Phenolic profile and Sensory Attributes of New Zealand 'Frantoio' Extra Virgin Olive Oil (EVOO)

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Abstract

Commercial production of premium extra virgin olive oil (EVOO) in New Zealand (NZ) is gaining international reputation due to distinctive composition and flavour characteristics of the oils. There were two main objectives of this research. The first was to characterise ‘Frantoio’ olive oil produced from olives from three orchards in different NZ growing regions (Hawke’s Bay, Bombay and Waiheke Island) in terms of phenolic profile, sensory attributes and composition of fatty acids and tocopherols. The oil was also analysed for specific EVOO chemical quality index required by the International Olive Council (IOC). The second objective of this study was to investigate the potential application of Maturity Index (MI), dry matter, total solids and fruit firmness as measures of the olive maturity specific to NZ growing climate. The olives were harvested at different maturities after full bloom and oil was extracted by accelerated solvent extraction (ASE) throughout the season and by cold pressed (CP) extraction at two defined harvest maturities. The CP oils were evaluated by a trained panel for sensory attributes and a chemical test for the intensity of bitterness was carried out.

Total phenolics were found to decrease with maturity in the oil from the three orchards. Several simple phenols, hydroxytyrosol, tyrosol, vanillic acid, vanillin, *p*-coumaric acid and ferulic acid were quantified in oils using HPLC. Hydroxytyrosol and tyrosol declined with fruit maturity. Luteolin was the main flavonoid identified. Significant quantitative differences between the orchards was found in the concentration of secoiridoids, which were identified as the main phenolic compounds 3,4-DHPEA-EDA, *p*-HPEA-EDA, 3,4-DHPEA-EA, *p*-HPEA-EA (p-value<0.05). The total phenolic content (R^2=0.79), intensity of bitterness (IB) (R^2=0.81) and oleuropein bitter index (OBI) (R^2=0.93) were found to be highly correlated to the perceived sensory bitterness. Strong positive correlations between sensory bitterness, pungency and concentration of individual secoiridoids demonstrated the important role played by these compounds in the flavour of virgin olive oil (R^2>0.73). Oils from the different orchards were found to have different distinctive aroma and flavour attributes for NZ ‘Frantoio’ VOO which were described as ‘bitter salad’, fresh ‘green bean’, ‘vanilla toffee’, ‘walnut’ and ‘black pepper’. A strong correlation was also found between total phenolics and oxidative stability determined by a Rancimat® (R^2=0.96). All the CP oils were classified as ‘extra virgin’ by the chemical and sensory tests, except for the Hawke’s Bay late harvest that was found to be rancid due to prolonged effect of frost. Orchard differences in % fatty acid composition were observed. Oleic acid was the lowest in Waiheke (77.3 %), compared to Hawke’s Bay (80.5 %) and Bombay (81.3 %) while α-linolenic acid was (1.01 %) in the Bombay VOOs. The concentration of tocopherols was similar in VOOs from the three orchards. Oil accumulation (% dry weight) showed a good indication of olive maturation. Total solids, maturity index and firmness showed strong correlations with % oil content (R^2>0.6). This study revealed orchard differences in phenolic content and sensory attributes of the oils studied. In conclusion, climate and location influenced fruit colouration, growth, accumulation of oil, total solids and composition of phenolics and fatty acids in the oil. To achieve balanced oils with acceptable sensory levels of bitterness, pungency and fruitiness, olives should be harvested at the point of maturity which will provide not only maximum yield but also balanced chemical composition, particularly phenolics.

Dedication

I dedicate this work to my beloved wife, Rosemary Achola

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I am indebted to my supervisor A/Prof. Marie Wong for her guidance from the beginning to the end of this project. I am particularly grateful for her enthusiasm in this research and her broad expertise that has been a priceless inspiration to me.

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I am very humbled and grateful to my heavenly father for giving me the strength and wisdom in my education. Amen.

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Acronyms and Abbreviations

3, 4- DHPEA	3,4-dihydroxyphenyl ethanol or hydroxytyrosol
3,4-DHPEA-AC	3,4-dihydroxyphenylethanol acetate or hydroxytyrosol acetate
3,4-DHPEA-EA	3,4-dihydroxyphenyl-ethanol linked to elenolic or dialdehydic form of oleuropein aglycone
3,4-DHPEA-EDA	3,4-dihydroxyphenylethanol linked to dialdehydic form of elenolic acid or dialdehydic form of decarboxymethyl oleuropein aglycone
AOCS	The American oil chemists' society
ASE	Accelerated solvent extraction
C12:0	Lauric acid
C14:0	Myristic acid
C16:0	Palmitic acid
C16:1	Palmitoleic acid
C17:0	Heptadecanoic acid
C17:1	Heptadecenoic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
C20:0	Arachidic acid
C20:1	Eicosenoic acid
C22:0	Behenic acid
C22:1	Erucic acid
C24:0	Lignoceric acid
CP	Cold pressed
DAFB	Days after full bloom
EA	Elenolic acid
EVOO	Extra virgin olive oil
FAME	Fatty acid methyl ester
FFA	Free fatty acids
g	Grams
HPLC	High performance liquid chromatography
IOC	International olive council

LLE	Liquid-liquid- extraction
M	Molar
meq/kg	Milli equivalents per kilogram
mg	Milligram
MI	Maturity index
mL	Millilitre
MUFA	Monounsaturated fatty acid
p- HPEA	*p*-hydroxyphenyl ethanol or tyrosol
p-HPEA-EDA	*p*-hydroxyphenylethanol linked to dialdehydic form of elenolic acid or dialdehydic form of decarboxymethyl ligstroside aglycone
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
SFA	Saturated fatty acid
VOO	Virgin olive oil
µL	Microlitre

Chapter One: Introduction

1.1 Background

Virgin olive oil (VOO) is the oil obtained only by mechanical processing of whole healthy fruit of the olive tree (*Olea europaea* L.)*,* without further treatment other than washing, filtration, decantation or centrifugation (Angerosa, 2002; Inarejos-García *et al.*, 2010; Kiritsakis, 1998g; Therios, 2009d). This extraction process maintains the composition, nutritional quality and also ensures the distinctive aroma and flavour of the oil (Andjelkovic *et al.*, 2009; Bendini *et al.*, 2007; Cicerale *et al.*, 2009; Haddada *et al.*, 2007; Salvador *et al.*, 1998; Servili *et al.*, 2004). Over the past two decades, numerous studies have been reported on the role of olive composition on the nutritional benefits, sensory attributes and shelf life of the olive oil (Andjelkovic *et al.*, 2009; Aparicio *et al.*, 1997; Ayton *et al.*, 2001; Lercker *et al.*, 2007; Nergiz & Engez, 2000; Ranalli *et al.*, 2000; Salvador *et al.*, 1998; Sinesio *et al.*, 2005; Stefanoudaki *et al.*, 2000). Specifically, there have been vibrant research interests on phenolic composition of olive oil (Amiot *et al.*, 1986; Bendini *et al.*, 2007; Bianco *et al.*, 2002; Bonoli *et al.*, 2004; Boskou *et al.*, 2005; Cinquanta *et al.*, 1997; Damak *et al.*, 2008; Hajimahmoodi *et al.*, 2008; Ocakoglu *et al.*, 2009; Oliveras-Lopez *et al.*, 2007; Servili *et al.*, 2007; Servili & Montedoro, 2002; Silva *et al.*, 2006; Tovar *et al.*, 2001; Yousfi *et al.*, 2006; Youssef *et al.*, 2010). Analytical studies have revealed that olive oil contains over thirty phenolic compounds, the highest level among all other vegetable oils (Hrncirik & Fritsche, 2004; Servili *et al.*, 2009; Silva *et al.*, 2006).

The increasing interests in olive oil phenolic compounds are a result of their diverse role in defining the quality of olive oil. For example, phenolic compounds contribute to specific positive sensory characteristics such as bitterness, pungency, astringency and green-leaf aroma of the oil (Andrewes *et al.*, 2003; Aparicio *et al.*, 1997; Bendini *et al.*, 2007; Ranalli *et al.*, 2000; Rotondi *et al.*, 2008). Phenolic compounds also contribute to oxidative stability of the oil, an important role in extending shelf life of the oil (Bendini *et al.*, 2007; Servili *et al.*, 2009). Several journals have reported that consumption of olive oil lowered the incidence of atherosclerosis, cardiovascular disease, and certain types of cancer (breast, ovarian, colon, endometrial), due to high content of oleic acid and antioxidants, namely, phenolics, tocopherols and carotenes in olive oil (Garcia-Gonzalez *et al.*, 2008; Giugliano & Esposito, 2005; Pérez-Jiménez *et al.*, 2006; Shahtahmasebi, 2003; Therios, 2009c; Tripoli *et al.*, 2005; Visioli & Galli, 1998).

However, phenolic compounds are a very diverse group of compounds and their qualitative and quantitative composition can be influenced by several factors (Dai & Mumper, 2010). In olives, the content and composition of phenolic compounds can be significantly influenced by the olive growing climate, harvest maturity and agronomic practices (Aguilera *et al.*, 2005; Aparicio *et al.*, 1994; Ben Temime *et al.*, 2006; Cinquanta *et al.*, 2001; Esti *et al.*, 1998; Guerfel *et al.*, 2009; Issaoui *et al.*, 2010; Krichene *et al.*, 2009). Records on how these factors impact on phenolic composition and other chemical composition, notably fatty acids, tocopherols and sterols of olives growing under New Zealand climate are very scarce, especially, in comparison to rigorous research reported on these aspects in the Northern hemisphere (Aparicio *et al.*, 1994; Ben Temime *et al.*, 2006; Kalua *et al.*, 2005; Krichene *et al.*, 2009; Lazzez *et al.*, 2008; Mousa *et al.*, 1996; Ranalli *et al.*, 1999; Vinha *et al.*, 2005; Youssef *et al.*, 2010). There is no study reported on the impact of New Zealand growing climate and harvest maturity on phenolics and chemical composition of olive oil. A comprehensive study on these factors specific to New Zealand growing conditions is therefore necessary for various reasons.

Firstly, the knowledge can be crucial for olive growers in New Zealand to consistently produce high quality olive oils with the right balance of bitterness, pungency and of excellent stability. Such knowledge will also be pivotal to ensure New Zealand olive oils continue to win international awards, and to ensure NZ growers remain very competitive in the market. Secondly, an understanding of the fruitiness, bitterness and pungency profiles of the oils can be particularly important to define the style and how appropriately the oil can be used by consumers. This knowledge can also enable diversification of the oil blends (Gawel & Rogers, 2009). Finally, the study of olive composition in NZ is vital to reveal other important components of olive oil such as fatty acids and tocopherols and how their contents are influenced by the growing environment.

1.2 Aims of the study

1.2.1 Hypotheses

- Regional climatic differences in New Zealand can significantly influence the growth physiology and maturation of olive, and the resultant composition of both major and minor components of the oil, particularly, phenolic composition.

- Phenolic composition is influenced by the growing environment and harvest maturity.

- Phenolic composition influences sensory characteristics and oxidative stability of olive oil.

1.2.2 Main study objectives

- The principle aim of this research was to determine the impact of harvest maturity on the individual phenolic composition and their influence on the sensory profile of NZ virgin olive oil (VOO). VOO was obtained from the olive cultivar 'Frantoio' growing from three different locations (Hawke's Bay, Bombay-South Auckland and Waiheke Island) in the North Island of New Zealand.

- To characterise the individual phenolic compounds and their concentrations which are known to contribute to bitterness, pungency and astringency of the oil

- To correlate the characteristic sensory profile evaluated by a trained panel to chemical tests for bitterness intensity.

- To determine the oxidative stability of the oils and correlate this to phenolic composition

- To characterise the oils in terms of composition (fatty acids and tocopherols) and examine quality indices of the oil (free fatty acids, % FFA and peroxide value, PV).

1.2.3 Practical applications

- NZ olive growers and processers (especially from the selected orchards) will be more informed on how their particular growing climate, location and or agronomic practices can impact on the individual phenolic profiles, fatty acid composition, sensory and stability of the oil.

- The collective knowledge obtained from this study will be invaluable to producing high quality olive oils with the right balance of bitterness, pungency and fresh flavour, which is characteristic of the premium olive oils from NZ.

- In the future, olive growers will be more informed on the locations to plant olives by considering impact of climatic conditions.

Chapter Two: Literature Review

2.1 Definitions and classification of olive oil

Olive oil can be classified into different categories following the specific chemical parameters and sensory standards established by the International Olive Council (IOC) (Aparicio *et al.*, 1997; Edwards, 2007; Kiritsakis, 1998d; Mailer, 2007b; Monteleone *et al.*, 1998; Nam & Lee, 2007). The chemical tests are required for percentage of free fatty acids (% FFA), peroxide value (PV) and specific extinction coefficients K_{232} and K_{270} measured at wavelengths 232 nm and 270 nm, respectively. FFA test indicates the level of hydrolytic break down of triglycerides and is expressed as the percentage of oleic acid in the oil (Kiritsakis, 1998d). PV test is a measure of primary oxidation products (hydroperoxides) in the oil while spectrophotometric measurements at 232 nm and 270 nm indicate the degree of primary and secondary oxidation products in the oil (Kiritsakis, 1998d). In all the categories, the olive oil must have no sensory defects when evaluated by an IOC certified panel. Based on these guidelines, olive oils can be categorised as follows:

2.1.1 Virgin Olive Oil (VOO)

Olive oil is classified as ‘virgin’ when the oil is obtained from the healthy fruit of the olive tree *(Olea europaea L.)* only by mechanical means under conditions including washing, decantation, centrifugation and filtration that do not lead to alterations in the oil (IOC, 2006). The VOO can be consumed in the crude form without any refining process (Caponio *et al.*, 2001; Kiritsakis, 1998a). On the basis of chemical tests, VOO must have a maximum acidity, of no more than 1.5 to 2.0 g/100 g and meets the requirements for the organoleptic characteristics of this oil category (IOC, 2006). VOO is further classified into the following categories:

2.1.2 Extra Virgin Olive Oil (EVOO)

EVOO is the best grade of olive oil (IOC, 2006). The VOO is classified as ‘extra virgin’ when it has a maximum acidity, of no more than 0.8 g/100 g and when it meets the requirements for the organoleptic characteristics of the EVOO category (IOC, 2006). In New Zealand and Australia, EVOO must have FFA level of less than 0.8 g/100 g (Edwards, 2007; Mailer, 2007a; Mailer, 2005; Mailer, 2007b).

2.1.3 Ordinary VOO

Ordinary VOO has a maximum acidity expressed as oleic acid, of no more than 3.3 g/100 g and must meet the requirements for the organoleptic characteristics and other quality criteria of this oil category (IOC, 2006).

For all the classes of VOO mentioned above, the IOC standard requires PV of less than 20 meqO_2/kg olive oil. A summary of the specific chemical limits including the limits for other classes of olive oil not mentioned above are shown in Table 2.1.

Table 2.1: The International Olive Council (IOC) limits for percentage of free fatty acidity (% FFA), peroxide value (PV) and K-values applied to different categories of olive oil.

	IOC limit				
Category	FFA (%w/w as oleic acid)	PV(meqO_2/kg oil)	K232	K270	∆K
Extra virgin olive oil	≤ 0.8	≤ 20.0	≤ 2.50	≤ 0.22	≤ 0.01
Virgin olive oil	≤ 2.0	≤ 20.0	≤ 2.60	≤ 0.25	≤ 0.01
Ordinary virgin olive oil	≤ 3.3	≤ 20.0	nl	≤ 0.30	≤ 0.01
Lampante oil	≤ 3.3	nl	nl	nl	nl
Refined olive oil	≤ 0.3	≤ 5.0	nl	≤ 1.10	≤ 0.16
Olive oil	≤ 1.0	≤ 15.0	nl	≤ 0.90	≤ 0.15
Crude olive oil residue	nl	≤ 15.0	nl	nl	nl
Refined olive residue oil	≤ 0.3	≤ 15.0	nl	≤ 2.0	≤ 0.20
Olive residue oil	≤ 1.0	≤ 15.0	nl	≤ 1.70	≤ 0.18

nl No IOC limits: Source: Angerosa *et al.* (2006) and Kiritsakis *et al.* (1998)

2.2 History of olive production in New Zealand

On a global basis, most olive trees are cultivated in the Mediterranean region with more than 75 % of the global production in Europe which cultivates over 500 million trees (Therios, 2009b). World production of olive oil is dominated by the EU countries (60 %), Greece (17 %), Tunisia (10 %), Syria (4 %), Turkey (3 %), Morocco (2 %), Algeria (1 %) and others (3 %) (Therios, 2009b). This global statistics puts the commercial production of olive oil in New Zealand at a very infant stage. Recent data from Olives New Zealand suggested that just over one million olive trees exist in New Zealand, planted across the North and South Island of the country (ONZ, 2006).

The origin of olive production in New Zealand can be dated back to the era of Charles Darwin in 1830. Early records documented by Charles Darwin when he visited the Northern region of New Zealand showed that olives existed in the country as early as 1830 (Edwards, 2006b). Generally, details about the existence of olives in New Zealand are very limited, although some documents dated in 1843 can reveal that olives were growing successfully in the Auckland region (Edwards, 2006b). Moreover, at about the same period, an early colonist guide reported that New Zealand climatic condition could be suitable for growing the early olive varieties, and records at the time revealed that early olive varieties such as ‘Caillet’, ‘Blanquette’ and ‘Picholene’ were planted in Auckland (Edwards, 2007).

It took nearly a century for the New Zealand olive industry to experience some growth, although very slow growth with barely any commercial operations at the time. In the early 1990s, olive production made some notable progress when the first association of olive growers was formed to undertake comprehensive research within the industry and to capture global networking opportunities (Edwards, 2006a; Edwards, 2007). The association which is now called Olives New Zealand (ONZ) was named New Zealand Olive Association at the time of its formation. In 1996 the industry attained recognition by the then International Olive Oil Council (IOOC) ‘observer status’, and a further achievement in the industry was in 1998 when the first oil tasting panel for virgin olive oil was established (Edwards, 2007).

Over the past decade, the New Zealand olive industry has shown consistent expansion in olive cultivation across both North and South Islands of the country (ONZ, 2006). Recent data from Olives New Zealand (ONZ), indicates over one million olive trees now exist in New Zealand (ONZ, 2006), with most plantations aiming at commercial production of extra virgin olive oil. The same organisation has also reported a continuous rise in extra virgin olive oil produced in New Zealand between 2004 (120,000 litres) and 2006 (190,000 litres). From this date onwards, production of EVOO in NZ has nearly doubled and this has also been accompanied by notable distinctions in the quality of the EVOO produced. For example, reports have revealed that these oils have intense flavours that distinguish them from oils produced overseas, including Australia (Edwards, 2006a; Edwards, 2007).

However, there are very scarce studies relating to olive maturation, growing climate, cultivar selection, harvest maturity, oil composition, genetic diversification and organoleptic attributes specific to New Zealand growing conditions while there are sound scientific research from overseas, including Australia, (Aguilera *et al.*, 2005; Allalout *et al.*, 2009; Andjelkovic *et al.*, 2009; Ayton *et al.*, 2007; Ayton *et al.*, 2001; Brenes *et al.*, 1999; Criado *et al.*, 2004; Diraman & Dibeklioglu, 2009; Gomez-Rico *et al.*, 2008; Mailer, 2007a; Mailer, 2005; Mailer *et al.*, 2002; Tous *et al.*, 1997). Most importantly, there is no scientific work reported on the impact of New Zealand growing climate and or location on phenolics and composition of olive oil. Moreover, preliminary results from research carried out by Plant and Food Research and Massey University in New Zealand have found that Northern hemisphere data on olive maturity cannot be applied to New Zealand (Requejo-Jackman *et al.*, 2009; Wong *et al.*, 2009).

2.3 Significance of phenolic profile and composition database for NZ EVOO

About 2.7 million tonnes of olive oil is produced globally, with major contribution from Spain, Italy, Greece, Turkey and France as well as Australia, California and Tunisia (Brenes *et al.*, 1999; Therios, 2009b). The expansion of global olive oil trade have also led to a rising concern regarding adulteration of olive oil with either lower quality olive oil (refined or pomace) or other vegetable oils such as corn, peanut, cottonseed, sunflower, soybean and poppy seed oils (Gurdeniz & Ozen, 2009). Obviously, the infiltration of cheaper adulterated olive oil from overseas can be harmful to the NZ olive industry and could also threaten the health of consumers (Flores *et al.*, 2006; Gurdeniz & Ozen, 2009; Wesley *et al.*, 1995; Wesley *et al.*, 1996).

For this reason, the authentic quality of EVOO is monitored by the International Olive Council (IOC) to ensure that olive oil sold to consumers are extra virgin and have the composition and sensory profile that falls within the IOC standards (Fritsche & Hrncirik, 2007; Kiritsakis, 1998d; Mailer, 2007b; Pajuelo, 2004; Psomiadou *et al.*, 2003; Tsimidou, 2006).

The international regulations on quality of olive oil have prompted several researchers to establish compositional databases for EVOO obtained from different olive varieties and growing regions that would enable easy authentication of olive oil (Aparicio *et al.*, 1994; Aparicio & Luna, 2002; Beltrán *et al.*, 2005; Bouaziz *et al.*, 2004; Caponio *et al.*, 2001; Criado *et al.*, 2004; Gallina-Toschi *et al.*, 2005; Guerfel *et al.*, 2009; Gurdeniz *et al.*, 2008; Iacumin *et al.*, 2009; Issaoui *et al.*, 2010; Rotondi *et al.*, 2004; Salvador *et al.*, 2003; Tura *et al.*, 2009). Results from most of these studies are in fact projecting that the phenolic profiles could be used as a marker for regional and varietal authenticity of EVOO. For example, significant qualitative and quantitative differences in individual phenolic compounds have been reported due to both cultivar differences (Aguilera *et al.*, 2005; Dabbou *et al.*, 2009a; Vinha *et al.*, 2005) and growing regions (Criado *et al.*, 2004; Guerfel *et al.*, 2009; Issaoui *et al.*, 2010; Mousa *et al.*, 1996; Ranalli *et al.*, 1997; Ranalli *et al.*, 1999; Ranalli *et al.*, 2005; Ravetti, 2009; Romero *et al.*, 2004b).

EVOO authentication is increasingly becoming very common in the Northern hemisphere. To date, over 79 extra virgin olive oils have received the European Protected Origin Denomination (POD) mark, as a result of their organoleptic characteristics and particular composition profile, Italy alone has 30 POD oils (Cerretani *et al.*, 2006). Obviously, such a trend in regional authentication of EVOO can place New Zealand at high commercial risk, because, there is no chemical composition data published for EVOO produced in New Zealand. It is important that New Zealand closes this knowledge gap on the chemical composition of EVOO it produces. It is also vital that New Zealand growers are informed on the effect of NZ climate, growing location and several agronomic practices that are equally necessary for producing EVOO of consistent and superior quality.

2.4 The morphology and composition of olives

2.4.1 Characteristics and major composition

Olive fruit are solely obtained from the olive tree, *Olea europaea.* The fruit shown in (Figure 2.1) is characterised as an oval-shaped drupe, consisting of two main parts: pericarp (epicarp and mesocarp) and endocarp (kernel or pit) (Boskou, 2006; Kiritsakis, 1998f). The pericarp accounts for up to 83 percent of the whole fruit size while the size of the endocarp may vary between 13 percent and 30 percent of the fruit (Boskou, 2006; Therios, 2009f).

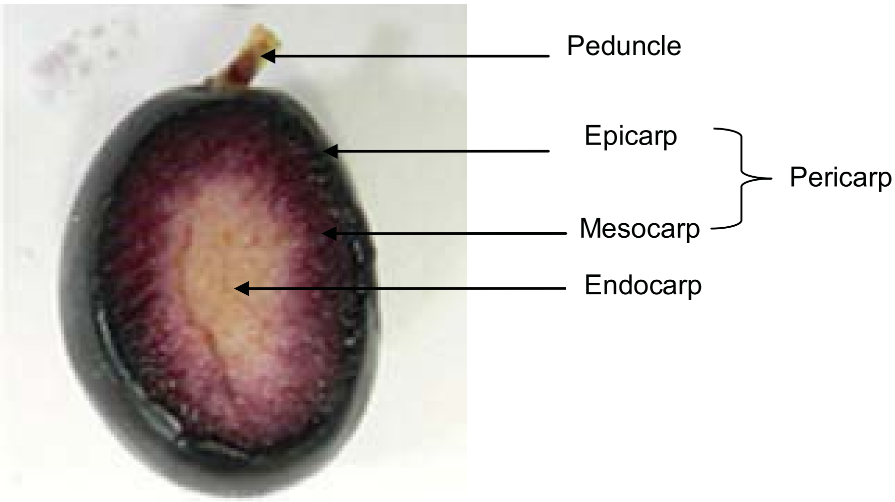


Figure 2.1: Transverse section of a ripe olive showing major components.

The average chemical composition of fresh ripe olive includes water 50%, protein 1.6%, oil 22%, carbohydrates 19%, cellulose 5.8% and ash 1.5% by dry weight of the fruit (Boskou, 2006; Conde *et al.*, 2008). In terms of the location of oil in the drupe, the pericarp contains 96 to 98 percent of the total amount of oil while the remaining 2 to 4 percent of oil is in the endocarp (Kiritsakis, 1998f). Boskou (2006) suggested that the unique flavour and fragrance of olive oil was a result of the formation and vast concentration of oil in the drupe. The other important constituents of olive fruit are pectins, organic acids, oleuropein, pigments, simple sugars, vitamins and glycosides (Boskou, 2006; Conde *et al.*, 2008; Kiritsakis, 1998f).

The weight and shape of an olive is often an important characteristic of the olive cultivar (Therios, 2009f). Although some varieties of olives may weigh as much as 20 grams, most varieties including ‘Frantoio’ weigh from 1.5 to 12 grams. During maturation, fruit weight gradually increases to an optimum level, after which any reduction in weight is due to loss of moisture (Boskou, 2006; Conde *et al.*, 2008).

2.4.2 Phenolic compounds and their distribution in olive

Phenolic compounds can be broadly defined as the secondary metabolites with an aromatic ring bearing one or more hydroxy substituents (Ryan *et al.*, 2002a; Ryan & Robards, 1998). Plant phenolic compounds are however, specifically defined as the secondary aromatic compounds characteristically derived from the shikimate pathway and phenylpropanoid metabolism shown in Figure 2.2 (Ryan & Robards, 1998). The phenolic compounds in olive are generally referred to as ‘polyphenols’, however, this terminology is pinned only on the metabolic pathway of these compounds; it unsatisfactorily defines certain phenolic compounds in olive because not all phenolic compounds in olive have the phenolic group and or the aromatic ring in their structure (Ryan *et al.*, 2002b; Ryan & Robards, 1998).

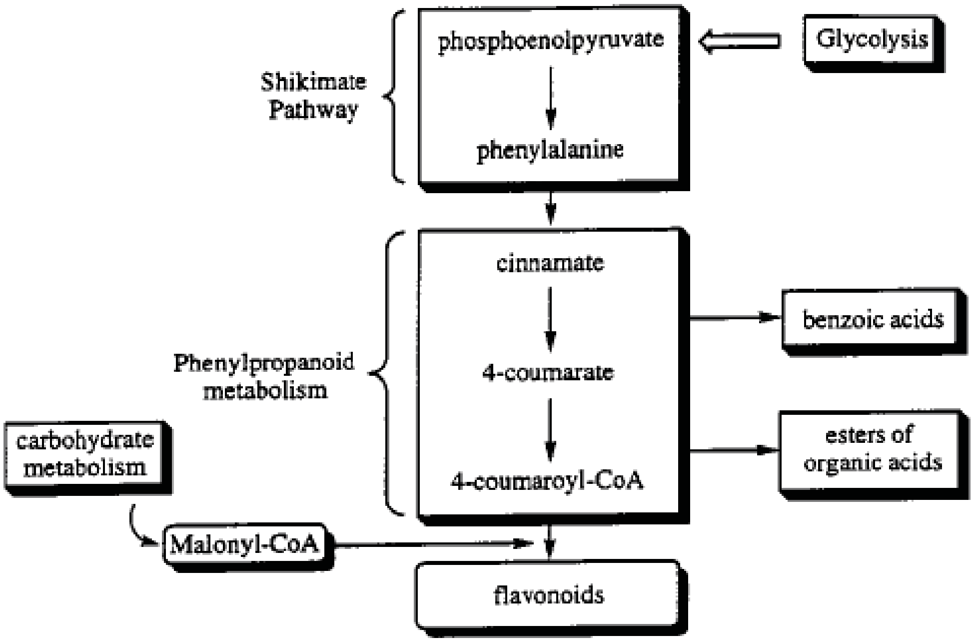
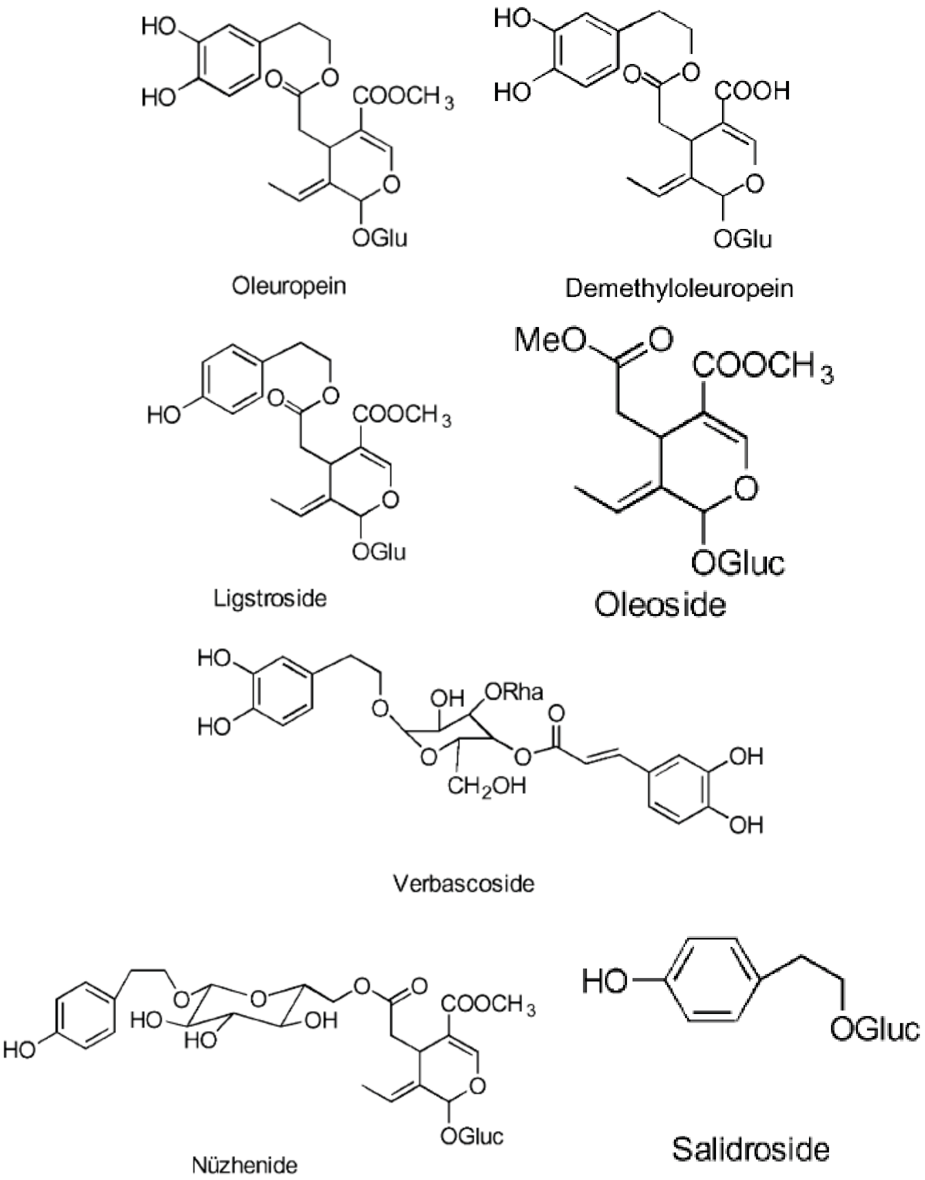


Figure 2.2: The shikimate and phenylpropanoid metabolic pathways of phenolic compounds. Source: Ryan and Robards (1998).

Phenolic compounds are virtually distributed in all parts of the olive fruit however, there are significant differences in concentration and nature of the compounds in different parts of olive; the greatest concentration being in the pulp and oil (Montedoro *et al.*, 1992b; Obied *et al.*, 2008b; Romero *et al.*, 2004a; Ryan *et al.*, 2002a; Ryan & Robards, 1998; Ryan *et al.*, 1999; Therios, 2009f). The main phenolic oleosides in the mesocarp of olive includes oleuropein, demethyloleuropein and ligstroside, whereas verbascoside is the main hydroxycinnamic phenolic derivative of the olive fruit (Servili *et al.*, 1999a; Soler-Rivas *et al.*, 2000; Therios, 2009f). Oleuropein is an ester which consists of hydroxytyrosol and elenolic acid. Oleuropein is the major phenolic compound in olive fruit, which can be as much as 14 % in dried fruit (Therios, 2009f). As the olive fruit matures the concentration of oleuropein decreases and hydroxytyrosol, a hydrolysis product of oleuropein increases (Amiot *et al.*, 1986; Amiot *et al.*, 1989). The oleuropein content also depends on the variety and origin of the olive, hence, the Greek and Italian cultivars contain greater amounts of oleuropein in comparison with Spanish and Portuguese (Therios, 2009f). In addition, salidroside, nüzhenide and nüzhenide oleoside have been reported in the seed of olive at all stages of maturity (Ryan *et al.*, 2002a; Ryan *et al.*, 2002b; Ryan *et al.*, 2003). Figure 2.3 illustrates the structure of main phenolic compounds in olive fruit.



Oleuropein

Demethyloleuropein

Ligstroside

Oleoside

Verbascoside

Nüzhenide

Salidroside

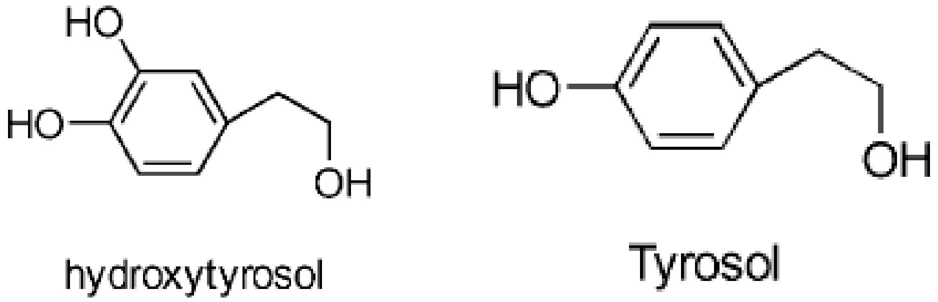
Figure 2.3: Main phenolic compounds in the pulp and seed of olive. Source: Ryan *et al.* (2002).

2.4.3 Phenolic compounds in virgin olive oil (VOO)

The phenolic compounds found in virgin olive oil originate in the fruit. Phenolic compounds of virgin olive oils are the most important minor components in the oil responsible for key organoleptic characteristics of bitterness and pungency and for oxidative stability of the oil through radical scavenging antioxidative activity (Amiot *et al.*, 1986; Andrewes *et al.*, 2003; Angerosa & Di Giovacchino, 1996; Angerosa *et al.*, 2004; Bendini *et al.*, 2007; Oliveras-Lopez *et al.*, 2007; Ryan & Robards, 1998; Soler-Rivas *et al.*, 2000). The phenolic compounds are essentially the heterogeneous mixture of compounds that are formed from the hydrolysis of main phenolic constituents of olive fruit, namely, oleuropein, ligstroside, aglycones and related compounds during maturation and processing of the olives (Carrasco-Pancorbo *et al.*, 2005; Conde *et al.*, 2008; Hrncirik & Fritsche, 2004).

Several researchers have attempted to identify the full phenolic profile in virgin olive oil in order to characterise them into various classes and functionalities (Bonoli *et al.*, 2004; Boskou *et al.*, 2006b; Carrasco-Pancorbo *et al.*, 2006a; Gomez-Alonso *et al.*, 2002; Haddada *et al.*, 2008; Owen *et al.*, 2003; Owen *et al.*, 2000a; Paiva-Martins & Pinto, 2008; Servili *et al.*, 1999b; Sivakumar *et al.*, 2005; Stefanoudaki *et al.*, 2000; Vinha *et al.*, 2005). Continued research on this topic coupled with recent advancement in technologies has also led to the discoveries of new phenolic molecules while there are still more unknown compounds of phenolic nature being discovered in virgin olive oil (Brenes *et al.*, 2000; Owen *et al.*, 2003; Paiva-Martins & Pinto, 2008; Selvaggini *et al.*, 2006). These studies have therefore enabled identification of phenolic compounds into different classes.

Three of the phenolic compounds found in virgin olive oil are the glycoside oleuropein, hydroxytyrosol (3,4-dihydroxyphenyl ethanol (3,4-DHPEA)) and tyrosol (4-hydroxyphenyl ethanol (p-HPEA)). These compounds are related structurally (Figure 2.4). Hydroxytyrosol and tyrosol are structurally identical except that hydroxytyrosol posses an extra hydroxy group in the *meta* position.



hydroxytyrosol Tyrosol

Figure 2.4: Structures of hydroxytyrosol and tyrosol commonly found in virgin olive oil. Source: Ryan *et al.* (2002).

2.4.4 Classification of phenolic compounds in VOO

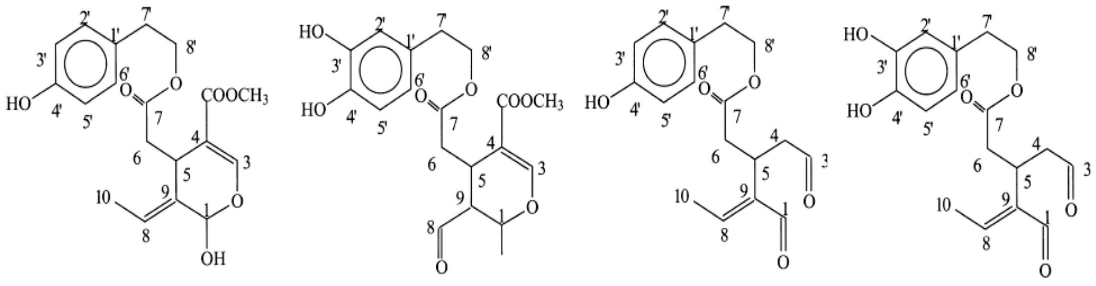
On the basis of their chemical structures, phenolic compounds in olive oil can be grouped into five major classes.

2.4.4.1 Secoiridoids

Secoiridoids are compounds that are usually glycosidically bound and produced from the secondary metabolism of terpenes (Obied *et al.*, 2008b). The secoiridoids in olive oil are formed by the hydrolysis of oleuropein, demethyloleuropein and ligstroside in a reaction catalysed by endogenous *β*-glucosidases during crushing of olives and malaxation of the paste (Conde *et al.*, 2008; Obied *et al.*, 2008b). The secoiridoids formed are partitioned between the oily layer and the vegetation water due to their amphiphilic characteristics, albeit they are more concentrated in the vegetation fraction because of their polar functional groups (Bendini *et al.*, 2007). Oleuropein, demethyloleuropein, ligstroside and nüzhenide (Figure 2.3) are the most abundant secoiridoid glucosides in the olive fruit (Servili *et al.*, 1999a; Servili *et al.*, 1999b). From the secoiridoids, simple phenols such as hydroxytyrosol and tyrosol are released by hydrolytic mechanisms, especially during storage of the oil (Bendini *et al.*, 2007; Gutfinger, 1981; Tsimidou, 1998).

Montedoro *et al.* (1972) cited in Montedoro *et al.* (1992) first identified secoiridoids as the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol 3,4-DHPEA (3,4-DHPEA-EDA) or tyrosol *p*-HPEA (*p*-HPEA-EDA). They also identified secoiridoids as an isomer of oleuropein aglycone (3,4-DHPEA-EA) and the ligstroside aglycone (*p*-HPEA-EA). Figure 2.5 illustrates the structural configuration of these secoiridoids. The existence of these compounds in VOO was later confirmed by other authors (Bendini *et al.*, 2007; Brenes *et al.*, 1999; Dabbou *et al.*, 2009b; De La Torre-Carbot *et al.*, 2005; Haddada *et al.*, 2008; Montedoro *et al.*, 1992b; Montedoro *et al.*, 1993; Owen *et al.*, 2000b; Servili *et al.*, 1999b).

The concentration of secoiridoids varies significantly in olive oils (Baiano *et al.*, 2009b; Boskou *et al.*, 2005; Brenes *et al.*, 1999). These variations have been attributed to the olive cultivar potential, maturity stage fruit was harvested, seasonal variations, technological conditions applied during extraction and storage condition of the oil (Soler-Rivas *et al.*, 2000). Small fruit cultivars had high oleuropein and low verbascoside contents, while large fruit cultivars are characterised by low oleuropein and high verbascoside contents, indicating different cultivars have different phenolic compositions (Amiot *et al.*, 1989; Soler-Rivas *et al.*, 2000; Therios, 2009f).



I II III IV

Figure 2.5: Structural configuration of secoiridoid derivatives and phenyl alcohols identified in olive oil. (I) ligstroside aglycon (*p*-HPEA-EA) (II) isomer of oleuropein aglycon (3,4-DHPEA-EA) (III) the dialdehydic form of elenolic acid linked to *p*-DHPEA (*p*-DHPEA-EDA) (IV) the dialdehydic form of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA). Source: (Servili & Montedoro, 2002).

Another finding by Esti *et al.* (1998) suggested that the occurrence of demethyloleuropein only in 'Leccino' and 'Coratina' could be of particular interest because it was not present in all the other six varieties they tested; the olives were obtained in the same area over 2 years. They thus proposed this group of phenolics can be used as a varietal marker. The other secoiridoids, salidroside, nüzhenide, nüzhenide oleoside (Figure 2.3) are less reported in olive oil because of their very low concentrations and because they are only found in the seed (stone) at all ripening stages (Amiot *et al.*, 1986; Obied *et al.*, 2008b; Servili *et al.*, 1999a).

2.4.4.2 Phenolic acid and acid derivatives

Phenolic acids were among the first group of phenolic compounds identified in olive oil (Montedoro *et al.*, 1992a; Servili *et al.*, 1999b; Servili *et al.*, 2009; Servili *et al.*, 2008; Tsimidou *et al.*, 1996). The phenolic acids in olive oil include caffeic acid, vanillic acid, syringic acid, *p*-coumaric acid, *o*-coumaric acid, protocatechuic acid, sinapic acid, *p*-hydroxybenzoic and gallic acid (Figure 2.6). Phenolic acids contain two distinguishing structural frameworks of carbon consisting of hydroxycinnamic acid and hydroxybenzoic acid. Recent research findings have suggested phenolic acids can be used as potential markers of geographical origin or the olive variety (Buiarelli *et al.*, 2004; Carrasco-Pancorbo *et al.*, 2005; Cartoni *et al.*, 2000; Pancorbo *et al.*, 2004). With this view, Pancorbo *et al.*(2004) studied the phenolic acids from several monovarietal olive oils of six Spanish olive cultivars and found that trans-cinnamic acid, sinapinic acid, caffeic acid and 3,4-dihydroxyphenylacetic acid appeared as possible potential markers of geographical origin or olive variety.

Phenolic acids have also been associated with colour and sensory qualities, as well as with the health-related and antioxidant properties of foods (Nergiz & Unal, 1991). The quantity of phenolic acids in olive oils is however relatively low. In a recent review Bendini *et al.* (2007) reported that the quantity of several phenolic acids in olive oils was less than 1 mg of analyte per kilogram of olive oil.

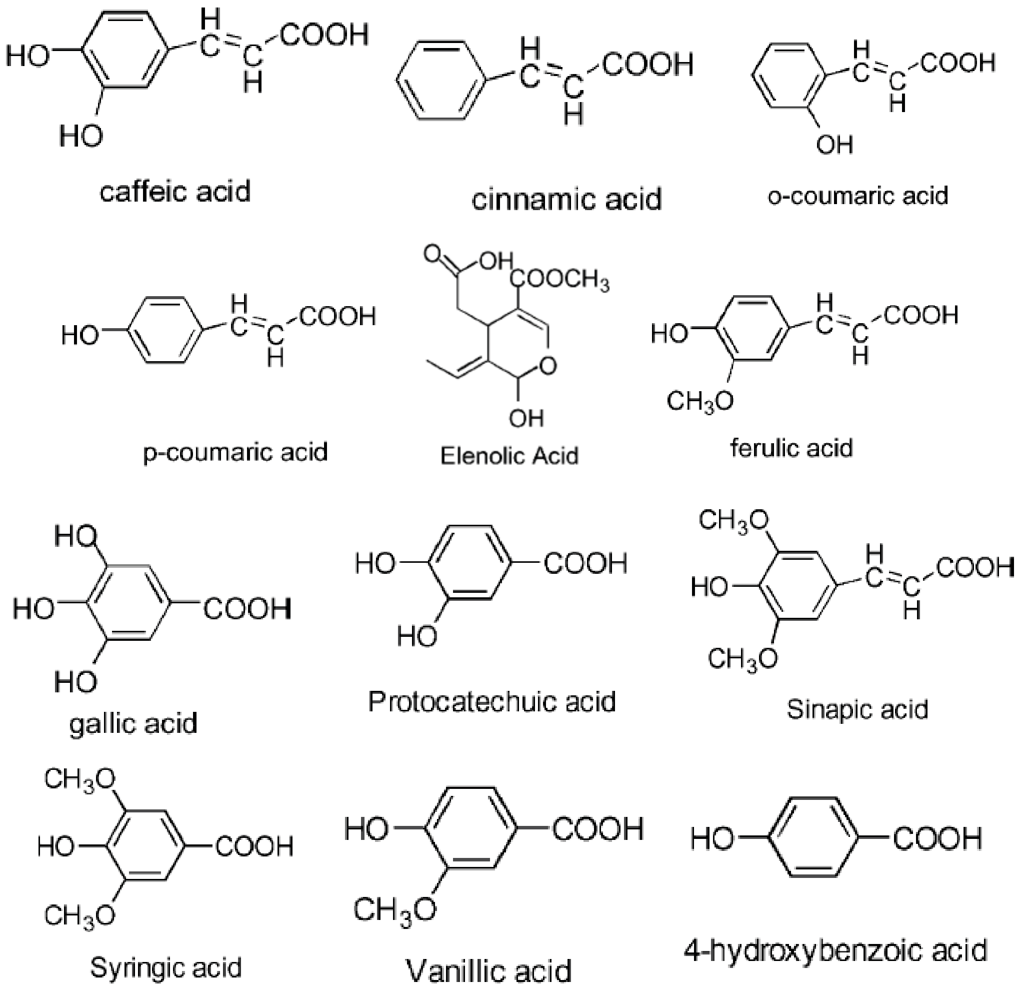


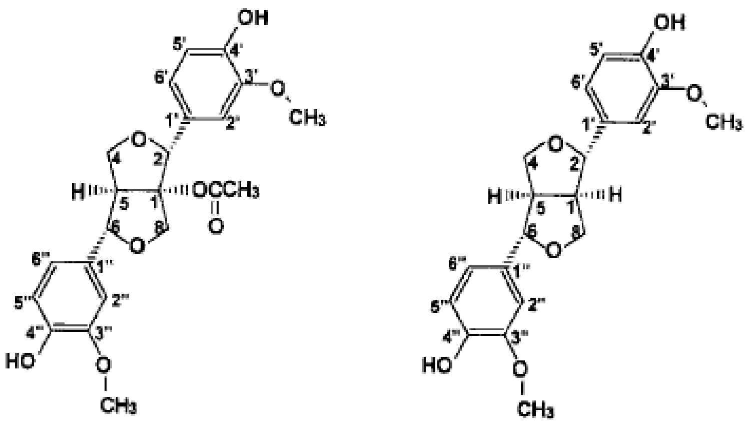
Figure 2.6: The main phenolic acids in olive oil. Source: Ryan *et al.* (2002)

2.4.4.3 Phenolic alcohols

Phenolic alcohols in olive oil include 3,4-dihydroxyphenyl ethanol (3,4-DHPEA) and *p*-hydroxyphenyl ethanol (*p*-HPEA) (Kiritsakis, 1998a). The concentration of phenolic alcohols is generally low in fresh oils but increases during oil storage due to the hydrolysis of secoiridoids such as 3,4-DHPEA-EDA, *p*-HPEA-EDA and 3,4-DHPEA-EA containing 3,4-DHPEA and *p*-HPEA in their molecular structures (Brenes *et al.*, 1999; Montedoro *et al.*, 1992a).

