Department of Environment and Agriculture

Mechanical harvesting, fruit and oil quality in olives influenced by harvest time and exogenous application of ethylene

Bassam Fares Alowaiesh

This thesis is presented for the degree of

Doctor of Philosophy

of

Curtin University

August 2015

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: _____ Date: _____

Dedicated

To my father (Fares)

My mother (Fatimah)

My wife (Qammar)

My children (Latifah, Eyad and Ibrahim)

My brothers and my sisters

For their endless love, support and encouragement

Acknowledgements

I would like to express my deep gratitude to Professor Zora Singh, Foundation Professor of Horticulture, Department of Environment and Agriculture, Curtin University, Perth, Western Australia, for his valuable advice and assistance and for his generous scholarly guidance during my research as well as thesis writing and affectionate support which was a colossal source of inspiration until I completed my PhD studies. Particularly, I am grateful to his infectious supervision and help particularly in weekends and holidays during my PhD research and thesis writing. Also I am expressing my gratitude to my associate supervisor, Professor Stan Kailis, The University of Western Australia, Crawley, Western Australia, for his help during my research and for critically reading my thesis. I sincerely acknowledge my profound gratitude to Dr. Zhongxiang Fang, Senior Research Fellow, School of Public Health, Curtin University, Perth, Western Australia for help in various oil quality analyses and for critically reading the manuscript. I sincerely acknowledge financial support from the Food and Agriculture Organization of the United Nations (FAO) along with the Ministry of Agriculture in Saudi Arabia (Cairo and Riyadh offices) during the whole study period. In addition, I gratefully thank the Department of Environment and Agriculture, Curtin University, Western Australia for all support., I am also thankful to Mr. Haitham Naser, Royal Scientific Society, Amman, Jordan for help in various oil quality analyses. I would like also to acknowledge Ms. Susan Petersen, Manager, Horticulture Research Laboratory for her technical support and assistance during my laboratory work. I take this opportunity to express my thanks to Mr. Frederik Altenstadt, Talbot Grove for generously providing olive trees and fruit for all my experiments. I also wish to thank my lab-mates Dr. Mubarak Alrashedi, Dr.Shamim Khan, Dr. Zahoor Hussain, Youssef Azzu, Muneer Rehman, Sabah abdulgani, Mekhala Vithana, Mehwish Yaseenand and my friend, Mr. Suliman Alsawi, for their help and support during my stay in Australia. I am also thankful to my relatives, well-wishers and friends for their good wishes and for their encouragement and moral support, which boosted my morale to fly high to accomplish my goals. I am thankful to Allah Almighty, without His help it would have been impossible to complete my research and thesis.

Bassam Alowaiesh

Abstract

Manzanilla and Frantoio olive cultivars are highly productive, produce quality fruit and oil and are widely cultivated in various olive growing countries in the world including Australia. Information is very limited on the effects of different factors, especially the growth changes in ripening fruit, effect of harvesting time, concentration and application time of ethephon on the quality attributes of cvs. Frantoio and Manzanilla olives. Considering this view, the current study was conducted on 15 year old fully productive, self-rooted, olive trees during 2013 and 2014 in the same grove to observe the growth and development of olive fruit, to explore the effects of different harvesting times, different concentrations and time of application of ethephon on the physical, biochemical and sensory attributes of cvs. Frantoio and Manzanilla olives grown in south-western Australia. All experimental trees received supplementary irrigation depending upon rainfall. The physical parameters such as fruit weight, fruit volume, fruit length, fruit width, pulp weight, stone weight, pulp/stone ratio and fruit ripening index increased significantly ($P \leq 0.05$) until 150 to 175 days after full bloom with the progress of the growth and development period, irrespective of the cultivar. The cv. Manzanilla showed higher average values for these parameters and they were also higher in 2013 than in 2014. The physiological parameters (production of ethylene and respiration) and fruit firmness declined significantly with the progress of fruit growth. Ethylene peak was observed after 190 days after full bloom. The respiration peak appears after 175 days in cv. Manzanilla and after 190 days of full bloom in cv. Frantoio in both years, and cv. Frantoio showed significantly higher respiration rate than cv. Manzanilla.

Manzanilla cv. showed higher fruit removal force, moisture content (%) and oil content (% dry weight) than cv. Frantoio irrespective of the harvesting period. Furthermore, lowest moisture and oil content were observed in the driest harvest year, 2014. The fatty acids showed significant increase (free fatty acid, palmitic acid, stearic acid, linoleic acid) or decrease (peroxide value, oleic acid, MUFA, PUFA and MUFA:PUFA ratio) with the delay of harvesting from first (mid-April) to fifth (mid-June) periods in both of the years, irrespective of the cultivar. A significant gradual decrease was noted in major polyphenol compounds from first to fifth harvest. The concentration of phenolic compounds was comparatively high in the fruit harvested in 2014. The sensory attributes deteriorated with the delay of harvesting. Water stress possibly influenced the bitterness in the fruit in 2014.

Application of single spray of ethephon significantly increased the level of ethylene production, ripening index, fruit and leaf abscission and peroxide value of olive oil with the increase of applied ethephon concentration in comparison to the control treatment. Ethephon treatments also significantly increased the level of most of the fatty acids, however, oleic acid, MUFA and MUFA/PUFA ratio decreased with the increase of ethephon concentration. Concentration of different polyphenols (hydroxytyrosol, tyrosol, oleuropein, and total polyphenol) and levels of sensory attributes (fruitiness, bitterness and pungency) decreased significantly with the increase of ethephon concentration. Effect of ethephon on the fruit moisture (%) and oil (% fresh and dry weight basis) content of the olive fruit were non-significant. Among the applied concentrations of ethephon, 1000 to 2000 mg L^{-1} in 2013 and 1000 to 1500 mg L^{-1} in 2014 did not show significant differences for the studied parameters.

Significantly increased ripening index (RI) (4.84), fruit and leaf abscission (95.92% and 27.44% respectively), free fatty acids (0.42%), peroxide value (11.02 meqO_2 kg^{-1}), palmitic acid (13.19%), stearic acid (4.19%), linoleic acid (11.12%) and PUFA (11.60%) were observed when the olive trees were sprayed with ethephon at four weeks before harvesting. Significantly reduced phenolic compounds (3.91, 6.05 and 59.54 mg/kg^{-1} hydroxytyrosol, tyrosol and oleuropein respectively) and sensory attributes (1.74, 1.51 and 1.72 scores for fruitiness, bitterness and pungency respectively) were also noted from this treatment. However, the ethephon application periods did not differ significantly effects on the parameters.

It could be concluded, that the growth parameters increase with the progress of fruit growth until 175 days after full bloom and physiological parameters show a declining trend during this period with a peak at 175 to 190 days after full bloom. The harvesting of olive fruit during the early part of winter delivered olive oil with better attributes while climatic conditions such as water stress negatively influences the quality attributes of olive fruit. The most suitable concentration of ethephon to treat olive trees would be considered as 1000 – 1500 mg L^{-1} and the suitable period of ethephon spray to olive trees would be at least two weeks before harvesting the fruit.

Table of contents

List of figures

List of tables

List of symbols and abbreviations

×	Multiply / interaction between
+	Plus
-	Minus
>	Greater than
<	Less than
/	Interaction between
≤	Less than or equal to
±	Plus minus
/	Divide
=	Equal to
´	Minutes
~	Approximately
°	Degree
°C	Degree Celsius
%	Percent
β	Beta
P	Picomole (s)
µg	Microgram(s)
µL	Microlitre (s)
µmol	Micromole(s)
ABA	S-(+)-cis, trans-abscisic acid
ABS	Australian Bureau of Statistics
ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	Analysis of Variance
AOA	Aminooxyacetic acid
C:16:0	Palmitic acid
C:18:0	Stearic acid
C:18:1	Oleic acid
C:18:2	Linoleic acid
C:18:3	Linolenic acid
C:20:0	Archidic acid
C_2H_4	Ethylene
Co	Company

CO_2	Carbon dioxide / respiration
CoSO4	Cobalt sulphate
cv.	Cultivar
cvs.	Cultivars
d	day(s)
DAFB	Days after full bloom
DH_2O	Distilled water
DI	Deficit irrigation
EVOO	Extra virgin olive oil
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Statistics
FeSO4	Ferrous sulphate
FID	Flame Ionization Detector
FFA	Free fatty acids
FRF	Fruit removal force
FW	Fruit fresh weight
GA3	Gibberellic acid
GR	Glutathione reductase
h	Hour(s)
h°	Hue angle
ha	Hectare(s)
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
IAA	Indole-3-acetic acid
JAs	Jasmonates
kg	kilogram(s)
KOH	Potassium Hydroxide
kPa	kilo Pascals
$KMnO_4$	Potassium permanganate
L	Litre
L*	Lightness
LSD	Least significant difference
Ltd.	Limited
LOX	Lipoxygenase
MUFA	Monounsaturated fatty acid
MUFA/PUFA	monounsaturated fatty acids/ polyunsaturated fatty acids

m	metre
mm	Millimetre
M	Molar
MA	Modified atmosphere
MeOH	Methanol
mg	milligram(s)
$MgCO_3$	Magnesium carbonate
Min	minute(s)
ml	millilitre(s)
mM	millimolar(s)
mmol	millimole(s)
Mt	Metric tonnes
N	Newton
NA	Not available
NAA	Naphthalene acetic acid
NaCl	Sodium chloride
NaF	Sodium fluoride
$NaHSO_3$	Sodium hydrogen sulphite
NaOH	Sodium hydroxide
NaOCl	Sodium hypochlorite
N_a2SO_4	Sodium sulphate(s)
Nl	nanolitre(s)
Nmol	nanomole(s)
NO	Nitric oxide
ns	Not significant
NSW	New South Wales
O_2	Oxygen
PUFA	Polyunsaturated fatty acids
p.s.i.	Pounds per square inch
PGRs	Plant growth regulators
pH	Symbol denoting hydrogen ion in a solution
PL	Pectin Lyase
POD	Peroxidase
PP	Polyphenols
PPO	Polyphenol oxidase
ppb	Parts per billion (10^{-9})

ppm	Parts per million (10^{-6})
PV	Peroxide value
PVP	Polyvinylpyrolidone
r	Correlation coefficient
rcf	Relative centrifugation force
RH	Relative humidity
RDI	Regulated deficient irrigation
ROS	Radical oxygen species
rpm	Rounds per minute
S	South
s	second(s)
SAM	S-adenosyl methionine
S.E.	Standard error
sp	Species
SPD	Spermidine
SPM	Spermine
USA	United States of America
UV	Ultra-violet
VOO	Virgin olive oil
VIC	Victoria
v/v	volume by volume
WA	Western Australia
WAFB	Weeks After Full Bloom
WPH	Weeks Prior Harvest
w/v	weight by volume
w/w	weight by weight

Chapter 1

General Introduction

Olive (*Olea europaea* L.) is a member of the Oleaceae family which is considered as monophyletic on the basis of several morphological synapomorphies. The olive trees have opposite, simple or compound leaves without stipules. The flowers are hypogenous and tetramerous, with two stamens. The corolla is actinomorphic and usually sympetalous (Wallander and Albert, 2000). Olive is one of the most important and widely grown fruit trees in the Mediterranean basin which is an emblematic species (Loumou and Giourga, 2003). Domestication of olive may have occurred in many places around the Mediterranean basin and at different times, however early evidence suggests the Levant coast some 8000 years ago. Also olive cultivation is believed to have taken place on the island of Crete during the Minoan period around 1500–3000 BC (Riley, 2002). Currently olive is one of the most extensively cultivated fruit crops in the world and its cultivation area has tripled in the past 45 years, from 2.6 to 8.6 million hectares. About 73% of the global olive oil production comes from European Union countries where Spain, Italy, and Greece contribute 97% of European production and Spain alone accounts for more than 40% of the world's olive oil production (Conde et al., 2008). Olive is grown over an area of 10.31 million ha in the world with about 95% in the Mediterranean basins (FAOSTAT, 2014) such as Southern Australia, Americas, South Africa and part of New Zealand (Kailis and Harris, 2007).

Olive is one of the oldest cultivated fruit from which oil has been extracted. It provides perfect balance of aroma, taste, flavour and health benefits (La Lastra et al., 2001). Olive was introduced in Australia as early as 1800 with the start of European settlement (Spenemann, 2000). In Australia, there are around 10 million olive trees grown on over 800 orchards and spread over an area of 30,000 hectares. About 90% of olive oil in Australia is produced from 10 major cultivars of olive, which are Coratina, Picual, Arbequina, Frantoio, Barnea, Leccino, Corregiola, Manzanilla, Koroneiki, and Pendolino, and the major olive production areas are North-central Victoria and north of Perth, Western Australia with an estimated 70% of Australia's current production (Anon, 2010). In Western Australia, nearly 2

million olive trees were counted in 2007 and these produced 1.4 tonnes of oil in 2006 (Department of Agriculture and Food WA, 2007). Export of olive oil from Australia has increased sharply from 500 tonnes in 2003 to 8000 tonnes in 2010. On the other hand, Australian import of olive oil stabilised in the last decade with 30,000 tonnes in 2000 and an estimated 29,000 tonnes in 2010. Australian extra virgin olive oil production is valued at around $AUD 68.4 M in 2010 (IOC, 2010). Extra virgin olive oil represents 95% of the whole Australian olive oil production and is considered top quality having gained awards in national and international competitions. Moreover, olive production value in retail markets is estimated at over $185 M' in 2009 (Anon, 2010). Among the cultivated olives in Australia, Frantoio and Manzanilla are two major cultivars originating in Italy and Spain respectively. Frantoio produces heavy crops of small olives with a very high oil content which is well known as the benchmark for olive oil in Italy. It is an extremely adaptable crop to diverse and harsh climatic conditions and is an excellent source of quality oil. The fresh oil from cv. Frantoio is quite strong/bitter and is therefore used widely often in Tuscan extra virgin olive oil and as a blending oil to increase the flavour and shelf life of less distinct cultivars. cv. Manzanilla is a medium sized olive with a medium to high oil content. It is well known as a green pickling fruit, though considered as the best dual purpose olive cultivar worldwide. cv. Manzanilla gives a heavy yield in mild climates and due to size and its firm flesh it processes easily (http://www.oliveaustralia.com.au).

Generally, olive oil and table olives contain fatty acids, polyphenols, tocopherols, Vitamin E, sterols and carotenoids and minerals which are considered to benefit human health when consumed (La Lastra et al., 2001). Intake of olive oil reduces harmful cholesterol of Low Density Lipoprotein (LDL) (Psaltopoulou et al., 2004) and this is related withe contain of antioxidant (polyphenols and tocopherols) and oleic acid in olive oil. Furthermore ,oleic acid is the major olive oil fatty acid, is monounsaturated fat and occupies around 55% to 83% of total fatty acids in virgin olive oil (VOO) the IOC stander range (Aparicio, 1999; Beltran, 2000; Mailer et al., 2005) depending on cultivar, growing conditions and possibly technological intervention. Total polyphenols in olive oil ranges between 50-1000 mg L^{-1} (Salvador et al 2001; Aguilera et al., 2005; Youssef et al., 2010). Phenolic antioxidants present in extra virgin olive oil are potent inhibitors of oxidation and

reduce cancer risk (Owen et al., 2000). The o-diphenol family has been identified as the major antioxidant component and maintains the sensorial properties of extra virgin olive oils (Lavelli, 2002). The concentration of health benefit compounds in virgin olive oil is affected by different agro-ecological factors and cultural practices including cultivar, maturity stage, location, soil, irrigation systems, environmental factors, production and oil extraction process (Nergiz, 2000; Ranalli et al., 2001; Patuumi et al., 2002; Kalua et al., 2005; Baccouri et al., 2008; Dag et al., 2011).

Quality indexes and fatty acid composition of the oil are mostly affected by maturity stage at harvest of olive fruit (Dabbou et al., 2011). Early ripening fruit contain significantly higher amounts of oil (%) (Salvador et al., 2001; Lavee and Wodner, 2004), though crop year and maturation phases of olive fruit affect the amount of total polyphenols in olive oil (Anastasopoulos et al., 2011). Maximum oil content was noted between the 60^{th} and 75^{th} day after the start of the maturation process (Camposeo et al., 2013). Lavee and Wonder (2004) reported uniform oil content in the mesocarp of black matured fruit of Barnea and Manzaillla cultivars. Beltran et al., (2004) also observed similar results and reported that the oil content may vary due to climatic conditions such as lower rainfall which may cause lower oil and higher dry matter content in olive fruit. Reduction in the value of peroxide, pigments, sensory scores, oleic acid and total sterols; and increase in the free fatty acids and linoleic acid were observed during ripening of cv. Cornicabra olive (Salvador et al., 2001).

Cultivation of olive oil is a labour and cost intensive task where harvesting of olive fruit consumes 50–80% of the total cost of production (Metzidakis, 1999). In many countries the harvesting of olive is done by hand over a period of two months. The ratio between fruit mass and pedicel's strength of olive fruit is relatively small as compared with other fruits and thereby a large force is required to remove the fruit by hand or be shaken off with a mechanical harvester (Ben-Tal and Wodner, 1994). To minimise the cost of olive production, mechanical harvesting systems combined with use of abscission inducing agent has gained popularity (Burns et al., 2005). Ethephon has been used to promote fruit abscission for easy picking or mechanical fruit harvesting of cherries (Bukovac et al., 1969), apple (Edgerton, 1968), olive (Hartmann et al., 1970), citrus (Young and Jahn, 1972), macadamia (Kadman and

Ben-Tal, 1983) and many others (Kays and Beaudry, 1987). Ethephon is a synthetic plant growth regulator which releases ethylene when it penetrates into plant tissues (Royer et al., 2006) and promotes pedicel's loosening to enable easy mechanical harvesting of olive fruit (Martin et al., 1981; Denney and Martin, 1994; Metzidakis, 1999). However, a considerable loss of leaves is coupled with fruit loosening due to the effect of applied ethephon (Burns et al., 2008). To maintain the balance between fruit and leaf drop, researchers trialled alteration of doses, application timing and duration of ethephon (Lang and Martin, 1985, 1989). However, the results were unpredictable and variable in field conditions (Martin, 1994). Touss et al. (1995) applied different concentrations of ethephon and found that 1250 and 1875 mg L^{-1} ethephon applied on conventional Arbequina olive trees increased the yield from mechanical harvesting by 20%. Additionally, ethephon also increased the amount of harvested fruit without significantly enhancing pre-harvest leaf drop and did not adversely affect flowering in the subsequent year and it showed little effect on oil acidity, peroxide value, and oil fatty acid composition. The extent of ethylene penetration into the plant cells and rate of ethylene evolution from the decomposition of ethephon depends on the growth stage of the fruit and ambient conditions including temperature and relative humidity (Flore and Bukovac, 1982; Olien and Bukovac,1978, 1982; Kays and Beaudry, 1987; Beaudry and Kays, 1987). Application of ethephon (1250 mg L^{-1}) was found to be effective in reducing the fruit removal force (FRF) (maximum of 73%) while the olive trees were treated two weeks before harvesting (El-Tamzini et al., 1980). However, Touss et al. (1995) reported non-significant effect of different concentrations of ethephon treatments when applied 12 days before harvest onto the trees of mechanically harvested Arbequina olives.

Olive oil from the major cultivars grown in Australia does not meet some of the chemical limits set by international standards (Mailer et al., 2010). There are limited studies reported from New South Wales (NSW), Australia, where it has been claimed that the olive fruit oil content increases rapidly until fruit maturity with maximum oil level differing with cultivar (Mailer et al., 2007; Zeleke et al., 2012). Significant effect of cultivation year and harvesting time on total polyphenols, chlorophyll concentration, palmitic acid and linoleic acid in oil from the olives grown in NSW, Australia, was also reported by Ayton et al. (2007) and Obied et al.

(2008). There is an enormous potentiality and economic importance of growing olives in Western Australia (WA) (Kailis, 1999). However, no information is available on fruit maturation stage, chemical composition and properties of the olives and extracted oil according to the ripening stages of the fruit grown in WA conditions characterised by a long, hot summer and a cold-wet winter. Olive fruit is a non-climacteric and the role of ethylene in olive fruit growth development and maturation has not yet been investigated in WA. Information is also scarce on the response of Frantoio and Manzanilla olive to different ethephon concentrations and time of application in Western Australian conditions. Information on the effects of ethephon on olive oil composition is also limited (Cimato, 1988). Considering these views, the current study was conducted to investigate the growth, development and maturation of cvs. Frantoio and Manzanilla olives grown under south-western Australian conditions. It also aimed to facilitate mechanical harvesting by underpinning the effects of different harvesting times/stages of ripening, different concentrations and time of application of ethephon on physical (fruit removal force, fruit moisture and oil content), biochemical (level of fatty acids and polyphenols) and sensory attributes (fruitiness, bitterness and pungency) of cvs. Frantoio and Manzanilla olives in south-western Australian conditions.

Therefore, investigations were conducted with the following objectives

1. To investigate fruit growth, development and maturation in relation to endogenous ethylene production in cvs. Frantoio and Manzanilla olives grown in south-western Australian conditions.
2. To evaluate the effects of different harvesting times on physical, biochemical and sensory attributes of cvs. Frantoio and Manzanilla olives and oil in south-western Australian conditions.
3. To underpin the effects of different concentrations and application time of ethephon on physical, biochemical and sensory attributes of cvs. Frantoio and Manzanilla olives and oil in south-western Australian conditions.

Chapter 2

General Literature Review

2.1. Introduction

Olive (*Olea europaea* L.) is native to the Mediterranean region, central Asia and some parts of Africa. It is a member of the Olea genus which includes 30-35 species under the Oleaceae family. There are more than 2600 olive cultivars (Therios, 2009). Archaeological excavation has discovered that there are olive leaf fossils dating from the Stone Age eras (37,000BC) on Santorini Island, Greece (Therios, 2005). The native olive, *Olea europaea* L. ssp. europaea (var. sylvestris), and the cultivated olive *Olea europaea* L. ssp. europaea (var. sativa), are the main olive species in Mediterranean regions (Breton et al., 2006). Olives grow and fruit well under Mediterranean climates and in similar conditions such as experienced in southern Australia, The Americas, South Africa and part of New Zealand (Kailis and Harris, 2007).

The olive tree is a medium sized, long-lived evergreen tree which can grow up to a height of 15-20 m. It has a cylindrical trunk with an uneven surface bearing numerous swellings. The wood is a yellowish colour with darker features towards the centre of the trunk and the growing tips are characterized by apical dominance. Leaves are grey-green in colour and associated with each leaf is an axial bud that can develop into vegetative growth with some induced to become inflorescences after a winter chilling period. Each inflorescence contains 15-30 small white flowers that emerge in the spring. These flowers can be self and/or cross pollinated (Martin, 1994). The olive fruit is a drupe, oval in shape consisting of epicarp (peel -1.0-3.0%), mesocarp (pulp- 70-80%) and endocarp (pit-10-27%). Collectively the pericarp consists of the skin and pulp. The young fruits are green in colour and become purplish-black through to the stone when completely ripe. The size of olive fruit is variable depending on cultivar, soil fertility, available water, fruit load and cultural practices (Therios, 2009). For oil extraction, the olive harvest time is determined by the pattern of oil accumulation that changes with growing conditions (Inglese et at., 1996).

Olive oil is one of the oldest produced edible oils which provide perfect balance of aroma, taste, flavour, nutritional and health benefits. Olive fruit are rarely consumed directly from the tree because of their extreme bitterness due to oleuropein but are readily processed into edible products such as virgin olive oil or table olives (Menz and Vriesekoop, 2010).

Among the vegetable oils, virgin olive oil is unique as it contains high levels of antioxidants, such as polyphenols and vitamin E derivatives as well as numerous unsaturated fatty acids attributing to beneficial nutritional and health effects when consumed (Visioli and Galli, 1998; Menz and Vriesekoop, 2010) especially with a Mediterranean type diet. Moreover, table olives and olive oil provide additional nutrients, beta carotene, minerals and dietary fibre. The oil fraction of olives and olive oil contain a high level of monounsaturated fatty acids (up to 83% total of fat) and are rich in antioxidants that help to prevent body cell aging (Reichelt and Burr, 2000). Intake of olive oil reduces harmful low-density lipoprotein (LDL) without reduction in beneficial high-density lipoprotein (HDL) (Psaltopoulou et al., 2004). Extra virgin olive oil contains numerous phenolic antioxidants which are potent inhibitors of oxidation that have been reported to reduce the cancer risk (Owen et al., 2000). The physical, chemical, biochemical and physiological parameters of olive fruit and oil are largely determined by different factors including cultivar, growth stage of fruit or harvesting time, environmental factors, cultural practices and oil processing methods (Ayton et al., 2001). This review, relevant to the research in this thesis will focus on the growth and development habit of olive fruit, effect of harvesting time and application of an abscission agent (ethephon) on physical, biochemical and sensory attributes of olive fruit and oil.

2.2. Global olive and olive oil production and trade

The olive industry experienced buoyant growth in Australia in the past decade 2003 - 2013, the harvest area of olive rose from 2000 hectares (ha) to 42000 hectares and the olive production from 3000 to 94000 tonnes respectively (Fig. 2.1).

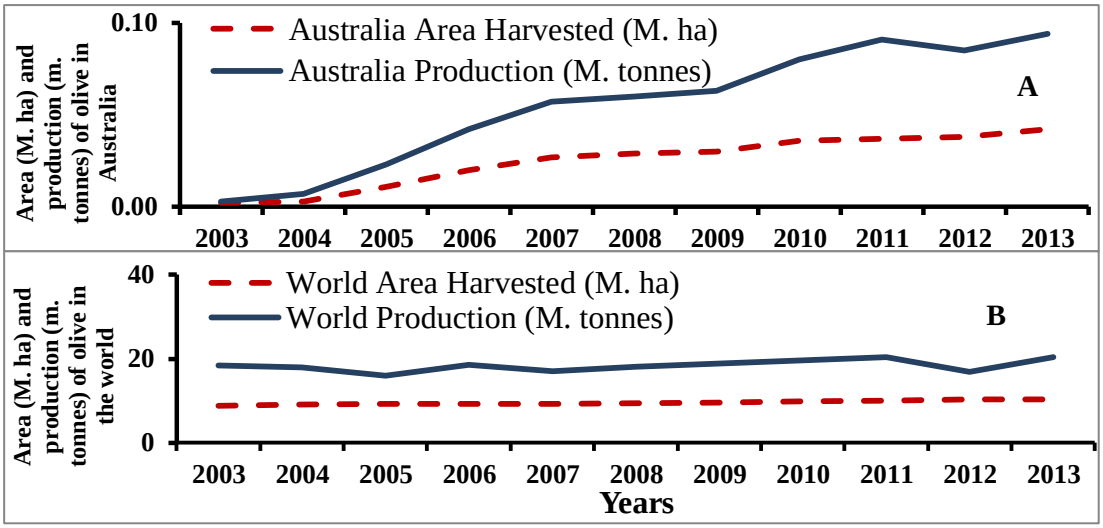


Fig. 2.1 Area (M. ha) and production (M. tonnes) of olive in Australia (A) and in the world (B) from 2003 to 2013. (FAOSTAT, 2014)

Whilst the world harvest area of olives rose from 8.85 million (M) ha to 10.31 M ha, olives production rose from 18.4 M ton to 20.39 M ton (FAOSTAT, 2014). In addition, the total olive oil production in the world in 2013 was 3.27 M ton with the global olive oil production dominated by Spain in the last five years which produce (44% of total world production), followed by Italy 15% and Greece at 10% (Fig.2.2). Moreover, The European Union produced 71.7% of total olive oil production in the world in the same period (IOC, 2014).

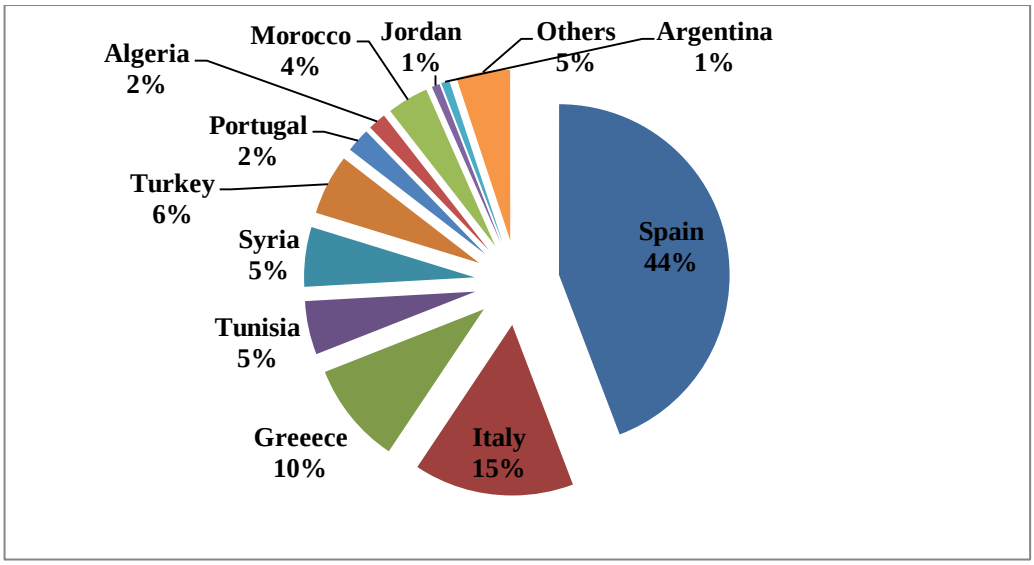


Fig.2.2. Major olive oil producing countries in the world during 2006- 2013 (IOC, 2014).

World olive oil exports have increased in the last decade from 667,500 tonnes to 817,500 tonnes. Furthermore, Spain is the main exporter of olive oil with 310,000 tonnes in 2013/2014 followed by Italy with 245,000 tonnes; Tunisia, Portugal and Turkey follow respectively (Table. 2.1). In contrast, the major importer of olive oil in the world in 2013/2014 was the United States of America (USA) with 302,500 tonnes, followed by Brazil 72,000 tonnes, Italy 70,000 tonnes, Japan, Canada follow respectively and then Australia with 28,000 tonnes in 2013/2014. In addition, worldwide consumption of olive oil has grown slowly from 2,882,500 tonnes in 2003 to 3,030,000 tonnes in 2013 (IOC, 2014).

Table. 2.1. Major olive oil exporting countries of the world during 2013/2014 (IOC, 2014).

Country	Exportation (1000 tonnes)	% Total world exportation
Spain	310.0	37.9
Italy	245.0	29.9
Tunisia	65.0	7.9
Portugal	54.3	6.6
Turkey	35.0	4.2
Syria	25	3.0
Argentina	21.5	2.6
Morocco	11.0	1.3
Chili	10.0	1.2
USA	6.0	0.7
Others	34.7	4.2
Total World	817.5	100%

2.3. Growth and development of olive fruit

Olive fruit is a drupe containing the exocarp or skin, the mesocarp or flesh, and the endocarp or pit consisting of a woody shell enclosing one or, rarely, two seeds. Mesocarp comprises 70–90% of total fruit weight followed by endocarp (9–27%) and seed (2–3%). In a mature olive fruit, the mesocarp contains about 60% water, 30% oil, 4% sugars, 3% protein, and the rest is primarily fibre and ash. The endocarp contains 10% water, 30% cellulose, 40% other carbohydrates and about 1% oil. The seed has 30% water, 27% oil, 27% carbohydrates and 10% protein (Connor and Fereres, 2005). As olives develop, they display changes in size, composition, colour, texture and flavour. The composition of chemical and biochemical elements in olive

fruit along with other physical and physiological parameters varies according to the growth phases described as follows.

2.3.1. Growth phases

The phases of olive fruit growth and development are a combination of biochemical and physiological events that vary according to cultivar and to the influence of growing conditions (Beltrán, 2000; Connor and Fereres, 2005). Olive fruit grow fast in the early stages, followed by a slower growth period and finally return again to accelerated rate of growth and thus exhibit double sigmoid growth curve (Shulman and Lavee, 1979). Growth and development occurs over 4–5 months and includes five main phases (Lavee, 1996; Manrique et al., 1999; Proietti et al., 1999) in the following order:

(i) Fertilization and fruit-set rapid cell division occurs to promote embryo growth which continues from flowering to approximately 30 d afterwards.

(ii) Seed development- due to intense cell division and enlargement rapid fruit growth occurs mainly with the growth and development of the endocarp (seed/pit).

(iii) Seed/pit hardening- fruit grows slowly as the endocarp cells stop dividing and become sclerified.

(iv) Mesocarp development- the second major period of fruit growth by the expansion of pre-existing flesh cells and intense oil accumulation.

(v) Ripening- fruit changes from dark lime green to lighter green/purple becoming soft and the pulp is purple in most cultivars.

2.3.2. Physical changes during olive fruit growth and development

The weight of olive fruit relies on crop load, cultivar, growing conditions and management practices (Beltran et al., 2004; Trentacoste et al., 2010). Fruit weight increases during the first part of the season and before seed hardening. Fruit weight then increases slightly, becomes stable or even decreases during the latter stages of ripening (Grattan, et al. 2006; Mailer et al., 2007). However, dry fruit weight continues to increase throughout the late season (Mailer et al., 2007). Weight of fruit mostly increases concurrently with progression of the harvest season until maturation (Bouaziz et al, 2004; Menz and Vieskoop, 2010). Beltran et al., (2004) found that the

olive fruit weight increases during maturation stage where around 56% of the total fruit weight is water. However, the moisture percentage of fruit decreases during the late season which reduces the total fresh fruit weight (Mailer et al., 2007). Beltran et al., (2004) studied 14 olive cultivars and found cvs. Frantoio and Picual fruit with lowest mean values for moisture.

The olive ripening stage lasts for several months and development varies depending on the region, olive variety, temperature and farming practices (Salvador et al., 2001). A number of changes observed during olive ripening include fruit weight, pulp/stone ratio, colour, oil accumulation, chemical composition, enzyme activity, fruit firmness and sensory characteristics. Optimal ripening period has been defined as the time between the first purple colouration of olive fruit and black skin for olive oil yield and for table olive purposes a green maturation while the olives are green just before collaring to purple (Ryan et al., 2002; Beltrán et al., 2004). However, the reliable ripening indices are based on the most significant variations of the physiological, physical and biochemical characteristics occurring during the fruit ripening process (Beltrán et al., 2004).

2.3.3. Biochemical changes during olive fruit growth and development

Percentage of oil in olive fruit increases significantly early in the fruit ripening period (Salvador et al., 2001; Lavee and Wodner, 2004). However oil content in fruit reduces after the green mature stage (Wodner et al., 1988) which is recognized as the most appropriate stage for fruit harvesting for olive oil production (Luaces et al., 2007). Issaoui et al (2008) also reported that the growth stage for harvesting time of olive fruit can significantly affect oil quality. The quality of fruit and expressed oil is directly related to the growth stage of the fruit (Garcia et al., 2007). Extra virgin olive oil produced from olive fruit, to meet trade standards, must have a free acidity percentage of 0.8% or less, a low peroxide value and clear flavour characteristics along with other qualitative and quantitative standards that reflect the fruit from which it was made (IOC, 2001).

The acidity level of oil from high quality olives can be less than 0.02%. Overall the quality of the oil fraction depends on numerous factors related to pre and post-harvest operations, as well as processing technique and post processing

handling. Oils extracted from green olives show excessive bitterness that may result in rejection by some consumers. Also, oil yield obtained by physical processing increases with the advancement of fruit maturity Garcia et al., (2007).

The peroxide values of olive oil changes according to crop year and cultivation method but slightly decreases through the ripening process (Gutierrez et al., 1999; Salvador et al., 2003; Baccouri et al., 2008 and Anastasopoulos., et al 2011). Matos et al. (2007) did not find any significant change in peroxide value during ripening. In contrast, Dag et al., (2011) found significantly reduced peroxide values during different maturity stages and attributed to reduced activity of lipoxygenase enzyme. (Gutiérrez et al., 1999; De Mendoza et al., 2013).

The fatty acid composition of olive oil is significantly affected by different factors such as cultivar, crop yield, fruit ripeness and growing medium (Forina and Tiscornia, 1982). During ripening of olive fruit, many chemical composition changes occur due to activation and inhibition of different enzymatic activities and such changes affect the fatty acids composition also reported as well by Gutierrez et al. (1999).

Polyphenols accumulate gradually in the fruit reaching a maximum level just as the olive skin begins to change colour (Chimi and Atouati, 1994; Monteleone et al., 1995; Anastasopoulos et al., 2011). This increase has been attributed to a decrease in the fruit's water content, and it contributes to increased oxidative stability (Salvador et al., 2001). Phenolic compounds play the main role in growth, reproduction and providing protection against pathogens and predators due to involvement in defence strategies and signalling properties, particularly in the interaction between plants and their environment. Therefore these compounds, called secondary metabolites, are essential for a number of important functional aspects of plant life (Bravo, 1998; Garcia-Salas et al, 2010). As the fruit matures, the oil becomes less stable due to an increase in polyunsaturated fatty acids and a decrease in total polyphenol content (Caponio et al., 2001; Morello et al., 2004; Rotondi et al., 2004; Ayton et al., 2007). These changes are of major commercial importance as they dramatically influence the sensory characteristics of the oil, as well as its shelf life (Dag et al., 2011). As the fruit ripens, oleuropein, the main bitterness-producing component in olives, progressively decreases (Amiot et al., 1986, 1989) and phenolic

compounds such as dimethyl oleuropein and 3,4-dihydroxyphenylethanol hydroxytyrosol accumulate (Brenes et al., 1995). Oleuropein concentration varies with olive cultivar and decreases during fruit development (Jemai et al., 2009; Damak, 2008).

2.3.4. Physiological changes during olive fruit growth and development

Kitsaki et al. (1999) reported higher rate of respiration and ethylene production during the first three weeks after bud burst (ABB). They reported that accumulation of inflorescence ABA (abscisic acid) on 6 and 4 days before full bloom (FB) was associated with the minimum values of respiration and ethylene production on the same dates. ABA concentration declines sharply during FB and 3 days later a rise in ethylene and an increase in respiration rate occurs. These rose further one week after full bloom (AFB). Kitsaki et al. (1999) suggested that there is a possible correlation between ABA with the early stage of floral abscission and ethylene production correlate with the terminal respiratory activity in olive inflorescence abscission processes. Similar results have also been reported for young fruit development in cherry (Blanpied 1972) which reflects the high respiratory levels in the meristematic cells of young fruit.

Photosynthesis occurs in the green fruit cells of olives in the presence of chlorophyll in the exocarp and the mesocarp. These contain significant amounts of phosphoenol pyruvate carboxylase (Sánchez, 1994), the CO_2 fixation enzyme. During fruit development, CO_2 produced from the mitochondrial respiration of photoassimilates becomes photosynthetically fixed into triose-phosphate in the fruit chloroplasts during the light period with the result that the growing fruit releases lower levels of CO_2 (Sánchez and Harwood, 2002).

2.3.5. Effect of water stress on fruit growth and quality

Olive trees can cope with severe and prolonged drought, however a lack of water decreases yield and fruit size (Pastor et al., 1999; Iniesta et al., 2009). Lower amounts of rainfall or lack of irrigation during the growth period, leading to water stress, negatively affects the fruit growing process (Lavee et al., 1990 and Tombesi, 1994) and ultimately influences the physiology of the fruit ripening process (Lavee et al., 1982, 1991; Barone et al., 1994; Inglese et al., 1996; Barranco et al., 2000).

Where continuous deficit irrigation (DI) has been applied, oil yield is reduced by only 25% as compared to maximum irrigation application (Wahbi et al., 2005; Gucci et al., 2007 and Fernandes-Silva et al., 2010) without marked deterioration in olive oil quality. The lowest oil content was reported to occur in a low rainfall crop year by Ortega et al. (2001). However, some researchers reported no effect of irrigation on oil quality with respect to the fatty acids profile (Patumi et al., 1999; Motilva et al., 2000; Magliulo et al., 2003) while others indicated that the profile can be modified with irrigation (Faci et al., 2002; Tognetti et al., 2006) especially in differential responses of cultivars to environmental factors. A negative relationship has been reported between irrigation levels and polyphenol content (Patumi et al., 1999; Gómez-Rico et al., 2007). Irrigation also affects the polyphenol and the volatile profiles of olive oil (Romero et al., 2002). Yousfi et al. (2006) noted higher amounts of polyphenols compounds in picked oil obtained from fruit harvested in the low rainfall season than those picked in the season with higher rainfall.

2.4. Effect of harvesting period on olive fruit and oil quality

Harvesting period or the ripening status of the fruit is one of the important factors affecting the quality of olive fruit and its oil fraction (Koutsaftakis et al., 1999). The fruit removal force (FRF) is linearly correlated to the stage of fruit maturity and reduces with the advancement of the ripening period (Lavee et al., 1973, 1982). The reduction of FRF is also associated with the level of endogenous ethylene which increases with the development of the fruit and contributes in reducing the FRF through the release of ethylene (Lavee et al., 1982). Genotypic differences also cause variability in FRF among cultivars (Lavee and Haskal, 1976) as does stalk thickness (Lavee et al., 1982).

Water is a major component of olive fruit comprising more than half of the total fruit weight and varies according to the growth stage of the fruit, variation of seasonal rainfall and cultivar (Beltrán et al., 2004). A decrease in fruit moisture content is related to the progressive increase in the oil content during fruit maturation (Sánchez and Fernández, 1991).

The maturity status of the olive fruit and growing conditions influence the polyphenol compounds of virgin olive oil (Cinquanta et al., 1997). Over-ripe fruit give higher oil yield with increased level of acidity. On the other hand, limited

amounts of oil can be extracted from the fruit harvested too early (Anastasopoulos et al., 2011).

A higher amount of oil (%) was reported in early ripening fruit (Salvador et al., 2001; Lavee and Wodner, 2004). Crop year and maturation phases of olive fruit affect the amount of total polyphenols in olive oil (Anastasopoulos et al., 2011). Lower peroxide values, pigments, sensory scores, oleic acid and total sterols; and higher levels of free acidity and linoleic acid were observed by Salvador et al. (2001) during ripening of cv. Cornicabra olive. Uniform oil content in the mesocarp of black matured fruit of Barnea and Manzanilla cultivars was also observed by Lavee and Wonder (2004) regardless of size and level of fruit yield. Beltran et al. (2004) reported a similar observation where the oil content may vary due to climatic conditions such as lower rainfall possibly causing lower oil and higher dry matter content in olive fruit. Quality indices and fatty acid composition are mostly influenced by maturity of olives (Dabbou et al., 2011) and maximum oil content has been reported to occur between the 60^{th} and 75^{th} day after the start of the ripening process (Camposeo et al., 2013).

2.4.1. Effect of harvest time on biochemical parameters

2.4.1.1. Fatty acids

The concentration of fatty acids in olive oil may differ due to the effect of growing conditions in the cultivation year and stage of fruit growth or maturation. Salvador et al. (2003) and Anastasopoulos et al. (2011) also reported similar variations in fatty acids according to crop year and maturation of fruit. The free fatty acids at the later stage of ripening may increase with the increased activity of lypolytic enzymes in the flesh and decreased level of peroxide values may be due to decreased activity of lipoxygenase enzymes (Gutierrez et al., 1999; Salvador et al., 2003; Baccouri et al., 2008; Anastasopoulos et al., 2011). The level of oleic acid decreases and linoleic acid increases in the matured fruit ready to harvest due to the activity of the enzyme oleate desaturase which converts oleic acid into linoleic acid (Gutierrez et al., 1999). This inter-conversion of oleic and linoleic acid is accelerated by water stress which ultimately reduces the monounsaturated fatty acid /polyunsaturated fatty acid ratio (MUFA: PUFA ratio) as reported by Gómez-Rico et al. (2007) and Dag et al. (2014).

2.4.1.2. Polyphenols

The polyphenols in olive oil improve its resistance to oxidation as well as being responsible for its sharp bitter taste (Bendini et al., 2007). Tyrosol, hydroxytyrosol and oleuropein are the dominant polyphenols and vanillic acid, caffeic acid, synergic acid; para-coumaric acid and ferulic acid are minor polyphenols present in olive oils (Mulinacci et al., 2005; Damak et al., 2008; Manai-Djebali et al., 2012; Dağdelen et al., 2013). The concentration of polyphenols varies according to the maturation phase of the fruit and ultimately by the harvesting time (Anastasopoulos et al., 2011). The concentration of total polyphenols increases progressively and decreases in the final ripening stage (Baccouri et al., 2008). The level of polyphenols also varies according to the crop year with respect to water availability (Patumi et al., 2002 and Gómez-Rico et al., 2007 and Anastasopoulos et al., 2011). Differences in the level of water content of the fruit imply a different solubilisation of phenols (Allogio and Caponio, 1997). The amount of water in the fruit also controls the activity of enzymes responsible for polyphenol synthesis, such as L-phenylalanine ammonia-lyase, that differs according to water conditions (Morello et al., 2005). A linear correlation between polyphenols and water stress was reported by Tovar et al. (2002), Gómez-Rico et al. (2006), Dag et al. (2008), Ben-Gal et al. (2011) Vita Serman et al. (2011) and Caruso et al. (2014) and the variation of polyphenols in different olive cultivars reported by researchers has been attributed to a genetically controlled trait (Aguilera et al., 2005; Vinha et al., 2005; Manai-Djebali et al., 2012 and Dağdelen et al., 2013).

2.4.2. Effect of harvest period on sensory attributes

The sensory properties of olive fruit are influenced by the cultivar and ripeness status of the fruit (Angerosa et al., 2004; Rotondi et al., 2004; Servili et al., 2004; Tripoli et al., 2004; Kalua et al., 2007; Delgado and Guinard, 2011). Chemical composition of the fruit also influences the sensory properties. Higher polyphenol content in the fruit may cause greater bitterness and pungency (Bendini et al., 2007). Environmental conditions such as water stress also influence the bitterness of the fruit. Greater bitterness was observed in the fruit harvested during the year with a lower amount of rainfall (Cinquanta et al., 1997) which indicates that the ripeness of the olives along with pedoclimatic conditions influence the quality attributes of the expressed virgin olive oil.

2.5. Effect of abscission agents on olive fruit and oil

Use of abscission agents shows a positive effect on the harvest efficiency when applied at correct rates, times and conditions. This efficiency is reflected through faster harvesting, reduced length of harvest period as well as, lower costs and risks associated with late harvest (Ravetti and McClelland, 2008). Harvesting of olive fruit consumes 50–80% of the total cost of production and traditionally it is done by hand labour over a period of two months (Metzidakis, 1999). Increased labour costs and low availability of labour during harvesting time have intensified industry interest in mechanical harvesting. Mechanical harvesting systems combined with application of an abscission agent have gained popularity in recent years (Burns et al., 2005). Use of an abscission agent reduces the required mechanical forces to remove olives during harvest and minimizes fruit damage. Ethephon, an ethylene releasing chemical, is one of the abscission agents used to promote fruit abscission and to enable easy picking or mechanical fruit harvesting in cherries (Bukovac et al., 1969), apple (Edgerton, 1968), olive (Hartmann et al., 1970), citrus (Young and Jahn, 1972), macadamia (Kadman and Ben-Tal, 1983) and many others (Kays and Beaudry, 1987). Ethephon and other abscission agents are used especially with high cropping levels, or to harvest greener olive fruit early in the season, or to lower the FRF of certain difficult to harvest cultivars Frantoio, Koroneiki and Arbequina (Burns et al., 2008).

A large force is required to shake off fruit from olive trees where the ratio between fruit mass and pedicel's strength of olive fruit is relatively small compared with large olive cultivars and other fruits (Ben-Tal and Wodner, 1994). Among the different types of chemicals tested to promote pedicel loosening, positive results have been obtained by using ethylene releasing compounds like ethephon (2-chloroethyl phosphonic acid) which is a synthetic plant growth regulator and acts by releasing ethylene when it penetrates into plant tissues (Royer et al., 2006). Ethephon promotes pedicel loosening and increases the natural ratio between fruit mass and pedicel strength and as a result the mechanical harvesting of olive fruit is facilitated (Martin et al., 1981; Denney and Martin, 1994; Metzidakis, 1999). On the other hand, a disadvantage of ethephon application is a considerable loss of leaves coincident with fruit loosening (Burns et al., 2008). To keep a balance between leaf and fruit loss, investigators have paid attention to alteration in ethephon application

timing and duration (Lang and Martin, 1985, 1989) with promising results under laboratory conditions but is unpredictable in the field (Martin, 1994). A positive effect of ethephon application on fruit yield was observed by Touss et al. (1995). They applied ethephon at 1250 and 1875 mg L^{-1} on conventionally farmed Arbequina cultivar olive trees and obtained 20% increased yield from mechanical harvesting. They also reported that ethephon increased the amount of harvested fruit without significantly enhancing preharvest leaf drop and with no adverse effects on flowering in the following year as well as having little effect on oil acidity, peroxide value and fatty acid composition.

Growth stage of the fruit and environmental conditions including temperature and relative humidity affect the extent of ethylene penetration into the plant cells and rate of ethylene evolution from the applied ethephon (Olien and Bukovac, 1978, 1982; Flore and Bukovac, 1982; Beaudry and Kays, 1987 and Kays and Beaudry, 1987). El-Tamzini et al. (1980) suggested the use of ethephon (1250 mg L^{-1}) at two weeks before harvesting to reduce the FRF (maximum of 73%). However, Touss et al. (1995) reported non-significant effect of ethephon treatments on mechanical harvesting of Arbequina olive trees at 12 days before harvest with different concentrations of ethephon.

2.6. Effect of different concentrations and time of application of ethephon

2.6.1. On fruit removal force

Higher concentration of ethephon increases the ethylene production from the treated olive fruit and the increase in ethylene influences the ripening index of the olive fruit (Chaves and De Mello-Farias 2006; Nath et al. 2006 and Tharanathan et al., 2006). On the other hand, the fruit removal force is reduced significantly with the increase of the applied ethephon concentration. Ethephon penetrates the pedicels and releases ethylene to reduce the FRF (Ben-Tal, 1992). Fruit and leaf abscission (%) also increases significantly with the increase of applied ethephon concentration. (Barranco et al., 2004; Ferguson et al., 2010). Abscission of leaf and fruit is directly related to the level of endogenous ethylene in leaf and fruit. Ethylene evolution seems to parallel the effects of applied ethephon (Banno et al., 1993). Ethephon induces fruit abscission through its accumulation in the pedicel-fruit basin and leaf surface which ultimately penetrates into the plant system to enhance the ethylene

production (Reed and Hartmann, 1976; Polito and Lavee, 1980 and Weis et al., 1988, 199l). Ethephon treatments have also been reported to have positive effects on mechanical harvesting efficiency (Touss et al., 1995; Yousefi et al., 2010; Ninot et al., 2012 and Zahra, 2014).

2.6.2. On physiological parameters

As indicated earlier, ethephon is an ethylene-releasing chemical (Martin et al., 1981) which induces ethylene from the fruit of treated plants (Banno et al., 1993). Ben-Tal (1992) also reported that a small portion of applied ethephon penetrates the pedicels and releases ethylene responsible for increased ethylene in the treated fruit. However, Ferrante (2005) reported that ethephon shows a marked effect on ripening (early pigmentation, increased fructose content) when applied before the olives respire at their maximum rate, and when it is applied after the maximal respiration rate, there is only weakening of the pedicel. This observation signifies that the ethephon treatment influences the rate of respiration in accordance to the increase of ethylene production from the treated fruit.

2.6.3. On biochemical parameters

Ethephon treatment enhances the fruit olive maturation and affects oil quality (Ismail et al., 1999). However, the effect of ethephon on fatty acid content of olive oil depends on local climatic conditions (Ranalli et al., 1999; Faila et al., 2000). Ethephon enhances the ethylene production in treated fruit together with increased peroxide value (Griffiths et al., 1999; Sheng et al., 2003 and Yousfi et al., 2009). However, Salvador et al. (2001); Tovar et al. (2001) and Baccouri et al. (2008) reported decreased peroxide values due to the activity of lipoxygenase enzyme which also decreases as the fruit ripening process advances. Levels of polyphenols in olive oil depend on genotype, agronomic, environmental and technological factors (Montedoro and Garofolo, 1984; Lavee and Wodner, 1991). Decrease of the major polyphenols with the progress of olive fruit maturity has been reported in different studies (Skevin et al., 2003; Rotondi et al., 2004; Yousfi et al. 2006; Baccouri et al., 2007; Riachy et al., 2012) and this is correlated with the increased activity of hydrolytic enzymes during ripening (Amiot et al., 1989).

The application time of ethephon has no effect on the oil content, free fatty acids and peroxide values of olive fruit. Ahmed et al. (1981) observed that spraying of ethephon two weeks before harvesting the fruit did not affect the biochemical properties of the extracted olive oil. Touss et al. (1995) also reported non-significant effect of ethephon treatments on cv. Arbequina olive trees at 12 days before harvest with different concentrations of ethephon. Moderate effect of ethephon on palmitic, linoleic and oleic acids was also reported by Cimato (1988). Lavee and Haskal (1975) treated cv. Nabali olives with ethephon at 1500 mg L^{-1} and did not observe any effect of ethephon on either colour or taste of the oil. The early application of ethephon (4-weeks prior to harvest) showed lower concentration of polyphenols (Amiot et al., 1989) which resembles the effect of the evolved ethylene from the applied ethephon. This correlated with the increased activity of hydrolytic enzymes during ripening reducing the concentration of polyphenols (Yousfi et al. 2006; Baccouri et al., 2007 and Riachy et al., 2012). Early exposure to ethylene results in higher PPO (polyphenol oxidase) activity (Couture et al. 1993; Peng and Yamauchi, 1993) which readily oxidises soluble polyphenols (Ke and Saltveit., 1988) and later application of ethephon does not show significant changes on this declining trend.

2.6.4. On sensory attributes

Phenolic compounds are highly correlated to sensory attributes of olive oil (Andrewes et al., 2003; Beltrán et al., 2007). The amount of polyphenols decreases with the progress of maturity (Yousfi et al. 2006; Riachy et al., 2012) and the effect of ethylene involved the decomposition of applied ethephon (Couture et al., 1993; Peng and Yamauchi, 1993). Differences in the sensory attributes are related to the chemical reactions and enzymatic activities, such as glycosidases, phenol oxidases or phenol polymerases (Ke and Saltveit, 1988). Yousfi et al., (2009) also reported decreased bitterness while he exposed modified atmosphere (MA) stored olive fruit to 30 $\mu 1L^{-1}$ ethylene.

2.7. Conclusion

The literature reviewed here indicates that the physical, physiological and biochemical characteristics of olive fruit vary depending on the growth and developmental stages of olive fruit. Literatures references also indicate that the harvesting time affects the quality attributes of olive fruit. Investigators have also recommended the use of abscission agents like ethephon when harvesting olives and there are substantial effects at different concentrations of ethephon and time of application of ethephon on quality attributes of olive fruit and oil. cvs. Frantoio and Manzanilla are two of the important cultivars grown commercially in Australia. However, information on the Australian grown olive cultivars in respect of fruit growth habit, effect of harvesting period and effects of ethephon concentration and its time of application on fruit and oil qualities is scarce. Therefore, the current studies have been designed to understand the morphological (fruit weight, fruit volume, pulp weight, stone weight, pulp/stone ratio and ripening index) and physiological (production of ethylene and rate of respiration) changes during fruit growth development and ripening in cvs. Frantoio and Manzanilla. An attempt has also been made to evaluate the effect of harvesting periods, concentrations and time of application of ethephon on the physical, physiological, biochemical and sensory attributes of oil in Frantoio and Manzanilla olive cultivars commercially grown in south-western Australian conditions.

Chapter 3

General Materials and Methods

The current study includes four experiments conducted during olive crop season in 2013 and 2014 to investigate the fruit growth; development; and maturation and ripening; in relation to ethylene and to explore the effects of different harvesting times, various concentrations and time of spray application of ethephon on various physiological, biochemical, quality and sensory attributes of olives and oil. The materials and methods used in these experiments have been presented in this chapter.

3.1. Plant material, experimental location and climatic conditions

All the experiments were conducted on the olive trees grown at Talbot Grove, York (31°52′44″ S, 116°45′57″ E), located at 120 km east of Perth city, Western Australia (WA) (Fig. 3.1.).

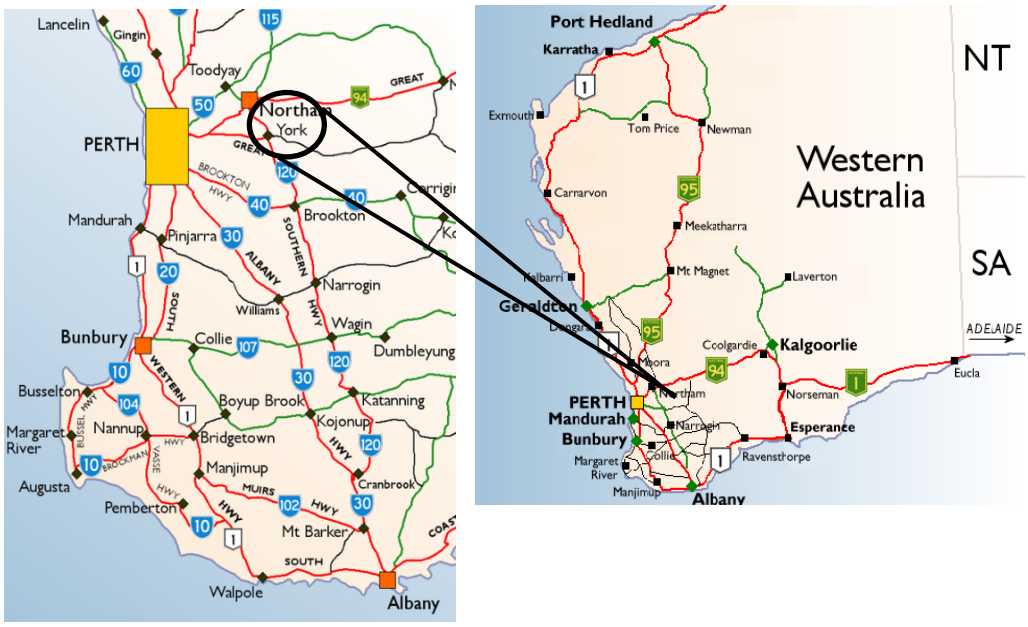


Fig. 3.1. Geographic location of the experimental field (https://maps.google.com.au)

York is located in a Mediterranian climate zone of WA which experiences distinctly dry, long, hot summers and cool, wet winters. The mean annual daily minimum temperature is 10.5 °C (50.9 °F). The hottest month is January with a mean

maximum temperature of 33.6 °C (92.5 °F), while the coolest month is July with a mean minimum temperature of 5.3 °C (41.5 °F). York has a mean annual rainfall of 403.6 mm. The wettest month is July with 71.8 millimetres and the driest is December with 11.3 millimetres rainfall. The temperatures and rainfall are based on data from 1996 to 2014 (Australian Bureau of Meteorology, 2015, http://www.bom.gov.au). During the growing period of olive fruit, the lowest rainfall (0 to 5.5 mm) was observed from December to March 2014, when the rainfall ranged from 2.8 to 56.7 mm in 2013. The amount of rainfall increased during the development and maturation phase of the fruit from April to June when it ranged from 46.6 to 89.3 mm and 4.8 to 68.8 mm in 2014 and 2013 respectively. Higher levels of maximum and minimum temperature (about 34°C and 16°C respectively) were observed from December to March in both years (Fig. 3.2).

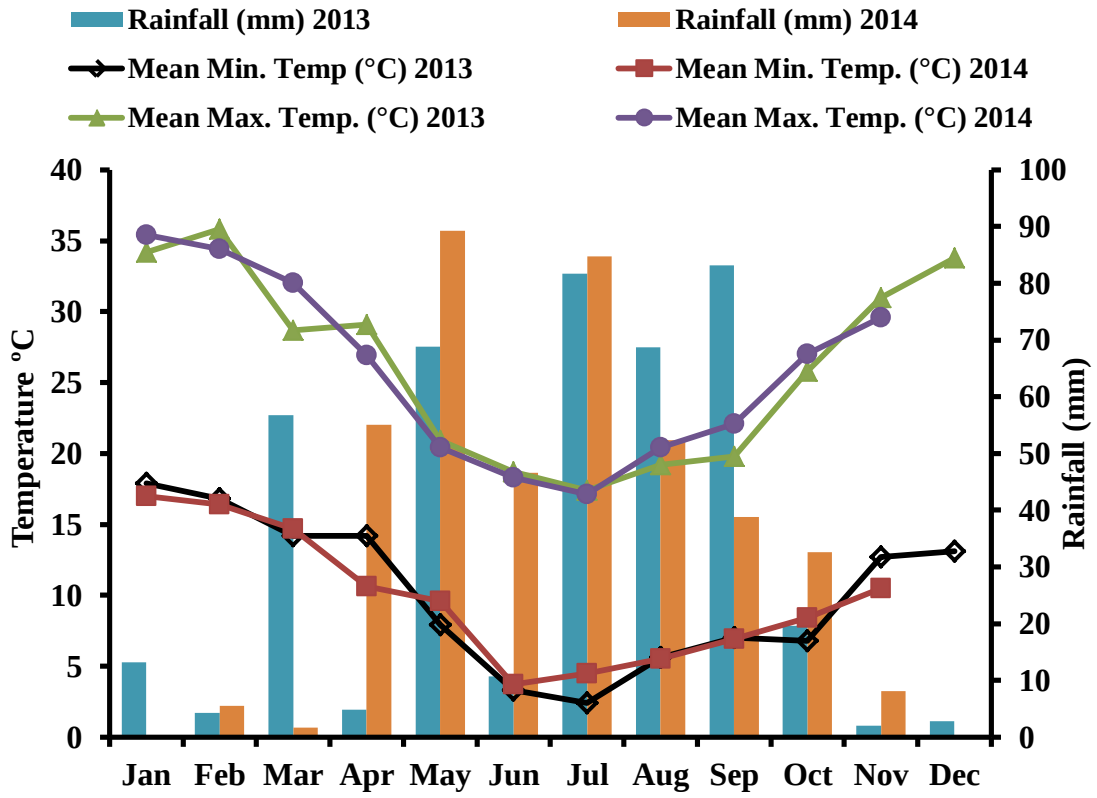


Fig.3.2. Climatic conditions in experimental location, York, WA, during 2013-2014. (Australian Bureau of Meteorology, 2015, http://www.bom.gov.au).

3.2. Design of experiment and treatments

The experiments were conducted by following one- or two-factor factorial Randomized Complete Block Design (RCBD) with four replications. Depending upon experiment, an experimental unit included one to five olive trees. Different

harvesting times or different concentrations of ethephon or different spray periods of ethephon were considered as the treatments. The cultivars included in these experiments were cvs. Manzanilla and Frantoio. The experimental design and treatments have also been explained in relevant individual chapters also.

3.3. Experimental olive trees and their maintenance

The study was carried out on 15 year old olive trees of two cultivars including cvs. Frantoio and Manzanilla. The trees were trained to a central leader, supplementary irrigated and spaced at 7 m × 7 m with a tree density of 358 trees ha^{-1}. The irrigation period was for five months per year (from December to May, 200-600 L/tree) with daily irrigation using drippers placed around the trees delivering water flow of 1.2 L/h.

3.4. Collection of olives and extraction of olive oil

Olive fruit (composite sample of 1.5 to 2 Kg) were taken harvested from four representative trees included in four replications of cvs. Frantoio and Manzanilla which were harvested using a commercial trunk shaker (Sicma F3 Umbrella Olive Harvester, Catanzaro, Italy) (Fig.3.3).

Fig.3.3. Commercial trunk shaker Sicma F3 umbrella olive harvester

Virgin olive oil was extracted from the collected fruit by following the method explained by Rivas et al. (2013) with some modifications. The collected fruit were immediately transported to the Horticulture Research Laboratory, Curtin University,

Perth, Western Australia and oil was extracted within 24 h. To extract oil, the leaves were removed from the fruit prior to cleaning; the fruit were crushed with a hammer mill and then homogenized slowly for 30 min by using a mechanical mixer (Breville, the Wizz Mix, BEM200, China) at 25-27 °C. Then the obtained paste was centrifuged (Eppendorf Centrifuge 5810R, Hamburg, Germany) at 3500 rpm for 10 min and the extracted oil was stored at 4°C in darkness until analysis. Dark glass bottles were used and the head space was filled with nitrogen gas (Fig.3.4).

