baseline.S2	1	7.049	7.049	0.71	0.402
baseline.T2.S2	1	24.684	24.684	2.48	0.118
T1.S1. baseline.T2	2	34.738	17.369	1.74	0.179
T1.S1. baseline.S2	2	33.675	16.838	1.69	0.188
T1. baseline.T2.S2	2	8.190	4.095	0.41	0.664
S1. baseline.T2.S2	1	38.275	38.275	3.84	0.052
T1.S1. baseline.T2.S2	2	24.942	12.471	1.25	0.289
Residual	149 (1)	1485.510	9.970		
Total	178 (1)	2895.446			

Table A. 151. H°

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
T1	2	2758.2	1379.1	12.35	<.001
S1	1	8418.0	8418.0	75.40	<.001
Baseline*	1	8826.0	8826.0	79.06	<.001
T1.S1	2	285.9	142.9	1.28	0.281
T1.baseline	2	263.6	131.8	1.18	0.310
S1.baseline	1	1297.4	1297.4	11.62	<.001
baseline.T2	1	666.0	666.0	5.97	0.016
baseline.S2	1	3706.2	3706.2	33.20	<.001
T1.S1.baseline	2	159.4	79.7	0.71	0.491
T1.baseline.T2	2	12.3	6.2	0.06	0.946
S1.baseline.T2	1	670.8	670.8	6.01	0.015
T1.baseline.S2	2	529.6	264.8	2.37	0.097
S1. baseline.S2	1	81.4	81.4	0.73	0.395
baseline.T2.S2	1	53.0	53.0	0.48	0.492
T1.S1. baseline.T2	2	418.9	209.4	1.88	0.157
T1.S1. baseline.S2	2	883.2	441.6	3.96	0.021
T1. baseline.T2.S2	2	2.5	1.2	0.01	0.989
S1. baseline.T2.S2	1	511.7	511.7	4.58	0.034
T1.S1. baseline.T2.S2	2	765.2	382.6	3.43	0.035
Residual	149 (1)	16634.1	111.6		
Total	178 (1)	46806.2			

Table A.152.-A.153. Effect of storage S1, treatment in S1 (T1) and treatment in S2 (T2) on firmness and H of avocado after 2 days ripening at 20°C following S2 (Exp.1, section 5.3.2.1, Table 5.4).

Table A.152. Firmness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
S1	1	385.3	385.3	2.41	0.126
T1	2	7086.9	3543.5	22.17	<.001
T2	1	174.2	174.2	1.09	0.301
S1.T1	2	883.0	441.5	2.76	0.071
S1.T2	1	35.6	35.6	0.22	0.638
T1.T2	2	236.7	118.4	0.74	0.481
S1.T1.T2	2	62.0	31.0	0.19	0.824
Residual	60	9588.7	159.8		
Total	71	18452.5			

Table A.153. H°

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
S1	1	1449.6	1449.6	5.69	0.020
T1	2	3631.5	1815.7	7.12	0.002
T2	1	206.7	206.7	0.81	0.371
S1.T1	2	3640.0	1820.0	7.14	0.002
S1.T2	1	346.5	346.5	1.36	0.248
T1.T2	2	186.6	93.3	0.37	0.695
S1.T1.T2	2	144.4	72.2	0.28	0.754
Residual	60	15293.0	254.9		
Total	71	24898.2			

Table A.154.-A.157. Effect of trigger time (Trigger), storage time after trigger (Storage) and treatment on firmness, L*, C* and H° (Exp.2, section 5.3.2.2, Table 5.5).* baseline separates sampling following trigger (but before packing) from after packing.

Table A.154. Firmness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline*	1	55170.1	55170.1	68.56	<.001
Baseline.Trigger	2	68765.7	34382.9	42.72	<.001
Baseline.Storage	1	8003.5	8003.5	9.95	0.002
Baseline.Treatment	2	6546.7	3273.4	4.07	0.020
Baseline.Trigger. Storage	1	135.8	135.8	0.17	0.682
Baseline.Trigger.Treatment	2	1109.7	554.9	0.69	0.504
Baseline. Storage.Treatment	2	327.9	164.0	0.20	0.816
Baseline.Trigger. Storage.Treatment	2	1124.4	562.2	0.70	0.499
Residual	112	90131.9	804.7		
Total	125	231315.8			

Table A.155. L*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	32.005	32.005	6.45	0.012
Baseline.Trigger	2	4.196	2.098	0.42	0.656
Baseline.Storage	1	64.883	64.883	13.09	<.001
Baseline.Treatment	2	68.201	34.101	6.88	0.002
Baseline.Trigger. Storage	1	34.059	34.059	6.87	0.010
Baseline.Trigger.Treatment	2	19.209	9.604	1.94	0.149
Baseline. Storage.Treatment	2	10.483	5.241	1.06	0.351
Baseline.Trigger. Storage.Treatment	2	7.727	3.863	0.78	0.461
Residual	112	555.349	4.958		
Total	125	796.112			