2.4.4.4 Lignans

Lignans can be found in the olive pulp and in the woody portion of the seed. The two most important lignans (Figure 2.7) present in virgin olive oil include (+)-1-acetoxypinoresinol and (+)-1-pinoresinol (Brenes *et al.*, 2000; Owen *et al.*, 2000a). Lignans are released in VOO without any biochemical modification during the mechanical extraction process (Carrasco-Pancorbo *et al.*, 2006b; Mulinacci *et al.*, 2006; Oliveras-Lopez *et al.*, 2007; Owen *et al.*, 2000a). In fact, one major difference between secoiridoids and lignans is that the concentrations of lignans show less variation because their occurrence in the oil is mainly affected by agronomic conditions for cultivating the olive trees and parameters of the oil extraction process have negligible effect on their concentrations in the oil (Servili *et al.*, 2004). Owen *et al.* (2000a) found the concentrations of lignans in VOO may be up to 100 mg/kg of oil. Similar to phenolic acids, Brenes *et al.* (2002) suggested that lignans can be used as varietal markers for VOO when they reported a method to authenticate the VOO of Picual olives based on the very low content of the lignan 1-acetoxypinoresinol compared to those obtained in other olive varieties.



(+)-1-ACETOXYPINORESINOL (+)-1-PINORESINOL

Figure 2.7: Structure of lignans in VOO. Source: (Servili & Montedoro, 2002)

2.4.4.5 Flavonoids

Flavonoids are a group of polyphenolic plant, secondary metabolites (Schijlen *et al.*, 2004). They can be classified into different subclasses, namely, flavones, flavanones, flavonols, isoflavones, flavanonols, flavanols, chalcones and anthocyanins depending on the degree of unsaturation and degree of oxidation of the three-carbon skeleton (Robards & Antolovich, 1997). In olive oil, luteolin and apigenin (Figure 2.8) are the two main flavonoids that have been identified (Brenes *et al.*, 1999; García *et al.*, 2001a; Mateos *et al.*, 2001; Montedoro *et al.*, 1993; Morello *et al.*, 2005; Ocakoglu *et al.*, 2009; Romani *et al.*, 1999; Tasioula-Margari & Okogeri, 2001a; Tovar *et al.*, 2001). Moreover, (+)-Taxifolin, a flavanol has also been identified in most Spanish virgin olive oils (Carrasco-Pancorbo *et al.*, 2005). Some other flavonol glycosides such as luteolin 7-glucoside, rutin and anthocyanins such as cyanidin and delphinidin glycosides were detected in olive fruit (Soler-Rivas *et al.*, 2000).

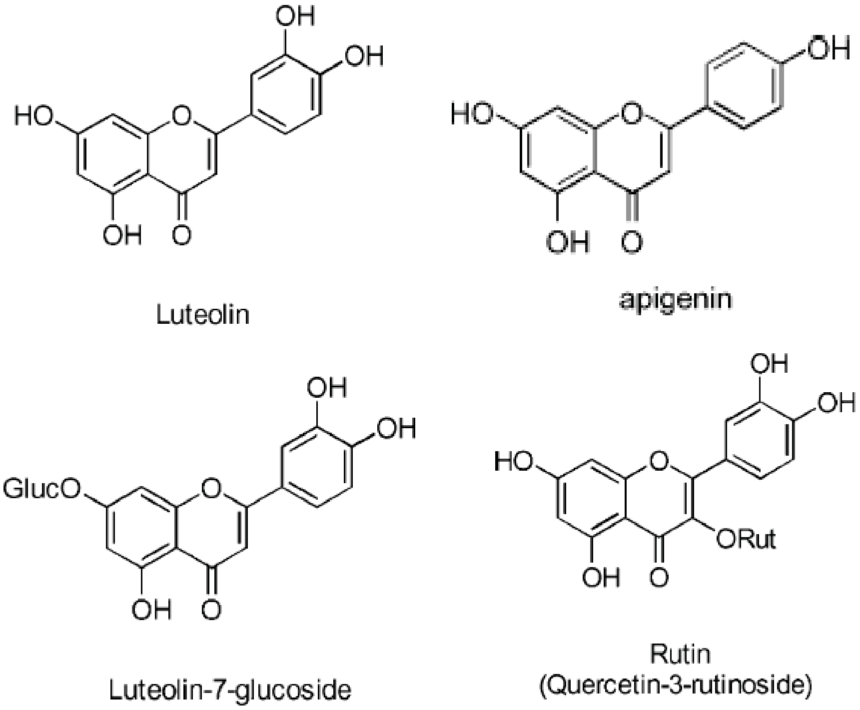


Figure 2.8: The main flavonoids in olive oil. Source: (Carrasco-Pancorbo *et al.*, 2005)

2.5 Role of phenolic compounds to sensory attributes of VOO

Phenolic compounds are responsible for the desirable characteristic organoleptic attributes of bitterness, astringency, pungency and metallic sensations in virgin olive oils (Angerosa *et al.*, 2000; Beltrán *et al.*, 2007; Inarejos-Garcia *et al.*, 2009a; Koprivnjak *et al.*, 2009; Servili *et al.*, 2004; Yousfi *et al.*, 2008). Astringency results from the interaction of phenolic compounds with proteins of mouth and saliva. The extent of this interaction depends on the level of phenolics present in the oil as well as to the number of 1,2 and 1,2,3 trihydroxy groups attached to the aromatic moieties of the phenolic molecule (Inarejos-Garcia *et al.*, 2009a; Lule & Xia, 2005). Pungency in olive oils is noted as the peppery, burning and hot sensations just after consumption of the oil (Tsimidou, 1998). Recent studies have attributed pungency in olive oil mainly due to the presence of dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol (Andrewes *et al.*, 2003), moreover, Beauchamp *et al.*(2005) attributed pungency to the ibuprofen-like compound called oleocanthal. Pungency, astringency and metallic attributes are collectively the chemesthetic perceptions that result from stimulation of the free endings of the trigeminal nerves located in the palate and gustative buds (Bendini *et al.*, 2007).

Although bitterness is a desirable characteristic of olive oil, a bitterness of high intensity can largely be unacceptable by consumers (Aparicio *et al.*, 1997; Gawel & Rogers, 2009). Studies on sensory attributes of olive oil have established that the intensity of bitterness, astringency and pungency in the olive oil largely depends on the total phenolic content of the oil; olive oils with higher total content of phenolic compounds are more bitter than those with lower content (Andrewes *et al.*, 2003; García *et al.*, 2001b; Gawel & Rogers, 2009; Skevin *et al.*, 2003). Beltran *et al.* (2007) classified VOO by its total phenolic content into four categories, in regards to bitterness perception: Phenol contents equal or lower than 220 mg/kg corresponded to non-bitter oils or oils with almost imperceptible bitterness; light bitterness corresponds to 220–340 mg/kg; bitter oils have phenol contents ranging from 340 to 410 mg/kg; and phenol contents higher than 410 mg/kg correspond to quite bitter or very bitter oils. A similar classification of the perception of bitterness in olive oil by its total phenolic content was reported (Gawel & Rogers, 2009).

For this reason, some researchers have suggested that the level of phenolic compounds can be used as a major sensory parameter when evaluating sensory quality of extra virgin olive oil (Andrewes *et al.*, 2003; Gawel & Rogers, 2009; Rotondi *et al.*, 2008; Stefanoudaki *et al.*, 2000).

However, it is still unclear what total contribution the individual phenolics make to each of these sensory attributes. For example, it is uncertain whether all bitter polyphenols are also astringent or pungent (Bendini *et al.*, 2007; Rotondi *et al.*, 2008; Tous *et al.*, 1997). Therefore, some studies have attempted to characterise the individual phenolics in VOO and their specific role to bitterness, pungency and or astringency. For example, a strong correlation has been reported between bitterness and the concentration of secoiridoid derivatives of hydroxytyrosol, and also a strong correlation between pungent and bitter attributes and the concentration of secoiridoid derivative of tyrosol (García *et al.*, 2001b; Gawel & Rogers, 2009; Skevin *et al.*, 2003; Yousfi *et al.*, 2008). Andrews *et al.* (2003) conducted sensory evaluation on individual polyphenols isolated from VOO by a group of four sensory panellists. The individual polyphenols were also analysed by HPLC. Although all the phenolic fractions they isolated were described as bitter and astringent, they concluded that the fraction containing *p*-HPEA-EDA had the most pronounced burning pungent sensation relative to the fraction containing 3,4-DHPEA-EDA that produced only a slight burning or pungent sensation on the tongue. Andrews *et al*. (2003) also indicated that the secoiridoid derivatives of hydroxytyrosol and tyrosol (i.e. 3.4-DHPEA-EDA and *p*-HPEA-EDA) have different sensory properties. Moreover, no other polyphenols from the isolated fractions produced similar intense sensations. The phenolic molecule *p*-HPEA-EDA was also reported by other authors as the principal agent for pungency of VOO (Beauchamp *et al.*, 2005; Galli, 2006).

Other studies have suggested the perceived bitterness in VOO could be due to oleuropein aglycone and isomers of oleuropein aglycone, rather than oleuropein, because their concentrations are higher than that of oleuropein in VOO (Gutiérrez-Rosales *et al.*, 1992; Gutiérrez-Rosales *et al.*, 2003; Inarejos-Garcia *et al.*, 2009a; Koprivnjak *et al.*, 2009; Mateos *et al.*, 2004; Skevin *et al.*, 2003). For example, Mateos *et al*. (2004) evaluated VOO bitterness in different olive varieties by quantifying secoiridoid derivatives. They reported the aldehydic form of oleuropein aglycones was responsible for VOO bitterness and they also found a strong correlation (r=0.96) between sensory bitterness and concentration of the secoiridoid derivatives (0.03-0.5

mmol/kg). On the other hand, when they evaluated the simple components of phenolic fraction of the oils such as hydroxytyrosol, tyrosol, vanillic acid, vanillin, *p*-coumaric acid, ferulic acid, cinnamic acid, elenolic acid, hydroxytyrosol acetate, tyrosol acetate, luteolin and apigenin, they found none to show a bitter taste. This finding confirms similar reports that bitterness was due to secoiridoid derivatives of hydroxytyrosol and tyrosol (Caponio *et al.*, 2001; García *et al.*, 2001b; Gutiérrez- Rosales *et al.*, 1992; Gutiérrez-Rosales *et al.*, 2003)

Gutiérrez-Rosales *et al.* (2003) analysed secoiridoid derivative of 20 different samples of VOO by HPLC and evaluated their intensity of bitterness on a sensory score scale of 1-5 (1 indicated imperceptible, 2 indicated slight, 3 indicated moderate, 4 indicated great, and 5 indicated extreme) by 12 sensory panellists. Their study demonstrated a strong correlation between the content of 3,4-DHPEA-EDA ($r=0.9819$ $p \leq 10^{-3}$), p-HPEA-EDA ($r=0.9830$ $p \leq 10^{-3}$) and 3,4-DHPEA-EA ($r=0.7929$ $p \leq 10^{-2}$) and bitterness intensity. By this result they were able to conclude that the bitter taste of the VOO samples was due to concentrations of 3,4-DHPEA-EDA, 3,4-DHPEA-EA and *p*-HPEA-EDA. The finding was consistent to the previous study by Mateos *et al.* (2004) who reported a good correlation between the aldehydic form of the oleuropein aglycone and bitterness.

The perceived sensory attributes in VOO can be influenced by both harvest time (maturity) and the olive variety as well the olive growing climate (Skevin *et al.*, 2003). Harvest maturity is an important factor because several chemical transformations, including the degradation of oleuropein by esterase activity lead to formation of compounds that are both phenolic and non-phenolic in nature, hence affecting the intensity of bitterness of the resultant oil (Amiot *et al.*, 1989; Psomiadou & Tsimidou, 2002; Skevin *et al.*, 2003). Rotondi *et al.* (2004) found significant reduction in bitterness and pungency with olive maturity, and this finding was particularly related to the content of secoiridoids at each stage of maturity the olive was harvested. In regards to olive variety, Amiot *et al.* (1989) reported that olive varieties with smaller drupes were more bitter and had a higher level of oleuropein. Other studies have reported pronounced difference in bitterness and phenolic content of olive oils are due to climate conditions in which the fruit was grown (Aguilera *et al.*, 2005; Aparicio *et al.*, 1994; Criado *et al.*, 2004; Guerfel *et al.*, 2009; Issaoui *et al.*, 2010; Lazzez *et al.*, 2008).

Therefore, although there is still a milestone to reach in order to fully understand the contribution of individual phenols to specific sensory attributes, it is becoming clearer that specific groups of phenolics are responsible for each of the sensory attributes of bitterness, pungency and astringency. More so, the recent advancement in technologies can in fact suggest a possible combination of an electronic-nose, an electronic-tongue and an electronic-eye for the characterisation of olive oils with different degrees of bitterness (Apetrei *et al.*, 2010).

2.6 Role of phenolic compounds to oxidative stability of VOO

Oxidation of olive oil is a gradual process that starts immediately after the oil has been extracted, but the process becomes more pronounced during storage, especially, in the presence of light or at high temperatures (Owen *et al.*, 2000b; Papadopoulos & Boskou, 1991). However, the high level of phenolic compounds in VOO ensures oxidative stability of the oil. The antioxidative properties of VOO are mainly attributed to the presence of hydrophilic phenols in the oil (Angerosa & Di Giovacchino, 1996; Baccouri *et al.*, 2008b; Baiano *et al.*, 2009a; Beltrán *et al.*, 2005; Ben Othman *et al.*, 2008; Boskou *et al.*, 2006b; Briante *et al.*, 2002b; Conte *et al.*, 2002; Franconi *et al.*, 2006; Galvano *et al.*, 2007; Hajimahmoodi *et al.*, 2008; Servili *et al.*, 2009; Servili & Montedoro, 2002; Servili *et al.*, 2004). In 1996, Baldioli *et al.* (1996) reported the oxidative capacity of *o*-diphenols such as 3,4-DHPEA, 3,4-DHPEA-EDA and 3,4-DHPEA-EA, present in olive oil was higher than that of *p*-HPEA and alpha tocopherol. In a similar study using the Rancimat test Artajo *et al*. (2006) reported in general terms, 3,4-dihydroxy and 3,4,5-trihydroxy structures linked to an aromatic ring such as oleuropein, 3,4-DHPEA-EDA, and the methylated form of 3,4-DHPEA-EA to have the highest antioxidant capacity in VOO.

Servili *et al.* (2004) found a similar result in refined olive oil and in sunflower oil which showed that the oxidative power was due to 3,4-DHPEA and the phenolic acids, namely, caffeic, *p*-coumaric, ferulic, syringic and vanillic. Carrasco-Pancarbo *et al*. (2005) also demonstrated the strongest antioxidant activity with 3,4-DHPEA, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA using three different antioxidant techniques, namely, diphenylpicrylhydrazyl-radical essay (DPPH), accelerated oxidation in a lipid model system (OSI-oxidative stability index) and an electrochemical method using flow injection analysis (FIA amperometry) and cyclic voltammetry. They found elenolic acid and lignans (+)-1-acetoxypinoresinol and (+)-1-pinoresinol as pro-oxidants. They also reported limited antioxidant activity due to the presence of a single hydroxyl group, and the presence of $–COOCH_3$ in structures such as oleuropein aglycones caused a reduction in antioxidant activity because of their inability to donate electrons.

Guerfel *et al.* (2009) found oxidative stability in VOO was positively correlated to hydroxytyrosol (r=0.77), tyrosol (r=0.66), 3,4-DHPEA-EDA (r=0.62) and 3,4-DHPEA-EA (r=0.88), and negatively correlated to vanillic acid (r=–0.72) and caffeic acid (r=–0.71). These studies can suggest that olive oils with very high levels of 3,4-DHPEA and the secoiridoids containing this compound in their molecular structures (3,4-DHPEA-EDA and 3,4-DHPEA-EA) are the natural phenolics with the highest antioxidant capacity in VOO (Artajo *et al.*, 2006; Baldioli *et al.*, 1996; McDonald *et al.*, 2001; Ninfali *et al.*, 2001; Oliveras-Lopez *et al.*, 2008; Owen *et al.*, 2003; Owen *et al.*, 2000b; Papadopoulos & Boskou, 1991; Romani *et al.*, 2007).

2.7 Factors affecting the phenolic composition of olive oil

The phenolic composition of olive oil can be influenced by different factors such as the growing climate, harvest maturity, olive cultivar, agronomic practices including irrigation or application of fertilizers, ripening hormones and the techniques employed to process and extract the oil (Abramovic *et al.*, 2007; Baccouri *et al.*, 2008c; Dabbou *et al.*, 2010b; Oliveras-Lopez *et al.*, 2007; Salvador *et al.*, 1998; Servili *et al.*, 2007; Tovar *et al.*, 2001).

2.7.1 Growing environment (climate, rainfall, humidity, altitude)

Growing environment and climatic factors such as rainfall, humidity, the duration of sunlight, temperature, altitude and latitude have a significant effect on plant physiology, and consequently on composition of the fruit and the oil extracted (Conde *et al.*, 2008; Ripa *et al.*, 2008; Ryan & Robards, 1998). These factors are also known to have influence on the development of taste and the aroma characteristics of olive oil. For example, dry climates with a lot of sunshine produce oil of good flavour and oils produced in dry seasons are characteristically sharp and bitter (Kiritsakis, 1998b).

The impact of growing climate on composition and sensory attributes of VOO has been vigorously investigated (Aguilera *et al.*, 2005; Aparicio *et al.*, 1994; Brenes *et al.*, 1999; Criado *et al.*, 2004; Giacometti & Milin, 2001; Guerfel *et al.*, 2009; Issaoui *et al.*, 2010; Krichene *et al.*, 2009; Mousa *et al.*, 1996; Zarrouk *et al.*, 2008). Criado *et al.* (2004) reported significant quantitative differences ($P<0.01$) in a wide number of phenolic compounds in VOO from 'Arbequina' variety growing in three different areas in Spain (Jaén, Lleida and Tarragona). The VOO obtained from Jaén grown at higher altitude (899 m above sea level) had significantly higher contents of hydroxytyrosol and tyrosol than oils from Tarragona growing at low altitude (between 400 and 450 m above sea level). Criado *et al.* (2004) suggested the higher levels of simple phenols could be related to more advanced maturation indexes of olive drupes in the olives from Jaén. On the other hand, Tarragona oils had significant concentration of secoiridoid derivatives, DHPEA-EDA and DHPEA-EA, which they attributed to the bitter sensory attribute and oxidative stability of the oil. The oils from Tarragona region also had the highest total phenolic content (between 92 and 111 mg/kg), oxidative stability (8.80 h) and the highest bitterness values (0.158). In another related study, (Mousa *et al.*, 1996) found olives grown in lower altitudes (100 m above sea level) produced VOO with higher phenolic contents than from oils at higher altitude (800 m above sea level).

Lower altitude has a more suitable temperature and sunlight intensity leading to an increase of carbohydrate biosynthesis and acyl building blocks for polyphenol biosynthesis (Criado *et al.*, 2004; Kiritsakis, 1998a). It also follows that within the same growing area, as the temperature decreases (higher elevation) the percentage of unsaturated fatty acids increases (Mousa *et al.*, 1996). However, a recent study on the main Tunisian cultivars, 'Chemlali' and 'Chétoui', cultivated in North and South Tunisia with different climatic conditions found higher total phenolic content in the oils obtained at higher altitude rather than lower altitude. Oils obtained from 'Chemlali' cultivated in the North (at an altitude of about 222 m) had 3 times greater phenol contents (573 mg/kg) than those in the same cultivar cultivated from the South (13 m) (173 mg/kg). A similar trend was obtained for 'Chétoui' in which the olive oil from the North had twice the phenols value (551 mg/kg), compared to the same oil from the South (274 mg/ kg). The oils from the North with higher phenolic content also had higher oxidative stability, a finding similar to what other researchers have reported (Aguilera *et al.*, 2005; Aparicio *et al.*, 1999). The high content of total phenolics and the secoiridoids, 3,4-DHPEA-EDA and 3,4-DHPEA-EA found in the VOOs at high altitude was similar to another recent study in Tunisia using VOO from the ‘Sigoise’ variety (Dabbou *et al.*, 2010a).

Another influence of growing environment on olive oil phenolics was illustrated by Aguilera *et al*. (2005) when they characterised ‘Frantoio’ and ‘Leccino’ virgin olive oil grown in two different locations, Mengibar in the open (280 m above sea level) and Cabra close to the hills (440 m above sea level), both locations in Andalusia, Spain. Their study found that the level of phenolics in both ‘Leccino’ and ‘Frantoio’ was hugely influenced by the growing environment with both cultivars responding in different ways; ‘Frantoio’ had a higher phenolic content in Cabra while ‘Leccino’ had a higher phenolic content in Mengibar. Sensorial characteristics also showed significant differences between the oils from each cultivar and location. Other than variation in phenolics, Aguilera *et al.* (2005) reported the oils at higher altitude, showed a greater content of oleic acid and higher stability, while from trees grown in the open the oils had higher tocopherol and linoleic acid contents.

The impact of growing environment seems to be more important for certain classes of phenolics and less important for others. Lignans and flavonoids have been reported as the group of phenolic compounds greatly affected by growing environment (Aparicio *et al.*, 1994; Criado *et al.*, 2004; Dabbou *et al.*, 2009a; Giacometti & Milin, 2001; Morello *et al.*, 2006; Mousa *et al.*, 1996; Tovar *et al.*, 2001). Criado *et al.* (2004) found significant differences in the concentrations of flavonoids among the different growing regions, they obtained *p*-HPEA-EDA/lignans ratio of 1.4 in Lleida region olive oils whereas the ratio in oils from Jaén and Tarragona regions was 0.7. Lignans are thus suggested important for olive characterisation based on growing region and as a varietal marker in VOO produced from different regions (Carrasco-Pancorbo *et al.*, 2006b; Criado *et al.*, 2004; Mulinacci *et al.*, 2006; Oliveras-Lopez *et al.*, 2007; Owen *et al.*, 2000a). Vinha *et al.* (2005) showed the impact of geographical location on phenolic composition in olives when they reported the phenolic profiles of Portuguese olive fruit using high performance liquid chromatography (HPLC). Their study found considerable variations in hydroxytyrosol, oleuropein and flavonoids in the olives obtained from different regions.

The above findings on the effect of growing environments on phenolic profiles have prompted the suggestion that phenolic profiles can potentially be used as markers of geographical origins (Guerfel *et al.*, 2009). Other authors have already suggested that HPLC phenolic profile could be used to classify VOO according to the fruit cultivar

(Gomez-Alonso *et al.*, 2002; Salvador *et al.*, 2003). These studies acknowledge that climate and growing region has an impact on phenolic composition of VOO (Aguilera *et al.*, 2005; Aparicio *et al.*, 1994; Criado *et al.*, 2004; Dabbou *et al.*, 2009b; Giacometti & Milin, 2001; Guerfel *et al.*, 2009; Mousa *et al.*, 1996; Vinha *et al.*, 2005).

2.7.2 The harvest maturity

The maturity stage at which an olive is harvested is one of the most important factors associated with the final composition, sensorial and quality characteristics of olive oil (Al-Maaitah *et al.*, 2009; Beltrán *et al.*, 2004a; Beltrán *et al.*, 2004b; Kalua *et al.*, 2005; Requejo-Jackman *et al.*, 2009). The olive maturation is marked by a series of physiological events and transformations, including biosynthesis of organic compounds, especially, triglycerides and aroma compounds (Ayton *et al.*, 2001; Beltrán *et al.*, 2005; Bianchi, 2003; Briante *et al.*, 2002a; Conde *et al.*, 2008; Criado *et al.*, 2004; Matos *et al.*, 2007; Salvador *et al.*, 2001a). The VOO obtained from overripe olives are characterised by low total phenols antioxidants and aromatic compounds, which leads to olive oil of inferior sensory attributes, higher peroxidation and free fatty acid content (Harwood & Sanchez, 2000). Therefore, it is crucial that olive harvest is made at the optimum maturity. However, defining optimum harvest maturity is still a major challenge for olive growers in New Zealand and overseas because of lack of clear maturity guidelines and each olive variety and growing region tends to have different profile of maturity (Ayton *et al.*, 2007; Beltrán *et al.*, 2004a; Gimeno *et al.*, 2002; Mailer *et al.*, 2007; Requejo-Jackman *et al.*, 2009; Skevin *et al.*, 2003; Wong *et al.*, 2009).

2.7.3 Importance of harvest maturity on phenolic composition

The phenolic composition of olives is dependent on its stage of maturity (Amiot *et al.*, 1986). The phenolic content decreases rapidly in an inverse relationship to oil in the fruit, as ripening progresses (Beltrán *et al.*, 2005; Conde *et al.*, 2008; Rotondi *et al.*, 2004). Specifically, the content of oleuropein decreases during olive maturation while demethyloleuropein and 2,4-dihydroxyphenylethanol increases (Amiot *et al.*, 1986; Amiot *et al.*, 1989; Brenes *et al.*, 1999; Caponio *et al.*, 2001; Esti *et al.*, 1998). Figure 2.9 shows typical relationship between phenolic content and % oil during olive ripening. The change in phenolic composition during maturation impacts on the quality of olive oil in different ways. The olives harvested at late maturity tend to have lower oxidative stability than olives harvested at early maturity (Baccouri *et al.*, 2007; Baccouri *et al.*, 2008b; Baccouri *et al.*, 2008c; Briante *et al.*, 2002a; Esti *et al.*, 1998; García *et al.*, 1996b; Gutierrez *et al.*, 1999; Rotondi *et al.*, 2004).

The rapid decline in phenolic content that occurs during ripening is due to increased hydrolytic activities of esterase enzymes that cause the degradation of oleuropein, the main phenolic compound in the unripened fruit, leading to the formation of compounds which are both phenolic and non phenolic in nature (Amiot *et al.*, 1989; Caponio *et al.*, 2001; Garcia *et al.*, 1996; Skevin *et al.*, 2003). In addition to esterase activity, Briante *et al.* (2002a) identified β-glucosidase activity to be responsible for the degradation of oleuropein during olive maturation.

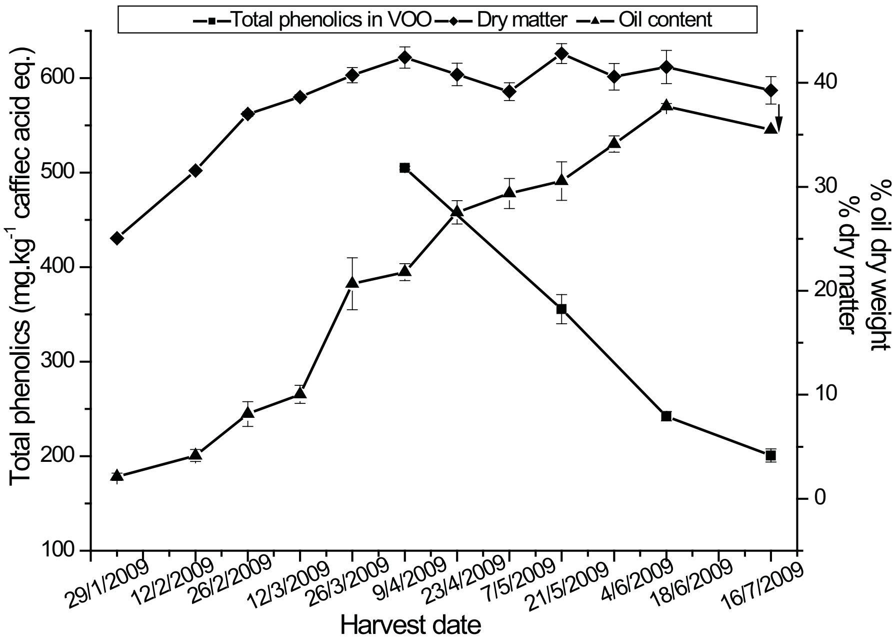


Figure 2.9: Changes in % oil, % dry matter and total phenol content of 'Frantoio' olive fruit with harvest time. Source: (Requejo-Jackman *et al.,* 2009).

2.7.4 Effect of agronomic practice and age of tree on phenolic content

Little has been reported on the effect of agronomic practice on phenolic profile of olive oils. The majority of reports on this topic have discussed the influence of irrigation on phenolic content during growth of olive fruit (Ayton *et al.*, 2007; Cicerale *et* *al.*, 2009; GóMez-Rico *et al.*, 2006; Romero *et al.*, 2002a; Romero *et al.*, 2002b; Tovar *et al.*, 2001). Tovar *et al.* (2002) reported the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol decreased from 442.8±73.5 mg/kg with minimal irrigation to 183.0±12.2 mg/kg with extensive irrigation. Furthermore, the concentration of the dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol and 4-acetoxy-ethyl-1-1,2 dihydroxybenzene decreased linearly with increased irrigation from 50.9±6.5 mg/kg to 23.1±1.3 mg/kg and 75.9±12.3 mg/kg to 51.8±5.7 mg/kg respectively. Conversely, the concentration of lignans increased with extensive irrigation from 168.5±10.7 mg/kg to 228.1±8.0 mg/kg (Tovar *et al.*, 2001).

Phenolic content in olive oil has also been closely linked to the age of the olive tree. For example, (Ayton *et al.*, 2007) reported lower phenolic concentrations in young trees of 4-5 years (100–200 mg/kg) than older trees of 6-7 years (300–500 mg/kg).

2.7.5 Processing techniques for extraction of oil

The processing of virgin olive oil involves standard procedures which include leaf removal, washing, crushing, malaxation, pressing, centrifugation and bottling (Kiritsakis, 1998g). Each of these stages needs careful execution in order to maintain superior and consistent organoleptic characteristics and oxidative stability of the oil.

2.7.5.1 Leaf removal and washing of olives

The insufficient removal of leaf can lead to crushing olives with large amount of leaves, causing revelation of organoleptic attributes of “fresh-cut” or “green” grass aroma in the oil (Di Giovacchino *et al.*, 1996; Di Giovacchino *et al.*, 2002c). The extent of this effect however depends on the type of crusher used; olives processed with leaves using more violent metallic crushers have very strong organoleptic characteristics of colour, flavour and taste than same olives crushed by stone mills which produce olive oil with less character of colour, aroma and taste from the leaves (Di Giovacchino *et al.*, 2002c). In addition, oils produced by metallic crushers with high percentage of leaves are higher in contents of chlorophyll pigment and *cis*-3-hexenal and *trans*-2-hexenal that are responsible for the higher intensity of fresh-cut grass aroma (Di Giovacchino *et al.* 2002c). High percentage of leaves does not have significant impact on the phenolic content of olive oil because the content of phenolic compounds in the leaves is similar to that of the olive flesh (Di Giovacchino *et al.*, 1996).

2.7.5.2 Olive crushing

During olive crushing it is important to break vegetative cells and to release the oil droplets in order to improve extraction yields (Kiritsakis, 1998g). The effect of olive crushing and the techniques used on yield, composition and quality indices of olive oil has been thoroughly investigated (Caponio & Gomes, 2001; Gallina Toschi *et al.*, 2004; Servili *et al.*, 2002; Veillet *et al.*, 2009). It is generally found that metallic crushers lead to more oil yield due to violent crushing than stone mill crushers, although Ranalli *et al.* (2005) reported the latter method gave a higher oil yield. Stone mill or metallic crushers do not influence the qualitative parameters, like free fatty acids, peroxide value, specific absorptions in the UV region and organoleptic assessment because these parameters depend on the cultivar, harvest time and fruit quality at the time of harvest (Di Giovacchino *et al.*, 2002c).

However, the crushing method has a significant effect on the phenolic composition of olive oil (Brenes *et al.*, 1995; Di Giovacchino *et al.*, 2002c; Obied *et al.*, 2008a). Di Giovacchino *et al.* (2002c) found the metallic crushers yielded more phenolic compounds because of more complete breakage of olive flesh that liberates higher quantities of phenolic substances, which are bound to the different cellular tissues of the olive flesh. It is however still not clear whether the higher relative increase in temperature (compared to ambient) during olive crushing by metallic crushers (13 – 14°C) compared to stone mill crushers (4 - 5°C) contributes to the observed difference in phenolic extraction (Di Giovacchino *et al.*, 2002c).

2.7.5.3 Malaxation

Malaxation is a fundamental stage for increasing extraction yields by aiding the release of oil from mesocarp cells, vacuoles and cytoplasm (Kiritsakis, 1998g). Malaxation is also important to increase the percentage of free oil as well as to aid the coalescence of small oil drops into larger ones and to aid breaking up of oil/water emulsion droplets. The efficiency of malaxation depends on several factors including rheological state of the paste, but most importantly, temperature and malaxation time are critical to maintaining both quality and yield of the oil for (Angerosa *et al.*, 2001; Di Giovacchino *et al.*, 2002a; Gallina Toschi *et al.*, 2004; Inarejos-Garcia *et al.*, 2009b; Kalua *et al.*, 2006; Parenti *et al.*, 2008; Servili *et al.*, 2008). Di Giovacchino *et al.* (2002a) found increased malaxation temperature and time led to increase in oil extraction yield (Figure 2.10). However, other studies have reported that longer malaxation time (more than 60 minutes) and higher malaxation temperature (higher than 27°C) can cause reduction in total phenolics in the oil, although without significant change in commercial qualitative parameters such as free acidity, peroxide value (PV), specific spectroscopic parameters and sensory attributes (Di Giovacchino *et al.*, 2002a; Di Giovacchino *et al.*, 2002c; Di Giovacchino *et al.*, 1994). A possible explanation to this finding is because the amounts of these qualitative parameters are mainly influenced by factors such as cultivar and quality of the olive prior to harvesting and also due to protective effect against oxidation by the phenolic compounds. These studies have found malaxation temperature and time does not affect the composition of fatty acids, sterols, aliphatic and triterpene alcohols, triterpene di-alcohols, waxes, diglycerides and triglycerides fractions of virgin olive oil. In most commercial processing plants malaxation of olive pastes occurs at 27°C for one hour (Boselli *et al.*, 2009).

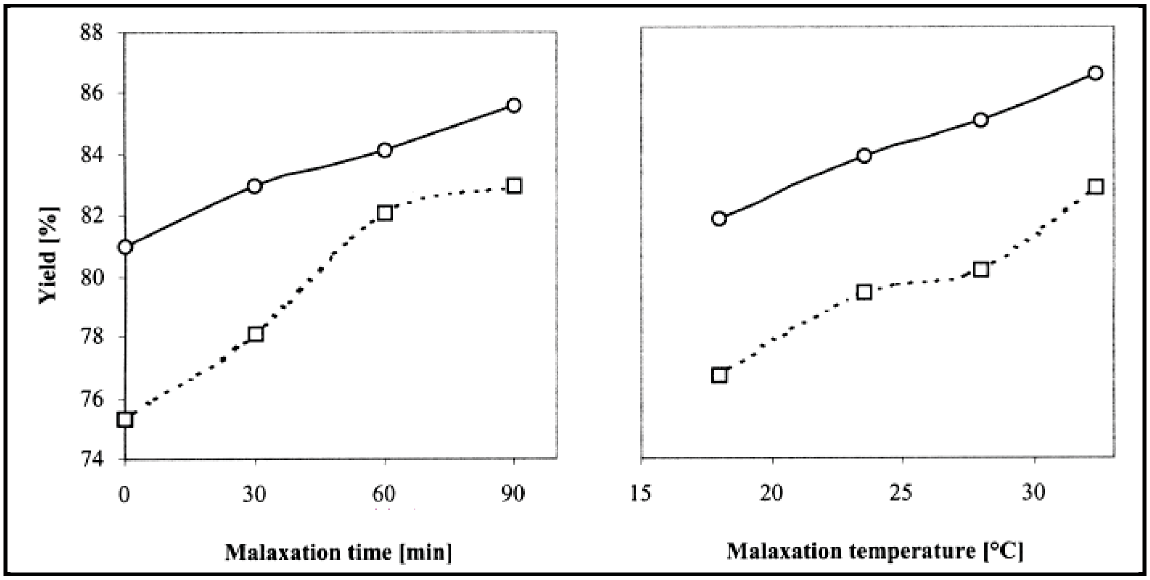


Figure 2.10: Oil extraction yields (% of oil) obtained with the 3-phases centrifugal decanter from “easy” (–o–) and “difficult” (----) olive pastes malaxed at different times and temperatures. Source: (Di Giovacchino *et al.*, 2002c)

2.7.5.4 Biochemical events of phenolic degradation during crushing and malaxation

Analysis of olive oil after processing can reveal the presence of compounds such as 3,4-DHPEA-EDA, 3,4-DHPEA-EA, *p*-HPEA–EDA and 4-HPEA-EDA that are usually not present in olive fruit prior to processing (Obied *et al.*, 2008b). It is known that these compounds are formed by the hydrolysis of oleuropein, demethyloleuropein and ligstroside as result of cellular destruction and the mixing of cellular content during crushing of olives and malaxation of paste.

During crushing and malaxation, oleuropein and demethyloleuropein are hydrolysed by endogenous glycosidase enzymes to the dialdehydic form of decarboxymethyl oleuropein aglycones (3,4-DHPEA-EDA) and the aldehydic form of oleuropein aglycones (3,4-DHPEA-EA) (Brenes *et al.*, 1995; Veillet *et al.*, 2009). Specifically, crushing and malaxation enhances endogenous hydrolysis by β-glucosidases and esterases of the glycosides in the fruit resulting in an increase in the secoiridoids of olive oil (Bendini *et al.*, 2007; García *et al.*, 2001b). The secoiridoids being amphiphilic are partitioned between the oily layer and the vegetation water, but because of their polar functional group, they tend to be more concentrated in the vegetation layer (Bendini *et al.*, 2007). Secoiridoids can also be hydrolysed during storage to release simple phenolics such as hydroxytyrosol and tyrosol (Tsimidou, 1998). Other researchers have found losses of secoiridoids and phenyl alcohol contents, possibly due to the enzymatic oxidation by the endogenous oxireductases and non enzymatic oxidation, as well as their polymerisation with polysaccharides (McManus *et al.*, 1985; Sciancalepore & Longone, 1984; Servili *et al.*, 1999b). In addition, the activities of peroxidase (POD) and polyphenoloxidase (PPO) have been reported during olive malaxing (Angerosa *et al.*, 2001; Ranalli *et al.*, 2003; Servili *et al.*, 2007; Servili *et al.*, 2002). For this reason, it is a common practice to flush olive paste with nitrogen during malaxation and the final oil product is bottled under nitrogen (Di Giovacchino *et al.*, 2002b).

2.7.5.5 Separation of oil

After malaxation, the oil is obtained from the olive paste by mechanical means which separates the liquid phase from the solid phase of the paste. Pressure is the oldest and widely used system in liquid-solid phase extraction. It is based on the principle that when a combination of solid and or liquid mass such as the olive paste is subjected to pressure, the volume of the mass decreases because the liquid phase is forced out of the mats leaving a dry solid phase called pomace (Petrakis, 2006). Percolation is another system for oil separation that takes the advantage of the different surface tensions of the liquid phases in the paste when a steel plate is plunged and subsequently withdrawn from the paste (Di Giovacchino *et al.*, 2002c). Most current percolation systems are equipped with centrifugal decanters as opposed to pressure systems. Lastly, liquid-solid phase extraction by centrifugation uses centrifugal force applied to the olive paste when diluted with lukewarm water to increase the difference between the densities of the immiscible liquids and the solid material. This separation is commonly referred to as 3-phases centrifugation as it involves separation of oil, vegetable water and the solid matter. Centrifugal separators can also be equipped with centrifugal decanters and other designs using pressure centrifugation (Di Giovacchino *et al.*, 2002c). An investigation on the efficiency of each type of separator (pressure and 3-phase) found both systems to give good yields (Di Giovacchino *et al.*, 2002c).

In recent years interests have emerged to establish whether the different systems of liquid-solid phase extraction impact on major quality attributes of the olive oil. The majority of the studies have found no significant difference in the content of free fatty acids, PV, UV absorptions and organoleptic attributes when oils are obtained from the different systems (Di Giovacchino *et al.*, 1994). When the content of phenolics was investigated between the different systems, it was found that olive oil extracted by the three-phase centrifugation had significantly lower levels of phenolics than pressure and percolation extraction methods (Bianchi, 1999; Di Giovacchino *et al.*, 1994; Welsh & Williams, 1989). As an example, Gimeno *et al.* (2002) reported that two-phase extraction yielded a phenolic concentration of 123.82 mg/kg for green olives and 80.88 mg/kg for ripe olives, while the three-phase centrifuge method yielded a phenolic concentration of 72.93 mg/kg for green olives and 42.07 mg/kg for ripe olives.

This difference in phenolic concentration was attributed to the addition of water in the three-phase centrifugation method reducing the more water-soluble phenolics from the oil phase during processing. The hydrophilic nature of phenols means that water easily lowers phenolic concentration in the aqueous phase. However, some other studies did not find the same trend between the different centrifugal systems. Such findings could be attributed to the fact that the total phenolic content of olive oils during processing stages depends mainly on the crushing method and the malaxation conditions and the addition of water may only have a small effect (Di Giovacchino *et al.*, 2002c).

2.8 Composition of fatty acid in olive oil

The fatty acid composition is one of the most invaluable components of VOO because of its widely established role in reduction of cardiovascular diseases in the so-called Mediterranean diet (Caravita *et al.*, 2007; Cicerale *et al.*, 2009; Finotti *et al.*, 2007). The greatest composition of fatty acids in VOO is monounsaturated oleic acid (C18:1) which accounts up to 80 % of the total lipids, polyunsaturated linoleic acid (C18:2) and linolenic acid (C18:3) which accounts for 2.5-20 % of the total lipids and saturated palmitic acid (C16:0) and stearic acid (C18:0) and which accounts for 10-20 % of the total lipids (Baccouri *et al.*, 2008a; Baccouri *et al.*, 2008c; Boskou *et al.*, 2006a; Conde *et al.*, 2008; Donaire *et al.*, 1984; Haddada *et al.*, 2008; Haddada *et al.*, 2007; Kiritsakis, 1998a; Salvador *et al.*, 1998). The composition of individual fatty acids in VOO is regulated by the International Olive Council as shown in Table 2.2. This composition can be used as one of the many fingerprints to verify that the oil is olive oil (Mailer, 2007b). As with other chemical standards regulated by the IOC, olive growers will always want to ensure their oil is of the best sensory and nutritional quality to consumers. In particular, analysing the fatty acid profile will provide the nutritive value of the oil and the levels of unsaturated and saturated fatty acids. Specific to this study, analysis of fatty acid profile will provide the authentic fatty composition of New Zealand 'Frantoio' VOO.

Studies conducted overseas have shown fatty acid composition of VOO can vary depending on maturity of the fruit at harvest and the growing environment and climate (Aguilera *et al.*, 2005; Finotti *et al.*, 2001; Salvador *et al.*, 1998; Salvador *et al.*, 2001a; Shibasaki, 2005; Tous *et al.*, 1997). Aparicio *et al.* (1994) and Kiritsakis (1998a) obtained an increase in oleic acid and a reduction in linolenic acid of olive fruit as the climate where olives are grown become cooler. Reduced growth temperatures increases membrane lipid unsaturation in order to maintain membrane fluidity at the low growth temperatures (Beltrán *et al.*, 2004a; Harwood *et al.*, 1994). Plants growing in cold climates have the mechanism to retain membranous fluidity by changing the fatty acid composition of membranous lipids in order to be relieved from the cold stress (Ichihara, 1999). The cold stress causes changes in the lipid biosynthetic pathways of phosphatidylglycerol synthesis and glycerol 3-phosphoric acid (G3P) acyltransferase activity in the chloroplast to maintain the photosynthetic function under low temperature condition. The difference in climate and growing environment can explain variation in fatty acid composition in VOO from the same cultivars growing in different environments. Other than the growing climate, the effect of latitude has also been reported, where the olives growing at higher altitudes consistently have higher percentage of oleic acid than those growing at lower altitudes (Aguilera *et al.*, 2005; Tous *et al.*, 1997).

Table 2.2: Fatty acid composition determined by gas chromatography (% m/m methyl esters)

Fatty acid	**Symbol**	**% (m/m methyl esters)**
Lauric acid	C12:0	Not specified
Myristic acid	C14:0	≤ 0.05
Palmitic acid	C16:0	7.5-20.0
Palmitoleic acid	C16:1	0.3-3.5
Heptadecanoic acid	C17:0	≤ 0.3
Heptadecenoic acid	C17:1	≤ 0.3
Stearic acid	C18:0	0.5-5.0
Oleic acid	C18:1	55.0-80.0
Linoleic acid	C18:2	3.5-21.0
Linolenic acid	C18:3	≤ 1.0
Arachidic acid	C20:0	≤ 0.6
eicosenoic acid	C20:1	≤ 0.4
Behenic acid	C22:0	≤ 0.2
Erucic acid	C22:1	Not specified
Lignoceric acid	C24:0	≤ 0.2

Adopted (symbol inserted) from COI/T.15/NC no.3/Rev.1. Source:(Mailer, 2007b)

The fatty acid composition also depends on the harvest maturity (Boskou *et al.*, 2006a; Paganuzzi & Leoni, 1979). During olive maturation, oleic acid is formed first and its content remains constant or shows a slight increase. As ripening progresses, the fatty acid profile shows antagonistic relationship between oleic and palmitic, palmitoleic and linoleic acids (Beltrán *et al.*, 2004a; Conde *et al.*, 2008). Kiritsakis (1998a) reported the delay in olive harvest increased the content of unsaturated fatty acids, especially linoleic acids relative to palmitic acid. Other studies have shown that the ratio between polyunsaturated and monounsaturated fatty acids (PUFA/MUFA) falls as the fruit ripens (Beltrán *et al.*, 2004a; Conde *et al.*, 2008). Olive oils with higher percentage of MUFA (oleic acid) and PUFA are more important to human health than those with higher percentage of SFA.

2.9 The composition of tocopherols in olive oil

Olive oil is composed of alpha (α), beta (β), gama (γ) and delta (δ) tocopherols as shown in Figure 2.11. The amount of α-tocopherol is the highest in olive oil while the concentrations of β, γ and δ do not exceed 100 mg/kg of oil (Boskou *et al.*, 2006a; Kiritsakis, 1998a). Like total phenols, the level of tocopherol in olive oil have been found to vary considerably mainly due to the degree of ripeness (Angerosa *et al.*, 2006). The amount of tocopherols in some Italian and Spanish olive oils is reported between 55-320 mg/kg of oil (Boskou *et al.*, 2006a) and 55-234 mg/kg (Salvador et al., 1998), respectively. Greek olive oil however, has among the highest levels of α-tocopherol ranging between 98 mg/kg to 370 mg/kg, and 60 % of the olives have values higher than 200 mg/kg (Psomiadou et al., 2000).

A high level of alpha tocopherol together with total phenolics contribute to the oxidative stability of the oil by protecting the PUFA from autoxidation (Baldioli *et al.*, 1996; Kiralan *et al.*, 2008). Tocopherols constitute the lipophilic antioxidant group and are noted for their effective inhibition of lipid oxidation in all vegetable oils (Allalout *et al.,* 2009). Tocopherols also exert vitamin potency (Baccouri *et al.*, 2008a). Although there is no IOC limit on the amount of tocopherol it should contain, a good quality olive oil should contain tocopherol concentration between 100 and 300 mg/kg of the oil (Baccouri *et al.*, 2008a).

