

CRANFIELD UNIVERSITY

MANUELA DONETTI

**POSTHARVEST BIOCHEMICAL AND PHYSIOLOGICAL
CHARACTERISATION OF IMPORTED AVOCADO FRUIT**

**CRANFIELD HEALTH
Plant Science Laboratory**

**PhD THESIS
Academic Year: 2008-2011**

**Supervisor: Dr. Leon A. Terry
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degree of Doctor of Philosophy

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ABSTRACT

Difficulties in controlling and forecasting avocado fruit ripening and the highly perishable nature of the crop once harvested, are the major causes of concern for avocado traders. In particular, the simultaneous presence of many suppliers may account for increased fruit variability during ripening. Avocado is a climacteric fruit with consistent ethylene production after harvest which is also related to high perishability. However, the mechanisms regulating ethylene biosynthesis and mesocarp softening are not completely understood.

In order to study such effects, avocado fruit from different growing areas and harvested at various maturity stages, were investigated and the biochemical and physiological changes during ripening at both 18 and 23°C were studied. Mesocarp softening and fatty acid content discriminated fruit maturity and growing area, respectively, whereas C7 sugars (D-mannoheptulose and perseitol) discriminated length of fruit shelf life. For the first time, oleic acid content presents in the oil mesocarp was found to depend on fruit sources making of this a suitable indicator of avocado fruit growing area. In contrast, sugar content declined along fruit maturity and ripening. In particular the mannoheptulose presents in avocado mesocarp might be use to estimate avocado fruit shelf life. Indeed, fruit harvested late in season were found to have a lower C7 content than earlier harvest fruit and a faster softening, regardless fruit source. However, sugars content changed between growing area, thus a general C7 threshold defining fruit storability seems to be not definable. Furthermore, other possible indicators of fruit maturity and/or ripening stage have been searched in the cell wall constituents of avocado mesocarp. Thus, the structural carbohydrates profile of avocado mesocarp investigated with a new immunological method changed during ripening and harvest time (early and late season), suggesting a possible effect of cell wall composition on fruit ripening regulation.

Also, the possible use of ethylene application in reducing the high heterogeneity noted on imported fruit from South Africa was also evaluated through different consignments. Results showed ethylene efficacy changed depending on harvest time and fruit dimension with less efficacy of the treatment on fruit harvested at the end of the season and characterised by smaller size.

One of the most commercialized avocado cultivars, Hass, is peculiar in that its skin colour changes from green to deep purple as ripening progresses. The most common ripening indicator of avocado fruit is the mesocarp firmness and the destructive nature of this evaluation increases losses in the avocado industry. The availability of a non-destructive indicator of fruit ripening represents an important advantage for avocado consumers and importers. Thus, the possible relationship between mesocarp softening, skin colour were objectively evaluated (C^* , L^* , and H°), and the main pigment, cyanidin 3-*O*-glucoside, was investigated. Cyanidin 3-*O*-glucoside was confirmed to be the main anthocyanin present in avocado cv. Hass peel, regardless of preharvest factors. However, differences in its content were noted between shelf life temperatures. A higher relationship between hue angle and firmness was detected in late harvest fruit, whereas no correlation was found between anthocyanin content and firmness. Avocado skin is also involved in defence mechanisms due to the presence of antifungal and phenolic compounds. These phenolic compounds represent a natural protection against pathogenic infections and seem to be down regulated during ripening. The main phenolics were identified and quantified, using a new analytical method which was validated and optimised. Epicatechin, chlorogenic acid and procyanidin B2 were found to be present in the skin tissue and quantified using this assay and found to vary during shelf life and seasons. Although phenolics were present in minor amounts, in avocado pulp they are involved in mesocarp discoloration incidence, and therefore with fruit postharvest quality. Due to a lack of information, a new straightforward method for the identification and quantification of the main phenolics present in avocado mesocarp was developed. Finally, a commercial trial was undertaken to ensure that the results obtained in the laboratory can be reproduced in the market place.

In conclusion, postharvest markers can define avocado fruit maturity and growing area and give guidelines in the control of avocado shelf life. Moreover, new methods for the investigation of the phenolic profiles (peel and mesocarp) and the characterisation of cell wall structures can be further tools in the management of avocado fruit postharvest quality.

Keywords: firmness, colour, fatty acids, sugars, ethylene, cell wall composition

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LIST OF ABBREVIATIONS

<	less than
>	greater than
±	plus or minus
%	per cent
α	alpha
β	beta
Δ	delta
μg	microgram
μl	microlitre
μm	micrometer
=	equals
*	times
°C	degree Celsius
1-MCP	1-methylcyclopropene
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
ACP	acyl carrier protein
AE	ethyl acetate
AIM	alcohol insoluble material
ANOVA	analysis of variance
ANS	anthocyanins synthase
AT	anthocyanin acyltransferase
ATP	adenosine triphosphate
BHT	butylated hydroxytoluene
BOC	British Oxygen Company
C.	Cucumis
C*	chroma

<i>ca.</i>	approximately
Ca ²⁺	calcium
CDTA	1,2-diaminocyclohexanetetraacetic acid tetrasodium
CeSA	cellulose synthase
CH ₃	methyl group
CHS	chalcone synthase
CHI	chalcone isomerase
cm	centimetre
Co.	company
CO ₂	carbon dioxide
CoA	coenzyme A
Csl	cellulose synthase-like
CTR	constitutive triple response
cv.	cultivar
C7	seven carbon
DE	diethyl ether
d.f.	degrees of freedom
DFR	dihydroflavonol 4 reductase
DHK	dihydrokaempferol
DHM	dyhidromyricetin
DHQ	dyhydroquercetin
DM	dry matter
DW	dry weight
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ERS1	ethylene response sensor
ESI	electrospray ionization
ETR	ethylene response
e ⁺ /e ⁻	ethylene treated/non ethylene treated
EtOH	ethanol
<i>et al.</i>	and others

Exp.	experiment
F3H	flavonones-3-hydroxylase
FAMEs	fatty acid methyl esters
FAO	Food and Agriculture Organisation of the United Nations
Fe ²⁺	iron
FID	flame ionisation detection
FLS	flavonol synthase
FNS	flavonone synthase
ft	foot
FW	fresh weight
<i>g</i>	gravity
g	gram
GC	gas chromatography
GT	anthocyanins glucosyltransferase
h	hours
H°	hue angle
ha	hectares
HCl	hydrochloric acid
HDL	high density lipoprotein
HGA	homogalacturonan
H ₂ O	water
H ₂ O ₂	hydrogen peroxide
HPLC	high performance liquid chromatography
HRP	horseradish peroxidase
HWD	hot wire detector
IAA	indole-3-acetic acid
i.e.	in example
<i>in vivo</i>	inside a living organism
<i>in situ</i>	in position
IU	international unit
kg	kilogram

KOH	potassium hydroxide
l	litre
L.	Linnaeus
L*	lightness
LDL	low density lipoprotein
LM	Leeds monoclonal
Ltd.	limited
LSD	least significant difference
m	metre
M	molar
MACC	malonyl-ACC
MAT	5-methylthioadenosine
MeOH	methanol
mg	milligram
Mg ²⁺	magnesium
Mill.	Miller
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
MS	mass spectrometry
MT	million tons
<i>n</i>	number
N	Newton
NaBH ₄	sodium borohydrate
Na ₂ CO ₃	sodium carbonate
NAOH	sodium hydroxyde
nm	nanometer
N ₂	nitrogen
O ₂	oxygen
OECD	Organisation for Economic Co-operation and Development

<i>P</i>	probabiltity
p	para
ppm	part per million
<i>Pa</i>	<i>Persea americana</i>
PAL	l-phenylalanine-ammonia-lyase
PBS	phosphate buffer solution
PCA	principal component analysis
PG	polygalacturonase
PME	pectin methyl esterase
PPO	polyphenol oxidase
R ²	regression index
RG-I	rhamnogalacturonan-I
RG-II	rhamnogalacturonan-II
ROS	reactive oxygen species
SAAGA	South African growr's association
SAM	S-adenosyl-L-methionine
S.E.	standard error
SPE	solid phase extraction
T	tons
TLC	thin layer chromatography
UK	United Kingdom
USA	United States of America
UV	ultra violet
var.	variety
vis.	visible
vs.	versus
<i>viz.</i>	namely
v/v	volume by volume
w/v	weight by volume
XET	xyloglucan endotransglycosylase

1 CHAPTER ONE

INTRODUCTION

1.1 Project background

The consumption of the tropical-subtropical fruit, avocado, has increased considerably in the last century throughout Europe, and particularly in the UK. There seems to be potential for further increases in consumption in the future. The shipping time required and difficulties in the control of ripening are the main problems for European avocado traders. Controlling ethylene levels during storage can extend shelf life in a commercial setting. However, avocado ripening remains unpredictable and there are many postharvest problems which still need to be resolved in order to improve quality for consumers.

Chilling injury, temperature, growing conditions and maturity at harvest all seem to have an affect on avocado fruit ripening and postharvest quality (Arpaia *et al.*, 2004, Arpaia, 2005). In the UK, imported fruit dominates the market and it is likely that fruit sold at any point in time has been sourced from different geographical locations. Thus, fruit grown in different conditions, such as soil composition, water supplied, and agricultural practises, can behave differently postharvest. Additionally, unless in exceptional cases (HersHKovitz *et al.*, 2009b), avocados do not ripen until harvested although maturity does increase while the fruit remains on the tree. The presence of a ‘tree factor’ (Adato and Gatiz, 1974) and C7 sugars (Liu *et al.*, 2002) have been hypothesised to act as inhibitors in the repining process of avocado fruit. Additionally, the content of sugars seem to decrease with fruit maturity (Bertling and Bower, 2006), an occurrence which is also characterised by a shorter shelf life (Dixon *et al.*, 2003b). However, it is not clear yet which mechanisms regulate avocado fruit ripening.

Due to commercial requirements, the delay of ripening in avocado fruit is controlled by application of ethylene scavengers (Terry *et al.*, 2007) or “binding site blockers” such as 1-methylcyclopropene (1-MCP) (Feng *et al.*, 2000). Indeed, the role of ethylene on avocado ripening has been demonstrated (Lelièvre *et al.*, 1997; Watkins, 2002; Lin *et al.*, 2009) and other metabolites such as ABA (Cowan *et al.*, 1998; Riching

et al., 2000; Cowan *et al.*, 2001), C7 sugars (Liu *et al.*, 1999b), phenolics (Barasundram *et al.*, 2006; Rinaldo *et al.*, 2010, Coi *et al.*, 2011) and cell wall components (Pesis *et al.*, 1978; Brummell and Harpster, 2001) may all have possible synergistic roles in modulating the ripening of avocados and in the determination of fruit quality.

1.2 Aim and objectives

1.2.1 Aim

The aim of this PhD project was to identify physiological and/or biochemical markers able to distinguish growing area or maturity stages in order to give guidelines to UK traders for the postharvest handling of imported avocado fruit cv. Hass. This study also investigated the main changes occurring in avocado fruit mesocarp and peel tissues during shelf life in order to identify a possible non-destructive indicator of fruit ripening. Moreover, the purpose of this work was to evaluate the role of plant metabolites such as ethylene, C7 sugars, phenolics, and structural carbohydrates on the ripening process.

1.2.2 Objectives

- To investigate the physiological and biochemical mesocarp changes in avocado fruit cv. Hass, imported from Spain, South Africa, Peru and Chile and harvested in three main seasons (early, middle and late), during postharvest.
- To compare the influence of temperature and ethylene treatment on imported avocado fruit from different consignments.
- To develop an efficient method to investigate the main phenolics present in avocado mesocarp cv. Hass.
- To investigate the relationship between skin colour changes and mesocarp softening during postharvest life in avocado fruit cv. Hass with different maturity stages (early, middle and late season).

- To investigate the structural carbohydrates present in avocado mesocarp through a new immunological detection method and discuss the possible influence of cell wall composition on ripening.

1.3 Thesis structure

This thesis is organised in 9 chapters. The current literature on avocado fruit is presented in Chapter 2. The main botanical and morphological traits of avocado fruit are initially described, followed by a market overview which gives particular consideration to UK trading. Literature pertaining to avocado fruit composition is reviewed with an additional description of the structure of the cell wall and its involvement in fruit ripening. The most important preharvest factors influencing the growth, development and ripening of avocado fruit are also described. Following this, the peculiar behaviour of avocado fruit during postharvest storage is presented with particular emphasis on the role of ethylene in fruit physiology and in ripening regulation. This chapter also briefly describes the health related properties for which avocado is primarily known.

The methodologies used in this work are described in Chapter 3. This chapter consists of a description of physiological (i.e. respiration rate, ethylene production, colour changes and mesocarp softening) and biochemical (i.e. fatty acids, sugars and phenolics, and structural carbohydrates compounds) measurements of avocado tissues (pulp and/or peel) during postharvest life. A new method for the extraction and quantification of the main phenolics present in avocado mesocarp is also described (part B).

The various preharvest factors influencing fruit quality (Woolf and Ferguson, 2000; Arpaia *et al.*, 2004; Arpaia 2005) could be responsible for the inconsistent behaviour during ripening. These inconsistencies, emphasized by a wide range of suppliers from all over the world required to meet market needs, are often the cause of UK consumer's complaints (Adam Shaw, personal communication). The identification of markers related to a specific growing region or harvest time could help in the postharvest handling of imported avocado fruit. In Chapter 4, avocado fruit cv. Hass originating from Spain, South Africa, Peru and Chile, with three different maturity stages for each country, were characterised by the main physiological and biochemical

changes during shelf life at 18 and 23°C. The temperatures chosen here are representative of the full range of possible conditions in which avocados are usually ripened by costumers and traders. Results from chapter 4 were presented as poster

- Poster presentation- **Manuela Donetti** and Leon A. Terry, “Seasonal effects on postharvest quality of imported Spanish avocado cv. Hass”, **6th International Postharvest Symposium, 8-12 April 2009, Antalya, Turkey.**

and will be submitted for publication as follow:

- **Variations in the ripening and biochemistry of imported avocado cv. Hass fruit from different origins and maturity.** **Manuela Donetti** and Leon A. Terry (Food Research International).

The influences of fruit maturity and postharvest condition were also investigated by examining the relationship between skin colour changes and mesocarp softening (*cf.* Chapter 5). Due to the role of the phenolic compounds in the fruit response to postharvest diseases (Prusky *et al.*, 1982; Ardi *et al.*, 1998) and of the anthocyanins in the determination of skin colour (Cox *et al.*, 2004; Asthon *et al.*, 2006) a new method for their simultaneous extraction and quantification was developed (*cf.* Chapter 5). Results from this work will be submitted to a peer reviewed journal as follow:

- **Influence of fruit maturity in the relationship between skin colour changes and mesocarp softening of imported avocado fruit cv. Hass.** **Manuela Donetti** and Leon A. Terry (Horticultural Science and Biotechnology).

The development of a new immunological method for the detection of main structural carbohydrate (chapter 7) will be submitted as short communication:

- **Immunodetection of plant cell wall polymers in the mesocarp of avocado fruit cv. Hass during ripening.** **Donetti Manuela**, Jose’ Ordaz-Ortiz and Leon A. Terry (Journal of Food Composition and Analysis).

The higher variability noted in the first experiment in fruit coming from South Africa was further investigated in Chapter 6. The postharvest application of ethylene, commercially used to accelerate and induce a more uniform ripening behaviour, was tested on three different consignments of fruit grown in Mpumalanga, South Africa, (*cf.* Chapter 6). Finally, a new approach for the investigation of the cell wall components of avocado mesocarp and their possible influence on fruit ripening is detailed in Chapter 7.

The main structural carbohydrates of avocado mesocarp were immunologically detected during ripening in fruit from Spain and were compared with/between different levels of maturity at harvest (early and late season). A final General Discussion with proposals for future work as presented in Chapter 8 and the literature cited is in Chapter 9. A case study on the comparison of ethylene application in commercial and experimental conditions is presented in Appendix A. A list of ANOVA tables is presented in Appendix B, and Appendix C details all knowledge transfer activities through this project.

2 CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical description of *Persea americana*

The avocado tree was classified by Linnaeus as Lauraceae. The commercial avocado, *Persea americana* Mill., belongs to the *Persea* subgenus and takes the name from Miller, a Scottish botanist who in the 17th century published a description of the genus in his “Gardeners Dictionary” (Bergh and Ellstrand, 1986). The avocado tree is native to the tropical area of the Central America and subsequently adapted to subtropical conditions. Basic requirements for the crop are a sufficient numbers of hours of sun (at least 2,000 per year), temperature of around 25°C in summer and not under 15°C in winter, and a rainfall average of between 1,200 and 1,600 mm per year. Depending on the varieties and on environmental conditions, an avocado tree can reach 10 to 30 m high. However, trees are usually kept at relatively low height (8 m) in order to facilitate the orchard management and picking (Gaillard and Godefroy, 1995) (Figure 2.1).



Figure 2.1: Avocado tree (ca. 2.5 meters high) with flower panicles in Tropics orchard, Malaga, Spain (source M. Donetti).

The avocado tree is characterised by a rapid growth rate, especially in the first year. The most productive age is around the first 3-4 years yet the tree can survive for more than 50 years (Gaillard and Godefroy, 1995; Requejo-Tapaia *et al.*, 1999). The root system is usually shallow and finds suitable conditions in moist soil (Scora and Bergh, 1992). A characteristic of avocado is the small flower size (5-8 mm) grouped in a panicle that can have up to 100 inflorescences. However, only 1% of the total flowers will reach the fruit stage (Gaillard and Godefroy, 1995; Scora and Bergh, 1992). Other peculiarities are the sexual organs which are present in the same flower (dichogamous) but not at the same time. The flower can expose the female organ in the morning for a couple of hours, close itself and re-open as male in the afternoon of the day after (type A) or (type B) opens as female in the afternoon and as male in the morning of the following day. This mechanism is temperature regulated: when the conditions are not opportune (i.e. excessive cold temperature) the opening time is delayed, the flowers in the plant are no longer synchronised and pollinated (Bergh, 1973; Scora and Bergh, 1992).

2.2 Main races and cultivars

There are three main races or botanical varieties of avocado: americana (West Indian), *drymifolia* (Mexican) and *guatemalensis* (Guatemalan) (Berg and Ellstrand, 1986; Newett, *et al.*, 2002). The Guatemala race generally has larger size fruits covered by a thick and hard skin. The oil content is generally low, between 10 and 20% of the fruit fresh weight. Guatemala fruit types prefer altitudes between 800 and 1,800 m above sea level, but can adapt to 2,000 m, yet they are particularly sensitive to low temperatures. The best conditions are warm and humid climates in tropical rainfall forest and acid soils with low or high fertility (Newett *et al.*, 2002). The West Indian avocado (var. *americana*) also has large fruit size between 400-900 g but has a large seed. The fruit again has a low oil content (less than 10%) and consequently a watery taste. The skin is thin and smooth, with a dark green colour turning into red/brown at maturity. This type is typical in tropic-subtropical climates of an altitude < 1,200 m, where the fruit is also susceptible to chilling injury. The Mexican race (*drymifolia*) is characterised by small fruit (under 250 g) with a big seed, high oil content in the

mesocarp and a fibrous flesh consistency. The skin is thin and smooth, turning purple at maturity. It is usually tolerant of high and low temperatures but sensitive to soil salinity. The optimal latitude of growth is over 1,000 m.

These three varieties are compatible between each other and their hybrids nowadays represent the dominant cultivars in the market (Gaillard and Godefroy, 1995; Newett *et al.*, 2002). The target of the breeding programmes is to improve the fruit characters which make an avocado more valuable for the trade i.e. fruit size, flesh quality, skin colour, tolerance and storage capacity. In this regard the most common subtropical cultivars are hybrids derived from Mexican and Guatemala crosses.

2.2.1 The cultivar Hass

The most grown cultivar in the last century in subtropical climates is cv. Hass. This variety is a Guatemalan fruit with Mexican genes (Figure 2.2) and was created by Rudolph Hass in the 1935 in California.



Figure 2.2: Unripe avocado fruit cv. Hass from Chile.

In this cultivar, the flowering and the fruit set is less affected by low temperature than others, i.e. Fuerte. The fruit is rounded pear shape (weight from 140 to 400 g) with a medium size seed. The flesh is yellow in colour with a characteristic nutty flavour. The thick knobby skin is the main peculiarity of this cultivar and represents a good protection from pests and disease. Indeed, the peel tissues turn in colour changing from green to deep purple with the progress of ripening. Additionally the fruit's suitability for shipping and for the long term storage makes Hass a perfect variety for international trade (Newett *et al.*, 2002).

2.3 Economies of avocado world-wide

Avocado (*Persea americana* Mill.) is a tropical-subtropical fruit whose demand has risen considerably in the last decade. It is still considered a minor commodity. However, consumption has increased from 1960 until 2008 by more than 4 fold, reaching 2,621.7 thousand tons per annum (FAO, 2009). The major world areas involved in this expansion are Latin America (Mexico, Brazil, Colombia and Chile) and more recent growth in Asian countries (Indonesia, China) (Figure 2.3) (FAO, 2009).

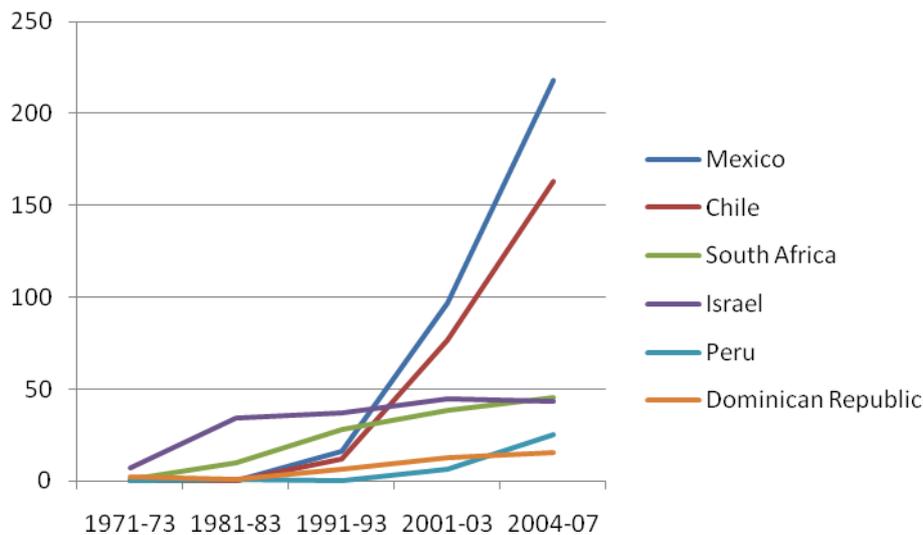


Figure 2.3: Exported avocado fruit (MT) in the world. Data from FAO (2009).

Most avocado production is located in Latin American countries, lead by Mexico, USA and Asia. Mexicans are the main purchasers, with 1,100 thousand tons of avocado fruit consumed in 2007-08. In the USA, only half of the market demand is met by home production, the remainder is imported from Mexico, and the Dominican Republic. Only in recent times, has the European consumer become more familiar with the peculiar taste of avocado fruit and its health benefits which has resulted in increased demand, mainly from France, Netherlands and UK (Table 2.1) (FAO, 2009). In particular, France and Netherlands are the main redistributors of avocado fruit for Europe whereas UK imports are exclusive for internal consumption (Mc Grath *et al.*, 2008).

Table 2.1: Values in tonnes/year for the main world's avocado producers (2006-07) and importers (2004-07) (FAO, 2009).

Country	Production (T/year)	Country	Import (T/year)
Mexico	1.138,600	U.S.A.	237,800
Chile	235,000	France	102,900
Indonesia	220,500	Netherlands	48,000
U.S.A.	217,600	U.K.	47,900
Dominic Republic	200,000	Japan	28,200
Colombia	177,000	Canada	20,600

The all year round requirements in the European market are provided by a wide range of suppliers. South Africa (35,753 T), Chile (34,088 T), Israel (31,855 T), Peru (30,508 T) and Mexico (16,455 T) are the dominant exporters to Europe. Israel and Spain share the winter and spring market with Mexico providing all year round supply. The summer season is controlled by South Africa, Kenya and Peru, mainly suppliers of cv. Hass, with a small participation of Argentina. Chile is responsible for the end of summer and beginning of winter period (Mc Grath *et al.*, 2008) (Figure 2.4).

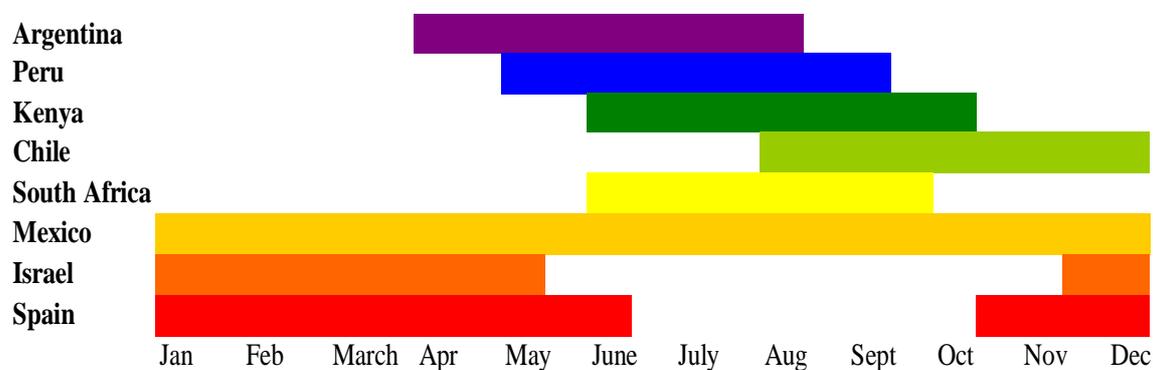


Figure 2.4: Main suppliers of avocado fruit cv. Hass in Europe. The graph indicates in which period of the year each country exports avocado to Europe (Mc Grath *et al.*, 2008).

Avocado is mainly consumed as a fresh product, with minor uses in the cosmetics industry (Evans and Nalampang, 2009; FAO, 2009). The high perishability of the fruit

and the long shipping time required before reaching most of the main exporter represents the main problem in increasing avocado availability through extending postharvest life.

2.4 Leading export countries in to the UK avocado trade

UK is the third country in Europe for the import of avocado with triple the consumption from 1980 to 2004, followed by a rise in successive two years when it reached 60,000 tons imported in 2006 (Figure 2.5). Despite a slight decrease in 2007-08, the lower per capita consumption (circa 0.5 kg) compared with other European countries, such as France or Portugal with more than 1 kg capita, represents a positive indicator of potential growth in the near future. In the UK, due to the all year round demand, various suppliers are used. Fruit are imported from many different countries but principally from Spain (6,000 T), Chile (4,848 T), Peru (4,869 T) and South Africa (11,753 T) depending on the fruit availability during the year (Eurostat, 2008).

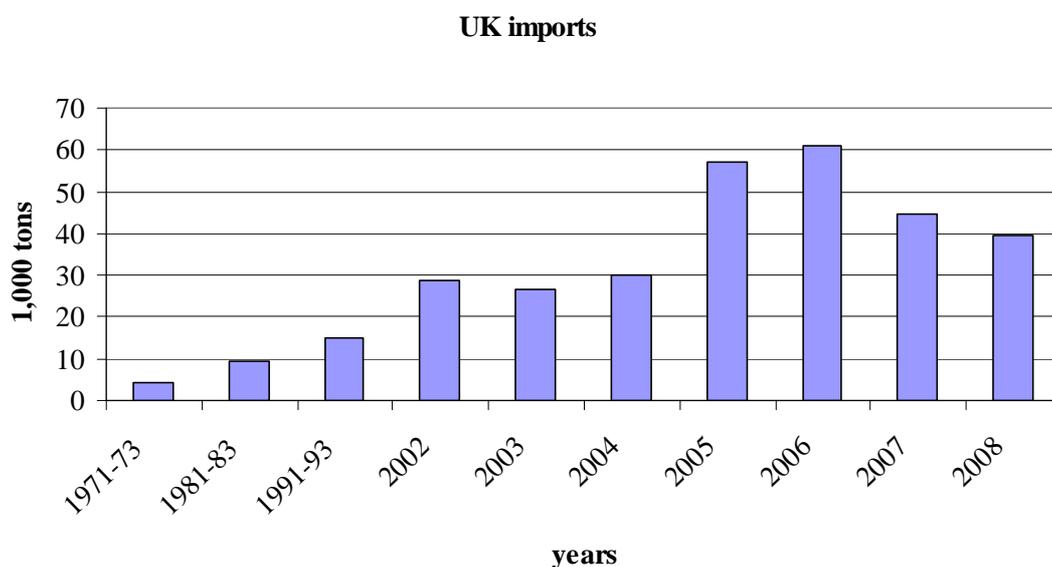


Figure 2.5: Data showing the increased exports of avocado fruit into the UK in the last few decades. Data expressed as thousand tons (Eurostat, 2008; FAO, 2009).

2.4.1 Spain

Spain is the main avocado producer in Europe (FAO, 2009). Already present in the early 16th century, avocado became commercially cultivated in the south of Spain only after 1930 (Farri and Pliego, 1987). The main cultivated areas are the region of Andalusia with the provinces of Granada and Malaga (66,000 ha), Canarias (764 ha) and Valencia (40 ha) (Anonymous, 2006). The avocado plantations are usually located on hills or on artificial terraces furnished with irrigation systems. Depending on the variety, the picking time can extend from October to June. Due to the market preference and the good yield, cv. Hass is the most cultivated variety. In this area *Protopulvinana pyriformis*, thrips, and the diseases *Phytophthora cinnamomi* and *Phytophthora armillaria* are the main problems that can reduce the production (Farri and Pliego, 1987).

2.4.2 Chile

In the beginning of the 17th Century, avocado was introduced by the Spanish to the country. The Chilean avocado industry began in the area of Quillota and from here diffused into La Ligua, Cabildo, Buin, Maipo and Peumos town. From these orchards, new varieties arose which are commercially known as “Chilean paltas”. However, nowadays cv. Hass and Fuerte are the main cultivated cultivars (Irazabal, 2001). The main risks facing the Chilean producers are the lower temperatures due to cold winds coming from the Pacific (Irazabal, 2001).

Avocado is economically relevant in Chile, representing the third most important horticultural crop, after grapes and apples. The internal consumption is high all year round with a most productive period between April and November (Lemus *et al.*, 2005). Local consumption and research have been supported since 1991 by an internal organisation known as the Palta Committee (Irazabal, 2001).

2.4.3 Peru

Avocado was introduced in Peru by the Incas from Central America before 1492 (Knight, 2002). The local varieties, known as “Topa Topa”, with a black skin, belong to the Mexican variety. Throughout the year, rainfalls are not frequent in Peru (less than 19

mm per year), especially if the plantation is located in the equatorial zone. Hence, the avocado growing areas are located near the rivers due to the greater water availability. The best conditions for plantations are at 300-1000 m (990-3300 ft) above sea level. The cv. Hass was successfully introduced alongside the Peruvian varieties for export purposes, taking advantage of the good growing conditions available in the country. A common practice to help the production is the grafting of cv. Hass on Topa Topa seedling. Production and market are organized by ProHass, a organization involved in the promotion of Peruvian Hass (Hofshi, 2003).

2.4.4 South Africa

The avocado crop in South Africa was initially introduced only in the 19th century but was not particularly successful. Only with the introduction of hybrid Mexican-Guatemala from California, could the avocado industry expand. More recently, after the introduction of cv. Fuerte, did cv. Hass become the main cultivar. Most of the avocado plantations are in the province of Mpumalanga, Limpopo and KwaZuluNatal (Knight, 2002; Donkin, 2007). Infection from *Phytophthora cinnamomi* represents one of the main problems for the South African avocado industry. The high temperatures and frequent rainfalls during the summer season give optimal conditions for the fungi, which is mainly suppressed using resistant rootstocks and with phosphoric acid trunk injections (Donkin, 2007).

An internal association, named SAAGA, South African Avocado Growers' Association, aimed at improving both internal production and export, mostly for the European market (Donkin, 2007).

2.5 The morphology of avocado fruit

Avocado fruit is commonly classified as a berry (Figure 2.6) with one big seed located in the basal part of the mesocarp. The pericarp is composed of the skin (exocarp), mesocarp (flesh) and the endocarp, a thin layer around the seed coat (Cumming and Schroeder, 1942). The peel, constitutes three main tissues (epidermal, parenchyma and sclerenchyma) coated with a thin wax-like layer, which physically protects the berry from the environment (Cummings and Schroeder, 1942).

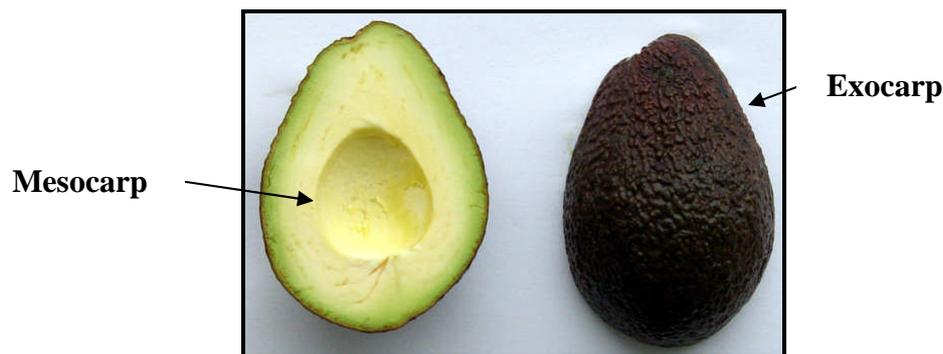


Figure 2.6: Ripe avocado fruit cv. Hass cut in half without seed. Pulp (mesocarp) and peel (exocarp) are indicated (source M. Donetti).

The edible part of fruit is the mesocarp and primarily is composed of parenchyma cells, some of which are specialised in nature, such as idioblasts containing oil (Platt-Aloia and Thomson, 1981; Priego *et al.*, 1996; Scora and Bergh, 1992). The increase in the fruit dimensions during growth is due to intense cell division that continues whilst the fruit is on the tree (Schroeder, 1958). Depending on the variety and cultivar, the avocado fruit can take between 6 to 12 months to fully develop. Finally, the seed is composed of two cotyledons, hypocotyl and radical, connected by a vascular system to the pedicel (Cummings and Schroeder, 1942; Priego *et al.*, 1996). Besides the involvement in fruit growth, a recent investigation on seedless fruit hypothesised the seed as having a role in fruit ripening regulation (Hershkovitz *et al.*, 2010).

2.6 Main biochemical components of avocado fruit

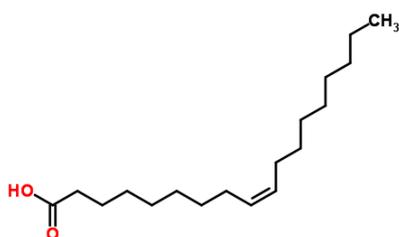
The main constituents of the avocado fruit are water (70-80%), fat (15-18%), carbohydrates (4-5%), protein (1-2%), fibre (1%) and ash (1%) (Slater *et al.*, 1975; Moreno *et al.*, 2003). A brief description of the main compounds are given below.

2.6.1 Lipids

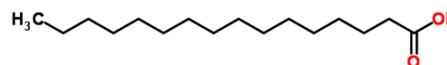
Most of the lipid fraction is synthesised in the fruit during the growth phase and the maturation stage while the fruit is still attached to the tree (Platt-Aloia and Thomson, 1981). The lipids are mainly concentrated in the mesocarp as the seed is usually low in fat content (only 1%). The oil in avocado is predominantly composed of neutral lipids,

which are mainly formed of triglycerides, phospholipids, glycolipids and free fatty acids (Table 2.2).

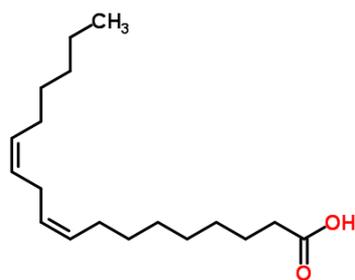
Constituents of cellular membranes such as glycosilglycerides and phosphoglycerides, or storage molecules, such as triacylglycerol, lipids are mostly based on fatty acids structures. Briefly, the main steps in the biosynthesis of fatty acids are the production of acetyl-CoA and its conversion in malonyl CoA, used as source of two carbons structure in further condensations in longer chains. There are different condensing enzymes depending on to the length of the chain that they produce. For instance, short chain are formed by the β -ketoacyl ACP synthase III; 14 carbons chains, such as palmitoyl ACP, by β -ketoacyl ACP synthase I, and the stearyl ACP (18 carbons) is formed by the β -ketoacyl ACP synthase II. The final products of the fatty acids synthesis are the saturated palmitate and stearic acids. A desaturation by the stearyl-ACP $\Delta 9$ desaturase forms the oleate, incorporated in the membranes as phosphatidylcoline, with a further desaturation *in situ* this is converted to linoleate (Figure 2.7). The addition of double bonds decreases the melting point of the molecule and consequently increases the membrane fluidity and the tolerance to cold temperature. A following step by the $\Delta 15$ desaturase can convert the linoleate into linolenic acid (Harwood, 1997). Fatty acids chains longer than 16 or 18 carbons are formed by elongase as precursor of waxes, cutin or suberin.



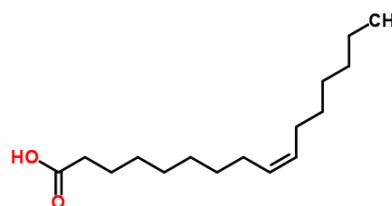
1. Oleic acid



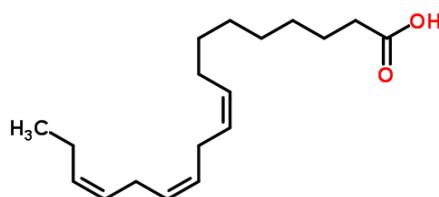
2. Palmitic acid



3. Linoleic acid



4. Palmitoleic acid



5. Linolenic acid

Figure 2.7: Structures of the main fatty acids identified in the oil mesocarp of avocado. In order of abundance are: 1.Oleic acid, 2.Palmitic acid, 3.Linoleic acid, 4.Palmitoleic acid, 5.Linolenic acid.

Variation in the oil content and composition can be within the fruit (Landahl *et al.*, 2009) but also affected by cultivar, cultural practises, environmental conditions and fruit maturity. Changes in the oil content are mainly related with fruit development while few modifications have been detected postharvest (Ozdemir and Topuz, 2004; Landahl *et al.*, 2009). Indeed, the oil fraction of the mesocarp has been used for many years as indicator of fruit maturity (Kader, 2002). However, it has recently been replaced by the cheaper and simpler method represented by the evaluation of the dry matter content (Lee *et al.*, 1983; Requejo-Tapaia *et al.*, 1999). Additionally, the variability of the oil content through the mesocarp sections adds doubt over its feasibility as universal indicator of avocado fruit maturity (Woolf *et al.*, 2003b; Landahl *et al.*, 2009).

Table 2.2: Principal fatty acid compounds in avocado oil. The level of saturation and the presence in avocado mesocarp are indicated as a percentage (Requejo-Tapaia *et al.*, 1999; Inoue and Tateishi, 1995; Ozdemir and Topuz, 2004; Meyer and Terry, 2008; Landahl *et al.*, 2009).

Fatty acid	Structure and saturation level	Percentage in avocado mesocarp	Concentration (mg g ⁻¹ oil)
Oleic	18:1	60-50%	86
Palmitic	16:0	20-15%	32
Linolenic	18:2	11-16%	19
Palmitoleic	16:1	6-10%	14
Linolenic	18:3	0.1-0.9%	2

The oil of avocado fruit is well known for its similarity to olive oil and for its health proprieties which are related to the predominant ratio of monounsaturated and polyunsaturated in the total fatty acids content (Bergh, 1992; Colquhoun *et al.*, 1992; Brescia *et al.*, 2007; Ikhuoria and Maliki, 2007).

2.6.2 Non-Structural Carbohydrates

The primary source of energy in a plant system consists of soluble sugars, mostly present as six-carbon compounds (i.e. sucrose and glucose). Avocado is an exception in this regard. Indeed, the common six-carbon sugars are predominant in the flower and in the seed (Liu *et al.*, 2002) whereas in the most of the other tissues, such as leaves, shoots, trunk, root and fruit, are mainly present structures of 7 carbons, *D*-mannoheptulose (ketosugar) and perseitol (polyol) (Liu *et al.*, 1999, 1999b; 2002) (Figure 2.8).

The concentration of the main sugars present in avocado fruit changes according to the type of cultivar, tissue, and the part of the tissues investigated. For instance, significant variability in the perseitol and *D*-mannoheptulose content was found between different mesocarp sections (Landahl *et al.*, 2009). Similarly, the mesocarp of avocado

cv. Hass seems to have a higher concentration of mannoheptulose than in other cvs. such as Fuerte and Pickerton.

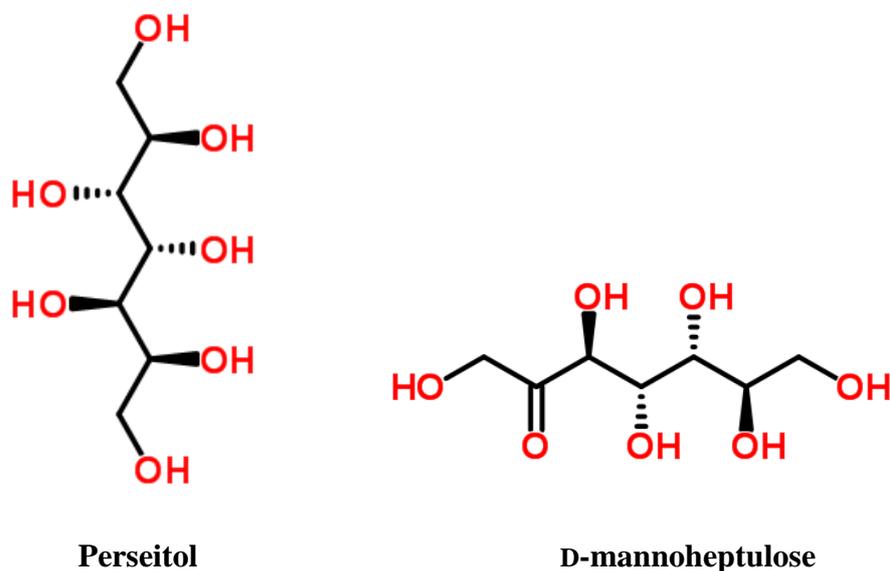


Figure 2.8: Perseitol and D-mannoheptulose structures (Royal Society of Chemistry).

The peculiar structure of C7 sugars and their predominance in most of the avocado tissues has resulted in extensive investigation. Recent studies found that C7 sugars accumulated in the seed, in the cotyledons (Tesfay *et al.*, 2010) and are major products of the photosynthetic process (Liu *et al.*, 2002). Their presence in the phloem, and their reduced content as fruit maturity increases, suggests that these C7 structure can represent the main energy source in avocado (Liu *et al.*, 1999b). A reduction in the C7 sugars concentration has also been reported in fruit after storage and ripening (Liu *et al.*, 1999b, 2002; Blakey *et al.*, 2009). Specifically, the decrease in C7 sugars content as fruit soften, suggests a possible inhibitor role of these sugars in fruit ripening (Liu *et al.*, 2002). Moreover, a recent investigation identified mannoheptulose as the main antioxidant source in the mesocarp besides the low presence of other antioxidant compounds (Tesfay *et al.*, 2010). The multiple functions of the C7 sugars is proof of their importance in avocado metabolism, however, the mechanisms by which they are involved are still not completely understood.

2.7 Pigmentation

Colour plays a key role in the appearance of fruit and vegetables and therefore can influence the costumer's perception of the products quality. Different pigments such as chlorophylls, carotenoids, and anthocyanins, present in the plant tissues are the main cause defining the tissues colour (Brouillard *et al.*, 1997). However, it is wise to consider that the physical shape of the exocarp is also a factor, which can determine the colour. Indeed, pigments and surface absorb and reflects light giving a specific spectrum (Lancaster *et al.*, 1997). Changes in colour are representative of physiological stages of fruit and vegetables and its measurement in postharvest can be an indicator of quality (Tomás-Barberán and Robins, 1997).

In avocado fruit carotenoids and chlorophylls are the main accountable for the green-yellow colour of the mesocarp (pulp) and its extracted oil (Cox *et al.*, 2004; Asthon *et al.*, 2006). As previously mentioned (*cf.* section 2.2.1), a distinctive character of cv. Hass is the temporal change of the skin colour from green to deep purple during ripening. The purple coloration develops with the increase of the cyanidin 3-*O*-glucoside content as fruit ripen (Cox *et al.*, 2004; Asthon *et al.*, 2006). The possible use of skin colour changes as indicator of avocado cv. Hass fruit ripening will be further investigated (*cf.* Chapter 5).

2.7.1 Carotenoids

Carotenoids belong to the class of terpenoids present in chloroplasts serve a protective function for the photosynthetic pigments from excessive sunlight (Tomás-Barberán and Robins, 1997). The avocado mesocarp is particularly rich in carotenoids of which lutein represents 70% (Slater *et al.*, 1975). The major carotenoids found in the ripe pulp of cv. Hass are as follows: lutein ($2.93 \mu\text{g g}^{-1}$), zeaxanthin ($0.11 \mu\text{g g}^{-1}$), α -cryptoxanthin ($0.25 \mu\text{g g}^{-1}$), β -carotene ($0.60 \mu\text{g g}^{-1}$) and α -carotene ($0.25 \mu\text{g g}^{-1}$) (Lu *et al.*, 2005).

Carotenoid metabolism is modulated by fruit physiology: where the maturation process acts as a positive feedback on the carotenoids synthesis (Tomás-Barberán and Robins, 1997); the ripening tends to accelerate carotenoid degradation (Ashton *et al.*,

conditions (Lichtenthaler, 1987). Although few significant changes in the chlorophyll content have been detected in flesh tissues during ripening (Asthon *et al.*, 2006) the skin darkening in cv. Hass has been also associated with an accentuated chlorophyll breakdown (Cox *et al.*, 2004; Asthon *et al.*, 2006).

2.7.3 Anthocyanins

The anthocyanins are a class of flavonoids based on an heterocyclic structure with 15 carbons units bound to sugars. They are stored in cell vacuoles with a characteristic low pH (around 3-3.5) (Hosseinian and Beta, 2007). They are visible to the human eye, as they absorb light in the visible range (Brouillard *et al.*, 1997). Anthocyanins colour depends mainly on the chemical structures of the compounds, environmental pH, and substitution groups (Brouillard *et al.*, 1997). The biosynthetic pathway of anthocyanins starts from the condensation of three malonyl-CoA units with p-coumaroyl-CoA in a tetrahydrochalcone molecule by the chalcone synthase (CHS) (Figure 2.10). A follows isomeration by a chalcone isomerase (CHI) gives the naringenin, a colourless molecule. Further hydroxylation by flavanone-3-hydroxylase (F3H) formed the dihydrokaempferol (DHK). Hydroxylation on different groups results in the formation of dihydroquercetin (DHQ) or dihydromyricetin (DHM) that can be converted in the different anthocyanins (Holton and Cornish, 1995). One main anthocyanin has been detected in the skin of ripe avocado fruit cv. Hass, the cyanidin 3-*O*-glucoside, yet others minor anthocyanins still not identified might also contribute to the colour development (Cox *et al.*, 2004). This anthocyanin is generated from the leucocyanidin by glycosilation (Holton and Cornish, 1995).

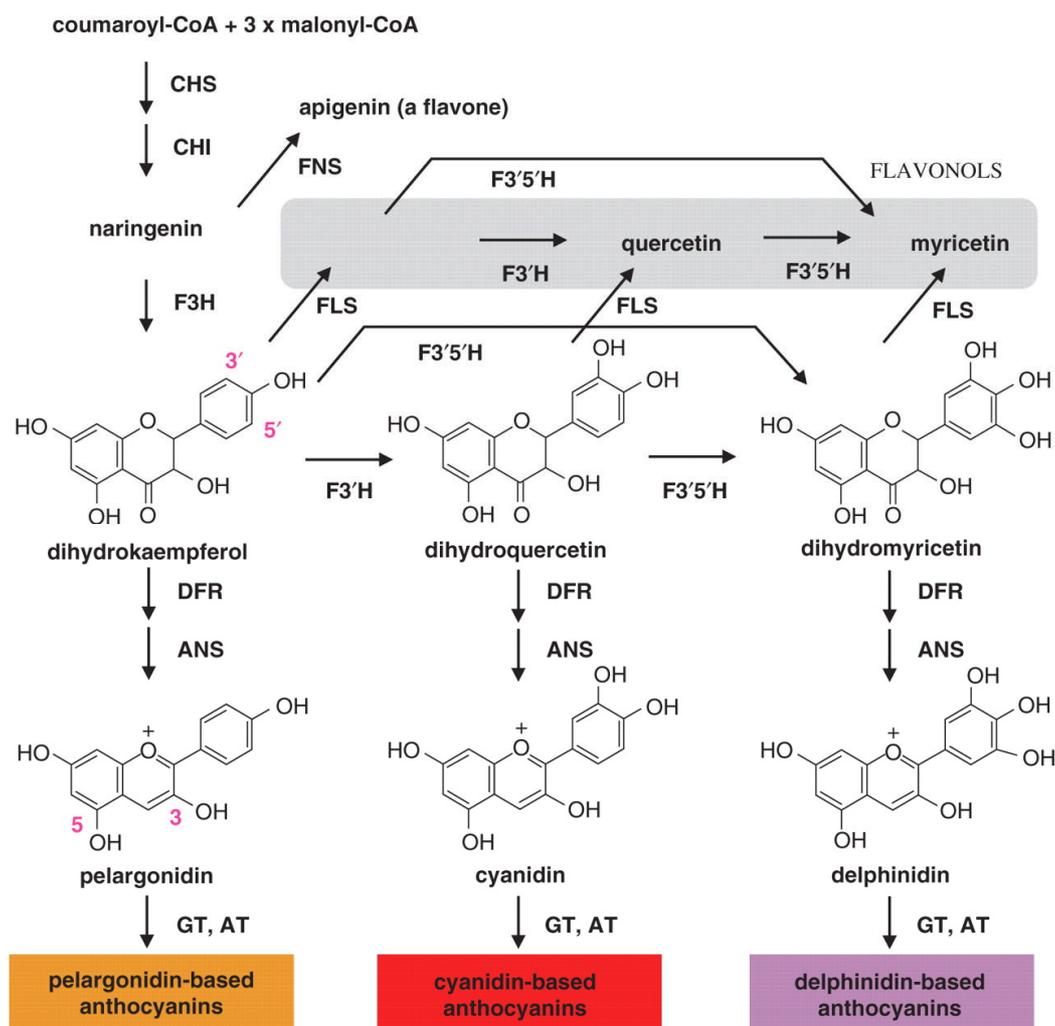


Figure 2.10: Biosynthetic pathway of flavonoids detected in flower and main enzymes involved (CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3',5'-hydroxylase; FLS, flavonol synthase; FNS, flavone synthase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; GT, anthocyanidin glucosyltransferase; AT, anthocyanin acyltransferase) (Katsumoto *et al.*, 2007).

2.8 Minor compounds

2.8.1 Hormones

The modulation of the different hormone ratios, i.e. indole-3-acetic acid (IAA)/ abscisic acid (ABA), plays a central role in the control of cell division and fruit growth

(Taylor and Cowan, 2001). In particular, ABA and cytokinin have been reported to be involved in the determination of avocado fruit size and with carbohydrate metabolism (Cowan *et al.*, 1998; Riching *et al.*, 2000; Cowan *et al.*, 2001). Small avocado fruit showed lower levels of sucrose and higher glucose content, higher respiration rate and ABA metabolism (Riching *et al.*, 2000). Indeed, higher levels of ABA during fruit development inhibits cytokinin and sterol synthesis with a consequently arresting of the cell cycle (Cowan *et al.*, 1998). ABA, with a precursor on the carotenoids pathway, is also involved in seed development, dormancy, and water stress responses (Chernys, and Zeevaart, 2000; Seo and Koshiha, 2002). Recently, investigation on tomato fruit highlighted the possible key role of ABA on the regulation of fruit ripening (Zhang *et al.*, 2009). Results showed that exogenous ABA increased internal ABA content, ethylene precursors, and subsequently the climacteric, first in the seed and after in the fleshy tissues of the tomato fruit. In contrast, in non-climacteric commodities, such as onion, the ABA content decreased during storage and seemed to be an indicator of onion storability (Chope *et al.*, 2006).

In avocado fruit, ABA content increases in the mesocarp with maturity and ripening, particularly, an increase in its content follows the peak of released ethylene (Adato and Gatiz, 1977; Cutting *et al.*, 1989; Chernys and Zeevaart, 2000). Additionally, application of ABA in unripe avocado fruit induces climacteric and accelerated ripening (Blakey *et al.*, 2009). Implications of ABA metabolism on ethylene synthesis or tissues sensitivity is still to be clarified. Besides, the importance of ethylene in the onset of ripening, particularly in climacteric fruit has been largely documented (Adato and Gatiz, 1977; Pesis *et al.*, 1987; Wang *et al.*, 2002; Giovannoni, 2004) and due to its importance on avocado fruit physiology will be discussed in a specific section (*Cf.* session 2.12.3).

2.8.2 Phytosterols

Phytosterols are minor compounds present in avocado fruit with potential anticancer activity (Awad and Fink, 2000). They belong to the terpenoids and most of them have a structure consisting of 28-29 carbon atoms (Piironen *et al.*, 2003). Their concentration in the fruit can be different depending on pre-harvest factors such as

harvest time and growing area (Lu *et al.*, 2009). In the plant tissues the phytosterols act as molecular carriers in the regulation of membrane fluidity, or can be precursors of secondary metabolites (Piironen *et al.*, 2003). The most abundant phytosterols in avocado are β -sitosterol and 4-desmethylsterol with a concentration that can reach 70 mg g^{-1} in the raw pulp, a relatively high content compared to other fruits such as orange (17 mg g^{-1}), grapefruit (13 mg g^{-1}) or banana (11 mg g^{-1}) (Piironen *et al.*, 2003).

2.8.3 Phenolic compounds

The phenolic compounds present in plants are responsible for the taste and often the colour of fruit and vegetables and can also be involved in growth and disease resistance (Balasundram *et al.*, 2006).

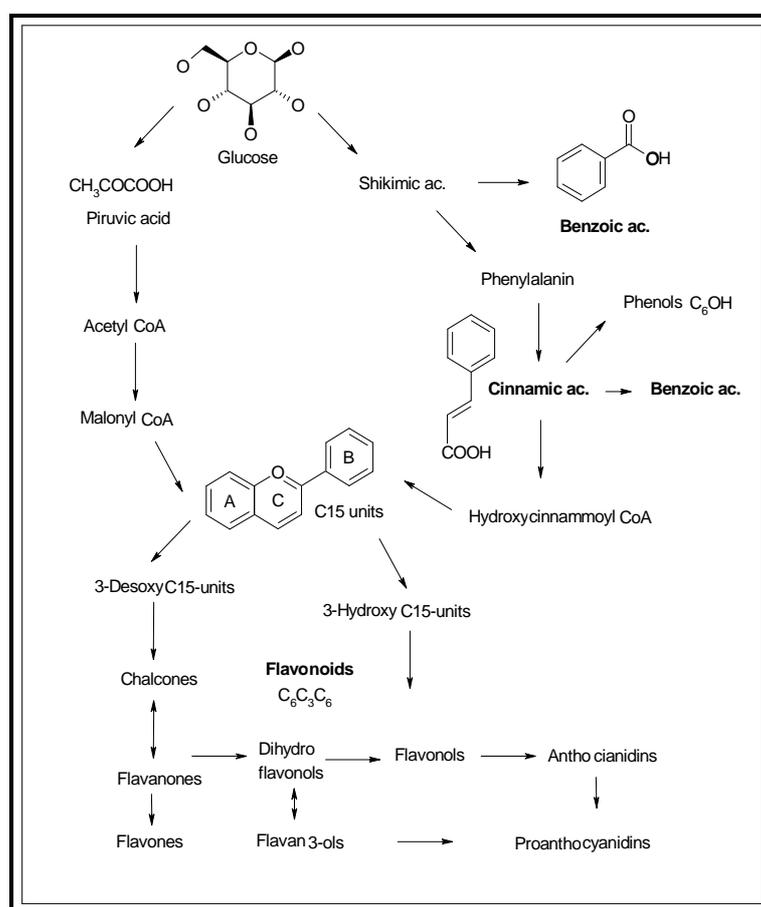


Figure 2.11: Synthetic pathway of the main phenylpropanoid compounds.

Phenolics have a wide range of structures (Figure 2.11) present in simple forms such as the phenolic acids or in polymeric structures (Bravo, 1998). Phenolic acids are mainly composed of a benzene ring with hydroxyl group derived from shikimic acid (Rice-Evans *et al.*, 1996) and are generally located in the cell vacuole. The polyphenols have a wider range of structures (i.e. flavonols, flavonones) (Pietta, 2000). The largest group by size is the class of flavonoids, with a basic structure composed of two benzene rings bound by a 3 carbons chain (Harborne and Williams, 1995). Tannins are a group of phenolic compounds with high molecular weight present as hydrolysable or condensed structures. They can be divided into two classes: one derived by esters of ellagic acids which give form to the ellagitannins, and a second one derived from flavonoids (Tomás-Barberán and Robins, 1997).

In avocado fruit, and in order of abundance, the phenolic compounds are mainly present in the seed, peel and flesh (Wang *et al.*, 2010). The total phenolic content of the mesocarp has been reported to be between 130-240 µg of gallic acid equivalents (Luximon-Ramma *et al.*, 2003; Soong and Barlow, 2004); however, as previously mentioned for other components (i.e. lipids, sugars) phenolic content can differ with variety, fruit maturity and growing conditions. For instance, the total phenolic content in the avocado mesocarp seems to increase with fruit maturity (Cutting *et al.*, 1992). Individual phenolic compounds have been identified in different avocado cultivars. Ramirez-Martinez and Luh (1973) isolated epicatechins, p-coumaric and isoflavones and at lower concentration chlorogenic, leuco-anthocyanins and p-coumarylquinic acids in the mesocarp of cv. Fuerte. Successive investigations on several avocado cultivars reported the presence of caffeic and p-coumaric acid (Prabha and Patwardhan, 1980), benzoic acid derivatives (p-coumaric, ferulic, p-hydroxi, protocatechic, vanillic, syringic acids) and cinnamic derivatives (caffeic, sinapic acids) (Torres *et al.*, 1987). Flavonoids have been found in seed (procyanidin) (Geissman, 1965) and peel tissues (catechins, epicatechin and procyanidin) (Ardi *et al.*, 1998; Terasawa *et al.*, 2006; Wang *et al.*, 2010). A recent investigation showed a different phenolic acids composition in the mesocarp of avocado fruit from various varieties (Rugoro, Lamb-Hass, and Hass) (Hurtado-Fernández *et al.*, 2011).

2.8.4 Phenolics and mesocarp discoloration

The phenolic content together with the activity of the polyphenol oxidase (PPO) are the main causes of postharvest browning for many fruit and vegetable (Rinaldo *et al.*, 2010, Ciou *et al.*, 2011). PPO supposedly acts in plant system as defensive mechanism against wounding (Constabel *et al.*, 2000) and disease resistance (Li and Steffens, 2002). This enzyme oxidises the phenolic compounds released from the cells vacuoles in *o*-quinones that then polymerised to melanins, causing the browning of the tissues (Schmitz *et al.*, 2008, Rinaldo *et al.*, 2010). This reaction is influenced by H₂O₂ levels, pH, the phenolic structure, and the rapidity of the oxidative reactions can influence the incidence of the browning (Tomás-Barberán and Robins, 1997). The dark appearance of the fruit and vegetable is a cause of rejection from the customer as a consequence of a perceived reduction in quality.

In avocado fruit the browning of the mesocarp is related to postharvest disorders such as mesocarp discoloration or pulpspot (Bower and Cutting, 1988). Mesocarp discoloration and peel injury are commonly consequent to chilling injury caused by exposition at low temperature (Pesis *et al.*, 2002). Besides the PPO activity and the phenolic content (Kahn, 1983; Van Rooyen and Bower, 2003) others factors can determinate the browning process. For instance, reduction of water loss during storage seems to reduce incidence of browning (Bower and Bertlinger, 2008). The mineral composition of the mesocarp seems also to be influent in the incidence of mesocarp discoloration, whit a negative effect by high nitrogen levels (Van Rooyen and Bower, 2003). Recent study highlights the importance of fruit growing area more than storage temperature on the browning incidence of Pinkerton avocado (Van Rooyen and Bower, 2003). However, at low temperature the PPO activity increased whereas the phenolic content reduces (Van Rooyen and Bower, 2003). Increase of l-phenylalanine-ammonia-lyase (PAL) activity is also been related with low temperature of storage in others subtropical fruit with the hypotised stimulation of phenylpropanoid pathway as protective response. In agreement, in cold resistant mandarin variety PAL (l-phenylalanine-ammonia-lyase) biosynthesis was not stimulated by low temperature (Sanchez-Ballesta *et al.*, 2000). Additionally, ethylene and embryo development seem also to play a role on PPO activity. Increased incidence of mesocarp discoloration and

higher PPO transcripts was detected in seeded ethylene treated fruit cvs. Ettinger and Arad after cold storage (5°C for 3-4 weeks). Application of ethylene inhibitor (1-MCP) reduced incidence of mesocarp discoloration, embryo development and PPO activity (HersHKovitz *et al.*, 2009). Beside, the use of ethylene repressor in postharvest is still controversial for the controversial results. An earlier development of browning with higher PPO activity was detected in apple fruit 1-MCP treated during control atmosphere storage (Jung and Watkins, 2011). Fruit maturity on chilling injury development is still controversial. Whether late harvest fruit are more susceptible to extended cold storage and with higher total phenolic content (Cutting *et al.*, 1992; Van Rooyen and Bower, 2003) seems to be not always valid depending on the year of production (Dixon *et al.*, 2004).

Pre-storage treatment at moderate temperature (38°C) (Woolf *et al.*, 2004) and preharvest sun exposure (Woolf and Ferguson, 2000) are effective in the reduction of skin chilling injury. This response has been related with higher levels of hot shock proteins in the tissues for an extended period after the treatment (Woolf and Ferguson, 2000; Woolf *et al.*, 2004). Still not completely understood are the mechanisms inducing postharvest browning in avocado fruit, however the perception of stress by the fruit seems to lead the process.

Browning is also a major problem for process avocado (purée) and diverse techniques are used nowadays to limit the activity of PPO on process food such as application of antioxidant (i.e. EDTA), and limited oxygen conditions (under nitrogen) (Soliva *et al.*, 2001).

2.9 Antifungal compounds

The susceptibility to pathogen infections tends to increase with the metabolic changes occurring during the ripening process due to a decline of natural defence (Prusky, 1996; Terry and Joyce, 2004). Responsibility for this increased susceptibility during ripening seems to be the reduction in diene content (1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene) which have antifungal and antibacterial activities (Prusky *et al.*, 1991, Prusky and Kobiler, 1992). Supporting this hypothesis, it has been shown that there is a correlation between the diene concentration and the resistance to

Colletotrichum gloeosporioides infections in avocado (Prusky *et al.*, 1991). Indeed, the diene seems to inhibit the elongation of the fungal germ tube used during the progress of the infection (Prusky *et al.*, 1991). Of secondary importance for efficacy is another diene known as monoene 1-acetoxy-2,4-dihydroxy-n-heptadeca-16-ene. Most of the antifungal activity of the diene and the monoene has referred to the presence of hydroxyl and acetate groups (Prusky and Kobiler, 1992).

The possible modulation of diene concentration in avocado fruit could be used as natural postharvest defence. In the understanding of the mechanisms acting in the diene synthesis, investigations revealed a possible involvement in it of lipoxygenase and epicatechin (Prusky and Kobiler, 1992; Ardi *et al.*, 1998; Guetsky *et al.*, 2005; Adikaram *et al.*, 2010). The lipoxygenase seems to be responsible for the degradation of the diene and be down regulated by the epicatechin. Hence, the decrease of diene during ripening has been reported to be related to the decline of epicatechin content (Prusky and Kobiler, 1992; Ardi *et al.*, 1998; Guetsky *et al.*, 2005; Adikaram *et al.*, 2010). Additionally, ethylene seems to stimulate diene and the monoene synthesis (Wang *et al.*, 2006).

2.10 Plant cell walls

Plant cell walls are a complex and dynamic structures which determine the growth, stability and defence of the cell. Plant cells have an internal osmotic pressure that gives rigidity and tension to the cell wall. Nevertheless, the cell wall is also flexible and coordinates cell growth. During cell division, a new cell wall is formed on the old one, allowing cell to expand. Least, plant cell wall is also involved in the defence program. Release of cell wall fragments after a pathogenesis attack seems to stimulate a defensive response (Somerville *et al.*, 2004). The plant cell wall composition change regarding cell type, tissues and species and developmental stage. Nevertheless, generally plant cell walls are defined tubes of cellulose microfibrils immersed in a non-cellulosic polysaccharides matrix in which can be present proteins, glycoproteins and phenolic compounds (Boudjeko *et al.*, 2009). It is possible to distinguish three main layers: middle lamella, primary cell wall and secondary cell wall (Somerville *et al.*, 2004; Popper, 2008; Marcus *et al.*, 2010) (Figure 2.12). The middle lamella is the first

layer created after mitosis on which the primary cell wall develops along cell growth and differentiation. The secondary cell wall is not present in all cell type (i.e. collenchyma and parenchyma) and only after the cell has differentiated, therefore, most of the research on cell wall has concentrated on the identification of the primary cell wall (Popper, 2008).

2.10.1 Cell wall components

The main polymers identified in cell wall structures are cellulose, hemicelluloses and pectic polysaccharides (Popper, 2008). The primary cell wall is usually composed by cellulose microfibrils, tubes of 3,000 up to 10,000 (β -1-4) glucose units, kept tight by hydrogen bonds (Somerville *et al.*, 2004). Cellulose structures formed in the plasma membrane are insoluble due to the presence of side glucans chains forming a crystalline structure (Somerville *et al.*, 2004). The others components, hemicellulose and pectins, are secreted as soluble polymers and presumably modified on site. The hemicelluloses present in the primary cell wall are polysaccharides formed by neutral sugars which can be linked to the cellulose microfibrils with hydrogen bonds (Somerville *et al.*, 2004). The linkage by the hemicellulose to the cellulose decreases the strength of the cell wall structure and confers mobility. Others principal components of cell wall are the pectins, polysaccharides rich in galacturonic acid involved in cell wall growth, adhesion, and wall porosity. Typical structures present in all the pectins are homogalacturonan (HGA) chains of α 1-4 D-galacturonic acid, rhamnogalacturonan-I (RG-I), chain of D-galacturonic and L-rhamnose connected with neutral sugars such as arabinan and galactan, and rhamnogalacturonan-II (RG-II). RG-II is formed by galacturonic chain (α -1-4 linked) with 4 side groups (Willats, *et al.*, 2001; Somerville *et al.*, 2004). Both RG-I and RG-II have been suggested to bound HGA chains (Willats, *et al.*, 2001). Thus, changes in the substitution groups on the pectic backbone can alter the mentioned properties of the cell wall (Somerville *et al.*, 2004).

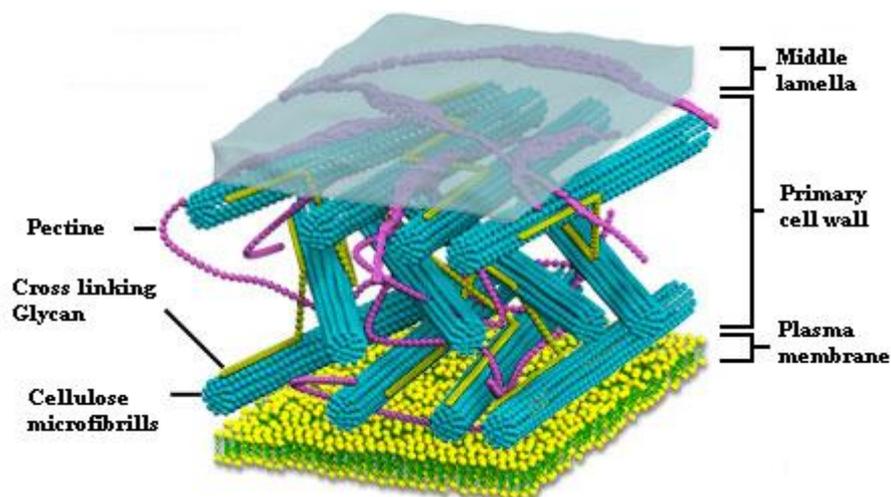


Figure 2.12: Description of the main structures present in a plant cell wall. Middle lamella, primary cell wall and plasma membrane are indicated on the right side, whereas the single components, pectins and cellulose microfibrils, are on the left side (Davidson, 2011).

2.10.2 Biosynthesis

Most of the processes involved in the synthesis of the cell wall are still unknown. Enzymes implicated in the synthesis of the structural carbohydrate are been grouped in families as cellulose synthase (CeSA) related with the synthesis of cellulose, or the cellulose synthase-like (Csl) involved in the arrangement of heteroxylans, xyloglucans and pectins (Popper, 2008). The cellulose synthesis is active during the cell expansion and the orientation of the fibrils is also controlled to avoid cells elongation and avoid later enlargement. Thus, genes required for the cellulose constitution might be controlled by factors regulating the cell cycle. Besides, changes in the structural polysaccharides composition between cells from different tissues might indicate that differentiated cells still modify the wall structures for its specific needs (Richmond and Somerville, 2001). Two additional protein classes are been identified as xyloglucan endotransglycosylase (XET) and expansins both involved in the cell wall expansion, acting on the recombination of xyloglucan and the disruption of cell wall interactions, respectively (Somerville *et al.*, 2004).

Substantial modifications in the cell wall structures are related to the ripening process of fruit and with the softening of the mesocarp (Pesis *et al.*, 1978). Cellulase, α - β -galactosidase, polygalacturonase (PG) and pectinmethylesterase (PME) are the enzymes involved in the depolymerization of cell wall structures identified until now in avocado fruit. Polygalacturonase removes de-methylated polygalacturonic acids from homogalacturonans structures, and PME de-esterified mainly polygalacturonans by removing methyl groups from the galacturonic residue of pectins (Brummel and Harpster, 2001). Cellulose also undergoes depolymerisation process by cellulase enzymes which might act on xyloglucan and glucomannan. However, one of the main frequent modifications on cell wall during ripening is the cleavage of galactosyl residues from side chains of RG-I or RG-II (Brummel and Hapster, 2001). Cellulase (endo- β -1,4-glucanase) activity has been reported to be very low or even not detected immediately after harvest (24 hours) but its content was constantly increasing along ripening (Pesis *et al.*, 1978; Jeong *et al.*, 2002). The PG levels were also very low after picking and during the preclimacteric, increasing as ethylene and respiration rate arise with higher activity in the overripe stage of the fruit. Opposite trend was detected in α - β galactosidase activity, decreasing after harvest and during the first days of shelf life (Jeong *et al.*, 2002). Similar behaviour in the PME levels, reported to be highly active already after harvest and to decrease during shelf life (Awad and Young, 1979; Jeong *et al.*, 2002).

A method to investigate the main component from plant cell wall consist in multi-step extractions starting with the isolation of water-soluble polysaccharides, follows by a solution of chelating agent (CDTA) that extract polymers binding with Ca^{2+} and Mg^{2+} such as pectins. Successive steps with alkali solvent can solubilize pectins and hemicellulose breaking down the hydrogen bounds with the cellulose (Selvendran *et al.*, 1987). In recent study on avocado fruit, the delay of ripening induced with applications of 1-MCP was retarding the activity of PG enzymes. Treated fruit had also a delay in the increase of the water soluble material and the depolymerization of the cell wall structures compared with control fruit. In both extracts, water and CDTA, the molecular mass of the polyuronides recovered was decreasing with the ripening more in control than in treated fruit, might consequence of a lower breakdown activity when 1-MCP has

been applied (Jeong *et al.*, 2002; Jeong and Huber, 2004). However, at the fully ripe stage, both treated and control fruit had a similar extracts composition and arabinose, galactose, glucose, mannose, rhamnose, and xylose were the main sugars identified (Jeong and Huber, 2004).

2.11 Preharvest factors

Climate and environment, rootstock, planting design, pruning practices, pest management, irrigation, plant growth regulators, and plant nutrition are some of the preharvest factors responsible for the determination of avocado fruit postharvest quality (Arpaia *et al.*, 2004). The identification of the optimal preharvest conditions can minimise cost of production and enhance fruit characteristics. However, it is wise to deem that the three commercial races respond differently to the same ecotype or growing practises. According to this, improvement of avocado fruit quality results from the accurate choice of cultivar and growing practice for each environment (Ferguson *et al.*, 1999; Shaffer and Whiley, 2003).

2.11.1 Crop management

Mulching, pruning and control of the orchard dimensions are some of the strategies used to improve the plantation's yield. It is advisable to consider that these horticultural practices can give different results regarding the environmental conditions in which they are applied.

Mulching is an old practise still used for its sustainability and usually good results obtained. It consists of apply organic matter around the root system. The choice of the material is related to the soil characteristic in order to avoid excess or deficiencies of some nutrients (Whiley *et al.*, 2002). Pruning is used to control tree size, shape, and to give enough light exposure in high density plantations. This practise is also used to reduce the vegetative growth and promotes fruit set (Thorp and Sedgley, 1993; Snijder *et al.*, 2000; Whiley *et al.*, 2002). It is important to choose the most convenient rootstock and the right time to do the pruning to avoid reduction of crop yield (Whiley *et al.*, 2002). The control of fruit size and tree productivity can be done with the

cincturing, consisting in the incision of the trunk with the purpose of interrupt the phloem (Whiley *et al.*, 2002).

2.11.2 Temperature and sun exposure

Temperature and sun exposure, also if closely related, can differently bear upon avocado fruit quality. For instance, different environmental temperatures during fruit development can influence fruit shape, inducing a round conformation in cooler climate and a more elongate one in warmer conditions (Arpaia *et al.*, 2004). A study on the lipid content and composition of New Zealand avocado fruit (Requejio-Tapaia *et al.*, 1999) hypothesised a possible influence of growing temperatures on the membrane composition and consequently on the oil composition.