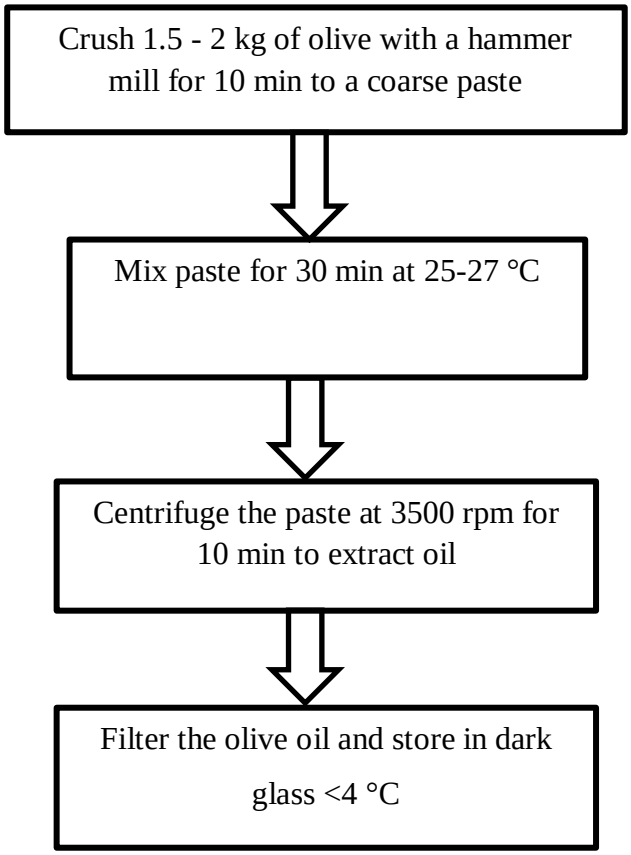


Fig.3.4. Flow chart of extraction of olive oil from the collected fruit.

3.5. Observation recorded:

3.5.1. Physical parameters

3.5.1.1. Fruit, stone and pulp weight, pulp/stone ratio

The fruit, stone and pulp weight was recorded using a digital balance (A&D Electronic Balance, FX-2000, Japan) with ±0.01g accuracy. The weight of fruit, pulp and stone was expressed in grams (g). The pulp and stone ratio was calculated by dividing the pulp weight with corresponding stone weight. A sample of 100 fruit per

replication was used to measure the fruit weight and the pulp and stone weight was measured by using 10 fruit from each replication and average values were calculated.

3.5.1.2. Fruit dimensions

The dimensions (length and width) of randomly selected 100 fruit per replication were measured using a digital Vernier calliper and the average values were expressed as millimetres (mm).

3.5.1.3. Fruit volume (water displacement method)

To measure the fruit volume, 100 fruit per replication were submerged in 500 mL of water contained in a graduated one litre measuring cylinder (Fortuna, Germany) and the volume was recorded as the volume of the displaced water in cubic centimetres cc^3. Fruit volume was expressed as cubic centimetres per fruit.

3.5.2. Ethylene production

The endogenous level of ethylene was determined by using a Sensor Sense (Sensor Sense B.V, Nijmegen, The Netherlands) (Fig. 3.5) following the method described by Pranamornkith et al. (2012). The Sensor Sense includes an ETD 300 ethylene detector, a set of valve controllers with an option of six valves connected to six separate cuvettes [1.0 L air-tight jar, fitted with a rubber septum (SubaSeal®, Sigma-Aldrich Co., St. Louis, USA)]. The "continuous flow" method was used with coarse mode (conversion factor 99818, capacity to measure ethylene concentration at 0 - 500 $\mu 1L^{-1}$, sensitivity at <1%) of analysis. A fruit sample of 100 g was used to determine the ethylene production. All the cuvettes were kept airtight to prevent leakage. Before connecting flow to the cuvettes, it was ensured that the output from the cuvettes was not blocked, in order to avoid pressure build-up in the cuvette. Each sample was run for 20 minutes with a flow rate of 4.0 L hr^{-1} and the average reading of the last 15 minutes was considered to calculate the amount of ethylene. The ethylene production was expressed as $\mu lkg^{-1}hr^{-1}$.

The concentration of produced ethylene was converted from $\mu L\ kg^{-1}\ h^{-1}$ to $nmol\ kg^{-1}\ h^{-1}$ using Ideal Gas Law, $PV = nRT$, where P is pressure (kPa), V is volume (L), n is the number of moles, R = 8.314 (the ideal gas constant) and T is temperature (Kelvin) (Bower et al., 1998).

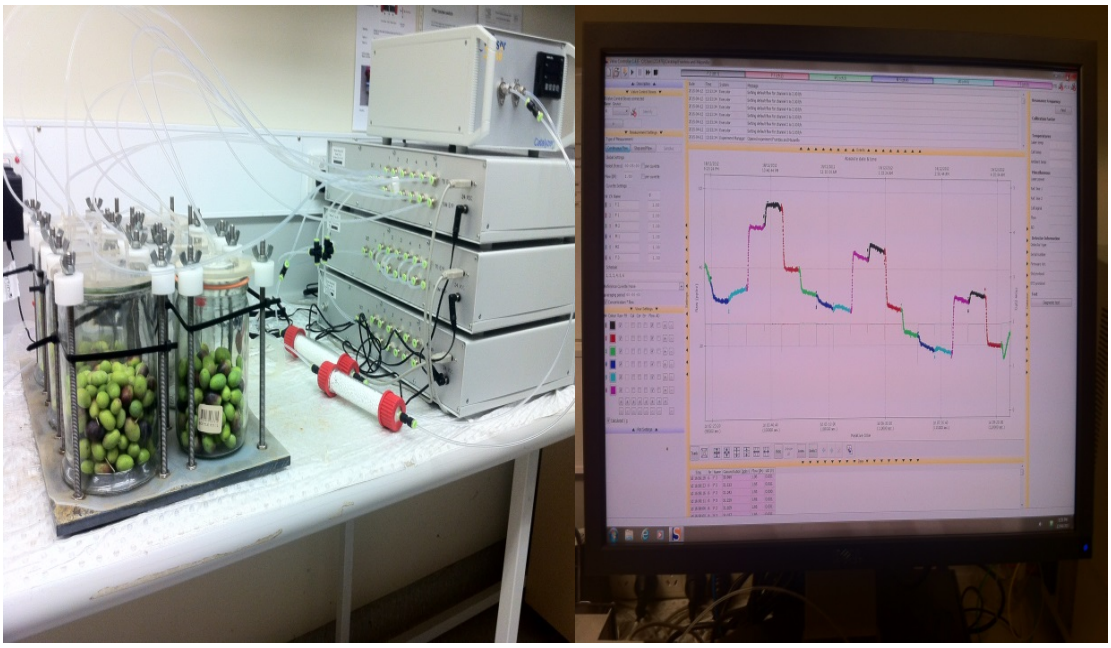


Fig.3.5. Determination of ethylene production in olive fruit using ETD 300 ethylene detector.

Data of barometric pressure during the ethylene measurement were collected from Bureau of Meteorology Australia, WA. The relevant calculation is as follows-

Under standard conditions-

The atmospheric pressure, P= 1 atm

Temperature, T = 273.15 K

Universal gas constant, R= 0.0821 L atm $mol^{-1}K^{-1}$

V= volume

n= Number of moles

The olive fruit used for this part of the study were kept at 20°C, the temperature needed for calculation is

T= 273.15+20= 293.15K

Now, PV= nRT

=> V/n = RT/P

=> (0.0821 L atm $mol^{-1}K^{-1}$*293.15 K)/1 atm = 24.07 $Lmol^{-1}$

i.e., 1 mol gas = 24.07 L

or, 1 mmol gas = 24.07 ml

or, in 1 ml gas it has = 1/24.07 mmol = 0.0415 mmol

So, for example, if the measured ethylene gas is 2.5 ml $kg^{-1}hr^{-1}$, then there will be 2.5X0.0415= 0.104 mmol ethylene $kg^{-1}hr^{-1}$.

3.5.3. Determination of respiration rate

Respiration rate from olive fruit was determined as CO_2 production by following the method described by Zaharah (2011). The headspace gas sample (2.0 ml) was taken through a rubber septum (SubaSeal®, Sigma-Aldrich Co., St. Louis, USA) using a syringe from the hermetically sealed 1L jars with sample fruit (100 g) for an hour and injected into an infrared gas analyzer [Servomex Gas Analyzer, Analyzer series 1450 Food Package Analyzer, Servomex (UK) Ltd., East Sussex, UK]. The respiration rate was calculated on the basis of the peak areas of 2.0 mL gas sample (CO_2) and CO_2 standard (StdCO_2, 8.52 ± 0.17%). The standard CO_2 was purchased from BOC Gases, Australia Ltd., Perth, Australia. All the estimations were performed twice. Respiration rate was calculated using the following formula and expressed as mL CO_2 kg^{-1} h^{-1}.

$$\text{Respiration rate (ml } CO_2 \text{ kg}^{-1} \text{ h}^{-1}\text{)} = \frac{\text{Changes in } CO_2 \text{ concentration (\%)} \times \text{Vol. of container (L)}}{\text{Fruit weight (kg)} \times \text{Incubation time (h)}}$$

Respiration rates were converted from mL CO_2 kg^{-1} h^{-1} to mmol CO_2 kg^{-1} h^{-1} using the Ideal Gas Law, PV = nRT as explained in Section 3.5.2. To check the possibility of CO_2 emission from the rubber septum or normal air, a blank injection from the headspace of the empty jar or air was also run under the similar conditions of analysis.

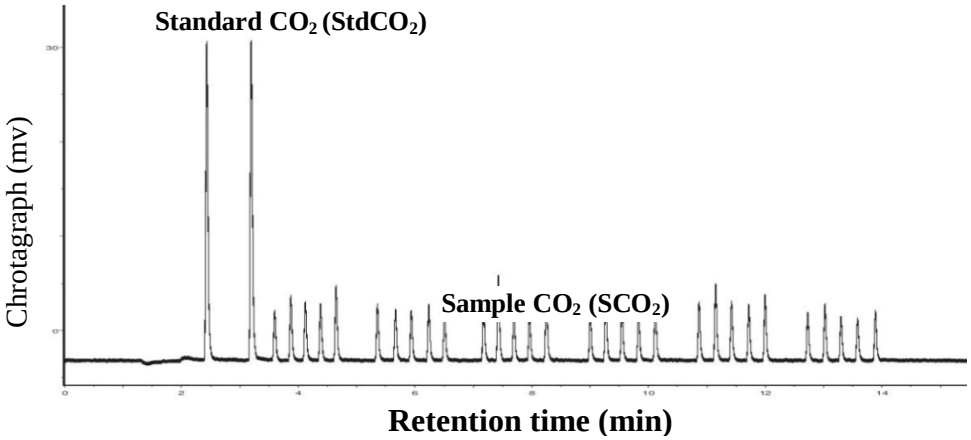


Fig.3.6. Servomex Series 1400 (Sussex, England) chromatographic profile of respiration peak of standard ($StdCO_2$) and fruit sample peak (SCO_2).

3.5.4. Determination of fruit firmness

The firmness of olive fruit was determined using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK), equipped with horizontal square base table (15 cm × 15 cm) and interfaced to a personal computer with Nexygen® software following the method explained earlier by Singh et al. (2009). Twenty randomly selected fruit per replicate were used for this test after a small slice (< 2 mm thick) of fruit skin was removed and the firmness was recorded from the opposite sides of equatorial region of individual fruit by puncturing a 2 mm Magness-Taylor probe. The crosshead speed, depth, trigger and compression were maintained at 3 mm s^{-1}, 3 mm, 0.5 N and 70% respectively for all determinations, as per the following example (Fig.3.7).

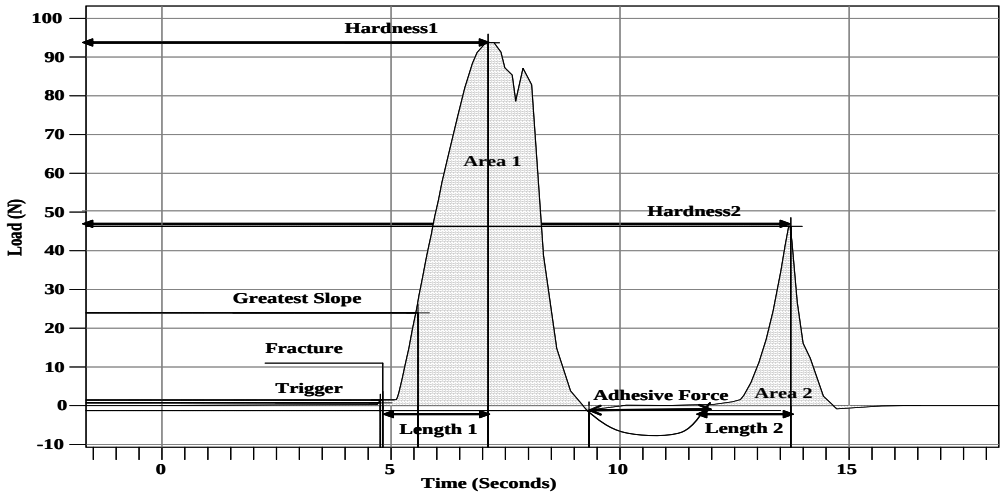


Fig.3.7. An example of chromatographic profile of rheological properties of a fruit using texture profile analyzer (TPA) (Zaharah, 2011).

3.5.5. Fruit removal force (FRF)

The FRF was determined by using the texture profile analyser (TPA Plus), described in Section 3.5.4. The olive fruit with pedicel was placed under the base and the pedicel clamped to the moveable load cell as shown in Figure.3.8. Twenty randomly selected fruit per replicate were used for determining FRF. The fruit pedicel was subjected to tensile speed of 150 mm min^{-1} and preload of 0.5 N. The tensile speed and preload strength were adjusted after trial and error with due reference to the dynamometer (Ravetti and McClelland, 2008) used for evaluating the removal force in field conditions. The fruit pedicel removal strength was calculated at the maximum load and limit points where the pedicel removal occurred and expressed as N.

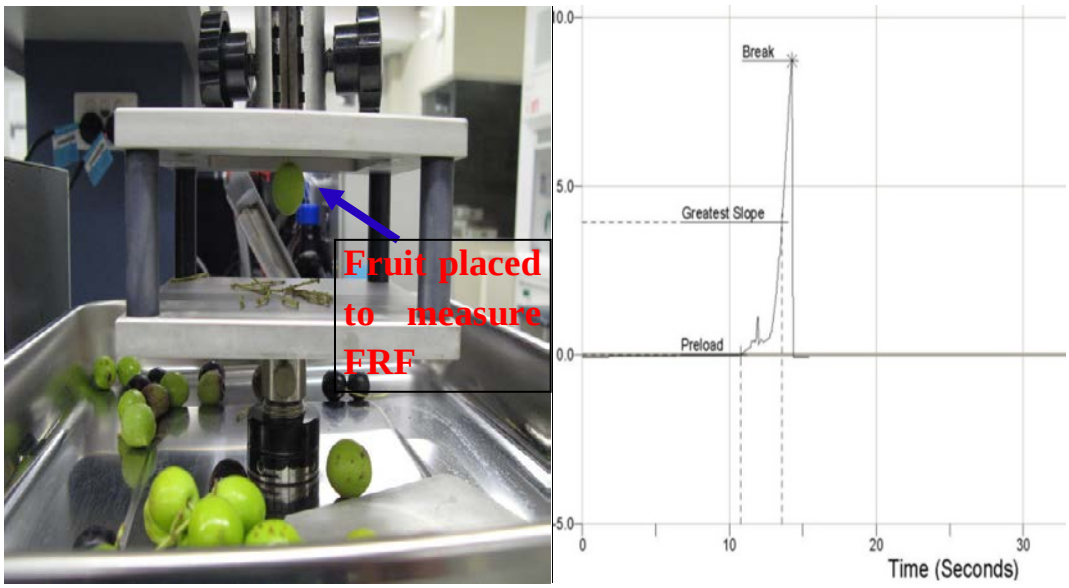


Fig.3.8. Determination of fruit removal force by using a texture profile analyser.

3.5.6. Ripening Index

Ripening index (RI) of olive fruit sample was determined according to the method described by Uceda and Frias (1975). One hundred randomly selected healthy fruit were cut in half to expose the internal flesh for grading in eight groups. The groups were categorized as- '0' deep green skin; '1' yellow-green skin; '2' half skin turning red ; '3' reddish-brown skin; '4' black skin with white flesh; '5' black skin with < 50% purple flesh; '6' black skin with ≥50% purple flesh and '7' black skin with

100% purple flesh. The total number of olives in each category was counted and the ripening index was calculated by using the following equation-

$$RI = \frac{A \times 0 + B \times 1 + C \times 2 + D \times 3 + E \times 4 + F \times 5 + G \times 6 + H \times 7}{100}$$

Letters (A-H) = number of fruit in each category.

3.5.7. Fruit moisture (%) and dry matter (%)

The olive fruit moisture content was determined by using healthy and randomly selected olive fruit (60g). The fruit were crushed by hammer mill in a pre-calibrated dish and the paste was dried in a forced air oven at 80°C for approximately 24 hours or until the weight was constant. The sample was then cooled in a desiccator and the moisture content and dry matter of the fruit calculated as a percentage of the fruit weight by using the following formula (International Olive Council, 2011).

Moisture in the sample (%) = loss in weight x 100/weight of the fresh fruit sample

Fruit dry matter (%) = weight of the dried fruit x 100/weight of the fresh fruit sample

3.5.8. Olive oil content (%) in the fruit

Olive oil content in fresh fruit was determined by following the method described by Avidan et al. (1999) with some modifications. Olive fruit paste (10g) was taken in each replication into small scintillation vials after crushing the olive fruit. The content was dried in an oven at 80°C for 24 h and the dry weight of each replicate was recorded. The dried sample (5g) was transferred separately to 25×100 mm glass tubes and 10 ml of petroleum ether at 60-80°C was added, homogenised at medium speed for 30 sec with a vortex (Heidolph, Reax Top, VIC, Australia). The content left in the original scintillation vial was rinsed with 5 ml petroleum ether and crude extract in vials were stoppered and agitated by a rotator shaker (Ratek Orbital Mixer, VIC, Australia) overnight. Next day the crude extract was passed through a filter paper and the pellet was rinsed again with 5 ml petroleum ether. This final solution was used to extract oil and petroleum ether was evaporated at 40°C. The oil residue was weighted as percent of oil on fresh and dry weight basis by using the follow formula.

$$\text{Oil \% (Dry basis)} = \frac{\text{Oil Weight}}{\text{Dry weight}} \times 100$$

$$\text{Oil \% (Fresh basis)} = \frac{\text{Oil Weight}}{\text{Fresh weight}} \times 100$$

3.5.9. Fruit and leaf abscission

Three branches from each replicate were selected to count the leaf and fruit abscission. The number of leaf and fruit were noted down before applying the treatments and observation on the abscission of leaf and fruit was recorded after harvesting the fruit. The percentage of leaf and fruit abscission was calculated by following the equation suggested by Dung (2013).

$$\text{Abscission of leaf or fruit (\%)} = \frac{\text{Number of leaf or fruit after harvest X 100}}{\text{Initial number of leaf or fruit}}$$

3.5.10. Determination of free fatty acids

Olive oil sample (10 g) was weighted into a 250ml conical flask and then dissolved in 50 ml of the solvent mixture (1:1 of 95% (V/V) ethanol and diethyl ether). The mixture was titrated while shaking with a solution of 0.1N potassium hydroxide (KOH_4) to the end point of the indicator pink colour of phenolphthalein persisting for at least 10 sec (EC 2568/1991).

Free fatty acid (FFA) expressed as (% of oleic acid):

FFA (%) = volume, in ml, of solution titrated with potassium hydroxide.

3.5.11. Determination of peroxide value

Peroxide value was determined under artificial or diffused daylight by using 1-2 g of the olive oil sample taken in a 250 ml flask with ground neck and stopper (EC 2568/1991). The sample dissolved rapidly by stirring with the addition of 10 ml of chloroform, 15 ml of acetic acid and 1 ml of 0.5 N potassium iodide solution. The stopper was immediately inserted, shaken for 1 minute and then left for 5 minutes at a temperature of 20°C in the dark. Then 75 ml of distilled water was added and titrated with the sodium thiosulphate solution (0.002 N for expected values less than 12, and 0.01 N for expected values above 12). During titration the flask was under continuous shaking and starch was used as an indicator (10 g/l aqueous dispersion). The solution was titrated from a purplish to yellowish or colourless

endpoint. All the chemicals used were of analytical grade. The peroxide value was calculated as follows:

Peroxide Value (P.V.) expressed in milli equivalents of active oxygen per kilogram of olive oil (meq O_2 kg^{-1}) and calculated by using the formula as below (EC 2568/1991)-

(P.V.) = (V * T / m) * 1000

Where:

V = ml of solution of titrant (sodium thiosulphate).

T = Normality of sodium thiosulphate solution.

m = weight of sample of olive oil, in grams.

3.5.12. Determination of fatty acid composition

Fatty acid composition of olive oil as fatty acid methyl esters (FAMEs) was determined by gas chromatograph following the method prescribed by the International Olive Council (2001). Methanol contained not more than 0.5% (v/v) water with heptane and potassium hydroxide. All chemicals used were analysed grade. A methanolic solution of potassium hydroxide was prepared (dissolve 11.2 g of potassium hydroxide in 100 mL of methanol = to 2N) and used to trans-methylate fatty acids of triacylglycerol present in olive oil. In a 5 mL screw-top test tube 0.1 g of the oil sample was added to 2 mL of heptane and homogenized followed by addition of 0.2 mL of 2 N methanolic potassium hydroxide solution. The mixture was homogenized vigorously for 30 seconds and left undisturbed until the upper solution became clear. The upper layer containing FAMEs was decanted and injected into the gas chromatograph.

The gas chromatograph was fitted with a fused silica column (50m length × 0.25mm i.d.) coated with SGL-1000 phase (0.25µm thickness Suger labour, Spain) and containing a FID detector (HP 6890, Agilent Technologies). Temperature of the injector and detector was maintained at 250 °C and the oven temperature was 210 °C. Trans-C18:1 percentage was calculated as corresponding to methyl esters of fatty acid and trans-C18:2 + trans-C18:3 percentage. All the peaks corresponding to trans-isomers were summed. The amount of individual FAMEs as a relative percentage was calculated according to the formula:

% Fatty acid = (Area X 100) / (total area).

3.5.13. Total polyphenols

The total phenols were quantified by following the method of Ranalli et al., (1999). Olive oil (10 g) was dissolved in 50 ml of hexane and phenolic compounds were isolated by triple extraction of a solution with a water/methanol mixture (60:40, v/v), and made up to 100 ml with water and left to stand overnight. After that 5ml Folin-Ciocalteau phenol reagent was added to1 ml aliquot of the extract with shaking for 5 minutes and addition of 1 ml of saturated sodium carbonate (Na_2CO_3) then 2 hours later analysed using a spectrophotometer (Model SECOMAM ANTHELIE Advanced, France). The absorption of extracts was read at 725 nm and a 1cm rectangular glass cuvette was used. Total phenols concentration was calculated according to the response factors determined by Mateos et al. (2001).

3.5.14. Determination of polyphenolic compounds

Individual polyphenolic compounds in the olive oil were extracted and determined following the method previously detailed by the International Olive Council (COI/T.20/Doc NO 29, 2009) using a HPLC system coupled with an infinity 1260 diode array detector (HPLC-DAD). Virgin olive oil (5.0 g) was weighed in a 10 ml screw-cap test tube and 1 ml of the internal standard solution (syringic acid) was added and homogenized for 30 sec. Methanol/water 80/20 (v/v) extraction solution (5 ml) was added and homogenized again for another 1 min and the mixture was left in an ultrasonic bath for 15 minutes at room temperature. Then the samples were centrifuged at 5000 rpm for 25 minutes and the supernatant was filtered through a 0.45 µm polyvinylidene fluoride (PVDF) filter prior to HPLC analysis (COI/T.20/Doc No 29, 2009).

The phenolic compounds were quantified at 235-280 nm using syringic acid as an internal standard. Polyphenols standards, 3,4 DHPEA-EA (methyl-4-(2-(3,4-dihydroxyphenethoxy)-2-oxoethyl)-3-formyl-2-methyl-3,4-dihydro-2H-pyran-5-carboxylate), tyrosol, hydroxytyrosol, P-coumaric acid, vanillic acid, ferulic acid, caffeic acid and cinnamic acid of 0.015 g each were weighed into 10 ml flasks and made up to volume with the methanol/water solution (80/20). Then 1 ml of standard was transferred to a 100 ml volumetric flask and made up to volume with the methanol/water solution to obtain a concentration of 0.015 mg/ml.

3.5.14.1. HPLC-DAD conditions and quantification

Chromatographic separation was achieved on an Agilent 1200 HPLC system coupled with an infinity 1260 diode array detector (DAD), using a C-18 reversed-phase Spherisorb ODS-2 (4.6 mm x 25 cm, 5µm) column (Waters Corporation, Milford, MA, USA). The column was conditioned for 15 minutes with the elution solvent (initial composition) (0.2 % H_3PO_4 in water (v/v) /methanol/acetonitrile (96/2/2, v/v/v) and then the gradient elution program was used as shown in (Table.3.1) (International Olive Council, COI/T.20/Doc No 29, 2009). A preliminary empty gradient chromatographic run was undertaken (to make sure there were no interfering co-elution peaks) by injecting 20 µl of methanol/water 80/20 (v/v) into the HPLC-DAD system. Then 20 µl of the external calibration standard solution was injected and the chromatogram at 235-280 nm was recorded. Afterward, (20 µl) of the sample solution was also injected into the HPLC system and the chromatogram at 235-280 nm was recorded. Two independent determinations on the same sample were performed. The external calibration standard curves and the peak areas were used to calculate the polyphenol concentrations in the olive oil sample. Individual polyphenol compounds in the olive oil were expressed as mg kg^{-1}.

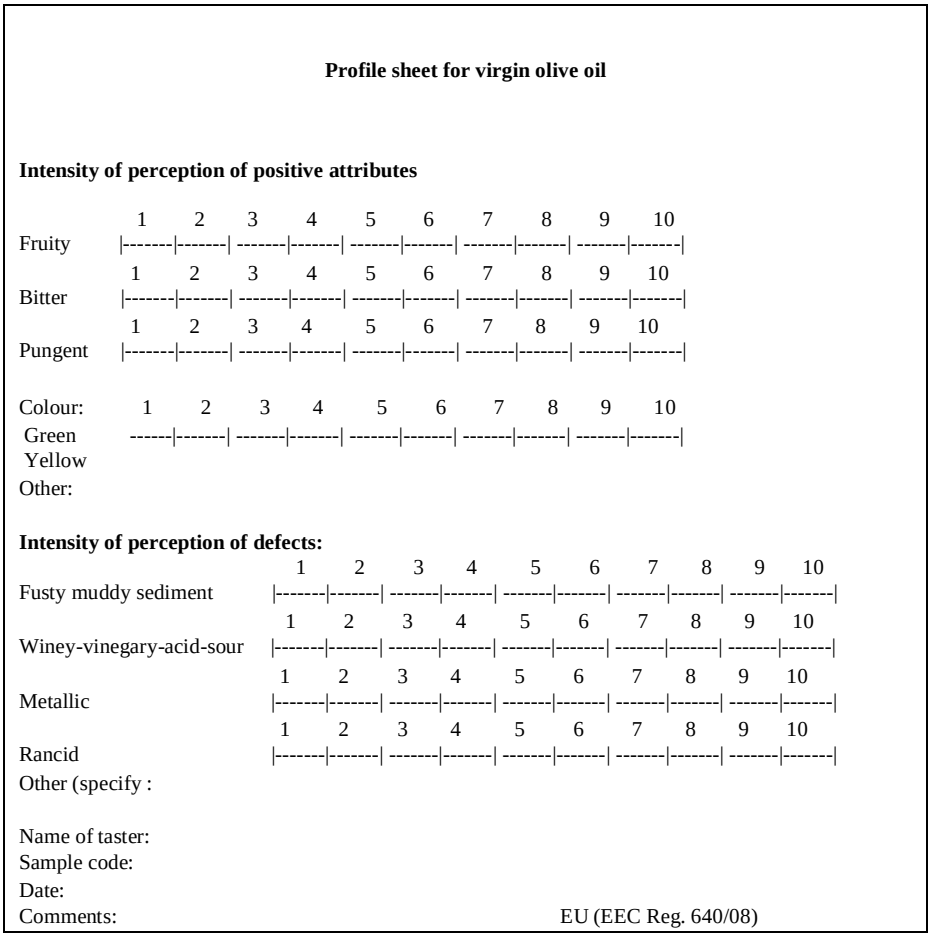
Table.3.1. Gradient elution of the HPLC analysis of olive oil polyphenols (COI/T.20/Doc No 29, 2009)

Time min	Flow ml/min	A %	B%	C%
0	1.00	96	2	2
40	1.00	50	25	25
45	1.00	40	30	30
60	1.00	0	50	50
70	1.00	0	50	50
72	1.00	96	2	2
82	1.00	96	2	2

3.5.15. Olive oil sensory attributes

A tasting panel composed of seven trained tasters distinguished 30 olive oil samples according to the standard procedure (EC Reg. 796/2002). The tests were scheduled in two different days with ½ hour breaks between two tests. The taste panel was supplied with scaled sheets for the sensory attributes such as fruitiness, bitterness and pungency and for recording defects if any, including fusty, musty and metallic natures. Each attribute was rated using a rating scale from 1 to 10 where 1

represented the value for the poorest and 10 the best possible quality for the sample (Fig. 3.9) Between every two samples the tasters ate a piece of apple to refresh their palate (Favati et al., 2013).



Profile sheet for virgin olive oil

Intensity of perception of positive attributes

	1	2	3	4	5	6	7	8	9	10
Fruity	|-------|-------|									
	1	2	3	4	5	6	7	8	9	10
Bitter	|-------|-------|									
	1	2	3	4	5	6	7	8	9	10
Pungent	|-------|-------|									

	1	2	3	4	5	6	7	8	9	10
Colour: Green Yellow	------|-------|									

Other:

Intensity of perception of defects:

	1	2	3	4	5	6	7	8	9	10
Fusty muddy sediment	|-------|-------|									
	1	2	3	4	5	6	7	8	9	10
Winey-vinegary-acid-sour	|-------|-------|									
	1	2	3	4	5	6	7	8	9	10
Metallic	|-------|-------|									
	1	2	3	4	5	6	7	8	9	10
Rancid	|-------|-------|									

Other (specify :

Name of taster:
Sample code:
Date:
Comments:

EU (EEC Reg. 640/08)

Fig.3.9. Profile sheet panel for virgin olive oil

6.3. Statistical analysis

All the experimental data were subjected to one- or two-way analysis of variance (ANOVA) using Genstat 14 (release 14.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK). The effects of various treatments and their interactions were assessed within ANOVA and least significant differences (Fisher's LSD) were calculated following significant ($P \leq 0.05$) F test. To ensure validity of statistical analysis all the assumptions of analysis of variance were checked. The data over two years were not pooled when error mean squares were found to be heterogonous.

Chapter 4

Physical and physiological changes in cvs. Frantoio and Manzanilla olive fruit during growth and development

Abstract

The physical and physiological growth parameters of olive fruit are related to the improvement of commercial and qualitative characteristics of fruit and extracted oil. The current experiment was conducted to observe the morphological and physiological changes in Frantoio and Manzanilla olive cultivars grown in south-western Australian conditions. The physical parameters of fruit weight (g), fruit volume (cc), fruit length (cm), fruit width (cm), pulp weight (g), stone weight (g), pulp/stone ratio and fruit ripening index increased significantly ($P \leq 0.05$) until 150 to 175 days after full bloom with the progress of growth period, irrespective of the cultivar. The cv. Manzanilla showed higher average values then cv. Frantoio for these parameters. The physiological parameters (production of ethylene and rate of respiration) and fruit firmness declined significantly with the progress of fruit growth. After 190 days of full bloom, the ethylene peak was observed. The respiration peak was observed after 175 days in cv. Manzanilla and after 190 days of full bloom in cv. Frantoio in both years. The cv. Frantoio olive showed significantly higher respiration rate than cv. Manzanilla. It could be concluded that the physical growth parameters increase with the progress of fruit growth until 175 days after full bloom and physiological parameters showed a declining trend during this period with a peak at 175 to 190 days after full bloom and ethylene plays an important role in fruit ripening.

4.1. Introduction

Olive fruit is one of the oldest cultivated fruit for extracting oil and preparing different types of food items such as pickles with perfect balance of aroma, taste, flavour and health benefits (La Lastra et al., 2001). It provides many nutrients including: fatty acid, tocopherols (vitamin E), protein, fibre, beta-carotene and minerals (Reichelt and Burr, 2000). Botanically olive fruit is a drupe consisting of the exocarp or skin, the mesocarp or flesh, and the endocarp or pit having a woody

shell enclosing one or, rarely, two seeds. Olive fruit weight comprises 70–90% mesocarp, 9–27% endocarp and 2–3% seed. In mature or ready to harvest olive fruit, the mesocarp contains about 60% water, 30% oil, 4% sugars, 3% protein, and the rest is primarily fibre and ash. The endocarp contains 10% water, 30% cellulose, 40% other carbohydrates and about 1% oil. The seed has 30% water, 27% oil, 27% carbohydrates and 10% protein (Connor and Fereres, 2005). During the period of growth and development, the olive fruit display changes in size, colour, texture and flavour. A number of biochemical and physiological events occur during this period under strict genetic control and the influence of several growing conditions (Connor and Fereres, 2005).

Olive fruit growth and development lasts for 4–5 months which includes 5 main phases (Lavee, 1996; Manrique et al., 1999 and Proietti et al., 1999) in the following order: (i) fertilization and fruit set which comprises the period from flowering to approximately 30 d afterwards when rapid early cell division promotes embryo growth, (ii) seed development, a period of rapid fruit growth with intense cell division and enlargement involving mainly growth and development of the endocarp (seed/pit), with little flesh (mesocarp) development, (iii) seed/pit hardening, during which fruit growth slows down as the endocarp cells stop dividing and become Sclerified, (iv) mesocarp development, representing the second major period of fruit growth when mesocarp develops mainly by the expansion of pre-existing flesh cells, and intense oil accumulation, and (v) ripening, when the fruit changes from darklime-green to lighter green/purple and fruit become soft. During the ripening phase, the rapid change in the fruit texture takes place over a period of 1–2 weeks and can be observed as a change from hard to softer texture when the fruit is easily squashed and some juice is released. With the progress of ripening, dry matter continues to increase along with oil synthesis at a slower rate than in the previous phase (Rotondi et al., 2004). Higher concentration of phenolic substances at this phase enhances the nutritional properties of the resulting oil. At overripe or black stage of maturation, the total phenol content significantly decreases, reaching half of the initial values and the ratio of oleic acid to linoleic acid also decrease considerably. The oils produced from overripe black olives are more prone to auto-oxidation during storage due to synchronized increase in fatty acid unsaturation and decrease in antioxidants. Moreover, the olive oil from overripe fruit is devoid of

some attractive attributes such as bitterness, pungency, green-leaf aroma and pleasant flavours (Rotondi et al., 2004).

Olive oil quality is greatly affected by the maturity stage of the fruit and harvesting time (Garcia et al., 1996). However, some investigations have undertaken studies to understand the physical and physiological changes in olive fruit during growth and development (Beltran et al., 2004). Changes occurring during this phase have high commercial importance as they dramatically influence the sensory characteristics and storage time of the oil. Early harvested fruit produces oil with high polyphenol content reflected by a higher level of bitterness and pungency with oil extracted from these fruit organoleptically unacceptable (Osman et al., 1994; Dıraman and Dibeklio˘glu., 2009). The percentage of oil increases significantly during early fruit ripening (Lavee and Wodner, 1991, 2004; Salvador et al., 2001) and oil quality improvement is initially associated with the increase in oil content (Tombesi et al., 1994). Therefore, a clear understanding of the physical and physiological changes during growth and development of olive fruit is necessary (Famiani et al., 1991; Proietti et al., 1994 and Tombesi et al., 1994).

Olives are being cultivated in Australia on a commercial basis and Frantoio and Manzanilla are two of the important cultivars (Mailer et al., 2007). However, there is limited information available on morphological and physiological changes as well as ethylene production during growth and developmental of olive fruit cultivated in Australian conditions. The current experiment was conducted to find out the morphological (fruit weight, fruit volume, pulp weight, stone weight, pulp/stone ratio and ripening index) and physiological (production of ethylene and rate of respiration) changes in Frantoio and Manzanilla olive cultivars grown in south-western Australian conditions.

4.2. Materials and methods

The current experiment which was conducted during the olive crop season in 2013 observed the olive fruit growth processes by including two olive cultivars (cvs.). Frantoio and Manzanilla grown at York (31°52′44″ S, 116°45′57 E) under south-western Australian conditions.

4.2.1. Design of experiment

The experiment was conducted by following two-factor (cultivar and days after full bloom) factorial Randomized Complete Block Design (RCBD) with 4 replications the experimental unit. Five olive trees was considered as an experimental unit.

4.2.2. Collection of olives and observations recorded

Olive fruit (composite sample of 1.5 to 2 Kg) were harvested on the selected days after full bloom (DAFB) from five trees included in four replications of cvs. Frantoio and Manzanilla each. Observations on physical (fruit weight, fruit volume, fruit length, fruit width, pulp weight, stone weight and pulp/stone ratio, and fruit firmness) and physiological (production of ethylene and rate of respiration) parameters were recorded from 30 DAFB to 205 DAFB at an interval of 15 to 30 days. The ripening index data were recorded from 120 to 205 DAFB at an interval of 15 days.

4.2.3. Measuring fruit, stone and pulp weight, pulp/stone ratio and fruit volume

The fruit weight, stone weight and pulp weight were expressed in grams (g) by measuring with a digital balance as explained in Chapter 3, Section 3.5.1.1. The pulp and stone ratio was calculated by dividing the pulp weight with corresponding stone weight. A sample of 100 fruits was used to measure the fruit weight and the pulp as well as stone weight which was measured by using 10 randomly selected fruits from each replication. To measure the fruit volume, 100 fruits were submerged in 500 mL of water contained in a graduated one litre measuring cylinder (Fortuna, Germany) and the volume was recorded as the volume of displaced water in cm^3.

4.2.4. Measuring fruit length and width

The fruit length and width of 100 fruits per replication randomly selected fruits were measured in millimetres (mm) by using a digital calliper and the average value was calculated.

4.2.5. Determining the production of ethylene

A sample of fruit (100 g) per replication was used to determine the endogenous level of ethylene by using the Sensor Sense (Sensor Sense B.V, Nijmegen, The Netherlands) following the method described in Chapter 3, Section 3.5.2. The

Sensor Sense is comprised of an ETD 300 ethylene detector and a set of valve controllers with connected cuvettes for holding the fruit sample. The "continuous flow" method was used with coarse mode (conversion factor 99818, capacity to measure ethylene concentration at 0-500 μlL^{-1}, sensitivity at <1%) of analysis. Each sample was run for 20 minutes with a flow rate of 4.0 L $hour^{-1}$ and the average reading of the last 15 minutes was considered to measure the amount of ethylene. The levels of ethylene were expressed as $\mu mol\ kg^{-1}hour^{-1}$.

4.2.6. Determining the rate of respiration

Respiration rate of olive fruit was determined by following the method described by Zaharah (2011) and detailed in Chapter 3, Section 3.5.3. The headspace gas sample (2.0 ml) was taken through a rubber septum (SubaSeal®, Sigma-Aldrich Co., St. Louis, USA) using a syringe from the hermetically sealed 1L jars with sample fruit (100 g) for an hour and injected into an infrared gas analyzer [Servomex Gas Analyzer, Analyzer series 1450 Food Package Analyzer, Servomex (UK) Ltd., East Sussex, UK]. The respiration rate was calculated on the basis of the peak areas of 2.0 mL gas sample of CO_2 as standard ($StdCO_2$, 8.52 ± 0.17%) (Fig.3.6). Respiration rate was expressed as mmol CO_2 kg^{-1} h^{-1}.

4.2.7. Determining the ripening index

Ripening index of olive fruit sample was determined according to the method described by Uceda and Frias (1975) and detailed in Chapter 3, Section 3.5.6. One hundred randomly selected healthy fruit were cut in half to expose the internal flesh for grading in eight groups. The total number of olives in each category was counted and the ripening index was calculated by using the designated formula.

4.2.8. Determination of fruit firmness

Olive fruit firmness was determined using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK), equipped with horizontal square base table (15 cm × 15 cm) and interfaced to a personal computer with Nexygen® software by following the methods explained earlier by Singh et al. (2009) and. this test has been done only on cv. Manzanilla because cv. Frantoio is too small for TPA Plus. The detail has been described in Chapter 3, Section 3.5.4.

4.2.9. Statistical Analysis

The collected data were analysed by following two-way analysis of variance (ANOVA) using Genstat 14 (release 14.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK). The significance of various cultivars, growth periods and their interactions were assessed within ANOVA and least significant differences (Fisher's LSD) were calculated following significant ($P \leq 0.05$) F test. All the assumptions of analysis were checked to ensure validity of statistical analysis.

4.3. Results

The results obtained from this experiment have been presented in the following figures and explained accordingly.

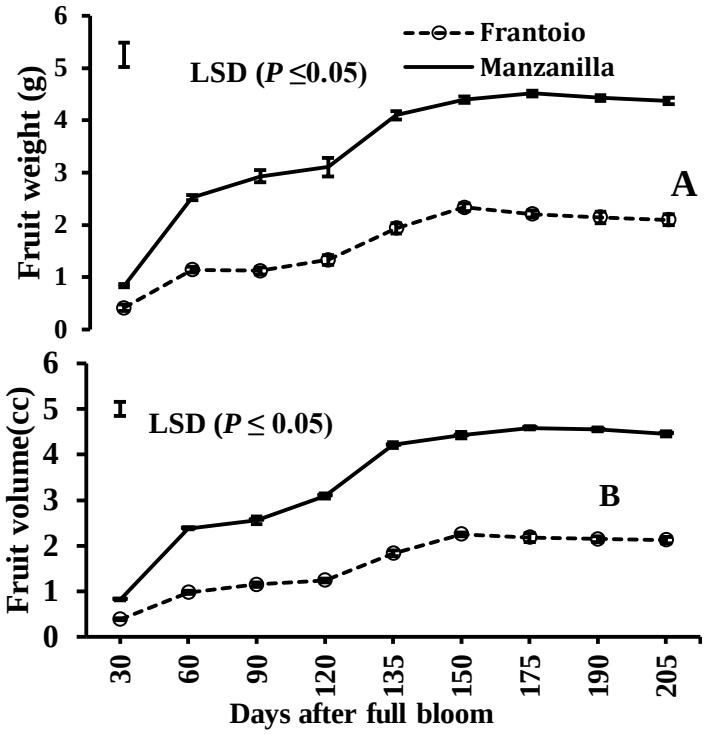


Fig. 4.1. The changes in weight (A) and volume (B) during fruit growth and development (DAFB) in cvs. Frantoio and Manzanilla olives during 2013. The vertical bars representing the SE of means (n = 4 replicates each one 100 fruit) and are invisible when the values are smaller than the symbol. LSD ($P \leq 0.05$) for fruit weight; DAFB = 0.33, cv.0.16, DAFB x cv. = 0.47 and for fruit volume; DAFB = 0.21, cv.0.10, DAFB x cv. = 0.30.

4.3.1. Fruit weight

The fruit weight showed a double sigmoid growth during its growth and development period in Manzanilla and Frantoio cvs (Fig.4.1A). The fruit weight (g) increased significantly ($P \leq 0.05$) irrespective of the cultivars with the growth period of the fruit until 150 days after flowering and then it plateaued. The average fruit weight was higher in cv. Manzanilla than Frantoio (Fig. 4.1A). There was a significant ($P \leq 0.05$) interaction between growth development time and cultivars for fruit weight.

4.3.2. Fruit volume

The increase in fruit volume exhibited a double sigmoid pattern during fruit growth and development period in cvs. Manzanilla and Frantoio (Fig.4.1A). The mean fruit volume (cc) in both cultivars increased significantly ($P \leq 0.05$) with the fruit growth from 30 days to 175 days after flowering (5.54-fold) (Fig.4.1B). The fruit volume increased significantly (from 0.82 to 4.58cc) in cv. Manzanilla from 30 to 175 days after flowering. However it is similar in cv. Frantoio but the highest was for 150 days after flowering (2.25 cc). The Manzanilla fruit showed significantly higher fruit volume (2.16- fold) than Frantoio (Fig.4.1B). There was a significant ($P \leq 0.05$) interaction between growth period and cultivars for fruit volume.

4.3.3. Fruit length

The growth in fruit length showed a double sigmoid pattern during growth and development period in both the cultivars (Fig.4.2A). The fruit length (cm) increased significantly in both cultivars with the progress of fruit growth from 30 days to 135 days after flowering. The Manzanilla fruit showed significantly higher fruit length (1.17-fold) than Frantoio. The highest fruit length was noticed at 205 days after flowering of Manzaillla (2.23 cm) and the lowest was at 30 days after flowering of Frantoio (1.28 cm). There was a significant ($P \leq 0.05$) interaction between growth period and cultivars for fruit length.

4.3.4. Fruit width

A double sigmoid pattern during growth and development period was also noticed for fruit width in cvs. Manzanilla and Frantoio (Fig.4.2 B).The mean fruit width (cm) in both cultivars increased significantly ($P \leq 0.05$) with growth period from 30 days to 175 days after flowering (2.06-fold). In cv. Manzanilla, the fruit width increased significantly from 30 to 175 days after flowering (from 0.97 to 1.92cm). However, in cv. Frantoio the highest width (1.40 cm) was recorded 175 days after flowering. There was a significant ($P \leq 0.05$) interaction between growth period and cultivars for fruit width.

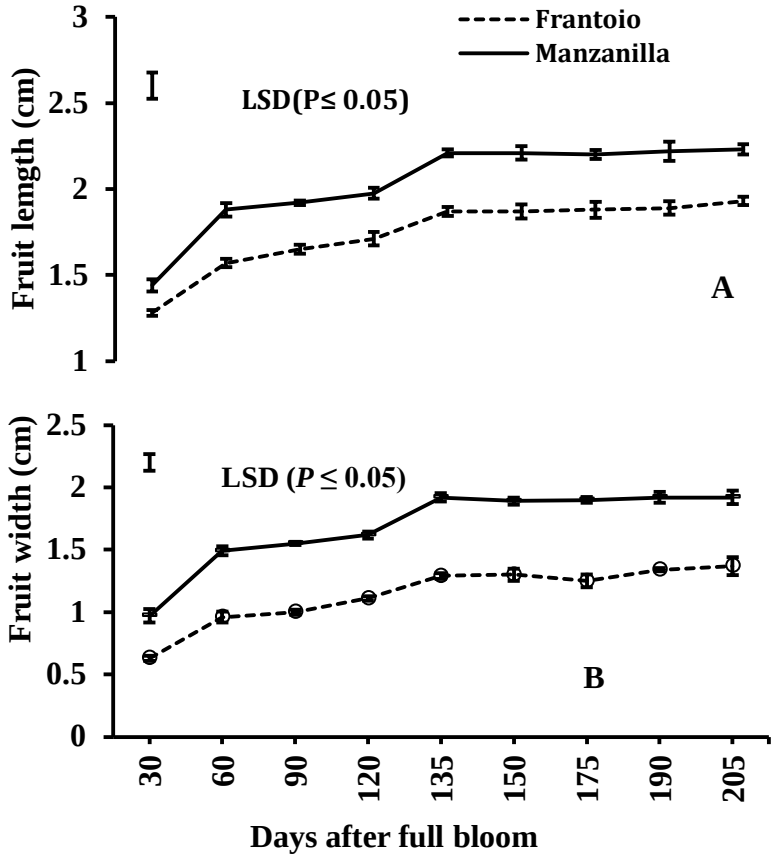


Fig. 4.2. The changes in fruit length (A) and width (B) during fruit growth and development (days after full bloom, DAFB) in cvs. Frantoio and Manzanilla of olive in 2013. The vertical bars representing the SE of means (n = 4 replicates each one 100 fruit) and are invisible when the values are smaller than the symbol. LSD ($P \leq 0.05$) for fruit length; DAFB = 0.0.15, cv.0.07, DAFB x cv. = 0.22 and for fruit width; DAFB = 0.13, cv.0.06, DAFB x cv. = 0.19.

4.3.5. Pulp weight

Irrespective of the cultivars, the pulp weight (g) increased significantly ($P \leq 0.05$) with the progress of growth period from 60 days to 150 days after flowering to (2.06-fold) (Fig. 4.3A). The cv. Manzanilla showed significantly higher pulp weight (2.85-fold) than Frantoio. Highest pulp weight was observed in cv. Manzanilla at 150 days after flowering (3.78g) and the lowest was in cv. Frantoio at 60 days after flowering. There was a significant ($P \leq 0.05$) interaction between growth period and cultivars for pulp weight.

4.3.6. Stone weight

The mean fruit stone weight (g) in both cultivars increased significantly ($P \leq 0.05$) with the advancement of growth period from 30 days to 175 days after flowering (1.17-fold) (Fig.4.3B). The cv. Manzanilla fruit showed significantly higher stone weight than cv. Frantoio (1.19- fold). There was a significant ($P \leq 0.05$) interaction between growth period and both cultivars for fruit stone weight.

4.3.7. Pulp/stone ratio

Irrespective of the cultivars, the pulp/stone ratio increased significantly ($P \leq 0.05$) with the progress of fruit growth from 60 days to 205 days after flowering (1.77-fold). The cv. Manzanilla showed significantly higher pulp/stone ratio (2.42-fold) than cv. Frantoio (Fig. 4.3C). The highest pulp/stone ratio (5.01) was observed in cv. Manzanilla at 150 days after flowering and the lowest (1.02) was in cv. Frantoio at 60 days after flowering. There was a significant ($P \leq 0.05$) interaction between growth period and cultivars for pulp/stone ration.

4.3.8. Ethylene production

Production of ethylene (nmol Kg^{-1} h^{-1}) declined significantly in both fruit cultivars with the progress of fruit growth which continued until 120 days after full bloom and then plateaued (Fig. 4.4A). At 190 days after full bloom, a small rise in ethylene production was noted in both cultivars. The cv. Frantoio showed significantly higher level of ethylene production (1.45-fold) than cv. Manzanilla. The highest production of ethylene was observed in 30 days after full bloom in Frantoio (3.36 nmol Kg^{-1} h^{-1}) and was lowest (0.10 nmol Kg^{-1} h^{-1}) in cv. Manzanilla at 205 days after full bloom.

There was a significant ($P \leq 0.05$) interaction between growth period and cultivars for production of ethylene during fruit growth and development.

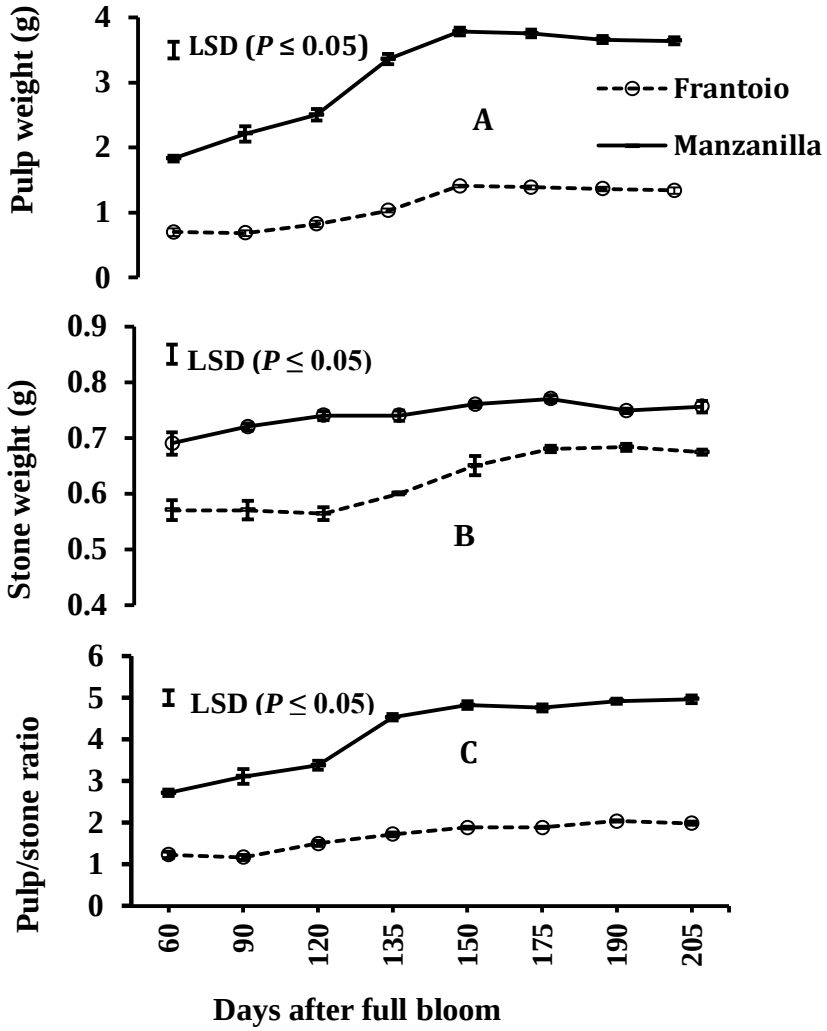


Fig.4.3. pulp weight (A), stone weight (B), pulp and stone weight ratio (C) (A) during fruit growth and development (days after full bloom, DAFB) in cvs. Frantoio and Manzanilla. The vertical bars representing the SE of means (n = 4 replicates each one 100 fruit) and are invisible when the values are smaller than the symbol. LSD ($P \leq 0.05$) for f pulp weight; DAFB = 0.0.26, cv.0.13, DAFB x cv. = 0.36, for stone weight; DAFB = 0.03, cv.0.02, DAFB x cv. = 0.05 and for pulp/stone ratio; DAFB = 0.34, cv.0.17, DAFB x cv. = 0.49.

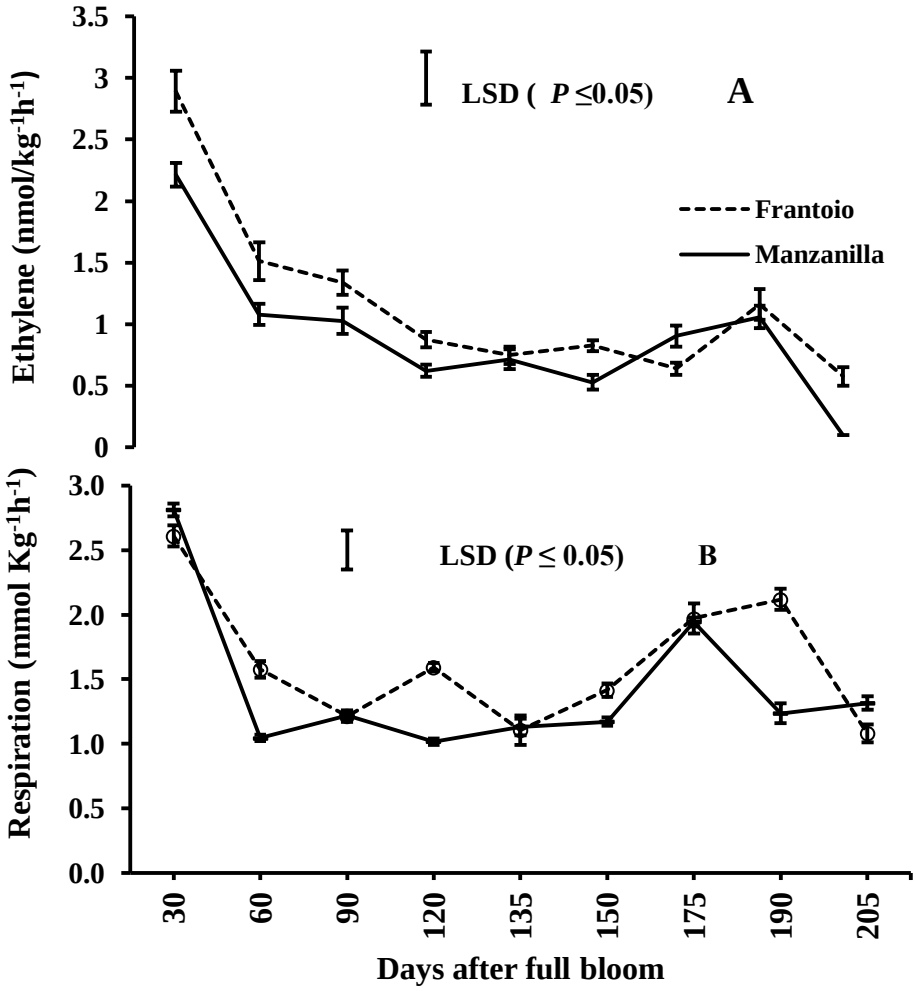


Fig.4.4. The changes in Production of ethylene (A) and rate of respiration (B) during fruit growth and development (days after full bloom, DAFB) in cvs. Frantoio and Manzanilla olives during 2013. The vertical bars representing the SE of means (n = 4 replicates each one 100 fruit) and are invisible when the values are smaller than the symbol. LSD ($P \leq 0.05$) for ethylene; DAFB = 0.46, cv.0.22, DAFB x cv. = 0.66, for respiration; DAFB = 0.30, cv.0.14, DAFB x cv. = 0.43.

4.3.9. Rate of respiration

A sharp decrease in the rate of respiration (2.05-fold) was observed from 30 to 60 days after full bloom and the respiration peak was observed after 175 days in cv. Manzanilla and after 190 days of full bloom in cv. Frantoio. Furthermore, cv. Frantoio showed significantly higher respiration rate (1.2-fold) than cv. Manzanilla (Fig. 4.4B). The highest respiration rate (2.89 mmol kg^{-1} h^{-1}) was observed in Frantoio at 30 days after full bloom and the lowest (0.72 mmol kg^{-1} h^{-1}) was in cv. Manzanilla at 120 days after full bloom. There was a significant ($P \leq 0.05$) interaction between growth period and cultivars for respiration rate.

4.3.10. Fruit ripening index

The fruit ripening index (0-7) increased significantly in both cultivars with the advancement of fruit development and ripening from 120 days to 205 days after full bloom (fig. 4.5). The cv. Frantoio showed significantly higher ripening index (1.36-fold) than Manzanilla. (Fig.4.5). Both the cultivars showed highest ripening index on 205 days after full bloom and the lowest ripening index was noticed at 120 days after full bloom. There was significant ($P \leq 0.05$) interaction between growth period and cultivars for fruit ripening index.

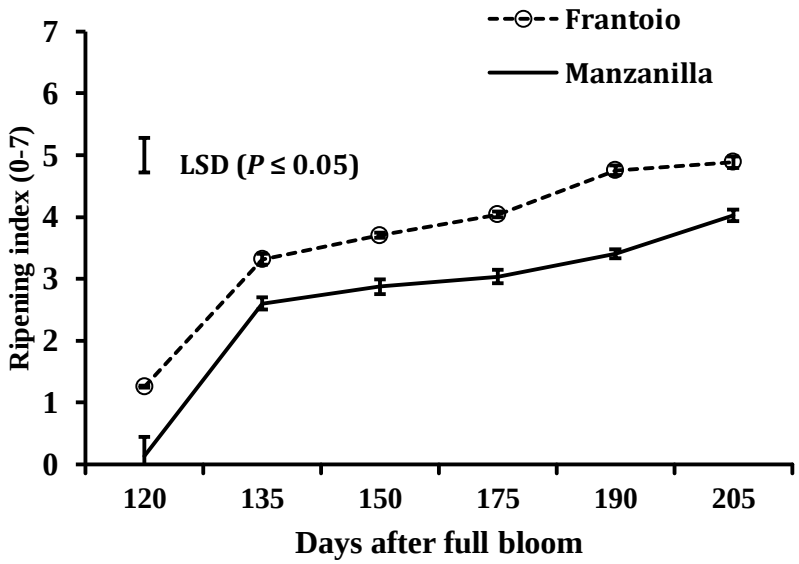


Fig.4.5. The changes in ripening index during fruit development and maturation (days after full bloom) in cvs. Frantoio and Manzanilla of olive during 2013. The vertical bars representing the SE of means (n = 4 replicates each one 100 fruit) and are invisible when the values are smaller than the symbol. LSD ($P \leq 0.05$) for ripening index DAFB = 0.55, cv.0.32, DAFB x cv. = 0.78.

4.3.11. Fruit firmness of Manzanilla

The fruit firmness (N) in Manzanilla cultivar decreased significantly (4.36-fold) with the advancement of fruit development and ripening from 60 days to 205 days after full bloom (Fig.4.6). The highest fruit firmness (7.03 N) was observed at 60 days after full bloom and the lowest (1.15 N) was at 205 DAFB. Fruit firmness in cv. Frantoio was not recorded because the fruits were very small.

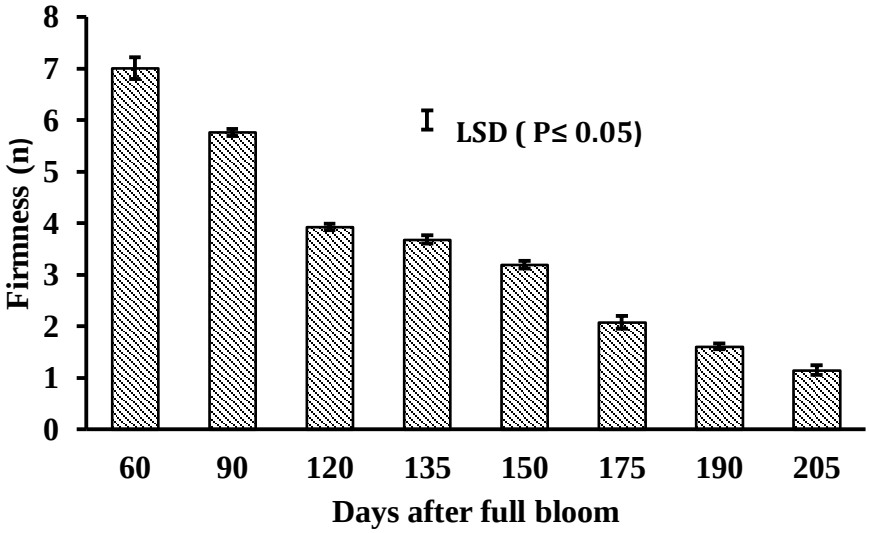


Fig.4.6. Changes in the fruit firmness during fruit development and maturation (days after full bloom) in Manzanilla cv olive during 2013. The vertical bars representing the SE of means (n = 4 replicates each one 100 fruit) and are invisible when the values are smaller than the symbol. LSD ($P \leq 0.05$) for fruit firmness = 0.38.

4.4. Discussion

The knowledge on the physical and physiological changes during development of olive fruit growth is necessary to understand the relationship between the level of maturity and different chemical compositions including oil content (Famiani et al., 1991; Proietti et al., 1994 and Tombesi et al., 1994). The physical and physiological growth parameters observed in this study included ethylene production, rate of respiration, fruit weight, fruit volume, pulp weight, stone weight, pulp/stone ratio and ripening index. Growth and development of olive fruit are influenced by cultivar, growing conditions and cultural practices (Tombesi et al., 1994). A better understanding of the morphological and physiological changes during growth and development of olive fruit can help to improve commercial and qualitative characteristics of fruit. In Australia olives are being grown on a commercial basis and Frantoio and Manzanilla are two of the important cultivars under cultivation (http://www.oliveaustralia.com.au). There is limited information available on morphological and physiological changes during growth and developmental of olive fruit cultivated in Australian conditions. The current experiment was conducted with the goal of finding out the morphological (fruit weight, fruit volume, pulp weight, stone weight, pulp/stone ratio and ripening index) and physiological (production of

ethylene and rate of respiration) changes in Frantoio and Manzanilla olive cultivars grown in south-western Australian conditions. The results obtained from this experiment have been discussed here in light of the available relevant information from studies of other investigators.