Table A.156. C*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	11.85	11.85	0.67	0.415
Baseline.Trigger	2	201.15	100.57	5.68	0.004
Baseline.Storage	1	68.21	68.21	3.85	0.052
Baseline.Treatment	2	385.61	192.80	10.89	<.001
Baseline.Trigger. Storage	1	72.21	72.21	4.08	0.046
Baseline.Trigger.Treatment	2	67.77	33.89	1.91	0.152
Baseline. Storage.Treatment	2	34.25	17.12	0.97	0.383
Baseline.Trigger. Storage.Treatment	2	61.54	30.77	1.74	0.181
Residual	112	1983.69	17.71		
Total	125	2886.27			

Table A.157. H°

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	211.97	211.97	9.38	0.003
Baseline.Trigger	2	1216.49	608.24	26.91	<.001
Baseline.Storage	1	400.86	400.86	17.74	<.001
Baseline.Treatment	2	1057.50	528.75	23.40	<.001
Baseline.Trigger. Storage	1	185.37	185.37	8.20	0.005
Baseline.Trigger.Treatment	2	661.67	330.84	14.64	<.001
Baseline. Storage.Treatment	2	325.79	162.90	7.21	0.001
Baseline.Trigger. Storage.Treatment	2	349.56	174.78	7.73	<.001
Residual	112	2531.21	22.60		
Total	125	6940.42			

Table A.158.-A.163. Effect of storage time S1 (S1), treatment in S1 (T1), storage time S2 (S2) and treatment in S2 (T2) on mannoheptulose, perseitol and sucrose content (Exp.1, section 5.3.3.1, Table 5.6-5.7).*baseline separate values in S1 from values in S2.

Table A.158. Mannoheptulose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
S1	1	91.49	91.49	3.55	0.063
T1	1	3.01	3.01	0.12	0.733
Baseline	1	1029.93	1029.93	39.98	<.001
S1.T1	1	29.38	29.38	1.14	0.288
S1.Baseline	1	25.18	25.18	0.98	0.325
T1. Baseline	1	62.02	62.02	2.41	0.124
Baseline.T2	1	0.01	0.01	0.00	0.986
Baseline.S2	1	48.89	48.89	1.90	0.172
S1.T1. Baseline	1	15.96	15.96	0.62	0.433
S1. Baseline.T2	1	13.26	13.26	0.51	0.475
T1. Baseline.T2	1	0.02	0.02	0.00	0.978
S1. Baseline.S2	1	117.55	117.55	4.56	0.035
T1. Baseline.S2	1	16.56	16.56	0.64	0.425
Baseline.T2.S2	1	35.05	35.05	1.36	0.246
S1.T1. Baseline.T2	1	0.28	0.28	0.01	0.917
S1.T1. Baseline.S2	1	0.00	0.00	0.00	0.991
S1. Baseline.T2.S2	1	0.01	0.01	0.00	0.983
T1. Baseline.T2.S2	1	0.78	0.78	0.03	0.863
S1.T1. Baseline.T2.S2	1	0.18	0.18	0.01	0.934
Residual	94 (6)	2421.56	25.76		
Total	113 (6)	3847.32			

Table A.159. Mannoheptulose per DW

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
S1	1	8.075	8.075	2.79	0.098
T1	1	0.427	0.427	0.15	0.702
Baseline	1	166.640	166.640	57.50	<.001
S1.T1	1	8.102	8.102	2.80	0.098
S1.Baseline	1	5.748	5.748	1.98	0.162
T1. Baseline	1	6.403	6.403	2.21	0.141
Baseline.T2	1	0.004	0.004	0.00	0.969
Baseline.S2	1	14.137	14.137	4.88	0.030
S1.T1. Baseline	1	3.037	3.037	1.05	0.309
S1. Baseline.T2	1	2.385	2.385	0.82	0.367
T1. Baseline.T2	1	0.002	0.002	0.00	0.981
S1. Baseline.S2	1	20.888	20.888	7.21	0.009
T1. Baseline.S2	1	2.703	2.703	0.93	0.337
Baseline.T2.S2	1	3.743	3.743	1.29	0.259
S1.T1. Baseline.T2	1	0.067	0.067	0.02	0.879
S1.T1. Baseline.S2	1	0.336	0.336	0.12	0.734
S1. Baseline.T2.S2	1	0.004	0.004	0.00	0.972
T1. Baseline.T2.S2	1	1.253	1.253	0.43	0.512
S1.T1. Baseline.T2.S2	1	0.346	0.346	0.12	0.730
Residual	93 (7)	269.534	2.898		
Total	112(7)	503.112			