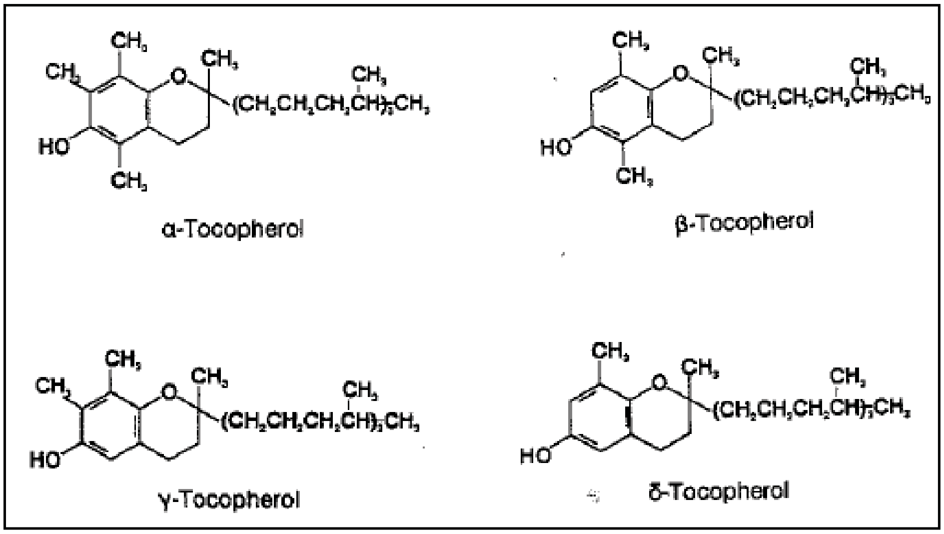


Figure 2.11: Structures of tocopherols present in olive oil (Kiritsakis, 1998g, p.123)

2.10 Conclusions from literature review

The authentic quality of olive oil is strongly attributed to the chemical composition of the olives at the time of harvest. Olive maturation is accompanied by significant changes in its major composition, notably, phenolics, triglycerides and tocopherols. The best olive oil is obtained at the time of harvest which incorporates optimum and a balanced chemical composition of the fruit.

Phenolic compounds are the most important minor components in olive oil responsible for the perceived sensory attributes (aroma and flavour), particularly bitterness and pungency. The perceived sensory attributes are influenced by specific groups of phenolic compounds, the secoiridoids, 3,4-DHPEA-EDA, 3,4-DHPEA-EA, *p*-HPEA–EDA and 4-HPEA-EDA. Phenolic compounds are also important for oxidative stability of the oil and numerous health benefits.

The composition of phenolic compounds, triglycerides and tocopherols in olive oil is influenced by the growing climate, harvest maturity and the olive variety, as well as the processing technology used in extraction of the oil.

Understanding effects of growing climate and harvest maturity on composition and sensory attributes will be fundamental for olive growers in NZ to producing consistent quality of VOO.

This project aimed to investigate the impact of different growing regions in New Zealand and the olive maturity at harvest in these regions on chemical composition and sensory attributes of the oils. Particular interest was on phenolic composition of the oil. The measure of physicochemical changes such as firmness, percent dry matter, percent oil, fruit weight and colour will be investigated as potential measures of olive maturity.

Chapter Three: Materials and Methods

3.1 Cultivar selection and fruit assessments

3.1.1 Selection of olive cultivar and growing regions adapt

The ‘Frantoio’ cultivar was selected because it is one of the most widely grown olive variety in New Zealand and due to its excellent adaptability to New Zealand growing conditions (Taber, 2005). The choice of olive growing regions and the orchards was according to their geographical location, climate and the existing agronomic practices in the orchard. Olive samples were obtained from three commercial orchards located in the North Island, Matapiro orchard in Hawke’s Bay, Simunovich orchard in Bombay (South Auckland) and Matiatia grove on Waiheke Island. Throughout this report, Matapiro orchard, Simunovich orchard and Matiatia grove will be referred to by their growing regions: Hawke’s Bay, Bombay and Waiheke, respectively. Table 3.1 shows climatic summary of the three orchards.

Table 3.1 Climatic summary showing total annual rainfall, mean daily maximum and minimum air temperatures, total growing degree days (GDD) and elevation for Hawke’s Bay, Bombay and Waiheke orchards (2010 season).

Orchard	Rainfall (mm)	Max. air temp. (°C)	Min. air temp. (°C)	Total GDD	Altitude(m)
Hawke’s Bay	1470	24 to 30	-0.7 to -3.8	1168.3	156
Bombay	1163.4	22 to 24	4 to 13	1623.4	194
Waiheke	1131	24 to 26	9 to 16	2024.1	44

Source of Data: The national climatic data base-NIWA. Data were obtained from stations nearest to the orchard: Waiheke orchard (NIWA Waiheke Island, Awaroa Valley), Bombay orchard (NIWA Pukekohe) and Hawke’s Bay orchard (Pernod Ricard, Metwatch.net.nz). Altitude was obtained from Google earth (March, 2011). Air temperatures are a range between the harvest times, January to August, 2010. GDD data was obtained from NIWA for the Waiheke and Bombay orchards and http://waterwatch.net.nz for the Hawke’s Bay orchard.

3.1.2 Tree selection in the orchards

Nine olive trees older than eight years were selected at variable locations in each orchard on varying days after full bloom (DAFB). The trees selected were a good representation of fruit load and size on trees in the orchard. The trees were tagged and grouped into three replicates (three trees per replicate).

Fruit samples were randomly obtained from the same tagged trees for the duration of this study. The harvest dates, full bloom dates and the number of DAFB fruit were harvested from each orchard are shown in Table 3.2.

Table 3.2: The harvest dates, full bloom dates and days after full bloom fruit were obtained from Hawke's Bay, Bombay and Waiheke olive orchards in 2010.

Hawke's Bay	Full bloom date	**Bombay**	Full bloom date	**Waiheke**	Full bloom date
	10/12/2009		14/12/2009		1/11/2009
Harvest date	**DAFB**	Harvest date	**DAFB**	Harvest date	**DAFB**
29th April	139	29th March	105	12th April	162
11th May	151	12th April	119	27th April	176
24th May	164	26th April	133	10th May*	190
1st June	172	10th May	147	25th May**	204
10th June	181	24th May	161	-	-
22nd June	193	3rd June	171	-	-
30th June*	201	22nd June*	190	-	-
14th July**	215	6th July**	204	-	-

*indicates the commercial harvest (CH); **late harvest (LH); DAFB days after full bloom

3.1.3 Fruit sampling (harvests) categories

The olives were harvested in three main categories; pre-commercial, commercial (CH) and two weeks after the commercial harvest (LH). At each harvest, fruit samples were couriered within 12 hours to the New Zealand Institute for Plant and Food Research located at Mt Albert, Auckland, where the fruit was immediately processed. The fruit was assessed for indices of maturity by evaluating the maturity index (MI-skin and flesh colour), dry matter, firmness, percent oil content and weight. These data were collected in order to better understand the olive maturation profile and future harvest timing for olives grown in these orchards. Oil was also extracted from fruit obtained at CH and LH using cold pressed extraction procedures (section 3.3). The grinder, malaxer and presser used were manufactured by Aquarius Axis Industrial Ltd. N.Z.

Pre-commercial harvest: About 1.5 kg of olive per replicate was picked by hand according to harvest dates in Table 3.2.

Commercial harvest (CH): Olive growers from these orchards usually estimate the commercial stage of maturity from the percentage of oil and dry matter content in fruit, also, in reference to other data, such as colour index that were obtained from the previous seasons. The growers then decide to harvest the fruit for commercial processing, although this may not necessarily be the optimum harvest time. Thus, in this harvest category, fruit sample (~10 kg per replicate) was obtained at the same time the orchards harvested their fruit for commercial processing.

Late harvest (CH): A batch of fruit sample (~10 kg per replicate) was harvested, two weeks after the commercial harvest.

3.2 At harvest assessments of fruit

3.2.1 Maturity index (MI)

Maturity index (MI), also called visual colour rating is a numerical measure of olive maturity that growers can use to predict the optimum stage to make harvest (Boskou, 2006; Kiritsakis, 1998c). MI was determined according to the method described by Kiritsakis (1998c) and Boskou (2006). One hundred olives were randomly drawn from the one kilogram olive sample. The fruit were then graded for both external and internal (pulp) colour according to the colour scale shown in Figure 3.1 and Table 3.3, respectively. The internal colour was assessed for all the olives with MI score of four after the fruit were cut in half.

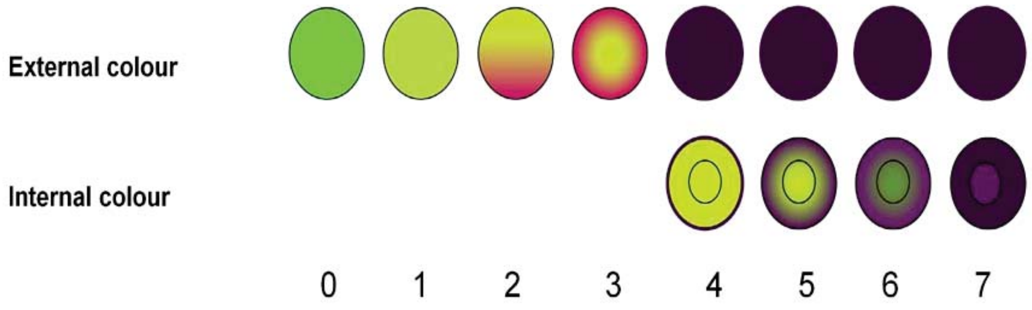


Figure 3.1: Olive colour maturity index (MI) scale. Source: Requejo-Jackman *et al.* (2009).

Table 3.3: Description of external and internal colour rating of olive fruit

External colour scale	**Description**
0	Intense green skin
1	Green yellowish skin
2	Green skin with red-purple gradient in less than 50% of fruit
3	Red-purple skin in more than 50% fruit
Internal colour scale	
4	Purple/black skin with white or green pulp
5	Purple/black skin and less than 50% of the pulp is purple
6	Purple/black skin and more than 50% of pulp is purple
7	Purple/black skin and purple pulp all the way to the stone (100%)

The maturity index (MI) was calculated by the following formula

Where: A, B, C, D, E, F, G and H represents the number of fruit in each maturity class 0, 1, 2 3, 4, 5, 6 and 7 respectively on the MI scale (Figure 3.1).

3.2.2 Fruit Firmness

Non-destructive firmness (NDF) was measured on 150 individual fruit randomly obtained from the one kilogram fruit sample using *FirmTech2* (BioWorks Inc., USA).). The *FirmTech2* software generated firmness in grams/mm using the force deformation response from gradual compression of each fruit. Firmness of each fruit was calculated and reported in kg/mm. Similar technique has been extensively used on blueberries and cherries (Changying *et al.*, 2011; Mitcham *et al.*, 1998).

3.2.3 Fruit weight

The weight in grams of 150 individual fruit was measured by an automatic balance (Mettler Toledo PB-L).

3.2.4 Dry matter determination

Dry matter was determined by drying whole olives in an oven according to the method reported for avocado (Clark *et al.*, 2003; Clark *et al.*, 2007). Twenty even sized whole olives were placed in a dish and dried in an oven at 65±0.5°C for 120 hours. Samples were dried in triplicate. The percentage dry matter was calculated.

In addition to % dry matter, the amount of ‘total solids’ in the fruit was calculated. ‘Total solids’ is the weight (grams) of dry matter on a per fruit basis. Twenty average-size fruit was dried in a pre-weighed dish in an oven at 65°C to a constant weight. The dry weight obtained was divided by twenty to obtain the ‘total solids per fruit’ (in grams). The test was done in triplicate.

3.2.5 Total oil content (% dry weight and % wet weight)

Oil was chemically extracted from freeze dried ground olive tissue using the Accelerated Solvent Extractor (ASE) (ASE® 300, Dionex Corporation, USA). Approximately 20 g of the freeze dried olive tissue was finely ground and placed in a 66 mL stainless steel closed cell fitted with ASE grade cellulose filter (Millipore Dura pore membrane filters, 0.22 μm GV) to collect any organic materials. Oil in the tissue was extracted using 95 percent n-hexane (HPLC grade, Ajax chemicals). The extraction conditions were as follows: five minutes sample heating time to 60°C followed by 100 minutes total extraction time at 1500 psi. The run was split into five cycles, each consisting of 20 minutes static extraction with solvent followed by purge cycle of 90 seconds with nitrogen gas (oxygen free, 99.9 % purity, BOC gases). The oil dissolved in the solvent was auto-collected in glass bottles. The glass bottles were nitrogen flushed during and after extraction. The extractions were carried out in duplicate.

3.2.6 Oil recovery after ASE

The oil extracted in hexane was recovered (concentrated) by evaporating the hexane component using a RapidVap unit (RapidVap N_2 Evaporation Systems, Labconco® Corp., USA) for 75 minutes at 47°C with the system oscillating at the speed of 45 % for the first 40 minutes then 70 % for the remaining 35 minutes. The oil was weighed and the yield obtained was expressed as the percentage of oil on dry weight and wet weight basis.

The oil extracted was collected into 20 mL dark brown glass vials, flushed with nitrogen and held at -80°C prior to chemical analysis.

3.3 Cold press (CP) extraction procedure for olive oil

The cold press (CP) extraction followed the commercial procedure used to obtain extra virgin olive oil (Kiritsakis, 1998g; Petrakis, 2006). Each step involved in the CP extraction is described below.

3.3.1 Washing

Fruit was washed in clean water (25±0.5°C) to remove leaves and other impurities. Fruit damaged during harvesting or by rots were removed.

3.3.2 Grinding/crushing

The washed olive was crushed using a 1.5 horse power (1.1 KW) hammer mill grinder fitted with 5 mm mesh grid (Aquarius Axis Industrial Ltd. N.Z.). The ground paste was collected in a 15 litre stainless steel malaxer pots.

3.3.3 Malaxing

Malaxation is the process of slow mixing of the olive paste under controlled conditions to increase extraction yields by aiding the release of oil from mesocarp cells, vacuoles and cytoplasm (Kiritsakis, 1998g). A motor driven stirrer blade was placed into the malaxer pots and the olive paste was slowly mixed by revolving the pot at about 14 rpm on a turntable (Figure 3.2) for sixty minutes. The malaxing temperature was controlled to approximately 27±0.5°C using a temperature regulator fan. Food grade nitrogen gas (BOC Ltd.) was flushed on top of the paste surface throughout malaxing to provide an inert gas blanket in order to minimise oxidation of the paste and to minimise enzymatic activity mainly due to lipases and lipoxygenases (Petrakis, 2006).

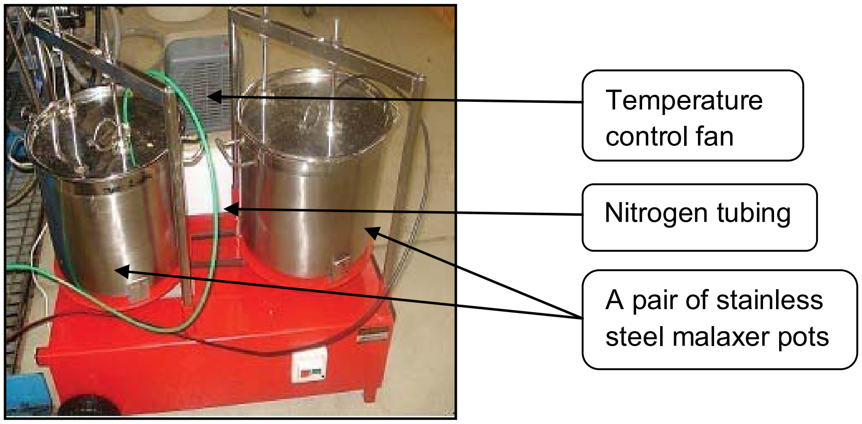


Figure 3.2: A pair of the stainless steel malaxer pots with purge nitrogen tubing and temperature fan (Aquarius Axis Industrial Ltd. Auckland).

3.3.4 Pressing

Olive paste was pressed in a filter press with the paste placed between 2 mm nylon drainage mats inside the pressing cylinder (Aquarius Axis Industrial Ltd. Auckland, New Zealand). The press was carried out for approximately 10 minutes. The liquid phase collected consisting of oil and water was constantly purged with food grade nitrogen gas.

3.3.5 Settling

The liquid phase was flushed with food grade nitrogen, covered with aluminium foil and left to settle for approximately one hour at 20°C. Two layers were obtained; bottom layer consisting of water and tissue debris and the top layer of oil.

3.3.6 Centrifugation

The oil was separated from the water layer in a disc centrifuge (GEA Westfalia Separator Model LWA 205, Germany). Oil was poured off the top of the settled extract and put into the bowl of the centrifuge operating at the speed 2000 rpm. The oil obtained was flushed with nitrogen and settled in the dark for 12 hours at 20±0.5°C.

3.3.7 Bottling

Olive oil was bottled in 250 mL dark green glass bottles, flushed with N_2 and stored at 10°C prior to sensory evaluations and at -80°C prior chemical analysis.

3.4 Chemical quality indices of cold pressed oil

3.4.1 Analysis of free fatty acids (FFA)

The measure of acidity normally reflects the amount of free fatty acids hydrolysed from triglycerides (TGs). The percentage of FFA (expressed as oleic acid), is defined as milligram of potassium hydroxide required to neutralize the FFA present in 1 gram of the oil (Pike, 1998). The FFA content is often used as an important marker of oil degradation , 2005). Olive oil is classified as extra virgin if its FFA level is less than or equal to 0.8 % (Kiritsakis, 1998d).

3.4.1.1 Reagents

The following reagents were used for the analyses of free fatty acids: phenolphthalein indicator (Analytical grade, Sigma-Aldrich), ethanol (HGAA, AnchorEthanol), sodium hydroxide (NaOH) and potassium hydrogen phthalate were all Analytical grade (LabServe^{TM}).

3.4.1.2 Preparation of reagents

Phenolphthalein indicator (1 %) was prepared by dissolving one gram of phenolphthalein in 100 mL 95 % ethanol. Sodium hydroxide solution (0.05 M) was obtained by dissolving two grams of sodium hydroxide pellets in 1 litre of carbon dioxide free distilled water. The sodium hydroxide solution was standardised following the AOCS official method Ca 5a-40 (AOCS, 1998). Potassium hydrogen phthalate required for the standardisation was dried in an oven at 120±0.5°C for two hours and cooled in a desicator.

3.4.1.3 Standardisation of sodium hydroxide (0.05 M)

Dried potassium hydrogen phthalate (0.2 g) was accurately weighed in an Erlenmeyer flask. Distilled water (25 mL) was added and the mixture was heated to dissolve. Three drops of phenolphthalein indicator was added to this solution. The solution was titrated with the NaOH solution being standardised until the first persistent faint pink colour was obtained with. The titre volume (mL) of NaOH was recorded. Titration was repeated in triplicate. Given, the molarity of NaOH (mol/L) was calculated.

Where, 204.229 g/mol is the molecular mass of potassium phthalate. The standardised NaOH solution (0.05 M±0.01) was used as the titrant in determination of FFA.

3.4.1.4 Procedure for the determination of FFA

Free fatty acid in the oil was determined following the AOCS official method Ca 5a-40 (AOCS, 1998a). Neutralised 95 % ethanol is required for titration of oil samples. Three drops of phenolphthalein indicator was added to 50 mL 95 % ethanol in a conical flask. The solution was immediately titrated with standardised NaOH solution (0.05 M) until the ethanol produced a faint but permanent pink colour.

The above neutralised ethanol was then added to an accurately weighed sample of olive oil (2.5 ± 0.05 g) in another 250 mL conical flask followed by addition of 1-2 drops of phenolphthalein indicator. The solution was heated to boiling then immediately titrated with vigorous shaking using the standardised 0.05 M NaOH until a persistent faint pink colour remained in the alcohol layer for 30 seconds. Titration was carried out in triplicate.

3.4.1.5 Expression of the results

The amount of free fatty acids was calculated by the formula

* The molarity of standardised NaOH (section **3.4.1.3**). Result was reported as mean FFA (expressed as % oleic acid) ± standard error mean.

3.4.2 Analysis of peroxide value (PV)

The peroxide value (PV) is a measure of oxidative stability of fats and oils (P , (2005). It is measured by the liberation of iodine (I_2) from potassium iodide by the hydroperoxides present in the oil (Hahm & Min, 1995; Kiritsakis, 1998e). Hydroperoxides are the primary oxidation products in lipids (Angerosa *et al.*, 2006; Aparicio *et al.*, 1997; Kiritsakis, 1998d). The mechanism of iodometric determination of hydroperoxide has been investigated (Pokorn´y, 2005). In the first step of the reaction mechanism, peroxide compounds react with iodide ions where they are reduced to hydroxy derivatives, and the iodide is oxidised into free iodine.

$\mathbf{R\text{-}OOH + 2I^- + 2H^+ \longrightarrow R\text{-}OH + I_2 + H_2O}$ (Reaction of hydroperoxides with iodide ions)

When the iodine is titrated with a solution of sodium thiosulfate (a reducing agent), the thiosulfate is oxidized into a tetrathionate.

$\mathbf{I_2 + 2S_2O_3^{2-} \longrightarrow S_4O_6^{2-} + 2I^-}$ (Reduction of free iodine with thiosulfate)

Addition of a starch indicator forms a deep violet product due to a complex formed with iodine. The reaction reaches an end point when sodium thiosulfate reduces the iodine to iodide, resulting in a colourless reaction solution.

3.4.2.1 Reagents

Potassium iodide, isooctane (2,2,4-trimethyl pentane), hydrochloric acid, glacial acetic acid and sodium carbonate used were analytical grade reagents (LabServe™) while sodium thiosulfate ($Na_2S_2O_3.5H_2O$) and potassium iodate, KIO_3 were reagent grade, ACS and the starch used was an AnalaR grade reagent, BDH.

3.4.2.2 Preparation of reagents

Saturated potassium iodide (KI) solution was made fresh daily. Ten grams of the potassium iodide was added to 5 mL distilled water and stirred thoroughly to dissolve. Dissolution was enhanced through ultrasonication for 5 minutes. The presence of un-dissolved crystals after stirring and ultrasonication was an indication of a saturated solution. A 1 % (w/v) solution of starch indicator was prepared by adding 1 g unmodified starch to 100 mL boiled distilled water and the solution was boiled for a further two minutes. This solution was stored at 4-10°C no longer than three weeks. Glacial acetic acid and isooctane solution was prepared in 3:2 volume ratios. The potassium iodate, KIO_3 was dried for two hours at 110°C and cooled in a desicator.

3.4.2.3 Standardisation of sodium thiosulfate (0.1 N)

To make 0.1 N sodium thiosulfate solution, 24.817 g $Na_2S_2O_3.5H_2O$ and 0.05 g sodium carbonate were dissolved in 1 litre freshly boiled and cooled distilled water. The standardisation procedure for 0.1 N $Na_2S_2O_3.5H_2O$ solution was as follows: accurately weighed (0.10 - 0.15 g) dried KIO_3 was dissolved in 75 mL distilled water in a 250 mL conical flask and two grams iodate free KI was added. An aliquot of 2 mL 6 M HCL was added and the solution was immediately titrated with vigorous shaking using sodium thiosulfate solution being standardised until the colour of the solution turned pale yellow. A solution containing 1 % starch (0.5 mL) was added and the solution was titrated until the violet colour disappeared. The final volume of sodium thiosulfate was recorded and a blank titration without KIO_3 was conducted. Titrations were carried out in triplicate. The normality (N) of sodium thiosulfate was calculated.

Where W is the weight of KIO_3 in grams, *S* and *B* are the volume of sodium thiosulfate required to titrate the sample and the blank, respectively.

The standardised 0.1 N sodium thiosulfate solution was diluted to 0.002 N by transferring 2 mL of the 0.1N standardised sodium thiosulfate solution into 100 mL volumetric flask and was made to volume with distilled water. This solution (0.002 N) was used as the titrant for iodometric titration of oil sample.

3.4.2.4 Procedure for determination of PV

Peroxide value (PV) was determined following the AOCS Official Method Cd 8b-90, acetic acid-isooctane method (AOCS, 1998b). A sample of olive oil was accurately weighed (2 ± 0.05 g) in a 250 mL stoppered Erlenmeyer flask. The acetic acid-isooctane solution (30 mL) was added. The solution was swirled to facilitate dissolving of oil. Saturated potassium iodide solution (0.5 mL) was added to the flask in the dark, and after exactly 1 minute, 30 mL distilled water was added. The solution was titrated with 0.002 N sodium thiosulfate, with constant and vigorous shaking, until the yellow colour of the solution almost disappeared. Then 1 % starch indicator (0.5 mL) was added to this solution. Titration was continued with vigorous shaking of solution near the end point to liberate all iodine from the acetic acid-isooctane layer. Sodium thiosulfate solution was added drop wise until the violet colour disappeared. Titrations were carried out in triplicate and a blank titration was conducted.

3.4.2.5 Expression of result

The peroxide value (PV) was calculated as

Where S and B are titration volumes (mL) of the sample and blank respectively, and N is the calculated normality of sodium thiosulfate solution. The result was reported and expressed as the milliequivalents of active oxygen per kg olive oil (meqO_2/kg) ± SEM

3.4.3 Specific extinction coefficient at K232 and K270

Specific extinction coefficient at K_{232} and K_{270} provides an indication of the oxidation process in unsaturated oils such as olive oil (Nam & Lee, 2007; Tsimidou, 2006; Velasco & Dobarganes, 2002). Absorbance in the ultra violet (UV) region at the wavelengths K_{232} corresponds to the maximum absorptions of conjugated dienes and it gives an indication of conjugated hydroperoxides from oxidation of polyunsaturated fatty acids (PUFA). Whereas, absorbance in the UV region at the wavelengths K_{270} corresponds to the maximum absorptions of conjugated triene and it gives an indication of the presence of carbonylic compounds, mainly aldehydes and ketones formed during oxidation of oil (Angerosa *et al.*, 2006; Kiritsakis, 1998e). The spectrophotometric examination was carried out following the International Olive Council (IOC) methodology (COI/T20/Doc.no. 19/Rev.1) (IOC, 2001).

3.4.3.1 Reagents

Cyclohexane (HPLC grade, UltimAR) was purchased from Ajax FineChem Pty Ltd. The spectrophotometric transmittance of the cyclohexane was not less than 40% at 220 nm and not less than 95 % at 250 nm in reference to distilled water.

3.4.3.2 Procedure

Accurately weighed olive oil sample (0.25±0.05g) was placed into a 25 mL volumetric flask and made up to volume with the cyclohexane solvent. The solution was gently swirled to ensure homogeneity. The clear solution was filled in a standard rectangular quartz cuvette cell (I-Q-100 mm, Scientific Supplies) having an optimal length of 1 cm. The specific extinction coefficient of the clear solution was measured at the wavelengths 232 nm and 270 nm respectively (Shimadzu UV-Visible spectrometer, UV-1601, Japan). Absorbance of the pure cyclohexane solvent was used as the reference.

3.4.3.3 Expression of the results

The specific extinctions at 232 nm and 270 nm were calculated using the formula

—

Where K is the specific extinction, *A* is the specific absorbance measured at wavelength 270 nm and 232 nm, b thickness of the cuvette in cm and *c* is concentration of the solution in g/100 mL.

The extinction value at K_{232nm} was corrected for esters using 0.07 as the correction factor (Kiritsakis, 1998e). By this correction factor, the percentage conjugated diene was expressed as

Kiritsakis (1998) reported this correction factor to provide an indication of the degree of oxidation of olive oil. All results are reported as mean values ± SEM

3.5 Determination of total phenolics

Total phenolic content was determined spectrophotometrically by the method of Folin-Ciocalteu (Singleton *et al.*, 1998). Folin-Ciocalteu reagent is a complex mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants (Singleton *et al.*, 1998). The assay works by measuring the amount of test compounds needed to inhibit the oxidation of the Folin-Ciocalteau reagent (Vinson *et al.*, 2005).

3.5.1 Reagents

The following reagents were used for the analyses of total phenolics: Folin-Ciocalteu reagent (2 M acid Bio-reagent grade, Sigma), caffeic acid (Analytical grade, Aldrich), methanol and Formic acid (analytical grade, LabServe^{TM}), n-hexane (HPLC grade, UltimAR) and sodium carbonate (ACS reagent).

3.5.2 Preparation of reagents and caffeic acid standard

Acidified water (pH 2.5) was prepared by gradually adding formic acid to distilled water to adjust the pH to 2.5±0.5. A mixture of methanol and acidified water solution (80:20) was prepared. A stock of Folin-Ciocalteu reagent was made by diluting it 10 times with milli-Q water. Sodium carbonate solution (75 g/L) was prepared in milli-Q water. Standard caffeic acid at concentration 0.5 mg/mL was prepared in absolute methanol. This standard stock solution was stable for several weeks at -20°C.

3.5.3 Extraction of phenolic compounds in oil

Ten millilitres of the oil was weighed followed by addition of 10 mL of n-hexane. The oil-hexane mixture was then transferred into a 100 mL separating funnel where phenolic compounds were extracted by adding 30 mL methanol-acidified water mixture and shaken vigorously for three minutes. Pressure build up in the separating funnel was minimised by releasing gas intermittently. The aqueous layer at the bottom of the separating funnel was then collected in a 250 mL round bottomed flask. This extraction cycle was repeated twice because the preliminary extraction analysis indicated that almost all phenolics in the oil were extracted by the third cycle and also a similar extraction cycle has been reported elsewhere (Abramovic *et al.*, 2007; Baccouri *et al.*, 2007; Oliveras-Lopez *et al.*, 2007; Salvador *et al.*, 2001b). The combined aqueous fraction was dried in the rotary evaporator (Büchi - RE 111 Rotavapor, Germany) with the fraction in the round bottomed flask submerged in a water bath set at 40°C. The dry residue was dissolved in 3 mL of absolute methanol and gently mixed thoroughly. This extract was used for the total phenolic and antioxidant assays following appropriate dilutions.

3.5.4 Procedure for total phenolic essay

Two hundred microlitres of the appropriately diluted aliquots were prepared in 15 mL falcon tubes. Folin-Ciocalteu reagent (1 mL) was added into each sample aliquot followed by thorough mixing. Sodium carbonate solution (0.8 mL) was then added three to eight minutes after of addition of Folin-Ciocalteu reagent. The tubes were then sealed and incubated for 90 minutes in the dark at 20°C. A portion of this solution (200 µL) was pipetted into a microplate (Griner bio-one 96 well microplate, Germany) and the absorbance was read at 760 nm in triplicate using the Spectra-Max microplate reader (SPECTRA-Max PLUS^{384}, MDS Analytical Devices, USA).

A range of dilutions of standard caffeic acid solution (0.5 mg/mL) was prepared by pipetting the following volumes of the stock standard solution and making up to 1 mL with absolute methanol: 0.05 mL, 0.075 mL, 0.1 mL, 0.125 mL, 0.15 mL, 0.2 mL and 0.25 mL. Folin-Ciocalteu reagent (5 mL) was added into each of the caffeic acid standard aliquots followed by the addition of 4 mL sodium carbonate in the same manner as mentioned previously. The capped tubes were incubated in the dark at 20°C and absorbance in triplicate at 760 nm was read after 90 minutes

3.5.6 Calculations and presentation of results

The total phenolic content was calculated using the equation for the straight line standard curve obtained from plotting the absorbance values (at 760 nm) against concentration of caffeic acid.

Thus from the standard curve mg caffeic acid/mL MeOH was obtained and the total phenolic content was calculated as below

The result was reported as milligram of total phenolics (caffeic acid equivalent) per kilogram of the oil.

3.6 Determination of the Bitter Index (K_{225})

3.6.1 Method of analysis

The bitterness perceived in olive oil is attributed to polar phenols that are extracted from the oils with methanol or a methanol and water mixtures (Andrewes *et al.*, 2003; Bendini *et al.*, 2007; Esti *et al.*, 2009; García-Mesa *et al.*, 2008; Gutiérrez-Rosales *et al.*, 1992; Gutierrez-Rosales *et al.*, 2003; Gutiérrez-Rosales *et al.*, 2003; Koprivnjak *et al.*, 2009; Mateos *et al.*, 2004; Mateos *et al.*, 2003; Ocakoglu *et al.*, 2009; Rotondi *et al.*, 2008; Servili *et al.*, 2009). The absorbance of this polar extract when measured at 225 nm can provide a good estimation of intensity of bitterness of the oil (Andrewes *et al.*, 2003; Gutiérrez-Rosales *et al.*, 2003; Gutiérrez- Rosales *et al.*, 1992).

In this study, the analytical method proposed by Gutiérrez-Rosales *et al.* (1992) for testing intensity of bitterness (IB) was used. This method evaluates bitterness in olive oil by measurement of absorbance of the polar extract at wavelength 225 nm (K_{225}). The K_{225} test has been reported to have a strong relationship with total phenol content and the calculated value for intensity of bitterness provided an easy and strong tool for bitterness assessment without the use of more expensive sensory evaluation (Beltrán *et al.*, 2007; Inarejos-Garcia *et al.*, 2009a; Mateos *et al.*, 2004; Skevin *et al.*, 2003).

3.6.2 Reagents

The reagents used were HPLC grade hexane (UltimAR) and methanol (Analytical, LabServe^{TM}).

3.6.3 Extraction of polar phenolic compounds

The polar phenolic compound was extracted by liquid-liquid extraction (LLE) as opposed to solid phase extraction (SPE) used in the original method of Gutiérrez *et al.* (1992). Hrncirik & Fritsche (2004) reported that the recovery of total phenolic compounds with LLE was higher than that using SPE C18 cartridge. Similar results obtained by Carrasco Pancorbo *et al.* (2004) suggested that the LLE procedure permitted the extraction of a higher number of phenolic acids, which give a greater potential for the analysis of these compounds in any kind of olive oil. Their findings showed that the SPE procedures do not allow a sufficient amount of oil to be passed across the cartridge to produce more diluted extracts. Therefore, while LLE is time consuming compared to SPE, it was the preferred method for the extraction of the polar phenolic compounds.

3.6.4 The procedure of phenolic extraction

The LLE was carried out according to the method reported by Inarejos-Garcia *et al.* (2009a). One gram of olive oil was accurately weighed and dissolved in 5 mL n-hexane. To the oil-hexane mixture was added 5 mL of the extraction solvent MeOH/H2O (60:40 v/v). The mixture was vortexed for two minutes and centrifuged at 2470 x *g* for 10 minutes. The hexane layer was removed, leaving the polar fraction which was transferred in 50 mL volumetric flask; this dilution was corrected in the calculation of K_{225}. The extraction was repeated three times because preliminary results showed greater than 96% of polar phenolics were extracted by the third extraction cycle. The combined polar fraction in a 50 mL volumetric flask was made up to volume (50 mL) with MeOH/H2O (60:40, v/v). The absorbance of this fraction was recorded at 225 nm using a UV-1601 spectrophotometer (Shimadzu Co., Kyoto, Japan). The absorbance of the extract was measured against methanol/water (60:40) in a 1 cm quartz cuvette cell.

3.6.5 Calculation of bitter index (K_{225})

K_{225} was calculated from the formula

$$K_{225=}\frac{\qquad}{\qquad}$$

Where *As* is absorbance of the polar extract; *Ws* is weight of oil in grams and *d* is the dilution factor corresponding to absorbance *As*, since the final result is expressed as 1% or absorbance of 1 g in 100 mL. The intensity of bitterness (IB) was then calculated from the expression reported by Gutiérrez-Rosales *et al.* (1992)

3.6.6 Objective evaluation of bitter index

Inarejos-Garcia *et al.* (2009a) proposed a more objective method to report bitter index using oleuropein as the reference compound. The use of a reference compound eliminates drawbacks arising from different laboratory equipments and it would also allow reproducibility and comparison of results across different researchers. The choice of oleuropein as the reference compound was because this glycoside is the only bitter compound in olive oil that is commercially available (Inarejos-Garcia *et al.*, 2009a). An oleuropein standard curve was generated for concentration ranges of oleuropein 0 to 0.1 mg/mL versus absorbance at 225 nm. The bitter index was calculated using the oleuropein standard curve $R^2 = 0.999$) using linear model (y = ax + b). Where 'y' is the absorbance at 225 nm, ‘a’ and ‘b’ are designate constants determined from the oleuropein standard curve and ‘x’ is concentration of oleuropein (mg/mL). The value obtained was referred to as oleuropein bitter index (OBI) and was reported as milligram of bitter phenolics (oleuropein equivalent) per kilogram of the oil.

3.7 Determination of oxidative stability by Rancimat

The Rancimat test is an accelerated measure of oxidative stability and shelf life. In this study, the oxidative stability of olive oil was measured with a 743 Rancimat (Metrohm & Co) using 5 g of olive oil sample, heated to120°C with an air flow rate of 9 L/h. The run was conducted in duplicates and the results were expressed in hours. Olive oil samples stored at -80°C were couriered to Bakels Edible Oils Laboratory (Mount Maunganui, New Zealand) who conducted the Rancimat test.

3.8 Sensory evaluation of cold pressed oil

Sensory evaluation was conducted at the Sensory and Consumer Science Facility at Plant and Food Research Institute, Mt Albert Research Centre, Auckland. Eight trained panellists consisting of two males and six females in the age range between 26 and 65 participated in the sensory study. Seven of the panellists carry out regular sensory evaluation and certification of olive oil in New Zealand. All the panellists attended five training sessions over five day’s period with each session lasting for about two hours. The panel were trained following the conventional descriptive analysis methods as described in Lawless & Heymann (1998). During the training sessions, the panel generated sensory attributes specific to olive oil in this study through discussions, concept alignment and agreement on the attributes. The list of sensory attributes the panellists generated for aroma and flavour and their reference standards and intensities is shown in Table 3.4. These attributes were evaluated using a line scale of 0 mm to 150 mm, where 0 reflects the lowest intensity and 150 the highest intensity of the attribute. Panellists were given these reference standards at the beginning of each day in order to anchor their responses and the intensities of the attributes already defined. In addition, the panellists were trained in the recognition of the standard International Olive Council (IOC) attributes for fruity, bitter pungent and the sensory defects (COI T.20 Doc. No 15 September 2007) following the IOC profile sheet (Appendix 1). The panellists agreed on the definition of these IOC attributes as outlined by the IOC document (COI T.20 Doc. No 15 September 2007) and rated their intensities on a line scale of 0 cm to 10 cm, where 0 reflects the lowest intensity and 10 the highest intensity (fruity, bitter and pungent). In order to anchor the panellist's responses and to specify the attributes intensity, they were given a reference sample (Fresh 'Frantoio' VOO), of high bitterness and pungency, at the beginning of each day. This reference sample was also included in the set of test samples for sensory evaluations. Sensory evaluation was conducted after all the panellists were well calibrated with the reference standards (panel calibration was evaluated using PanelCheck® programme).

3.8.1 Sensory evaluation of oil samples

The olive oil samples were assessed in individual booths with green lighting and positive airflow to reduce any biases from colour or non-product odours. Assessments occurred between 11 am and 1 pm and samples were served in standard blue oil tasting glasses covered with watch glasses. Each blue glass was labelled with a three-digit code and 15 mL of oil was poured into it. Samples were prepared 30 minutes prior to the assessment and served at 20 ± 3^{o}C using thermostatically controlled electric heating mats. Evaluation of the oil samples using the lexicons generated in Table 3.4 was carried out first orthonasally for the aroma intensities and then retronasally for the flavour intensities. Panellists were instructed to sip water or rinse their mouths with 0.9% pectin solution and or chew fresh carrot between samples.

The intensities of all attributes (lexicons) generated were rated on a 150 mm line scale anchored at 0 (absent) and 150 (extreme). However, the IOC standard rating scale of 0 (absent) to 10 (extreme) was used for the IOC standard descriptors, fruity, bitter, pungent and faults. Panellists were also asked to describe and rate any other attributes that were present in the oils.

A total of six virgin olive oil samples were evaluated; two samples each obtained from Hawkes Bay, Bombay and Waiheke Island orchards. Each panellist evaluated the six oil samples in one session with five minutes break after tasting the third oil sample. There were a total of three sessions spread over three days, thus each sample was evaluated in triplicate. In each session, panellists were presented with samples following a randomised block design balanced for presentation order and carry-over effects. Data were collected using the Compusense® (V.5.1) sensory data collection programme.

Table 3.4 Sensory descriptors (aroma and flavour) and their corresponding reference intensity generated by a trained panel

ATTRIBUTE	REFERENCE STANDARD	INTENSITY
Aroma		
Fresh green olive leaves & stalks	11 g fresh olive leaf and stalk crushed in a blender was made fresh daily	100
Fresh green/Cut grass	600 µL/L Cis-3-hexen-1-ol in sunflower oil; ~120 µL/200 mL was prepared	150
Bitter salad	Fresh 1 mizuna, 1 wong bok and 1 red mustard leaf	120
Vanilla toffee	Half-cut 'Werther's' original cream candies prepared in lidded plastic dish 30 minutes prior to sensory	150
Fresh Nutty/Fresh walnuts & husks	1 crushed raw fresh walnut and 5 crushed raw almonds with skin on	150
Buttery	1 crushed walnut husk	150
Floral	80 µL /L Linalool in sunflower oil» 16 µL /200 mL	90
Stewed/Canned apricots	Tinned apricot (commercial)	150
Flavour		
Fresh green beans/ Grassy	600 µL/L Cis-3-hexen-1-ol in sunflower oil; ~120 µL/200 mL was prepared for sensory	150
Bitter salad	1 leaf each from radicchio, mizuna, wong bok and red mustard	105
Black pepper	10% Gregg's ground black pepper in oil grape seed oil* ~5 g/50 mL	150
Vanilla toffee	Half-cut 'Werther's' original cream candies prepared in lidded plastic dish 30 minutes prior to sensory	150
Fresh Nutty/Fresh walnuts & husks	1 crushed raw fresh walnut and 5 crushed raw almonds (Sunlife brand) with skin on.	150
Buttery	1 crushed walnut husk	150
Fresh herb	2 cm piece each of parsley, sage and marjoram	150
IOC standard descriptors		
Fruity	Fresh 2010 Frantoio olive oil	7
Bitter	Fresh 2010 Frantoio olive oil	7
Pungent	Fresh 2010 Frantoio olive oil	7
IOC Faults		
Rancid		
Other defects	Fusty/muddy sediment, Musty, Winey, Metallic, Rancid, dirty or stewed apricot, very old peanuts	

*Azalea® 100% grape seed oil

3.8.2 Analysis of Sensory data

A Principal Component Analysis (PCA) was performed, using the first two principal components to explain the differences and to identify the sensory attributes influencing the overall characterisation of each of the olive oils. The relationship between sensory attributes and individual phenolic composition (3.9.1) was analysed using Pearson's correlation coefficient.

3.9 Analysis of chemical composition

3.9.1 Analysis of phenolic compounds by High Performance Liquid Chromatography (HPLC)

Separation of phenolic compounds by HPLC can be conducted in either normal phase (adsorption mode) or reverse phase (partition mode) (Carrasco-Pancorbo *et al.*, 2005). Normal phase chromatography separates phenolic constituents according to the nature and number of polar functional groups using silica gel as the absorbent and a non-polar solvent as the eluent (Carrasco-Pancorbo *et al.*, 2005). Whereas, reverse phase HPLC separates individual components that belong to one constituent class, usually a non-polar octadecylsilane (C18) bonded phase as the stationary phase while the mobile phase is a polar solvent (Carrasco-Pancorbo *et al.*, 2005).

RP-HPLC is the most preferred because of its sound replication of retention times and separation of most polar phenolic compounds (Aturki *et al.*, 2008; Tasioula-Margari & Okogeri, 2001b; Tsimidou *et al.*, 1996; Tsimidou *et al.*, 1992). Therefore, in this study the RP-HPLC technique was used to characterise and quantify phenolics in the olive oil studied.

3.9.1.1 HPLC apparatus, column and operating condition

The Shimadzu RP-HPLC apparatus (Model SCL-10A, Japan) was used to analyse the phenolic compounds in the olive oil extracts. The HPLC was equipped with an auto sampler and auto injector (Shimadzu SIL-10AF), a pump (Shimadzu LC-10ATvp), a degasser (Shimadzu DGU-14A) and a UV-visible diode array detector (Shimadzu SPD-M 10Avp). The column used was Synergi Fusion RP 80 C18 (3.5 µm 100 x 4 mm i.d). Gradient elution was employed and the mobile phase consisted of water/acetic acid (97.5:2.5 v/v) as solvent A and acetonitrile as solvent B (Benavente-Garcia *et al.*, 2000). Acetic acid was added to maintain a constant acid concentration during gradient runs. The acidic pH of the mobile phase helps to avoid the dissociation of phenolic compounds, and also improves the asymmetry of the peaks and reduces peak-tailing (Harwood & Aparacio, 2000). Table 3.5 shows the elution gradient used. The flow rate was set at 0.8 mL/min and the column temperature was 30 ± 0.5°C.

Table 3.5: The Elution gradient for phenolic separation by HPLC

Time (minutes)	**Solvent A (%) Water/acetic acid (97.5:2.5 v/v)**	**Solvent B (%) Acetonitrile**
0	95	5
20	75	25
40	50	50
50	20	80
60	95	5

3.9.1.2 Reagents

The reagents used were methanol and glacial acetic acid (Analytical grade, LabServe^{TM}) and acetonitrile (LiChrosolv, Mercek). The solvents were filtered (Millipore Durapore membrane filter, 0.22 μm GV) then degassed using ultrasonication.

3.9.1.3 Reference phenolic standards

Table 3.6 shows the commercial grade phenolic reference standards used in identification of phenolic compounds in the studied oils

9.1.1.4 Procedure for phenolic extraction

Extraction of phenolic compounds in olive oil was performed by Liquid-liquid extraction (LLE) using a mixture of methanol/water (80:20 v/v) as the extraction solvent (Montedoro et al., 1992). Moreover, several authors have used this same extraction solvent ratio in LLE extraction of phenolics from VOO (Brenes *et al.*, 1999; Brenes *et al.*, 2000; Morello *et al.*, 2004; Ocakoglu *et al.*, 2009; Selvaggini *et al.*, 2006; Tasioula-Margari & Okogeri, 2001a; Tovar *et al.*, 2001). The extraction procedure is outlined below:

Extraction: Olive oil was accurately weighed (2.5 g) in a centrifuge tube. Five milliliters of MeOH/H_2O (80:20 v/v) was added to the oil then vortexed for 2 minutes (Montedoro *et al.*, 1992a; Pirisi *et al.*, 2000; Tovar *et al.*, 2001), followed by centrifugation for 10 minutes at 2470 x *g* to separate the two phases (Montedoro *et al.*, 1992a). The methanol layer was collected. This extraction step was repeated twice with another 5 mL MeOH/H_2O (Montedoro *et al.*, 1992a; Pirisi *et al.*, 2000). The extract was combined and concentrated under vacuum at <35°C to near dryness.

Purification: Acetonitrile (0.5 mL) followed by 2 mL of hexane was added to the concentrated phenolic extract (Tovar *et al.*, 2001). The resulting solution was evaporated under vacuum to dryness.

Solubilisation of the phenolic extract: The phenolic extract was dissolved in 1 mL methanol (Tovar *et al.*, 2001). An aliquot (20 µL) of this extract was injected into the HPLC system.