Fruit directly exposes to sun light can reach an internal temperature 10-15°C higher than the external level (Woolf *et al.*, 1999). Direct sun exposure can influence fruit metabolism. Indeed, while the fruit is on the tree, the light exposure is a fundamental component for the photosynthetic activity and the trees productivity (Schaffer and Whiley, 2003). Woolf and colleagues also reported higher tolerance to heat or cold postharvest treatments in sun exposed fruit (Woolf *et al.*, 1999). Particularly, in the same fruit the sun exposed side better responded to low temperature of storage than the shaded one (Woolf *et al.*, 1999). Thus, the direct sun light and the consequently high temperature reached in the exposed tissues stimulate the synthesis of heat shock proteins responsible for temperatures tolerance mechanism (Woolf *et al.*, 1999). Direct exposure to the sun light seems also to stimulate the production of the diene previously described (*cf.* section 2.9) and induces higher resistance to pathogens attack (Woolf *et al.*, 2000). Though there are positive effects, excessive sun exposure can damage the photosynthetic process and cause sunburn or injure fruit tissues (Woolf and Ferguson, 2000; Schroeder and Kay, 1961). On the fruit appearance, more browning in the skin tissue was consequent to heat water treatment (Woolf *et al.*, 1999), and has will be specify later, higher postharvest temperature induces increased anthocyanin level (Cox *et al.*, 2004).

As previously mentioned, oil ratio can changes regarding preharvest temperature with higher oil content found in exposed fruit and with higher content of palmitic acid

(Woolf and Ferguson, 2000). Additionally, sun exposed avocado fruit showed higher firmness than shaded fruit, with a possible implication of the cell wall composition (Woolf *et al.*, 2000, Woolf and Ferguson, 2000).

2.11.3 Irrigation

The water regimes adopted in an avocado plantation can influence tree height, trunk dimensions, shoot growth and is crucial particularly in two development stages: flowering, influencing the fruit set, and fruit growth, affecting fruit size (Lahav and Whiley, 2002). Indeed, frequent but controlled irrigation can raise fruit size and flesh oil content (Whiley *et al.*, 2002). Also the root system can be influenced by water availability; indeed a deeper root system can be consequent to water paucity (Whiley *et al.*, 2002).

Water availability is also directly involved in defining fruit quality, influencing the assimilation of mineral elements, the regulation of PPO activity and the incidence of pulp browning (Bower, 1988). Usually excess of water in drained soil can induce chlorosis and increase *Phytophthora* incidence (Ploetz and Schaffer, 1992; Whiley *et al.*, 2002). On the other hand, moist rather than dry soil easily accumulates heat during the day helping to safeguard against frost damage (Lahav and Whiley, 2002).

The identification of the better water regime and the application systems for an avocado orchard is related to various factors such as soil composition, fruit developmental stage and water availability. Depending on the irrigation system, the frequency of the water application can be different: a drip system (Figure 2.13) requires more frequent application (every 1-3 days) rather than the sprinkler one (every 7-12 days) and will be more sustainable in areas with good water availability (Whiley *et al.*, 2002) whereas an overhead irrigation can be optimal in hot climates helping to decrease the air temperature. The use of micro-irrigation is the most efficient method for the application of soluble nutrients as directly applied on the root system (Lahav and Whiley, 2002).



Figure 2.13: Drip irrigation system in Trops’s Malaga plantation (source M. Donetti).

2.11.4 Mineral nutrition

In fruit and vegetable, excesses, deficiencies or imbalance of mineral nutrients can result in an increased incidence of postharvest disorders (Kader, 2002). The additional application of micro and macro minerals is due to the soil structure and nutrient availability (Whiley *et al.*, 2002).

Calcium is one of the most important elements in plant physiology and fruit postharvest quality, influencing membrane stability, enzyme activity and membrane ions exchange (Bower and Cutting, 1988). Particularly in an avocado orchard, adequate calcium concentrations can limit root infection by draining soil and reducing cell membrane permeability (Messenger *et al.*, 1997). The plant calcium up-take should be monitor in the most critical developmental stages, such as flowering or fruit set. Calcium finds applications also during avocado shelf life where seems to help retarding fruit ripening by limiting ethylene production and respiration rate (Eacks, 1985).

In conclusion, besides the action that a single mineral can have on plant metabolism it is advisable to consider the general mineral composition of the soil (Du Plessis and Koen, 1992; Kremer-Kohen *et al.*, 1993). For instance, calcium can positively affect fruit development, whereas in the concomitant presence of potassium and magnesium can increase the incidence of avocado postharvest disorders such as grey pulp, vascular browning and pulp spot (Eaks, 1985; Bower and Cutting, 1988; Du Plessis and Koen, 1992, Ferguson *et al.*, 1999).

2.12 Factors influencing avocado postharvest physiology

During postharvest external conditions such as temperature, air composition, handling, packaging or diseases can be sources of stress for the fruit reducing its quality (Kays, 1991). The losses occurring during storage and/or shelf life are economically relevant for the entire food industry (Lester, 2003). Therefore, it is wise to identify the most suitable handling conditions to extend fruit shelf life without reducing its quality, particularly when dealing with a high perishable product which is avocado (Kader, 2002; Woolf, 2004). This section aims to review the current knowledge about the postharvest behaviour of avocado fruit.

2.12.1 Maturity stage in avocado

“Mature” and “ripe” are terms used to identify horticultural stages that may or may not coincide with each other. For instance, many fruits need to ripe properly after maturation before being edible. In avocado fruit there are many external observations that indicate maturity. However, it is important to harvest the fruit at the right time to allow the development of the proper flavours and taste (Lee *et al.*, 1983). It is known that dry matter (after oven or freeze drying) and oil content increase during the development of avocado fruit (Lee *et al.*, 1983). A worldwide maturity indicator used for avocado is the dry matter or oil content of the fruit mesocarp (Woolf *et al.*, 2003a). In 1925, the California Avocado Standardization Bill defined that to be acceptable for the commercialization the mesocarp oil level has to be at least 8 % of the fruit weight. Recently, the suitability of DM or oil content as indicator of fruit maturity founds some criticism (Hofman *et al.*, 2000) as these two parameters are not always closely related through the same fruit (Landahl *et al.*, 2009).

The DM content can be inconsistent within the fruit (Woolf *et al.*, 2003a; Landahl *et al.*, 2009) and possibly between growing areas. Though, late harvest fruit have been reported to be more susceptible to cold storage with higher incidence of vascular browning and mesocarp discoloration (Cutting *et al.*, 1992). However, any correlation has been found yet between oil content/DM and fruit quality (Hofman *et al.*, 2000).

2.12.2 The ripening process

Ripening is the later stage of fruit maturity and/or an early stage of senescence (Lee, *et al.*, 1983). At this stage fruit metabolism involves, generally, variation in the respiration rate, development of flavours, colour and textural changes (Kays, 1991; Seymour *et al.*, 1993). However, fruit and vegetables behaved differently during ripening and a first classification exists regarding changes in the respiration rate. The presence or absence of respiration peak accompanied by arise of released ethylene are the main terms which discriminate climacteric (i.e. avocado) and non climacteric commodities (i.e. orange), respectively (Lelièvre *et al.*, 1997; Watckins, 2006; Pech *et al.*, 2008) (Table 2.3).

Table 2.3: List of the more common climacteric and non climacteric fruit and vegetable (Watckins, 2006).

Non-climacteric		Climacteric	
Cherry (<i>Prunus avium</i> L.)	Broccoli (<i>Brassica oleracea</i> L.)	Apple (<i>Malus sylvestris</i> (L) Mill.)	Apricot (<i>Prunus armeniaca</i> L.)
Clementine mandarin (<i>Citrus reticulata</i> L.)	Carrot (<i>Daucus carota</i> L.)	Avocado (<i>Persea americana</i> Mill.)	Banana (<i>Musa</i> L.)
Cucumber (<i>Cucumis sativu</i> L.)	Chrysanthemum , garland (<i>Chrysanthemum coronarium</i>)	Blueberry (<i>Vaccinium corymbosum</i> L.)	Chinese bayberry (<i>Myrica rubra</i>)
Grape (<i>Vitis vinifera</i> L.)	Coriander (<i>Coriandrum sativum</i> L.)	Chinese jujube (<i>Zizyphus jujube</i> M.)	Custard apple (<i>Amnona squamosa</i> L.)
Grapefruit (<i>Citrus paradisi</i> Macf.)	Pepper (<i>Capsicum frutescens</i> L.)	Figs (<i>Ficus carica</i> L.)	Guava (<i>Psidium guajava</i> L.)
Peach (<i>Prunus persica</i> L. Batsch)	Lettuce (<i>Lactuca sativa</i> L.)	Kiwifruit (<i>Actinidia deliciosa</i>)	Lychee (<i>Litchi chinensis</i>)
Lime (<i>Citrus latifolia</i> Tanaka)	Parsley (<i>Petroselinum crispum</i> Mill.)	Melon (<i>Cucumis melo</i> L.)	Mango (<i>Mangifera indica</i> L.)
Orange (<i>Citrus sinensis</i> L. Osbeck)	Potato (<i>Solanum tuberosum</i>)	Nectarine (<i>Prunus persica</i> Lindl.)	Papaya (<i>Carica papaya</i> L.)
Pineapple (<i>Ananas comosus</i> L.)		Pear (<i>Pyrus communis</i> L.)	Pear (<i>Pyrus pyrifolia</i> Nakai)
Strawberry (<i>Fragaria x ananassa</i> Duch)		Persimmon (<i>Diospyros khaki</i> L.)	Plum (<i>Prunus salicina</i> L.; <i>Prunus x domestica</i> L.)
Watermelon (<i>Citrullus lanatus</i>)		Tomato (<i>Solanum esculentum</i> Mill)	

Avocado fruit are well known for the higher internal production of ethylene (100-80 $\mu\text{l l}^{-1}$) (Adato and Gatiz, 1974; Hershkovitz *et al.*, 2009b) compared to others climacteric (i.e. banana 40 $\mu\text{l l}^{-1}$; mango 3 $\mu\text{l l}^{-1}$) and non-climacteric fruit (i.e. lemon 0.1 $\mu\text{l l}^{-1}$; pineapple 0.4 $\mu\text{l l}^{-1}$) (Seymour *et al.*, 1999). The climacteric peak increased internal production of ethylene stimulates the autocatalytic production leading to metabolic changes associated with faster ripening and consequently shorter shelf life (Lelièvre *et al.*, 1997). For instance, in avocado fruit ethylene burst have been related with increased activity of cellulase and consequently softening of the mesocarp (Pesis *et al.*, 1978). Besides, in non-climacteric fruit an ethylene-independent system control the most of the ripening process (Lelièvre *et al.*, 1997). Nevertheless, the regulation of the ripening seems to be a more complex process. Recent investigation on melon (*Cucumis melo*) characterised by two opposite genotypes, one with a typical climacteric behaviour (*C. cantalupensis*) and non climacteric one (*C. inodorus*), showed the possible concomitant presence of the two systems, ethylene-dependent and independent, and their control on different metabolic pathways (Pech *et al.*, 2008).

2.12.3 Ethylene as plant hormone

The biosynthetic pathway of ethylene was described first by Yang and Hoffman (1984) (Figure 2.14). Main enzymatic reactions in the ethylene pathway are the conversion of methionine to S-adenosylmethionine (SAM) by AdoMet synthase and the successive formation of 1-amminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS). Finally, ACC is converted to ethylene by the ACC oxidase (ACO) enzyme (Yang and Hoffman, 1984). Factors limiting the synthesis of ethylene are the availability of CH_3 group for the synthesis of methionine; the presence of cofactors such as Fe^{2+} , for the ACO activity (Yang and Hoffman, 1984) and adequate oxygen level necessary for the activation of ACS and ACO enzymes (Gorny and Kader, 1996).

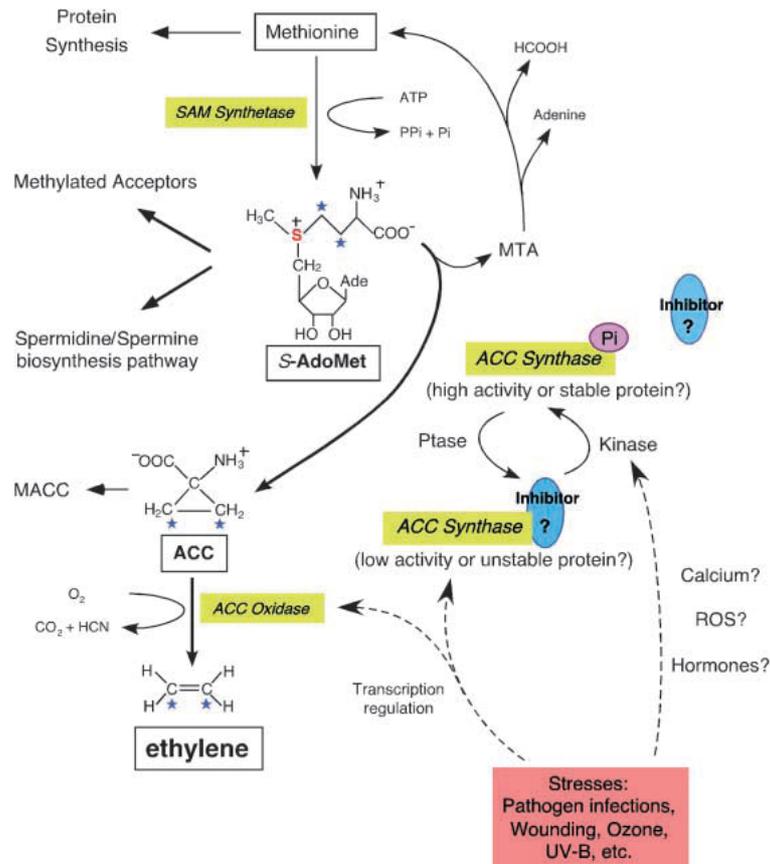


Figure 2.14: Description of ethylene biosynthetic pathway known as the “Yang cycle”. Sam synthetase converts methionine to S-AdoMet with the use of one ATP molecule for each S-AdoMet. The conversion of S-AdoMet in ACC is the limiting step in the ethylene biosynthesis. S-AdoMet is also a methyl donor in others biosynthetic process (i.e. nucleic acids, proteins, lipids) and precursor of polyamines. During the formation of ACC, the methionine group is recycled as 5-methylthioadenosine (MAT). The control of ethylene production is possible by the limitation of ACC transformed in malonyl-ACC (MACC). Finally, the ACO produces ethylene from a molecule of ACC with release of CO₂ and cyanide. Arrows indicate possible transcriptional regulation factors for these enzymes (i.e. stress conditions) (Wang *et al.*, 2002).

Ethylene can act on the plant system through two mechanisms. One (system I) is a characteristic of all plant tissues including pre-climacteric fruit in which ethylene is present in low levels and has a negative control on its own biosynthesis (Owino *et al.*, 2002; Lin *et al.*, 2009). A second one (system II) is an autocatalytic system and it is active during climacteric fruit ripening and during flower senescence (Lelièvre *et al.*,

1997; Lin *et al.*, 2009). Studies on *Arabidopsis thaliana* showed the presence of two main subfamilies of proteins regulating the transmission of the ethylene signal (Stepanova and Alonso, 2005; Chen *et al.*, 2005). The receptors structures are different among the various subfamilies but a common attribute is the presence of transmembranes regions and kinase activity (Hua and Meyerowitz, 1998; Stepanova and Alonso, 2005). The receptors are down-regulated by ethylene that gives a switch-off conformation of the protein and induces its degradation (Zhu and Guo, 2008). Cofactors, such as copper, or inhibitors, such as silver, adjust the transmission of the signal (Rodriguez *et al.*, 1999). The response to ethylene in the plant system is modulated by a complex system of positive and negative controls. In absence of ethylene constitutive triple response (CTR) proteins are activated inducing suppression of the ethylene response (Lin *et al.*, 2009). In avocado fruit, genes involved in ethylene biosynthesis (*PaACS*, *PaACO* (Owino *et al.*, 2002; Hershkovitz *et al.*, 2009)) and response (*PaERS1* (Owino *et al.*, 2002); *PaETR* and *PaERS1* (Hershkovitz *et al.*, 2009)) had very low activity at harvest showing an accumulation of their transcripts as ethylene levels increased. Particularly in avocado, ACC content and ACS activity reached their maximum levels before the ethylene burst whereas ACO activity the *PaACO* transcript arise with the climacteric peak (Owino *et al.*, 2002).

Ethylene is a plant hormone involved in many regulative systems. It stimulates ripening, phenylproprionoid metabolism, chlorophyll degradation, seed germination, respiration, senescence, sex determination and plant defence (Lelièvre *et al.*, 1997; Watkins, 2006; Lin *et al.*, 2009). The use of transgenic plant with suppressed ethylene production is the most used tools to investigate the influence of ethylene on fruit metabolism. For instance, accumulation of ACC and increased activity of ACS were detected in ACO antisense melon suggesting an ethylene-independent regulation. The softening process seems have a ethylene-dependent system in the regulation of PG levels with a high sensitivity to ethylene (Sitrit *et al.*, 1998) whereas other cell wall enzymes can be ethylene-dependent, independent or partially dependent regarding to the family which they belong to (Pech *et al.*, 2008). Similarly, colour changes are defined by a ethylene-dependent system regulating chlorophyll degradation (Jacob-Wilk *et al.*,

1999) and anthocyanin synthesis (El-Kereamy *et al.*, 2003) whereas carotenoids synthesis seems to be ethylene-independent system (Alba *et al.*, 2005).

Control of ethylene in avocado postharvest is become a common practise increase fruit shelf life. Reduction in the avocado ripening can be performed by application of ethylene scavengers (Terry *et al.*, 2007) or “binding site blockers” like 1-methylcyclopropene (1-MCP) (Feng *et al.*, 2000; Hershkovitz *et al.*, 2005, Woolf *et al.*, 2005; Meyer and Terry, 2010). Avocado fruit exposed to 1-MCP showed reduced PG and cellulase levels, delays colour changes, and mesocarp softening (Feng *et al.*, 2000), delay in respiration and climacteric peak (Hershkovitz *et al.*, 2005) and with controversial results influences postharvest disorders (Adkins *et al.*, 2005; Woolf *et al.*, 2005).

2.12.4 Avocado fruit ripening and ethylene

The ripening in avocado fruit occurs generally after the fruit has been harvested suggesting the presence of a ripening inhibitor factor released by the tree. After the harvest and the depletion of the “inhibitor” from the fruit, the ripening process occurs (Adato and Gatiz, 1974) with a maximum respiration rate occurring in the first 3-4 days of shelf life, depending on temperature (Van Rooyen and Bower, 2006). Softening of the mesocarp and, with a restriction to some cultivars, changes in skin colour, are the main manifestations of ripening (Cox *et al.*, 2004; Choi *et al.*, 2008). Cellulase activity was very low or even not detected immediately after harvest (24 hours) but its content was constantly increasing along ripening (Pesis *et al.*, 1978; Jeong *et al.*, 2002). The PG levels were also very low after picking and during the preclimacteric, increasing as ethylene and respiration rate arise with higher activity in the overripe stage of the fruit (Jeong and Huber, 2004).

Exceptionally, chilling injured avocado fruit showed increase respiration and ethylene production on tree, higher level of ACS, ACO and ethylene response genes (*PaETR*, *PaERS1*), and a premature softening during shelf life (Hershkovitz *et al.*, 2009b). Authors suggested that cold stress can stimulate the ethylene pathway and possibly inactivate the tree inhibitor factor (Hershkovitz *et al.*, 2009b). Additionally, symptoms related with chilling injury seem to increase their gravity during cold storage

in ethylene-treated avocado seeded fruit cv. Arad (HersHKovitz *et al.*, 2010). In this regard, PPO levels were higher in the area close to the seed and its activity increased by ethylene treatment. In seedless cv. Arad fruit ethylene biosynthesis (*PaACSI*, *PaACO*) and response transcripts (*PaETR*, *PaERS1* and *PaCTR1*) were in much higher level than in seeded fruit (HersHKovitz *et al.*, 2010). This suggested a possible role of embryo in the ethylene-response of avocado fruit with a consequent influence on fruit postharvest quality (HersHKovitz *et al.*, 2010).

Others metabolites of avocado fruit with a suggested role on fruit ripening are the C7 sugars (Liu *et al.*, 1999b). D-mannoheptulose and perseitol, such as the minor sugars (glucose, fructose and sucrose) decreased along fruit ripening in mesocarp (Liu *et al.*, 1999b; Meyer and Terry, 2008; Blakey, *et al.*, 2009; Landahl *et al.*, 2009; Meyer and Terry, 2010) and peel (Liu *et al.*, 1999b). Results suggest that whereas during storage the seed is the main source of energy, during ripening pulp and skin are the main carbon suppliers (Liu *et al.*, 1999). Possible role of the C7 sugars in avocado fruit metabolism has been suggested as inhibitor of ripening (Liu *et al.*, 1999). The decrease of the C7 sugars with fruit maturity (Bertling and Bower, 2006) and the variations in the sugars content between cultivars (Bertlinger and Bower, 2005) and growing area (Landahl *et al.*, 2009) might involve a relation with avocado fruit postharvest quality (Bertling and Bower, 2006).

2.13 Health related properties of avocado

Avocado fruit is well known to be highly nutritious and a good source of minerals, vitamins and fibre (Bergh, 1992) and its regular consumption has been associated with potential health properties (Board, 1995; Lu *et al.*, 2005; Ding *et al.*, 2009). Researchers on avocado fruit have reported a possible prevention against cardiovascular risk and anti-cancer activity (Ding *et al.*, 2007). Extracts from avocado showed antioxidant activity (Soong and Barlow, 2004; Bertling *et al.*, 2007), radical suppressing (Kim *et al.*, 2000; Vinson *et al.*, 2001), antifungal (Prusky *et al.*, 1991) and chemopreventive activity (Lu *et al.*, 2005; Ding *et al.*, 2007; Ding *et al.*, 2009).

The caloric value associated with the high oil content of avocado fruit might be a factor affecting its consumption. Nevertheless, regular consumption of avocado could

reduced the energy intake (Walker and O’Dea, 2001). Scientific evidence has shown that eating avocado does not compromise good weight control (Pieterse *et al.*, 2003) but ameliorates the uptake of nutrients from other foodstuffs (Unlu, 2004). For instance, some carotenoids can be better assimilated when ingested in combination with lipids, which stimulate the production of bile acids and increase their bioavailability (Roodenburg *et al.*, 2000). Even more important is the high ratio of unsaturated fatty acids present in avocado oil related with decrease of risk of heart disease and high blood pressure (Coulston 1999; Ascherio, 2002). Minor lipid compounds such as carotenoids potentially interfere with reactive oxygen species (ROS) in the cell system (Jennings *et al.*, 1998; Tapiero *et al.*, 2004), protect the LDL (low density lipoproteins) proteins from oxidative process and are involved in the prevention against cardiovascular disease and atherogenic process (Kritchevsky *et al.*, 2003; Tapiero *et al.*, 2004).

Predominant vitamins such as the lipo-soluble vitamin A (present in the form of its precursor, β -carotene) and E (α -tocopherol) are present whereas the water soluble vitamin C (ascorbic acid) only in low amounts compared with other commodities (Slater *et al.*, 1975). Vitamins content varies with cultivars and maturity (Slater *et al.*, 1975; Lu *et al.*, 2009). For instance, cv. Hass (740 IU of vitamin A, 1.6 IU vitamin E and 0.1 mg g⁻¹ vitamin C) has been reported with higher vitamins content compared to cv. Fuerte (484 IU, 2.4 IU and 0.058 mg g⁻¹) (Slater *et al.*, 1975). The vitamin A content is relatively high compared to other common fruit such as peach, apple, banana and grape (Smith *et al.* 1983), can be toxic for the human cell if in excess, but the β -carotene forms present in avocado prevents this eventuality (Bergh, 1992). Other important vitamins present in avocado are riboflavine (vitamin B2), thiamine (vitamin B1) and folacin (Slater *et al.*, 1975).

Avocado fruit is generally low in phenolics content (Luximon-Ramma *et al.*, 2003; Soong and Barlow, 2004), metabolite related with antioxidant activity (Rice-Evans *et al.*, 1995; Amiot *et al.*, 1997). However, a recent report relates most of the antioxidant potential in avocado fruit to the mannoheptulose (Tefady *et al.*, 2010). The C7 sugars have been studied for a possible action against the proliferation of cancer cells lines (Board *et al.*, 1995; Ishizu *et al.*, 2002) and, specifically for D-mannoheptulose, insulin secretion inhibitory effect (Ferrer *et al.*, 1993).

2.14 Industry's standards

The UK avocado market is mainly represented by suppliers, importers, retailers and consumers. For a successful trade, the fruit needs to satisfy the requirement of each market's component. The first parameter evaluated is the fruit maturity level at harvest. As previously mentioned (*Cf.* section 2.12.1), the international criteria for the determination of avocado fruit maturity stage is done through the evaluation of the dry matter content. This parameter, together with the evaluation of internal disorders, firmness and internal fruit temperature, is usually checked by importers as fruit arrived (Adam Shaw, personal communication). Avocado is then located in specific controlled temperature rooms until the fruit has the required characteristics to be sent to retailers. At this stage the main parameter evaluated is the firmness used as indicator of ripening stage of the fruit. Once the fruit reached the required softness (ca. 7 psi/31 N), it can be sent to the retailer and displayed on shelf.

2.15 Conclusions

The increased popularity of avocado fruit in European countries forces importers to solve problems related to prolonged storage and the control of ripening. The peculiarity of avocado fruit is due to the inability to ripen until harvest, along with the presence of C7 carbohydrates and a high degree of variability between fruits during ripening process. As climacteric fruit, avocado's shelf life is generally extended by controlling the action of ethylene on the fruit. However, the network regulating ripening seems to extend to other metabolites which made of action is still not completely understood. The C7 sugars and the possible presence of a tree factor have been suggested as possibly inhibitors of ripening metabolism.

The inconstant behaviour during ripening might be due to preharvest factors. The influence that maturity at harvest, sun exposure, water availability or soil composition have on fruit development and on its postharvest quality have been previously discussed. Whether oil composition or sugar content can be used as discriminators for fruit preharvest conditions and postharvest quality is still a suggestion. Additionally, one of the main avocado cultivars present on the market, cv.

Hass, popular for its good storage attributes, nutty pulp flavour and thick skin, is unusual in that it turns from green to purple as the fruits ripen. This characteristic has been studied for a possible application as a non destructive indicator of fruit ripening. Changes in skin colour (H°) have been related to variation in the cyanidin 3-*O*-glucoside, as far as we know, no consistent correlation has yet been proved between skin colour changes and mesocarp softening. Better knowledge of the main postharvest metabolic regulators could help controlling inconsistency during ripening. The identification of biochemical or physiological markers distinguishing fruit growing conditions or maturity at harvest could be a helpful tool for traders.

3 CHAPTER THREE

METHODOLOGY AND METHOD DEVELOPMENT

PART A: METHODOLOGY

3.1 Plant material and experimental design

3.1.1 Chapter 4

Avocado fruit cv. Hass ($n = 1200$) of commercial size 16 (236-265 g) were sourced in 2008 from Malaga, Spain, in February, March and April; from South Africa, in April from Limpopo, in June and July, from Mpumalanga; from Viru, Peru, in May, June and July 2008, and from Chile, in August, October and January 2009 from the Region de Valparaiso. All fruits were imported by Mack Multiples (Kent, UK) and were held at 5-6°C until they reached the UK. Due to the different geographical locations of the suppliers, fruit had different transit times, Spanish fruit had a transit time of < 10 days, South African 24 - 36 days, Peruvian 33 - 39 days and Chilean fruit 35 - 37 days. Once in the laboratory, the fruit were held at 5°C overnight until experiments commenced. Fruits were not pretreated with 1-MCP.

A total number of 12 experiments, one experiment for each harvest time (3) for each country (4), were carrying out during one year (February 2008- January 2009). For each experiment 100 fruit were ripened at either 18 or 23°C in two Sanyo incubators (model MLR-350HT, Ltd, Japan). For each experiment, fruit were randomly divided in two different groups: 80 fruit (Experiment A) were used for the assessment of firmness, colour and the analysis of fatty acids and sugars content throughout shelf-life; 20 fruit (Experiment B) were used for the measurement of respiration, ethylene production and weight. Experiment A: at day 0 (after cold storage) 16 fruit were randomly chosen and measured. The rest of the fruit ($n = 36$) were arrange in two separated test chambers with an internal temperature of 18 or 23 ± 0.5 °C and sampled at specific intervals (day 1, 2, 4 and 7) of shelf life ($n= 4 \times 8 = 32$). Experiment B: for each temperature of shelf life (18 and 23°C) the same 10 fruit were measured each day of shelf life (day 0, 1, 2, 4 and 7).

3.1.2 Chapter 5

Avocado fruit cv. Hass of commercial size 16 (236-265 g) were sourced in 2008 from Spain, Malaga, in February, March and April. All fruits were imported by Mack Multiples (Kent, UK) and were held at 5-6°C until they reached the UK. Once in the laboratory, the fruit were held at 5°C overnight before being analysed. Fruits were not pretreated with 1-MCP.

For each harvest time, 16 fruit were randomly chosen at the beginning of shelf life (day 0) and colour and mesocarp firmness measured. The remaining fruit ($n = 36$) were arranged into two separated incubators (Sanyo, model MLR-350HT, Ltd, Japan) with an internal temperature of 18 or 23 ± 0.5 °C. Fruits ($n = 8$) held in each shelf life condition were sampled at specific intervals (day 1, 2, 4 and 7). At each interval, objective colour (Minolta), mesocarp firmness (Instron) were measured and the fruits peeled. The removed skin was snap-frozen in liquid nitrogen and freeze-dried for 3 days in an Edwards Modulyo (W. Sussex, UK). The samples were stored at -40°C until analysed. Additional samples (8 fruits each day for each shelf life temperature) were used to assess the climacteric behaviour throughout shelf life.

3.1.3 Chapter 6

Avocado fruit cv. Hass was harvested from Green Edwin farm, Mpumalanga, South Africa, at three different maturity stages corresponding to early, middle and late season. Fruit collected during the year were from different commercial sizes regarding season: early season from size 16 (236-265 g); middle seasons from size 18 (211-235 g) and late season fruit from size 22 (171-190 g). After harvest, all fruit were kept at 5°C and shipped for a month until they reached the UK. All fruit were imported by Mack Multiplies. Ethylene, supplied by SIP analytical Ltd. (Kent, London), was applied as compressed gas (100%).

3.1.4 Experiment I and II

Avocado fruit harvested in May (size 16) ($n = 144$) and June (size 18) ($n = 144$) from Mpumalanga, South Africa, arrived in UK held at 5°C and the day after

transferred to Cranfield. Once in the laboratories, fruit were kept at 12, 16 and 20°C for 2-4 hours until they internally reached the required temperature. Fruit were then subsampled ($n = 72$) and distributed between two polyester boxes (320 l) and, for each temperature of shelf life, one box was injected with 100 ppm of ethylene and a second one was used as control (non-treated fruit). Ethylene was applied as gas and injected (35 ml) with a plastic syringe. After application, the ethylene level was measured to ensure that the concentration reached 100 ± 5 ppm and the boxes were kept sealed for 12 hours. Small fans inside each box were used to circulate the internal air (Figure 3.1). At sampling day (day 1), fruits ($n = 18$) from each treatment (control and ethylene) and each shelf life condition (12, 16 and 20°C) were moved to a higher and/or lower shelf life temperatures (Figure 3.2).

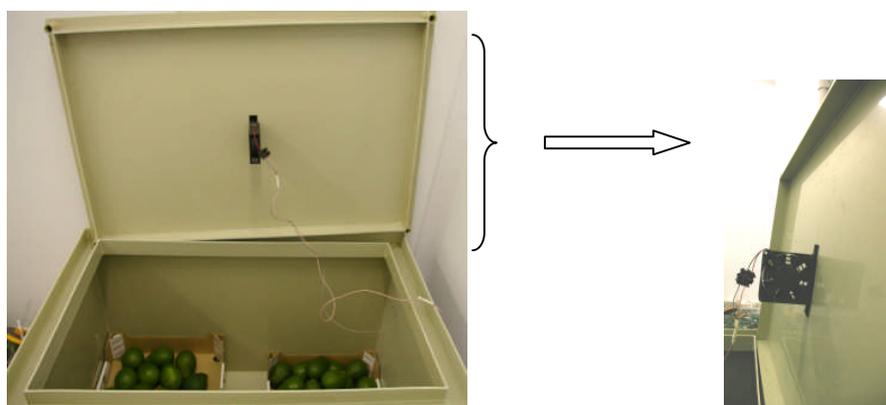


Figure 3.1: Avocado fruit in plastic box with a fan on the top of the lid's box.

Fruit were assessed for weight, colour, respiration and climacteric rate at day 0, 1, 2, 4 and 7 of postharvest life. The progress of ripening was assessed as a decrease in mesocarp firmness at specific intervals (day 0, 2 and 7). Respiration and ethylene production were measured at each temperature (12, 16 and 20°C) and at 20°C.

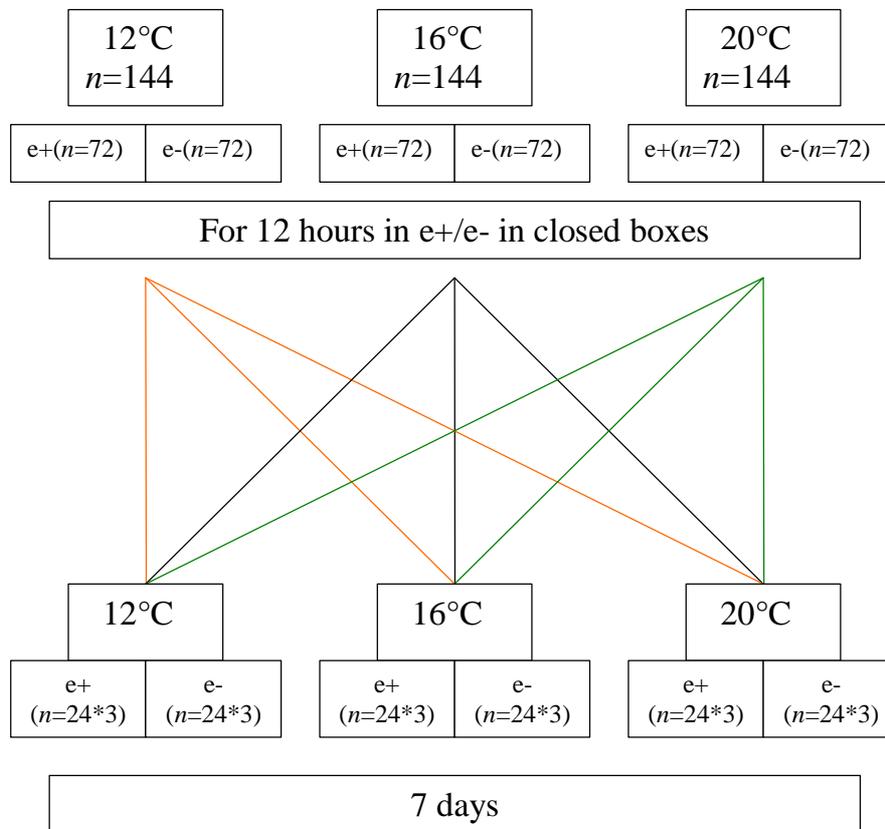


Figure 3.2: Experimental design applied to experiment I and II. After their arrival in the laboratory, fruits ($n = 432$) were distributed between three temperatures (12, 16 and 20°C). For each temperature, fruit were divided between boxes and treated with ethylene (e+) ($n = 72$). An equal number of fruit ($n = 72$) was not treated (e⁻) and closed in separated boxes. After 12 hours all boxes were opened and for each treatment (e+, e⁻) and each conditions (12, 16 and 20°C), fruits ($n = 24$) were moved to another temperature and/or kept at the same temperature. Fruit were analysed for respiration rate, climacteric, colour and firmness at specific intervals along 7 days.

3.1.5 Experiment III

Avocado fruit cv. Hass from commercial size 22 were harvested in July from Mpumalanga, South Africa. Once in the laboratory the fruit were held for one night at 5°C. The day after (day 0) the fruit ($n = 288$) were kept in polyester boxes (320 l) at 12

and 20°C. For each temperature, fruit ($n = 36$) were treated for 12 and 24 hours. A same number of fruit ($n = 36 \times 2$) were held closed in the boxes without been treated, as control. The application of ethylene was done as in the previous experiment (section 3.1.4). Briefly, ethylene gas (standard 100%) was applied in the sealed boxes with a plastic syringe until the concentration reached 100 ± 5 ppm. After the treatment, fruit were equally divided between two temperatures ($n = 18$ per temperature) (Figure 3.3). Fruits were measured every day for respiration, climacteric, weight, colour and mesocarp softening, as described in the following sections.

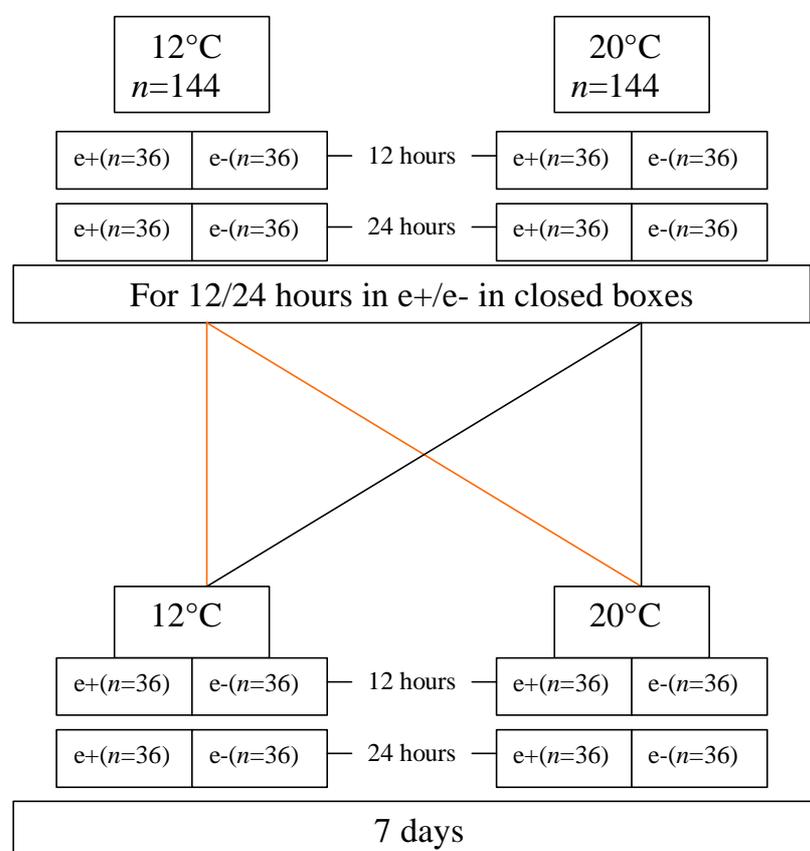


Figure 3.3: Experimental design for experiment III. After their arrival in the laboratory, fruits ($n = 324$) were distributed between two temperatures (12 and 20°C). For each temperature fruit were disposed in boxes and $\frac{1}{4}$ of the fruit ($n = 36$) were ethylene treated (e+) for 12 and another $\frac{1}{4}$ ($n = 36$) for 24 hours. An equal number of fruit was not treated (e⁻) and closed in boxes for 12 ($n = 36$) and 24 hours ($n = 36$). For each

condition ((e+, e⁻ for 12 hours and (e+, e⁻) for 24 hours) after 1 day half of the fruits were moved to another temperature (12 or 20°C). Until fully ripe, fruit were analysed for respiration rate, climacteric, colour and firmness at each day.

3.1.6 Chapter 7

The investigation of the structural carbohydrate present in the mesocarp of avocado fruit was performed on defatted powder (section 3.2.2) of fruit coming from Spain, Malaga, harvested in early and late season (section 3.1.1). For each season fruit were choose regarding day of shelf life (Table 3.1). Three samples were selected from day 0, 2 and 7 of shelf life at 18°C.

Table 3.1: Fruit coming from Spain, early and late seasons, ripen for 7 days at 18°C. Three fruits for each season were choose at day 0, 2 and 7 and analysed in the structural carbohydrate composition of the mesocarp. For each sample is indicated the firmness (N) of the mesocarp.

T 18°C		Spain early		Spain late	
day	sample	Firmness (N)	sample	Firmness (N)	
0	3	252.02	2	188.69	
	5	268.92	7	220.82	
	7	251.65	9	237.43	
2	35	88.26	34	6.755	
	37	25.25	36	10.54	
	39	229.675	38	12.95	
7	67	2.825	69	1.945	
	68	3.615	70	1.84	
	72	2.195	71	1.91	

3.1.7 Weight and colour

Each day of sampling fruit weight (Sartorius laboratory scale, WIB-SAR-019) and objective colour were recorded. The skin colour was measured with Minolta DP-400

chromameter (Minolta Co. Ltd., Japan) with an 8 mm light path aperture, as previously in Meyer and Terry (2008). Each fruit was measured in three points around the equatorial axis. The mean of the three readings was recorded and the lightness (L^*), chroma (C^*) and hue angle (H°) automatically calculated. The instrument was calibrated each day with a Minolta standard white tile CR-400 ($Y = 93.5$, $x = 0.3114$, $y = 0.3190$). The colour measurement was taken every sampling day before others evaluations.

3.1.8 Firmness

Fruit mesocarp softening was measured with an Instron Universal Testing Machine (Model 5542, Bucks., UK) coupled to a 500 N load cell with a flat-head 8 mm probe set at a crosshead speed of 20 mm min^{-1} . Firmness was expressed as the maximum force required for mesocarp tissue failure and recorded as maximum load (N). For each fruit measurements were taken on two opposite sides located in the equatorial zone after a small part of the skin was carefully removed. Fruit was secure on a sand bath located under the probe. Data analysis was performed with Bluehill 2, version 2.11, Instron (Terry *et al.*, 2007).

3.1.9 Carbon dioxide and ethylene quantification

In the present work, the CO_2 and ethylene production were measured by incubating two fruits in a 3 l glass jar for a period of 2 hours at room temperature (20°C) (Terry *et al.*, 2007) (Figure 3.4). In chapter 4, for each temperature of shelf life used (18 and 23°C) five measurements ($n = 10$ fruit) were taken at each sampling day (day 0, 1, 2, 4 and 7). Gas samples were withdrawn with a 60 ml plastic syringe and injected into a gas chromatograph (GC) (Terry *et al.*, 2007). The respiration rate of middle and late season fruit sourced from South Africa and Peru, early and middle season fruit from Chile was analysed using an Agilent 6890N GC (Network GC System, IL, USA) coupled with a hot wire detector (HWD) and a capillary column (30 m long x $530 \mu\text{m}$ x $0.25 \mu\text{m}$ film thickness, Supelco 36245-010A Carboxen 1006 PLOT; Sigma-Aldrich Company Ltd., Dorset, UK). Oven and detector temperatures

were set at 200°C with column temperature programmed at 100°C for 2 min, then increased to 200°C for 4 min.

In all the other experiments the gasses were measured with a GC 8340 (Carlo Erba Instruments, Herts., UK) fitted with an HWD and DP800 integrator (Thermoquest, Herts., UK) in 2 m long stainless steel column packed with Porapak P mesh range 60-80 (Jones Chromatography, Mid Glamorgan, UK). Oven and detector temperatures were set at 80 and 120°C respectively. For all fruit, ethylene production was quantified with a Carlo Erba gas chromatograph (GC 8340) with an EL 980 flame ionisation detector (FID) and DP800 integrator (Thermoquest, Herts., UK) as previously described (Terry *et al.* 2007). Certified standards of 10.06% CO₂ (10% CO₂, 2% O₂, 88% N₂) and 10.6 µl l⁻¹ ethylene (10 µl l⁻¹ ethylene in nitrogen) were used for calibration (Certified Standard from BOC, Surrey, UK).



Figure 3.4: Avocado fruit incubated in jars for the respiration and climacteric measurement (source M. Donetti).

3.2 Biochemical analysis

Fatty acids structures and sugars (non structural carbohydrate) from avocado mesocarp were investigated in chapter 4, whereas chlorophylls, carotenoids, anthocyanins and phenolic compounds from avocado peel tissues is documented in chapter 5.

After the physiological measurements (respiration, weight, colour and firmness), fruit were cut vertically in half as previously described (Landahl *et al.*, 2009). Stone and

peel were removed manually and the mesocarp was immediately chopped into small chunks, mixed and then pooled. Approximately 30 g of pooled sample was snap-frozen in liquid nitrogen and held at -40°C before being freeze-dried for 7 days in Edwards Modulyo (W. Sussex, UK). Dry matter content (DM) was recorded and samples were stored at -40°C until analysed. The peel tissue was snap-frozen separately in liquid nitrogen and held at -40°C before being freeze-dried for 3-4 days.

3.2.1 Chemicals

All chemicals were of analytical grade. Acetone, acetic acid, hexane and methanol were purchased from Fisher Scientific Chemicals (Leics, UK); methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, methyl linolenate, sucrose, glucose, fructose, mannoheptulose, catechin, chlorogenic acid and procyanidin B2 external standards were from Sigma (Dorset, UK). Perseitol (D-glycero-D-galactohexitol) was provided by Industrial Research Ltd. (IRL- Fine Chemical, New Zealand). Cyanidin 3-*O*-glucoside was purchased by Extrasynthese (Genay Cedex, France).

3.2.2 Oil extraction

The oil fraction was extracted from the freeze-dried mesocarp of avocado fruit with a previously described method (Meyer and Terry, 2008). Briefly, lyophilized mesocarp tissue was ground to a powder (1 g DW), homogenized repeatedly in hexane and then filtered through a 5.5 cm diameter Fisherbrand QL 100 filter paper (Fisher Scientific, Leics, UK). The lipid fraction was recovered using a rotary evaporator (Buchi Rotovapor, Büchi Labortechnik AG, Flawil, Switzerland) under vacuum at 40°C. The oil was stored in amber vials under nitrogen at -40°C until use.

3.2.3 Non-structural carbohydrates extraction

The avocado mesocarp reduced to a fine powder from the defatted process was recovered and stored at -40°C. The sugar compounds present in this were analysed according to Meyer and Terry (2008) with modification. Briefly, 100 mg of powder residue was combined with 3 ml of 62.5% aqueous methanol (v/v) and placed in a shaking water bath at 55°C for 15 min and vortexed (Vortex Genie 2, Scientific

Industries, NY, USA) for 20 seconds at 5 min intervals. Samples were filtered through syringe filters (0.2 µm pore diameter; Millipore, MA, USA) and stored at -40°C until needed. Before analysis extracts were diluted 1:10 with water (HPLC grade).

3.2.4 Total chlorophylls and carotenoids

The chlorophylls content of avocado skin was measured as previously described by Lancaster and colleagues (1997), with some modifications. The freeze-dried peel was ground into powder and 150 mg of this was extracted with a solution of 80% acetone in HPLC water (v/v) in a 15 ml volume dark vial glass at room temperature. The powder was mixed with 5 ml of solution, vortex for 10 seconds and left at room temperature for 5 minutes. The powder was re-extracted twice leaving the mixture standing for 3 minutes each time. After each extraction the solution was filtered (0.2 µm diameter size). The fractions extracted were collected, mixed and measured with a Camspec M501 UV/vis spectrophotometer (Camspec Ltd., Cambs., UK) at 663.2, 646.8 and 470 nm respectively for chlorophyll *a*, *b* and total carotenoids (Lichtenthaler, 1987) (Figure 3.5). The respective concentrations were calculated by the equations described by Lichtenthaler (1987).

3.2.5 Anthocyanin and main phenolics

The peel tissue of avocado fruit cv. Hass coming from Spain, harvested in the three main seasons, was investigated in its anthocyanin (cyanidin 3-*O*-glucoside) content. The freeze-dried avocado peel powder (150 mg) was mixed with 3 ml of 70:29.5:0.5 methanol: water: hydrochloric acid (v/v/v) and vortexed to mix thoroughly (Giné Bordonaba and Terry, 2008). The dark vials were incubated at 37°C for 1.5 h in a shaking water bath vortexing for 20 seconds every 15 min. Cooled samples were filtered through a 0.2 µm Millex-GV syringe driven filter unit and stored at -40°C until required (Giné Bordonaba and Terry, 2008) (Figure 3.5).

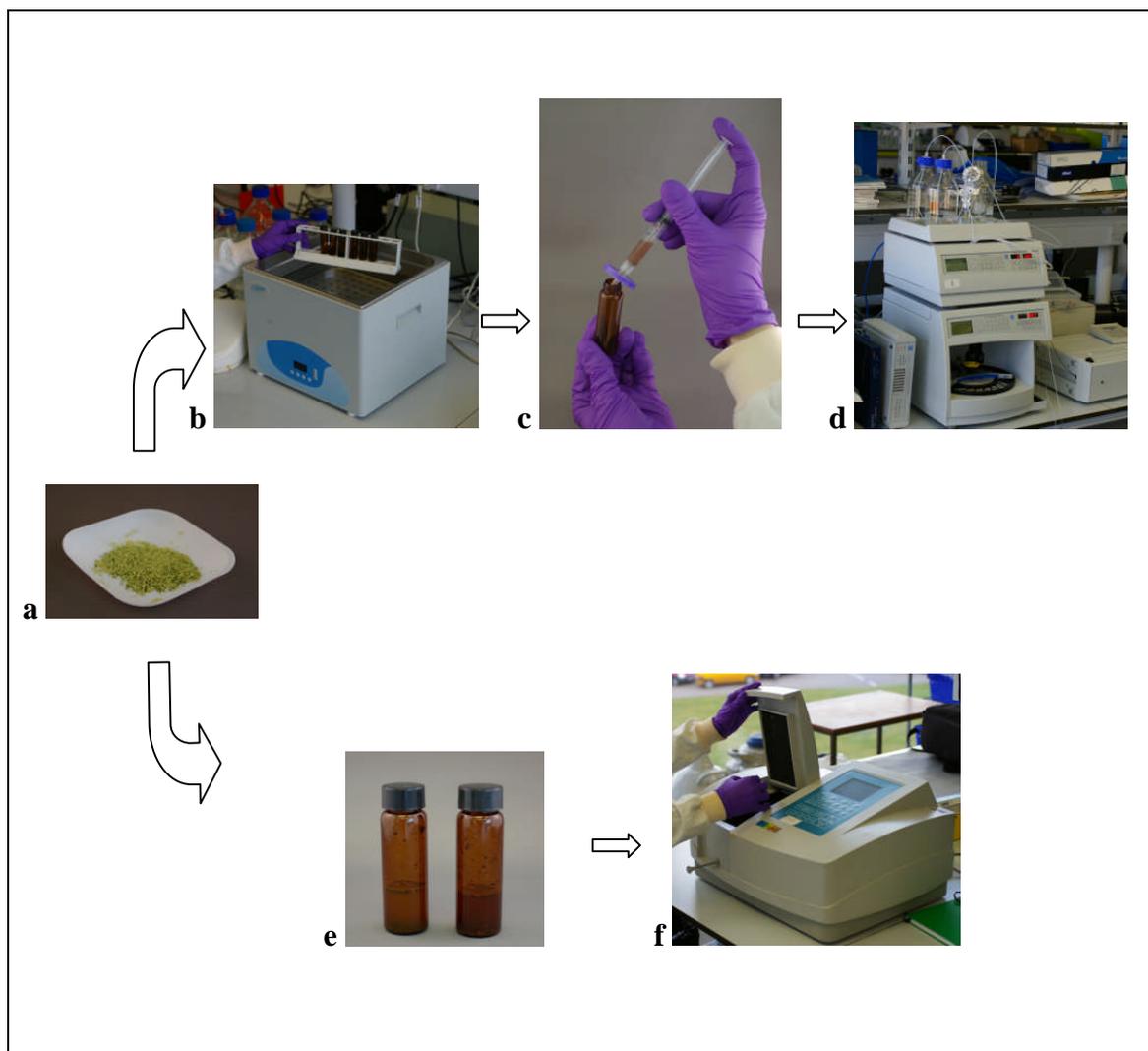


Figure 3.5: Description of the procedures for the extraction and analysis of anthocyanin and others pigments (chlorophylls and carotenoids) from avocado skin. For the anthocyanin extraction the freeze-dried skin reduced to powder (a) was extracted with the solvent at 70°C (b) for 1.30 h. The mixture was than filtered (c) and the extract analysed with HPLC Dionex (d). The same powder (a) was used and extracted in a solution of acetone (e) the total carotenoids, chlorophyll *a* and *b* were quantified with the spectrophotometer (f).

3.2.6 Structural carbohydrate

The alcohol insoluble material (AIM) was extracted as previously described (Ordaz-Ortiz *et al.*, 2009) with modifications. Defatted freeze dried avocado mesocarp

(1 g) (3.2.2) was mixed in 10 ml of 80% (v/v) ethanol solution in a falcon tube of a known weight. After being cooled down, the tube was centrifuge for 20 min at $4,193 \times g$ at room temperature (Heraeus Labofuge 200R, Thermo Scientific). The supernatant was discarded and another 10 ml of ethanol 80% (v/v) was added and the sample centrifuged again. The same procedure was repeated five times until the sample was free from soluble sugars. The detection of the soluble sugars was done with the phenol-sulphuric acid test (Dubois *et al.*, 1956) (Figure 3.6). The sample was washed once with 10 ml of absolute ethanol, centrifuged, and finally with 10 ml 100% acetone. The residue was freeze dried overnight and weighed.

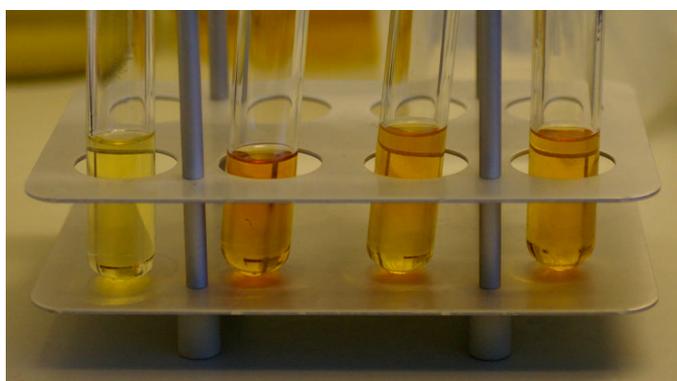


Figure 3.6: Phenol-sulphuric acid test (Dubois *et al.*, 1956). In the first tube to 0.5 ml of a known concentration of mannoheptulose ($10 \mu\text{g ml}^{-1}$ concentration) is added to 0.5 ml of phenol solution (6.25 ml of phenol 5% in 93.75 ml water (v/v)) and 2.5 ml of sulphuric acid (96%) and used as standard. In the following tubes are present samples (0.5 ml) with the phenol-sulphuric acid mixture. The different coloration between tubes indicates that in the samples are still detectable sugars.

The water soluble solids were extracted by adding 10 ml of deionised water to the previous residue, followed by gentle stirring for a duration of 30 minutes at room temperature. The mixture was centrifuged, the supernatant recovered and freeze-dried. The residue was recovered and 10 ml of CDTA (0.1 M in water) (Idranal IV, 1,2-diaminocyclohexanetetraacetic acid tetrasodium salt solution) in 0.1 M sodium acetate, (pH 6.5) were added, centrifuged as previously and left for 6 hours shaking at room temperature. After being centrifuged, the extraction was repeated overnight at 4°C . The supernatant from each extraction was collected and dialysed in membranes (3 Kdalton

cut off, SnakeSkin Plated dialysis tubing, Thermo Scientific, USA) against deionised water in gentle stirring at 4°C for 36 h changing the water 3 times per day. The residue from the previous extraction was mixed with 10 ml of Na₂CO₃ (0.05 M) in 20 mM of NaBH₄ overnight at 4°C for 3 h at room temperature. After each extraction the sample was centrifuged, the supernatant collected, the pH adjusted to 7 with glacial acetic acid (HPLC grade), and dialysed as previously described. On the residue, was performed a series of extractions with different concentration of KOH. At first 8 ml of 0.05 M KOH were added to the residue and left for 1 h at 4°C. In the second step, 1 M solution of KOH (1 hour at 20°C) was used and the last extraction was performed with 4 M KOH (1 hour at 20°C). After each extraction the slurry was centrifuged, the supernatant collected and the pH adjusted to 7 with addition of glacial acetic acid (HPLC grade). All the extracts were reduced under vacuum with rotary evaporator in water bath (70°C) and subsequently freeze dried (-120°C) for two days. The residue was weighed and diluted in ultrapure water before been stored at -40°C.

3.3 Quantification methods

3.3.1 Fatty acids

The fatty acid methyl esters (FAMES) were obtained according to Meyer and Terry (2008). From the previously extracted oil (3.2.2), 0.1 g was weighed out and dissolved in 2 ml of hexane and mixed 0.2 ml of methanolic KOH (0.2 M) in a plastic vial which was manually shaken for 30 seconds. The upper layer containing the methyl esters was decanted in a separate vial and analysed on the same day. After dilution 1:100 with hexane (GC grade), the extract was analysed using an Agilent 6890N GC equipped with a flame ionisation detector and a 7683B autosampler (Agilent Technologies). Fatty acids were identified and profiled on a CP-Sil 88 fused silica capillary column (30 m x 0.25 mm i.d., 0.2 µm film thickness; Varian, CA, USA). The run parameters were in accordance with that of Meyer and Terry (2008). Fatty acids were identified and concentrations calculated by comparing the peak area of the sample with the peak area of a known concentration standards (methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, methyl linolenate).

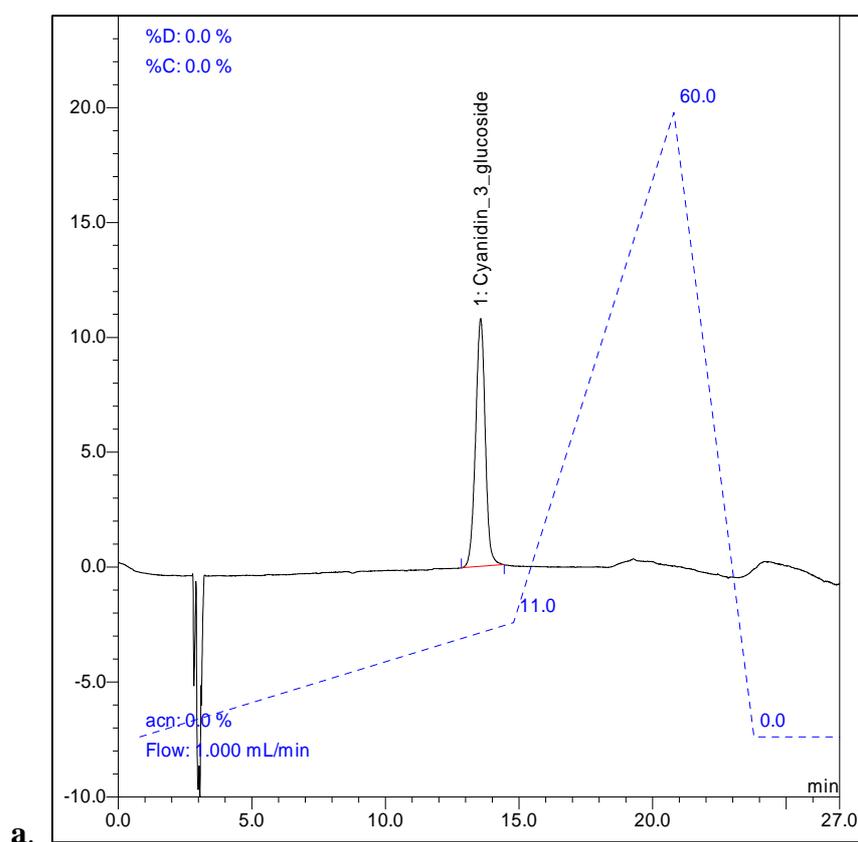
3.3.2 Non-structural carbohydrates

The main avocado sugars (mannoheptulose, perseitol and sucrose) were identified and quantified according to Meyer and Terry (2008), with slight modifications, using an Agilent 1200 series HPLC. Avocado extract (20 μ l) and standard sugar solutions were injected into a Rezex RCM monosaccharide Ca^+ (8%) (300 mm x 7.8 mm diameter, 8 μ m particle size; Phenomenex, Torrance, CA; part no.00H-0130-K0) with a Carbo- Ca^{2+} security guard cartridge (4 mm x 3 mm diameter; Phenomenex). The elution of the non-structural carbohydrates from the sugars extract was monitored using a refractive index detector (Agilent Technologies 1200 Series, G1362A). The presence and the abundance of the selected soluble sugars were automatically calculated by comparison with the standards peak areas.

3.3.3 Optimization of the parameters for the detection and quantification of anthocyanin

The quantification of the anthocyanin and the main phenolic compounds was done referring to the work of Tsao and Yang (2003). The filtrate obtained from the previous extraction (section 3.1.9) (10 μ l) was injected into an Agilent ZORBAX Eclipse (XDB-C18 column, 4.6 mm x 150 mm, 5 μ m particle size) with an Agilent ZORBAX Eclipse XDB guard column (1.0 mm x 17 mm). The binary mobile phase was represented by acetonitrile 100% (solvent B) and 2 mM sodium acetate in water with acetic acid (solvent A). Solvent A was changed from 6 up to 8% acetic acid in order to reduce the elution time of the peaks. All solvents were filtered and degassed before use. The system was run with a Dionex pump (P 680 HPLC) at a constant flow rate of 1 ml min^{-1} . The UV detector (Photodiode Array, PDA-100) was set up at 280 and 530 nm for the detection of phenolic compounds and anthocyanin, respectively. The gradient between solvent A and B was given different elution times to the compounds. Starting from a 20% of solvent A with a better definition of the peaks but with higher retention time, the more suitable conditions were found in: 0-11% of B in 14 min, 14-60% of B in 20 min, 60-0% B in 23 min with 4 min of post-run at 0% B. These parameters allowed the identification of the cyanidin 3-*O*-glucoside in shorter time (23

min.) compared with previous work (Cox *et al.*, 2004, 30 minutes) and gives additionally the advantage to separate all the phenolic compounds identified with the relative standard using an additional wavelength in the detector (280 nm) (Figure 3.7). In case the peaks here did not identify with specific standard compounds, it will be appropriate to modify the parameters to allow a better separation of the unknown peaks.



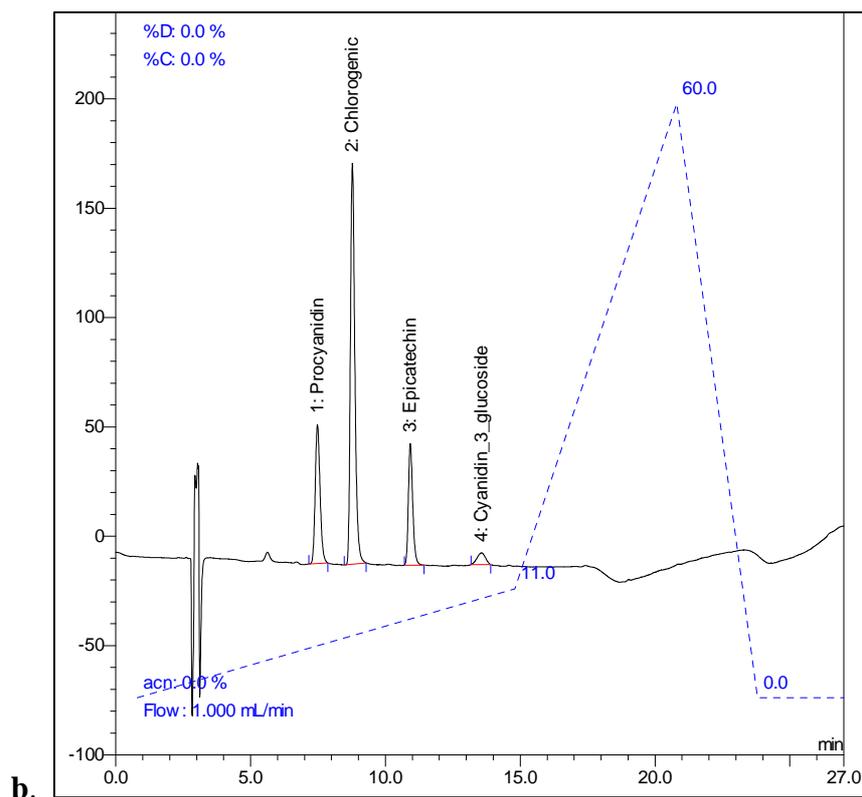


Figure 3.7: Chromatograms of cyanidin 3-*O*-glucoside (a), procyanidin B2, chlorogenic acid and epicatechin (b) standard detected at 360 and 280 nm, respectively. The gradient of solvent B (%) used for the detection is indicated (blue line) with a flow rate (1 ml per minute).

3.3.4 Total chlorophylls and carotenoids

The total chlorophylls and carotenoids content was measured with a Camspec M501 UV/vis spectrophotometer (Camspec Ltd., Cambs., UK) at 663.2, 646.8 and 470 nm respectively for chlorophyll *a*, *b* and total carotenoids (Lichtenthaler, 1987). The respective concentrations were calculated by the equations described by Lichtenthaler (1987).

3.3.5 Immunodetection of structural carbohydrate (ELISA)

The polysaccharides extracted were detected with immunodetection method (ELISA) as previously described (Ordaz-Ortiz *et al.*, 2009). In principle the method

aims to identify main structural carbohydrate using primary antibody specifically designed to recognise a site distinguishing the carbohydrate. The sample was adjusted to a concentration of $500 \mu\text{g ml}^{-1}$ in water and was used as the immobilized antigen. The antigen ($100 \mu\text{l}$) was added in each well of a microtitre plate (Immuno MaxiSorb 96 well, Thermo Scientific Nunc, USA) and incubated overnight at 4°C . After the removal of the antigen, with repetitive washing using water, all the binding site were blocked with $200 \mu\text{l}$ per well of 3% low fat powder milk in phosphate-buffered saline solution (w/v) (3% PBS milk) for 2 h at room temperature. Plates were washed with water and $100 \mu\text{l}$ of primary antibody diluted (1/25) in 3% PBS milk was added in each well and incubated for 1.5 hr. The primary antibodies tested are listed in the table below (Table 3.2).

Table 3.2: List of the primary antibodies used in the characterization of the structural carbohydrate present in the mesocarp of avocado fruit. For each antibody is indicated the binding site, the polysaccharide recognised and the class which they belong to.

Reference	Antibody	Site recognized	Polysaccharides	Class
Jones <i>et al.</i> 1997	LM 5	(1→4) β -D-galactan	Rhamnogalacturonan I	pectins
Willats <i>et al.</i> 1998	LM 6	(1→5) α -L-arabinan anti-arabinogalactan- protein	Rhamnogalacturonan I	pectins
McCartney <i>et al.</i> 2005	LM 10	(1→4)- β -D-xylan	Xylan	hemicellulose
McCartney <i>et al.</i> 2005	LM 11	(1→4)- β -D-xylan	Arabino-xylan	hemicellulose
Marcus <i>et al.</i> 2008	LM 15	XXXG motif of xyloglucans	Anti-xyloglucan	hemicellulose
Verhertbruggen <i>et al.</i> 2009	LM 19	partially Me-HG/de- esterified HG	Homogalacturonan (HG)	pectins
Verhertbruggen <i>et al.</i> 2009	LM 20	partially Me-HG	Homogalacturonan (HG)	pectins
Marcus <i>et al.</i> 2010	LM 21	(galacto)(gluco)mannan	Mannan	hemicellulose
Marcus <i>et al.</i> 2010	LM 22	(gluco)mannan	Mannan	hemicellulose

The plate was washed and $100 \mu\text{l}$ /well of secondary antibody (anti-rat IgG (whole molecule) coupled to horseradish peroxidase (HRP), Sigma-Aldrich) diluted in

the region of 1000-fold in 3% PBS milk was added and incubated for an additional 1.5 h at room temperature. The presence of antibody attached to the sample was detected by adding 150 μl per well of HRP substrate prepared immediately before use (18 ml de-ionized water; 2 ml of 1 M sodium acetate buffer, pH 6.0; 200 μl tetramethylbenzidine; 20 μl of 6% (v/v) hydrogen peroxide) with development of a blue coloration. The reaction was stopped by addition of 120 μl per well of 2.5 M sulphuric acid after three minutes (Figure 3.8). The absorbances were determined at 450 nm in ELISA plate reader.

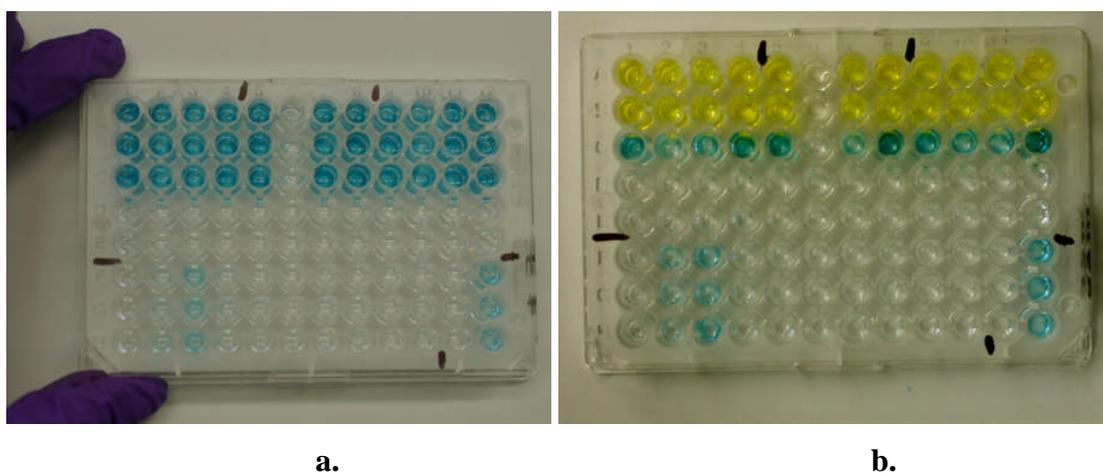


Figure 3.8: The presence of the secondary antibody attached to the primary antibody is revealed with the addition of 100 μl per wells of HRP solution (18 ml de-ionized water; 2 ml of 1 M sodium acetate buffer, pH 6.0; 200 μl tetramethylbenzidine; 20 μl of 6% (v/v) hydrogen peroxide) (a) and the reaction is successively stopped with the addition of 35 μl of sulphuric acid (2.5 M) (b).

3.4 Statistical analysis

All statistical analyses were carried out using Genstat for Windows, version 9.1.0.147 (VSN International Ltd., Herts, UK). Least significant difference values (LSD; $P < 0.05$) were calculated for mean separation using critical values of t for two-tailed tests. In Chapter 4 each value was a mean of 8 or 10 measurements for experiment A (firmness, colour measurement and the biochemical analysis) and B (weight, ethylene and CO_2 production), respectively. The ethylene released and

respiration rate were evaluated from the same fruit and thus the statistical analysis for experiment B was carried out considering 5 replicates (jars) for each temperature for each day of measurement. A Principal Component Analysis (PCA) was calculated with a full cross validation as previously done (Landahl *et al.*, 2009) using Unscramble version 9.8 (CAMO Software, AS, Norway). A regression between firmness, hue angle and cyanidin 3-*O*-glucoside was calculated with Sigma with an exponential growth curve (*cf.* Chapter 5). In Chapter 6, the statistical analysis was used to identify the main effects and interactions between factors such as shelf life temperature, ethylene application, days of shelf life and, in the experiment III, also the exposure time to ethylene (12-24 hours). Due to an imbalance in the number of samples between day 0, day 1 and the following days, two baselines were used; baseline 1 (day 0) and baseline 2 (day 1), to which the remaining time points were compared.

PART B: METHOD DEVELOPMENT

Development of a novel extraction, identification and quantification method for the main phenolic compounds in the mesocarp of avocado fruit cv. Hass.

3.5 Introduction

Phenolics are secondary plant metabolites which have been studied in many fruits and vegetables. Consumption in some socioeconomic groupings has increased as a result of consumers' awareness of their related health properties (i.e. antioxidant, antiallergic, cardioprotective activity) (Ascherio *et al.*, 1992; Lodovici *et al.*, 2008; Zhao and Moghadasian, 2008). In particular, phenolic derivatives are well known for their attributed antioxidant activity (Heim *et al.*, 2002; Seeram *et al.*, 2006; Sultana and Anwar, 2008) and their regular consumption has been associated with decreased incidence of human diseases (Ascherio *et al.*, 1992; Lodovici *et al.*, 2008; Zhao and Moghadasian, 2008). The effective benefit of these compounds depends to their absorption (i.e. bioavailability) through the human body (Holst and Williamson, 2008) and their structure (Balasundram *et al.*, 2006). However, it is still unclear and difficult

to establish the role of each single ingredient on human metabolism considering the impact of genetics and individual response to certain compounds (Holst and Williamson, 2008).

In plant systems, phenolics are responsible for the determination of colour, taste and are involved in the response to stress conditions (Balasundram *et al.*, 2006). Phenolics are one of the most widespread groups of plant metabolites with a wide range of structures (Naczki and Shahidi, 2006). They can be present as free phenolic acids or bound with other molecules such as sugars, or polymerized into more complex structures such as flavonoids, tannins or lignin (Rice-Evans *et al.*, 1996; Tomás-Barberán and Robins, 1997). Phenolics can have different locations in the cell depending on their structure. For instance, insoluble compounds are mainly present in cell walls whereas in the vacuoles more soluble phenolics are found (Balasundram *et al.*, 2006; Naczki and Shahidi, 2006). The type and quantity of phenolics present in the plant change in response to environmental conditions. For instance, in strawberry fruit increased phenolic content has been related to water stress (Terry *et al.*, 2007b).

The identification of phenolics present in fruits and vegetables is often a complex task due to their widespread nature. The identification of a more efficient method to extract phenolics from the natural matrix is a first task. Indeed, depending on their structure, phenolics can be more or less soluble in a solvent and respond differently to extraction conditions, such as acidity or temperature (Bravo, 1998; Kalt *et al.*, 2001; Naczki and Shahidi, 2006).