Table A.160. Perseitol per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
S1	1	514.34	514.34	5.68	0.019
T1	1	129.61	129.61	1.43	0.235
Baseline	1	2435.63	2435.63	26.89	<.001
S1.T1	1	114.48	114.48	1.26	0.264
S1.Baseline	1	64.60	64.60	0.71	0.401
T1. Baseline	1	139.47	139.47	1.54	0.218
Baseline.T2	1	51.10	51.10	0.56	0.454
Baseline.S2	1	3229.93	3229.93	35.66	<.001
S1.T1. Baseline	1	1.95	1.95	0.02	0.884
S1. Baseline.T2	1	99.92	99.92	1.10	0.296
T1. Baseline.T2	1	285.40	285.40	3.15	0.079
S1. Baseline.S2	1	826.60	826.60	9.13	0.003
T1. Baseline.S2	1	170.85	170.85	1.89	0.173
Baseline.T2.S2	1	33.92	33.92	0.37	0.542
S1.T1. Baseline.T2	1	53.72	53.72	0.59	0.443
S1.T1. Baseline.S2	1	63.07	63.07	0.70	0.406
S1. Baseline.T2.S2	1	21.75	21.75	0.24	0.625
T1. Baseline.T2.S2	1	4.29	4.29	0.05	0.828
S1.T1. Baseline.T2.S2	1	29.75	29.75	0.33	0.568
Residual	94 (6)	8514.55	90.58		
Total	113 (6)	16259.96			

Table A.161. Perseitol per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
S1	1	50.42	50.42	3.52	0.064
T1	1	9.31	9.31	0.65	0.422
Baseline	1	506.04	506.04	35.31	<.001
S1.T1	1	50.75	50.75	3.54	0.063
S1.Baseline	1	17.65	17.65	1.23	0.270
T1. Baseline	1	18.65	18.65	1.30	0.257
Baseline.T2	1	6.35	6.35	0.44	0.507
Baseline.S2	1	526.44	526.44	36.73	<.001
S1.T1. Baseline	1	1.55	1.55	0.11	0.743
S1. Baseline.T2	1	17.38	17.38	1.21	0.274
T1. Baseline.T2	1	20.44	20.44	1.43	0.235
S1. Baseline.S2	1	136.67	136.67	9.54	0.003
T1. Baseline.S2	1	15.82	15.82	1.10	0.296
Baseline.T2.S2	1	9.00	9.00	0.63	0.430
S1.T1. Baseline.T2	1	0.50	0.50	0.03	0.852
S1.T1. Baseline.S2	1	0.04	0.04	0.00	0.960
S1. Baseline.T2.S2	1	0.71	0.71	0.05	0.824
T1. Baseline.T2.S2	1	3.74	3.74	0.26	0.611
S1.T1. Baseline.T2.S2	1	0.98	0.98	0.07	0.794
Residual	93 (7)	1332.97	14.33		
Total	112 (7)	2637.89			

Table A.162. Sucrose per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
S1	1	20.5	20.5	0.05	0.817
T1	1	56.4	56.4	0.15	0.701
Baseline	1	14751.1	14751.1	38.91	<.001
S1.T1	1	0.1	0.1	0.00	0.985
S1.Baseline	1	69.3	69.3	0.18	0.670
T1. Baseline	1	50.9	50.9	0.13	0.715
Baseline.T2	1	1128.0	1128.0	2.98	0.088
Baseline.S2	1	471.1	471.1	1.24	0.268
S1.T1. Baseline	1	995.7	995.7	2.63	0.108
S1. Baseline.T2	1	0.5	0.5	0.00	0.972
T1. Baseline.T2	1	1103.0	1103.0	2.91	0.091
S1. Baseline.S2	1	3.4	3.4	0.01	0.925
T1. Baseline.S2	1	390.7	390.7	1.03	0.313
Baseline.T2.S2	1	538.1	538.1	1.42	0.236
S1.T1. Baseline.T2	1	20.2	20.2	0.05	0.818
S1.T1. Baseline.S2	1	816.9	816.9	2.15	0.145
S1. Baseline.T2.S2	1	10.1	10.1	0.03	0.871
T1. Baseline.T2.S2	1	795.5	795.5	2.10	0.151
S1.T1. Baseline.T2.S2	1	2253.1	2253.1	5.94	0.017
Residual	94 (6)	35632.8	379.1		
Total	113 (6)	58875.7			

Table A. 163. Sucrose per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
S1	1	4.43	4.43	0.11	0.739
T1	1	0.43	0.43	0.01	0.917
Baseline	1	1197.36	1197.36	30.15	<.001
S1.T1	1	10.39	10.39	0.26	0.610
S1.Baseline	1	16.99	16.99	0.43	0.515
T1. Baseline	1	15.67	15.67	0.39	0.531
Baseline.T2	1	101.82	101.82	2.56	0.113
Baseline.S2	1	0.90	0.90	0.02	0.881
S1.T1. Baseline	1	162.48	162.48	4.09	0.046
S1. Baseline.T2	1	0.75	0.75	0.02	0.891
T1. Baseline.T2	1	84.39	84.39	2.12	0.148
S1. Baseline.S2	1	5.85	5.85	0.15	0.702
T1. Baseline.S2	1	28.56	28.56	0.72	0.399
Baseline.T2.S2	1	39.83	39.83	1.00	0.319
S1.T1. Baseline.T2	1	0.03	0.03	0.00	0.979
S1.T1. Baseline.S2	1	36.29	36.29	0.91	0.342
S1. Baseline.T2.S2	1	4.71	4.71	0.12	0.731
T1. Baseline.T2.S2	1	34.27	34.27	0.86	0.355
S1.T1. Baseline.T2.S2	1	115.89	115.89	2.92	0.091
Residual	93 (7)	3693.70	39.72		
Total	112 (7)	5528.96			

Table A.164.-A.166. Effect of sampling day (Day) and treatment on mannoheptulose, perseitol and sucrose (Exp.2, section 5.3.3.2, Figure 5.3).* BL1 separates sampling on arrival at laboratory (baseline) from all other sampling; BL2 separates sampling at arrival+after trigger but before packing from after packing.