4.4.1. Fruit growth parameters (fruit weight, fruit volume, fruit length, fruit width, pulp weight, stone weight and pulp/stone ratio)

As expected, fruit weight, volume, length and width exhibited double sigmoid growth pattern in both the cultivars. The fruit weight (g) and volume (cc) increased significantly ($P \leq 0.05$) irrespective of the cultivars with the progress of growth period. The average fruit weight and volume were higher in Manzanilla than Frantoio (Fig 4.1A and B). Changes in fruit growth parameters such as fruit weight and volume do not depend on the cultivar (Lavee et al., 1982, 1991; Barone et al., 1994; Inglese et al., 1996; Barranco et al., 2000 and Iniesta et al., 2009), however, the fresh fruit weight differs for cultivars and it is genetically determined (Beltrán et al., 2004). Concurrent increase of fruit weight with progression of fruit growth until maturation has also been reported from recent works on olive fruit (Lavee and Wodner, 2004; Menz and Vriesekoop, 2010 and Dag et al, 2011). Mailer et al. (2007) observed significant year effect on the physical measurements (e.g. maturity index, moisture content, oil content and fruit weight) of olive fruit cvs. Corregiolla, Mission and Paragon (all members of the Frantoio group) grown in the south western region of New South Wales, Australia, and harvested at six different times during the season, over three years.

Similarly, it was also observed that the fruit length (cm) and width increased significantly in both cultivars with the progress of fruit growth irrespective of the cultivars. cv. Manzanilla fruit showed significantly higher fruit length and width than Frantoio (Fig. 4.2A and B). It was also noted that the pulp weight (g), stone weight (g) and their ratio increased significantly ($P \leq 0.05$) with the progress of growth period and cv. Manzanilla showed significantly higher values than cv. Frantoio (Fig. 4.3A, B and C). Our observations support the observations of Lavee et al. (1990) and Tombesi (1994) that fruit growth exhibit double sigmoid growth curve and the growth and development of olive fruit are influenced by cultivar, growing conditions and cultural practices.

4.4.2. Production of ethylene and rate of respiration

Production of ethylene and rate of respiration were higher at the initial stages of fruit growth and declined significantly in both cultivars from 30 days after full bloom. On 190 days after full bloom, increased ethylene production was observed. The increased ethylene production was observed 175 days after full bloom in cv. Manzanilla and after 190 days of full bloom in cv. Frantoio. Kitasaki et al. (1999) also reported higher rate of respiration and ethylene production during first three weeks after bud burst and then a decline in both. Similar results have also been reported for young fruit development in cherry (Blanpied, 1972) which reflects the high respiratory levels in the meristematic cells of young fruit. Both ethylene and respiration follow a similar pattern of changes suggesting a possible interaction between them (Kitasaki et al., 1999). Generally photosynthesis occurs in the presence of chlorophyll in the exocarp and the mesocarp contains significant amounts of phosphoenol pyruvate carboxylase (Sánchez, 1994), the CO_2 fixation enzyme. During the fruit development, CO_2 produced from the mitochondrial respiration of photoassimilates becomes photosynthetically fixed into triose-phosphate in the fruit chloroplasts in the light period and thereby the growing fruit expresses a lower level of CO_2 as an indicator during the measurement of respiration (Sánchez and Harwood, 2002). Cv. Frantoio showed a significantly higher level of ethylene production and rate of respiration than cv. Manzanilla which might have been genetically determined.

4.4.3. Fruit ripening index

The fruit ripening index increased exponentially in both cultivars with the progress of fruit growth (Fig. 4.5). The cv. Frantoio showed significantly higher ripening index (1.36- fold) than cv. Manzanilla. Barranco et al. (2000) also reported varied pattern of ripening index for different cultivars including Frantoio. Photosynthetic activity in the fruit tissue decreases with the progress of fruit growth and ripening which reduces the concentrations of both chlorophylls and carotenoids (Salvador et al., 2001). The fruit becomes violet or purple due to the accumulation of anthocyanins at its black ripe stage (Roca and Minguez-Mosquera, 2001). A number of changes occur during the ripening of olive and these changes influence fruit

firmness, chemical composition and sensory characteristics of the fruit and oil (Beltrán, 2000).

4.4.4. Fruit firmness

The fruit firmness (N) in cv. Manzanilla decreased significantly (4.36-fold) with the advancement of fruit development and maturation (Fig.4.6). During the ripening phase, the rapid change in the fruit texture takes place over a period of 1–2 weeks and can be observed as a change from hard to softer texture when the fruit is easily squashed and some juice is released (Rotondi et al., 2004). The composition of chemical and biochemical components including fatty acids, polyphenols, tocopherols and sterols change with maturation and the magnitude of these changes depend on the cultivar, climate and growing conditions (Gutierrez et al., 2000). These changes are consequent with the textural changes as well and the flesh firmness reduces with progress of fruit maturity (Nanaos et al., 1999). Firmness of olive pulp decreases with the loss of uronic acids in the cell wall as reported earlier by Jimenez et al. (2001) in Hojiblanca olives during ripening. A decrease in methyl esterification of olive pulp cell wall pectic polysaccharides during ripening causes the loosening of complexation between galacturonic acid and Ca (Mafra et al., 2001 and Ferreira et al., 2006) which ultimately decreases the olive pulp firmness. The increased ethylene production at 175 days after full bloom in Manzanilla and 190 days after full bloom in Frantoio may also be ascribed to the loss of fruit firmness. Ethylene plays an important role in fruit softening (Menniti et al., 2004), whilst exogenous application of ethylene receptor blockers such as 1-methylcyclopropene, has been reported to delay fruit softening in climacteric fruit (Sisler and Serek, 1997).

4.5. Conclusion

Changes occur during the growth and development of olive fruit including different physiological and physical changes. The current experiment was conducted to understand these changes in respect of days after full bloom on two olive cultivars Frantoio and Manzanilla grown in south-western Australian condition. The fruit weight (g), fruit volume (cc), fruit length (cm), fruit width (cm), pulp weight (g), stone weight (g), pulp/stone ratio and fruit ripening index increased significantly ($P \leq 0.05$) irrespective of the cultivars with the progress of growth period. The increase of

these parameters continued until 150 to 175 days after full bloom and then plateaued. Higher average values for these parameters were noted in Manzanilla than Frantoio. Production of ethylene, rate of respiration and fruit firmness declined significantly in both cultivars with the progress of fruit growth. At 190 days after full bloom, the ethylene peak was observed suggesting that ethylene modalities olive fruit ripening. Moreover, exogenous application of ethylene has also hastened fruit ripening in Frantoio and Manzanilla cultivars. It could be concluded that the value of physical parameters related to olive fruit growth and development increased with the progress of fruit growth after full bloom until 175 days and physiological parameters showed a declining trend during this period with a peak at 175 to 190 days after full bloom. Ethylene seems to be involved in initiation of olive fruit ripening.

Chapter 5

Effect of harvesting time on the physical, biochemical and sensory attributes of olive fruit and oil from cvs. Frantoio and Manzanilla in south-western Australia

Abstract

Well reported are factors influencing cultural practices, application of technologies, oil extraction method, storage conditions and quality of olive oil. Effect of harvesting time at different ripening stages of fruit is also an influential factor. There is a scarcity of published reports on the effects of harvesting time on the quality attributes of olives grown in south-western Australia. Therefore, the current investigations reported in this thesis were conducted during 2013 and 2014 to explore the effects of five different harvesting times (mid- and late-April, mid- and late-May and mid-June) on the physical, biochemical and sensory attributes of cvs. Frantoio and Manzanilla olives grown in south-western Australia and their respective oils. The fruit of cv. Manzanilla showed higher fruit removal force, moisture content (%) and oil content in dry weight (%) than cv. Frantoio. Furthermore, lowest moisture and oil content were observed in the driest harvest year, 2014. The fatty acids showed significant increase (free fatty acid, palmitic acid, stearic acid, linoleic acid) or decrease (peroxide value, oleic acid, MUFA, PUFA and MUFA:PUFA ratio) with the delay of harvesting from first to fifth periods in both of the years, irrespective of the cultivars. A significant gradual decrease was noted in major polyphenol compounds from first to fifth harvest. The concentration of phenolic compounds was comparatively high in the fruit harvested in 2014. The sensory attributes of cvs. Frantoio and Manzanilla deteriorated with the delay of harvesting, with water stress possibly influencing the bitterness of the fruit in 2014. In conclusion, the harvesting of olive fruit during the early part of winter delivered olive oil with better physical, biochemical and sensory attributes and climatic conditions such as water stress negatively influences the quality attributes of olive fruit. Higher concentrations of phenolic compounds were observed in 2014 with less rainfall; however the trend of declining concentration from first to fifth harvest was comparatively prominent in 2014 than 2013.

5.1. Introduction

Olive oil is one of the oldest produced foods providing perfect balance of aroma, taste, flavour and health benefits. The dietary importance of olive oil and health benefits are due to both fatty acid composition and minor compounds like polyphenols, tocopherols, sterols and carotenoids (La Lastra et al., 2001). It also provides many nutrients including vitamin E, beta-carotene and minerals (Reichelt and Burr, 2000). Intake of olive oil reduces harmful cholesterol LDL (Low Density Lipoprotein) without reduction in beneficial HDL (High Density Lipoprotein) cholesterol (Psaltopoulou et al., 2004). Olive oil fatty acids contain 16 to 18 carbon atoms with a carboxyl group (COOH) at one end (Mailer et al., 2005). The major olive oil fatty acid is oleic acid which is monounsaturated and accounts for 55% to 83% of total fatty acids (Aparicio, 1999 and Beltran, 2000).

Extra virgin olive oil contains numerous phenolic antioxidants which are potent inhibitors of oxidation and reduce the cancer risk (Owen et al., 2000). Furthermore, o-diphenol family is identified as the major source contributing to the overall antioxidant activity and sensorial properties of extra virgin olive oils (Lavelli, 2002). The total phenol in olive oil ranges between 50-1000 mg L^{-1} (Aguilera et al., 2005; Salvador et al 2001; Youssef et al., 2010). The concentration of phenolic compounds in virgin olive oil is significantly affected by many agronomical and technological factors such as cultivar, maturity stage, location, soil, irrigation systems, environmental factors and production process (Nergiz, 2000; Ranalli et al., 2001; Patuumi et al., 2002; Kalua et al., 2005; Baccouri et al., 2008; Dag et al., 2011).

A significantly higher amount of oil (%) was reported in early ripening fruit (Salvador et al., 2001; Lavee and Wodner, 2004). Anastasopoulos et al. (2011) reported that crop year and maturation phases of olive fruit affect the amount of total phenol in olive oil. Lavee and Wonder (2004) observed uniform oil content in the mesocarp of black matured fruit of Barnea and Manzanilla cultivars regardless of size and level of fruit yield. Similar observation was reported by Beltran et al. (2004), however they claimed that the oil content may vary due to climatic conditions such as lower rainfall, which may cause lower oil and higher dry matter content in olive fruit. Quality indexes and fatty acid composition are most

significantly affected by maturity fruit of olives (Dabbou et al., 2011). Maximum oil content was reported between 60^{th} to 75^{th} days after the start of the ripening process (Camposeo et al., 2013). Reduction in the value of most of the analytical parameters such as peroxide value, pigments, sensory scores, oleic acid and total sterols; and increase in the free acidity and linoleic acid were observed during ripening of cv. Cornicabra olive (Salvador et al., 2001).

Olive oil from the major cultivars grown in Australia does not meet some of the limits set by international standards for some parameters in some cases (Mailer et al., 2010). There are limited studies reported from New South Wales (NSW), Australia, on the effect of harvesting time on oil content. From these reports it has been noted that olive fruit oil content increases rapidly until fruit maturity with different rate between cultivars to reach a maximum percentage of oil content (Mailer et al., 2007 and Zeleke et al., 2012). Ayton et al. (2007) also reported significant effect of cultivation year and harvesting time on total polyphenols, chlorophyll concentration, palmitic acid and linoleic acid in oil from the olives grown in NSW, Australia. Qualitative effects of harvesting time on polyphenol profile in olives grown in NSW conditions were also reported by Obied et al. (2008). Despite the enormous potentiality and economic importance of growing olives in Western Australia (WA) (Kailis, 1999), there is no information available on chemical composition and properties of the olives and extracted oil according to the ripening stages of the fruit grown under south-western Australian conditions. Therefore, the current study was conducted with the aim of determining the optimal harvest time of olive cvs. Frantoio and Manzanilla in south-western Australia and finding out the effect of different harvesting times/stages (April to June) of ripening on olives based on the physical (fruit removal force, fruit moisture and oil content), biochemical (level of fatty acids and polyphenols) and sensory attributes (fruitiness, bitterness and pungency).

5.2. Materials and Methods

5.2.1. Study location and climatic conditions

The experiment was conducted in the olive field at Talbot Grove, York (31°52′44″ S, 116°45′57″ E), located at 120 km east of Perth, Western Australia. Details on the

study location and its climatic conditions have been described in Chapter 3, Section 3.1.

5.2.2. Design of experiment and treatments

The studies were conducted by following two-factor (harvesting time X cultivar) factorial Randomized Complete Block Design (RCBD) with 4 replication units. Five olive trees were considered as an experimental unit. Different harvesting times were in autumn as mid- and late-April, mid- and late-May and early winter, mid-June. The cultivars included in this experiment were cvs. Frantoio and Manzanilla.

5.2.3. Experimental olive trees and their maintenance

The experiment was conducted with 15 year old central leader-shaped olive trees of cvs. Frantoio and Manzanilla and the planting pattern and other management practices have been detailed in Chapter 3, Section 3.1.

5.2.4. Collection of olives and preparation of virgin olive oil

Olive fruit (composite sample of 1.5 to 2 Kg) were harvested by hand from five representative trees included in each replicate of cvs. Frantoio and Manzanilla The fruit were harvested at five different ripening times from mid-autumn to early winter (April to mid-June) (Table.5.1) at fortnightly intervals in 2013 and 2014. Virgin olive oil was prepared from the collected fruit by following the method of Rivas et al. (2013) and as described in Chapter 3, Section 3.4.

Table. 5.1. Harvest time of cvs. Frantoio and Manzanilla olive fruit during 2013 and 2014.

Harvest Time	Harvest time 2013	Harvest time 2014
First	17th April	15th April
Second	30th April	29th April
Third	14th May	13th May
Fourth	28th May	29th May
Fifth	11th Jun	12th June

5.2.5. Observations recorded:

5.2.5.1. Fruit removal force (FRF)

The FRF was determined by using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK), equipped with horizontal square base table (15 cm × 15 cm) and interfaced to a personal computer with Nexygen® software. The procedure of determining FRF has been detailed in Chapter 3, Section 3.5.5.

5.2.5.2. Moisture content (%) of olive fruit

The olive fruit moisture content was determined according to (COI/OH/Doc. No 1 November 2011) by using 60g healthy and randomly selected olive fruit. The fruit were ground with a hammer mill and dried in a forced air oven at 105°C for approximately 8–10 hours until the weight was constant. Details of determining moisture content in olive samples have been described in Chapter 3, Section 3.5.7.

5.2.5.3. Olive oil content (% dry basis)

Olive oil percentage was determined from fresh olive fruit by following the method described by Avidan et al. (1999) with some modifications. Ten grams of olive fruit paste from each replicate of cvs. Frantoio and Manzanilla was taken in small scintillation vials and dried in an oven at 80°C for 24h to constant weight. Then 5g of each dried sample was transferred to 25×100 mm glass tubes and 10 ml of petroleum ether at 60-80°C was added, and the mixture homogenised at medium speed for 30 sec with a vortex mixer (Heidolph, Reax Top, Australia). The content was rinsed with 5 ml petroleum ether and agitated overnight with a rotator shaker (Ratek Orbital Mixer, Australia). The following day each extract was passed through a paper filter and again rinsed with 5 ml petroleum ether. This dissolved oil was recovered by evaporating off the petroleum ether at 40°C. The detail of determining oil content has been described in Chapter 3, Section 3.5.8.

5.2.5.4. Determination of free fatty acid

The free fatty acid (%) was determined according to the EC (2568/91) method and as described in Chapter 3, Section 3.5.11. Only 10 g of virgin olive oil was dissolved in 50 ml of the solvent mixture (1:1 of 95% (V/V) ethanol and diethyl ether) and

titrated with a solution of potassium hydroxide to a pink colour that persisted for at least 10 sec.

5.2.5.5. Peroxide value of oil

Peroxide value of the virgin olive oil fraction was determined according to EC 2568/1991 method under artificial or diffused daylight. Only 1-2 g of the virgin olive oil sample was added to 10 ml of chloroform, 15 ml of acetic acid and 1 ml of potassium iodide (KI) in a 250 ml flask. The mixture was shaken for 1 minute and then left for 5 minutes at a temperature of 15 to 25°C in the dark. Then 75 ml of distilled water was added and the mixture titrated with the sodium thiosulphate solution (0.002 N). During titration, the flask and contents were shaken. The detailed procedure with calculation for determining peroxide value has been presented in Chapter 3, Section 3.5.11.

5.2.5.6. Determination of fatty acid composition

Fatty acid composition of virgin olive oil samples was determined by using a gas chromatograph following the method prescribed by the International Olive Council (2001). Methanol with heptane and methanolic potassium hydroxide (2M) were mixed with the oil sample and the mixture was homogenized vigorously for 30 seconds. The upper layer containing methyl esters was decanted and injected into the gas chromatograph with heptane solution. The gas chromatograph was fitted with a fused silica column (50m length × 0.25mm i.d.) coated with SGL-1000 phase (0.25µm thickness Suger labour, Spain) and containing a FID detector (HP 6890, Agilent Technologies). Detailed procedure of determining fatty acid composition has been described in Chapter 3, Section 3.5.12.

5.2.5.7. Total polyphenols

The total phenols were quantified by following the method of (Ranalli et al., 1999). Olive oil (10g) was isolated and dissolved in hexane by triple extraction of a solution with a water/methanol mixture (60:40, v/v). Folin-Ciocalteu phenol reagent was absorbed later using a spectrophotometer (Model SECOMAM ANTHELIE Advanced, France). The absorption of extracts was read at 725 nm, and then calculated according to Mateos et al. (2001), as described in Chapter 3, Section 3.5.13.

5.2.5.8. Determination of polyphenol compounds

Polyphenol compounds were determined by adding only 5 ml of methanol/water (80/20, v/v) with 5 g of virgin olive oil and analysing the mixture by HPLC-DAD. The phenolic compounds were quantified at 235-280 nm using syringic acid as internal standard. Phenolic standards (3,4 DHPEA-EA, Tyrosol and hydroxytyrosol) of 0.015 mg/ml strength were prepared and used to determine the level of polyphenols and calculation as described in Chapter 3, Section 3.5.14. using the official method of the International Olive Council, COI/T.20/Doc. No 29.

5.2.5.9. Olive oil sensory attributes

A tasting panel of seven trained tasters examined olive oil samples on two different days with short breaks between each two tests. The members were supplied with scaled sheets for sensory attributes such as fruitiness, bitterness and pungency. Each attribute was scaled from 0 to 10 where 1 represented the value for the poorest and 10 the best possible quality for the sample. The procedure of measuring sensory attributes has been detailed in Chapter 3, Section 3.5.15.

5.2.6. Statistical Analysis

All the experimental data were subjected to two-way analysis of variance (ANOVA) using Genstat 14 (release 14.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK). The effects of various treatments and their interactions were assessed within ANOVA and least significant differences (Fisher's LSD) were calculated following significant ($P \leq 0.05$) F test. All the assumptions of analysis were checked to ensure validity of statistical analysis. The data on various parameters over two years were not pooled because error mean squares over years were found to be heterogeneous.

5.3. Results:

5.3.1. Physical properties

5.3.1.1. Fruit removal force

The mean fruit removal force in olive fruit significantly reduced irrespective of the cultivar from first to fifth harvest (5.85 N to 4.00 N) in 2013 (Fig.5.1). A similar trend in reduction of fruit removal force was also noticed in 2014. The highest

removal force (6.20 N) was in first harvest time of cv. Manzanilla in 2013 and the lowest (2.47N) was in fifth harvest time of cv. Frantoio in 2014. When averaged over treatments, the mean fruit removal force was significantly higher in cv. Manzanilla than cv. Frantoio (1.71- and 1.43-fold in 2013 and 2014 respectively). There was a significant ($P \leq 0.05$) interaction between harvest time and cultivars for fruit removal force in both years.

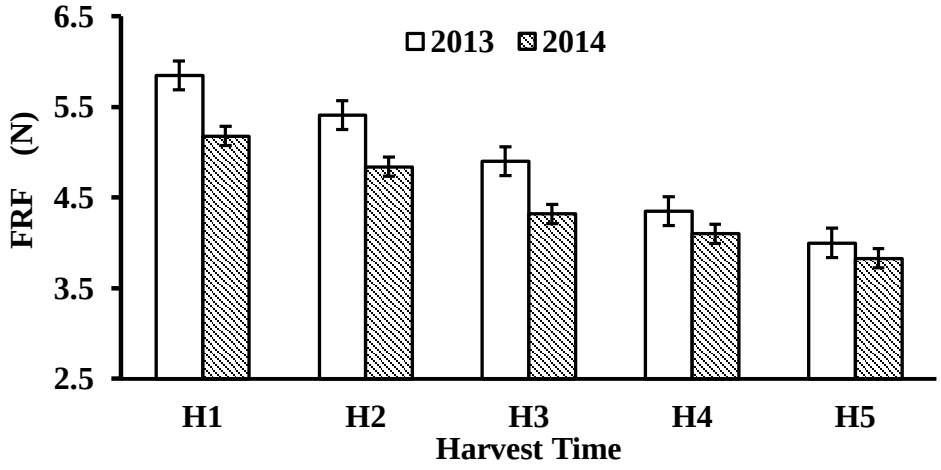


Fig. 5.1. Effects of different harvest time on mean fruit removal force (FRF) in both olive cultivars during 2013 and 2014 .Vertical bars represent LSD ($P \leq 0.05$**).**

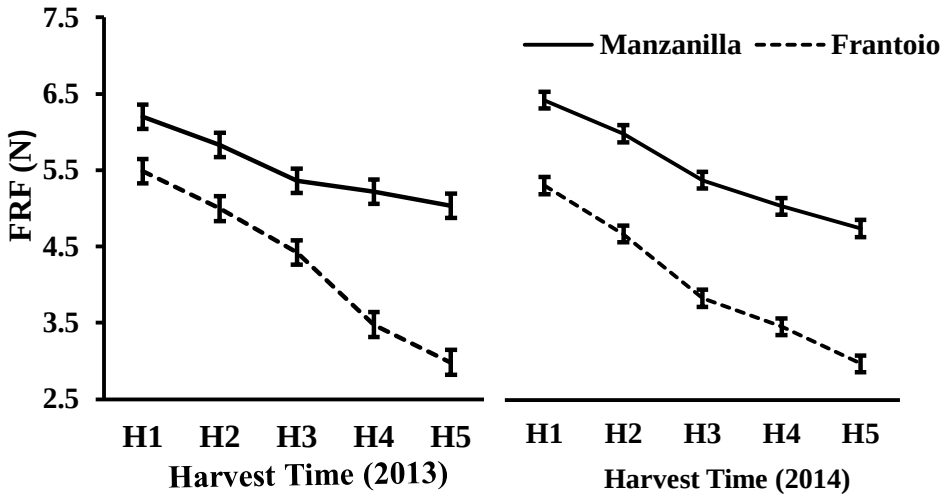


Fig.5.2. Effects of different harvest time on the fruit removal force in cvs. Frantoio and Manzanilla olives during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$).

5.3.1.2. Fruit Moisture (%)

When averaged over cultivars, mean moisture percentage in the fruit declined from first to fifth harvest in 2013 and 2014 (from 57.57 to 54.08% and from 54.69 to 51.48% respectively) (Figure. 5.3). The highest moisture was in first harvest time of Manzanilla in 2013 (62.35%) and the lowest was in fifth harvest time of cv. Frantoio in 2014 (47.25 %) (Fig.5.4). When averaged over treatments, the mean fruit moisture (%) was significantly higher in cvs. Manzanilla than Frantoio (1.19-fold in both years). There was a significant ($P \leq 0.05$) interaction between harvest time and cultivars for fruit moisture (%) in both years.

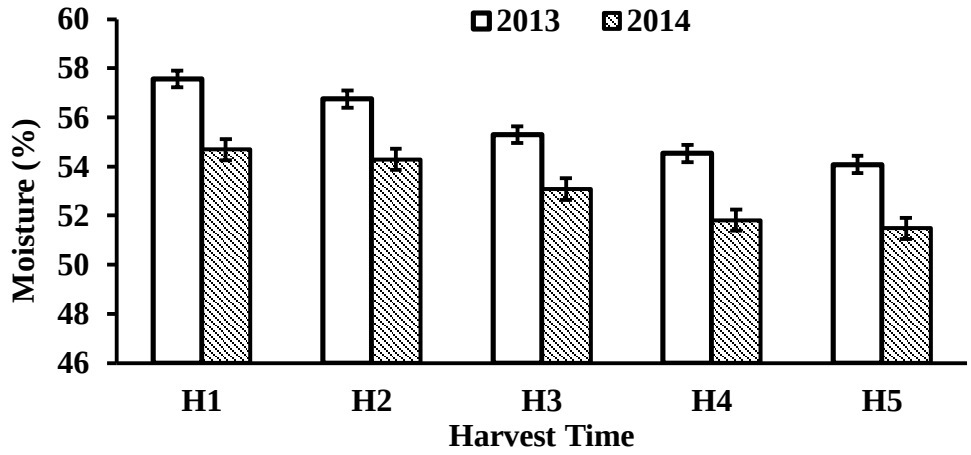


Fig.5.3. Effects of different harvest time on mean level of moisture (%) in the olive fruit for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$)

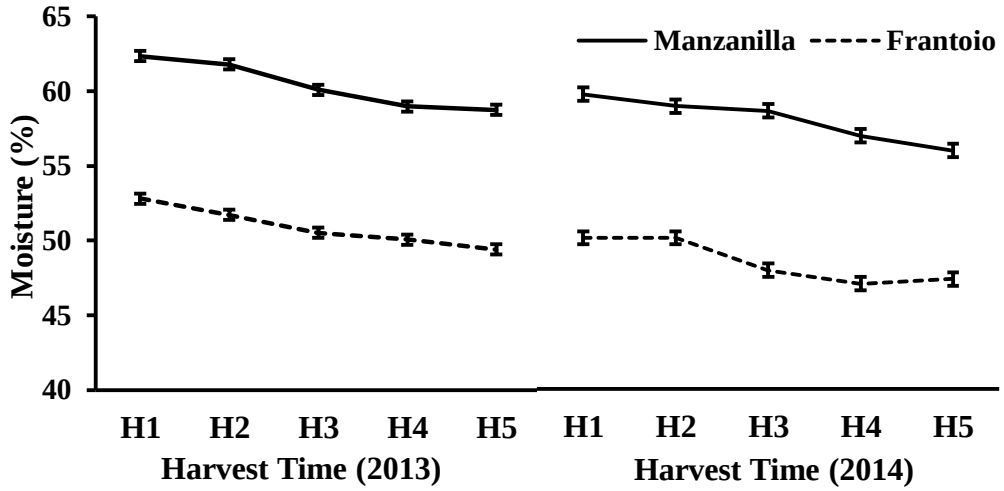


Fig.5.4. Effects of different harvest time on the levels of fruit moisture (%) of cvs. Frantoio and Manzanilla olives during 2013 and 2014.Vertical bars represent LSD ($P \leq 0.05$).

5.3.1.3. Oil content (% dry weight)

The mean oil percentage in olive fruit significantly increased (1.07-fold and 1.10-fold respectively) irrespective of cultivars from first to fifth harvest time in 2013 and 2014 (Fig. 5.5). The oil accumulates faster in cvs. Manzanilla than Frantoio from first to fifth harvest time in both years (Figure 5.6). However the highest oil percentage was noted in fifth harvest time in cv. Frantoio during 2013 and 2014 (39.05% and 38.55% respectively). Meanwhile, in cv. Manzanilla, fourth and fifth harvest time resulted in higher oil percentage both years (38.65%, 38.58% in 2013 and 38.05%, 37.85% in 2014 respectively). Irrespective of the harvest time, the mean oil percentage in cv. Manzanilla was significantly higher than Frantoio (1.01-fold) in both the years. There was a significant ($P \leq 0.05$) interaction between harvest time and cultivars in both years for percentage of oil (% dry weight).

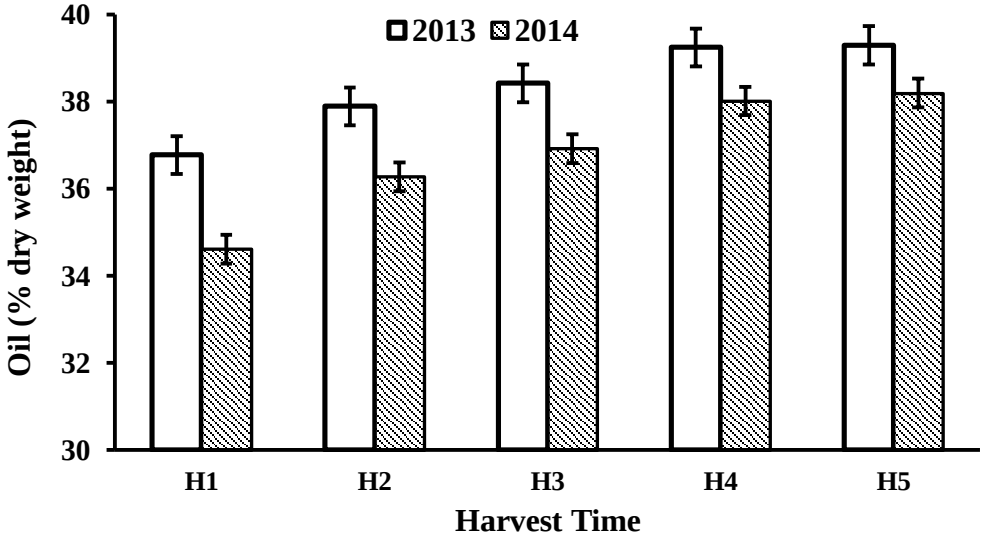


Fig.5.5. Effects of different harvest time on mean oil percentage (% dry weight) in olive fruit for both cultivars in 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**)**

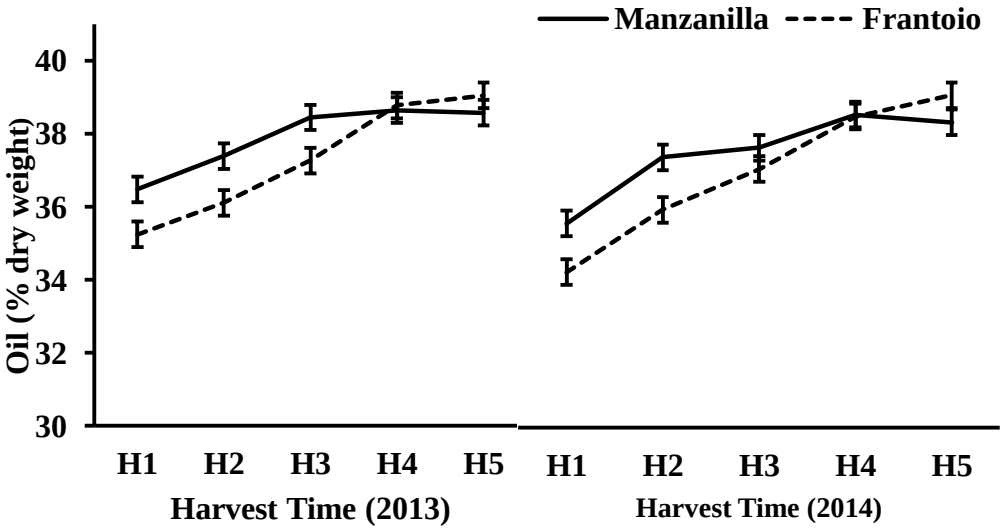


Fig.5.6. Effects of different harvest time on concentration of oil (% dry weight) in cvs. Frantoio and Manzanilla olives during 2013 and 2014. Vertical bars represent as LSD ($P \leq 0.05$**).**

5.3.2. Chemical aspects of virgin olive oil

5.3.2.1. Free fatty acid (%)

Irrespective of the cultivars, the mean free fatty acid (%) in virgin olive oil increased significantly from first to fifth harvest in 2013 (from 0.27% to 0.37%) and in 2014 (from 0.28% to 0.38%) (Fig.5.7). The concentration of free fatty acid (%) increased significantly with delay in harvest from first to fifth in cv. Manzanilla (from 0.28% to 0.37% in 2013 and 0.32% to 0.37% in 2014) and cv. Frantoio (from 0.25% to 0.37% in 2013 and 0.24% to 0.38% in 2014) (Figure 5.8). When averaged over harvest treatments, the mean free fatty acid (%) was significantly higher in cv. Manzanilla (0.33% and 0.34%) than cv. Frantoio (0.32 % and 0.30%) during 2013 and 2014 respectively. The interaction between harvest time and cultivars for free fatty acid in the oil during 2013 was non-significant; whilst in 2014, the interaction was significant ($P \leq 0.05$).

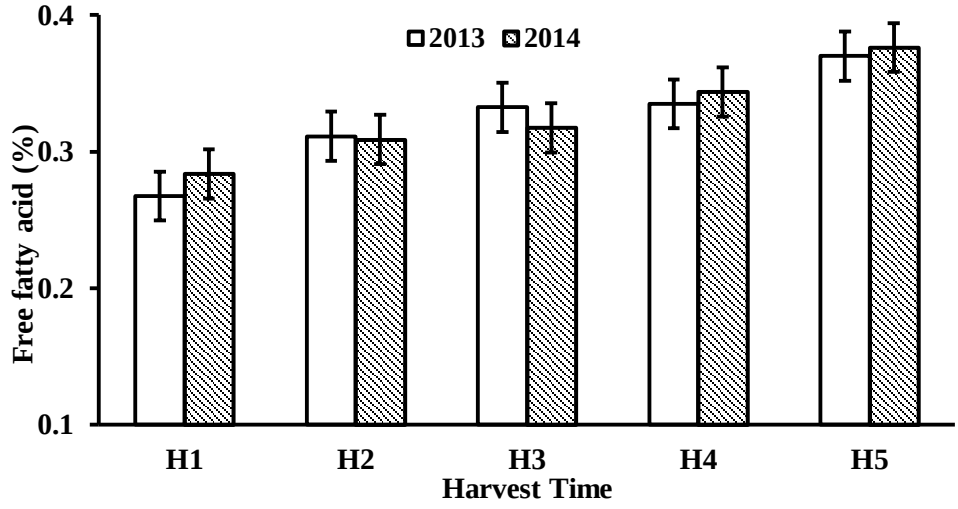


Fig. 5.7. Effects of different harvest time on mean free fatty acid (%) in virgin olive oils for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$).

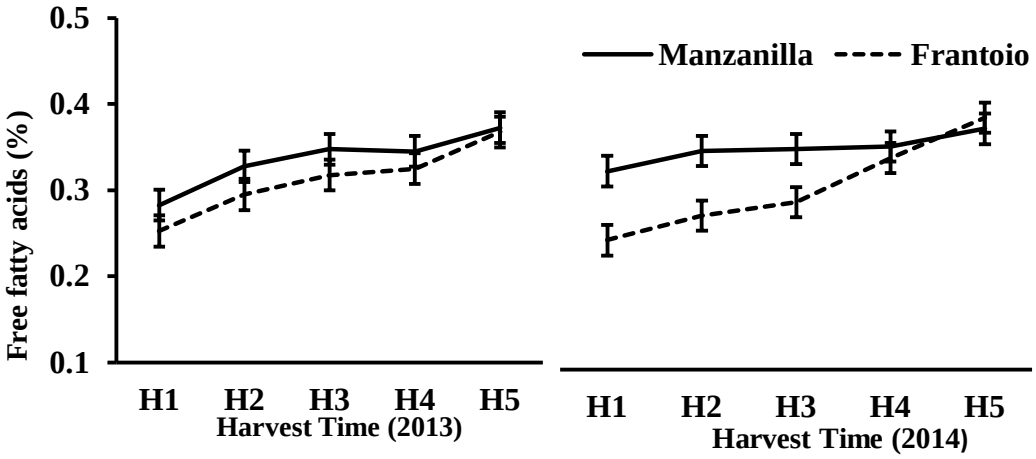


Fig. 5.8. Effects of different harvest time on the free fatty acids (%) in the oil of cvs. cvs. Frantoio and Manzanilla olives during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.2. Peroxide value

The peroxide value in olive cultivars decreased significantly from first to fifth harvest (0.47-fold in 2013 and 0.59-fold in 2014) (Fig. 5.9). The peroxide value in virgin olive oil of cv. Manzanilla decreased significantly with delay in harvest from first to fifth (from 6.65 to 3.28 meq O_2 kg^{-1} in 2013 and from 3.28 to 5.31 meq O_2 kg^{-1} in 2014) (Figure 5.7). Similar trend was also observed in cv. Frantoio in both years. The Frantoio virgin olive oil showed significantly higher peroxide value than cv. Manzanilla (1.32- and 1.08-fold in 2013 and 2014 respectively). There was a

significant ($P \leq 0.05$) interaction between harvest time and cultivars in both years for peroxide value in oil.

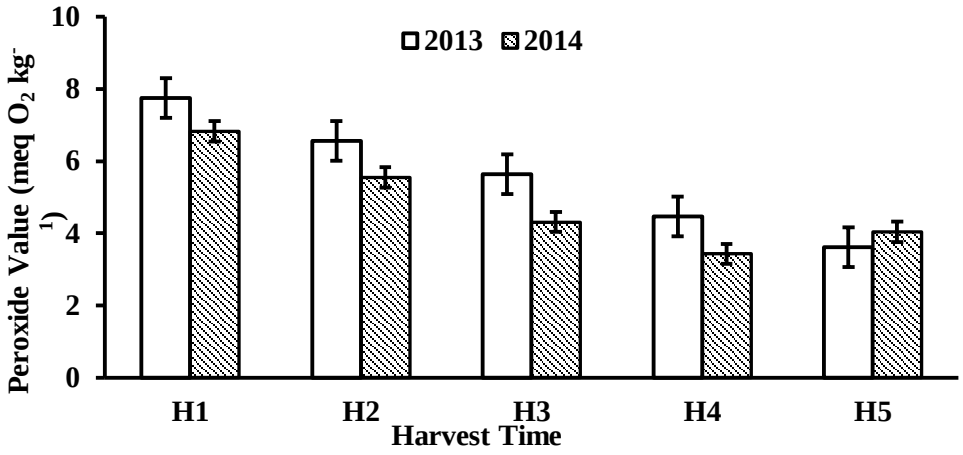


Fig. 5.9. Effects of different harvest time on mean peroxide value (meq O_2 kg^{-1}) in virgin olive oils during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$).

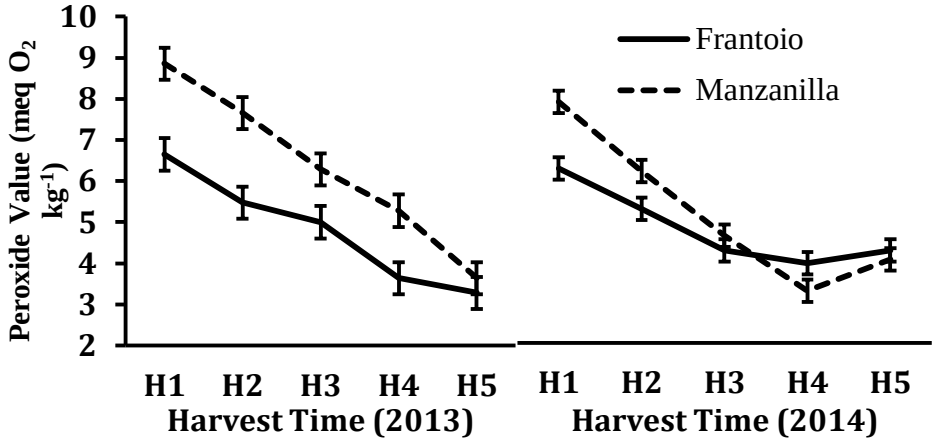


Fig. 5.10. Effects of different harvest time on peroxide value (meq $O_2 kg^{-1}$) in virgin olive oils of cvs. Frantoio and Manzanilla olives during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.3. The fatty acids:

5.3.2.3.1. Palmitic acid (C16:0):

The concentration of palmitic acid (C16:0) in virgin olive oil increased significantly with delay in harvest from first to fifth (1.08-fold in 2013 and 1.12-fold in 2014) in both years (Fig. 5.11). Significant increase of palmitic acid (C16:0) in virgin olive oil was observed from first to fifth harvest in cv. Manzanilla (from 12.07 to 12.84 % in

2013 and from 12.00 to 13.96 % in 2014) in cv. Frantoio (from 12.54 to 13.64 % in 2013 and from 13.74 to 15.04 % in 2014). The cv. Frantoio virgin olive oil showed significantly higher amount of palmitic acid (C16:0) than Manzanilla (1.05- fold in 2013 and 1.08-fold in 2014) (Fig. 5.11).

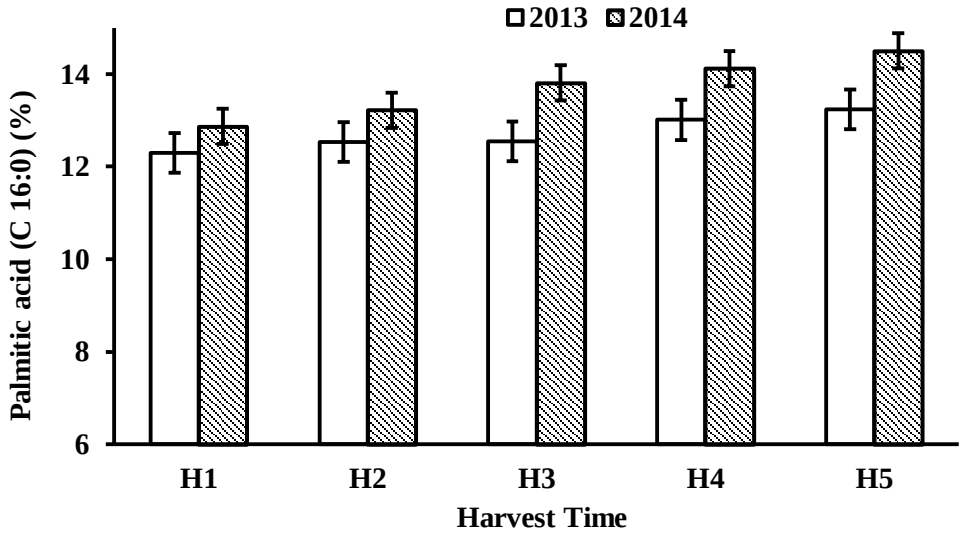


Fig. 5.11. Effects of harvest time on mean levels of palmitic acid (C 16:0) (%) in olive oil during for both cultivars in 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

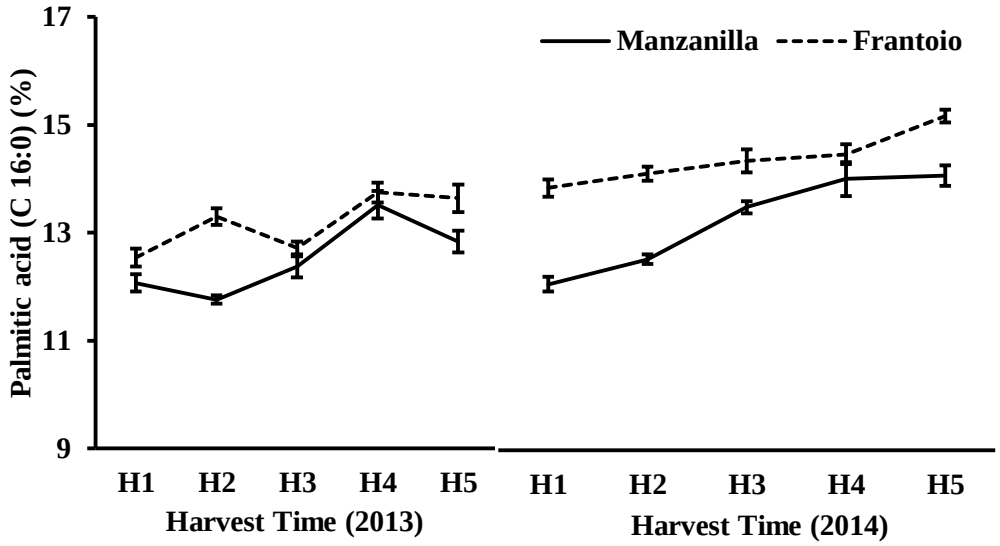


Fig. 5.12. Effects of different harvest time on the level of palmitic acid (C 16:0) (%) in the virgin olive oil of cvs. Frantoio and Manzanilla olives during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.3.2. Stearic acid (C18:0)

The stearic acid (C18:0) in virgin olive oil increased significantly with delay in harvest from first to fifth (1.39-fold in 2013 and 1.51-fold in 2014). Irrespective of the harvest time and cultivars, the stearic acid was higher in 2013 than 2014 (1.34-fold) (Fig. 5.13). The Manzanilla virgin olive oil showed significantly higher amount of stearic acid than Frantoio in both 2013 and 2014 (1.42- fold) (Fig. 5.14). Moreover, the highest stearic acid (C18:0) was in fifth harvest time of Manzanilla in 2013 (3.31%) and the lowest was in first harvest time of cv. Frantoio in 2014 (1.23%) (Fig.5.14). Significant ($P \leq 0.05$) interaction between harvest time and cultivars in both years was observed for stearic acid.

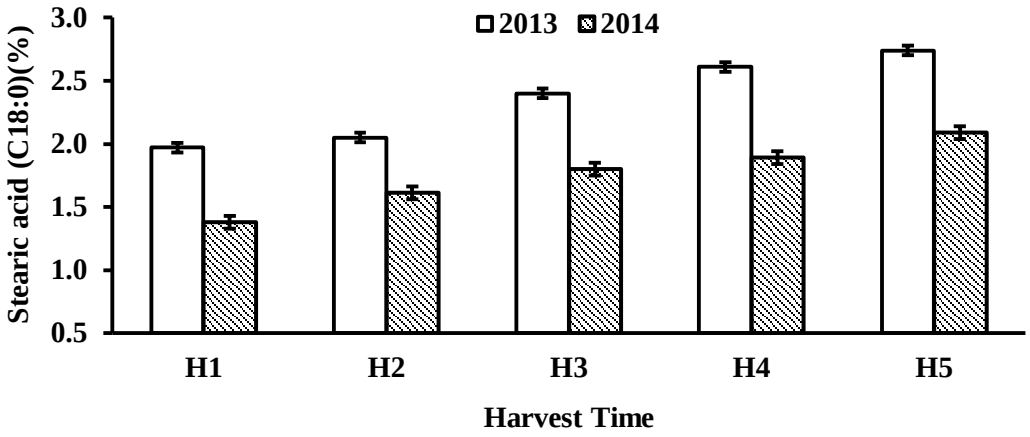


Fig. 5.13. Effects of harvest time on mean levels of stearic acid (C 18:0) (%) in virgin olive oil for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$).

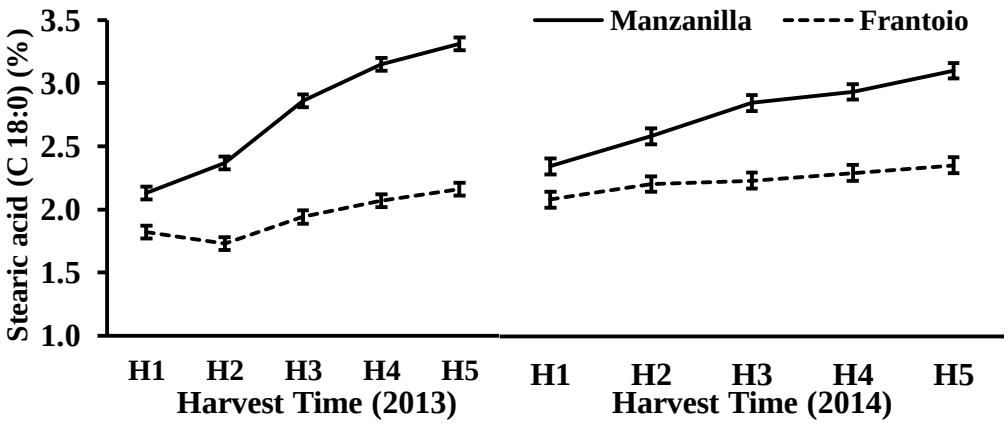


Fig. 5.14. Effects of different harvest time on the level of stearic acid (C 18:0) (%) in the virgin olive oil of cvs. Frantoio and Manzanilla olives during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**)**

5.3.2.3.3. Oleic acid (C18:1)

The mean oleic acid (C18:1) content in virgin olive oil decreased significantly with delay in harvest time from first to fifth (0.96-fold in 2013 and 0.97-fold in 2014) in both years (Fig. 5.15). The virgin olive oil oleic acid (C18:1) decreased significantly from first to fifth harvest in cv. Manzanilla (from 78.75 to 76.29% in 2013 and from 76.58 to 74.53% in 2014). Similarly, in cv. Frantoio it also decreased in both years (Fig. 5.16). The cv. Manzanilla virgin olive oil showed significantly higher amount of oleic acid (C18:1) than cv. Frantoio (1.08- fold in 2013 and 1.06- fold in 2014). There was significant ($P \leq 0.05$) interactions between harvest time and cultivars for oleic acid (C18:1) in oil during 2013 and 2014.

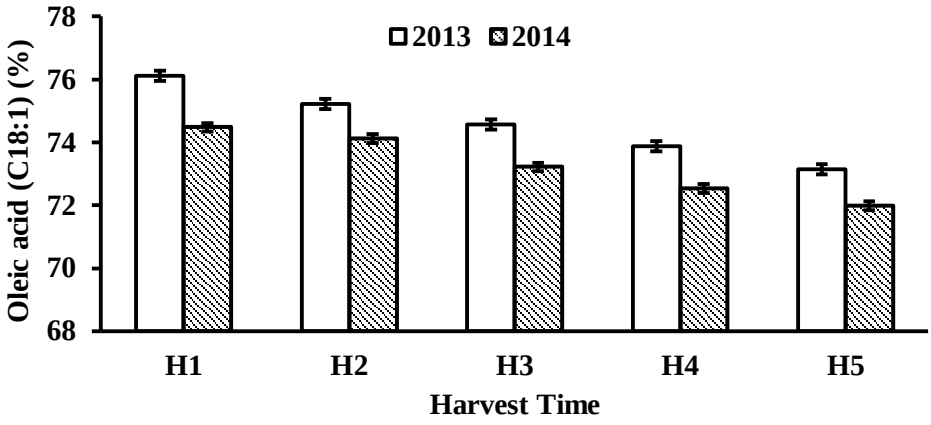


Fig. 5.15. Effects of harvest time on mean of oleic acid (C18:1) (%) in virgin olive oil for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

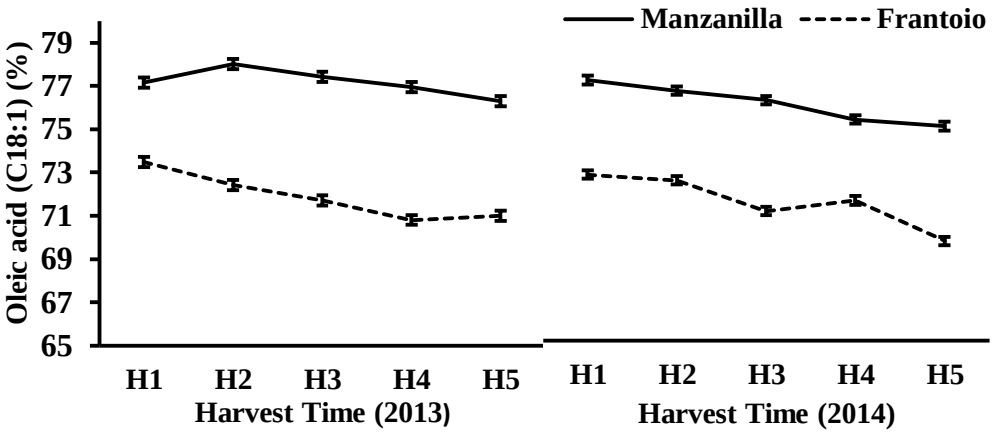


Fig. 5.16. Effects of different harvest time on the level of oleic acid (C18:1) (%) in the virgin olive oil of cvs. Frantoio and Manzanilla during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.3.4. Linoleic acid (C18:2)

Irrespective of the cultivars, the linoleic acid (C18:2) content in virgin olive oil increased significantly with delay in harvest from first to fifth (1.19- fold in 2013 and 1.14-fold in 2014). The level of linoleic acid (C18:2) was higher in 2013 than 2014 (1.08-fold) (Fig. 5.17). The cv. Frantoio virgin olive oil showed significantly higher amount of linoleic acid (C18:2) than cv. Manzanilla (1.16- fold and 1.12 fold in 2013 and 2014 respectively) (Fig.5.18). Highest concentration of linoleic acid (C18:2) was observed in fifth harvest of Frantoio in 2014 (10.94%) and the lowest was in first harvest of cv. Manzanilla in 2013 (7.69%) Significant ($P \leq 0.05$) interaction between harvest time and cultivars in both years was observed for linoleic acid.

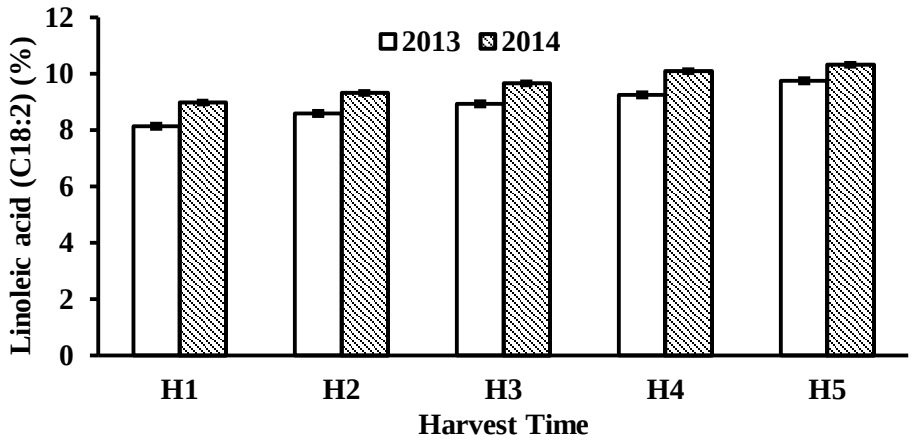


Fig. 5.17. Effects of harvest time on mean level of linoleic acid (C 18:2) (%) in virgin olive oil for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$).

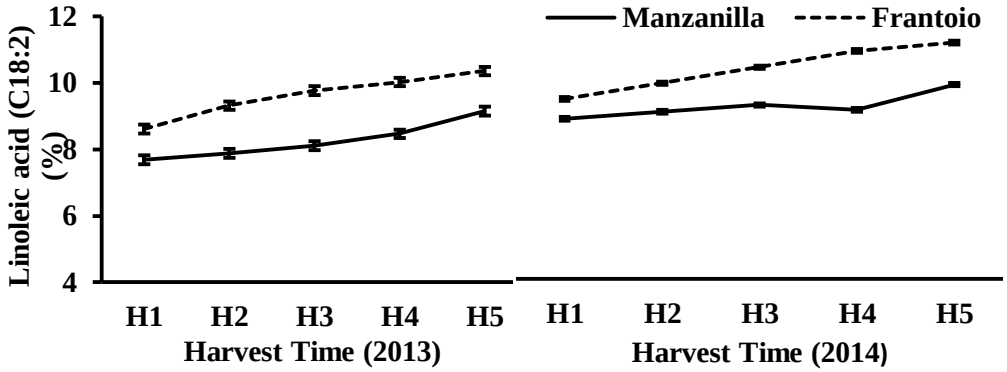


Fig. 5.18. Effects of different harvest time on the level of linoleic acid (C18:2) (%) in the virgin olive oil of cvs. Frantoio and Manzanilla during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.3.5. Monounsaturated fatty acids (MUFA)

Irrespective of the cultivars, the concentration of MUFA (%) in virgin olive oil decreased significantly from first to fifth harvest (from 77.99 to 75.90% in 2013 and from 75.86 to 74.44% in 2014) (Fig. 5.19). The virgin olive oil MUFA (%) also decreased significantly from first to fifth harvest in cv. Manzanilla (from 80.90 to 79.51 % in 2013 and from 78.12 to 76.94 % in 2014) and Frantoio (from 75.08 to 72.30 % in 2013 and from 73.61 to 71.94 % in 2014) (Fig. 5.20). When averaged over treatments, the mean of MUFA percentage was significantly higher in Manzanilla than Frantoio cvs. in 2013 (1.08-fold) and in 2014 (1.06-fold). Significant ($P \leq 0.05$) interactions were noticed between harvest time and cultivars for MUFA in virgin olive oil in both years.

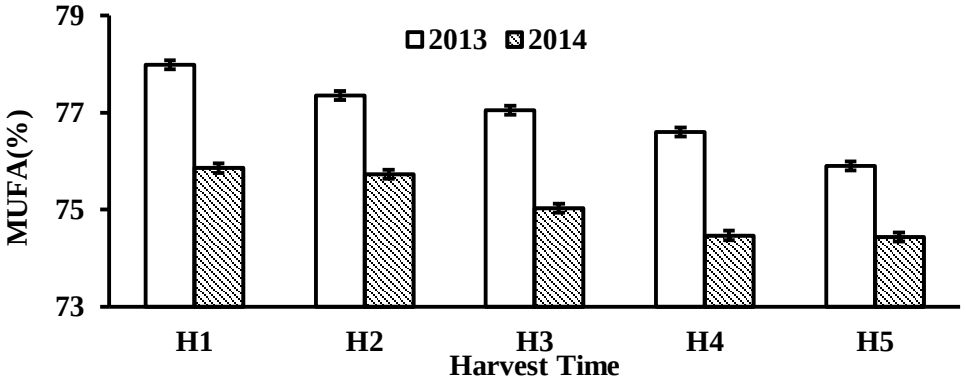


Fig. 5.19. Effects of harvest time on the level of mean monounsaturated fatty acids (MUFA %) in virgin olive oil for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**)**

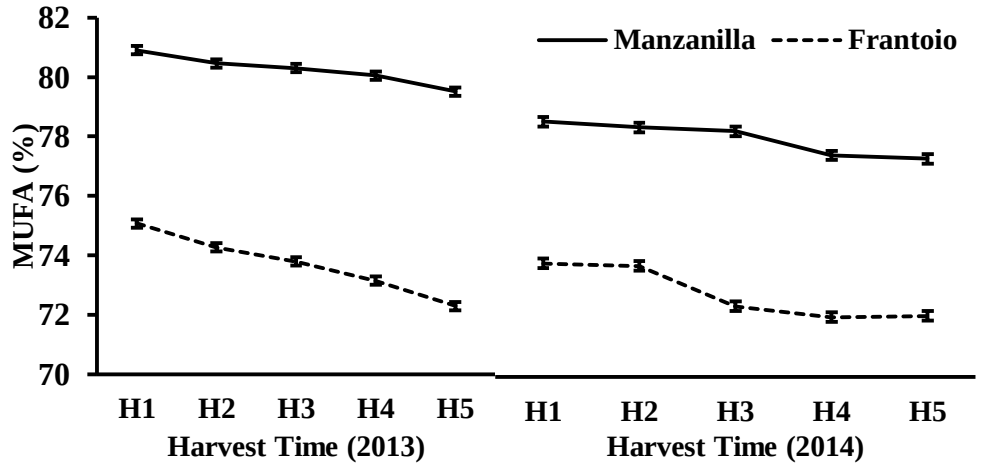


Fig. 5.20. Effects of different harvest time on the level of monounsaturated fatty acid (MUFA %) in the virgin olive oil of cvs. Frantoio and Manzanilla olives during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.3.6. Polyunsaturated Fatty Acids (PUFA %)

The polyunsaturated fatty acids (PUFA) content in virgin olive oil decreased significantly with delay in harvest from first to fifth (1.15- fold in 2013 and 1.12-fold in 2014). Higher concentration of PUFA (%) was recorded in 2013 than 2014 (1.02-fold) (Fig. 5.21). The cv. Frantoio virgin olive oil showed significantly higher amount of PUFA than cv. Manzanilla (1.32- and 1.10-fold in 2013 and 2014 respectively) (Fig. 5.22). Moreover, the highest PUFA (%) was in fifth harvest time of Frantoio in 2013 (11.49 %) and the lowest was in first harvest of cv. Manzanilla in 2013 (8.39 %) (Fig.5.21). The interactions between harvest time and cultivars in both years were significant ($P \leq 0.05$) for PUFA (%).

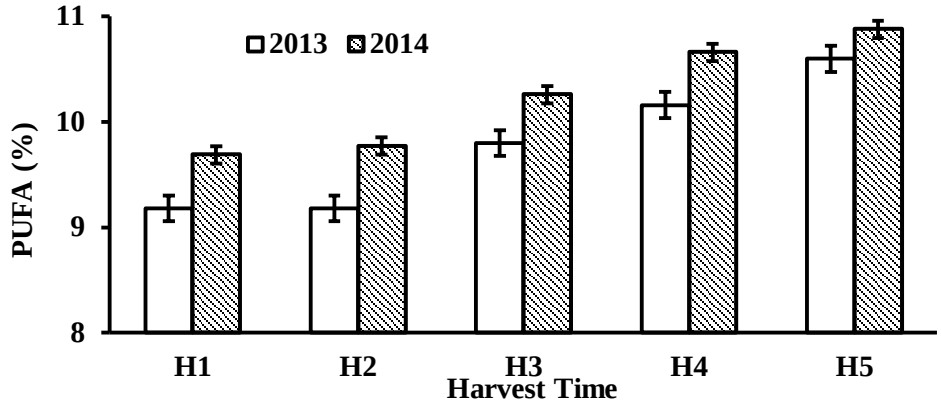


Fig. 5.21. Effects of harvest time on mean of level of polyunsaturated fatty acids (PUFA %) in virgin olive oil for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$)

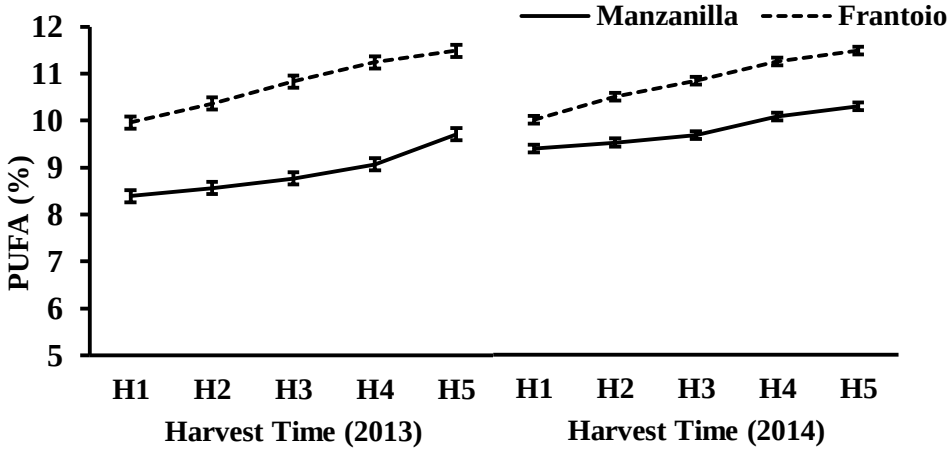


Fig. 5.22. Effects of different harvest time on polyunsaturated fatty acid (PUFA %) in the virgin olive oil of cvs. Frantoio and Manzanilla during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.3.7. Ratio of mono- and polyunsaturated fatty acid (MUFA:PUFA)

The ratio of MUFA:PUFA in virgin olive oil decreased significantly from first harvest to fifth harvest in 2013 (from 8.56 to 7.24) and 2014 (from 7.84 to 6.87) (Fig. 5.23). The ratio also decreased significantly from first to fifth harvest (9.65 to 8.20 in 2013 and 8.32 to 7.48 in 2014) in cvs. Manzanilla than Frantoio (7.54 to 6.29 in 2013 and 7.36 to 6.27 in 2014) (Fig. 5.24). When averaged over treatments, the ratio was significantly higher in cvs. Manzanilla than Frantoio in 2013 (1.31-fold) and 2014 (1.17-fold). Significant ($P \leq 0.05$) interactions were noticed between harvest time and cultivars for MUFA:PUFA ratio in both years.