Table 3.6: The commercial grade phenolic standards

Compound name % purity specification	**Grade, brand**
Gentistic acid ≥98% (2,5-dihydroxybenzoic acid)	HPLC, Aldrich
Benzoic acid ≥99.5%	ACS reagent, Sigma-Aldrich
Vanillic acid (4-hydroxy-3-methoxybenzoic acid) ≥97%	HPLC, Fluka
Vanillin(4-hydroxy-3-methoxybenzaldehyde)	HPLC, Fluka
Protocatechuic acid ≥97% (3,4-dihydroxybenzoic acid)	Purum, Aldrich
p-hydroxybenzoic acid ≥99%	HPLC, Aldrich
Syringic acid ≥95%	HPLC, Sigma
Caffeic acid	Analytical, Sigma
p-coumaric acid (4-hydroxycinnamic acid)	HPLC, Sigma
o-coumaric acid ≥97% (trans 2-hydroxycinnamic acid)	HPLC, Sigma
Ferulic acid ≥99%	HPLC, Aldrich
Sinapinic acid ≥99%	HPLC, Aldrich
(3,4-dihydroxyphenyl)ethanol (3,4-DHPEA) ≥97%	HPLC, Sigma-Aldrich
p-hydroxyphenylethanol (p-HPEA) or tyrosol ≥90%	HPLC, Sigma-Aldrich
Verbascoside	HPLC, Extrasynthese Genay France
Oleuropein ≥80%	HPLC, Extrasynthese Genay France
Apigenin ≥95%	HPLC, Sigma-Aldrich
Luteolin ≥99%	HPLC, Extrasynthese Genay France
Quercetin-3-O-glucoside ≥99%	HPLC, Extrasynthese Genay France
Rutin ≥99%	HPLC, Extrasynthese Genay France
Luteolin-7-O-glucoside ≥98%	HPLC, Extrasynthese Genay France
Quercetin dehydrate ≥98%	HPLC, Extrasynthese Genay France
Luteolin-4-O-glucoside ≥95%	HPLC, Extrasynthese Genay France

3.9.1.5 Analysis of results

The phenolic compounds were identified on the basis of their retention times and UV spectra in comparison to external reference standards (Table 3.4). The chromatograms were read at 280 nm, except flavonoids, luteolin and apigenin that were identified at 340 nm. The identified phenolic compounds were calculated from a regression equation obtained from the regression curve of peak areas and known concentrations of the external reference standards from the same batch. Complex phenolic compounds and secoiridoids were identified in reference to their elution profile from previous papers (Tover *et al.,* 2003; Salvaggini *et al.,* 2006; Guiterez-Rosales *et al.,* 2003; Servilli *et al.,* 1999) and quantified from regression equation of oleuropein standard curve. Their concentrations in the oil were thus expressed as mg/kg equivalence of oleuropein.

3.9.2 Analysis of fatty acid composition

The composition of fatty acids in the oil was quantified by capillary gas chromatography (GC).

3.9.2.1 Reagents

The reagents used were ammonium chloride (ACS reagent grade, Scharlau) and the absolute methanol, potassium hydroxide and concentrated sulphuric acid (98 %) used were Analytical grade (LabServe^{TM}).

3.9.2.2 Reference fatty acid standard

The following methylated mix fatty acid standards (analytical grade, Supelco) were obtained: Palmitic acid (C16:0), Palmitoleic acid (C16:1), Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2), Linolenic acid (C18:3), cis-11-eicosenoic acid (C20:1) and Erucic acid (C22:1). The standards were prepared in 95 % HPLC grade hexane (Mallinckrodt)

3.9.2.3 Preparation of reagents

Ammonium chloride, absolute methanol and concentrated sulphuric acid were used to prepare the Hartman reagent. To make the Hartman reagent, 2 g ammonium chloride was dissolved in 60 mL of absolute methanol in a round bottom flask. Concentrated sulphuric acid (3 mL) was added and the mixture was refluxed for 15 minutes. Methanolic potassium hydroxide (0.5 M) was prepared by dissolving 2.8 g of potassium hydroxide in 100 mL of absolute methanol.

3.9.2.4 Saponification and methylation procedure

Saponification and methylation procedure of fatty acid to fatty acid methyl ester (FAME) was according to the method described by Hartman and Lago (1973). An olive oil sample was accurately weighed (0.1 ± 0.05 g) in a Kimax tube. To the oil sample, 0.5 mL of methanolic potassium hydroxide solution (0.5 M) was added and the tube was placed in a water bath at 70±0.5°C for 10 minutes. The mixture was methylated with Hartman reagent (5 mL) and heated for another 10 minutes at 70±0.5°C. Hexane (2 mL) and distilled water (5 mL) was added to the extract to separate the fatty acid methyl esters. The mixture was thoroughly shaken to enhance this separation. A portion of the resultant supernatant layer (0.5 µL) was injected into the gas chromatography, GC (Shimadzu GC-17A).

3.9.2.5 The GC operating conditions (Shimadzu GC-17A)

The Shimadzu GC-17A (Shimadzu, Japan) gas chromatography was equipped with an auto injector, AOC-20i and an auto sampler, AOC-20s. The operating column used was a DB-wax capillary column of length 30 m with an internal diameter of 0.32 mm and film thickness 0.25 microns. The column temperature was 200^{o}C, flame ionising detector (FID) temperature was 300°C and the injection temperature was 250°C. Total pressure and purge pressure were 33 kPa and 100 kPa, respectively, with a total purge flow of 0.8 mL/min. A split injection mode was used with the nitrogen gas carrier total flow rate of 16 mL/min. Total running time of each sample was 25 minutes.

3.9.2.6 Calculation of individual fatty acid

Standard calibration curve was obtained from peak areas and known concentration of the methylated standards. The individual fatty acids were first identified in reference to retention times of the known standards and calculated from the standard curve equation. Arachidic acid (C:20), Heptadecanoic acid (C17:0) and Heptadecanoic (C17:1) were quantified in terms of oleic acid.

3.9.3 Analysis of tocopherols by HPLC

The individual tocopherols, alpha, beta, gamma and delta (α, β, γ and δ) were quantified by high performance liquid chromatography (HPLC) based on the method described by Indyk (1988). The external standard method was used to quantify the individual tocopherols.

3.9.3.1 Reagents

The reagents used were Pyrogallol (reagent grade, Sigma), absolute ethanol (HGAA, AnchorEthanol), HPLC grade n-hexane (Mallinckrodt Chemicals). Diisopropyl ether and isopropanol were all HPLC grade (Mallinckrodt Chemicals).

3.9.3.2 Reference tocopherol standards

The following external standards were obtained: α-tocopherol (≥98 %), (±) γ-tocopherol (≥97 %) (Fluka) and 1Rac-β-tocopherol (+90 %) and (±) δ-tocopherol (~90 %) (Supelco) were all HPLC grade. The standards were prepared in 95 % HPLC grade hexane (Mallinckrodt)

3.9.3.3 Preparation of reagents

The extraction solvent was prepared by mixing HPLC grade n-hexane and diisopropyl ether in the ratio 3:1. A 1 % (w/v) pyrogallol solution was made fresh in absolute ethanol. Potassium hydroxide 50 % (w/v) was prepared in distilled water. The mobile phase (n-hexane and isopropanol 98.9:1.1) was prepared in a 500 mL volumetric flask from the mixture of n-hexane and isopropanol in the volume ratio 98.9:1.1. The mobile phase was degassed and filtered with a 0.22 µm filter (Millipore Durapore membrane filter, 0.22 µm GV) before use.

3.9.3.4 The HPLC chromatographic condition (Shimadzu Model SCL-10A)

The Shimadzu HPLC system (Shimadzu Model SCL-10A, Japan) was used for quantification of tocopherols. The system was equipped with a model LC-10A pump, a model SPD-10A UV-Vis detector and a Rheodyne loop injector. The HPLC column used was a 5 µm Lichrospher Si-60 (250 X 40 mm) column with a guard column (Silica 4 X 3.0 mm AJ0 -4348). UV detection was at a wavelength 294 nm. The volume of sample injected was 20 µL at a flow rate of 0.7 mL/min under isocratic condition.

3.9.3.5 Sample preparation procedure

The oil sample was saponified according to the method described by Indyk (1988). About 0.25 g of oil was weighed in a Pyrex centrifuge tube (Pyrex, Mexico). Working quickly, 10 mL pyrogallol and 2 mL 50% (w/v) potassium hydroxide solution was added to the oil mixture. The centrifuge tube was shaken in a mechanical shaker (Grant Instruments, Cambridge Ltd.) while at the same time submerged in water bath at 70 ± 0.5°C for exactly eight minutes. The tube was immediately cooled on ice water for about five minutes and 20 mL of the extraction solvent (hexane and diisopropyl ether 3:1) was added. The tube was capped securely and put on the mechanical shaker for a further five minutes. Distilled water (30 mL) was added to the centrifuge tube and the tube was re-capped, inverted 10 times and centrifuged (International Equipment Company, IEC Centra CL3R) at 2470 x *g* for 10 minutes. After centrifugation, exactly 10 mL of the hexane layer was carefully transferred into a 250 mL round bottom flask using an auto-pipette. The extract was evaporated under a vacuum drying system. During vacuum drying, dry ice (BOC Limited, Auckland) was used to condense the evaporating solvent. The dried residue was purged with nitrogen for at least 60 seconds and working quickly, 1 mL of n-hexane was added to dissolve the dried residue. An aliquot (20 µL) of this extract was injected into the HPLC system under full lighting. The sample was injected in duplicate. The HPLC system was run under full lighting.

3.9.3.6 Analysis of results

The individual tocopherols were identified based on peak areas and retention times of their corresponding external standards. A three point calibration curve was obtained from known concentrations of the mixed external standards, α, β, γ and δ-tocopherol for each batch of sample processed. The amount of each tocopherol in the oil sample was then calculated against the linear regression equation obtained from calibration curve. Result was presented as mean ± SEM calculated from duplicate injection.

Chapter Four: Results and Discussions

4.1 Fruit maturation

Olive maturation is a slow process that involves several physical and chemical changes taking place within the fruit. Understanding this process is crucial because it allows the grower to make an informed decision on the optimum harvest maturity. In this study, the indices of olive maturity were determined by evaluating the changes in percent oil content, percent dry matter, weight, maturity index (MI) and firmness of fruit. Fruit from Hawke's Bay, Waiheke and Bombay orchards were harvested at different stages of maturity after full bloom, usually two weeks apart. The harvest details (harvest dates and categories) were reported in Table 3.1. Oil in the fruit was extracted by accelerated solvent extractor (ASE) so as to give total available oil. Olives from the last two harvests: (commercial harvest-CH and two weeks after the commercial harvest-LH) were cold pressed (CP) according to the procedure previously described (Section 3.3). The results from this study revealed appreciable differences in olive maturation between the three orchards in terms of % oil content, % dry matter, fruit ripening time, fruit weight and other physiological changes such as softening (firmness).

4.1.1 Changes in oil content during olive maturation

The percentage (%) of oil in olive fruit is an important factor that influences harvest decision for most olive growers (Kiritsakis, 1998a; Boskou, 2006). The % of oil was expressed as % dry weight and % wet (fresh) weight. Results shown in Figure 4.1 indicate that the % of oil generally increased between 110 days to about 185 days after full bloom (DAFB), beyond which the % accumulation started to cease or only showed a slight increase. Several authors have suggested that the % of oil in olive first continuously increases in the mesocarp of the fruit as the olive ripens due to intense triglyceride biosynthesis and enzymatic activities, thereafter it levels off as the ripening progresses (Beltrán *et al.*, 2004b; Kiritsakis, 1998f; Mailer *et al.*, 2007; Petrakis, 2006).

The trend in % oil accumulation (% dry and % wet weight) was similar in the Hawke's Bay and Bombay orchards, these orchards attained lower oil content than the Waiheke orchard, overall. Hawke's Bay and Bombay both attained a maximum oil content of about 40 % and 17 % by dry weight and wet weight, respectively, at 190 days after bloom. Throughout the season, Waiheke orchard attained the highest % of oil both on a dry and wet weight basis (Figure 4.1). The % of oil (dry weight) increased from about 39 % to 44 % between 160 and 190 DAFB, after which it increased very slowly to 45 % at 204 days after full bloom. During the same period, the % of oil by wet weight increased from 18.2 % to 25.2 %, after which then sharply declined to 21.70 % at 204 DAFB. The sharp decline can be attributed to heavy rainfall recorded on the last three days prior to this harvest (personal communication Edwards M, orchard manager, 2010). Figure 4.2 shows the rainfall for this orchard related to days after full bloom when fruit was harvested. The effect of the amount of rainfall on % oil (wet weight) and % dry matter for all the orchards is presented in Section 4.1.3. A low rainfall climate (dry) can also affect the % wet weight of oil in fruit, especially at late maturity. The dry climate can lead to a false increase in % oil (wet weight) as a result of moisture loss rather than triglyceride biosynthesis Kiritsakis (1998c). For this reason it is recommended that oil content in the fruit is best determined on percent dry weight of the fruit (Kiritsakis, 1998c).

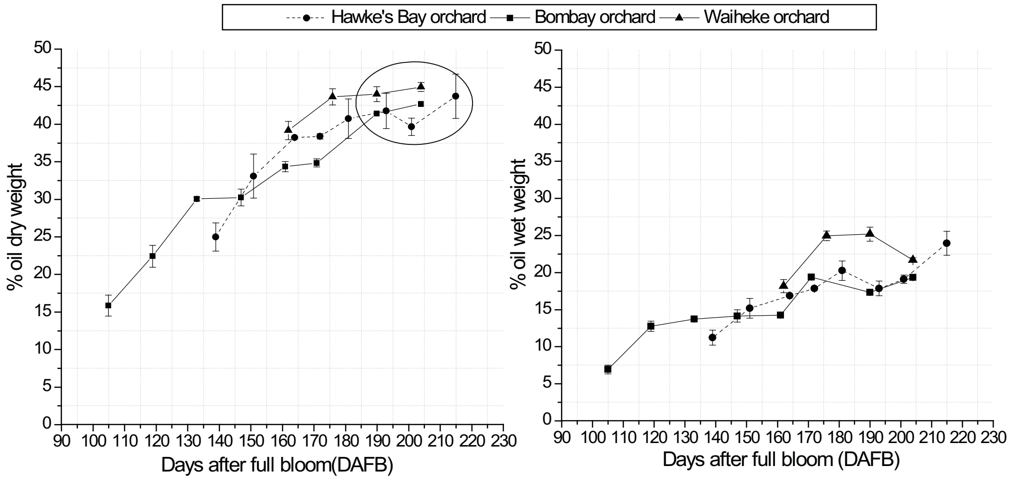


Figure 4.1: Oil percentage at different harvest dates after full bloom (DAFB) from the Hawke's Bay, Bombay and Waiheke orchards. Each point on the graph is the mean of three replicates. Error bars represents the standard error of mean. The circle indicates the time commercial harvests were made.

Generally, the % of oil at commercial harvest (190 days-circled in Figure 4.1) in all the three orchards was similar to 'Frantoio' olives grown in the Northern hemisphere. For example, Beltrán *et al.* (2004) reported the percentage of oil in 'Frantoio' grown in Spain between 21.1 % to 23.14 % by fresh weight and 40 % to 42.8 % by dry weight. Allalout *et al.* (2009) also reported % oil on fresh matter basis and dry matter basis in the range between 18.3 % to 25.4 % and 48.6 % and 56.1 % for some Spanish and Greek olive cultivars, respectively, grown in Tunisia. At the ideal ripe stage, the oil content in olive is generally greater than 22 % by dry weight, however, significant variations have been reported among growing regions and olive cultivars (Boskou, 2006).

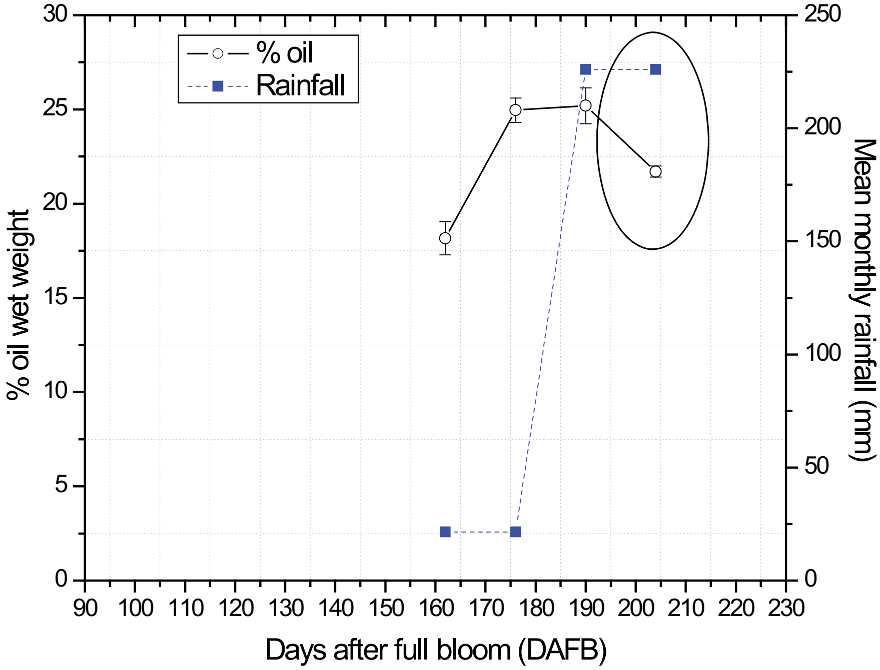


Figure 4.2: Effect of rainfall on % oil (wet weight) in Waiheke orchard. Each point on the graph is the mean of three replicates. Error bars represents the standard error of mean. Rainfall data was obtained from NIWA, Awaroa Valley Station in Waiheke Island.

If the aim is to achieve maximum oil yield, then these results can suggest that the best time to harvest olives in Hawke's Bay, Bombay and Waiheke orchards is between 170 and 195 days after full bloom. This agrees with the optimum harvest time reported by Conde *et al.* (2004) who suggested the best harvest time is between 150 to

210 days after full bloom. At this stage of maturity, the olive pulp has attained an optimum fatty acid balance, in terms of both the nutritional and oxidative stability, as well as antioxidants from total phenols and tocopherols in the oil (Rotondi *et al.*, 2004).

This confirms reports from many researchers who have found that delaying olive harvest in fact impacts more significantly on the quality of the oil than on yield, including loss of some attractive attributes such as bitterness, pungency and other flavours (Bouaziz *et al.*, 2004; Yousfi *et al.*, 2008; Youssef *et al.*, 2010). This loss is due to significant reduction of important compounds such as phenolics and aroma volatiles as ripening progresses (Rotondi *et al.,* 2004; Ayton *et al.,* 2001).

Therefore, although oil accumulation (% dry weight) showed a good indication of olive maturation, the harvest decision cannot be based on the oil content alone, since a range of intrinsic chemical components such as phenolics, tocopherols, sterols and flavour volatiles all contribute to the overall quality of olive oil. In addition, olive growers often require faster means of predicting the level of oils in fruit. As a result other parameters such as % dry matter, fruit colour, and firmness changes taking place during fruit maturation were evaluated to provide data that can be vital to defining the optimum harvest maturity.

4.1.2 Changes in fruit weight with oil content

Figure 4.3 shows the changes in fruit weight with % oil (dry weight) after full bloom for Hawke's Bay, Bombay and Waiheke orchards, respectively. The developmental phases, A, B and C on the graph are included to highlight stages of major changes in weight and % oil during fruit maturation. The weight of fruit in the Hawke's Bay orchard increased gradually to about 1.6 g at 165 DAFB, and then it declined in weight until the end of the season. Similar to % oil content, the fruit weight in Bombay and Waiheke orchards increased gradually with olive maturity to about 160 DAFB, thereafter, fruit maintained nearly constant weight for the next 20 DAFB. The most rapid increase in fruit weight (stage A-Figure 4.3) continued until about 165, 155, and 175 DAFB in Hawke's Bay, Bombay, and Waiheke orchards, respectively. The average fruit weights at the time of the two cold pressed harvests (CP1 & CP2) varied between 1.3 g to 1.9 g in the three orchards.

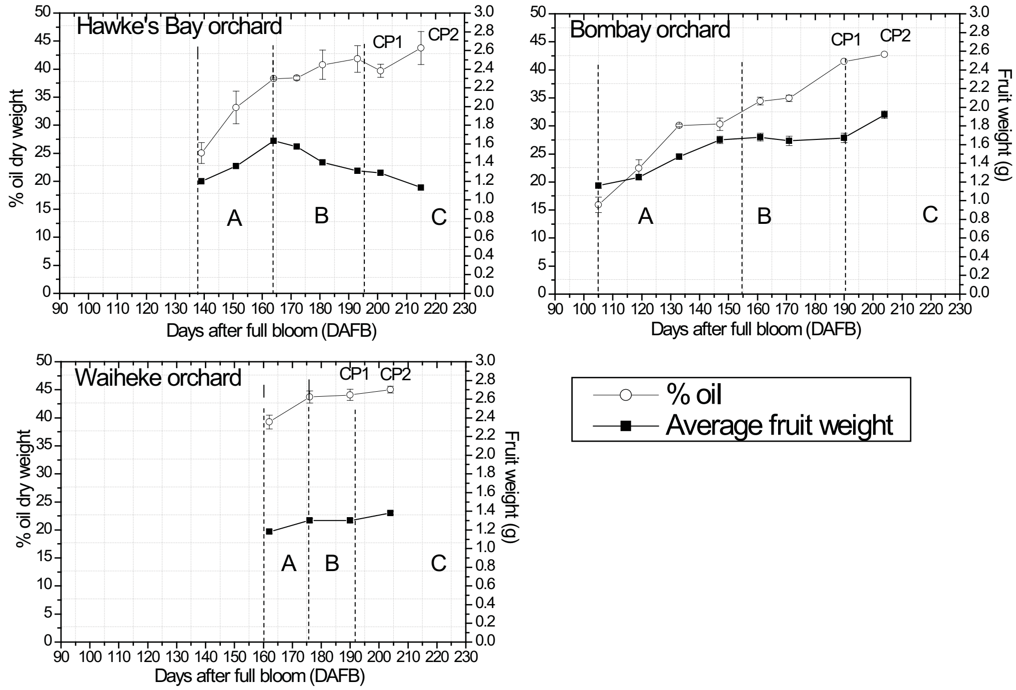


Figure 4.3: Changes in average fruit weight and % oil at different harvest maturity after full bloom for Hawke's Bay, Bombay and Waiheke orchards. Error bars represent the standard error of mean. Stage A shows the period for rapid increase in fruit weight and % oil; B the period for slow increase in weight, rapid increase in % oil and C optimum ripening, very slow increase in weight and % oil.

The rapid increase in the weight of olives at stage A can be attributed to mesocarp development in the fruit, which is characterised by the expansion of pre-existing cells and intense oil accumulation (Conde *et al.*, 2008). This event is also supported by the steep rise in oil content between the same periods (stage A), although % oil continued to rise past this stage as previously mentioned in section 4.1.1. Stage B in Figure 4.3 illustrates the stationary phase in fruit weight, but at this stage oil continues to be synthesised in the mesocarp cells. The sudden increase in fruit weight after the stationary growth phase in the fruit from the Waiheke and Bombay orchard can be due to rainfall recorded prior to the harvest. Rain increases moisture content with a concomitant increase in the absolute wet weight of the fruit.

The continuous decline in fruit weight in the Hawke's Bay orchard after 165 DAFB can be attributed to negative minimum temperatures recorded in the orchard and remarkably low average monthly growing degree days (GDD) recorded during the harvest period (Figure 4.4). Figure 4.4 revealed the minimum monthly temperatures in the Hawke's Bay orchard were below 0°C from April to July, reaching as low as -3.8°C in June. The low temperature can explain the lowest cumulative heat build up (GDD) in the orchard, compared to Waiheke and Bombay orchards. GDD is a measure of daily ambient heat accumulation above the base average temperature, 10°C (for this study). GDD is an important parameter because plant growth occurs in a cumulative stepwise manner which is strongly influenced by the ambient temperature. The low GDD and minimum temperatures recorded could mean that fruit development was hindered as reflected by the continuous reduction in fruit weight during this period. The fruit from Hawke's Bay also looked physically dehydrated and shrivelled at the harvests made after 165 DAFB. A typical example of frost damaged fruit (shrivelling and dehydration) from Hawke's Bay orchard is shown in Figure 4.5, and Table 4.1 shows the harvest dates and accumulated days of the frost damage during the study period. Shrivelling and dehydration are classical examples of frost effect on olives (Guillaume *et al.* *et al.*, 2003). The incidence of frost in the Hawke's Bay orchard first occurred on May 01, 2010, 143 days after full bloom (Personal communication with orchard manager). Ice crystals are formed in the apoplast (space after plasma membrane where water and solutes are transported) during a frost event and when this occurs, the cell maintains its permeability to water and a significant difference in water potential causes water loss from the symplast (inner section of the plasma membrane where diffusion of water and low molecular weight solutes occurs), hence causing dehydration and shrivelling of the fruit (Therios, 2009a). As shown in Figure 4.3, the frost event did not significantly impact on oil biosynthesis, although frost damage can affect composition of phenolics, sensory attributes and quality parameters, such as peroxide value (Guillaume *et al.* *et al.*, 2003).

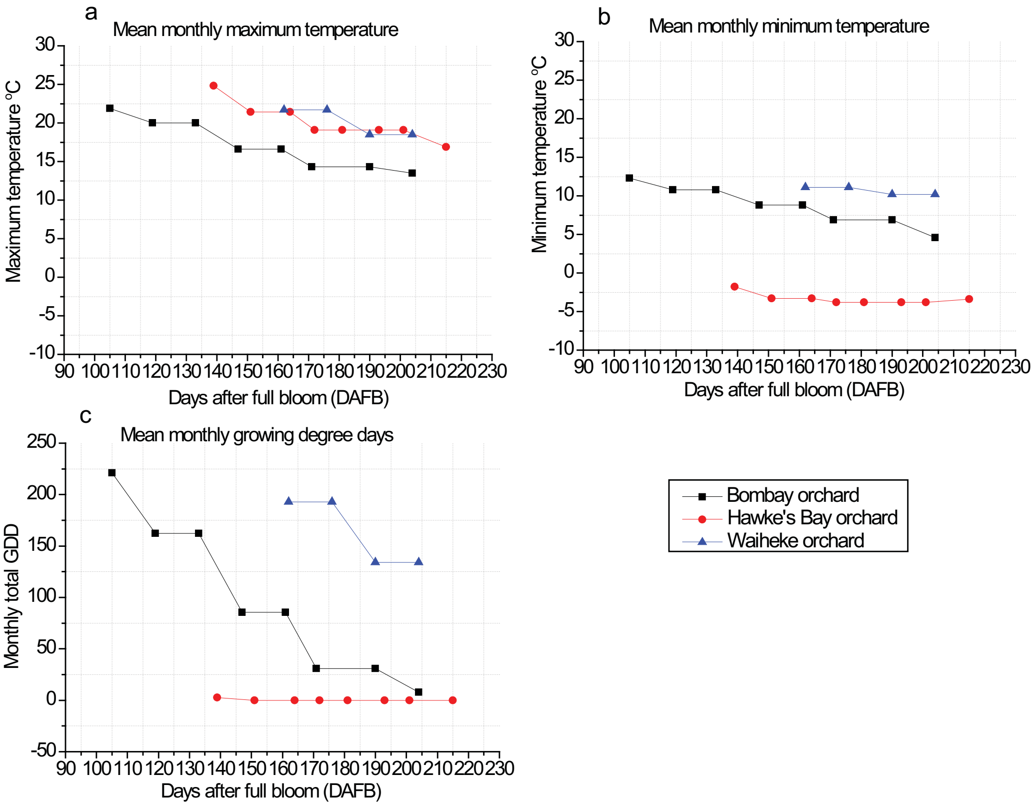


Figure 4.4: Monthly maximum temperature (a), minimum temperature (b) and growing degree days (c) relative to specific harvest days after bloom for Hawke's Bay, Bombay and Waiheke orchards. GDD data was obtained from NIWA for the Waiheke and Bombay orchards and http://waterwatch.net.nz for the Hawke's Bay orchard.

Figure 4.5: Typical example of the effect of frost damage on fruit from Hawke's Bay orchard Source: Personal elaboration (July, 2010).

Table 4.1 Harvest dates and progression of frost damage (days after the first occurrence) during olive ripening in the Hawke's Bay orchard.

Harvest date	Days after full bloom 10th December 2009	Days after the first incidence of frost on 1st May 2010
24th May	164	23
1st June	172	32
10th June	181	41
22nd June	193	53
30th June*	201	61
14th July**	215	75

*Commercial harvest (CH) **Late harvest (LH)

The weights of fruit at the cold pressed harvests (CP1 and CP2, Figure 4.3) are similar to what Requejo-Jackman *et al.* (2009) found in the 2009 season (for Hawke's Bay, 1.2 g and Bombay, 2.1 g). In the 2008 season, Requejo-Jackman *et al.* (2009) reported the mean fruit weight of 'Frantoio' olive from the following orchards located on the North Island: Bombay (1.55 g), Helensville (2.4 g), Mangawhai (1.6 g) and Waiheke (1.8 g).

A similar study on 'Frantoio' in Spain reported that fruit weight varied between 1.9 g and 2.3 g (Beltrán *et al.*, 2004b). The results shown in Figure 4.3 and those of Requejo-Jackman *et al.* (2009) and Beltrán *et al.* (2004) show there is considerable variation in fruit weight between different seasons and growing regions. Several factors can contribute to the variation in fruit weights, for example, tree age and vigour, where young trees will produce larger fruits and their ripening is more rapid (Therios, 2009e). The olive orchard orientation can also influence fruit weight. Olive orchards oriented in a westerly direction are more exposed to sunshine and their fruits ripen earlier (Therios, 2009e). Other factors that can affect the olive fruit size include fruit load on the trees, plant nutrition and physiological stress due to changes in climate, rain or irrigation (Kiritsakis, 1998c).

4.1.3 Changes in dry matter with oil content

The measure of dry matter gives the percentage of all solids in the fruit, including oil content, which is the second highest component of mature olive tissue. Dry matter can be obtained as a percentage (that is, the dry weight of fruit divided by the wet weight multiplied by 100) or as 'total solids' per fruit. Both measures were described under methods and materials Section 3.2.4. The former technique does not account for the moisture content in the fruit. This means that adverse changes in climate such as rainy and dry periods prior to the harvest can cause ambiguous variations in the data. However, calculation of the total solids per fruit (grams of total solids on a per fruit basis) is not influenced by moisture in the fruit (Requejo-Jackman *et al.,* 2009). Throughout this report, dry matter will refer to % dry matter while total solids will refer to grams of total solids on a per fruit basis.

A similar result to Requejo-Jackman *et al.* (2009) was found in this study (Figure 4.6). A good example in this figure is for fruit in the Bombay orchard, where the trend in % dry matter showed a decline at the stage of fruit maturity where % oil accumulation was at its fastest rate (between 120 and 190 DAFB). A similar trend was observed in fruit from Waiheke, where a sharp decline in % dry matter was evident after 190 DAFB, irrespective of the observed increase in % oil content. No clear trend was observed in fruit from Hawke's Bay; a decline in % dry matter as % oil increased was first observed, but after 160 DAFB the % dry matter showed a gradual increase with % oil.

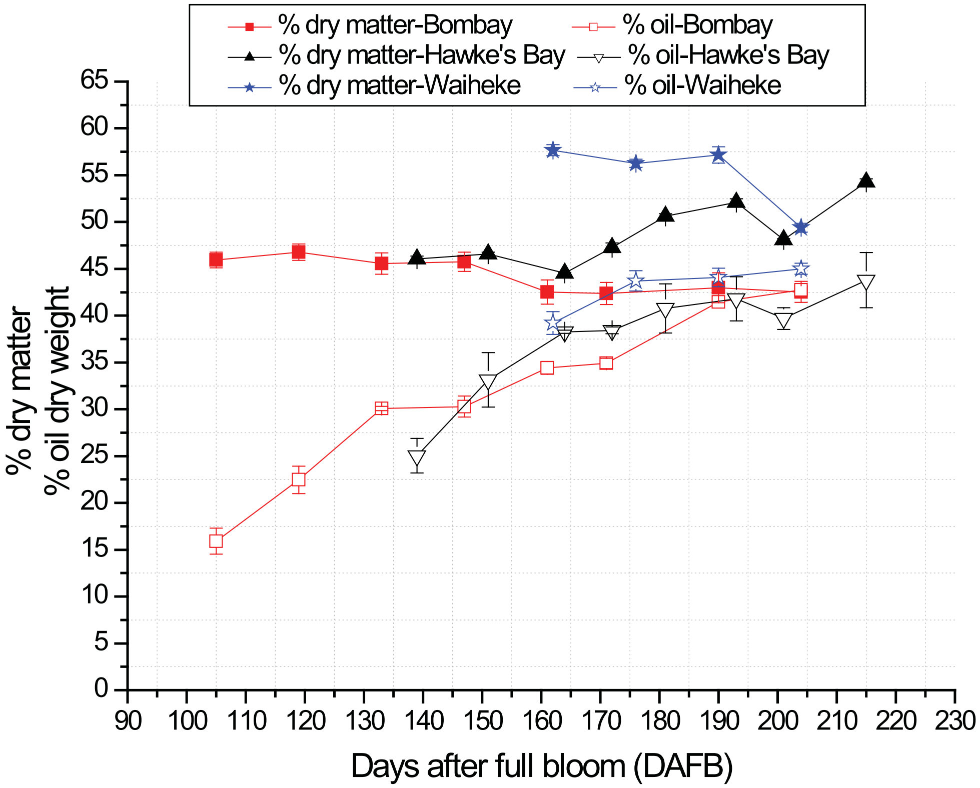


Figure 4.6: Changes in % dry matter and % oil at different harvest maturity after full bloom for Hawke's Bay, Bombay and Waiheke orchards. Error bars represent the standard error of mean.

Figure 4.7 is presented to illustrate the effect of rainfall on dry matter when expressed as percentage. In all the orchards, % dry matter declined as rainfall increased while % oil dry weight continued to rise as suggested by Kiritsakis (1998a). When rain was relatively constant in Hawke's Bay orchard, the dry matter started to increase again with increase in % oil between 170 and 200 DAFB and the sharp increase in % dry matter after 200 days could be attributed to the fall in the monthly rainfall (Figure 4.7). A similar decline in % dry matter was observed in Waiheke and Bombay orchards when rainfall increased.

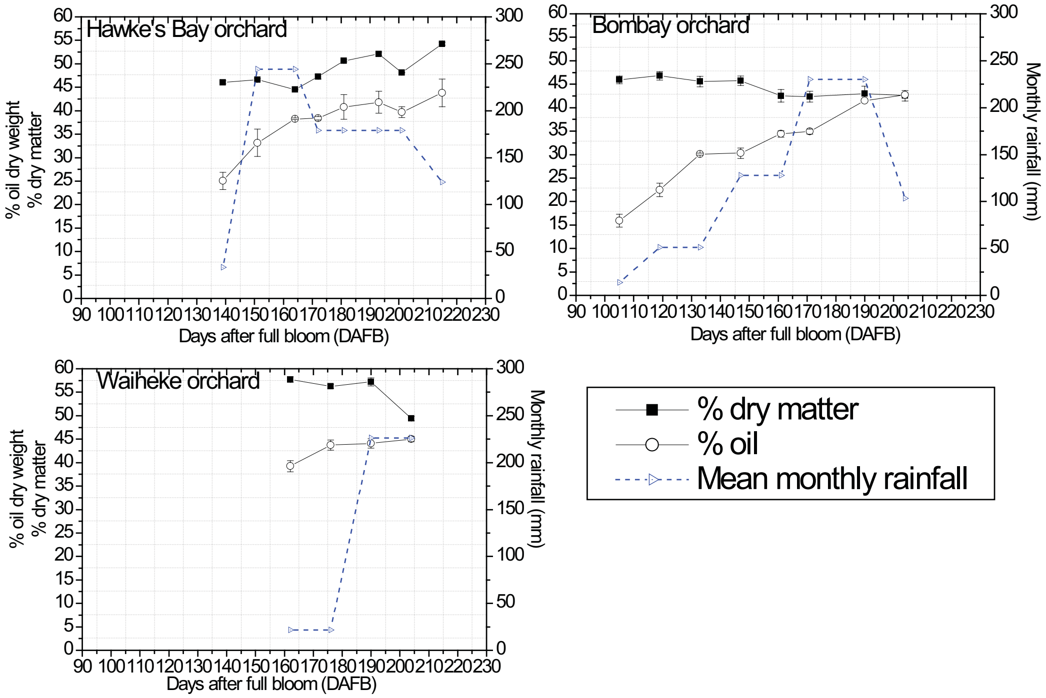


Figure 4.7: Changes in percent dry matter and percent oil with mean monthly rainfall relative to specific harvest days after bloom for Hawke's Bay, Bombay and Simunovich orchards.

However, a better way to measure dry matter is by expressing it as grams of total solids on a per fruit basis. This measure of dry matter is less influenced by weather conditions such as rain and drought. It is also more closely related to oil accumulation and therefore possibly more reliable to predict fruit maturity as shown in Figure 4.8, especially for the Bombay and Waiheke orchards. Figure 4.8 shows that total solids increased gradually with % of oil as the fruit ripens. In the Bombay orchard the total solids consistently increased with oil content from 0.6 to 0.8 g. Similarly, in the Waiheke orchard the total solids increased with % oil from 0.5 to 0.7 g. However, the total solids from Hawke's Bay orchard, started to gradually decline from the highest level of 0.7 g to 0.6 between 180 to 215 DAFB. As shown in Figure 4.5 fruit harvested in the Hawke's bay orchard after 165 DAFB started to show signs of dehydration revealed by shrivelling on the skin due to frost damage on the fruit. This observation is consistent with the result previously shown in Figure 4.3 in which fruit weight in the Hawke's Bay orchard decreased continuously after 165 DAFB.

The total solids showed a good correlation with % oil content for Bombay orchard (R^2=0.92) and Waiheke orchard (R^2=0.71) compared to the Hawke's Bay orchard (R^2=0.56), (Figure 4.9). The correlations of total solids with % oil obtained was stronger than % dry matter with % oil in all the three orchards (Hawke's Bay R^2=0.44, Bombay R^2=0.67 and Waiheke R^2=0.37) (Figure 4.10).

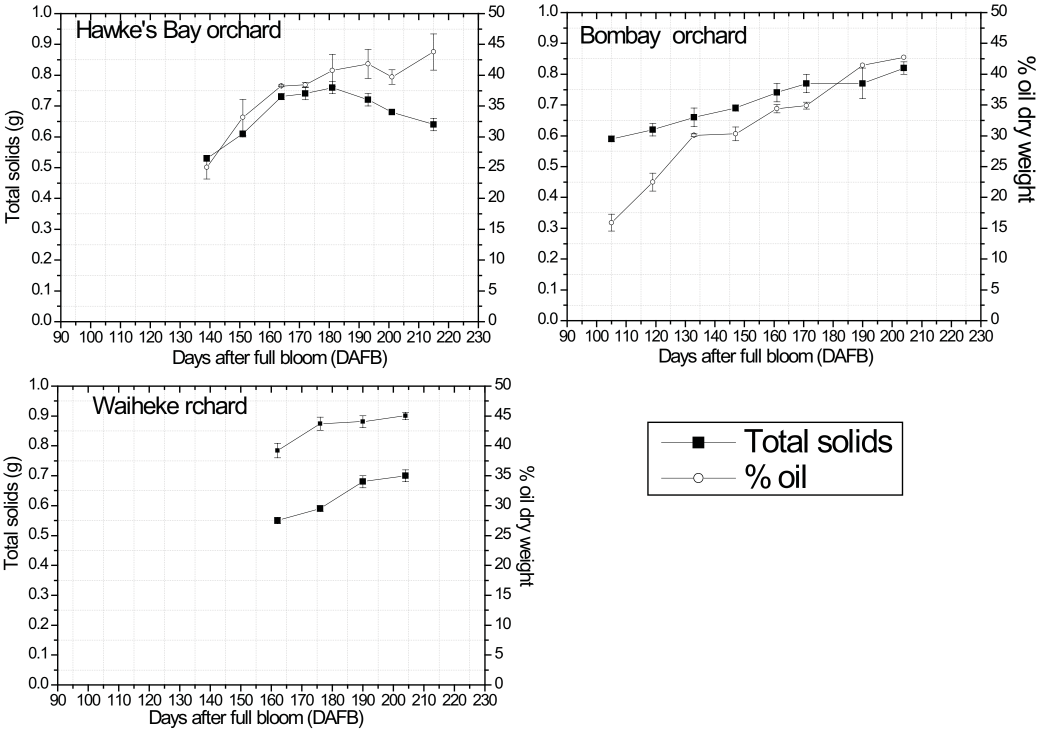


Figure 4.8: Changes in total solids and % oil at different harvest maturity after full bloom for Hawke's Bay, Bombay and Waiheke orchards. Error bars represent the standard error of mean.

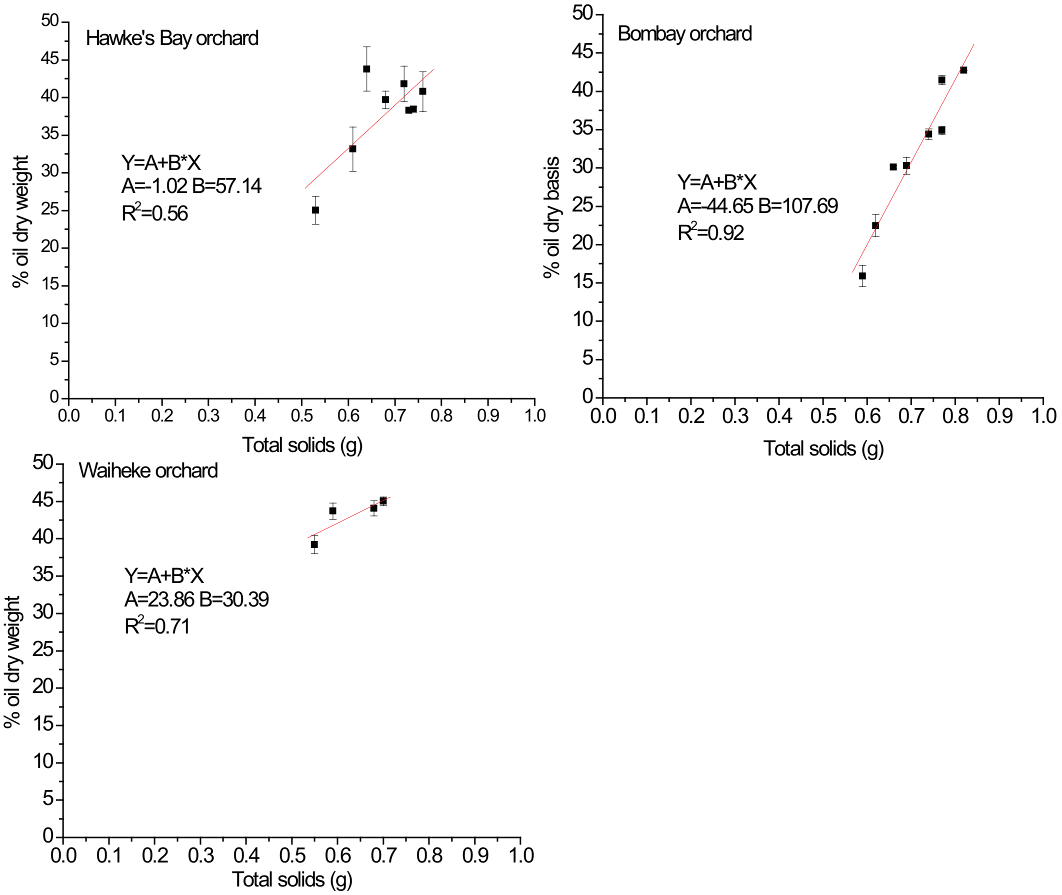


Figure 4.9: Correlation between total solids (g) versus % oil for the olives from Hawke's Bay, Bombay and Waiheke orchards. Data plotted are the means of triplicate values. The error bars are standard errors of the means.

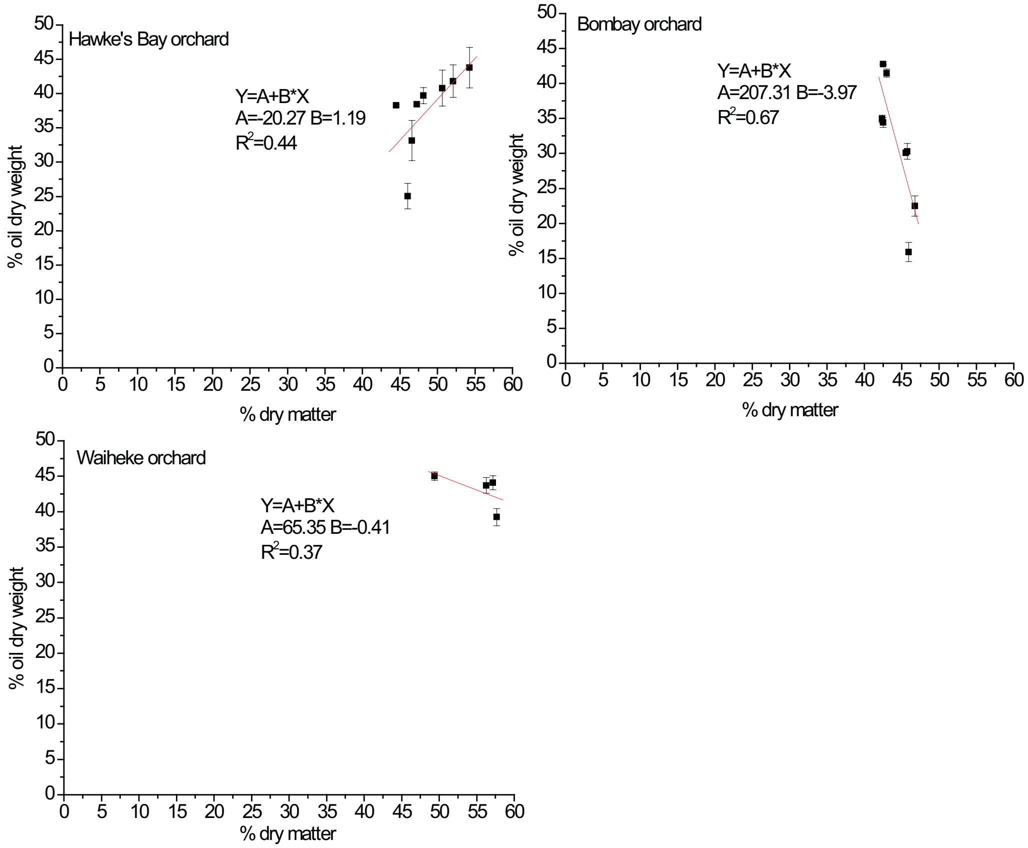


Figure 4.10: Correlation between % dry matter versus % oil for the olives obtained from Hawke's Bay, Bombay and Waiheke orchards. Data plotted are the means of triplicate values. The error bars are standard errors of the means.

In summary, the present findings on the measures of dry matter can reveal two things. Firstly, measurement of the total solids can indicate the level of oil content in the fruit better than percent dry matter. Secondly, as illustrated in Figure 4.8, dry matter expressed as % can be influenced by the climatic conditions, such as dry and rainy conditions prior to the harvest. High moisture in the fruit increases wet weight, hence causes a reduction in the percent dry matter. Therefore, it can be suggested that % dry matter is not a good indicator of oil content in olives as it does not reflect advancement in maturity. However, the measure of total solids is a better indicator of oil content. Although these findings indicate less suitability of % dry matter as a measure of % oil in olives, similar data has been reliably used to determine the maturity in other fruit including oil content in avocado fruit (Clark *et al.,* 2003).

The correlation equation such as shown in Figure 4.9 can be useful to predict the % of oil in the fruit, albeit at any stage of maturity. For example, the correlation between % oil and total solids for Bombay was described by the equation: y = -44.65 + 107.69x, where y is the % oil and x is grams of total solids. From the known value of total solids (x), the % of oil (y) can be calculated as shown in Table 4.2. The R^2 value 0.92 obtained from this equation means that a one unit change in total solids (x) can explain 92 % change in % oil in the fruit (y).