Table A.164. Mannoheptulose per residue

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
BL1	1	26649.	26649.	11.38	0.001
BL1.BL2	1	1174.	1174.	0.50	0.482
BL1.BL2.Day	1	9.	9.	0.00	0.951
BL1.BL2.Treatment	2	170.	85.	0.04	0.964
BL1.BL2.Day.Treatment	2	672.	336.	0.14	0.867
Residual	63 (1)	147489.	2341.		
Total	70 (1)	173535.			

Table A.165. Perseitol per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
BL1	1	62.71	62.71	0.69	0.409
BL1.BL2	1	0.67	0.67	0.01	0.932
BL1.BL2.Outurn	1	550.08	550.08	6.06	0.017
BL1.BL2.Treatment	2	5.99	2.99	0.03	0.968
BL1.BL2.Outurn.Treatment	2	29.09	14.54	0.16	0.852
Residual	63 (1)	5719.25	90.78		
Total	70 (1)	6361.61			

Table A.166. Sucrose per per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
BL1	1	31.16	31.16	0.54	0.464
BL1.BL2	1	228.39	228.39	3.98	0.051
BL1.BL2.Outurn	1	355.64	355.64	6.19	0.016
BL1.BL2.Treatment	2	285.36	142.68	2.48	0.092
BL1.BL2.Outurn.Treatment	2	154.68	77.34	1.35	0.268
Residual	63 (1)	3619.67	57.46		
Total	70 (1)	4671.83			

9.1.4 ANOVA tables for Chapter 6

Table A.167. Effect of storage day and treatment on atmospheric ethylene inside chambers (section 6.3.1 Figure 6.2).

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	4	7.7075423	1.9268856	4663.23	<.001
Treatment	4	40.7749191	10.1937298	24669.71	<.001
Day.Treatment	16	27.7672809	1.7354551	4199.95	<.001
Residual	48 (2)	0.0198340	0.0004132		
Total	72 (2)	75.2947520			

Table A.168.-A.169. Effect of storage days and treatment on respiration rate at 12°C and 20°C (section 6.3.2, Table 6.1)

Table A.168. 12°C

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5	2399.8	480.0	3.44	0.009
Treatment	4	2380.1	595.0	4.26	0.004
Day.Treatment	20	1358.2	67.9	0.49	0.962
Residual	59 (1)	8236.1	139.6		
Total	88 (1)	14373.9			

Table A. 169. 20°C

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5	2204.9	441.0	1.39	0.240
Treatment	4	2025.9	506.5	1.60	0.186
Day.Treatment	20	4928.4	246.4	0.78	0.727
Residual	59 (1)	18663.8	316.3		
Total	88 (1)	27800.1			

Table A.170. Effect of treatment, storage day and temperature (12°C vs. 20°C) on ethylene production rate (section 6.3.2, Table 6.2)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5	6223.68	1244.74	19.86	<.001
Treatment	4	1171.55	292.89	4.67	0.002
temp	1	76.61	76.61	1.22	0.271
Day.Treatment	20	1916.92	95.85	1.53	0.084
Day.temp	5	348.99	69.80	1.11	0.357
Treatment.temp	4	306.66	76.66	1.22	0.305
Day.Treatment.temp	20	907.13	45.36	0.72	0.795
Residual	117 (3)	7332.90	62.67		
Total	176 (3)	18125.57			

Table A.171.-A.175. Effect of treatment and days storage on firmness, L*, C*, H° and log-transformed firmness (section 6.3.2, Table 6.3-6.4)

Table A.171. Firmness

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
time	5	408371.	81674.	79.53	<.001
treatment	4	28239.	7060.	6.87	<.001
time.treatment	20	25753.	1288.	1.25	0.220
Residual	147 (3)	150972.	1027.		
Total	176 (3)	572973.			