The phenolic content of avocado fruit is particularly low, compared with others fruit (Arts *et al.*, 2000; du Pascual-Teresa *et al.*, 2000). However, due to the importance of these structures on plant metabolism and on the human diet, some studies focussed on their identification in different tissues (Ramirez and Luh, 1973; Golan *et al.*, 1977; Torres *et al.*, 1987; Arts *et al.*, 2000; du Pascual *et al.*, 2000; Harnly *et al.*, 2006). Additionally, phenolics are the main substrate for postharvest browning in avocado fruit (Kahn, 1983; Van Rooyen and Bower, 2003) and one of the major problems for processed avocado (Soliva *et al.*, 2001). Previous investigations have found p-coumaric, epicatechin, caffeic acid (Ramirez-Martinez and Luh, 1973; Golan *et al.*, 1977), protocatechin and ferulic acids (Golan *et al.*, 1977) in cv. Fuerte (Ramirez-Martinez and

Luh, 1973; Golan *et al.*, 1977) as well as in an unnamed variety (Prabah and Patwardhan, 1980). Ramirez-Martinez and Luh (1973) also identified low amounts of chlorogenic acid, leuco-anthocyanins and p-coumarylquinic acid in the mesocarp of cv. Fuerte. These results have been supported by Torres and colleagues (1987) who identified by means of standard HPLC, benzoic acid derivatives (p-coumaric, ferulic, p-hydroxi, protocatechic, vanillic, synergic acids) and cinnamic derivatives (caffeic and sinapic acids). More recently, investigations by Arts (2000) and du Pascual Teresa groups (2000) identified epicatechins ($0.06\text{-}0.08 \mu\text{g g}^{-1}$ FW) in the edible part of avocado (cv. not indicated). In contrast, Mattila and Hellström (2006) extracted and quantified mainly phenolic acids in the avocado mesocarp of an unspecific cultivar. In a more recent study, 17 different phenolic compounds were identified from the methanol extract of the mesocarp of cv. Hass, Lamb Hass and Rugoro (Hurtado-Fernàndez *et al.*, in press) but these were unfortunately not quantified.

The extraction and quantification of the phenolics present in plant tissues needs to be made easier and more rapid for routine assessment. Simple methods are already present in the literature for fruits and vegetables with higher phenolic content (Hertog *et al.*, 1992; Hakkinen *et al.*, 1998; Nutilla *et al.*, 2002, Giné Bordonaba and Terry, 2008). Unfortunately, due to the only recent popularity and low antioxidant content of avocado, few works have described the identification of phenolic compounds in avocado fruit. Moreover, the use of different methods by previous investigations (Ramirez-Martinez and Luh, 1973; Golan *et al.*, 1977; Mattila and Hellström, 2006; Hurtado-Fernàndez *et al.*, in press) make the comparison of results more problematic. This said, the aim of this work was to optimize the extraction and the analytical method for the identification and quantification of the main phenolics present in the mesocarp of avocado fruit cv. Hass.

Based on the work of Hertog and colleagues (1992) who aimed to identify and optimize the extraction method for the analysis of individual phenolics in berries, different solvents and extractive conditions were compared. Methanol (Sultana and Anwar, 2008; Giné Bordonaba and Terry, 2008), ethanol (Soong and Barlow, 2004), acetone (Aaby *et al.*, 2007), and ether derivatives (Torres *et al.*, 1987) are the most common solvents used to isolate phenolics from many commodities. In this work, we

compared the efficiency of extraction with three different solvents (methanol-, ethanol- and ether-based), different temperature, incubation time, acidity and addition of an antioxidant. The extracts were analysed by HPLC and compared with external standards.

3.6 Material

Avocado fruits cv. Hass harvested in Spain, in February, were ripened for 7 days at 18°C (*cf.* section 3.1.1). Ripe avocado fruit was peeled, and the mesocarp cut into small pieces, snap frozen, freeze-dried and kept at -40°C until analysed. The oil fraction was separated with a previously published method (Meyer and Terry, 2008) and the powder residue was kept at -40°C until analysed.

Caffeic, ferulic, gallic, p-coumaric, sinapic acids were used as standards provided by Sigma Aldrich. Epicatechin and procyanidin were provided by Fluka Analytical. All standards were provided as powders and dissolved in 50% methanol: water (v/v) HPLC grade.

3.7 Extractive method

The oil fraction was excluded from the freeze-dried sample by a previous method (Meyer and Terry, 2008) (*cf.* section 3.2.2) to avoid interference of fatty compounds in the further analysis. The powder residue was used to perform different extractions.

A. 5 mL of methanol: water: HCl (80:19.5:0.5 v/v/v) and/or ethanol: water: HCl (80:29.05:0.5 v/v/v) were mixed thoroughly with avocado mesocarp powder (100 mg) for 15 seconds using a vortex mixer. For each solvent the extraction was performed at 5°C overnight and at 70°C for 2 hours in a water bath, shaking for 15 seconds every 30 minutes. Addition of 0.1% BHT (butylated hydroxytoluene) to the methanol-based solvent (methanol: water: HCl (80:19.5:0.5 v/v/v)) was also performed at 70°C for 2 hours.

B. A recent methodology (Mattila and Hellström, 2006) (Table 3.3) was compared with previous extractions. Briefly, 150 mg powder was mixed with 7 ml of methanol, acetic acid and BHT, homogenized for 10 seconds and ultrasonicated for 30

minutes. Water (HPLC grade) was added to a final volume of 10 mL and mixed. The solution (1 ml) was filtered (0.2 μm) and kept under nitrogen until analysed for free phenolics. Water (12 ml, HPLC grade), 1% ascorbic acid and 0.415 % EDTA (ethylenediaminetetraacetic acid) was added to the remaining solution and mixed with 5 mL of NaOH (10 M) for a basic hydrolysis. The mixture was left in agitation at room temperature for 16 hours. The solution was adjusted to pH 2 and was washed three times with 15 mL of diethyl ether/ethyl acetate (DE/AE) (1:1 v/v). The three organic layers were mixed, purged under nitrogen and dissolved in 1 ml of methanol. The inorganic residue was mixed with 2.5 ml of concentrate (36%) HCl and left at 85°C for 30 minutes in a water bath. The pH was adjusted to 2 and the sample was washed three times with DE/AE (1:1 v/v). As previously mentioned, the three organic layers were pooled, reduced and stored in 1 ml of methanol. Before analysis, the three extracts containing free phenolics and phenolics from basic and acid extracts were pooled.

All extracts were filtered (0.2 μm diameter) and kept at -20°C in dark glass vials until analysed.

Table 3.3: List of the main studies on the phenolic characterization of avocado fruit. For each work the solvent is indicated and the analytical method used and the list of the compounds identified.

Authors	Extraction	Identification	Compounds identified
Ramirez-Martinez and Luh, 1973	70% methyl alcohol + 0.005 M-L-cysteine, at 4°C. Successive extractions in chloroform and petroleum ether. The aqueous phase was saturated with NaCl and extracted 4 times with ethyl acetate.	TLC	chlorogenic; p-coumarylquinic; catechins and epicatechins; caffeic; p-coumaric; leucoanthocyanidins; proanthocyanidins; isoflavones
Golan <i>et al.</i> , 1977	Cold acetone (-20°C) ratio 1:3, chloroform and petroleum ether (60-80°C). The aqueous phase saturated with NaCl and extracted 5 times with ethyl acetate.	TLC	chlorogenic acid; p-coumarylquinic; catechins; epicatechins; caffeic; p-coumaric; leucoanthocyanidins; proanthocyanidins; isoflavones; cinnamic; quercetin; 4-methylcatechol

Torres <i>et al.</i> , 1987	Methanol 100% with NaOH added for hydrolysis (overnight). Wash with ether and aqueous extract acidified with HCl at pH2; extracted 3 times with ether. Aqueous layer was acidified to pH 2 with HCl and wash with ethyl ether 3 times.	HPLC	p-hydroxybenzoic (medium), pyrocatechin (low), β - γ resorcylic (low), protocatechin (low), α - resorcylic medium, gallic (low), isovanillic (low), vanillic (low), syringic (low), coumaric o-m- (medium), p-coumaric (high), caffeic (low), sinapic (medium), ferulic.
Arts <i>et al.</i> , 2000	Extraction in aqueous methanol (70-90%) 1 h at room temperature.	HPLC	epicatechin
du Pascual-Teresa, 2000	Cold methanol 3 times and centrifuged.	HPLC	($\mu\text{g/g FW}$): epicatechins (0.8) procyanidin (dimer B2) (0.2)
Mattila and Hellström, 2006	Methanol/BHA (2 g/L) and 10% acetic acid homogenate 30 minutes (soluble phenolic) 12 mL water + 1% ascorbic acid + EDTA and NaOH (10M) (16h at 20°C). Adjust to pH 2 and extracted three times with cold diethyl ether and	HPLC	Soluble phenolic acid ($\mu\text{g/g FW}$) chlorogenic (5.6), ferulic (3.20), sinapic (2.2), p-coumaric (4), unknown (4.2) Total phenolic acids (free, bound, insoluble) ($\mu\text{g/g FW}$)

	ethyl acetate (1:1). Organic layer combined with HCl for acid hydrolysis (85°C for 30 minutes).		caffeic (4.2), ferulic (11), sinapic (9.7), vanillic (2.1), p-coumaric (8.1), p-hydroxybenzoic (1.3), syringic (1.5)
Hurtado-Fernández <i>et al.</i> , in press	Freezed dried sample (4 g) in 40 mL of methanol vortex for 30 minutes and centrifuged for 10 min. Evaporated by dryness and dissolved in methanol.	HPLC/ESI/MS	protocatechic, gentisic, 4-hydroxybenzoic, chlorogenic acids; catechins, caffeic acid, epicatechin, vanillic, p-coumaric, ferulic, sinapic, trans-cinnamic acids; laricitrin, naringenin, chrysin, and kaempferide.

3.8 HPLC parameters

Identification and quantification of the phenolic compounds was carried out using a Dionex P-680 HPLC pump coupled with an ASI-100 automated sample injector. An Agilent Zorbax eclipse XDB-C18 column 5 μm particle size, 250 mm x 44 mm diameter, with a guard column Opti-Guard 1 mm was used. Temperature of the column was set at 30°C and the flow rate at 1 ml min⁻¹. A PDA-100 (photodiode array detector) was set up at wavelengths of 280 and 320 nm. Two solvents were used in a binary system: solvent A (8% acetic acid in 2 mM sodium acetate in water (92:8 v/v)) and solvent B (100% acetonitrile). For initial conditions, solvent A was set up at 100%, with increase of solvent B to 10% after 12 min, to 1% at 22 min and to 80% at 26 min. A post-run condition with 100% of solvent A was held for 5 min. One main unknown peak was detected in the first 5 min. and minor compounds were also detected between the chlorogenic acid (8.25) and the epicatechin (10.35) but they did not match with any of the external standards (Figure 3.9).

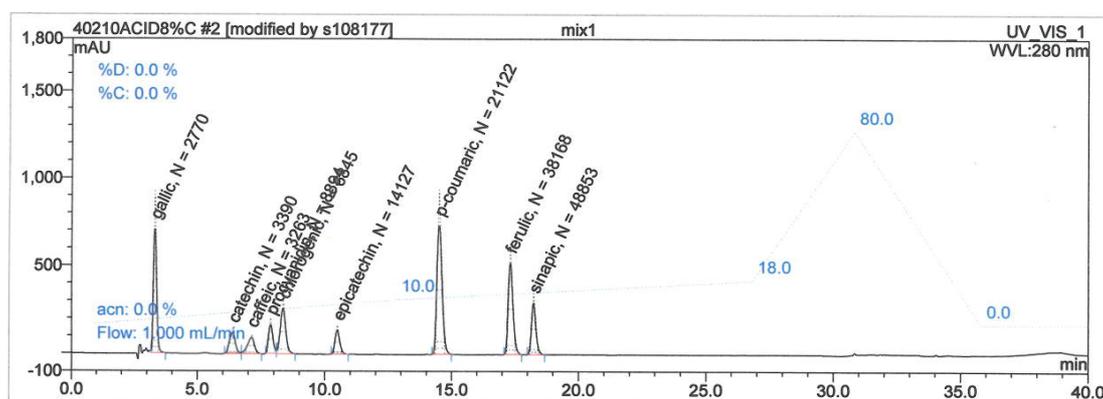


Figure 3.9: Chromatogram of a mixed standard (gallic acid, catechins, caffeic acid, procyanidin, chlorogenic, p-coumaric, ferulic, sinapic acids).

Table 3.4: Summary of the extractive methods used for the investigation of individual phenolics in avocado cv. Hass mesocarp. For each method is indicated the compounds identified expressed in mg on fresh weight.

Extraction method	Phenolics mg/mg FW							
	gallic acid	catechin	caffeic acid	chlorogenic acid	epicatechin	p-coumaric acid	ferulic acid	sinapic acid
EtOH:H₂O:HCl								
(80:19.5:0.5) 5°C	1.63±0.41	0.43±0.43	1.12±0.43	0±0	5.73±0.46	3.38±0.28	1.1±0.06	0±0
MetOH:H₂O:HCl								
(80:19.5:0.5) 5°C	1.25±0.13	0±0	2.82±0.26	0.46±0.46	5.46±2.75	4.5±0.56	1.4±0.22	0±0
EtOH:H₂O:HCl								
(80:19.5:0.5) 70°C	1.31±0.13	0±0	2.19±0.32	2.65±0-39	0±0	22.9±3.7	4.2±0.52	0.45±0.45
MetOH:H₂O:HCl								
(80:19.5:0.5) 70°C	1.25±0.13	1.17±1.17	3.01±1.5	2.78±0.35	5.55±0.24	27.9±0.82	5.2±0.16	2.02±0.22
MetOH:H₂O+0.1%BHT								
(80:20) 70°C	0.86±0.23	0.81±0.81	0±0	1.73±0.97	4.21±0.63	16.1±3.7	2.6±1.41	0±0
MetOH:H₂O:HCl+0.1%								
BHT(80:19.5:0.5) 70°C	1.3±0.58	1.07±0.1	0±0	1.6±0.2	3.6±0.72	14.9±2.1	2.9±0.4	0±0
Hydrolysis	0±0	0±0	0±0	0±0	0±0	4.7±1.5	3±0.41	0±0

Each method was performed in triplicate ± S.E.

3.9 Results and discussion

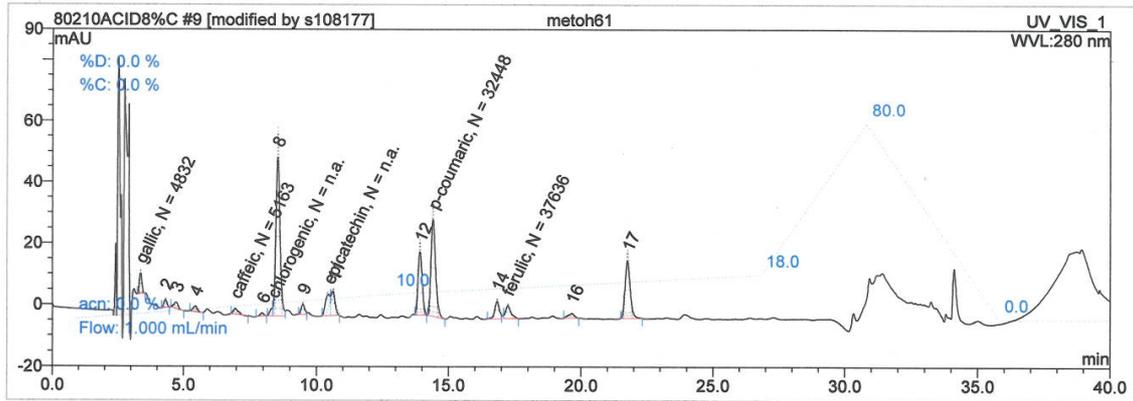
Phenolics present in fruit and vegetable can have various structures from the simple hydroxybenzoic acids to the more complex tannins (Tomás-Barberán and Espin, 2001) which confer them different properties (Rice-Evans *et al.*, 1995). The type and content of phenolics depends on genotype, tissues, but also agronomic treatment, and postharvest handling (Tomás-Barberán and Espin, 2001). The characterization of the phenolic content has been performed for the most common fruit commodities (Arts *et al.*, 2000; du Pascual-Teresa *et al.*, 2000; Harnly *et al.*, 2006). Due to a recent increase in consumption, avocado fruit has become of more scientific interest. Despite the oil and sugar content have been thoroughly investigated (Meyer and Terry, 2008; Landahl *et al.*, 2009), there is still little known about the phenolic composition of avocado fruit (Hurtado-Fernández *et al.*, in press). Additionally, the different methods used for the extraction and the quantification results make the comparison of results more problematic.

Different solvents and extractive procedures were herein compared (Table 3.4). Previous works identified the presence of phenolics acids (Torres *et al.*, 1987; Mattila and Hellström, 2006), procyanidin and epicatechin (Arts *et al.*, 2000; du Pascual-Teresa, 2000; Hurtado-Fernández *et al.*, in press). Between the many methods available in the literature for the phenolics analysis the most used solvents such as methanol, ethanol and diethyl ether were herein tested. The comparison between solvents highlights higher efficiency in recovering phenolic acids when using acid methanol in water. The chemical properties of phenolics compounds depend not only on the structure itself but also to the polymerization with others plant metabolites such as carbohydrates or proteins with a decrease in solubility (Naczka and Shahidi, 2006). The better performance of methanol in water characterised by high polarity could indicate that phenolics compounds in avocado fruit do not have high degree of polymerization. The highest amount of phenolics isolated from the mesocarp of avocado fruit cv. Hass was achieved with an acid methanol solvent (methanol: water: HCl (80:29.05:05 v/v)) at high temperature (70°C for 2 hours). In comparison, extraction with ethanol, characterised by a lower polarity, showed less efficacy particularly for the catechins and epicatechin. Acidified methanol is already a used method for the extraction of phenolics

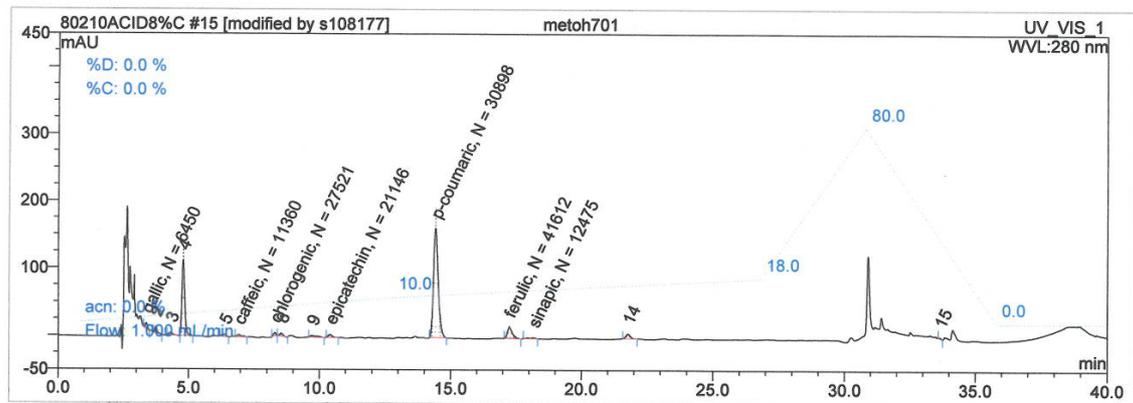
and flavonoids in many commodities (Nuutila *et al.*, 2002; Sultana and Anwar, 2008). Regarding temperature, extraction at 5°C overnight (16 hours) was less efficient than higher temperature for a shorter time (2 hours at 70°C). The breakdown of cell membranes by methanol (Naczka and Shahidi, 2006) might be more effective at 70°C rather than at 5°C. Higher temperatures were not tested due to the possible degradation of compounds such as ferulic, coumaric and cinnamic acids, as previously stated by Nuutila and co-workers (2002).

The addition of an antioxidant (BHT) seemed to have a negative effect on the efficiency of the extraction. This was previously reported with the use of ascorbic acid on onion extracts (Nuutila *et al.*, 2002). Nuutila and colleagues (2002) suggested a possible pro-oxidant action of the antioxidant and indicated it as not being suitable for samples with a predominant content of phenolic acids, such as avocado. Finally, the efficacy of both alkali and acid hydrolysis with successive extraction in diethyl ether and ethyl acetate as previously reported by Mattila and co-worker (2006) was tested. However, using this method, only p-coumaric and ferulic acids could be detected. In this case the hydrolysis conditions might have been too strong and the phenolics identified in the previous extractions may have degraded.

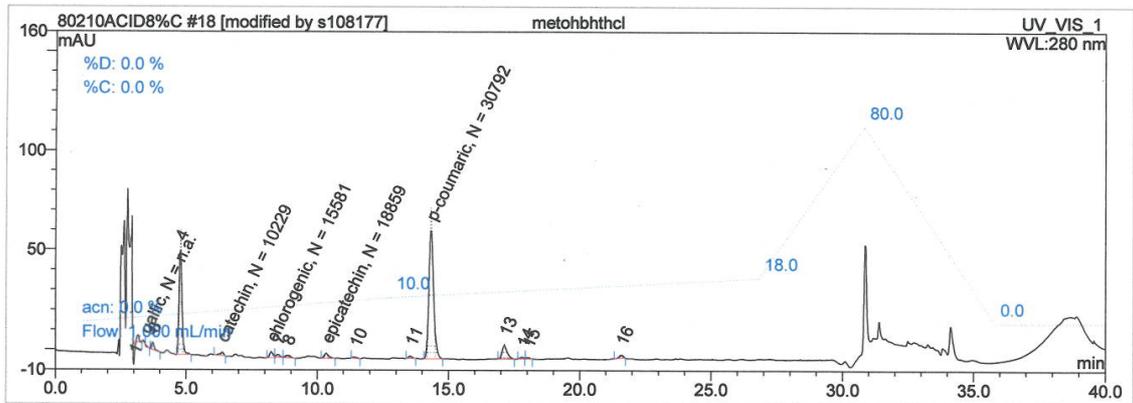
Once the better extractive conditions were identified, an optimization process of HPLC parameters was also performed. The comparison of the chromatograms, with external standards, identified 8 main compounds present in the mesocarp of avocado fruit cv. Hass (Figure 3.10). In order of abundance, p-coumaric (27.9 mg mg⁻¹ FW), epicatechin (5.6), ferulic (5.3), caffeic (3), chlorogenic (3), sinapic (2), gallic acids (1.3), and also with inconsistency between samples, small amounts of catechins were identified in ripe mesocarp. The compounds here identified were in the range of previous work (Mattila and Hellström, 2006) who reported chlorogenic (5.6 µg g⁻¹ FW) ferulic (3.2) sinapic (2.2) p-coumaric (4) as free phenolics and caffeic (4.2) ferulic (11) sinapic (9.7) vanillic (2.1) p-coumaric (8), p-hydroxybenzoic (3) and syringic (1.5) acids in the bound form. In comparison p-coumaric acid was detected in higher concentration whereas ferulic acid was lower. In contrast, in the work presented herein the following were not detected *viz.* vanillic, p-hydroxybenzoic and syringic acids but gallic acid, epicatechin and, not consistently, catechins.



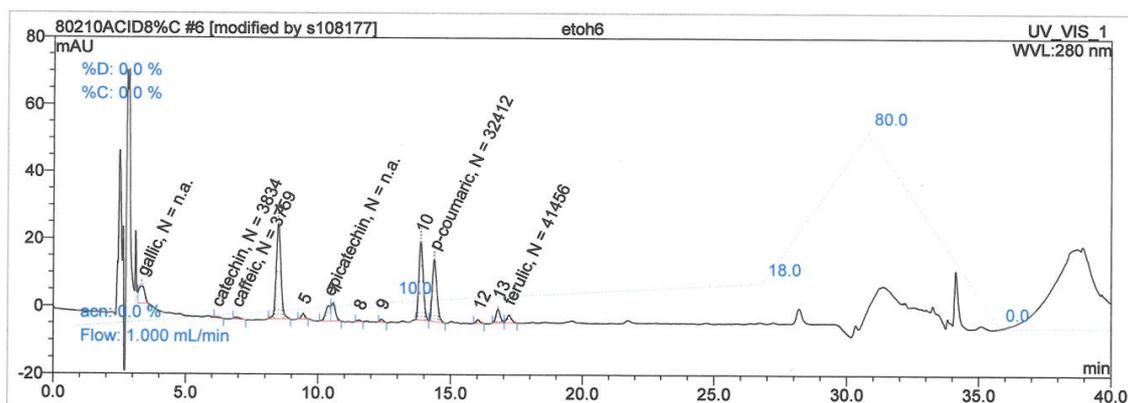
a.



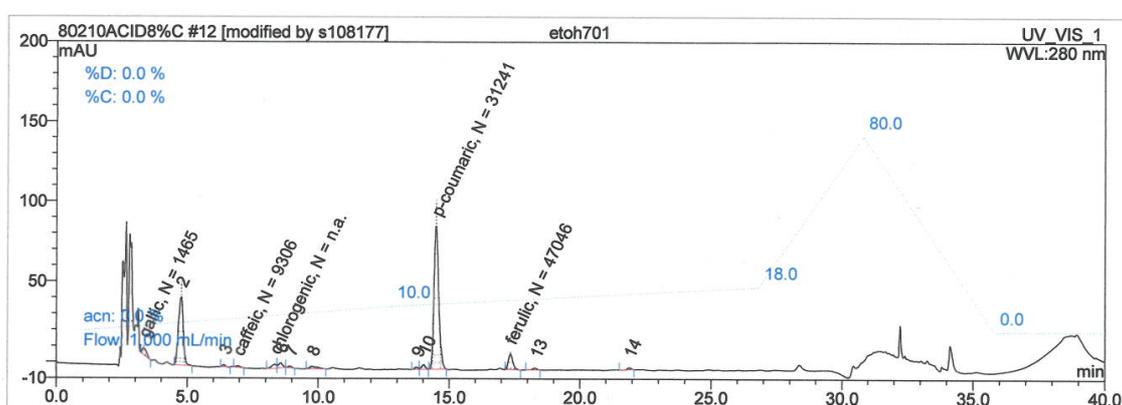
b.



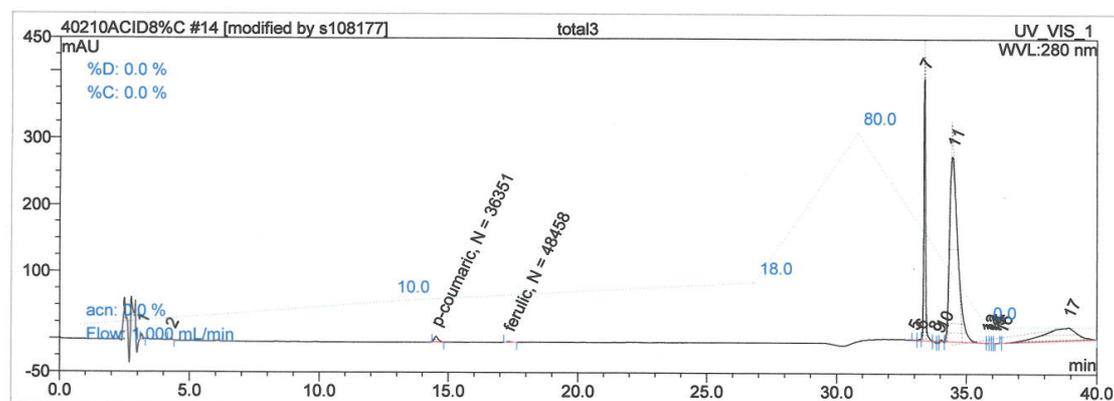
c.



d.



e.



f.

Figure 3.10: Chromatograms for HPLC analysis of ripen avocado fruit cv. Hass mesocarp. Each extractive method (a. methanol: water: HCl (80:19.5:0.5 v/v) at 6°C for 16 hours; b. methanol: water: HCl (80:19.5:0.5 v/v) at 70°C for 2 hours; c. methanol: water: HCl (80:19.05:0.05 v/v/v) +0.1%BHT at 70°C for 2 hours; d. ethanol: water: HCl

(80:19.5:0.5 v/v) at 6°C for 16 hours; e. ethanol: water: HCl (80:19.5:0.5 v/v) at 70°C for 2 hours; f. (Mattila *et al.*, 2006)) shows a different phenolics profile.

A recent investigation on different cultivars of avocado fruit from Spain (Hurtado-Fernández *et al.*, in press) identified without quantification protocatechuic and gentisic acids, yet the absence of chlorogenic acid, in cv. Hass was reported. The different results compared to this work obtained could be due not only to the different methodology used, postharvest conditions and to the possibly different ripening stage of the fruit, not indicated in the previously named work.

3.10 Conclusions

In avocado fruit, phenolics are important secondary metabolites not only for their health related properties but for their involvement in the incidence of pulp browning causing significant losses during postharvest storage. Thus, to have a better understanding of this phenomenon it becomes necessary to investigate changes in the phenolics profile of avocado fruit mesocarp during shelf life. The optimised and validated methodology presented herein is a novel, fast and straightforward procedure to extract and quantify the main phenolics of avocado fruit mesocarp. In ripened avocado cv. Hass fruit the main phenolics were identified as p-coumaric and ferulic acids. In comparison to various methods previously performed acidic methanol seems to be the more efficient solvent for the extraction of individual phenolics from avocado mesocarp cv. Hass, confirming recent work (Hurtado-Fernández *et al.*, in press). A previous separation of the oil fraction is advisable in order to eliminate lipids that could interfere with the phenolics detection. Further analysis with mass spectrometry would be useful to identify the peaks in the chromatogram that did not match the external standards tested. Moreover, it will be of interest to characterise the phenolic content in avocado mesocarp considering different postharvest conditions or treatments to evaluate changes in individual phenolics.

4 CHAPTER FOUR

VARIATION IN THE RIPENING AND BIOCHEMISTRY OF IMPORTED AVOCADO CV. HASS FRUIT FROM DIFFERENT ORIGINS AND MATURITY

4.1 Introduction

Avocado fruit is mainly produced in tropical-subtropical climates. Under normal growth conditions, the ripening process is usually inhibited until the fruit is detached from the tree (HersHKovitz *et al.*, 2009b). As a climacteric fruit, onset of ripening in avocado is accompanied by a rise in ethylene production and respiration rate followed by softening of mesocarp tissue (Adato and Gatiz, 1974) and in the specific case of cv. Hass changes in skin colour (Cox *et al.*, 2004).

Preharvest factors have an important role in determining postharvest behaviour and fruit quality (Ferguson *et al.*, 1999; Woolf *et al.*, 1999, 1999b). Avocado fruit harvested late in the season tend to soften faster (Cutting and Wolstenholme, 1992) and have higher total phenolic content which has been associated with greater susceptibility to physiological disorders (Cutting *et al.*, 1992). Late harvest is also associated with fruits having higher oil content in the mesocarp (Ozdemir and Topuz, 2004). However, no specific relationship has been found between the less quality of late season fruit and the mesocarp dry matter or oil content (Hofman *et al.*, 2000). Generally, the oil fraction of the mesocarp can be up to 70% of the mesocarp dry weight depending on fruit origin, grown location, mesocarp section, and ripening stage (Landahl *et al.*, 2009) and can vary with cultivar (Gomez-Lòpez, 1999), and time (Ozdemir and Topuz, 2004). Avocado oil is mainly composed of the monounsaturated oleic acid (from 50 to 60% of the fatty acid content), saturated palmitic acid (15-20%), unsaturated palmitoleic (6-10%), polyunsaturated linoleic (11-15%) and linolenic acid (*ca.* 1%) (Ozdemir and Topuz, 2004; Meyer and Terry, 2008; Landahl *et al.*, 2009). Differences in the oil composition can be seen across growing region (Landahl *et al.*, 2009), cultivar (Gomez-

López, 1999) and harvest time (Ozdemir and Topuz, 2004) and also differ spatially within the fruit (Landahl *et al.*, 2009) and over time (Ozdemir and Topuz, 2004; Meyer and Terry, 2008). Hence, fruit quality is dependent upon multiple interactions between preharvest variables and postharvest management (Arpaia *et al.*, 2004).

Different postharvest handling techniques for avocado aim to extend shelf life and preserve fruit quality. Limiting factors in the storability of avocado fruit include their highly perishable nature, and their susceptibility to cold storage induced disorders (Cutting and Wolstenholme, 1992). Possible biomarkers related with fruit storability are the non structural carbohydrates, as these represent the main energy source present in the mesocarp tissue (Bertling and Bower, 2005). A peculiarity of avocado is the predominance of C7 rather than the more common C6 carbohydrates (Liu *et al.*, 1999b, 2002). Harvest season, cultivar, tissue type and tissues region can influence the sugars content within the fruit (Bertling and Bower, 2005; Landahl *et al.*, 2009). Regardless of their content, C7 compounds tend to decrease during cold storage and during shelf life suggesting a possible role of these carbohydrates in the fruit postharvest metabolism (Liu *et al.*, 1999b, 2002).

Most of the literature on postharvest handling of avocado has been conducted on fruits with similar preharvest conditions from one country of origin. However, UK retailers depend on a wide range of suppliers for year round supply. Consequently, fruits grown in different parts of the world can be on the market simultaneously. Previous investigations have shown that multiple sources lead to undesired variability in fruit quality at market (Landahl *et al.*, 2009). Rootstocks, growing practices, soil composition and climatic conditions vary between countries. Market research conducted between 2008 - 2009 highlighted an increase in UK customer's complaints (to supermarkets) during the spring/summer season when demand for avocado fruit is highest and this coincides with the South African and Peruvian season. Most of the unsatisfactory responses from customers were related to inconsistency in ripening within a consignment for twin pack and baby avocado (A. Shaw, Mack Multiples Ltd. personal communication).

The aim of this work was to investigate the main physiological and biochemical variables of avocado fruit cv. Hass imported into the UK in the year 2008-09 with

specific emphasis on possible quality differentiation between suppliers. The identification of markers related to fruit origin and season could be helpful in understanding the lack of postharvest uniformity throughout the year. As such, this study compared the effect of preharvest variables (such as origin and harvest time) and postharvest conditioning (such as cold storage and ripening temperatures) on the main ripening indicators and mesocarp constituents of imported avocado cv. Hass fruit. Accordingly, fruits imported to the UK from the main supplier nations *viz.* Spain, South Africa, Peru, and Chile, harvested in each of the three main commercial periods (early, middle and late season) were investigated for differences in physiological parameters (changes in skin colour, mesocarp firmness, respiration rate and ethylene production) and fatty acids and sugars profiles. Potential commercial guidelines distinguishing the characteristic ripening behaviour of avocado fruit associated with origin and/or harvest season were identified in order to provide useful criteria for better postharvest management.

4.2 Plant material

Avocado fruit cv. Hass ($n = 1200$) of commercial size 16 (236-265 g) were sourced in 2008 from Malaga, Spain, in February, March and April; from South Africa, in April from Limpopo, in June and July, from Mpumalanga; from Viru, Peru, in May, June and July 2008, and from Chile, in August, October and January 2009 from the Region de Valparaiso. All fruits were imported by Mack Multiples (Kent, UK) and were held at 5 - 6°C until they reached the UK. Due to the different geographical locations of the suppliers, fruit had different transit times. Spanish fruit had a transit time of < 10 days, South African 24 - 36 days, Peruvian 33 - 39 days and Chilean fruit 35 - 37 days. Once in the laboratory, the fruit were held at 5°C overnight until experiments commenced. Fruits were not pretreated with 1-methylcyclopropene.

4.3 Experimental design

A total number of 12 experiments, one experiment for each harvest time (3) for each country (4), were carried out during one year (February 2008- January 2009). For each experiment 100 fruit were ripened at either 18 or 23°C in two Sanyo incubators

(model MLR-350HT, Ltd, Japan). For each experiment, fruit were randomly divided in two different groups: 80 fruit (Experiment A) were used for the assessment of firmness, colour and the analysis of fatty acids and sugars content throughout shelf-life; 20 fruit (Experiment B) were used for the measurement of respiration, ethylene production and weight. Experiment A: at day 0 (after cold storage) 16 fruit were randomly chosen and measured. The rest of the fruit ($n = 36$) were arranged in two separated test chambers with an internal temperature of 18 or 23 \pm 0.5°C and sampled at specific intervals (day 1, 2, 4 and 7) of shelf life ($n = 4 \times 8 = 32$). Experiment B: for each temperature of shelf life (18 and 23°C) the same 10 fruit were measured each day of shelf life (day 0, 1, 2, 4 and 7).

4.4 Results and discussion

Most previous postharvest research on avocado fruit has not considered the fate and physiological changes which occur within individual avocado consignments at certain times of the year when they reach their intended overseas market. Modern consumers increasingly expect year round consistency even though they may not fully appreciate that avocado fruit are sourced from a diverse set of suppliers and countries. A greater understanding of both the physiological and biochemical variability as related to origin and season might better inform importers on how to achieve greater consistency and reduce customer complaints.

4.4.1 Firmness and colour

The softening process of the mesocarp tissue was significantly affected by day, origin and season ($P < 0.001$) (Figure 4.1, 2). Generally, mesocarp firmness decreased during the first days, and mostly reached values < 100 N at day 4 in fruit from Spain, Chile and Peru. In contrast, mesocarp firmness of early season fruit from South Africa remained high after four days at both temperatures (179.6 and 154 N at 18 and 23°C, respectively). A general trend was observed in fruit harvested late in the season, characterised by higher maturity with concomitant faster softening of the mesocarp regardless of origin. Postharvest temperature significantly affected mesocarp firmness with the higher temperature inducing faster softening particularly for Chilean and late

season fruit from South Africa. Transit time had no particular influence on the firmness changes during ripening. For instance, long storage time (29 days cold storage) of South African early season fruit did not induce a faster softening process compared to Spanish fruit (7 days cold storage/transit).

Changes in the mesocarp firmness were accompanied by variation in skin colour, which confirms results reported by Cox *et al.* (2004). In all experiments skin lightness (L^*) and chroma (C^*) decreased (Table 4.1), and the colour changed from green to dark purple during ripening. Significant differences in hue angle (H°) were found between temperature, day, origin and season (Figure 4.3-4), while C^* and L^* differed mainly between day, origin and season. As expected, a higher ripening temperature significantly accelerated skin colour change, in agreement with previous reports (Cox *et al.*, 2004; Perez *et al.*, 2004). The skin colour of early and middle season fruit from Spain was darker than for fruits from other origins at the end of shelf life as indicated by lower H° values, whereas early season fruits from South Africa maintained greener skin with higher H° , 107.3 and 57.7 at 18 and 23°C, respectively. In general, late season fruit were characterised by a more rapid decrease in H° when compared with fruit from other seasons regardless of origin.

Generally, mesocarp softening was noted to be more consistent in fruit from Spain as indicated by a lower standard error of the firmness measurements at day 7 (0.262, 0.075 and 0.084 in early, middle and late season fruit, respectively). In contrast, fruits from South Africa showed the highest variability in the ripening process, (S.E. = 13.97, 17.31 and 6.00 for early, middle and late season fruit, respectively) accordingly have prone to higher costumers complain (A. Shaw Mack Multiples Ltd. personal communication)

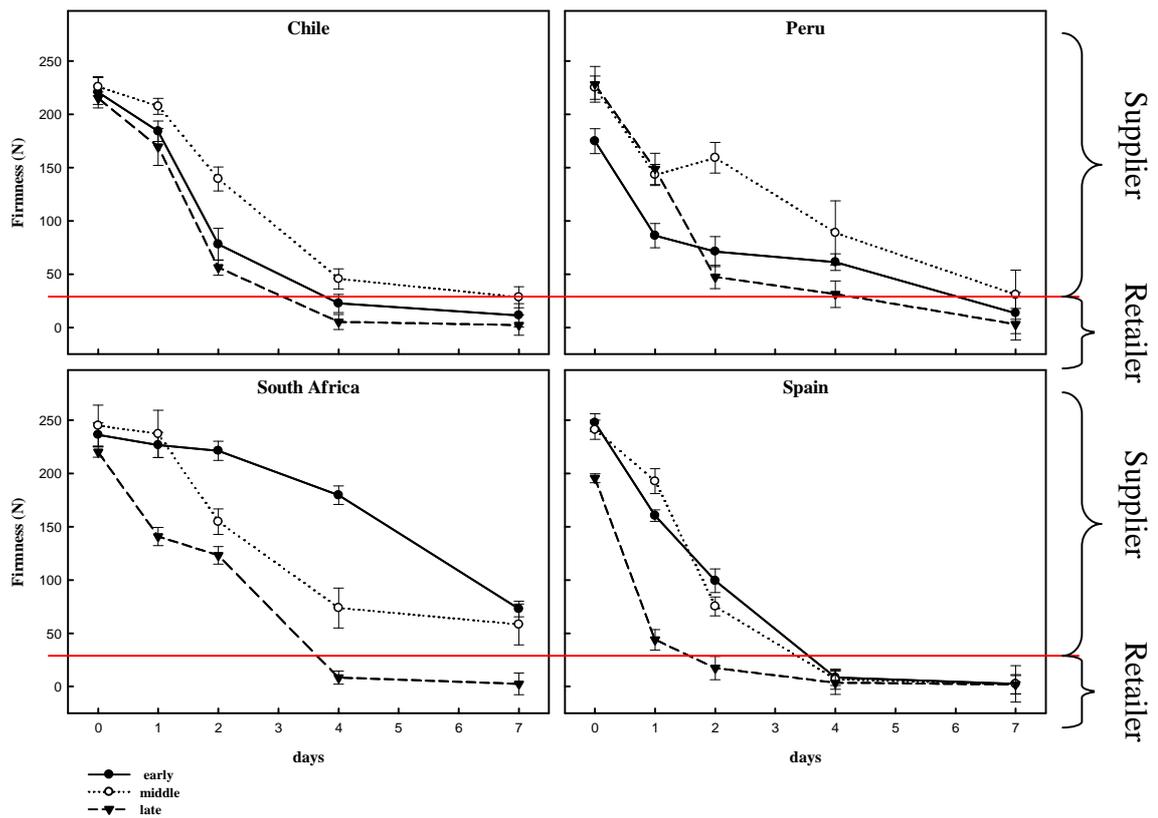


Figure 4.1: Mesocarp firmness values of avocado cv. Hass fruit from Chile, Peru, South Africa and Spain harvested in early (●), middle (○) and late (▲) season during 7 days at 18°C. Each point is a mean of 8 fruit measurement; general LSD ($P < 0.05$) is indicated.

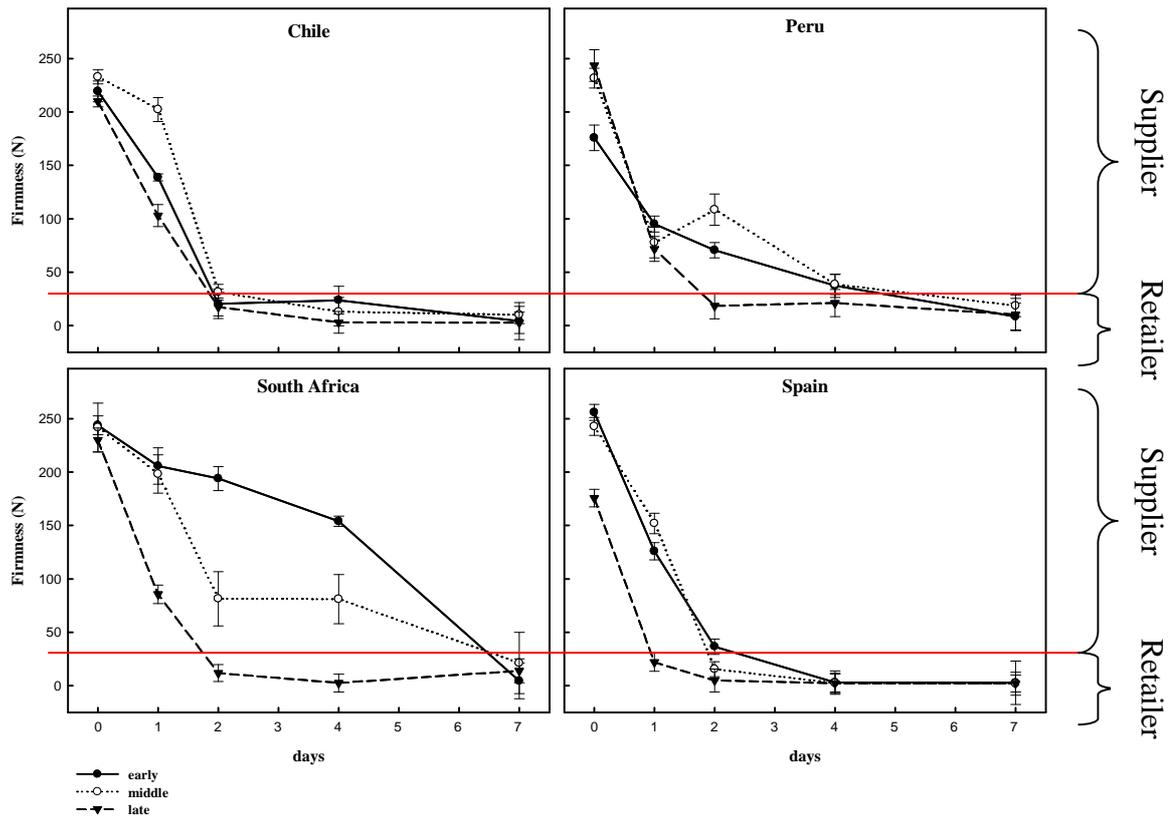


Figure 4.2: Mesocarp firmness values of avocado cv. Hass fruit from Chile, Peru, South Africa and Spain harvested in early (●), middle (○) and late (▲) season during 7 days at 23°C. Each point is a mean of 8 fruit measurement; general LSD ($P < 0.05$) is indicated.

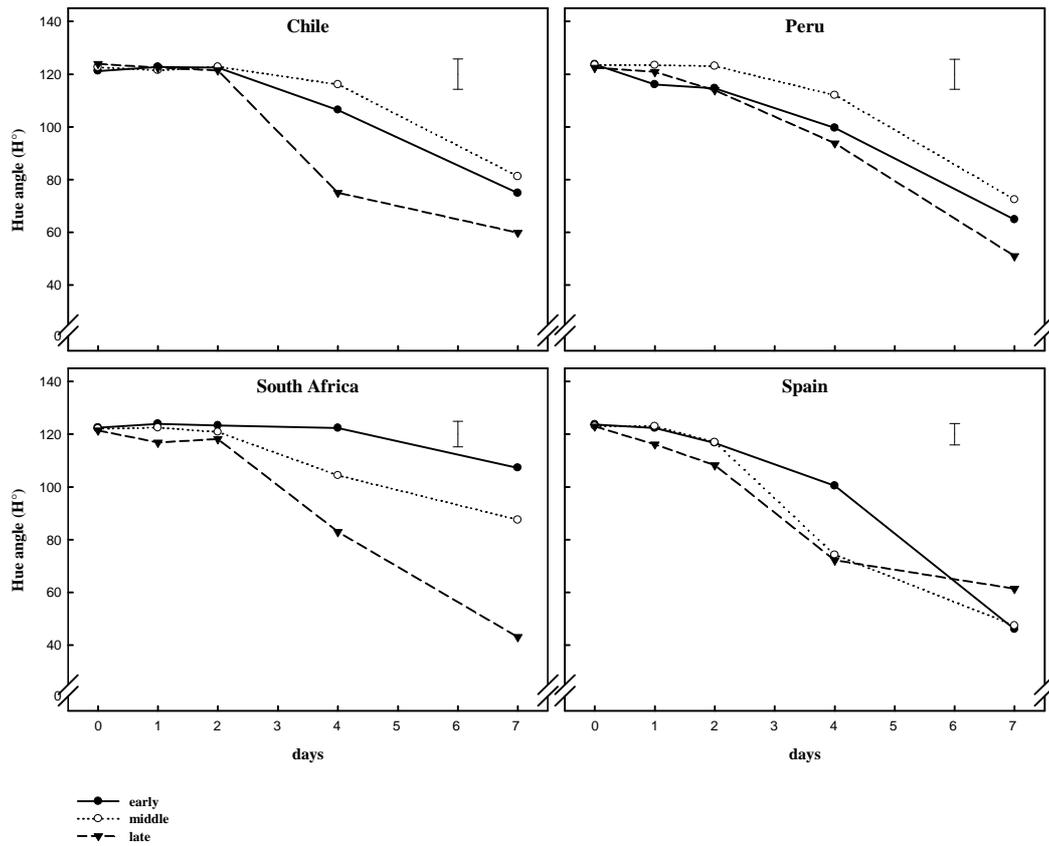


Figure 4.3: Hue angle values of avocado cv. Hass fruit from Chile, Peru, South Africa and Spain harvested in early (●), middle (○) and late (▲) season during 7 days at 18°C. Each point is a mean of 8 fruit measurement; standard deviation is indicated.

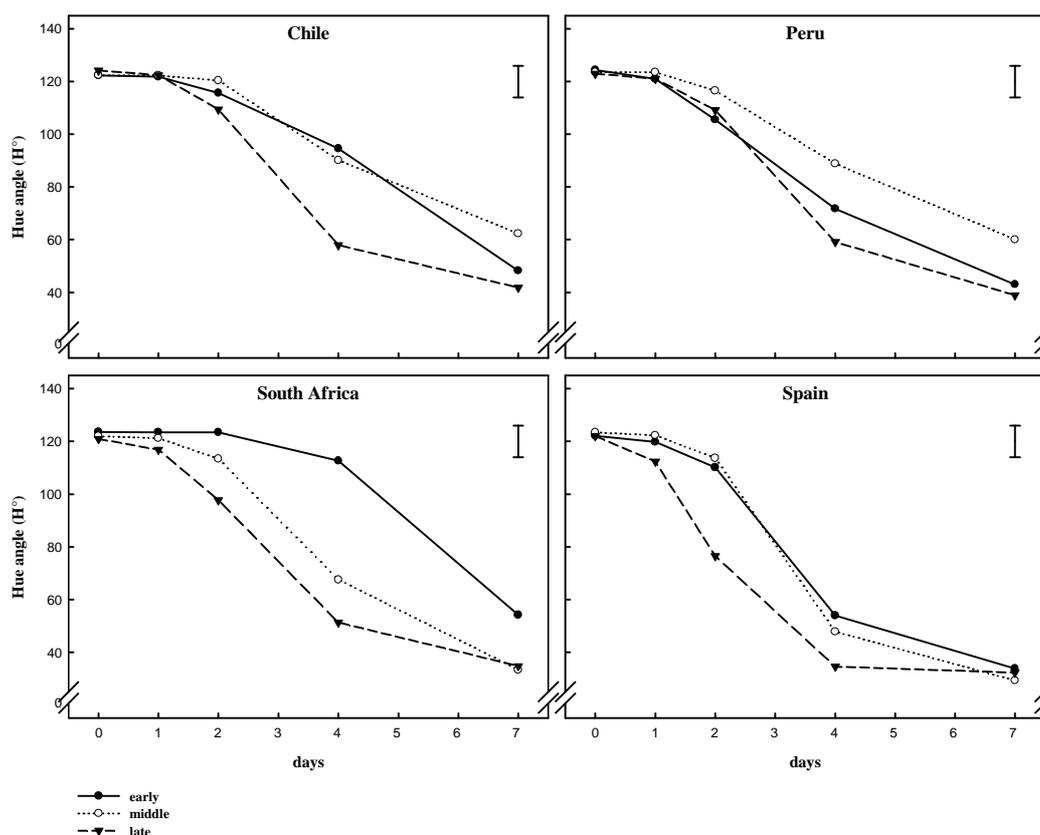


Figure 4.4: Hue angle values of avocado cv. Hass fruit from Chile, Peru, South Africa and Spain harvested in early (●), middle (○) and late (▲) season during 7 days at 23°C. Each point is a mean of 8 fruit measurement; standard deviation is indicated.

4.4.2 Weight loss

At the beginning of shelf life, day 0, all fruits weighed between *ca.* 235-260 g as they were previously selected as commercial size 16 (OECD, 2004). As expected, fruit weight decreased during postharvest life. Previous work showed together with mesocarp softening, peel darkening, increase in respiration and climacteric, avocado ripening is also characterised by increased water loss (Jeong *et al.*, 2003). In this study fruit water loss showed a similar trend to mesocarp softening and was significantly higher at 23°C. Yet, greater water loss was measured in Chilean fruit, with higher respiration rate, and least in South African fruit, with low production of CO₂ (Table 4.1). Length of transit time did not seem to be related to degree of water loss during ripening; for example

Peruvian fruit, with the longest cold storage duration (33 - 39 days) did not lose more water than Spanish fruit which has the shortest transit time (< 10 days).

4.4.3 Ethylene production and respiration rate

The involvement of ethylene in regulating ripening has been extensively reviewed (Watkins, 2006), however, the mechanisms involved in this process have not been completely resolved for avocado. Previous investigations demonstrated a correlation of ethylene production, respiration rate, mesocarp softening and cellulose activity in the mesocarp of detached avocado fruit (Pesis *et al.*, 1978) and the involvement of the seed in the regulation of ethylene response during ripening (HersHKovitz *et al.*, 2010). In this work, the climacteric peak was generally detected in the first two days of shelf life as previously shown for Mexican cv. Hass avocado fruit (Perez *et al.*, 2004) accompanied by a reduction in mesocarp firmness and skin colour changes. However, the timing and magnitude of the climacteric peak in ethylene concentration varied according to origin and harvest season (Figure 4.5-7). Generally, in fruit from Chile and Spain the ethylene peak preceded a pronounced decrease in firmness which is in agreement with Adato and Gatiz (1974). In Chilean fruit, the climacteric peak occurred in the first two days in late season fruit (37.5 and 39.5 $\mu\text{l kg}^{-1}\text{h}^{-1}$ at 18 and 23°C respectively) whilst in the early season higher ethylene levels were detected at day 7 at both temperatures. In middle season fruits ethylene production increased slightly until end of shelf life. Spanish fruit evolved the highest quantity of ethylene released, with the climacteric peak occurring in the first two days (respectively 52, 111 and 57 $\mu\text{l kg}^{-1}\text{h}^{-1}$, for early, middle and late season at 18°C) followed by a subsequent decrease. In particular, Spanish middle season fruit produced a higher amount of ethylene than early and late season fruit under both 18 and 23°C (135 $\mu\text{l kg}^{-1}\text{h}^{-1}$ in the first day of shelf life at 23°C).

Table 4.1: Chroma (C*) and lightness (L*), weight loss (%) values at day 0 and 7 at 18 and 23°C of cv. Hass avocado fruit from different origins (Spain, South Africa, Peru and Chile) and from different seasons (early, middle and late). Dry matter (%) content is also indicated at day 0 for each experiment.

	day	Chile			Peru			South Africa			Spain		
		early	middle	Late	early	middle	late	early	middle	late	early	middle	late
Chroma (C*)	0	24.34	19.88	16.34	21.15	21.72	23.08	24.72	25.05	22.2	21.41	21.12	23.86
	7 (18°C)	12.98	12.75	5.4	10.61	10.85	8.67	14.58	13.07	7.77	6.94	5.85	7.57
	7 (23°C)	6.42	6.44	3.55	7.99	5.28	6.05	6.49	6.62	3.75	5.13	3.79	5.15
	LSD		3.721			3.799			3.424			3.645	
Lightness (L*)	0	35.36	33.77	33.9	31.29	28.55	30.28	34.77	34.03	34.98	33.63	32.11	34.42
	7 (18°C)	30.21	30.28	26.93	28.07	25.74	27.61	32.68	31.33	28.48	25.11	25.13	25.84
	7 (23°C)	24.55	25.84	24.49	25.69	23.83	24.51	27.54	26.26	26.57	24.15	23.88	25.04
	LSD		2.337			2.531			2.125			2.183	
Weight loss (%)	0	-	-	-	-	-	-	-	-	-	-	-	-
	7 (18°C)	9.41	9.23	8.08	6.05	7.8	5.43	4.60	6.43	4.58	7.07	7.40	6.43
	7 (23°C)	12.76	12.09	10.07	8.66	8.97	6.71	6.83	7.69	5.99	9.80	10.21	8.05
	LSD		0.680			1.054			0.6999			0.695	
Dry matter (%)	0	27.72	26.58	31.65	21.77	22.21	26.67	24.79	28.08	34.16	29.81	28.18	33.03
	LSD						1.219						

Each value is the mean of 8 fruit measurement. Weight loss has been calculated measuring weight of same 10 fruit each from day 0 until the end of shelf life (day 7). For each parameter is indicated LSD ($P < 0.05$) calculated for each origin.

Early season fruit from South Africa showed a different pattern in ethylene production with a slow increase reaching a maximum value at the end of shelf life ($75 \mu\text{l kg}^{-1}\text{h}^{-1}$ at 18°C and $49 \mu\text{l kg}^{-1}\text{h}^{-1}$ at 23°C), while ethylene production by fruit from other harvest times (middle and late season) increased more rapidly when kept at 23°C compared with 18°C . Surprisingly, the climacteric peak was not evident in Peruvian fruit, even though the transit time was similar to Chilean fruit (> 30 days) and typically the ethylene production was consistently lower than in fruit from other origins. Indeed, values did not exceed the $25 \mu\text{l kg}^{-1}\text{h}^{-1}$ reached by early season fruit held at 18°C and did not increase at higher shelf life temperature. Nevertheless, mesocarp softening still occurred mainly in the first two days of shelf life. This could suggest, as previously studied, that the transcription of polygalacturonase involved in the softening process had been stimulated by low levels of ethylene (Sitrit and Bennet, 1998) or the presence of an ethylene independent softening mechanism (Pech *et al.*, 2008). More simply, the presence of the climacteric may not have been detected given the testing intervals used.

In fruit from Chile and Spain the climacteric was accompanied by an increase in the CO_2 production with peaks at 23°C of shelf life for early, middle and late season fruit, respectively 110 , 129 and $104 \text{ ml of CO}_2 \text{ kg}^{-1}\text{h}^{-1}$ and 96 , 95 and $109 \text{ ml of CO}_2 \text{ kg}^{-1}\text{h}^{-1}$ (Figure 4.6-8). These results on imported avocado fruit are in agreement with previous works where fruits from local orchards showed an increase in respiration rate in the first days of shelf life (Choi *et al.*, 2008; Hershkovitz *et al.*, 2009).

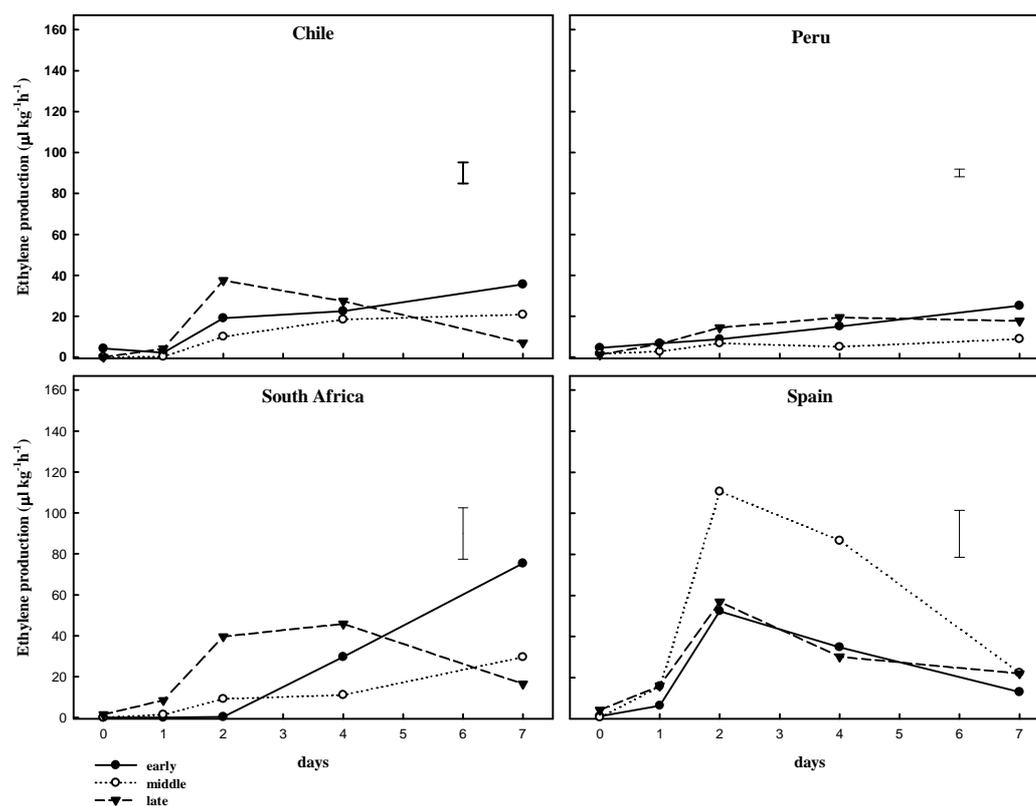


Figure 4.5: Ethylene production ($\mu\text{l kg}^{-1}\text{h}^{-1}$) during ripening at 18°C in cv. Hass avocado fruit from Chile, Peru, South Africa and Spain and harvested in early (●), middle (○) and late (▲) season. Each point is a mean value of 10 fruit. LSD ($P < 0.05$) per each origin is indicated. Data for the respiration rate of Peru early season are missing.

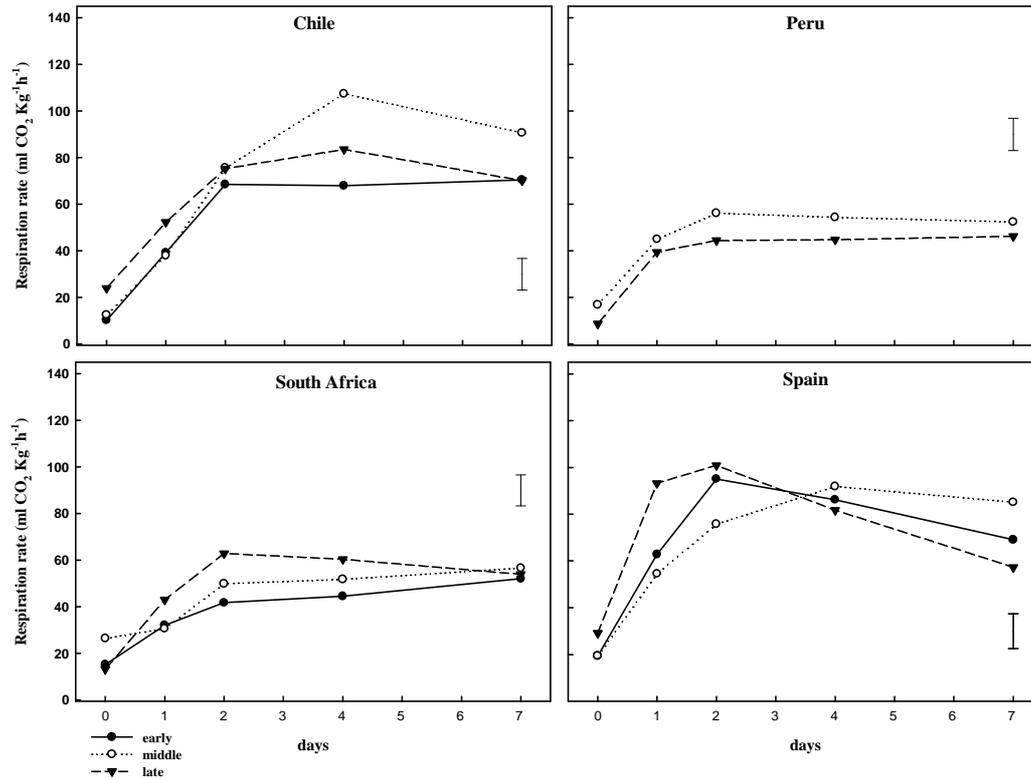


Figure 4.6: Respiration rate (ml of CO₂ kg⁻¹h⁻¹) during ripening at 18°C in cv. Hass avocado fruit from Chile, Peru, South Africa and Spain and harvested in early (●), middle (○) and late (▲) season. Each point is a mean value of 10 fruit. LSD ($P < 0.05$) per each origin is indicated. Data for the respiration rate of Peru early season are missing.

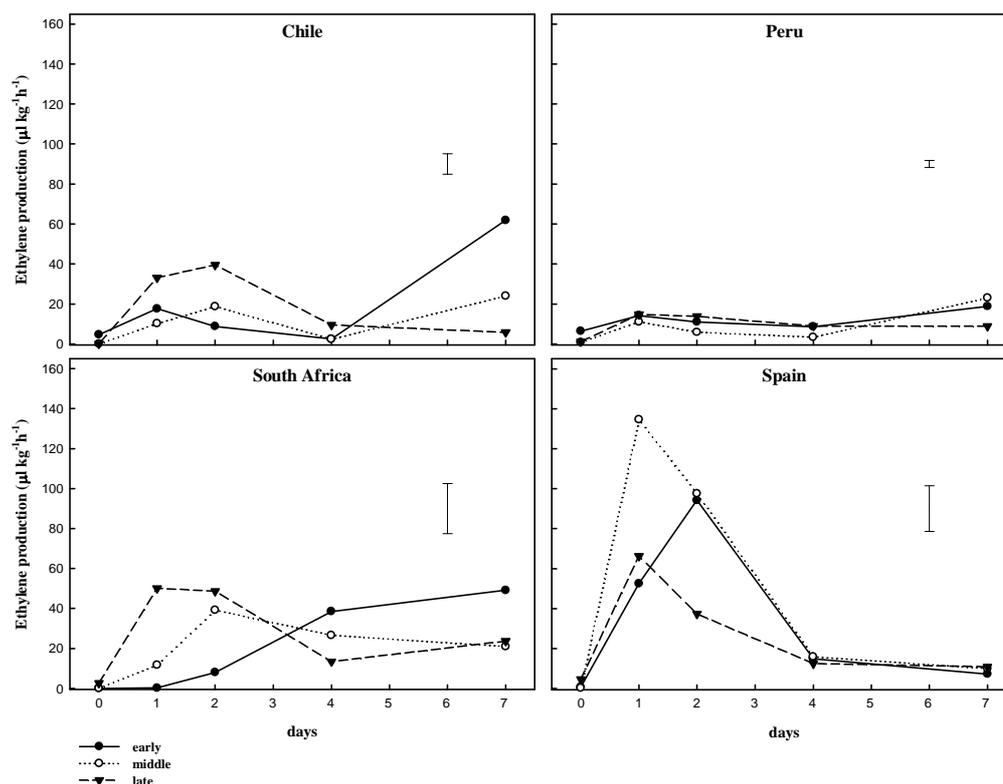


Figure 4.7: Ethylene production ($\mu\text{l kg}^{-1}\text{h}^{-1}$) during ripening at 23°C in cv. Hass avocado fruit from Chile, Peru, South Africa and Spain and harvested in early (●), middle (○) and late (▲) season. Each point is a mean value of 10 fruit. LSD ($P < 0.05$) per each origin is indicated. Data for the respiration rate of Peru early season are missing.

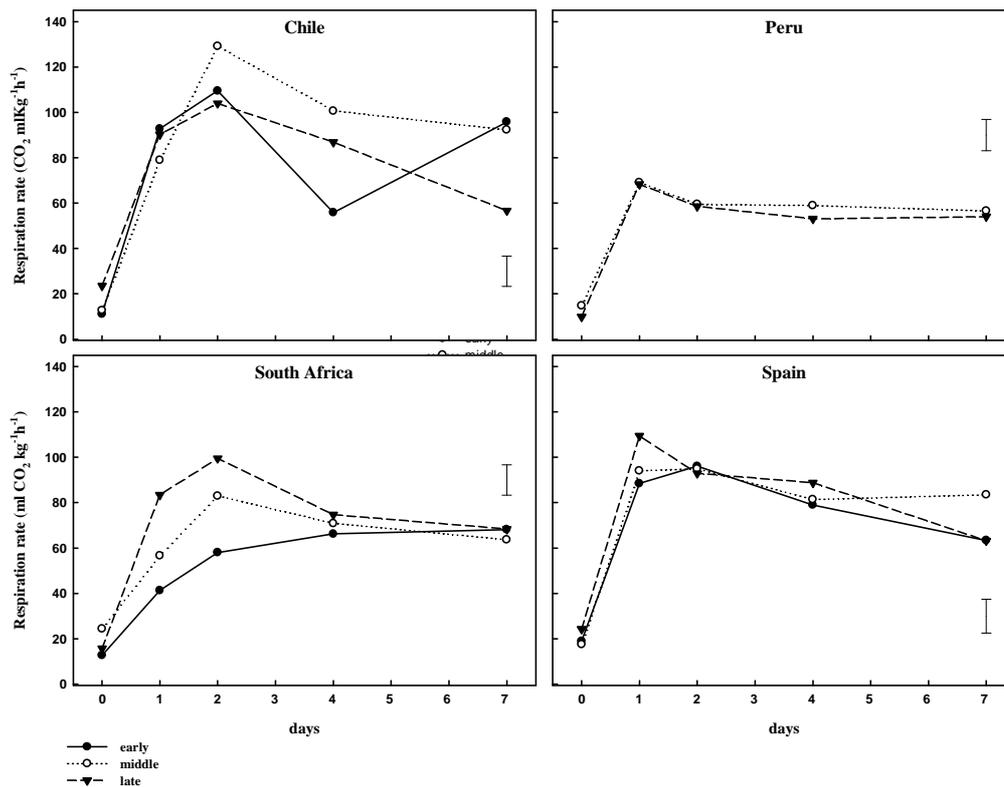


Figure 4.8: Respiration rate (ml of CO₂ kg⁻¹h⁻¹) during ripening at 23°C in cv. Hass avocado fruit from Chile, Peru, South Africa and Spain and harvested in early (●), middle (○) and late (▲) season. Each point is a mean value of 10 fruit. LSD ($P < 0.05$) per each origin is indicated. Data for the respiration rate of Peru early season are missing.

4.4.4 Dry matter and oil analysis

Significant differences in mesocarp dry matter (DM) content were found across different growing areas and harvest seasons (Table 4.1) and were in agreement with others (Ozdemir and Topuz, 2004; Lu *et al.*, 2009). Before ripen (day 0), fruit from the early harvest had a DM content, in order of dominance, 30% of the fresh weight in Spanish, 28% in Chilean, 25% in South African and 22% in Peruvian fruit. In late season the DM content (measured by lyophilization rather than oven drying) was higher with less disparity between fruit from different origin *viz.* South Africa (34%), Spain (33%) and Chile (32%), with the exception of Peruvian fruit with dry matter content

under 30% (Table 4.1). Only in fruit from South Africa did the DM content constantly increase with maturity. Therefore, the differences noted between origins showed also that it is not appropriate to define a general DM content for a certain maturity stage when considering fruits coming from different growing areas. Regardless of origin, the use of the DM content as an indicator of maturity seems to be more appropriate for fruits harvested in early and late season. Few changes were noted in the mesocarp DM content during ripening.

As expected from previous research (Ozdemir and Topuz, 2004; Meyer and Terry, 2008; Landahl *et al.*, 2009), the most abundant fatty acids was oleic acid (53%) followed by, in decreasing order of abundance, palmitic (21%), linoleic (14%), palmitoleic (7%) and linoleic acid (4%) (Figure 4.9). Specifically, the main differences in oleic acid content were seen across origin and harvest times. South African and Chilean fruit had higher oleic content ranging between 4.2 and 5.6 $\mu\text{g mg}^{-1}$ of oil. In agreement with the investigation of Ozdemir and Topuz (2004) on Turkish avocado, the oleic content in Spanish fruit increased with fruit maturity from 54 (3.8 $\mu\text{g mg}^{-1}$ of oil) up to 60% (5.2 $\mu\text{g mg}^{-1}$ of oil) from early to late season fruit. In contrast, fruit from Peru had higher oleic acid content in the middle season (46%). Generally, lower oleic acid content was detected in Peruvian fruit (42, 46 and 38% in early, middle and late season fruit, respectively). The relative concentrations detected here were in the range of those from previous investigations on fruits from Chile (middle season), Spain (late season) and Peru (early season) (Landahl *et al.*, 2009). Second in abundance was palmitic acid which showed a general decrease in its content with increase of fruit maturity in all origins. Contrary to oleic, palmitic acid concentration was higher in Peruvian (27, 26 and 23% respectively for the three seasons) and lower in Chilean fruit (17, 16 and 14%). These differences between countries in the oil composition could be in part a consequence of the different climatic conditions of the areas where fruit were sourced or criteria during harvesting and demonstrate the diversity in quality of imported fruit entering the UK. As previously suggested, cooler growing temperatures could stimulate higher ratios of monounsaturated fatty acids and consequently increase the membrane fluidity rather than higher temperatures average (Kaiser and Wolstenholme, 1993). Generally, the interaction between days, temperature, origin and season significantly

influenced content of palmitic, linoleic and linolenic acid, but overall most of the variability was noted between origin and harvest time. Any interaction with temperature of ripening was generally detected in the oleic acid content. These results support, in part, previous work (Ozdemir and Topuz, 2004) where the main significant differences in avocado oil composition were due to harvest season rather than postharvest ripening time.

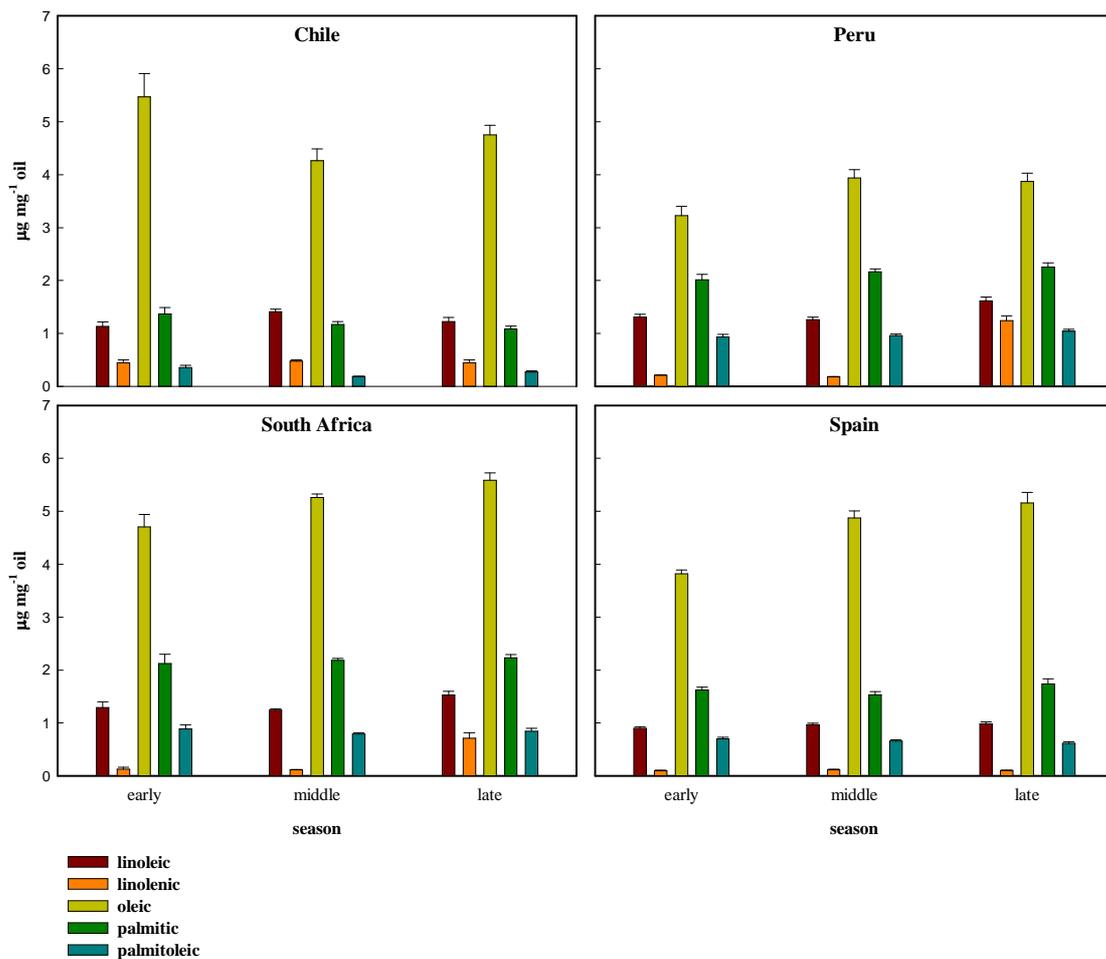


Figure 4.9: Main fatty acid (mg ml^{-1} oil)(oleic ■, palmitic ■, linoleic ■, palmitoleic ■, linolenic ■ acid) present in the mesocarp of avocado oil cv. Hass from Chile, Peru, South Africa and Spain, harvested in early, middle and late season. Each bar, with S.E., is a mean value of 16 fruit measurements before shelf life (day 0).