Table 4.2: The percentage of oil in olive fruit estimated from correlation equation between % oil and total solids for Bombay orchard in Figure 4.9

Correlation equation: Y= A+B*X	**Total solids (g)**	**Estimated % of oil from equation Y= A+B*X**
Y = % oil in fruit (unknown)	0.6	19.96
A = -44.65; B = 107.69	0.7	30.73
X = total solids (known)	0.8	41.50

4.1.4 Changes in fruit colour (maturity index) and oil content

Figure 4.11 shows the changes in maturity index (MI) with percent oil at different harvest times. The MI increased with increase in percent oil however, the MI values were variable between the three orchards. At the point of the first commercial harvest (CH1, Figure 4.11) MI was 3.02, 2.25, and 3.81 for Hawke's Bay, Bombay and Waiheke orchards, respectively. The MI values at CH1 for the three orchards are all below the recommended MI values for commercial harvesting of olives grown in the Northern hemisphere (MI≥4) such as Spain, Italy, Tunisia (Kiritsakis, 1998 c). This finding suggests that the MI value used in Northern hemisphere may not necessarily be suitable for predicting olive maturity under New Zealand growing conditions, as this would mean leaving olives on the tree for too long to reach MI ≥4. One possible reason for the difference in MI values between NZ and Northern hemisphere is climate. Olives in the Northern hemisphere such as Spain, Tunisia, Italy are grown in hot and dry climates (Therios, 2009a), and this possibly favours rapid changes in fruit colour relative to percent oil biosynthesis (Conde *et al.,* 2008). As a result, colour maturity index in the Northern hemisphere can predict the percent oil content in the fruit. However, the olive growing regions in NZ are composed of different microclimates ranging from dry to wet and cool to warm environments (Requejo-Jackman *et al.*, 2009). As found in this study, even olives from the three NZ regions studied here revealed different colour profiles at similar harvest times. Kiritsakis (1998c) and Boskou (2006) suggested that MI is a parameter that is influenced by several factors including, geographical location and climate. Ideally these climates are different to NZ and can explain the difference in fruit colouration observed in this study.

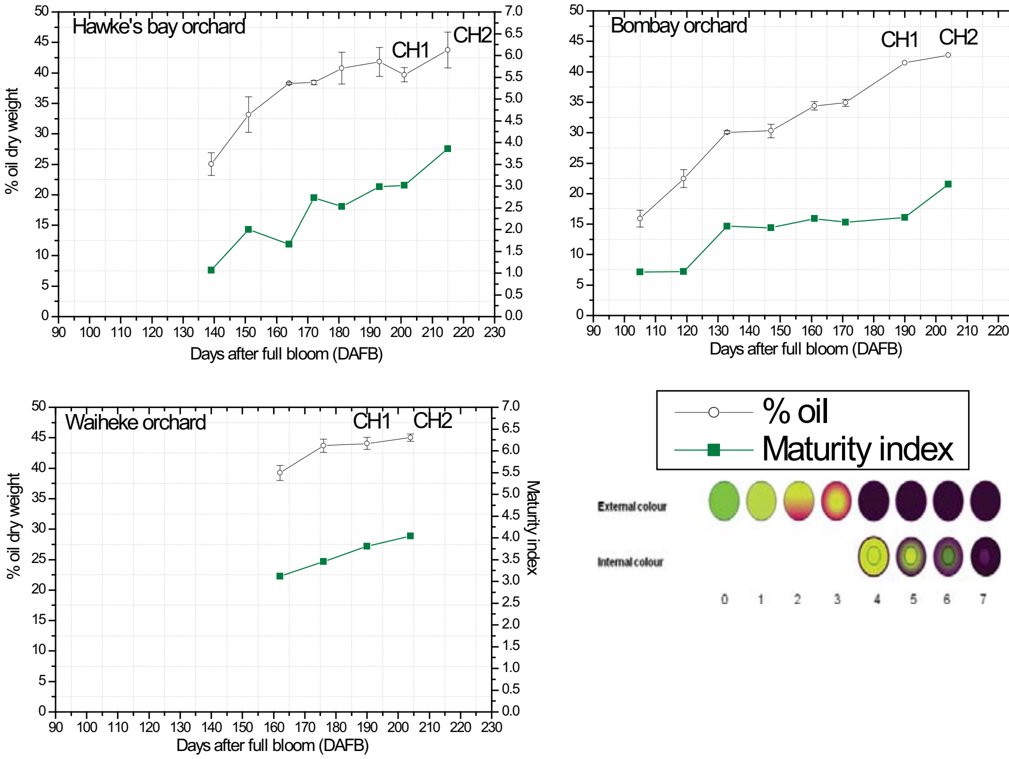


Figure 4.11: Changes in % oil and maturity index during olive maturation obtained for Hawkes Bay, Bombay and Waiheke orchards. Error bars represent the standard error of mean.

The % of oil in the fruit obtained at the commercial harvest (CH1 Figure 4.11) was similar to those found in the Northern hemisphere (as already discussed in section 4.1), although the MI values in New Zealand are below recommended MIs to harvest at. The correlation drawn between MI and % oil in this study (Figure 4.12) demonstrated good positive relationships for Hawke's Bay, Bombay and Waiheke orchards (R^2 =0.73, R^2 =0.87, R^2 =0.81, respectively). The linear correlation obtained can possibly suggest that a 'grower specific' MI may still be used as an index for maturity. A 'grower specific' index is recommended since in this study and others reported by Requejo-Jackman *et al.* (2009) found appreciable variation in MI values between different growing regions as well as between different cultivars grown in New Zealand.

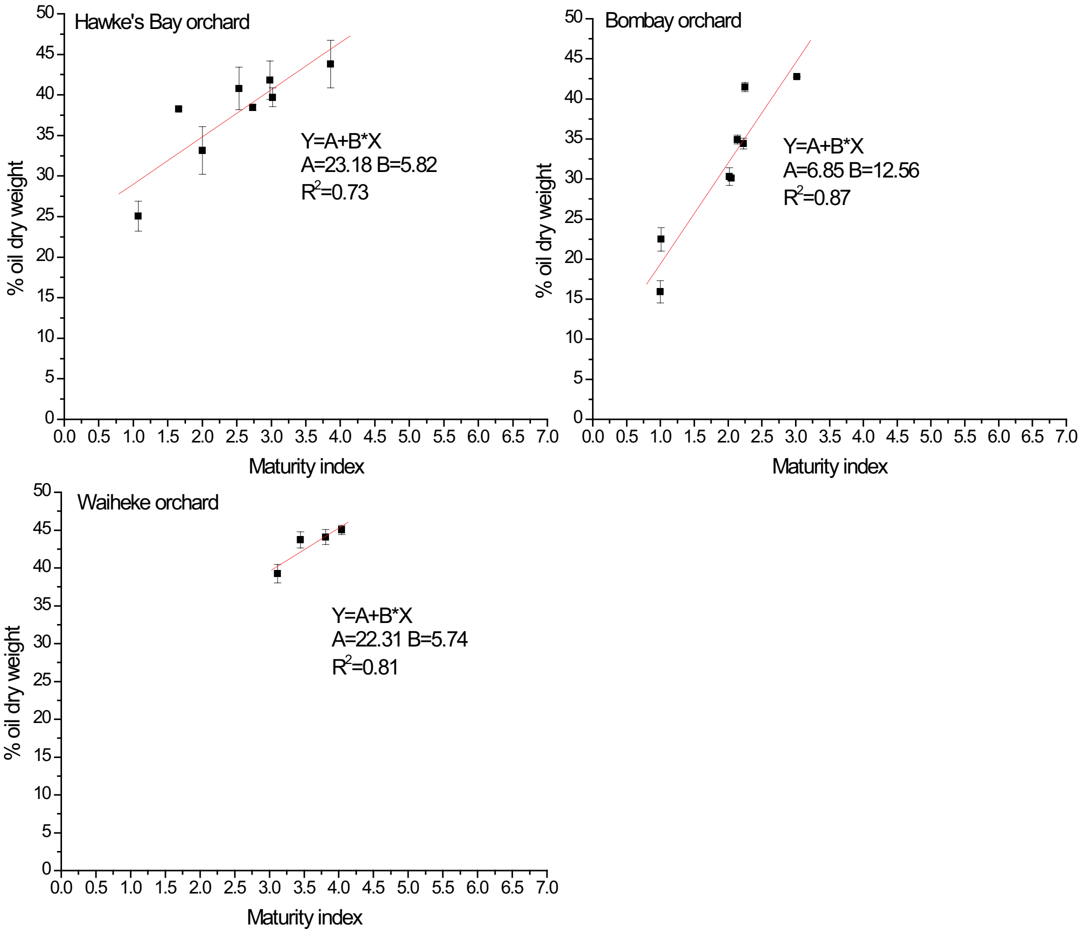


Figure 4.12: Correlations between % oil and maturity index during olive maturation obtained for Hawke’s Bay, Bombay and Waiheke orchards. Data plotted are the mean values. The error bars are standard errors of the means.

4.1.5 Changes in fruit firmness in relation to oil accumulation

Fruit firmness was measured non-destructively using a *FirmTech2*. The results shown in Figure 4.13 show a reduction in firmness with olive maturity and an inverse relationship of firmness with % oil in the fruit. The most rapid decline in firmness occurred between 140 and 200 DAFB, the same period at which rapid accumulation in the % oil occurred. In fruit from the Waiheke orchard, fruit firmness declined rapidly from about 0.8 to 0.6 kg/mm within 42 DAFB. Similarly, the firmness in the fruit from Hawke's Bay rapidly declined from 1.1 to 0.6 kg/mm in 62 DAFB. The firmness in fruit from Bombay orchard declined from about 1 to 0.7 kg/mm in 71 DAFB. The observed reduction in fruit firmness can be attributed to a series of physiological events taking place within the fruit. As the fruit ripens, firmness decreases as a result of endogenous enzymatic solubilisation and depolymerisation of pectins, hemicelluloses and other cell wall components (Huisman *et al.*, 1996). Fernandez-Bolanos *et al.* (1995) found large amounts of cell wall bound enzymes in green olive fruit, but the activities of *β*-galactosidase and cellulases increased as the fruit colour changed during ripening to cause significant reduction in firmness.

The inverse relationship between firmness and oil in the fruit may suggest a possible physiological relationship between the two parameters. As with cell wall changes, it is known that lipid biosynthesis increases during ripening in a very complex process controlled by specific enzymes (Donaire *et al.,* 1984; Harwood *et al.,* 2000). As such, a good correlation was obtained between fruit firmness and % oil content in the present study (Figure 4.14) (Hawke's Bay R^2=0.82, Bombay R^2=0.78 and Waiheke R^2=0.79).

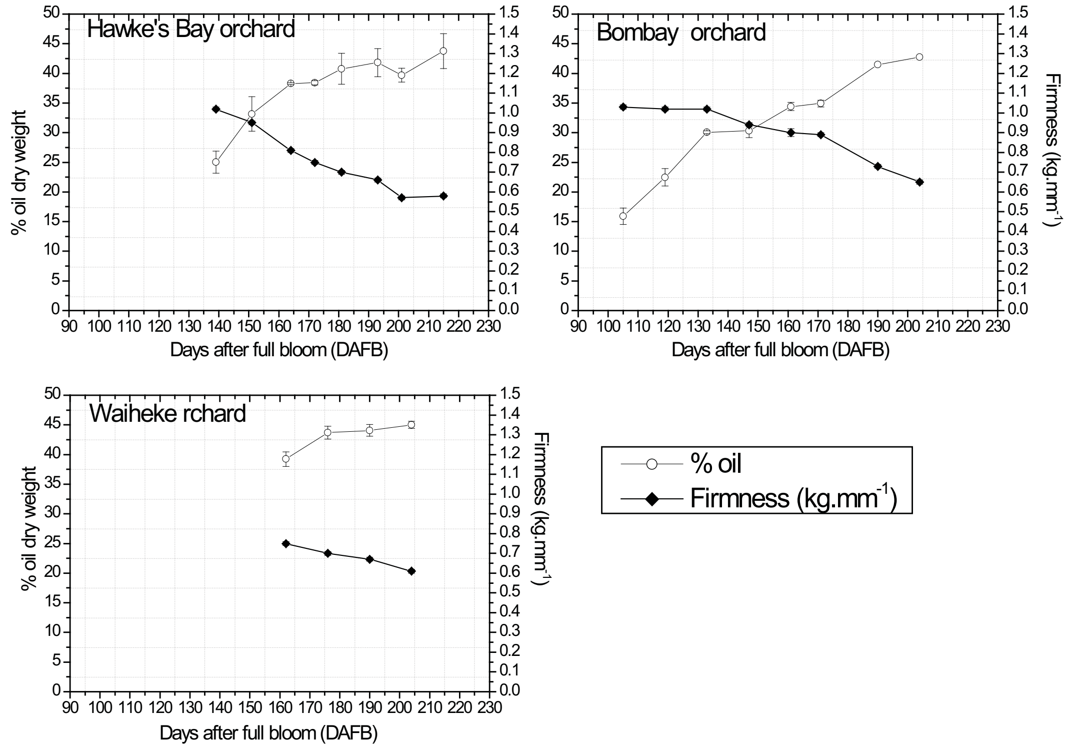


Figure 4.13: Changes in % oil and fruit firmness during olive maturation obtained for Hawke's Bay, Bombay and Waiheke orchards. Error bars represent the standard error of mean.

Results obtained in this study may suggest that firmness measured by *FirmTech2* can provide a non-destructive tool to estimate the oil content in olives at any stage of maturity. In other fruit such as apple, kiwifruit and apricot, firmness measured by different techniques is being extensively used as a useful predictor of the degree of fruit maturity and harvest time (McGlone & Kawano, 1998; Peng & Lu, 2007). However, application of firmness measures to predict oil content is not yet exploited in the olive industry. This study may suggest a possible application of firmness as a measure of oil content in olives. Therefore, further studies are recommended to reinforce the present finding.

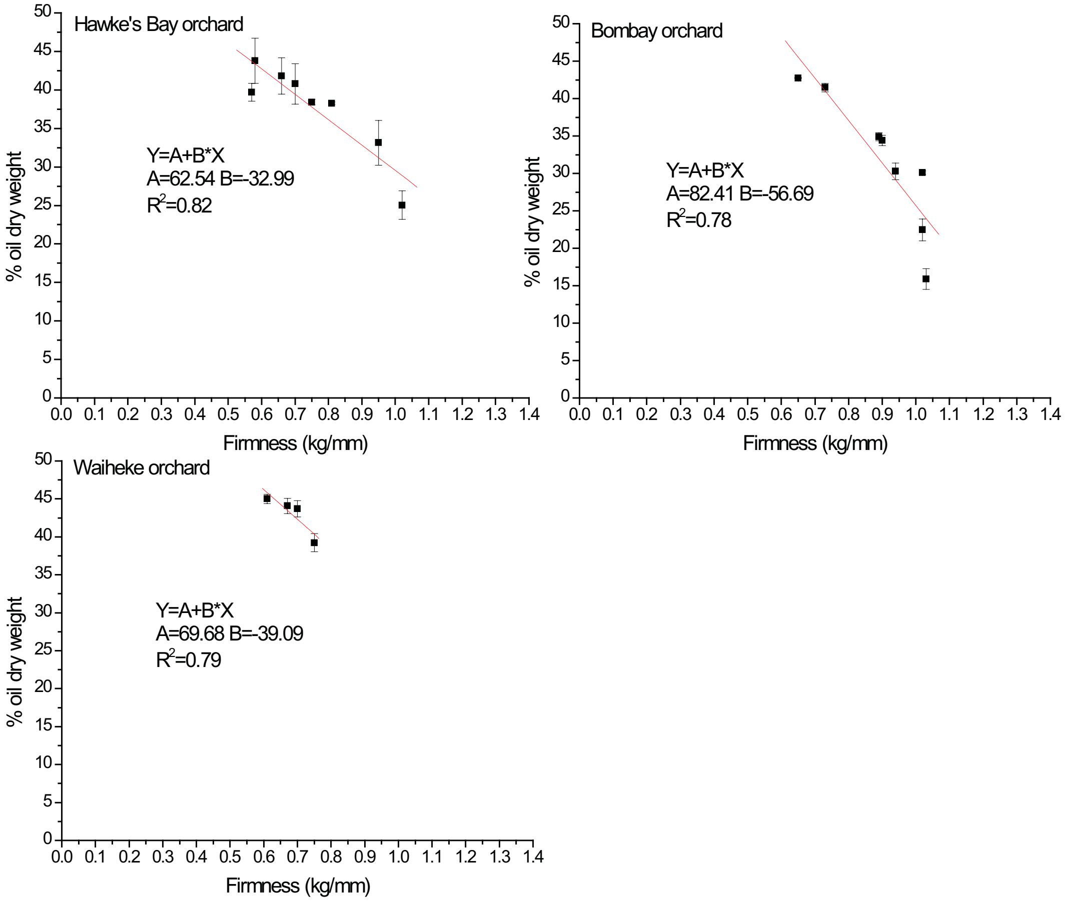


Figure 4.14: Correlations between fruit firmness and % oil during olive maturation obtained for Hawke's Bay, Bombay and Waiheke orchards.

4.2 Quality indices of olive oil

4.2.1 Free fatty acids, peroxide value and extinction coefficients

Table 4.3 shows results for the quality indices, percentage of free fatty acids (% FFA), peroxide value (PV) and extinction coefficients (K_{232} and K_{270}) for the VOOs (cold pressed) obtained in this study. Percentage of free fatty acids is often considered the basic analytical quality index of olive oil (Al-Maaitah *et al.,* 2009). The values of % FFA for all the oils analysed were well below the International Olive Council upper limit (≤ 0.8 %) for extra virgin olive oil (EVOO). Baccouri *et al.* (2007) suggested that in order to obtain VOO of low FFA it is recommended that only healthy olives are processed immediately after the harvest. Delaying olive processing and or extracting olives from damaged fruit can lead to high level of acidity as this allows enough time for enzymatic activities, especially the activity of lypolytic enzymes to cause breakdown of triglycerides to the free fatty acids García *et al.* (1996a).There was no significant difference in % FFA between the VOO obtained at commercial harvest (CH) and two weeks later (LH) for all orchards studied (*p*-value>0.05). This indicates the oil was obtained from healthy undamaged fruit and processed immediately. The cold pressed oils were then stored in dark bottles, flushed with nitrogen and held at -80°C prior to chemical analysis.

Table 4.3 Analytical quality parameters of 'Frantoio' virgin olive oils (cold pressed) from Hawke's Bay, Bombay and Waiheke orchards obtained at commercial harvest (CH) and two weeks later (LH). Results are the mean of three analytical values (± standard error of the mean).

Orchard/harvest category	**DAFB**	**Harvest dates 2010**	**%FFA(±SEM) % w/w as oleic acid**	**PV (±SEM) $MeqO_2$/kg oil**	**K_{232}(±SEM)**	**K_{270}(±SEM)**
Hawke's Bay CH	201	29th Jun	0.10(0.01)	2.71(0.06)	1.57(0.01)	0.03(0.00)
Hawke's Bay LH	215	15th Jul	0.08(0.01)	11.61(0.29)	1.57(0.00)	0.09(0.00)
Bombay CH	190	3rd Jun	0.04(0.01)	3.89(0.11)	1.44(0.03)	0.04(0.00)
Bombay LH	204	7th Jul	0.09(0.01)	4.04(0.06)	1.48(0.00)	0.07(0.00)
Waiheke CH	190	11th May	0.13(0.02)	2.86(0.11)	1.79(0.01)	0.08(0.01)
Waiheke LH	204	26th May	0.08(0.02)	2.80(0.11)	1.76(0.02)	0.07(0.01)
IOC* limit (EVOO)	N/A		≤ 0.8 %	≤ 20	≤ 2.5	Max. ≤ 0.22

*IOC International Olive Council, DAFB days after full bloom

Peroxide value (PV) is another quality parameter for EVOO. PV results (Table 4.3) for all the orchards was within the IOC limit for EVOO, however, a significant increase (*p*-value 0.000) in PV was recorded for Hawke's Bay late harvest (LH). The PV significantly increased from about 2.7 meqO_2/kg oil at CH to 11.6 meqO_2/kg oil at the LH (*p*-value 0.000). This increase was because the fruit obtained at the late harvest LH had been extensively damaged by frost. Cell freezing due to frost damage can cause serious destruction of cell membranes, cell death and oxidation of cell contents *et al.*, 2003). Morello *et al.* (2003) reported a non significant increase in PV in VOO from 6.7 to 8.7 meqO_2/kg oil and a non significant increase in % FFA from 0.11 to 0.14 before and after frost events, respectively. In another recent study, Guillaume *et al.* (2009) found that FFA was the most significantly affected by frost (an increase from 0.25 % before frost to 0.72 % four weeks later), however, their result is largely in disagreement with the findings of Poerio *et al.* (2008) and the present study for the Hawke's Bay VOO (Table 4.3), which showed that PV in the oils was the most significantly affected by frost damage (*p*-value 0.000). However, none of the values obtained in this study were above the IOC limit of 20 meqO_2/kg oil. PV is used as an indicator of oxidative deterioration due to poor harvest and processing technologies applied to extract the oil or poor storage conditions (Angerosa *et al.*, 2006; Ayton *et al.*, 2007; Tsimidou, 2006).

Similar to PV, the measure of extinction coefficients K_{232} and K_{270} indicates the degree of oxidation which has occurred in the oil. K_{232} indicates primary oxidation products (conjugated hydroperoxides) and K_{270} indicates secondary oxidation products such as aldehydes and ketones that are formed during oxidation of the oil (Angerosa *et al.*, 2006; Kiritsakis, 1998e). As reported in Table 4.3, both parameters are within the IOC limits for EVOO (K_{232} ≤0.25, K_{270} ≤0.22). However, a significant increase in K_{270} was noted in the Hawke's Bay orchard from 0.03 at CH to 0.09 at LH (*p*- *et al.*, 2003; Poerio *et al.*, 2008). On the other hand, there was no significant change in K_{232} in all the oils between the two harvest times (*p*-value >0.05).

4.3 Analysis of chemical composition

4.3.1 Changes in composition of fatty acids

The composition of fatty acid was analysed by capillary gas chromatograph (GC) under the experimental conditions outlined in section 3.9.2. Individual fatty acids were identified from retention times of known commercial standards and their concentration in the oils was expressed both as the percentage of total fatty acids and absolute concentrations present, milligram of the fatty acid per gram of oil. Concentrations of all except three fatty acids were expressed as mg fatty acid/g oil, while arachidic acid, C20:0; heptadecanoic acid, C17:0 and heptadecanoic C17:1 were quantified as mg/g oleic acid because their commercial standards were not obtained. Data will be reported for olive oils extracted by solvent (accelerated solvent extraction, ASE) and cold pressing (CP). Figure 4.15 shows the typical chromatograms of the fatty acid standards (a, b) and the virgin olive oil sample (c) obtained in this study.

The most predominant fatty acids (in the order of most present) identified in all the oils were oleic (C18:1), palmitic (C16:0), linoleic (C18:2), palmitoleic acid (C16:1), stearic acid (C18:0) and alpha linolenic (C18:3). Other minor fatty acid components identified were heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), arachidic acid (C20:0), cis-11-eicosenoic acid (C20:1) and behenic acid (C22:0).

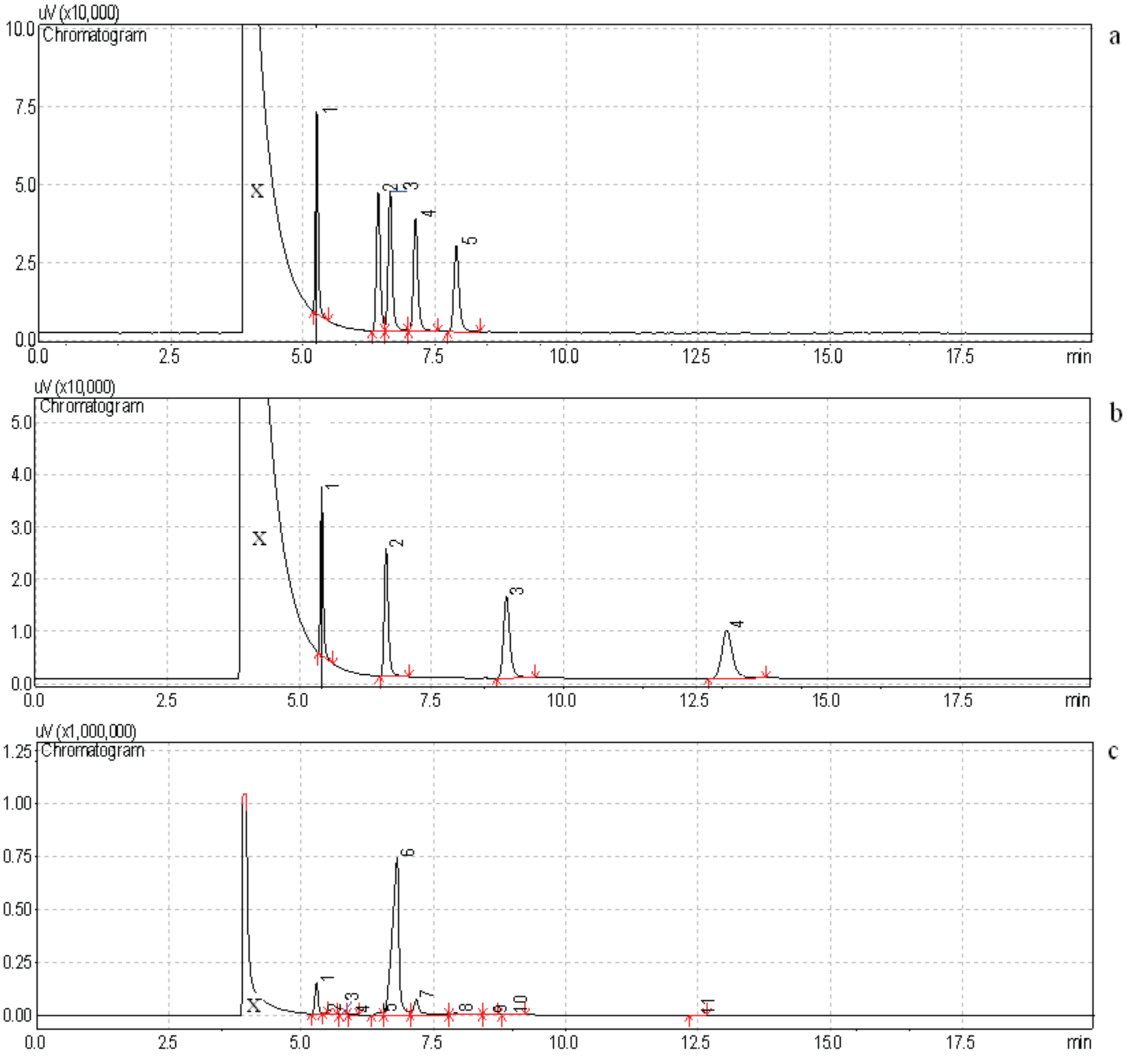


Figure 4.15: Typical chromatograms of mixed fatty acid standards (a & b) and the virgin olive oil sample (c) obtained at commercial harvest in the Bombay orchard. Peaks 1, 2, 3, 4 and 5 in the chromatograms of standard (a) palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and alpha linolenic acid (C18:3), respectively. Peaks 1, 2, 3 and 4 in the chromatograms of standard (b) are palmitoleic acid (C16:1), oleic acid (C18:1), cis-11-eicosenoic acid (C20:1) and erucic acid (C22:1). Peaks (1-11) identified in the sample are palmitic, palmitoleic, heptadecanoic, heptadecenoic, stearic, oleic, linoleic, linolenic, arachidic, eicosenoic and behenic acids, respectively. Peak (x) is the solvent peak.

Figure 4.16 illustrates the changes in percentage composition of major fatty acids in olive oils extracted by solvent from the three orchards. Full data for all the fatty acids identified are reported in Appendix 2. In the Hawke's Bay orchard, the percentage of oleic acid increased from about 79.3 % to 81.2 % between 139 and 215 days after full bloom (DAFB). The percentage of linoleic acid and linolenic varied between 7.4 % and 9.1 % and 0.5 % and 0.9 %, respectively, during fruit maturity. The percentage of oleic acid in the Bombay orchard was similar to Hawke's Bay orchard. Oleic acid remained relatively constant at about 80.4 % throughout the harvest period and then it increased to 81.1 % at 190 DAFB. Within the same period, linoleic acid in the Bombay orchard increased from about 7.1 % to 8.2 %. Palmitic acid decreased consistently from 9.9 % to 8.9 % between 105 and 204 DAFB. For oil from the Waiheke orchard, the percentage of oleic acid remained stable at about 77.3 % between 162 to 190 DAFB, thereafter, it increased slightly to 77.9 % at 204 DAFB. Within the same period, linoleic acid increased significantly from about 8.7 % to 9.4 % (*p*-value 0.03), while alpha-linolenic acid remained stable at about 0.4 %. Meanwhile, the most abundant saturated fatty acid, palmitic acid (C16:0) decreased gradually from 10.7 % to 9.9%.

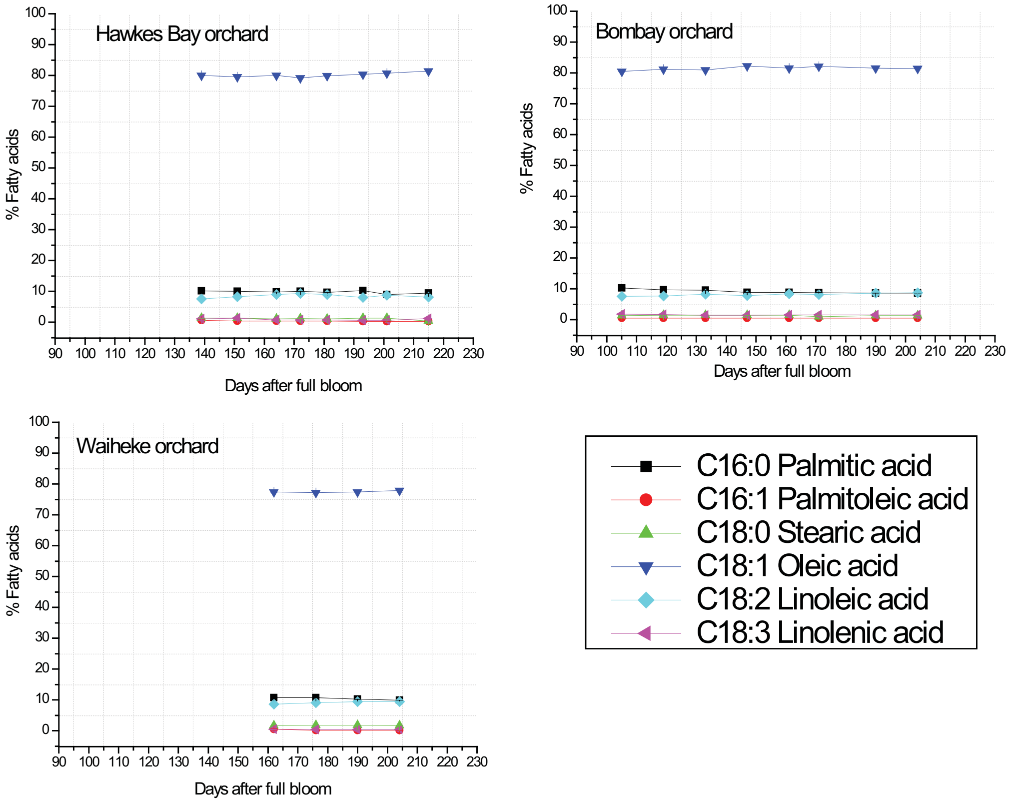


Figure 4.16: The composition of fatty acid expressed as a percentage of total lipids in 'Frantoio' olive oils extracted by solvent at different maturities from Hawke's bay, Bombay and Waiheke orchards. Values are the means of two measurements ± standard errors of the means.

The composition of fatty acids expressed in mg/g of oil (Figure 4.17) followed a similar trend to % total lipids (Figure 4.16), except for oleic acid (C18:1) in the Hawke's Bay orchard which varied considerably between 180 and 200 days after full bloom. Appendix 3 shows the full data, mg/g of individual fatty acids identified in the oils at different maturities. A similar study reported for avocado oil, Requejo-Tapia (1999) observed a steady accumulation of oleic acid in avocado fruit expressed as mg/g.

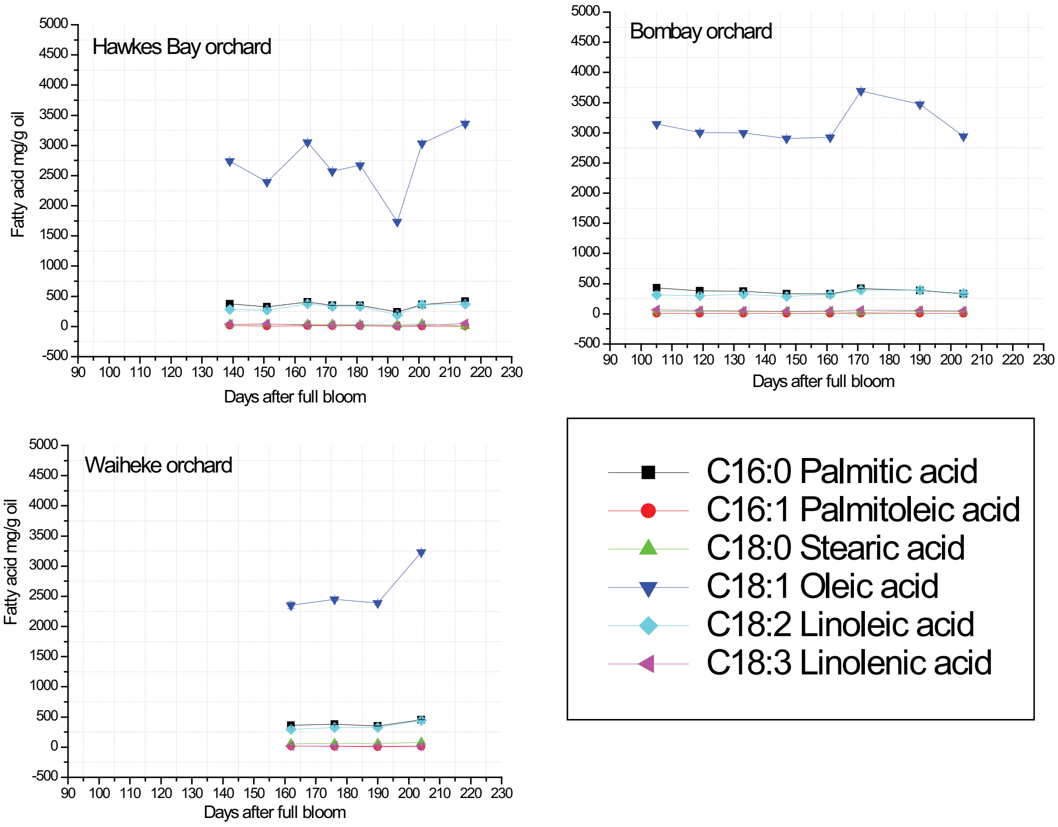


Figure 4.17: The composition of fatty acid expressed as mg/g in ‘Frantoio’ olive oils extracted by solvent at different maturity from Hawke’s bay, Bombay and Waiheke orchards. Values are the means of two measurements ± standard errors of the means.

Oleic acid concentration is the highest in olive oil among all the fatty acids (Aguilera *et al.*, 2005; Beltrán *et al.*, 2004b; Damak *et al.*, 2008; Mailer, 2005). During olive ripening, oleic acid is formed first and its content remains constant or only shows a slight or gradual increase (Beltrán *et al.,* 2004; Conde *et al.,* 2008). Oleic acid increases during ripening because of active triglyceride biosynthesis (Gutiérrez *et al.*, 1999). Similarly, the observed increase in linoleic acid can be explained by the fact that, besides the continuing biosynthesis of triglycerides (with the formation of oleic acid) the enzyme oleate desaturase remains relatively active during ripening and continues to transform the oleic acid into linoleic acid (Gutiérrez *et al.,* 1999). The change in concentration of alpha-linolenic acid varied in the three orchards during the ripening process (Appendix 2).

On the other hand, the observed fall in percentage of palmitic acid during olive ripening can be explained by the dilution effect or a relative increase in concentration of oleic acid and linoleic acid during ripening (Baccouri *et al.*, 2008c; Gutiérrez *et al.*, 1999). The fall in palmitic acid and rise in oleic acid that occurs during olive ripening has been found by other researchers (Ayton *et al.*, 2001; Giacometti & Milin, 2001; Navarro Ruiz *et al.*, 1990).

Analysis of the ASE oils revealed the changes in individual fatty acids during ripening of olive. However, it is the fatty acid composition in VOO (cold pressed oil) that is of paramount interest because of the IOC limits on their % composition. Table 4.4 shows the % of individual fatty acids of the cold pressed oils (obtained at CH and LH). The VOOs from Bombay obtained at CH and LH had slightly higher % of alpha-linolenic acid (C18:3) than the required IOC legal limit of 1 % (1.01 % and 1.04 %, respectively). The high % of alpha linolenic acid found in the VOO from Bombay orchard is of significance to international market access because high levels of alpha-linolenic acid could indicate adulteration of the oil with cheaper oils such as canola, which contains higher % of alpha-linolenic acid.

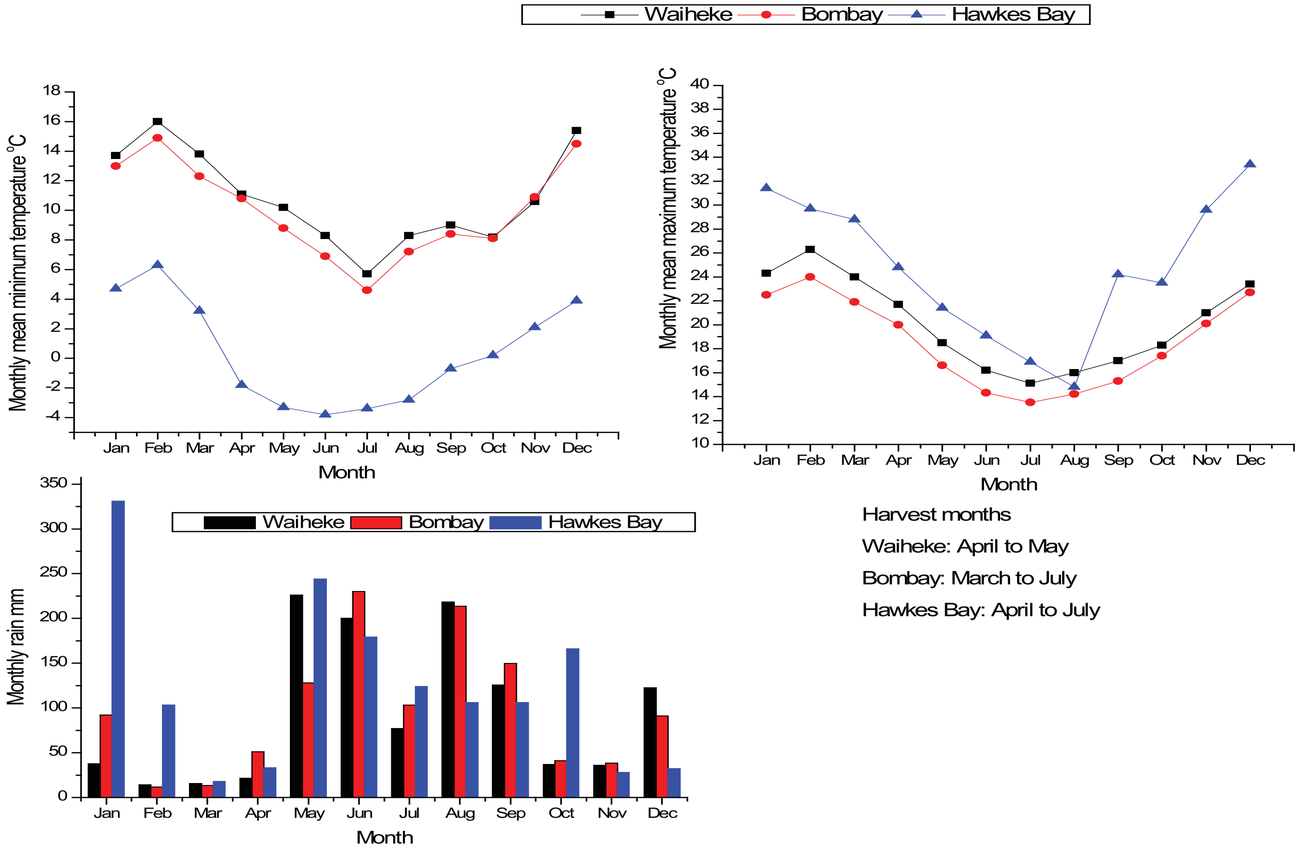
Aparicio & Luna (2002) reviewed the major fatty acid composition of EVOO obtained from ‘Frantoio’ olives grown in the Northern hemisphere. They reported the following mean compositions: palmitic acid (9.5 %), stearic acid (2.9 %), oleic acid (78.2 %), linoleic acid (7.4 %) and alpha-linolenic acid (0.6%). Comparing these results to the present study (Table 4.4), the fatty acid compositions are similar to those reported in the Northern hemisphere, with the VOO from Hawke’s Bay and Bombay obtained at CH having high % of oleic acid ≥ (80%) and Waiheke orchard at LH at about 80 %.

Table 4.4 Fatty acid compositions (expressed as % m/m methyl esters) in ‘Frantoio’ VOO oils obtained at commercial maturity (CH) and two weeks later (LH) in Hawke’s Bay orchards, Bombay and Waiheke. Values are the means of duplicate GC injections (±standard error of the mean).

Fatty acids		**Hawke’s Bay CH(±SEM)**	**Hawke’s Bay LH(±SEM)**	**Bombay CH(±SEM)**	**Bombay LH(±SEM)**	**Waiheke CH(±SEM)**	**Waiheke LH(±SEM)**	**IOC limit**
Palmitic	C16:0	8.22(0.01)	8.51(0.00)	7.97(0.18)	9.69(0.00)	9.44(0.01)	8.51(0.00)	7.5-20
Palmitoleic	C16:1	0.61(0.01)	0.68(0.00)	0.31(0.19)	0.74(0.01)	0.74(0.00)	0.68(0.00)	0.3-3.5
Heptadecanoic	C17:0	0.02(0.00)	0.02(0.00)	0.03(0.00)	0.02(0.00)	0.02(0.00)	0.02(0.00)	≤0.3
Heptadecenoic	C17:1	0.07(0.00)	0.07(0.00)	0.08(0.00)	0.05(0.00)	0.05(0.00)	0.07(0.00)	≤0.3
Stearic	C18:0	1.42(0.00)	1.30(0.00)	1.27(0.01)	2.09(0.00)	2.06(0.00)	1.30(0.00)	0.5-5
Oleic	C18:1	80.59(0.0)	80.56(0.01)	81.30(0.01)	77.33(0.09)	77.59(0.07)	80.56(0.01)	55-83
Linoleic	C18:2	7.66(0.01)	7.36(0.01)	7.45(0.00)	9.08(0.10)	9.27(0.04)	7.36(0.01)	3.5-21
Linolenic	C18:3	0.91(0.00)	0.95(0.00)	1.01(0.00)	0.43(0.00)	0.41(0.00)	0.95(0.00)	≤ 1.0
Arachidic	C20:0	0.24(0.00)	0.25(0.00)	0.27(0.00)	0.28(0.00)	0.27(0.00)	0.25(0.00)	≤ 0.6
Eicosenoic	C20:1	0.25(0.00)	0.27(0.00)	0.34(0.00)	0.22(0.00)	0.22(0.00)	0.27(0.00)	≤ 0.4
Behenic	C22:0	0.09(0.00)	0.09(0.00)	0.09(0.01)	0.06(0.01)	0.07(0.00)	0.09(0.00)	≤ 0.2
	Total	100.07	100.06	100.12	100.00	100.14	100.06	
	ΣSFA	9.98	10.16	9.63	12.14	11.86	10.16	
	ΣUFA	90.09	89.90	90.49	87.86	88.28	89.90	
	ΣPUFA	8.57	8.31	8.46	9.52	9.68	8.31	
	ΣPUFA/ΣSFA	0.86	0.82	0.88	0.95	0.86	0.82	
	ΣMUFA/ΣSFA	8.17	8.03	8.52	8.62	8.17	8.03	

SFA: saturated fatty acid; UFA: unsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Comparing the fatty acid composition in the VOO from the three orchards (Table 4.4), there are some notable differences, mainly in the content of saturated palmitic acid (C16:0), unsaturated oleic acid (C18:1) and linoleic acid (C18:2). At the commercial harvest CH, Waiheke had significantly higher percentage of palmitic acid (9.7 %) compared to Bombay (8.0 %) and Hawke's Bay (8.2 %) orchards (*p*-value<0.05). At the same harvest, Waiheke orchard was significantly lower in oleic acid (77.3 %) compared to Hawke's Bay (80.6 %) and Bombay (81.3 %) orchards (*p*-value<0.01). The observed variations in oleic acid and palmitic acid between the three orchards can be attributed to their climatic differences (rainfall and minimum and maximum air temperatures) shown in Figure 4.18. This figure shows that the Waiheke orchard was relatively warmer than Hawke's Bay and Bombay orchards, but it received similar amounts of rainfall to the Bombay orchard. The mean maximum air temperature in the Hawke's Bay orchard was the highest overall, however, its lowest minimum temperature between March and September means it recorded the lowest mean temperature, overall. Annual climatic data of all the orchards are reported in Appendix 4.



Harvest months

Waiheke: April to May

Bombay: March to July

Hawkes Bay: April to July

Figure 4.18: Annual mean maximum and minimum air temperature and rainfall for Hawke’s Bay, Bombay and Waiheke orchards. Source of weather data: The National Climatic Data Base-NIWA (Waiheke and Bombay) and http://waterwatch.net.nz (Hawke’s Bay), 2010 data.

Climate plays a vital part in lipid biosynthesis and composition of olive oil (Aparicio *et al.*, 1994; Cossignani *et al.*, 2001; Damak *et al.*, 2008; Donaire *et al.*, 1984; Harwood & Sanchez, 2000; Hernández *et al.*, 2008; Ichihara, 1999; Oueslati *et al.*, 2009). Cold climate in particular enhances more production of unsaturated fatty acids relative to saturated fatty acids (Beltrán *et al.*, 2004a; Harwood *et al.*, 1994). Both Beltrán *et al.* (2004a) and Harwood *et al.* (1994) in their study on avocado and olive oil, respectively, suggested that reduced growth temperatures increases the unsaturation of membrane lipids in order to maintain membrane fluidity at the low growth temperatures. Similarly, Ichihara *et al.* (1999) proposed that that plants growing in cold climates have the mechanism to retain membranous fluidity by changing the fatty acid composition of the membranous lipids in order to be relieved from the cold stress. Kaiser and Wolstenholme (1994) found the content of oleic acid in avocado oil from a warmer site was 20 % lower than the oil obtained from a cooler site, while the sum of monounsaturated fatty acids were 10 % higher in the oils from the cooler site. Therefore, in the light of weather and air temperature data (Figure 4.18) and similar studies already discussed, it can be suggested that the cold climate, particularly in the Hawke's Bay orchard may favour biosynthesis of unsaturated fatty acids such as oleic acid more than the saturated fatty acids palmitic acid. In fact, the sum of saturated fatty acid at the commercial harvest CH was higher in Waiheke orchard (12.14 %) than in Bombay orchard (9.6 %) and Hawke's Bay orchard (9.9 %) (Table 4.4). There was no significant difference in the fatty acid composition of frost damaged and non frost damaged fruit from Hawke's Bay orchard (*p*-value 1.000). This is in agreement with other authors who found frost did not have an impact on the fatty acid composition in olive oil *et al.*, 2003).