Table A.172. L*

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
time	5	711.533	142.307	35.36	<.001
treatment	4	17.080	4.270	1.06	0.378
time.treatment	20	101.243	5.062	1.26	0.217
Residual	147 (3)	591.625	4.025		
Total	176 (3)	1374.832			

Table A.173. C*

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
time	5	1989.255	397.851	42.77	<.001
treatment	4	54.059	13.515	1.45	0.220
time.treatment	20	300.108	15.005	1.61	0.056
Residual	147 (3)	1367.398	9.302		
Total	176 (3)	3570.855			

Table A. 174. H°

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
time	5	54881.08	10976.22	119.16	<.001
treatment	4	1846.18	461.55	5.01	<.001
time.treatment	20	1127.97	56.40	0.61	0.899
Residual	147 (3)	13540.24	92.11		
Total	176 (3)	69895.70			

Table A. 175. log_transformed firmness

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
time	5	183.2533	36.6507	118.10	<.001
treatment	4	24.1200	6.0300	19.43	<.001
time.treatment	20	11.8922	0.5946	1.92	0.015
Residual	147 (3)	45.6196	0.3103		
Total	176 (3)	254.8229			

Table A.176-A.178. Effect of treatment and storage day on sucrose, mannoheptulose and perseitol (mg g^{-1} residue) (section 6.3.4).

Table A.176. Sucrose

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
day	2	601.2	300.6	2.01	0.144
treatment	3	128.9	43.0	0.29	0.835
day.treatment	6	730.6	121.8	0.81	0.564
Residual	59 (1)	8838.3	149.8		
Total	70 (1)	10287.3			

Table A.177. Mannoheptulose

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
day	2	11804.	5902.	4.97	0.010
treatment	3	3843.	1281.	1.08	0.365
day.treatment	6	8786.	1464.	1.23	0.302
Residual	59 (1)	70000.	1186.		
Total	70 (1)	94376.			

Table A.178. Perseitol

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
day	2	124.54	62.27	0.78	0.461
treatment	3	405.61	135.20	1.70	0.176
day.treatment	6	830.62	138.44	1.74	0.127
Residual	59 (1)	4684.58	79.40		
Total	70 (1)	6032.86			

Table A.179-A.181. Effect of treatment and days storage on ABA (section 6.3.5, table 6.5).

Table A.179. ABA per residue

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	18092249.	9046124.	60.35	<.001
Treatment	3	5756646.	1918882.	12.80	<.001
Day.Treatment	6	2371108.	395185.	2.64	0.044
Residual	22 (2)	3297852.	149902.		
Total	33 (2)	28809229.			

Table A.180. ABA per DW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	2415525.	1207763.	35.30	<.001
Treatment	3	724471.	241490.	7.06	0.002
Day.Treatment	6	342016.	57003.	1.67	0.177
Residual	22 (2)	752753.	34216.		
Total	33 (2)	4142761.			

Table A.181. ABA per FW

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	2	221939.	110970.	46.36	<.001
Treatment	3	55959.	18653.	7.79	0.001
Day.Treatment	6	28100.	4683.	1.96	0.118
ResiduaL*	21 (3)	50265.	2394.		
TotaL*	32 (3)	331055.			

9.1.5 Correlation matrices for Chapter 3

Table A.182.-A.184. Correlations for mannoheptulose, perseitol and sucrose between method (viz. homogenization + ethanol (EtOH), homogenization +methanol (MeOH), Soxhlet + methanol (Soxhlet) and ripening stages (viz. early (E), mid (M) and late ripe (L)) (Sections 3.3.3).

Table A.182. Mannoheptulose

EtOH E	1								
EtOH L	-0.483	1							
EtOH M	0.467	-0.983	1						
MeOH E	0.917	-0.450	0.417	1					
MeOH L	-0.433	0.917	-0.933	-0.483	1				
MeOH M	0.433	-0.967	0.983	0.433	-0.950	1			
Soxhlet E	0.850	-0.483	0.467	0.867	-0.433	0.483	1		
Soxhlet L	-0.450	0.917	-0.883	-0.433	0.817	-0.867	-0.500	1	
Soxhlet M	0.383	-0.900	0.883	0.500	-0.917	0.917	0.483	-0.917	1
	EtOH_E	EtOH_L	EtOH_M	MeOH_E	MeOH_L	MeOH_M	Soxhlet_E	Soxhlet_L	Soxhlet_M

Table A.183. Perseitol

EtOH E	1								
EtOH L	0.283	1							
EtOH M	0.983	0.267	1						
MeOH E	0.900	0.400	0.867	1					
MeOH L	0.267	0.800	0.283	0.150	1				
MeOH M	0.800	0.417	0.867	0.717	0.433	1			
Soxhlet E	0.833	0.367	0.850	0.867	0.283	0.750	1		
Soxhlet L	-0.383	0.533	-0.350	-0.350	0.467	-0.183	-0.300	1	
Soxhlet M	0.767	0.400	0.783	0.867	0.200	0.850	0.850	-0.250	1
	EtOH_E	EtOH_L	EtOH_M	MeOH_E	MeOH_L	MeOH_M	Soxhlet_E	Soxhlet_L	Soxhlet_M

Table A.184. Sucrose

EtOH E	1								
EtOH L	0.867	1							
EtOH M	-0.583	-0.850	1						
MeOH E	0.883	0.933	-0.817	1					
MeOH L	0.950	0.933	-0.683	0.867	1				
MeOH M	-0.483	-0.500	0.700	-0.467	-0.517	1			
Soxhlet E	0.867	0.867	-0.683	0.883	0.883	-0.433	1		
Soxhlet L	0.433	0.483	-0.167	0.500	0.483	0.367	0.517	1	
Soxhlet M	-0.717	-0.567	0.533	-0.550	-0.733	0.833	-0.567	0.017	1
	EtOH E	EtOH L	EtOH M	MeOH E	MeOH L	MeOH M	Soxhlet E	Soxhlet L	Soxhlet M

9.1.6 Correlation matrices for Chapter 4

Table A.185-A.186. Correlation matrices for Exp 2 and 3 (Sections 4.3.3).