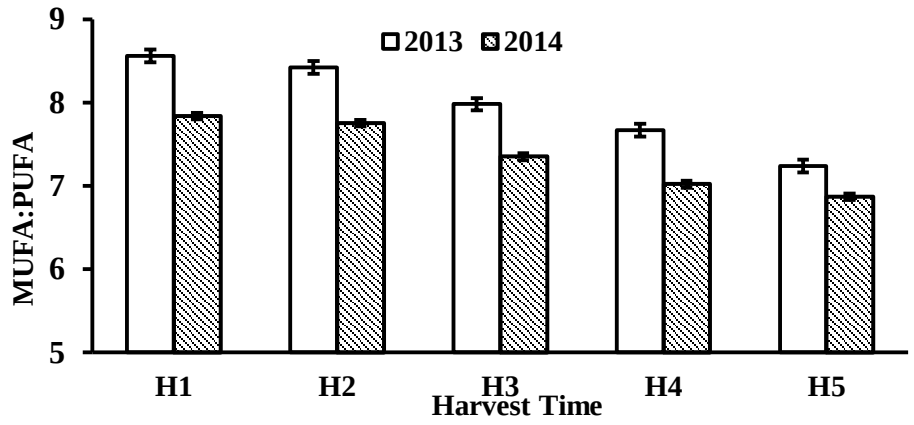


Fig.5.23. Effects of harvest time on mean level of the ratio of mono- and polyunsaturated fatty acid (MUFA:PUFA) in virgin olive oil for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$)

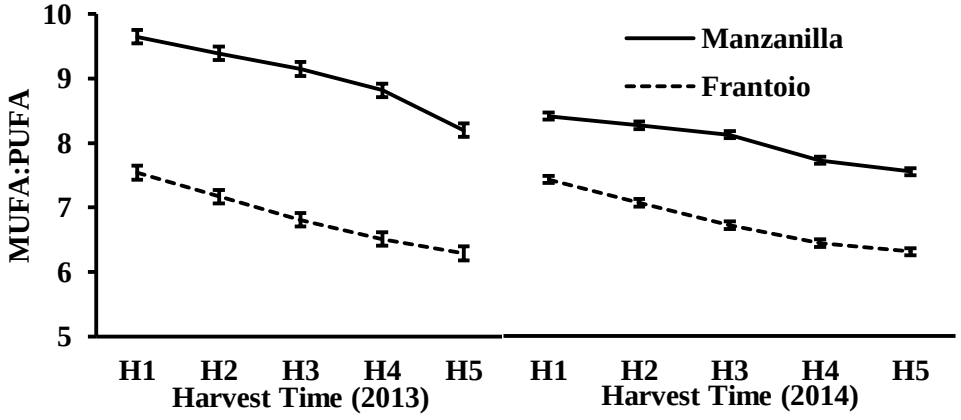


Fig. 5.24. Effects of different harvest time on ratio of mono- and polyunsaturated fatty acid (MUFA:PUFA) in the virgin olive oil of cvs. Frantoio and Manzanilla during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.4. Polyphenolic compounds:

5.3.2.4.1. Hydroxytyrosol

The level of hydroxytyrosol in virgin olive oil was significantly higher in 2014 than 2013 (1.41-fold) and a decreasing trend was observed in both years with the delay of harvesting (from 6.24 to 3.83 mg kg^{-1} and from 8.52 to 5.86 mg kg^{-1} in 2013 and 2014 respectively) (Fig. 5.24). In 2013, a 0.71-fold decrease in hydroxytyrosol compound in virgin olive oil was observed from first to fifth harvest in cv. Manzanilla which was 0.66-fold in 2014. Similarly, the cv. Frantoio showed a 0.48-fold decline in hydroxytyrosol level from first to fifth harvest during 2013 and 0.72-fold decline during 2014 (Fig. 5.25). The highest hydroxytyrosol level 9.59 (mg kg^{-1}) was in first harvest time of cv. Manzanilla in 2014 and the lowest hydroxytyrosol level was 2.63 (mg kg^{-1}) in fifth harvest time of Frantoio in 2013 (Fig. 26).

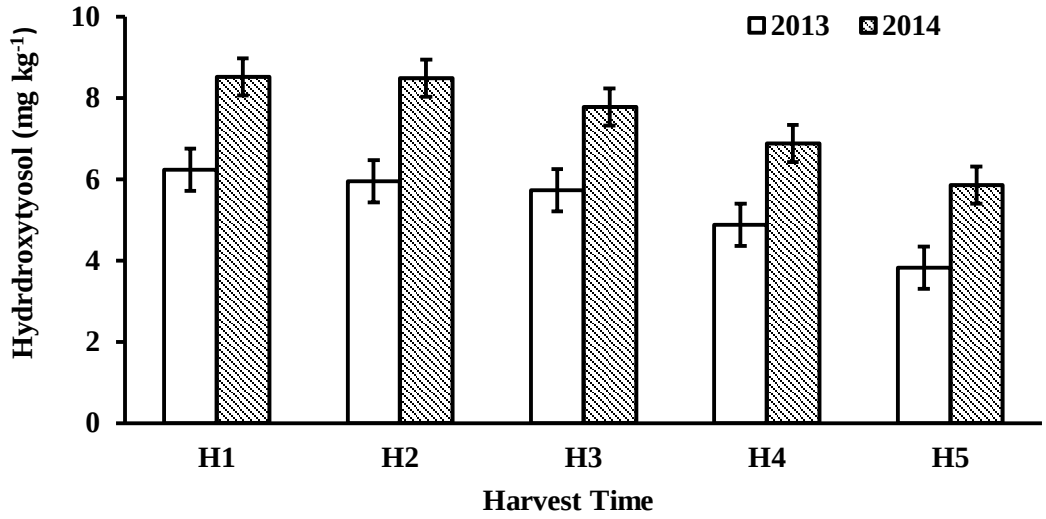


Fig. 5.25. Effects of harvest time on mean level of hydroxytyrosol (mg kg^{-1}) in virgin olive oil for both cultivars during 2013 and 2014. Vertical bars represent as LSD ($P \leq 0.05$**).**

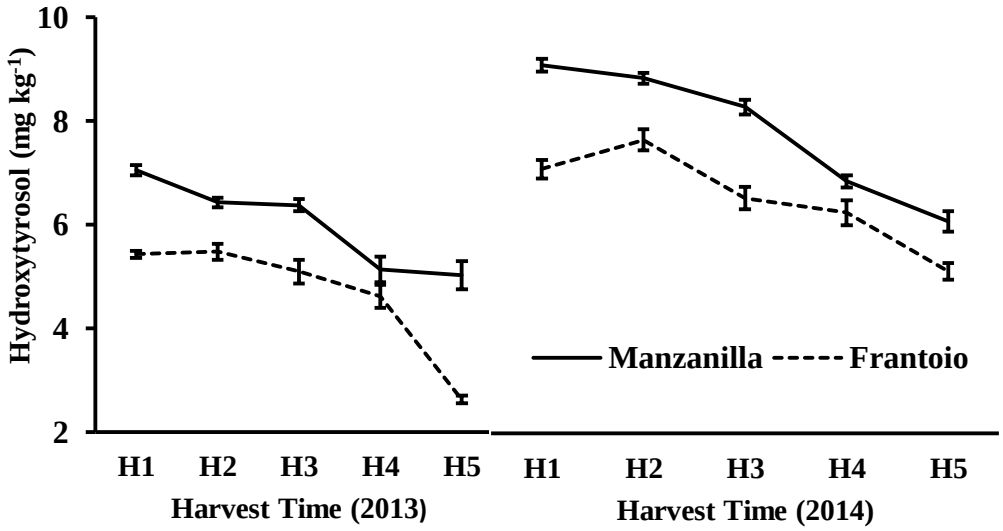


Fig. 5.26. Effects of different harvest time on the hydroxytyrosol (mg kg^{-1}) in the virgin olive oil of cvs. Frantoio and Manzanilla during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.4.2. Tyrosol

The level of tyrosol was also significantly higher (1.21-fold) in 2014 than 2013 and was observed to decrease in both years with the delay of harvesting (from 9.34 to 6.29 mg kg^{-1} and from 10.38 to 7.98 mg kg^{-1} in 2013 and 2014 respectively) (Fig. 5.26). It declined from first to fifth harvest at 0.82-fold in cv. Manzanilla in both harvest years and in cv. Frantoio the level of decrease was 0.49- and 0.63-fold in 2013 and 2014 respectively (Fig. 28). Higher concentration of tyrosol was observed in cv. Manzanilla (9.05 and 10.88 mg kg^{-1} in 2013 and 2014 respectively) which was significantly different from cv. Frantoio (6.93 and 8.51 mg kg^{-1} in 2013 and 2014 respectively). Moreover, the highest tyrosol level 12.00 (mg kg^{-1}) was in first harvest time of cv. Manzanilla in 2014 and the lowest was 4.04 (mg kg^{-1}) in fifth harvest time of cv. Frantoio in 2013 (Fig. 5.27). There was a significant ($P \leq 0.05$) interaction between harvest time and cultivars for tyrosol concentration during 2013 and 2014.

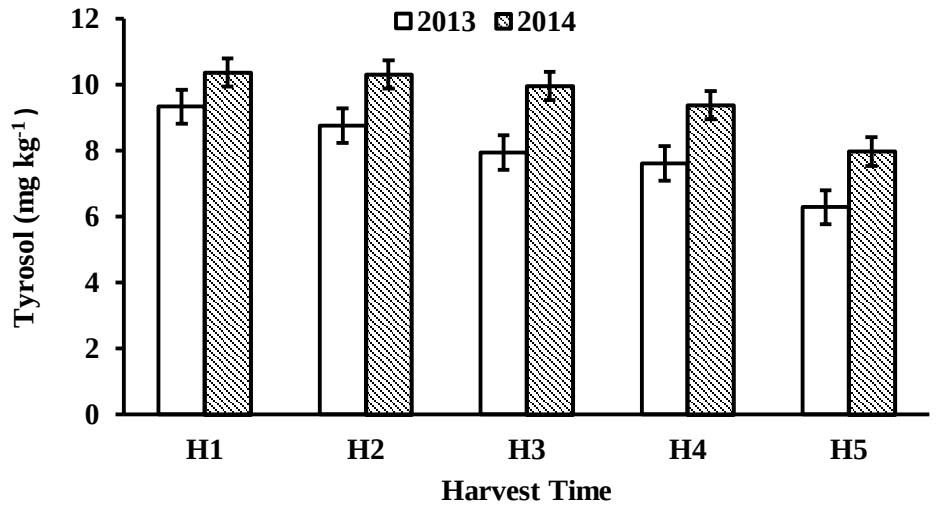


Fig. 5.27. Effects of harvest time on mean level of tyrosol (mg kg^{-1}) in virgin olive oil for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$).

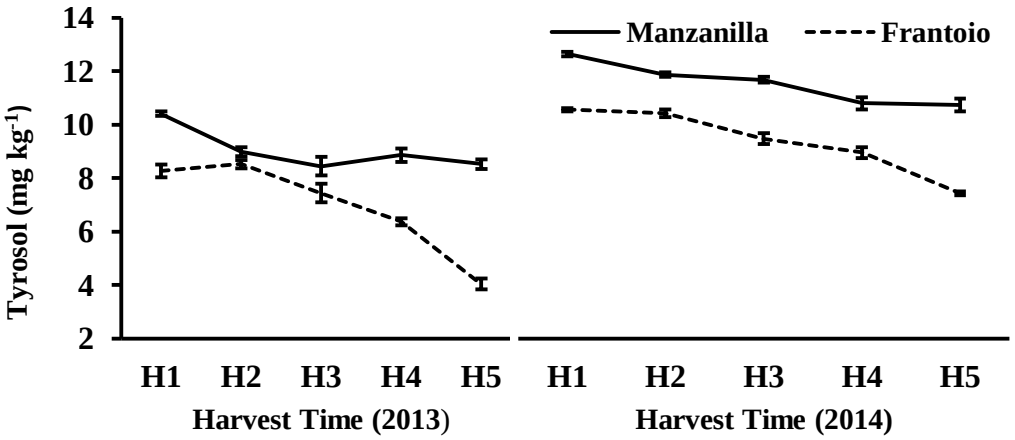


Fig. 5.28. Effects of different harvest time on the level of tyrosol (mg kg^{-1}) in the virgin olive oil of cvs. Frantoio and Manzanilla during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.4.3. Oleuropein aglycon (3,4 DHPEA-EA)

The delay in harvesting of olive fruit from first to fifth harvest showed significant decrease in 3,4 DHPEA-EA (1.44- and 1.53-fold in 2013 and 2014 respectively) and significantly higher concentration (1.1-fold) of 3,4 DHPEA-EA was observed in 2014 than 2013 (Fig. 5.29). The level of 3,4 DHPEA-EA in virgin olive oil decreased significantly from first to fifth which was noted as 1.4- and 1.29-fold for cv.

Manzanilla in 2013 and 2014 respectively. For Frantoio, the level of decrease was 1.5 and 1.8 -fold in 2013 and 2014 respectively. The level of DHPEA-EA was significantly higher (109.85 and 117.46.46 mg kg^{-1}) in Frantoio than Manzanilla cvs. (93.46 and 103.30 mg kg^{-1}) during 2013 and 2014 respectively (Fig. 5.30). There was a significant ($P \leq 0.05$) interaction between harvest time and cultivars for the level of oleuropein in the fruit during the both years.

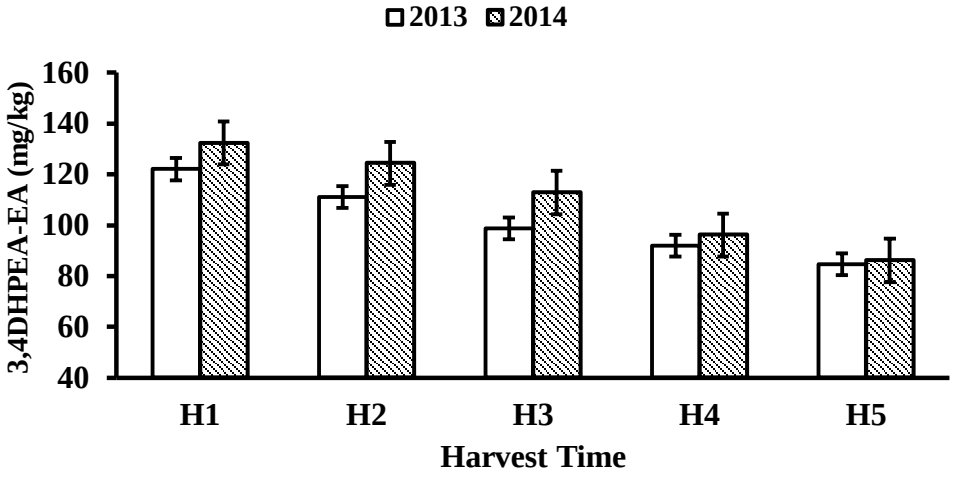


Fig. 5.29. Effects of harvest time on mean level of oleuropein aglycon (3,4 DHPEA-EA) (mg kg^{-1}) in virgin olive oil for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$).

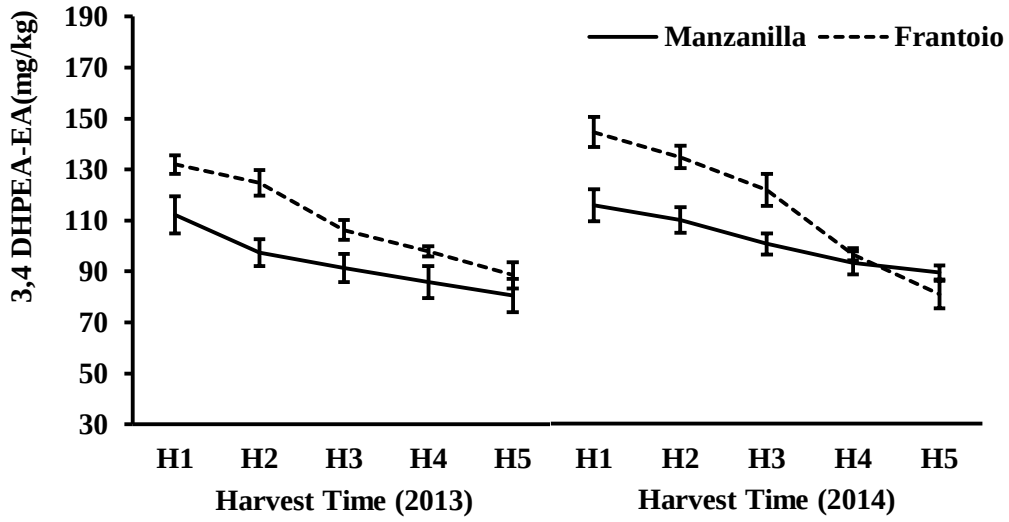


Fig. 5.30. Effects of different harvest time on mean level of oleuropein aglycon (3,4 DHPEA-EA) (mg kg^{-1}) in the virgin olive oil of cvs. Frantoio and Manzanilla during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.4.4. Total polyphenols

The level of total polyphenols in olive oil was also significantly higher (1.17-fold) in 2014 than 2013 and a gradual decrease was observed here from first to fifth harvest in both years (from 360.60 to 272.90 mg kg^{-1} in 2013 and from 423.70 to 320.30 mg kg^{-1} in 2014) (Fig. 5.31). The highest total polyphenols was 462.6 mg kg^{-1} in first harvest time of cv. Manzanilla in 2014 and the lowest total polyphenols level was 234.6 mg kg^{-1} in fifth harvest time of cv. Frantoio in 2013 (fig. 5.32).

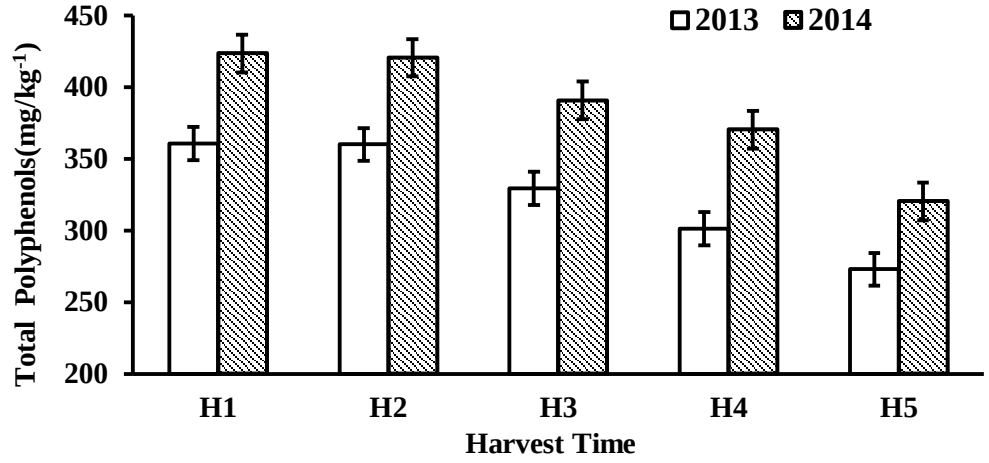


Fig. 5.31. Effects of harvest time on mean levels of total polyphenols (mg kg^{-1}) in olive oil for both cultivars during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**)**

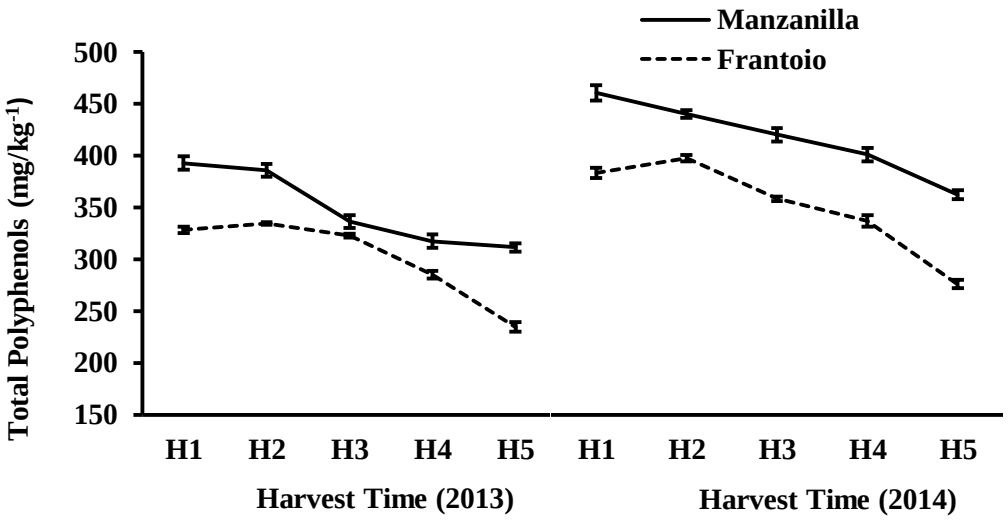


Fig. 5.32. Effects of different harvest time on levels of total polyphenols (mg kg^{-1}) in the virgin olive oil of cvs. Frantoio and Manzanilla during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.2.4.5. Phenolic acids

A gradual decrease was observed in the level of phenolic acids from first to fifth harvest in both olive cultivars in 2013 and 2014 (Table 5.1). An alternative increase and decrease in the level of vanillic acid was observed from second to fifth harvest in both cultivars during 2013 and 2014. However, a gradual decrease in the level of caffeic acid was observed in the olive cultivars (0.74- and 0.76-fold in cv. Manzanilla in 2013 and 2014 respectively; 0.36- and 0.40-fold in cv. Frantoio in 2013 and 2014 respectively). An initial slight decline in second harvest, static condition in third harvest and a slight increase and decrease was observed in last two harvests in 2013 in the level of syringic acid in cv. Frantoio Fluctuations in the level of syringic acid among different harvest time in cv. Manzanilla were also noted in both years. A similar inconsistency in the level of para-coumaric acid was observed with a 0.93-fold and 0.50-fold decrease from first to fifth harvest of cv. Manzanilla during 2013 and 2014 respectively. In Frantoio cv. the level of decrease in para-coumaric acid was 0.44- and 0.60-fold from first to fifth harvest in 2013 and 2014 respectively. Except a slight increase (1.07-fold) from third to fourth harvest during 2013, the level of ferulic acid in cv. Manzanilla showed a gradual decline in both harvest years from first to fifth harvest. Similarly, the cv. Frantoio also showed a gradual decline in ferulic acid with an exception of slight increase (1.31-fold) from first to second harvest in 2013 and static condition between these two harvests in 2014.

Table.5.2. Effects of different harvest time on the levels of different phenolic acids ($mgkg^{-1}$) in cvs. Frantoio and Manzanilla virgin olive oil during 2013, 2014.

	Harvest time																					
Phenolic acids ($mg\ kg^{-1}$)	**Fists (mid- April)**				**Second (late-April)**				**Third (mid- May)**				**Fourth (late- May)**				**Fifth (mid-June)**					
	Manzanilla		Frantoio		Manzanilla		Frantoio		Manzanilla		Frantoio		Manzanilla		Frantoio		Manzanilla		Frantoio		**LSD ($P \leq$ 0.05) T×Cv**	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Vanilic acid	0.40	0.43	0.21	0.24	0.43	0.53	0.26	0.26	0.36	0.37	0.26	0.20	0.41	0.55	0.28	0.32	0.33	0.46	0.26	0.29	NS	NS
Caffeic acid	0.43	0.62	0.44	0.53	0.43	0.56	0.24	0.62	0.46	0.52	0.17	0.52	0.33	0.48	0.28	0.27	0.32	0.47	0.16	0.21	NS	0.11
Syringic acid	0.15	0.30	0.15	0.25	0.22	0.15	0.13	0.25	0.13	0.15	0.13	0.15	0.13	0.18	0.14	0.13	0.12	0.15	0.12	0.15	0.05	0.07
Para-coumaric acid	0.28	0.58	0.39	0.43	0.28	0.46	0.44	0.40	0.26	0.30	0.21	0.33	0.29	0.27	0.35	0.30	0.26	0.29	0.17	0.26	NS	NS
Ferulic acid	3.97	5.08	1.75	2.73	3.67	4.95	2.30	2.73	3.55	4.82	1.90	1.67	3.80	4.48	1.71	1.68	2.77	4.11	1.25	1.58	NS	NS

5.3.3. Sensory attributes of virgin olive oils

5.3.3.1. Fruitiness attribute

The mean fruitiness (0-10 cm) in virgin olive oil decreased significantly from the first to fifth harvest time (2.85 to 2.39 in 2013 and 3.31 to 2.54 in 2014) (Fig. 5.33). With the delay of harvest time from first to fifth the fruity attribute in virgin olive oil decreased significantly with cv. Frantoio (from 2.36 to 2.11 in 2013 and from 2.94 to 2.38 in 2014) and cv. Manzanilla (from 3.34 to 2.67 in 2013 and from 3.67 to 2.71 in 2014) (Fig. 5.34). Irrespective of the harvest time, the mean virgin olive oil fruity attribute score was significantly higher in Manzanilla than Frantoio cvs. (1.7- and 1.24-fold in 2013 and 2014 respectively). There was a significant ($P \leq 0.05$) interaction between harvest time and cultivars for fruity attributes in the oil during 2013 and 2014.

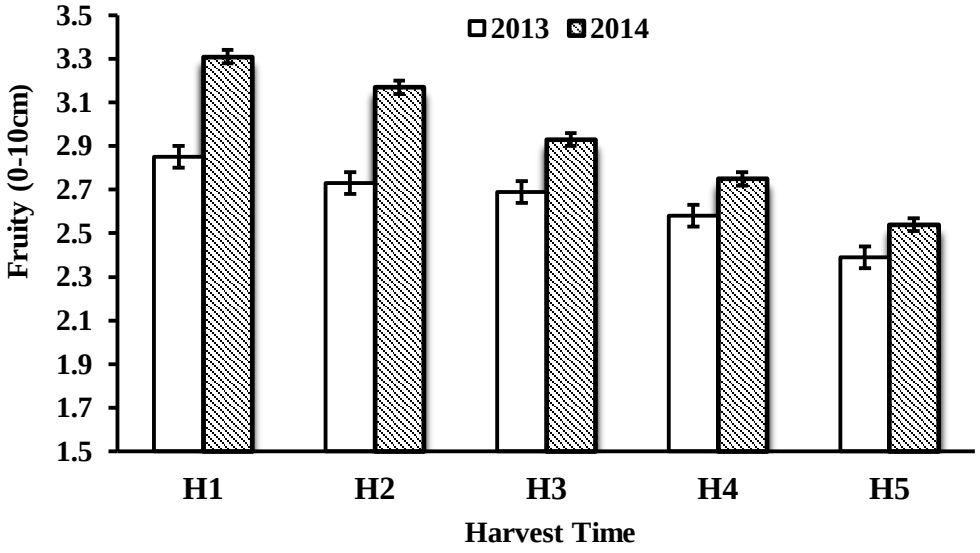


Fig. 5.33. Effects of harvest time on the mean fruitiness (0-10 cm) in olive oil during 2013 and 2014. Vertical bars represent as LSD ($P \leq 0.05$**).**

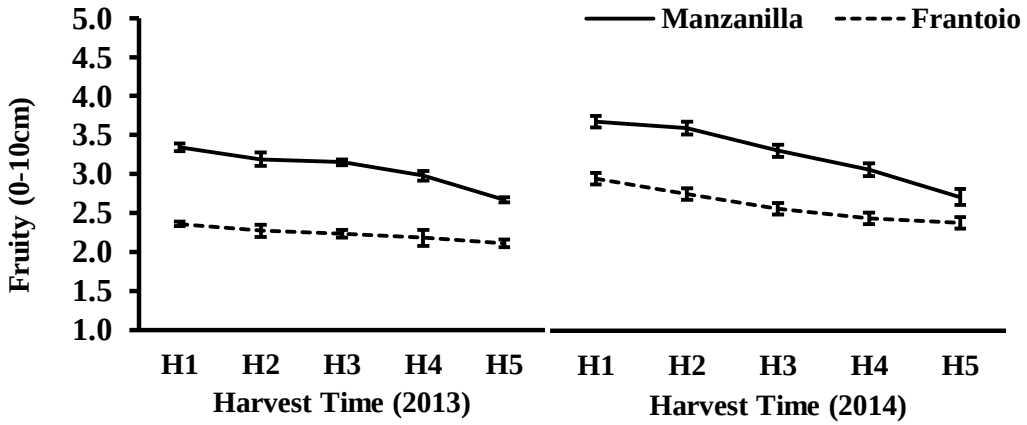


Fig. 5.34. Effects of different harvest time on mean fruitiness (0-10 cm) in the olives during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**)**

5.3.3.2. Bitterness attribute

The bitterness score (0-10 cm) decreased significantly with the delay in harvest time of the fruit. Irrespective of the cultivars, the bitterness score was 1.25 and 1.21-fold higher in first harvest than the fifth harvest during 2013 and 2014 respectively (Fig.5. 35). The decrease of bitterness score from first to fifth harvest of cv. Manzanilla (from 3.24 to 2.67 in 2013 and from 3.56 to 3.17 in 2014) and for cv. Frantoio (from 2.98 to 2.27 in 2013 and from 3.21 to 2.61 in 2014) was also recorded (Fig.5.36). When averaged over treatments, the mean virgin olive oil bitterness score was significantly higher with cv. Manzanilla than for cv. Frantoio (1.12- and 1.08-fold in 2013 and 2014 respectively).

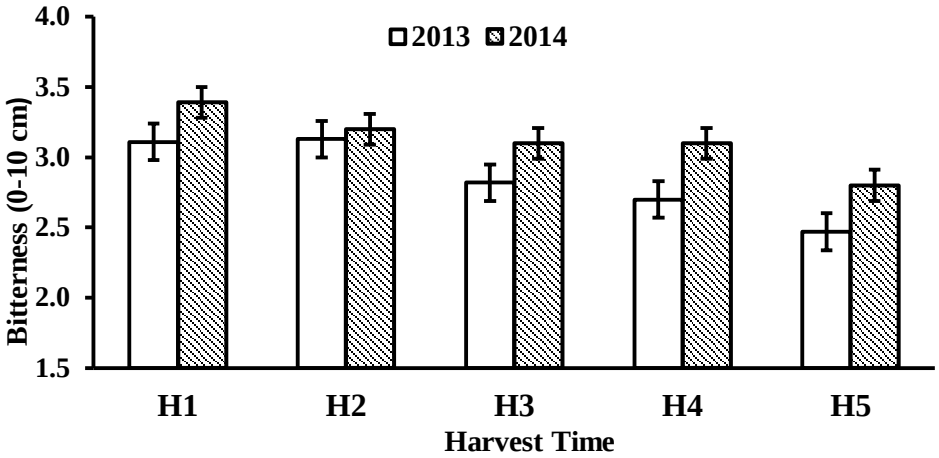


Fig. 5.35: Effects of harvest time on the bitterness (0-10 cm) in olive oil during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$).

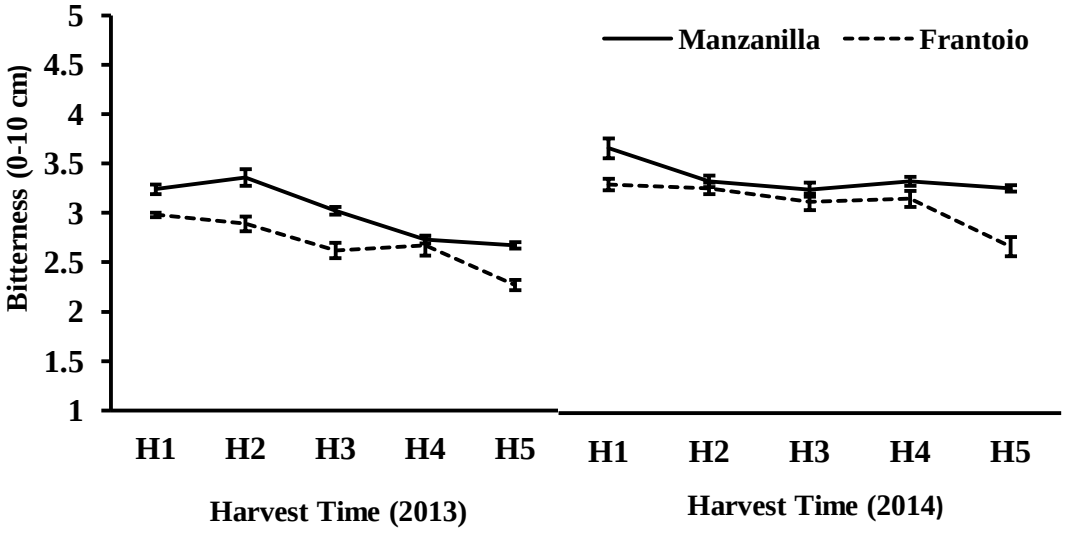


Fig.5.36. Effects of different harvest time on the bitterness (0-10 cm) of virgin olive oil of cvs. Frantoio and Manzanilla during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.3.3.3. Pungency attribute

Virgin olive oil from cv. Manzanilla was significantly less pungent as compared to the cv. Frantoio and a gradual significant decrease of pungency score (scale 0-10) was also observed in 2013 (from 3.60 to 3.01) and 2014 (from 3.34 to 2.69) (Fig. 5.37). The highest pungency score was 3.78 in first harvest time of cv. Frantoio in 2014 and the lowest was 2.38 in fifth harvest time of cv. Manzanilla in 2013 (fig.5. 38).

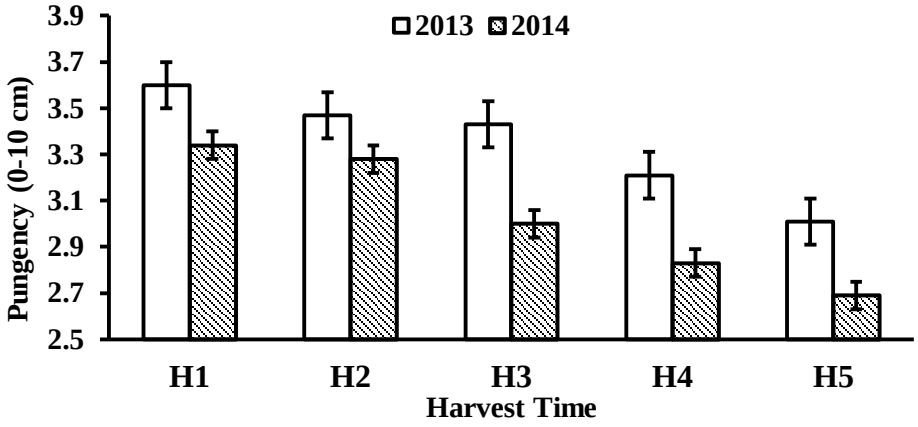


Fig. 5.37. Effects of harvest time on the pungency (0-10 cm) in virgin olive oil during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

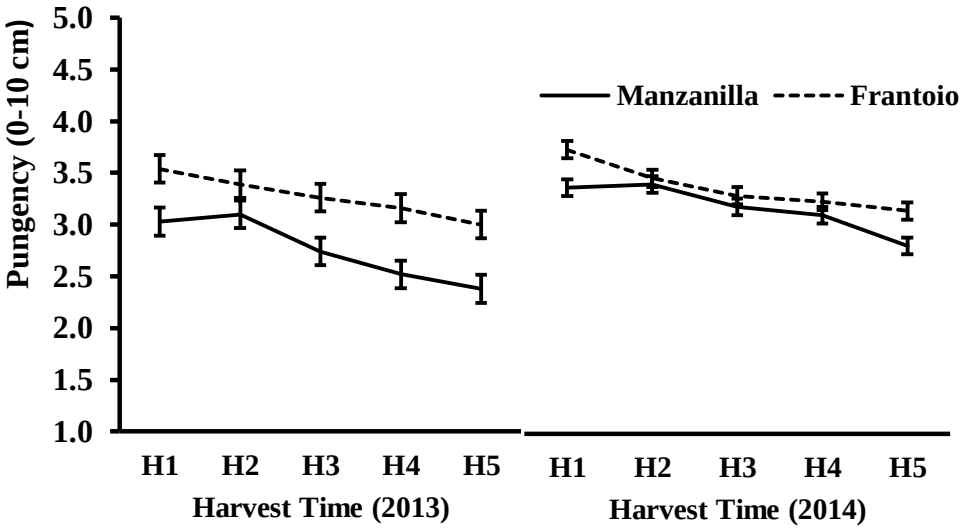


Fig. 5.38. Effects of different harvest time on the pungency (0-10 cm) in the virgin olive oil of cvs. Frantoio and Manzanilla olives during 2013 and 2014. Vertical bars represent LSD ($P \leq 0.05$**).**

5.4. Discussions

Frantoio and Manzanilla are two olive cultivars originating from Spain and Italy respectively. They are widely cultivated in the olive growing countries of the world including Australia due to their high productivity, quality fruit and oil and their agronomic adaptability. These cultivars have also been cultivated in south-western Australia for several decades (Taylor and Burt, 2007 and Olives, WA, 2015). Different factors influence the quality of virgin olive oil. Amongst these are agronomic practices (Lercker et al., 1994 and Motilva et al., 2000), application of technologies (Di Giovacchino et al., 2002 and Salvador et al., 2003), storage conditions (Procida and Cichelli, 1999) and virgin olive processing methods (Issaoui et al., 2009). Harvesting time or the ripening status of the fruit was suggested as one of the important factors as well (Koutsaftakis et al., 1999). Cinquanta et al. (1997) suggested that, the ripeness of the olives along with pedoclimatic conditions influence the phenolic composition of virgin olive oil. Over-ripening of olive fruit increases both oil yield and the acidity level, but limited amounts of oil can be removed from the fruit if harvested too early. Moreover, the sterol composition also depends on the ripening stage of the fruit (Anastasopoulos et al., 2011). There is a scarcity of published reports on the effects of different factors, especially the

harvesting time on the quality attributes of cvs. Manzanilla and Frantoio olives in the context of south-western Australia. Therefore, the current study was conducted during 2013 and 2014 to explore the effects of five different harvesting times (mid- and late-April, mid- and late-May and mid-June of 2013 and 2014) on the physical, biochemical and sensory attributes of cvs. Frantoio and Manzanilla olives oil grown in south-western Australia.

5.4.1. Effect of harvesting time on physical parameters

Reduction of fruit removal force (FRF) (from 5.85 N to 4.00 N) in olive fruit was observed irrespective of the cultivars from first to fifth harvest in both years and the least fruit removal force was observed in the fruit harvested during mid-June or in fifth harvest (Table.5.1). cv. Manzanilla showed higher removal force than cv. Frantoio in both of the harvesting years (1.71- and 1.43-fold in 2013 and 2014 respectively) (Fig.5.1 and fig.5. 2). The FRF is linearly correlated to the stage of fruit growth and reduces with the advancement of ripening (Lavee et al., 1973 and Lavee et al., 1982). The reduction of FRF could also be ascribed to the level of endogenous ethylene which increases with the development of the fruit and contributes in reducing the FRF through the release of ethylene (Lavee et al., 1982). The genotypic differences cause the variability in FRF between cultivars which has also been claimed by early researchers (Lavee and Haskal, 1976). The thickness of stalks differs in cultivars that also differ according to the size of the fruit within the cultivar which shows a declining trend with the progress of fruit growth (Lavee et al., 1982).
Moisture content of olive fruit decreased from first to fifth harvest (from 57.57% to 51.48%) and cv. Manzanilla showed higher moisture content than cv. Frantoio (1.19-fold in both years) (Fig.5.3 and fig.5.4) grown under the same conditions. Water is a major component of olive fruit comprising more than half of the total fruit weight and varies according to the variation of seasonal rainfall and cultivar (Beltrán et al., 2004). From the current study it was revealed that the level of moisture was low in 2014 when the rainfall was low during the growing period of the fruit in that year. The ripening of olive is affected by the cultivar and the environmental factors (Lavee et al., 1990). The lowest moisture content was observed in the driest harvest year (2014) due to water stress conditions which is supported by Lavee et al. (1991) and Ortega et al. (2001). On the other hand, a decrease of moisture content can also be

related to the progressive increase of the oil content during fruit maturation (Sánchez and Fernández, 1991).

Oil content (% dry weight) in olive fruit significantly increased (1.07- to 1.10-fold) from first to fifth harvest in both years and cv. Manzanilla showed higher oil content (%) than cv. Frantoio (1.01-fold) (Fig. 5.5 and fig.5.6). Maximum oil content was noted in fruit harvested in 4th harvest or during late May (Table. 5.1). Availability of water or its stress largely influences the development and oil content of the olive fruit (Lavee et al., 1982, 1990; Barone et al., 1994; Tombesi, 1994 and Inglese et al., 1996). The olive cultivars also show differences in oil content for growing area and year of cultivation (Barranco et al., 2000). Oil content in olive fruit showed an increasing trend until late harvest time in both cvs. Frantoio and Manzanilla (Fig. 5.5). A similar observation was reported by Beltrán et al. (2004). Oil content at the end of the ripening period showed insignificant differences and plateaued due to lower temperature during that period in both years while the autumn rains reduced the relative oil content due to rapid changes in water content of fruit and flesh (Beltrán et al., 2004). The two cultivars also differed significantly for oil content on a dry weight basis which is a genotypic characteristic (Beltrán et al., 2004). The lowest oil content was found in the low rainfall crop year, 2014, which is similar to the findings reported by Ortega et al. (2001) for water stress conditions.

5.4.2. Effect of harvest time on biochemical parameters fatty acid compositions

The free fatty acid and fatty acids of olive oil showed significant increase (palmitic acid, stearic acid, linoleic acid) or decrease in peroxide (value, oleic acid, MUFA, PUFA and MUFA/PUFA ratio) with the delay of harvesting from first to fifth in both years and irrespective of the cultivars (Fig.5.7, 5.9, 5.11, 5.13, 5.15, 5.17, 5.19, 5.21 and 5.23). Higher levels of oleic acid and MUFA/PUFA ratio were recorded from early harvested fruit (mid- to late-April) (Table.5.1). The concentration of fatty acids may differ due to the effect of environmental factors in the cultivation year and stage of fruit growth or maturation. Salvador et al. (2003) and Anastasopoulos et al. (2011) also reported similar variations in fatty acids according to crop year and maturation of fruit. The free fatty acids at the later stage of ripening may also increase with the increase of lypolytic enzyme activity within the flesh (Anastasopoulos et al., 2011). The trend of lowering peroxide value might be ascribed to the decreased activity of

lipoxygenase enzymes which have been reported by Gutierrez et al. (1999), Salvador et al. (2003), Baccouri et al. (2008) and Anastasopoulos et al. (2011). The decrease in the level of oleic acid and increase in linoleic acid were observed due to the activity of the enzyme oleate desaturase which converts oleic acid into linoleic acid (Gutierrez et al., 1999). This inter-conversion of oleic and linoleic acid is accelerated by water stress which ultimately reduces the MUFA:PUFA ratio as reported by Gómez-Rico et al. (2007) and Dag et al. (2014). The present study also revealed similar results where the fruit harvest in 2014 faced a water stress due to low rainfall during its growing period. The two cultivars also showed significant differences for fatty acid profiles. The oils from cv. Manzanilla showed higher levels of free fatty acid, stearic acid, oleic acid, MUFA and MUFA:PUFA ratio (Fig.5.10, 5.14, 5.16, 5.20 and 5.24). Higher levels of peroxide value, PUFA, palmitic acid and linoleic acid were observed in cv. Frantoio (Fig. 5.8, 5.12, 5.18 and 5.22). The variation between the two cultivars in respect of the fatty acid profiles is due to their genetic differences which were reported by early researchers as well (Stefanoudaki et al., 1999; EEC, 2003; Gómez-González et al., 2011and Manai-Djebali et al., 2012).

5.4.3. Polyphenol compounds

Tyrosol, hydroxytyrosol and oleuropein are the dominant phenolic compounds and vanillic acid, caffeic acid, synergic acid, para-coumaric acid and ferulic acid were also found as minor phenolic compounds in the cvs. Frantoio and Manzanilla They have also been reported as major and minor phenolic compounds in other olive cultivars by Mulinacci et al. (2005), Damak et al. (2008), Manai-Djebali et al. (2012) and Dağdelen et al. (2013) The phenols in olive oil improve its resistance to oxidation and are responsible for its sharp bitter taste (Bendini et al., 2007). In the current study, a significant gradual decrease was noted in major (Fig.5.25, 5.27and 5. 29) and minor polyphenolic compounds (Table.5.2) from first to fifth harvest in both harvesting years irrespective of the cultivars. Higher total polyphenol was noted in the fruit harvested during mid-April to late-April (Table.5.1). The concentration of phenolic compounds varies according to the maturation phase of the fruit (Anastasopoulos et al., 2011). Baccouri et al. (2008) reported that the total phenols increase progressively and decrease in the final ripening stage. Some fluctuations were also observed in most of the phenolic asids (syringic acid, vanillic acid, para-coumaric acid, ferulic acid) in the current study. The level of phenolic compounds

also varies according to the crop year with respect to water availability. The concentration of phenolic compounds were comparatively high in the fruit harvested in 2014 (Fig. 25, 27, 29, table 1) when less rainfall was recorded (0.00 to 8.10 mm) than 2013 (2.00 to 56.5 mm) during the growing period of the olive fruit. Similar observation was reported by Anastasopoulos et al. (2011), Patumi et al. (2002) and Gómez-Rico et al. (2007). Differences in the level of water content of the fruit could imply a different solubilisation of phenols (Allogio and Caponio, 1997). The amount of water in the fruit also controls the activity of enzymes responsible for phenolic compound synthesis, such as L-phenylalanine ammonia-lyase, and differs according to water conditions (Morello et al., 2005). A linear correlation between polyphenols and water stress was also observed by Tovar et al. (2002), Gómez-Rico et al. (2006), Dag et al. (2008), Ben-Gal et al. (2011), Vita Serman et al. (2011) and Caruso et al. (2014). The two cultivars also showed significant differences where cv. Manzanilla showed higher concentration of hydroxytyrosol, tyrosol and oleuropein (Fig.5. 26, 28, 30); vanillic acid, caffeic acid and ferulic acid (Table .5.2). The variation of phenolic compounds in different cultivars is also related to the genetic variations among them which were reported by Aguilera et al. (2005), Vinha et al. (2005), Manai-Djebali et al. (2012) and Dağdelen et al. (2013).

5.4.4. Effect of harvest time on sensory attributes

The sensory attributes namely fruitiness, bitterness and pungency, decreased gradually from the first to fifth harvest irrespective of the cultivars in both harvesting years. Higher levels of fruitiness and bitterness were recorded in the fruit harvested in 2014 and pungency was greater in the fruit harvested in 2013 (Fig. 5.33, 5.35 and 5.37). Virgin olive oil from the fruit of cv. Manzanilla had greater bitterness and pungency and lower fruitiness (Fig.5.34, 5.36 and 5.38) than that from cv. Frantoio. The lowest bitterness and pungency were recorded during mid-June and the most fruity oil was obtained from the fruit in early harvest of cv. Frantoio (mid-April) and late harvest of cv. Manzanilla (mid-June) (Table 5.1). The sensory properties of olive fruit are influenced by the ripeness status and variety of the fruit. Similar variations in sensory profile have been reported by Angerosa et al. (2004), Rotondi et al. (2004), Servili et al. (2004), Tripoli et al. (2004), Kalua et al. (2007) and Delgado and Guinard (2011). Chemical composition of the fruit also influences the sensory properties. Higher phenol content in the fruit of cv. Manzanilla may be ascribed for

its higher bitterness and pungency, a view supported by Bendini et al. (2007). The environmental conditions such as water stress may have influenced the bitterness of the fruit. More bitterness was observed in the fruit harvested in 2014 where there was a lower amount of rainfall during the growing period of fruit. Similarly, the findings of Cinquanta et al. (1997) indicate that the ripeness of the olives along with pedoclimatic conditions influence the quality attributes of virgin olive oil even though none of any defects were found.

Table.5.3. Summary table reflecting the best values for some selected parameters with respective period of harvesting

	Cultivar							
	Frantoio				Manzanilla			
Parameter	Best value		Harvesting period		Best value		Harvesting period	
	2013	2014	2013	2014	2013	2014	2013	2014
Least FRF (N)	2.98	2.47	Mid-June	Mid-June	5.03	4.23	Mid-June	Mid-June
Higher oil content (% dry weight)	39.05	38.55	late-May	late-May	38.58	37.85	late-May	late-May
Higher oleic acid (%)	73.49	72.38	Mid-April	Late-April	78.75	76.58	Mid-April	Late-April
Higher MUFA/PUFA ratio	7.54	7.36	Mid-April	Late-April	9.65	8.32	Mid-April	Late-April
Higher total phenols (mg kg^{-1})	334.3	399.3	Late-April	late-May	392.8	462.6	Mid-April	Late-April
Least bitterness (0-10)	2.27	2.61	Mid-June	Mid-June	2.67	3.17	Mid-June	Mid-June
Least pungent oil (0-10	2.38	3.18	Mid-June	Mid-June	3	2.84	Mid-June	Mid-June
Most fruity oil (0-10)	2.36	2.94	Mid-April	Mid-April	3.34	3.67	Mid-June	Mid-June

5.5. Conclusion

The physical, biochemical and sensory properties of the olive fruit and oil showed variations according to the delay of harvesting, genetic differences between the cultivars and environmental factors such as water stress during the growing period of the fruit. The fruit of cv. Manzanilla showed higher levels of fruit removal force, moisture content (%) and mean oil content in dry weight (%). Lowest moisture and oil content were observed in the relatively drier harvest year, 2014. At the later stage of ripening the increase of free fatty acids was observed which may be ascribed to the increase of lipolytic enzyme activity and lowering trend of peroxide value may be due to the decreased activity of lipoxygenase enzymes. A significant gradual decrease was noted in major polyphenolic compounds. The concentration of phenolic compounds was comparatively high in the fruit harvested in 2014. The sensory attributes varied from the first to the fifth harvest time. They degraded with the delay of harvesting; and water stress may have influenced the bitterness of the fruit in 2014. It could be concluded that the most suitable time for olive harvesting is late-May to mid-June.

Chapter 6

Effect of different concentrations of ethephon on physicochemical, biochemical and organoleptic properties of olive fruit and virgin oil of cv. Frantoio and Manzanilla in south-western Australia

Abstract

Ethephon, an ethylene producing compound, is used as an agent to promote fruit abscission for easy picking or mechanical harvesting of different fruit including olive. Its application can also result in a considerable loss of leaves. To achieve a balance between fruit and leaf abscission, the concentration of ethephon needs to be optimized so the current study was conducted with this goal through observing the effect of different concentrations of ethephon on physico-chemical, biochemical and organoleptic properties of Frantoio and Manzanilla olive cultivars grown in south-western Australia. Application of ethephon significantly increased the level of fruit ethylene production, fruit ripening index, fruit and leaf abscission and peroxide value of olive oil with the increased concentration of applied ethephon in comparison to control treatment. Ethephon also significantly increased the level of most of the fatty acids, however, oleic acid, MUFA and MUFA/PUFA ratio decreased with the increase of ethephon concentration. Concentration of different polyphenols (hydroxytyrosol, tyrosol, oleuropein, and total polyphenol) and levels of sensory attributes (fruitiness, bitterness and pungency) decreased significantly with the increase of ethephon concentration. Effect of ethephon on the fruit moisture (%) and oil (% fresh and dry weight basis) content of the olive fruit was non-significant. The applied concentrations of ethephon, 1000 to 2000 mg L^{-1} in 2013 and 1000 to 1500 mg L^{-1} in 2014 did not show significant differences for the studied parameters. It could be concluded that the most suitable concentration of ethephon to treat olive trees is 1000 – 1500 mg L^{-1}.

6.1. Introduction

Olive (*Olea europaea*) is one of the oldest cultivated fruits. Harvesting of olive fruit consumes 50–80 % of the total cost of production (Metzidakis, 1999) so any strategy that decreases this cost is of commercial importance. Its harvest is undertaken by

hand labour over a period of two months. Increased labour costs and low availability of labour during harvesting time have intensified industry interest in mechanical harvesting. To ensure economic stability for olive growers, mechanical harvesting systems combined with application of an abscission agent has gained popularity (Burns et al., 2005). Use of an abscission agent enables lower mechanical forces to be applied during harvest hence minimization of fruit damage. Ethephon, an ethylene producing compound, is one of the agents that has been used to promote fruit abscission and to enable easy picking or mechanical fruit harvesting in apple (Edgerton, 1968), cherries (Bukovac et al., 1969), olive (Hartmann et al., 1970), citrus (Young and Jahn, 1972), macadamia (Kadman and Ben-Tal, 1983) and many other species (Kays and Beaudry, 1987).

The ratio between fruit mass and pedicel strength of olive fruit is relatively small as compared with other fruits and thereby a huge force is required to shake off the fruits from olive trees (Ben-Tal and Wodner, 1994). Among the different types of chemicals tested to promote pedicel's loosening, positive results were only obtained by using the ethylene releasing compounds including ethephon (2-chloroethyl phosphonic acid). Ethephon is a synthetic plant growth regulator which acts by releasing ethylene when it penetrates plant tissues (Royer et al., 2006). It promotes pedicel loosening and increases the natural ratio between fruit mass and pedicel strength which leads to easy mechanical harvesting of olive fruit (Martin et al., 1981; Denney and Martin, 1994; and Metzidakis, 1999). However, ethephon application can result in a considerable loss of leaves coincident with fruit loosening (Burns et al., 2008). Alteration in ethephon application timing and duration (Lang and Martin, 1985, 1989), to minimize unwanted defoliation, was promising in laboratory conditions but unpredictable in the field (Martin, 1994).

Touss et al. (1995) reported that ethephon at 1250 and 1875 mg L^{-1} on conventional Arbequina olive trees increased the yield from mechanical harvesting by 20%. They also claimed that ethephon increased the amount of harvested fruit without significantly enhancing preharvest leaf drop and did not adversely affect flowering in the following year and it showed little effect on oil acidity, peroxide value, and oil fatty acid composition. However, there is no information on the response of cvs. Frantoio and Manzanilla olive to ethephon in south-western

Australian conditions. Information on the effects of ethephon on oil composition is also limited (Cimato, 1988). The current study was conducted to find out a suitable concentration of ethephon for cvs. Frantoio and Manzanilla olives grown in south-western Australian conditions for two consecutive years. Observations were recorded on ethylene production, ripening index, fruit removal force, fruit and leaf abscission, oil content (%), concentration of fatty acids and phenolic compounds, and sensory attributes of olive oil.

6.2. Materials and methods:

6.2.1. Plant material, experimental location and climatic conditions

Olive fruit of cvs. Frantoio and Manzanilla were used as experimental material. Disease free, mature and uniform sized fruit were collected from the control and ethephon treated olive trees grown in the olive field at Talbot Grove, York (31°52′44″ S, 116°45′57″ E), located at 120 km east of Perth, WA. Details on the plant material, their maintenance, experimental location and climatic conditions have been presented in Chapter 3, Section 3.1.

6.2.2. Design of experiment and treatments

The experiment was conducted by following two factors (treatments of ethephon × cultivar) factorial Randomized Complete Block Design (RCBD) with 4 replications of the experimental unit (a single olive tree). Different concentrations (0 to 3000 mg L^{-1} in 2013 and 0 to 2000 mg L^{-1} in 2014) of ethephon [2-chloroethylphosphonic acid (Rhone-Poulenc Rural Australia Pty Ltd, Baulkham Hills, NSW, Australia)] and 0.05% ‘Tween 20’ as a surfactant was sprayed by using a sprayer (The Selecta Trolleypak Mk II, Acacia Ridge, Australia) was applied as the treatment.

6.2.3. Collection of olives and extraction of olive oil

Olive fruit (composite sample of 1.5 to 2 Kg) were harvested from four representative trees included in four replications. The fruit were picked by hand after one week of treating the fruit trees with different concentrations of ethephon. Virgin

olive oil was obtained from the collected fruit by following the method explained by Rivas et al. (2013) with some modifications. The collection of fruit and extraction of oil has been described in detail in Chapter 3, Section 3.4.

6.2.4. Observations recorded:

6.2.4.1. Ethylene production

The endogenous level of ethylene was determined by using the Sensor Sense (Sensor Sense B.V, Nijmegen, The Netherlands) following the method described by Pranamornkith et al. (2012) and detailed in Chapter 3, section3.5.2. The Sensor Sense includes an ETD 300 ethylene detector, a set of valve controllers with an option of six valves connected to six separate cuvettes [1.0 L air-tight jar, fitted with a rubber septum (SubaSeal®, Sigma-Aldrich Co., St. Louis, USA)]. The "continuous flow" method was used with coarse mode (conversion factor 99818, capacity to measure ethylene concentration at 0-500 ppm, sensitivity at <1%) of analysis and only 100 g of sample fruit was used to determine the amount of evolved ethylene from the sample. The measured ethylene was expressed in $\mu lkg^{-1}hour^{-1}$ and converted to $\mu molkg^{-1}hour^{-1}$.

6.2.4.2. Determination of ripening index (RI)

Determination of ripening index of the olive fruit sample was conducted according to the method described by Uceda and Frias (1975). One hundred randomly selected healthy fruit were cut in half to expose the internal flesh for grading in eight groups and the ripening index value was calculated as described in Chapter 3, Section 3.5.6.

6.2.4.3. Determination of fruit removal force (FRF)

A texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK) was used to determine the FRF. The analyser was equipped with horizontal square base table (15 cm × 15 cm) and interfaced to a personal computer with Nexygen® software. Twenty randomly selected fruit per replication were used for this test. The procedure of determining the FRF has been detailed in Chapter 3, Section 3.5.5.

6.2.4.4. Fruit and leaf abscission

Abscission of leaf and fruit was determined from three selected branches from each replicate. The numbers of leaves and fruit before applying the treatments and after harvesting the fruit were used to calculate the percentage of abscission as described in Chapter 3, Section 3.5.9.

6.2.4.5. Fruit moisture

The olive fruit moisture content was determined by using healthy and randomly selected olive fruit (60g). The fruit were crashed by hammer mill in a pre-calibrated dish and the paste was dried in a forced air oven at 105°C for approximately 8–10 hours until the weight was constant. The detailed procedure has been described in Chapter 3, Section 3.5.7.

6.2.4.6. Olive oil content

Olive oil content was determined from fresh fruit by following the method described by Avidan et al. (1999) and detailed in Chapter 3, Section 3.5.8. Only 10g of olive fruit paste was dried in an oven at 80°C for 24 h and the dry weight of each replicate was recorded. Then petroleum ether was used and homogenised at medium speed for 30 sec with a vortex (Heidolph, Reax Top, VIC, Australia). After that a rotator shaker (Ratek Orbital Mixer, VIC, Australia) was used overnight. The following day the extract oil and petroleum ether was evaporated at 40°C. The oil residue was weighted as percentage of oil on fresh and dry weight basis.

6.2.4.7. Determination of free fatty acid

Ten grams of the olive oil sample was dissolved in 50 ml of the solvent mixture (1:1 of 95% (V/V) ethanol (C_2H_6O) and diethyl ether $(C_2H_5)_2O$). The mixture was titrated while shaking with a solution of potassium hydroxide (KOH_4) to the end point of the indicator pink colour of phenolphthalein ($C_{20}H_{14}O_4$) persisting for at least 10 sec. The details of calculating the amount of free fatty acids have been presented in Chapter 3, Section 3.5.11.

6.2.4.8. Peroxide value

To determine the peroxide value of olive oil (meq O_2 kg^{-1}) 1-2 g of the sample oil was dissolved with 10 ml of chloroform ($CHCl_3$), 15 ml of acetic acid ($C_2H_4O_2$) and

1 ml of potassium iodide (KI) solution. Then 75 ml of distilled water was added and titrated with the sodium thiosulphate solution; starch solution was used as an indicator (10 g/l aqueous dispersion) from a purplish to yellowish or colourless endpoint. The procedure for determining peroxide value has been detailed in Chapter 3, Section 3.5.12.

6.2.4.9. Determination of fatty acids composition

Fatty acid composition of virgin olive oils was determined by gas chromatograph following the method prescribed by the International Olive Council (2001). Methanol with heptane and methanolic potassium hydroxide (2M) were mixed with the oil sample and the mixture was homogenized vigorously for 30 seconds. The upper layer containing methyl esters was decanted and injected into the gas chromatograph with heptane solution. The gas chromatograph was fitted with a fused silica column (50 m length × 0.25 mm i.d.) coated with SGL-1000 phase (0.25µm thickness Suger labour, Spain) and containing a FID detector (HP 6890, Agilent Technologies). Detailed procedure of determining fatty acid composition has been described in Chapter 3, Section 3.5.13.

6.2.4.10. Total of polyphenol

The total phenols were quantified by following the method of Ranalli et al., (1999). Olive oil (10g) was isolated and dissolved in hexane by triple extraction of a solution with a water/methanol mixture (60:40, v/v). Folin-ciocalteus phenol reagent, the absorbance was recorded was using a uv /vis spectrophotometer (Model SECOMAM ANTHELIE Advanced, France). The absorption of extracts was read at 725 nm, and then calculated according to Mateos et al. (2001) as described in Chapter 3, Section 3.5.14.

6.2.4.11. Determination of polyphenol compounds

Composition of polyphenolic compounds was determined by adding 5 ml of methanol/water (80/20, v/v) with 5 g of virgin olive oil and analysing the mixture by HPLC-DAD. The phenolic compounds were quantified at 235-280 nm using syringic acid as internal standard. Phenolic standards (3,4 DHPEA-EA, Tyrosol and hydroxytyrosol) of 0.015 mg/ml strength were prepared and used to determine the level of polyphenols as described in Chapter 3, Section 3.5.15.

6.2.4.12. Sensory attributes

A tasting panel comprised of seven well trained tasters was recruited to distinguish 30 olive oil samples according to the standard procedure (EC Reg. 796/2002) which has been described in Chapter 3, Section 3.5.10. The tests were scheduled in two different days with ½ hour breaks between two tests. The test panel was supplied with scaled sheets for the sensory attributes such as fruitiness, bitterness and pungency. Each attribute was scaled from 1 to 10 where 1 represented the value for the poorest and 10 the best possible quality for the sample.

6.3. Results:

6.3.1. Ethylene production

Ethephon treatments significantly influenced the production of ethylene from the cv. Frantoio fruit in 2013. A greater level of ethylene was recorded from the fruit treated with higher concentration of ethephon for both cultivars cvs. Frantoio and Manzanilla the average concentrations of ethylene were 1.3, 1.0, 0.8 and 0.7 nmol kg^{-1} h^{-1}, from 3000, 2000, 1000 and 500 mg L^{-1} ethephon treated fruit respectively. The concentration of ethylene between the 500 mg L^{-1} ethephon treated fruit and control fruit (0.5nmol kg^{-1} h^{-1}) was non-significant. The concentration of ethylene showed a continuous steady increase in all ethephon treated fruit in comparison to the control fruit (Fig. 6.1). Significant effect of ethephon treatments were also observed in cv. Manzanilla olive in 2013. A constant level of ethylene production was observed in the 500 mg L^{-1} ethephon treated fruit; however the control, 1000 and 3000 mg L^{-1} ethephon treated fruit showed an increase and 2000 mg L^{-1} ethephon treated fruit showed a decrease in the level of ethylene in cv. Manzanilla after 9 to 12 days of treatment in 2013 (Fig. 6.1 B).