Finally, fatty acids are an important nutritional component of olive oil. It has been suggested that the ratio of polyunsaturated fatty acid to saturated fatty acid (PUFA/SFA) can be used as a measure for numerous health benefits, especially for coronary heart diseases (Dabbou *et al.*, 2009b). No specific data could be obtained on the ratio of PUFA/SFA required for healthy nutrition in New Zealand, but according to the dietary guideline for healthy nutrition reported by Ribarova *et al.* (2003), a PUFA/SFA ratio above 1.5 is associated with good health. Kiritsakis (1990) reported the ratio of PUFA/SFA in olive oil in the range 0.14 to 1.19 depending on the growing location. In the present study the ratio between PUFA/SFA in VOO was between 0.78 to 0.95 across the three orchards, slightly higher than the ratio reported for 'Seyali' VOO (0.4-0.6), but lower than the ratio obtained for 'Meski' VOO (1.7) and 'Picholine'

VOO (1.6) grown in Spain (Sakouhi et al., 2008). Other studies have suggested better health benefits from a high dietary ratio of monounsaturated fatty acids (MUFA), especially oleic acid to saturated fatty acid (SFA), than from a diet with a high PUFA/SFA (Elmadfa & Kornsteiner, 2009; Escrich *et al.*, 2006; Lorgeril & Salen, 2008; Lunn & Theobald, 2006; Min *et al.*, 2005; Pauwels & Covas, 2009; Ros, 2008; Ruiz-Canela & Martínez-González, 2011; Shahar & Grotto, 2006). In the present study, the ratio of MUFA/SFA was in the range of 6.5 to 8.6. However, it is important to note that the nutritional benefits of olive oil cannot be derived only from the PUFA/SFA or MUFA/SFA ratio, but rather from a combination of fatty acids and other compounds in the oil, such as the antioxidants (phenolics, tocopherols) and sterols. It is the combination of all components in the oils that are known to contribute to several health claims, especially in reduction of cancer and CVD (Jones & Abumweis, 2009; Woyengo *et al.*, 2009).

4.3.2 Composition of tocopherols

The quantity of individual tocopherols (alpha α, gamma γ, and beta β) and total tocopherols obtained by HPLC for the VOO is shown in Table 4.5. No delta tocopherol was detected in the oils.

Table 4.5 Tocopherol content in 'Frantoio' VOO obtained at commercial harvest (CH) and two weeks later (LH) in Waiheke, Bombay and Hawke's Bay orchards. Values are the means of duplicate HPLC injections (±standard error of the mean).

Tocopherol mg/kg oil	**Hawke's Bay CH(±SEM)**	**Hawke's Bay LH(±SEM)**	**Bombay CH(±SEM)**	**Bombay LH(±SEM)**	**Waiheke CH(±SEM)**	**Waiheke LH(±SEM)**
Alpha α	146.22(0.75)	77.09(2.97)	137.24(0.12)	126.04(2.23)	156.57(2.55)	145.79(0.36)
Beta β	2.92(0.41)	2.04(0.09)	1.04(0.06)	1.34(0.18)	0.98(0.31)	0.69(0.01)
Gamma γ	6.91(0.29)	2.08(0.39)	3.26(0.44)	3.49(0.07)	5.15(0.68)	3.13(0.22)
∑α β γ δ	156.06	81.20	141.55	130.87	162.70	149.60

Figure 4.19 shows the typical chromatograms of the mixed tocopherol standard (a) and those identified in the olive oil samples (b). Total tocopherols in the VOO was the highest in Waiheke CH (162.70 mg/kg), followed by Hawke's Bay CH (156.06 mg/kg) and Bombay CH (141.55 mg/kg). There is no IOC limit for the levels of tocopherol in extra virgin olive oil. However, a good quality cold pressed olive oil has been suggested to contain tocopherol levels between 100 and 300 mg/kg oil (Baccouri *et al.*, 2008a). The data for individual tocopherols for oils extracted by solvent at different maturity is reported in Appendix 5. The oils were found to contain the highest content of alpha (α) tocopherol, followed by gamma (γ) and beta (β). No delta (δ) tocopherol was detected in all the oils extracted by solvent. Concentrations of the individual tocopherols decreased with the degree of ripening in accordance with other researchers (Abramovic *et al.*, 2007; Krichene *et al.*, 2007; Slover *et al.*, 1983), and the significant reduction in alpha tocopherol in Hawke's Bay LH can be attributed to frost damage.

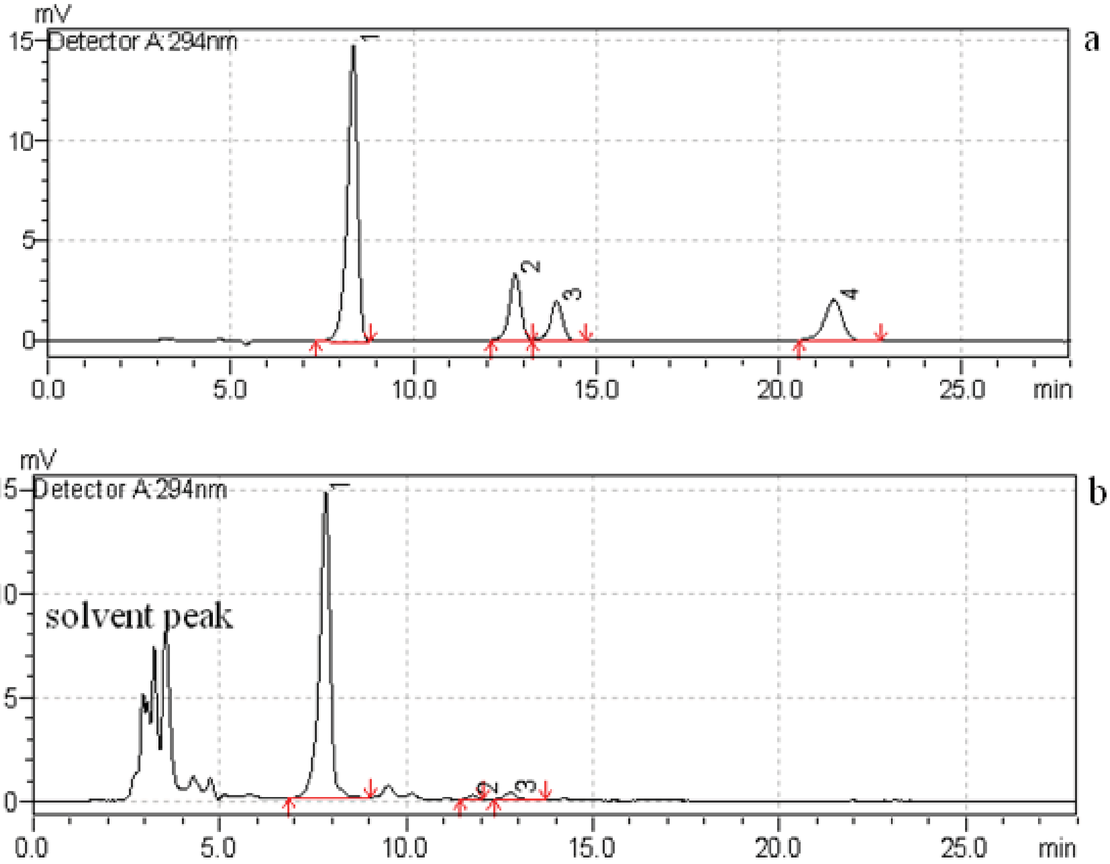


Figure 4.19: Typical chromatograms of mixed tocopherol standards (a) and identified tocopherols in the virgin olive oil sample (b) obtained at commercial maturity in Waiheke orchard. The peaks were obtained at 294 nm. Peaks: (1) alpha α, (2) beta β, (3) gamma γ and (4) delta δ tocopherols.

4.3.3 Total phenolic content

Figure 4.20 illustrates the changes in total phenolic content (TPC) in the oils extracted by solvent (ASE) at different stages of maturity and in the cold pressed (CP) oils. This figure shows total phenolics declined during olive ripening. Unlike Waiheke and Bombay orchards, there were sudden changes in concentration of total phenolics in the oils from Hawke's Bay orchard during the maturation of the olives. This could be the result of dilution effects due to increased moisture uptake or lack of water as the TPC in olives is influenced by rainfall and irrigation (Dabbou *et al.*, 2010a; Tovar *et al.*, 2001). At commercial harvest, the TPC in the oil from Hawke's Bay orchard was similar to Waiheke ASE and CP oils (668 and 514.8 mg/kg oil, respectively). However, there was a sharp decline in the TPC at 215 days after full bloom in the Hawke's Bay orchard. The fruit obtained after 215 days after bloom was significantly affected by frost (75 days from first frost incidence). Cell freezing as the result of frost damage can cause serious destruction of cell membranes, cell death and a high oxidation of cell contents due to direct contact of endogenous polyphenol oxidase (PPO) enzymes and phenolic substrates causing deterioration of phenolic compounds *et al.*, 2003).

In the Bombay orchard, TPC started to increase at the early stages of maturity, but declined gradually from 133 days after full bloom. At the commercial harvest, the TPC was 426.7 and 122.1 mg/kg in ASE and CP oils, respectively. In the Waiheke orchard, TPC declined gradually from about 1220.6 to 713.6 mg/kg oil. At the commercial harvest, TPC was 818.4 and 620.2 mg/kg in ASE and CP oils, respectively.

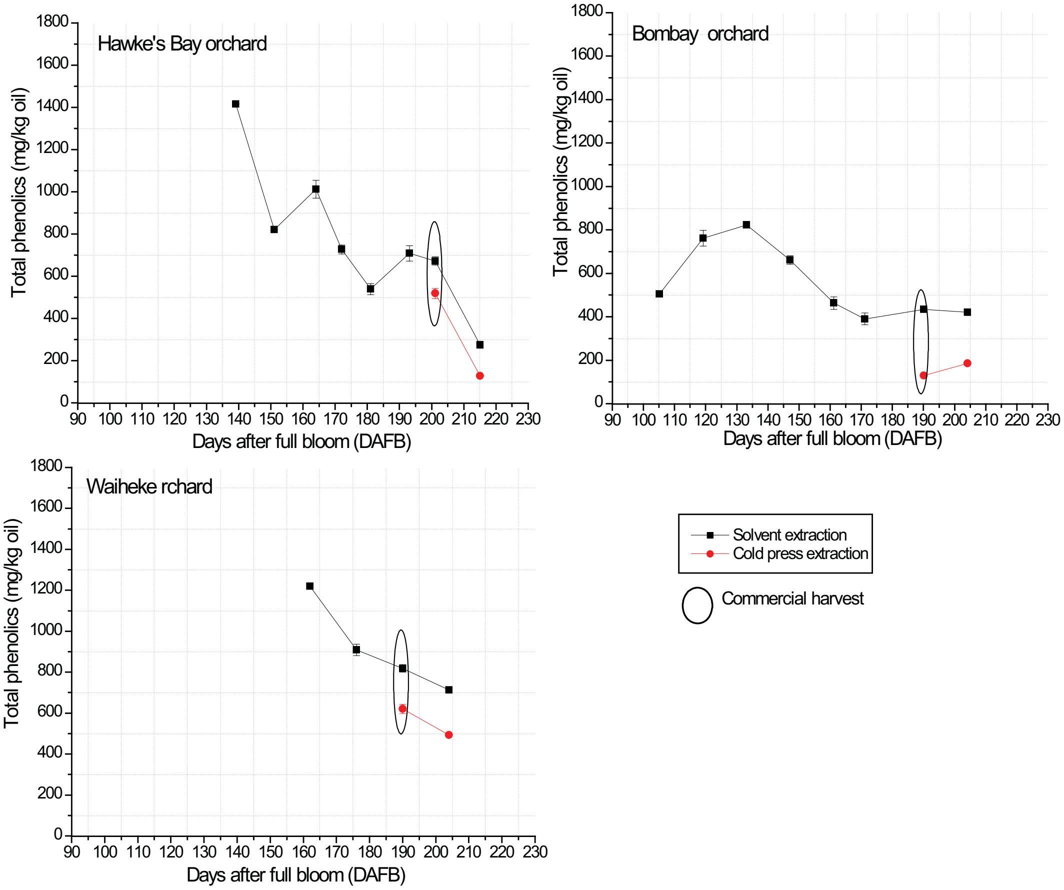


Figure 4.20: Changes in total phenol content in ‘Frantoio’ olive oil at different stages of maturity; obtained by solvent extraction. Circle shows the time olive was harvested for commercial processing in each orchard. Error bars is standard error of mean.

The reduction in TPC during olive ripening can be explained by increased hydrolytic activity of esterase enzymes that causes degradation of oleuropein, the main phenolic compound in the unripened olive, into small molecular weight compounds which are both phenolic and non phenolic in nature (Amiot *et al.*, 1989; Caponio *et al.*, 2001; Malik & Bradford, 2006). In addition to esterase activity, Briante *et al.* (2002a) identified *β*-glucosidase activity in degradation of oleuropein. Several authors have also reported a reduction in total phenolics during olive ripening (Amiot *et al.*, 1986; Amiot *et al.*, 1989; Angerosa *et al.*, 1995; Baiano *et al.*, 2009a; Beltrán *et al.*, 2005; Conde *et al.*, 2008; Garcia *et al.*, 1996; García *et al.*, 1996b).

At similar stages of maturity (between 160 and 215 days after full bloom) the TPC was the highest in Waiheke and lowest in Bombay. Many factors can contribute to this difference, for example, difference in growing climate, orchard location and difference in agronomic practice, especially tree irrigation and pruning. The olive trees in Bombay were irrigated frequently compared to Waiheke orchard whose trees were not irrigated (Personal communication with the orchard Managers). Frequent irrigation hinders the activity of phenylalanine ammonia-lyase (PAL) a key enzyme in the biosynthetic pathway and accumulation of phenolic compounds in olive fruit and olive trees with deficit water tend to have higher phenolics (Servili *et al.*, 2007; Tovar *et al.*, 2002). Olives with high PAL activity have been found to have highest total phenolic concentration (Tovar *et al.,* 2002; Gomez-Rico *et al.,* 2006). Another factor that can influence TPC in olive oil is seasonal variation. An example of seasonal variability can be extreme environmental conditions such as low water availability, extreme sunshine, frost, that can create a stress situation which induces the production of phenolics (Ryan & Robards, 1998). Table 4.6 shows a summary of TPC obtained in the VOO from Hawke's Bay and Bombay orchards over two seasons (2009/2010), and from Waiheke orchard for 2010 season. The data for 2009 seasons were obtained from Requejo-Jackman *et al.* (2009). No data for the 2009 season was available for Waiheke. The harvest dates and colour index are included to further establish the state of maturity the olive was at harvest.

Table 4.6: Total phenolic content in VOO obtained over two seasons at commercial harvest maturity (CH) and late maturity (LH) in Hawke's Bay and Bombay orchards.

Orchard	**Year/harvest category**		**Harvest date**	**Maturity index**	**TPC mg/kg oil (±SEM)**
Hawke's Bay	2009	CH	4-Jun	2.5	332.95(5.63)
		LH	25-Jun	2.7	183.12(3.46)
	2010	CH	30-Jun	3.0	514.81(24.52)
		LH	14-Jul	3.7	123.35(3.25)
Bombay	2009	CH	1-Jul	2.5	242.37(4.90)
		LH	31-Jul	3.0	200.77(7.05)
	2010	CH	22-Jun	2.3	122.09(0.72)
		LH	6-Jul	3.0	178.66(3.70)
Waiheke	2010	CH	10-May	3.8	620.21(22.04)
		LH	25-May	4.0	492.41(2.27)

The summary for Hawke's Bay 2009 LH and 2010 CH shows the TPC in the VOO in 2010 season (514.81 mg/kg) was higher than that obtained in 2009 (183.12 mg/kg) although fruit were obtained at a similar maturity index and harvest date. The opposite was observed in the Bombay orchard, TPC in the VOO in 2009 season (242.37 mg/kg) was higher than in 2010 season (122.09 mg/kg). The influence of seasonal variation can also be observed by noting the different date fruit were harvested at the commercial maturity over the two seasons in both orchards. Phenolics can vary from one season to the next , for example, Salvador *et al.* (2001b) reported variation in TPC between 180 to 614 mg/kg caffeic acid equivalent in 'Cornicabra' virgin olive oil over five crop seasons. Romero *et al.*(2003) found varied levels of phenolics over three growing seasons, 1996/97, 1997/98 and 1998/99. In their study, the lowest total phenol concentration was in 1997/98 crop season (between 106.3 to 84.0 mg/kg), and the highest values were in oils from the 1996/97 and 1998/99 seasons (between 272.5 to 215.2 and 242.0 to 171.9 mg/kg for each year and harvest period, respectively).

4.3.4 Phenolic composition determined by HPLC

The individual phenolic composition was analysed by a reversed phase high performance liquid chromatography (RP-HPLC) under the experimental conditions described in section 3.9.1. The simple phenolic compounds, phenolic acids and secoiridoids were identified at 280 nm, while the flavonoids were identified at 340 nm. Figure 4.21 shows the typical chromatograms of phenolic compounds identified in 'Frantoio' olive oil extracted by solvent (a) and cold press (b). The peaks relating to phenolic compounds identified are shown in Table 4.7. Peaks 1-8, 10, 13 and 16 were identified on the basis of their retention times with commercial standards. Peaks 9, 11-12, 14 and 16 were identified in reference to their elution profile from previously published papers (Brenes *et al.*, 1999; Gutierrez-Rosales *et al.*, 2003; Montedoro *et al.*, 1992a; Montedoro *et al.*, 1992b; Montedoro *et al.*, 1993; Servili *et al.*, 1999b; Tovar *et al.*, 2001) and quantified against an oleuropein standard curve. Their concentrations in the oil were thus expressed as mg/kg equivalence of oleuropein. The unidentified peak 17 was also quantified against the oleuropein standard curve.

Table 4.7: Phenolic compounds identified in the 'Frantoio' olive oils studied from Hawke's Bay, Bombay and Waiheke orchards. For compound structures refer to Figures 2.3 to 2.6.

Peak	**Abbreviation**	**Full name**
1	3, 4- DHPEA	3,4-dihydroxyphenyl ethanol (hydroxytyrosol)
2	*p*- HPEA	*p*-hydroxyphenyl ethanol (tyrosol)
3		Vanillic acid
4		Vanillin
5		*p*-coumaric acid
6		Ferulic acid
7		*o*-coumaric acid
8		Verbascoside
9	3,4-DHPEA-EDA	3,4-dihydroxyphenylethanol linked to dialdehydic form of elenolic acid or dialdehydic form of decarboxymethyl oleuropein aglycone
10		Oleuropein
11		Isomer form of 3,4-DHPEA-EA
12	*p*-HPEA-EDA	*p*-hydroxyphenylethanol linked to dialdehydic form of elenolic acid or dialdehydic form of decarboxymethyl ligstroside aglycone
13		Luteolin
14	3,4-DHPEA-EA	3,4-dihydroxyphenyl-ethanol linked to elenolic or dialdehydic form of oleuropein aglycone
15		Apigenin
16	*p*-HPEA-EA	*p*-hydroxyphenyl-ethanol linked to elenolic acid or aldehydic form of ligstroside aglycone
17		Unidentified

Several simple phenols such as hydroxytyrosol (peak 1), tyrosol (peak 2), vanillic acid (peak 3), vanillin (peak 4), *p*-coumaric acid (peak 5), and ferulic acid (peak 6) were quantified in low concentrations in the phenolic extracts, whereas quantifiable amounts of *o*-coumaric acid (peak 7) was found only in the Bombay orchard. These simple phenols and phenolic acids have been reported in olive oil by previous researchers (Montedoro *et al.,* 1993; Brenes *et al.,* 1999; Pirisi *et al.,* 1997; Tovar *et al.,* 2001). Other phenolic acids such as caffeic acid, syringic acid and homovanillic acid were not present in the oils studied although they have been found present in some olive oils, other than 'Frantoio' (Brenes *et al.,* 1999; Tovar *et al.,* 2001; Montedoro *et al.,* 1992; Tsmidou *et al.,* 1996).

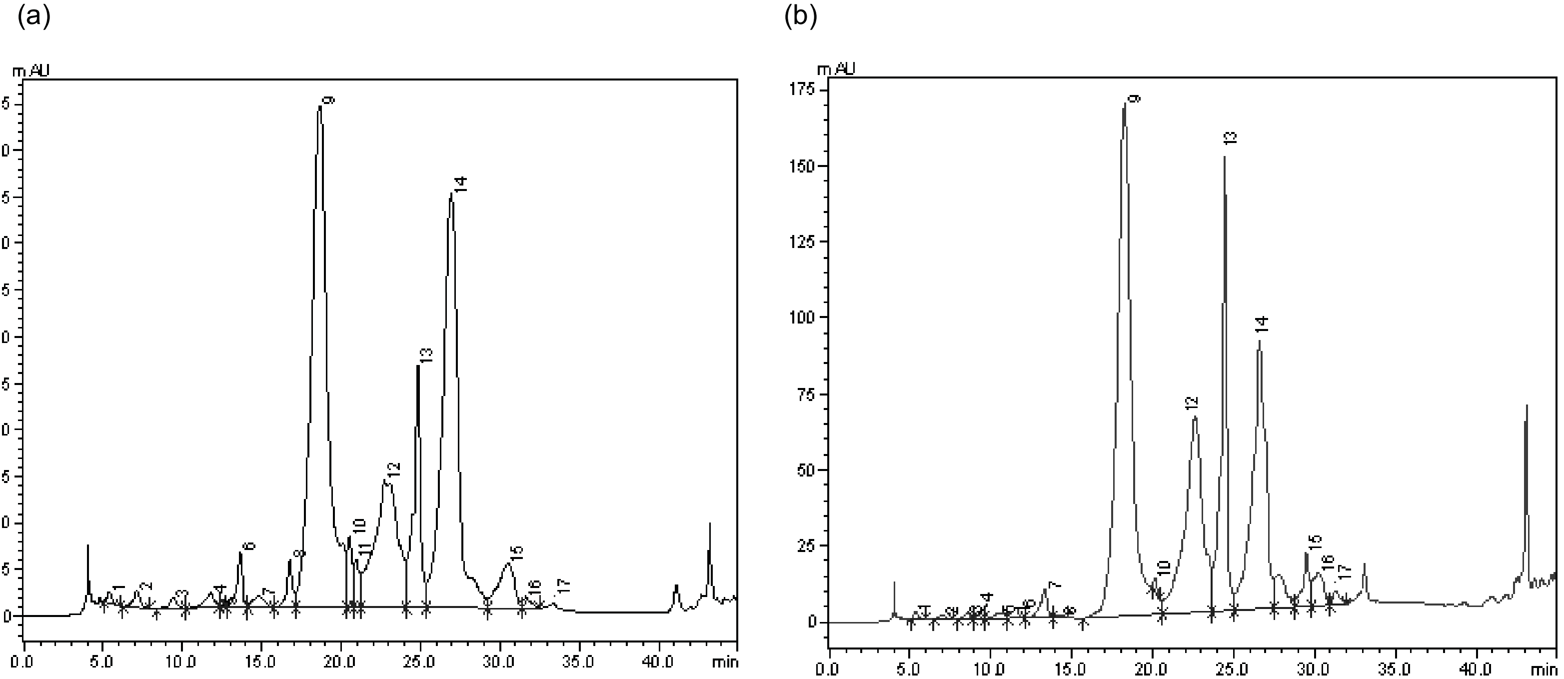


Figure 4.21: Typical HPLC chromatogram of phenolic compounds isolated from ‘Frantoio’ olive oil extracted by solvent (a) and cold press (b) from Waiheke orchard at the same harvest. The shown chromatograms were obtained at 280 nm. Peaks: (1) 3,4-DHPEA , (2) *p*-HPEA, (3) vanillic acid, (4) vanillin, (5) *p*-coumaric acid, (6) ferulic acid, (7) *o*-coumaric acid, (8) verbascoside (9) 3,4-DHPEA-EDA, (10) oleuropein, (11) isomer form of 3,4-DHPEA-EA (12) *p*-HPEA-EDA, (13) Luteolin, (14) 3,4-DHPEA-EA , (15) apigenin, (16) *p*-HPEA-EA, (17) unidentified.

Tables 4.8, 4.9 and 4.10 summarise the phenolic compounds identified and quantified in ‘Frantoio’ olive oil at different maturities from Hawke’s Bay, Bombay and Waiheke orchards, respectively. The results are for olive oils extracted by solvent and by cold press (VOO). Hydroxytyrosol (3,4-DHPEA) and tyrosol (*p*-HPEA) are the two major phenolic alcohols in olive oil. They are not usually found free in non-mature fruits but their concentrations increases with maturation of the fruit due to chemical degradation of their corresponding glucosides (Montedoro *et al.*, 1992; Brenes *et al.*, 2001). However, in this study, the concentrations of hydroxytyrosol and tyrosol generally decreased with olive ripening in the Hawke’s Bay and Waiheke orchards, while a slight increase in concentration of hydroxytyrosol was observed at 190 and 204 DAFB in the Bombay orchard. The content of tyrosol was always higher than hydroxytyrosol in all the orchards. Brenes *et al.,* (1999) also found a higher concentration of tyrosol than hydroxytyrosol in some Spanish olive oils.

In the Hawke’s Bay orchard (Table 4.8), hydroxytyrosol decreased from about 5.6 to 1.0 mg/kg in the oils extracted by solvent and from 1.2 to 0.9 mg/kg in the VOO. The concentration of tyrosol in the VOO decreased from 0.8 to 0.2 mg/kg. The VOO from Bombay orchard (Table 4.9) was found to contain the highest concentrations of tyrosol (2.1 mg/kg) and this decreased to about 1.7 mg/kg in the oil obtained at late harvest (LH). Meanwhile, the concentration of hydroxytyrosol in the VOO from Bombay orchard increased slightly from 0.2 to 0.3 mg/kg. In the Waiheke orchard (Table 4.10), the concentration of hydroxytyrosol in the oils extracted by solvent decreased from 3.6 to 2.3 mg/kg and in the VOO from 1.2 to 0.7 mg/kg at commercial (CH) and late harvests (LH), respectively. Similarly, the concentration of tyrosol in Waiheke decreased from 7.3 to 3.4 mg/kg in the oils extracted by solvent and from 1.67 to 1.17 mg/kg in the VOO.

Quantifiable amounts of the phenolic acid, *o*-coumaric was found only in the Bombay oils, at all stages of maturity (Table 4.9). The concentration of *o*-coumaric acid increased from about 0.2 to 1.3 mg/kg at 133 DAFB and then it decreased to about 0.3 mg/kg at 204 days after full bloom in the oil extracted by solvent. In the cold pressed oils, its concentration ranged between 0.2 to 0.3 mg/kg oil. The concentration of vanillin increased with fruit ripening in Waiheke orchard, however it did not increase in oils from Bombay and Hawke’s Bay orchards. The ferulic acid concentration fluctuated throughout the period of olive maturity. The data for oils extracted by solvent show that vanillic acid decreased with ripening in the Waiheke and Hawke’s Bay orchards but increased slightly in the Bombay orchard. More so, there was no significant change in concentrations of vanillic acid (*p*-value>0.05) in the cold pressed oils, except for Hawke's Bay late cold press (LH) were it increased from about 0.2 to 0.4 mg/kg oil (Table 4.8). This increase can be attributed to frost damage on the fruit obtained at this harvest. Guillaume *et al.* (2009) investigated the effect of frost on olive oil and found a significant increase ($p<0.005$) in vanillin as a percentage of total phenols with the frost damage (from 1.37 % before frost to 3.14 % two weeks after frost) and an increase in vanillic acid (from 1.20 % before frost to 2.13 % two weeks after frost).

Table 4.8: Phenolic composition (mg/kg) obtained at different stages of maturity (days after full bloom) in ‘Frantoio’ olive oil from Hawke’s Bay orchard. Each value is the mean of two measurements followed by the standard error of mean.

		mg phenolics/kg oil (±SEM)									
		Solvent extraction								**Cold pressed oil (VOO)**	
	DAFB	**139**	**151**	**164**	**172**	**181**	**193**	**201**	**215**	**201* CH**	**215* LH**
Peak	**Harvest date**	**29th Apr**	**11th May**	**24th May**	**1st Jun**	**10th Jun**	**22nd Jun**	**30th Jun**	**14th Jul**	**30th Jun**	**14th Jul**
1	3,4-DHPEA	5.60(0.08)	1.92(0.00)	3.68(0.00)	2.43(0.00)	1.77(0.02)	2.65(0.06)	1.93(0.02)	0.96(0.00)	1.19(0.00)	0.92(0.00)
2	p-HPEA	3.63(0.02)	4.85(0.02)	5.64(0.00)	4.45(0.02)	3.16(0.02)	5.26(0.04)	3.02(0.04)	1.74(0.02)	0.76(0.00)	0.20(0.00)
3	Vanillic acid	3.10(0.20)	2.30(0.02)	2.22(0.14)	1.42(0.02)	1.17(0.02)	0.90(0.02)	0.79(0.00)	0.36(0.00)	0.16(0.00)	0.44(0.00)
4	Vanillin	1.29(0.02)	1.08(0.00)	1.54(0.02)	1.24(0.00)	0.95(0.00)	0.48(0.00)	0.76(0.00)	0.52(0.00)	0.50(0.02)	0.16(0.00)
5	*p*-Coumaric acid	1.77(0.02)	1.14(0.02)	4.40(0.00)	5.16(0.02)	4.84(0.00)	3.47(0.00)	2.44(0.02)	1.26(0.02)	0.84(0.00)	1.46(0.02)
6	Ferulic acid	1.35(0.04)	0.22(0.02)	1.84(0.00)	1.65(0.02)	1.03(0.00)	1.58(0.02)	0.99(0.00)	0.60(0.00)	0.08(0.02)	0.10(0.00)
7	*o*-coumaric acid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
8	Verbascoside	2.62(0.48)	0.50(0.18)	1.02(0.02)	0.76(0.00)	0.60(0.00)	0.32(0.00)	0.44(0.00)	0.28(0.00)	ND	ND
9	3,4-DHPEA-EDA	1476.76(14.5)	752.13(0.88)	1104.75(1.02)	828.06(2.42)	391.79(0.91)	1060.6(0.68)	1046.85(0.77)	314.23(0.5)	801.95(11.1)	25.71(0.3)
10	Oleuropein	73.38(0.04)	24.45(1.28)	29.06(0.10)	22.35(0.14)	12.21(0.06)	0.74(0.02)	14.07(0.04)	5.45(0.02)	12.68(8.14)	ND
11	Isomer form of 3,4-DHPEA-EA	148.94(12.91)	66.72(0.66)	59.66(0.46)	35.93(0.14)	22.65(0.68)	0.12(0.04)	48.03(0.06)	9.87(0.10)	ND	ND
12	*p*-HPEA-EDA	310.50(4.25)	209.80(1.22)	212.91(0.87)	164.70(0.33)	94.51(0.85)	143.46(0.05)	129.90(0.63)	48.75(0.03)	138.07(0.09)	30.69(0.2)
13	Luteolin	425.66(0.20)	358.08(0.16)	308.29(0.12)	263.96(0.40)	180.38(0.97)	219.87(0.31)	221.58(0.62)	158.19(0.2)	289.53(0.99)	276.3(0.0)
14	3,4-DHPEA-EA	18.91(1.11)	10.15(0.08)	14.78(0.02)	12.40(0.04)	408.91(1.22)	908.96(0.73)	713.74(0.36)	278.24(0.1)	395.88(0.12)	45.27(0.1)
15	Apigenin	0.36(0.08)	0.40(0.00)	0.36(0.00)	0.36(0.00)	0.40(0.00)	0.28(0.00)	0.56(0.00)	0.36(0.00)	1.95(0.00)	3.07(0.00)
16	*p*-HPEA-EA	22.49(0.05)	28.29(4.77)	20.79(0.35)	11.55(0.19)	7.83(0.03)	48.15(0.11)	3.02(0.14)	3.31(0.04)	11.58(2.55)	15.89(0.7)
17	Unidentified	8.91(0.06)	6.75(1.72)	16.66(0.02)	22.18(0.01)	40.10(0.06)	41.00(0.17)	51.1(9.76)	23.31(0.11)	21.62(0.00)	32.29(0.0)

DAFB days after full bloom; *VOO obtained at commercial maturity (CH) and late maturity (LH); ND not detected

Table 4.9: Phenolic composition (mg/kg) obtained at different stages of maturity (days after full bloom) in ‘Frantoio’ olive oil from Bombay orchard. Each value is the mean of two measurements followed by the standard error of mean.

		mg phenolics/kg oil (±SEM)									
		Solvent extraction								**Cold pressed oil (VOO)**	
	DAFB	**105**	**119**	**133**	**147**	**161**	**171**	**190**	**204**	**190* CH**	**204* LH**
	Harvest date	**29th Mar**	**12th Apr**	**26th Apr**	**10th May**	**24th May**	**3rd Jun**	**22nd Jun**	6th Jul	**22nd Jun**	6th Jul
Peak											
1	3,4-DHPEA	0.90(0.06)	3.71(0.36)	4.04(0.02)	2.66(0.02)	2.20(0.00)	1.41(0.02)	3.96(0.02)	5.21(0.02)	0.22(0.22)	0.28(0.00)
2	p-HPEA	5.71(0.04)	9.71(0.06)	6.29(0.00)	6.75(0.08)	7.51(0.04)	4.82(0.00)	7.03(0.02)	6.31(0.04)	2.10(0.22)	1.73(0.06)
3	Vanillic acid	2.22(0.02)	3.11(0.04)	2.97(0.02)	3.36(0.08)	3.45(0.02)	2.43(0.00)	2.37(0.02)	2.08(0.00)	1.10(0.06)	0.92(0.00)
4	Vanillin	1.74(0.02)	2.81(0.02)	1.97(0.02)	1.04(0.20)	1.04(0.00)	0.48(0.00)	0.84(0.00)	0.92(0.00)	0.80(0.00)	0.84(0.00)
5	p-Coumaric acid	5.17(0.02)	6.38(0.00)	3.42(0.00)	2.94(0.06)	3.79(0.00)	2.51(0.00)	3.50(0.00)	4.99(0.00)	0.52(0.00)	2.43(0.00)
6	Ferulic acid	0.48(0.04)	0.46(0.02)	1.79(0.00)	0.16(0.04)	0.18(0.02)	0.08(0.00)	0.80(0.00)	0.52(0.52)	0.08(0.00)	2.37(0.02)
7	o-coumaric acid	0.24(0.00)	0.66(0.02)	1.25(0.02)	0.42(0.02)	0.50(0.02)	0.44(0.03)	0.32(0.00)	0.44(0.00)	0.16(0.00)	0.28(0.00)
8	Verbascoside	1.87(0.20)	0.68(0.00)	0.80(0.00)	0.54(0.02)	0.24(0.00)	0.66(0.02)	1.25(0.02)	0.42(0.02)	ND	ND
9	3,4-DHPEA-EDA	703.97(1.40)	649.88(8.04)	742.80(2.36)	527.15(1.96)	399.19(0.16)	327.36(0.19)	417.44(2.05)	453.52(0.87)	54.00(2.09)	234.38(0.73)
10	Oleuropein	2.64(0.08)	36.19(1.00)	37.31(0.14)	38.01(0.26)	17.49(0.72)	7.61(0.08)	12.50(0.24)	11.84(0.02)	ND	ND
11	Isomer form of 3,4-DHPEA-EA		53.05(8.79)	58.90(4.32)	63.90(0.60)	27.94(15.39)	15.93(0.08)	27.58(1.12)	19.44(0.00)	ND	ND
12	p-HPEA-EDA	233.70(1.79)	167.11(93.00)	192.81(1.27)	168.10(0.69)	137.91(0.04)	80.69(0.15)	97.80(0.46)	85.27(0.00)	75.05(2.05)	80.49(0.00)
13	Luteolin	510.86(2.32)	578.01(0.81)	336.51(11.26)	311.34(0.46)	306.86(0.63)	222.76(0.22)	206.88(0.32)	198.35(0.00)	363.39(0.75)	385.12(0.82)
14	3,4-DHPEA-EA	236.81(5.93)	493.89(14.43)	665.04(0.36)	574.86(0.77)	347.15(50.04)	221.57(0.69)	325.56(4.64)	235.32(0.00)	42.80(0.08)	87.25(0.17)
15	Apigenin	0.02(0.02)	0.08(0.00)	0.24(0.00)	0.20(0.04)	0.12(0.00)	0.16(0.00)	0.20(0.00)	0.32(0.00)	1.42(0.02)	2.71(0.00)
16	p-HPEA-EA	30.51(0.29)	66.81(0.31)	17.49(0.08)	24.39(0.10)	40.71(0.04)	30.53(6.10)	23.82(0.14)	20.12(0.00)	17.53(0.07)	11.40(0.20)
17	Unidentified	31.40(0.21)	25.44(0.00)	21.60(0.03)	12.28(0.27)	79.47(0.26)	36.18(24.19)	49.02(0.24)	68.05(0.00)	47.24(0.53)	73.93(0.17)

DAFB days after full bloom; *VOO obtained at commercial maturity (CH) and late maturity (LH); ND not detected

Table 4.10: Phenolic composition (mg/kg) obtained at different stages of maturity (days after full bloom) in ‘Frantoio’ olive oil from Waiheke orchard. Each value is the mean of two measurements followed by the standard error of mean.

		mg phenolics/kg oil (±SEM)					
		Solvent extraction				**Cold pressed oil (VOO)**	
	DAFB	**162**	**172**	**190**	**204**	**190* CH**	**204* LH**
	Harvest date	**12th Apr**	**27th Apr**	**10th May**	**25th May**	**10th May**	**25th May**
Peak							
1	3,4-DHPEA	3.59(0.12)	1.73(0.02)	2.77(0.06)	2.27(0.16)	1.21(0.02)	0.74(0.06)
2	p-HPEA	7.26(0.20)	5.30(0.00)	3.37(0.02)	3.41(0.02)	1.67(0.00)	1.17(0.42)
3	Vanillic acid	1.91(0.00)	1.12(0.00)	0.84(0.00)	1.14(0.02)	0.20(0.00)	0.24(0.00)
4	Vanillin	1.56(0.00)	1.60(0.00)	1.77(0.02)	1.83(0.00)	0.40(0.00)	0.44(0.00)
5	p-coumaric acid	2.19(0.00)	1.69(0.02)	2.37(0.02)	2.55(0.00)	0.95(0.00)	1.21(0.02)
6	Ferulic acid	2.09(0.02)	1.77(0.02)	1.87(0.00)	1.81(0.02)	0.08(0.00)	0.10(0.02)
7	o-coumaric acid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
8	Verbascoside	1.87(0.20)	0.68(0.00)	0.80(0.00)	0.54(0.00)	ND	ND
9	3,4-DHPEA-EDA	1949.69(3.66)	1684.65(0.10)	1889.05(1.73)	1546.41(2.23)	1044.90(0.23)	1010.94(1.85)
10	Oleuropein	74.54(0.17)	60.35(0.46)	4.90(0.20)	1.04(0.04)	3.35(0.06)	2.03(0.08)
11	Isomer form of 3,4-DHPEA-EA	38.85(1.87)	30.64(2.14)	ND	ND	ND	ND
12	p-HPEA-EDA	706.45(0.04)	559.02(0.35)	440.01(0.61)	343.11(0.16)	486.72(1.14)	469.3(0.31)
13	Luteolin	382.01(0.96)	300.65(2.24)	203.20(0.08)	243.97(0.20)	338.60(0.69)	370.88(2.42)
14	3,4-DHPEA-EA	1528.86(0.43)	987.97(0.17)	883.61(11.88)	703.47(6.69)	489.16(0.53)	461.52
15	Apigenin	0.54(0.02)	0.44(0.00)	0.40(0.04)	0.32(0.00)	1.90(0.00)	2.42(0.00)
16	*p*-HPEA-EA	161.04(0.37)	94.55(0.17)	45.50(14.84)	42.00(0.34)	42.03(0.14)	48.42(1.32)
17	Unidentified	11.12(0.30)	9.25(0.07)	7.63(1.93)	26.84(0.28)	7.44(0.08)	20.00(0.48)

DAFB days after full bloom; *VOO obtained at commercial maturity (CH) and late maturity (LH); ND not detected

The highest concentration of phenolic compounds identified in the olive oils were the secoiridoids, 3,4-DHPEA-EDA (3,4-dihydroxyphenyl-ethanol linked to dialdehydic form of elenolic acid, peak 9), *p*-HPEA-EDA (*p*-hydroxyphenyl ethanol linked to dialdehydic form of elenolic acid, peak 12), 3,4-DHPEA-EA (3,4-dihydroxyphenyl ethanol linked to elenolic acid, peak 14) and *p*-HPEA-EA (*p*-hydroxyphenyl ethanol linked to elenolic acid, peak 17). This is consistent with other published literature who found secoiridoids the highest phenolic compounds in olive oil (Tovar *et al.,* 2001; Vinha *et al.,* 2005; Servili *et al.,* 1999; Dabou *et al.,* 2009). Figure 4.22 shows the changes in concentrations of the main phenolic compounds and secoiridoids (3,4-DHPEA-EDA, *p*-HPEA-EDA, 3,4-DHPEA-EA and *p*-HPEA-EA) identified in the studied oils at different stages of maturity.

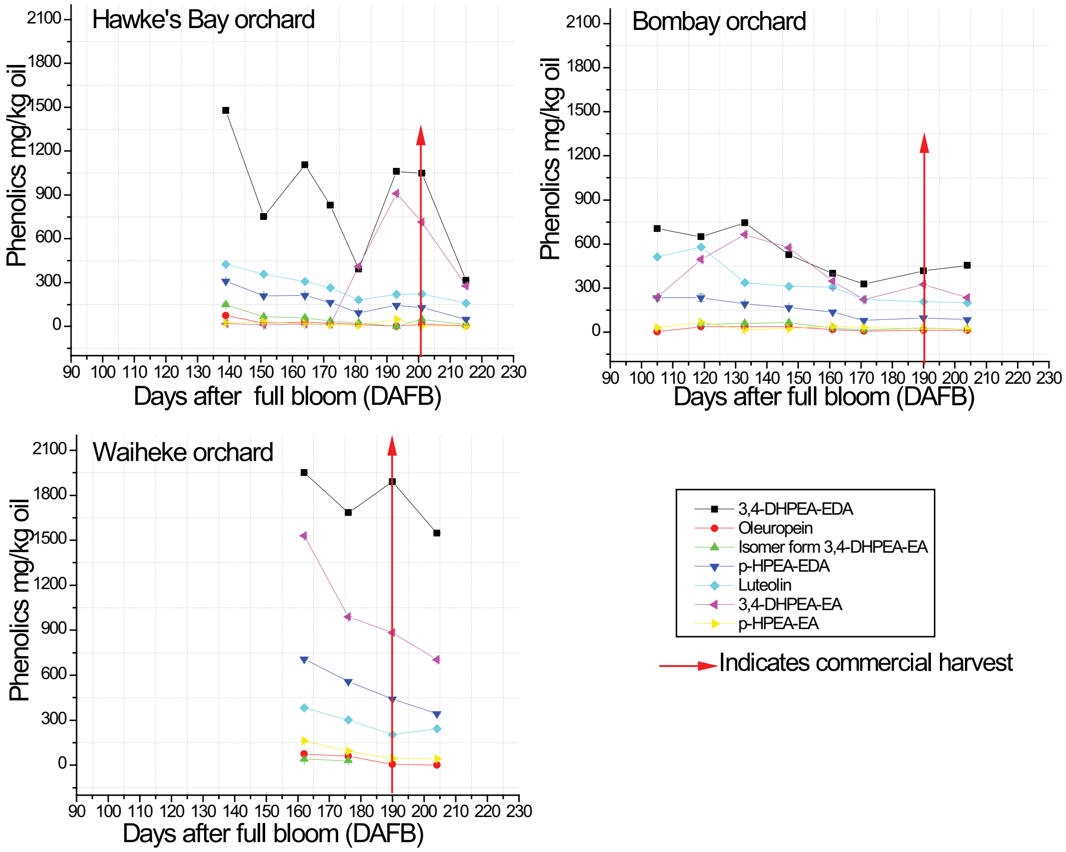


Figure 4.22: Changes in concentration of major phenolic compounds and secoiridoids in identified in 'Frantoio' olive oil extracted by solvent from Hawke's Bay, Bombay and Waiheke orchards at different harvest maturity. Each data point is the mean of two measurements followed by standard error of the mean.

The concentration of the secoiridoids generally decreased with olive ripening in all the three orchards. Between 164 and 215 DAFB the concentration of the secoiridoids in the Hawke's Bay orchard decreased as follows in the ASE oil; 3,4-DHPEA-EDA (from 1104.7 to 314.2 mg/kg), *p*-HPEA-EDA (from 212.9 to 48.8) and *p*-HPEA-EA (20.76 to 23.3 mg/kg), however 3,4-DHPEA-EA showed a very sharp increase just after 170 days after bloom. In the VOO, the concentrations of the secoiridoids were significantly lower in the late harvest (LH, 215 DAFB) VOO due to extensive effect of frost damage (*p*-value 0.01) (Table 4.8). The concentration of 3,4-DHPEA-EDA decreased from about 801.9 to 25.7 mg/kg oil, while *p*-HPEA-EDA decreased from 38.1 to 30.7 mg/kg oil and 3,4-DHPEA-EA decreased from 395.9 to 45.27 mg/kg oil. Oleuropein, (peak 11) isomer form of 3,4-DHPEA-EA (peak 12) were not detected in the VOO. Figure 4.23 shows the HPLC chromatograms of VOO obtained from Hawke's Bay orchard at commercial harvest (61 days after the first incidence of frost) and late harvest (75 days after the first incidence of frost). Only peak 13 (luteolin) did not significantly change (*p*-value>0.05) at the late harvest while a insignificant increase in vanillin was also evident at the late harvest (*p*-value>0.05).

The Bombay orchard had the lowest concentrations of these secoiridoids between similar harvest times (161 and 204 DAFB). Major reductions in concentrations of secoiridoids in Bombay orchard occurred between 130 and 170 days after bloom. The concentration of 3,4-DHPEA-EDA decreased from about 700 mg/kg to about 400 mg/kg after which it appeared to increase slightly. The other secoiridoids declined gradually as follows: *p*-HPEA-EDA from 137.9 to 85.3 mg/kg; 3,4-DHPEA-EA from 347.2 to 235.3 mg/kg and *p*-HPEA-EA from 40.7 to 20.1 mg/kg. The oils from Waiheke orchard had the highest concentration of the secoiridoids, 3,4-DHPEA-EDA (1949.7 mg/kg), *p*-HPEA-EDA (706.5 mg/kg), 3,4-DHPEA-EA (1528.9 mg/kg) and *p*-HPEA-EA (161.0 mg/kg) at 162 days after full bloom. Their respective concentrations decreased to 1546.4, 243.1, 703.5 and 42.00 mg/kg oil at 204 days after full bloom. Reduction in concentration of the secoiridoids with olive maturation has been reported by several authors (Amiot *et al.,* 1986; Owen *et al.,* 2000; Servilli *et al.* 1999; Tsmidou *et al.,* 1992).