Table A.185. Experiment 2: 1, 13, 26 days cold storage, all treatments (Section 4.3.3)

Firmness	1						
L*	0.556	1					
C*	0.771	0.808	1				
H°	0.819	0.579	0.733	1			
Sucrose	-0.484	-0.419	-0.489	-0.370	1		
Manno.	0.032	0.033	0.069	0.108	0.064	1	
perseitol	0.670	0.400	0.636	0.621	0.253	0.140	1
	Firmness	L*	C*	H°	Sucrose	Manno.	perseitol

Table A.186**.** Experiment 3: 0, 7 and 21 days cold storage, 0 and 3 days shelf life, all treatments (Section 4.3.3)

Firmness	1						
L*	0.682	1					
C*	0.765	0.895	1				
H°	0.815	0.631	0.652	1			
Sucrose	-0.372	-0.328	-0.389	-0.368	1		
Manno.	0.703	0.404	0.492	0.636	-0.127	1	
perseitol	0.566	0.381	0.430	0.488	-0.079	0.621	1
	Firmness	L*	C*	H°	Sucrose	Manno.	perseitol

9.1.7 Correlation matrices for Chapter 6

Table A.187. Correlation matrix. Days 1, 3 and 5, treatment with e+® Ethylene Remover, 1-MCP, 1MCP/ e+® Ethylene Remover, control; n = 6 fruit replicate per treatment (Sections 6.3.5).

Ethylene	1								
RR	0.310	1							
L*	-0.445	-0.274	1						
C*	-0.496	-0.323	0.935	1					
H°	-0.700	-0.366	0.727	0.790	1				
Firmness	-0.594	-0.354	0.590	0.674	0.716	1			
Sucrose	-0.040	0.099	-0.311	-0.337	-0.164	-0.284	1		
Manno.	-0.205	0.077	0.254	0.365	0.393	0.519	-0.240	1	
perseitol	0.042	0.287	0.136	0.218	0.186	0.128	-0.079	0.542	1
	Ethylene	RR	L*	C*	H°	Firmness	Sucrose	Manno.	perseitol

RR= Respiration rate

Table A.188. Correlation matrix**.** Days 1, 3 and 5, treatment with e+® Ethylene Remover, 1-MCP, 1MCP/ e+® Ethylene Remover, control; n = 3 fruit replicates per treatments (Sections 6.3.5).

ABA	1									
L*	-0.662	1								
C*	-0.760	0.934	1							
H°	-0.820	0.722	0.793	1						
Firmness	-0.693	0.669	0.751	0.752	1					
RR	0.378	-0.128	-0.236	-0.294	-0.231	1				
ethylene	0.561	-0.457	-0.531	-0.653	-0.582	0.292	1			
Manno.	-0.354	0.424	0.481	0.536	0.502	0.247	-0.194	1		
perseitol	-0.320	0.331	0.408	0.447	0.313	0.311	-0.102	0.710	1	
Sucrose	0.353	-0.467	-0.441	-0.319	-0.474	-0.005	0.181	-0.423	-0.204	1
	ABA	L*	C*	H°	Firmness	RR	Ethylene	Manno.	perseitol	Sucrose

9.2 APPENDIX B: Published literature

- 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.* 56, 7439–7445

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 7439–7445 **7439**

Development of a Rapid Method for the Sequential Extraction and Subsequent Quantification of Fatty Acids and Sugars from Avocado Mesocarp Tissue