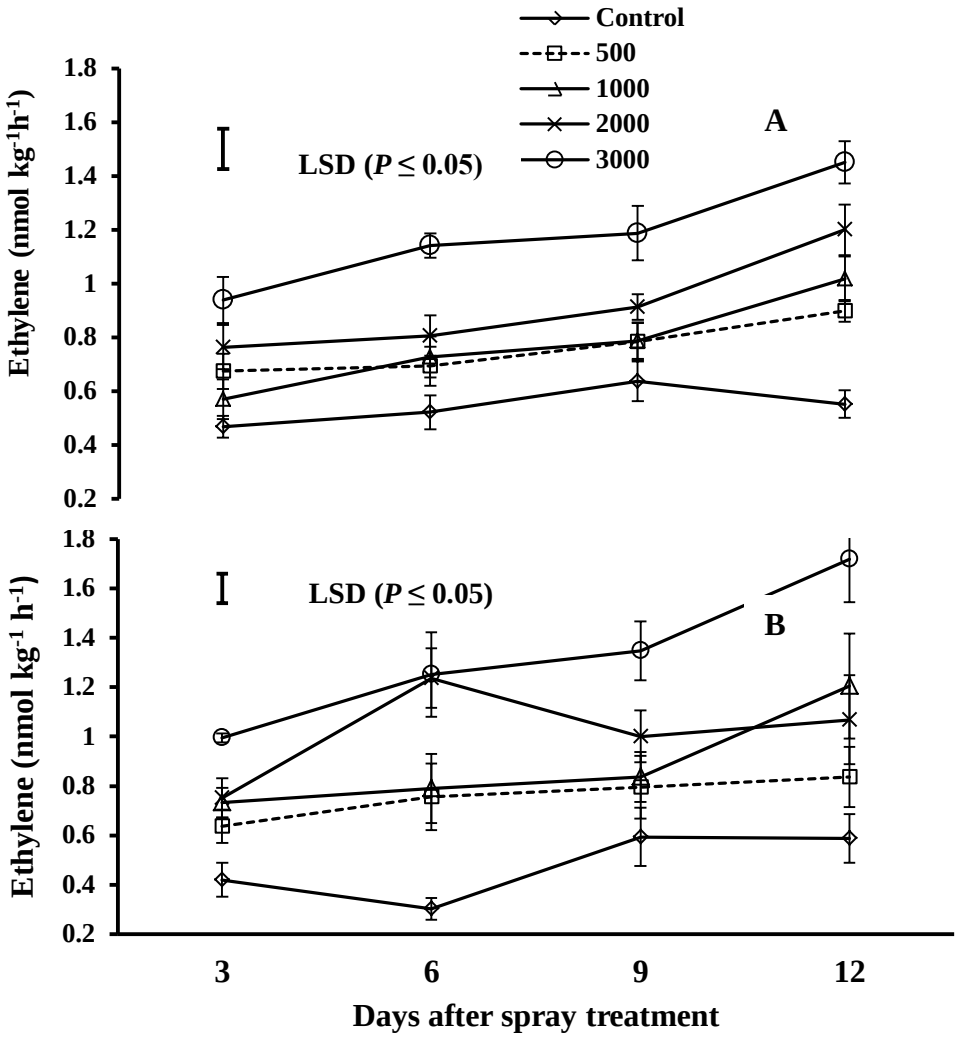


Fig.6.1. Effects of different concentrations of spray application of ethephon (mg L^{-1}) and days after spray treatment on ethylene production in fruit of cvs. Frantoio (A) and Manzanilla (B) olives in 2013. Vertical bar represent SE.

6.3.2. Ripening index

The spray application of different concentrations of ethephon showed significant ($P \leq 0.05$) effect on the ripening index of cvs. Frantoio and Manzanilla olive fruit in 2013 and 2014. The ripening index increased significantly when the olive trees were treated with higher concentrations (1000 – 3000 mg L^{-1}) of ethephon than the lower

concentration (500 mg L^{-1}) of ethephon and control (Fig. 6.2 A, B and 6.3 A, B). Highest ripening index was observed after 12 days of spray treatment in the fruit treated with 3000 mg L^{-1} (4.92) ethephon followed by 2000 mg L^{-1} (4.40), 1000 mg L^{-1} (4.04) and 500 mg L^{-1} (3.89) treatments. The interaction between treatments and the number of days after treatments for ripening index was also significant (Fig.6.2 A). A similar trend of ripening index of cv. Frantoio was observed and cv. Manzanilla was during 2013. However, the fruit treated with lower concentration of ethephon (500-1000 mg L^{-1}) did not differ significantly for ripening index from 6 to 12 days after spray treatment in 2013 (Fig. 6. 2 B).

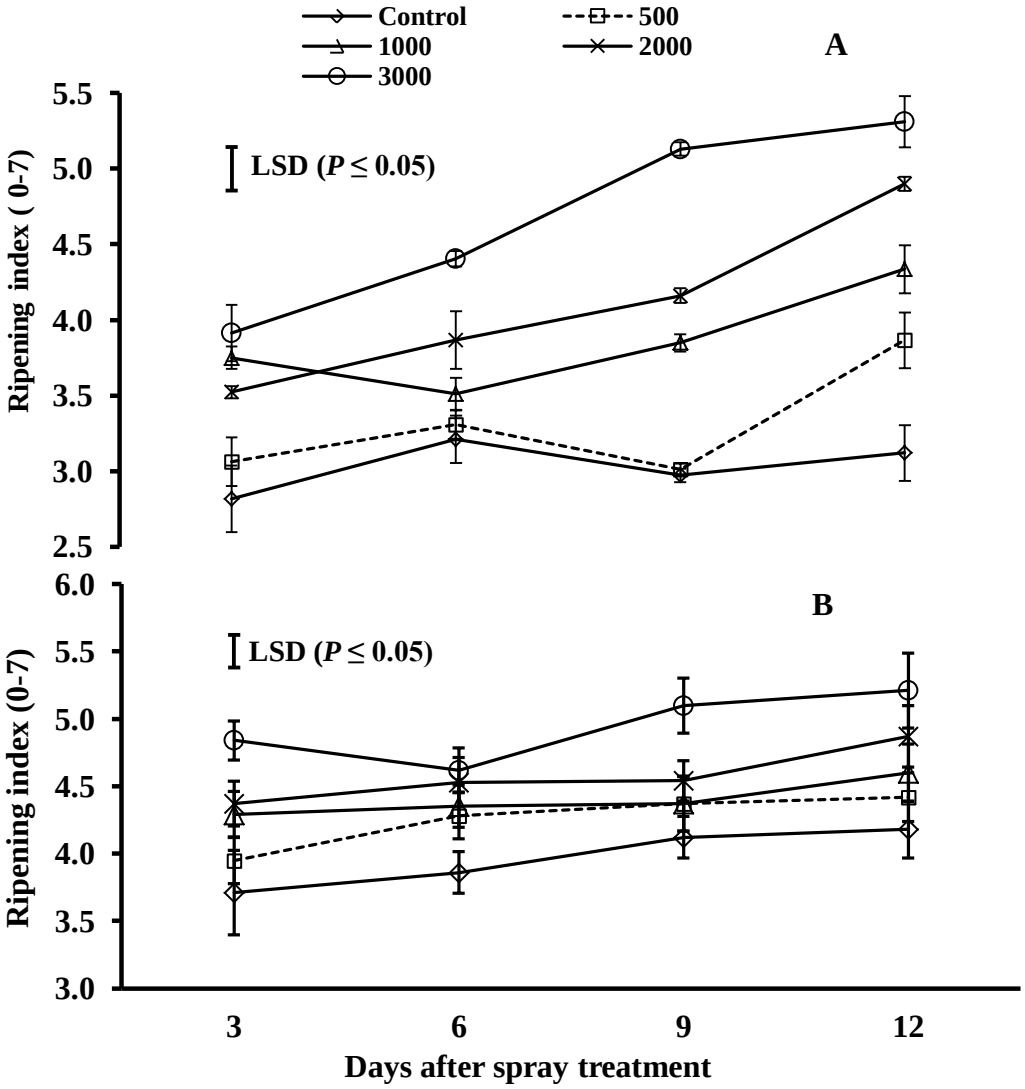


Fig.6.2. Effects of different concentrations of spray application of ethephon (mg L^{-1}) and days after spray treatment on ripening index in fruit of cvs. Frantoio (A) and Manzanilla (B) olives in 2013. Vertical bar represent SE.

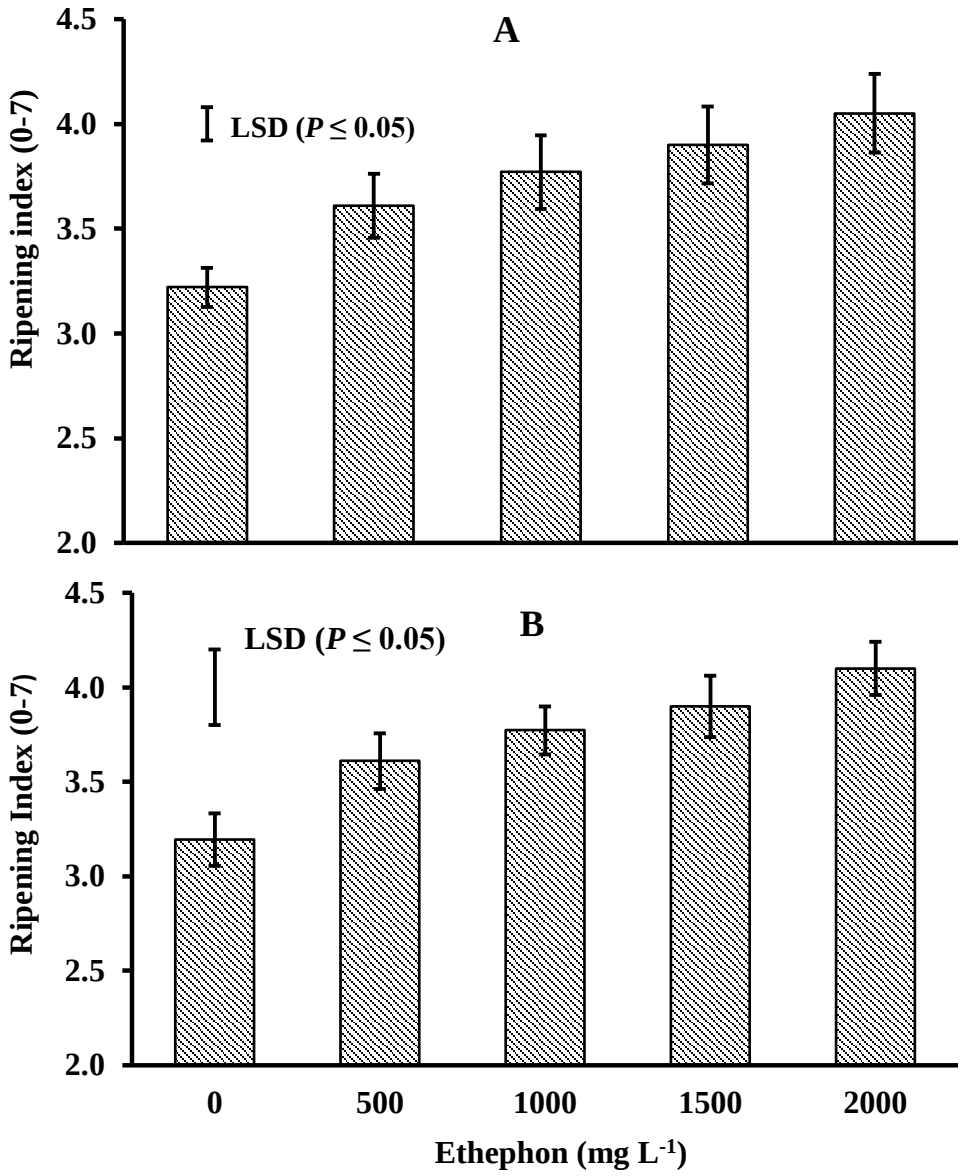


Fig.6.3. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on ripening index of Frantoio (A) and Manzanilla (B) cvs. of olives in 2014. Vertical bar represent SE.

6.3.3. Fruit removal force (FRF)

The ethephon treatment significantly ($P \leq 0.05$) reduced the fruit removal force in comparison to the control in cvs. Frantoio and Manzanilla of olive in 2013 and 2014. The highest average fruit removal force was observed in the control fruit and it

reduced with the increase of the applied ethephon concentration (Fig 6.4 A, B and 6.5 A, B). However, in 2014 from 9 days of spray treatment, fruit from the 500 and 1000 mg L^{-1} ethephon treated plants did not show significant differences for the fruit removal force (Fig. 6.5). The cv. Manzanilla olive fruit treated with higher concentration of ethephon (1000 – 3000 mg L^{-1}) showed a sharp decline in fruit removal force as compared to the control and 500 mg L^{-1} ethephon treated fruit (Fig. 6.4).

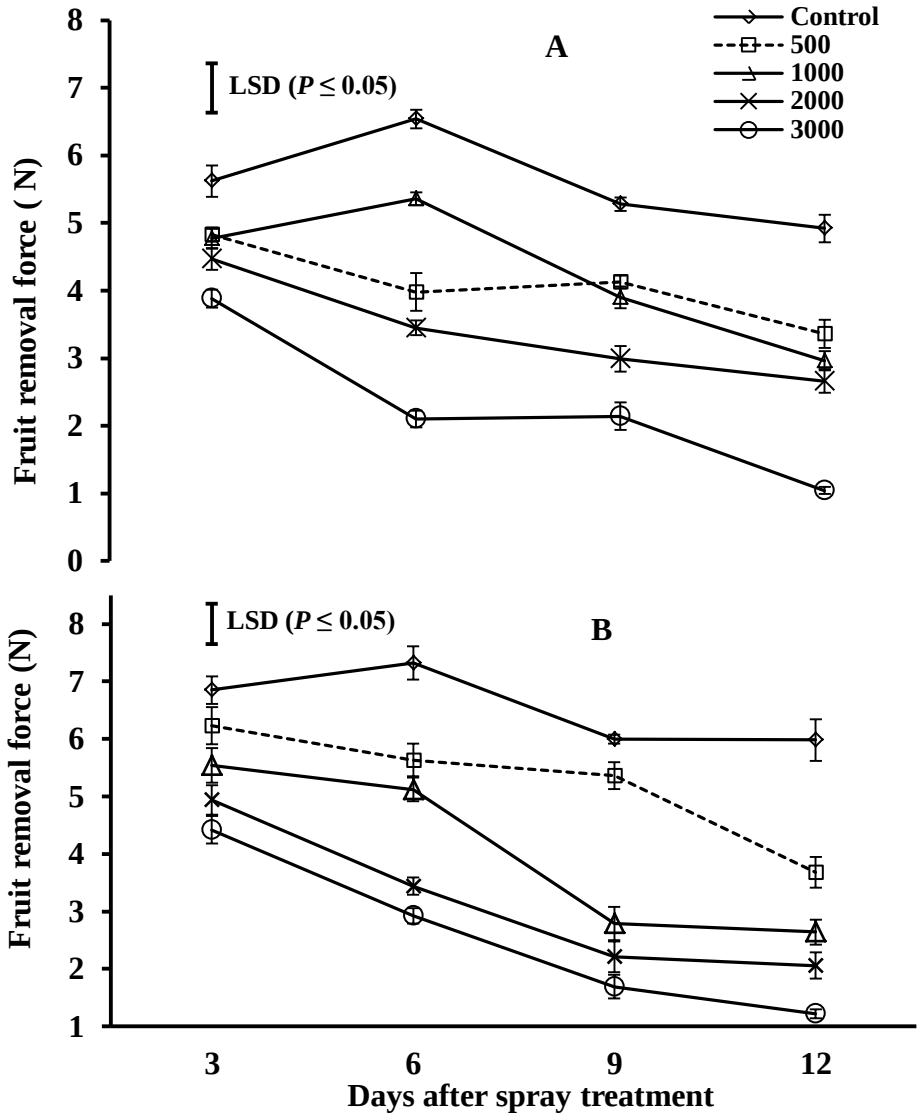


Fig.6.4. Effects of different concentrations of spray application of ethephon (mg L^{-1}) and days after spray treatment on fruit removal force of cvs. Frantoio (A) and Manzanilla (B) olives in 2013. Vertical bar represent SE.

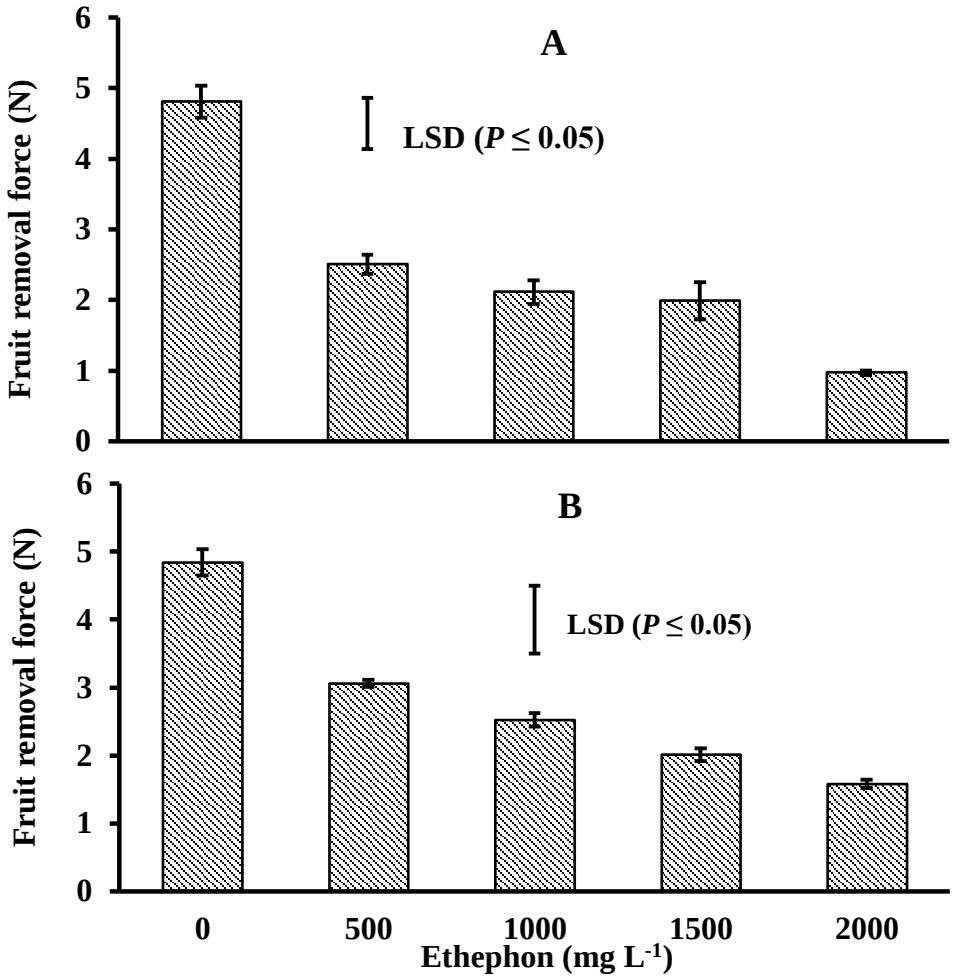


Fig.6.5. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on fruit removal force of cvs. Frantoio (A) and Manzanilla (B) olives in 2014. Vertical bar represent SE.

6.3.4. Fruit and leaf abscission

The rate of fruit abscission increased significantly ($P \leq 0.05$) the concentration of applied ethephon was increased in both cvs. Frantoio and Manzanilla in 2013 and 2014. Lowest percentage of fruit abscission was observed in control fruit (71.04%, 74.91%) in cv. Frantoio and (67.02%, 65.61) in cv. Manzanilla in 2013 and 2014 respectively) (Fig. 6.6 A, B and 6.7 A, B). However, the fruit collected from the trees treated with higher concentrations of ethephon (1000 - 3000 mg L^{-1}) did not show

significant difference for fruit abscission rate in cv. Frantoio (Fig. 6.6A and 6.7 A) and in cv. Manzanilla fruit abscission rate was non-significantly different between 2000 and 3000 mg L^{-1} treated trees (Fig. 6.6 B and 6.7 B).

The percentage of leaf abscission was significantly higher in the olive trees treated with higher concentrations (1000-3000 mg L^{-1}) ethephon in cvs. Frantoio and Manzanilla olive in both years (2013 and 2014). However, leaf abscission rate was not significantly different between control (5.68% and 4.40%) and 500 mg L^{-1} of ethephon treatment (9.91% and 5.61%) in cv. Frantoio during 2013 and 2014 respectively (Fig.6.8 A and Fig6.9 A). A similar trend of leaf abscission was also observed by cv. Manzanilla in 2013 and 2014 (Fig. 6.8 B and 6.9 B).

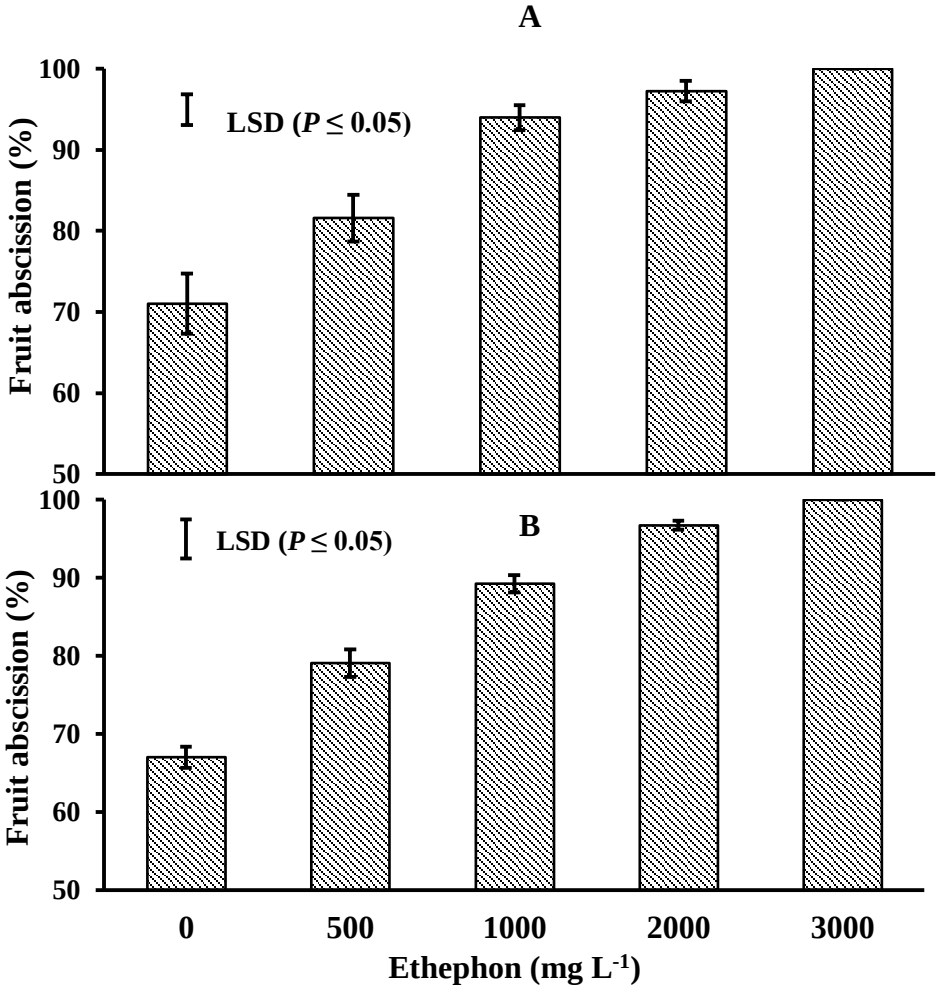


Fig.6.6. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on fruit abscission of cvs. Frantoio (A) and Manzanilla (B) cvs. olives in 2013. Vertical bar represent SE.

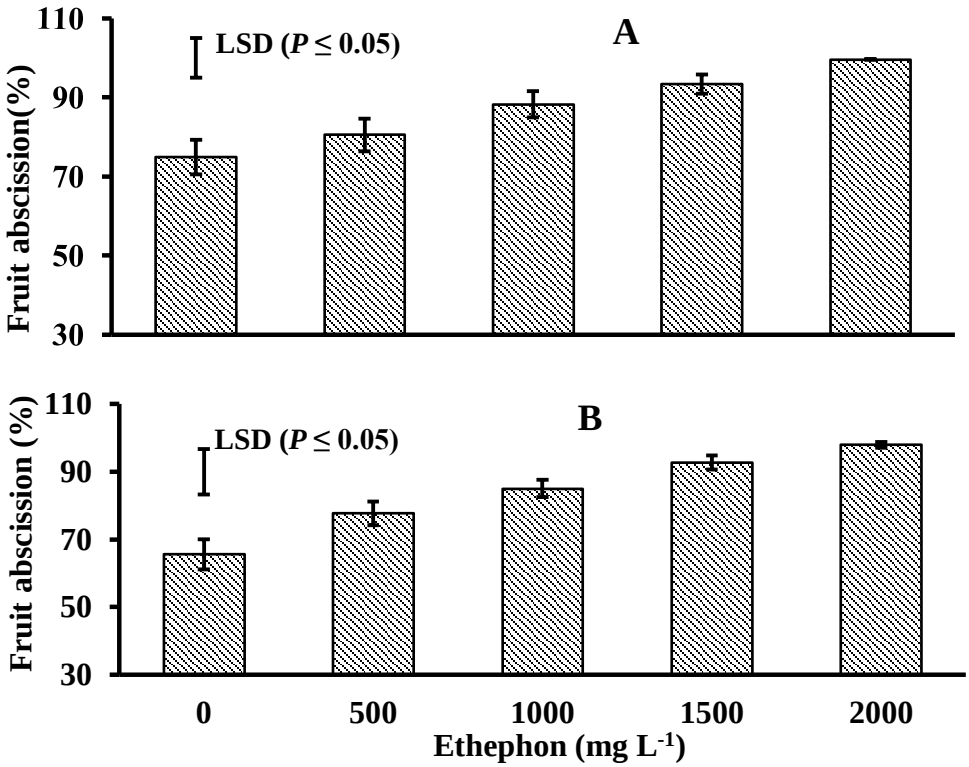


Fig.6.7. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on fruit abscission of cvs. Frantoio (A) and Manzanilla (B) cvs. olives in 2014. Vertical bar represent SE.

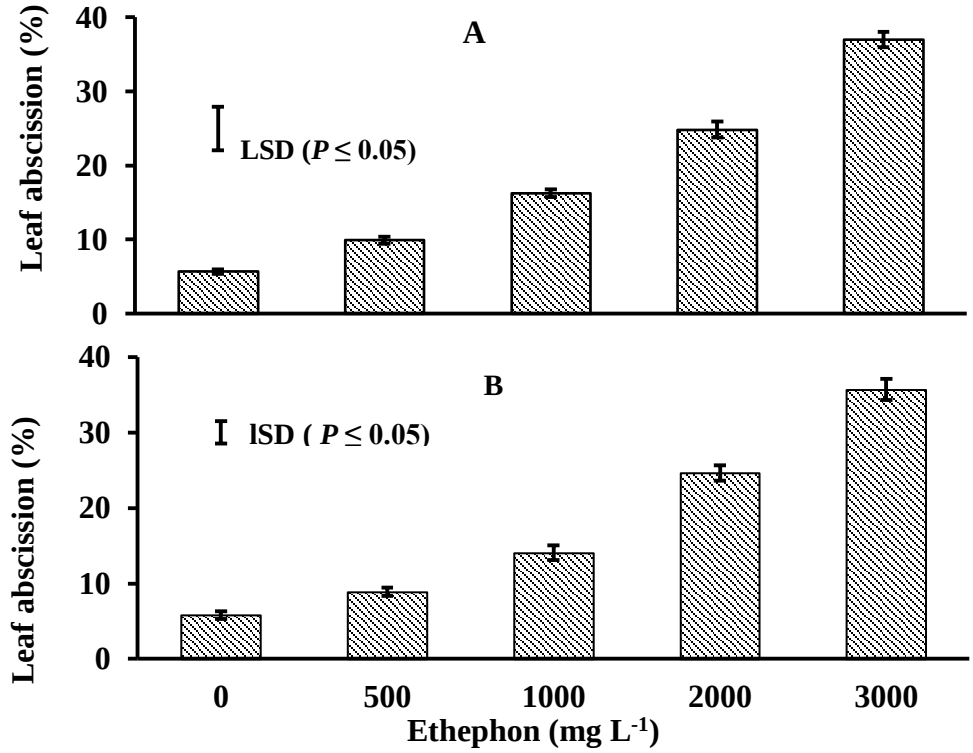


Fig.6.8. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on leaves abscission of cvs. Frantoio (A) and Manzanilla (B) olive in 2013. Vertical bar represent SE.

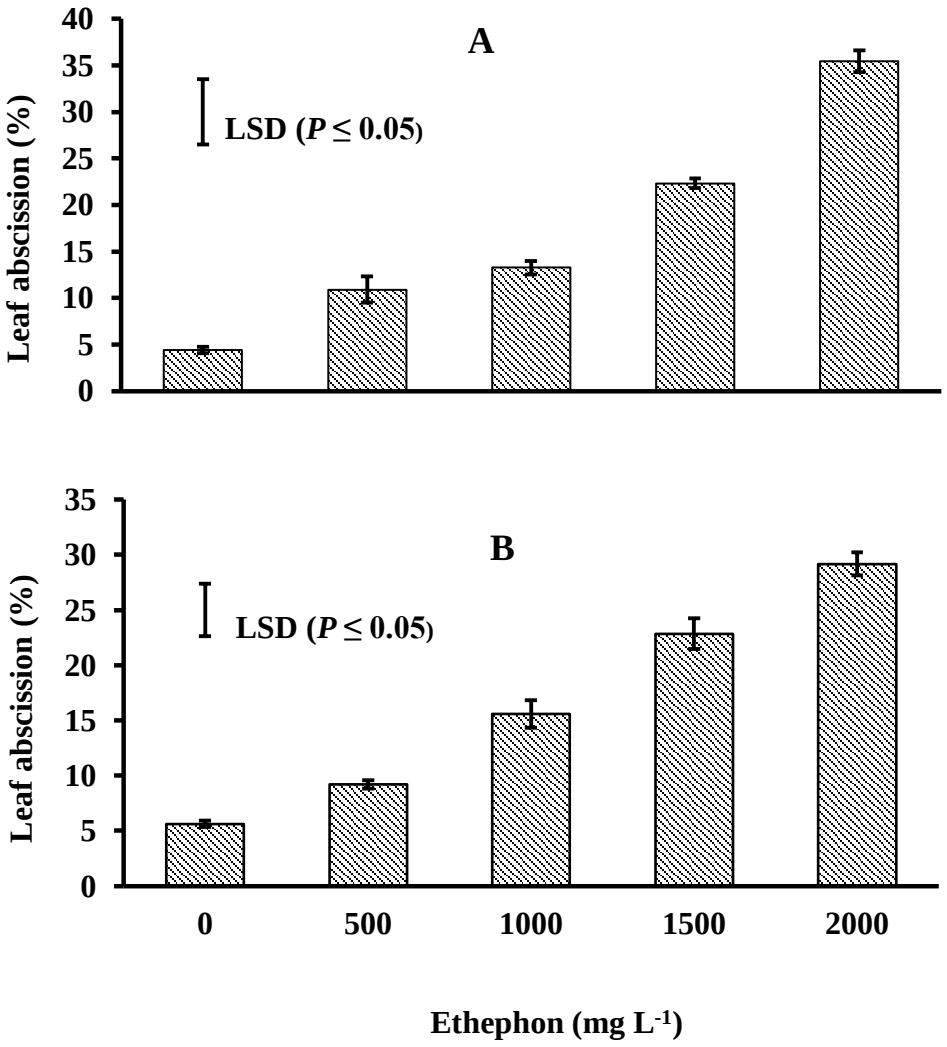


Fig.6.9. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on leaves abscission of cvs. Frantoio (A) and Manzanilla (B) cvs. olive leaves in 2014. Vertical bar represent SE.

6.3.5. Moisture and oil content (dry and fresh weight basis %)

The ethephon concentration did not affect the moisture (%) and oil content of the olive fruit (Fig 6.10)

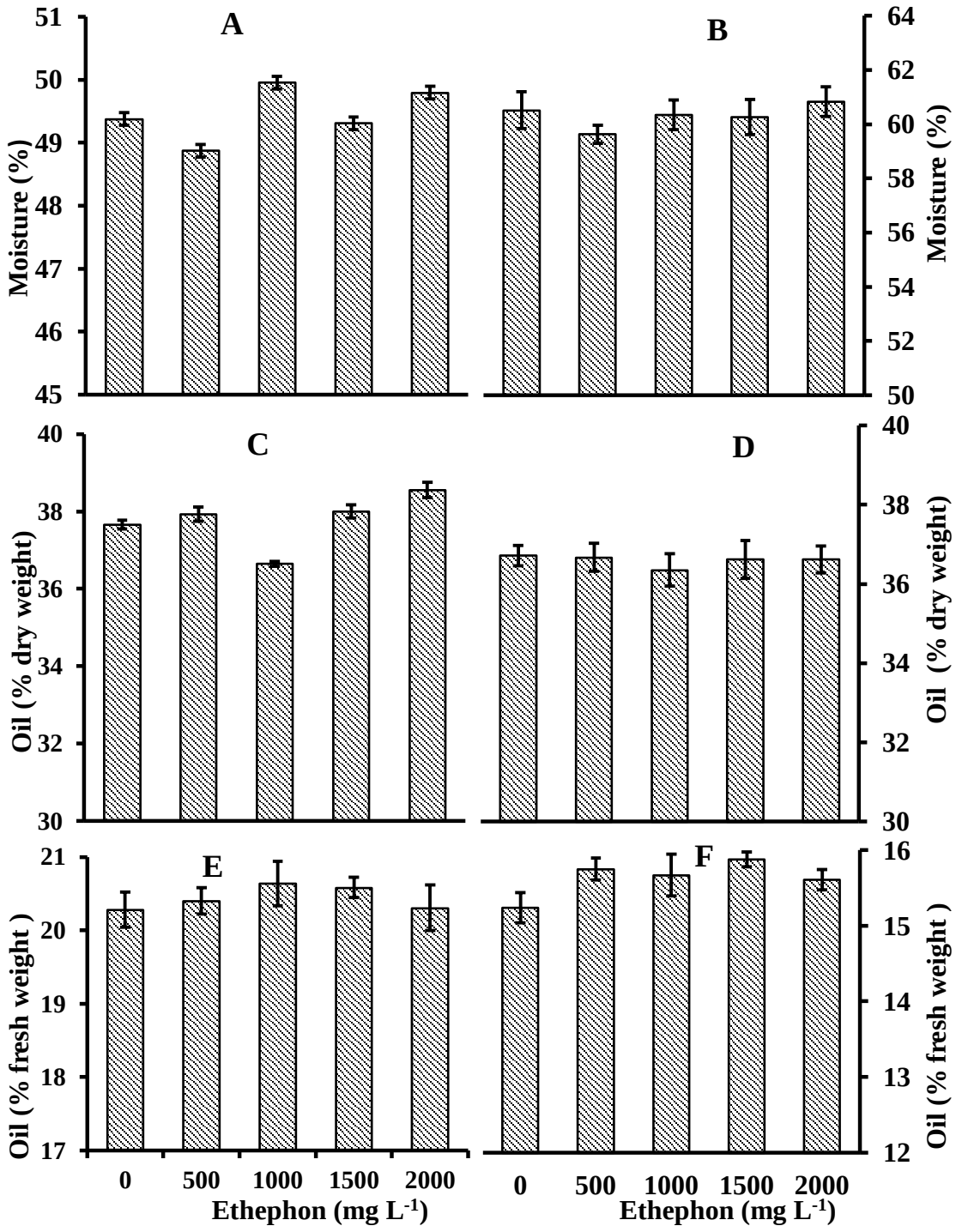


Fig.6.10. Effects of different concentrations of ethephon (mg L^{-1}) on moisture (A- Frantoio, B- Manzanilla cvs.) and oil content on the basis of dry weight (C- Frantoio, D- Manzanilla cvs.) and fresh weight (E- Frantoio , F- Manzanilla cvs.) in 2014. Vertical bar represent SE.

6.3.6. Free fatty acids

Frantoio olive fruit collected from the trees treated with higher concentrations of ethephon (2000-3000 mg L^{-1}) showed significantly higher level of free fatty acids (0.38% and 0.45% respectively) than the fruit collected from the control and other concentrations of ethephon treated trees. The percentage of free fatty acids in control, 500 and 1000 mg L^{-1} treatments (0.22%) did not differ significantly in 2013 (Fig. 6.11 A). Similarly, Manzanilla olive fruit showed significantly higher level of free fatty acids when treated with higher concentrations (2000-3000 mg L^{-1}) of ethephon while other concentrations of ethephon treatments and control fruit did not show significant difference for free fatty acids in 2013 (Fig.6.11 B). In 2014, the higher concentration of ethephon treatment also significantly increased the concentration of free fatty acid in cvs. Frantoio and Manzanilla

However, there was no significant difference among the 1000 to 2000 mg L^{-1} ethephon treatments in Frantoio and among the 500 to 2000 mg L^{-1} ethephon treatments in Manzanilla (Fig. 6.12 A and B).

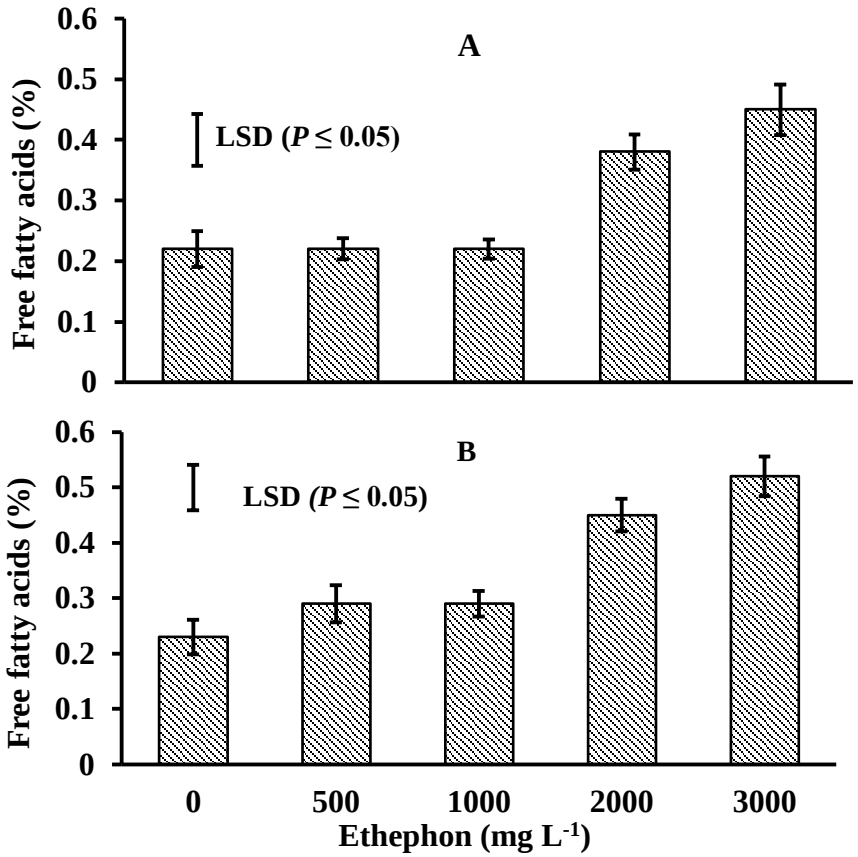


Fig.6.11. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on free fatty acids of cvs. Frantoio (A) and Manzanilla (B) in virgin olive oil in 2013. Vertical bar represent SE.

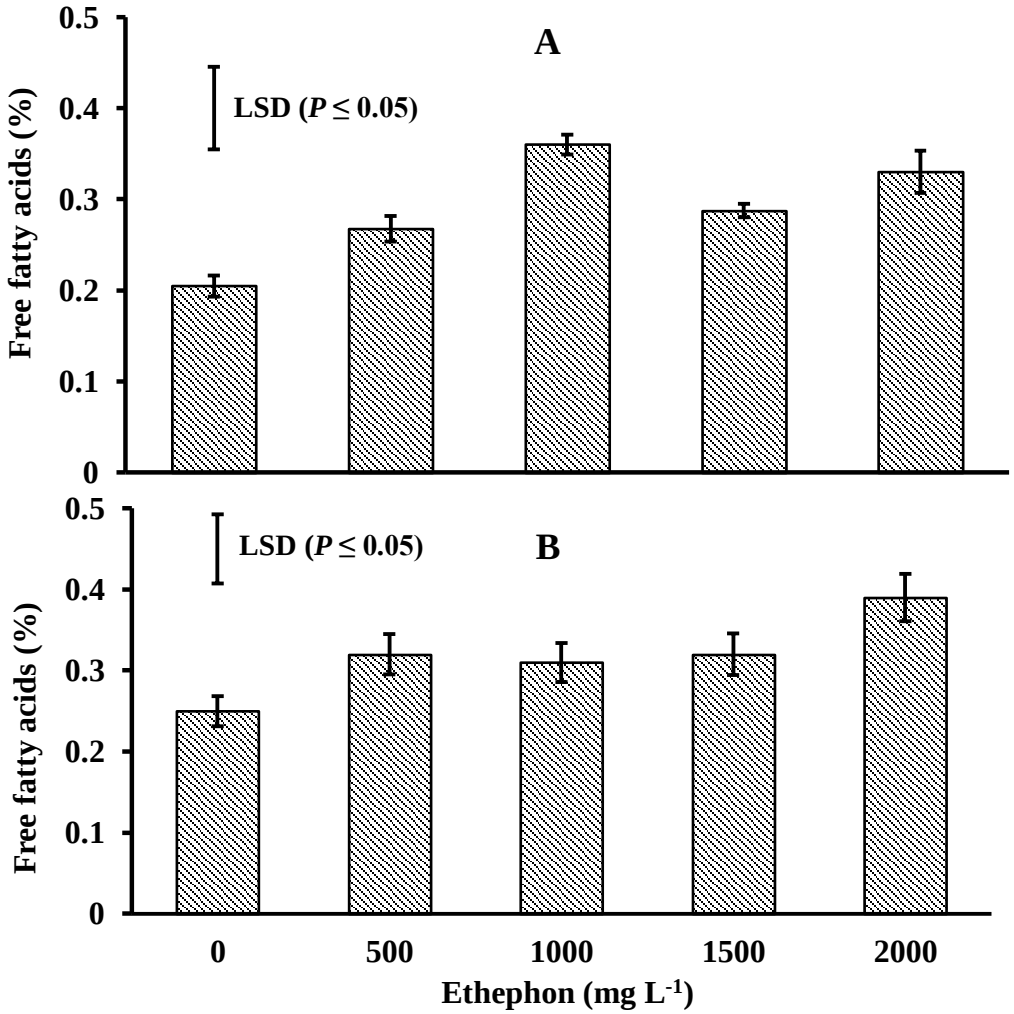


Fig.6.12. Effects of different concentration of spray application of ethephon (mg L^{-1}) on free fatty acids of cvs. Frantoio (A) and Manzanilla (B) in virgin olive oil in 2014. Vertical bar represent SE.

6.3.7. Peroxide value

The peroxide value (meq O_2 kg^{-1}) of the cvs. Frantoio and Manzanilla olive oil in 2013 and 2014 showed significant differences for different concentrations of ethephon treatment applied. It increased with the increase of applied ethephon concentration. The highest peroxide value was observed in 2013 for cvs. Frantoio and Manzanilla treated with 3000 mg L^{-1} ethephon (11.41 and 11.79 meq O_2 kg^{-1}) respectively and the lowest was in control fruit (6.25and 8.65 meq O_2 kg^{-1}) respectively in 2013 (Fig. 6.13). A similar trend was observed in 2014 where the highest peroxide value of olive oil in Frantoio and Manzanilla was noted in 2000 mg

L^{-1} ethephon treated fruit (10.13 and 11.70 meq O_2 kg^{-1}) and the lowest in control fruit (5.61 and 7.50 meq O_2 kg^{-1}) respectively (Fig. 6.14). However, there was no significant difference between the 1500 and 2000 mg L^{-1} ethephon treated fruit for peroxide value in both the cultivars in 2014 (6.14).

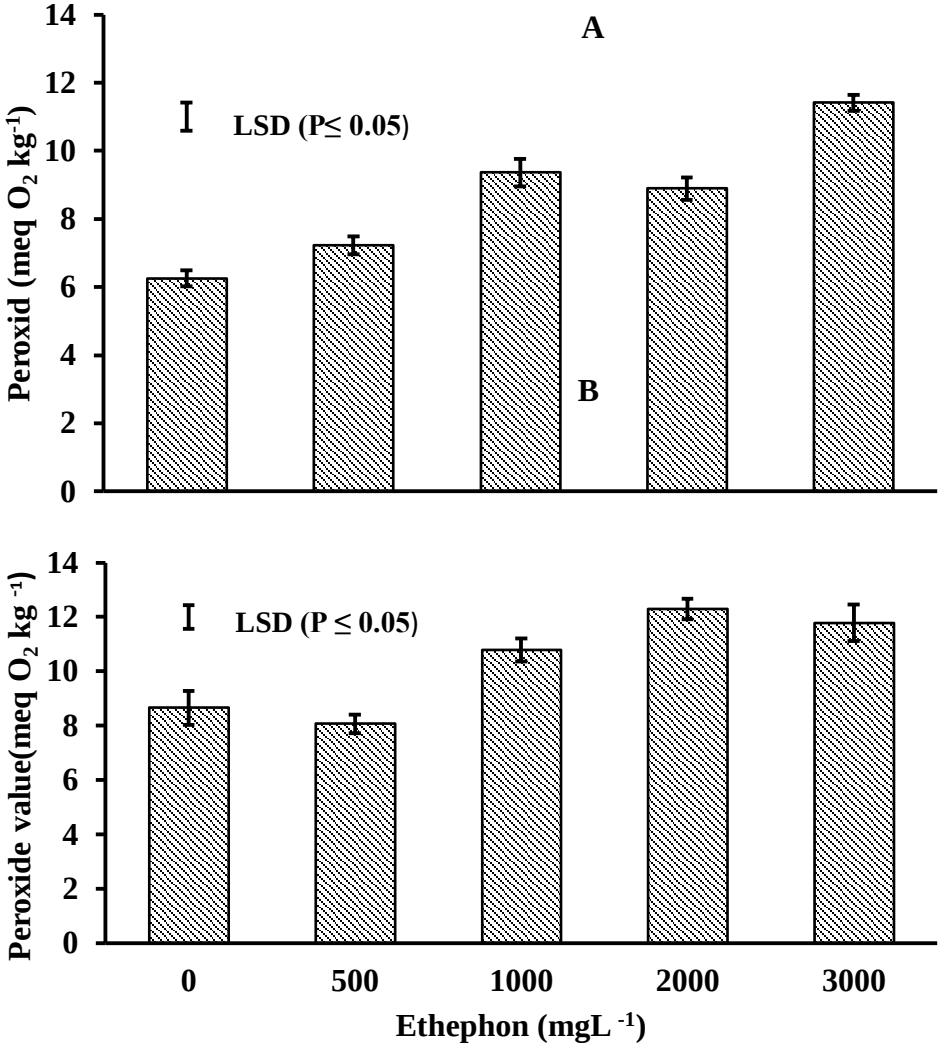


Fig.6.13. Effects of different concentration of spray application of ethephon (mg L^{-1}) on peroxide value (meq O_2 kg^{-1}) of cvs. Frantoio (A) and Manzanilla (B) in virgin olive oil in 2013. Vertical bar represent SE.

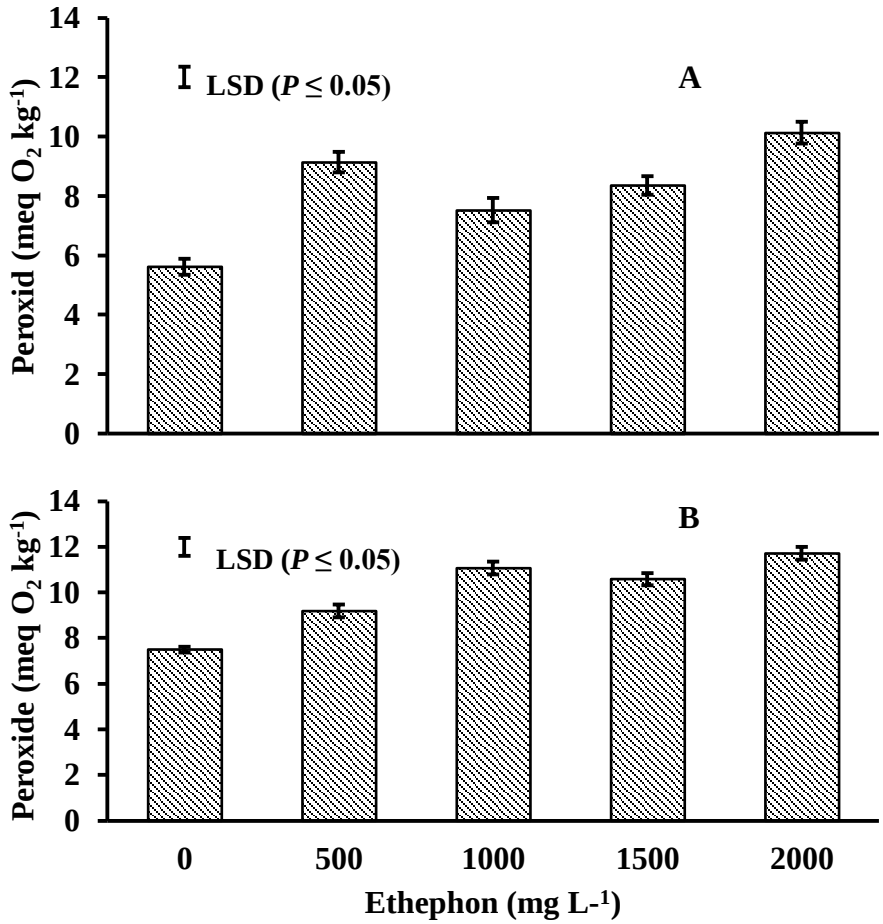


Fig.6.14. Effects of different concentration of spray application of ethephon (mg L^{-1}) on peroxide value (meq O_2 kg^{-1}) of cvs. Frantoio (A) and Manzanilla (B) in virgin olive oil in 2014. Vertical bar represent SE.

6.3.8. The fatty acids:

6.3.8.1. Palmitic acid (C 16:0)

Ethephon treatment significantly ($P \leq 0.05$) influenced the concentration of palmitic acid (%) in comparison to the control in cvs. Frantoio and Manzanilla olive oil in 2013 and 2014. The higher concentration of palmitic acid (12.50% to 12.61% in 2013 and 12.64% to 13.52% in 2014) was observed in the oil extracted from the Frantoio fruit treated with higher concentration (2000 and 3000 mg L^{-1}) of ethephon. The Manzanilla olive also showed a similar effect for palmitic acid in response to the ethephon treatments (Fig. 6.15, Fig 6.16).

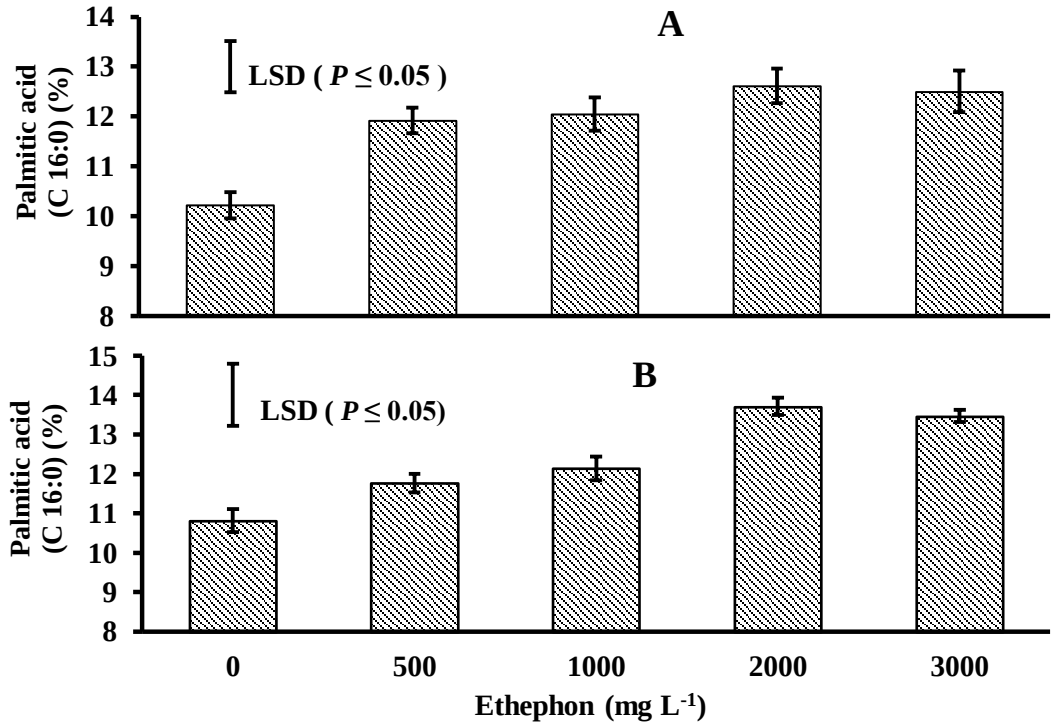


Fig.6.15. Effects of different concentration of spray application of ethephon (mg L^{-1}) on palmitic acid (C 16:0) (%) of cvs. Frantoio (A) and Manzanilla (B) in virgin olive oil in 2013. Vertical bar represent SE.

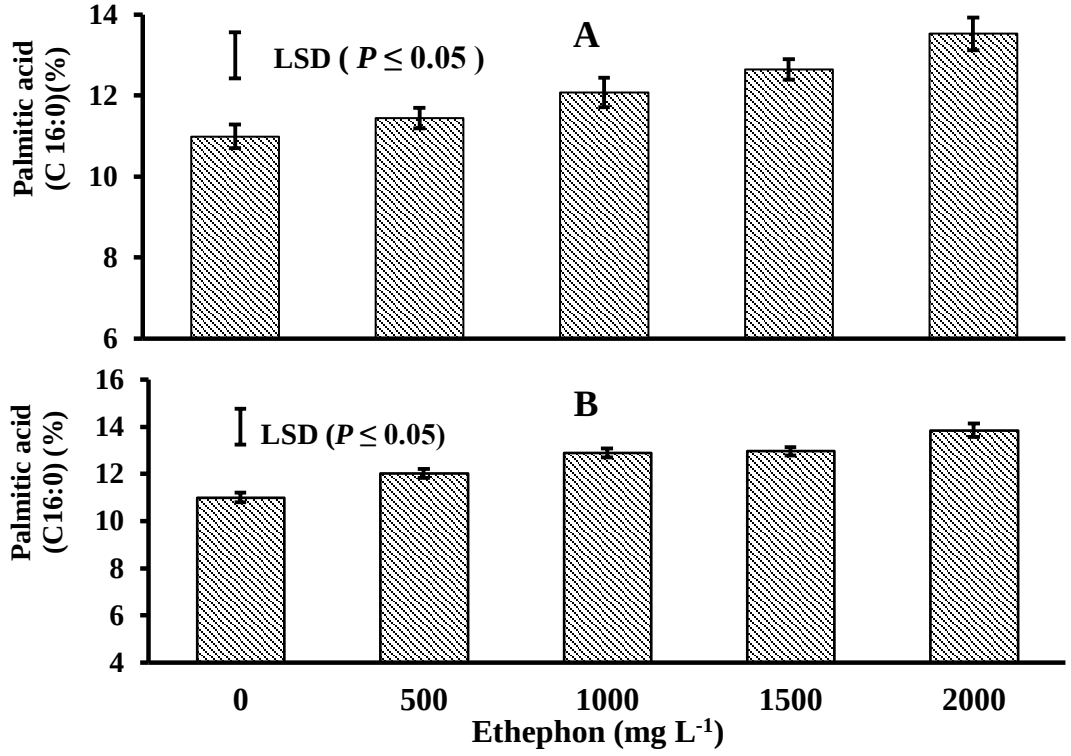


Fig. 6. 16. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on palmitic acid (C 16:0) (%) in virgin olive oil of Frantoio (A) and Manzanilla (B) cvs. in 2014. Vertical bar represent SE.

6.3.8.2. Stearic acid (C 18:0)

The concentrations of stearic acid in virgin olive oil was also significantly ($P \leq 0.05$) influenced by ethephon treatments in comparison to the control cvs. Frantoio and Manzanilla olive oil in 2013 and 2014. It increased with the increase of ethephon concentrations applied. The highest concentration of stearic acid (4.42%, 4.28% and 4.02%, 3.91 in cvs. Frantoio and Manzanilla in 2013 and 2014 respectively) was observed in the 3000 mg L^{-1} ethephon treated fruit (Fig. 6.17). However, the higher concentration of ethephon treatments (1500-2000 mg L^{-1}) did not show significant differences for stearic acid concentration in 2014 in both cultivars (Fig. 6.18).

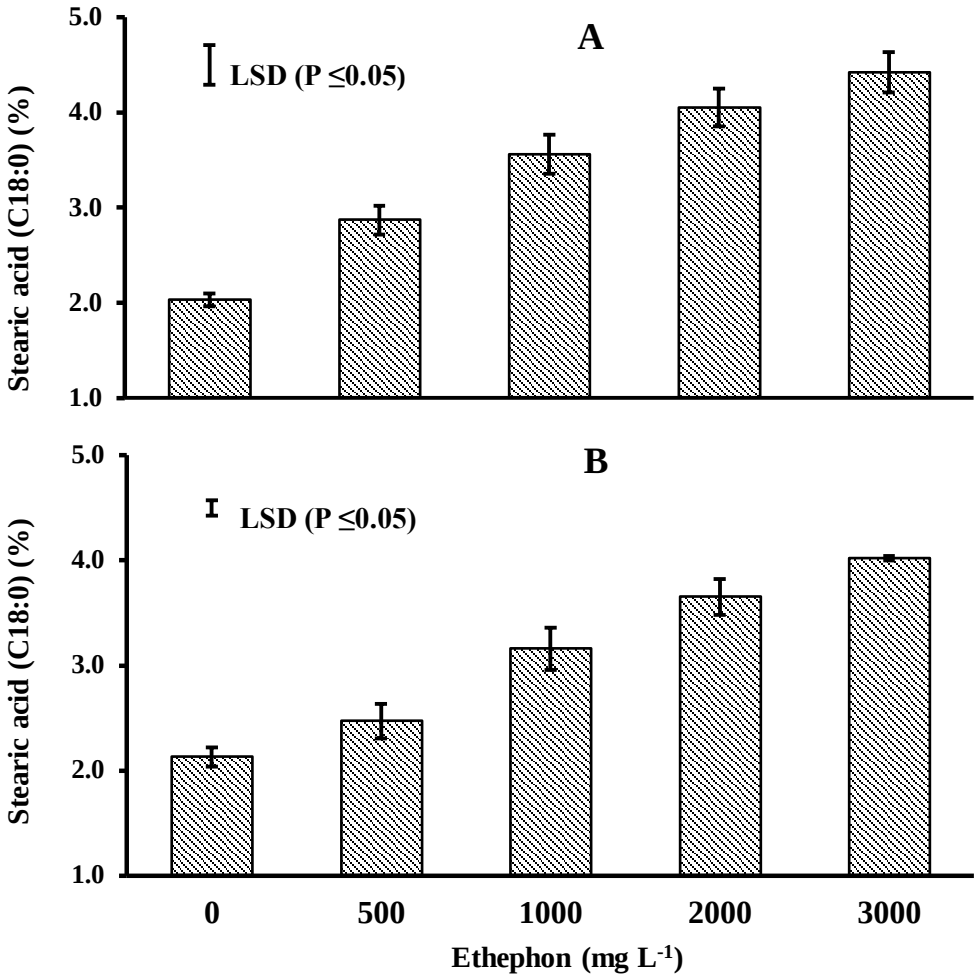


Fig.6.17. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on stearic acid (C 18:0) (%) in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2013. Vertical bar represent SE.

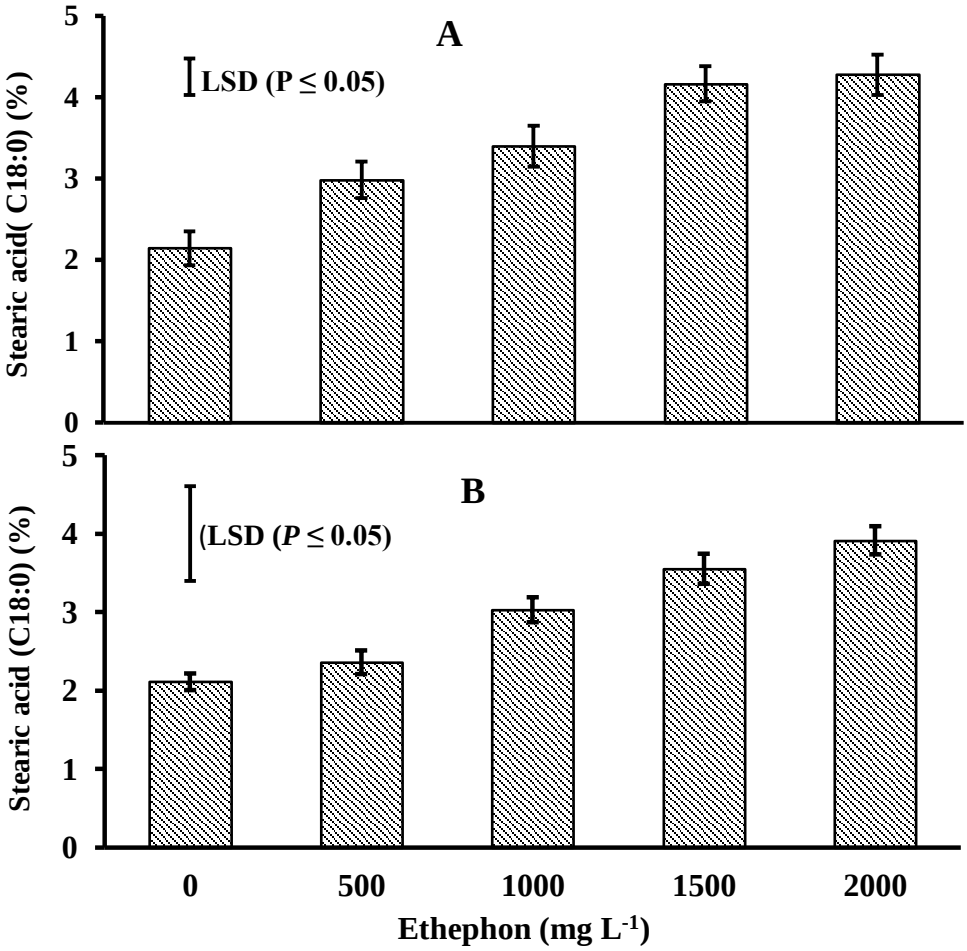


Fig.6.18. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on stearic acid (C 18:0) (%) in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2014. Vertical bar represent SE.

6.3.8.3. Oleic acid (C 18:1)

The application of ethephon reduced the concentration of oleic acid in in virgin olive oil for both olive cultivars in 2013 and 2014. Higher concentration of oleic acid was noted from the control fruit in 2013 (73% and 78.75% in cvs. Frantoio and Manzanilla respectively) which was significantly different from the ethephon treated fruit. It reduced slightly with the increase of ethephon concentration without significant differences in 2013 (Fig.6.19). In 2014, ethephon treatments did not show any significant effect on Manzanilla, however, they significantly reduced oleic acid in cv. Frantoio (Fig. 6.20 A and B).