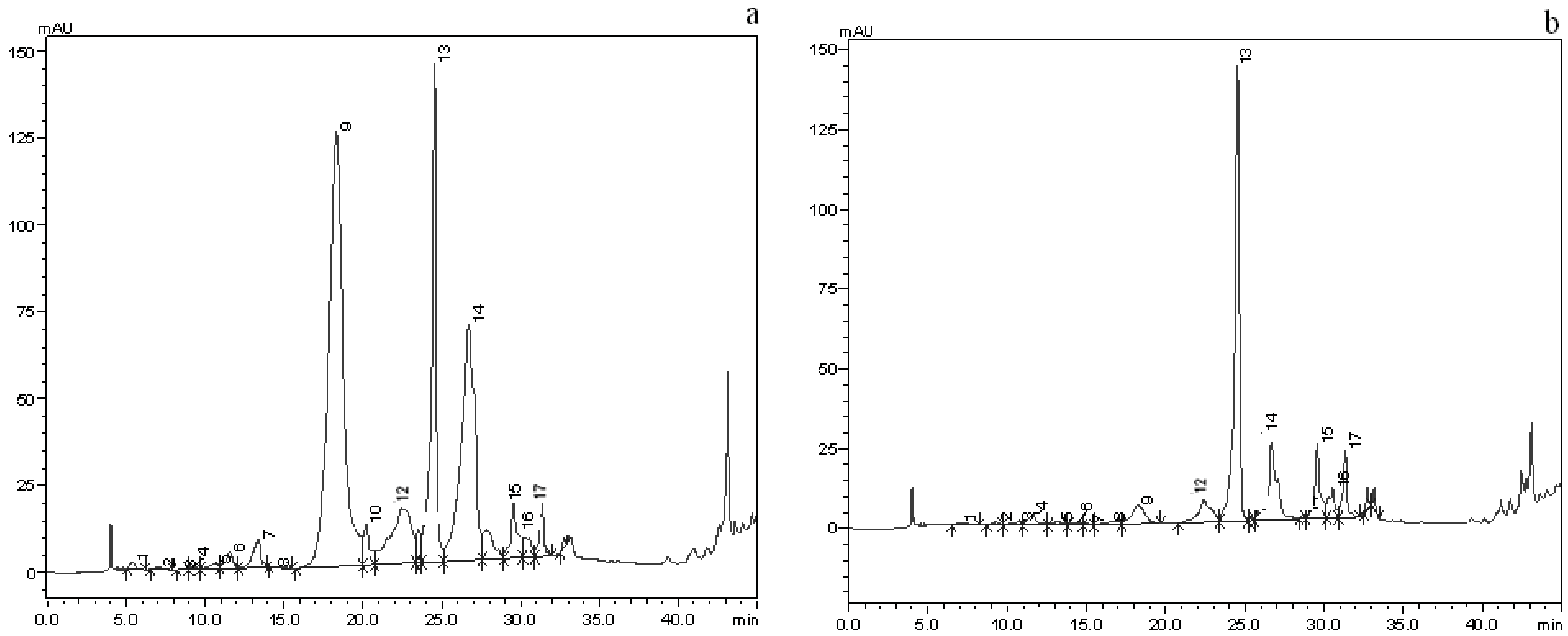


Figure 4.23: HPLC chromatograms of Hawke's Bay VOO showing phenolic profiles in the oils extracted at (a) commercial harvest (61 days after first incidence of frost) and (b) late harvest (75 days after first incidence of frost). The shown chromatograms were obtained at 280 nm. Peaks: (1) 3,4-DHPEA , (2) *p*-HPEA, (3) vanillic acid, (4) vanillin, (5) *p*-coumaric acid, (6) ferulic acid, (7) *o*-coumaric acid, (8) verbascoside (9) 3, 4-DHPEA-EDA, (10) oleuropein, (11) isomer form of 3,4-DHPEA-EA (12) *p*-HPEA-EDA, (13) Luteolin, (14) 3,4-DHPEA-EA, (15)apigenin, (16)*p*-HPEA-EA, (17)unidentified.

The concentration of the secoiridoids in the VOO (cold pressed) obtained at commercial harvest (CH) was the highest in Waiheke followed by Hawke's Bay and Bombay orchards (Table 4.10, 4.8 and 4.9, respectively). However, a significant reduction in concentration of the secoiridoids in the VOO was evident mainly in the oil from Hawke's Bay orchard late harvest. For example, the concentration of 3,4-DHPEA-EDA decreased from 801.95 to 25.708 mg/kg, p-HPEA-EDA from 138.07 to 30.69 mg/kg and 3, 4-DHPEA-EA declined from 395.88 to 45.27 mg/kg. These significant reductions can again reveal the impact of frost damage on these phenolic compounds. There was no appreciable change in luteolin in the VOO at commercial harvest and late harvest.

Secoiridoids are compounds that are usually glycosidically bound and produced from secondary metabolism of terpenes (Obied *et al.*, 2008b). The secoiridoids in olive oil are formed by the hydrolysis of oleuropein, demethyloleuropein and ligstroside in a reaction catalysed by endogenous *β*-glucosidases during crushing of olives and malaxation of the paste (Conde *et al.*, 2008; Obied *et al.*, 2008b). The secoiridoids formed are partitioned between the oily layer and the vegetation water due to their amphiphilic characteristics, albeit they are more concentrated in the vegetation fraction because of their polar functional groups (Bendini *et al.,* 2007). This can explain low concentration of the secoiridoids in the cold pressed oils compared to oils extracted by solvent.

Another significant reduction of phenolic compounds during ripening was in the content of oleuropein (Figure 4.22). The level of oleuropein reduced from 73.4 to 5.5 mg/kg in the Hawke's Bay orchard and from 74.5 to 1.0 mg/kg in the Waiheke and. Oleuropein is the main phenolic compound in olive fruits and its content decreases with fruit maturation due to increased activity of hydrolytic enzymes (Amiot *et al.,* 1986). In particular, glycosidases catalyse the hydrolysis of oleuropein with the formation of oleuropein aglycones and the dialdehydic form of elenolic acid linked to 3,4-DHPEA (Montedoro *et al.,*1993; Limiroli *et al.,* 1995). Concentration of the isomer oleuropein aglycon 3,4-DHPEA-EA and verbascoside followed a similar trend to oleuropein, and both compounds were not detected in the cold pressed oils.

The two major flavonoids identified by HPLC at 340 nm were luteolin and apigenin. Luteolin in particular was identified at high concentrations in the cold pressed oils. Hawke's Bay and Bombay VOO was found to contain 289 and 363.39 mg/kg oil respectively while Waiheke VOO had 338.6 mg/kg. A similar level of apigenin was detected in the oils from Hawke's Bay (1.95 mg/kg), Bombay and (1.42 mg/kg) Waiheke (1.9 mg/kg) orchards. The concentrations of both flavonoids declined with olive maturation. This is consistent with findings of Amiot *et al.* (1986). The two flavonoids have also been described in some Italian virgin olive oils (Rovellini *et al.,* 1997). It is suggested that luteolin may originate from rutin or luteolin 7-glucoside, while apigenin from apigenin glucosides (Amiot *et al.,* 1986, Rovelini *et al.,* 1997). Peak 17 was unidentified and was present in olive oil extracted by solvent and cold press oils (Table 4.8, 4.9, 4.10 for Hawke's Bay Bombay and Waiheke orchards, respectively). The concentration of this unknown phenolic compound decreased in a similar way to the secoiridoids, which can indicate that it could be a secoiridoid phenolic compound.

4.3.5 Phenolic composition and oxidative stability of VOO

The accelerated oxidative stability of the VOO samples was evaluated by using a Rancimat® under the experimental conditions described in section 3.7. The results from the Rancimat are expressed as the induction time (IT), in hours. IT typically characterises the resistance of the oils to oxidation. Table 4.11 shows the IT and concentrations of total phenolics and secoiridoids in VOO obtained in this study. The IT in the Hawke's Bay VOO obtained at CH (24.2 hrs) was similar to Waiheke CH and LH VOO, while both the CH and LH virgin olive oils of the Bombay orchard were characterised by lower induction time (13.8 and 8.5 hrs, respectively) which correlated to the lower concentration of total phenols in the oils. A remarkably sharp reduction in IT was obtained in the Hawke's Bay LH VOO (24.2 hrs to 7 hours). As mentioned previously, this can be attributed to frost damage on the fruit. The frost damage was accompanied by significant reductions in total phenolics and secoiridoids in the oil and hence a reduction in oil oxidative stability. Similar effects of frost damage on oxidative stability have been previously reported (Guillaume *et al.,* 2009). The VOO from Waiheke commercial harvest (CH) and the late harvest (LH) had the highest oxidative stability, expressed as the induction time (25.7 and 22.4 hours, respectively). This can be attributed to the highest content of total phenolics and secoiridoids in these oils.

Table 4.11 Rancimat® induction time (hrs) and concentration of total phenolics and secoiridoids in VOO from Waiheke, Bombay and Hawke's Bay obtained at commercial harvest (CH) and late harvest (LH).

VOO sample	**Induction time (hrs)**	**Total phenolics mg/kg oil(±SEM)**	**3,4-DHPEA-EDA (±SEM)**	***p*-HPEA-EDA (±SEM)**	**3,4-DHPEA-EA (±SEM)**	***p*-HPEA-EA (±SEM)**
Hawke's Bay CH	24.20	514.81(24.52)	801.95(11.09)	138.07(0.09)	395.88(0.12)	11.58(2.55)
Hawke's Bay LH	7.00	123.35(3.25)	25.71(0.27)	30.69(0.17)	45.27(0.06)	15.89(0.67)
Bombay CH	8.50	122.09(0.72)	54.00(0.73)	75.05(0.00)	42.80(0.17)	17.53(0.20)
Bombay LH	13.80	178.66(3.70)	234.38(0.01)	80.49(0.01)	87.25(0.02)	11.40(0.02)
Waiheke CH	25.70	620.21(22.04)	1044.90(0.23)	486.72(1.14)	489.16(0.53)	42.03(0.14)
Waiheke LH	22.40	492.40(2.27)	1010.94(1.85)	469.34(0.31)	461.52(3.20)	48.42(1.32)

Care must be taken when using Rancimat® IT results to estimate the shelf life of virgin olive oil because the predictions may result in overestimation or underestimation, but often acceptable results of the actual shelf-life (Farhoosh, 2007a; Farhoosh, 2007b). Several factors need to be considered, for example, the air temperature, flow rate and sample size used, let alone the type or make of the Rancimat used to conduct the test (Farhoosh, 2007a; Farhoosh, 2007b). This study shows that oils with higher IT will have higher oxidative stability which is primarily due to the higher total phenolics and secoiridoids present in the oil.

A strong correlation was found between Rancimat induction time and total phenolics (R^2=0.96) and the secoiridoids, particularly, 3,4-DHPEA-EDA (R^2=0.94), 3,4-DHPEA-EA (R^2=0.93), *p*-HPEA-EDA (R^2=0.60) in the VOO studied (Figure 4.24). However, weak correlation was found between Rancimat IT with *p*-HPEA-EA (R^2=0.31). This finding is in agreement with the report of Tovar *et al.* (2001) who found a good correlation between the concentration of 3,4-DHPEA-EDA (R=0.76), *p*-HPEA-EDA (R=0.68) and 3,4-DHPEA-EA (R=0.73) and oxidative stability of the oils. Several authors have also confirmed the role of these compounds as the main antioxidants in olive oil responsible for shelf life of the oil (Abramovic *et al.*, 2007; Baldioli *et al.*, 1996; Beltrán *et al.*, 2005; Cioffi *et al.*, 2010; McDonald *et al.*, 2001; Owen *et al.*, 2000b; Papadopoulos & Boskou, 1991; Romani *et al.*, 2007).

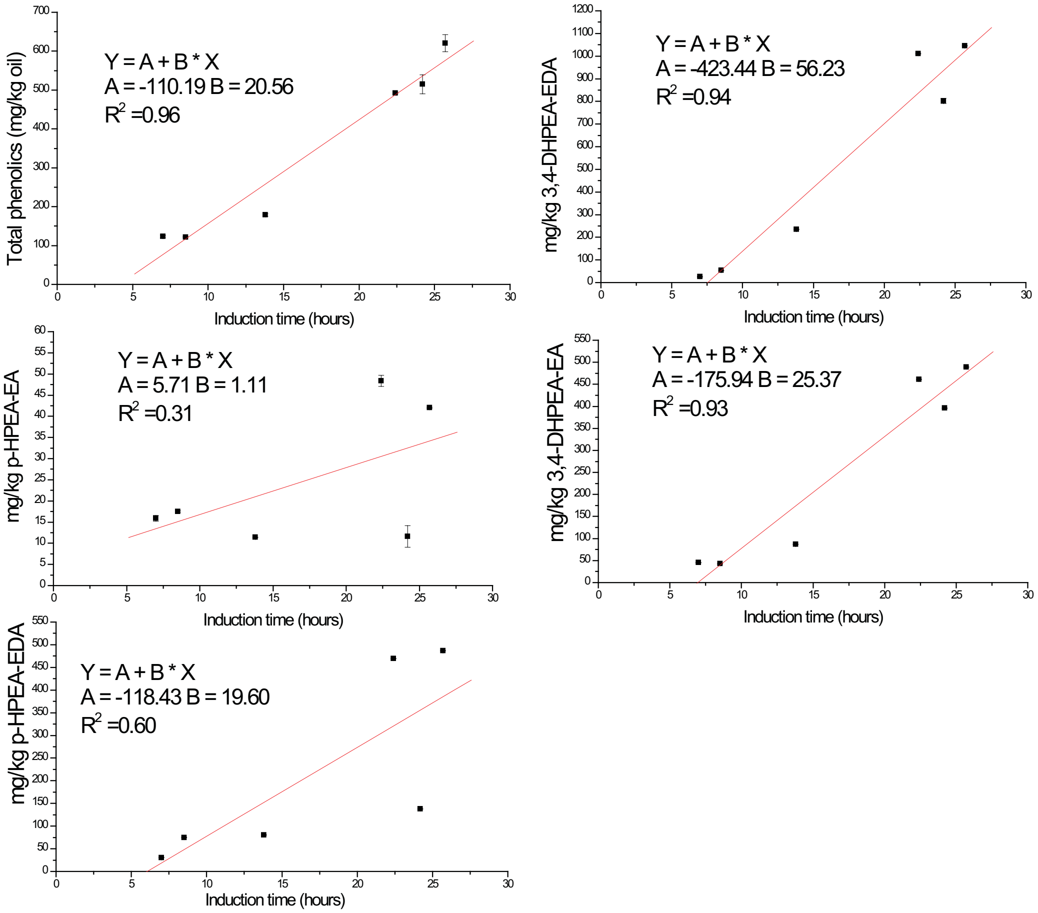


Figure 4.24: Correlations between total phenolics and secoiridoids with the induction time in ‘Frantoio’ VOO obtained in this study. Values are the means of triplicate Rancimat measurements and duplicate HPLC phenolics. Error bars are standard error of the means.

4.4 Sensory evaluation of VOO

The sensory evaluation was conducted following the procedure described in section 3.8. Two VOO samples (CH-obtained at commercial maturity and LH-obtained two weeks after CH) from each of the three orchards were evaluated for their sensory characteristics by a trained panel. A descriptive sensory analysis (DSA) was used to describe the intensities of the sensory attributes (aroma and flavour) generated in Table 3.4 (Methods and materials). The oils were also evaluated for defects and the standard IOC attributes (bitterness, fruitiness and pungency). In order to anchor the panellists' responses and to specify the attributes intensity, the panellists were given reference standards at the beginning of each day. The reference standards and the intensities for all the sensory attributes were stated in Table 3.3. Data used to conduct DSA were checked for consistency and agreement between the panellists using the software PanelCheck (V.1.3.2).

The spider plot in Figure 4.25 shows overall differences in flavour and aroma characteristics of VOO obtained from Hawke's Bay, Bombay and Waiheke orchards. The strongest positive flavour and aroma attributes obtained in the 'Frantoio' VOO were 'fresh green bean', 'fresh green leaves', 'bitter salad', 'fresh nutty flavour' and 'vanilla toffee'. These attributes were dominant in the Waiheke CH and LH VOO. Hawke's Bay CH VOO was similar in flavour and aroma attributes to Waiheke LH. The intensity of all the flavour and aroma attributes was the least in the Hawke's Bay LH VOO. Both the CH and LH VOO from Bombay also had low scores for the flavour and aroma characteristics reported in Figure 4.25. A reduction in all the flavour and aroma attributes was found in the oils from the late harvest (LH) for all the orchards.

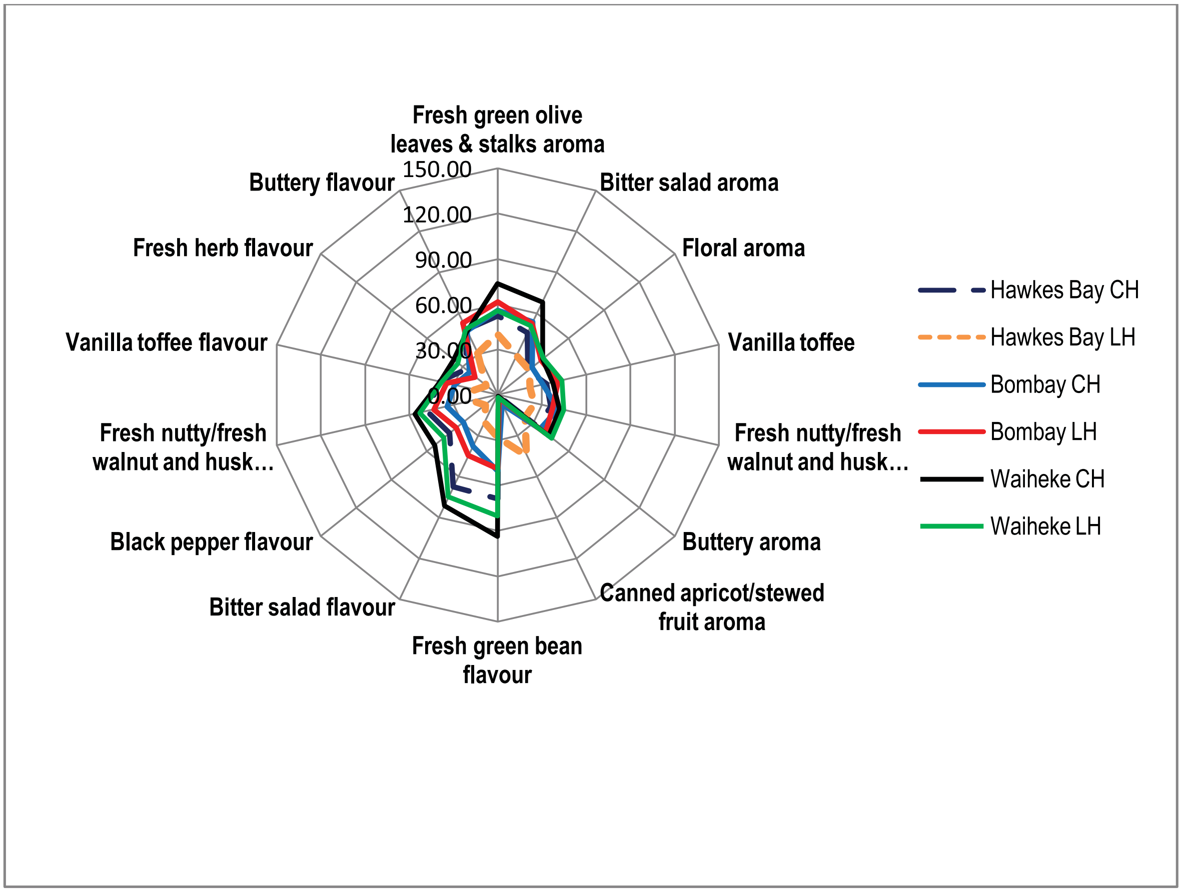


Figure 4.25: The aroma and flavour profiles in VOO obtained at commercial maturity (CH) and late harvest (LH) in Hawke's Bay, Bombay and Waiheke orchards.

In Figure 4.26, the positive attributes of bitterness, pungency and fruitiness were scored using the IOC recommended procedure. The VOO from the Waiheke orchard obtained at CH and LH had the highest sensory scores for bitterness (5.9 and 5.1), fruitiness (6.0 and 5.3) and pungency (6.3 and 5.3), in comparison to Hawke's Bay CH and Bombay CH and LH VOO, respectively. The scores for these attributes decreased at the late harvest (LH) for the three orchards, with Waiheke orchard having the highest scores of the attributes at both CH and LH. As found with the DSA aroma and flavour profiles, the IOC scores for positive attributes obtained in the Hawke's Bay LH were significantly (*p*-value 0.000) the lowest of all the samples evaluated (bitterness 1.3, fruitiness 1.4 and pungency 3.2). Some panellists reported sensory defects such as musty or muddy sediment in this oil and all the panellists evaluated the oil as rancid. This oil was also described to have a strong stewed apricot or stewed fruit aroma.

According to the IOC guidelines for sensory evaluation, olive oil can be classified as 'extra virgin' when it has no sensory defects and when its results from chemical tests are within the set limits for quality and composition (IOC, 2006). Therefore, Hawke's Bay LH VOO cannot be classed as an extra virgin olive oil (EVOO), although the results for chemical tests in Table 4.3 were within the IOC chemical limits. This finding can highlight the effect of frost damage on sensory quality of olive oil, mainly on the aroma and mouth feel perception. A similar study reported by Morello *et al.* (2006) described frost damaged oils thicker with a similar attribute of rancid tallow. Guillaume *et al.* (2009) reported clear hay and or stewed fruit sensory perception in frosted olives and the intensity of defects increased with the duration of frost damage on the fruit. The oils obtained from frosted olives in all these studies, including the present study resulted in oil that had less bitter and pungent attributes, which correlates to the reduction of secoiridoids in the freeze damaged olive.

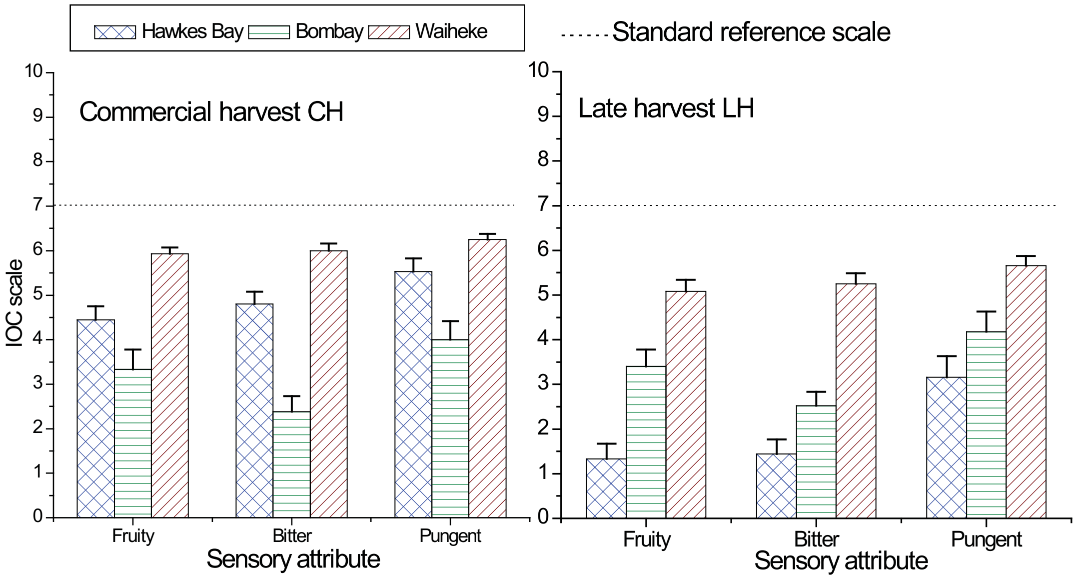


Figure 4.26: The IOC scores for positive attributes for 'Frantoio' VOO obtained at commercial harvest (CH) and late harvest (LH) for Hawke's Bay, Bombay and Waiheke orchard.

4.4.1 Principle Component Analysis (PCA) of sensory data

The DSA sensory data obtained from the oils were subjected to a Principal Component Analysis (PCA). PCA is an exploratory tool that helps uncover unknown trends within data (Baum *et al.*, 2010). The PCA was used to identify any variations between the VOO samples, thereby highlighting the attributes having the most effect on the sensory profiles found in the VOO from each of the three orchards. The PCA results are shown on two dimensional scale plots (or bi-plots) in Figure 4.27 and Figure 4.28, which both should be looked at concurrently. The first principal component (PC1 or dimension one) in both PCA plots accounts for 81.84 % of the variations in the sensory profiles while the second dimension accounts for only 8.94 %.

The PCA confidence ellipse plot in Figure 4.27 shows how the perceived sensory attributes defined by Figure 4.28 were distributed between the three orchards. It can be seen that the location of the orchard and harvest maturity (CH or LH) influenced the olive oil sensory profile substantially. Overall, the sensory profile of Bombay orchard is very different from Waiheke and Hawke’s Bay CH VOO that are very similar and overlapping in their perceived sensory attributes (Figure 4.27). The negative co-variation of sensory attributes of Hawke’s Bay LH can well be explained by frost effect at this harvest rather than the influence of orchard location and harvest maturity.

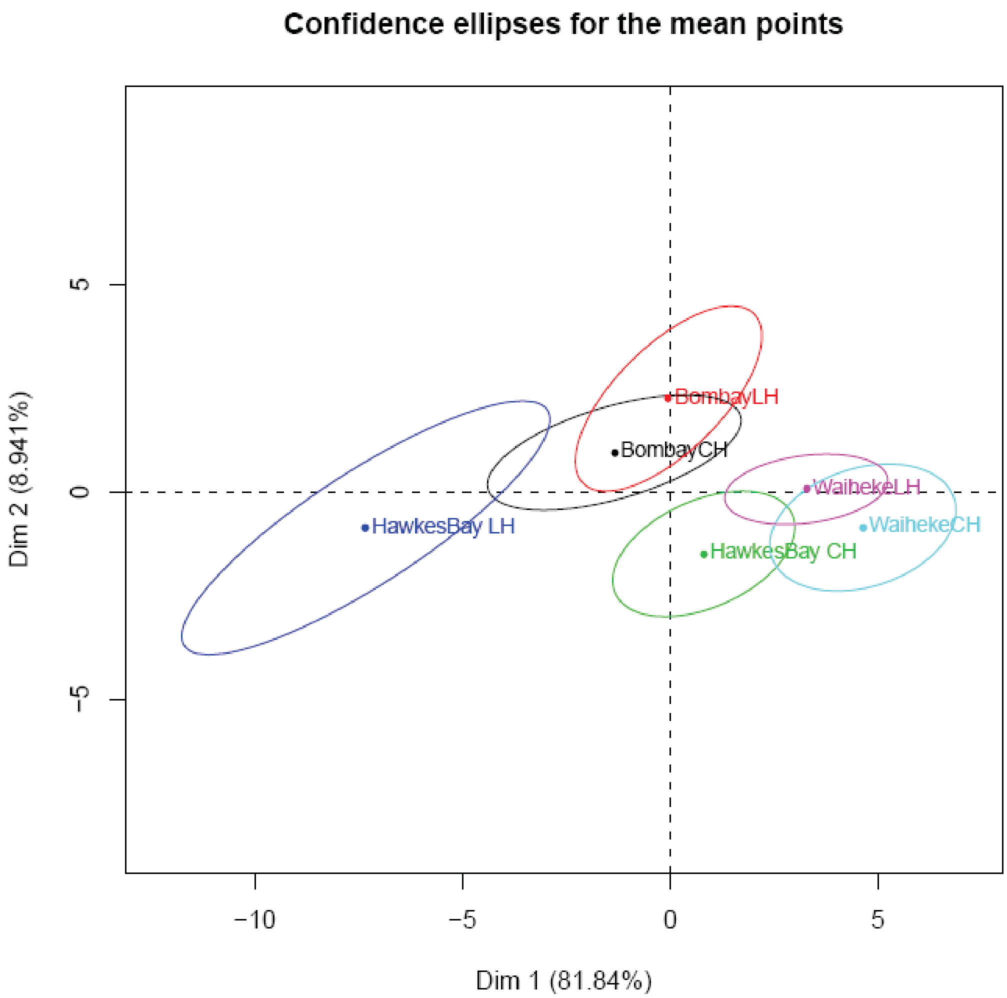


Figure 4.27: Principle component analysis plot showing effect of location and harvest maturity (commercial or late) on sensory profile of the VOO from Hawke's Bay, Bombay and Waiheke orchards. This figure is concurrent with Figure 4.28, outlining the distribution of sensory attributes.

The main sensory attributes influencing the VOO from the three orchards are illustrated in Figure 4.28. It shows the VOO from Waiheke CH, LH and Hawke's Bay CH were similarly characterised by bitter, pungent, fruity, 'bitter salad' aroma and flavour, fresh 'green olive leaves' aroma, 'black pepper' flavour, fresh 'nutty' or 'walnut' flavour and 'vanilla toffee' flavour. Whereas both Bombay CH and LH VOO were strongly influenced by fresh 'olives leaves' and 'stalk' aroma, fresh 'nutty', 'buttery' aroma, 'vanilla toffee', 'floral' aroma and 'buttery' flavour and a low level of the sensory attributes that defined the profile of Waiheke CH, LH and Hawke's Bay VOO (bitter, pungent, fruity, ‘bitter salad’ aroma and flavour, fresh ‘green olive leaves’ aroma, ‘black pepper’ flavour, fresh ‘nutty’ or ‘walnut’ flavour and ‘vanilla toffee’ flavour). The fresh green flavour and bitter salad flavour had similar intensities in the Waiheke CH and Hawke’s Bay CH VOO (Figure 4.28). In addition, two of the attributes (fresh green olive leaves and stalk aroma and bitter salad aroma) had similar sensorial intensities in both the Bombay CH and LH VOO as shown by the overlapping in Figure 4.28. The Hawke’s Bay LH VOO located on the positive axis of PC2 is more defined by stewed apricot and a lack of all the sensory attributes identified in the other oils. This is as expected for the Hawke’s Bay LH oils that was found with sensory defects. The Hawke’s Bay LH oil was also described by the overripe attribute more than the rest of the oil samples. The PCA plots therefore indicated differences in the sensory profile of VOO from the three orchards.

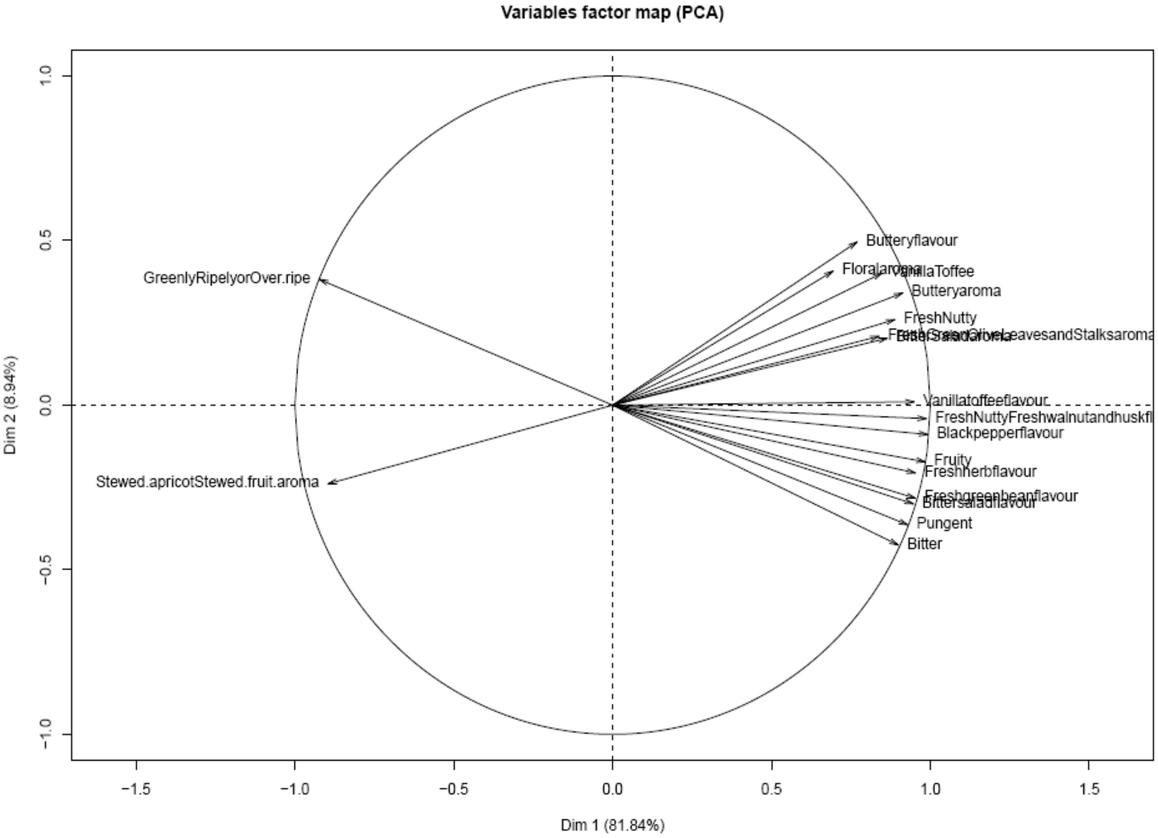


Figure 4.28: Variable factor map showing the distribution of specific aroma and flavour variables influencing the VOO obtained at commercial maturity and late maturity in olive oils from Hawke’s Bay, Bombay and Waiheke. This figure is to be viewed concurrent with Figure 4.27.

4.4.2 Phenolics and perceived sensory attributes of VOO

The perceived sensory attributes of olive oil, particularly, bitterness and pungency has been attributed to the concentration of phenolics in the oil, in that olive oils with high concentrations of phenolic compounds are bitter and pungent (Caponio *et al.*, 2001; Gawel & Rogers, 2009; Skevin *et al.*, 2003). As shown in Figure 4.29, a strong positive correlation was found between total phenolics and sensory bitterness (R^2=0.79), pungency (R^2=0.94) and fruitiness (R^2=0.96), although it is not clear whether phenolics contribute to the fruity sensory perception of VOO. Several studies have suggested that the fruity perception and unique aroma of VOO are due to C6 and C5 aromatic volatile compounds in the oils, whereas bitterness and pungency are attributed by secoiridoid compounds (Angerosa, 2002; Angerosa *et al.*, 2000; Beltrán *et al.*, 2007; Caporale *et al.*, 2004; Inarejos-Garcia *et al.*, 2009a; Koprivnjak *et al.*, 2009; Mateos *et al.*, 2004; Morales *et al.* *et al.*, 1993).

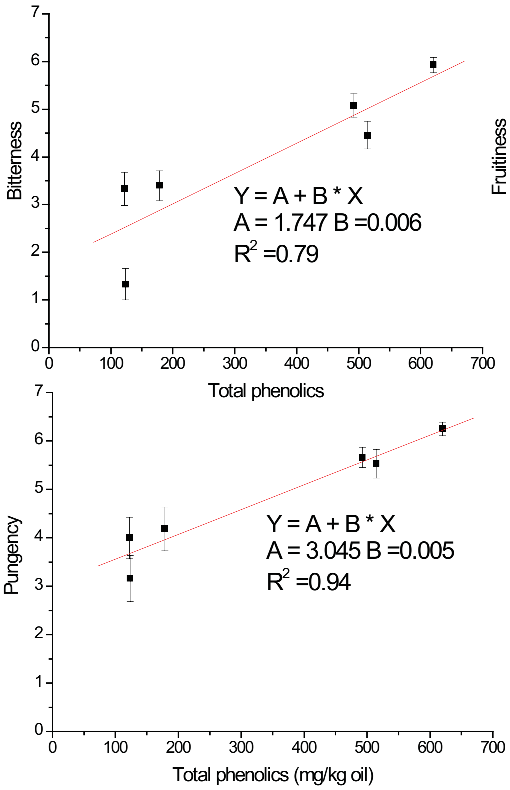


Figure 4.29: Correlations between the IOC sensory attributes (pungency, bitterness and fruitiness) versus total phenolics in 'Frantoio' VOO studied. Data plotted are the mean of triplicate measurements. Error bars are standard error of the means.

Secoiridoids are the major compounds in olive oil known to contribute to the perceived bitterness and pungency in the oil (Gawel & Rogers, 2009; Mateos *et al.*, 2004). Figures 4.30 and 4.31 show the correlations obtained between the secoiridoids and perceived sensory bitterness and pungency, respectively, in the virgin olive oils. The strongest correlations for bitterness were found for the secoiridoid 3,4-DHPEA-EDA (R^2=0.82), 3,4-DHPEA-EA (R^2=0.78), *p*-HPEA-EDA (R^2=0.73) while weak correlation was obtained with *p*-HPEA-EA (R^2=0.46). Similarly, the strongest correlations for pungency were found for the secoiridoid 3,4-DHPEA-EDA (R^2=0.94), 3,4-DHPEA-EA (R^2=0.92), *p*-HPEA-EDA (R^2=0.73) while weak correlation was obtained with *p*-HPEA-EA (R^2=0.44). A related finding was reported by Gutiérrez-Rosales *et al.* (2003) who studied the main polyphenols in the bitter taste of VOO and found a strong correlation for bitterness with 3,4-DHPEA-EDA (R=0.98), *p*-HPEA-EDA (R =0.98), 3,4-DHPEA-EA (R=0.79) and *p*-HPEA-EA (R=0.55). This finding confirms similar reports that bitterness and pungency in VOO was due to secoiridoid derivative of hydroxytyrosol and tyrosol (Caponio *et al.*, 2001; García *et al.*, 2001b; Gutiérrez-Rosales *et al.*, 1992; Gutiérrez-Rosales *et al.*, 2003).

As shown in Table 4.12, the secoiridoids also showed good positive relationship with specific lexicons generated by the sensory panellists for the 'Frantoio' VOO studied, in addition to the IOC attributes (bitterness and pungency). Strong correlations was obtained for *p*-HPEA-EA and *p*-HPEA-EDA with 'bitter salad', 'black pepper', 'fresh herb' and 'fresh green bean' flavour (R^2>0.46). The secoiridoid 3,4-DHPEA-EA also positively correlated with the aroma attributes, 'bitter salad', 'buttery', 'floral', fresh 'nutty' and 'fresh green olive leaves' & 'stalks' (R^2>0.52), whereas 3,4-DHPEA-EDA only showed a good positive correlation with fresh herb flavour (R^2>0.5), similar to *p*-HPEA-EA and *p*-HPEA-EDA. The unidentified compound (Peak 17) had very good correlations with all the aroma attributes, particularly, bitter salad aroma (R^2 0.91), buttery aroma (R^2 0.82), fresh nutty aroma (R^2 0.89) and fresh green olive leaves and stalk aroma (R^2 0.78), as well as fresh herb flavour (R^2 0.72) and black pepper flavour (R^2 0.67).

Simple phenolics compounds (3,4-DHPEA, *p*-HPEA, vanillic acid, vanillin, *p*-coumaric acid, ferulic acid, *o*-coumaric acid) generally had weak correlations with specific flavour attributes identified in the oils. Only *o*-coumaric acid, *p*-coumaric acid and ferulic acid showed good correlation with buttery flavour (R^2 0.69, R^2 0.98 and R^2 0.71, respectively) and vanilla toffee flavour (R^2 0.46, R^2 0.72 and R^2 0.57, respectively). The coumaric acids also had strong correlations to the aroma attributes. Vanillin, tyrosol (*p*-HPEA) and the two flavonoids, luteolin and apigenin showed the weakest correlations with any of sensory attributes (R^2<0.31). Hydroxytyrosol (3,4-DHPEA) was more correlated to bitter salad (R^2 0.8) and fresh green olive leaves & stalks (R^2 0.69). Majority of the aroma and flavour attributes did not strongly correlate to the phenolics. This is because most of aroma and flavour attributes perceived in olive oil are the result of C_5-C_{11} aroma and flavour volatiles rather than phenolics (Angerosa, 2002; Morales *et al.*, 1996).This result showed bitter and pungent attributes were mainly attributed to the concentration of secoiridoids, whereas, the simple phenolics contributed to some of the flavour and aroma attributes. The result agrees with Mateos *et al.* (2004), Inarejos-Garcia *et al.* (2009) and Beltran *et al.* (2007) who reported the bitter and pungent sensory perception of VOO was due to secoiridoids and that the simple phenolics fractions did not contribute to bitter taste of the VOO.

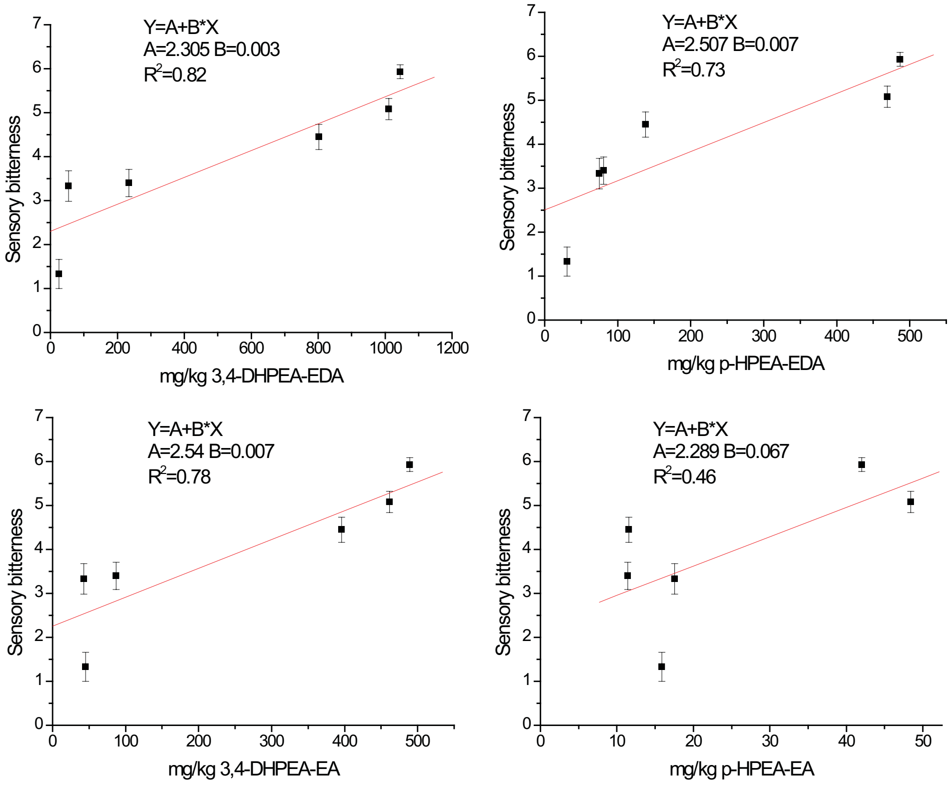


Figure 4.30: Correlation between the IOC sensory attribute of bitterness versus the secoiridoids (3,4-DHPEA-EDA, p-HPEA-EDA, 3,4-DHPEA-EA and p-HPEA-EA) in the 'Frantoio' VOO studied. Data plotted are the means of triplicate values. Error bars are standard error of the means.

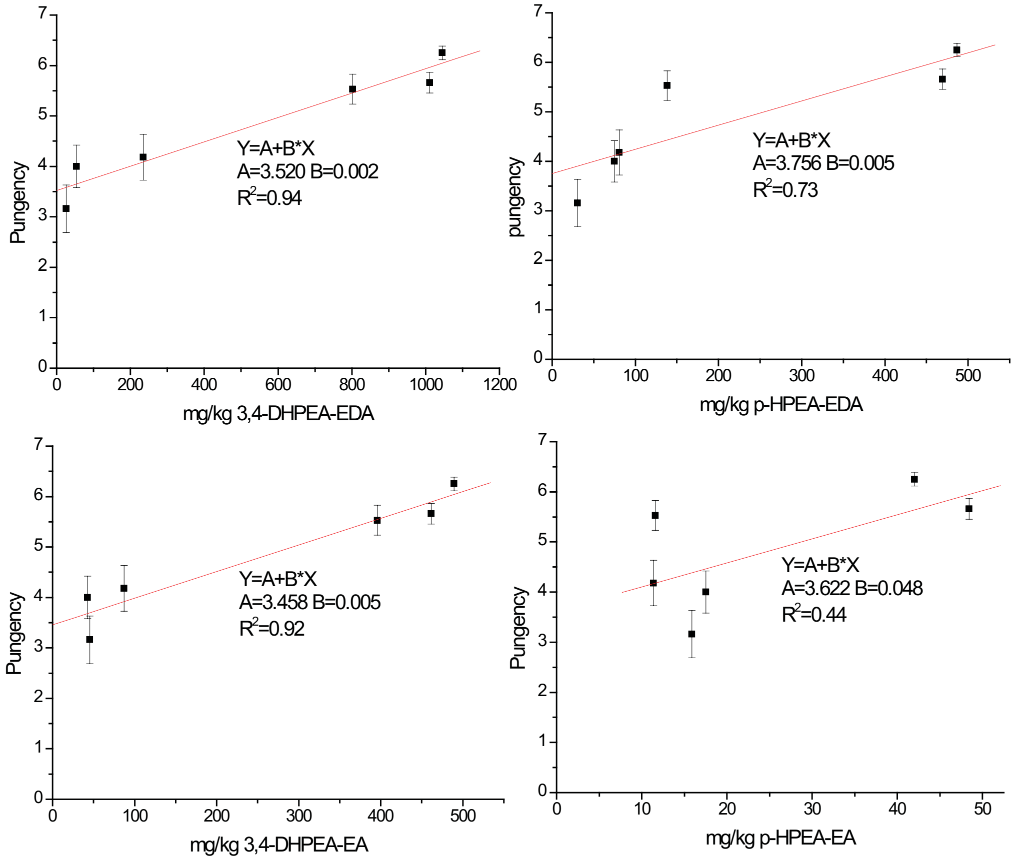


Figure 4.31: Correlation between the IOC sensory attribute of pungency versus the secoiridoids (3,4-DHPEA-EDA, p-HPEA-EDA, 3,4-DHPEA-EA and p-HPEA-EA) in the ‘Frantoio’ VOO studied. Data plotted are the means of triplicate values. Error bars are standard error of the means.

Table 4.12 Pearson's correlations between individual phenolics and sensory attributes of 'Frantoio' Virgin olive oil

Sensory attributes	Pearson's correlations (R^2) of phenolics versus sensory attributes													
	3,4-DHPEA	p-HPEA	3,4-DHPEA-EA	3,4-DHPEA-EDA	*p*-HPEA-EA	*p*-HPEA-EDA	Apigenin	Ferulic acid	Luteolin	*o*-coumaric acid	Unidentified	Vanillic acid	Vanillin	*p*-coumaric acid
Flavour														
Bitter salad	0.12	0.07	0.23	0.24	0.68	0.55	0.25	0.23	0.04	0.27	0.48	0.08	0.14	0.33
Black pepper	0.29	0.00	0.33	0.23	0.58	0.46	0.19	0.43	0.00	0.48	0.67	0.18	0.07	0.49
Fresh herb	0.31	0.03	0.47	0.50	0.68	0.68	0.28	0.28	0.05	0.44	0.72	0.27	0.31	0.30
Buttery	0.36	0.25	0.20	0.01	0.09	0.03	0.03	0.71	0.31	0.69	0.54	0.20	0.04	0.98
Fresh green bean	0.22	0.06	0.35	0.38	0.71	0.66	0.26	0.25	0.05	0.34	0.60	0.17	0.23	0.26
Vanilla toffee	0.21	0.00	0.18	0.08	0.36	0.22	0.21	0.57	0.02	0.46	0.55	0.09	0.01	0.72
Greenly, ripely or Overripe	0.11	0.11	0.20	0.24	0.64	0.54	0.31	0.24	0.06	0.23	0.45	0.06	0.17	0.27
Fresh Nutty, fresh walnut	0.23	0.00	0.29	0.17	0.55	0.40	0.16	0.43	0.00	0.48	0.62	0.15	0.04	0.58
Aroma														
Vanilla Toffee	0.22	0.18	0.38	0.11	0.47	0.26	0.01	0.25	0.15	0.66	0.56	0.29	0.01	0.74
Bitter salad	0.80	0.06	0.61	0.35	0.31	0.36	0.09	0.59	0.06	0.74	0.91	0.53	0.08	0.37
Buttery	0.44	0.15	0.65	0.34	0.59	0.46	0.00	0.30	0.07	0.82	0.82	0.53	0.02	0.61
Floral	0.31	0.20	0.66	0.31	0.60	0.49	0.13	0.04	0.11	0.57	0.54	0.56	0.00	0.24
Fresh Nutty	0.54	0.10	0.66	0.46	0.45	0.43	0.08	0.39	0.01	0.83	0.89	0.57	0.14	0.57
Fresh green olive leaves & stalks	0.69	0.06	0.52	0.24	0.30	0.32	0.04	0.52	0.10	0.64	0.78	0.43	0.02	0.33

3, 4- DHPEA-hydroxytyrosol; *p*- HPEA-tyrosol; 3,4-DHPEA-EDA-dialdehydic form of decarboxymethyl oleuropein aglycone; *p*-HPEA-EDA-dialdehydic form of decarboxymethyl ligstroside aglycone; 3,4-DHPEA-EAdialdehydic form of oleuropein aglycone; *p*-HPEA-EA-aldehydic form of ligstroside aglycone.