Besides seasonal variability, results showed that it is possible to define a general profile of the avocado oil according to origin. Using Principal Component Analysis (PCA) the oleic acid content explained most of the differences between fruit origin (PC1, 56%) (Figure 4.11). The minor compounds such as palmitic and linolenic acid, explained 20% of the variability along PC2 which separated fruit grown in Chile and Spain. Fruit from Peru and South Africa, rather than Chile and Spain, were characterised by higher heterogeneity in oil composition, but this may be an artefact of selection.

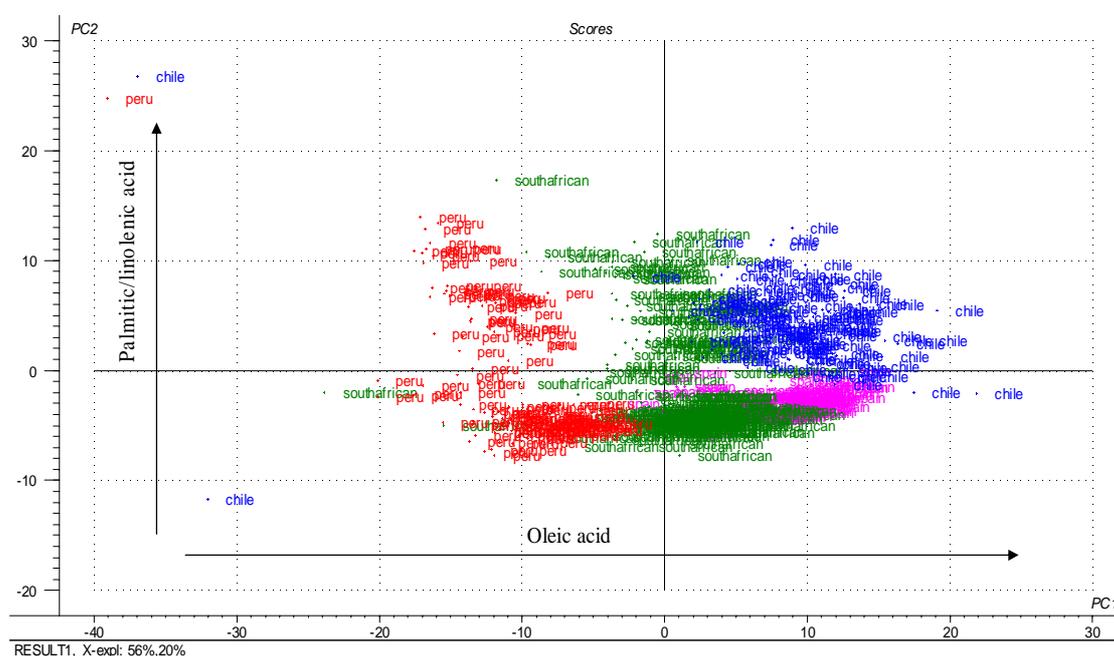


Figure 4.10: Score plot of PCA analysis of the main variables (dry matter, oleic, palmitic, palmitoleic, linoleic and linolenic acid) measured in the mesocarp of avocado fruit from different origin (Chile, Peru, South Africa and Spain) and harvest season (early, middle and late). Different origins are indicated with different colours (Chile=blue, Peru=red, South Africa=green, Spain=violet).

4.4.5 Sugar analyse

At the beginning of the shelf life (day 0), fruit from South Africa and Spain had similar content of mannoheptulose and perseitol whereas Chilean early and Peruvian

early and middle season fruit, according with a recent investigation (Landahl *et al.*, 2009), had predominant concentration of mannoheptulose (Table 4.2). However, previous investigations on unripe avocado fruit cv. Hass from California had detected a predominance of perseitol in the mesocarp (Liu *et al.*, 2002). With increased maturity, mannoheptulose content declined in fruit from Chile, Peru and Spain, possibly indicating a depletion of this energy source during fruit grown, and is in agreement with the hypothesis that C7 sugars are related to ripening (Liu *et al.*, 2002). Only in South African fruit was mannoheptulose content higher in middle season and lower in early season fruit. It is considered that fruits from this source were not from the same growing region and the variability in the climatic conditions between orchards could explain the different concentration of sugars in the fruit mesocarp. Sucrose was the main hexose sugar present (Liu *et al.*, 2002; Landahl *et al.*, 2009) with higher content in early season fruit from Chile when compared with fruit from other origins. Fructose and glucose were also detected but in minor amounts and their concentrations were not always over the detection limit.

A general decrease in the main sugars content during ripening supports previous works (Liu *et al.*, 1999b; Meyer and Terry, 2008, 2010). Indeed, the ripening process marked by increase in respiration rate and ethylene production was also accompanied by decrease in the C7 carbohydrate structures (Liu *et al.*, 2002). The depletion of mannoheptulose from the fruit mesocarp during ripening (Table 4.2) might confirm the involvement of this sugar as an inhibiting factor of ripening in avocado fruit, as previously stated (Liu *et al.*, 2002).

When considering the main biochemical compounds (fatty acids and non structural carbohydrates), temporal changes in mannoheptulose and perseitol content were the main discriminatory factors between days, respectively, explaining the 70% (PC1) and the 17% (PC2) of fruits variability using chemometric analysis (Figure 4.12). Previous reports have reported a threshold of mannoheptulose in fruit mesocarp of about 20 mg g⁻¹ of the fresh weight, above which fruit ripening was inhibited (Liu *et al.*, 2002). These results were not confirmed by more recent study (Landahl *et al.*, 2009) and by this work. Yet, what the results do suggest is that the concentration of mannoheptulose detected in the fruit mesocarp after harvest, significantly changes

according to growing condition (in this specific case represented by countries). In agreement with Liu and colleagues (2002) results generally confirm the predominance of C7 structures as the main sugars in the mesocarp of avocado fruit cv. Hass, and confirm that they may be used as biomarkers to establish avocado fruit ripening stage, regardless of fruit origin or harvest season.

Table 4.2: Main non structural carbohydrates identified in avocado mesocarp cv. Hass during ripening at 18 and 23°C.

origins	Chile			Peru			South Africa			Spain			
	day	early	Middle	late	early	middle	late	early	middle	late	early	middle	late
Mannohep.	0	128±8.5	85.9±9.7	39.7±3.9	115±10.3	96.6±8	53.4±6.9	52.1±6	84.1±7.6	67.3±8.3	65.2±5	72±8	25.4±2.5
	2	104±11.6	68.4±10.6	42.1±6.7	82.9±9.6	85.2±8.8	51.4±4.2	39.5±5.7	68±7.9	68.4±8.2	30.4±2.7	37.1±5.7	15.7±1.3
	7	23.5±5.6	18.4±4.2	13.2±2.7	33.6±4.1	29.2±2.8	37.3±2.1	16.2±2	38.2±6.4	21.6±3.4	23.5±3	21.9±2.6	7.6±0.8
Perseitol	0	73.98±5.5	69.65±3.3	65.34±3.3	41.79±2	39.3±1.2	34.47±1.5	44.4±3.3	64.48±2.8	56.58±3.7	66.46±2.6	66.02±2.8	44.84±2.8
	2	71.98±2.7	56.04±3.6	45.29±2.6	42.11±1.2	40±1.2	43.52±2.7	37.27±2.1	52.82±2.4	56.78±4.9	52.26±3.2	41.62±2	27.41±1.6
	7	47.5±2.8	38.96±5.1	21.38±3.4	29.96±2.7	35.41±1.4	37.38±3.2	22.33±2.5	35.28±3.9	58.56±3.9	32.65±4.6	22.55±2.1	23.76±2.7
Sucrose	0	55.62±2.4	28.49±1.1	18.33±1.6	33.77±1.8	40.86±1.2	15.89±1.3	12.41±0.9	39.61±1.2	33.03±3.5	25.83±1.4	39.61±1.8	24.6±2.2
	2	42.35±2.7	27.51±2.5	28.81±2	34.19±3.7	26.67±2.7	23.37±2.3	12.64±1.1	38.02±2.7	45.83±3.6	23.17±2.3	25.98±1.9	17.78±0.9
	7	17.62±2.9	19.54±2	34.02±2.6	31.56±6	20.34±2.7	25.57±2.3	20.21±2.3	31.79±4	33.12±3.1	52.99±3.7	48.51±2.2	21.84±1.6

Each value is a mean of 16 fruit ± S.E. Significant differences were found between origins (*viz.* Chile, Peru, South Africa and Spain), seasons (*viz.* early, middle and late) and days (*viz.* day 0, 2 and 7). Concentrations are expressed in mg g⁻¹ of powder residue.

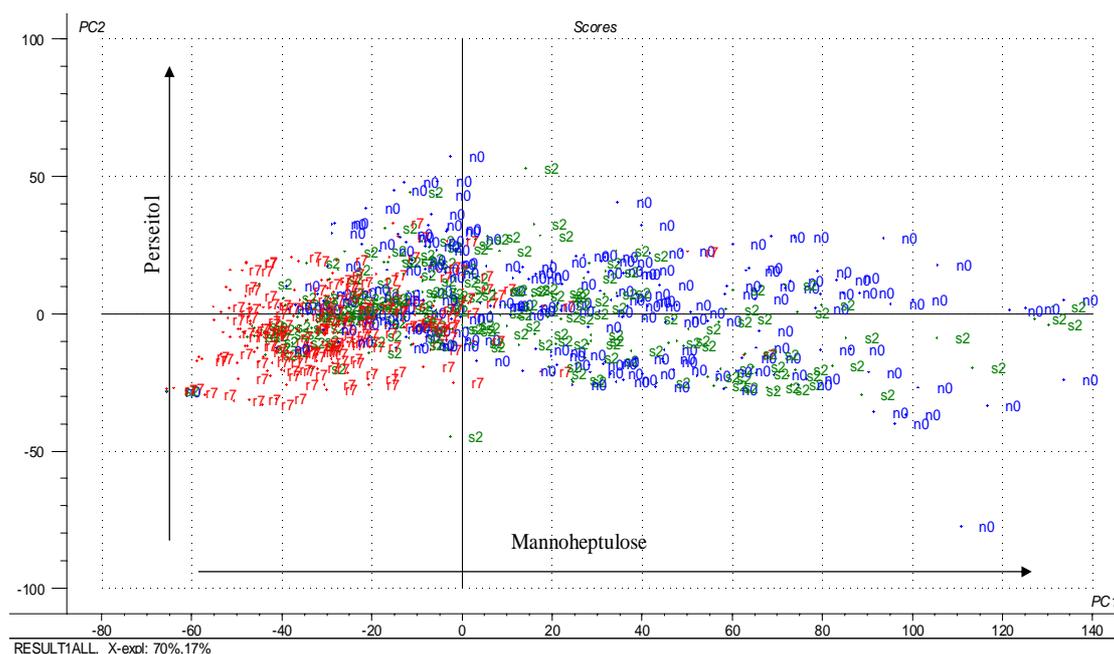


Figure 4.11: Score plot of PCA analysis of the main variables (dry matter content, oleic, palmitate, palmitoleic, linoleic, linolenic acid, mannoheptulose, perseitol and sucrose content) measured in the mesocarp of avocado fruit from different origin (Chile, Peru, South Africa and Spain) and harvest season (early, middle and late). Different colours indicate different days (blue=day 0; green=day 2; red=day 7).

4.5 Conclusions

Avocado fruit (*Persea americana* Mill.) is highly appreciated by European consumers, who nowadays increasingly expect guaranteed consistency in quality throughout the year. This demand is fulfilled by a wide range of importers, yet this necessary supplier diversity still leads to undesired differences in fruit quality throughout the year and results in seemingly sporadic customer complaints to supermarkets. Most previous postharvest research on avocado fruit has not considered the fate of individual consignments when they reach their intended overseas market. A better knowledge of variability in imported avocado fruit physiology across the year is thus required in order to strive for better consistency.

Major changes in avocado metabolism (climacteric, respiration rate and firmness parameters) were detected in the first two days. Predictably, higher ripening temperature induced a more rapid response, with faster mesocarp softening, a decrease in hue angle values and a higher amount of ethylene production. In all twelve experiments, late season fruits tended to soften faster than early and middle season fruit, respectively. Ethylene production did not increase in more mature fruit and the climacteric rise was not detected in fruit from Peru, however, this did not affect softening. Cold storage transit time did not appear to have a consistent effect on postharvest behaviour. Changes in the dry matter and fatty acids content were mostly influenced by origin and harvest season rather than length of cold storage transit or ripening conditions, with oleic acid as main discriminate factors between different origins. Chemometric analysis of sugar profiles changed during shelf life with a decrease in the mannoheptulose content during ripening regardless of origin or harvest time. Results suggest that biomarkers of origin and season could be used to identify postharvest variability during ripening in imported avocado cv. Hass fruit.

There has been a dearth of research which has actually surveyed the postharvest physiological and biochemical differences of imported avocado fruit coming from diverse origins and suppliers. This work has demonstrated that it is possible to use simple biomarkers related to fruit origin and possibly to consistency during fruit ripening. In addition, this work further supports the hypothesised role of C7 sugars in the ripening process of avocado fruit and their possible use as biomarkers of avocado ripening stage, regardless of fruit origin and/or harvest time.

5 CHAPTER FIVE

INFLUENCE OF FRUIT MATURITY IN THE RELATIONSHIP BETWEEN SKIN COLOUR CHANGES AND MESOCARP SOFTENING OF IMPORTED AVOCADO FRUIT CV. HASS

5.1 Introduction

The external aspect of fruits and vegetables is the first immediate parameter used by costumers in the choice of a product and in some cases in the identification of the ripening stage. In the specific case of avocado fruit, the peel remains green and only some cultivars (i.e. cv. Hass) does the skin colour change as the fruit ripens. This peculiarity, beside the good storage aptitude, the characteristic nutty flavour and the medium seed size (Newett *et al.*, 2002) makes cv. Hass one of the most demanded avocado fruit types on the market (FAO, 2009). Consequently, there is a scientific and commercial interest to know whether the skin colour can be a reliable indicator of fruit ripening as there is much conflicting evidence. For instance, the skin darkening process seems to be differently influenced by shelf life temperature and growing area than the mesocarp softening. A darker coloration has been achieved when fruit ripen at higher temperature even though the fruit reached the eating stage (Cox *et al.*, 2004). However, fruit at the ripe stage can develop a purple or more black coloration regarding source area (Cox *et al.*, 2004). Additionally, skin colour changes have been noted in late season fruit even before harvest resulting in dark fruit with firm mesocarp (Cox *et al.*, 2004). Due to the unclear relationship between skin colour changes and mesocarp softening, avocado fruit ripening stage is evaluated through the measurement of the mesocarp firmness with consequently product loss. Therefore, the possible use of a non destructive parameter such skin colour could limit industry waste. Besides, consumers could take advantage from such indicator to determine when the fruit has reached its eating stage.

With this aim in mind, previous works have investigated changes in skin darkening as fruit ripen at different temperature (Cox *et al.*, 2004) and the change in main pigments present in avocado skin cv. Hass during ripening (Ashton *et al.*, 2006). Both works agreed on the identification of cyanidin 3-*O*-glucoside as the main compound responsible for skin darkening. The cyanidin content is highly correlated with fruit colour changes and influenced by ripening temperature (Cox *et al.*, 2004). The initial changes in skin colour in avocado fruit have been reported to be also due to chlorophyll degradation (Cox *et al.*, 2004; Ashton *et al.*, 2006). Additionally, an exponential decline in the main carotenoid content, lutein, after fruit harvest (Ashton *et al.*, 2006) suggested its potential implication in the colour determination of avocado peel.

The correlation found between skin colour changes and cyanidin content seems to be influenced by temperature of ripening. Indeed, peel of late season avocado fruit from New Zealand did not achieve the same dark colour when ripened at 15°C rather than at higher temperatures (20 or 25°C) (Cox *et al.*, 2004).

Not completely understood and investigated is the relationship between skin colour changes and mesocarp softening, though both appear to be modulated by an endogenous release of ethylene (Pesis *et al.*, 1978; Saltveit, 1999). The purpose of this work is to investigate whether it is possible to define a general relationship between avocado fruit parameters (i.e. mesocarp firmness, skin hue angle and cyanidin content) and if this relationship might be used as a reliable fruit ripening indicator. Additionally, the maturity stage at harvest of avocado fruit has been shown to influence fruit composition (Ozdemir and Topuz, 2004) and postharvest behaviour (Ozdemir and Topuz, 2004; Liu *et al.*, 1999b). Specifically for avocado fruit, late harvest fruit are associated with faster ripening (Dixon *et al.*, 2003b). This study also aimed to identify a relationship, if any, between both fruit maturity and postharvest conditions and the parameters herein mentioned.

An important aspect of avocado skin is the presence of compounds involved in resistance to fungi. Particularly, infections by the anthracnose causing fungus, *Colletotrichum gloeosporioides*, can cause significant postharvest losses (Pegg *et al.*, 2002). Biochemical changes during fruit ripening seem to be account for the increased

fungi susceptibility. Studies on avocado peel tissues revealed the presence of a compound with a potential antifungal activity (1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene) (Prusky *et al.* 1991). The diene seems to inhibit spore germination of *Colletotrichum gloeosporioides* and its content decreases as ripening arises, increasing the potential for a fungus infection (Prusky *et al.* 1991). Responsible for the diene degradation is a lipoxygenase, which activity is inhibited by epicatechins. Consequently, the decrease in the epicatechin content during fruit ripening has been suggested to be indirectly responsible for greater susceptibility of the fruit to fungal infection (Prusky *et al.*, 1992; Ardi *et al.*, 1998; Guetsky *et al.*, 2005). Besides, other phenolic compounds have been detected in avocado peel. Particularly, most of the antioxidant potential present in avocado peel refers to procyanidin content (Wang *et al.*, 2010). Thus, this work reports the variation of individual phenolics here identified, distinguishing between fruit maturity and postharvest conditions in avocado fruit cv. Hass imported from Spain.

5.2 Materials and methods

The plant material and the experimental design are described in section 3.1.2. Briefly, peel of avocado fruit cv. Hass sourced from a same orchard in Malaga, Spain, in the three main harvest season was investigated during ripening. The methodology used to measure weight, colour, firmness, climacteric behaviour, total chlorophylls and carotenoids, individual anthocyanin and phenolics are described in Chapter 3. The statistical analysis is described in section 3.4.

5.3 Results

5.3.1 Colour

At the beginning of shelf life (day 0) the skin colour of fruit harvested in early, middle and late seasons showed similarity in the chroma (C*), lightness (L*) and hue angle (H°) parameters. Nevertheless, chroma and hue angle values significantly differed amongst harvest seasons (season*day). In particular, for middle and late season fruit C* and H° values decreased faster between day 2 and 4 of shelf life. These differences

gradually reduced as ripening progressed (Figure 5.1). Regarding skin lightness, slight differences were detected for the interaction between temperature*day. Additionally, the postharvest conditions seemed to influence colour changes with a faster decrease in chroma and hue angle values at higher temperatures.

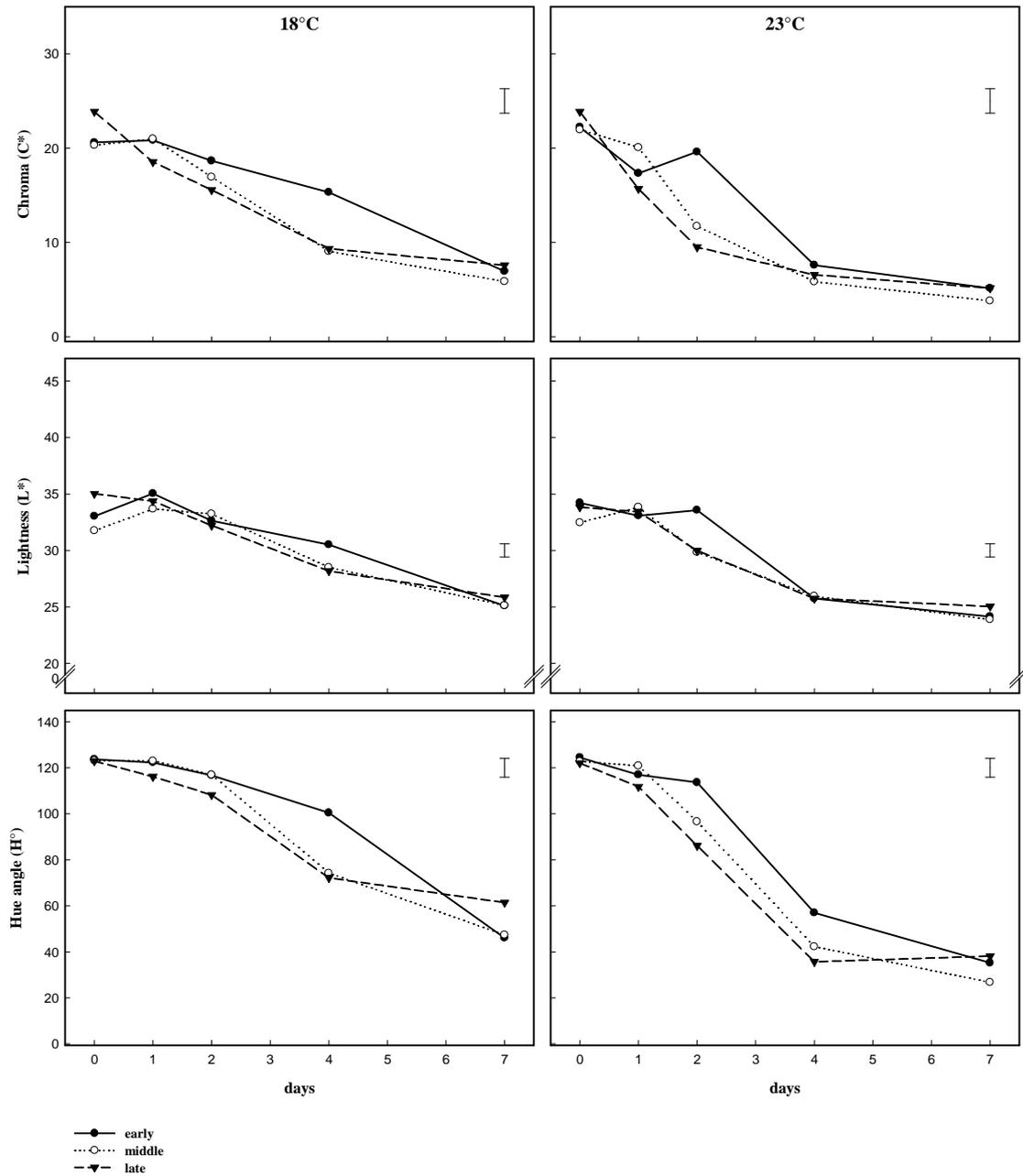


Figure 5.1: Changes in colour parameters (chroma (C*), lightness (L*) and hue angle (H°)) in avocado fruit cv. Hass during ripening at 18 (left) and 23°C (right). Fruit were

harvested in February, March and April indicated by early, middle and late season, respectively. For each parameter the measurements were taken at day 0, 1, 2, 4 and 7. Each point is a mean value of 8 fruit measurement. LSD bar is showed for the interaction season*days for C* and H° and temperatures*day for L*.

5.3.2 Firmness

The softening of the mesocarp measured as a decrease in firmness showed significant differences for the interaction season with day. Differences were noted at day 0 with a softer mesocarp in late season than earlier harvested fruit. This difference remained over the ripening process. However, on day 4 all fruits were ripe, regardless of maturity. Besides, higher ripening temperature induced a faster decrease in firmness values (Figure 5.2).

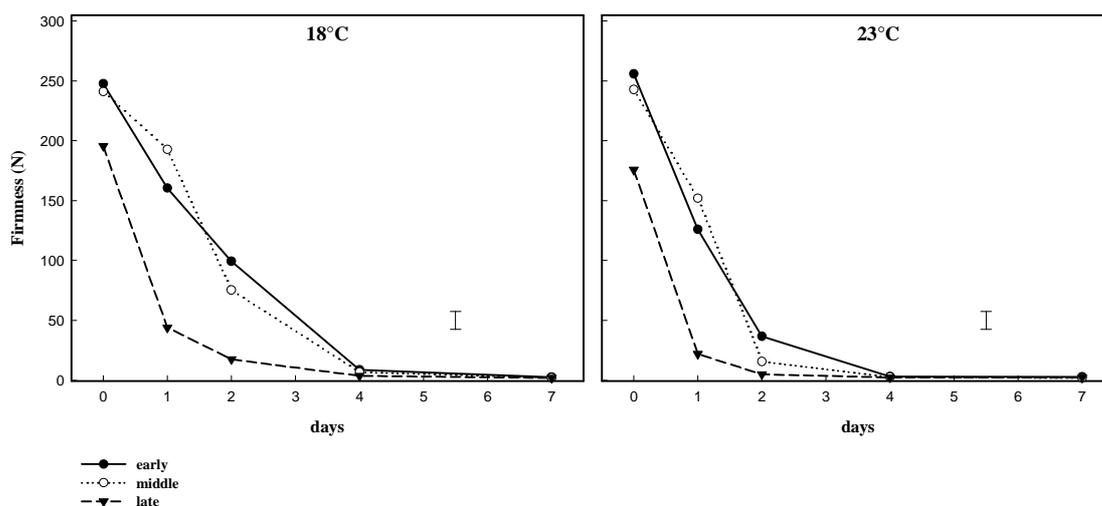


Figure 5.2: Changes in mesocarp firmness (N) in avocado fruit cv. Hass along ripening at 18 (left) and 23°C (right). Fruit were from three different maturity stage: early, middle and late harvest. Measurements were taken at day 0, 1, 2, 4 and 7. Each point is a mean value of 8 fruit measurement. LSD bar is showed for the interaction season*day.

5.3.3 Ethylene production

Fruit evolved the highest quantity of ethylene, known as the climacteric peak, in the first two days (52, 111 and 57 $\mu\text{l kg}^{-1}\text{h}^{-1}$, for early, middle and late season,

respectively) followed by a subsequent decrease. In particular, middle season fruit produced a higher amount of ethylene than early and late season fruit in both ripening conditions ($110 \mu\text{l kg}^{-1}\text{h}^{-1}$ at 18°C and $135 \mu\text{l kg}^{-1}\text{h}^{-1}$ at 23°C) (Figure 5.3).

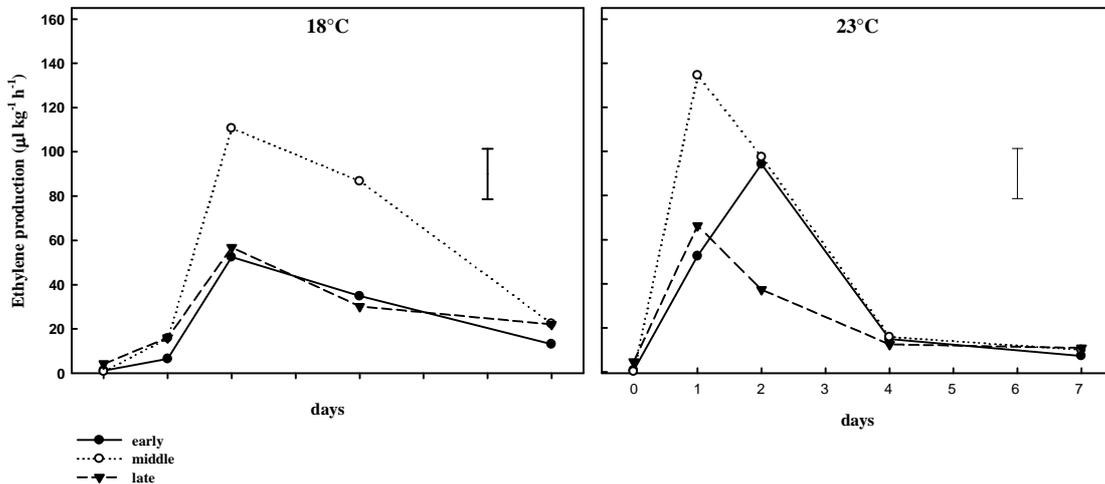


Figure 5.3: Ethylene production ($\text{ml kg}^{-1} \text{h}^{-1}$) in avocado fruit cv. Hass harvested in early, middle and late season fruit and ripen at 18 and 23°C . Each point is a mean value of 8 fruit measurement. LSD ($P < 0.05$) is indicated for the interaction day*temperature*season.

5.3.4 Total carotenoids and chlorophylls content

A significant interaction was observed between day with temperature and day with season in total carotenoids content. Specifically, the carotenoids content differed between seasons mainly at the beginning of postharvest life. Early season fruit tends to have higher carotenoid content (mean $173.1 \mu\text{g g}^{-1} \text{DW}$) than fruit harvested late in the season (mean $157.3 \mu\text{g g}^{-1} \text{DW}$). A common tendency was that carotenoid content decreased throughout ripening when fruit were held at 18°C . At higher temperature fruit from middle season showed the lower carotenoids content (Figure 5.4).

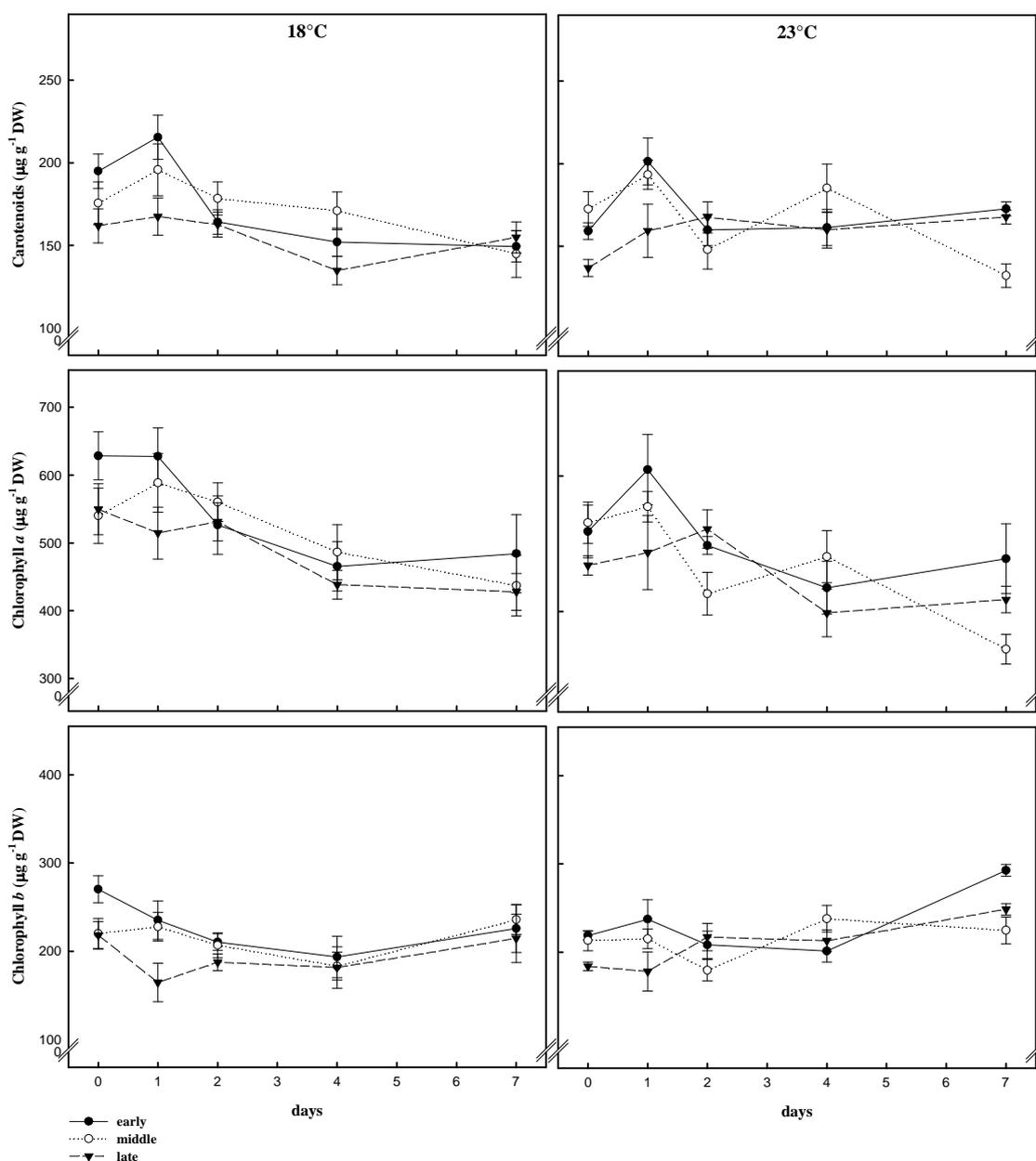


Figure 5.4: Variation in the total carotenoids, chlorophyll *a* and chlorophyll *b* content in the peel of avocado fruit cv. Hass for each harvest seasons (early, middle and late) during ripening at 18 and 23°C. For each parameter the measurements were taken at day 0, 1, 2, 4 and 7. Each value is a mean of 8 fruit measurements \pm S.E.

Most of the chlorophyll present in the peel of avocado fruit was represented by chlorophyll *a*, with at day 0 mean values of 573.2, 535.5, 508.9 $\mu\text{g g}^{-1}$ DW in early, middle and late season, respectively. Chlorophyll *a* content decreased during ripening,

regardless of harvest time and postharvest conditions. At the end of shelf life lower values were reached by more mature fruit and at higher temperature with 484.3, 437.1 and 427.9 at 18°C and 478.1, 344.6, 417.7 $\mu\text{g g}^{-1}$ DW at 23°C for early, middle and late season, respectively. Chlorophyll *b* was between two and half times lower than the chlorophyll *a* content, 244.6, 216.6, 201.1 $\mu\text{g g}^{-1}$ DW in early, middle and late season, respectively. A significant interaction was observed between temperature with day. In particular, when fruit were held at 18°C, the chlorophyll *b* content slowly decreased followed by an afterwards increase at day 4. A more uniform trend was detected at higher temperature with a general increase in chlorophyll *b* levels (Figure 5.4).

5.3.5 Anthocyanin content

Cyanidin 3-*O*-glucoside was the main anthocyanin identified in the skin tissues of avocado fruit cv. Hass. Its content significantly differed between days, temperatures and seasons. The anthocyanin level increased after two days reaching higher level by holding fruit at 23 rather than 18°C, with 1,060 and 637 $\mu\text{g g}^{-1}$ in early, 1,899 and 746 $\mu\text{g g}^{-1}$ in middle, 1,079 and 499 $\mu\text{g g}^{-1}$ in late season fruit. Additionally, the differences between harvest time were particularly marked at higher temperature of ripening with middle season fruit reaching higher levels in cyanidin 3-*O*-glucoside content compared with the other seasons (Figure 5.5).

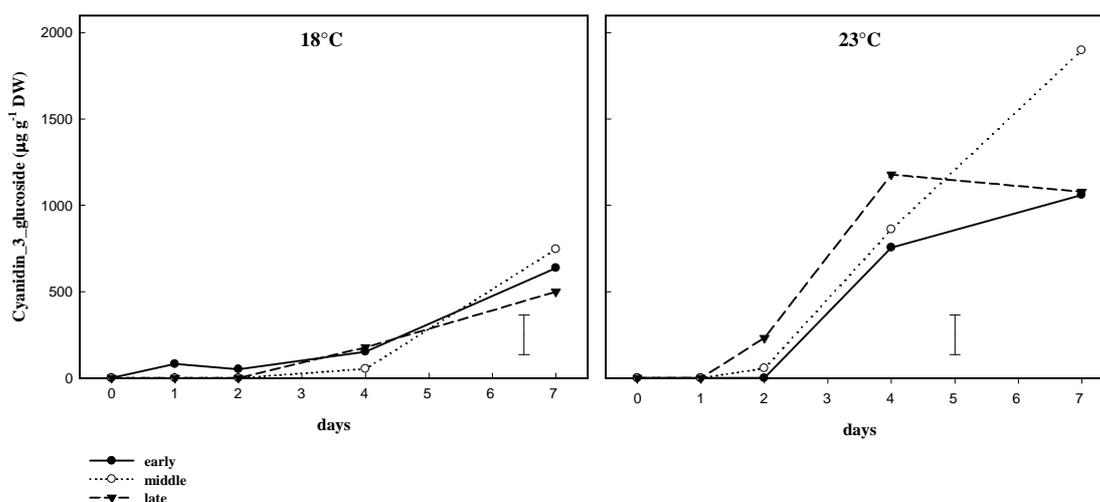


Figure 5.5: Changes in the cyanidin 3-*O*-glucoside content in the peel of avocado fruit cv. Hass harvested in early, middle and late season. For each compound are showed values related to seven days at 18 and 23°C. Each point is a mean of 8 fruit measurement. LSD bar ($P < 0.05$) is indicated for the interaction day*temperature*season.

5.3.6 Phenolic compounds

Epicatechin, procyanidin B2 and chlorogenic acid were identified as the main phenolic compounds, in order of abundance. Epicatechin and procyanidin B2 were influenced in their content by the interaction between treatment day*temperature*season. The herein mentioned phenolic compounds showed a similar trend regarding postharvest temperatures. In early and late harvested fruit, their content increased at day 1 (29.68 $\mu\text{g mg}^{-1}$ for epicatechin, 17.24 $\mu\text{g mg}^{-1}$ for procyanidin B2) when fruit were held at 18°C. Though, middle season fruit showed a drastic decrease after 1 day at 18°C, dropping from 18.61 to 10.13 $\mu\text{g mg}^{-1}$ and from 11.37 to 7.23 $\mu\text{g mg}^{-1}$ for epicatechin and procyanidin, respectively. Few changes were detected along ripening at higher temperature (23°C). After 4 days increased epicatechin content was detected in early season fruit (25.91 $\mu\text{g mg}^{-1}$), and higher procyanidin production in middle season fruit (16.6 $\mu\text{g mg}^{-1}$). The individual phenolics content showed a general decrease during ripening at 18°C. At the end of shelf life (day 7) the epicatechin content

results similar with 14.39, 13.53, 17.93 $\mu\text{g mg}^{-1}$ and 16.82, 16.21, 15.09 $\mu\text{g mg}^{-1}$ at 18 and 23°C in early, middle and late season, respectively (Figure 5.6).

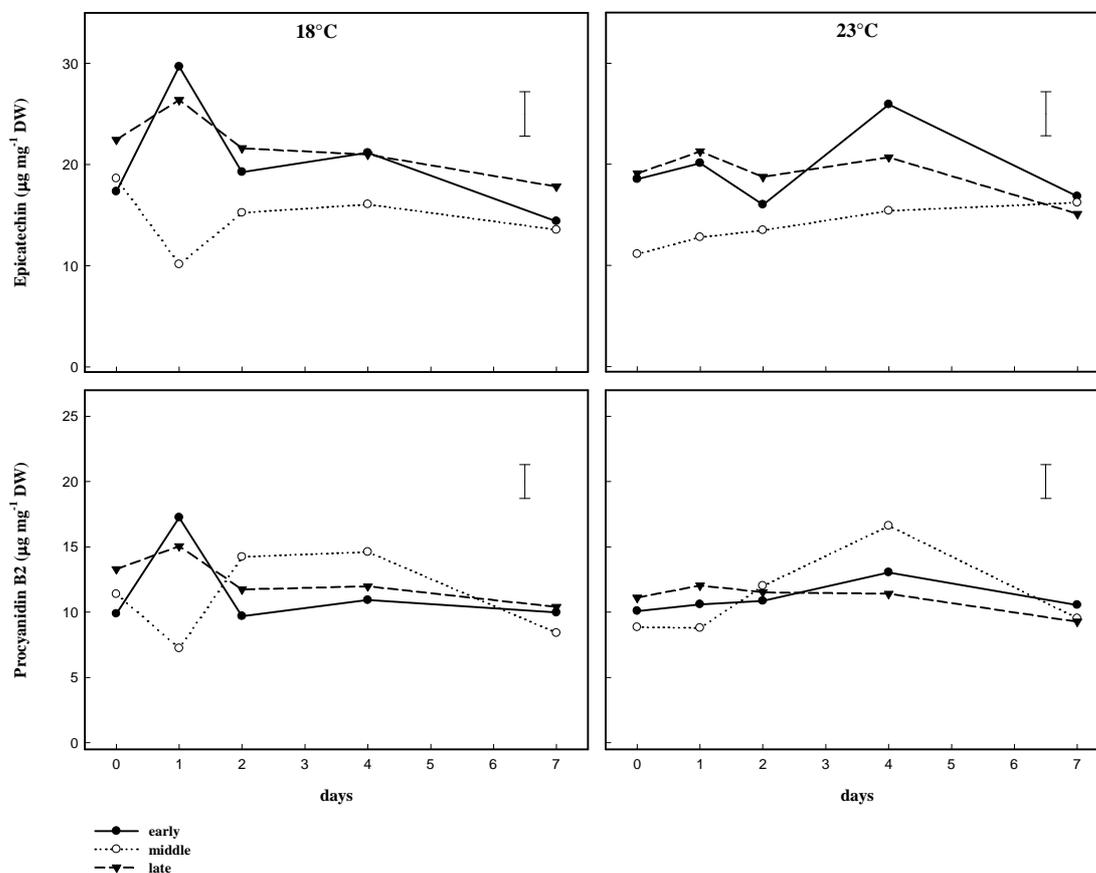


Figure 5.6: Changes in the epicatechin and procyanidin B2 content in the peel of avocado fruit cv. Hass harvested in early, middle and late season. For each compound are showed values related to seven days at 18 and 23°C. Each point is a mean of 8 fruit measurement. LSD bar ($P < 0.05$) is indicated for the interaction day*temperature*season.

The chlorogenic acid was significantly influenced by the interaction between day*season with a similar trend showed by procyanidin B2 at 18°C for middle season fruit. Indeed, after a slow decrease at day 1, chlorogenic acid levels increased in the following days. After 4 days the concentration drop, from 6.1 to 2.9 $\mu\text{g mg}^{-1}$, at day 4 (Figure 5.7). In contrast to other phenolics, any significant differences were noted between seasons and between days and not temperatures.

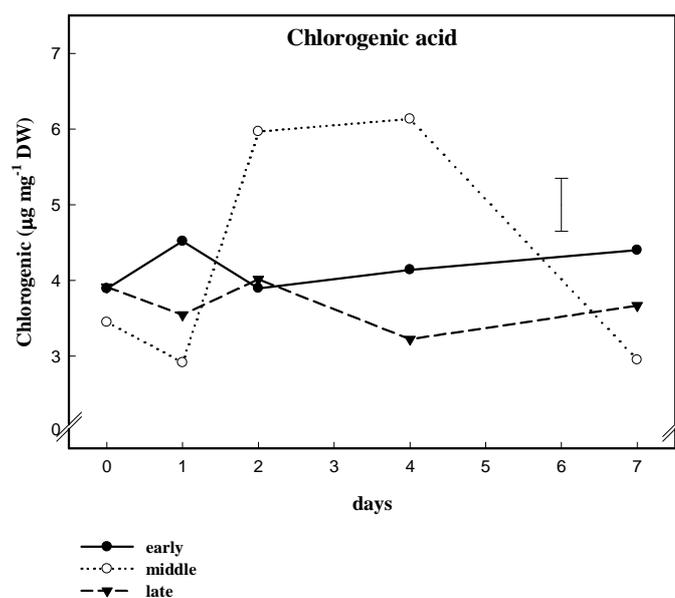


Figure 5.7: Changes in the chlorogenic acid content along ripening in avocado fruit cv. Hass harvested in early, middle and late season. Each point is a mean value of 8 fruit measurement. LSD bar ($P < 0.05$) is indicated for the interaction season*day.

5.3.7 Regression analysis

The interaction between parameters, used to measure mesocarp softening (firmness), objective colour changes (hue angle) and anthocyanin content (cyanidin 3-*O*-glucoside concentration), were studied herein. Results from exponential growth curve showed higher R^2 values for the interactions hue angle*cyanidin3-*O*-glucoside (Figure 5.8), hue angle*firmness (Figure 5.9) yet a low R^2 value was observed for the interaction firmness*cyanidin 3-*O*-glucoside, with $R^2 = 0.319, 0.340, 0.199$ for early, middle and late season fruit, respectively. However, early and middle season showed the better regression between hue angle and cyanidin 3-*O*-glucoside content, late and middle season fruits showed higher R^2 in the hue angle*firmness interaction. Results for the interactions cyanidin 3-*O*-glucoside vs. firmness showed that fruit from late harvested had the lower R^2 in comparison with others seasons. Additionally, an analysis on the effect of temperature showed higher R^2 in the interactions hue angle* cyanidin3-*O*-glucoside and firmness*hue angle when fruit were held at 23 ($R^2=0.931$ and 0.707 ,

respectively) rather than 18°C ($R^2 = 0.782$ and 0.613 , respectively) regardless fruit maturity.

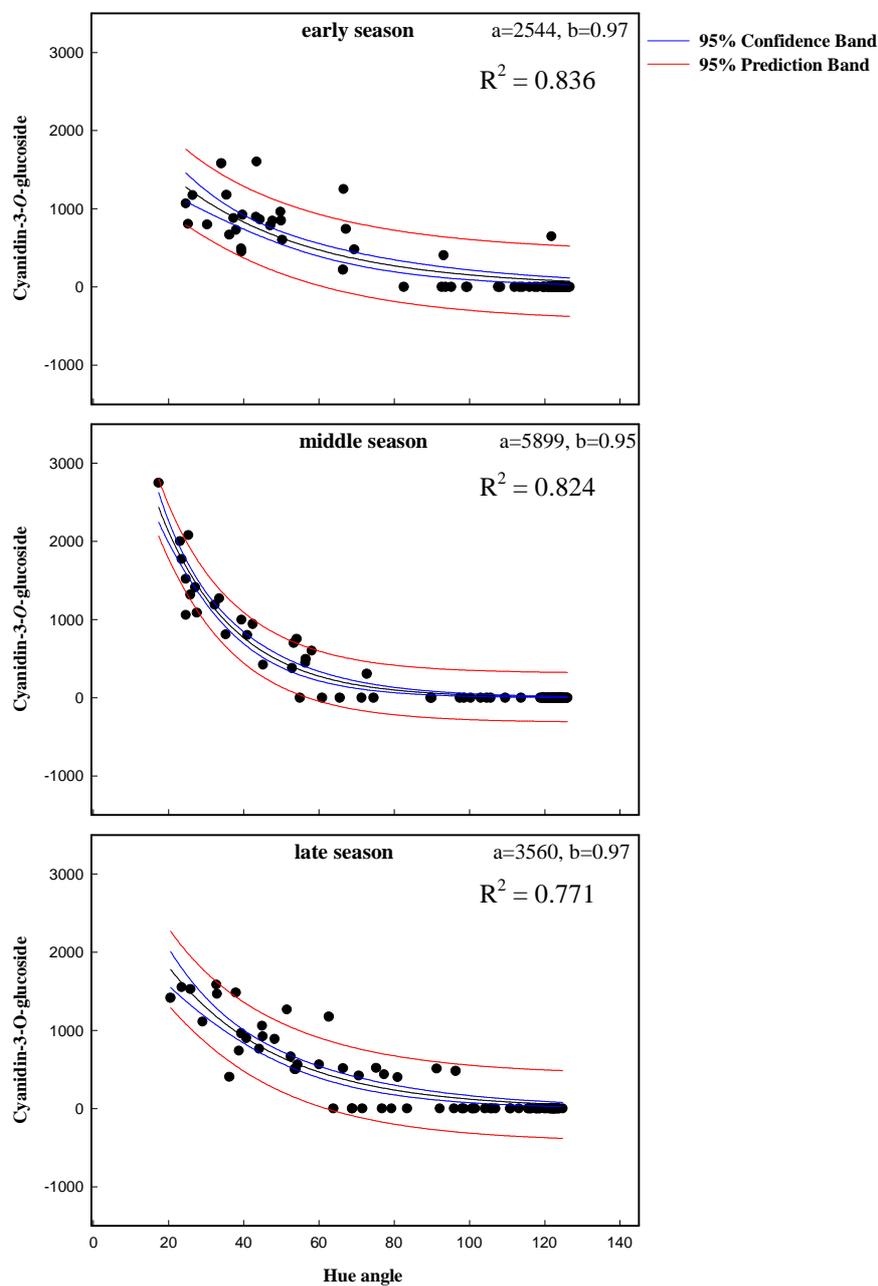


Figure 5.8: Regression ($f = a \cdot b^x$) for the exponential growth curves calculated between the parameters hue angle vs. cyanidin 3-O-glucoside for each harvest season (early, middle and late).

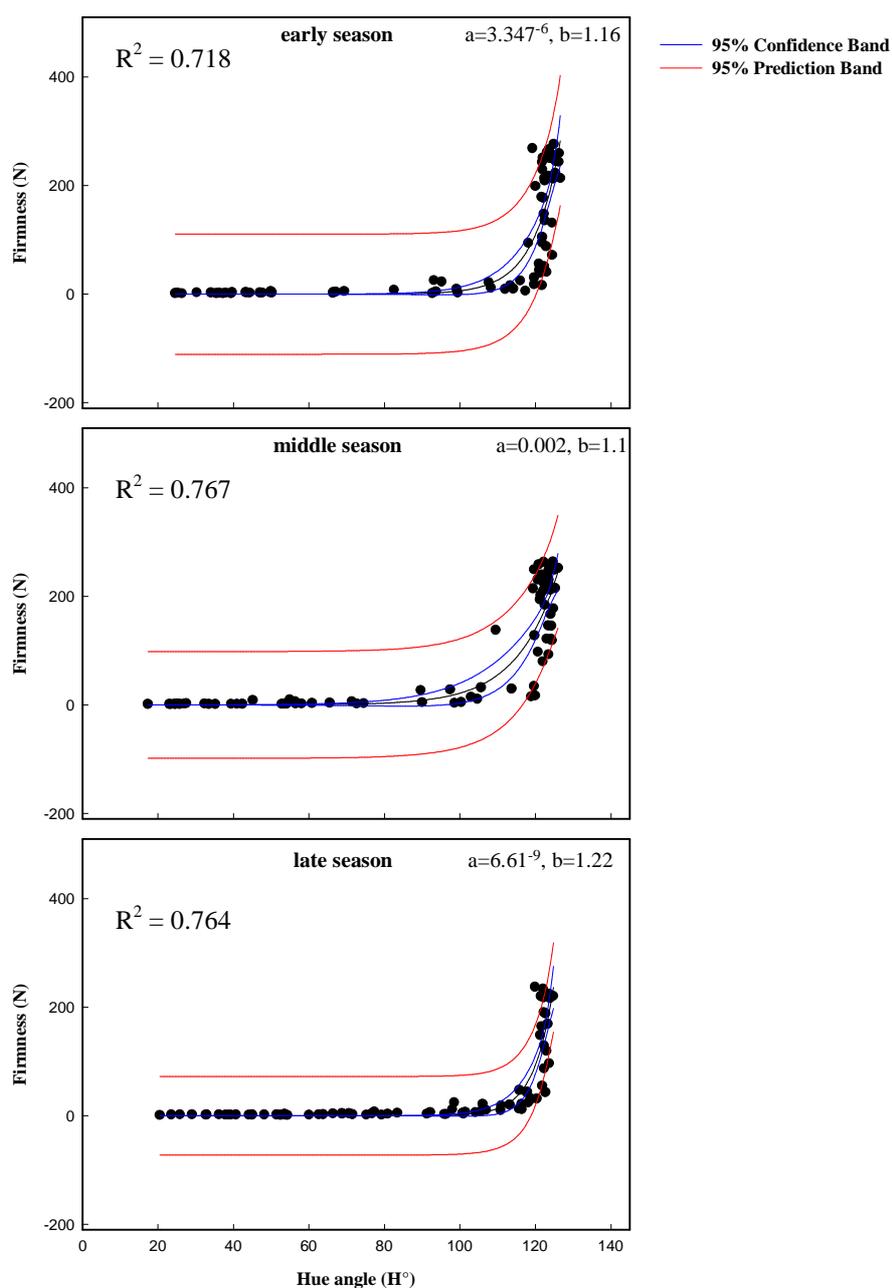


Figure 5.9: Regression ($f = a \cdot b^x$) for the exponential growth curves calculated between the parameters firmness vs. hue angle for each harvest season (early, middle and late).

5.4 Discussion and conclusions

The change in skin colour from green to deep purple in avocado fruit cv. Hass harvested in Malaga, Spain, was influenced by shelf life temperature and fruit maturity.

In particular, at the beginning of the ripening, a faster decrease in chroma and hue angle was observed in late season fruit, also accompanied by faster mesocarp softening. These results are in agreement with previous investigations suggesting that fruit that is more mature ripens faster (Dixon *et al.*, 2003b). However, late season fruit had a similar trend in hue angle values to middle season fruit whereas the mesocarp softening was more comparable between middle and early season fruit.

Nowadays, the variation of colour in fruit and vegetable has been reported to be due to a change in pigment concentrations such as chlorophylls, carotenoids and anthocyanins (Brouillard *et al.*, 1997; Lancaster *et al.*, 1997). In this study, chlorophyll *a* represents the main chlorophyll in avocado peel and decreased during ripening with values between 573-508 $\mu\text{g g}^{-1}$ DW comparable with the 0.4 mg g^{-1} FW found by Cox and colleagues (2004). Chlorophyll *b* almost 2.5 times lower, showed slight increase along ripening. In this work the decrease in the total carotenoids content detected at 18°C was in agreement with previous work (Asthon *et al.*, 2006). However, in late season fruit, the initial carotenoids content was lower than in other seasons and only slight changes were detected during ripening, with an increase noted at 23°C. Nevertheless, in avocado fruit cv. Hass it has been previously suggested, and here confirmed, that there is a predominant role of the cyanidin 3-*O*-glucoside in skin colour changes during fruit ripening (Cox *et al.*, 2004; Asthon *et al.*, 2006). The increase in anthocyanin content was influenced by temperature during ripening. Indeed, the cyanidin 3-*O*-glucoside synthesis was stimulated by higher temperature with a faster and more consistent increase in its content. Higher cyanidin 3-*O*-glucoside were reached in middle season (1,899 $\mu\text{g g}^{-1}$ DW) fruit after 7 days at 23°C which support the lower H° values detected for these fruit. Lower cyanidin 3-*O*-glucoside content was detected in fruit from other seasons. It seems to be difficult to detect a general range of anthocyanin content able to distinguish fruit harvest season. Indeed, the cyanidin 3-*O*-glucoside showed a wide range influenced by temperature of ripening with values between 499 and 1,079 $\mu\text{g g}^{-1}$ DW in late season in fruit held at 18 and 23°C, respectively). This said, considering that the DW was almost the 30% of the peel weight, this results are in the range with those reported by Cox and colleagues (2004) using the same cultivar of avocado grown in New Zealand harvested late in the season

and ripen at a different range of temperature (15, 20 and 25°C), with 321 mg kg⁻¹ FW in ripe fruit. Similar results were also obtained by Asthon and collaborators (2006) on early season avocado fruit from New Zealand detecting *ca.* 250 µg g⁻¹ FW in ripe cv. Hass fruit.

In conclusion, changes in the peel tissue colour, objectively evaluated as decrease in C*, L* and H° values, here coincide with the increase in cyanidin 3-*O*-glucoside content, decrease in chlorophyll *a* and total carotenoids. Indeed, lower H° in middle season fruit at 23°C correspond with the lower chlorophyll *a* and total carotenoids content and the higher cyanidin 3-*O*-glucoside levels. The darker colour achieved by fruit with higher maturity or when fruit are held at higher temperature (Cox *et al.*, 2004), could be a result of a higher content of anthocyanin and lower levels of chlorophyll *a* and carotenoids.

However, to better understand the relationship between objective colour measurement (H°), cyanidin 3-*O*-glucoside content and mesocarp softening, a regression studied was applied at the three parameters. The relationship between hue angle and cyanidin 3-*O*-glucoside, here calculated as regression, showed relatively higher values in fruit harvested early in season. This variation might be explained by observing how the two parameters are distributed: unless the cyanidin content follows a similar pattern between maturity stages, the hue angle is more predictable in fruit with low maturity. A similar trend was found between firmness and cyanidin, which was also poorly correlated. Both hue angle and firmness showed higher predictability in fruit with higher maturity with a possible use of skin colour as ripening indicator of middle-late season fruit. Regarding the influence of temperature, the interactions cyanidin*hue angle and hue angle*firmness showed better correlation at 23 rather than at 18°C. Cox and colleagues (2004) also found increased R² index in response to higher ripening temperature. The temperature in postharvest is an important factor in the synthesis of the cyanidin 3-*O*-glucoside as previously stated.

Besides the anthocyanins, other phenolic derivatives were detected and quantified. The most abundant present in the skin tissues was the epicatechin, with a peculiar trend during ripening. Indeed, early and late season fruit was marked by a burst in the epicatechin content at day 1 at 18°C whereas in middle season fruit drastically

decreased. A similar trend was found in procyanidin B2 content. At a higher temperature, the epicatechin and procyanidin B2 content which differed between seasons was less accentuated and slightly increases later at day 4 in early and late season fruit. The procyanidin B2 content reported for cv. Hass around 38 mg g⁻¹ FW at not specified ripening stage (Wang *et al.*, 2010) are comparable with the one here identified. Ardi and colleagues (1998) reported 1,447 µg g⁻¹ FW in unripe cv. Hass fruit.

As climacteric fruit, the ripening process of avocado is recognizable by a rise in ethylene production that is involved in many molecular pathways, one of which is the synthesis of phenolic compounds. Indeed, the stimulation of epicatechin and procyanidin synthesis seems to precede, as at 18°C, or follow, as at 23°C, the climacteric peak. However, the drop in the mentioned compounds in middle season fruit with a higher ethylene release might be an indicator of a threshold of ethylene or its precursor that inhibits the phenolic production and induces their reduction. Differently, the chlorogenic acid, with no consistent changes during ripening in early and late season fruit, showed a drastic increase, almost the double of day 0, with a drop at the end of shelf life in middle season fruit. In avocado fruit a decrease in epicatechin content during ripening has been detected from the pericarp in cv. Fuerte (Ardi *et al.*, 1998; Guestky *et al.*, 2005). These results link ethylene production with the phenolic pathway in avocado fruit, with a possible different action on the synthesis of each single compound. Exogenous ethylene application seems to increase epicatechin biosynthesis in unripe cv. Fuerte avocado in the follows 24 hours (Ardi *et al.*, 1998) and to induce gene expression in the anthocyanin biosynthesis (El-Kereamy *et al.*, 2003). Besides, as previously stated, the presence of compounds with anti-fungal activity is an important tool in the management of avocado fruit postharvest. Specifically, epicatechins seems to have a role in the metabolism of the antifungal diene (Ardi *et al.*, 1998) identified in avocado peel involved in the protection against *C. gleosporioides* (Prusky, 1991). If results here are confirmed, external ethylene levels and ripening temperature can be used to increase avocado fruit resistance to infection.

To conclude, this work has shown in avocado fruit cv. Hass there to be a correlation between skin colour changes and mesocarp softening which is strongly influenced by fruit maturity at harvest and ripening temperature. A good interaction has

been found between hue angle and cyanidin-3-*O*-glucoside content and between hue angle and firmness. The hue angle parameters seem to be better related with the firmness changes as fruit maturity increase. Therefore, the possible use of objective colour (H°) as indicator of avocado fruit cv. Hass ripening stage has to take in account fruit maturity at harvest. Additionally, the influence of temperature has to be taken in consideration, with the use of higher temperature causing a faster ripening. However, the cyanidin content and the mesocarp softening are poorly correlated and it needs further investigations. This work also confirms the predominant role of cyanidin 3-*O*-glucoside and chlorophyll *a* in defining of the change in skin colour from green to purple during ripening, regardless of fruit maturity and temperature. Furthermore, this work underlined the possible interaction of other metabolite such as internal ethylene and total carotenoids content in the determination of avocado fruit skin colour.

Concluding, the skin colour changes could be an additional indicator of avocado cv. Hass ripening when fruit has a faster ripening behaviour as in higher maturity fruit and when fruit is held at higher temperature. The understanding of the regulation systems among pigments metabolism, anthocyanins biosynthesis, mesocarp softening and ethylene released will be an important achievement in the management of avocado fruit ripening. Furthermore, preharvest factors or postharvest treatments (i.e. ethylene application) might have an effect on the relationship between skin and mesocarp parameters.

6 CHAPTER SIX

INVESTIGATION ON THE EFFECT OF ETHYLENE AND CONTROLLED TEMPERATURE ON THE RIPENING OF AVOCADO FRUIT CV. HASS IMPORTED FROM SOUTH AFRICA

6.1 Introduction

The importance of ethylene in the metabolism of climacteric fruit has been extensively reported (Faubion *et al.*, 1992; Pesis *et al.*, 2002; Hershkovitz *et al.*, 2009; Hershkovitz *et al.*, 2010). Usually, in fruit such as avocado, ripening is marked by a burst of endogenous ethylene followed by the activation of an ethylene response system (Biale *et al.*, 1954; Adato and Gatiz, 1977; Lin *et al.*, 2009). This phytohormone seems to play a key role in the control of the ripening process by regulating softening and colour changes (Awad and Young, 1979; Pesis *et al.*, 1978). Exogenous ethylene is commercially used to trigger ripening. Commercially, ethylene (from 10 up to 1000 $\mu\text{l l}^{-1}$) is usually applied from a gas cylinder diluted in air or from ethylene generators for 12-24 hours at different temperatures (15-25°C) (Saltveit, 1999). Differences in the response to exogenous ethylene have been found regarding fruit maturity and storage time. For instance, the treatment can be ineffective if ethylene is applied immediately after harvest possible due to the presence of inhibitor factors released from the tree (Adato and Gatiz, 1974) or by the action of C7 sugars (Liu *et al.*, 1999b). In contrast, late season fruit seem to respond to exogenous ethylene when it is applied immediately after picking (Starrett and Laties, 1991). This differential behaviour is possible due to the lower level of the inhibitory factors in fruit with higher maturity (Adato and Gatiz, 1974) and could also be related to the depletion of C7 sugars (Liu *et al.*, 1999b, 2002).

An additional problem in the application of ethylene on avocado is the negative effect noted on fruit quality when a long cold storage is also applied. The use of low temperature to delay fruit ripening becomes necessary when long term shipping is required. A limited range of low temperatures (between 4 to 12°C) are suitable to delay

the softening of unripe avocado fruit. However, the optimal combination between time and temperature of storage can be different depending on cultivar and/or maturity stage; for instance, cv. Hass is usually stored at temperatures between 5 and 7°C (Vorster *et al.*, 1990; Faubion *et al.*, 1992; Cutting and Wolstenholme, 1992; Dixon *et al.*, 2004). Ethylene application before cold storage induces an increase in the incidence of pulp browning in various cultivars (Pesis *et al.*, 2002). Similarly, irrespective of the application time, the ethylene treatment during cold storage has a negative effect on avocado fruit quality (Pesis *et al.*, 2002; Dixon *et al.*, 2003). Good results have been obtained with ethylene treatments applied just before ripening (Köhne, 1985). Though ethylene applied on late season fruit after long term cold storage seems not to affect fruit ripening, non stored fruit ripen faster and result in better quality fruit (Dixon *et al.*, 2003).

Besides, the UK market demands avocado fruit exist mainly as a “ready to eat” product (Terry *et al.*, in press) and inconsistency in the ripening process result in negative feedback from the customer (Mack Multiplies, private communication). As previously said, the use of ethylene as ripening trigger might not always be effective. Therefore, it is compulsory to further investigate whether ethylene application is useful for accelerating avocado fruit ripening

This said, the response of avocado fruit to ethylene treatment can depend on fruit maturity, temperature and storage time. The aim of this work was to assess the possible synergic action of ethylene treatment and temperature on the ripening of stored avocado fruit from different consignments. Specifically, it was of interest to investigate whether exogenous ethylene applied to fruit at different stages of maturity in association with low or high temperature can result in more uniform ripening. Thus, avocado fruit imported from Mpumalanga, South Africa harvested through the year were ethylene treated at different temperatures (12, 16 and 20°C) for 12 hours or at two temperature (12 and 20°C) for 12 or 24 hours. Treated fruit was compared against control fruit for physiological changes (i.e. respiration rate, ethylene production, colour, firmness) during ripening. A possible temperature effect on the treatment was also investigated by changing temperature after the ethylene treatment.

6.2 Methodology

Plant material and experiment design are described further in Chapter 3. Briefly, fruit harvest in May and June in Mampulanga, South Africa were ethylene treated at 12, 16 and 20°C (T2) for 2 hours (Experiment I and II) and transfer at specific temperature (T1) (section 3.1.4). Fruit from the same grower harvested in July were ethylene treated at 12 and 20°C for 12 or 24 hours (Experiment III) (section 3.1.5). The measurement of weight, colour, respiration rate, ethylene production and firmness are described in Chapter 3. Statistical analysis is presented in section 3.4.

6.3 Results

6.3.1 Respiration and ethylene production

In the first experiment, the fruit respiration rate mainly differed between ethylene treatments and ripening temperatures. Indeed, treated fruit had higher CO₂ production when ripened at 20°C. A similar respiration rate between treated and control fruit was noted when fruit were held at 12 and 16°C (Figure 6.1). A rise in the climacteric ethylene was not always detected, with a general low level of ethylene released. Any specific trend was induced by ethylene treatment. Instead, there was a tendency for low ethylene production at cooler conditions (12°C) (Figure 6.2).

In the follows experiment, the temperature was the factor that most affected the respiration rate with no significant difference between other treatments (Figure 6.3). Indeed, fruit held at 20°C showed a faster increase in respiration rate with the maximum rate detected between day 2 and 4. In contrast, in fruit held at lower temperatures (12 and 16°C) the CO₂ production slowly increased with maximum values reached at day 7. Ethylene production was generally low although slightly higher ethylene was released at 20°C marked by a constant increase during ripening (Figure 6.4). In contrast, ethylene levels in fruit held at lower temperatures was generally low at the end of shelf life.

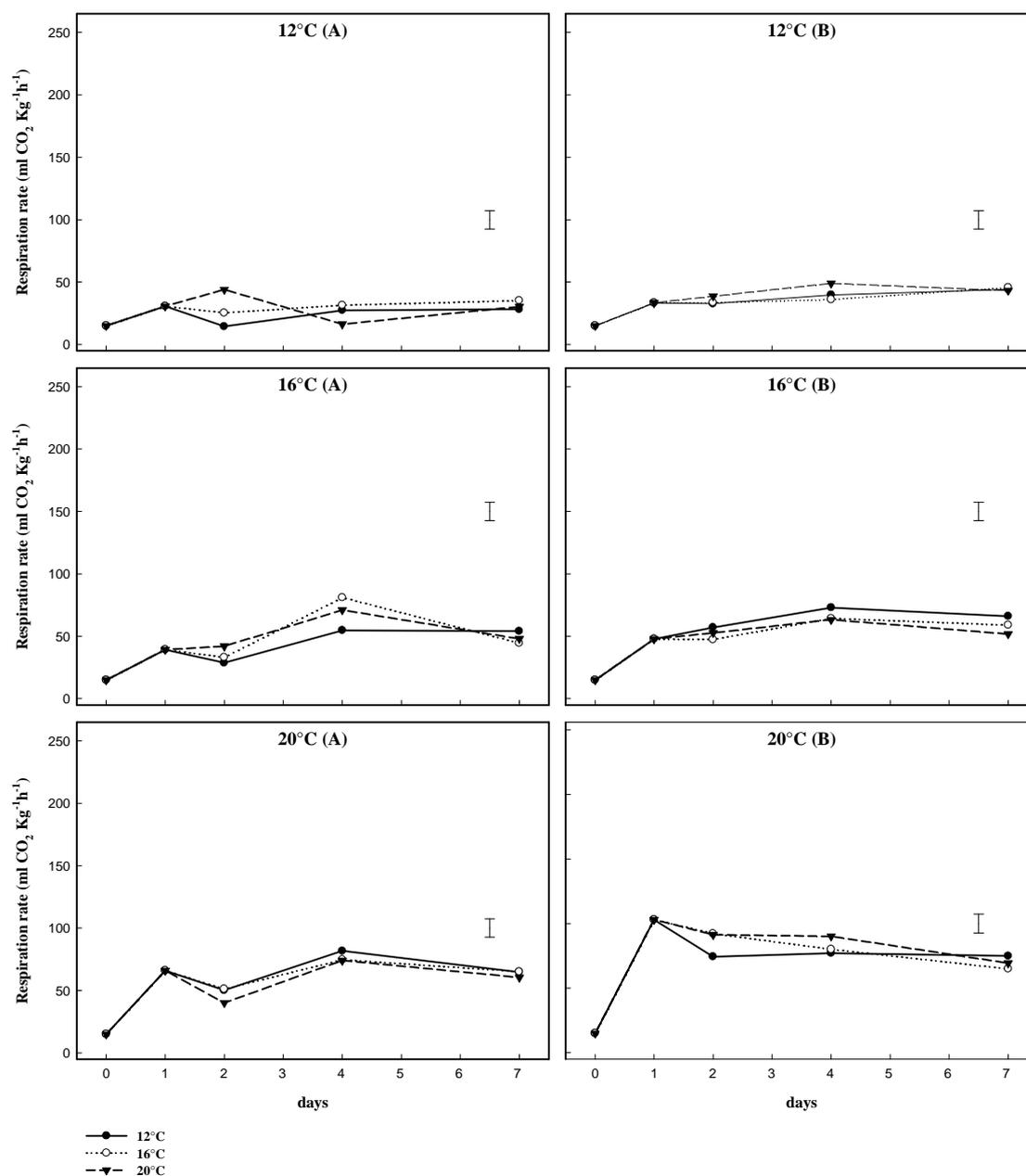


Figure 6.1: Respiration rate (ml of CO₂ kg⁻¹h⁻¹) of early season avocado fruit (cv. Hass) held at different temperatures (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (●), 16°C (○), or 20°C (▼). LSD ($P < 0.05$) = 14.8 for the interaction Baseline 1_treatment*T1*baseline 2*days is indicated for each temperature of ripening.

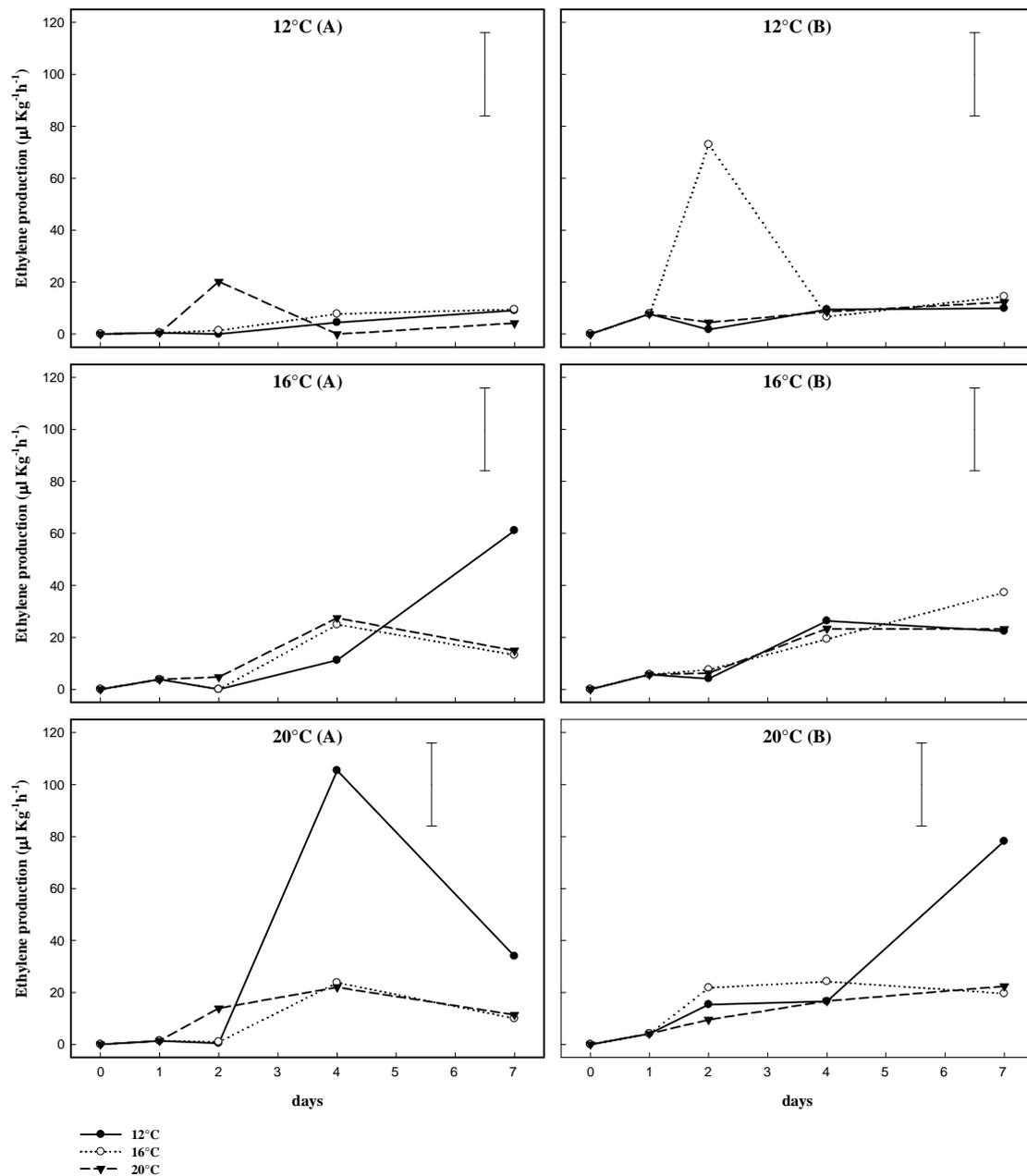


Figure 6.2: Ethylene production ($\mu\text{l kg}^{-1}\text{h}^{-1}$) of early season avocado fruit (cv. Hass) held at different temperature (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (●), 16°C (○), or 20°C (▼). LSD ($P < 0.05$) = 31.99 for the interactions Baseline 1_treatment*T1*Baseline 2_T2*days is indicated for each temperature of ripening.