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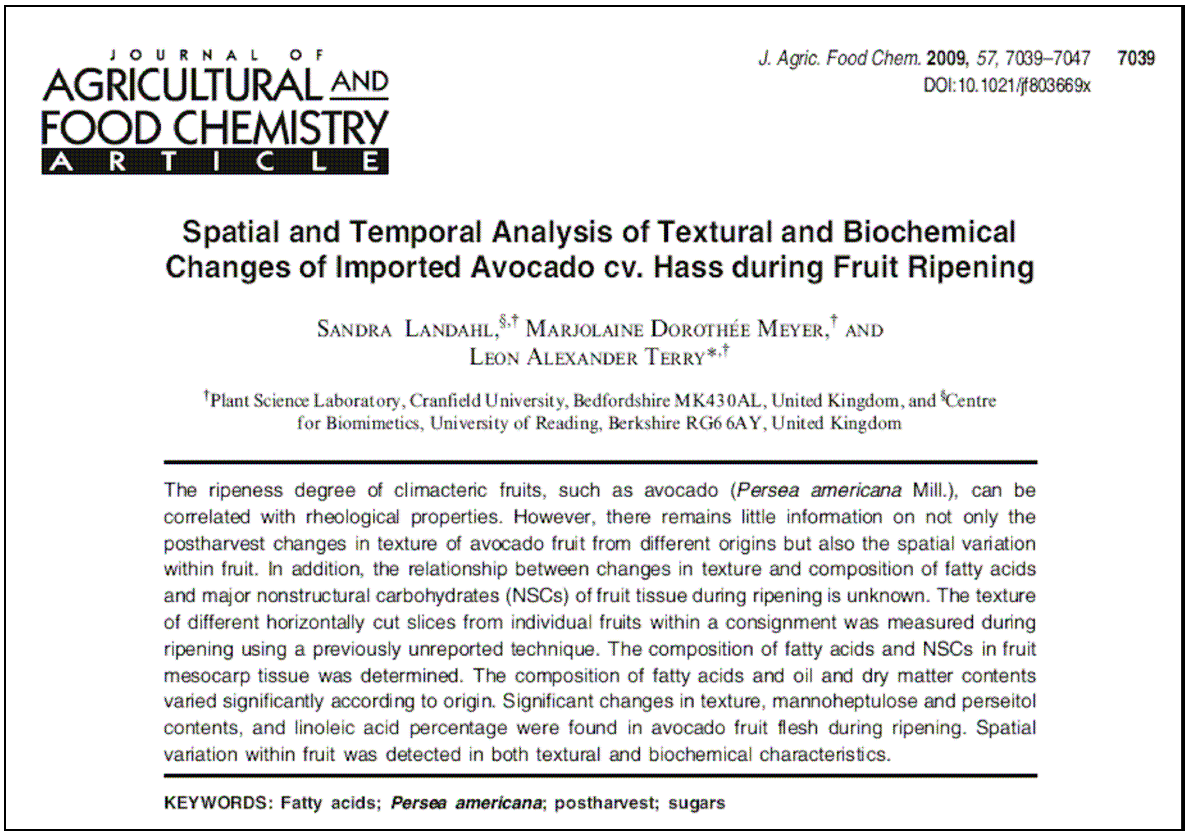
Methods devised for oil extraction from avocado (*Persea americana* Mill.) mesocarp (e.g., Soxhlet) are usually lengthy and require operation at high temperature. Moreover, methods for extracting sugars from avocado tissue (e.g., 80% ethanol, v/v) do not allow for lipids to be easily measured from the same sample. This study describes a new simple method that enabled sequential extraction and subsequent quantification of both fatty acids and sugars from the same avocado mesocarp tissue sample. Freeze-dried mesocarp samples of avocado cv. Hass fruit of different ripening stages were extracted by homogenization with hexane and the oil extracts quantified for fatty acid composition by GC. The resulting filter residues were readily usable for sugar extraction with methanol (62.5%, v/v). For comparison, oil was also extracted using the standard Soxhlet technique and the resulting thimble residue extracted for sugars as before. An additional experiment was carried out whereby filter residues were also extracted using ethanol. Average oil yield using the Soxhlet technique was significantly ($P < 0.05$) higher than that obtained by homogenization with hexane, although the difference remained very slight, and fatty acid profiles of the oil extracts following both methods were very similar. Oil recovery improved with increasing ripeness of the fruit with minor differences observed in the fatty acid composition during postharvest ripening. After lipid removal, methanolic extraction was superior in recovering sucrose and perseitol as compared to 80% ethanol (v/v), whereas mannoheptulose recovery was not affected by solvent used. The method presented has the benefits of shorter extraction time, lower extraction temperature, and reduced amount of solvent and can be used for sequential extraction of fatty acids and sugars from the same sample.

KEYWORDS: *Persea americana*; lipids; Soxhlet

- 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.* 57, 7039–7047



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. **2009,** *57,* 7039–7047 **7039**
DOI:10.1021/jf803669x

Spatial and Temporal Analysis of Textural and Biochemical Changes of Imported Avocado cv. Hass during Fruit Ripening