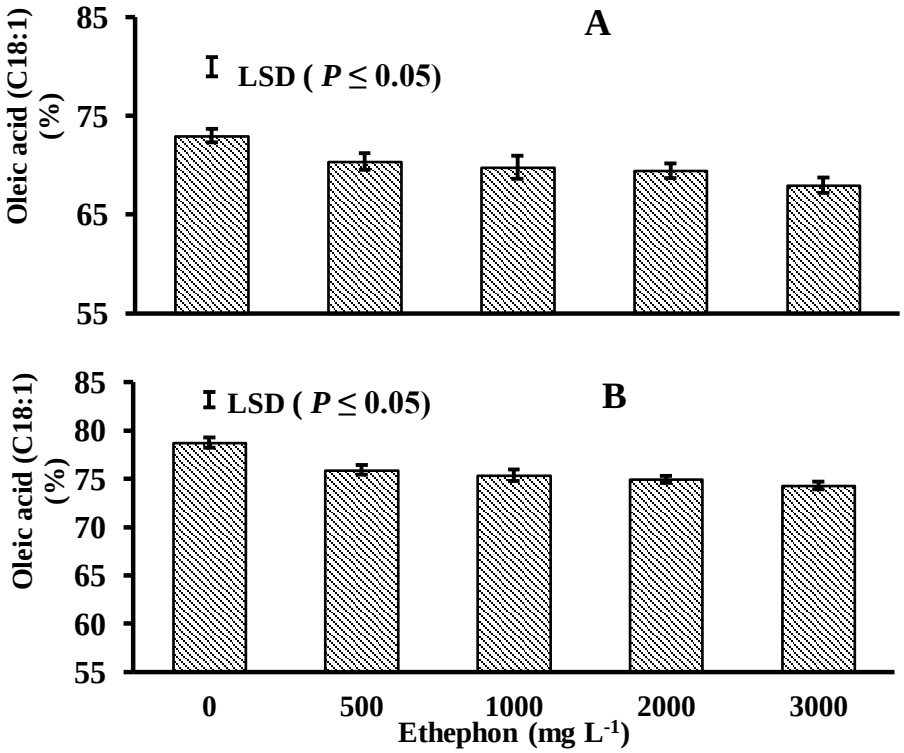


Fig.6.19. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on oleic acid (C 18:1) (%) in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2013. Vertical bar represent SE.

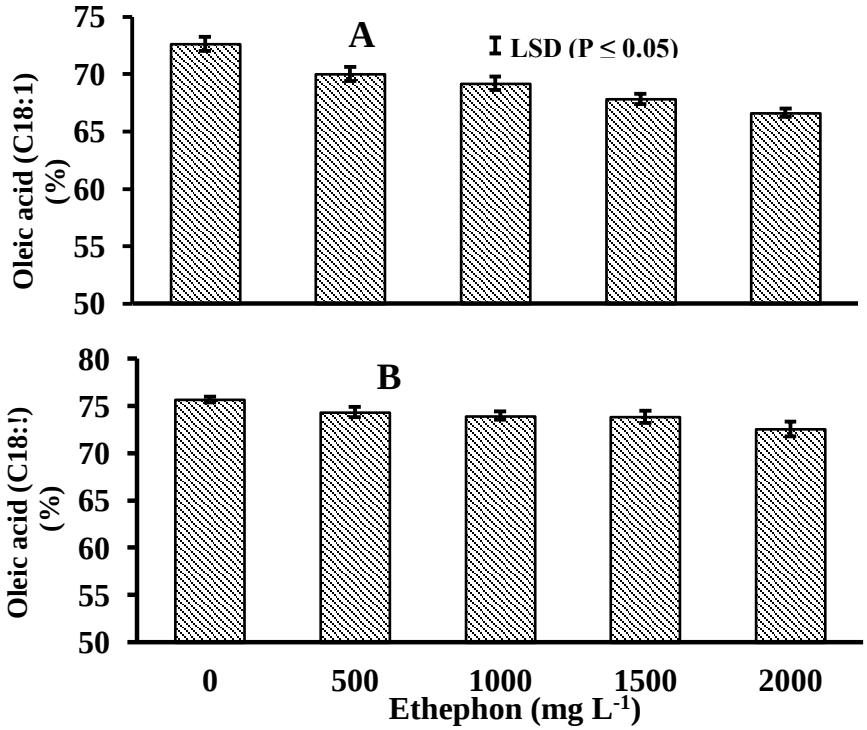


Fig.6.20. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on oleic acid (C 18:1) (%) in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2014. Vertical bar represent SE.

6.3.8.4. Linoleic acid (C 18:2)

Level of A reverse trend of oleic acid levels was observed for linoleic acid in virgin olive oil for both cvs. Frantoio and Manzanilla in 2013 and 2014. The ethephon treated fruit significantly differed from the control fruit for linoleic acid in extracted oil, where the higher concentration of linoleic acid was noted from higher concentration ethephon (3000 mg L^{-1}) treated olive fruit (10.91% and 9.65% in cvs. Frantoio and Manzanilla respectively) in 2013. A similar effect of higher concentration of ethephon (2000 mg L^{-1}) was also observed in 2014 (11.03% and 11.16% in cvs. Frantoio and Manzanilla respectively) (Fig. 6.21, Fig. 6.22)

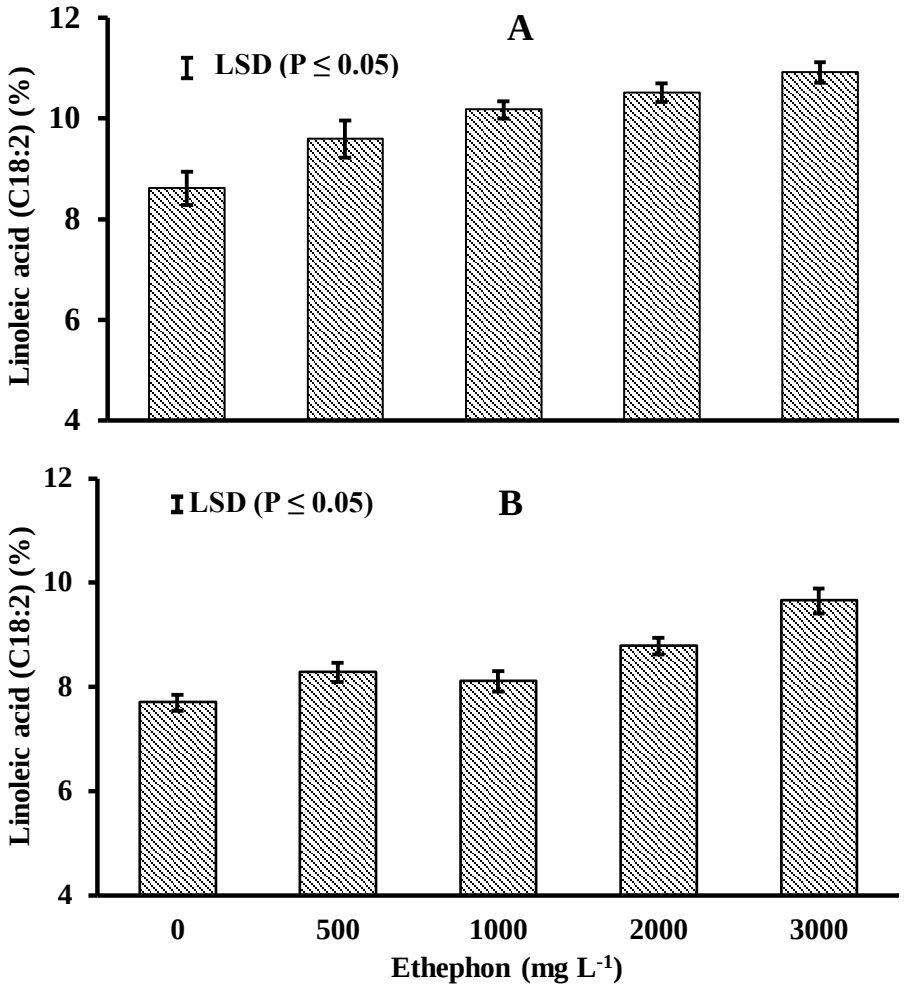


Fig.6.21. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on linoleic acid (C 18:2) (%) in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2013. Vertical bar represent SE.

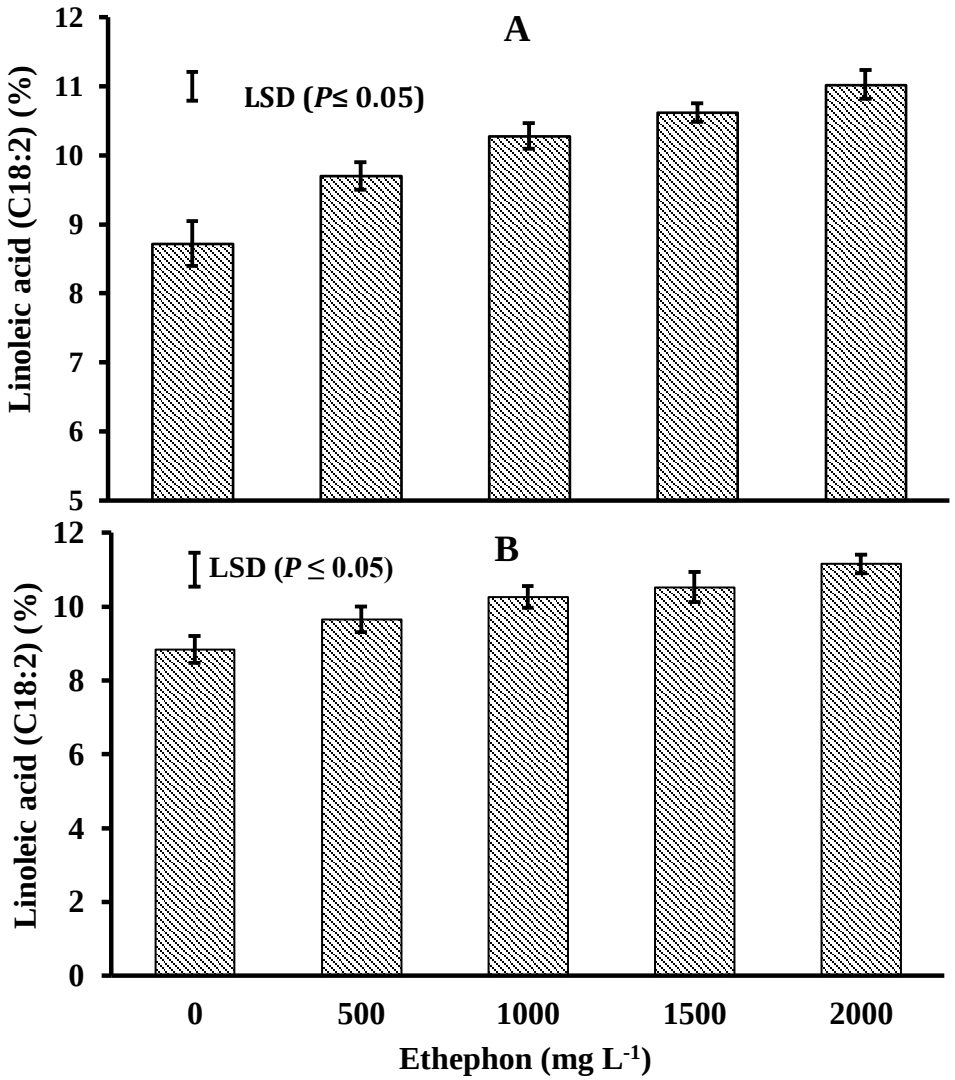


Fig.6.22. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on linoleic acid (C 18:2) (%) in virgin olive oil of Frantoio (A) and Manzanilla (B) cvs. in 2014. Vertical bar represent SE.

6.3.8.5. Monounsaturated fatty acids (MUFA)

All the ethephon spray treatments reduced the level of MUFA (%) in virgin olive oil of Frantoio and Manzanilla cultivars in both years (Fig. 6.23 and 6.24) than the control. The effects of different ethephon treatments on the level of MUFA (%) in virgin olive oil were not significant in cv. Frantoio in 2013 and in cultivar Manzanllia in 2014. Whilst, ethephon treatments exhibited significant reductions in the levels of MUFA (%) in virgin olive oil of cv. Manzanllia during 2014 (Fig. 6.23B) and in cv. Frantoio during 2014 (Fig. 6.23B).

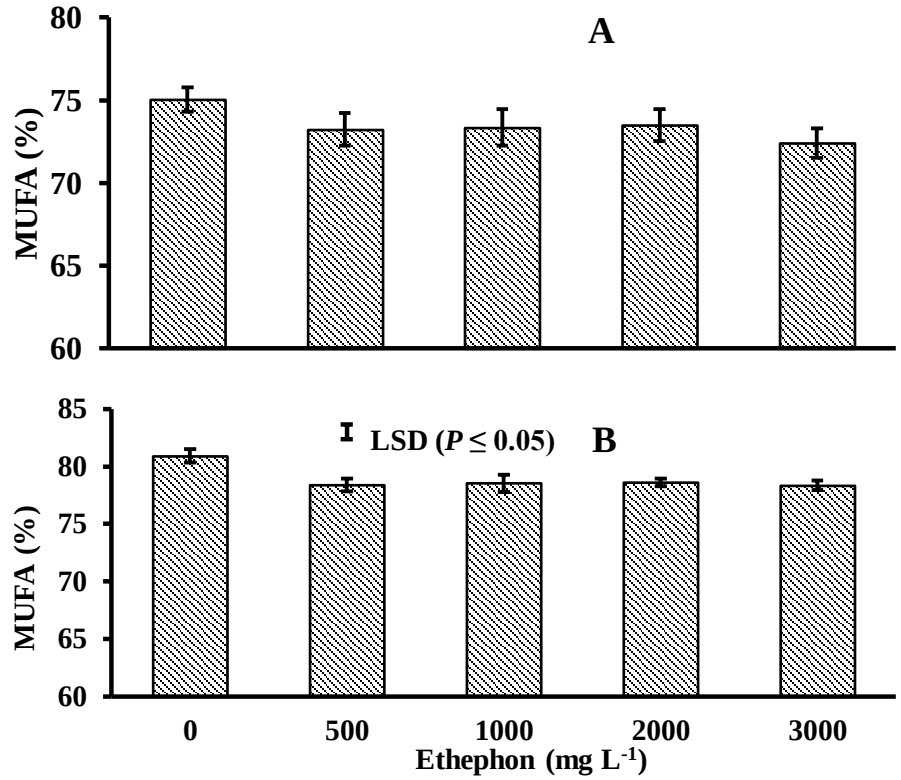


Fig.6.23. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on MUFA (%) in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2013. Vertical bar represent SE.

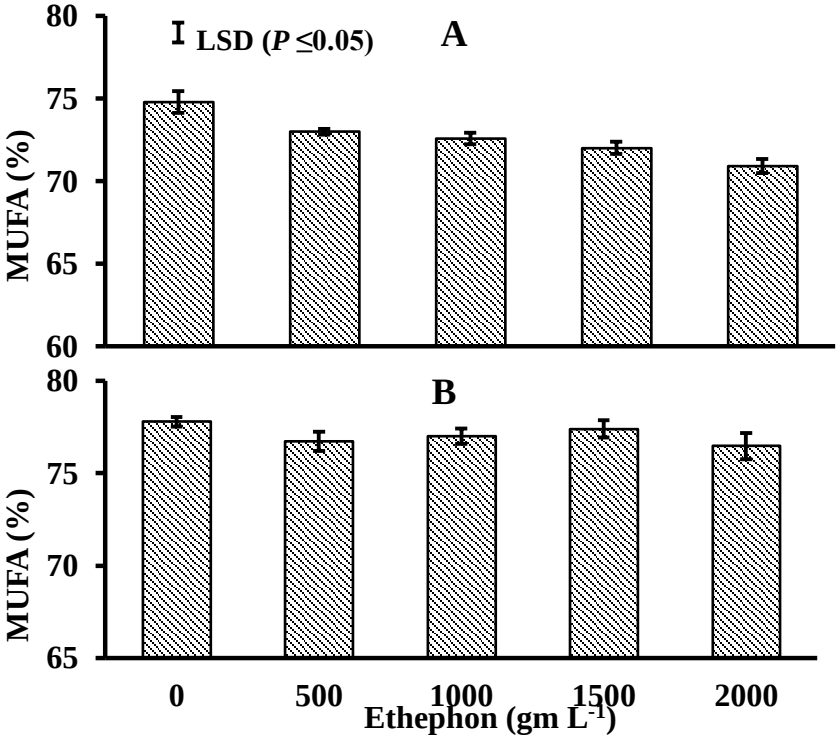


Fig. 6.24. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on MUFA (%) in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2014. Vertical bar represent SE.

6.3.8.6. Polyunsaturated fatty acids (PUFA) (%)

The level of PUFA (%) increased with the increase of ethephon concentration applied and it was highest (11.46% and 10.93% in cvs. Frantoio and Manzanilla respectively) in the 3000 mg L^{-1} treated fruit in 2013 (Fig.6.24 A and B). A similar trend was also obtained in 2014 (11.57% and 11.53% in cvs. Frantoio and Manzanilla respectively). Lower percentage of PUFA was noted from the control Frantoio (9.27%, 9.39%) and cv. Manzanilla (8.39%, 9.42) olive in 2013 and 2014 respectively and were significantly lower compared to the ethephon treated fruit in both cultivars (Fig. 6.25 A, B and 6.26 A, B).

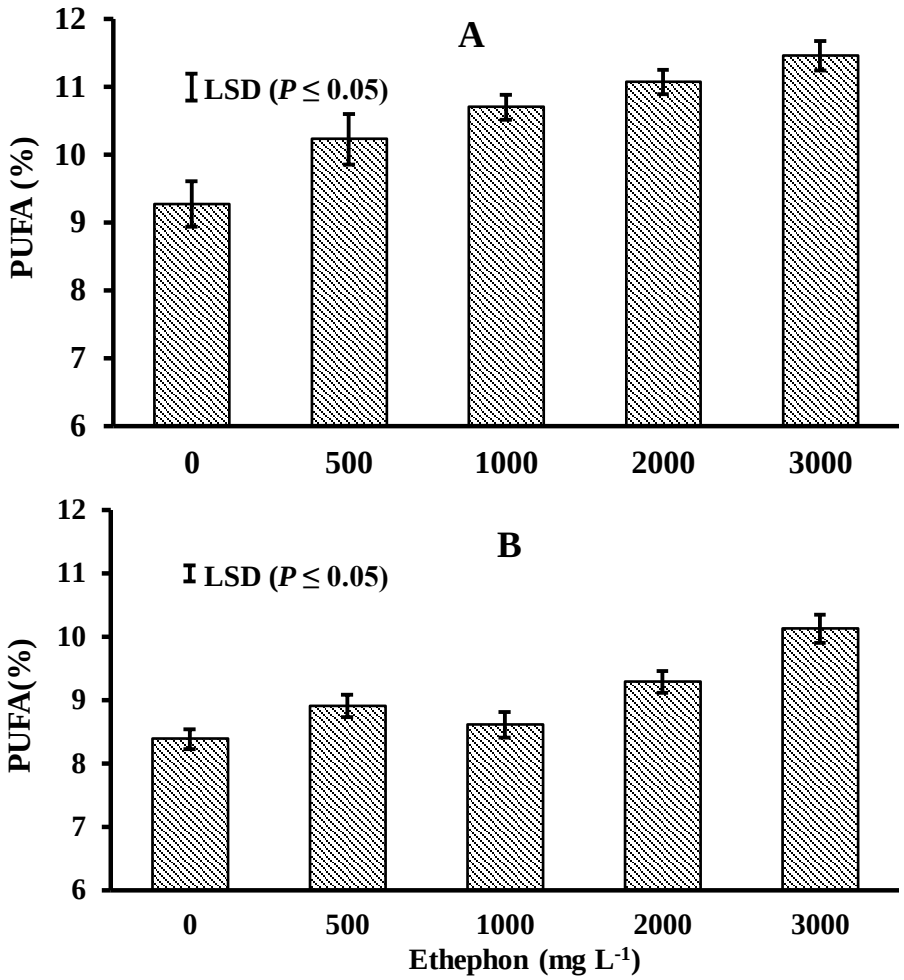


Fig.6.25. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on PUFA (%) in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2013. Vertical bar represent SE.

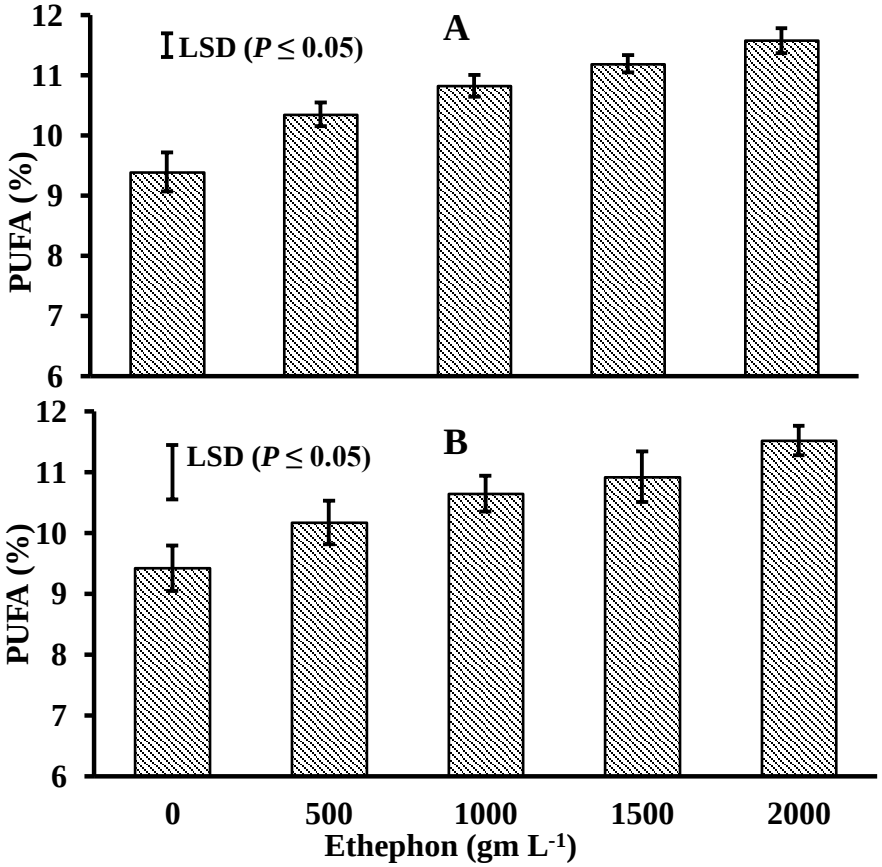


Fig.6.26. Effects of different concentrations of spray application of ethephon (mg L^{-1}) spray on PUFA (%) in virgin olive oil of cv. Frantoio (A) and Manzanilla (B) in 2014. Vertical bar represent SE.

6.3.8.7. MUFA/PUFA ratio

The MUFA/PUFA ratios in both cultivars were significantly reduced by ethephon treatments in both years. However, higher ratio was observed in 500 mg L^{-1} ethephon treated and control cv. Frantoio olive fruit (10.07 and 9.65 respectively) than other treated fruit in 2013 (Fig.6.27A). The ratio declined with the increase of the ethephon concentration where the lowest value was observed in the cv. Frantoio olive fruit treated with 3000 mg L^{-1} ethephon in 2013 (8.20) and 2014 (6.16). On the other hand, control and 1000 mg L^{-1} ethephon treated cv. Manzanilla olive fruit showed significantly higher MUFA/PUFA ratio (9.64 and 9.12 respectively) in 2013 (Fig. 6.28 B). Similar observation was recorded in 2014 with control and 1000 mg L^{-1} ethephon treated Manzanilla olive fruit for MUFA/PUFA ratio (8.49 and 7.69 respectively) (Fig. 6.28B).

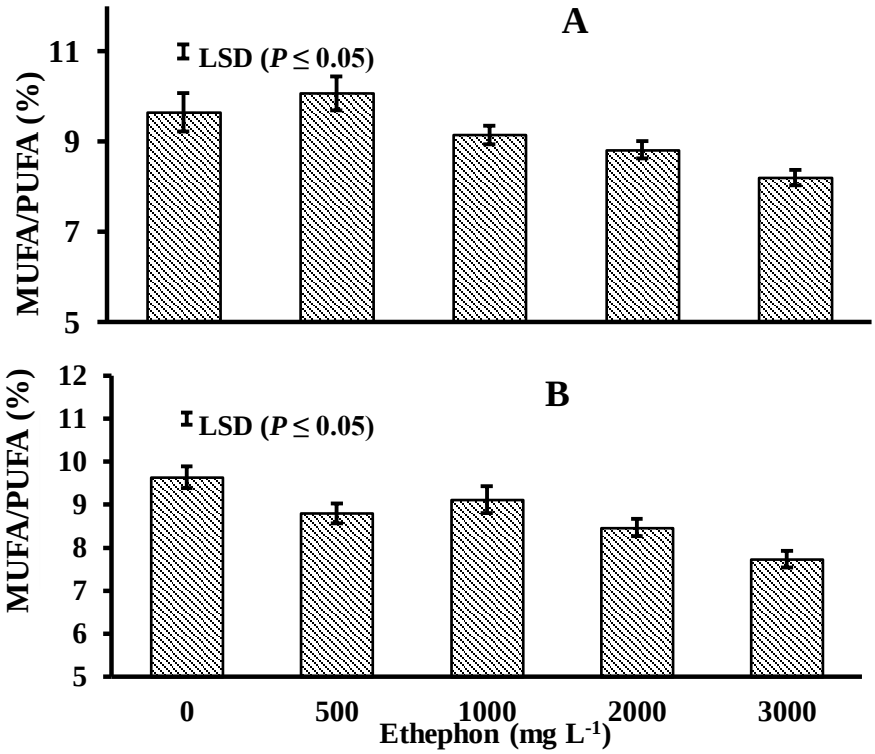


Fig.6.27. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on MUFA: PUFA ratio in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2013. Vertical bar represent SE.

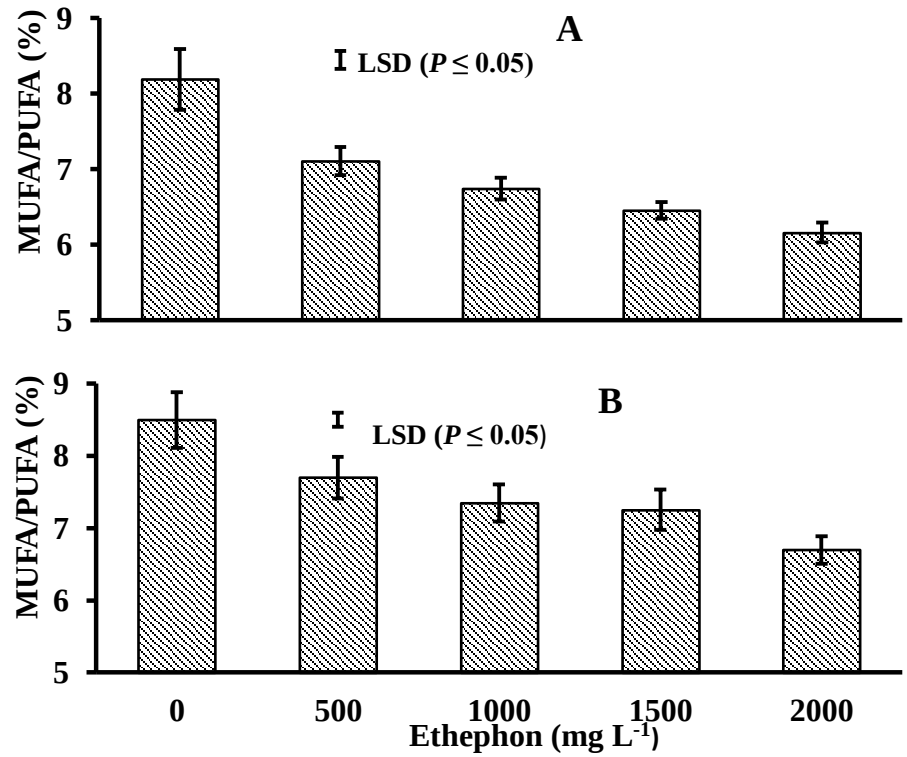


Fig.6.28. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on MUFA: PUFA ratio in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2014**.** Vertical bar represent SE.

6.3.9. Polyphenolic compounds

6.3.9.1. Tyrosol

The level of tyrosol (mg Kg-1) in virgin olive oil reduced significantly with the ethephon application and was more pronounced as the concentrations of applied ethephon increased in both cultivars during both the years (Fig. 6.29 and 6.30). Higher concentration of tyrosol was observed in the control fruit (8.28 and 12 mg Kg^{-1} in cvs. Frantoio and Manzanilla respectively during 2013) and the lowest was in the fruit treated with higher concentration (3000 mg L^{-1}) of ethephon (3.02 and 7.50 mg kg^{-1} in cvs. Frantoio and Manzanilla respectively in 2013) (Fig. 6.29 A and B). Similar trend was also observed in 2014 in both varieties (Fig.6.30).

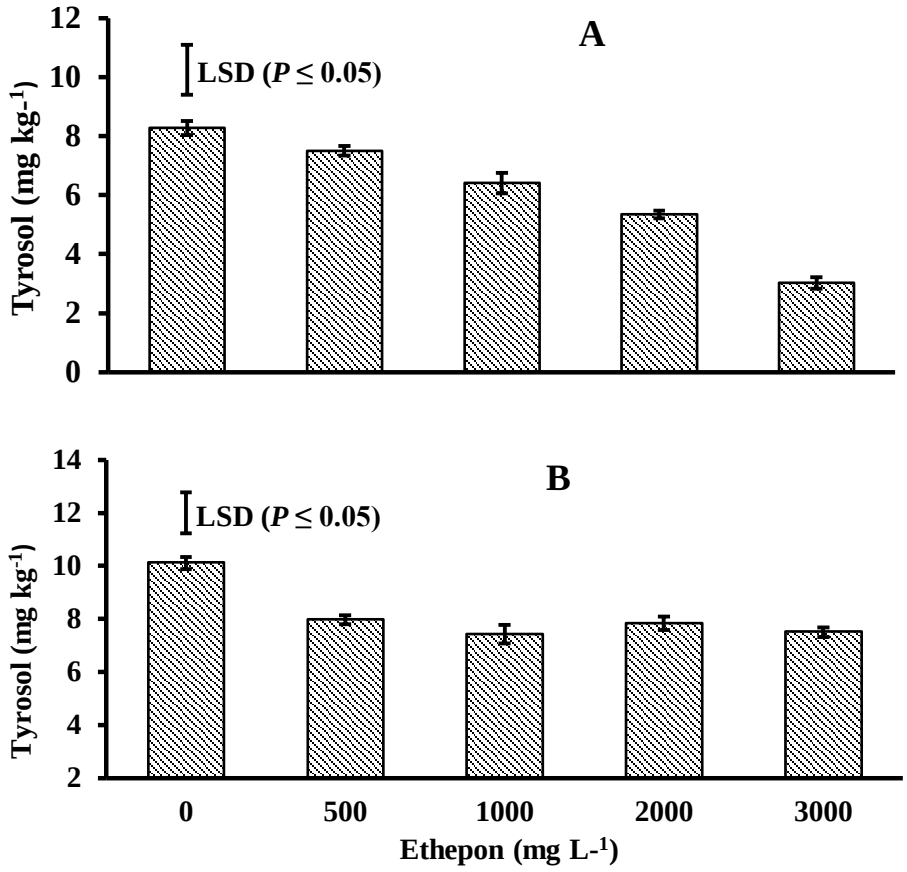


Fig.6.29. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on levels of tyrosol in the virgin olive oil of cv. Frantoio (A) and Manzanilla (B) during 2013. Vertical bar represent SE.

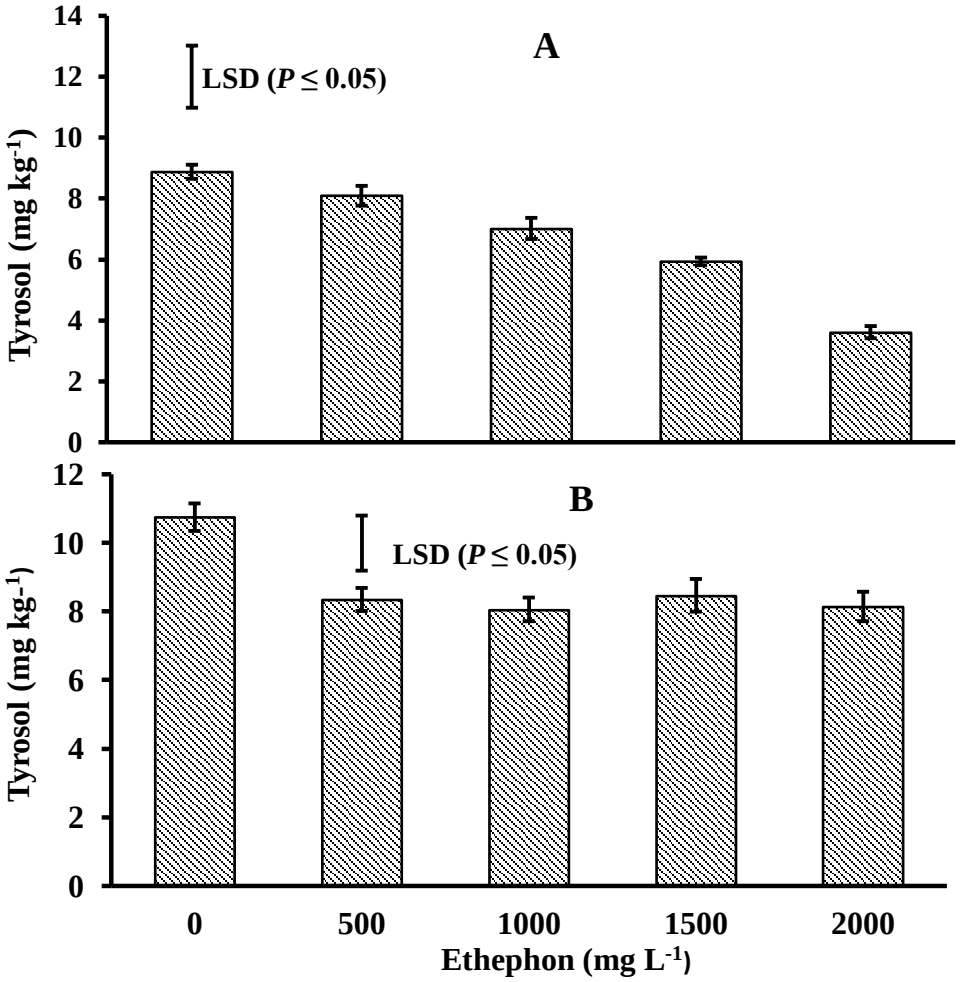


Fig.6.30. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on levels of tyrosol in the virgin olive oil of cv. Frantoio (A) and Manzanilla (B) during 2014. Vertical bars represent SE.

6.3.9.2. Hydroxytyrosol

The level of hydroxytyrosol (mg Kg^{-1}) in the virgin olive oil also reduced significantly with ethephon treatments and the decreased was more pronounced as the concentrations of ethepon increased in both cvs. Frantoio and Manzanilla during 2013 and 2014 (Fig. 6.31 and 6.32). The unsprayed fruit contained higher concentration of hydroxytyrosol (5.43 mg Kg^{-1}) and the lowest (1.53 mg Kg^{-1}) was observed in the cv. Frantoio olive fruit treated with higher concentration (3000 mg L^{-1}) of ethephon during 2013. Similarly, higher concentration of hydroxytyrosol was noted from control cv. Manzanilla fruit (7.05 mg Kg^{-1}) and lowest from the fruit treated with 2000 mg L^{-1} ethephon during 2013 (Fig. 6.31 B). In 2014, a similar trend was also observed with ethephon treatments in both the cultivars (Fig 6.32A and B).

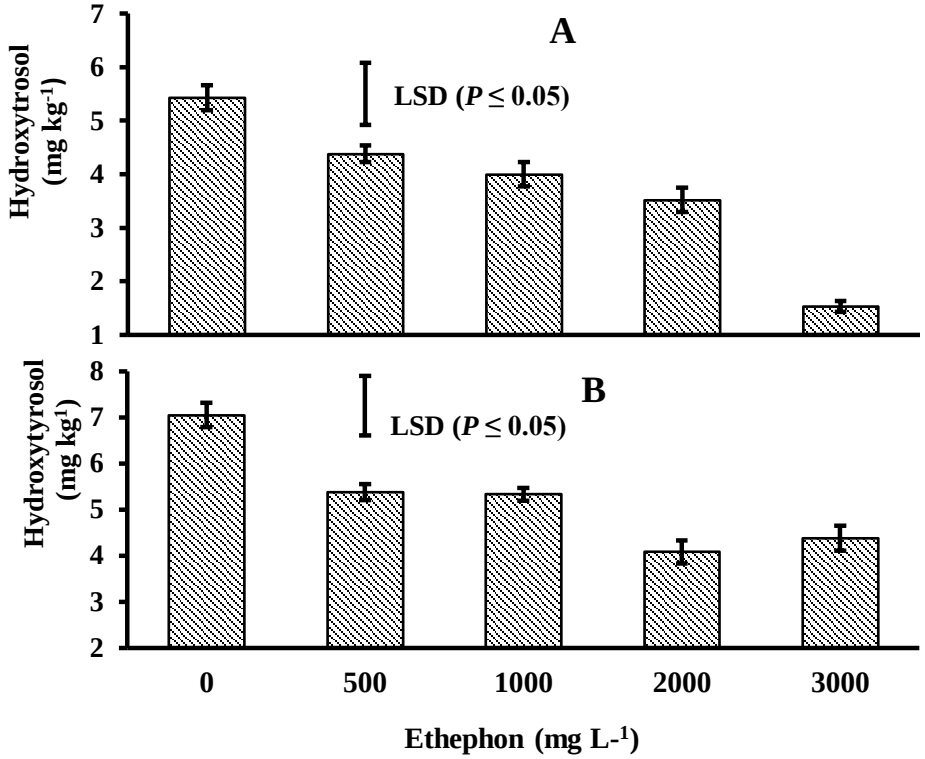


Fig.6.31. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on hydroxytyrosol in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2013. Vertical bars represent SE.

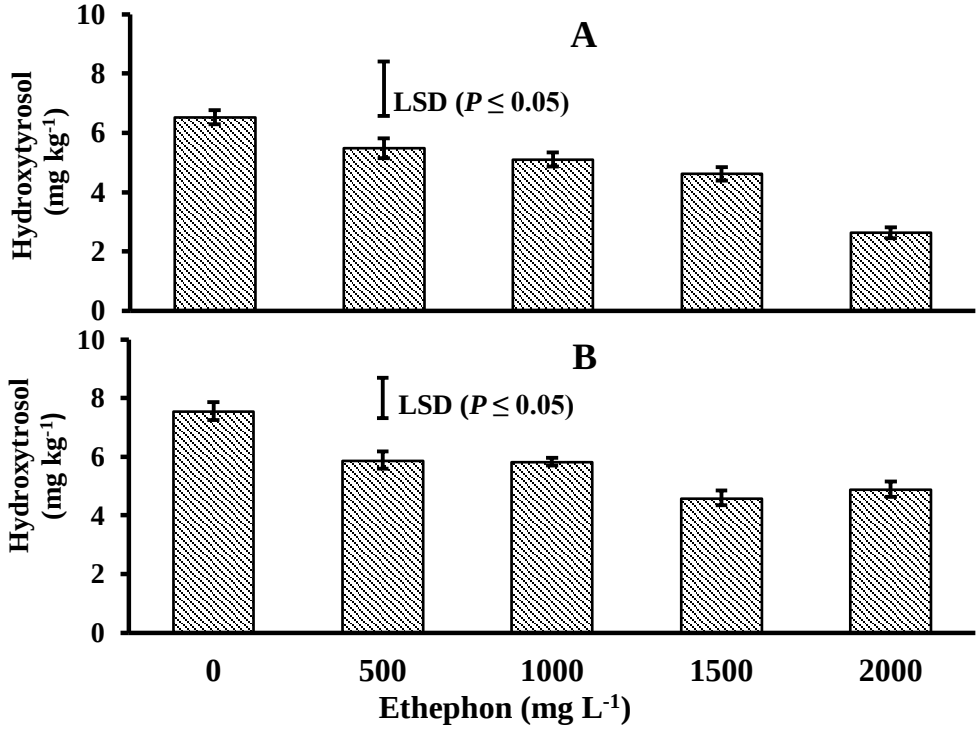


Fig.6.32. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on hydroxytyrosol in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2014. Vertical bars represent SE.

6.3.9.3. Oleuropein aglycon (3, 4 DHPEA-EA)

The spray treatments of ethephon significantly reduced concentrations of 3, 4 DHPEA-EA (mg Kg^{-1}) in the virgin olive oil as compared to the control in both cvs. Frantoio and Manzanilla in 2013 and 2014 (Fig. 6.33 and 6.34). The spray application of ethephon (3000 mgL^{-1}) significantly reduced the level of 3,4 DHPEA-EA in the virgin olive oil as compared to the control and all other treatments in both the cultivars during 2013 (Fig 6.33 A and B). Similarly, in 2014 the application of ethephon has also insignificantly reduced the levels of oleuropein in the oil compared to the untreated fruit of cv.Frantoio and Manzanilla in 2014 (Fig. 6.34 A and B).

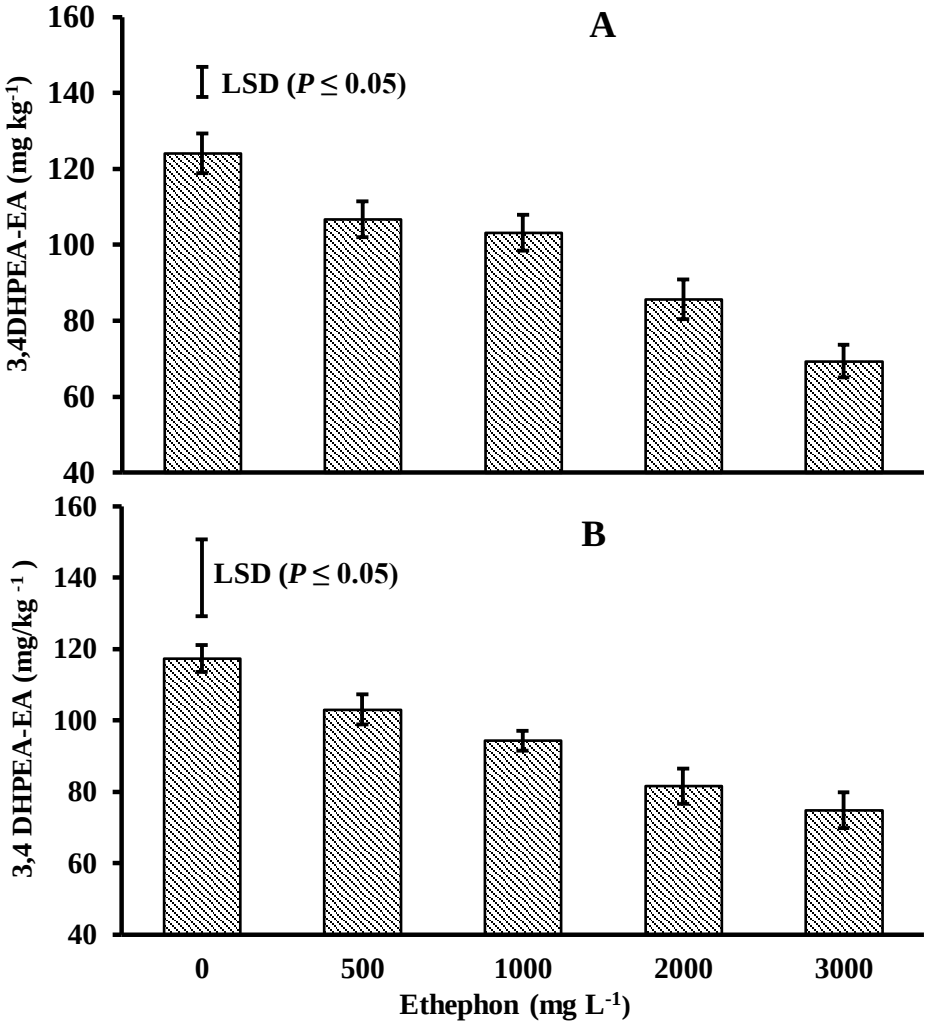


Fig.6.33. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on oleuropein in Frantoio (A) and Manzanilla (B) cvs. olive oil in 2013. Vertical bars represent SE.

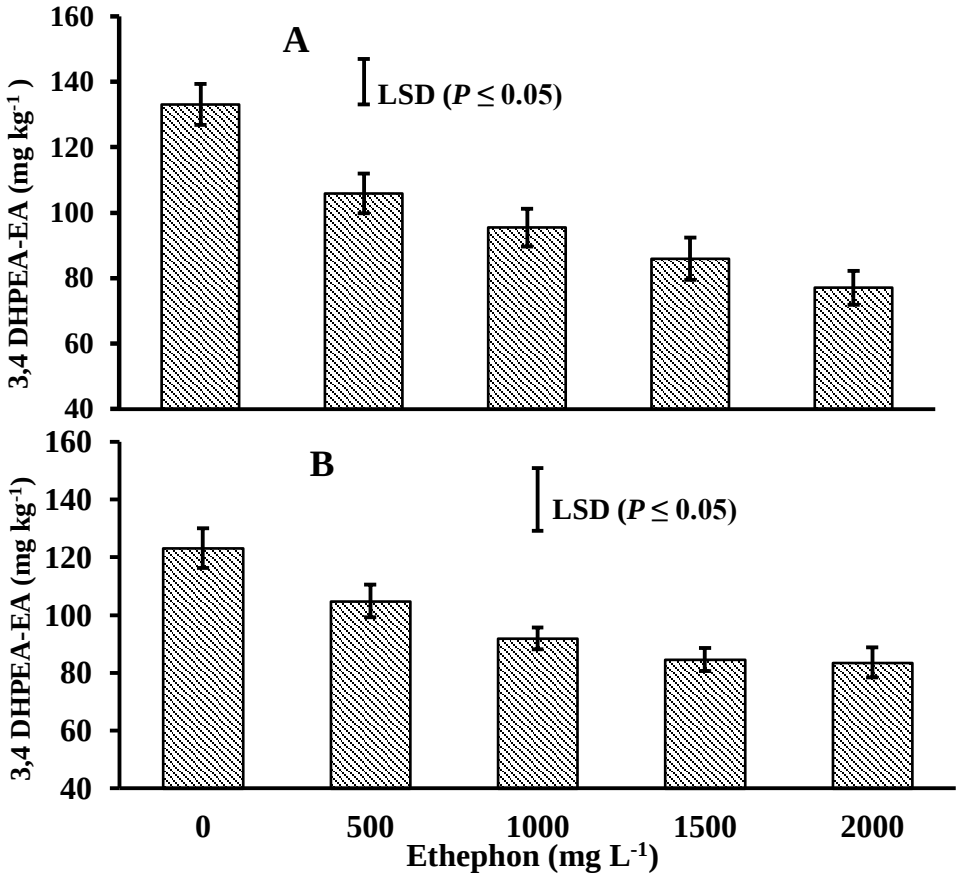


Fig.6.34. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on oleuropein in Frantoio (A) and Manzanilla (B) cvs. olive oil in 2014.Vertical bars represent SE.

6.3.9.4. Total polyphenols

The level of total phenols in the oil also showed a similar trend observed in different polyphenolic compounds such as tyrosol, hydroxytyrosol and 3,4 DHPEA-EA in cvs. Frantoio and Manzanilla olive during 2013 and 2014. Total phenols in the oil decreased significantly with the ethephon application and the lowest levels of total phenol was observed in the 3000 mg L^{-1} ethephon treated olive fruit (216.06 and 286.89 mg Kg^{-1} in cvs. Frantoio and Manzanilla respectively during 2013) (Fig. 6.35 A and B). Similar observations were recorded during 2014. However, the ethephon treatments show similar trends in both cultivars in 2014 (Fig.6.35 and 6.36).

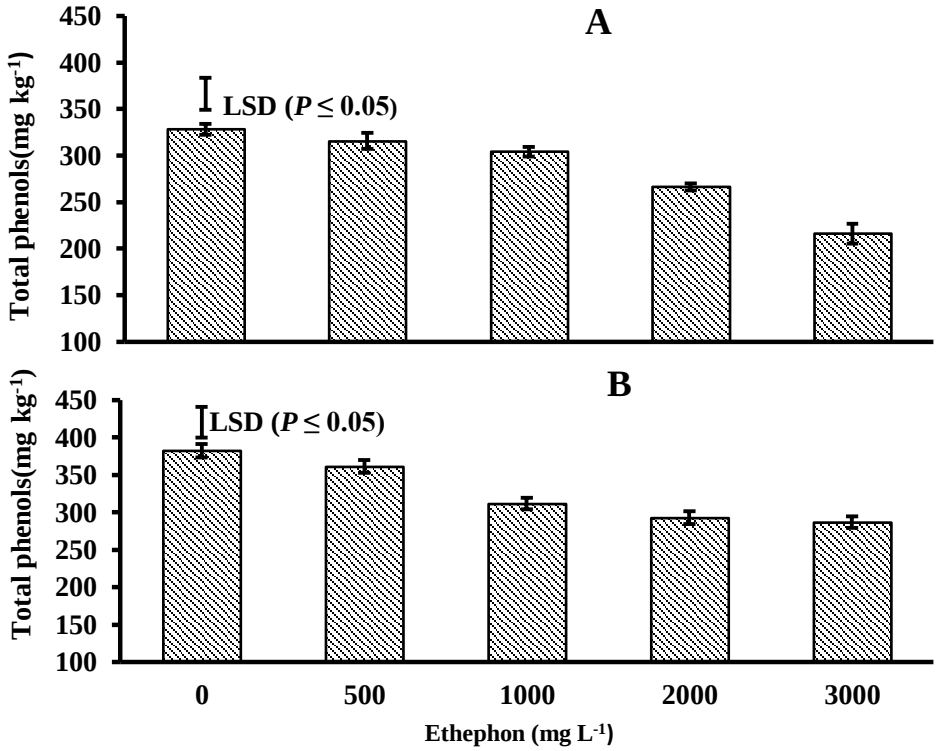


Fig.6.35. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on total polyphenols in Frantoio (A) and Manzanilla (B) cvs. olive oil in 2013. Vertical bars represent SE.

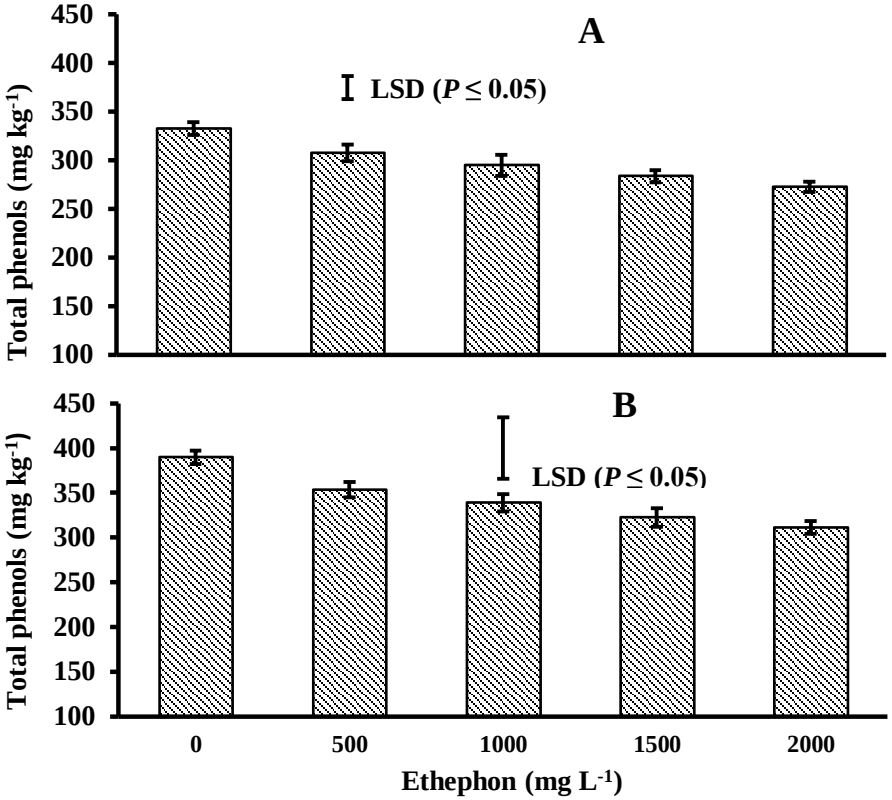


Fig.6.36. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on total polyphenols in Frantoio (A) and Manzanilla (B) cvs. olive oil in 2014. Vertical bars represent SE.

6.3.10. Sensory attributes

The sensory attributes (fruitiness, bitterness and pungency) of the virgin oil significantly decreased with the increased concentration of applied ethephon in both cultivars cvs. Frantoio and Manzanilla during 2014 (Fig. 6.37, 6.38 and 6.39). Higher fruitiness, bitterness and pungency were recorded in control cv. Frantoio (3.14, 3.21 and 3.78) and cv. Manzanilla (3.67, 3.56 and 3.40). Lower values of these attributes were observed when higher concentrations of ethephon were applied. However, the ethephon treatments from 500 to 1500 mg L^{-1} did not show apparent significant differences for fruitiness and bitterness in both cultivars (Fig. 6.37, Fig. 6.38) and for pungency in cv. Manzanilla only (Fig. 6.39).

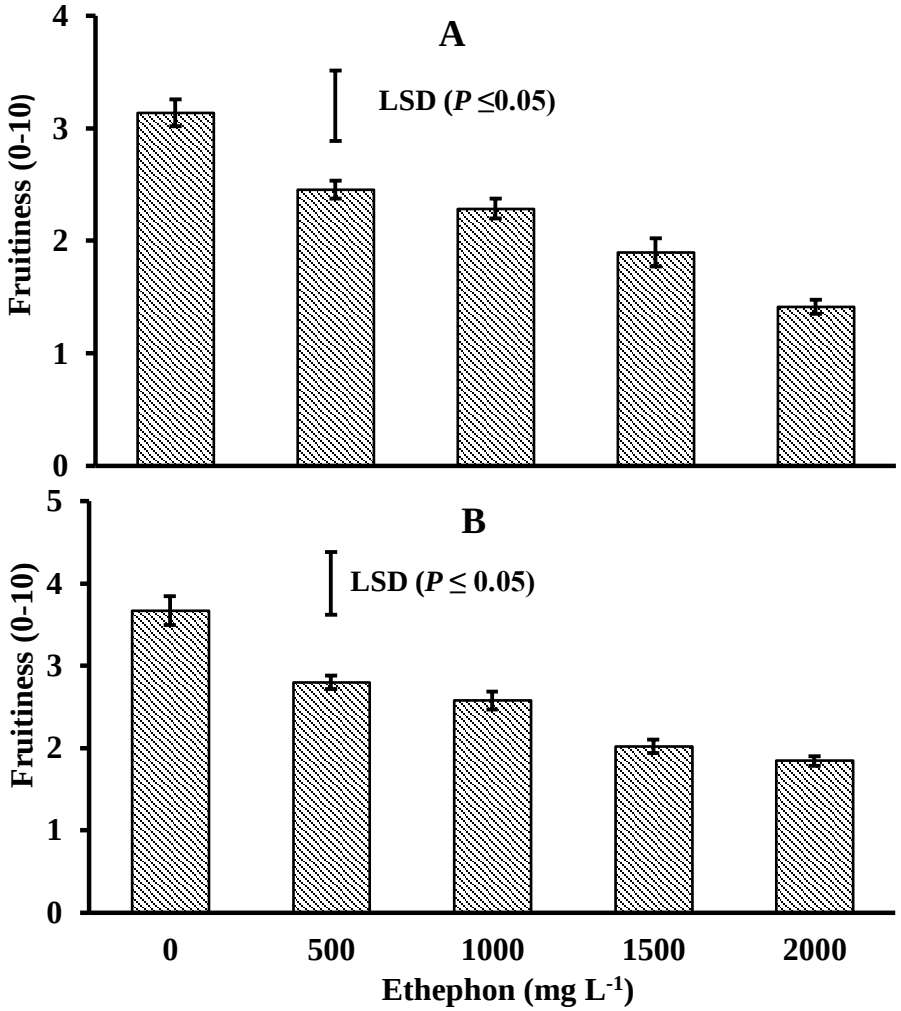


Fig.6.37. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on fruitiness in Frantoio (A) and Manzanilla (B) cvs. olive oil in 2014. Vertical bars represent SE.

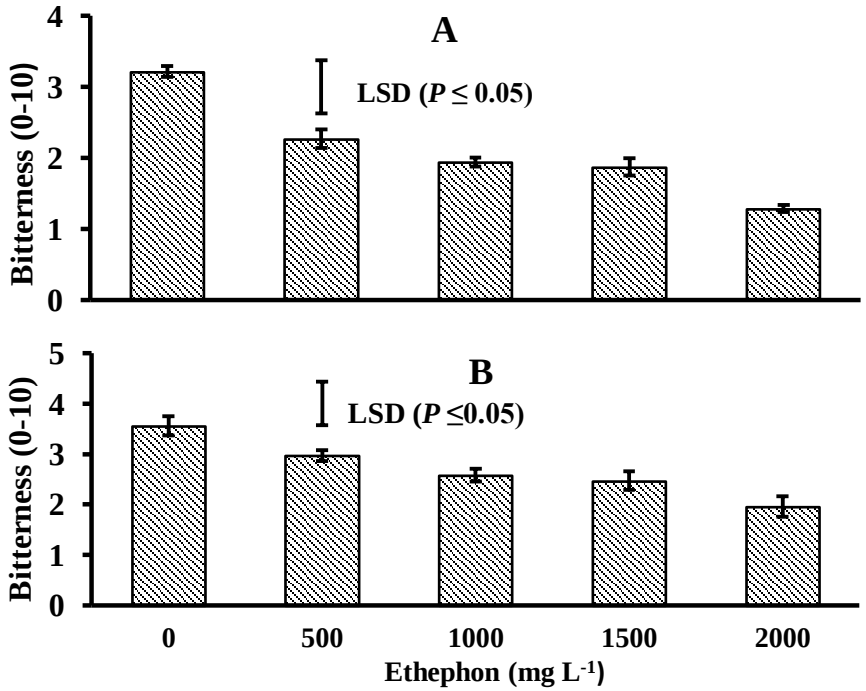


Fig.6.38. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on bitterness in Frantoio (A) and Manzanilla (B) cvs. olive oil in 2014. Vertical bars represent SE.

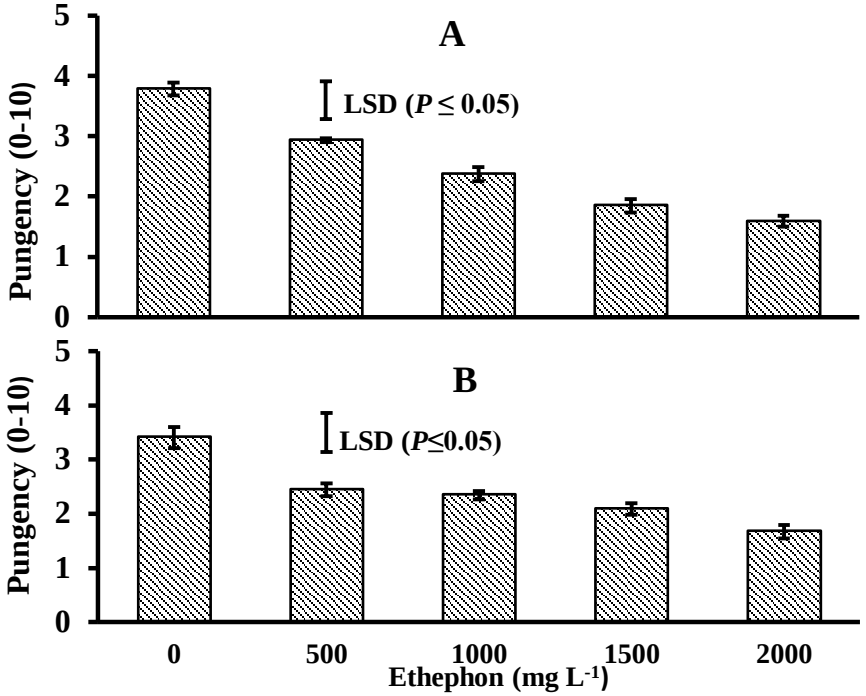


Fig.6.39. Effects of different concentrations of spray application of ethephon (mg L^{-1}) on pungency in Frantoio (A) and Manzanilla (B) cvs. virgin olive oil in 2014. Vertical bars represent SE.

6.4. Discussion

Mechanical harvesting of olive removes only 60% of the fruit and the remaining fruit can lead to alternate bearing (Martin et al., 1981). Different ethylene-releasing compounds, including ethephon have been tested as fruit thinning chemicals but injudicious use of ethephon can cause excessive leaf loss which allows the entry of olive knot bacteria (*Pseudomonas syringae* pv. savastanoi) and reduces the crop yield in the following year (Martin, 1986). Ethephon is the most widely used compound to induce fruit abscission (Wilkinson, 1972; Ben-Tal and Lavee, 1976a ; Daniel and Martin et al., 1981) and thereby, it is better in further attention to ascertain the effective concentration of it to be applied on olive trees for better maintenance of the physicochemical properties of the fruit and extracted oil from them. It is also necessary to maintain a balance between the fruit and leaf abscission to avoid possible hindrances for fruiting. Considering these views the current study was conducted to find out the suitable concentration of ethephon which will facilitate mechanical harvesting for cvs. Frantoio and Manzanilla in south-western Australian conditions in two consecutive years. Observations were recorded on the effects of different concentrations of ethephon on ethylene production, ripening index, fruit removal force, fruit and leaf abscission, oil content (%), concentration of fatty acids and phenolic compounds and sensory attributes of olive oil.

6.4.1. Ethylene production

As expected, the ethephon treatments significantly increased the production of ethylene in the fruit of cv. Frantoio and Manzanilla. Higher concentration of ethylene was recorded from the fruit treated with higher concentration of ethephon (Fig.6.1 A and B). Ethephon is an ethylene-releasing chemical (Martin et al., 1981) which might have induced ethylene production in the fruit of treated plants (Banno et al., 1993). Ben-Tal (1992) also reported that, a small portion of applied ethephon penetrates the pedicels and releases ethylene responsible for increased ethylene in the treated fruit.

6.4.2. Ripening index

The ripening index increased significantly with the higher concentrations (1000 – 3000 mg L^{-1}) of ethephon applied than the lower concentration (500 mg L^{-1}) and control in both cvs. Frantoio and Manzanilla in 2013 and 2014 (Fig.6.2 A, B and

Fig.6.3 A, B). Higher concentration of ethephon spray increased the ethylene production in the treated olive fruit and the increased ethylene may have influenced the ripening index of the olive fruit. Ethylene is a fruit ripening hormone (Chaves and De Mello-Farias 2006; Nath et al. 2006 and Tharanathan et al., 2006) which worked similarly to induce the ripening in olive.

6.4.3. Fruit removal force (FRF)

The fruit removal force reduced significantly with the increase in concentration of the applied ethephon in both cvs. Frantoio and Manzanilla in 2013 and 2014 (Fig.6.4 A, B and Fig.6.5 A, B). Ethephon penetrates the pedicels and releases ethylene to reduce the FRF (Ben-Tal, 1992). Higher concentrations of ethephon were assumed to be required to penetrate the thick waxy layer on the olive leaves or fruit as well as the overlapping peltate trichomes on the leaves (Weis et al., 1988). Ethylene is known to induce abscission of plant organs (Abeles et al., 1971).

6.4.4. Fruit and leaf abscission

Fruit and leaf abscission (%) increased significantly ($P \leq 0.05$) according to the increased concentration of applied ethephon in both cvs. Frantoio and Manzanilla in 2013 and 2014 (Fig. 6.8 and Fig.6.9). The effect of abscission agents has been reported to reduce fruit-detachment force (FDF) and increase harvest efficiency (Barranco et al., 2004; Ferguson et al., 2010). Abscission of leaf and fruit is directly related to the level of endogenous ethylene in leaf and fruit. Ethylene evolution seemed to parallel the level of applied ethephon, an observation also reported by Banno et al. (1993). They also found extensive leaf and fruit abscission due to the effect of applied ethephon. Ethephon induces fruit abscission through accumulation in the pedicel-fruit basin and leaf surface which ultimately penetrates into the plant system to enhance the ethylene production (Reed and Hartmann, 1976; Polito and Lavee, 1980 and Weis et al., 1988, 199l). Ethephon treatments have also been reported to have a positive effect on mechanical harvesting efficiency of the fruit (Yousefi et al., 2010; Ninot et al., 2012 and Zahra, 2014) and Touss et al. (1995) also claimed that the best suitable concentration of ethephon is 1250 to 1875 mg L^{-1} which is in agreement with the observation from the present study.