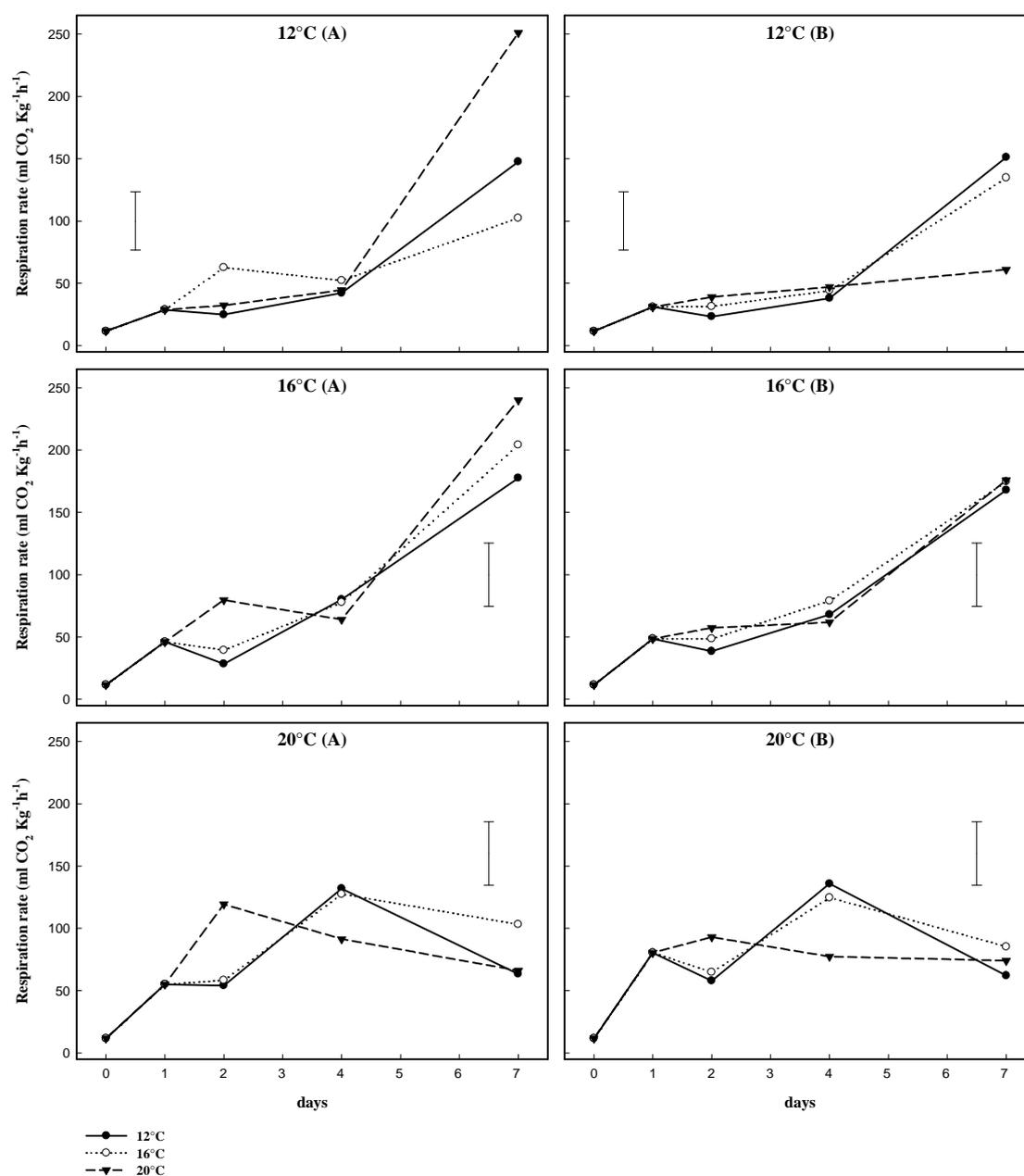


Figure 6.3: Respiration (ml of CO₂ kg⁻¹h⁻¹) of middle season avocado fruit (cv. Hass) held at different temperature (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (●), 16°C (○), or 20°C (▼). LSD ($P < 0.05$) = 50.79 Baseline 1_T1*Baseline 2*days is indicated for each temperature of ripening.

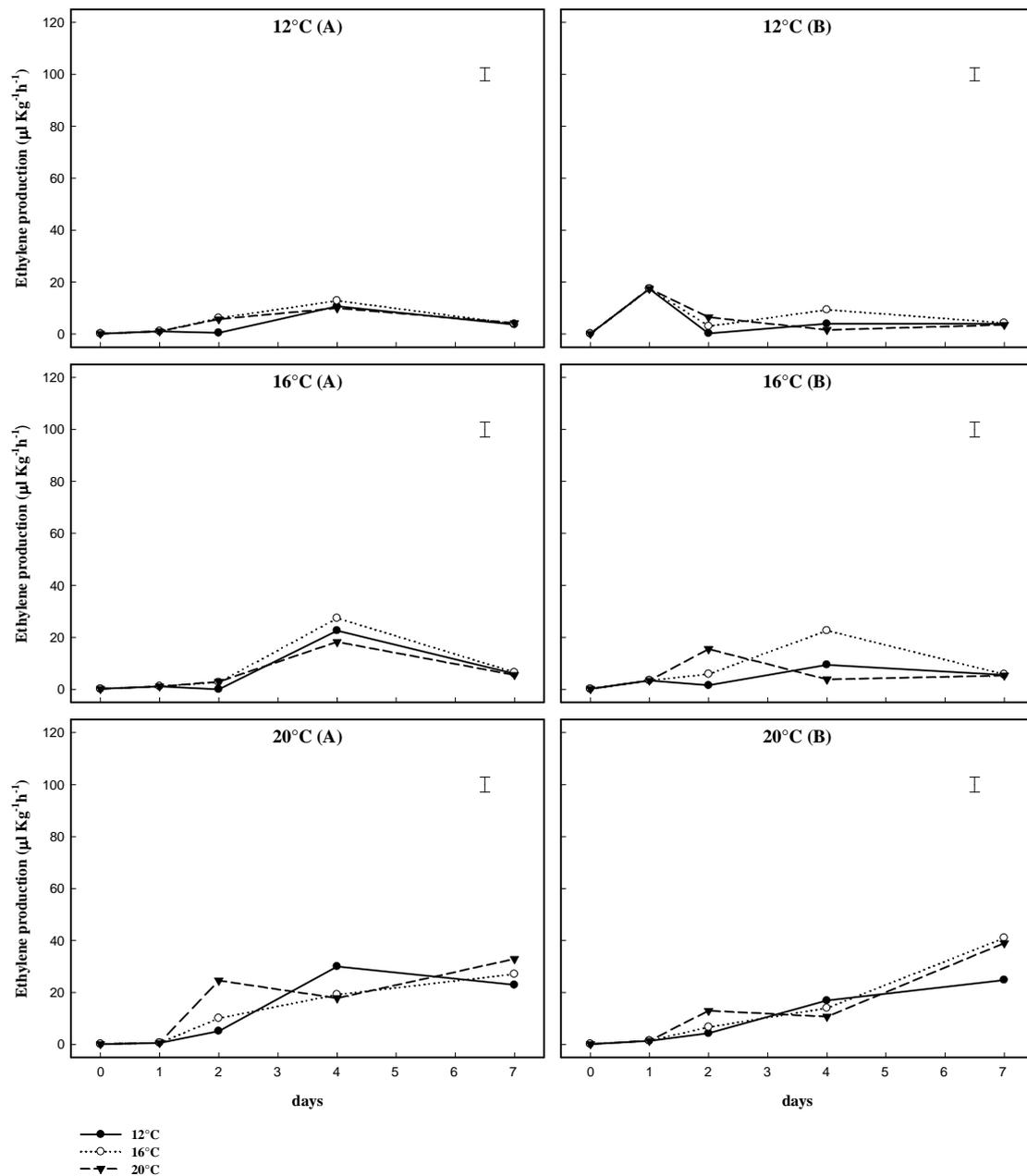


Figure 6.4: Ethylene production ($\mu\text{l kg}^{-1}\text{h}^{-1}$) of middle season avocado fruit (cv. Hass) held at different temperature (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (●), 16°C (○), or 20°C (▼). LSD ($P < 0.05$) = 5.77 for the interactions Baseline 1_T1*treatment*Baseline 2_T2*days is indicated for each temperature of ripening.

6.3.2 Colour

Colour parameters (L^* , C^* and H°) decreased throughout ripening in all experiments. In fruit from the first harvest chroma (C^*) was influenced by treatment and days whereas lightness (L^*) and hue angle (H°) were mainly changing regarding the interaction days*temperature. In particular, H° decreased faster in fruit kept at higher temperature (20°C) with significantly lower values at the end of shelf life compared to other temperatures (Figure 6.5). C^* decreased faster in ethylene treated fruit and at 20°C regardless of treatment (Figure 6.6) whereas L^* showed similar behaviour in all treatments (Figure 6.7).

A similar trend in fruit colour was observed in the following experiment (Experiment II). Fruit harvested in June showed faster colour changes than early season fruit with lower C^* and H° values reached at day 7. L^* (Figure 6.10) and H° decreased throughout ripening influenced by postharvest condition, with faster changes at higher temperatures. In particular, lower H° characterized fruit held at 16 and 20°C (Figure 6.8). In contrast to the previous experiment, C^* significantly changed regarding the interaction days*temperature with a faster decrease at 20°C (Figure 6.9). L^* had a similar behaviour in all conditions. Generally ethylene treatment did not significantly influence skin colour changes.

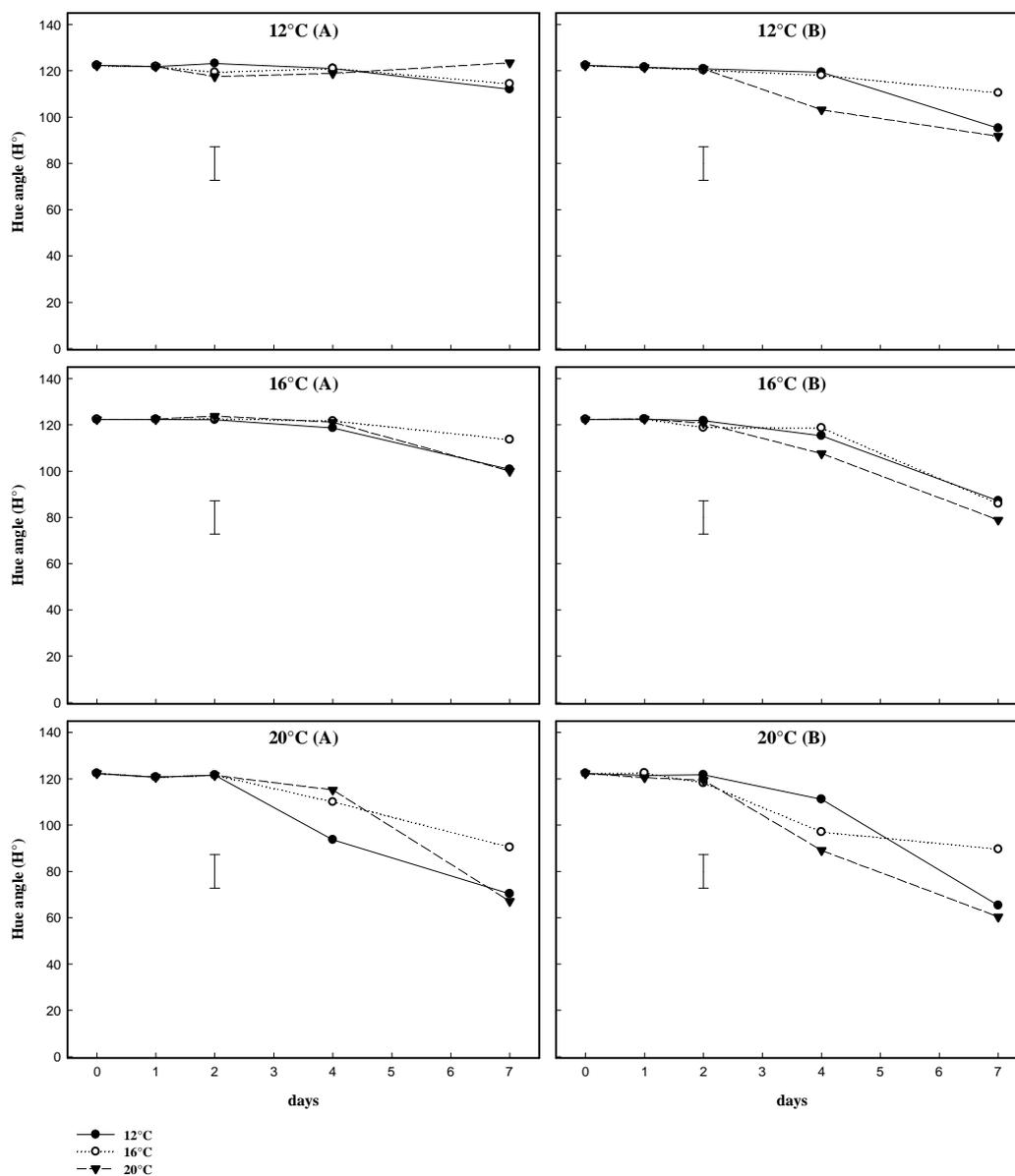


Figure 6.5: Hue angle (H°) measured in early season avocado fruit held at different temperatures (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (\bullet), 16°C (\circ), or 20°C (\blacktriangledown). LSD ($P < 0.05$) = 13.47 for the interactions Baseline 1_T1*Baseline 2_day is indicated.

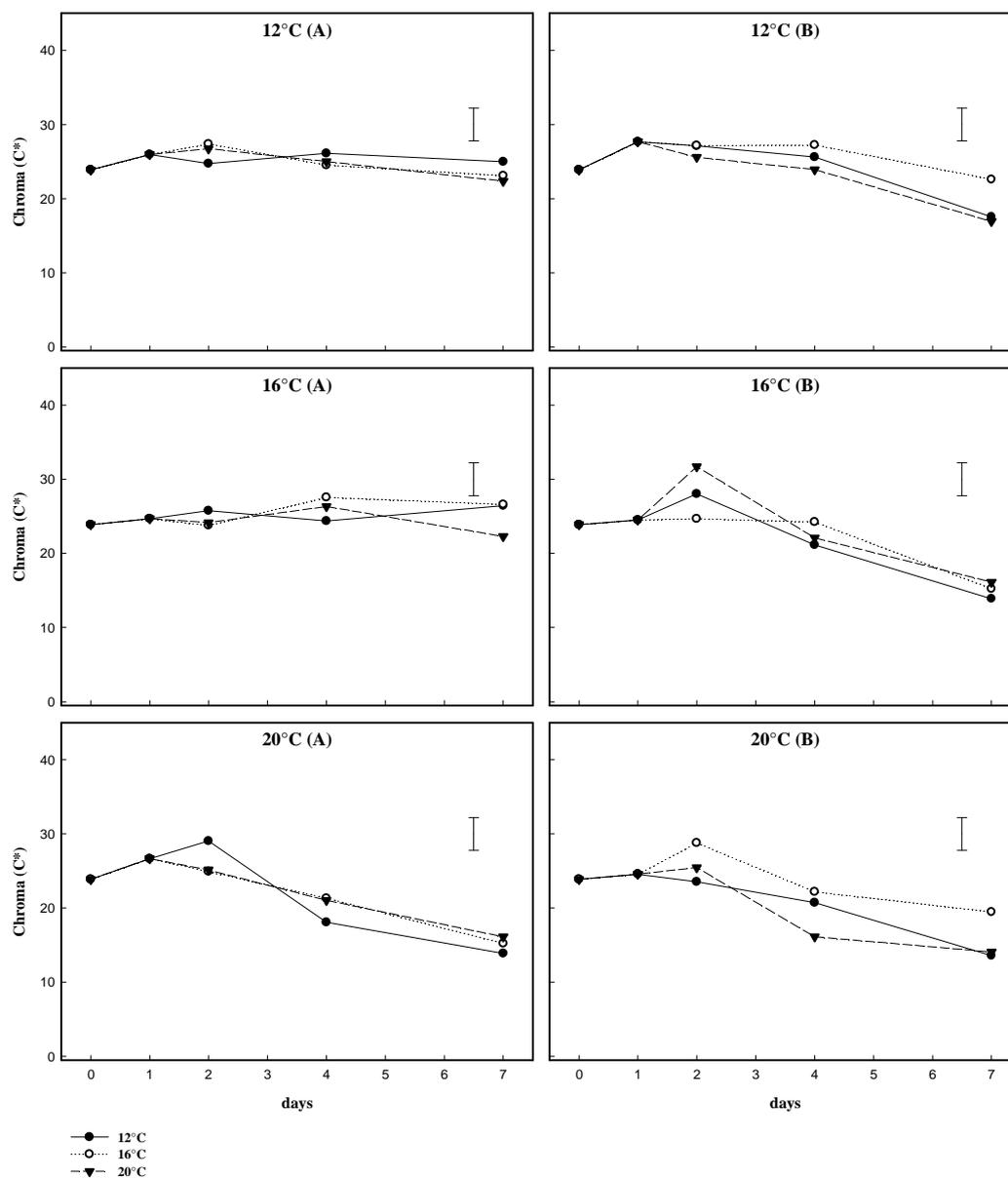


Figure 6.6: Chroma (C^*) measured in early season avocado fruit held at different temperatures (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (●), 16°C (○), or 20°C (▼). LSD ($P < 0.05$) = 4.4 for the interactions Baseline 1_treatment*Baseline 2_day is indicated.

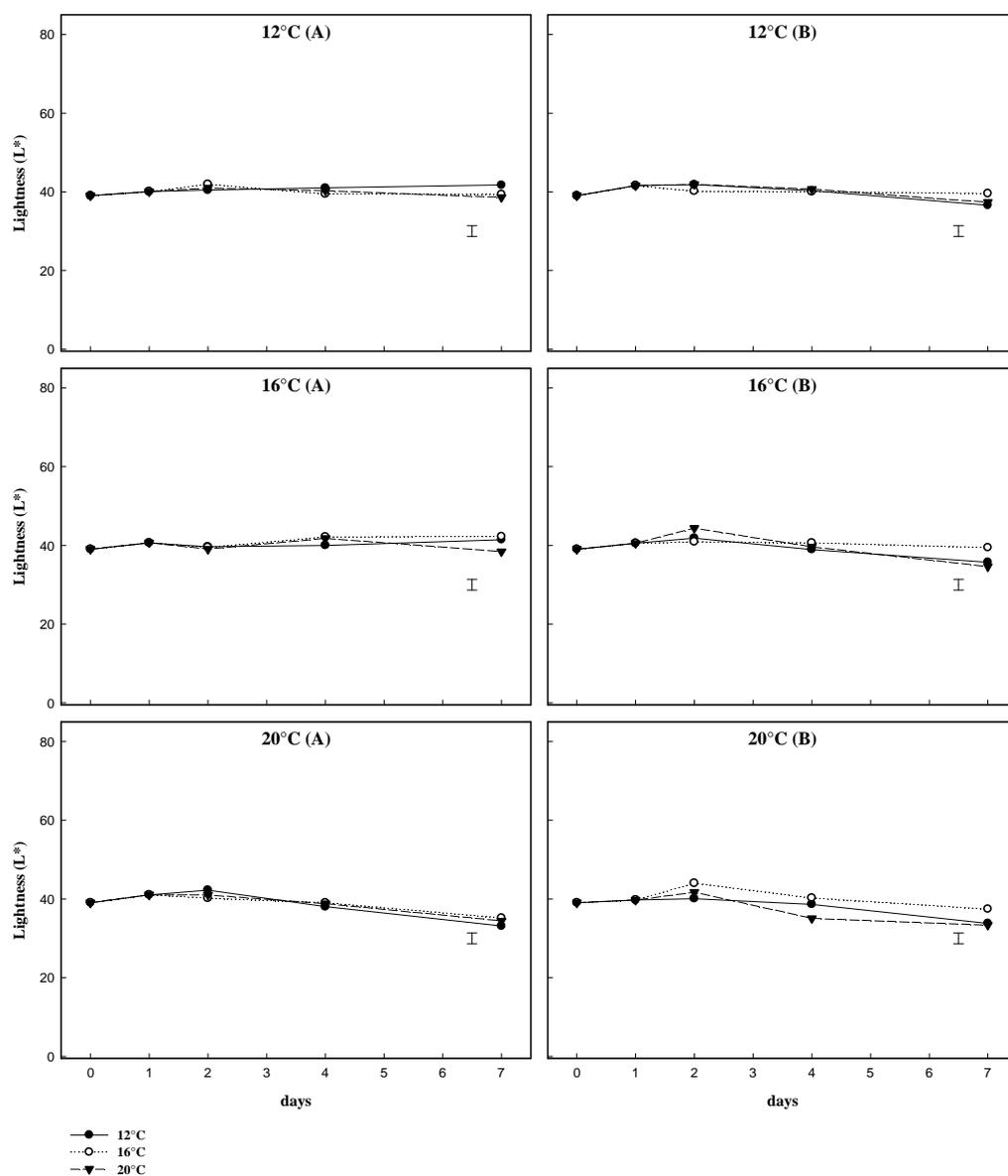


Figure 6.7: Lightness (L^*) measured in early season avocado fruit held at different temperatures (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (●), 16°C (○), or 20°C (▼). LSD ($P < 0.05$) = 2.8 for the interactions Baseline 1_T1*Baseline 2_day is indicated.

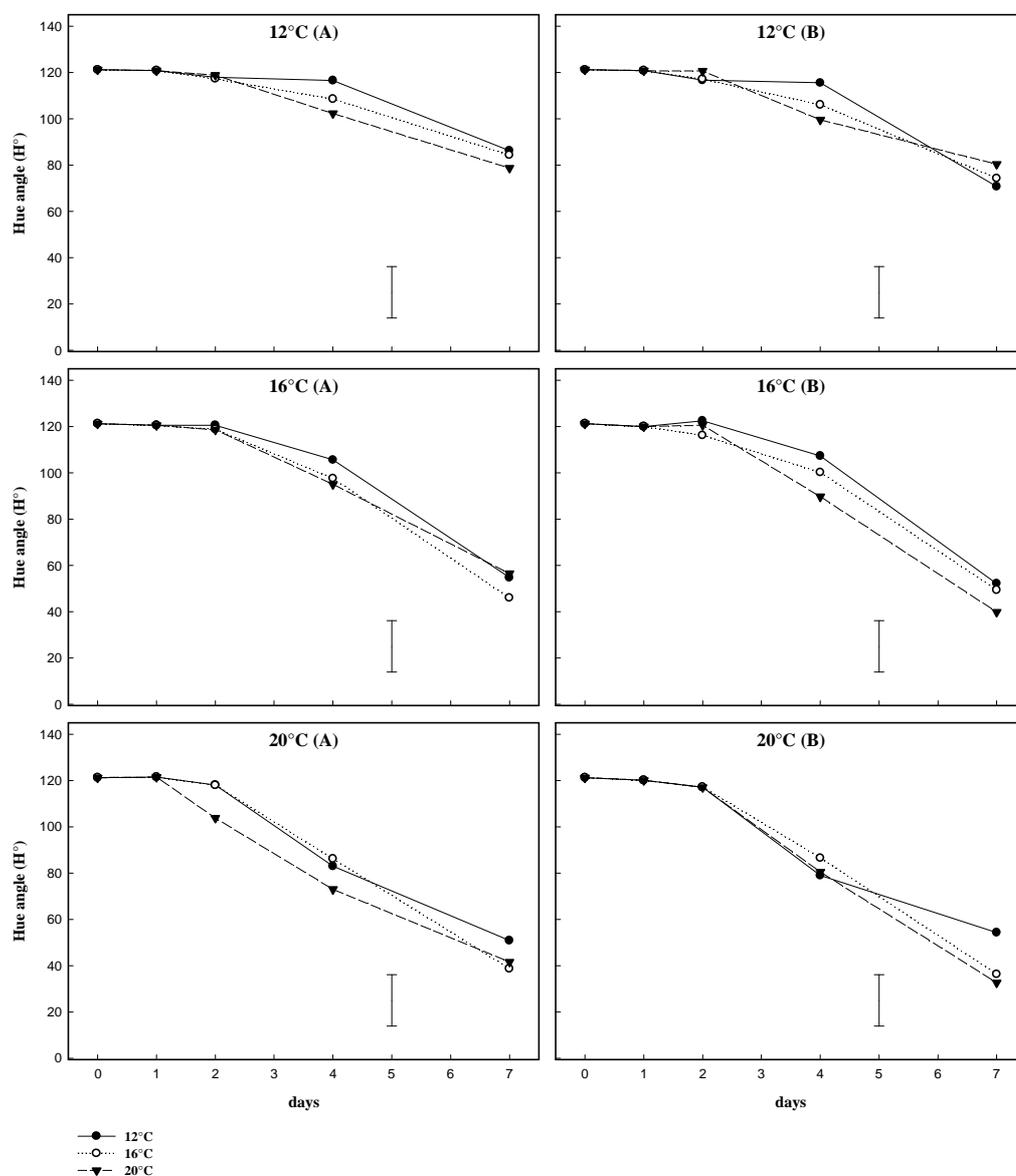


Figure 6.8: Hue angle (H°) measured in middle season avocado fruit held at different temperatures (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (●), 16°C (○), or 20°C (▼). LSD ($P < 0.05$) = 9.06 for the interactions Baseline 1_T1*Baseline 2_day is indicated.

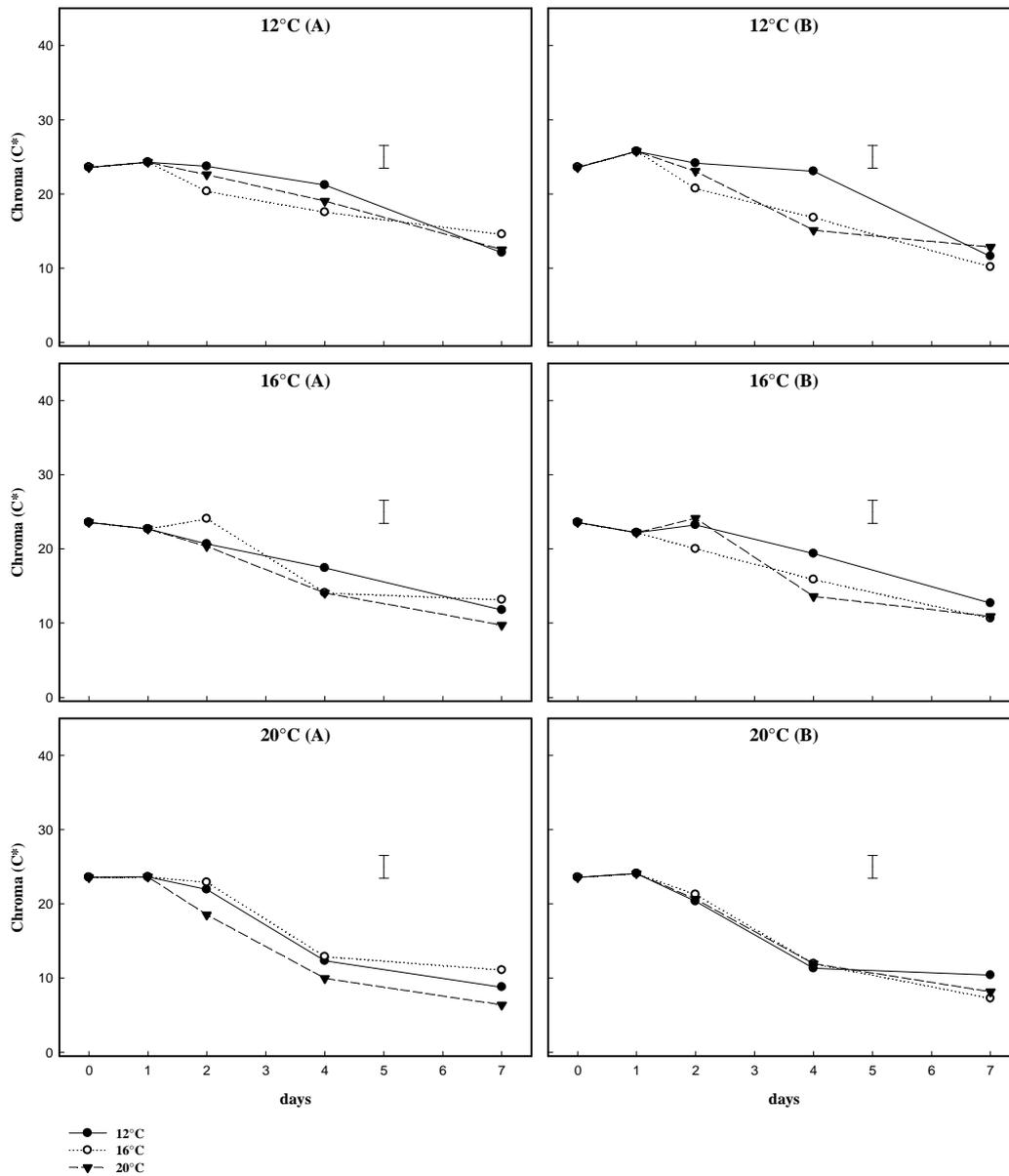


Figure 6.9: Chroma (C^*) measured in middle season avocado fruit held at different temperatures (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (●), 16°C (○), or 20°C (▼). LSD ($P < 0.05$) = 3.1 for the interactions Baseline 1_T1*Baseline 2_day is indicated.

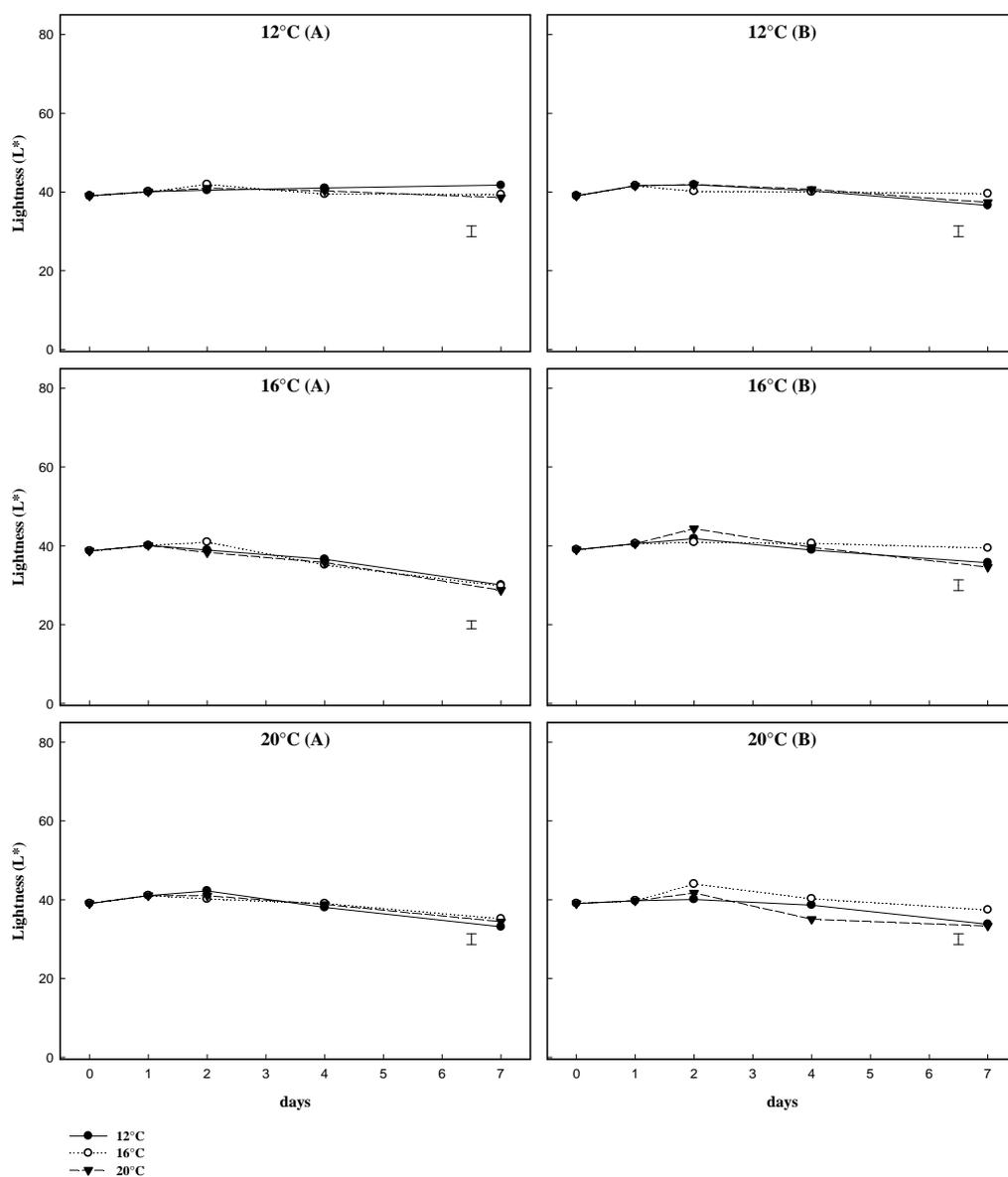


Figure 6.10: Lightness (L^*) measured in middle season avocado fruit held at different temperatures (12, 16 and 20°C). For each temperature half of the fruit were ethylene treated (B) and the other half used as control (A). Fruit were ethylene treated or held without ethylene at 12°C (●), 16°C (○), or 20°C (▼). LSD ($P < 0.05$) = 1.97 for the interactions Baseline 1_T1*Baseline 2_day is indicated.

6.3.3 Firmness

In the first experiment, fruit mesocarp softening was significantly different regarding ethylene treatment and days. Indeed, ethylene treated fruit showed a faster softening than control fruit which remained firmer at the end of shelf life. However, there was no evidence that the temperature at which ethylene was applied or ripening temperature had any effect on firmness. Higher variability between samples was detected regardless of the conditions (Table 6.1). In contrast, in the following experiment (Exp. II), temperature and ethylene affected mesocarp softening. The firmness was influenced by the interactions between temperature of treatment*days and by ripening temperature and ethylene treatment. Fruit held at 12°C and treated with ethylene at low temperatures (12 and 16°C) showed a faster softening process than non-treated. At higher temperatures (16 and 20°C) ethylene treated and control fruit had similar firmness measured at day 7. Fruit treated at 20°C and after held at 12 and 16°C were firmer than control fruit. The variability between fruit was less compared to previous experiment, as indicated by lower S.E. (Table 6.2).

Table 6.1: Firmness values in treated (e+) and control (e-) fruit through seven days (Exp. I). The table indicated the temperature at which the ethylene was applied (12, 16 and 20°C) and the ripening temperature (12, 16 or 20°C). Fruit were significant different for the factors days and treatment.

Firmness (N)		Temperature of shelf life					
		12°C		16°C		20°C	
Day 0	Treatment	Day 2	Day7	Day 2	Day7	Day 2	Day7
218.91 ± 6.76	12°C (e+)	335±19.07	77±63.94	182±88.29	70±58.36	298±48.13	56±46.97
	12°C (e-)	364±2.37	162±76.21	351±4.72	129±112.90	330±40.88	114±111.39
	16°C (e+)	295±67.89	21±8.46	275±36.83	9±2.63	218±94.16	18±7.83
	16°C (e-)	329±38.71	206±101.59	353±12.56	189±93.68	377±10.75	136±69.89
	20°C (e+)	287±39.47	13±5.13	210±20.37	73±69.13	219±61.17	61±57.45
	20°C (e-)	246±55.57	184±87.81	306±58.19	124±119.21	213±90.64	85±82.60

Each value is a mean of six (day 0) and three (day 2 and 7) fruits measurement with S.E. indicated. LSD ($P < 0.05$) = 94.1 for the interaction Baseline 1_treatment and Baseline 1_Baseline 2_days is indicated.

Table 6.2: Firmness values in treated (e+) and control (e-) fruit through seven days (Exp. II). The table indicated the temperature at which the ethylene was applied (12, 16 and 20°C) and the ripening temperature (12, 16 or 20°C). Mesocarp softening was significantly affected by the interactions temperature of treatment*days and by treatment and ripening temperature.

Firmness (N)		Temperature of shelf life					
		12°C		16°C		20°C	
Day 0	Treatment	Day 2	Day7	Day 2	Day7	Day 2	Day7
230.17 ± 8.14	12°C (e+)	155.9±43.90	7.1±0.26	129.7±30.51	3.9±0.38	70.6±7.61	2.6±0.31
	12°C (e-)	145.7±41.79	55.6±48.27	215.6±39.68	3.1±0.13	71.8±11.37	2.5±0.18
	16°C (e+)	118.9±40.18	5.2±0.82	83.9±20.10	2.6±0.13	72.6±23.03	2.4±0.24
	16°C (e-)	150.3±46.08	43.8±38.09	132.1±52.85	58.4±55.79	114.7±31.92	1.9±0.05
	20°C (e+)	66.3±41.61	34±28.02	31.4±4.43	14±11.17	33±3.05	2.8±0.28
	20°C (e-)	100.6±66.03	6.5±1.12	106.3±32.15	2.2±0.20	36.9±16.90	2.5±0.27

Each value is a mean of six (day 0) and three (day 2 and 7) fruits measurement with S.E. indicated. LSD ($P < 0.05$) = 45.94 (temperature of treatment*day) and 41.94 (treatment).

6.4 Influence of temperature on the ethylene production and respiration rate perception

In the first experiment, the respiration rate was significantly affected by the interactions between temperature at which the measurement was taken (T3) and days and treatment*T3*Baseline 2_temperature of treatment*day. Fruit measured at higher temperatures had higher CO₂ production (Figure 6.11-13). Generally, the ethylene released was very low at 12°C and slightly increased at 16°C. Significant differences were detected in the interactions treatment*day*temperature of treatment (12°C) and T3*temperature of treatment*days (16°C) (Figure 6.12-14).

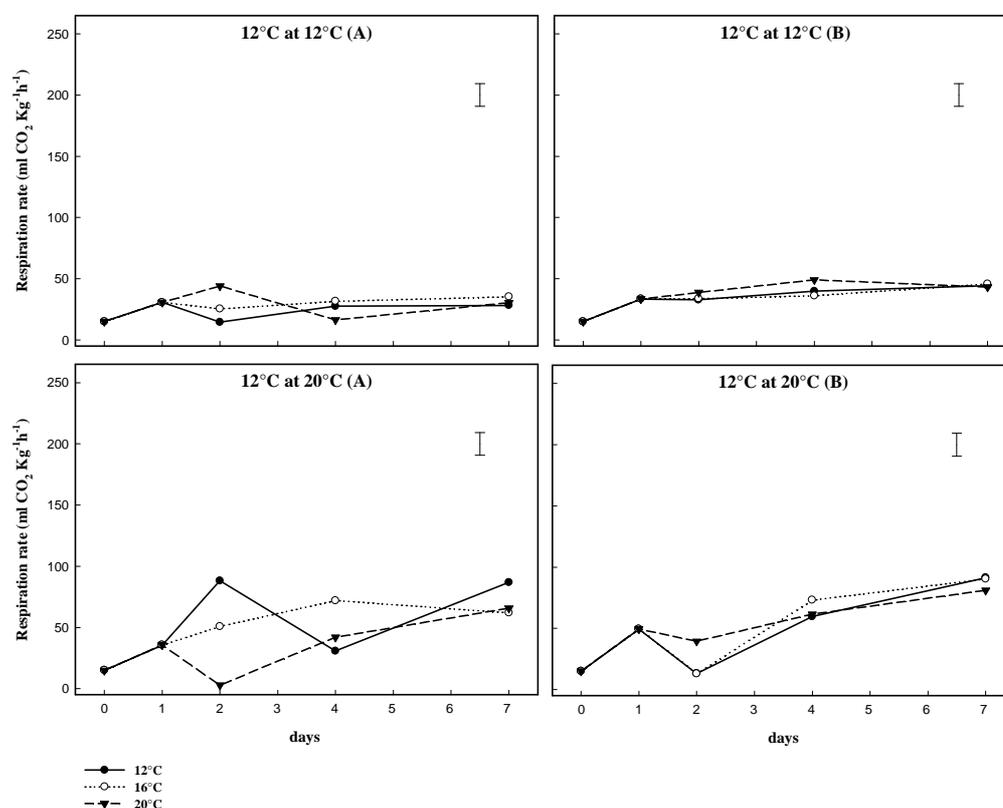


Figure 6.11: Respiration rate (ml of CO₂ kg⁻¹h⁻¹) measured in fruit ethylene treated (B) at 12°C (●), 16°C (○), and 20°C (▼) (Exp. I). The CO₂ production was recorded in control (A) and ethylene treated fruit (B) at the ripening temperature (12°C) and at

20°C. LSD ($P < 0.05$) = 18.6 for the interaction Baseline 1_T3*Baseline 2_day is indicated.

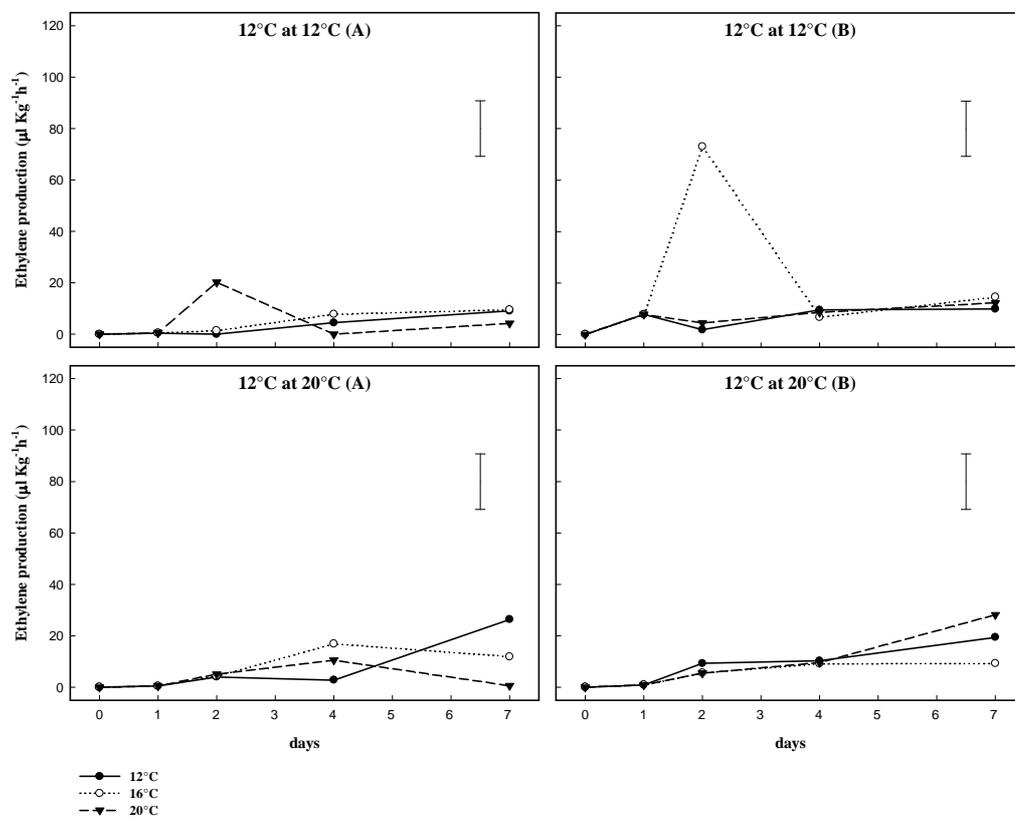


Figure 6.12: Ethylene production ($\mu\text{l kg}^{-1}\text{h}^{-1}$) measured in fruit ethylene treated (B) at 12°C (●), 16°C (○), and 20°C (▼) (Exp. I). The ethylene released was measured in control (A) and ethylene treated fruit (B) at the ripening temperature (12°C) and at 20°C. LSD ($P < 0.05$) = 17.62 for the Baseline 1_treatment*Baseline 2_temperature of treatment*day is indicated.

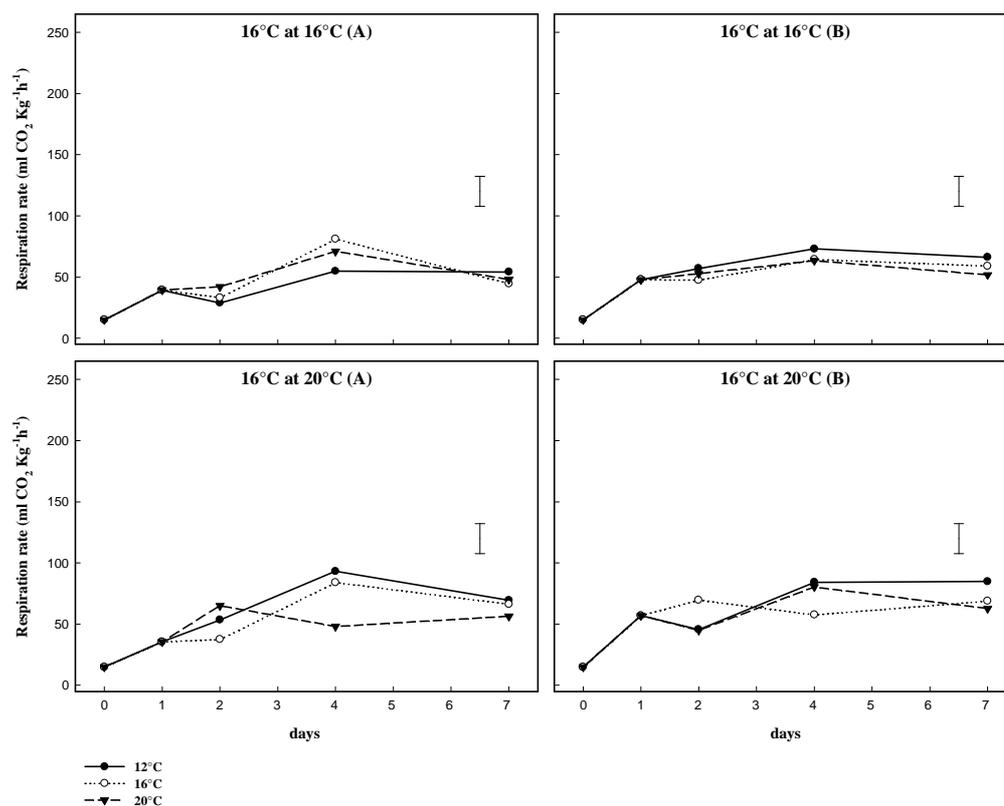


Figure 6.13: Respiration rate (ml of CO₂ Kg⁻¹h⁻¹) measured in fruit ethylene treated (B) at 12°C (●), 16°C (○), and 20°C (▼) (Exp. I). The CO₂ production was recorded in control (A) and ethylene treated fruit (B) at the ripening temperature (16°C) and at 20°C. LSD ($P < 0.05$) = 20.08 for the interaction Baseline 1_treatment*T3*Baseline 2_temperature of treatment*day is indicated.

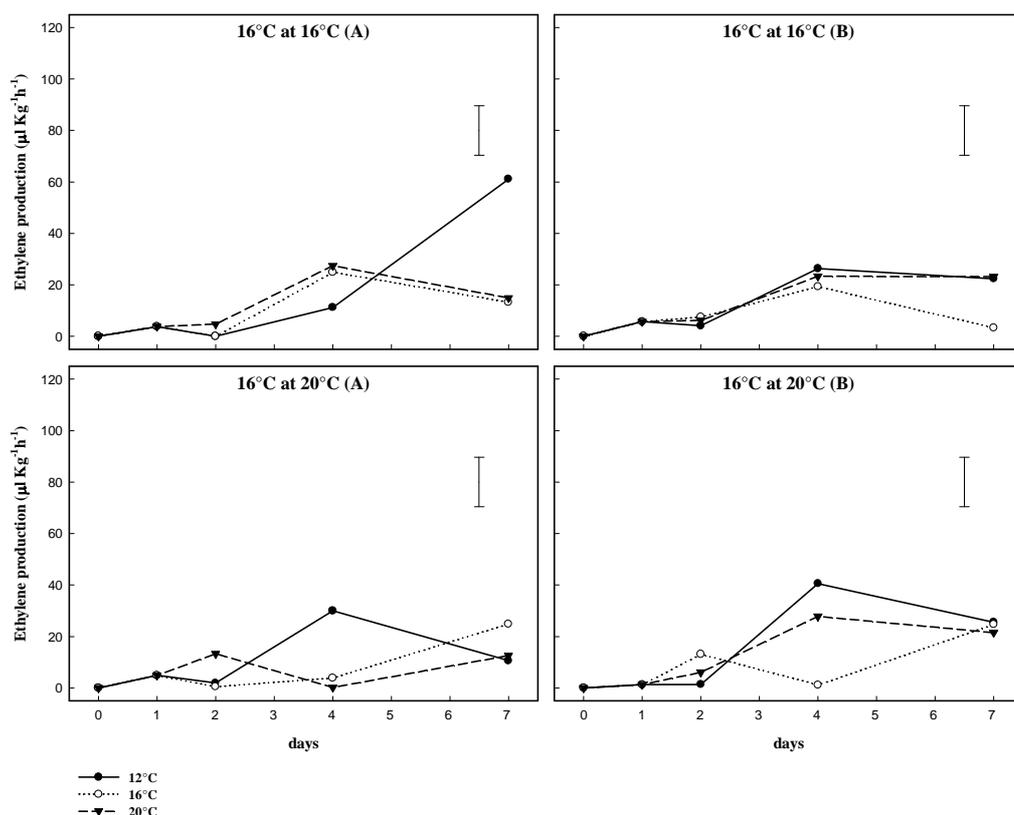


Figure 6.14: Ethylene production ($\mu\text{l kg}^{-1}\text{h}^{-1}$) measured in fruit ethylene treated (B) at 12°C (\bullet), 16°C (\circ), and 20°C (\blacktriangledown) (Exp. I). Ethylene released was measured in control (A) and ethylene treated fruit (B) at the ripening temperature (16°C) and at 20°C . LSD ($P < 0.05$) = 15.72 for the interaction Baseline 1_T3*Baseline 2_temperature of treatment*day is indicated.

In the following experiment (Exp. II), ripening temperature along with days (temperature of treatment*days) influenced fruit respiration rate. In particular, CO_2 production measured at 12 and 16°C increased after 4 days that said, when the measurement was taken at room temperature (20°C) no peak in CO_2 production was observed (Figure 6.15-17). Besides, the climacteric behaviour showed similar results to the previous experiment (Exp. I) (Figure 6.16-18). Indeed, the ethylene released was significantly higher when measured at 20°C rather than at 12 or 16°C . However, other parameters such as temperature at which the ethylene was applied and days, influenced the climacteric behaviour of the fruit.

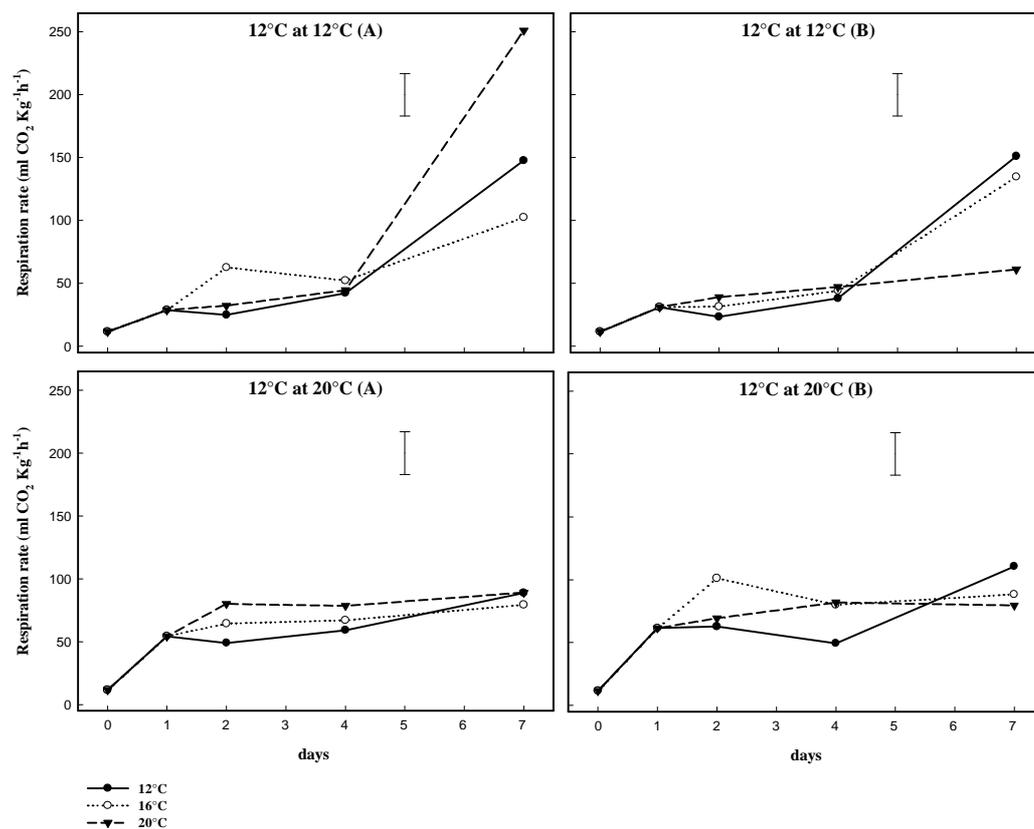


Figure 6.15: Respiration rate (ml of CO₂ kg⁻¹h⁻¹) measured in avocado fruit ethylene treated (B) at 12°C (●), 16°C (○), and 20°C (▼) (Exp. II). The CO₂ production was recorded in control (A) and ethylene treated fruit (B) at the ripening temperature (12°C) and at 20°C. LSD ($P < 0.05$) = 33.88 for the interaction Baseline 1_T3*Baseline 2_day is indicated.

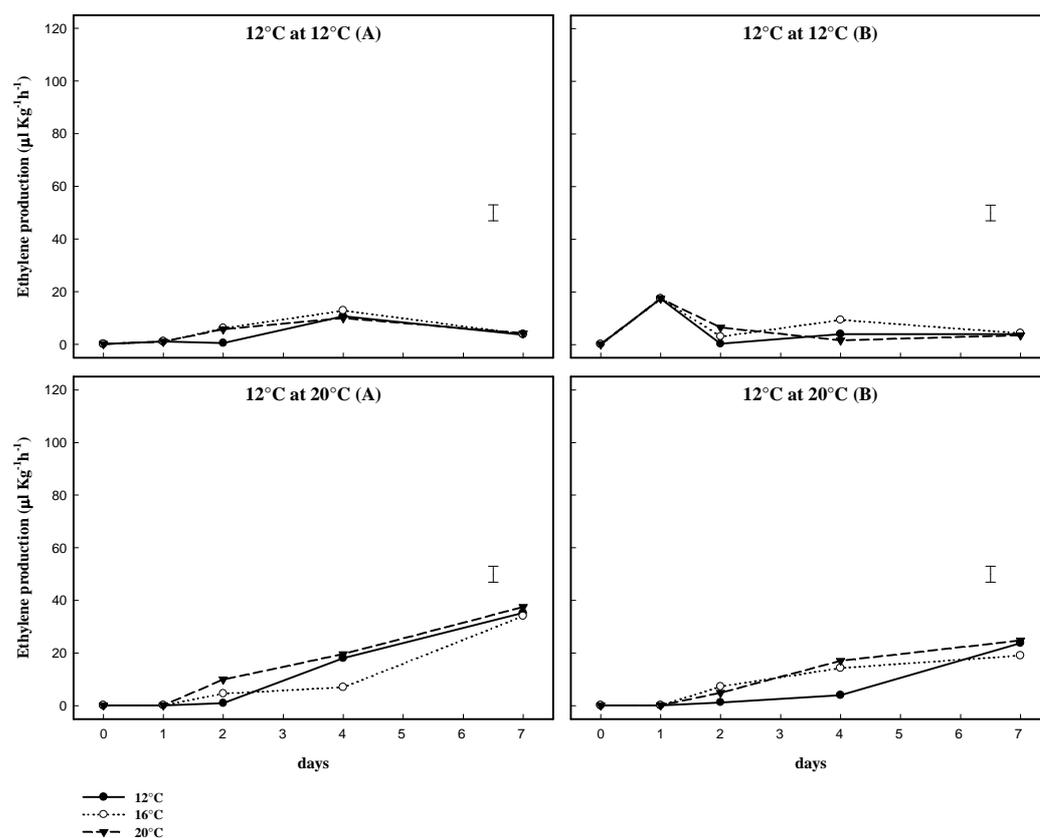


Figure 6.16: Ethylene production ($\mu\text{l kg}^{-1}\text{h}^{-1}$) measured in fruit ethylene treated (B) at 12°C (●), 16°C (○), and 20°C (▼) (Exp. II). The ethylene released was measured in control (A) and ethylene treated fruit (B) at the ripening temperature (12°C) and at 20°C . LSD ($P < 0.05$) = 6 for the interaction Baseline 1_T3*Baseline 2_days is indicated.

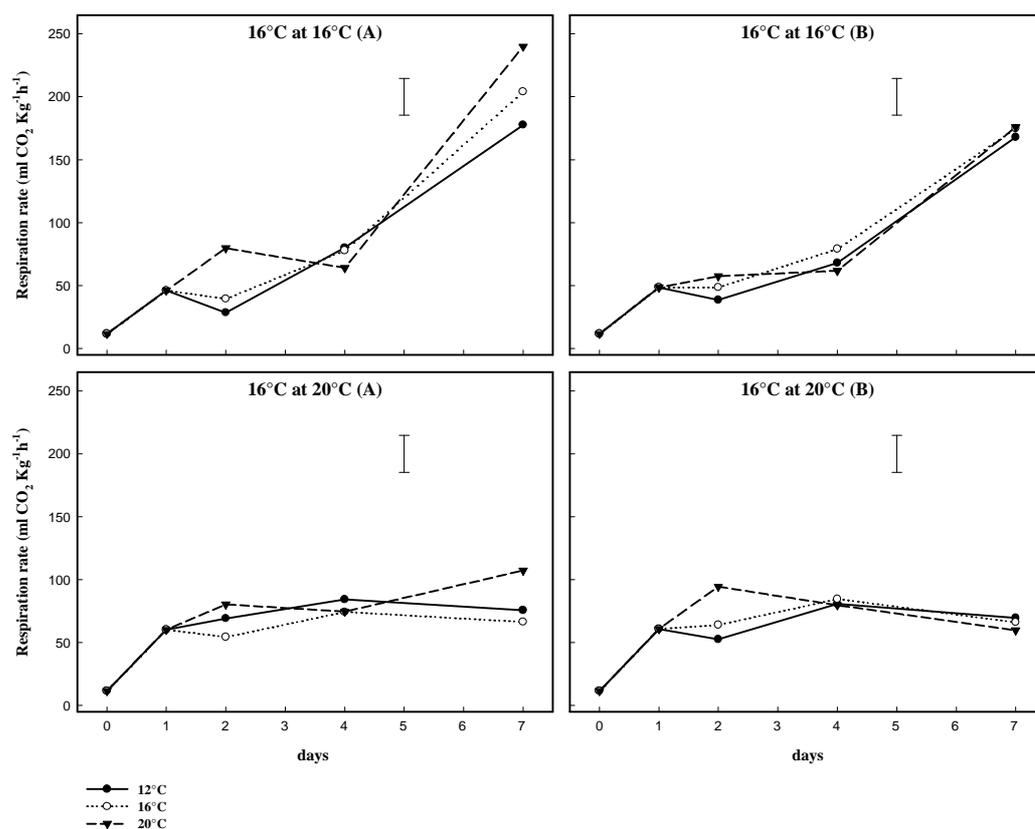


Figure 6.17: Respiration rate (ml of CO₂ kg⁻¹h⁻¹) measured in fruit ethylene treated (B) at 12°C (●), 16°C (○), and 20°C (▼) (Exp. II). The CO₂ production was recorded in control (A) and ethylene treated fruit (B) at the ripening temperature (16°C) and at 20°C. LSD ($P < 0.05$) = 29.31 for the interaction Baseline 1_T3*Baseline 2_day is indicated.

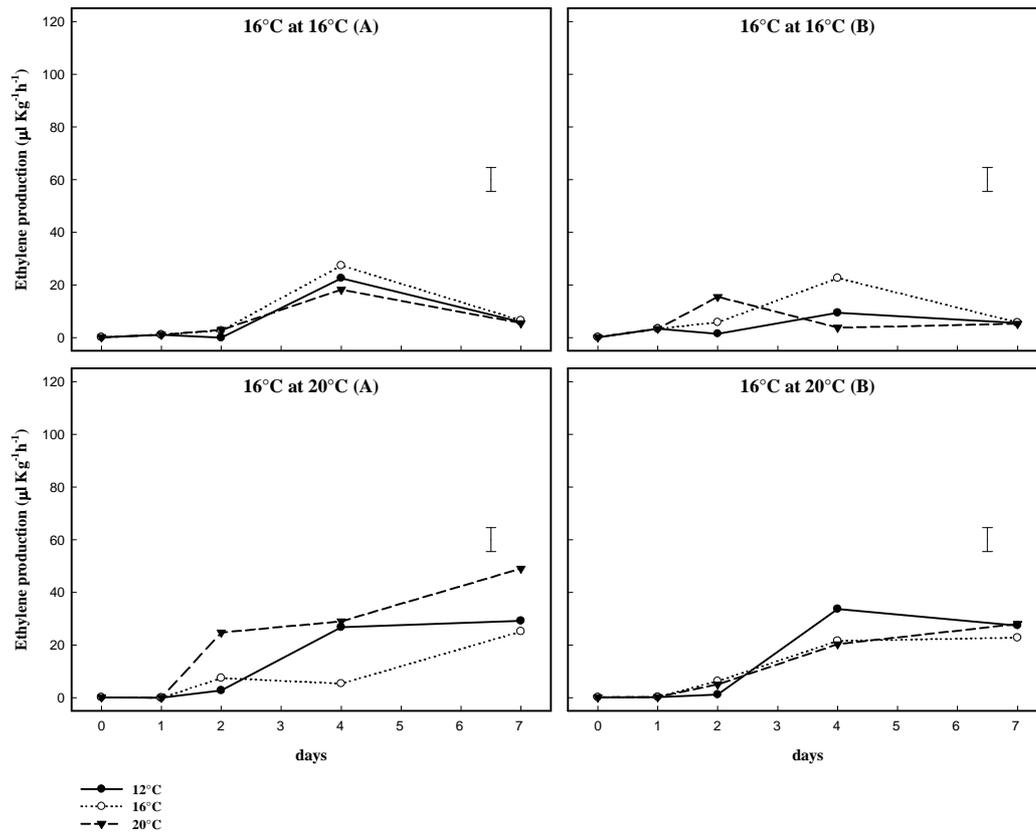


Figure 6.18: Ethylene production ($\mu\text{l kg}^{-1}\text{h}^{-1}$) measured in fruit ethylene treated (B) at 12°C (\bullet), 16°C (\circ), and 20°C (\blacktriangledown) (Exp. II). The ethylene released was measured in control (A) and ethylene treated fruit (B) at the ripening temperature (16°C) and at 20°C . LSD ($P < 0.05$) = 7.4 for the interaction Baseline 1_T3*Baseline 2_temperature of treatment*day is indicated.

6.5 Experiment III

A different experiment was conducted on late season fruit to investigate the efficacy of prolonged ethylene exposure. As previous research showed (Dixon *et al.*, 2003), stored late season fruit can be less responsive to ethylene applications. This experiment aimed to evaluate the effect that prolonged exposure to ethylene (12 and 24 hours) applied at low (12°C) or high (20°C) temperature can have on the ripening of avocado fruit cv. Hass (*cf.* Chapter 3, section 3.1.5).

6.6 Results

6.6.1 Colour

The main changes in the fruit skin colour were between ripening temperatures and days. Generally, ethylene treatment had little effect on colour changes during ripening. Indeed, C* (Figure 6.19) and H° (Figure 6.20) values significantly differed for the interaction temperature*day. The L* parameter was influenced by temperature of treatment and days only at 20°C (data not shown). Generally, fruit held at 20°C showed faster changes in skin colour, indicated by a faster decrease in the C* and H° values, compared with lower ripening temperature. No significant changes were related to ethylene treatment or exposure time. However, at 12°C the time of exposure and temperature of treatment were factors influencing H°, resulting in a slightly faster decrease in this parameter in fruit treated at 20 rather than 12°C. Nevertheless, at higher temperature (20°C) H° changed only along days.

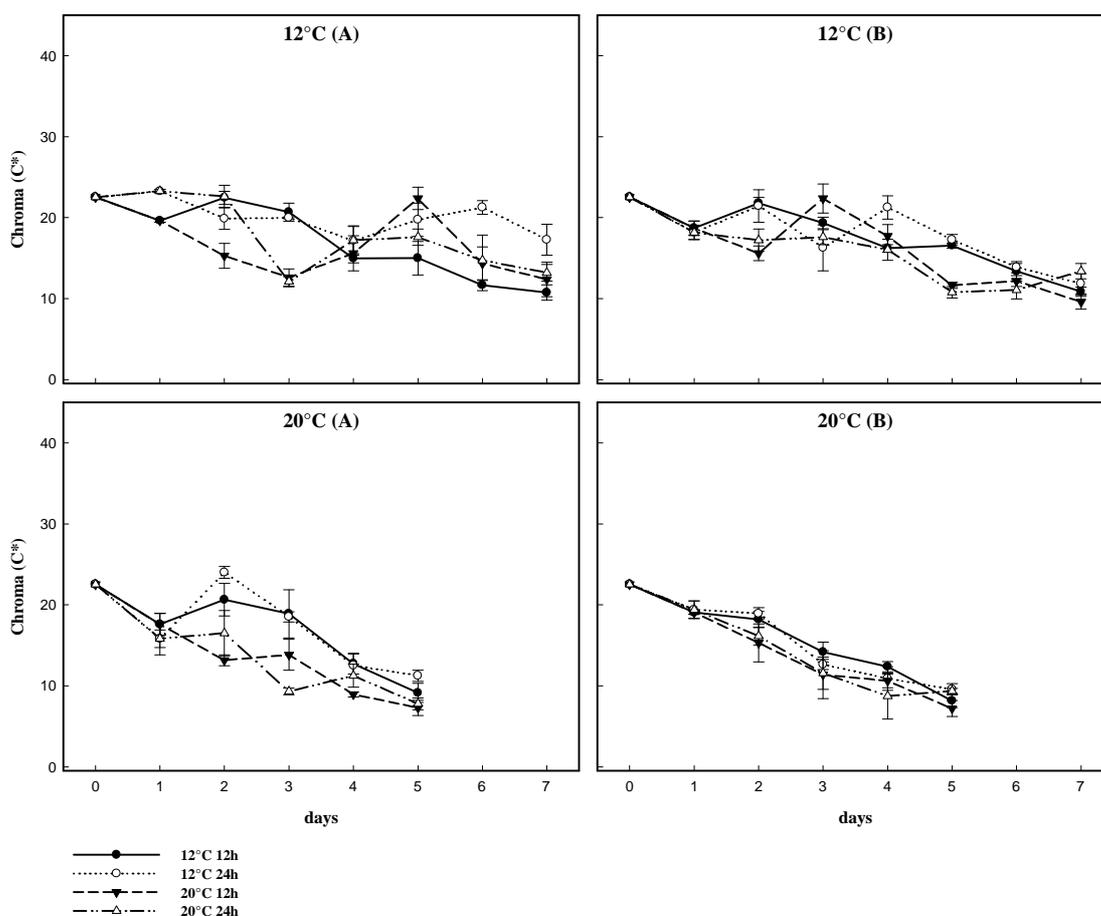


Figure 6.19: Changes in chroma (C*) parameter through ripening in late harvest avocado fruit. Fruit ($n = 36+36$) were ethylene treated (e+) for 12 and 24 hours while the remaining fruit ($n = 36+36$) were used as control (e-). For each temperature (12 and 20°C) after the ethylene application fruit were moved at higher (20°C) or lower (12°C) ripening temperature. In each graph is indicated the temperature at which ethylene has been applied and the length of application ((●) 12°C for 12 hours; (○) 12°C for 24 hours; (▼) 20°C for 12 hours; (△) 20°C for 24 hours)). Each point is a mean value of three fruit measurement \pm S.E.

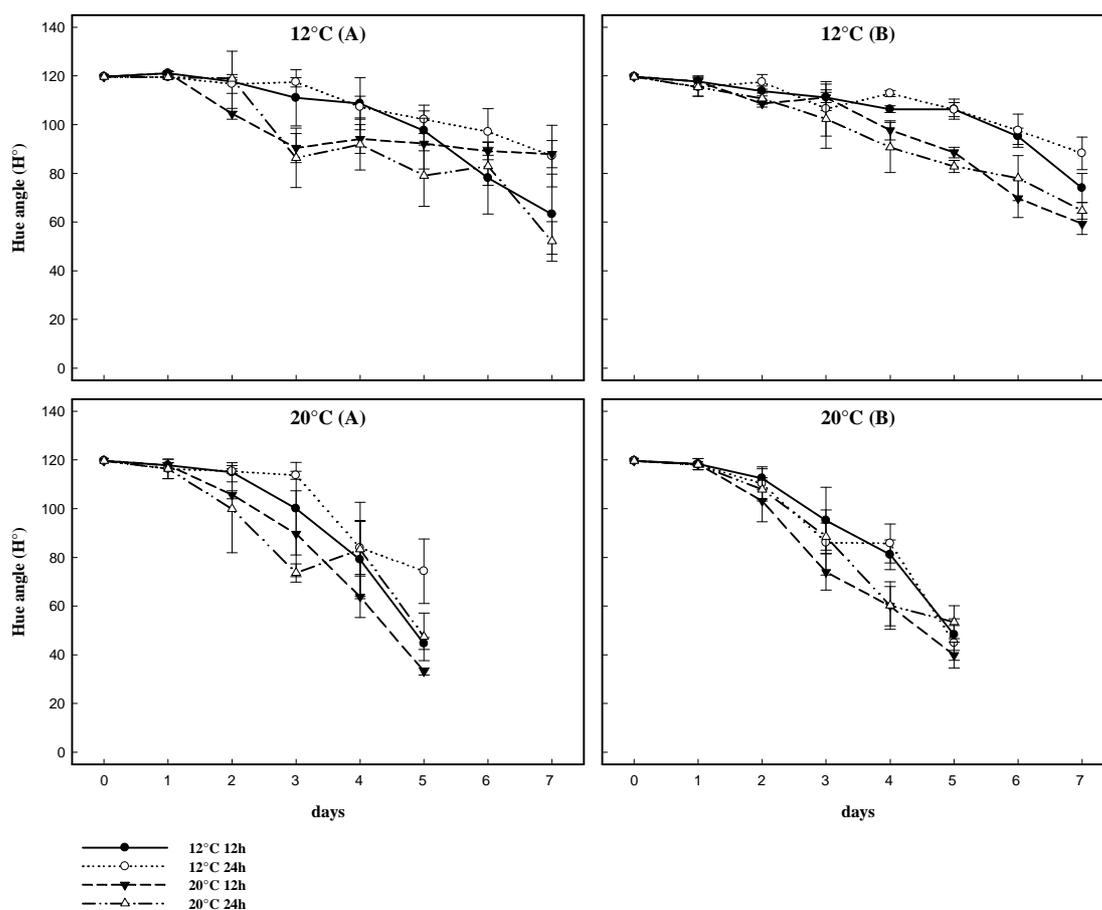


Figure 6.20: Hue angle (H°) decreased through ripening in late season avocado fruit cv. Hass. Fruit ($n = 36+36$) were ethylene treated (e+) for 12 and 24 hours while the remaining fruit ($n = 36+36$) were used as control (e-). For each temperature (12 and 20°C) after the ethylene application fruit were moved at higher (20°C) or lower (12°C) ripening temperature. In each graph is indicated the temperature at which ethylene has been applied and the length of application ((●) 12°C for 12 hours; (○) 12°C for 24 hours; (▼) 20°C for 12 hours; (Δ) 20°C for 24 hours)). Each point is a mean value of three fruit measurement \pm S.E.

6.6.2 Firmness

Avocado fruit softening was influenced by the interactions between ethylene treatment*temperature of the treatment*day. In detail, the effect of the ripening temperature was evident, with fruit held at 20°C already ripe after 5 days. Similarly,

ethylene-treated fruit showed a faster softening than control fruit (Figure 6.21). In particular, as earlier described for the H°, fruit treated at 20°C showed faster mesocarp softening than fruit treated at lower temperature (12°C) during ripening at 12°C. Results showed higher uniformity in the ripening in ethylene treated fruit compared to control one regardless temperature.