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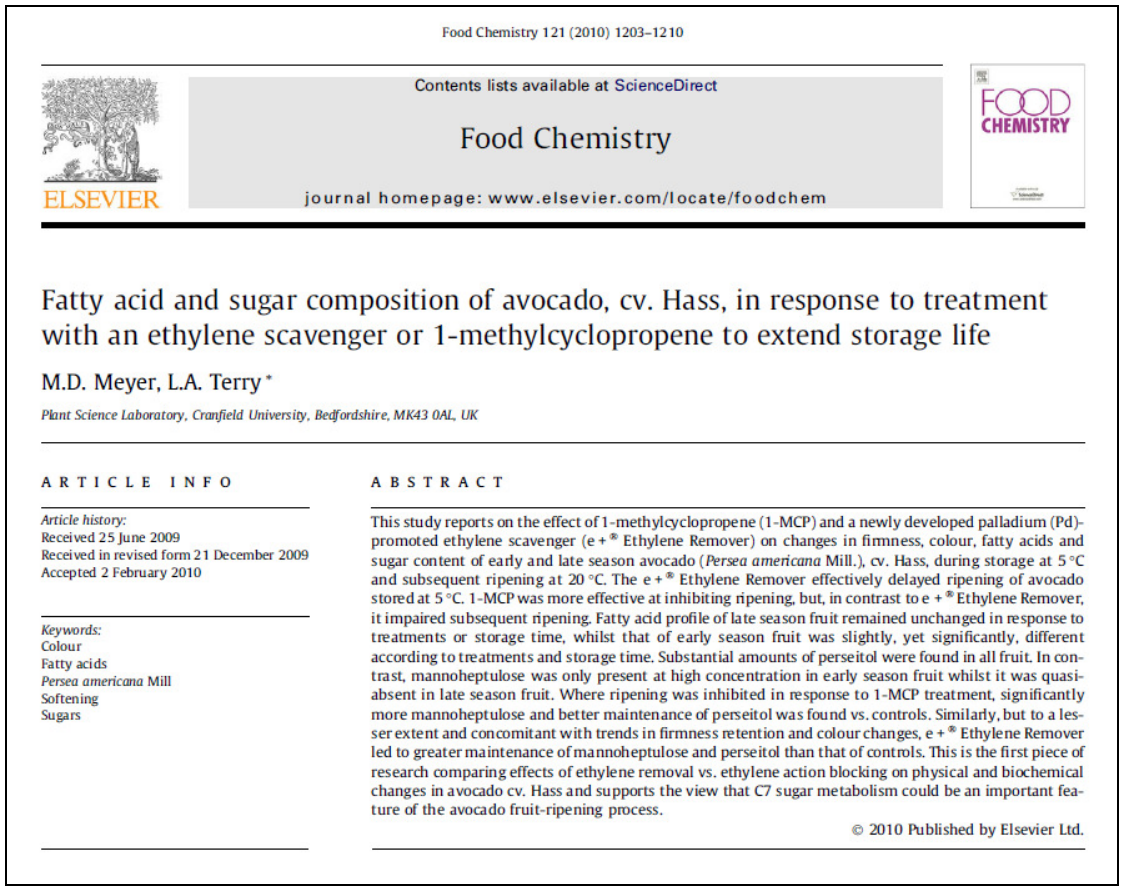
The ripeness degree of climacteric fruits, such as avocado (*Persea americana* Mill.), can be correlated with rheological properties. However, there remains little information on not only the postharvest changes in texture of avocado fruit from different origins but also the spatial variation within fruit. In addition, the relationship between changes in texture and composition of fatty acids and major nonstructural carbohydrates (NSCs) of fruit tissue during ripening is unknown. The texture of different horizontally cut slices from individual fruits within a consignment was measured during ripening using a previously unreported technique. The composition of fatty acids and NSCs in fruit mesocarp tissue was determined. The composition of fatty acids and oil and dry matter contents varied significantly according to origin. Significant changes in texture, mannoheptulose and perseitol contents, and linoleic acid percentage were found in avocado fruit flesh during ripening. Spatial variation within fruit was detected in both textural and biochemical characteristics.

KEYWORDS: Fatty acids; *Persea americana*; postharvest; sugars

- Meyer, M.D., and Terry, L. A. (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*. 121, 1203-1210.



Food Chemistry 121 (2010) 1203–1210

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Fatty acid and sugar composition of avocado, cv. Hass, in response to treatment with an ethylene scavenger or 1-methylcyclopropene to extend storage life

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ARTICLE INFO

Article history:
Received 25 June 2009
Received in revised form 21 December 2009
Accepted 2 February 2010

Keywords:
Colour
Fatty acids
Persea americana Mill
Softening
Sugars

ABSTRACT

This study reports on the effect of 1-methylcyclopropene (1-MCP) and a newly developed palladium (Pd)-promoted ethylene scavenger (e + ® Ethylene Remover) on changes in firmness, colour, fatty acids and sugar content of early and late season avocado (*Persea americana* Mill.), cv. Hass, during storage at 5 °C and subsequent ripening at 20 °C. The e + ® Ethylene Remover effectively delayed ripening of avocado stored at 5 °C. 1-MCP was more effective at inhibiting ripening, but, in contrast to e + ® Ethylene Remover, it impaired subsequent ripening. Fatty acid profile of late season fruit remained unchanged in response to treatments or storage time, whilst that of early season fruit was slightly, yet significantly, different according to treatments and storage time. Substantial amounts of perseitol were found in all fruit. In contrast, mannoheptulose was only present at high concentration in early season fruit whilst it was quasi-absent in late season fruit. Where ripening was inhibited in response to 1-MCP treatment, significantly more mannoheptulose and better maintenance of perseitol was found vs. controls. Similarly, but to a lesser extent and concomitant with trends in firmness retention and colour changes, e + ® Ethylene Remover led to greater maintenance of mannoheptulose and perseitol than that of controls. This is the first piece of research comparing effects of ethylene removal vs. ethylene action blocking on physical and biochemical changes in avocado cv. Hass and supports the view that C7 sugar metabolism could be an important feature of the avocado fruit-ripening process.