6.4.5. Free fatty acids

The trees when treated with higher ethephon concentration produced greater amounts of free fatty acids in the olive oil comparing to the control samples in both years and in both cultivars. The ethephon enhanced the ethylene production from the treated fruit which possibly may have caused the increased free fatty acids in olive oil. Similarly, Yousfi et al. (2009) reported that ethephon application increased the concentrations of fatty acids in the olive oil.

6.4.6. Peroxide value

The peroxide value of extracted oils from the treated olive trees increased with the increase of applied ethephon concentration in both years and in both cultivars. This may indicate a direct or indirect effect by the presence of ethylene. The findings of Yousfi et al. (2009) support the findings of the current study. However, decrease in peroxide value has been reported by Tovar et al. (2001), Salvador et al. (2001) and Baccouri et al. (2008) where they claimed that the activity of lipoxygenase enzyme decreases as the fruit ripening process advances. This finding is in agreement with the finding of Sheng et al. (2003) and Griffiths et al. (1999).

6.4.7. Fatty acids compositions

Fruit of both cultivars collected from the trees treated with higher concentrations of ethephon (2000-3000 mg L^{-1}) showed significantly higher level of fatty acids (%) in olive oil than the control and other concentrations of ethephon treated trees in both years. Ethephon treatment enhances the fruit maturation and ultimately affects the oil quality (Ismail et al., 1999) which has been expressed through increased (palmitic acid, stearic acid, linoleic acid and PUFA in both cultivars in both years; MUFA in cv. Manzanilla in 2013) or decreased (oleic acid in both cultivars in 2013 and in cv. Frantoio in 2013; MUFA/PUFA ratio in both cultivars in both years) levels of fatty acids from the increased concentration of ethephon treatment in the current study. However, some stable or non-significant changes in some of the fatty acids (oleic acid in cv. Manzanilla in 2014; MUFA in Frantoio in 2013 and in Manzanilla cvs. in 2014) were also observed with the ethephon treatments. This might be due to similar climatic conditions for the treated olive trees and this observation is in agreement with Faila et al. (2002) and Ranali et al. (1999) findings.

6.4.8. Polyphenolic compounds

The concentration of polyphenols (tyrosol, hydroxytyrosol and 3, 4 DHPEA-EA) decreased in both cultivars and both years with the increased concentrations of of ethephon applied (Fig. 28 to 35). Phenolic composition of olive oil is affected by genotype, agronomic, environmental and technological factors (Montedoro and Garofolo, 1984; Lavee and Wodner, 1991). Decrease of the major phenolic compounds with the progress of olive fruit maturity is well reported by other investigators (Skevin et al., 2003; Rotondi et al., 2004; Yousfi et al. 2006; Baccouri et al., 2007; and Riachy et al., 2012). According to Amiot et al. (1989), this decrease is correlated with the increased activity of hydrolytic enzymes during ripening. Exposure to ethylene results in higher PPO (polyphenol oxidase activity) (Couture et al. 1993; Peng and Yamauchi 1993) which readily oxidises the soluble phenolic compounds (Ke and Saltveith 1988). The effects of ethephon in the current study are similar to ethylene effects on PPO activity. The concentration of individual and total phenols in 2014 was comparatively high when the average rainfall was less than in 2013. Water availability has a large effect on the phenolic profile of virgin olive oil (VOO) (Gómez-Rico et al., 2007; Servili et al., 2007; Ripa et al., 2008 and Tura et al., 2008). Yousfi et al. (2006) noted a higher amount of different phenolic compounds in the oils obtained from the fruit harvested in the low rainfall season than those obtained in the season with double rainfall.

6.4.9. Sensory attributes

The sensory attributes of olive oil (fruitiness, bitterness and pungency) showed significant decrease in both years and cultivars (Frantoio and Manzanilla) with the higher concentrations ethephon applied (Fig. 36, 37 and 38). However, none of any olive oil defects were found in any samples. Phenolic compounds are highly correlated to organoleptic characteristics of olive oil (Andrewes et al., 2003 and Beltrán et al., 2007). The amount of phenolic compounds decreases with the progress of maturity (Yousfi et al. 2006 and Riachy et al., 2012) and is due to the effect of ethylene enhanced by the ethephon treatment (Couture et al., 1993 and Peng and Yamauchi, 1993). These differences are attributed to chemical reactions and enzymatic activities, such as glycosidases, phenol oxidases or phenol polymerases

(Ke and Saltveith, 1988). Yousfi et al. (2009) also reported decreased bitterness while he exposed ripening index (RI) stored olive fruit to 30 mgL^{-1} ethylene.

6.5. Conclusion

Ethephon is one of the ethylene releasing compounds commonly used to improve mechanical harvesting yield of olive fruit. However, injudicious use of ethephon can cause excessive leaf loss. To find out a suitable concentration of ethephon the current study was conducted on Frantoio and Manzanilla olive cultivars in two consecutive years in south-western Australian conditions. From the study it was observed that, application of ethephon significantly influences the physicochemical and biochemical attributes of the olive fruit. The level of ethylene production, ripening index, fruit and leaf abscission and free fatty acid of olive oil increased significantly with the increase of applied ethephon concentrations in comparison to the control treatment. Among different fatty acids, significant increase was observed in most of the cases, however, the level of oleic acid, MUFA and MUFA/PUFA ratio decreased with the increase of ethephon concentration. Concentration of different polyphenols (hydroxytyrosol, tyrosol, oleuropein, and total polyphenol) and level of sensory attributes (fruitiness, bitterness and pungency) of the virgin oil decreased significantly with the increase of ethephon concentrations applied. However, there was no effect of ethephon on the fruit moisture (%) and oil (% fresh and dry weight basis) content of the olive fruit. Among the applied concentrations of ethephon, 1000 to 2000 mg L^{-1} in 2013 and 1000 to 1500 mg L^{-1} in 2014 did not show significant differences for the studied parameters. In conclusion, exogenous spray application ethephon (1000 – 1500 mg L^{-1}) one week before harvest seems to be promising to facilitate mechanical harvesting and maintain quality of virgin olive oil.

Chapter 7

Effect of time of ethephon spray application on the physicochemical, biochemical and organoleptic properties of olive fruit and virgin oil cv. Frantoio and Manzanilla grown in south-western Australia

Abstract

The use of ethephon, ethylene precursor promotes fruit abscission and also results in a considerable loss of leaves if not used judiciously or at the proper stage of fruit growth. There is limited information on the effect of time of application of ethephon on fruit and oil of olives grown in Australia. The current study was conducted to observe the effect of different application period (1, 2, 3, or 4 weeks before harvesting) of single ethephon spray application on physico-chemical, biochemical and organoleptic properties of cvs. Frantoio and Manzanilla olives grown in south-western Australia. Ethephon treatment periods showed significant effects on the studied parameters in comparison to the control fruit which increased RI (4.84), fruit and leaf abscission (95.92% and 27.44% respectively), free fatty acids (0.42%), peroxide value (11.02 meqO_2 kg^{-1}), palmitic acid (13.19%), stearic acid (4.19%), linoleic acid (11.12%) and PUFA (11.60%) were observed when the olive trees were sprayed with ethephon at four weeks before harvesting compared to control. Significantly reduced phenolic compounds (3.91, 6.05 and 59.54 mg Kg^{-1} hydroxytyrosol, tyrosol and oleuropein respectively) and sensory attributes (1.74, 1.51 and 1.72 scores for fruitiness, bitterness and pungency respectively) of virgin oil were also noted from this treatment. However, the ethephon application periods did not differ significantly among themselves in respect of their effects on the parameters, it could be concluded that the suitable period of single ethephon spray to olive trees is at least two weeks before harvesting the fruit.

7.1. Introduction

Harvesting of olive (*Olea europaea* L.) fruit make up the major portion (50–80 %) of its cultivation cost (Metzidakis, 1999). For ensuring better return from olive cultivation, use of an abscission agent like ethephon gained enormous attention of the growers (Burns et al., 2005). Ethephon promotes fruit abscission, minimizes fruit

damage and enables easy picking or mechanical fruit harvesting by lowering the level of mechanical forces (FRF) necessary during harvest (Edgerton, 1968; Bukovac et al., 1969; Hartmann et al., 1970; Young and Jahn, 1972; and Kadman and Ben-Tal, 1983). Ethephon and other abscission agents are used especially with high cropping levels, or to harvest greener fruit earlier in the season, or to lower the FRF on certain varieties difficult to harvest (e.g. Frantoio, Koroneiki and Arbequina). However, ethephon application results in a considerable loss of leaves coincident with fruit loosening (Burns et al., 2008). Alteration in ethephon application timing and duration were tested by Lang and Martin (1985, 1989) with promising results to minimize unwanted defoliation.

Growth stage of the fruit and ambient conditions including temperature and relative humidity affect the extent of ethylene penetration into the fruit cells and rate of ethylene evolution from the decomposition of ethephon (Olien and Bukovac, 1978, 1982; Flore and Bukovac, 1982; Beaudry and Kays, 1987; and Kays and Beaudry, 1987). El-Tamzini et al. (1980) reported the use of ethrel (1250 mg L^{-1}) as an effective agent to reduce the FRF (maximum of 73%) while olive trees were treated two weeks before harvesting. However, Touss et al. (1995) reported non-significant effect of ethephon treatments on mechanical harvesting of olive while they treated Arbequina cv. olive trees at 12 days before harvest with different concentrations of ethephon. There are limited published reports on the effect of ethephon application period on the fruit and oil quality of olives grown in Australia. Therefore, determination of suitable period of ethephon treatment bears importance to investigate the effect of application period on the quality of harvested product under south-western Australian conditions. It is also important to observe the effect on maintaining the balance between fruit and leaf loss for avoiding potential risks with future fruiting in olive. The present study was conducted with the goal of finding out a suitable period of treating the Frantoio and Manzanilla olive cultivars grown in south-western Australian conditions and the ethephon application periods were sequenced as four, three, two and one week before harvesting of the olive fruit. The effect of the ethephon application period was observed on ripening index (RI), fruit removal force (FRF), fruit and leaf abscission (%), oil content (% dry weight), content of free and individual fatty acids, phenolic compounds and changes in sensory attributes.

7.2. Materials and methods:

7.2.1. Plant material, experimental location and climatic conditions

Frantoio and Manzanilla cvs. olive fruit grown in the olive field at Talbot Grove, York (31°52′44″ S, 116°45′57″ E), located at 120 km East of Perth, WA, were used as experimental material. Details on the quality of plant material, their maintenance, experimental location and climatic conditions have been presented in Chapter 3, Section 3.1. Disease free, matured and uniform sized fruit were collected from the control and ethephon treated olive trees.

7.2.2. Design of experiment and treatments

The experiment was designed as two factor (ethephon spray period X cultivar) factorial Randomized Complete Block Design (RCBD) with 4 replications of the experimental unit (a single olive tree). Different spray periods (1, 2, 3 and 4 weeks before harvesting) of ethephon (1500 mg L^{-1}) [2-chloroethylphosphonic acid (Rhone-Poulenc Rural Australia Pty Ltd, Baulkham Hills, NSW, Australia)] and 0.05% 'Tween 20' as a surfactant was sprayed by using a sprayer (The Selecta Trolleypak Mk II, Acacia Ridge, Australia) were considered as the treatments. Untreated olive trees were considered as control.

7.2.3. Collection of olives and extraction of olive oil

Olive fruit (composite samples of 1.5 to 2 Kg per replicate) were harvested from four representative trees included in four replications. The fruit were picked by hand and virgin olive oil was extracted from the collected fruit by following the method explained by Rivas et al. (2013) with some modifications. The details of extraction of oil have been described in details in Chapter 3, Section 3.4

7.2.4. Determination of ripening index

Ripening index (RI) of olive fruit sample was determined according to the method described by Uceda and Frias (1975). One hundred randomly selected healthy fruit per replicate were cut in half to expose the internal flesh for grading in eight groups and for calculation of the ripening index value as described in Chapter 3, Section 3.5.6.

7.2.5. Determination of fruit removal force (FRF)

The fruit removal force was determined by using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK) as described in Chapter 3, Section 3.5.5. The analyser was equipped with horizontal square base table (15 cm × 15 cm) and interfaced to a personal computer with Nexygen® software. Twenty randomly selected fruit per replication were used for determining fruit removal force.

7.2.6. Fruit and leaf abscission

Abscission of leaf and fruit was determined from three selected branches of each replicate. The numbers of leaves and fruit before applying the treatments and after harvesting the fruit were used to calculate the percentage of abscission as described in Chapter 3, Section 3.5.9.

7.2.7. Olive oil content (% dry weight)

Ten grams of olive fruit paste was dried in an oven at 80°C for 24 h and the dry weight of each replicate was recorded and oil percentage was determined by following the method described by Avidan et al. (1999) and detailed in Chapter 3, Section 3.5.8.

7.2.8. Determination of free fatty acids

A mixture of 10 g of the olive oil sample and 50 ml of the solvent (1:1 of 95% (V/V) ethanol (C_2H_6O) and diethyl ether) was titrated with a solution of potassium hydroxide (KOH_4) to the end point of the indicator pink colour. Three drops of phenolphthalein were used an indicator. The details of calculating the amount of free fatty acids have been presented in Chapter 3, Section 3.5.11.

7.2.9. Peroxide value

Sample olive oil (1-2 g) was dissolved with 10 ml of chloroform ($CHCl_3$), 15 ml of acetic acid ($C_2H_4O_2$) and 1 ml of potassium iodide (KI) solution. The mixture was titrated with the sodium thiosulphate solution (0.002 N). During titration the flask was under continuous shaking and starch solution was used as an indicator (10 g/l aqueous dispersion) from a purplish to yellowish or colourless endpoint. The detailed

procedure for determining peroxide value (meq O_2 kg^{-1}) has been described in Chapter 3, Section 3.5.12.

7.2.10. Determination of fatty acids composition

Composition of fatty acids in olive oil was determined by a gas chromatograph following the method prescribed by the International Olive Council (2001) as described in Chapter 3 Section 3.5.13. Only 0.1 g of the oil sample was added to 2 mL of heptane and homogenized followed by addition of 0.2 mL of 2 N methanolic potassium hydroxide solution. The gas chromatograph was fitted with a fused silica column (50m length × 0.25mm i.d.) coated with SGL-1000 phase (0.25µm thickness sugar labour, Spain) and containing a FID detector (HP 6890, Agilent Technologies). Temperature of the injector and detector was maintained at 250° C and the oven temperature was maintained at 210° C.

7.2.11. Total polyphenols

The total phenols from the oil were quantified by following the method of Ranalli et al. (1999) using a spectrophotometer (Model SECOMAM ANTHELIE Advanced, France) and calculated according to method described by Mateos et al. (2001). The detailed method has been described in Chapter 3, Section 3.5.13.

7.2.12. Determination of polyphenolic compounds

Composition of polyphenolic compounds was determined by mixing 5 ml of methanol/water (80/20, v/v) with 5 g of virgin olive oil and analysing the mixture by HPLC-DAD. The phenolic compounds were quantified at 235-280 nm using syringic acid as internal standard. Phenolic standards (3,4 DHPEA-EA, Tyrosol and hydroxytyrosol) of 0.015 mg/ml strength were prepared and used to determine the level of polyphenols as described in Chapter 3, Section 3.5.14. COI/T.20DocNo 29 (2009).

7.2.13. Determining the sensory attributes

A tasting panel of seven trained tasters worked to determine sensory attributes from olive oil samples according to the standard procedure (EC Reg. 796/2002) which has also been described in Chapter 3, Section 3.5.10. The tests were scheduled in two different days with ½ hour breaks between two tests. The test panel was supplied

with scaled sheets for the sensory attributes such as fruitiness, bitterness and pungency. Each attribute was scaled from 0 to 10 where 1 represented the value for the poorest and 10 the best possible quality for the sample (Fig.3.9).

7.2.14. Statistical analysis of data

The experimental data were analysed employing two ways analysis of variance (ANOVA) using Genstat 14 (release 14.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK). Within the analysis of variance, the effects of time of application of ethephon treatments, cultivars and their interactions were assessed. Least significant differences (Fisher's LSD) were calculated following significant ($P \leq 0.05$) F test. To ensure the validity of statistical analysis all the assumptions of analysis were examined.

7.3. Results:

7.3.1. Ripening index (RI)

The ripening index increased significantly ($P \leq 0.05$) when the olive trees of both cultivars were sprayed with 1500 mg L^{-1} ethephon from one to four weeks before fruit harvest. Highest average RI value (4.85) was observed while the trees were sprayed four weeks prior to the harvest and the lowest average RI (3.35) was recorded in control fruit. The cultivars also showed significant differences for RI with an average value of 4.65 and 3.95 for cv. Frantoio and Manzanilla respectively. There was a non-significant ($P \leq 0.05$) interaction between time of application and cultivars for fruit ripening index (Table.7.1).

7.3.2. Fruit removal force (FRF)

The mean fruit removal force (FRF) in olive fruit significantly ($P \leq 0.05$) reduced with the spray application of ethephon (1500 mg L-1) one- to four-week prior to harvest, irrespective of the cultivar (Table 7.1). The highest average FRF was observed in the control (4.88N) and the lowest average FRF (1.58N) was noted when the trees were sprayed four weeks before harvest. Manzanilla olive fruit showed significantly higher FRF than Frantoio cultivar and the mean FRF values were 2.86N and 2.22 N respectively. The interaction between time of application of ethephon and cultivars for fruit removal force was non-significant (Table. 7.1).

7.3.3. Fruit and leaf abscission

The fruit and leaf abscission increased significantly in both cultivars with the advancement of ethephon application from one- to four-weeks before harvest. The average fruit abscission increased from 69.39% in control to 95.92% on the trees sprayed four weeks prior to harvest and the average leaf abscission increased from 4.33% in control to 27.44% on the trees sprayed four weeks before harvest. The rate of fruit and leaf abscission in cv. Frantoio (91.46% and 20.11% respectively) was higher than cv. Manzanilla (84.53% and 17.18% respectively). The interaction between the treatments of time of application of ethephon and cultivars for fruit and leaf abscission was non-significant (Table. 7.1).

7.3.4. Oil content (% dry weight)

The ethephon treatments and the interaction between the treatments and cultivars did not differ significantly for oil content in both cultivars as percentage of dry weight of olive fruit. Higher concentration of oil was observed in cv. Frantoio (37.66%) which is significantly different than the content of cv. Manzanilla (36.62%) (Table.7.2).

7.3.5. Free fatty acids

The virgin olive oil extracted from olives of the trees sprayed with ethephon (1500 mg L^{-1}) two weeks prior to harvest showed significantly higher levels of free fatty acids compared with the control and other treatments in both cultivars (0.36% in Frantoio and 0.52% in Manzanilla cvs.). Irrespective of timing of spraying, the cv. Manzanilla olive oil showed higher average free fatty acid (0.40%) than the cv. Frantoio (0.32%). There was a non-significant ($P \leq 0.05$) interaction between time of application and cultivars for levels of free fatty acids in the oil (Table. 7.2).

7.3.6. Peroxide value

The peroxide value (meq O_2 kg^{-1}) of virgin olive oil significantly increased from one- to four-weeks before spraying the trees with ethephon (1500 mg L^{-1}) then the control fruit irrespective of the cultivar. Higher peroxide values in the oil were observed in both cultivars when ethephon spray was applied four weeks before harvest (10.70 and 11.34 meq O_2 kg^{-1} respectively). The cultivars and the interaction between time of application and cultivars for peroxide value did not show significant

differences. However, peroxide value of cv. Manzanilla (10.16 meq O_2 kg^{-1}) was higher than the cv. Frantoio (9.18 meq O_2 kg^{-1}) (Table. 7.2).

7.3.7. Fatty acids compositions:

7.3.7.1. Palmitic acid (C 16:0)

The average concentration of palmitic acid (C:16) (%) of virgin olive oil significantly ($P \leq 0.05$) increased in both cultivars with ethephon (1500 mg L^{-1}) spray from one- to four-weeks prior to harvest (from 12.78% to 13.19%). The interaction between cultivars and time of application of ethephon for palmitic acid (%) were found to be non-significant. However, palmitic acid in cv. Manzanilla (12.50%) was higher than the cv. Frantoio (12.41%) (Table.7.3).

7.3.7.2. Stearic acid (C18:0)

Virgin olive oil extracted from Frantoio and Manzanilla cv. fruit treated with ethephon (1500 mg L^{-1}) four weeks before harvesting showed significantly higher concentration of stearic acid (4.28% and 4.10% respectively) than the fruit collected from the control (Table.7.3). Irrespective of the time of spraying, higher average stearic acid (%) was observed in Frantoio (3.80%) than cv. Manzanilla (3.03%). There was a non-significant ($P \leq 0.05$) interaction between time of spraying ethephon and cultivars for stearic acid (%).

7.3.7.3. Oleic acid (C 18:1)

The concentration of oleic acid of virgin olive oil samples significantly ($P \leq 0.05$) decreased with ethephon treatment (1500 mg L^{-1}) when applied one- to four-weeks prior to harvest than the control fruit in both cultivars (Table 7.3). The value of oleic acid decreased from 72.65% in control cv. Frantoio to 66.64% in four-week prior ethephon sprayed Frantoio and from 75.68% in control cv. Manzanillato 72.66% in four-week prior ethephon sprayed cv. Manzanilla Irrespective of treatments, higher average concentration of oleic acid (1.08-fold) was observed in Manzanilla than Frantoio. There was a non-significant ($P \leq 0.05$) interaction between ethephon treatments and cultivars for oleic acid (C 18:1) (%) (Table 7.3).

7.3.7.4. Linoleic acid (C 18:2)

The average concentration of linoleic acid (C 18:2) (%) of virgin olive oil in cv. Frantoio and Manzanilla increased significantly ($P \leq 0.05$) from the control (8.78 %) to ethephon (1500 mg L^{-1}) treated fruit in four weeks before harvest (11.12%) (Table. 7.3). Irrespective of timing of ethephon spray, the cv. Frantoio olive oil showed higher average linoleic acid (%) (10.61%) than cv. Manzanilla (10.45%). There was a non-significant ($P \leq 0.05$) interaction between time of ethephon application treatments and cultivars for linoleic acid (%).

7.3.7.5. Monounsaturated fatty acids (MUFA)

Virgin olive oil extracted from Frantoio and Manzanilla cv. treated with ethephon (1500 mg L^{-1}), irrespective of time of treatment, showed significantly lower levels of MUFA (%) than the fruit collected from control trees (Table. 7.3). Higher average of MUFA (%) was observed in cv. Manzanilla (77.10%) which was significantly ($P \leq 0.05$) higher than cv. Frantoio (72.20 %). There was a non-significant ($P \leq 0.05$) interaction between the time of ethephon spray treatments and cultivars for MUFA (%) of virgin olive oil.

7.3.7.6. Polyunsaturated fatty acids (PUFA)

The treatment, cultivars and their interactions did not show significant differences for PUFA (%) of virgin olive oil. However, higher average PUFA (%) was observed in the olive oil extracted from the fruit treated with ethephon (1500 mg L^{-1}) four weeks before harvesting (11.60%) than the control fruit (9.41) irrespective of the cultivars. The average of PUFA (%) in cv. Frantoio was higher than in cv. Manzanilla (1.03-fold) (Table. 7.3).

7.3.7.7. MUFA/PUFA ratio

The average of the MUFA/PUFA ratio of virgin olive oil in both cultivars was significantly ($P \leq 0.05$) reduced from the control to the ethephon treated (1500 mg L^{-1}) fruit four weeks prior to harvest (8.36 to 6.47 %). The interactions between the ethephon treatments and cultivars were found to be non-significant MUFA/PUFA ratio of virgin olive oil. However, irrespective of timing of treatments, average of MUFA/PUFA ratio in cv. Manzanilla (7.26 %) was slightly higher than cv. Frantoio (6.61 %) (Table 7.3).

7.3.8. Polyphenolic compounds:

7.3.8.1. Hydroxytyrosol

When averaged over cultivars, the mean level of hydroxytyrosol (mg kg^{-1}) of virgin olive oil decreased significantly ($P \leq 0.05$) with ethephon (1500 mg L^{-1}) application four weeks prior to harvest (3.91 mg kg-1) than control (7.05 mg L^{-1}). Manzanilla cv. showed significantly higher concentration of hydroxytyrosol (5.21 mg kg^{-1}) than the Frantoio (4.49 mg kg^{-1}). There was a non-significant ($P \leq 0.05$) interaction between the treatments of time of application of ethephon and cultivars for hydroxytyrosol of virgin olive oil (Table 7.4).

7.3.8.2. Tyrosol

When averaged over cultivars, the treatment of ethephon (1500 mg L^{-1}) four weeks prior to harvest significantly reduced the levels of tyrosol (6.05 mg kg^{-1}) of virgin olive oil than control fruit (9.37 mg kg^{-1}). Manzanilla olives also showed significantly higher levels of tyrosol (8.42 mg kg^{-1}) than cv. Frantoio (5.70 mg kg^{-1}). There was no significant ($P \leq 0.05$) interaction between time of ethephon treatment and cultivars for tyrosol content of virgin olive oil (Table 7.4).

7.3.8.3. Oleuropein aglycon (3,4 DHPEA-EA)

The mean level of 3,4 DHPEA-EA of virgin olive oil decreased (59.54 mg kg^{-1}) significantly in ethephon (1500 mg L^{-1}) treated olives four weeks prior to harvest than the control olives (124.40 mg kg^{-1}). Irrespective of timing of ethephon treatments, the average of oleuropein in cv. Manzanilla was slightly higher (85.53 mg kg^{-1}) than Frantoio (81.25 mg kg^{-1}). There was a non-significant ($P \leq 0.05$) interaction between time of ethephon treatments and cultivars for 3,4 DHPEA-EA (Table 7.4).

7.3.8.4. Total polyphenols

When averaged over cultivars, the spray treatment of ethephon (1500 mg L^{-1}) four weeks prior to harvest has significantly reduced mean levels of total phenols in the virgin olive oil (264.00 mg kg^{-1}) compared with control (400.05 to 264.00 mg kg^{-1}). When averaged over treatments, mean levels of total phenols in the virgin olive oil were higher in cv. Manzanilla (333.42 mg kg-1) than cv. Frantoio olives (277.41 mg

Kg^{-1}). The interaction between time of application of ethephon treatments and cultivars was found to non-significant ($P \leq 0.05$) for levels of total phenols in the virgin olive oil (Table 7.4).

7.3.9. Sensory attributes

The sensory attributes of virgin olive oil including fruitiness, bitterness and pungency showed significant ($P \leq 0.05$) decrease in both olive cultivars with the application of ethephon (1500 mg L^{-1}) from one- to four-week prior to the harvest (Table 7.5). Higher fruitiness, bitterness and pungency were recorded in control Frantoio (3.14, 3.21 and 3.78 respectively) and Manzanilla (3.67, 3.56 and 3.41) cultivar. The lower values of fruitiness, bitterness and pungency were observed in the fruit sprayed four weeks before harvest (Frantoio- 1.35, 1.17and 1.64 and Manzanilla- 2.13, 1.85 and 1.81 respectively). When averaged over treatments, the mean values of sensory attributes of virgin olive oil including fruitiness, bitterness and pungency were higher in cv. Manzanilla (2.76, 2.42 and 2.35) than cv. Frantoio (1.97, 1.80 and 2.27) (Table 7.5).

Table. 7.1. Effects of pre-harvest times of ethephon (1500 mg L^{-1}) spray treatment on ripening index (RI), fruit removal force, fruit and leaf abscission in cvs. Frantoio and Manzanilla olives grown in south-western Australia.

Parameters	Cultivars (cv)	Ethephon spray (week before harvest) (T)					Mean (cv)	LSD ($P < 0.05$)
		Control	1	2	3	4		
RI (0-7)	Frantoio	3.508	4.648	4.773	5.033	5.275	4.647b	T = 0.62
	Manzanilla	3.195	3.90	4.223	4.038	4.415	3.954a	CV = 0.39
	Mean (T)	3.351a	4.274b	4.498b	4.535b	4.845b		T x CV = NS
Fruit removal force (N)	Frantoio	4.56	2.09	1.80	1.39	1.26	2.22a	T = 0.67
	Manzanilla	5.21	2.76	2.26	2.19	1.90	2.86b	CV= 0.42
	Mean (T)	4.88a	2.43b	2.03b	1.79b	1.58b		T x CV =NS
Fruit abscission (%)	Frantoio	75.66	92.16	94.64	96.30	98.56	91.46b	T = 10.31
	Manzanilla	63.11	85.52	89.86	90.87	93.28	84.53a	CV = 6.52
	Mean (T)	69.39a	88.84b	92.25b	93.58b	95.92b		T x CV = NS
Leaf abscission (%)	Frantoio	5.36	18.83	20.18	26.16	30.02	20.11	T = 6.51
	Manzanilla	3.31	19.61	17.18	20.96	24.85	17.18	CV = NS
	Mean (T)	4.33a	19.22b	18.68b	23.56bc	27.44c	18.65	T x CV = NS

*Any two mean within a column and within a row followed by different letters are significantly different at $P < 0.05$. n = 4 replications

Table.7.2. Effects of pre-harvest times of ethephon (1500 mg L^{-1}) spray treatment on oil content, free fatty acids and peroxide value in virgin olive oil of cvs. Frantoio and Manzanilla grown in south-western Australia.

Parameter	Cultivars (cv)	Ethephon spray (week before harvest) (T)					Mean (cv)	LSD ($P < 0.05$)
		Control	1	2	3	4		
Oil (% dry weight)	Frantoio	37.66	37.50	38.58	37.93	36.65	37.66b	T = NS
	Manzanilla	36.72	36.62	36.62	36.72	36.40	36.62a	CV = 0.815
	Mean	37.19	37.06	37.60	37.33	36.53		T x cv = NS
Free fatty acid (%)	Frantoio	0.23	0.32	0.36	0.30	0.39	0.32a	T = 0.086
	Manzanilla	0.25	0.32	0.52	0.45	0.44	0.40b	CV = 0.055
	Mean	0.24a	0.32ab	0.44c	0.37c	0.42c		T x CV = NS
Peroxide (meqO2 kg^{-1})	Frantoio	6.62	9.62	8.75	10.21	10.70	9.18	T = 1.55
	Manzanilla	8.485	10.68	11.13	9.19	11.34	10.16	CV = NS
	Mean	7.55a	10.15b	9.94b	9.7b	11.02bc		T x CV = NS

*Any two mean within a column and within a row followed by different letters are significantly different at $P \leq 0.05$. n = 4 replications

Table.7.3. Effects of pre-harvest times of ethephon (1500 mg L^{-1}) spray treatment on fatty acid composition of virgin olive oil of cvs. Frantoio and Manzanilla olives grown in south-western Australia

Fatty acids (%)	Cultivars (cv)	Ethephon spray (week before harvest) (T)					Mean (cv)	LSD ($P < 0.05$)
		Control	1	2	3	4		
Palmitic acid (C16:0)	Frantoio	9.87	12.64	12.72	13.32	13.52	12.41	T = 1.261
	Manzanilla	10.99	12.91	13.79	11.96	12.84	12.50	CV = NS
	Mean	10.43a	12.78b	13.26b	12.65b	13.19b		T x CV = NS
Stearic acid (C18:0)	Frantoio	2.14	4.16	4.24	4.16	4.28	3.80b	T = 0.859
	Manzanilla	2.55	2.93	2.35	3.24	4.10	3.03a	CV = 0.543
	Mean	2.35a	3.54b	3.29b	3.70b	4.19c	3.42	T x CV = NS
Oleic acid (C18:1)	Frantoio	72.65	67.85	67.65	67.25	66.64	68.41a	T = 2.373
	Manzanilla	75.68	74.46	74.08	73.45	72.66	74.06b	CV = 1.501
	Mean	74.16b	71.15a	70.86a	70.35a	69.65a		T x CV = NS
Linoleic acid (C18:2)	Frantoio	8.72	10.62	10.77	11.42	11.52	10.61	T = 1.416
	Manzanilla	8.84	10.72	11.36	10.61	10.71	10.45	CV = NS
	Mean	8.78a	10.67b	11.06b	11.01b	11.12b		T x CV = NS
Monounsaturated fatty acid (MUFA)	Frantoio	74.79	72.01	71.88	71.41	70.91	72.20a	T = NS
	Manzanilla	78.23	77.38	76.43	76.69	76.76	77.10b	CV = 1.494
	Mean	76.51	74.70	74.16	74.05	73.84		T x CV = NS
Polyunsaturated fatty acid (PUFA)	Frantoio	9.39	11.19	11.36	11.79	12.08	11.16	T = NS
	Manzanilla	9.42	11.15	11.75	11.15	11.12	10.91	CV = NS
	Mean	9.41	11.17	11.55	11.47	11.60		T x CV = NS
MUFA/ PUFA	Frantoio	8.19	6.46	6.39	6.08	5.92	6.61	T = 10.311
	Manzanilla	8.54	7.11	6.55	7.09	7.02	7.26	CV = NS
	Mean	8.36b	6.78a	6.47a	6.58a	6.47a		T x CV = NS

*Any two mean within a column and within a row followed by different letters are significantly different at $P \leq 0.05$. n = 4 replications

Table. 7.4. Effects of pre-harvest times of ethephon (1500 mg L^{-1}) spray treatment on phenolic compounds of virgin olive oil of cvs. Frantoio and Manzanilla olives grown in south-western Australia.

Polyphenols compounds (mg/kg^{-1})	Cultivars (cv)	Ethephon spray (week before harvest) (T)					Mean (cv)	LSD ($P < 0.05$)
		Control	1	2	3	4		
Hydroxytyrosol	Frantoio	6.535	4.618	4.118	3.818	3.318	4.49a	T = 0.87
	Manzanilla	7.56	4.60	4.50	4.90	4.50	5.21b	CV = 0.55
	Mean	7.05b	4.61a	4.31a	4.38a	3.91a		T x CV = NS
Tyrosol	Frantoio	8.88	5.94	5.23	4.84	4.54	5.70a	T = 1.24
	Manzanilla	10.85	8.10	7.80	7.74	7.54	8.42b	CV = 0.79
	Mean	9.37b	7.04a	6.53a	6.29a	6.05a		T x CV = NS
3,4 DHPEA-EA	Frantoio	133.08	78.43	78.62	67.32	48.82	81.25	T =28.48
	Manzanilla	115.72	82.12	88.21	71.37	70.27	85.53	CV =NS
	Mean	124.40b	80.27a	83.41a	69.34a	59.54a		T x CV= NS
Total phenols	Frantoio	385..31	277.81	267.31	236.56	221.06	277.41a	T =48.43
	Manzanilla	414.79	322.50	313.68	310.63	304.00	333.42b	CV= 30.63
	Mean	400.05b	299.03a	290.52a	273.47a	264.00a		T x CV= NS

*Any two mean within a column and within a row followed by different letters are significantly different at $P \leq 0.05$. n = 4 replications

Table.7.5. Effects of pre-harvest times of ethephon (1500 mg L^{-1}) spray treatment on sensory attributes of virgin olive oil of cvs. Frantoio and Manzanilla olives grown in south-western Australia

Sensory attributes	Cultivars (cv)	Ethephon spray (week before harvest) (T)					Mean (cv)	LSD ($P < 0.05$)
		Control	1	2	3	4		
Fruitiness (0-10)	Frantoio	3.14	2.15	1.70	1.51	1.35	1.97a	T = 0.516
	Manzanilla	3.67	2.92	2.71	2.34	2.13	2.76b	CV = 0.326
	Mean	3.40c	2.53b	2.21ab	1.93a	1.74a		T x CV = NS
Bitterness (0-10)	Frantoio	3.21	1.87	1.47	1.28	1.17	1.80a	T = 0.470
	Manzanilla	3.56	2.47	2.25	1.96	1.85	2.42b	CV = 0.297
	Mean	3.39c	2.17b	1.86b	1.62a	1.51a		T x CV = NS
Pungency (0-10)	Frantoio	3.78	2.12	2.00	1.81	1.64	2.27	T = NS
	Manzanilla	3.41	2.42	2.11	2.00	1.81	2.35	CV = 0.501
	Mean	3.59c	2.27b	2.05ab	1.90ab	1.72a		T x CV = NS

*Any two mean within a column and within a row followed by different letters are significantly different at $P \leq 0.05$. n = 4 replications

7.4. Discussion

Use of abscission agents shows a positive effect on decreasing the FRF hence increasing harvest efficiency when applied at correct rates, times and conditions. This efficiency is reflected through faster harvesting, reduced length of harvest, as well as costs and risks associated with late harvest (Ravetti and McClelland, 2008). As a potential aid in the harvesting of olives, the abscission agents are used especially during high cropping levels, or to harvest greener fruit earlier in the season, or to lower the FRF on certain varieties that prove difficult to harvest (for example Frantoio, Koroneiki and Arbequina cvs.) (Ravetti and McClelland, 2008). Ethephon is a hormonal product and there is always a potential risk of undesirable fruit losses and/or defoliation. Therefore, it is crucial to determine the suitable period of ethephon treatment to ensure better quality harvested product and maintain the balance between fruit and leaf loss for avoiding potential risks with future fruiting in olives. There is limited information available on the effect of ethephon application period on the olives growing in south-western Australian conditions. The current study was conducted with the goal of finding out a suitable period of treating the Frantoio and Manzanilla olive cultivars grown in south-western Australian conditions. The ethephon treatments were sequenced as four, three, two and one week before harvesting of the olive fruit. Control fruit trees were maintained without any treatment of ethephon. The results obtained from this experiment have been discussed here in light of the available relevant information from studies of other researchers.

7.4.1. Physical parameters (RI, FRF, fruit and leaf abscission)

The ripening index and the rate of fruit abscission (%) of both olive cultivars were significantly ($P \leq 0.05$) promoted in comparison to control by pre-harvest ethephon treatment (four weeks before fruit harvest). On the other hand, FRF significantly reduced irrespective of the cultivar from one- to four-week advanced spray of ethephon (1500 mg L^{-1}). However, there were no significant differences among the ethephon treatments for RI and the treatments also did not differ significantly with control for leaf abscission (Table.7.1). The ethylene produced by the ethephon treatment at four weeks prior to harvest was effective on the fruit tissues to increase the RI, fruit abscission (%) and to reduce the FRF in both cvs. Frantoio and

Manzanilla Ethylene induce the abscission of leaves, flowers and fruits (Abeles et al., 1971; Jackson and Osborne, 1972 and Henry et al., 1973). The growth stage and ambient conditions including temperature and relative humidity affect the extent of ethylene penetration into the plant cells and rate of ethylene evolution from the decomposition of ethephon (Olien and Bukovac, 1978, 1982; Flore and Bukovac, 1982; Beaudry and Kays, 1987 and Kays and Beaudry, 1987). El-Tamzini et al. (1980) also reported a maximum of 73% reduced FRF in olives with the application of 1250 mg L^{-1} ethrel two weeks before harvest.

7.4.2. Oil content, free fatty acids and peroxide value

Irrespective of the cultivars, the ethephon treatments and the interaction between the treatments and cultivars did not differ significantly for oil content. On the other hand, the treatments differed significantly from control fruit for free fatty acids and peroxide values. But there was no significant difference among the treatments for these parameters (Table.7.2). The application time of ethephon has no effect on the oil content, free fatty acids and peroxide values of cvs. Frantoio and Manzanilla olives. Similar findings were reported by Ahmed et al. (1981) while they conducted a study with Coratina cv. olive by spraying ethrel at two weeks before harvesting the fruit.

7.4.3. Fatty acid compositions

The average concentration of palmitic acid, stearic acid, linoleic acid and PUFA (%) significantly ($P \leq 0.05$) increased in both cultivars by ethephon (1500 mg L^{-1}) spray than control from one- to four-week prior to harvest. On the other hand, oleic acid, MUFA and MUFA/PUFA ratio showed significant decrease due to the effect of ethephon spray. However, the ethephon application periods did not show significant differences among them for individual fatty acids (Table.7.3). From the study there is indication that the ethephon spray affects the concentration of fatty acids in comparison to the control fruit but there is little or no significant effect of ethephon application time on fatty acid concentrations. Touss et al. (1995) also reported non-significant effect of ethephon treatments while they treated cv. Arbequina olive trees at 12 days before harvest with different concentrations of ethephon. Moderate effect of ethephon on palmitic, linoleic and oleic acids was also reported by Cimato (1988).

Lavee and Haskal (1975) treated cv. Nabali olives with ethephon at 1500 mg L^{-1} and did not observe any effect of ethephon on neither colour nor taste of the oil.

7.4.4. Polyphenolic compounds

The average levels of polyphenols (hydroxytyrosol, tyrosol, and oleuropein) in Frantoio and Manzanilla olive oil ($mgkg^{-1}$) significantly ($P \leq 0.05$) decreased from the control to ethephon (1500 mg L^{-1}) treated (four weeks prior to harvest) olives. Manzanilla showed significantly higher concentration of these phenolic compounds than the Frantoio. However, the ethephon application periods did not show any significant effect on the levels of phenolic compounds (Table 7.4). Significant differences among the cultivars for phenolic compounds were also reported by Mania-Djebali et al. (2012). The phenolic compounds differ mainly according to crop year, maturation phase and cultivation method (Anastasopoulos et al., 2011). There are limited published reports on the effect of time of application of ethephon on phenolic composition of virgin olive oil. The pre-harvest application of ethephon (4-weeks prior to harvest) showed lower concentration of phenolic compounds. This resembles the effect of the evolved ethylene from the applied ethephon that correlates with the increased activity of hydrolytic enzymes during ripening to reduce the concentration of phenolic compounds (Amiot et al., 1989; Yousfi et al. 2006 Baccouri et al., 2007 and Riachy et al., 2012). Early exposure to ethylene results in higher PPO (polyphenol oxidase) activity (Peng and Yamauchi 1993) which readily oxidises the soluble phenolic compounds (Ke and Saltveith 1988) and later application of ethephon does not show significant changes on this declining trend.

7.4.5. Sensory attributes

The sensory attributes (fruitiness, bitterness and pungency) virgin olive oil showed significant ($P \leq 0.05$) decrease in both olive cultivars from one- to four-week pre-harvest spray of ethephon (1500 mg L^{-1}). Higher average values of these attributes were also noted in cv. Manzanilla virgin olive oil than cv. Frantoio (Table.7.5). However, the ethephon application period did show significant differences among them for sensory attributes. There is no published report on the effect of spray period of ethephon on the sensory attributes of virgin olive oil. It has been claimed that, the sensory attributes of virgin olive oil are dependent on chemical composition including concentrations of polyphenols and sterols (Andrewes et al., 2003 and

Beltrán et al., 2007) which decreases with the progress of maturity (Yousfi et al. 2006 and Riachy et al., 2012). Moreover, the ethylene evolved from sprayed ethephon causes the reduction of phenolic compounds through oxidation by enzymatic activities (Couture et al. 1993). The early application of ethephon at four weeks before harvest significantly reduces the concentration of phenolic compounds and consequently the reduced values of sensory attributes are also observed from that treatment. Decreased bitterness of virgin olive oil was also reported by Yousfi et al. (2009) while he exposed of atmosphere stored olive fruit to 30 mgL^{-1} ethylene. However, the effect of ethephon spray period on fruitiness is not clear which arrests further investigation.

7.5. Conclusion

Ethephon is used as an abscission agent in olive cultivation to make its harvesting easier in high cropping levels, or to harvest greener fruit earlier in the season, or to lower the FRF on certain varieties difficult to harvest. There is limited information available on the effect of pre-harvest application times of ethephon on the olives growing in Australian conditions. Therefore, the current study was conducted to find out a suitable period of ethephon treatment on physico-chemical, biochemical and organoleptic properties of olive fruit and oil of Frantoio and Manzanilla cultivars grown in south-western Australian conditions. The spray of ethephon showed significant differences for the studied parameters in comparison to the control fruit. Significantly increased RI (4.84), fruit and leaf abscission (95.92% and 27.44%), free fatty acids (0.42%), peroxide value (11.02 $meqO_2\ kg^{-1}$), palmitic acid (13.19%), stearic acid (4.19%), linoleic acid (11.12%) and PUFA (11.60%) were observed when the olive trees were sprayed with ethephon at four weeks before harvesting. Significantly reduced phenolic compounds and sensory attributes were also noted from this treatment. However, the treatments did not differ significantly among themselves in respect of their effects on the parameters. Therefore, it could be concluded that the suitable period of ethephon spray to olive trees is at least two weeks before harvesting the fruit to produce virgin olive oil acceptable for trade quality.

Chapter 8

General Discussion

Frantoio and Manzanilla are widely cultivated cultivars in the olive growing countries of the world including Australia. Both cultivars are highly productive and produce quality fruit and oil. These cultivars also show agronomic adaptability and have been cultivated in south-western Australia for several decades (Taylor and Burt, 2007 and Olives, WA, 2015;). These cultivars also show variations in ripening due to variations in agro-climatic conditions which ultimately influence the phenolic composition of virgin olive oil (Cinquanta et al., 1997). Other different factors influence the quality of virgin olive oil including agronomic practices (Lercker et al., 1994 and Motilva et al., 2000), application of technologies (Di Giovacchino et al., 2002 and Salvador et al., 2003), storage conditions (Procida and Cichelli, 1999) and virgin olive processing methods (Issaoui et al., 2009). Harvesting time or the ripening status of olive fruit was suggested as one of the important factors as well (Koutsaftakis et al., 1999).

Harvesting of olive fruit at over-ripe or at early stage show negative impact on the quality and quantity of the extracted oil (Anastasopoulos et al., 2011). Moreover, this harvesting operation consumes 50–80 % of the total cost of production (Metzidakis, 1999). To minimise the cost of olive production, mechanical harvesting systems combined with use of an abscission inducing agent have gained popularity (Burns et al., 2005) and ethephon has been used to promote fruit abscission for easy picking or mechanical fruit harvesting of different fruit (Edgerton, 1968; Bukovac et al., 1969; Young and Jahn, 1972 and Kadman and Ben-Tal, 1983) includingolive (Hartmann et al., 1970). Injudicious use of ethephon can cause excessive leaf loss which allows the entry of olive knot bacteria (*Pseudomonas syringae* pv. savastanoi) and reduces the crop yield in the following year (Martin, 1986). There is a scarcity of published reports on the effects of different factors, especially the growth changes in ripening fruit, effect of harvesting time, concentration and application time of ethephon on the quality attributes of cvs. Frantoio and Manzanilla olives in the context of south-western Australia. Therefore, the current study was conducted during 2013 and 2014 to investigate the growth and development of olive fruit, to

determine the effects of five different harvesting times (mid- and late-April, mid- and late-May and mid-June of 2013 and 2014), different concentration and time of application of ethephon on the physical, biochemical and sensory attributes of olive fruit and virgin olive oil in cvs. Frantoio and Manzanilla grown in south-western Australia. The data obtained from the current study is presented and discussed in chapter 4 to chapter 8 along with relevant discussion. An attempt has been made here to place a generalized discussion on the major findings from the conducted experiments of the study.

8.1. Fruit growth and development

Growth and development of olive fruit are influenced by cultivar, climatic conditions and cultural practices (Tombesi et al., 1994). A better understanding of the physical and physiological changes during growth and development of olive fruit is necessary to improve commercial and qualitative characteristics of fruit.

8.1.1. Changes in the physical parameters during fruit growth and development

The fruit weight (g) and volume (cc) increased significantly ($P \leq 0.05$) and exhibited double sigmoid growth curve and were higher in Manzanilla than Frantoio (Fig.4.1). Changes in fruit growth parameters do not depend on the cultivar (Lavee et al., 1982, 1991; Barone et al., 1994; Inglese et al., 1996; Barranco et al., 2000 and Iniesta et al., 2009), however, the fruit fresh weight differs for cultivars and it is genetically determined (Beltrán et al., 2004). Increase of fruit weight in accordance to the fruit growth until maturation has also been reported by different researchers (Lavee and Wodner, 2004; Menz and Vriesekoop, 2010 and Dag et al, 2011). Significant effect of cropping year on the physical measurements (e.g. maturity index, moisture content, oil content and fruit weight) of the fruit in different olive cultivars such as Corregiolla, Mission and Paragon cvs. have been reported by Mailer et al. (2007) in the south western region of New South Wales, Australia, which also supports the observation from the current study.

Fruit length (cm) and width increased significantly in both cultivars with the progress of fruit growth and exhibited double sigmoid growth curve as well as higher values for them was noted in 2013. The cv. Manzanilla fruit showed significantly higher fruit length and width than Frantoio in both years (Fig. 4.2).

Similar observations were noted for pulp weight (g), stone weight (g) and their ratio (Fig.4.3). There was a lesser amount of rainfall in 2014 during the growing period of olive fruit which might have affected the growth parameters of the fruit in that year. This observation supports the observation of Lavee et al. (1990) and Tombesi (1994).

8.1.2. Changes in production of ethylene and rate of respiration

Production of ethylene and rate of respiration were high at the initial stage of fruit growth and declined significantly in both cultivars from 30 days after full bloom. Kitasaki et al. (1999) also reported higher rate of respiration and ethylene production during first three weeks after bud burst and then a decline in both. Similar results have been reported for cherry (Blanpied, 1972) which reflects the high respiratory levels in the meristematic cells of young fruit. Both ethylene and respiration follow a similar pattern of changes suggesting a possible interaction between them (Kitasaki et al., 1999). The exocarp and the mesocarp contains significant amounts of phosphoenol pyruvate carboxylase (Sánchez, 1994), the CO_2 fixation enzyme. During the fruit development, CO_2 produced from the mitochondrial respiration of photoassimilates becomes photosynthetically fixed into triose-phosphate in the fruit chloroplasts in the light period and thereby the growing fruit expresses lower level of CO_2 as an indicator during the measurement of respiration (Sánchez and Harwood, 2002).

It was observed that, irrespective of the growth period and cultivars the production of ethylene and rate of respiration were higher in 2013 than 2014. Lower level of rainfall was noted in 2014 during the growth period of the fruit which affected the fruit growing process (Lavee et al., 1990 and Tombesi, 1994) and ultimately affected the physiological and ripening process of the fruit (Lavee et al., 1982, 1991; Barone et al., 1994; Inglese et al., 1996 and Barranco et al., 2000). Higher ethylene production at later stages of olive fruit development (175-190 days after full bloom) in both cultivars suggests that ethylene seems to be involved in olive fruit ripening (Fig. 4.4 A). Exogenous application of ethephon four weeks prior to harvest in both the cultivars resulted in higher ethylene production in the fruit (Fig.6.1) consequently promoted of olive fruit ripening as reflected by higher ripening index (Fig 6.2) in both cultivars during two consecutive years. Higher levels of endogenous ethylene production in olive fruit at later stages of development (175-

190 days after full bloom) and promotion of fruit ripening with the exogenous pre-harvest application of ethephon in both cultivars suggest the involvement of ethylene in hastening ripening of a non-climacteric olive fruit.

8.1.3. Changes in fruit ripening index during olive fruit development and maturation

The fruit ripening index increased exponentially in both cultivars with the progress of fruit maturation (Fig.4.5). The cv. Frantoio showed significantly higher ripening index (1.36- fold) than Manzanilla. Varied pattern of ripening index for different cultivars were reported by Barranco et al. (2000) where they noted stepped pattern for cv. Frantoio. Photosynthetic activity in the fruit tissue decreases with the progress of fruit growth and ripening which lowers down the concentrations of both chlorophylls and carotenoids (Salvadoret al., 2001). The fruit becomes violet or purple due to the accumulation of anthocyanin at its ripe stage (Roca and Minguez-Mosquera, 2001). A number of changes occur during the ripening of olive and these changes influence fruit firmness, chemical composition and sensory characteristics of the fruit and oil (Beltrán, 2000).

8.1.4. Changes in fruit firmness during fruit growth and development

The fruit firmness (N) in cv. Manzanilla decreased (4.36- fold) significantly ($P \leq 0.05$) with the progress of fruit growth (Fig.4.6). Rotondi et al. (2004) reported that the rapid change in the fruit texture takes place over a period of 1–2 weeks during fruit ripening and can be observed as a change from hard to softer texture. The composition of chemical and biochemical components change with maturation and the magnitude of these changes depend on the cultivar, climate and growing conditions (Gutierrez et al., 2000). These changes are consequent with the textural changes as well and the flesh firmness reduces with progress of fruit maturity (Nanaos et al., 1999). Firmness of olive pulp decreases with the loss of uronic acids in the cell wall as reported earlier by Jimenez et al. (2001) in Hojiblanca cv. olives during ripening. A decrease in methyl esterification of olive pulp cell wall pectic polysaccharides during ripening causes the loosening of complexation between galacturonic acid and Ca (Mafra et al., 2001 and Ferreira et al., 2006) which ultimately decrease the olive pulp firmness.

8.2. Effect of harvesting time, concentration and time of application of ethephon on physical parameters of olive fruit

Fruit removal force (FRF) was reduced (from 5.85 N to 4.00 N) in olive fruit irrespective of the cultivars from first to fifth harvest in both years and the least fruit removal force was observed in the fruit harvested during mid-June or in fifth harvest (Table.5.1). The FRF is linearly correlated to the stage of fruit growth and reduces with the advancement of ripening (Lavee et al., 1973 and Lavee et al., 1982). The reduction of FRF has also been influenced by the level of endogenous ethylene which increases with the development of the fruit (Lavee et al., 1982). cv. Manzanilla showed higher FRF than cv. Frantoio in both of the harvesting years (1.71- and 1.43-fold in 2013 and 2014 respectively) (Fig.5.2). The genotypic differences cause the variability in FRF between cultivars (Lavee and Haskal, 1976). The thickness of stalks differs in cultivars that also differ according to the size of the fruit within the cultivar which shows a declining trend with the progress of fruit growth (Lavee et al., 1982).

The fruit removal force reduced significantly with the increase of the applied ethephon concentration. Ethephon penetrates the pedicels and releases ethylene to reduce the FRF (Ben-Tal, 1992). Higher concentrations of ethephon assumed to be required to penetrate the thick waxy overlapping peltate trichomes on the olive leaves or fruit (Weis et al., 1988). FRF significantly reduced irrespective of the cultivar from one- to four-week advanced spray of ethephon (1500 mg L^{-1}).

Higher concentrations (1000 – 3000 mg L^{-1}) of ethephon significantly increased the ripening index of olive fruit (Fig.6.4 and Fig.6.5) through increased ethylene production (Chaves and De Mello-Farias 2006; Nath et al. 2006 and Tharanathan et al., 2006). The ripening index of both olive cultivars were significantly ($P \leq 0.05$) increased in comparison to controls, by early ethephon treatment (four weeks before fruit harvest) (Fig.6.2). The ethylene produced by ethephon treatment at four weeks prior to harvest was effective on the fruit tissues to increase the RI, fruit abscission (%) and to reduce the FRF in both cvs. Frantoio and Manzanilla. Ethylene induces the abscission of leaves, flowers and fruits (Abeles et al., 1971; Jackson and Osborne, 1972; and Henry et al., 1973). The growth stage and ambient conditions including temperature and relative humidity affect the extent of

ethylene penetration into the plant cells and rate of ethylene evolution from the decomposition of ethephon (Ben-Tal and Lavee, 1976a, b; Flore and Bukovac, 1982; Olien and Bukovac, 1978, 1982; Beaudry Kays, 1987; and Kays and Beaudry, 1987). El-Tamzini et al. (1980) also reported a maximum of 73% reduced FRF when olive trees were treated with 1250 mg L^{-1} ethephon two weeks before harvesting.

Moisture content of olive fruit decreased from first to fifth harvest (from 57.57% to 51.48%) and cv. Manzanilla showed higher moisture content than cv. Frantoio (1.19-fold in both years, Fig.5.3). From the current study it was revealed that the percentage rainfall was low in 2014 during the growing period of the fruit in that year. Water is a major component of olive fruit comprising more than half of the total fruit weight and varies according to the variation of seasonal rainfall and cultivar (Beltrán et al., 2004). The ripening of olive is affected by the cultivar and the environmental factors (Lavee et al., 1990). Lowest moisture content in the driest harvest year (2014) due to water stress conditions is supported by Lavee et al. (1991) and Ortega et al. (2001). On the other hand, a decrease of moisture content can also be related to the progressive increase of the oil content during fruit maturation (Sánchez and Fernández, 1991).

Fruit and leaf abscission (%) increased significantly ($P \leq 0.05$) with the increase of applied ethephon concentration in both cvs. Frantoio and Manzanilla in 2013, 2014. The effect of abscission agents has been reported to reduce fruit-detachment force and increase harvest efficiency with ethephon (Barranco et al., 2004 and Ferguson et al., 2010). Ethylene evolution seems to parallel to the applied concentration (150 mgL^{-1}) ethephon (Banno et al., 1993). Ethephon induces fruit abscission through accumulation in the pedicel-fruit basin and leaf surface which ultimately penetrates into the plant system to enhance the ethylene production (Reed and Hartmann, 1976; Polito and Lavee, 1980; and Weis et al., 1988, 199l). Touss et al. (1995) claimed that the most suitable concentration of ethephon is 1250 to 1875 mg L^{-1} which is in agreement with the observation from the current study.

Oil content (% dry weight) in olive fruit significantly increased (1.07- to 1.10-fold) from first to fifth harvest in both years and cv. Manzanilla showed higher oil content (%) than cv. Frantoio (1.01-fold) (Fig. 5.4). Availability of water or its stress largely influences the development and oil content of the olive fruit (Lavee et

al., 1982 and 1990; Barone et al., 1994; Tombesi, 1994; Inglese et al., 1996). Oil content in olive fruit showed an increasing trend until late harvest time in both cvs. Frantoio and Manzanilla (Fig.5.4).A similar observation was reported by Beltrán et al. (2004). The two cultivars also differed significantly for oil content on a dry weight basis which is a genotypic characteristic (Beltrán et al., 2004). The lowest oil content was found in the low rainfall crop year, 2014 which is similar to the findings reported by Ortega et al. (2001) for water stress conditions.

The time of ethephon treatments did not show significant effect on the free fatty acids and peroxide values of cvs. Frantoio and Manzanilla olives (Table.7.2). Similar findings were reported by Ahmed et al. (1981) while they conducted a study with cv. Coratina olive by spraying ethrel at two weeks before harvesting the fruit.

8.2.1. Effect of ethephon concentration on ethylene production

Ethephon treatments significantly increased the production of ethylene in both cultivars and higher concentration of ethylene was recorded from the fruit treated with higher concentration of ethephon (Fig.6.1). Ethephon is an ethylene-releasing chemical (Martin et al., 1981) which induces ethylene from the fruit of treated plants (Banno et al., 1993). Ben-Tal (1992) also reported that, a small portion of applied ethephon penetrates the pedicels and releases ethylene responsible for increased ethylene in the treated fruit.

8.2.2. Effect of harvesting time, concentration and time of application of ethephon on biochemical parameters:

8.2.2.1. Free fatty acid and fatty acids compositions

The fatty acids showed significant increase or decrease with the delay of harvesting from first to fifth in both of the years and irrespective of the cultivars (Fig. 5.11, 5.13, 5.15 and 5.17). Higher level of oleic acid and MUFA/PUFA ratio was recorded from early harvested fruit (mid- to late-April) (Fig.5.23). The concentration of fatty acids in the oil may differ due to the effect of environmental factors in the cultivation year and stage of fruit growth or maturation (Salvador et al., 2003 and Anastasopoulos et al., 2011). The free fatty acids at the later stage of ripening increase with the increase of lypolytic enzyme activity in the flesh (Anastasopoulos et al., 2011). The decrease in the level of oleic acid and increase in linoleic acid were

observed due to the activity of the enzyme oleate desaturase which converts oleic acid into linoleic acid (Gutierrez et al., 1999). This inter-conversion of oleic and linoleic acid is accelerated by water stress which ultimately reduces the MUFA: PUFA ratio as reported by Gómez-Rico et al. (2007) and Dag et al. (2014). The present study also observed similar results where the fruit harvest in 2014 faced a water stress due to low rainfall. The oils from cv. Manzanilla showed higher levels of free fatty acid, stearic acid, oleic acid, MUFA and MUFA:PUFA ratio (Fig. 5.8, 5.14, 5.16, 5.20 and 5.24). Higher levels of peroxide value, palmitic acid, linoleic acid and PUFA, were observed in cv. Frantoio (Fig.5.10, 5.12, 5.18 and 5.22). The variation between the two cultivars in respect of the fatty acid profiles is due to their genetic differences (Stefanoudaki et al., 1999; EEC, 2003; Gómez-González et al., 2011; and Manai-Djebali et al., 2012;).

Ethephon treatment (2000-3000 mg L^{-1}) significantly increased the level of fatty acids than the control in both years. Ethephon treatment enhances the fruit maturation and ultimately affects the oil quality (Ismail et al., 1999) which has been expressed through increased (palmitic acid, stearic acid, linoleic acid and PUFA in both cultivars in both years; MUFA in cv. Manzanillain in 2013) or decreased (oleic acid in both cultivars in 2013 and in cv. Frantoio in 2013; MUFA/PUFA ratio in both cultivars in both years) levels of fatty acids from the increased concentration of ethephon treatment. However, some stable or non-significant changes in some of the fatty acids (oleic acid in cv. Manzanilla in 2014; MUFA in cv. Frantoio in 2013 and cv. Manzanillain in 2014) were also observed due to the effect of ethephon treatments. This might be due to similar macroclimatic conditions for the treated olive trees and this observation is in agreement with Ranali et al. (1999) and Faila et al. (2002) findings.

The average concentration of palmitic acid, stearic acid, linoleic acid and PUFA significantly ($P \leq 0.05$) increased in both cultivars by ethephon (1500 mg L^{-1}) spray application from one- to four-week prior to harvest than control. However, the ethephon application periods did not show significant differences among them for individual fatty acids (Fig.6.15, 6.17, 6.21 and 6.25). From the study it resembles that the ethephon spray affects the concentration of fatty acids in comparison to the control fruit but there is little or no significant effect of ethephon application time on

fatty acid concentrations. Touss et al. (1995) also reported non-significant effect of ethephon treatments while they treated Arbequina cv. olive trees at 12 days before harvest with different concentrations of ethephon. Moderate effect of ethephon on palmitic, linoleic and oleic acids was also reported by Cimato (1988). Lavee and Haskal (1975) treated cv. Nabali olives with ethephon at 1500 mg L^{-1} and did not observe any effect of ethephon on neither colour nor taste of the oil.

8.2.2.2. Peroxide value

The peroxide value increased with the increase of applied ethephon concentration in both years and in both cultivars. The ethephon enhanced the ethylene production from the treated fruit which ultimately increased the peroxide value (Yousfi et al., 2009). However, decrease in peroxide value has been reported by Tovar et al. (2001), Salvador et al. (2001) and Baccouri et al. (2008) where they claimed that the activity of lipoxygenase enzyme decreases as the fruit ripening process advances. The increased level of ethylene produced from the fruit has increased the activity of lipoxygenase enzyme activity in ripening olive fruit (Griffiths et al., 1999 and Sheng et al., 2003).

8.2.2.3. Polyphenolic compounds

A significant gradual decrease was noted in (Fig.5.25, 5.27 and 5.29) polyphenolic compounds from first to fifth harvest in both harvesting years irrespective of the cultivars (Table.5.2). Higher total polyphenols was noted in the fruit harvested during mid-April to late-April (Fig.5.31). The concentration of phenolic compounds varies according to the maturation phase of the fruit (Anastasopoulos et al., 2011). The total phenols increase progressively and decrease in the final ripening stage (Baccouri et al., 2008). The concentration of polyphenolic compounds was comparatively high in the fruit harvested in 2014 (Fig.5.30) when less rainfall was recorded (0.00 to 8.10 mm) than 2013 (2.00 to 56.5 mm) during the growing period of the olive fruit. Similar observation was reported by Patumi et al. (2002) and Gómez-Rico et al. (2007) and Anastasopoulos et al. (2011). Differences in the level of water content of the fruit could imply a different solubilisation of phenols (Allogio and Caponio, 1997). The amount of water in the fruit also controls the activity of enzymes responsible for phenolic compound synthesis (Morello et al., 2005). A linear correlation between polyphenols and water stress was also observed by Tovar

et al. (2002), Gómez-Rico et al. (2006), Dag et al. (2008), Ben-Gal et al. (2011) Vita Serman et al. (2011) and Caruso et al. (2014). The two cultivars also showed significant differences for phenolic compounds. The variation of phenolic compounds in different cultivars is also related to the genetic variations among them which were reported by Aguilera et al. (2005), Vinha et al.(2005), Manai-Djebali et al. (2012) and Dağdelen et al. (2013).