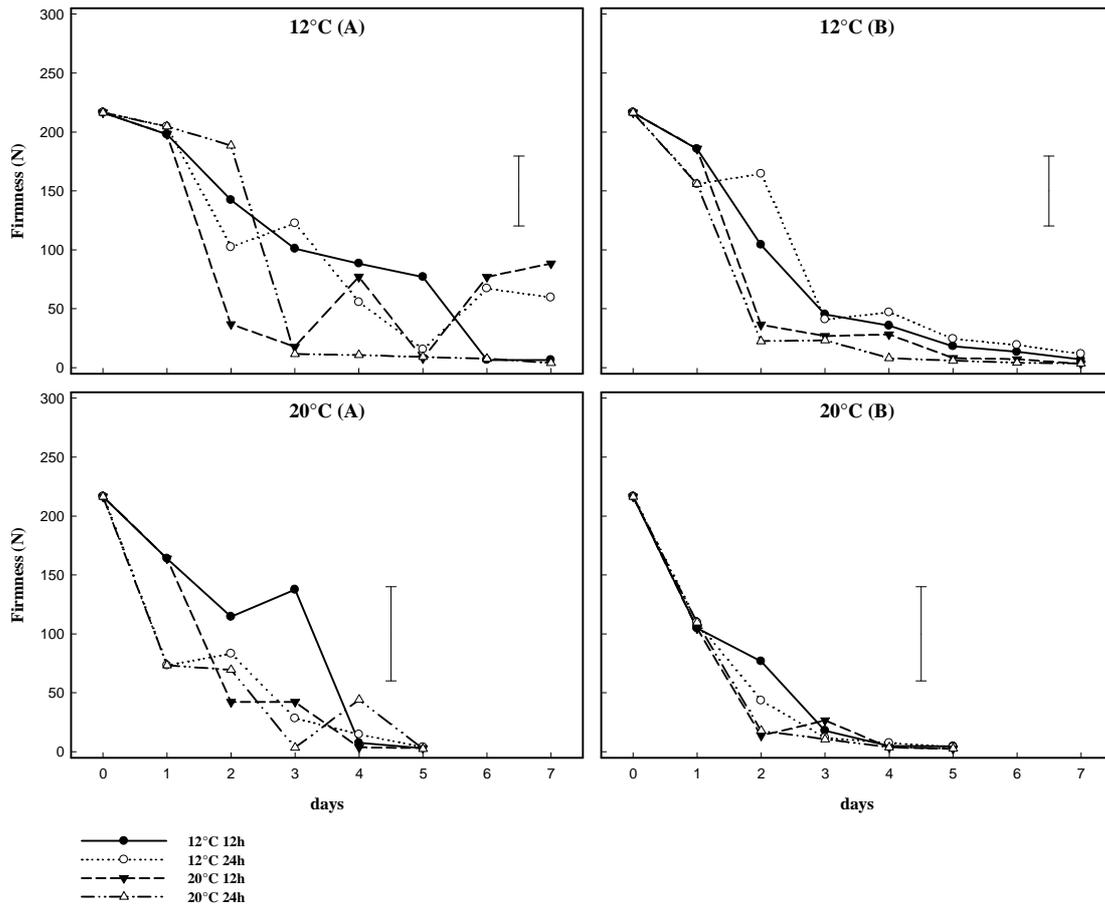


Figure 6.21: Mesocarp softening (N) in late season avocado fruit cv. Hass through ripening at 12 and 20°C. Fruit were partially ($n = 36+36$) ethylene treated (e+) for 12 and 24 hours and in part ($n = 36+36$) used as control (e-). For each temperature (12 and 20°C) after the ethylene application fruit were moved at higher (20°C) or lower (12°C) temperature. In each graph is indicated the temperature at which ethylene has been applied and the length of application ((●) 12°C for 12 hours; (○) 12°C for 24 hours; (▼) 20°C for 12 hours; (△) 20°C for 24 hours)). Each point is a mean value of three fruit measurement with LSD ($P < 0.05$) = 59.35 (12°C) and 38.31 (20°C) for the interactions

Baseline 1_treatment*treatment-time*Baseline 2_day and Baseline 1_Baseline 2_day, respectively.

6.6.3 Respiration and ethylene production

Most of the variability in the CO₂ production was between different ripening temperatures with higher respiration rate when fruit were held at 20°C rather than 12°C. However, CO₂ production was also influenced by the interaction between ethylene application*length of the treatment*temperature (Figure 6.22). Fruit held at 20°C showed higher LSD values than ethylene treated or control fruit held at 12°C.

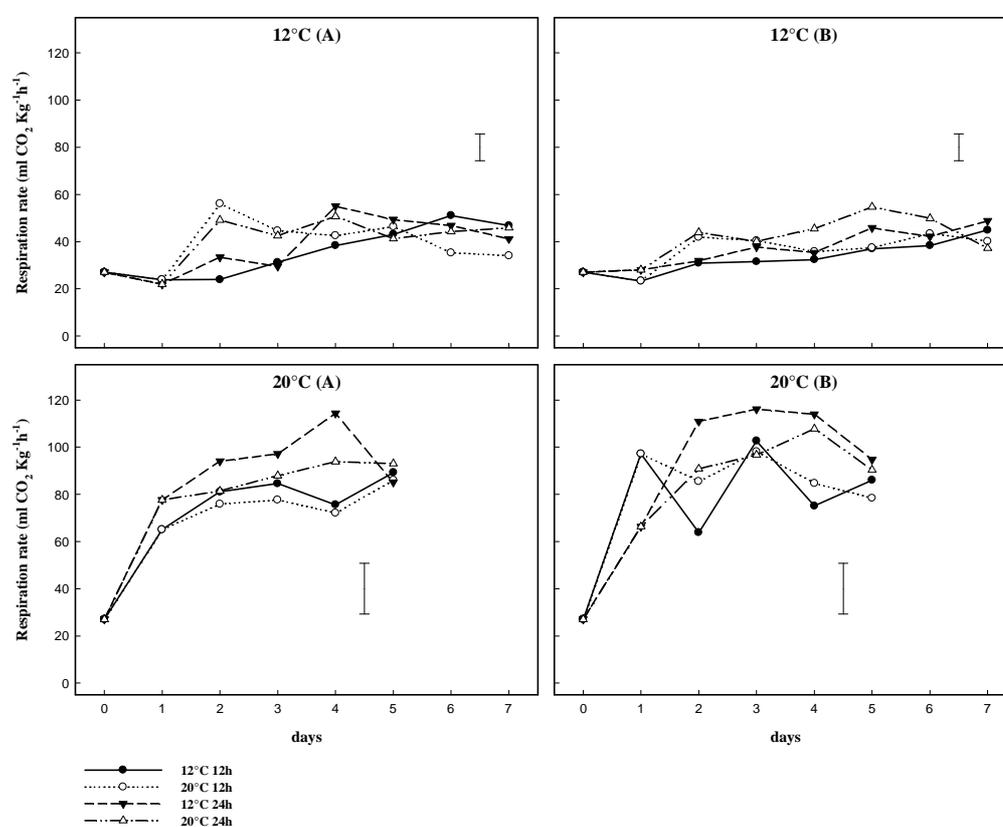


Figure 6.22: Respiration rate (ml of CO₂ kg⁻¹h⁻¹) in avocado fruit grown in South Africa and harvested late season during ripening at 12 and 20°C. In each graph is indicated the temperature at which ethylene has been applied and the length of application ((●) 12°C for 12 hours; (○) 12°C for 24 hours; (▼) 20°C for 12 hours; (Δ) 20°C for 24 hours)). Each point is a mean value of three measurements. LSD ($P < 0.05$)

= 9.15 (12°C) and 22.74 (20°C) for the interactions Baseline 1_treatment*Baseline 2_swap*days and Baseline 1_treatment*treatment time*Baseline 2 are indicated.

Ethylene production was intensified at higher temperature (20°C). In detail, the climacteric peak was not detected in fruit ethylene treated at 12°C, regardless exposure time. However, similar behaviour was also detected in control fruit kept closed in boxes for 24 hours at 12°C (Figure 6.23).

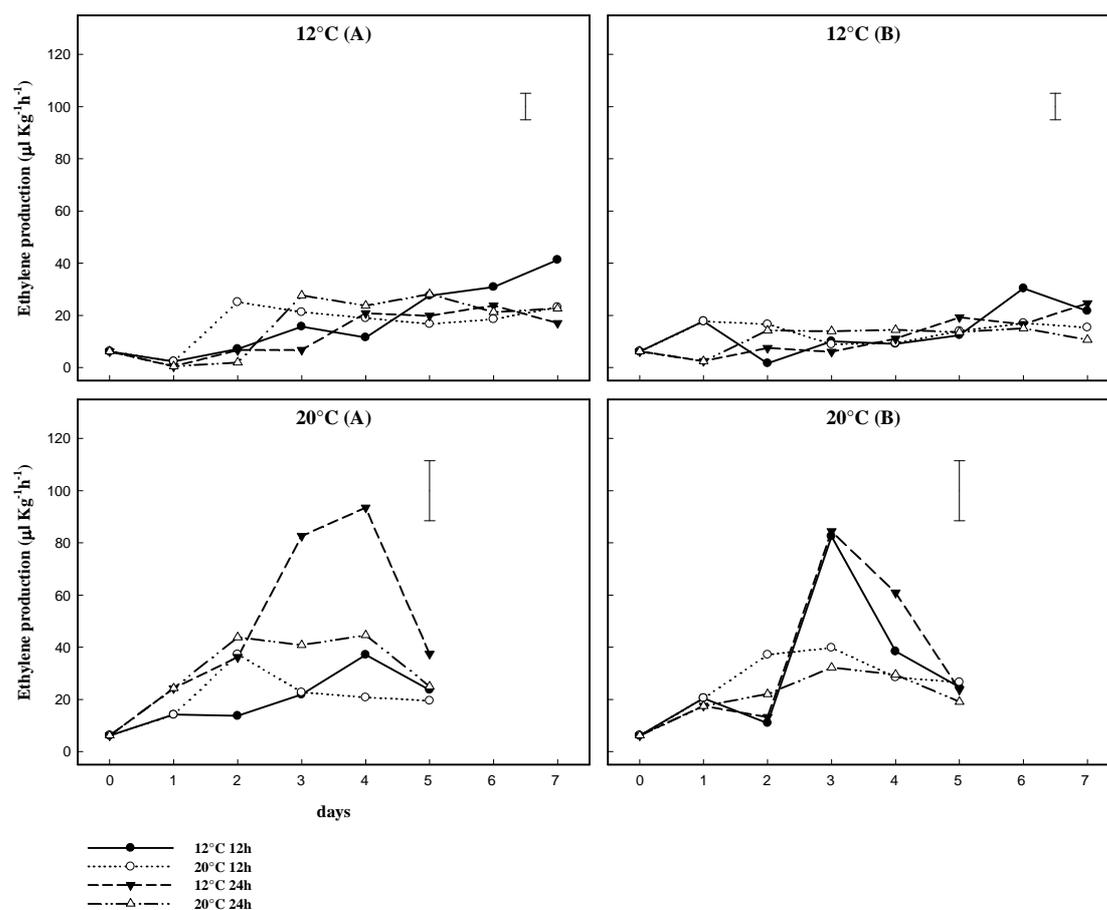


Figure 6.23: Ethylene production ($\mu\text{l kg}^{-1}\text{h}^{-1}$) measured in late season avocado fruit through ripening at 12 and 20°C. In each graph is indicated the temperature at which ethylene has been applied and the length of application ((●) 12°C for 12 hours; (○) 12°C for 24 hours; (▼) 20°C for 12 hours; (△) 20°C for 24 hours)). LSD ($P < 0.05$) = 10.2 (12°C) and 23.04 (20°C) for the interactions Baseline 1*Baseline 2_swap*days and Baseline 1*Baseline 2_swap*day are indicated.

6.7 Discussion

Ethylene treatment on avocado fruit is used in postharvest systems to stimulate faster softening and to induce more homogeneity in the ripening. However, differences in the response to ethylene treatment are due not only to the application length but also to the fruit maturity stage at harvest (Starrett and Laties, 1991). Results from this investigation confirm the different response to ethylene application depending upon fruit maturity.

Generally fruit in the first experiment, commercial size 16, harvested in May took longer to soften and were more affected by ethylene treatment than fruit in experiment III, commercial size 22 and harvested in July. The faster softening in ethylene treated fruit was more evident at low ripening temperature in experiment I. The following experiment (Exp. II) fruit harvest in June, commercial size 18, showed faster mesocarp softening consequently to ethylene treatment and high temperature (20°C). Finally, in the last study (Exp. III), fruit harvested in July with smaller size (22) had shelf life two days shorter when ripen at 20°C rather than at 12°C. However, it is not to exclude a possible synergic effect between temperature and exogenous ethylene as ripening accelerators. Prolonged ethylene treatment did not show significant changes in the fruit ripening of late season fruit. Thus, postharvest temperature was more influent on the ripening of the fruit harvested in middle and late season.

As previously mentioned (*cf.* Chapter 2), a peculiar characteristic of avocado fruit cv. Hass is the darkening of the skin colour during ripening (Cox *et al.*, 2004; Ashton *et al.*, 2006). This process has been related with an increase of the cyanidin-3-*O*-glucoside content (Cox *et al.*, 2004). The anthocyanin content seemed to be influenced by temperature (Cox *et al.*, 2004) (*cf.* Chapter 5) and by ethylene in its biosynthesis (*cf.* section 2.8.3) (El-Kereamy *et al.*, 2003). However, whether there is a good relationship between firmness and hue angle, firmness and cyanidin-3-*O*-glucoside are poorly correlated (*cf.* Chapter 5). In this work skin darkening was not significantly affected by ethylene treatment even though an effect was observed on the softening (Exp. I) whether, confirming previous work (Cox *et al.*, 2004), higher temperature induced faster decrease in the hue angle parameter. It would be wise to investigate the anthocyanin content in the skin tissues of ethylene treated fruit to investigate whether

the treatment improve the relationship with firmness. Nevertheless, only in the first experiment (Exp. I) the ripening was clearly triggered by the ethylene treatment. In contrast, in the following investigation (Exp. II) fruit had a faster softening of the mesocarp mainly due to the interaction of various factors (i.e. treatment, days, temperatures) and the effect of the ethylene treatment was less evident. The efficacy of the ethylene treatment decreased even more in the last experiment, where fruit harvested at the end of the season (July) and of smaller size were mainly affected by ripening temperature.

Respiration rate and the ethylene production also showed differences between experiments. The respiration rate and the ethylene production detected in the first experiment were lower than when measured in fruit in experiment II. In those fruit, it was also evident that a faster increase in the respiration rate and ethylene was released at high ripening temperature. Additionally, the results obtained in the measurement of respiration and climacteric behaviour at different external temperatures should be taken into consideration in further work. Indeed, the temperature seems to affect the measurement of the gasses also in a short exposure. The evaluation of the ethylene released and the respiration rate of avocado fruit is commonly measured leaving the fruit in glass jars (Feng *et al.*, 2000; Hershkovitz *et al.*, 2009, 2010) or plastic containers (Jeong *et al.*, 2003) for a specific time at room temperature, usually at 20°C, independently of previous storage or postharvest conditions. However, the temperature can influence fruit physiology and consequently could affect the measurement. In agreement, results from the first experiment (early season) showed significant differences in the respiration rate when measured at 20°C in fruit previously held at lower temperature (12 or 16°C). The influence of the temperature during the measurement was particularly evident when fruit were previously held at 12 rather than at 16°C. It will be advisable to measure the gasses at the actual ripening temperature avoiding possible alteration in the fruit metabolism. Besides, no long term evidence was noted regarding the change of “one day temperature” on fruit ripening. Indeed, the temperature at which the ethylene has been applied seems not to influence the treatment.

In conclusion, ethylene application following extended cold storage can have a different effect on avocado fruit ripening. The different response of the fruit to the

ethylene treatment could be due to the different harvest time of the fruit or to the different size therefore both hypotheses need future validation. Besides the induction of a faster ripening, ethylene treatment is supposed to reduce fruit heterogeneity. This effect was noted in the last experiment where ethylene treated fruit had more uniform ripening. Any particular response was related with longer exposure to ethylene. This work shows that the efficacy of ethylene in avocado depends on fruit preharvest conditions such as fruit harvest time and fruit size. Moreover, ethylene application should be carried out commercially when it can be of real benefit and not to be considered as a standard procedure.

The present work is a first example of screening in the evaluation of the combined effect between temperature and ethylene on the ripening behaviour of imported avocado fruit cv. Hass. However, due to the high variability in the ripening behaviour of avocado fruit, it would be wise to further investigate the conditions tested herein with a higher number of replicates and better control of fruit source.

7 CHAPTER SEVEN

IMPLICATIONS OF MESOCARP STRUCTURAL CARBOHYDRATE COMPOSITION ON THE RIPENING BEHAVIOUR OF AVOCADO FRUIT CV. HASS

7.1 Introduction

The ripening process of fruit and vegetables includes multiple modifications in colour, texture, flavour (Kays, 1991; Seymour *et al.*, 1993). The increased softening of mesocarp tissue is one of the more evident processes occurring during the ripening stage of many commodities. Cell wall composition and its metabolism plays a main role in the softening process (Pesis *et al.*, 1978; Brummell and Harpster, 2001). Even though fruit ripening involves biosynthesis and incorporation of new components in the cell wall, most of the changes are thought to be related to the degenerative process of pectins and cellulose (Brummell and Harpster, 2001). After de-methylesterified, pectins can be de-polymerized by polygalacturonase (PG) with the release of polyuronide residues in the cell wall matrix. Even though most of these fragments are still linked to pectins by ionic bonds, the loss of strong linkages responsible for the cell adhesions (Crookes and Grierson, 1983) results in looser cell connections and increased porosity of the cell wall (Brummell and Harpster, 2001). The presence of short chains of polyuronides and neutral sugars residues such as xyloglucans, glucomannans mostly released from hemicelluloses, increases as a result of these degenerative processes (Brummell and Harpster, 2001) which involves changes in the cell wall environment. For instance, the acidity in the cell wall spaces increases which subsequently affects cell wall enzyme activity (Almeida and Huber, 1999) and the concentration of solutes increases reducing cell turgor (Shackel *et al.*, 1991).

Cell wall composition and activity of cell wall enzymes can be different between species. The texture of a commodity can be quite variable and have different changes during the ripening process. This leads to commodities having different consistency

when ripen. For instance, apple develops a crispy flesh whether a fully ripe avocado has a softer texture (Brummell and Harpster, 2001).

Avocado is a climacteric fruit with high perishability after harvest, able to fully ripen in 7-8 days (Jeong *et al.*, 2002). Application of an ethylene antagonist (i.e. 1-MCP) (Jeong *et al.*, 2003) or ethylene scavenger (i.e. palladium) (Terry *et al.*, 2007) are used nowadays to prolong avocado shelf life and delay softening. In climacteric fruit, the depolymerization of cellulose, hemicellulose and pectins has been associated with ethylene production (Adwad and Young, 1979; Pesis *et al.*, 1978). Pectins in plant cell wall are present mainly as homogalacturonan (α 1-4 D-galacturonic acid (HGA), rhamnogalacturonan-I (D-galacturonic and L-rhamnose chains, RG-I), or rhamnogalacturonan-II (galacturonic chains α -1-4 linked with 4 side groups, RG-II). The depolymerization of these structures is mainly due to the activity of the polygalacturonase (PG) enzyme which removes de-methylated polygalacturonic acids present on homogalacturonic chains. The methyl groups present on the galacturonic residues are previously removed by pectinmethylesterase (PME) which allows the pectins to be a substrate for the PG (Brummell and Harpster, 2001). In avocado fruit PG levels seem to be closely related to the climacteric rise (Jeong and Huber, 2004). PG activity has been reported to be low after harvest with a constant increase through ripening and maximum activity in overripe fruit (Jeong and Huber, 2004). Accordingly, PME activity is already high at harvest and declines with ripening (Awad and Young, 1979; Jeong *et al.*, 2002). The PG transcript accumulation seems to be an ethylene-dependent system with a high sensitivity to ethylene (Sitrit and Bennett, 1998). Indeed, in 1-MCP-treated avocado fruit with delayed softening the PG levels remained lower during shelf life whereas the PME was maintained at higher activity compared to control fruit (Jeong *et al.*, 2002). A further modification is the loss of (1-4) β -D-galactan or (1-3) (1-6) β -D-galactan residues from side chains of RG-I, or RG-II, respectively. The activity of galactosidase is responsible for this modification (Carpita and Gibeau, 1993). Two forms of these proteins have been detected in avocado fruit, α/β galactosidase, whose activity decreases after harvest and during the first days of shelf life (Jeong *et al.*, 2002). In 1-MCP-treated avocado fruit the activity α -galactosidase decreased more slowly than in control fruit whereas no significant changes were

detected for the β -galactosidase (Jeong *et al.*, 2002). The softening of the mesocarp is also due to the depolymerization of glycans from the cell wall matrix (O'Donoghue and Huber, 1992) possibly hydrolyzed in the (1-4) β -D-linkages by cellulase (Brummel and Harpster, 2001). The cellulase (endo- β -1,4-glucanase) activity is very low in unripe fruit or even not detected immediately after harvest (24 hours) but increases with the ethylene and CO₂ peaks during ripening (Pesis *et al.*, 1978; O'Donoghue and Huber, 1992; Jeong *et al.*, 2002). Whether these enzymes in avocado fruit are responsible for the xyloglucans (XG) depolymerization or not, it is still controversial (O'Donoghue and Huber, 1992). The main sugars monomers previously found in avocado mesocarp extract are pectic associated arabinose, galactose and xylose (Jeong and Huber, 2004). These monomers are mainly substitution groups of more complex structures such homogalacturonans, RG-I or RG-II. In avocado literature is not present yet a study considering the nature of these chains polymers and the linkages present between carbohydrate structures.

The different types of linkage between chains and the high polymerization of the cell wall carbohydrates require for their characterization a multi-steps procedure. At first the free soluble carbohydrate released from biosynthesis or degradation of longer chains can be isolated using a water extract. Afterwards it is necessary to use chelating agents (i.e. CDTA) to allow the separation of pectins binding Ca²⁺ and Mg²⁺ whereas polymers bound with covalent linkages are separated by de-esterification with a sodium carbonate solution. Successive steps with alkali solvents (e.i. KOH) can solubilize the stronger hydrogen bounds found in pectins and hemicellulose (Selvendran *et al.*, 1987; Brummell and Harpster, 2001). Once isolated, the structural carbohydrates can be identified with a sheparose column, eluting the sample according to its molecular mass, or alternatively, hydrolysing in alditol acetate derivate and analysing through gas chromatography (Jeong and Huber, 2004). Alternatively, the use of monoclonal antibodies has been recently applied to the study of structural carbohydrates. The antibodies have been designed for the epitopes characterising the more diffuse carbohydrate chains in the plant cell wall (Jones *et al.* 1997; Willats *et al.* 1998; McCartney *et al.* 2005; Verhertbruggen *et al.* 2008; Marcus *et al.* 2010). This technique gives faster and more accurate results on the structures of cellulose, hemicellulose and

pectins present in extracts obtained with the previously described procedures (Jones *et al.*, 1997; Willats *et al.*, 1998). The immunodetection of structural carbohydrates has been already used on vegetal matrixes such as tomato (Jones *et al.*, 1997; Ordaz-Ortiz *et al.*, 2009) and wheat (Ordaz-Ortiz *et al.*, 2005); yet this technique, to date, has not been tested on avocado.

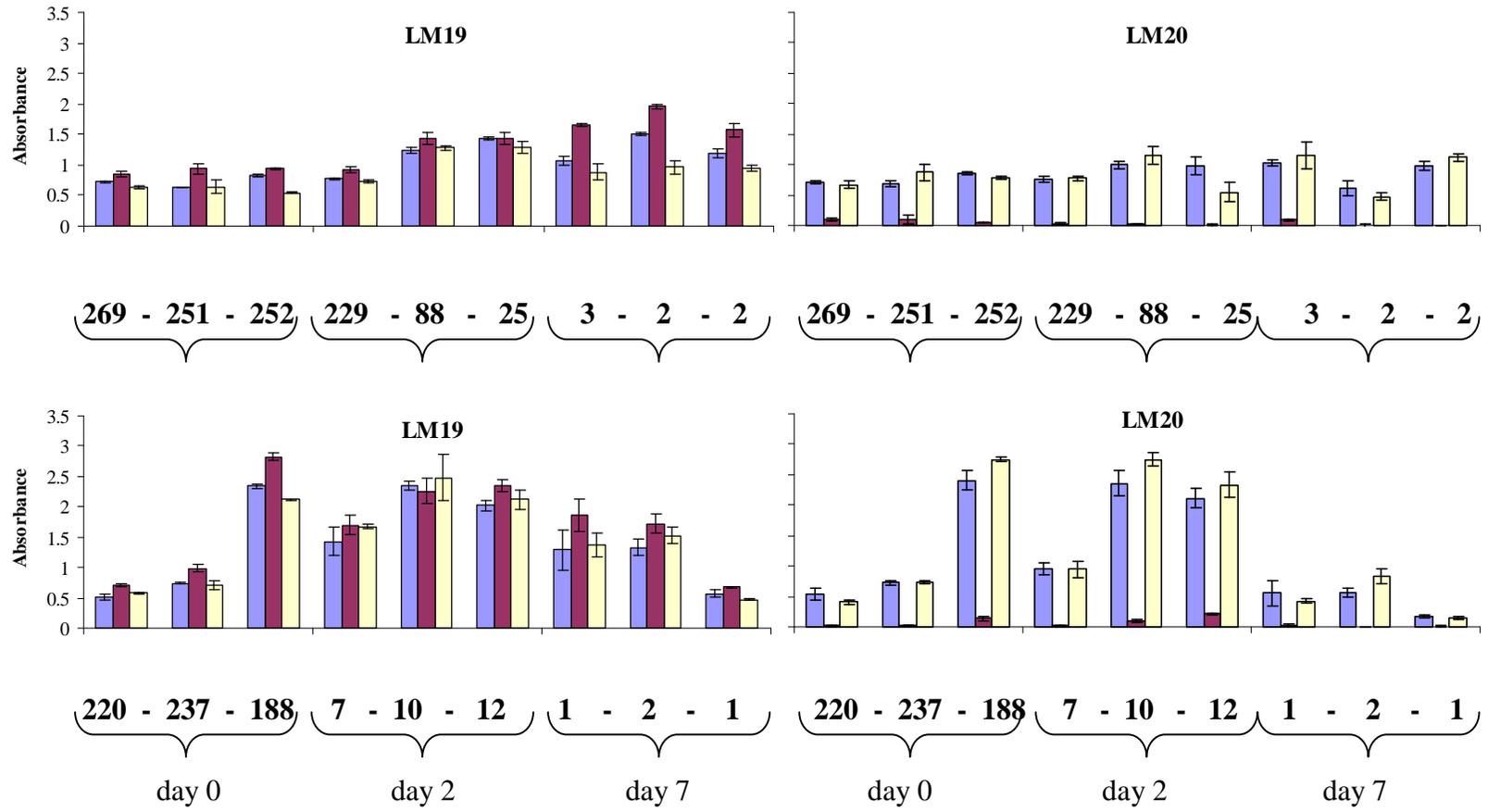
Aim of this work is to adapt the monoclonal antibody technique to avocado and to investigate the profile of the main structural carbohydrate present in the fruit mesocarp during ripening. Differences in the carbohydrate profile through ripening between different harvest time might establish a relationship with the faster softening of late harvest fruit previously reported (Dixon *et al.*, 2003b, *cf.* Chapter 4).

7.2 Methodology

The plant material (sections 3.1.6) and the methods used in the extraction (section 3.2.6) and the identification (section 3.3.5) of the structural carbohydrate are described in Chapter 3. The statistical analysis is described in section 3.4. Briefly, avocado fruit from Spain harvested at different maturity stage (early, 27.72 % DM, and late season, 31.65 % DM) were ripened at 18°C for seven days. Fruit were measured for main physiological parameters (firmness, respiration rate, ethylene production) (*cf.* Chapter 4) and investigated for the main cell wall structures at day 0, 2 and 7. Pectins possibly present in the water, CDTA and Na₂CO₃ extracts were detected with LM19, specific for homogalacturonic chains (Verhertbruggen *et al.* 2008), LM20, recognising homogalacturonic chains with methyl-esterificated (Verhertbruggen *et al.* 2008); LM5, specific for the (1-4)-β-galactosyl residues (Jones *et al.* 1997); LM6, recognising chains of (1-5)-α-arabinosyl which are usually bind to RGI polymers (Willats *et al.* 1998). The presence of hemicellulose chains were detected in KOH extract with LM15, specific for xyloglucans (Marcus *et al.* 2008), LM10 specific for xylans with few substitutions (McCartney *et al.* 2005); whereas galacto-glucomannans, glucomannans and (1→4)-β-D-xylan residue were investigated with LM21 (Marcus *et al.* 2010), LM22 (Marcus *et al.* 2010) and LM11 (McCartney *et al.* 2005), respectively.

7.3 Results

In water, CDTA and Na₂CO₃ extracts from early season fruit were present homogalacturonic (LM19), (1-4)- β -galactosyl residues (LM5), and chains of (1-5)- α -arabinosyl which are usually attached to RG-I polymers (LM6). LM20 recognising methyl-esterificated homogalacturonic chains was absent in Na₂CO₃ extract due to the de-methylation action of the sodium carbonate on the polymers, as previously reported (Verherbruggen *et al.* 2008). In particular, LM19 and LM6 levels increased through ripening. LM20 did not show any specific trend according to degree of fruit softening (Figure 7.1). Water and CDTA extracts aim to separate the main pectins residues present in the cell wall. Some of them have been already released from the cell wall as the one present in the water extract, others still cation-linked to cellulose or hemicellulose, in the CDTA extract. The KOH extract can break stronger linkages characteristic of hemicellulose. Nevertheless, this is an approximated classification and in each extract can be present all the classes of carbohydrates. However, to have a general indication about the cell wall composition this classification has been already used in previous studies (Ordaz-Ortiz, *et al.*, 2009). In the KOH extracts, LM21 and LM11 showed higher levels in softer fruit, binding mannans and xylans or glucuronarabinoxylans, respectively. LM22, specific for galactomannans, was not detected in all the samples and if present was in very low amount with no consistency through ripening. LM15, specific for xyloglucans, and LM10, specific for xylans, were not always present. LM10 was mainly detected in soft fruit (Figure 7.2).



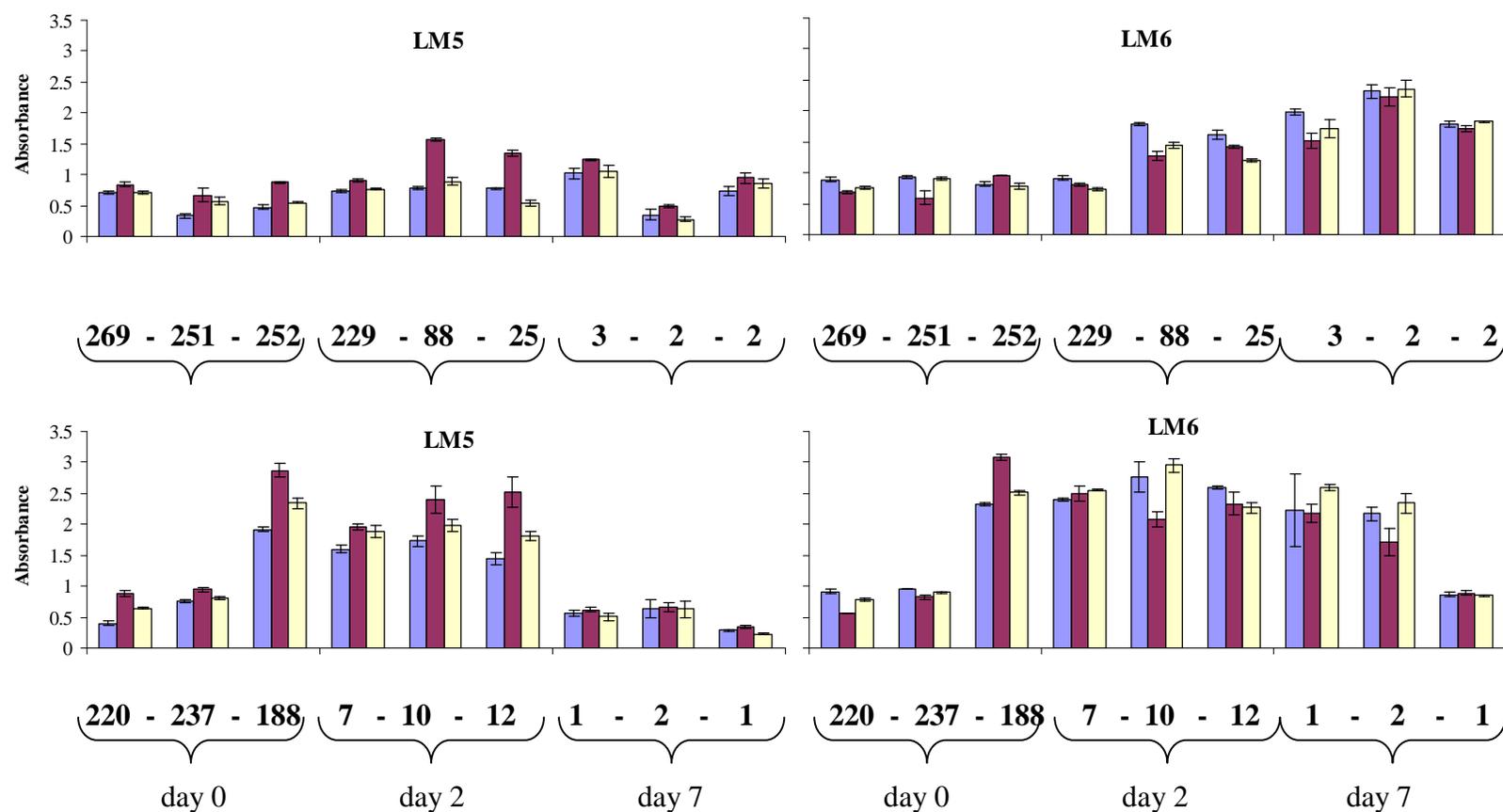


Figure 7.1: Detection of the polysaccharides epitopes isolated by sequential extraction in water (amber), CDTA (blue), and Na₂CO₃ (red) from the mesocarp of avocado fruit cv. Hass harvested in early and late season. Pectins (LM19, LM20, LM5 and LM6) were detected in fruit sampled at day 0, 2 and 7 with different mesocarp firmness (N). Each bar is a mean of three readings at 450 nm (absorbance) \pm S.E.

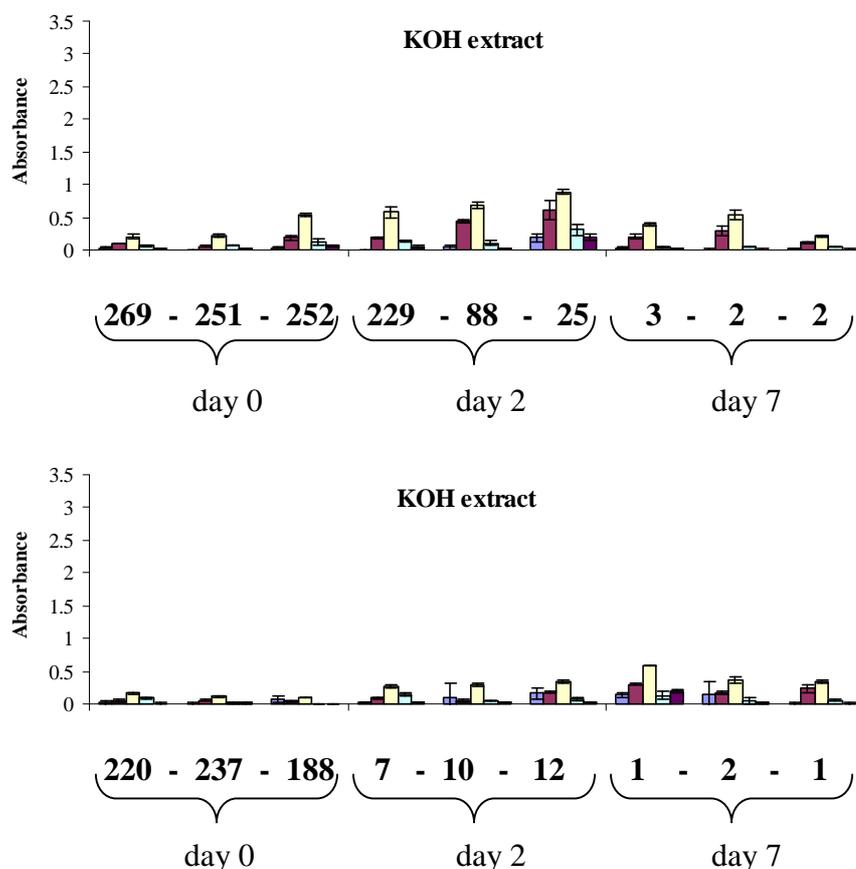


Figure 7.2: Detection of the hemicellulose (LM10, LM11, LM21, LM15 and LM22) epitopes isolated by sequential extraction in KOH from the mesocarp of avocado fruit cv. Hass harvested in early and late season. Fruit were sampled at day 0, 2 and 7 at different ripening stage (N). Each bar is a mean of three readings at 450 nm (absorbance) \pm S.E.

In fruit from late harvest LM19 and LM6 epitopes increased at day 2 with a successive decrease towards the end of shelf life. LM20 levels changes regardless fruit firmness. Indeed, methyl-esterificated homogalacturonic residues were higher in fruit with firmness measured at 118, 10 and 12 N rather than in fruit measuring 220, 237, 2 and 1 N. LM5 residues slightly increase with the arise of the softening process with an afterward decrease in soft fruit. Xyloglucans residues were higher in fruit with firmness values between 118 and 12 N and was lower in firm (220 and 237 N) and overripe fruit (2 and 1 N) (Figure 7.1). In alkali extracts, LM21 and LM11 epitopes slightly increased

at day 2 with a subsequent decrease at day 7. LM15 and LM22 were not always detected where LM10 was almost absent in all samples (Figure 7.2). The increase or decrease of a single epitope was generally comparable in water, CDTA and Na₂CO₃ extracts.

7.4 Discussion and conclusions

Textural changes occurring during ripening imply cell wall modifications. The different composition and activity of the cell wall related enzymes determine fruit texture and softening (Brummell and Harpster, 2001). The plant cell wall is a complex dynamic structure and acts to extend, sustain and protect the cell. Even accounting for the differences between species, the cell wall is generally formed of cellulose, hemicellulose and pectins. Cellulose microfibrilles with side glucans chains forms a tight matrix which hemicellulose binds, conferring mobility. Pectins are chains of galacturonic acid which can have side chains of various neutral sugars, i.e. arabinan and galactan, involved in cell growth, adhesion and porosity (Sommerville *et al.*, 2004; Willats, *et al.*, 2011). The presence of different substitution groups on the main chain confers different characteristics to the cell wall. The involvement of each structure and the role that modifications on their structures mean in the cell wall organization is still not completely understood (Brummell and Harpster, 2001).

Specifically, extended fruit shelf life is required due to the high perishability of avocado fruit and the long shipping time required. The main factor implicated in the postharvest handling of many commodities is the softening of the mesocarp (Brummell and Harpster, 2001). As a climacteric fruit, textural changes during ripening are related with increased ethylene production during ripening (Pesis *et al.*, 1978). Thus, avocado fruit usually soften after 7-8 days after been harvested (Jeong *et al.*, 2002). However, preharvest conditions such as cold stress or higher maturity can result in variable behaviour. Indeed, avocado fruit subjected to chilling injury showed anticipated softening (Herskovitz *et al.*, 2009b) and late harvest fruit are usually recognised as having a shorter shelf life (Dixon *et al.*, 2003b).

The softening in avocado fruit has been related to changes in the activity of cell wall enzymes (Awad and Young, 1979; Jeong *et al.*, 2002; Jeong and Huber, 2004)

however, changes in the cell wall composition in avocado fruit is poorly understood. Previous studies report increased PG and endoglucanase activities (Awad and Young, 1978; Pesis *et al.*, 1978) during fruit softening also accompanied by increased solubility and depolymerization of pectins. However, delays in ripening enforced with application of 1-MCP showed that fruit were able to soften with very low level of PG (Jeong and Huber, 2004). Herein, confirming previous statements, residues from pectin degradation increased during ripening. In fruit harvested early in season the firmness decreased to around 2 N only at the end of shelf life and accordingly the homogalacturonic epitopes (LM19) slightly increased their levels at day 7. In particular, late season fruit showed higher epitopes level at day 2 of shelf life when the mesocarp firmness was already low (under 20 N) and slightly decreased in overripe fruit. This might imply a higher activity in late season fruit of PG, and consequently increased levels of PME necessary to de-esterificate the galacturonic residues in the beginning of ripening. In agreement, the methyl-esterificated epitopes recognised by LM 20 did not change through ripening in early season fruit whereas in late harvested fruit methyl-esterificated epitopes were higher. PME activity is usually high in the first days of shelf life follows by a decrease during ripening when the PG activity increases (Awad and Young, 1979; Jeong *et al.*, 2002). However, the regulation of PME transcripts is not clear yet. PME activity remains high in 1-MCP-treated fruit with a delayed softening (Jeong and Huber, 2004).

Further modification which can affect pectins is the loss of galactan residues from side chains. In this study LM5 content, recognising (1-4)- β -galactosyl residues, did not change in early harvest fruit with ripening stage. Concordantly, galactose sugars recovered in the water soluble extract decreased during ripening with higher ratio in 1-MCP treated fruit (Jeong and Huber, 2004). These results might indicate that the activity of β -galactosidase do not increase during softening. In agreement, α/β galactosidase showed reduced activity after harvest and during the first days of shelf life (Jeong *et al.*, 2002). External ethylene seems to have a negative control on β -galactosidase activity (Jeong and Huber, 2004) whereas 1-MCP treated avocado fruit showed a slower decrease of α -galactosidase levels in treated fruit and any significant changes were detected for the β -galactosidase (Jeong *et al.*, 2002; Jeong and Huber, 2004). However, higher LM5 levels were detected in fruit from late harvest but with no

consistency through ripening. This might implicate a constitutional higher content of galactosyl residues or β -galactosidase activity in those fruit.

Instead, differences during shelf life were detected in LM6 levels, specific for (1-5)- α -arabinosyl, which increased with fruit ripening. Arabinosyl groups are usually found as residues attached to RG-I chains (Willats *et al.*, 1998) and (1-4)- β -galactosyl residues changed in their content through fruit maturity with higher increase during softening in late season fruit. The presence of side chains in the RG-I structure increases the possibility of linkages with cellulose and confers flexibility to the cell wall structure (Somerville *et al.*, 2004). The higher levels of neutral sugars identified in late season fruit could be related to the higher depolymerisation of the carbohydrate chains and increased softness of the mesocarp or once again be due to a different cell wall composition of late season fruit. Indeed, arabinans epitopes were found abundantly in the oil gland of lemon fruit (Willats *et al.*, 1998). It will be interesting to localize these epitopes *in situ* in avocado fruit and investigate the possible implication that higher DM content of late season fruit on cell wall composition.

The hemicelluloses detected were mainly present as xylans (LM11) and mannans (LM21) chains. Their content was slightly higher in soft fruit and in late season fruit. The increase in hemicellulose residues as fruit soften might indicate a depolymerization of the chains as suggested previously (Jeong *et al.*, 2002). Minor side groups were xyloglucans (LM10 and LM15) and galactomannans (LM22) detected in very low levels regardless fruit maturity or ripening stage. Xyloglucans, which have been suggested to be involved in the linkage between cells in tomato fruit (Ordaz-Ortiz *et al.* 2009), were here detected at low levels and only in soft early season fruit. However, previous work showed higher levels of these polymers in the hemicellulose fraction of avocado cell wall (O'Donoghue and Huber, 1992). This might implicate a general low level of these polymers in the avocado cell walls or their slow degradation and consequently a secondary role in the mesocarp softening. Enzymes related with hemicellulose depolymerization are endo- β -(1-4)-glucanase which activity increased during storage and seems to be ethylene regulated with a suppressed activity in 1-MCP treated fruit (Jeong and Huber, 2004). This is in agreement with herein results, showing increased level of hemicellulose residues as softening arise.

Moreover, the higher levels of arabinans (LM6), methyl-esterified (LM20) or de-esterificated (LM19) residues homogalacturonan (HG) reached in late season fruit might indicate an advanced depolymerization process of the cell wall structures in fruit with higher maturity in agreement with the faster decline of firmness values.

To conclude for the first it has been showed that monoclonal antibodies can be used to investigate of structural carbohydrates in avocado fruit mesocarp has been demonstrated. Results showed that as fruit soften the neutral sugars residues increase confirming the involvement of depolymerisation of structural carbohydrate in the softening process, as previously suggested (Jeong *et al.*, 2002; Jeong and Huber, 2004). Indeed, in early season fruit the main epitopes levels increased at the end of shelf life whereas in the late season fruit with anticipated softening, the pectins residues were already higher at day 2 of shelf life. The slightly decreased of epitopes identified at day 7 when fruit were already overripe (1 N) might indicate that the depolymerization process of cell wall structures increase along ripening with a repression in the last stage of ripening. The presence of neutral sugars related with pectins units founded in previous work (Jeong and Huber, 2004) could also confirm the main role of pectins in the cell wall linkages and the role that the break down of this connections has on fruit softening. Pectins degradation could be one of the main processes responsible for avocado softening, confirming previous works (Jeong and Huber, 2004). A notable difference between harvest seasons on the methyl epitopes content, (1-4)- β -galactosyl and (1-5)- α -arabinosyl groups might be related to a different cell wall composition.

Further investigations with the use of molecular antibodies *in situ* could show where the cell wall structures are located in the layer of the mesocarp and might give more information about the modifications during ripening and revealed possible structural differences along maturity stage. It will be wise to detect the level of the main enzymes activity (i.e. PG, PME) to possible found differences along ripening and fruit maturity stage.

8 CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSIONS

8.1 Discussion

Consumption of exotic fruit has recently increased in many European countries; specifically, according to market research, avocado fruit demand is likely to become even more substantial in the next few years. The year round demand for avocado fruit in the UK has led to a wide range of importers. As a tropical-subtropical fruit, avocado is mainly a product of central-south American countries, Indonesia, USA, and South Africa. The agricultural practises and environmental conditions of each area can result in different postharvest fruit quality. Temperature, sun exposure (*cf.* section 2.11.2), irrigation (*cf.* section 2.11.3) and mineral nutrition (*cf.* section 2.11.4), are only some of the factors that can influence avocado fruit development and consequently effect postharvest behaviour. The difficulty in controlling of avocado fruit ripening is usually a source of discontent within traders and ultimately the resulting inconsistency can dissuade consumers from repeat purchases. Besides, avocado is appreciated particularly for its taste and the potential health benefiting properties related with its regular consumption (Bergh, 1992; Board, 1995; Lu *et al.*, 2005; Ding *et al.*, 2009). The high ratio of unsaturated fatty acids with potential benefits against cardiovascular diseases (Coulston 1999; Ascherio, 2002) and the presence of C7 sugars with effects on insulin inhibition (Ferrer *et al.*, Roth *et al.*, 2009) and possible anticancer activity (Board *et al.*, 1995; Ishizu *et al.*, 2002) are the most well known characteristics of avocado metabolites. The presence of unsaturated fatty acids in oil fractions (Ozdemir and Topuz, 2004; Landahl *et al.*, 2009; *cf.* Chapter 4) and the predominant C7 soluble sugars (Liu *et al.*, 2009; *cf.* Chapter 4) are the main biochemical traits of avocado fruit. The oil fraction seems to be at its highest during fruit development with minor changes noted postharvest (Ozdemir and Topuz, 2004), whereas the C7 sugars play a central role in fruit size (Cowan *et al.*, 2001) and ripening regulation (Liu *et al.*, 2009). The influence of agricultural practises and environmental conditions on postharvest fruit quality (Arpaia *et al.*, 2004) might be related to the unpredictable behaviour during

ripening. However, most of the literature presented on avocado reports on fruit from the same or nearby growing areas and therefore, a lack of information still exists on the physiological and biochemical characterization of imported avocado fruit. This is surprising given that the ripening process from import to sale is perhaps the most important step in the supply chain.

The novelty of this project involved the postharvest investigation of avocado fruit cv. Hass with the purpose of identifying general guidelines for distinguishing fruit ripening behaviour. With an aim to define general avocado fruit quality markers, this work characterized the postharvest behaviour of avocado fruit exported from the leading suppliers of avocado fruit to the UK market (i.e. Spain, South Africa, Peru and Chile), three different maturities at harvest, and two postharvest storage temperatures (*cf.* Chapter 4). Most of the previous research on avocado fruit considered mainly changes occurring in the edible part of the fruit. Few works (Liu *et al.*, 1999; Wang *et al.*, 2010) have considered the peel tissue. Herein, biochemical changes in peel (*cf.* Chapter 5) and mesocarp (*cf.* Chapter 4 and 7) were simultaneously compared with physiological parameters highlighting a possible role of the main metabolites on ripening regulation. This project aimed to elucidate the possible use of skin colour changes as a marker for better identifying avocado fruit softness and investigate, using a modern and specific technique, the main cell wall modifications that take place (*cf.* Chapter 6). Additionally, this work gives useful information regarding the efficacy of ethylene treatment in postharvest quality while taking into account preharvest factors in fruit from different consignments (*cf.* Chapter 6). In order to reduce fruit variability, ethylene is usually applied in commercial conditions. However, fruit can have a different response to the same postharvest conditions as a result of preharvest factors (i.e. harvest time, fruit size) (*cf.* Appendix A).

Avocado fruit has the inability to ripen while attached to the tree unless extreme environmental conditions (i.e. chilling injury) alters its behaviour (HersHKovitz *et al.*, 2009b). As a climacteric fruit, the ripening process in avocado is marked by a burst in ethylene production and respiration rate (Adato and Gatiz, 1974; HersHKovitz *et al.*, 2010). Ethylene is a plant hormone widely investigated due to its influence on plant metabolism. Cell wall enzymes, pigment degradation and biosynthesis are only some of

the pathways dependent on ethylene (Lelièvre *et al.*, 1997). Among climacteric fruit, avocado has one of the highest rate of internal ethylene production occurring in the first couple of days depending on the conditions (*cf.* Chapter 4). Higher sensitivity to ethylene is also connected with the higher perishability after harvest (Lelièvre *et al.*, 1997). Climacteric ethylene production and respiration rate in avocado fruit has been recorded after harvest but little is known on the gasses released from fruit after prolonged cold storage. Increased ethylene production was detected in the first few days of ripening (*cf.* Chapter 4) with different patterns according to fruit source, maturity and storage length. In fruit stored long-term, different ethylene production was noted between fruit source, with an absence of an ethylene climacteric peak in fruit from Peru (Figure 4.5, 4.7). Higher ethylene production was detected in fruit sourced from Spain harvested in middle season (111 and 135 $\mu\text{l kg}^{-1}\text{h}^{-1}$ at 18 and 23°C, respectively). However, fruit from Malaga were stored for no more than 10 days whereas fruit from other sources were shipped for at least a month. Thus, the lower ethylene detected in fruit from Chile, Peru and South Africa might be due to the long storage time. Nevertheless, higher climacteric ethylene peaks were not related with faster softening. Although differences were observed in the climacteric pattern, the mesocarp softening showed a similar trend in fruit from Spain and Chile. The monitoring of fruit gasses was carried out after fruit were cold stored and the detection during ripening was not continuous. Thus, the climacteric behaviour identified could be considered indicative but not accurate. In contrast, the respiration rate increased in the first two day regardless of fruit growing area, harvest time or storage time (Figure 4.6, 4.8). Due to the poor reliability of the gasses measured, a more common indicator of avocado fruit ripening is the softening of the mesocarp. As expected, fruit were soft after 7 days of shelf life, with a faster decrease in firmness at higher temperature (*cf.* Chapter 4). The mesocarp softening differed according to fruit maturity with generally faster ripening in late season fruit, as previously found in fruit from New Zealand (Dixon *et al.*, 2003b). The different behaviour of fruit from different maturities perhaps supports the depletion of a tree-derived inhibitor factor while fruit mature on the tree (Adato and Gatiz, 1974).

Soluble sugars seem to play a role as ripening inhibitors (Liu *et al.*, 2002) with a consistent decrease in the C7 content through fruit postharvest life (Liu *et al.*, 1999b;

Blakey *et al.*, 2009). This thesis provides additional proof of the importance of C7 sugars in avocado fruit metabolism. The decrease in the C7 carbohydrate levels through seasons progression and during storage suggests a possible implication in the ripening inhibition (Liu *et al.*, 2009b). In particular, D-mannoheptulose content constantly decreased through seasons and shelf life (Table 4.2). For the first time, chemometric analysis of sugar profiles of fruit coming from different areas and of different maturities at harvest revealed a systemic decrease of D-mannoheptulose as fruit ripened. In particular, when fruit were sourced from a similar region (Spain, Peru and Chile), late season fruit had lower D-mannoheptulose content between harvest times. Less uniformity in ripening was noted in fruit sourced from South Africa compared with other suppliers and thus might be due to the different location of the orchard where fruit were sourced. Indeed, whereas fruit from Spain, Peru and Chile were harvested from the same area for all three seasons, fruit from South Africa were collected from regions with different environmental conditions. Results confirm the possible use of D-mannoheptulose as an indicator of fruit shelf life between fruit from the same source. The presence of a general threshold of D-mannoheptulose content after which the ripening occurred (Liu *et al.*, 2002) was not however confirmed. Fruit harvested in Chile and Peru had the highest sugar content compared to fruit coming from Spain or South Africa and this might be related to growing and environmental conditions during fruit development.

The composition of avocado fruit, rich in unsaturated fatty acids and soluble sugars, the majority of which are C7 carbohydrates, seems to be a key factor involved in fruit metabolism. Whereas sugar content changes after harvest, the oil fraction is mainly defined during fruit development (Ozdemir and Topuz, 2004) and its composition is associated with membrane fluidity and cold tolerance (Harwood, 1997). A good indicator of fruit growing area was the fatty acid composition of the mesocarp oil, where oleic acid was the main compound identified. In agreement with previous works (Woolf *et al.*, 2003b; Landahl *et al.*, 2009) the feasibility of oil content or dry matter as a universal indicator of avocado fruit maturity stage is here questioned. Indeed, dry matter and oil content decreased as expected through the seasons but similar dry matter content could identify different seasons regarding growing area (i.e. 26% in middle

season in Chile and late harvest in Peru) (Table 4.1). Once again, results indicated the predominant impact of preharvest factors on the shelf life of avocado fruit. Traders might have to take postharvest conditions into more consideration. This said, it remains true that establishing the role of biomarkers related with fruit ripening behaviour such as C7 sugars or oil composition in order to use the more appropriate postharvest conditions would be useful.

As previously suggested, a possible indicator of avocado fruit cv. Hass ripening might be related to changes in skin colour (Cox *et al.*, 2004; Asthon *et al.*, 2006). Hass is a cultivar that dominates the market due to the high quality of the pulp, nutty flavour and good predisposition to storage. Additionally, a thick skin protects the fruit from external damage and turns from green to deep purple when approaching ripening; however this colour change is not universal for all fruit source and thus the relationship between ripening and peel colour is sometime problematic. The characteristic colour change in cv. Hass is highly appreciated by consumers who rely on the skin colour as a ripening indicator. That said, the relationship between skin colour changes and mesocarp softening is still uncertain (Cox *et al.*, 2004). The importance of knowing the regulation of flesh softening and skin colour darkening is due to the possible use of skin colour as an external and non destructive parameter to measure fruit ripening with a consequent reduction of industrial wastes. Indeed, recently the destructive measurement of mesocarp softness is the most reliable indicator used by industry whereas costumers tend to refer to the skin colour. However, inconsistencies in skin darkening and fruit softening have been previously reported (Cox *et al.*, 2004). Furthermore, it is still questionable whether the colour can be used as an indicator of fruit ripening stage in avocado fruit cv. Hass. Skin darkening has been related with increased anthocyanins levels, mainly cyanidin-3-*O*-glucoside, and with decreased chlorophyll *a* content (Cox *et al.*, 2002; Asthon *et al.*, 2006). Mesocarp softening is related to the activation of different cell wall enzymes (Pesis *et al.*, 1978). Both cyanidin-3-*O*-glucoside (El-Keramy *et al.*, 2003) and textural changes (Pesis *et al.*, 1978) seem to be ethylene regulated. That said, these two parameters showed different responses to temperature (*cf.* Chapter 5). Indeed, low temperature retards skin darkening even at the fully soft stage however, mature fruit can show darkening before harvest when the mesocarp is

still hard (Cox *et al.*, 2004). To the best of our knowledge, there is no literature which has specifically focused on systematically measuring the main skin metabolites of avocado during fruit ripening and across different maturity stages. For the first time, the skin of avocado fruit cv. Hass harvested at different maturity stages was characterised by assessing the changes in total chlorophylls, carotenoids, cyanidin-3-*O*-glucoside and individual phenolics throughout ripening at different temperatures. A straightforward method for the extraction and quantification of anthocyanin and phenolics was here developed (*cf.* Chapter 5). Results confirmed the main role of cyanidin-3-*O*-glucoside and chlorophyll *a* in the determination of skin colour changes (Cox *et al.*, 2004). Total chlorophyll *a* decreased with increasing maturity, from 573.2 to 508.9 $\mu\text{g g}^{-1}$ DW from early to late season, respectively. Carotenoids, also involved in colour determination of fruits and vegetables, also showed a decrease during ripening again in agreement with previous work (Asthon *et al.*, 2006) and a higher content in early (173.1 $\mu\text{g g}^{-1}$ DW) rather than late season fruit (157.3 $\mu\text{g g}^{-1}$ DW). The change of colour in avocado skin cv. Hass has been implicated as the initial degradation of chlorophylls and a second stage where anthocyanin content increased (Cox *et al.*, 2004). Herein, results showed a possible role of carotenoids too. The darker coloration of fruit with higher maturity might also be related with their lower content of carotenoids. Higher temperature of ripening was not particularly influential in determining of chlorophylls and carotenoid content, but at 23°C the anthocyanin content was higher in all harvest seasons at the end of shelf life; 1,060, 1,899, 1,079 $\mu\text{g g}^{-1}$ DW in early middle and late season, respectively. In this case, the darker skin coloration could be mainly due to the increased level of cyanidin-3-*O*-glucoside. At lower temperatures (18°C), the content was significantly lower at 637, 746 and 499 $\mu\text{g g}^{-1}$ DW for the three seasons (early, middle and late, respectively). Higher ripening temperature and higher fruit maturity induced faster skin darkening and mesocarp softening and increased the relationship between hue angle and mesocarp firmness. In contrast, hue angle and cyanidin-3-*O*-glucoside were better correlated in early season fruit. However, no substantive and reliable relationship was found between the cyanidin-3-*O*-glucoside content and mesocarp softening. This might be explained by different influences of preharvest factors affecting the fruit metabolites and consequently the relationship between

mesocarp softening and colour changes. For instance, external temperature seems to result in darker colour development than fruit softening (*cf.* Chapter 5). Generally, in fruit with faster ripening such as middle-late season fruit or fruit held at relatively high temperature (23°C), skin colour changes could be a good indicator of avocado softness. A new avocado fruit ripening indicator has been here identified, with the limitation to a specific maturity stage of the fruit and postharvest conditions. The mechanisms involved in the colour changes and mesocarp softening are not completely understood yet, though it is known that both anthocyanin biosynthesis and cell wall enzymes are regulated by ethylene levels. Thus, decreased fruit softening and increased cyanidin biosynthesis occur after the climacteric peak (*cf.* Chapter 5). After the climacteric rise, ethylene production decreases to initial levels whereas the mesocarp softening and the cyanidin-3-*O*-glucoside content still continue to change throughout ripening (Figure 8.1). Thus, increased ethylene levels during the climacteric phase might act on the stimulation of biosynthetic pathways. In contrast, a different trend was noted for other phenolics compounds detected in avocado peel (*cf.* Chapter 5). The main phenolics identified in the peel tissue (epicatechin, procyanidin B2 and chlorogenic acid) changed throughout shelf life. An increase in epicatechin and procyanidin B2 content in early and late season ripe fruit preceded, at 18°C, or followed at higher temperatures, the ethylene peak. A different trend was noted for chlorogenic acid. Chlorogenic acid content was mainly influenced by fruit maturity and the higher content was detected in middle season fruit characterised by a higher climacteric peak. This suggests further investigation is required to elucidate the role and involvement of ethylene on the phenolic pathway.

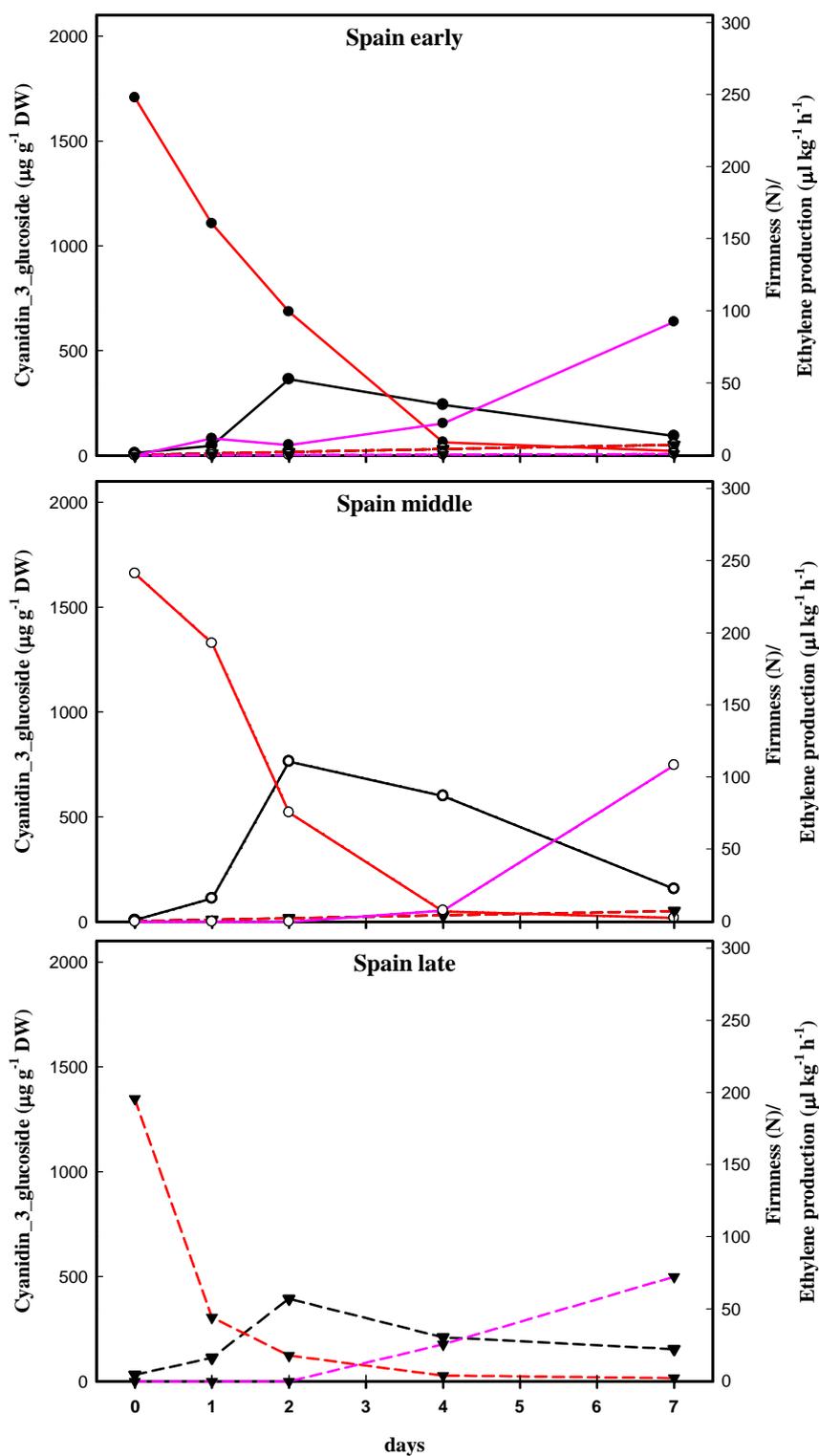


Figure 8.1: Description of firmness (N) (—), ethylene production $\mu\text{l kg}^{-1}\text{h}^{-1}$ (—) and cyanidin-3-*O*-glucoside $\mu\text{g g}^{-1}\text{ DW}$ (—) in avocado fruit cv. Hass harvested in early, middle and late season in Malaga, Spain.

Phenolics are known to be involved in the defence system (Barasundram *et al.*, 2006) and specifically in avocado fruit increase the susceptibility to fungal infection during ripening. In avocado, disease incidence has been related to the decrease in diene and epicatechin content (Prusky *et al.*, 1992; Ardi *et al.*, 1998). Thus, the possible postharvest modulation of phenolic content might help to reduce postharvest losses. Previous work (Ardi *et al.*, 1998) found that ethylene application on avocado fruit results in a rise in epicatechin content. Nevertheless, the increase was not stable, and the epicatechin level returned to normal levels (Ardi *et al.*, 1998) whereas the effect on the anthocyanins seemed to be more reliable (El-Keramy *et al.*, 2003). The ethylene regulation system and the relationship between different ripening parameters requires further investigation. Increased phenolic content together with PPO (polyphenol oxidase) activity can have a negative impact on fruit quality as it is related with higher incidence of internal mesocarp discoloration (Rinaldo *et al.*, 2010; Coi *et al.*, 2011). This might be associated to the possible negative effect that early application of exogenous ethylene can have on avocado fruit quality with an increased incidence of mesocarp browning (Pesis *et al.*, 2002).

Due to the generally low phenolic content in the pulp, the importance of phenolic profiles in avocado fruit is still uncertain. The few works present in the literature (Torres *et al.*, 1987; Arts *et al.*, 2000; du Pascual *et al.*, 2000; Harnly *et al.*, 2006; Hurtado-Fernández *et al.*, in press) and the inconstant conditions used (i.e. different extractive and quantitative methods) make a comparison of results difficult. The variability and the wide range of phenolics present in plants (Naczki and Shahidi, 2006) necessitates the development of a specific method for avocado fruit cv. Hass necessary. The possibility of routinely quantifying the phenolic content of avocado fruit requires the development of a rapid and improved methodology. A comparison of the main methods previously tested on avocado fruit found acidic water/methanol solution at high temperature (70°C for 2 hours) the most convenient method to extract the main phenolic compounds from cv. Hass mesocarp (*cf.* Chapter 3, part B). Due to the different structural and chemical characteristics of plant-derived phenolics, the use of solvents with different polarity resulted in different phenolics compounds being extracted and profiled from the same plant material. For instance, the use of ethanol,

with a lower polarity than methanol, and at low temperature reduced the amount of phenolics detected. The use of acidic and basic hydrolysis with ether derivatives possibly resulted in a greater release of phenolics from cellular vacuoles and improve break down of the linkages with sugars moieties or other molecules. However, this procedure might be regarded as too stringent for avocado as it limited the detection of other phenolic derivatives, such as epicatechin or catechins. The use of an antioxidant to protect the phenolics from the degenerative process which can occur during extraction at high temperature, is not always recommended. Indeed, it has been suggested that the antioxidant has a possible pro-oxidant action on the phenolic acids (Nuutila *et al.*, 2002). In agreement with recent investigations (Mattila and Hellström, 2006; Hurtado-Fernández *et al.*, in press), p-coumaric (27.9 mg mg⁻¹ FW), epicatechin (5.6), ferulic (5.3), caffeic (3), chlorogenic (3), sinapic (2), gallic acids (1.3), and also, although not in all samples, catechins were identified in ripe mesocarp cv. Hass. Similar results to the results presented herein were obtained by Mattila and Hellström (2006) whom also detected vanillic, p-hydroxybenzoic and syringic acids instead of gallic acid, epicatechin and catechins. However, the presence of peaks in the chromatogram which did not matching any of the internal standards tested herein suggests that the future use of mass spectrometry may be beneficial to help identify additional unknown compounds. Due to the relationship between phenolic content and avocado fruit postharvest quality as briefly reviewed (*cf.* Chapter 2, section 2.8.4), the method defined herein could be used in the characterization of phenolic profiles during fruit ripening. Indeed, it might be interesting to investigate a possible relation within fruit mesocarp discoloration and the relative concentration of individual phenolics.

Changes occurring during avocado fruit ripening are wide and involve many biosynthetic and degenerative pathways. The regulation of these processes seems to be mediated by the internal ethylene production. In particular, avocado can reach 100 µl kg⁻¹ h⁻¹ of ethylene released during the climacteric phase whereas other fruit range from 50 (i.e. banana) to 3 µl kg⁻¹ h⁻¹ (i.e. mango) (Seymour *et al.*, 1999). The high ethylene production is related with the higher perishability after harvest (Lelièvre *et al.*, 1997). In order to increase fruit storability, ethylene is usually removed from the atmosphere during storage (Woolf *et al.*, 2005; Terry *et al.*, 2007) whereas to accelerate fruit

ripening, ethylene can also be supplied as a ripening trigger (Saltveit *et al.*, 1999). Nevertheless, ethylene treatment does not always give the desired results. Ethylene application can be ineffective immediately after picking (Köhne, 1985) unless fruit have been harvested later in the season (Starrett and Laties, 1991). The depletion of an inhibitor factor from late harvest fruit seems to allow the action of the exogenous ethylene. Furthermore, application during cold storage seems to decrease fruit postharvest quality (Pesis *et al.*, 2002; Dixon *et al.*, 2003). Even though, the commercial use of ethylene treatment is in most cases a general practice with no consideration of fruit maturity or growing area. In this work (*cf.* Chapter 6), ethylene treatment was tested on different fruit consignments considering also the possible synergistic effect of the variable temperature regimes. Surprisingly, the ethylene treatment in cold stored fruit decreased its efficacy through harvest time and with the decrease of fruit size. Indeed, avocado fruit commercial size 16 (236-265 g) harvested in May from Mpumalanga, South Africa, responded with an observed accelerated softening. Fruit from the next harvest (June) and size 18 (211-235 g) were affected by both temperature and ethylene application whether in fruit harvested in July, size 22 (171-190), the ripening was mainly affected by increased temperature (*cf.* Chapter 6). Even with longer application (24 hours) the ethylene effect was not evident. Where ethylene treatment was not effective a relatively high temperature (20°C) accelerated the ripening process (*cf.* Chapter 6). The presence of small avocado fruit has been related to an alteration of ABA (abscisic acid), cytokinines (Cowan *et al.*, 1998) and sugars metabolism (Richings *et al.*, 1999) with a reduction of cell division and consequently reduced fruit dimension. ABA is also involved in the regulation of avocado fruit ripening and external application of ABA induces faster ripening and a rise in climacteric ethylene production (Blakey *et al.*, 2009). However, whether fruit maturity or fruit size can reduce or increase avocado fruit sensitivity to ethylene treatment is yet to be proven. Nevertheless, this work suggests the inefficacy of standard application of ethylene in the fruit trade and the importance of considering preharvest fruit characteristics, such as harvest time and fruit size. To elucidate these effects further a more systemic approach is required.

Ethylene is involved in many metabolic systems such as the phenylpropanoid pathway for phenolics, and in the softening of the mesocarp (Pesis *et al.*, 1978;

Lelièvre, *et al.*, 1997; Watkins 2006). As previously mentioned, mesocarp firmness is the main indicator of avocado fruit ripening stage. The textural changes occurring during ripening are a consequence of the activity of cell-wall related enzymes (Brummell and Harpster, 2001). The softening of the mesocarp can be influenced by fruit maturity (*cf.* Chapter 4), ethylene treatment (*cf.* Chapter 6) and temperature during ripening (*cf.* Chapter 4 and 6). The softening process has been previously related to the activation of cell wall enzymes (Pesis *et al.*, 1978; Jeong *et al.* 2002) some of these are known to be ethylene-dependent such as the PG transcript accumulation (Sitrit *et al.*, 1998). What is still unclear is the possible involvement of cell wall enzymes or structures to accelerate softening of high mature avocado fruit. For the first time a new technique for the characterization of structural carbohydrates has been applied and adapted to avocado fruit mesocarp (*cf.* Chapter 7). The detection of the main cell wall structures with the use of specific antibodies (Table 3.2) revealed the increased presence of neutral sugars residues as ripening occurring. This could be related to higher activity of PG and PME enzymes, previously found in avocado mesocarp (Awad and Young, 1979; Jeong *et al.*, 2002; Jeong and Huber, 2004). In particular, pectin monomers were observed as most abundant residues found in avocado cell wall extracts and their content was higher in fruit with higher maturity. Nevertheless, further investigations *in situ* may confirm that the faster softening in late season fruit avocado is due to a higher activity of cell wall enzymes rather than different cell wall composition.

In conclusion, this project identified and confirmed D-mannoheptulose and oleic acid as two markers for avocado ripening and possibly for growing area, respectively. Additionally, the decrease in D-mannoheptulose content throughout harvest seasons might be a further indicator of avocado fruit maturity. Furthermore, it has yet to be investigated the role agricultural practise and environmental conditions have on determining individual sugars content. Results herein have shown that fruit ripen faster when harvested at higher maturity regardless of the growing area. Moreover, a possible future marker in avocado softening stage could be the skin colour changes but this might be limited to certain fruit maturity stage (i.e. late season fruit) or postharvest conditions. Phenolics metabolism and its relationship with ethylene should be further investigated in the peel tissue of avocado. Since phenolics are important metabolites

involved in the fruit defence system modulation, their internal concentration might be a future postharvest research topic.

Unfortunately phenolics can also have a negative effect on avocado fruit after harvest as they may result in the increased incidence of pulp discoloration. Due to the lack of studies on the phenolic profile of avocado fruit, this work also validated and optimised a novel yet straightforward method for the identification and quantification of the main phenolic content in avocado fruit mesocarp. Investigation of the individual phenolic content through avocado storage and ripening could be useful in the improvement of avocado postharvest quality. Furthermore with the use of a novel technique (monoclonal antibodies) the main cell wall components have been here identified and related with the mesocarp softening during ripening.

8.1.1 Future work

Better uniformity and extended shelf life of high quality fruit are priorities for the international avocado trade. Thus, a better knowledge of the physiological mechanisms regulating fruit postharvest is necessary; this is especially true for the imported fruit. Ethylene has a main role on the ripening process (*cf.* Chapter 2, section 12.4) but how this plant hormone acts on individual metabolite pathway(s) is still unknown. For instance, the different regulation of anthocyanin synthesis and mesocarp softening needs further investigation with particular attention on the effect that temperature and exogenous ethylene can have. Since new techniques are already available (i.e. photoacoustic laser) (Iannetta *et al.* 2006), a real-time detection of ethylene released during ripening could give more information about the role of ethylene on postharvest metabolism. Molecular techniques to target anthocyanin precursors such as chalcone synthase (CHS) or flavanone hydroxylase (F3H) could reveal changes in the anthocyanin pathway related to the climacteric behaviour. Thus, higher temperature (Cox *et al.*, 2004) and ethylene treatment (El-Keramy *et al.*, 2003) could stimulate anthocyanin biosynthesis, as suggested by previous studies. It would be interesting to investigate whether ethylene (i.e. ACC) and cyanidin-3-*O*-glucoside precursor levels change throughout postharvest life. At the same time, it would be wise to detect changes in cell wall enzymes activity (i.e. PG and PME): higher PG and PME transcripts could

correspond to increased residues of pectins. The use of molecular antibodies *in situ* could be used to detect the main structural carbohydrates and highlight changes in cell wall linkages during fruit ripening.

Due to the importance of phenolics in avocado fruit quality (*cf.* Chapter 2, section 2.8.4) and the development of a new and rapid method for their detection and quantification (*cf.* Chapter 3, part B), a profile of individual phenolics at different ripening stages could be a future application. Nevertheless, more work has to be done on the identification of the phenolics present in the mesocarp of avocado fruit cv. Hass, and with this aim, the use of mass spectrometry could be a useful tool. The increase PPO activity during ripening, responsible for high incidence of pulp discoloration in avocado fruit (*cf.* Chapter 2, section 2.8.4), might be related with changes in individual phenolics content given that individual phenolics are the substrate to any browning reaction.

The differential effects that ethylene treatment (*cf.* Chapter 6) has on avocado fruit of different size and harvest time, suggests that further research is required. Indeed, fruit maturity at harvest induced faster ripening and this has been here related with a decrease in the D-mannoheptulose content (*cf.* Chapter 4). However, it remains unknown whether maturity at harvest can influence ethylene perception. Ethylene receptor transcripts might be influenced by fruit maturity and consequently influence fruit postharvest response to exogenous ethylene. If this is proven, it might be possible to show that also for avocado two systems regulate the ripening process, one ethylene-independent and an other ethylene sensitive, as previously found in melon (Pech *et al.*, 2008). Thus, C7 sugars and ethylene receptors might be under different regulatory systems during fruit development. Furthermore, the involvement of D-mannoheptulose as a ripening regulator previously suggested (Liu *et al.*, 1999b) and here further evidenced (*cf.* Chapter 4) needs more investigation as there is a dearth of knowledge about whether preharvest factor(s) influence and determinate fruit sugars content.

8.1.2 Project conclusions

The main objectives were listed in Chapter 1, section 1.2.2. In summary, the project conclusions in terms of objectives are indicated below.

a. To investigate the physiological and biochemical mesocarp changes in avocado fruit cv. Hass imported from Spain, South Africa, Peru and Chile harvested in three main seasons (early, middle and late) during shelf life. Most of the changes in the physiological behaviour of avocado fruit cv. Hass were detected in the first days of after fruit were induced to ripen. Nevertheless, fruit showed different behaviour regarding origin, harvest time or temperature of ripening. Increase in the ethylene production was influenced by ripening temperature and was higher in fruit from Spain, characterised by shorter cold storage length. In the softening process, late season fruit softened faster than earlier harvested, regardless of fruit source. Also in the characterization of the main biochemical compounds present in avocado mesocarp, fruit distinguished regarding origin and harvest time. In particular, for the first time two biomarkers have been suggested as able to distinguish fruit growing area and ripening stage. Indeed, oleic acid content was consistent in fruit from same growing area with a possible role as a marker of fruit source, with minor changes occurring during fruit ripening. In contrast, D-mannoheptulose decreased during seasons and as fruit ripen. In this case, the sugar content has been suggested as an indicator of fruit maturity and ripening stage.

b. To compare the influence of temperature and ethylene treatment on imported avocado fruit harvested at different time during the year (early, middle and late). Imported avocado fruit from Mpumalanga, South Africa, differentially responded to ethylene treatment according to fruit size and harvest time. Fruit from the first harvest period (May) showed faster ripening when ethylene treated whereas in other harvest seasons the effect of ethylene application was not evident. Indeed, fruit harvested in the following season (June) soften faster when the ethylene and higher ripening temperature were applied. Avocado fruit from the same orchard harvested in July had shorter shelf life at higher ripening temperature. This said, the different response to ethylene treatment and ripening temperature might be a consequence of the different maturity stage of the fruit. Besides, it is to consider that in this experiment fruit size decreased along seasons with a possible effect on the fruit postharvest behaviour.

c. To develop an efficient method to investigate the main phenolics present in avocado cv. Hass mesocarp and skin tissues. Better extractive conditions were validated and optimised in an aqueous solution of acidic methanol at high temperature (70°C) for the extraction of the main phenolic compounds present in avocado fruit mesocarp. Main phenolics were identified in p-coumaric and ferulic acid. Other compounds present in the chromatogram were not matching with external standards, and further investigation is suggested. In contrast, due to the susceptibility of anthocyanins to high temperatures, the extraction of phenolics from peel tissues has been done at lower temperature (35°C). Beside the main anthocyanin, cyanidin-3-*O*-glucoside, other phenolic compounds, such as epicatechin, procyanidin B2 and chlorogenic acid, were identified and quantified by HPLC.

d. To investigate the relationship between skin colour changes and mesocarp softening through shelf life in avocado fruit cv. Hass with different maturity stage (early, middle and late season). The use of hue angle as indicator of fruit softening is valid but only under specific fruit maturity and ripening conditions. Indeed, a better correlation between mesocarp softening and hue angle has been found in avocado fruit cv. Hass harvested late in season or when relatively higher ripening temperature was applied. Generally, a poor relationship was found between firmness and cyanidin-3-*O*-glucoside.

e. To investigate the structural carbohydrates present in avocado mesocarp through a new immunologic detection method and discuss the possible influence of cell wall composition on ripening. Specific epitopes present in carbohydrate structures were identified in the mesocarp of avocado fruit. Higher polymer residues were detected in softer fruit, ripe and in late season fruit. The faster softening characteristic of late season fruit might be due to a different enzyme activity or to a different cell wall composition. Further work on the identification in situ of the main cell wall structures might be helpful in a better understanding of the softening process during avocado fruit ripening.

9 CHAPTER NINE

9.1 Literature cited

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Appendix A

A.1 Comparison between commercial and experimental postharvest ethylene treatment on fruit from South Africa early season

The recent increased consumption of avocado fruit in European countries has aroused the interest of traders' of this fruit who, in an increasingly competitive market, aim for higher quality and more consistence produce. Avocado importers are faced with the extreme perishable nature of the fruit and the difficulties associated with controlling ripening. Indeed, a recent market investigation revealed that one of the most unwanted characteristics for UK consumers is poor uniformity during ripening (Mack Multiplies private communication).

It is common practise to apply ethylene before the fruit has been distributed to the supermarket to accelerate ripening and to satisfy the demand for the "ready to eat" avocado market (Mack Multiplies private communication). However, the ripening process of avocado fruit is not yet fully understood and the use of ethylene does not always give predictable results. For instance, ethylene treatment can be ineffective if applied immediately after harvest (Adato and Gatiz, 1974; Köhne, 1985) with the exception of late harvest fruit (Starrett and Laties, 1991). Ethylene application during cold storage showed in some cases, a negative effect on avocado fruit quality (Pesis *et al.*, 2002; Dixon *et al.*, 2003). Thus, it would be wise to further investigate the postharvest application of ethylene on avocado fruit. Moreover, parameters such as temperature or humidity in experimental conditions are usually well controlled to assess the possible influence of each factor on fruit metabolism. The real market prerequisites are usually less uniform and consequently not always comparable with experimental conditions. In this specific case, the dimensions and the characteristics of the room in which the fruit were commercially treated differed from the controlled room used in a more controlled experiment (*cf.* Chapter 6). Additionally, the treatment rooms used by importers have different ventilation systems and ethylene application methods compared with the techniques used at Cranfield University. Lastly, the quantity of fruit held in the commercial rooms was much higher than the capacity at Cranfield University and in experimental conditions the assessment of the fruit ripening stage (i.e.

objective colour, mesocarp firmness) differed from the commercial procedures (subjective colour, penetrometer). This said, the purpose of this study was to compare the efficacy of the ethylene application method used in experimental trials with those used under commercial practises.

After their arrival in UK, avocado fruit cv. Hass were placed in commercial ethylene treatment rooms. At the same time, fruit from the same consignment were transferred to Cranfield University and treated with ethylene as previously described (*cf.* Chapter 6). Previous work, conducted in the same commercial facilities, showed inconsistency in the temperature within the avocado's storage room with possible influence on water loss (Gamarra Saiz, 2004). Consequently, it was assumed that the possible variability between fruit was related to the different fruit location inside the room. The room was divided in two almost symmetrical sections. Within one room, sector fruit were taken from four different areas chosen between direct ventilation and higher or lower position on the shelf. Unfortunately, due to commercial priorities, it was not possible to have a control (non treated fruit) in the commercial facilities [hence this chapter is in the appendices].

A.2 Plant material and experimental desing

Avocado fruit cv. Hass were harvested in South Africa in May and transported to the UK under cold storage conditions (5°C). Fruit were organized into pallets and distributed between different rooms with controlled temperature (18°C). The maximum capacity of a room was 18 pallets. Each pallet comprised of *ca.* 240 boxes each containing between 16 to 22 fruits, depending on fruit size. After one day at 18°C, fruit were treated with ethylene at 20°C using an ethylene generator located inside the room. The system consisted of a solution of ethyl alcohol that released ethylene when heated (Health and Safety Executive, 2011). Approximately 1 l of solution was used in 24 hours, corresponding to 11.24 $\mu\text{l l}^{-1}$ per minute. The following day, treated fruit ($n = 256$) were randomly selected from four different promoties according to a ventilation system nearby the pallet located in a top (E), or low shelf (H), and the absence of the air generator in the top S and low (V) shelf (Figure A.1).

At the same time, fruit previously collected from a 5°C room were transported to Cranfield and were treated as described in Chapter 6. Briefly, fruit ($n = 32$) were kept in sealed plastic boxes with $100 \pm 5 \mu\text{l l}^{-1}$ of ethylene for 24 hours at 20°C. The same number of fruits was kept in a separate room without ethylene (control 1). Moreover, to evaluate the possible effect of the ethylene and the CO₂ released by the fruit whilst in the boxes, an additional fruit subsample ($n = 32$) was closed in boxes for 24 hours without ethylene (control 2). After the treatments, fruit were kept at 20°C at Cranfield until they were fully ripe. Fruit previously treated in commercial conditions were transferred to Cranfield University for shelf life assessment. Ethylene release, respiration rate, firmness and colour were measured during shelf life at specific intervals (day 2, 3, 4 and 6) as previously described (*cf.* Chapter 3). At each interval, for each treatment (ethylene, control 1 and control 2, $n = 24$) and for each location (E, H, S and V, $n = 36$) 8 fruit were analysed.

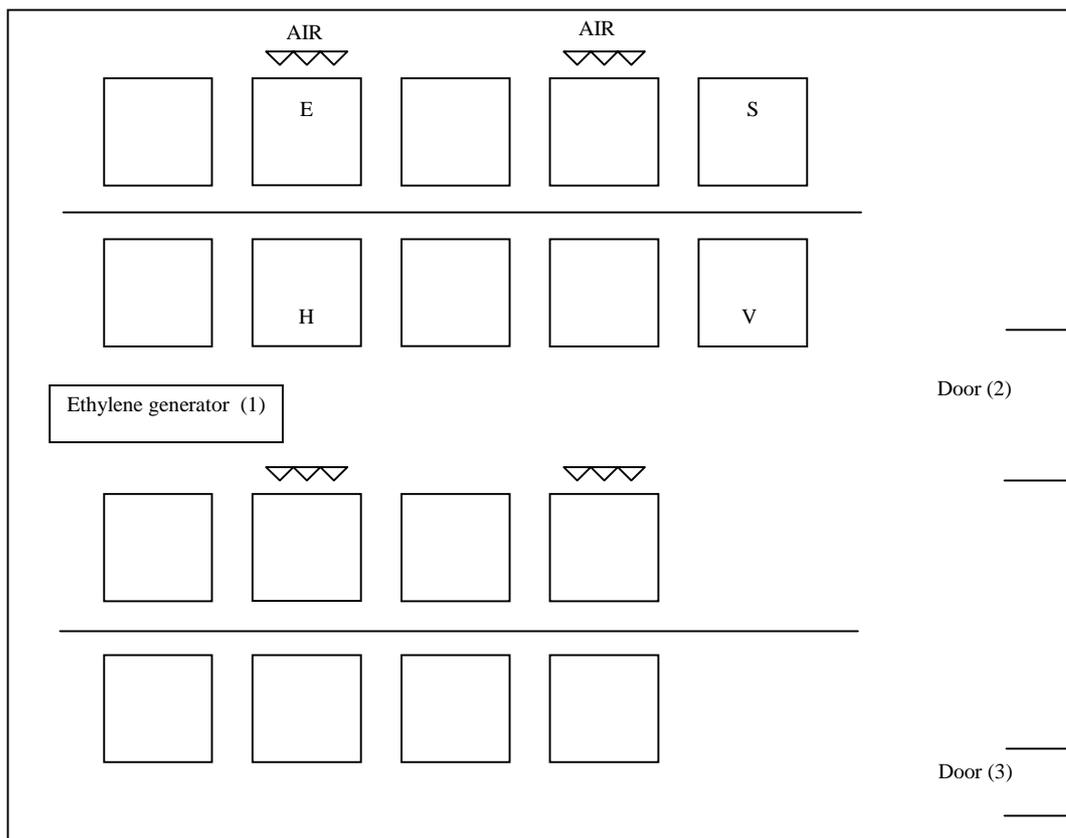


Figure A.1: Schematic representation of the room's structure in the commercial facilities. Fruit were taken from four different locations inside the room: close to

ventilation system in top (E) or in low shelf (H) and without direct air generator in top (S) and low shelf (V). The ethylene generator (1) and two doors (2, 3) are also shown.

A.3 Results

A.3.1 Weight

Fruit weight readings were recorded before other measurements. Results showed that fruit were of different sizes. As previously mention it (*cf.* Chapter 6), this might results in a different response to ethylene treatment. Fruit located in zone E and H were significantly smaller than fruit on zone S and V (Table A.1) and so could not be directly compared. However, fruit weight generally decreased during shelf life.

Table A.1: Weight measurement in avocado fruit from South Africa held at 20°C until fully ripe (six days). Column indicates fruit position in commercial room (E, H, S and V) and if fruit were ethylene-treated or used as control (control (1), control boxes) in the experimental conditions.

day	Commercial ethylene treatment				Experimental conditions		
	E	H	S	V	Ethylene	Control (1)	Control boxes
0	231.65	219.01	243.97	247.4	244	247.4	231.6
6	235.59	212.07	236.53	216.61	237	216.6	220.5
LSD		8.86				8.64	

Each value is a mean of three fruit measurement with LSD ($P < 0.05$) indicated.

A.3.2 Colour

Skin colour turned from green to dark purple, indicated by a general decrease in colour parameters (C^* , L^* and H°), with ripening. In detail, C^* values were not significantly different between treatments in commercial and experimental conditions and most of the changes were related with the ripening stage of the fruit. In contrast, L^* showed faster decrease in fruit treated in sector H and V and in ethylene-treated rather than in control fruit. No relationship was found regarding fruit size. Finally, the H°

value showed a general decrease throughout shelf life with no significant variation between treatments or fruit location (Figure A.2).

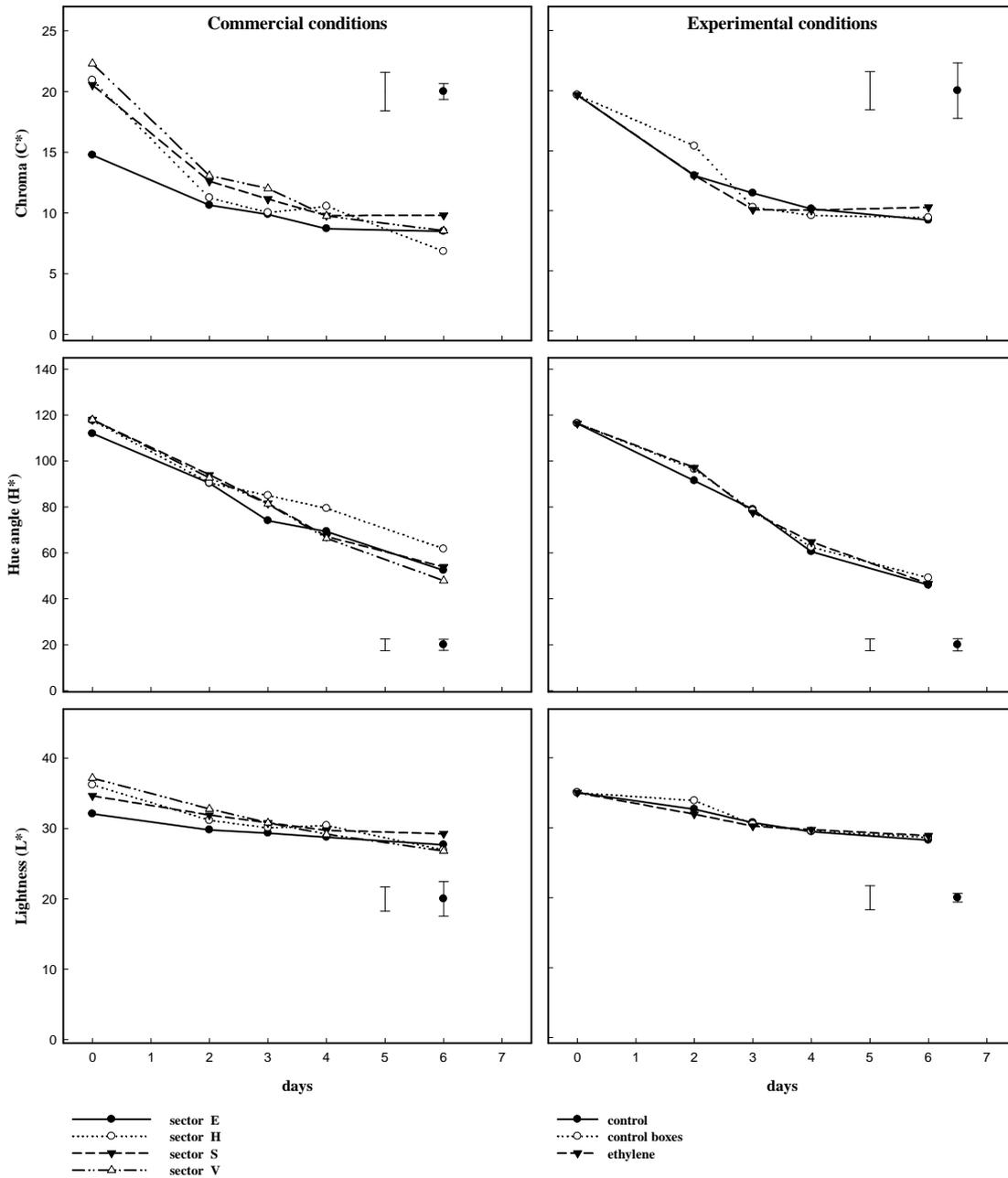


Figure A.2: Changes in colour parameters, chroma (C^*), hue angle (H°) and lightness (L^*) in skin of avocado cv. Hass fruit from South Africa. Measurements were taken during shelf life at 20°C . Fruit were ethylene treated in commercial facilities ((●) E, (○) H, (▼) S, (Δ) V) and then transferred to Cranfield University. From the same batch, fruit

were ethylene treated at Cranfield University (\blacktriangledown), used as control kept in boxes for the length of the treatment (\circ) or simple without treatment (\bullet). Individual values are means of 8 fruit measurements. LSD for each condition (\bullet) and for the interaction commercial vs. experimental conditions are indicated.

A.3.3 Firmness

Fruit firmness was generally lower than expected after cold storage (day 0) and fruit measured less than 150 N (Figure 3). Significant differences were detected mainly between location of fruit within the ripening room, with a softer mesocarp in fruit from sectors E and S compared with sectors H and V. Generally, fruit softened in the first two days of shelf life reaching values below 50 N and all fruit were already fully ripe by day 3 (Figure A.3). The main differences noted in fruit softening were between days of shelf life with no relevant variability between treatments or sectors.

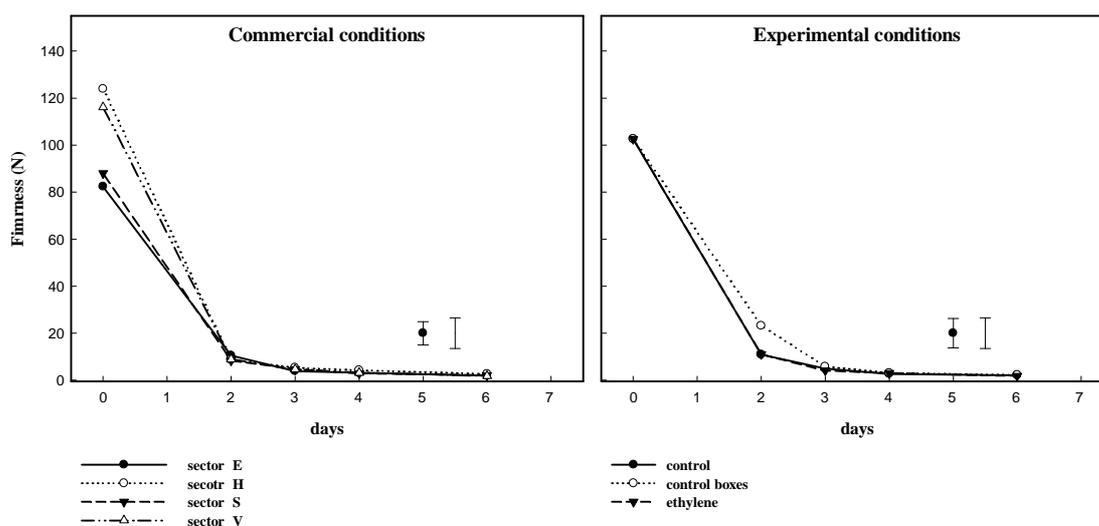


Figure A.3: Firmness of fruit from South Africa along 6 days of shelf life at 20°C. Fruit were ethylene treated before the shelf life by the fruit importer (\bullet E, \circ H, \blacktriangledown S, Δ V) and transferred to Cranfield University. From the same batch some fruit were ethylene treated at Cranfield University (\blacktriangledown), used as control kept in boxes for the length of the treatment (\circ) or simple without treatment (\bullet). Individual values are means of 8 fruit measurements. LSD for each condition (\bullet) and for the interaction commercial vs. experimental conditions are indicated.

A.3.4 Climacteric behaviour and respiration

Ethylene and CO₂ production occurred after two days at 20°C, regardless of location or treatment. The climacteric behaviour differed between days of shelf life and treatments or locations (Figure A.4). In contrast, fruit showed significantly different respiration rate with higher release of CO₂ in fruit treated in commercial conditions rather than in Cranfield facilities. Thus, no significant difference was noted between fruit locations or for the ethylene treatment (Figure A.5).

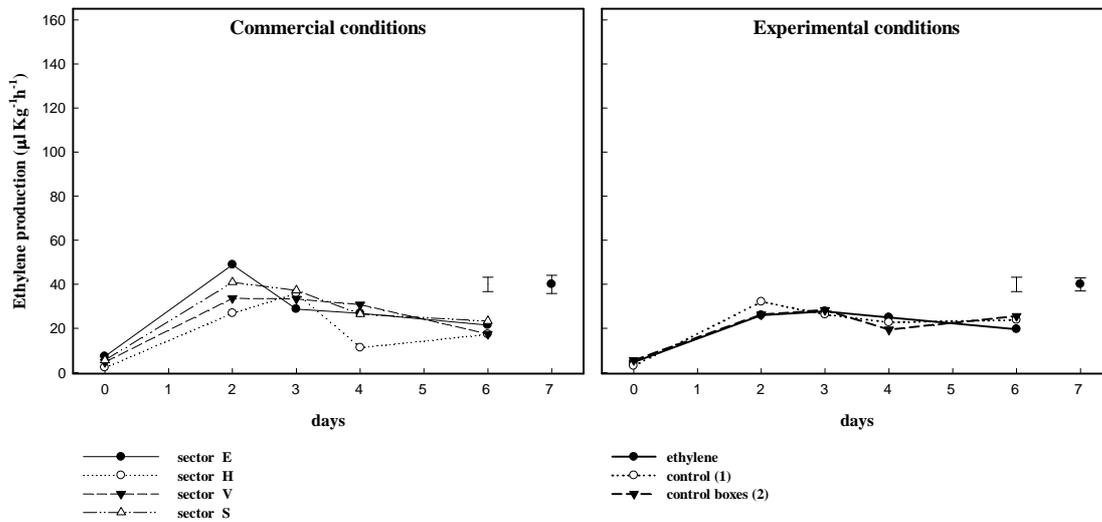


Figure A.4: Climacteric ($\mu\text{l ethylene kg}^{-1}\text{h}^{-1}$) behaviour in avocado cv. Hass fruit from South Africa during shelf life at 20°C. Fruit were ethylene treated in commercial conditions with four different locations inside the room: direct air generator and top (E) or low (H) shelf and away from air generator in top (V) or low shelf (S). At the same time fruit were also ethylene treated (●), non treated (○) and non treated but kept in closed boxes for the length of the treatment (▼) in Cranfield laboratory (experimental conditions). Individual values are means of 8 fruit measurements. LSD for each condition (●) and for the interaction commercial vs. experimental conditions are indicated.

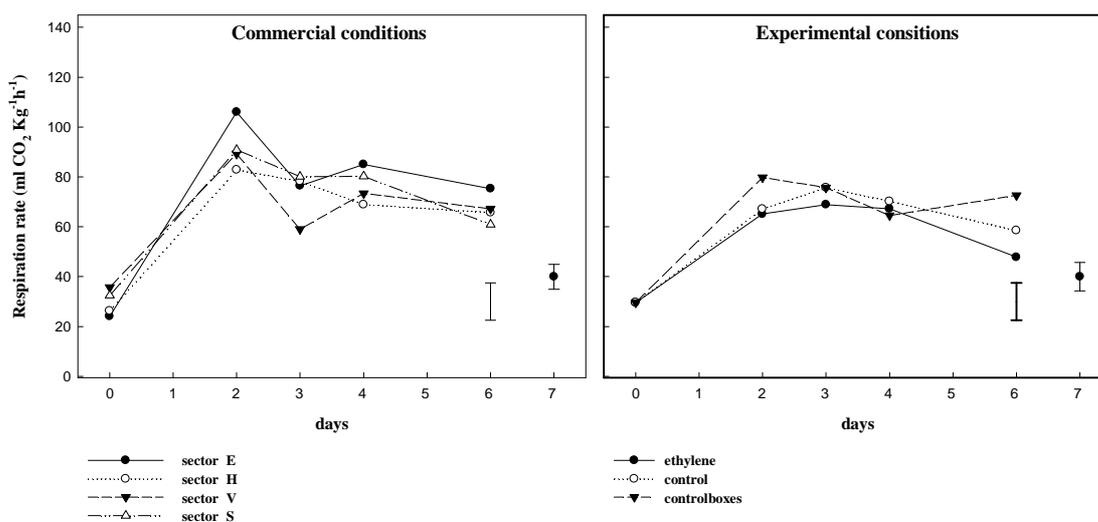


Figure A.5: Respiration rate (ml of CO₂ kg⁻¹h⁻¹) of avocado cv. Hass fruit from South Africa during shelf life at 20°C. Fruit were ethylene treated in commercial conditions in four different locations inside the room: close to ventilation system in top (E) or low (H) shelf and far from air generator in top (V) or low shelf (S). At the same time fruit were also ethylene treated (●), non treated (○) and non treated but kept in closed boxes for the length of the treatment (▼) in Cranfield laboratory (experimental conditions). Individual values are means of 8 fruit measurements. LSD for each condition (●) and for the interaction commercial vs. experimental conditions are indicated.

A.4 Discussion

In the present work, ethylene treatment in experimental conditions did not have a large effect on the ripening process of imported avocado fruit cv. Hass. However, it is important to consider a general faster ripening rate of the fruit after cold storage. As previously suggested in Chapter 6, ethylene application might be less effective on fruit with a faster ripening process, and thus might be the low response to the fruit to the ethylene treatment. Additionally, it will be wise to better control parameters such as air flow and humidity in the commercial conditions.

Differences were noted between fruit regarding their size with fruit from zone E and H significantly smaller than fruit from sector S and V. Different fruit size might have an impact on fruit postharvest metabolism and therefore response to ethylene

treatment (*cf.* Chapter 6). At the end of the shelf life, fruit with the higher water loss were from zone V and H. Fruit kept out of the boxes showed higher water loss as a possible consequence of less transpiration. Differences in the other measurements, i.e. colour and firmness, were mainly associated with fruit location but no specific trend was detected regarding fruit size.

The gas measurements confirmed previous results (*cf.* Chapter 4) with ethylene rising and increased CO₂ production in the second day of shelf life. Generally, levels of ethylene (around 40 µl kg⁻¹h⁻¹) and CO₂ (80-100 ml kg⁻¹h⁻¹) released were comparable with previous experiments (*cf.* Chapter 4 and 6).

In conclusion, this work gives additional support to previous investigations (*cf.* Chapter 6), and the effect of the ethylene treatment might be not evident due to the general faster softening of the fruit. However, the absence of a control (fruit not treated with ethylene) in the commercial conditions prevented the complete assessment of the ethylene treatment. Results suggest that when avocado fruit show a fast softening process the ethylene application is not needed. As previously discussed (*cf.* Chapter 2), a premature ripening could be the consequence of chilling injuries (HersHKovitz *et al.*, 2009). A better management of the avocado postharvest may be achieved with more consideration into preharvest conditions.

Appendix B

B.1 ANOVA TABLE

Table B.4.1: Influence of origin, season, temperature and shelf life on firmness changes of avocado fruit cv. Hass (Chapter 4).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
T	1		120681.	120681.	96.03	<.001
Residual	6		7540.	1257.	1.44	
blocks.T.pairs stratum	8		7004.	876.	0.48	
blocks.T.pairs.*Units* stratum						
days	4		5562770.	1390692.	766.28	<.001
origin	3		387681.	129227.	71.20	<.001
season	2		333668.	166834.	91.93	<.001
T.days	4		95964.	23991.	13.22	<.001
T.origin	3		5305.	1768.	0.97	0.404
days.origin	12		246214.	20518.	11.31	<.001
T.season	2		5255.	2627.	1.45	0.236
days.season	8		137787.	17223.	9.49	<.001
origin.season	6		213699.	35617.	19.62	<.001
T.days.origin	12		22845.	1904.	1.05	0.401
T.days.season	8		22085.	2761.	1.52	0.146
T.origin.season	6		6274.	1046.	0.58	0.750
days.origin.season	24		254501.	10604.	5.84	<.001
T.days.origin.season	24		56990.	2375.	1.31	0.147
Residual	825	(1)	1497265.	1815.		
Total	958	(1)	8968143.			

Table B.4.2-4: Influence of origin, season, temperature and shelf life on colour changes of avocado fruit cv. Hass.

Table B.4.2: Hue angle

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
T	1		44826.8	44826.8	165.66	<.001
Residual	6		1623.6	270.6	1.14	
blocks.T.pairs stratum	8		1890.8	236.4	1.07	
blocks.T.pairs.*Units* stratum						
days	4		667857.5	166964.4	753.31	<.001
origin	3		17896.5	5965.5	26.92	<.001
season	2		24962.1	12481.0	56.31	<.001
T.days	4		34428.0	8607.0	38.83	<.001
T.origin	3		864.4	288.1	1.30	0.273
days.origin	12		17284.4	1440.4	6.50	<.001

T.season	2		503.6	251.8	1.14	0.322
days.season	8		17485.0	2185.6	9.86	<.001
origin.season	6		17737.4	2956.2	13.34	<.001
T.days.origin	12		3881.9	323.5	1.46	0.134
T.days.season	8		3387.1	423.4	1.91	0.055
T.origin.season	6		775.4	129.2	0.58	0.744
days.origin.season	24		19985.2	832.7	3.76	<.001
T.days.origin.season	24		8508.6	354.5	1.60	0.035
Residual	825	(1)	182854.3	221.6		
Total	958	(1)	1066249.0			

Table B.4.3: Chroma

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
T	1		1514.39	1514.39	130.40	<.001
Residual	6		69.68	11.61	2.97	
blocks.T.pairs stratum	8		31.32	3.91	0.28	
blocks.T.pairs.*Units* stratum						
days	4		26278.00	6569.50	470.42	<.001
origin	3		1171.48	390.49	27.96	<.001
season	2		1984.99	992.49	71.07	<.001
T.days	4		787.69	196.92	14.10	<.001
T.origin	3		34.19	11.40	0.82	0.485
days.origin	12		607.15	50.60	3.62	<.001
T.season	2		8.58	4.29	0.31	0.736
days.season	8		500.47	62.56	4.48	<.001
origin.season	6		1224.34	204.06	14.61	<.001
T.days.origin	12		235.79	19.65	1.41	0.157
T.days.season	8		314.43	39.30	2.81	0.004
T.origin.season	6		30.92	5.15	0.37	0.899
days.origin.season	24		901.71	37.57	2.69	<.001
T.days.origin.season	24		309.85	12.91	0.92	0.568
Residual	825	(1)	11521.17	13.97		
Total	958	(1)	47490.95			

Table B.4.4: Lightness

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
T	1		628.248	628.248	137.72	<.001
Residual	6		27.370	4.562	1.90	
blocks.T.pairs stratum	8		19.248	2.406	0.43	
blocks.T.pairs.*Units* stratum						
days	4		7812.726	1953.182	352.04	<.001
origin	3		1847.683	615.894	111.01	<.001
season	2		249.891	124.946	22.52	<.001
T.days	4		460.638	115.159	20.76	<.001

T.origin	3		14.716	4.905	0.88	0.449
days.origin	12		426.706	35.559	6.41	<.001
T.season	2		1.465	0.732	0.13	0.876
days.season	8		130.590	16.324	2.94	0.003
origin.season	6		212.400	35.400	6.38	<.001
T.days.origin	12		119.590	9.966	1.80	0.045
T.days.season	8		114.616	14.327	2.58	0.009
T.origin.season	6		10.762	1.794	0.32	0.925
days.origin.season	24		231.316	9.638	1.74	0.016
T.days.origin.season	24		150.804	6.284	1.13	0.300
Residual	825	(1)	4577.247	5.548		
Total	958	(1)	17028.000			

Table B.4.5: Influence of origin, season, temperature and shelf life on weight changes of avocado fruit cv. Hass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
blocks stratum					
T	1	1233.1	1233.1	9.89	0.020
Residual	6	748.4	124.7	1.07	
blocks.T.pairs stratum	8	936.8	117.1	0.75	
blocks.T.pairs.*Units* stratum					
days	4	64225.4	16056.3	102.26	<.001
origin	3	11456.8	3818.9	24.32	<.001
season	2	3293.0	1646.5	10.49	<.001
T.days	4	1353.2	338.3	2.15	0.072
T.origin	3	999.0	333.0	2.12	0.096
days.origin	12	3856.4	321.4	2.05	0.018
T.season	2	39.6	19.8	0.13	0.882
days.season	8	1918.4	239.8	1.53	0.144
origin.season	6	42057.0	7009.5	44.64	<.001
T.days.origin	12	2957.0	246.4	1.57	0.095
T.days.season	8	369.2	46.2	0.29	0.968
T.origin.season	6	172.5	28.7	0.18	0.982
days.origin.season	24	3930.2	163.8	1.04	0.407
T.days.origin.season	24	4505.2	187.7	1.20	0.236
Residual	826	129698.9	157.0		
Total	959	273750.0			

Table B.4.6-7: Influence of origin, season, temperature and shelf life on ethylene production and respiration (CO₂) of avocado fruit cv. Hass.

Table B.4.6: Ethylene

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
jars stratum					
tempe	1	1614.0	1614.0	10.17	0.013
Residual	8	1269.4	158.7	0.66	

jars.*Units* stratum					
days	4	60557.5	15139.4	63.40	<.001
origin	3	47079.3	15693.1	65.72	<.001
season	2	238.4	119.2	0.50	0.607
tempe.days	4	31474.4	7868.6	32.95	<.001
tempe.origin	3	810.4	270.1	1.13	0.336
days.origin	12	80550.1	6712.5	28.11	<.001
tempe.season	2	179.5	89.8	0.38	0.687
days.season	8	19510.9	2438.9	10.21	<.001
origin.season	6	23660.8	3943.5	16.51	<.001
tempe.days.origin	12	25971.5	2164.3	9.06	<.001
tempe.days.season	8	3387.2	423.4	1.77	0.080
tempe.origin.season	6	1559.8	260.0	1.09	0.368
days.origin.season	24	30650.0	1277.1	5.35	<.001
tempe.days.origin.season	24	24427.4	1017.8	4.26	<.001
Residual	472	112714.2	238.8		
Total	599	465654.9			

Table B.4.7: CO₂

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
jars stratum						
tempe	1		20194.9	20194.9	88.44	<.001
Residual	8		1826.7	228.3	1.84	
jars.*Units* stratum						
days	4		269819.6	67454.9	545.03	<.001
origin	3		82343.2	27447.7	221.77	<.001
season	2		8224.0	4112.0	33.22	<.001
tempe.days	4		18118.3	4529.6	36.60	<.001
tempe.origin	3		4313.3	1437.8	11.62	<.001
days.origin	12		25096.7	2091.4	16.90	<.001
tempe.season	2		74.6	37.3	0.30	0.740
days.season	8		10573.7	1321.7	10.68	<.001
origin.season	5	(1)	4666.3	933.3	7.54	<.001
tempe.days.origin	12		8997.0	749.8	6.06	<.001
tempe.days.season	8		1942.8	242.8	1.96	0.050
tempe.origin.season	5	(1)	1547.1	309.4	2.50	0.030
days.origin.season	20	(4)	16028.2	801.4	6.48	<.001
tempe.days.origin.season	20	(4)	4677.9	233.9	1.89	0.012
Residual	432	(40)	53466.3	123.8		
Total	549	(50)	494751.1			

Table B.4.8-12: Influence of origin, season, temperature and shelf life on oil profile of avocado fruit cv. Hass

Table B.4.8: Linoleate

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum						
T	1		1.022	1.022	2.99	0.135
Residual	6		2.051	0.342	0.36	
block.T.pairs stratum	8		7.667	0.958	0.51	
block.T.pairs.*Units* stratum						
days	2		63.818	31.909	16.83	<.001
origin	3		1152.737	384.246	202.65	<.001
season	2		23.193	11.597	6.12	0.002
T.days	2		39.564	19.782	10.43	<.001
T.origin	3		22.260	7.420	3.91	0.009
days.origin	6		63.458	10.576	5.58	<.001
T.season	2		9.714	4.857	2.56	0.078
days.season	4		40.907	10.227	5.39	<.001
origin.season	6		114.533	19.089	10.07	<.001
T.days.origin	6		41.843	6.974	3.68	0.001
T.days.season	4		23.140	5.785	3.05	0.017
T.origin.season	6		24.428	4.071	2.15	0.047
days.origin.season	12		380.355	31.696	16.72	<.001
T.days.origin.season	12		64.208	5.351	2.82	<.001
Residual	487	(3)	923.384	1.896		
Total	572	(3)	2996.323			

Table B.4.9: Linolenate

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum						
T	1		0.412	0.412	0.11	0.751
Residual	6		22.404	3.734	2.89	
block.T.pairs stratum	8		10.349	1.294	0.53	
block.T.pairs.*Units* stratum						
days	2		55.966	27.983	11.37	<.001
origin	3		2173.408	724.469	294.46	<.001
season	2		2184.576	1092.288	443.96	<.001
T.days	2		6.975	3.488	1.42	0.243
T.origin	3		8.031	2.677	1.09	0.354
days.origin	6		114.364	19.061	7.75	<.001
T.season	2		11.739	5.869	2.39	0.093
days.season	4		26.215	6.554	2.66	0.032
origin.season	6		1937.375	322.896	131.24	<.001
T.days.origin	6		4.931	0.822	0.33	0.919
T.days.season	4		20.849	5.212	2.12	0.077
T.origin.season	6		14.281	2.380	0.97	0.447

days.origin.season	12		113.420	9.452	3.84	<.001
T.days.origin.season	12		58.526	4.877	1.98	0.024
Residual	487	(3)	1198.188	2.460		
Total	572	(3)	7949.569			

Table B.4.10: Oleate

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum						
T	1		3.492	3.492	0.31	0.596
Residual	6		66.878	11.146	1.46	
block.T.pairs stratum	8		61.170	7.646	1.04	
block.T.pairs.*Units* stratum						
days	2		55.344	27.672	3.76	0.024
origin	3		21491.398	7163.799	974.60	<.001
season	2		640.495	320.248	43.57	<.001
T.days	2		30.183	15.092	2.05	0.129
T.origin	3		86.862	28.954	3.94	0.009
days.origin	6		516.213	86.035	11.70	<.001
T.season	2		12.532	6.266	0.85	0.427
days.season	4		22.494	5.624	0.77	0.548
origin.season	6		2987.517	497.920	67.74	<.001
T.days.origin	6		37.730	6.288	0.86	0.528
T.days.season	4		30.208	7.552	1.03	0.393
T.origin.season	6		44.207	7.368	1.00	0.423
days.origin.season	12		435.806	36.317	4.94	<.001
T.days.origin.season	12		93.157	7.763	1.06	0.396
Residual	487	(3)	3579.711	7.351		
Total	572	(3)	30057.119			

Table B.4.11: Palmitate

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum						
T	1		4.575	4.575	1.06	0.342
Residual	6		25.815	4.303	1.17	
block.T.pairs stratum	8		29.354	3.669	1.31	
block.T.pairs.*Units* stratum						
days	2		38.166	19.083	6.79	0.001
origin	3		7190.320	2396.773	853.36	<.001
season	2		1156.274	578.137	205.84	<.001
T.days	2		5.317	2.659	0.95	0.389
T.origin	3		33.919	11.306	4.03	0.008
days.origin	6		99.766	16.628	5.92	<.001
T.season	2		30.538	15.269	5.44	0.005
days.season	4		29.180	7.295	2.60	0.036
origin.season	6		347.642	57.940	20.63	<.001
T.days.origin	6		51.544	8.591	3.06	0.006

T.days.season	4		51.413	12.853	4.58	0.001
T.origin.season	6		16.921	2.820	1.00	0.422
days.origin.season	12		98.804	8.234	2.93	<.001
T.days.origin.season	12		106.479	8.873	3.16	<.001
Residual	487	(3)	1367.801	2.809		
Total	572	(3)	10592.204			

Table B.4.12: Palmitoleate

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
block stratum						
T	1		0.232	0.232	0.04	0.843
Residual	6		32.520	5.420	1.73	
block.T.pairs stratum	8		25.044	3.131	0.86	
block.T.pairs.*Units* stratum						
days	2		71.205	35.602	9.80	<.001
origin	3		3399.320	1133.107	311.85	<.001
season	2		181.947	90.973	25.04	<.001
T.days	2		5.335	2.667	0.73	0.480
T.origin	3		48.826	16.275	4.48	0.004
days.origin	6		198.882	33.147	9.12	<.001
T.season	2		7.061	3.530	0.97	0.379
days.season	4		31.902	7.975	2.19	0.069
origin.season	6		79.339	13.223	3.64	0.002
T.days.origin	6		31.603	5.267	1.45	0.194
T.days.season	4		20.185	5.046	1.39	0.237
T.origin.season	6		16.470	2.745	0.76	0.605
days.origin.season	12		108.806	9.067	2.50	0.003
T.days.origin.season	12		44.173	3.681	1.01	0.435
Residual	487	(3)	1769.500	3.633		
Total	572	(3)	6054.220			

Table B.4.13-17: Influence of origin, season, temperature and shelf life on sugars profile of avocado fruit cv. Hass**Table B.4.13: Mannoheptulose**

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
t	1		2844.6	2844.6	10.17	0.019
Residual	6		1678.3	279.7	1.88	
blocks.t.pairs stratum	8		1187.9	148.5	0.54	
blocks.t.pairs.*Units* stratum						
days	2		116630.4	58315.2	212.26	<.001
origin	3		36155.9	12052.0	43.87	<.001
seasons	2		26832.3	13416.2	48.83	<.001
t.days	2		3507.0	1753.5	6.38	0.002
t.origin	3		518.1	172.7	0.63	0.597

days.origin	6		11472.0	1912.0	6.96	<.001
t.seasons	2		2669.5	1334.7	4.86	0.008
days.seasons	4		13243.5	3310.9	12.05	<.001
origin.seasons	6		29584.0	4930.7	17.95	<.001
t.days.origin	6		1192.6	198.8	0.72	0.631
t.days.seasons	4		1796.0	449.0	1.63	0.164
t.origin.seasons	6		1948.6	324.8	1.18	0.314
days.origin.seasons	12		11374.3	947.9	3.45	<.001
t.days.origin.seasons	12		2877.8	239.8	0.87	0.575
Residual	487	(3)	133794.2	274.7		
Total	572	(3)	398562.6			

Table B.4.14: Fructose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
t	1		17.716	17.716	7.16	0.037
Residual	6		14.836	2.473	0.68	
blocks.t.pairs stratum	8		29.256	3.657	1.24	
blocks.t.pairs.*Units* stratum						
days	2		57.326	28.663	9.73	<.001
origin	3		1667.262	555.754	188.63	<.001
seasons	2		189.593	94.797	32.18	<.001
t.days	2		53.309	26.654	9.05	<.001
t.origin	3		132.220	44.073	14.96	<.001
days.origin	6		100.434	16.739	5.68	<.001
t.seasons	2		49.962	24.981	8.48	<.001
days.seasons	4		212.328	53.082	18.02	<.001
origin.seasons	6		464.283	77.381	26.26	<.001
t.days.origin	6		54.525	9.088	3.08	0.006
t.days.seasons	4		51.902	12.975	4.40	0.002
t.origin.seasons	6		199.370	33.228	11.28	<.001
days.origin.seasons	12		511.903	42.659	14.48	<.001
t.days.origin.seasons	12		130.618	10.885	3.69	<.001
Residual	487	(3)	1434.824	2.946		
Total	572	(3)	5360.994			

Table B.4.15: Glucose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
t	1		1.46	1.46	0.05	0.826
Residual	6		164.75	27.46	2.27	
blocks.t.pairs stratum	8		96.66	12.08	0.87	
blocks.t.pairs.*Units* stratum						
days	2		274.50	137.25	9.83	<.001
origin	3		2024.37	674.79	48.31	<.001
seasons	2		905.65	452.82	32.42	<.001

t.days	2		2.12	1.06	0.08	0.927
t.origin	3		507.49	169.16	12.11	<.001
days.origin	6		352.73	58.79	4.21	<.001
t.seasons	2		210.53	105.27	7.54	<.001
days.seasons	4		94.15	23.54	1.69	0.152
origin.seasons	6		3623.61	603.94	43.24	<.001
t.days.origin	6		98.71	16.45	1.18	0.317
t.days.seasons	4		72.73	18.18	1.30	0.268
t.origin.seasons	6		814.15	135.69	9.71	<.001
days.origin.seasons	12		363.08	30.26	2.17	0.012
t.days.origin.seasons	12		173.14	14.43	1.03	0.417
Residual	487	(3)	6802.42	13.97		
Total	572	(3)	16555.21			

Table B.4.16: Perseitol

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
t	1		969.5	969.5	13.29	0.011
Residual	6		437.7	72.9	0.50	
blocks.t.pairs stratum	8		1156.6	144.6	1.14	
blocks.t.pairs.*Units* stratum						
days	2		21444.5	10722.3	84.43	<.001
origin	3		9289.8	3096.6	24.38	<.001
seasons	2		722.4	361.2	2.84	0.059
t.days	2		2373.7	1186.9	9.35	<.001
t.origin	3		1068.4	356.1	2.80	0.039
days.origin	6		7133.0	1188.8	9.36	<.001
t.seasons	2		187.4	93.7	0.74	0.479
days.seasons	4		1434.6	358.7	2.82	0.024
origin.seasons	6		12067.7	2011.3	15.84	<.001
t.days.origin	6		604.4	100.7	0.79	0.576
t.days.seasons	4		1211.4	302.8	2.38	0.050
t.origin.seasons	6		815.5	135.9	1.07	0.379
days.origin.seasons	12		3075.0	256.3	2.02	0.021
t.days.origin.seasons	12		3364.7	280.4	2.21	0.011
Residual	487	(3)	61844.1	127.0		
Total	572	(3)	129117.4			

Table B.4.17: Sucrose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
t	1		774.04	774.04	24.29	0.003
Residual	6		191.21	31.87	0.77	
blocks.t.pairs stratum	8		332.90	41.61	1.12	
blocks.t.pairs.*Units* stratum						
days	2		148.88	74.44	2.01	0.136

origin	3		354.37	118.12	3.18	0.024
seasons	2		1108.48	554.24	14.93	<.001
t.days	2		762.85	381.43	10.28	<.001
t.origin	3		531.51	177.17	4.77	0.003
days.origin	6		5572.20	928.70	25.02	<.001
t.seasons	2		183.23	91.62	2.47	0.086
days.seasons	4		1912.29	478.07	12.88	<.001
origin.seasons	6		12160.22	2026.70	54.61	<.001
t.days.origin	6		487.68	81.28	2.19	0.043
t.days.seasons	4		836.01	209.00	5.63	<.001
t.origin.seasons	6		396.14	66.02	1.78	0.102
days.origin.seasons	12		8459.67	704.97	18.99	<.001
t.days.origin.seasons	12		1090.10	90.84	2.45	0.004
Residual	487	(3)	18074.76	37.11		
Total	572	(3)	53065.62			

Table B.5.1-3: Influence of season, temperature and shelf life on colour changes of avocado fruit cv. Hass from Spain (Chapter 5)

Table B.5.1: Chroma

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum	3		3.06	1.02	0.10	
blocks.temp stratum						
temp	1		316.56	316.56	30.07	0.012
Residual	3		31.58	10.53	1.56	
blocks.temp.pairs stratum	8		53.93	6.74	0.49	
blocks.temp.pairs.*Units* stratum						
days	4		8888.27	2222.07	160.20	<.001
season	2		175.28	87.64	6.32	0.002
temp.days	4		218.83	54.71	3.94	0.004
temp.season	2		8.47	4.23	0.31	0.737
days.season	8		534.48	66.81	4.82	<.001
temp.days.season	8		189.82	23.73	1.71	0.098
Residual	195	(1)	2704.78	13.87		
Total	238	(1)	13108.33			

Table B.5.2: Hue angle

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum	3		505.4	168.5	0.78	
blocks.temp stratum						
temp	1		13444.2	13444.2	61.87	0.004
Residual	3		651.9	217.3	0.76	
blocks.temp.pairs stratum	8		2293.2	286.7	2.08	
blocks.temp.pairs.*Units* stratum						
days	4		246266.1	61566.5	447.12	<.001
season	2		2923.3	1461.7	10.62	<.001

temp.days	4		10251.5	2562.9	18.61	<.001
temp.season	2		258.0	129.0	0.94	0.394
days.season	8		7177.5	897.2	6.52	<.001
temp.days.season	8		1253.9	156.7	1.14	0.339
Residual	195	(1)	26850.6	137.7		
Total	238	(1)	311661.3			

Table B.5.3: Lightness

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum	3		7.318	2.439	3.46	
blocks.temp stratum						
temp	1		101.634	101.634	144.30	0.001
Residual	3		2.113	0.704	0.18	
blocks.temp.pairs stratum	8		31.490	3.936	0.76	
blocks.temp.pairs.*Units* stratum						
days	4		3017.033	754.258	145.04	<.001
season	2		31.474	15.737	3.03	0.051
temp.days	4		77.763	19.441	3.74	0.006
temp.season	2		1.674	0.837	0.16	0.851
days.season	8		69.245	8.656	1.66	0.109
temp.days.season	8		74.528	9.316	1.79	0.081
Residual	195	(1)	1014.046	5.200		
Total	238	(1)	4425.688			

Table B.5.4: Influence of season, temperature and shelf life on firmness of avocado fruit cv. Hass from Spain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
blocks stratum	3	3690.	1230.	1.27	
blocks.temp stratum					
temp	1	17096.	17096.	17.71	0.024
Residual	3	2896.	965.	1.38	
blocks.temp.pairs stratum	8	5594.	699.	0.61	
blocks.temp.pairs.*Units* stratum					
days	4	1726506.	431627.	373.52	<.001
season	2	116982.	58491.	50.62	<.001
temp.days	4	20088.	5022.	4.35	0.002
temp.season	2	1026.	513.	0.44	0.642
days.season	8	122970.	15371.	13.30	<.001
temp.days.season	8	7789.	974.	0.84	0.566
Residual	196	226493.	1156.		
Total	239	2251131.			

Table B.5.5: Influence of season, temperature and shelf life on ethylene production of avocado fruit cv. Hass from Spain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
JARS stratum					
TEM	1	1586.2	1586.2	5.72	0.044
Residual	8	2216.7	277.1	0.83	
JARS.*Units* stratum					
Days	4	104164.4	26041.1	77.63	<.001
Season	2	19157.4	9578.7	28.55	<.001
Tem.Days	4	47174.4	11793.6	35.16	<.001
Tem.Season	2	660.8	330.4	0.98	0.377
Days.Season	8	14497.3	1812.2	5.40	<.001
Tem.Days.Season	8	18040.8	2255.1	6.72	<.001
Residual	112	37571.3	335.5		
Total	149	245069.2			

Table B.5.6-9: Influence of season, temperature and shelf life on pigment content of avocado fruit cv. Hass from Spain

Table B.5.6: Carotenoids

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
temperature	1		527.	527.	0.58	0.476
Residual	6		5475.	913.	1.76	
blocks.pairs stratum	8		4159.	520.	0.47	
blocks.pairs.*Units* stratum						
day	4		33634.	8408.	7.66	<.001
season	2		11043.	5522.	5.03	0.007
day.season	8		26480.	3310.	3.02	0.003
day.temperature	4		10866.	2717.	2.48	0.046
season.temperature	2		790.	395.	0.36	0.698
day.season.temperature	8		7467.	933.	0.85	0.559
Residual	194	(2)	212896.	1097.		
Total	237	(2)	310727.			

Table B.7: Chlorophyll *a*

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
temperature	1		108477.	108477.	14.81	0.008
Residual	6		43959.	7326.	0.94	
blocks.pairs stratum	8		62042.	7755.	0.69	
blocks.pairs.*Units* stratum						
day	4		614388.	153597.	13.71	<.001
season	2		108268.	54134.	4.83	0.009
day.season	8		147293.	18412.	1.64	0.115
day.temperature	4		16804.	4201.	0.38	0.826

season.temperature	2		4852.	2426.	0.22	0.805
day.season.temperature	8		74798.	9350.	0.83	0.573
Residual	194	(2)	2173284.	11202.		
Total	237	(2)	3301181.			

Table B.8: Chlorophyll *b*

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
temperature	1		2346.	2346.	1.35	0.289
Residual	6		10431.	1739.	0.72	
blocks.pairs stratum	8		19320.	2415.	0.85	
blocks.pairs.*Units* stratum						
day	4		50830.	12707.	4.45	0.002
season	2		32749.	16375.	5.73	0.004
day.season	8		32242.	4030.	1.41	0.194
day.temperature	4		31488.	7872.	2.76	0.029
season.temperature	2		2485.	1243.	0.44	0.648
day.season.temperature	8		26113.	3264.	1.14	0.336
Residual	194	(2)	553932.	2855.		
Total	237	(2)	761444.			

Table B.5.9: Total chlorophylls

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
blocks stratum						
temperature	1		78916.	78916.	5.00	0.067
Residual	6		94683.	15781.	0.93	
blocks.pairs stratum	8		136243.	17030.	0.72	
blocks.pairs.*Units* stratum						
day	4		537266.	134316.	5.64	<.001
season	2		259355.	129677.	5.45	0.005
day.season	8		298032.	37254.	1.56	0.138
day.temperature	4		83875.	20969.	0.88	0.476
season.temperature	2		13535.	6767.	0.28	0.753
day.season.temperature	8		174528.	21816.	0.92	0.504
Residual	194	(2)	4618593.	23807.		
Total	237	(2)	6246372.			

Table B.5.10-13: Influence of season, temperature and shelf life on phenolic profile of avocado fruit cv. Hass from Spain**Table B.5.10: Chlorogenic acid**

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
pairs stratum	1		0.245	0.245	3.34	
pairs.t stratum						
t	1		5.287	5.287	71.95	0.075
Residual	1		0.073	0.073	0.07	

pairs.t.*Units* stratum						
days	4		43.981	10.995	10.93	<.001
season	2		16.784	8.392	8.35	<.001
t.days	4		6.284	1.571	1.56	0.186
t.season	2		0.660	0.330	0.33	0.720
days.season	8		137.123	17.140	17.05	<.001
t.days.season	8		15.771	1.971	1.96	0.053
Residual	204	(4)	205.129	1.006		
Total	235	(4)	429.472			

Table B.5.11: Epicatechin

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
pairs stratum	1		3.59	3.59	0.15	
pairs.t stratum						
t	1		146.30	146.30	6.11	0.245
Residual	1		23.95	23.95	1.26	
pairs.t.*Units* stratum						
days	4		672.86	168.21	8.87	<.001
season	2		1878.20	939.10	49.50	<.001
t.days	4		278.44	69.61	3.67	0.007
t.season	2		52.68	26.34	1.39	0.252
days.season	8		984.20	123.02	6.48	<.001
t.days.season	8		560.61	70.08	3.69	<.001
Residual	204	(4)	3870.48	18.97		
Total	235	(4)	8461.53			

Table B.5.12: Procyanidin B2

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
pairs stratum	1		0.345	0.345	0.04	
pairs.t stratum						
t	1		25.560	25.560	3.04	0.332
Residual	1		8.408	8.408	1.30	
pairs.t.*Units* stratum						
days	4		309.859	77.465	11.95	<.001
season	2		16.971	8.486	1.31	0.272
t.days	4		108.545	27.136	4.19	0.003
t.season	2		20.478	10.239	1.58	0.209
days.season	8		613.982	76.748	11.84	<.001
t.days.season	8		184.797	23.100	3.56	<.001
Residual	204	(4)	1322.529	6.483		
Total	235	(4)	2599.562			

Table B.5.13: Cyanidin-3-O-glucoside

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
pairs stratum	1		255797.	255797.	7.27	
pairs.t stratum						
t	1		5954607.	5954607.	169.34	0.049
Residual	1		35164.	35164.	0.64	
pairs.t.*Units* stratum						
days	4		36213125.	9053281.	163.94	<.001
season	2		310135.	155067.	2.81	0.063
t.days	4		8075138.	2018785.	36.56	<.001
t.season	2		568904.	284452.	5.15	0.007
days.season	8		3054673.	381834.	6.91	<.001
t.days.season	8		1110459.	138807.	2.51	0.013
Residual	205	(3)	11320794.	55223.		
Total	236	(3)	61741031			

Table B.5.14-16: Regression index in early season fruit cv. Hass from Spain. Nonlinear Regression.**Equation: Exponential Growth, 2 Parameter ($f=a*b^x$)****Table B.5.14: Cyanidin*firness**

	R	Rsqr	Adj Rsqr	Standard Error of Estimate	
	0.7790	0.6069	0.6017	277.6934	
Coefficient	Std. Error	t	P	VIF	
a	1212.2450	195.7457	6.1930	<0.0001	5.1504<
b	0.8416	0.0426	19.7711	<0.0001	5.1504<

Table B.5.15: Cyanidin-3-O-glucoside*hue angle

	R	Rsqr	Adj Rsqr	Standard Error of Estimate	
	0.8607	0.7408	0.7374	225.4921	
Coefficient	Std. Error	t	P	VIF	
a	2544.1098	350.8333	7.2516	<0.0001	6.4564<
b	0.9724	0.0032	308.5489	<0.0001	6.4564<

Table B.5.16: Firmness*hue angle

	R	Rsqr	Adj Rsqr	Standard Error of Estimate		
	0.849	0.7217	0.7181	55.5831		
Coefficient	Std. Error	t	P	VIF		
A	3.3471E-006	9.7394E-006	0.3437	0.7320	3661.5669<	
b	1.1551	0.0272	4	2.5345	<0.0001	3661.5669<

Table B.5.17-19: Regression index in middle season fruit cv. Hass from Spain (Chapter 5). Nonlinear Regression. Equation: Exponential Growth, 2 Parameter ($f=a*b^x$)

Table B.5.17: Cyanidin-3-O-glucoside*firmness

	R	Rsqr	Adj Rsqr	Standard Error of Estimate	
	0.8563	0.7332	0.7297	313.7795	
Coefficient	Std. Error	t	P	VIF	
a	6386.8827	2069.4659	3.0862	0.0028	32.7535<
b	0.4303	0.0696	6.1791	<0.0001	32.7535<

Table B.5.18: Firmness*hue angle

	R	Rsqr	Adj Rsqr	Standard Error of Estimate	
	0.8777	0.7703	0.7673	49.3986	
Coefficient	Std. Error	t	P	VIF	
a	0.0020	0.0044	0.4458	0.6570	2748.5976<
b	1.0976	0.0200	54.8839	<0.0001	2748.5976<

Table B.5.19: Cyanidin-3-O-glucoside*hue angle

	R	Rsqr	Adj Rsqr	Standard Error of Estimate	
	0.9655	0.9322	0.9313	158.1690	
Coefficient	Std. Error	t	P	VIF	
a	5899.3091	520.6675	11.3303	<0.0001	11.6236<
b	0.9503	0.0030	314.1227	<0.0001	11.6236<

Table B.5.20-22: Regression index in late season fruit cv. Hass from Spain (Chapter 5). Nonlinear Regression. Equation: Exponential Growth, 2 Parameter ($f=a*b^x$)

Table B.5.20: Cyanidin-3-O-glucoside*firmness

	R	Rsqr	Adj Rsqr	Standard Error of Estimate	
	0.7881	0.6211	0.6162	303.0012	
Coefficient	Std. Error	t	P	VIF	
a	2286.0928	546.3257	4.1845	<0.0001	12.7988<
b	0.6271	0.065	49.5873	<0.0001	12.7988<

Table B.5.21: Cyanidin-3-O-glucoside*hue angle

	R	Rsqr	Adj Rsqr	Standard Error of Estimate	
	0.8974	0.8054	0.8028	217.1607	
Coefficient	Std. Error	t	P	VIF	
a	3560.0337	408.0969	8.7235	<0.0001	6.5400<
b	0.9667	0.00283	48.0996	<0.0001	6.5400<

Table B.5.22: Firmness*hue angle

	R		Rsqr	Adj Rsqr	Standard Error of Estimate	
	0.8756	0.7667	0.7637		36.3895	
Coefficient	Std. Error	t		P	VIF	
A	6.6100E-009	2.3808E-008		0.2776	0.7820	5051.8215<
b	1.2151	0.0357		34.0219	<0.0001	5051.8215<

Chapter 6**Table B.6.1-2: Influence of ethylene treatment, temperature (T1, T2) and shelf life on respiration and ethylene production of avocado fruit cv. Hass from South Africa (Exp. I)****Table B.6.1: CO₂**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
rep stratum				2	1163.0	581.5	2.88
rep.*Units* stratum							
Baseline_1				1	3871.6	3871.6	19.17 <.001
Baseline_1.treat				1	9809.2	9809.2	48.58 <.001
Baseline_1.T1				2	59663.9	29832.0	147.74 <.001
Baseline_1.Baseline_2				1	0.2	0.2	0.00 0.977
Baseline_1.treat.T1				2	1953.5	976.7	4.84 0.009
Baseline_1.treat.Baseline_2				1	128.9	128.9	0.64 0.425
Baseline_1.T1.Baseline_2				2	4362.6	2181.3	10.80 <.001
Baseline_1.Baseline_2.T2				2	85.4	42.7	0.21 0.810
Baseline_1.Baseline_2.days				2	4687.9	2343.9	11.61 <.001
Baseline_1.treat.T1.Baseline_2				2	1547.0	773.5	3.83 0.024
Baseline_1.treat.Baseline_2.T2				2	260.6	130.3	0.65 0.526
Baseline_1.T1.Baseline_2.T2				4	230.5	57.6	0.29 0.887
Baseline_1.treat.Baseline_2.days				2	1581.0	790.5	3.92 0.022
Baseline_1.T1.Baseline_2.days				4	3139.0	784.7	3.89 0.005
Baseline_1.Baseline_2.T2.days				4	843.2	210.8	1.04 0.386
Baseline_1.treat.T1.Baseline_2.T2				4	1247.4	311.9	1.54 0.192
Baseline_1.treat.T1.Baseline_2.days				4	2769.7	692.4	3.43 0.010
Baseline_1.treat.Baseline_2.T2.days				4	444.4	111.1	0.55 0.699
Baseline_1.T1.Baseline_2.T2.days				8	1074.5	134.3	0.67 0.722
Baseline_1.treat.T1.Baseline_2.T2.days				8	1436.6	179.6	0.89 0.527
Residual				159	32105.1	201.9	
Total				221	132405.4		

Table B.6.2: Ethylene

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	584.8	292.4	0.50	
rep.*Units* stratum					
Baseline_1	1	1218.9	1218.9	2.06	0.153
Baseline_1.treat	1	748.3	748.3	1.27	0.262
Baseline_1.T1	2	3630.6	1815.3	3.07	0.049
Baseline_1.Baseline_2	1	8072.0	8072.0	13.67	<.001
Baseline_1.treat.T1	2	711.5	355.7	0.60	0.549
Baseline_1.treat.Baseline_2	1	0.9	0.9	0.00	0.969
Baseline_1.T1.Baseline_2	2	1569.1	784.5	1.33	0.268
Baseline_1.Baseline_2.T2	2	2277.4	1138.7	1.93	0.149
Baseline_1.Baseline_2.days	2	4844.9	2422.5	4.10	0.018
Baseline_1.treat.T1.Baseline_2	2	32.5	16.3	0.03	0.973
Baseline_1.treat.Baseline_2.T2	2	2677.8	1338.9	2.27	0.107
Baseline_1.T1.Baseline_2.T2	4	7338.6	1834.7	3.11	0.017
Baseline_1.treat.Baseline_2.days					
	2	3025.2	1512.6	2.56	0.080
Baseline_1.T1.Baseline_2.days	4	8121.7	2030.4	3.44	0.010
Baseline_1.Baseline_2.T2.days	4	5889.7	1472.4	2.49	0.045
Baseline_1.treat.T1.Baseline_2.T2					
	4	459.3	114.8	0.19	0.941
Baseline_1.treat.T1.Baseline_2.days					
	4	4713.5	1178.4	2.00	0.098
Baseline_1.treat.Baseline_2.T2.days					
	4	2651.9	663.0	1.12	0.348
Baseline_1.T1.Baseline_2.T2.days					
	8	3309.4	413.7	0.70	0.691
Baseline_1.treat.T1.Baseline_2.T2.days					
	8	13499.2	1687.4	2.86	0.005
Residual	159	93881.8	590.5		
Total	221	169259.0			

Table B.6.3-5: Influence of ethylene treatment, temperature (T1, T2) and shelf life on colour changes of avocado fruit cv. Hass from South Africa (Exp. I)**Table B.6.3: Chroma**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	204.60	102.30	4.15	
rep.*Units* stratum					
Baseline_1	1	0.64	0.64	0.03	0.872
Baseline_1.treat	1	76.08	76.08	3.09	0.081
Baseline_1.T1	2	439.33	219.66	8.91	<.001
Baseline_1.Baseline_2	1	325.59	325.59	13.20	<.001
Baseline_1.treat.T1	2	25.34	12.67	0.51	0.599
Baseline_1.treat.Baseline_2	1	18.24	18.24	0.74	0.391

Baseline_1.T1.Baseline_2	2	149.31	74.66	3.03	0.051
Baseline_1.Baseline_2.T2	2	88.33	44.16	1.79	0.170
Baseline_1.Baseline_2.days	2	1386.13	693.06	28.11	<.001
Baseline_1.treat.T1.Baseline_2	2	42.38	21.19	0.86	0.425
Baseline_1.treat.Baseline_2.T2	2	47.70	23.85	0.97	0.382
Baseline_1.T1.Baseline_2.T2	4	29.11	7.28	0.30	0.881
Baseline_1.treat.Baseline_2.days	2	198.59	99.30	4.03	0.020
Baseline_1.T1.Baseline_2.days	4	220.80	55.20	2.24	0.067
Baseline_1.Baseline_2.T2.days	4	83.87	20.97	0.85	0.495
Baseline_1.treat.T1.Baseline_2.T2	4	90.98	22.75	0.92	0.452
Baseline_1.treat.T1.Baseline_2.days	4	210.78	52.69	2.14	0.079
Baseline_1.treat.Baseline_2.T2.days	4	102.71	25.68	1.04	0.388
Baseline_1.T1.Baseline_2.T2.days	8	104.63	13.08	0.53	0.832
Baseline_1.treat.T1.Baseline_2.T2.days	8	93.58	11.70	0.47	0.873
Residual	159	3920.64	24.66		
Total	221	7859.36			

Table B.6.4: Hue angle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	958.0	479.0	1.99	
rep.*Units* stratum					
Baseline_1	1	657.4	657.4	2.73	0.100
Baseline_1.treat	1	1678.6	1678.6	6.97	0.009
Baseline_1.T1	2	5932.9	2966.4	12.32	<.001
Baseline_1.Baseline_2	1	7013.1	7013.1	29.12	<.001
Baseline_1.treat.T1	2	156.3	78.1	0.32	0.723
Baseline_1.treat.Baseline_2	1	540.6	540.6	2.25	0.136
Baseline_1.T1.Baseline_2	2	1571.4	785.7	3.26	0.041
Baseline_1.Baseline_2.T2	2	1060.1	530.1	2.20	0.114
Baseline_1.Baseline_2.days	2	23667.7	11833.8	49.15	<.001
Baseline_1.treat.T1.Baseline_2	2	56.8	28.4	0.12	0.889
Baseline_1.treat.Baseline_2.T2	2	709.4	354.7	1.47	0.232
Baseline_1.T1.Baseline_2.T2	4	171.7	42.9	0.18	0.949
Baseline_1.treat.Baseline_2.days	2	1137.4	568.7	2.36	0.098
Baseline_1.T1.Baseline_2.days	4	5592.0	1398.0	5.81	<.001
Baseline_1.Baseline_2.T2.days	4	1296.7	324.2	1.35	0.255
Baseline_1.treat.T1.Baseline_2.T2	4	356.4	89.1	0.37	0.830
Baseline_1.treat.T1.Baseline_2.days	4	506.4	126.6	0.53	0.717
Baseline_1.treat.Baseline_2.T2.days					

	4	665.4	166.4	0.69	0.599
Baseline_1.T1.Baseline_2.T2.days					
	8	883.4	110.4	0.46	0.884
Baseline_1.treat.T1.Baseline_2.T2.days					
	8	851.3	106.4	0.44	0.894
Residual	159	38286.2	240.8		
Total	221	93749.0			

Table B.6.5: Lightness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	67.604	33.802	3.84	
rep.*Units* stratum					
Baseline_1	1	2.730	2.730	0.31	0.578
Baseline_1.treat	1	5.236	5.236	0.59	0.442
Baseline_1.T1	2	115.056	57.528	6.53	0.002
Baseline_1.Baseline_2	1	55.575	55.575	6.31	0.013
Baseline_1.treat.T1	2	4.385	2.192	0.25	0.780
Baseline_1.treat.Baseline_2	1	1.859	1.859	0.21	0.646
Baseline_1.T1.Baseline_2	2	24.176	12.088	1.37	0.256
Baseline_1.Baseline_2.T2	2	36.377	18.188	2.07	0.130
Baseline_1.Baseline_2.days	2	408.098	204.049	23.18	<.001
Baseline_1.treat.T1.Baseline_2	2	23.438	11.719	1.33	0.267
Baseline_1.treat.Baseline_2.T2	2	14.932	7.466	0.85	0.430
Baseline_1.T1.Baseline_2.T2	4	22.463	5.616	0.64	0.636
Baseline_1.treat.Baseline_2.days	2	69.302	34.651	3.94	0.021
Baseline_1.T1.Baseline_2.days	4	135.038	33.760	3.83	0.005
Baseline_1.Baseline_2.T2.days	4	44.309	11.077	1.26	0.289
Baseline_1.treat.T1.Baseline_2.T2					
	4	28.875	7.219	0.82	0.514
Baseline_1.treat.T1.Baseline_2.days					
	4	66.961	16.740	1.90	0.113
Baseline_1.treat.Baseline_2.T2.days					
	4	25.948	6.487	0.74	0.568
Baseline_1.T1.Baseline_2.T2.days					
	8	35.171	4.396	0.50	0.855
Baseline_1.treat.T1.Baseline_2.T2.days					
	8	40.088	5.011	0.57	0.802
Residual	159	1399.818	8.804		
Total	221	2627.439			

Table B.6.6: Influence of ethylene treatment, temperature (T1, T2) and shelf life on weight changes of avocado fruit cv. Hass from South Africa (Exp. I)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	33750.	16875.	0.91	
rep.*Units* stratum					
Baseline_1	1	256.	256.	0.01	0.906

Baseline_1.treat	1	21931.	21931.	1.19	0.278
Baseline_1.T1	2	51739.	25870.	1.40	0.250
Baseline_1.Baseline_2	1	1417.	1417.	0.08	0.782
Baseline_1.treat.T1	2	23825.	11913.	0.64	0.527
Baseline_1.treat.Baseline_2	1	6323.	6323.	0.34	0.560
Baseline_1.T1.Baseline_2		11191.	5595.	0.30	0.739
Baseline_1.Baseline_2.T2	2	63162.	31581.	1.71	0.185
Baseline_1.Baseline_2.days	2	67823.	33911.	1.83	0.163
Baseline_1.treat.T1.Baseline_2	2	13754.	6877.	0.37	0.690
Baseline_1.treat.Baseline_2.T2	2	64673.	32336.	1.75	0.177
Baseline_1.T1.Baseline_2.T2	4	94874.	23719.	1.28	0.279
Baseline_1.treat.Baseline_2.days	2	58995.	29498.	1.59	0.206
Baseline_1.T1.Baseline_2.days	4	105959.	26490.	1.43	0.226
Baseline_1.Baseline_2.T2.days	4	122026.	30506.	1.65	0.165
Baseline_1.treat.T1.Baseline_2.T2	4	127638.	31909.	1.73	0.147
Baseline_1.treat.T1.Baseline_2.days	4	126636.	31659.	1.71	0.150
Baseline_1.treat.Baseline_2.T2.days	4	114480.	28620.	1.55	0.191
Baseline_1.T1.Baseline_2.T2.days	8	222002.	27750.	1.50	0.161
Baseline_1.treat.T1.Baseline_2.T2.days	8	217560.	27195.	1.47	0.172
Residual	159	2940877.	18496.		
Total	221	4490892.			

Table B.6.7: Influence of ethylene treatment, temperature (T1, T2) and shelf life on firmness of avocado fruit cv. Hass from South Africa (Exp. I)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	3467.	1733.	0.14	
rep.*Units* stratum					
Baseline_1	1	4733.	4733.	0.39	0.533
Baseline_1.treat	1	201242.	201242.	16.72	<.001
Baseline_1.T1	2	27207.	13603.	1.13	0.328
Baseline_1.treat.T1	2	4832.	2416.	0.20	0.819
Baseline_1.Baseline_2.T2	2	41865.	20932.	1.74	0.183
Baseline_1.Baseline_2.days	1	956734.	956734.	79.48	<.001
Baseline_1.treat.Baseline_2.T2	2	21379.	10689.	0.89	0.416
Baseline_1.T1.Baseline_2.T2	4	17130.	4283.	0.36	0.839
Baseline_1.treat.Baseline_2.days	1	7929.	7929.	0.66	0.420
Baseline_1.T1.Baseline_2.days	2	1654.	827.	0.07	0.934
Baseline_1.Baseline_2.T2.days	2	25834.	12917.	1.07	0.347
Baseline_1.treat.T1.Baseline_2.T2	4	5771.	1443.	0.12	0.975
Baseline_1.treat.T1.Baseline_2.days					

	2	37484.	18742.	1.56	0.217
Baseline_1.treat.Baseline_2.T2.days					
	2	7378.	3689.	0.31	0.737
Baseline_1.T1.Baseline_2.T2.days					
	4	13726.	3432.	0.29	0.887
Baseline_1.treat.T1.Baseline_2.T2.days					
	4	22003.	5501.	0.46	0.767
Residual	75	902787.	12037.		
Total	113	2303155.			