The concentration of polyphenols (tyrosol, hydroxytyrosol and oleuropein aglycon) decreased in both cultivars and both years with the increase of the applied ethephon concentration (Fig.6.29, 6.31, and 6.33). Decrease in the major phenolic compounds with the progress of olive fruit maturity has also been reported from different studies (Skevin et al., 2003; Rotondi et al., 2004; Yousfi et al. 2006; Baccouri et al., 2007 and Riachy et al., 2012). According to Amiot et al. (1989), this decrease is correlated with the increased activity of hydrolytic enzymes during ripening. Exposure to ethylene results in higher polyphenol oxidase (PPO) activity (Couture et al. 1993; Peng and Yamauchi 1993) which readily oxidises the soluble phenolic compounds (Ke and Saltveith 1988). The effects of ethephon in the current study were similar to ethylene effects on PPO activity. The concentration of individual and total phenols in 2014 was comparatively high when the average rainfall was less in 2013. Water availability has a large effect on the phenolic profile of virgin olive oil (Gómez-Rico et al., 2007; Servili et al., 2007; Ripa et al., 2008 and Tura et al., 2008). Yousfi et al. (2006) noted higher amounts of different phenolic compounds in the oils obtained from the fruit harvested in the low rainfall season than those obtained in the season with double rainfall.

The mean levels of polyphenols (hydroxytyrosol, tyrosol, and oleuropein aglycon) in cvs. Frantoio and Manzanilla significantly ($P \leq 0.05$) decreased with the pre-harvest spray application of ethephon (1500 mg L-1) four weeks prior to harvest compared to the control. However, the ethephon application periods did not show any significant effect on the concentration of phenolic compounds in the virgin olive oil (Table.7.4). The phenolic compounds differ mainly according to crop year, maturation phase and cultivation method (Anastasopoulos et al., 2011). There is limited number of published reports on the effect of ethephon application period on phenolic composition of virgin olive oil. The pre-harvest application of ethephon (4-

weeks prior to harvest) showed lower concentration of phenolic compounds in the virgin olive oil. This resembles the effect of the evolved ethylene from the applied ethephon and correlates with the increased activity of hydrolytic enzymes during ripening to reduce the concentration of phenolic compounds (Amiot et al., 1989; Yousfi et al. 2006 Baccouri et al., 2007 and Riachy et al., 2012). Early exposure to ethylene results in higher polyphenol oxidase (Peng and Yamauchi 1993) which readily oxidises the soluble phenolic compounds (Ke and Saltveith 1988) and later application of ethephon does not show significant changes on this declining trend.

8.2.3. Effect of harvesting time, concentration and time of application of ethephon on sensory attributes

The sensory attributes (fruitiness, bitterness and pungency) of the virgin olive oil decreased gradually from the first to fifth harvest time irrespective of the cultivars in both harvesting years (Fig.5.33, 5.35 and 5.37). Virgin olive oil from cv. Manzanilla showed greater bitterness and pungency and lower fruitiness than that from cv. Frantoio. The least bitterness and pungency were recorded during mid-June and the most fruity oil was obtained from the fruit in early harvest of Frantoio (mid-April) and late harvest of Manzanilla (mid-June). The sensory properties of olive fruit are influenced by the ripeness status and variety of the fruit. Similar variations in sensory profile have been reported by Angerosa et al. (2004), Rotondi et al. (2004), Servili et al. (2004), Tripoli et al. (2004) Kalua et al., (2007) and Delgado and Guinard (2011). Higher phenol content in the fruit of Manzanilla may be ascribed for its higher bitterness and pungency (Bendini et al., 2007). More bitterness was observed in the fruit harvested in 2014 while there was a lower amount of rainfall during the growing period of fruit. Similarly, the findings of Cinquanta et al. (1997) indicate that the ripeness of the olives along with pedoclimatic conditions influence the quality attributes of virgin olive oil.

The sensory attributes showed significant decrease in both years and cultivars cvs. Frantoio and Manzanilla with the increased concentration of ethephon applied (Fig.6 37, 38 and 39). Phenolic compounds are highly correlated to organoleptic characteristics of olive oil (Andrewes et al., 2003 and Beltrán et al., 2007). The amount of phenolic compounds decreases with the progress of maturity (Yousfi et al., 2006 and Riachy et al., 2012) and due to the effect of ethylene

enhanced by the ethephon treatment (Couture et al. 1993; Peng and Yamauchi 1993). These differences are attributed to chemical reactions and enzymatic activities, such as glycosidases, phenol oxidases or phenol polymerases (Ke and Saltveith, 1988). Yousfi et al. (2009) also reported decreased bitterness when he exposed modified atmosphere (MA) stored olive fruit to 30 mgL^{-1} ethylene.

The sensory attributes showed significant ($P \leq 0.05$) decrease in both olive cultivars from one- to four-week pre-harvest spray of ethephon (1500 mg L^{-1}). However, the ethephon application period treatments did show significant differences among them for sensory attributes. Higher average values of these attributes were also noted in Manzanilla olive oil than Frantoio (Table.7.5). There is no published report on the effect of spray period of ethephon on the sensory attributes of virgin olive oil. The pre-harvest application of ethephon at four weeks before harvest significantly reduces the concentration of phenolic compounds which consequently reduces the value of sensory attributes. Decreased bitterness of virgin olive oil was also reported by Yousfi et al. (2009), however, the effect of ethephon spray period on fruitiness is not clear which warrants further investigation.

8.3. Conclusion

The physical, biochemical and sensory properties of the olive fruit and oil showed variations according to the days after flowering, delay of harvesting, genetic differences between the cultivars, concentration of applied ethephon and the environmental factors such as water stress during the growing period of the fruit. The fruit of cv. Manzanilla showed higher levels of fruit removal force, moisture content (%) and oil content (% dry weight) than cv. Frantoio. Lowest moisture and oil content were observed in the driest harvest year, 2014. At the later stage of ripening the increase of free fatty acids was observed which may be ascribed to the increase of lipolytic enzyme activity and lowering trend of peroxide value may be due to the decreased activity of lipoxygenase enzymes. A significant gradual decrease was noted in major polyphenol compounds during the growing of olive fruit. The concentration of phenolic compounds was comparatively high in the fruit harvested in 2014. The sensory attributes varied from the first to fifth harvest. They degraded

with the delay of harvesting and water stress may have influenced the bitterness of the fruit in 2014.

The level of ethylene production, ripening index, fruit and leaf abscission and peroxide value of olive oil increased significantly with the increase of applied ethephon concentration in comparison to the control. Among different fatty acids, significant increase was observed in most of the cases, however, the level of oleic acid, MUFA and MUFA/PUFA ratio decreased with the increase of ethephon concentration. Concentration of different polyphenols (hydroxytyrosol, tyrosol, oleuropein aglycon, and total polyphenol) and level of sensory attributes (fruitiness, bitterness and pungency) decreased significantly with the increase of ethephon concentration and none of any virgin olive oil (VVO) defects were found. However, there was no effect of ethephon on the fruit moisture (%) and oil (% fresh and dry weight basis) content of the olive fruit. Among the applied concentrations of ethephon, 1000 to 2000 mg L^{-1} in 2013 and 1000 to 1500 mg L^{-1} in 2014 did not show significant differences for the studied parameters.

Significantly increased RI (4.84), fruit and leaf abscission (95.92% and 27.44%), free fatty acids (0.42%), peroxide value (11.02 meqO_2 kg^{-1}), palmitic acid (13.19%), stearic acid (4.19%), linoleic acid (11.12%) and PUFA (11.60%) were observed in VOO when the olive trees were sprayed with ethephon at four weeks before harvesting. Significantly reduced phenolic compounds and sensory attributes were also noted from this treatment. However, the treatments did not differ significantly among themselves in respect of their effects on the parameters.

In conclusion, this study indicated that the most suitable time for olive harvesting is late-May to mid-June, best suitable concentration of ethephon to treat olive trees is 1000 – 1500 mg L^{-1} and suitable period of ethephon spray to olive trees is at least two weeks before harvesting the fruit under south-western Australia.

8.4. Recommendations

From the conducted experiments the following recommendations may be made for the olive industries in south-western Australia-

- The most suitable time for cvs. Frantoio and Manzanilla olive harvesting in south-western Australia might be as late-May to mid-June for best quality of olive oil.
- The most suitable concentration of ethephon to treat olive trees in south-western Australia might be 1000 – 1500 mg L^{-1}
- And the most suitable period for ethephon treatment to olive trees in south-western Australia might be at least two weeks before harvesting the fruit.

8.5. Future Research

- Effects of harvest time on physical, biochemical and sensory attributes of olives and oil in other commercial cultivars such as Kalamata, Picual, Leccino, and Coratina, grown at different locations in south-western Australia are yet to be investigated.
- To facilitate mechanical harvesting, the effects of application time of ethephon on physical, biochemical and sensory attributes of olives and oil and reduction of leaf abscission induced ethephon application in other commercial cultivars grown at various locations in south-western Australian conditions warrants to be investigated.
- Ethephon application generally promotes flowering in fruit trees. Effects of application of ethephon on return bloom and alternate bearing in commercial cultivars of olive grown at various locations in south-western Australian conditions warrants to be investigated over a number of years.
- Influence of harvest time and application of ethephon on other quality parameters such as sterols and tocopherols in virgin olive oil are worthy of investigations in the future.

References

Abeles, F. B., L. E. Craker and G. R. Leather. 1971. Abscission: The phytogerontological effects of ethylene. Plant Physiol. 47: 7-9.

Aguilera, P. M., G. Beltran, D. Ortega, A. Fernandez, A. Jimenez and M. Uceda. 2005. Characterization of virgin olive oil of Italian olive cultivars: Frantoio and Leccino, grown in Andalusia. Food Chem. 89: 387-391.

Aguilera, M. C., M. D. Ramı´rez-Tortosa, A. Mesa and G. Angel. 2000. Do MUFA and PUFA have beneficial effects on development of cardiovascular disease? Rec. Res. Dev. Lipid. 4: 369-390.

Ahmed, H.S., M.Y. El-Shurfa and M. S. Shaladan. 1981. Effects of ethrel on quality of Coratina olive fruits. Libyan. J. Agric. 10: 97-102.

Allalout, A., D. K Richene, K. Methenni, A. Taamalli, D. Daoud and M. Zarrouk. 2011. Behavior of super-intensive Spanish and Greek olive cultivars grown in Northern Tunisia. J. Food Biochem. 35: 27-43.

Allogio, V. and F. Caponio. 1997. The influence of olive paste preparation techniques on the quality of olive oil. II. Evolution of phenolic substances and some quality parameters referred to the ripening of drupes in virgin olive oil from the Coratina cv. Riv. Ital. Sost. Grasse. 74: 443-447.

Amiot, M., A. Fleuriet and J. Macheix. 1986. Importance and evolution of phenolic compounds in olive during growth and maturation. J. Agric. Food Chem. 34: 823-6.

Amiot, M., A. Fleuriet, and J. Macheix.1989. Accumulation of oleuropein derivatives during olive maturation. Phytochemistry. 28: 67-69.

Anastasopoulos, E., N. Kalogeropoulos, A.C. Kaliora, A. Kountouri, and N.K. Andrikopoulos. 2011. The influence of ripening and crop year on quality indices, polyphenols, terpenic acids, squalene, fatty acid profile, and sterols in virgin olive oil (Koroneiki cv.) produced by organic versus non-organic cultivation method. J. Food Sci. Tech. 46: 170-178.

Andrewes, P., J.Busch, T. De Joode, A. Groenewegen and H. Alexandre. 2003. Sensory properties of virgin olive oil polyphenols: Identification of deacetoxy-ligstroside aglycons as a key contributor to pungency. J. Agric. Food Chem. 51: 1415-1420.

Angerosa, F., M. Servili, R. Selvaggini, A. Taticchi, S. Esposto and G. Montedoro. 2000. Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. J. Chromatogr. A. 1054(1-2): 17-31.

Anon, 2010. Australia Olive Industry RD&E Plan. Australian olive industry research, development and extension. Plan 2010-2015. No 10/155.

Aparicio, R., L. Roda, M. Albi and F. Gutiérrez. 1999. Effect of various compounds on virgin olive oil stability measured by Rancimat. J. Agric. Food Chem. 47: 4150-4155.

Australian Bureau of Meteorology, 2015. Australian Climatic Zones - All Climate Classes (Map). (http://www.bom.gov.au Retrieved 8 February 2015).

Australian Climatic Zones - All Climate Classes (Map). Bureau of Meteorology website. (http://www.bom.gov.au/iwk/climate_zones/). Retrieved 8 April 2015.

Avidan, B., A. Ogrodovitch and S. Lavee. 1999. A reliable and rapid shaking extraction system for determination of the oil content in olive fruit. Acta. Hortic. 474: 653-658.

Aviram, M and K. Eias, . 1993. Dietary olive oil reduces low-density lipoprotein uptake by macrophages and decreases the susceptibility of the lipoprotein to undergo lipid peroxidation. Ann. Nutr. Metab. 37: 75-84.

Ayton J., J. Mailer and K. Robards. 2001. Changes in oil content and composition of developing olives in a selection of Australian cultivars. Austr. J. Exp. Agric. 41: 815-21.

Ayton, J., J. Mailer, A. Haigh, D. Tronson and D. Conlan. 2007. Quality and oxidative stability of Australian olive oil according to harvest time and irrigation. J. Food Lipid. 14: 138-156.

Baccouri, O., M.Guerfel, B. Baccouri, L. Cerretani, A.Bendini, G. Lercker, E. Barone, G. Gullo, R. Zappia and P. Inglese. 1994. Effect of crop load on fruit ripening and olive oil (*Olea europaea* L.) quality. J. Hort. Sci. 69:67–73.

Baccouri, B., W. Zarrouk, D. Krichene, I. Nouairi , N. Ben Yousef, D. Daoud and M. Zarrouk. 2007. Influence of fruit ripening and crop yield on chemical properties of virgin olive oils from seven selected oleasters (*Olea europaea* L.). J. Agron. 6: 388-396.

Baccouri, O., M. Guerfel and B. Baccouri. 2008. Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. Food Chem. 109: 743-754.

Banno, K., G.C. Martin and R.M. Carlson. 1993. The role of phosphorus as an abscission-inducing agent for olive leaves and fruit. J. Amer. Soc. Hort. Sci. 118(5): 599-604.

Barone, E., Gullo, G., Zappia, R. and P. Inglese. 1994. Effect of crop load on fruit ripening and olive oil (*Olea europaea* L.) quality. J. Hort Sci. 69: 67-73.

Barranco, D., C. de Toro, M. Oria and H. Rapoport. 2000. Monopotassium phosphate (PO_4H_2K) for olive fruit abscission. Acta Hortic. 586: 263-266.

Barranco, D., O. Arquero, C. Navarro, and H. F. Rapoport. 2000. Monopotassium phosphate for olive fruit abscission. J. Hort. Sci. 39: 1313–1314.

Bartolini, S., C. Cantini and C. Vitagliano. 1992. Olive fruit abscission: anatomical observations following application of ethylene-releasing compound. Acta Hortic. 329: 249-251.

Beaudry, R. M. and S. J. Kays. 1987. Effects of physical and environmental factors on the release of ethylene from (2-chloroethyl) phosphonic acid and (2-chloroethyl)-methyl-bis-(phenylmethoxy) silane. J. Amer. Soc. Hort. Sci. 112: 352-359.

Beltrán, G. 2000. Influence of ripening process in *Olea europaea* L. fruits on the physicochemical characteristics of the oils. PhD. Thesis, Universidad de Jaén, Spain. 212p.

Beltrán, G., C. del Río, S. Sánchez and L. Martínez. 2000. Seasonal changes in olive fruit characteristics and oil accumulation during ripening process. J. Sci. Food Agri. 84: 1783-1790.

Beltrán G., C. del Rio, S. Sánchez , L. Martínez. 2004. Influence of harvest date and crop yield on the fatty acid composition of virgin olive oils from cv. Picual. J. Agric. food chem. 52: 3434-3440.

Beltrán, G., M. T. Ruano, A. Jiménez, M. Uceda and M. Aguilera. 2007. Evaluation of virgin olive oil bitterness by total phenol content analysis. Eur J. Lipid. Sci. Technol. 108: 193-197.

Bendini, A., L. Cerretani, A. Carrasco-Pancorbo, A. Gómez-Caravaca, A Segura-Carretero, A Fernández-Gutiérrez and G. Lercker. 2007. Phenolic molecules in virgin olive oils: A survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules. 12: 1679-1719.

Ben-Gal, A., A. Dag, L. Basheer , U. Yermiyahu, I. Zipori and Z. Kerem. 2011. The influence of bearing cycles on olive oil quality response to irrigation. J Agric. Food Chem. 59:11667-11675.

Ben-Tal, Y. and S. Lavee. 1976a. Increasing the effectiveness of ethephon for olive harvesting. J. Hort. Sci. 11:489-490.

Ben-Tal, Y. and S. Lavee. 1976b. Ethylene influence on leaf and fruit detachment in Manzaillla olive trees. Sci. Hortic. 4:337-344.

Ben-Tal, Y. 1992.Quantification of ethephon requirements for abscission in olive fruits. Plant Growth Reg. 11: 3 97-403.

Ben-Tal Y. and M Wodner. 1994. Chemical loosening of olive pedicels for mechanical harvesting. Acta. Hortic. 356: 382-387.

Bianchi, G. 2003. Lipids and phenols in table olives. Eur. J. Lipid Sci. Tech. 105: 229-242.

Blanpied, G. D. 1972. A study of ethylene in apple, red raspberry and cherry. Plant Physiol. 49: 627-630.

Bouaziz, M., M. Chamkha and S. Sayadi. 2004. Comparative study on phenolic content and antioxidant activity during maturation of the olive cultivar Chemlali from Tunisia. J. Agric. Food Chem. 52: 5476-5481.

Bower, J., J. Jobling, B. Patterson and D. Ryan. 1998. A method for measuring the respiration rate and respiratory quotient of detached plant tissues. Postharv. Biol. Tech. 13: 263-270.

Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr. Rev. 56:317-33.

Brenes, M., L. Rejano, P. Garcia, A. Sanchez and A. Garrido. 1995. Biochemical changes in phenolic compounds during Spanish-style green olive processing. J. Agric. Food Chem. 43: 2702-2706.

Brenes, M., A. Garcı´a, P. Garcıı´a, J. Rios and A. Garrido. 1999. Phenolic compounds in Spanish olive oils. J Agric. Food Chem. 47:3535-40.

Breton, C., F. Medail, C. Pinatel and A. Berville. 2006. From olive tree to oleaster: origin and domestication of *Olea europaea* L.in the Mediterranean basin. Cah Agric. 15: 329-336.

Bukovac, M. J, F. Zucconi, R. Larsen and C. D. Kesner. 1969. Chemical promotion of fruit abscission in cherries and plums with special reference to 2-chloroethylphosphonic acid. J Amer. Soc. Hortic. Sci. 94: 226-230.

Burns, J. K., R. S. Buker, and F. M. Roka. 2005. Mechanical harvesting capacity in sweet orange is increased with an abscission agent. Hort. Tech. 15:758-765.

Burns, J. K., L. Ferguson, K. Glozer, W. H. Krueger and R. C. Rosecrance. 2008. Screening fruit loosening agents for black ripe processed table olives. J. Hort. Sci. 43:1449-1453.

Camposeo, S., G. A. Vivaldi and C. E. Gattullo. 2013. Ripening indices and harvesting times of different olive cultivars for continuous harvest. Sci. Hort. 151: 1-10.

Caponio, F., T. Gomes and A. Pasqualone. 2001. Phenolic compounds in virgin olive oils: influence of the degree of olive ripeness on organoleptic characteristics and shelf-life. Eur. Food Res. Tech. 212: 329-333.

Caruso, G., R. Gucci, S. Urbani, S. Esposto, A. Taticchi, and I. Di Maio. 2014. Effect of different irrigation volumes during fruit development on quality of virgin olive oil of cv. Frantoio. Agric. Water Manag. 134:94-103.

Chaves, A.L and P.C. De Mello-Farias. 2006. Ethylene and fruit ripening: From illumination gas to the control of gene expression, more than a century of discoveries. Plant. Mol. Biol. 29: 508-515.

Chimi, H and Y. Atouati . 1994. Determination of the optimal stage for harvesting Moroccan picholine olives by monitoring change in total polyphenols. Olivae. 54: 56-60.

Cimato, A. 1988. La qualità dell'olio vergine di oliva ed i fattori agronomici. Informatore. Agrario. 45:63-69.

Cinquanta, L., M. Esti and E. La Notte. 1997. Evolution of phenolic compounds in virgin olive oil during storage. J. Amer. Oil Chem. Soc. 74: 1254-1261.

Conde, C., S. Delrot and G. Hernani. 2008. Physiological, biochemical and molecular changes occurring during olive development and ripening. J. Plant Physiol. 165: 1545-1562.

Connor, D. J. and E. Fereres. 2005. The physiology of adaptation and yield expression in olive. Hort. Rev. 34: 155–229.

Connor, D. J., M. Gómez-del-Campo, M. C. Rousseaux and P. S.Searles. 2014. Structure, management and productivity of hedgerow olive orchards: A review. Sci. Hort. 169:71-93.

Couture R., M. I. Cantwell, D. M. Ke and M. Saltveit. 1993. Physiological attributes related to quality attributes and storage life of minimally processed lettuce. HortSci. 28: 723-725.

Dabbou, S., S. Dabbou, H. Chehab, F. Brahmi, A. Taticchi, M. Servili and M. Hammami. 2011. Chemical composition of virgin olive oils from Koroneiki

cultivar grown in Tunisia with regard to fruit ripening and irrigation regimes. Int. J. Food Sci. Tech. 46: 577-585.

Dag, A., A. Ben-Gal, U. Yermiyahu, L. Basheer, N. Yogev and Z. Kerem. 2008. The effect of irrigation level and harvest mechanization on virgin olive oil quality in a traditional rain-fed 'Souri' olive orchard. J. Sci. Food Agric. 88: 1524-1528.

Dag, A., Z. Kerem, N. Yogev, I. Zipori, S. Lavee and E. Ben-David. 2011. Influence of time of harvest and maturity index on olive oil yield and quality. Sci. Hort. 127: 358-366.

Dag, A., A. Naor, A. Ben-Gal, G. Harlev, I. Zipori, D. Schneider, R. Birger, M. Peres, Y. Gal and Z. Kerem. 2014. The effect of water stress on super-high-density Koroneiki olive oil quality. J. Sci. Food Agri. 20:16-20.

Dağdelen, A., G. Tumen, M. M. Özcan and E. Dundar. 2013. Phenolic profiles of olive fruits (*Olea europaea* L.) and oils from Ayvalık, Domat and Gemlik varieties at different ripening stages. Food Chem. 136: 41-45.

Damak, N., M. Bouaziz, M. Ayadi, S. Sayadi and M. Damak 2008. Effect of the maturation process on the phenolic fractions, fatty acids, and antioxidant activity of the Chétoui olive fruit cultivar. J. Agric. Food Chem. 56: 1560-1566.

Daniell, J. W. and R. E. Wilkinson. 1972. Effect of ethephon-induced ethylene on abscission of leaves and fruits of peaches. J. Amer. Soc. Hort. Sci. 97:682-685.

De la Torre, M.C., M. V. Lopez and J. Colell. 1985. Evolucion de la fraccion esterolica durante la maduracion de las aceitunas. Grasas Aceites. 36: 198-202.

De Mendoza, M. F., C. D. Gordillo, J. M. Expóxito, J. S. Casas, M. M. Cano,D. M. Vertedor and M. N. Baltasar. 2013. Chemical composition of virgin olive oils according to the ripening in olives. Food Chem. 141: 2575-2581.

Delgado, C. and J. X. Guinard. 2011. Sensory properties of Californian and imported extra virgin olive oils. J. Food Sci. 76: 171-176.

Denney, J., and G. Martin. 1994. Ethephon tissue penetration and harvest effectiveness in olive as a function of solution pH, application time, and BA or NAA addition. J. Amer. Soc. Hort. Sci. 119: 1185-1192.

Di Giovacchino, L., S. Sestili and D. Di Vincenzo. 2002. Influence of olive processing on virgin olive oil quality. Eur. J. Lipid Sci. Tech. 104: 587-601.

Diraman, H. and H. Dibeklioglu. 2009. Characterization of Turkish virgin olive oils produced from early harvest olives. J. Amer. Oil Chem. Soc. 86: 663-674.

Dung, C. D. 2013. Factors controlling vase life of waxflowers (*Chamelaucium* Desf. Varieties and Hybrids). M. Phil. Thesis, Department of Environment and Agriculture, Curtin University of Technology, Western Australia. p333.

EEC. 1991. European Union Commission Regulation (2568/1991). Characteristics of olive and olive pomace oils and their analytical methods. Off. J. Eur. Commun. 248: 1-5

EEC. 2003. Characteristics of olive and olive pomace oils and their analytical methods EEC Regulation. 1989/2003. European Economic Community. Off. J. Eur. Commun. 298: 57-66.

Edgerton, L. 1968. New materials to loosen fruit for mechanical harvesting. Proc. NY State Hortic. Soc. 113: 99-102.

El-Abassy, R. M., P. Donfack and A. Materny. 2009. Rapid determination of free fatty acid in extra virgin olive oil by Raman spectroscopy and multivariate analysis. J. Amer. Oil Chem. 86: 507-511.

El-Tamzizni, M .I., M. Y. El-Shurfa, H. S. Ahmed and M. S. Shaladan. 1980. Use of ethrel and alsol as chemical aids for harvesting olives. Libyan J. Agric. 9: 85-90.

Esti, M., Cinquanta L, and E. La Notte. 1998. Phenolic compounds in different olive varieties. J Agric. Food Chem. 46: 32-5.

Faci, J. M., M. J. Berenguer, J. L. Espada and S. Garcia. 2002. Effect of variable water irrigation supply in olive (*Olea europaea* L.) cv. Arbequina in Aragon, Spain. II. Extra virgin oil quality parameters. Acta. Hortic. 586: 341-344.

Failla, O., D. Tura and D. Bassi. 2000. Genotype-environmental-year interaction on oil antioxidants in an olive district of northern Italy. Acta. Hortic. 586: 171-174.

Famiani, F., P. Proietti and A. Tombesi. 1991. The influence of some agronomic parameters on the efficiency of innovative vibration system used for mechanical harvesting. Olea: 21.

FAOSTAT. 2014. Food and Agriculture Organization of the United Nations Statistics Division. http://faostat.fao.org(Retrieved on 25/02/2015)

Favati, F., N. Condelli, F. Galgano and M. C. Caruso. 2013. Extra virgin olive oil bitterness evaluation by sensory and chemical analyses. Food Chem. 139: 949-954.

Fernandes-Silva, A. A., T. C. Ferreira, C. M. Correia, A. C. Malheiro and J. F. Villalobos. 2010. Influence of different irrigation regimes on crop yield and water use efficiency of olive. Plant and Soil. 333: 35-47.

Ferguson, L., U. A. Rosa, S. Castro-Garcia, S. M. Lee, J. X. Guinard, J. Burns, W. H. Krueger, N. V. O'Conell and K. Glozer. 2010. Mechanical harvest of California table and oil olives. Adv. Hort. Sci. 24:53–63.

Ferrante, A. 2005. Therapeutic properties of oils. US Patent 20: 123-479.

Ferreira, J. A., I. Mafra, M. R. Soares, D. V. Evtuguin and M. A. Coimbra. 2006. Dimeric calcium complexes of arabin-rich pectic polysaccharides from *Olea europaea* L. cell walls. Carb. Polym. 65:535-543.

Flore, J. A. and M. J. Bukovac. 1982. Factors influencing absorption of ^{14}C (2-chloroethyl) phosphonic acid by leaves of cherry. J. Amer. Soc. Hort. Sci. 107: 965-968.

Forina, M. and E. Tiscornia. 1982. Pattern-recognition methods in the prediction of Italian olive oil origin by their fatty-acid content. Annali di Chimica. 72: 143-155.

Ganino, T., G.Bartolini and A. Fabbri. 2006. The classification of olive germplasm- a review. J. Hort. Sci. Biotech 81: 319-334.

Garcia, J. M., S. Seller and M. C. Perez-Camino. 1996. Influence of fruit ripening on olive oil quality. J. Agric. Food Chem. 44: 3516-3520.

García, J., K. Yousfi, M. Martínez and M. Pérez-Camino. 2007. Use of ethylene to accelerate mill olive ripening. Acta. Hortic. 796: 111-117.

Garcia-Salas, P., A. Morales-Soto, A. Segura-Carretero and A. Fernández-Gutiérrez. 2010. Phenolic-compound-extraction systems for fruit and vegetable samples. Molecules. 15: 8813-8826.

Garg, A., A. Bonanone, S. M. Grundy, Z. Zhang and R. H. Unger. 1988. Comparison of high carbohydrate diet with a high mono- unsaturated-fat in patients with non-insulin-dependent diabetes mellitus. New Engl. J. Med. 319: 829-834.

Gómez-Rico A, M. D. Salvador, M. La Greca and G. Fregapane. 2006. Phenolic and volatile compounds of extra virgin olive oil (*Olea europaea* L. cv. Cornicabra) with regards to fruit ripening and irrigation management. J. Agric. Food Chem. 54:7130-7136.

Gómez-Rico A, M. D. Salvador, A. Moriana, D. Pérez, N. Olmedilla, F. Ribas and G. Fregapane. 2007. Influence of different irrigation strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. Food Chem. 100: 568-578.

Gómez-González, S., J. Ruiz-Jiménez and M. D Luque de Castro. 2011 . Oil content and fatty acid profile of Spanish cultivars during olive fruit ripening. J. Amer. Oil Chem. Soc. 88: 1737-1745.

Grattan, S. R., M. J. Berenguer, J. H. Connell, V. S. Polito and P. M. Vossen. 2006. Olive oil production as influenced by different quantities of applied water. J. Agric. Water Manage. 85: 133-140.

Griffiths, A., C. Barry, A. G. Alpuche-Solis and D. Grierson. 1999. Ethylene and developmental signals regulate expression of lipoxygenase genes during tomato fruit ripening. J. Exp. Bot. 50:793-798.

Gucci, R., E. Lodolini and H. Rapoport. 2007. Productivity of olive trees with different water status and crop load. J. Hort. Sci. Biotech. 82: 648-656.

Gutiérrez, F., B Jimenez, A. Ruiz, M. A. Albi. 1999. Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. J. Agric. Food Chem. 47: 121-127.

Gutierrez, F., I. Varona, and M. A. Albi. 2000. Relation of acidity and sensory quality with sterol content of olive oil from stored fruit. J. Agric. Food Chem. 48: 1106-1110.

Hartmann, H. T., A. Tombesi and J. Whisler. 1970. Promotion of ethylene evolution and fruit abscission in the olive by 2-chloroethanephosphonic acid and cycloheximide. J. Amer. Soc. Hort. Sci. 95:635-640.

Heim, K. E., A. R. Tagliaferro and D. J Bobilya. 2002. Flavonoid antioxidants: chemistry, metabolism and structure‐activity relationships. J. Nutr. Biochem. 13: 572‐584.

Henry, E. W. and T. E. Jensen. 1973. Peroxidases in tobacco abscission zone tissue. 1. Fine structural localization in cell walls during ethylene induced abscission. J. Cell Sci. 13: 591-601.

Hertog, M. G., E .J. Feskens, P. C. Hollman, M. B. Katan and D. Kromhout. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet. 342: 1007‐1011.

https://www.google.com.au/maps/@-31.9098304,116.6016322,10z (Retrieved on 8 February 2015)

Inglese, P., E. Barone and G. Gullo. 1996. The effect of complementary irrigation on fruit growth, ripening pattern and oil characteristics of olive (*Olea europaea* L) cv. Carolea. J. Hort. Sci. 71:257-263.

Iniesta, F., L. Testi, F. Orgaz and F. J. Villalobos. 2009. The effects of regulated and continuous deficit irrigation on the water use, growth and yield of olive trees. Europ. J. Agronomy. 30: 258–265.

IOC, 1996. Organoleptic assessment of virgin olive oil. COI/T.20/ Doc. No. 15/1st Review International Olive Oil Council, Madrid.

IOC, 2001. Trade standard applying to olive oil and olive pomace Oil. International Olive Oil Council, In COI/T.15/NC no. 2/Rev. 10; COI/T.20/Doc. no. 24. Madrid.

IOC, 2007. Sensory analysis of olive oil. Method for the organoleptic assessment of virgin olive oil. COI/T.20Doc.No15/Rev. 2. Madrid.

IOC, 2009. Trade standard applying to olive oils and olive-pomace oils. International Olive Council. Madrid.

ICO, 2011. COI/ OH/ Doc. No 1 November [on line date 1/3/14] (http://www.internationaloliveoil.org/). Madrid.

IOC, 2012. Table olives. International Olive Council. / [on line date 1/3/14].N<http:// www.internationaloliveoil.org>. Madrid.

IOC, 2014. World olive oil figures. International Olive Council. [online date 6/2/15] (http://www.internationaloliveoil.org/). Madrid

Ismail, A.S., G. Stavroulakis, D. Gerasopoulos, and J. Metzidakis. 1999. Effect of ethephon on the quality of cv. Koroneiki olive oil. Acta. Hortic. 474: 683-686.

Issaoui, M., B. Mechri, A. Echbili, S. Dabbou, A. Yangui, H. Belguith, A. Trigui, A and M. Hammami. 2008. Chemometric characterization of five Tunisian varietals of I L. olive fruit according to different maturation indices. J. Food Lipid. 15: 277-296.

Issaoui, M., S. Dabbou, F. Brahmi, K. Ben Hassine, M. H. Ellouze and M. Hammami. 2009. Effect of extraction systems and cultivar on the quality of virgin olive oils. J. Food Sci. Tech. 44: 1713-1720.

Jackson, M. B. and D .J. Osborne. 1972. Abscisic acid, auxin and ethylene in explants abscission. J. Exp. Bot. 23: 849-862.

Jemai, H., M. Bouaziz and S. Sayadi. 2009. Phenolic composition, sugar contents and antioxidant activity of Tunisian sweet olive cultivar with regard to fruit ripening. J. Agric. Food Chem. 57: 2961-2968.

Jimenez, A., R. I. Rodriguez, R. Fernadez-Caro, R. Guillen, J. Fernadez-Bolanos and A. Heredia. 2001. Olive fruit cell wall: Degradation of pectic polysaccharides during ripening. J. Agric. Food Chem. 49:409-415.

Kadman, A and Y. Ben-Tal. 1983. Inducing macadamia nut fruit drop with ethephon. J. Hort. Sci. 18: 240-242.

Kailis, S and D. J. Harris. 1999. Potential for European olive growing in Western Australia. Acta. Hortic. 474: 771-775.

Kailis, S. and D.J. Harris. 2007. *Producing table olives.* Linkpress Collingwood VIC Australia. pp.1-6.

Kalua, C.M., M. S.Allen, D. R.Bedgood, A.G. Bishop, P. D. Prenzler. 2005. Discrimination of olive oils and fruits into cultivars and maturity stages based on phenolic and volatile compounds. J. Agric. Food Chem. 53: 8054-8062.

Kalua, C. M, M. S. Allen, J. D. Bedgood, A. G. Bishop, P. Prenzler and K. Robards. 2007. Olive oil volatile compounds, flavour development and quality: A critical review. Food Chem. 100: 273-286.

Katan, M. V, C. Aravanis, R.P. Mensink. 1987. Serum lipoproteins in Cretan boys and men consuming a high olive oil diet. Circulation. 76: 530-536.

Kays, S. J. and R. M. Beaudry. 1987. Techniques for inducing ethylene effects.

Ke, D. and M. E. Saltveit. 1988. Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in Iceberg lettuce. Plant Physiol. 88: 1136-1140.

Kitsaki, C. K., S. N. Vemmos and C. G. Tzoutzoukou. 1999. Changes of respiration rate, ethylene evolution, and abscisic acid content in developing inflorescence and young fruit of olive (*Olea europaea* L. cv. Konservolia). J. Plant Growth Reg. 18: 1-7.

Koutsaftakis, A., F. Kotsifaki and E. Stefanoudaki. 1999. Effect of extraction system stage of ripeness and kneading temperature on the sterol composition of virgin olive oils. J. Amer. Oil Chem. Soc. 76: 1477-1481.

La Lastra, C., D. Alarcón, M. D. Barranco, V. Motilva and J. M. Herrerías. 2001. Mediterranean diet and health: Biological importance of olive oil. Curr. Pharm. Design. 7: 933-950.

Lang, G.A. and G.C. Martin. 1985. Ethylene releasing compounds and the laboratory modelling of olive fruit abscission vs. ethylene release. J. Amer. Soc. Hort. Sci. 110:207-211.

Lang, G. A. and G. C. Martin. 1989. Olive organ abscission: Fruit and leaf response to applied ethylene. J. Amer. Soc. Hort. Sci. 114: 134-138.

Lavee, S., G. Barshi and A. Haskal. 1973. Natural fruit drop and induced abscission to facilitate mechanical harvesting of Manzaillla and Souri olives. Sci. Hort. 1: 63-75.

Lavee, S. and A. Haskal. 1975. Studies with ethephon for facilitating olive harvest. Sci. Hort. 3: 163-171.

Lavee, S. and A. Haskal. 1976. Further field studies of the mode of application and efficiency of various ethylene-releasing chemicals to facilitate olive fruit harvest. Riv. Ortof. Ital. 60: 166-175.

Lavee, S., B. Avidan and Y. Ben-Tal. 1982. Effect of fruit size and yield on the fruit removal force within and between cultivars. Sci Hort. 286:441-451.

Lavee, S., M. Nashef, M. Wodner and H. Harshemesh. 1990. The effect of complementary irrigation added to old olive trees (*Olea europaea* L.) cv. Souri on fruit characteristics, yield and oil production. J. Hort. Sci. 4:135-138.

Lavee, S and M. Wodner. 1991. Factors affecting the nature of oil accumulation in fruit of olive. J. Hort. Sci. 66: 583-591.

Lavee, S. 1996. Biology and physiology of the olive. In: *World olive encyclopaedia*. 4: 181-192.

Lavee, S., and M. Wodner. 2000. The effect of yield, harvest time and fruit size on the oil content in fruits of irrigated olive trees (*Olea europaea*), cvs. Barnea and Manzaillla. Sci. Hort. 99: 267-277.

Lavelli, V., 2002. Comparison of the antioxidant activities of extra virgin olive oils. J. Agric. Food Chem. 50: 7704-7708.

Lazzez, A., E. Perri, M. A. Caravita, M. Khlif and M. Cosentino . 2008. Influence of olive maturity stage and geographical origin on some minor components in virgin olive oil of the Chemlali variety. J. Agric. Food Chem. 56: 982-988.

Lercker, G., N. Frega, F. Bocci, and G. Servidio. 1994. ‘‘Veiled’’ extra virgin olive oils: dispersion response related to oil quality. J. Amer. Oil Chem. Soci. 71: 657-658.

Luaces, P., C. Romero, F. Gutierrez, C. Sanz, A. G.Perez. 2007. Contribution of olive seed to the phenolic profile and related quality parameters of virgin olive oil. J. Sci. Food Agri. 87: 2721-2727.

Luna, G. 2002. Characterisation of monovarietal virgin olive oils. Eur. J. Lipid Sci. Tech. 104: 614-627.

Loumou A, and C. Giourga. 2003. “Olive groves: ‘The life and identity of the Mediterranean’’. Agric. Human Values. 20: 87-95.

Mafra, I., B. Lanza, A. Reis, V. Marsilio, C. Campestre, M. De Angelis and M. A. Coimbra. 2001. Effect of ripening on texture, microstructure and cell wall polysaccharide composition of olive fruit (*Olea europea*). Physiol. Plant. 111: 439-447.

Mailer, R., D. Conlan and J. Ayton. 2005. Olive harvest : harvest timing for optimal olive oil quality. RIRDC, Barton. pp. 56-58.

Mailer, R.J., J. Ayton and D. Conlan. 2007. Influence of harvest timing on olive (*Olea europaea*) oil accumulation and fruit characteristics under Australian conditions. J. Food Agric. Enviro. 5: 58 - 63.

Mailer, R. J., J. Ayton and K. Graham. 2010. The influence of growing region, cultivar and harvest timing on the diversity of Australian olive oil. J. Amer. Oil Chem. Soc. 87: 877-884.

Martin, G. C., S. Lavee and G. S. Sibbett. 1981. Chemical loosening agents to assist mechanical harvest of olive. J. Amer. Soc. Hort. Sci. 106: 325-330.

Martin, G. C. 1986. Olive harvest in California. Olivae. 3: 11-20.

Martin, G.C., 1993. Mechanical olive harvest: Use of fruit loosening agents. Acta. Hortic. 356: 284–291.

Martin, G. C.1994. Botany of the olive. In "Olive production Manual", University of California, Division of Agriculture and Natural Resources, publication. 3353: 19-21.

Manai-Djebali, H., D. Krichéne, Y. Ouni, L. Gallardo, J. Sánchez, E. Osorio, D. Daoud, F. Guido and M. Zarrouk. 2012. Chemical profiles of five minor olive oil varieties grown in central Tunisia. J. Food Comp. Anal. 27: 109-119.

Manrique, T., H. F. Rapoport, J. Castro and M. Pastor. . 1999. Mesocarp cell division and expansion in the growth of olive fruits. Acta. Hortic. 474: 301-314.

Matos, L.C., S. C. Cunha, J. S Amaral, J. A Pereira, P. B. Andrade, R. M. Seabra and B. P. Oliveira. 2007. Chemometric characterization of three varietal olive oils (Cvs. Cobrançosa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices. Food Chem. 102: 406-414.

Matson, F. M. and S. M. Grundy. 1985. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J. Lipid Res. 26: 194-202.

Mensink, R. P. and Katan, M. B., 1992. Effects of dietary fatty acids on serum lipids and lipoproteins. Arterioscler. Thromb. Vasc. Biol. 12: 911-919.

Menz, G., F. Vriesekoop. 2010. Physical and chemical changes during the maturation of Gordal Sevillana olives (*Olea europaea* L., cv. Gordal Sevillana). J. Agric. Food Chem. 58: 4934-4938.

Metzidakis, I. 1999. Field studies for mechanical harvesting by using chemicals for the loosening of olive pedicel on cv. Koroneiki. Acta. Hortic. 474: 112-117.

Montedoro, G. F. and L. Garofolo. 1984. Caratteristiche qualitative degli oli vergini di oliva. Influenza di alcune variabili: varietà, ambiente, conservazione, estrazione, condizionamento del prodotto finito. Riv It Sost Grasse LXI: 3-11. [In Italian].

Monteleone, E., G. Caporale, L. Lencioni , F. Favati, and M. Bertuccioli. 1995. Optimization of virgin olive oil quality in relation to fruit ripening and storage. Dev. Food Sci. 37:397-418.

Morello, J. R., M. J. Motilva, T. Ramo and M. P. Romero. 2003. Effect of freeze injuries in olive fruit on virgin olive oil composition. Food Chem. 81:547-53.

Morello, J. R., M. J. Motilva, M. Tovar and M. P. Romero. 2004. Changes in commercial virgin olive oil during storage, with special emphasis on the phenolic fraction. Food Chem. 85: 357-364.

Morello, J. R., M.P. Romero, T. Ramo and M. J. Motilva. 2005. Evaluation of L-phenylalanine ammonia-lyase activity and phenolic profile in olive drupe from fruit setting period to harvesting time. Plant Sci. 168: 65-72.

Motilva, M. J., J. M. Tovar, P. M. Romero, S. Alegre and J. Girona. 2000. Influence of regulated deficit irrigation strategies applied to olive trees (Arbequina cultivar) on oil yield and oil composition during the fruit ripening period. J. Sci. Food Agri. 80: 2037-2043.

Mousa, Y. M., D. Gerasopoulos, I. Metzidakis and A. Kiritsakis. 1996. Effect of altitude on fruit and oil quality characteristics of Mastoides olives. J. Sci. Food Agri. 71: 345-349.

Mulinacci, N., C. Giaccherini, M. Innocenti, A. Romani, F.Vincieri, A. Marotta and A. Mattei. 2005. Analysis of extra virgin olive oils from stoned olives. J. Sci. Food Agri. 85: 662-670.

Nanos, G. D., T. Thomai, E. M. Sfakiotakis and N. Fitsio. 1999. Maturity indices for green olives destined to be processed as 'Spanish-style' olives. Acta. Hortic. 474: 521-524.

Nath, P., P. K. Trivedi and V. A. Sane. 2006. Role of ethylene in fruit ripening. In: Khan, N.A. (ed.). *Ethylene Action in Plants*. Springer-Verlag, Berlin, Geidelberg, pp.151-185.

Nergiz, C. and Y. Engez. 2000. Compositional variation of olive fruit during ripening. Food Chem. 69: 55-59.

Ninot, A., A. Romero, J. Tous and I. Batlle. 2012. Effect of loosening agent sprays on the efficiency of the mechanical harvesting of Arbequina olives. Hort. Sci. 47: 1419-1423.

Obied, H., P. Prenzler, D. Ryan, M. Servili. 2008. Biosynthesis and biotransformations of phenol-conjugated oleosidic secoiridoids from *Olea europaea* L. Nat. Prod. Rep. 25: 1167-1179.

Olein, W. C. and M. J. Bukovac. 1978. The effect of temperature on rate of ethylene evolution from ethephon and from ethephon-treated leaves of sour cherry. J. Amer. Soc. Hort. Sci. 103:199-202.

Olien, W. C and M. J. Bukovac. 1982. Ethylene generation, temperature responses and relative biological activities of several compounds with potential for promoting abscission of sour cherry fruit. J. Amer. Soc. Hort. Sci. 107: 1085-1089.

Olives, WA, 2015. http://www.oliveaustralia.com.au (retrieved on 23 January, 2015)

Ortega, D, G. Beltr´and M. Uceda. 2001. Influencia del riego en lalipogénesis del cv 'Arbequina'. Proc Symp Cientifico-Tecnico Expoliva, Jaén, 63-71.

Osman, M., I. Metzidakis, D. Gerasopoulos and A. Kiritsakis. 1994. Qualitative changes in olive oil of fruits collected from trees grown at two altitudes. Riv. Ital. Sos. Grasse. 71: 187-190.

Owen, R. W., A. Giacosa, W. E. Hull, B. Haubner, B. Spiegelhalder and H. Bartsch. 2000. Identification of lignans as major components in the phenolic fraction of olive oil. Clin. Chem. 46: 976-988.

Parr, A.J and G. P. Bolwell. 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. J. Sci Food Agric. 80: 985-1012.

Pastor, M., J. Castro, M. J. Mariscal, V. Vega, F. Orgaz, E. Fereres and J. Hildalgo 1999. Respuesta del olivar tradicional a diferentes estategias y dosis de agua de riego. Invest. Agric. 14: 393-404.

Patumi, M., R.d'Andria , G. Fontanazza , G. Morelli, P. Giorio and G. Sorrentino. 1999. Yield and oil quality of intensively trained trees of three cultivars of olive (*Olea europaea* L.) under different irrigation regimes. J. Hort. Sci. Biotech. 74: 729-737.

Patumi, M., R.d'Andria, V. Marsilio, G. Fontanazza, G. Morelli and B.Lanza, 2002. Olive and olive oil quality after intensive monocone olive growing (*Olea europaea* L., cv. Kalamata) in different irrigation regimes. Food Chem. 77: 27-34.

Peng, X. X. and M. Yamauchi. 1993. Ethylene production in rice bronzing leaves induced by ferrous iron. Plant Soil. 149: 227-234.

Perin, C., M. C.Gomez-Jimenez, C. Hagen, L. Dogimont, J. C. Pech, A. Latche , M. Pitrat and J. M Lelievre. 2002. Molecular and genetic characterization of a non-climacteric phenotype in melon reveals two loci conferring altered ethylene response in fruit. Plant Physiol. 129: 300-309.

Perretti, G., E. Finotti, S. Adamuccio, R. Della Sera and L. Montanari. 2000. Composition of organic and conventionally produced sunflower seed oil. J. Amer. Oil Chem. Soc. 81:1119-1123.

Pitrat, M. and J. M. Lelièvre. 2002. Molecular and genetic characterization of a non-climacteric phenotype in melon reveals two loci conferring altered ethylene response in fruit. Plant Physiol. 129: 300-309.

Polito, V. S. and S. Lavee. 1980. Anatomical and histochemical aspects of ethephon-induced leaf abscission in olive (*Olea europaea* L). Bot. Gaz. 141: 413-417.

Pranamornkith, T., A. East and J. Heyes. 2012. Influence of exogenous ethylene during refrigerated storage on storability and quality of *Actinidia chinensis* (cv. Hort16A). Postharv. Biol Tech. 64: 1-8.

Procida, G. and A. Cichelli. 1999. Evolution of antioxidant fraction of extra virgin olive oils stored in different containers. J. Comm. Sci. 38: 215-228.

Proietti, P. and A.Tombesi and M. Boco. 1994. Influence of leaf shading and defoliation on oil synthesis and growth of olive fruits. Acta Hortic. 356: 272-277.

Proietti, P., F. Famiani and A. Tombesi. 1999. Gas exchange in olive fruit. Photosynthetica. 36: 423-32.

Psaltopoulou, T., A. Naska, P. Orfanos, D. Trichopoulos, T. Mountokalakis and A. Trichopoulou. 2000. Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European prospective investigation into cancer and nutrition (EPIC) study. Am. J. Clini. Nut. 80: 1012- 1018.

Ramirez-Tortosa, M. C., C. M. Aguilera, J. L. Quiles and Gil, A. 1998. Influence of dietary lipids on lipoprotein composition and LDL Cu^{2+} induced oxidation in rabbits with experimental atherosclerosis. Biofactors. 8: 79-85.

Ranalli, A., G. De Mattia, M. Ferrante, L. Giansante. 1997. Incidence of olive cultivation area on the analytical characteristics of the oil. Riv. Ital. Sostanze Gr. 74: 501-508.

Ranalli, A., G. De Mattia, M. Patumi and P. Proietti. 1999. Quality of virgin olive oil as influenced by origin area. Grasas Aceites. 50: 249–259.

Ranalli, A., S. Contento, C. Schiavone, N. Simone. 2001. Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. Eur. J. Lipid Sci. Tech. 103: 228-238.

Rashidi, M. and K. Seyfi. 2007. Determination of cantaloupe volume using image processing. World. Appl. Sci. J. 2: 646-651.

Ravetti, L and B. McClelland. 2008. Improving the efficiency of mechanical olive harvesting: Evaluation of fruit loosening agents. Rural Industries Research and Development Corporation (RIRDC) Publication No 08/052, RIRDC Project No MOD-1A. pp. 22-31.

Reed, N. R. and H. T. Hartmann. 1976. Histochemical and ultrastructural studies of fruit abscission in olive after treatment with 2-chlorethyl-tris-(2-methoxyethoxy)-silane. J. Amer. Soc. Hort. Sci. 101: 633-637.

Reichelt, K. and M. Burr. 2000. Extra virgin: An Australian companion to olives and olive oil. Wakefield. pp.10-14

Riachy, M. E., F. Priego-Capote, L. Rallo, M. D. Luque de Castro and L. León. 2012. Phenolic profile of virgin olive oil from advanced breeding selections. Span. J. Agric. Res. 10: 443-453.

Riley, F. R. 2002. Olive oil production on bronze age Crete: nutritional properties, processing methods and storage life of Minoan olive oil. Oxf. J. Archaeol. 21: 63-75.

Ripa, V., F. De Rose , M. L. Caravita, M. Parise, E. Perri, A. Rosati, S. Pandolfi, A. Paoletti, G. Pannelli, G. Padula , E. Giordani, E. Bellini, A. Buccoliero and C. Mennone. 2008. Qualitative evaluation of olive oils from new olive selections and effects of genotype and environment on oil quality. Adv. Hort. Sci. 22: 95-103.

Rivas, A., A. Sanchez-Ortiz, B. Jimenez, J. García-Moyano and M. L. Lorenzo. 2013. Phenolic acid content and sensory properties of two Spanish monovarietal virgin olive oils. Eur. J. Lipid Sci. Tech. 115: 621-630.

Roca, M. and M. I. Minguez-Mosquera. 2001. Change in the natural ratio between chlorophylls and carotenoids in olive fruit during processing for virgin olive oil. J. Amer. Oil Chem. Soc. 78: 133-138.

Romero, M. P., M. J. Tovar, J. Girona and M. J. Motilva. 2002. Changes in the HPLC phenolic profile of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under different deficit irrigation strategies. J. Agri. Food Chem. 50:5349–54.

Rotondi, A., A. Bendini, L.Cerretani, M. Mari, G. Lercker and T. Toschi. 2000. Effect of olive ripening degree on the oxidative stability and organoleptic properties of cv. Nostrana di Brisighella extra virgin olive oil. J. Agri. Food Chem. 52: 3649-3654.

Royer A., F. Laporte, S. Bouchonnet and Y. Communal. 2006. Determination of ethephon residues in water by gas chromatography with cubic mass spectrometry after ion-exchange purification and reprivatisation with N-(tertbutyl-dimethylsilyl)-N-methyl trifluoroacetamide. J. Chromatogr. 1108: 129-135.

Ryan, D., M. Antolovich, P.Prenzler, K.Robards and S. Lavee. 2002. Biotransformations of phenolic compounds in (*Olea europaea* L.). Sci. Hort. 92: 147-176.

Salas, J., J. Sánchez, U. S. Ramli, A. M. Manaf, M. Williams and J. L. Harwood. 2000. Biochemistry of lipid metabolism in olive and other oil fruits. Prog. lipid. Res. 39: 151-180.

Salvador, M., F. Aranda, and G. Fregapane. 2001. Influence of fruit ripening on Cornicabra virgin olive oil quality. A study of four successive crop seasons. Food Chem. 73: 45-53.

Salvador, M. D., F. Aranda, S. Gomez-Alonso and G. Fregapane. 2003. Influence of extraction system, production year and area on Cornicabra virgin olive oil: a study of five crop seasons. Food Chem.80: 359-366.

Sánchez, A. H and M. Fernández. 1991. Correlaci´on entre material grasa, azucares reductores humedad en la pulpa de aceitunas. Grasas Aceites. 42: 414-419.

Sanchez, J. and J. L. Harwood. 2002. Biosynthesis of triacylglycerols and volatiles in olives. Eur. J. Lipid Sci. Technol. 104:564-73.

Sanchez, J. 1994. Lipid photosynthesis in olive fruit. Progr. Lipid Res. 33: 97-104.

Servili, M. R., S. Selvaggini. A.Esposto, G. Taticchi, and G. Montedoro. 2004. Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. J Chromatogra. 1054: 113-27.

Servili, M., S. Esposto, E. Lodolini, R. Selvaggini, A. Taticchi, S. Urbani, G. Montedoro and M. Serravalle. 2007. Irrigation effects on quality, phenolic composition, and selected volatiles of virgin olive oils cv. Leccino. J. Agric. Food Chem. 55: 6609-6618.

Sessiz, A., M. Özcan. 2006. Olive removal with pneumatic branch shaker and abscission chemical. J. Food Eng. 76: 148-153.

Sheng, J., J. Ye, L. Shen and Y. Luo. 2003. Effect of lipoxygenase and jasmonic acid on ethylene biosynthesis during tomato fruit ripening. Acta. Hortic. 620: 119-125.

Shulman, Y. and S. Lavee. 1979. Fruit development and maturation as effected by treatments of auxin. Riv. Ortof. Ital. 63: 31-40.

Singh, S. P., Z. Singh and E. E. Swinny. 2009. Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina* Lindell). Postharv. Biol. Tech. 53: 101-108.

Skevin, D., D. Rade, D. Strucelj, Z. Mokrovcak , S. Nederal and D. Bencic. 2003. The influence of variety and harvest time on the bitterness and phenolic compounds of olive oil. Eur. J. Lipid Sci. Technol. 105: 536-541.

Spennemann, D. H. R. 2000. Centenary of olive processing at Charles Sturt University. Charles Sturt University, Wagga Wagga. 20p.

Stefanoudaki, E., F. Kotsifaki and A. Koutsaftakis. 1999. Classification of virgin olive oils of the two major Cretan cultivars based on their fatty acid composition. J. Amer. Oil Chem. Soc. 76: 623-626.

Taylor, R. and J. Burt. 2007. *Growing olives in Western Australia*. Department of Agriculture and Food, Western Australia, South Perth, WA. P 22.

Tharanathan, R. N., H. M. Yashoda and T. N. Praba. 2006. Mango (*Mangifera indica* L.), The King of Fruits - An Overview. Food Rev. Int. 22: 95-123.

Therios, I. 2005. *Olive Production*. Gartaganis Publications, Thessaloniki, Greece, p. 476 .

Therios, I. 2009. *Olives, crop production science in horticulture*,18 CABI, Oxfordshire, UK, pp: 9-15.

Tognetti, R., R. D'Andria, A. Lavini, A and G. Morelli, G. 2006. The effect of deficit irrigation on crop yield and vegetative development of *Olea europaea* L. (cvs. Frantoio and Leccino). Eur. J. Agron. 25: 356-364.

Tombesi, A. 1994. Olive fruit growth and metabolism. Acta. Hortic. 356:225-232.

Tombesi, A., M. Pilli, M. Boco and P. Proietti. 1994. Evolution of olive fruit respiration, photosynthesis and oil composition during ripening. Acta. Hortic. 356: 278-445.

Touss, J., J. Lloveras and A. Romero. 1995. Effect of ethephon spray treatments on mechanical harvesting and oil composition of Arbequina olives. J. Amer. Soc. Hort. Sci. 120: 558-561.

Tovar, M. J., M. J. Motilva and M. P. Romero. 2001. Changes in the phenolic composition of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under linear irrigation strategies. J Agric. Food Chem. 49: 2-8.

Tovar, M. J., M. P. Romero, J. Girona and M. J. Motilva. 2002. L-Phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L. cvArbequina) fruit grown under different irrigation regimes. J. Sci. Food Agric. 82: 892-898.

Trentacoste, E. R., C. M .Puertas and V. O. Sadras. 2010. Effect of fruit load on oil yield components and dynamics of fruit growth and oil accumulation in olive (*Olea europaea* L.). Eur. J. Agron. 32: 249-254.

Tripoli, E., M. Giammanco, G. Tabacchi, D. Di Majo, S. Giammanco and M. La Guardia. 2000. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nutr. Res. Rev. 18:98-112.

Tura, D. O. Failla, S. Pedò, C. Gigliotti, D. Bassi and A. Serraiocco. 2008. Effects of seasonal weather variability on olive oil composition in Northern Italy. Acta Hortic. 791: 769-776.

Tuyns, A., M. Haelterman and M. Kaaks. 1987. Colorectal cancer and the intake of nutrients: oligosaccharides are a risk factor, fats are not. A case control study in Belgium. Nutr. Cancer. 10: 81-85.

Uceda, M. and L. Frias. 1975. Harvest time. Evolution of the fruit oil content, oil composition and oil quality. Proceedings of Segundo Seminario Oleícola Internacional, Cordoba, Spain, 125-130.

U.S. National Research Council. 1989. Diet and Health: Implications for reducing chronic disease risk. National Academic Press: Washington, DC. pp. 67-82.

Vazquez, A., R. Maestro and E. Graciani.1971. Cambios en los polifenoles de la aceituna durante la maduracion. Grasas Aceites. 22: 366-370.

Vinha, A. F., F. Ferreres, B. M. Silva, P. Valentao, A. Goncalves and J. A. Pereira. 2005. Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): Influences of cultivar and geographical origin. Food Chem. 89: 561-568.

Visioli, F. and C. Galli. 1998. Olive oil phenols and their potential effects on human health. J. Agri. Food Chem. 46: 4292-4296.

Vita, F. D. Pacheco, A. Olguín Pringles, L. Bueno, A. Carelli and F. Capraro. 2011. Effect of regulated deficit irrigation strategies on productivity, quality and water use efficiency in a high-density Arbequina olive orchard located in an arid region of Argentina. Acta. Hortic. 888: 81-88.

Wahbi, S., R. Wakrim, B. Aganchich and R. Serraj. 2005. Effects of partial rootzone drying (PRD) on adult olive tree (*Olea europaea*) in field conditions under arid climate. Physiological and agronomic responses. Agric. Ecosyst. Environ. 106: 289-301.

Wallander, E. and V. A. Albert. 2000. Phylogeny and classification of Oleaceae based on rps16 and trnL-F sequence data. Am. J. Bot. 12:1827-41.

Weis, K. G., R. Goren, G. C. Martin and B. D. Webster. 1988. Leaf and inflorescence abscission in olive. I. Regulation by ethylene and ethephon. Bot. Gaz. 149: 391-397.

Weis, K. G., B. D. Webster, R. Goren and G. C. Martin. 1991. Inflorescence abscission in olive: Anatomy and histo-chemistry in response to ethylene and ethephon. Bot. Gaz. 152: 51-58.

Wodner, M., S. Lavee and E. Epstein. 1988 Identification and seasonal changes of glucose, fructose and mannitol in relation to oil accumulation during fruit development in *Olea europaea* L. Sci. Hortic. 36:47-54.

Young, R. H and O. L. Jahn. 1972. Degreening and abscission of citrus fruit with preharvest application of (2-chloroethyl)-phosphonic acid (ethephon). J. Amer. Soc. Hort. Sci. 97: 237-241.

Yousfi, K., R. M.Cert and J. M.García. 2006. Changes in quality and phenolic compounds of virgin olive oils during objectively described fruit maturation. Eur. Food Res.Technol. 223: 117-124.

Yousfi, K.,J. A. Cayuela and J. M. García. 2009. Effect of temperature, modified atmosphere and ethylene during olive storage on quality and bitterness level of the oil. J. Amer. Oil Chem. Soc. 86: 291-296.

Yousefi, Z., M. Almassi, A. A. Zeinanloo, R. Moghadasi and M. B. Khorshidi. 2010. A comparative study of olive removal techniques and their effects on harvest productivity. J. Food Agric. Environ. 8: 240 -243.

Youssef, N. B.,W. Zarrouk, A. Carrasco-Pancorbo, Y. Ouni, A. Segura-Carretero and A. Fernandez-Gutierrez. 2010. Effect of olive ripeness on chemicals properties and phenolic composition of Chetoui virgin olive oil. J. Sci. Food Agric. 90: 199-204.

Zaharah, S. S. 2011. Hormonal regulation of mango fruit ripening. PhD Thesis, Department of Environment and Agriculture, Curtin University of Technology, Western Australia. 333p.

Zahra, T. A. 2014. Effect of different ethephon concentrations on olive fruits harvesting at different orchard locations. Palest. Tech. Univ. Res. J. 1: 09-13.

Zarrouk, M. and D. Daoud Ben Miled. 2008. Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. Food Chem. 109: 743-754.

Zeleke, K., R. Mailer, P. Eberbach and J.Wünsche. 2012. Oil content and fruit quality of nine olive (*Olea europaea* L.) varieties affected by irrigation and harvest times. New Zeal. J. Crop. Hort. Sci. 40: 241-252.

Appendix 1: Quality standards of the four main classes of olive oil

Quality parameters	Classes of olive oil			
	Extra Virgin Olive Oil (EVOO)	Virgin Olive Oil (VOO)	Olive Oil (OO)	Pomace Olive Oil (POO)
Acidity level (% m/m oleic acid)	≤ 0.8	≤ 2.0	≤ 1.0	≤ 1.0
Peroxides value (mEq/kg oil)	≤ 20	≤ 20	≤ 15	≤ 15
K232 (%)	≤ 2.50	≤ 2.60	N/A	N/A
K270 (%)	≤ 0.22	≤ 0.25	≤ 0.90	≤ 1.70
C16:0 (%)	7.5-20.0	7.5-20.0	7.5-20.0	7.5-20.0
C18:0 (%)	0.5-5.0	0.5-5.0	0.5-5.0	0.5-5.0
C18:1 (%)	55.0-83.0	55.0-83.0	55.0-83.0	55.0-83.0
C18:2 (%)	3.5-21.0	3.5-21.0	3.5-21.0	3.5-21.0

Source: International Olive Council (2007)