Table B.6.8-9: Influence of temperature on the measurement of CO₂ and ethylene production of avocado fruit cv. Hass from South Africa (Exp. I). Gasses measured at 12°C and 20°C for 12°C shelf life.

Table B.6.8: CO₂

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
rep stratum	2		1515.5	757.7	0.96	
rep. *Units* stratum						
Baseline_1.treat	1		2048.1	2048.1	2.59	0.111
Baseline_1.Tresp	1		13875.3	13875.3	17.53	<.001
Baseline_1.Baseline_2	1		1847.3	1847.3	2.33	0.130
Baseline_1.treat.Tresp	1		195.0	195.0	0.25	0.621
Baseline_1.treat.Baseline_2	1		6.4	6.4	0.01	0.929
Baseline_1.Tresp.Baseline_2	1		997.9	997.9	1.26	0.264
Baseline_1.Baseline_2.T2	2		406.2	203.1	0.26	0.774
Baseline_1.Baseline_2.days	2		11853.4	5926.7	7.49	<.001
Baseline_1.treat.Tresp.Baseline_2						
	1		735.0	735.0	0.93	0.337
Baseline_1.treat.Baseline_2.T2	2		1736.7	868.4	1.10	0.338
Baseline_1.Tresp.Baseline_2.T2	2		1677.3	838.6	1.06	0.350
Baseline_1.treat.Baseline_2.days						
	2		3693.3	1846.7	2.33	0.102
Baseline_1.Tresp.Baseline_2.days						
	2		6727.8	3363.9	4.25	0.017
Baseline_1.Baseline_2.T2.days	4		1514.0	378.5	0.48	0.752
Baseline_1.treat.Tresp.Baseline_2.T2						
	2		1792.0	896.0	1.13	0.326
Baseline_1.treat.Tresp.Baseline_2.days						
	2		1745.5	872.7	1.10	0.336
Baseline_1.treat.Baseline_2.T2.days						
	4		2341.9	585.5	0.74	0.567
Baseline_1.Tresp.Baseline_2.T2.days						
	4		3407.1	851.8	1.08	0.372
Baseline_1.treat.Tresp.Baseline_2.T2.days						
	4		5964.5	1491.1	1.88	0.119
Residual	101	(1)	79921.7	791.3		
Total	142	(1)	142784.6			

Table B.6.9: Ethylene

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
rep stratum	2		354.7	177.3	0.50	
rep.*Units* stratum						
Baseline_1.treat	1		1073.0	1073.0	3.02	0.085
Baseline_1.Tresp	1		53.7	53.7	0.15	0.698
Baseline_1.Baseline_2	1		1862.1	1862.1	5.25	0.024
Baseline_1.treat.Tresp	1		411.9	411.9	1.16	0.284
Baseline_1.treat.Baseline_2	1		30.5	30.5	0.09	0.770
Baseline_1.Tresp.Baseline_2	1		57.1	57.1	0.16	0.689
Baseline_1.Baseline_2.T2	2		633.5	316.8	0.89	0.413
Baseline_1.Baseline_2.days	2		450.4	225.2	0.63	0.532
Baseline_1.treat.Tresp.Baseline_2						
	1		0.1	0.1	0.00	0.986
Baseline_1.treat.Baseline_2.T2	2		371.7	185.8	0.52	0.594
Baseline_1.Tresp.Baseline_2.T2	2		1145.9	573.0	1.61	0.204
Baseline_1.treat.Baseline_2.days	2		362.0	181.0	0.51	0.602
Baseline_1.Tresp.Baseline_2.days						
	2		1568.8	784.4	2.21	0.115
Baseline_1.Baseline_2.T2.days	4		1542.4	385.6	1.09	0.367
Baseline_1.treat.Tresp.Baseline_2.T2						
	2		1662.7	831.4	2.34	0.101
Baseline_1.treat.Tresp.Baseline_2.days						
	2		387.5	193.8	0.55	0.581
Baseline_1.treat.Baseline_2.T2.days						
	4		3686.6	921.6	2.60	0.041
Baseline_1.Tresp.Baseline_2.T2.days						
	4		1501.6	375.4	1.06	0.382
Baseline_1.treat.Tresp.Baseline_2.T2.days						
	4		2038.4	509.6	1.44	0.228
Residual	101	(1)	35855.7	355.0		
Total	142	(1)	55043.3			

Table B.6.10-11: Influence of temperature on the measurement of CO₂ and ethylene production of avocado fruit cv. Hass from South Africa (Exp. I). Gasses measured at 16°C and 20°C for 16°C shelf life.**Table B.6.10: CO₂**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	2816.3	1408.1	6.11	
rep.*Units* stratum					
Baseline_1.treat	1	2314.9	2314.9	10.04	0.002
Baseline_1.Tresp	1	2402.3	2402.3	10.42	0.002
Baseline_1.Baseline_2	1	6251.8	6251.8	27.12	<.001
Baseline_1.treat.Tresp	1	12.6	12.6	0.05	0.815
Baseline_1.treat.Baseline_2	1	588.9	588.9	2.55	0.113

Baseline_1.Tresp.Baseline_2	1	357.6	357.6	1.55	0.216
Baseline_1.Baseline_2.T2	2	799.3	399.7	1.73	0.182
Baseline_1.Baseline_2.days	2	9701.3	4850.6	21.04	<.001
Baseline_1.treat.Tresp.Baseline_2	1	589.4	589.4	2.56	0.113
Baseline_1.treat.Baseline_2.T2	2	199.4	99.7	0.43	0.650
Baseline_1.Tresp.Baseline_2.T2	2	590.1	295.0	1.28	0.282
Baseline_1.treat.Baseline_2.days	2	716.1	358.0	1.55	0.217
Baseline_1.Tresp.Baseline_2.days	2	264.0	132.0	0.57	0.566
Baseline_1.Baseline_2.T2.days	4	1272.5	318.1	1.38	0.246
Baseline_1.treat.Tresp.Baseline_2.T2	2	709.3	354.7	1.54	0.220
Baseline_1.treat.Tresp.Baseline_2.days	2	383.1	191.5	0.83	0.439
Baseline_1.treat.Baseline_2.T2.days	4	2978.2	744.5	3.23	0.015
Baseline_1.Tresp.Baseline_2.T2.days	4	1061.9	265.5	1.15	0.337
Baseline_1.treat.Tresp.Baseline_2.T2.days	4	2542.1	635.5	2.76	0.032
Residual	102	23510.4	230.5		
Total	143	60061.4			

Table B.11: Ethylene

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	1584.2	792.1	2.80	
rep.*Units* stratum					
Baseline_1.treat	1	321.2	321.2	1.14	0.289
Baseline_1.Tresp	1	366.3	366.3	1.30	0.257
Baseline_1.Baseline_2	1	4133.8	4133.8	14.63	<.001
Baseline_1.treat.Tresp	1	81.0	81.0	0.29	0.594
Baseline_1.treat.Baseline_2	1	177.6	177.6	0.63	0.430
Baseline_1.Tresp.Baseline_2	1	31.3	31.3	0.11	0.740
Baseline_1.Baseline_2.T2	2	607.7	303.8	1.08	0.345
Baseline_1.Baseline_2.days	2	7425.2	3712.6	13.14	<.001
Baseline_1.treat.Tresp.Baseline_2	1	215.5	215.5	0.76	0.384
Baseline_1.treat.Baseline_2.T2	2	145.2	72.6	0.26	0.774
Baseline_1.Tresp.Baseline_2.T2	2	51.2	25.6	0.09	0.913
Baseline_1.treat.Baseline_2.days	2	89.6	44.8	0.16	0.854
Baseline_1.Tresp.Baseline_2.days	2	556.9	278.5	0.99	0.377
Baseline_1.Baseline_2.T2.days	4	1752.9	438.2	1.55	0.193
Baseline_1.treat.Tresp.Baseline_2.T2	2	481.8	240.9	0.85	0.429

Baseline_1.treat.Tresp.Baseline_2.days	2	245.6	122.8	0.43	0.649
Baseline_1.treat.Baseline_2.T2.days	4	1642.1	410.5	1.45	0.222
Baseline_1.Tresp.Baseline_2.T2.days	4	3127.9	782.0	2.77	0.031
Baseline_1.treat.Tresp.Baseline_2.T2.days	4	2545.3	636.3	2.25	0.069
Residual	102	28812.7	282.5		
Total	143	54395.0			

Table B.12-13: Influence of ethylene treatment, temperature (T1, T2) and shelf life on respiration and ethylene production of avocado fruit cv. Hass from South Africa (Exp. II).

Table B.12: CO₂

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	2589.	1295.	0.43	
rep.*Units* stratum					
Baseline_1	1	17216.	17216.	5.78	0.017
Baseline_1.treat	1	2793.	2793.	0.94	0.334
Baseline_1.T1	2	27741.	13870.	4.66	0.011
Baseline_1.Baseline_2	1	65831.	65831.	22.12	<.001
Baseline_1.treat.T1	2	3124.	1562.	0.52	0.593
Baseline_1.treat.Baseline_2	1	5340.	5340.	1.79	0.182
Baseline_1.T1.Baseline_2	2	8791.	4395.	1.48	0.232
Baseline_1.Baseline_2.T2	2	2861.	1430.	0.48	0.619
Baseline_1.Baseline_2.days	2	195640.	97820.	32.86	<.001
Baseline_1.treat.T1.Baseline_2	2	336.	168.	0.06	0.945
Baseline_1.treat.Baseline_2.T2	2	8696.	4348.	1.46	0.235
Baseline_1.T1.Baseline_2.T2	4	2291.	573.	0.19	0.942
Baseline_1.treat.Baseline_2.days	2	5827.	2913.	0.98	0.378
Baseline_1.T1.Baseline_2.days	4	154001.	38500.	12.94	<.001
Baseline_1.Baseline_2.T2.days	4	13452.	3363.	1.13	0.344
Baseline_1.treat.T1.Baseline_2.T2	4	3862.	965.	0.32	0.861
Baseline_1.treat.T1.Baseline_2.days	4	3518.	879.	0.30	0.881
Baseline_1.treat.Baseline_2.T2.days	4	10465.	2616.	0.88	0.478
Baseline_1.T1.Baseline_2.T2.days	8	17123.	2140.	0.72	0.674
Baseline_1.treat.T1.Baseline_2.T2.days	8	27330.	3416.	1.15	0.334
Residual	159	473253.	2976.		
Total	221	1052081.			

Table B.13: Ethylene

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	62.16	31.08	0.81	
rep.*Units* stratum					
Baseline_1	1	519.61	519.61	13.53	<.001
Baseline_1.treat	1	0.10	0.10	0.00	0.960
Baseline_1.T1	2	3420.34	1710.17	44.54	<.001
Baseline_1.Baseline_2	1	2182.45	2182.45	56.83	<.001
Baseline_1.treat.T1	2	146.12	73.06	1.90	0.153
Baseline_1.treat.Baseline_2	1	743.37	743.37	19.36	<.001
Baseline_1.T1.Baseline_2	2	3581.62	1790.81	46.64	<.001
Baseline_1.Baseline_2.T2	2	309.79	154.89	4.03	0.020
Baseline_1.Baseline_2.days	2	2161.84	1080.92	28.15	<.001
Baseline_1.treat.T1.Baseline_2	2	526.62	263.31	6.86	0.001
Baseline_1.treat.Baseline_2.T2	2	66.61	33.30	0.87	0.422
Baseline_1.T1.Baseline_2.T2	4	231.83	57.96	1.51	0.202
Baseline_1.treat.Baseline_2.days	2	859.65	429.83	11.19	<.001
Baseline_1.T1.Baseline_2.days	4	3742.52	935.63	24.37	<.001
Baseline_1.Baseline_2.T2.days	4	1173.59	293.40	7.64	<.001
Baseline_1.treat.T1.Baseline_2.T2	4	71.09	17.77	0.46	0.763
Baseline_1.treat.T1.Baseline_2.days	4	491.62	122.91	3.20	0.015
Baseline_1.treat.Baseline_2.T2.days	4	87.90	21.97	0.57	0.683
Baseline_1.T1.Baseline_2.T2.days	8	734.50	91.81	2.39	0.018
Baseline_1.treat.T1.Baseline_2.T2.days	8	254.31	31.79	0.83	0.579
Residual	159	6105.66	38.40		
Total	221	27473.30			

Table B.14-16: Influence of ethylene treatment, temperature (T1, T2) and shelf life on colour changes of avocado fruit cv. Hass from South Africa (Exp. II)**Table B.14: Chroma**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	8.87	4.44	0.41	
rep.*Units* stratum					
Baseline_1	1	184.12	184.12	16.86	<.001
Baseline_1.treat	1	0.14	0.14	0.01	0.911
Baseline_1.T1	2	396.96	198.48	18.18	<.001
Baseline_1.Baseline_2	1	2430.32	2430.32	222.58	<.001
Baseline_1.treat.T1	2	1.88	0.94	0.09	0.917
Baseline_1.treat.Baseline_2	1	3.17	3.17	0.29	0.591
Baseline_1.T1.Baseline_2	2	118.86	59.43	5.44	0.005

Baseline_1.Baseline_2.T2	2	90.80	45.40	4.16	0.017
Baseline_1.Baseline_2.days	2	3293.43	1646.72	150.82	<.001
Baseline_1.treat.T1.Baseline_2	2	17.54	8.77	0.80	0.450
Baseline_1.treat.Baseline_2.T2	2	57.23	28.62	2.62	0.076
Baseline_1.T1.Baseline_2.T2	4	51.28	12.82	1.17	0.324
Baseline_1.treat.Baseline_2.days	2	5.53	2.77	0.25	0.777
Baseline_1.T1.Baseline_2.days	4	144.84	36.21	3.32	0.012
Baseline_1.Baseline_2.T2.days	4	49.82	12.45	1.14	0.339
Baseline_1.treat.T1.Baseline_2.T2	4	24.15	6.04	0.55	0.697
Baseline_1.treat.T1.Baseline_2.days	4	7.33	1.83	0.17	0.955
Baseline_1.treat.Baseline_2.T2.days	4	44.16	11.04	1.01	0.403
Baseline_1.T1.Baseline_2.T2.days	8	70.82	8.85	0.81	0.594
Baseline_1.treat.T1.Baseline_2.T2.days	8	43.31	5.41	0.50	0.858
Residual	159	1736.08	10.92		
Total	221	8780.65			

Table B.15: Hue angle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	143.46	71.73	0.76	
rep.*Units* stratum					
Baseline_1	1	3181.81	3181.81	33.58	<.001
Baseline_1.treat	1	79.72	79.72	0.84	0.360
Baseline_1.T1	2	9987.18	4993.59	52.70	<.001
Baseline_1.Baseline_2	1	37214.86	37214.86	392.71	<.001
Baseline_1.treat.T1	2	69.37	34.68	0.37	0.694
Baseline_1.treat.Baseline_2	1	5.61	5.61	0.06	0.808
Baseline_1.T1.Baseline_2	2	3297.91	1648.96	17.40	<.001
Baseline_1.Baseline_2.T2	2	1205.35	602.68	6.36	0.002
Baseline_1.Baseline_2.days	2	101653.81	50826.91	536.36	<.001
Baseline_1.treat.T1.Baseline_2	2	50.27	25.14	0.27	0.767
Baseline_1.treat.Baseline_2.T2	2	9.48	4.74	0.05	0.951
Baseline_1.T1.Baseline_2.T2	4	191.78	47.94	0.51	0.731
Baseline_1.treat.Baseline_2.days	2	330.89	165.44	1.75	0.178
Baseline_1.T1.Baseline_2.days	4	7197.52	1799.38	18.99	<.001
Baseline_1.Baseline_2.T2.days	4	533.57	133.39	1.41	0.234
Baseline_1.treat.T1.Baseline_2.T2	4	312.37	78.09	0.82	0.512
Baseline_1.treat.T1.Baseline_2.days	4	9.99	2.50	0.03	0.999
Baseline_1.treat.Baseline_2.T2.days					

	4	172.54	43.14	0.46	0.768
Baseline_1.T1.Baseline_2.T2.days					
	8	978.30	122.29	1.29	0.252
Baseline_1.treat.T1.Baseline_2.T2.days					
	8	559.87	69.98	0.74	0.657
Residual	159	15067.35	94.76		
Total	221	182253.00			

Table B.16: Lightness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	8.602	4.301	0.96	
rep.*Units* stratum					
Baseline_1	1	43.330	43.330	9.68	0.002
Baseline_1.treat	1	7.286	7.286	1.63	0.204
Baseline_1.T1	2	81.250	40.625	9.08	<.001
Baseline_1.Baseline_2	1	1162.986	1162.986	259.87	<.001
Baseline_1.treat.T1	2	5.021	2.511	0.56	0.572
Baseline_1.treat.Baseline_2	1	0.090	0.090	0.02	0.888
Baseline_1.T1.Baseline_2	2	30.624	15.312	3.42	0.035
Baseline_1.Baseline_2.T2	2	41.451	20.725	4.63	0.011
Baseline_1.Baseline_2.days	2	2722.908	1361.454	304.21	<.001
Baseline_1.treat.T1.Baseline_2	2	7.150	3.575	0.80	0.452
Baseline_1.treat.Baseline_2.T2	2	6.063	3.031	0.68	0.509
Baseline_1.T1.Baseline_2.T2	4	17.222	4.305	0.96	0.430
Baseline_1.treat.Baseline_2.days	2	9.898	4.949	1.11	0.333
Baseline_1.T1.Baseline_2.days	4	78.264	19.566	4.37	0.002
Baseline_1.Baseline_2.T2.days	4	27.965	6.991	1.56	0.187
Baseline_1.treat.T1.Baseline_2.T2					
	4	7.489	1.872	0.42	0.795
Baseline_1.treat.T1.Baseline_2.days					
	4	5.794	1.448	0.32	0.862
Baseline_1.treat.Baseline_2.T2.days					
	4	18.026	4.506	1.01	0.406
Baseline_1.T1.Baseline_2.T2.days					
	8	53.341	6.668	1.49	0.165
Baseline_1.treat.T1.Baseline_2.T2.days					
	8	52.904	6.613	1.48	0.169
Residual	159	711.577	4.475		
Total	221	5099.239			

Table B.17: Influence of ethylene treatment, temperature (T1, T2) and shelf life on weight changes of avocado fruit cv. Hass from South Africa (Exp. II)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	471.9	235.9	1.30	
rep.*Units* stratum					
Baseline_1	1	561.6	561.6	3.10	0.080

Baseline_1.treat	1	7.8	7.8	0.04	0.836
Baseline_1.T1	2	2633.2	1316.6	7.27	<.001
Baseline_1.Baseline_2	1	2411.7	2411.7	13.32	<.001
Baseline_1.treat.T1	2	177.3	88.7	0.49	0.614
Baseline_1.treat.Baseline_2	1	841.2	841.2	4.65	0.033
Baseline_1.T1.Baseline_2	2	242.3	121.1	0.67	0.514
Baseline_1.Baseline_2.T2	2	650.7	325.3	1.80	0.169
Baseline_1.Baseline_2.days	2	969.7	484.9	2.68	0.072
Baseline_1.treat.T1.Baseline_2	2	427.9	214.0	1.18	0.309
Baseline_1.treat.Baseline_2.T2	2	662.2	331.1	1.83	0.164
Baseline_1.T1.Baseline_2.T2	4	2393.7	598.4	3.31	0.012
Baseline_1.treat.Baseline_2.days	2	36.5	18.2	0.10	0.904
Baseline_1.T1.Baseline_2.days	4	823.7	205.9	1.14	0.341
Baseline_1.Baseline_2.T2.days	4	2041.7	510.4	2.82	0.027
Baseline_1.treat.T1.Baseline_2.T2	4	2948.0	737.0	4.07	0.004
Baseline_1.treat.T1.Baseline_2.days	4	495.5	123.9	0.68	0.604
Baseline_1.treat.Baseline_2.T2.days	4	1657.2	414.3	2.29	0.062
Baseline_1.T1.Baseline_2.T2.days	8	2765.1	345.6	1.91	0.062
Baseline_1.treat.T1.Baseline_2.T2.days	8	1100.9	137.6	0.76	0.638
Residual	159	28779.2	181.0		
Total	221	53098.8			

Table B.18: Influence of ethylene treatment, temperature (T1, T2) and shelf life on firmness changes of avocado fruit cv. Hass from South Africa (Exp. II).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	3066.	1533.	0.64	
rep.*Units* stratum					
Baseline_1	1	168554.	168554.	70.43	<.001
Baseline_1.treat	1	14254.	14254.	5.96	0.017
Baseline_1.T1	2	31123.	15561.	6.50	0.002
Baseline_1.treat.T1	2	5497.	2748.	1.15	0.323
Baseline_1.Baseline_2.T2	2	25935.	12968.	5.42	0.006
Baseline_1.Baseline_2.days	1	209398.	209398.	87.50	<.001
Baseline_1.treat.Baseline_2.T2	2	2597.	1299.	0.54	0.583
Baseline_1.T1.Baseline_2.T2	4	4019.	1005.	0.42	0.794
Baseline_1.treat.Baseline_2.days	1	3664.	3664.	1.53	0.220
Baseline_1.T1.Baseline_2.days	2	7842.	3921.	1.64	0.201
Baseline_1.Baseline_2.T2.days	2	20534.	10267.	4.29	0.017
Baseline_1.treat.T1.Baseline_2.T2	4	308.	77.	0.03	0.998
Baseline_1.treat.T1.Baseline_2.days					

	2	3792.	1896.	0.79	0.457
Baseline_1.treat.Baseline_2.T2.days					
	2	2568.	1284.	0.54	0.587
Baseline_1.T1.Baseline_2.T2.days					
	4	10680.	2670.	1.12	0.356
Baseline_1.treat.T1.Baseline_2.T2.days					
	4	8144.	2036.	0.85	0.498
Residual	75	179494.	2393.		
Total	113	701471.			

Table B.19-20: Influence of temperature on the measurement of CO₂ and ethylene production of avocado fruit cv. Hass from South Africa (Exp. II). Gasses measured at 12°C and 20°C for 12°C shelf life.

Table B.19: CO₂

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	3141.	1570.	0.60	
rep.*Units* stratum					
Baseline_1.treat	1	556.	556.	0.21	0.646
Baseline_1.Tresp	1	2919.	2919.	1.11	0.294
Baseline_1.Baseline_2	1	26484.	26484.	10.08	0.002
Baseline_1.treat.Tresp	1	4628.	4628.	1.76	0.187
Baseline_1.treat.Baseline_2	1	885.	885.	0.34	0.563
Baseline_1.Tresp.Baseline_2	1	4336.	4336.	1.65	0.202
Baseline_1.Baseline_2.T2	2	1417.	708.	0.27	0.764
Baseline_1.Baseline_2.days	2	87009.	43504.	16.56	<.001
Baseline_1.treat.Tresp.Baseline_2					
	1	947.	947.	0.36	0.550
Baseline_1.treat.Baseline_2.T2	2	9332.	4666.	1.78	0.174
Baseline_1.Tresp.Baseline_2.T2	2	513.	256.	0.10	0.907
Baseline_1.treat.Baseline_2.days	2	3236.	1618.	0.62	0.542
Baseline_1.Tresp.Baseline_2.days					
	2	40979.	20489.	7.80	<.001
Baseline_1.Baseline_2.T2.days	4	7828.	1957.	0.75	0.563
Baseline_1.treat.Tresp.Baseline_2.T2					
	2	2466.	1233.	0.47	0.627
Baseline_1.treat.Tresp.Baseline_2.days					
	2	3331.	1666.	0.63	0.532
Baseline_1.treat.Baseline_2.T2.days					
	4	18162.	4540.	1.73	0.149
Baseline_1.Tresp.Baseline_2.T2.days					
	4	2262.	566.	0.22	0.929
Baseline_1.treat.Tresp.Baseline_2.T2.days					
	4	18016.	4504.	1.71	0.152
Residual	102	267901.	2626.		
Total	143	506346.			

Table B.20: Ethylene

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
rep stratum	2		174.18	87.09	1.05	
rep.*Units* stratum						
Baseline_1.treat	1		31.50	31.50	0.38	0.539
Baseline_1.Tresp	1		1144.40	1144.40	13.82	<.001
Baseline_1.Baseline_2	1		902.10	902.10	10.89	0.001
Baseline_1.treat.Tresp	1		386.48	386.48	4.67	0.033
Baseline_1.treat.Baseline_2	1		999.79	999.79	12.07	<.001
Baseline_1.Tresp.Baseline_2	1		2590.34	2590.34	31.28	<.001
Baseline_1.Baseline_2.T2	2		190.29	95.15	1.15	0.321
Baseline_1.Baseline_2.days	2		2697.19	1348.60	16.28	<.001
Baseline_1.treat.Tresp.Baseline_2						
	1		291.55	291.55	3.52	0.064
Baseline_1.treat.Baseline_2.T2	2		61.14	30.57	0.37	0.692
Baseline_1.Tresp.Baseline_2.T2	2		159.84	79.92	0.96	0.384
Baseline_1.treat.Baseline_2.days						
	2		155.33	77.66	0.94	0.395
Baseline_1.Tresp.Baseline_2.days						
	2		2955.22	1477.61	17.84	<.001
Baseline_1.Baseline_2.T2.days	4		126.54	31.63	0.38	0.821
Baseline_1.treat.Tresp.Baseline_2.T2						
	2		50.41	25.20	0.30	0.738
Baseline_1.treat.Tresp.Baseline_2.days						
	2		333.48	166.74	2.01	0.139
Baseline_1.treat.Baseline_2.T2.days						
	4		182.01	45.50	0.55	0.700
Baseline_1.Tresp.Baseline_2.T2.days						
	4		140.22	35.05	0.42	0.792
Baseline_1.treat.Tresp.Baseline_2.T2.days						
	4		137.50	34.38	0.42	0.797
Residual	101	(1)	8364.75	82.82		
Total	142	(1)	22034.37			

Table B.6.21-22: Influence of temperature on the measurement of CO₂ and ethylene production of avocado fruit cv. Hass from South Africa (Exp. II). Gasses measured at 16°C and 20°C for 16°C shelf life.**Table B.6.21: CO₂**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	2785.	1392.	0.71	
rep.*Units* stratum					
Baseline_1.treat	1	1315.	1315.	0.67	0.415
Baseline_1.Tresp	1	12462.	12462.	6.34	0.013
Baseline_1.Baseline_2	1	33191.	33191.	16.89	<.001
Baseline_1.treat.Tresp	1	384.	384.	0.20	0.660

Baseline_1.treat.Baseline_2	1	697.	697.	0.35	0.553
Baseline_1.Tresp.Baseline_2	1	12110.	12110.	6.16	0.015
Baseline_1.Baseline_2.T2	2	4574.	2287.	1.16	0.316
Baseline_1.Baseline_2.days	2	105891.	52945.	26.94	<.001
Baseline_1.treat.Tresp.Baseline_2	1	211.	211.	0.11	0.744
Baseline_1.treat.Baseline_2.T2	2	1805.	903.	0.46	0.633
Baseline_1.Tresp.Baseline_2.T2	2	931.	465.	0.24	0.790
Baseline_1.treat.Baseline_2.days	2	4190.	2095.	1.07	0.348
Baseline_1.Tresp.Baseline_2.days	2	102303.	51151.	26.03	<.001
Baseline_1.Baseline_2.T2.days	4	6168.	1542.	0.78	0.538
Baseline_1.treat.Tresp.Baseline_2.T2	2	749.	374.	0.19	0.827
Baseline_1.treat.Tresp.Baseline_2.days	2	197.	99.	0.05	0.951
Baseline_1.treat.Baseline_2.T2.days	4	2842.	710.	0.36	0.835
Baseline_1.Tresp.Baseline_2.T2.days	4	692.	173.	0.09	0.986
Baseline_1.treat.Tresp.Baseline_2.T2.days	4	999.	250.	0.13	0.972
Residual	102	200456.	1965.		
Total	143	494951.			

Table B.6.22: Ethylene

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	108.17	54.08	0.86	
rep.*Units* stratum					
Baseline_1.treat	1	109.30	109.30	1.74	0.189
Baseline_1.Tresp	1	2165.75	2165.75	34.58	<.001
Baseline_1.Baseline_2	1	4994.55	4994.55	79.74	<.001
Baseline_1.treat.Tresp	1	31.95	31.95	0.51	0.477
Baseline_1.treat.Baseline_2	1	110.27	110.27	1.76	0.188
Baseline_1.Tresp.Baseline_2	1	1168.17	1168.17	18.65	<.001
Baseline_1.Baseline_2.T2	2	336.92	168.46	2.69	0.073
Baseline_1.Baseline_2.days	2	3947.22	1973.61	31.51	<.001
Baseline_1.treat.Tresp.Baseline_2	1	0.03	0.03	0.00	0.984
Baseline_1.treat.Baseline_2.T2	2	498.51	249.26	3.98	0.022
Baseline_1.Tresp.Baseline_2.T2	2	983.24	491.62	7.85	<.001
Baseline_1.treat.Baseline_2.days	2	58.14	29.07	0.46	0.630
Baseline_1.Tresp.Baseline_2.days					

	2	2467.43	1233.71	19.70	<.001
Baseline_1.Baseline_2.T2.days	4	841.55	210.39	3.36	0.013
Baseline_1.treat.Tresp.Baseline_2.T2					
	2	654.68	327.34	5.23	0.007
Baseline_1.treat.Tresp.Baseline_2.days					
	2	1056.53	528.26	8.43	<.001
Baseline_1.treat.Baseline_2.T2.days					
	4	153.67	38.42	0.61	0.654
Baseline_1.Tresp.Baseline_2.T2.days					
	4	713.52	178.38	2.85	0.028
Baseline_1.treat.Tresp.Baseline_2.T2.days					
	4	50.40	12.60	0.20	0.937
Residual	102	6389.14	62.64		
Total	143	26839.13			

Table B.6.23-25: Influence of ethylene treatment, length of treatment and shelf life on colour changes of avocado fruit cv. Hass from South Africa with shelf life at 12°C (Exp. III).

Table B.6.23: Chroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	23.93	11.97	0.83	
rep.*Units* stratum					
baseline1	1	246.43	246.43	17.00	<.001
baseline1.treatment	1	3.10	3.10	0.21	0.645
baseline1.treattime	1	30.45	30.45	2.10	0.150
baseline1.Baseline2	1	202.83	202.83	13.99	<.001
baseline1.treatment.treattime	1	28.83	28.83	1.99	0.161
baseline1.treatment.Baseline2	1	24.16	24.16	1.67	0.199
baseline1.treattime.Baseline2	1	1.46	1.46	0.10	0.751
baseline1.Baseline2.swap	1	198.54	198.54	13.70	<.001
baseline1.Baseline2.day	5	883.13	176.63	12.19	<.001
baseline1.treatment.treattime.Baseline2					
	1	4.91	4.91	0.34	0.562
baseline1.treatment.Baseline2.swap					
	1	2.62	2.62	0.18	0.672
baseline1.treattime.Baseline2.swap					
	1	11.83	11.83	0.82	0.368
baseline1.treatment.Baseline2.day					
	5	125.64	25.13	1.73	0.133
baseline1.treattime.Baseline2.day					
	5	70.66	14.13	0.98	0.436
baseline1.Baseline2.swap.day	5	121.86	24.37	1.68	0.145
baseline1.treatment.treattime.Baseline2.swap					
	1	0.00	0.00	0.00	0.993
baseline1.treatment.treattime.Baseline2.day					
	5	25.27	5.05	0.35	0.882
baseline1.treatment.Baseline2.swap.day					

	5	172.69	34.54	2.38	0.043
baseline1.treattime.Baseline2.swap.day					
	5	100.72	20.14	1.39	0.234
baseline1.treatment.treattime.Baseline2.swap.day					
	5	73.85	14.77	1.02	0.410
Residual	107	1550.79	14.49		
Total	161	3903.72			

Table B.6.24: Hue angle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	246.1	123.1	0.77	
rep.*Units* stratum					
baseline1	1	2926.2	2926.2	18.39	<.001
baseline1.treatment	1	21.1	21.1	0.13	0.717
baseline1.treattime	1	14.3	14.3	0.09	0.765
baseline1.Baseline2	1	5958.4	5958.4	37.45	<.001
baseline1.treatment.treattime	1	6.9	6.9	0.04	0.835
baseline1.treatment.Baseline2	1	65.3	65.3	0.41	0.523
baseline1.treattime.Baseline2	1	21.3	21.3	0.13	0.715
baseline1.Baseline2.swap	1	5832.5	5832.5	36.66	<.001
baseline1.Baseline2.day	5	25951.1	5190.2	32.62	<.001
baseline1.treatment.treattime.Baseline2					
	1	1.8	1.8	0.01	0.915
baseline1.treatment.Baseline2.swap					
	1	85.6	85.6	0.54	0.465
baseline1.treattime.Baseline2.swap					
	1	994.9	994.9	6.25	0.014
baseline1.treatment.Baseline2.day					
	5	327.4	65.5	0.41	0.840
baseline1.treattime.Baseline2.day					
	5	470.8	94.2	0.59	0.706
baseline1.Baseline2.swap.day	5	473.6	94.7	0.60	0.704
baseline1.treatment.treattime.Baseline2.swap					
	1	314.9	314.9	1.98	0.162
baseline1.treatment.treattime.Baseline2.day					
	5	488.4	97.7	0.61	0.689
baseline1.treatment.Baseline2.swap.day					
	5	1758.2	351.6	2.21	0.059
baseline1.treattime.Baseline2.swap.day					
	5	1339.9	268.0	1.68	0.145
baseline1.treatment.treattime.Baseline2.swap.day					
	5	1258.1	251.6	1.58	0.171
Residual	107	17024.2	159.1		
Total	161	65581.0			

Table B.6.25: Lightness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	988.3	494.1	0.62	
rep.*Units* stratum					
baseline1	1	6.2	6.2	0.01	0.930
baseline1.treatment	1	1786.1	1786.1	2.23	0.139
baseline1.treattime	1	1691.4	1691.4	2.11	0.149
baseline1.Baseline2	1	3.3	3.3	0.00	0.949
baseline1.treatment.treattime	1	1318.9	1318.9	1.64	0.203
baseline1.treatment.Baseline2	1	86.6	86.6	0.11	0.743
baseline1.treattime.Baseline2	1	197.8	197.8	0.25	0.621
baseline1.Baseline2.swap	1	386.5	386.5	0.48	0.489
baseline1.Baseline2.day	5	4503.4	900.7	1.12	0.353
baseline1.treatment.treattime.Baseline2	1	166.9	166.9	0.21	0.649
baseline1.treatment.Baseline2.swap	1	79.4	79.4	0.10	0.754
baseline1.treattime.Baseline2.swap	1	49.7	49.7	0.06	0.804
baseline1.treatment.Baseline2.day	5	3456.6	691.3	0.86	0.510
baseline1.treattime.Baseline2.day	5	4080.9	816.2	1.02	0.411
baseline1.Baseline2.swap.day	5	5534.1	1106.8	1.38	0.238
baseline1.treatment.treattime.Baseline2.swap	1	59.2	59.2	0.07	0.787
baseline1.treatment.treattime.Baseline2.day	5	3351.2	670.2	0.84	0.528
baseline1.treatment.Baseline2.swap.day	5	6548.6	1309.7	1.63	0.158
baseline1.treattime.Baseline2.swap.day	5	5572.3	1114.5	1.39	0.234
baseline1.treatment.treattime.Baseline2.swap.day	5	5437.5	1087.5	1.36	0.247
Residual	107	85863.6	802.5		
Total	161	131168.5			

Table B.6.26-27: Influence of ethylene treatment, length of treatment and shelf life on respiration and ethylene production of avocado fruit cv. Hass from South Africa with shelf life at 12°C (Exp. III).**Table B.6.26: CO₂**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.59	0.29	0.00	
rep.*Units* stratum					
baseline1	1	992.52	992.52	15.54	<.001

baseline1.treatment	1	140.76	140.76	2.20	0.141
baseline1.treattime	1	540.53	540.53	8.46	0.004
baseline1.Baseline2	1	3254.43	3254.43	50.96	<.001
baseline1.treatment.treattime	1	51.09	51.09	0.80	0.373
baseline1.treatment.Baseline2	1	72.43	72.43	1.13	0.289
baseline1.treattime.Baseline2	1	17.75	17.75	0.28	0.599
baseline1.Baseline2.swap	1	586.28	586.28	9.18	0.003
baseline1.Baseline2.day	5	968.12	193.62	3.03	0.013
baseline1.treatment.treattime.Baseline2	1	15.21	15.21	0.24	0.627
baseline1.treatment.Baseline2.swap	1	7.11	7.11	0.11	0.739
baseline1.treattime.Baseline2.swap	1	0.01	0.01	0.00	0.991
baseline1.treatment.Baseline2.day	5	430.81	86.16	1.35	0.249
baseline1.treattime.Baseline2.day	5	364.50	72.90	1.14	0.343
baseline1.Baseline2.swap.day	5	2147.44	429.49	6.73	<.001
baseline1.treatment.treattime.Baseline2.swap	1	8.04	8.04	0.13	0.723
baseline1.treatment.treattime.Baseline2.day	5	315.17	63.03	0.99	0.429
baseline1.treatment.Baseline2.swap.day	5	844.09	168.82	2.64	0.027
baseline1.treattime.Baseline2.swap.day	5	247.47	49.49	0.78	0.570
baseline1.treatment.treattime.Baseline2.swap.day	5	617.72	123.54	1.93	0.095
Residual	107	6833.14	63.86		
Total	161	18455.19			

Table B.6.27: Ethylene

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	111.1	55.6	0.53	
rep.*Units* stratum					
baseline1	1	563.0	563.0	5.33	0.023
baseline1.treatment	1	928.5	928.5	8.80	0.004
baseline1.treattime	1	208.4	208.4	1.97	0.163
baseline1.Baseline2	1	1379.3	1379.3	13.07	<.001
baseline1.treatment.treattime	1	19.9	19.9	0.19	0.665
baseline1.treatment.Baseline2	1	599.6	599.6	5.68	0.019
baseline1.treattime.Baseline2	1	128.0	128.0	1.21	0.273
baseline1.Baseline2.swap	1	10.3	10.3	0.10	0.755
baseline1.Baseline2.day	5	2731.0	546.2	5.17	<.001
baseline1.treatment.treattime.Baseline2					

	1	179.9	179.9	1.70	0.194
baseline1.treatment.Baseline2.swap	1	46.8	46.8	0.44	0.507
baseline1.treattime.Baseline2.swap	1	141.9	141.9	1.34	0.249
baseline1.treatment.Baseline2.day	5	326.7	65.3	0.62	0.685
baseline1.treattime.Baseline2.day	5	784.0	156.8	1.49	0.201
baseline1.Baseline2.swap.day	5	1683.0	336.6	3.19	0.010
baseline1.treatment.treattime.Baseline2.swap	1	72.8	72.8	0.69	0.408
baseline1.treatment.treattime.Baseline2.day	5	394.3	78.9	0.75	0.590
baseline1.treatment.Baseline2.swap.day	5	166.6	33.3	0.32	0.903
baseline1.treattime.Baseline2.swap.day	5	850.7	170.1	1.61	0.163
baseline1.treatment.treattime.Baseline2.swap.day	5	548.7	109.7	1.04	0.398
Residual	107	11293.7	105.5		
Total	161	23168.4			

Table B.6.28: Influence of ethylene treatment, length of treatment and shelf life on firmness changes of avocado fruit cv. Hass from South Africa with shelf life at 12°C (Exp. III).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	11261.	5631.	2.32	
rep.*Units* stratum					
baseline1	1	151421.	151421.	62.44	<.001
baseline1.treatment	1	30966.	30966.	12.77	<.001
baseline1.treattime	1	170.	170.	0.07	0.792
baseline1.Baseline2	1	224806.	224806.	92.69	<.001
baseline1.treatment.treattime	1	345.	345.	0.14	0.707
baseline1.treatment.Baseline2	1	18.	18.	0.01	0.931
baseline1.treattime.Baseline2	1	291.	291.	0.12	0.730
baseline1.Baseline2.swap	1	27350.	27350.	11.28	0.001
baseline1.Baseline2.day	5	106924.	21385.	8.82	<.001
baseline1.treatment.treattime.Baseline2	1	1458.	1458.	0.60	0.440
baseline1.treatment.Baseline2.swap	1	133.	133.	0.05	0.815
baseline1.treattime.Baseline2.swap	1	2539.	2539.	1.05	0.309
baseline1.treatment.Baseline2.day	5	1820.	364.	0.15	0.980
baseline1.treattime.Baseline2.day					

	5	15167.	3033.	1.25	0.291
baseline1.Baseline2.swap.day	5	20265.	4053.	1.67	0.148
baseline1.treatment.treattime.Baseline2.swap					
	1	167.	167.	0.07	0.793
baseline1.treatment.treattime.Baseline2.day					
	5	6139.	1228.	0.51	0.771
baseline1.treatment.Baseline2.swap.day					
	5	24662.	4932.	2.03	0.080
baseline1.treattime.Baseline2.swap.day					
	5	20295.	4059.	1.67	0.147
baseline1.treatment.treattime.Baseline2.swap.day					
	5	40572.	8114.	3.35	0.008
Residual	107	259501.	2425.		
Total	161	946271.			

Table B.6.29: Influence of ethylene treatment, length of treatment and shelf life on weight changes of avocado fruit cv. Hass from South Africa with shelf life at 12°C (Exp. III).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	235.4	117.7	0.61	
rep.*Units* stratum					
baseline1	1	23851.5	23851.5	123.59	<.001
baseline1.treatment	1	1314.6	1314.6	6.81	0.010
baseline1.treattime	1	653.7	653.7	3.39	0.068
baseline1.Baseline2	1	269.8	269.8	1.40	0.240
baseline1.treatment.treattime	1	865.0	865.0	4.48	0.037
baseline1.treatment.Baseline2	1	88.4	88.4	0.46	0.500
baseline1.treattime.Baseline2					
	1	321.3	321.3	1.66	0.200
baseline1.Baseline2.swap	1	12.9	12.9	0.07	0.797
baseline1.Baseline2.day	5	3998.9	799.8	4.14	0.002
baseline1.treatment.treattime.Baseline2					
	1	728.0	728.0	3.77	0.055
baseline1.treatment.Baseline2.swap					
	1	2117.0	2117.0	10.97	0.001
baseline1.treattime.Baseline2.swap					
	1	76.0	76.0	0.39	0.532
baseline1.treatment.Baseline2.day					
	5	578.3	115.7	0.60	0.701
baseline1.treattime.Baseline2.day					
	5	473.5	94.7	0.49	0.783
baseline1.Baseline2.swap.day	5	1217.7	243.5	1.26	0.286
baseline1.treatment.treattime.Baseline2.swap					
	1	405.2	405.2	2.10	0.150
baseline1.treatment.treattime.Baseline2.day					
	5	335.6	67.1	0.35	0.883
baseline1.treatment.Baseline2.swap.day					

	5	243.7	48.7	0.25	0.938
baseline1.treattime.Baseline2.swap.day					
	5	87.5	17.5	0.09	0.994
baseline1.treatment.treattime.Baseline2.swap.day					
	5	1485.2	297.0	1.54	0.184
Residual	107	20649.8	193.0		
Total	161	60009.0			

Table B.6.30-32: Influence of ethylene treatment, length of treatment and shelf life on colour changes of avocado fruit cv. Hass from South Africa with shelf life at 20°C (Exp. III, Chapter 6).

Table B.6.30: Chroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	24.06	12.03	0.73	
rep.*Units* stratum					
baseline1	1	475.87	475.87	29.07	<.001
baseline1.treat	1	16.72	16.72	1.02	0.315
baseline1.treatmenttime	1	1.54	1.54	0.09	0.760
baseline1.baseline2	1	284.18	284.18	17.36	<.001
baseline1.treat.treatmenttime	1	0.60	0.60	0.04	0.849
baseline1.treat.baseline2	1	36.98	36.98	2.26	0.137
baseline1.treatmenttime.baseline2					
	1	3.08	3.08	0.19	0.666
baseline1.baseline2.swap	1	263.71	263.71	16.11	<.001
baseline1.baseline2.day	3	1145.98	381.99	23.34	<.001
baseline1.treat.treatmenttime.baseline2					
	1	4.93	4.93	0.30	0.585
baseline1.treat.baseline2.swap	1	52.58	52.58	3.21	0.077
baseline1.treatmenttime.baseline2.swap					
	1	0.00	0.00	0.00	0.989
baseline1.treat.baseline2.day	3	24.52	8.17	0.50	0.684
baseline1.treatmenttime.baseline2.day					
	3	46.97	15.66	0.96	0.418
baseline1.baseline2.swap.day	3	60.59	20.20	1.23	0.303
baseline1.treat.treatmenttime.baseline2.swap					
	1	1.59	1.59	0.10	0.756
baseline1.treat.treatmenttime.baseline2.day					
	3	25.44	8.48	0.52	0.671
baseline1.treat.baseline2.swap.day					
	3	23.76	7.92	0.48	0.695
baseline1.treatmenttime.baseline2.swap.day					
	3	4.07	1.36	0.08	0.969
baseline1.treat.treatmenttime.baseline2.swap.day					
	3	15.09	5.03	0.31	0.820
Residual	75	1227.64	16.37		
Total	113	3739.92			

Table B.6.31: Hue angle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	20075.	10037.	0.96	
rep.*Units* stratum					
baseline1	1	3605.	3605.	0.34	0.559
baseline1.treat	1	14880.	14880.	1.42	0.237
baseline1.treatmenttime	1	15749.	15749.	1.50	0.224
baseline1.baseline2	1	7270.	7270.	0.69	0.408
baseline1.treat.treatmenttime	1	12655.	12655.	1.21	0.276
baseline1.treat.baseline2	1	2036.	2036.	0.19	0.661
baseline1.treatmenttime.baseline2	1	2116.	2116.	0.20	0.655
baseline1.baseline2.swap	1	2026.	2026.	0.19	0.662
baseline1.baseline2.day	3	142196.	47399.	4.52	0.006
baseline1.treat.treatmenttime.baseline2	1	1672.	1672.	0.16	0.691
baseline1.treat.baseline2.swap	1	8508.	8508.	0.81	0.371
baseline1.treatmenttime.baseline2.swap	1	12110.	12110.	1.15	0.286
baseline1.treat.baseline2.day	3	31126.	10375.	0.99	0.403
baseline1.treatmenttime.baseline2.day	3	29591.	9864.	0.94	0.425
baseline1.baseline2.swap.day	3	39446.	13149.	1.25	0.296
baseline1.treat.treatmenttime.baseline2.swap	1	6994.	6994.	0.67	0.417
baseline1.treat.treatmenttime.baseline2.day	3	31026.	10342.	0.99	0.404
baseline1.treat.baseline2.swap.day	3	36196.	12065.	1.15	0.334
baseline1.treatmenttime.baseline2.swap.day	3	34917.	11639.	1.11	0.351
baseline1.treat.treatmenttime.baseline2.swap.day	3	37930.	12643.	1.21	0.314
Residual	75	786533.	10487.		
Total	113	1278657.			

Table B.6.32: Lightness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	20.745	10.373	1.59	
rep.*Units* stratum					
baseline1	1	51.782	51.782	7.93	0.006
baseline1.treat	1	3.933	3.933	0.60	0.440
baseline1.treatmenttime	1	2.439	2.439	0.37	0.543
baseline1.baseline2	1	106.527	106.527	16.32	<.001
baseline1.treat.treatmenttime	1	3.994	3.994	0.61	0.436
baseline1.treat.baseline2	1	5.051	5.051	0.77	0.382

baseline1.treatmenttime.baseline2	1	4.276	4.276	0.66	0.421
baseline1.baseline2.swap	1	150.200	150.200	23.01	<.001
baseline1.baseline2.day	3	591.668	197.223	30.22	<.001
baseline1.treat.treatmenttime.baseline2	1	2.016	2.016	0.31	0.580
baseline1.treat.baseline2.swap	1	10.613	10.613	1.63	0.206
baseline1.treatmenttime.baseline2.swap	1	0.277	0.277	0.04	0.837
baseline1.treat.baseline2.day	3	12.457	4.152	0.64	0.594
baseline1.treatmenttime.baseline2.day	3	15.919	5.306	0.81	0.491
baseline1.baseline2.swap.day	3	21.355	7.118	1.09	0.358
baseline1.treat.treatmenttime.baseline2.swap	1	2.294	2.294	0.35	0.555
baseline1.treat.treatmenttime.baseline2.day	3	4.319	1.440	0.22	0.882
baseline1.treat.baseline2.swap.day	3	12.907	4.302	0.66	0.580
baseline1.treatmenttime.baseline2.swap.day	3	3.167	1.056	0.16	0.922
baseline1.treat.treatmenttime.baseline2.swap.day	3	9.291	3.097	0.47	0.701
Residual	75	489.477	6.526		
Total	113	1524.708			

Table B.6.33-34: Influence of ethylene treatment, length of treatment and shelf life on respiration and ethylene production of avocado fruit cv. Hass from South Africa with shelf life at 20°C (Exp. III).

Table B.6.33: CO₂

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	663.5	331.7	0.95	
rep.*Units* stratum					
baseline1	1	21565.4	21565.4	62.04	<.001
baseline1.treat	1	1338.5	1338.5	3.85	0.053
baseline1.treatmenttime	1	4566.4	4566.4	13.14	<.001
baseline1.baseline2	1	1963.0	1963.0	5.65	0.020
baseline1.treat.treatmenttime	1	0.0	0.0	0.00	0.997
baseline1.treat.baseline2	1	37.3	37.3	0.11	0.744
baseline1.treatmenttime.baseline2	1	1660.3	1660.3	4.78	0.032
baseline1.baseline2.swap	1	660.4	660.4	1.90	0.172
baseline1.baseline2.day	3	1326.9	442.3	1.27	0.290
baseline1.treat.treatmenttime.baseline2	1	1590.3	1590.3	4.58	0.036
baseline1.treat.baseline2.swap	1	47.7	47.7	0.14	0.712

baseline1.treatmenttime.baseline2.swap	1	681.3	681.3	1.96	0.166
baseline1.treat.baseline2.day	3	948.6	316.2	0.91	0.441
baseline1.treatmenttime.baseline2.day	3	2220.4	740.1	2.13	0.104
baseline1.baseline2.swap.day	3	210.6	70.2	0.20	0.895
baseline1.treat.treatmenttime.baseline2.swap	1	273.7	273.7	0.79	0.378
baseline1.treat.treatmenttime.baseline2.day	3	416.8	138.9	0.40	0.754
baseline1.treat.baseline2.swap.day	3	497.6	165.9	0.48	0.699
baseline1.treatmenttime.baseline2.swap.day	3	821.6	273.9	0.79	0.504
baseline1.treat.treatmenttime.baseline2.swap.day	3	256.1	85.4	0.25	0.864
Residual	75	26068.6	347.6		
Total	113	67815.2			

Table B.6.34: Ethylene

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	2989.2	1494.6	2.79	
rep.*Units* stratum					
baseline1	1	4640.9	4640.9	8.68	0.004
baseline1.treat	1	62.3	62.3	0.12	0.734
baseline1.treatmenttime	1	3711.7	3711.7	6.94	0.010
baseline1.baseline2	1	3301.5	3301.5	6.17	0.015
baseline1.treat.treatmenttime	1	4174.4	4174.4	7.80	0.007
baseline1.treat.baseline2	1	5.3	5.3	0.01	0.921
baseline1.treatmenttime.baseline2	1	222.9	222.9	0.42	0.521
baseline1.baseline2.swap	1	3590.6	3590.6	6.71	0.012
baseline1.baseline2.day	3	11780.1	3926.7	7.34	<.001
baseline1.treat.treatmenttime.baseline2	1	116.8	116.8	0.22	0.642
baseline1.treat.baseline2.swap	1	13.6	13.6	0.03	0.874
baseline1.treatmenttime.baseline2.swap	1	2225.2	2225.2	4.16	0.045
baseline1.treat.baseline2.day	3	3282.1	1094.0	2.05	0.115
baseline1.treatmenttime.baseline2.day	3	2293.0	764.3	1.43	0.241
baseline1.baseline2.swap.day	3	9374.9	3125.0	5.84	0.001
baseline1.treat.treatmenttime.baseline2.swap	1	189.4	189.4	0.35	0.554
baseline1.treat.treatmenttime.baseline2.day	3	659.7	219.9	0.41	0.746

baseline1.treat.baseline2.swap.day	3	1366.4	455.5	0.85	0.470
baseline1.treatmenttime.baseline2.swap.day	3	382.3	127.4	0.24	0.869
baseline1.treat.treatmenttime.baseline2.swap.day	3	273.2	91.1	0.17	0.916
Residual	75	40120.1	534.9		
Total	113	94775.4			

Table B.6.35: Influence of ethylene treatment, length of treatment and shelf life on firmness changes of avocado fruit cv. Hass from South Africa with shelf life at 20°C (Exp. III).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	3201.	1600.	0.90	
rep.*Units* stratum					
baseline1	1	184278.	184278.	103.81	<.001
baseline1.treat	1	11671.	11671.	6.57	0.012
baseline1.treatmenttime	1	4736.	4736.	2.67	0.107
baseline1.baseline2	1	79157.	79157.	44.59	<.001
baseline1.treat.treatmenttime	1	1894.	1894.	1.07	0.305
baseline1.treat.baseline2	1	303.	303.	0.17	0.681
baseline1.treatmenttime.baseline2	1	2934.	2934.	1.65	0.203
baseline1.baseline2.swap	1	6916.	6916.	3.90	0.052
baseline1.baseline2.day	3	43426.	14475.	8.15	<.001
baseline1.treat.treatmenttime.baseline2	1	5228.	5228.	2.95	0.090
baseline1.treat.baseline2.swap	1	801.	801.	0.45	0.504
baseline1.treatmenttime.baseline2.swap	1	3263.	3263.	1.84	0.179
baseline1.treat.baseline2.day	3	6549.	2183.	1.23	0.305
baseline1.treatmenttime.baseline2.day	3	9984.	3328.	1.87	0.141
baseline1.baseline2.swap.day	3	9462.	3154.	1.78	0.159
baseline1.treat.treatmenttime.baseline2.swap	1	1709.	1709.	0.96	0.330
baseline1.treat.treatmenttime.baseline2.day	3	6660.	2220.	1.25	0.298
baseline1.treat.baseline2.swap.day	3	5681.	1894.	1.07	0.368
baseline1.treatmenttime.baseline2.swap.day	3	1922.	641.	0.36	0.781
baseline1.treat.treatmenttime.baseline2.swap.day	3	1367.	456.	0.26	0.856
Residual	75	133138.	1775.		
Total	113	524279.			

Table B.6.36: Influence of ethylene treatment, length of treatment and shelf life on weight of avocado fruit cv. Hass from South Africa with shelf life at 20°C (Exp. III).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	897.3	448.7	2.21	
rep.*Units* stratum					
baseline1	1	22193.2	22193.2	109.50	<.001
baseline1.treat	1	1477.0	1477.0	7.29	0.009
baseline1.treatmenttime	1	62.7	62.7	0.31	0.580
baseline1.baseline2	1	369.4	369.4	1.82	0.181
baseline1.treat.treatmenttime	1	472.3	472.3	2.33	0.131
baseline1.treat.baseline2	1	384.8	384.8	1.90	0.172
baseline1.treatmenttime.baseline2	1	160.2	160.2	0.79	0.377
baseline1.baseline2.swap	1	396.0	396.0	1.95	0.166
baseline1.baseline2.day	3	204.6	68.2	0.34	0.799
baseline1.treat.treatmenttime.baseline2	1	195.1	195.1	0.96	0.330
baseline1.treat.baseline2.swap	1	196.5	196.5	0.97	0.328
baseline1.treatmenttime.baseline2.swap	1	43.7	43.7	0.22	0.644
baseline1.treat.baseline2.day	3	184.8	61.6	0.30	0.822
baseline1.treatmenttime.baseline2.day	3	68.0	22.7	0.11	0.953
baseline1.baseline2.swap.day	3	126.9	42.3	0.21	0.890
baseline1.treat.treatmenttime.baseline2.swap	1	146.7	146.7	0.72	0.398
baseline1.treat.treatmenttime.baseline2.day	3	406.8	135.6	0.67	0.574
baseline1.treat.baseline2.swap.day	3	167.1	55.7	0.27	0.843
baseline1.treatmenttime.baseline2.swap.day	3	970.1	323.4	1.60	0.198
baseline1.treat.treatmenttime.baseline2.swap.day	3	655.3	218.4	1.08	0.364
Residual	75	15200.8	202.7		
Total	113	44979.2			

Table B.A.1-2: influence of ethylene treatment on respiration and ethylene production of avocado fruit cv. Hass from South Africa (Appendix A).**Table B.A.1: CO₂**

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
baseline	1		26739.7	26739.7	147.97	<.001
baseline.days	3		4991.0	1663.7	9.21	<.001
baseline.treatment	9		5556.8	617.4	3.42	0.001
baseline.days.treatment	18		4233.3	235.2	1.30	0.205

Residual	95	(1)	17167.6	180.7
Total	126	(1)	58618.2	

Table B.A.2: Ethylene

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
baseline	1		6973.7	6973.7	64.51	<.001
baseline.days	3		2987.6	995.9	9.21	<.001
baseline.treatment	9		1274.1	141.6	1.31	0.242
baseline.days.treatment	18		2230.6	123.9	1.15	0.322
Residual	94	(2)	10162.0	108.1		
Total	125	(2)	23523.6			

Table B.A.3-5: influence of ethylene treatment on colour changes of avocado fruit cv. Hass from South Africa (Appendix A).**Table B.A.3: Chroma**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
blocks stratum					
baseline	1	2.35	2.35	0.05	0.839
Residual	5	257.36	51.47	1.56	
blocks.*Units* stratum					
baseline	1	2181.38	2181.38	66.05	<.001
days	4	397.03	99.26	3.01	0.019
blocks.baseline	3	81.07	27.02	0.82	0.485
blocks.baseline.days	18	651.93	36.22	1.10	0.357
Residual	223	7364.39	33.02		
Total	255	10935.52			

Table B.A.4: Hue angle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
blocks stratum					
baseline	1	8164.60	8164.60	35.52	0.002
Residual	5	1149.25	229.85	2.33	
blocks.*Units* stratum					
baseline	1	43280.28	43280.28	439.26	<.001
days	4	54090.44	13522.61	137.24	<.001
blocks.baseline	3	266.31	88.77	0.90	0.441
blocks.baseline.days	18	2304.14	128.01	1.30	0.190
Residual	223	21972.24	98.53		
Total	255	131227.25			

Table B.A.5: Lightness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
blocks stratum					
baseline	1	9.802	9.802	0.71	0.437

Residual	5	68.839	13.768	3.65	
blocks.*Units* stratum					
baseline	1	704.613	704.613	186.70	<.001
days	4	459.420	114.855	30.43	<.001
blocks.baseline	3	60.937	20.312	5.38	0.001
blocks.baseline.days	18	85.634	4.757	1.26	0.216
Residual	223	841.599	3.774		
Total	255	2230.845			

Table B.A.6: Influence of ethylene treatment on firmness changes of avocado fruit cv. Hass from South Africa (Appendix A).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
blocks stratum					
baseline	1	19714.3	19714.3	40.53	0.001
Residual	5	2432.1	486.4	1.71	
blocks.*Units* stratum					
baseline	1	244816.1	244816.1	862.73	<.001
days	4	3093.7	773.4	2.73	0.030
blocks.baseline	3	7913.3	2637.8	9.30	<.001
blocks.baseline.days	18	956.1	53.1	0.19	1.000
Residual	223	63280.6	283.8		
Total	255	342206.1			

Table B.A.7: Influence of ethylene treatment on weight changes of avocado fruit cv. Hass from South Africa (Appendix A).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
blocks stratum					
baseline	1	129.9	129.9	0.03	0.863
Residual	5	19741.4	3948.3	26.62	
blocks.*Units* stratum					
baseline	1	400.3	400.3	2.70	0.102
days	4	3082.0	770.5	5.19	<.001
blocks.baseline	3	5209.7	1736.6	11.71	<.001
blocks.baseline.days	18	2274.0	126.3	0.85	0.638
Residual	223	33078.6	148.3		
Total	255	63916.1			

Table B.A.8-9: Influence of commercial ethylene treatment on respiration and ethylene production of avocado fruit cv. Hass from South Africa (Appendix A).

Table B.A.8: CO₂

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
jars stratum	3	144.0	48.0	0.24	
jars.*Units* stratum					
day	4	34689.5	8672.4	43.24	<.001

position	3	1039.4	346.5	1.73	0.172
day.position	12	2641.9	220.2	1.10	0.380
Residual	57	11431.6	200.6		
Total	79	49946.4			

Table B.A.9: Ethylene

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
jars stratum	3		371.3	123.8	0.90	
jars.*Units* stratum						
day	4		10666.4	2666.6	19.43	<.001
position	3		841.4	280.5	2.04	0.118
day.position	12		1441.6	120.1	0.88	0.576
Residual	56	(1)	7685.1	137.2		
Total	78	(1)	20815.4			

Table B.A.10-12: Influence of commercial ethylene treatment on colour changes of avocado fruit cv. Hass from South Africa (Appendix A).**Table B.A.10: Chroma**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block	3	125.995	41.998	6.29	<.001
day	4	2388.087	597.022	89.35	<.001
block.day	12	132.778	11.065	1.66	0.083
Residual	140	935.408	6.681		
Total	159	3582.267			

Table B.A.11: Lightness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block	3	65.604	21.868	6.68	<.001
day	4	941.831	235.458	71.94	<.001
block.day	12	115.166	9.597	2.93	0.001
Residual	140	458.206	3.273		
Total	159	1580.809			

Table B.A.12: Hue angle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block	3	1034.66	344.89	3.54	0.016
day	4	68049.35	17012.34	174.59	<.001
block.day	12	1371.08	114.26	1.17	0.308
Residual	140	13641.66	97.44		
Total	159	84096.76			

Table B.A.13: Influence of commercial ethylene treatment on firmness changes of avocado fruit cv. Hass from South Africa (Appendix A).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block	3	2162.8	720.9	1.79	0.151
day	4	245701.3	61425.3	152.78	<.001
block.day	12	7944.3	662.0	1.65	0.085
Residual	140	56286.4	402.0		
Total	159	312094.8			

Table B.A.14: Influence of commercial ethylene treatment on weight of avocado fruit cv. Hass from South Africa (Appendix A).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block	3	16966.88	5655.63	70.43	<.001
day	4	2403.46	600.87	7.48	<.001
block.day	12	5508.87	459.07	5.72	<.001
Residual	140	11242.02	80.30		
Total	159	36121.24			

Table B.A.15-16: Influence of experimental ethylene treatment on respiration and ethylene production of avocado fruit cv. Hass from South Africa (Appendix A).

Table B.A.15: CO₂

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
jars stratum	3	144.0	48.0	0.24	
jars.*Units* stratum					
day	4	34689.5	8672.4	43.24	<.001
position	3	1039.4	346.5	1.73	0.172
day.position	12	2641.9	220.2	1.10	0.380
Residual	57	11431.6	200.6		
Total	79	49946.4			

Table B.A.16: Ethylene

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
jars stratum	3		371.3	123.8	0.90	
jars.*Units* stratum						
day	4		10666.4	2666.6	19.43	<.001
position	3		841.4	280.5	2.04	0.118
day.position	12		1441.6	120.1	0.88	0.576
Residual	56	(1)	7685.1	137.2		
Total	78	(1)	20815.4			

Table B.A.17-19: Influence of experimental ethylene treatment on colour changes of avocado fruit cv. Hass from South Africa (Appendix A).

Table B.A.17: Chroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	2	147.25	73.63	1.14	0.322
days	4	1331.09	332.77	5.17	<.001
treatment.days	8	628.78	78.60	1.22	0.294
Residual	105	6757.74	64.36		
Total	119	8864.87			

Table B.A.18: Hue angle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	2	133.98	66.99	0.78	0.459
days	4	67972.31	16993.08	199.03	<.001
treatment.days	8	282.01	35.25	0.41	0.911
Residual	105	8964.78	85.38		
Total	119	77353.08			

Table B.A.19: Lightness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	2	30.502	15.251	3.22	0.044
days	4	587.646	146.912	31.04	<.001
treatment.days	8	64.692	8.087	1.71	0.105
Residual	105	496.905	4.732		
Total	119	1179.745			

Table B.A.20: Influence of experimental ethylene treatment on weight changes of avocado fruit cv. Hass from South Africa (Appendix A).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	2	2342.9	1171.5	5.15	0.007
days	4	3648.3	912.1	4.01	0.005
treatment.days	8	2907.1	363.4	1.60	0.135
Residual	105	23905.6	227.7		
Total	119	32804.0			

Table B.A.21: Influence of experimental ethylene treatment on firmness of avocado fruit cv. Hass from South Africa (Appendix A).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	2	2196.3	1098.1	2.33	0.102
days	4	155498.4	38874.6	82.57	<.001
treatment.days	8	3827.6	478.4	1.02	0.429
Residual	105	49435.3	470.8		
Total	119	210957.6			

Appendix C

C.1 Conference proceedings and publications

- Oral presentation-**Manuela Donetti** and Leon A. Terry, “Investigation of skin colour changes as a indicator of fruit ripeness of avocado fruit cv. Hass”, **IV Postharvest Unlimited, 23-26 May 2011, Leavenworth, WA ,USA.**
- Oral presentation-**Manuela Donetti** and Leon A. Terry, “Evaluation of factors affecting shelf-life and quality biomarkers of imported avocado fruit”, **28th International Horticultural Conference, 22-27 August 2010, Lisbon, Portugal.**
- Oral presentation-**Manuela Donetti** and Leon A. Terry, “The influence of season and origin on physiology and biochemical composition of imported avocado cv. Hass fruit”,
- **From Field to Fork: How to improve the quality of fruits and vegetables, 17th October 2009, Cranfield University, UK.**
- Oral presentation-**Manuela Donetti** and Leon A. Terry, “Influence of season and origin on ripening of imported avocado cv. Hass fruit”, **The 8th International Symposium on the Plant Hormone Ethylene, 21-25 June 2009, Cornell University, Ithaca, New York, USA.**
- Oral presentation-**Manuela Donetti** and Leon A. Terry, “Bioactive profile of imported avocado fruit during ripening”, **III International Symposium on Human Health Effects of Fruits and Vegetables, FAVHEALTH, 18-21 October 2009, Avignon, France.**

Seasonal effects on postharvest quality of imported Spanish avocado cv. Hass

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Background

Avocado is a tropical and subtropical fruit characterised by a high oil concentration in mesocarp tissue. Previous work has already investigated the influence of picking time on the ripening process and on fatty acid composition^{1,2} mainly in fruits coming from overseas. In Europe Spain is the main avocado producing country. No comparison of seasonal variation has been conducted for Spanish avocado fruit. Accordingly, the aim of this work was to elucidate the influence of picking date on the ripening process as well as on the fatty acid composition of avocado cv. Hass fruit grown in the Spanish region of Malaga.

Materials and Methods

Three different commercial seasons (early, middle and late) of avocado cv. Hass fruit were collected in February, March and April 2008 from the same orchard in the region of Malaga. The fruit were imported to the UK by Mack Multiples Division and stored at 5°C for no longer than 9 days after picking as per the standard commercial practice. Ripening was induced by keeping the fruit at 18°C for 7 days. Objective colour (lightness L*, chroma C* and hue angle H°), firmness, respiration rate and ethylene production were measured at regular intervals (day 0, 2, 4 and 7). The softening process of the mesocarp was evaluated as the decrease in firmness measured with an Instron Universal Testing Machine (Model 1122, Bucks., UK) as described previously³. Respiration rate and ethylene were measured by placing two fruits in sealed glass jars for 2 hours at 20°C. Gas samples were analysed using gas chromatography. Oil from freeze-dried samples was extracted and analysed for fatty acid composition⁴.

Results

➤ Mesocarp firmness, as determined by the maximum load (N), in late season fruit held at 18°C decreased in the first 4 days. In all the seasons values under 100 N were reached after 2 days. Late season fruits were initially less firm than fruit picked in earlier seasons, and softened faster (Fig. 1). As a consequence of ripening, fruit cv. Hass skin turned from green to deep purple, with a decrease in colour parameters in all seasons (Fig. 5).

➤ In all seasons the increase in the respiration rate (Fig. 3) and climacteric behaviour (Fig. 4) was detected within the first 4 days of ripening. Early and late season fruit had similar patterns, with peaks observed at day 2. Middle season fruit reached a maximum respiration rate at day 4 and higher values in ethylene production were recorded during the climacteric peak (Figs. 3, 4).

➤ Oil was extracted from freeze-dried pulp⁴. Differences were detected in fatty acid composition between seasons, with a decrease in palmitic (16:0) acid and increase in oleic (18:1) acid from early to middle/late season (Fig. 2).

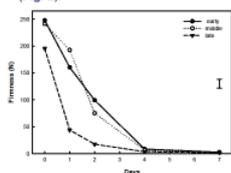


Figure 1. Firmness (N) values in early, middle and late season fruit during ripening.

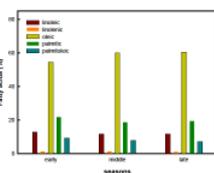


Figure 2. Change in fatty acid (%) in oil extract from freeze-dried avocado pulp during seasons.

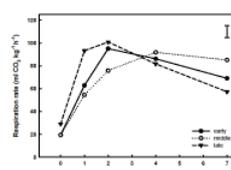


Figure 3. Respiration rate (ml kg⁻¹ h⁻¹) of avocado cv. Hass fruit during 7 days at 18°C.

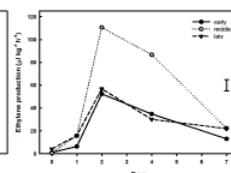


Figure 4. Ethylene production (µl kg⁻¹ h⁻¹) of avocado cv. Hass fruit during 7 days at 18°C.

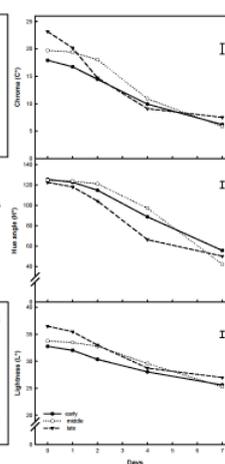


Figure 5. Change in colour parameters (L*, C* and H°) during 7 days at 18°C.

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Conclusion

✓ Early and late season fruit showed similar patterns for respiration and climacteric behaviour. Fruits picked during the middle season reached a higher rate of ethylene production, but similar respiration rates compared to those harvested during early and late season.

✓ Late season fruits were less firm after storage compared to fruits from earlier seasons. The softening process was accelerated faster in fruits picked later in the year with decrease in their shelf life.

✓ Fatty acid analysis indicated a change in oil composition, mainly between early and later seasons. Results showed a significant increase in the healthy components monounsaturated oleic acid and polyunsaturated linoleic acid, and a decrease in palmitic acid.

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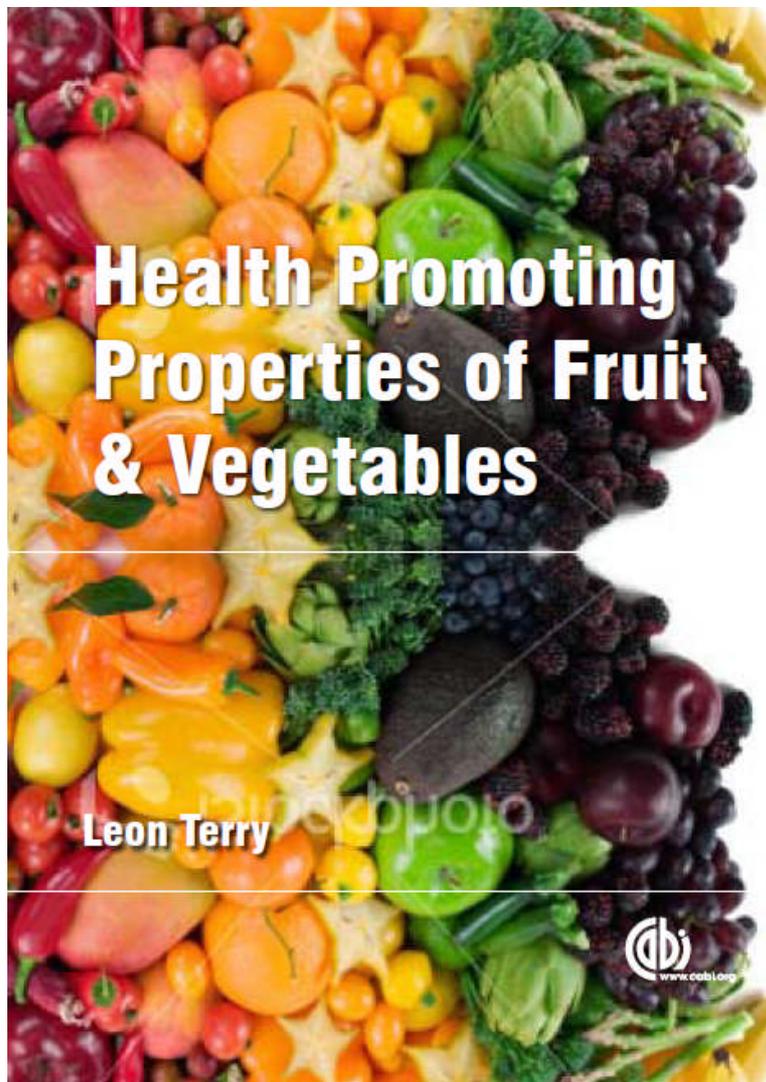
Papers in submission:

- Variations in the ripening and biochemistry of imported avocado cv. Hass fruit from different origins and maturity. Manuela Donetti and Leon A. Terry (Food Research International).

- **Influence of fruit maturity in the relationship between skin colour changes and mesocarp softening of imported avocado fruit cv. Hass.** **Manuela Donetti** and Leon A. Terry (Horticultural Science and Biotechnology).

Book chapter

Marjolaine D. Meyer, Dr. Sandra Landahl, **Manuela Donetti** and Dr. Leon A. Terry (*in press*). Avocado. In: Terry, L.A. (Ed.), Health promoting properties of fruits and vegetables, CABI publishing, Wallingford, UK.



Spain late



South Africa early



Peru late



Chile early