4.4.3 Intensity of bitterness (IB)

The colorimetric method for testing the intensity of bitterness (IB) proposed by Gutiérrez-Rosales *et al.* (1992) was used in this study. Absorbance of phenolic extracts was measured at a wavelength of 225nm (K_{225}), from which the IB was calculated. The IB was determined for the VOO and the oils extracted by solvent at different stages of maturity. Table 4.13 shows the intensity of bitterness (IB) relative to sensory scores and total phenolics for VOO obtained in this study. This result suggests a strong influence of phenolic compounds on perceived bitterness of VOO. High levels of IB and sensory bitterness scores corresponded with higher concentrations of total phenolics in the oil. When all the data for the VOO and the oil extracted by solvent was combined, a very strong correlation was also obtained between the intensity of bitterness and total phenolics (Figure 4.32b, R^2=0.93). A strong correlation was also obtained between sensory bitterness and calculated IB (Figure 4.32d, R^2=0.81). These results can suggest that data of IB obtained at 225 and phenol content obtained by Folin-Ciocalteau assay can be used in classification of oils based on their level of bitterness.

Table 4.13 Absorbance data (K_{225}) and intensity of bitterness (IB) relative to sensory scores and total phenolics for VOO obtained in this study

VOO sample	**K_{225}**	**IB (intensity of bitterness)**	**Sensory bitterness (Ref. score =7)**	**Total phenolics mg/kg oil (±SEM)**
Hawkes Bay CH	0.41	4.65	4.45	514.81(24.52)
Hawkes Bay LH	0.24	2.35	1.33	123.35(3.25)
Bombay CH	0.27	2.78	3.33	122.09(0.72)
Bombay LH	0.26	2.68	3.40	178.66(3.70)
Waiheke CH	0.68	8.25	5.93	620.21(22.04)
Waiheke LH	0.56	6.68	5.08	492.40(2.27)

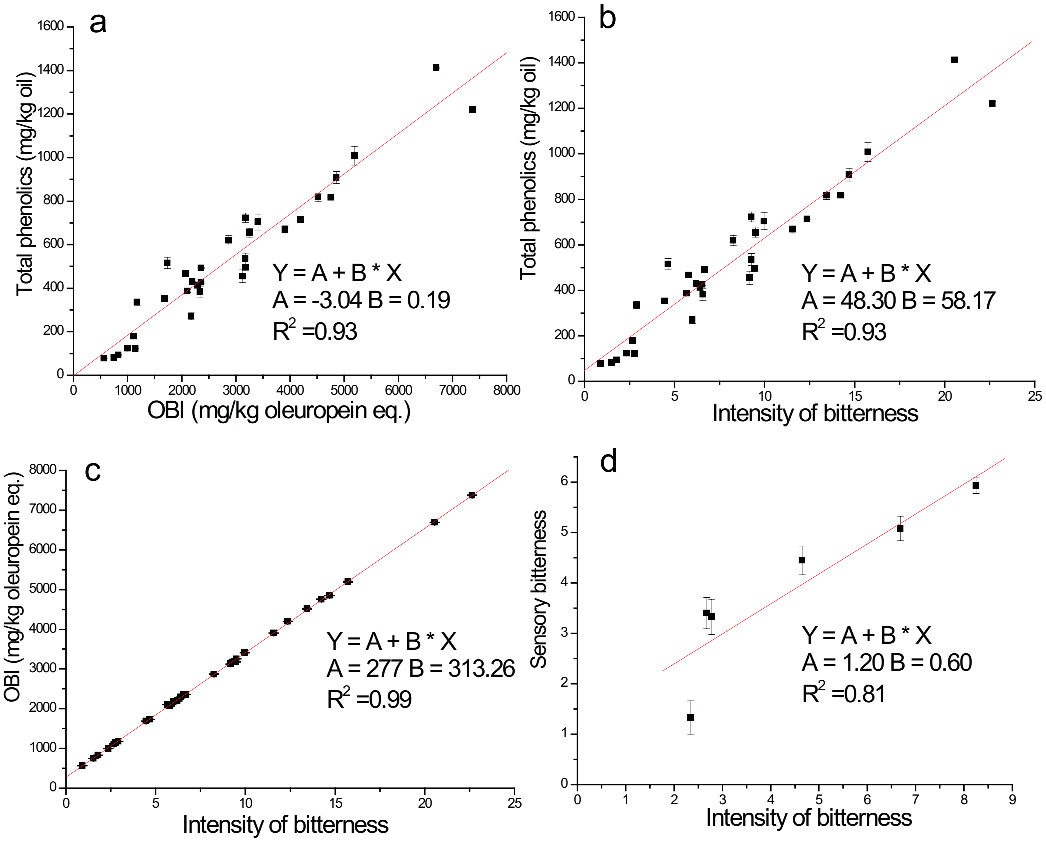


Figure 4.32: Correlations between total phenolics versus oleuropein bitter index OBI (a); total phenolics versus intensity of bitterness IB (b); OBI versus calculated intensity of bitterness (c) and sensory bitterness versus intensity of bitterness (d) in 'Frantoio' olive oils studied in Hawke's Bay, Bombay and Waiheke orchards. Error bars are the standard error of means.

Although the IB technique has shown to have a good correlation for bitterness and the traditional sensory evaluation method (Figure 4.32d), some authors have critically discussed on interpretation of result obtained by the method (Mateos *et al.*, 2004). The criticisms are mainly due to non-selectivity for only bitter phenolics when absorbance of the polar extract is measured at a wavelength of 225 nm. For example, non-bitter phenolic compounds such as elenolic acid, hydroxytyrosol acetate, hydroxytyrosol, tyrosol and lignans have been reported to also absorb at the same wavelength, as a result, the bitterness scores could be higher than their actual scores (Inarejos-Garcia *et al.*, 2009a; Mateos *et al.*, 2004; Romani *et al.*, 2001). This can potentially limit the method to compare olive oils with similar phenolic profiles (Mateos *et al.*, 2004).

The intensity of the K_{225} value can also depend on the spectrophotometer specifications so that it cannot be expected to be reproducible between equipments and laboratories (Inarejos-Garcia *et al.*, 2009a).

Therefore, to eliminate these issues, and to enable replication of results, a more objective method to report bitter index using oleuropein as the reference compound was used in this study. This index was originally proposed by (Inarejos-Garcia *et al.*, 2009a) and will be referred to as oleuropein bitter index (OBI). Oleuropein is a primary choice as the reference bitter phenolic compound because it is the only commercially available bitter phenol in olive oil. Figure 4.33 shows changes in bitterness expressed as oleuropein bitter index (OBI) and total phenolics in the oils obtained at different stages of maturity. Changes in OBI with olive maturity followed a similar trend with total phenolics for the three orchards, suggesting a relationship between the two parameters. Very good positive correlations were obtained in Figure 4.32a&c between OBI and total phenolics (R^2=0.93) and OBI and calculated intensity of bitterness (R^2=0.99). The present result can therefore suggest that the two chemical measures of bitterness (IB and OBI) can correctly predict the level of bitterness in olive oil as shown by their strong correlations to total phenolics and intensity of bitterness evaluated by sensory. The data for OBI, K_{225} and intensity of bitterness (IB) in the 'Frantoio' olive oils are included in Appendix 6.

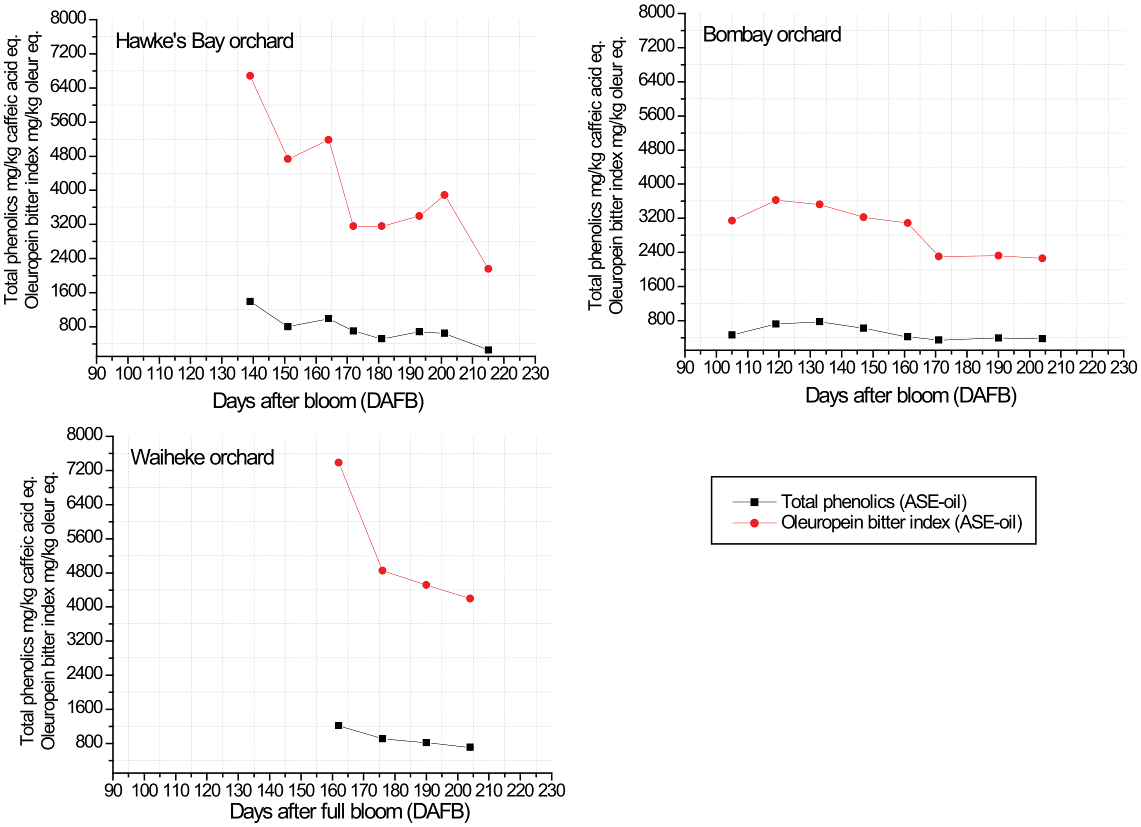


Figure 4.33: Changes in total phenolics (caffeic acid eq.) compared to bitter index (oleuropein eq.) in 'Frantoio' olive oils obtained at different stages of maturity from Hawke's Bay, Bombay and Waiheke. The oils were extracted by solvent.

Chapter Five: General Discussion

The aims of this study were to investigate the effect of NZ climatic conditions on the quality of 'Frantoio' virgin olive oil by characterising the phenolic profile, chemical composition and sensory attributes of the oil, and to investigate possible measures of olive maturity specific to New Zealand growing conditions.

The concentration of phenolic compounds in VOO is an important factor when evaluating the quality of olive oil. This is because phenolics can function as anti-oxidants in the oil and they have an important role specific to the sensory attributes of the oil such as bitterness and pungency (Cioffi *et al.,* 2010; Morello *et al.,* 2004; Bendini *et al.,* 2007; Baldioli *et al.,* 1996; Gawel *et al.,* 2009). Furthermore, phenolic compounds are vital for their numerous human health benefits in reduction of cardiovascular diseases and certain types of cancer (Battino *et al.,* 2004; Shahtahmasebi *et al.,* 2003).

This study revealed a significant reduction in the concentration of total phenolics with olive ripening in the three orchards, consistent with numerous studies reported overseas (Amiot *et al.*, 1986; Baccouri *et al.*, 2007; Beltrán *et al.*, 2005; Bouaziz *et al.*, 2004; Sakouhi *et al.*, 2008; Salvador *et al.*, 2001a). The concentration of individual phenolics varied significantly in the virgin olive oils between the three orchards (p-value<0.05). Specifically, concentration of the secoiridoids in the VOO, 3,4-DHPEA-EDA, *p*-HPEA-EDA, 3,4-DHPEA-EA, *p*-HPEA-EA were the highest in the Waiheke orchard and the lowest in the Bombay orchard. The majority of this work revealed phenolic compounds as the most important component of VOO contributing to the perceived sensory bitterness, pungency and oxidative stability of the oil.

The results presented found the secoiridoids responsible for the perceived sensory bitterness, pungency and oxidative stability of the oils. The virgin olive oils from Waiheke and Hawke's Bay with higher total phenolic content (>350 mg/kg oil caffeic acid equivalent) and secoiridoids (>800mg/kg oil oleuropein eq.) were described by the panellists with the most attractive sensory attributes (Figure 4.25). Whereas the VOO from Bombay orchard whose total phenolic contents were less than 200 mg/kg of oil were described with very low sensory attributes. Furthermore, the VOO from Waiheke CH, LH and Hawke's Bay CH with phenolic compounds higher than 350 mg/kg revealed higher oxidative stability measured by Rancimat®. The level of bitterness and pungency in olive oil is an important positive attribute of VOO, however, bitterness of high intensity can be rejected by some consumers (Gawel & Rogers, 2009; Gutiérrez-Rosales *et al.*, 1992; Gutierrez-Rosales *et al.*, 2003). As found in this study, both attributes depended on the total phenolic content in the oil. For this reason, some researchers have suggested that the level of phenolic compounds can be used as a major sensory parameter when evaluating the sensory quality and shelf life of extra virgin olive oil (Andrewes *et al.*, 2003; Ayton *et al.*, 2007; Baccouri *et al.*, 2008c; Bendini *et al.*, 2007; Caponio *et al.*, 2001; Gawel & Rogers, 2009; Keceli & Gordon, 2001; Kiralan *et al.*, 2008; Rotondi *et al.*, 2008; Sinesio *et al.*, 2005; Stefanoudaki *et al.*, 2000).

Overseas studies have reported varying contents of total phenolics (caffeic acid eq.) in VOO in different olive varieties, including 'Frantoio'. For example, 75-810 mg/kg oil (Ayton *et al.*, 2007), 190-800 mg/kg oil (Beltrán *et al.*, 2005), 180-726 mg/kg oil (Aguilera *et al.,* 2005). The result obtained in this study found phenolics levels within the ranges reported by others at commercial maturity from Hawke's Bay orchard and Waiheke orchard (514.8, and 620.2 mg/kg oil, respectively), however, relatively lower content of total phenolics was obtained in the VOO from Bombay orchard at commercial maturity (122.1mg/kg oil). Knowing the level of phenolics at harvest is vital for olive growers in order to produce olive oil with consistent, balanced chemical composition, sensorial attributes and shelf life of the oil. Phenolics significantly contributes to unique quality of VOO, and if the content is controlled it may provide the opportunity to diversify olive oil to suit different end use (Gawel & Rogers, 2009).

There are a number of factors that can influence the phenolic composition in VOO, for example, seasonal variability, including dry weather that can create stress situation on the trees leading to increased production of phenolics in the fruit (Beltrán *et al.*, 2004b; Romero *et al.*, 2003; Salvador *et al.*, 2001b; Tura *et al.*, 2009). Other factors include agronomic practice, including tree irrigation that can influence phenolic production either through dilution effect or by affecting the key enzymes involved in the phenolic biosynthesis (Dabbou *et al.*, 2010a; GóMez-Rico *et al.*, 2006; Jose Motilva *et al.*, 2000; Romero *et al.*, 2002c; Tovar *et al.*, 2001). However, out of all of these factors, it is the harvest maturity that can significantly dictate the final content of phenolic compounds in the fruit (Al-Maaitah *et al.*, 2009; Ayton *et al.*, 2007; Diraman & Dibeklioglu, 2009; Gimeno *et al.*, 2002; Lavee & Wodner, 2004; Lazzez *et al.*, 2008;

Mailer *et al.*, 2007; Requejo-Jackman *et al.*, 2009; Skevin *et al.*, 2003; Wong *et al.*, 2009). When olives are left longer on the tree, the phenolics can decrease significantly and this makes the oils less flavourful and more prone to oxidation. Leaving olives longer on the tree can also increase the risk of frost damage to the fruit, as revealed in this study in the fruit obtained from the Hawke's Bay orchard.

The longer fruit is harvested after frost event, the greater is the effect of frost damage on composition and sensory quality of the oil (Guillaume *et al.*, 2009).

From the present study, it appears that the concentration of phenolics continues to decline, first very sharply at which point the biosynthesis of oil is the most rapid, then phenolics concentration starts to decline gradually, at which point biosynthesis of oil also slows down (Figure 5.1). The reduction in phenolics concentration appears to continue, but at a slow rate as ripening progresses to the 'black' fruit stage of maturity. At the black stage, rapid degradation of phenolics occurs. The inverse relationship between phenolic degradation and oil biosynthesis may suggest physiological relationship between the two events during olive ripening. If olive growers wait to harvest olives at the advanced 'black' ripening stage, the resultant oil will have low content of total phenolics. If the olives were harvested a few weeks earlier the resultant fruit will have both high oil yield and total phenolics. As shown in Figure 5.1, the commercial harvests in the three orchards were made at the time when the % of oil accumulation had in fact started to level off. It is however, understandable that most commercial orchards want to leave fruit on the tree and wait until more than 80 % fruit has reached the 'black' maturity so that large percentage of fruit can be easily harvested by mechanical harvesters. However, leaving the fruit longer on the tree could lead to very poor quality olive oil with low shelf life and loss of the attractive sensory attributes (Ayton *et al.*, 2007; Mailer *et al.*, 2007; Skevin *et al.*, 2003).

Therefore, a possible time to harvest olives in the orchards studied is at the second phase of phenolic decline, where the oil is starting to level off and the total phenolics reduction is gradual. This time is at about 180±5 days after full bloom (Figure 5.1). As shown in the Figure 5.1, this time is shown to vary among the orchards due to different maturation profile and other factors such as climate, irrigation and orchard management coming into effect. Other climatic factors such as the occurrence of frost can also influence olive harvest time. As a result, it is recommended that the olive growers can consider harvesting olives twice in the season, the first harvest aiming for VOO with very high phenolics and the second for medium level of phenolics. The two

VOO can later be blended to achieve VOO with good level of phenolics and sensory attributes.

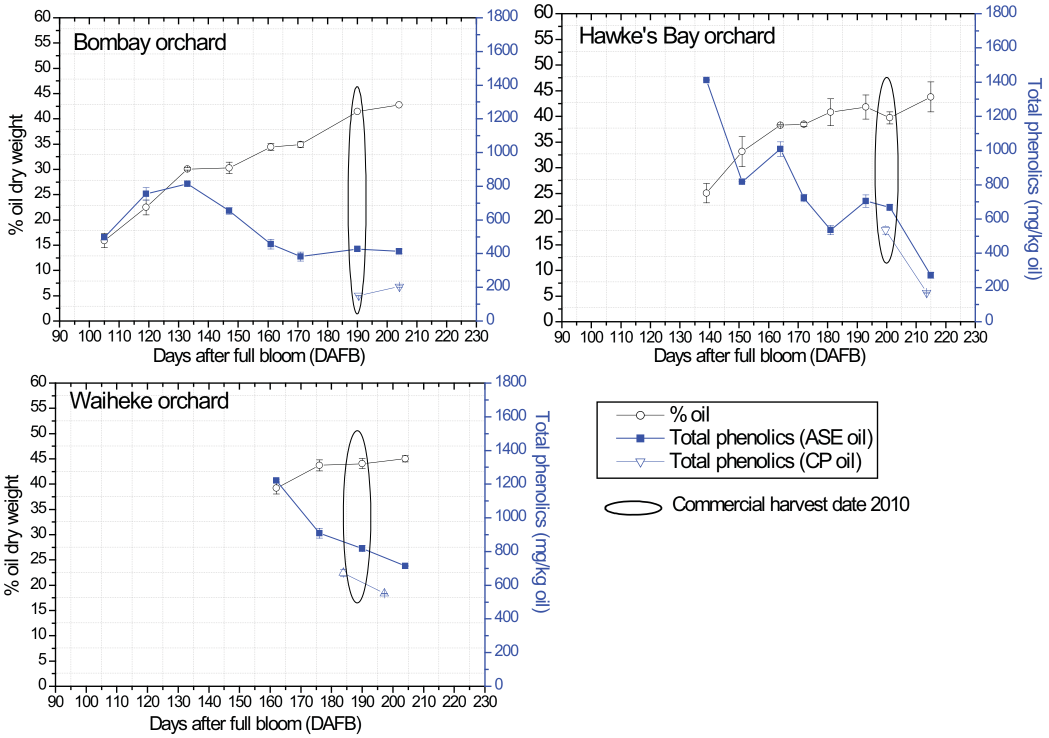


Figure 5.1: Changes in % oil and total phenolics in olive fruit (ASE) and cold pressed oils (CP) highlighting the harvest time in regards to DAFB in the respective orchards.

The present study also revealed the important effect of prolonged frost damage on the quality of VOO in the oils obtained from Hawke's Bay orchard at the late harvest (LH). Frost damage has direct influence on physiological development of the fruit, resulting in significant changes in the fruit physical quality, composition and eventually sensory attributes of the oil (Guillaume *et al.* *et al.*, 2003; Poerio *et al.*, 2008). Frost damage causes cell dehydration and destruction due to the formation of ice crystals inside parenchyma cells *et al.*, 2003). These changes cause serious destruction of cell membranes, cell death and a high oxidation of cell contents as a result of direct contact of endogenous enzymes and substrates. Specifically, frost effect leads to significant reductions in the contents of volatile and phenolic compounds in the oil (Guillaume *et al.* *et al.*, 2003; Morello *et al.*, 2006).

The effect of frost damage on volatiles and phenolics in olive oil is particularly important because these compounds are the major components responsible for flavour, oxidative stability and organoleptic characteristics, which in turn influences consumer acceptability of the oil.

In the present study, the VOO obtained from frosted fruit which remained the longest on the tree (75 days after frost incidence) had significantly higher PV 11 meq/kg (*p*-value 0.00), low oxidative stability and was characterised as rancid by a trained sensory panel. The frosted oil also had remarkably low total phenolics and individual phenolic contents. The secoiridoid derivatives which were found important in both oxidative stability and bitter taste of olive oil both decreased after the frost event, specifically in the late harvest VOO (75 days after frost incidence). Guillaume *et al.* (2009) found remarkable reduction in concentrations of secoiridoid derivatives after freeze injuries and significant reduction in these compounds ensued the longer the fruit was left on the tree before processing. The reduction in total phenolics and secoiridoids is the result of formation of ice crystals in the mesocarp and epicarp tissues during freezing events, thereby encouraging tissue destruction which would allow the phenolic substrates to mix with polyphenol oxidase enzymes, PPO (Morello *et al.*, 2006).

It is well known that high phenolic contents in olive oils are responsible for its sharp bitter taste and specific sensory attributes (Andrewes *et al.*, 2003; Beltrán *et al.*, 2007; Esti *et al.*, 2009; Gawel & Rogers, 2009). Considerable differences were evident in the sensory attributes, mainly in the aroma and taste perception between the Hawke's oil obtained after 61 days from frost incidence and 75 days from the frost incidence. The oils were particular described with intense stewed apricot flavour, as well as rancid. In some other studies, panellists have described the defects in oils obtained from frosted fruit as being thicker, softer and with the taste of rancid tallow (Morello *et al.*, 2006). Guillaume *et al.* (2009) reported clear hay and or stewed fruit sensory perception in frosted olives and the intensity of defects increased with the time in between the frost effect and processing of the oils. This study also found a very low bitter, fruity and pungent perceptions in the prolonged frost affected oils, which is a good indication of the reduction of secoiridoids in the frost damaged olives.

A laboratory based index for measuring the intensity of bitterness (IB) in olive oil was reported in section 4.4.1 and in Table 4.13. The test was based on measurement of absorbance of phenolic extracts at 225 (K_{225}), from which the IB was calculated. This test proved very significant in estimating the perceived level of bitterness in olive oil.

It showed a strong correlation to the intensity of bitterness evaluated sensorially (R^2=0.81) and to total phenolics in the oil (R^2=0.93) (Figure 4.32). The VOO with K_{225} value ≤0.25 and IB score ≤2.4 corresponded to total phenolics ≤ 130 mg/kg oil and VOO of low sensory scores for bitterness (≤1.4).

However, the VOO with high sensory scores (≥ 5.0) corresponded to high content of total phenolics (≥ 450 mg/kg oil) and high sensory scores for intensity of bitterness (≥ 5.5). The advantages of this test rests on its ability to measure bitterness in the olive oil sample and the shorter time and low running cost that is necessary to complete the test, compared to the traditional sensory evaluation method. For several decades, evaluation of bitterness in VOO has been dominated by the traditional sensory method using trained panel. Sensory evaluation is also the recommended method by the International Olive Council (IOC) for evaluating bitterness and other sensory attributes in VOO. However, there are a few limitations to the sensory method, for example, it requires a trained panel to conduct the test and training of the panellists is both time consuming and very expensive to run (Beltrán *et al.*, 2007). Inarejos-Garcia *et al.* (2009) also suggested other arguable drawbacks of the sensory method, such as, lack of reproducibility, bureaucracy in the design and implementation of the work. Some researchers have also argued that food odours and flavours can suppress, enhance or have no effect on sensory perception of most foods (Murphy & Cain, 1980; Murphy *et al.*, 1977; Prescott, 1999; Van Der Klaauw & Frank, 1996). In fact, the 'green' odour note in olive oil has been found to have a positive significant effect on the perception of bitterness, in that the presence of 'cut grass' odour enhanced the bitterness perception (Caporale *et al.*, 2004). Furthermore, the intrinsic variable composition of VOO, particularly the composition and degree of saturation of fatty acids have been shown to influence the perception of bitterness in VOO; in that the polyunsaturated matrices produces milder sensations and are less bitter than monounsaturated ones (García-Mesa *et al.*, 2008).

It is difficult for human subjects to pick contributions of various olive compositions towards bitterness. Such draw backs for use of sensory evaluation may warrant future use of chemical measures such as intensity of bitterness (IB) and oleuropein bitter index (OBI) reported in this study as an alternative in predicting intensity of bitterness in olive oil. The present result is similar to the work of Beltran *et al.* (2007) who found IB predicted the level of perceived sensory bitterness in the VOO they studied. In their study, Beltrán *et al.* (2007) demonstrated the usefulness of bitterness intensity obtained at K_{225} and phenol content obtained by Folin Ciocalteu assay in classification of olive oils based on their levels of bitterness. They classified VOO by its phenol content and IB values into four categories: Phenol contents equal or lower than 220 mg/kg correspond to non-bitter oils or oils with almost imperceptible bitterness (intensities 0–1.5); light bitterness corresponds to 220–340 mg/kg (intensities 1.6–2.5); bitter oils have phenol contents ranging from 340 to 410 mg/kg (intensities 2.5–2.99); and phenol contents higher than 410 mg/kg correspond to quite bitter or very bitter oils (intensities higher than 3). Gawel and Rogers (2009) reported a similar classification of perception of bitterness in olive oil by its total phenolic content. In the present study (Table 4.13), less bitter VOO (Hawke's Bay LH, Bombay CH and LH) as perceived by the trained panel corresponded to low sensory scores for bitterness (less than 3.5) and low phenolic content (less than 200 mg/kg oil). The bitter VOO (Hawke's Bay CH and Waiheke CH, LH) corresponded to very high sensory scores for bitterness (≥ 4.5) and high levels of total phenolics ≥ 450 mg/kg oil.

Although the present study and those already reported (Beltrán *et al.*, 2007; Gawel & Rogers, 2009) show strong usefulness of IB and OBI in predicting sensory bitterness in VOO, the test cannot replace the sensory evaluation by trained human panellists that is recommended by the IOC. It can however be used by the olive growers to evaluate the level of bitterness in their samples for many purposes such as diversification of the VOO blends.

The analysis of fatty acid composition with maturity revealed differences in percentage composition between the orchards, especially in the percentage of oleic acid and palmitic acid. The percentage of palmitic acid was higher in the Waiheke orchard than in Hawke's Bay and Bombay orchards, whereas linoleic acid was higher in Hawke's Bay and Bombay orchards than in Waiheke orchard. The VOO from the three orchards had greater than 77 % oleic acid, which is more stable to oxidation than the PUFAs such as linoleic and linolenic acid present in the oil (Beltrán *et al.*, 2004a). The content of alpha-linolenic acid that is restricted by the IOC to≤1 % was higher in the Bombay orchard (1.1 %) and close to the limit in Hawke's Bay orchard (>0.8 %). Such compositional variations can be attributed to climate in the growing regions. The climate in Waiheke orchard was generally warmer than Bombay and Hawke's Bay orchard. Fruit will produce more unsaturated fatty acids in colder climates due to cold stress in order to maintain membrane fluidity (Harwood & Sanchez, 2000; Ichihara, 1999). The cold climate enhances more production of unsaturated fatty acids (UFA) than saturated fatty acids (SFA), and this may explain the high levels of both oleic acid and alpha-linolenic acids in the Bombay and Hawke's Bay orchards that were relatively colder than Waiheke orchard. The fatty acid composition and the contents of natural antioxidants such as tocopherols and phenolics of olive oil are important for nutritional benefits and oxidative stability; olive oils high in polyunsaturated fatty acids (PUFA) and low in antioxidants will oxidise faster than monounsaturated fatty acids (MUFA) and SFA (Ayton *et al.*, 2007; Beltrán *et al.*, 2004a; Bendini *et al.*, 2006; Cinquanta *et al.*, 2001; Gutiérrez *et al.*, 2001; Jiang *et al.*, 2005; Kiralan *et al.*, 2008; Koski *et al.*, 2002; Lee *et al.*, 2007; Rotondi *et al.*, 2004). The high % of unsaturated oleic acid in olive oil contributes to its nutritional benefits such as the reduction of cardiovascular diseases (Battino & Ferreiro, 2004; Cicerale *et al.*, 2009; Shahtahmasebi, 2003; Tripoli *et al.*, 2005). As found in this study, the contents of these antioxidants (tocopherols and phenolics) significantly decline with olive maturity while the amount of unsaturated fatty acids (UFA) tends to increase. Tocopherols constitute the other natural antioxidants present in VOO (Baldioli *et al.*, 1996; Kiralan *et al.*, 2008).

The results for chemical composition (fatty acid, phenolics and tocopherols) reported in the results and discussion sections and in Appendix 4 were for the oils extracted by solvent (ASE) and by cold press extraction. In all cases, the concentrations of phenolics, tocopherols and fatty acids were higher in the oils extracted by solvent than by CP. Extraction of chemical compounds by ASE is a more exhaustive because it occurs under controlled conditions of temperature and pressure. For example, the high percentage of fatty acids, particularly alpha-linolenic acid obtained in the ASE oil can be explained by the fact that fatty acid extraction by solvent often leads to the extraction of total lipids in the olive fruit including polar lipids such as glycolipids, hence reflecting high levels of linolenic acid (Agar *et al.,* 1998). The same principle can explain the high occurrence of tocopherols in the oils extracted by solvent (ASE) because phytochemicals are more soluble in non-polar solvents such as hexane used in ASE extraction (Appendix 5). The use of ASE has proved efficient in extraction of chemical compounds for analytical purpose in this study. The results obtained by ASE extraction correlated well to the cold press extraction. For example, a strong correlation (R^2=0.93) was obtained between total phenolics in ASE oils and the cold pressed oil. This means the results obtained by ASE can be comparable to CP extraction. Olive growers can obtain small olive sample (e.g. 50 g) and the results can be analysed in the laboratory. Of course the use of other technologies such as near infrared spectroscopy (NIR) is proving beneficial to olive growers in determining % oil, composition moisture, dry matter, oil content, oil free acidity, fruit maturity index and total phenolics in the fruit (Cayuela & Camino, 2010).

Results on oil chemical quality tests on free fatty acids (FFA), peroxide value (PV) and *K*-values for the VOO obtained at commercial harvest maturity (CH) were all within the IOC limits for extra virgin olive oil (EVOO), however, the Hawke's Bay LH VOO was not EVOO due to the presence of sensory defects (rancid) detected in the oil. It is however important to note that processing over mature olives can influence on these quality parameters, therefore, in order to achieve consistent results on the chemical quality, it is important to harvest healthy mature olives and process them immediately (Al-Maaitah *et al.*, 2009).

Possible measures of olive maturity were investigated in this study. One of the biggest challenges the olive growers in NZ and overseas face is that of deciding on the best time to harvest olives. Until now, most olive growers in NZ employ very rudimentary measures of maturity such as squeezing the olives by hand to feel ripeness, visual external colour of the fruit, the availability of harvesters or labourers and or because other nearby orchards are also picking. However, these methods are not reliable and can lead to processing olives with inconsistent flavour, yield and chemical composition of the VOO. Understanding the changes in chemical composition of the fruit taking place with maturity, most importantly, the composition of phenolics, fatty acids (and percent oil accumulation) and dry matter are vital to reach an objective olive harvest decision. The grower also needs to decide on the end use of the VOO they intend to produce, that is, whether the grower wants mild, bitter or pungent olive oils. As obtained in the sensory results, this study revealed that the distinctive and flavourful olive oils should contain high content of total phenolics (greater than 350 mg/kg oil), tocopherols (greater than 100 mg/kg oil) and a good composition of fatty acids that contributes to its additional health benefits. These attributes must also be balanced and compromised with the % yield and the prevailing conditions such as bad weather (frost). Of course the grower's commercial capability in terms of the harvesting machineries and processing facilities is another factor that can possibly hinder olive harvest decision.

From the growers' perspective, the percent yield in the fruit is the most important index of maturity. In addition to percent oil content, the present study also investigated the applicability of colour index, dry matter and firmness to predict harvest maturity. The result obtained for MI was similar to previous studies conducted by

Massey University and the New Zealand Institute for Plant and Food Research, where the measure of maturity index used in the Northern hemisphere was not applicable to olives growing in NZ conditions. The maturity index, MI values varied appreciably between the orchards and the values obtained at late maturities in all the orchards were ≤4. In the Northern hemisphere, olives are recommended for harvest at MI>4 (Kiritsakis, 1998c). Nonetheless, a good correlation was found between the MI obtained in this study and % oil accumulation for Hawke's Bay, Bombay and Waiheke orchards (R^2=0.73, R^2=0.87 and R^2=0.81, respectively, Figure 4.12). This result can suggest that 'orchard specific' MI can be developed to determine the percentage of oil in the fruit. Another good correlation was obtained between % oil and firmness for Hawke's Bay, Bombay and Waiheke orchards (R^2=0.82, R^2=0.78 and R^2=0.79, respectively, Figure 4.14). The usefulness of firmness to measure olive maturity has also been reported for 'Picual' and 'Arbequina' olives in Spain (Yousfi *et al.,* 2006). This was the first time firmness measure was used to predict olive maturity in NZ. The good correlation obtained can suggest possible use of firmness to measure % oil in olives as it has been used in other industries such as kiwifruit, apple and stone fruit to predict harvest maturity (Blankenship *et al.*, 1997; Hopkirk *et al.*, 1996; Valero *et al.*, 2007). The firmness was measured by a bench-top machine called FirmTech. A possible future opportunity would be to develop a hand-held device, with similar capability to FirmTech that can allow measurement of firmness in the field to predict percentage of oil in the fruit. The present study demonstrated that dry matter evaluated as grams of total solids per gram of fruit was a better indicator of fruit maturity than % dry matter. Dry matter as total solids per fruit was not significantly influenced by weather conditions such as draught and heavy rainfall. As a result it showed a better correlation to % oil content than % dry matter.

In conclusion, the measure of firmness, orchard specific maturity index and total solids have shown potential application in predicting olive maturity. These measures warrant further investigations to refine their applicability into the NZ olive industry. The Maturity Index (colour) was found to be a parameter that is hugely influenced by the climatic condition the orchard is located. Other studies including those conducted in New Zealand have suggested different ripening potential between different olive cultivars growing on the same location (Requejo-Jackman *et al.,* 2009). For this reason, the Maturity Index has to be developed specific to the orchard and to the cultivar. It cannot be developed as a uniform index for all growing regions in New Zealand.

Chapter Six: Conclusions and Recommendations

6.1 Phenolic compounds

- Important phenolic composition in ‘Frantoio’ VOO was composed of several simple phenols such as hydroxytyrosol, tyrosol, vanillic acid, vanillin, *p*-coumaric acid, and ferulic acid.
- The major phenolic compounds constituted of the secoiridoids, 3,4-DHPEA-EDA, *p*-HPEA-EDA, 3,4-DHPEA-EA and *p*-HPEA-EA.
- The concentration of these secoiridoids generally decreased with olive ripening in all the three orchards.
- Luteolin was the major flavonoid identified in the oils.
- The concentration of oleuropein decreased significantly with olive maturity.
- The phenolic composition significantly impacted on perceived sensory bitterness and pungency of the VOO. The secoiridoids 3,4-DHPEA-ED, *p*-HPEA-EDA, 3,4-DHPEA-EA were the main phenolic compounds contributing to these sensory attributes.
- Oxidative stability of the oils was related to the total phenolic content and the concentration of secoiridoids 3,4-DHPEA-ED, *p*-HPEA-EDA, 3,4-DHPEA-EA.

6.2 Phenolic compounds and sensory profile

- The ‘Frantoio’ VOO was described with the main characteristic aroma and flavour attributes being ‘bitter salad’, ‘fresh green bean’, ‘vanilla toffee’, ‘walnut’ and ‘black pepper’.
- The ‘bitter salad’, ‘black pepper’, ‘fresh herb’ and ‘fresh green bean’ flavour were mainly due to *p*-HPEA-EA and *p*-HPEA-EDA.
- An unidentified phenolic compound had very good correlations with all the aroma attributes, particularly, ‘bitter salad’ aroma, ‘buttery’ aroma, fresh ‘nutty’

aroma and fresh ‘green olive leaves’ and ‘stalk’ aroma, as well as fresh ‘herb’ flavour and ‘black pepper’ flavour.

- Of the simple phenols, only *o*-coumaric acid, *p*-coumaric acid and ferulic acid showed good correlation with buttery flavour and vanilla toffee flavour.
- Hydroxytyrosol (3,4-DHPEA) was more correlated to bitter salad and fresh ‘green olive leaves’ and ‘stalks’.
- This study demonstrated that by measuring the total phenol content, VOO bitterness intensity can be estimated and the oils can be classified by their bitterness, providing an option to olive growers to efficiently determine the level of bitterness compared to using more expensive and time consuming sensory evaluation.

6.2 Quality characteristic and composition versus maturity

- The VOO obtained at commercial harvest (CH) were all classified as ‘extra virgin’ based on chemical tests (PV, FFA, K_{232} and K_{270}) and sensory evaluations.
- Prolonged frost damage significantly impacted on PV, sensory attributes (rancidity), phenolic concentration and oxidative stability of Hawke’s Bay late harvest VOO.
- There was no significant change in the composition of fatty acids with olive maturity, however, differences in fatty acid profile was evident especially for palmitic acid, oleic acid and linolenic acid between the three orchards.
- Similar to phenolics, tocopherols were found to decrease with maturity. Alpha tocopherol was the most dominant followed by gamma. No δ-tocopherol was detected in the cold pressed oils.

6.3 Olive harvest maturity

- Oil accumulation (% dry weight) gave a good indication of olive maturation.
- Progressive accumulation of oil occurred up to about 170 days after the full bloom, after which it started to level off. There was orchard difference in both the rate of oil accumulation and the final content of oil at the commercial harvest maturity.
- Total solids per fruit was found to increase with fruit maturity and showed better positive correlations with % oil content than percent dry matter.
- Percentage dry matter showed inconsistency in trends in all the three orchards and it appeared to be greatly affected by weather conditions such as rainfall.
- Maturity index (MI) increased slowly with olive development, but overall the scores obtained were below those reported for olives harvests in the Northern hemisphere.
- The inverse relationship found between % oil and fruit firmness may suggest firmness as an important measure of % oil content in olive.
- Climate and location appeared to influence fruit maturation, colouration, growth and accumulation of oil and dry matter.
- Fruit weight varied between the orchards with average of about 1.3 g. Fruit weight cannot be used as a measure of maturity as it is hugely influenced by climate and other agronomic factors such as irrigation and crop load.
- The best time to harvest olive with balanced chemical composition and acceptable sensory levels of bitterness and pungency is at about 180 ± 5 days after full bloom in all the three orchards.

6.4 Recommendations

- The present study revealed the role of phenolics in the key sensory attributes of bitterness and pungency. Future studies involving GCMS can characterise flavour volatiles in olive oil responsible for several distinct fruity flavours of NZ EVOO

- Future studies could aim to develop Maturity Index (MI) specific to the orchard and to the cultivar because the present study found MI cannot be developed as a uniform index for all the growing regions studied.

- Future studies could investigate the usefulness of growing degree days (GDD) data in predicting olive maturity.

- Future opportunity could aim to develop a hand-held device, with similar capability to FirmTech that can allow measurement of firmness in the field to predict percentage of oil in the fruit.

Chapter Seven: References

Abramovic, H., Butinar, B. & Nikolic, V. (2007). Changes occurring in phenolic content, tocopherol composition and oxidative stability of Camelina sativa oil during storage. *Food Chemistry,* 104, 903-909.

Agar, I.T., Hess-Pierce, B., Sourour, M.M. & Kader, A.A. (1998). Quality of fruit and oil of black-ripe olives is influenced by cultivar and storage period. *Journal of Agricultural and Food Chemistry,* 46, 3415-3421.

Aguilera, M.P., Beltrán, G., Ortega, D., Fernández, A., Jiménez, A. & Uceda, M. (2005). Characterisation of virgin olive oil of Italian olive cultivars: Frantoio' and Leccino', grown in Andalusia. *Food Chemistry,* 89, 387-391.

Al-Maaitah, M.I., Al-Absi, K.M. & Al-Rawashdeh, A. (2009). Oil quality and quantity of three olive cultivars as influenced by harvesting date in the middle and Southern parts of Jordan. *International Journal of Agriculture and Biology,* 11, 266-272.

Allalout, A., Krichène, D., Methenni, K., Taamalli, A., Oueslati, I., Daoud, D. & Zarrouk, M. (2009). Characterization of virgin olive oil from Super Intensive Spanish and Greek varieties grown in northern Tunisia. *Scientia Horticulturae,* 120, 77-83.

Amiot, M.J., Fleuriet, A. & Macheix, J.J. (1986). Importance and evolution of phenolic compounds in olive during growth and maturation. *Journal of Agricultural and Food Chemistry,* 34, 823-826.

Amiot, M.J., Fleuriet, A. & Macheix, J.J. (1989). Accumulation of oleuropein derivatives during olive maturation. *Phytochemistry,* 28, 67-69.

Andjelkovic, M., Acun, S., Van Hoed, V., Verhe', R. & Van Camp, J. (2009). Chemical composition of Turkish olive oil - Ayvalik. *JAOCS, Journal of the American Oil Chemists' Society,* 86, 135-140.

Andrewes, P., Busch, J.L.H.C., De Joode, T., Groenewegen, A. & Alexandre, H. (2003). Sensory properties of virgin olive oil polyphenols: Identification of deacetoxy-ligstroside aglycon as a key contributor to pungency. *Journal of Agricultural and Food Chemistry,* 51, 1415-1420.

Angerosa, F. (2002). Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. *European Journal of Lipid Science and Technology,* 104, 639-660.

Angerosa, F., Campestre, C. & Giansante, L. (2006). Analysis and Authentication. In Boskou, D. (Ed.), *Olive Oil Chemistry and Technology*, (pp. 113-135). AOCS Press Champaign, IL USA.

Angerosa, F., D'alessandro, N., Konstantinou, P. & Di Giacinto, L. (1995). GC-MS evaluation of phenolic compounds in virgin olive oil. *Journal of Agricultural and Food Chemistry,* 43, 1802-1807.

Angerosa, F. & Di Giovacchino, L. (1996). Natural antioxidants of virgin olive oil obtained by two and tri-phase centrifugal decanters. *Grasas y Aceites,* 47, 247-254.

Angerosa, F., Mostallino, R., Basti, C. & Vito, R. (2000). Virgin olive oil odour notes: Their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chemistry,* 68, 283-287.

Angerosa, F., Mostallino, R., Basti, C. & Vito, R. (2001). Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chemistry,* 72, 19-28.

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S. & Montedoro, G. (2004). Volatile compounds in virgin olive oil: Occurrence and their relationship with the quality. *Journal of Chromatography A,* 1054, 17-31.

AOCS. (1998a). Official methods and recommended practices of the AOCS, Ca 5a-40. Free Fatty Acid. 5th ed. American Oil Chemists' Society, Champaign, Illinois. [Electronic Version].

AOCS. (1998b). Official methods and recommended practices of the AOCS, Cd 8b-90.Peroxide Value, Acetic Acid-Isooctane Method. 5th ed. American Oil Chemists' Society, Champaign, Illinois. [Electronic Version].

Aparicio, R., Ferreiro, L. & Alonso, V. (1994). Effect of climate on the chemical composition of virgin olive oil. *Analytica Chimica Acta,* 292, 235-241.

Aparicio, R. & Luna, G. (2002). Characterisation of monovarietal virgin olive oils. *European Journal of Lipid Science and Technology,* 104, 614-627.

Aparicio, R., Morales, M.T. & Alonso, V. (1997). Authentication of european virgin olive oils by their chemical compounds, sensory attributes, and consumers' attitudes. *Journal of Agricultural and Food Chemistry,* 45, 1076-1083.

Aparicio, R., Roda, L., Albi, M.A. & Gutierrez, F. (1999). Effect of various compounds on virgin olive oil stability measured by Rancimat. *Journal of Agricultural and Food Chemistry,* 47, 4150-4155.

Apetrei, C., Apetrei, I.M., Villanueva, S., De Saja, J.A., Gutierrez-Rosales, F. & Rodriguez-Mendez, M.L. (2010). Combination of an e-nose, an e-tongue and an e-eye for the characterisation of olive oils with different degree of bitterness. *Analytica Chimica Acta*.

Artajo, L.S., Romero, M.P., Morelló, J.R. & Motilva, M.J. (2006). Enrichment of refined olive oil with phenolic compounds: Evaluation of their antioxidant activity and their effect on the bitter index. *Journal of Agricultural and Food Chemistry,* 54, 6079-6088.

Aturki, Z., Fanali, S., D'orazio, G., Rocco, A. & Rosati, C. (2008). Analysis of phenolic compounds in extra virgin olive oil by using reversed-phase capillary electrochromatography. *Electrophoresis,* 29, 1643-1650.

Ayton, J., Mailer, R.J., Haigh, A., Tronson, D. & Conlan, D. (2007). Quality and oxidative stability of australian olive oil according to harvest date and irrigation. *Journal of Food Lipids,* 14, 138-156.

Ayton, J., Mailer, R.J., Robards, K., Orchard, B. & Vonarx, M. (2001). Oil concentration and composition of olives during fruit maturation in south-western New South Wales. *Australian Journal of Experimental Agriculture,* 41, 815-821.

Baccouri, B., Zarrouk, W., Baccouri, O., Guerfel, M., Nouairi, I., Krichene, D., Daoud, D. & Zarrouk, M. (2008a). Composition, quality and oxidative stability of virgin olive oils from some selected wild olives (Olea europaea L. subsp. Oleaster). *Grasas y Aceites,* 59, 346-351.

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

Baccouri, O., Cerretani, L., Bendini, A., Lercker, G., Zarrouk, M. & Ben Miled, D.D. (2008b). Phenol content as correlated to antioxidant activity and gustative characteristics of Tunisian monovarietal virgin olive oils. *Rivista Italiana delle Sostanze Grasse,* 85, 189-195.

Baccouri, O., Guerfel, M., Baccouri, B., Cerretani, L., Bendini, A., Lercker, G., Zarrouk, M. & Daoud Ben Miled, D. (2008c). Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. *Food Chemistry,* 109, 743-754.

Baiano, A., Gambacorta, G., Terracone, C., Previtali, M.A. & La Notte, E. (2009a). Characteristics of drupes, phenolic content and antioxidant capacity of italian olive fruits. *Journal of Food Lipids,* 16, 209-226.

Baiano, A., Gambacorta, G., Terracone, C., Previtali, M.A., Lamacchia, C. & La Notte, E. (2009b). Changes in phenolic content and antioxidant activity of italian extra-virgin olive oils during storage. *Journal of Food Science,* 74, C177-C183.

Baldioli, M., Servili, M., Perretti, G. & Montedoro, G.F. (1996). Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *JAOCS, Journal of the American Oil Chemists' Society,* 73, 1589-1593.

Battino, M. & Ferreiro, M.S. (2004). Ageing and the Mediterranean diet: A review of the role of dietary fats. *Public Health Nutrition,* 7, 953-958.

Baum, A., Lu, Y., Muccio, Z., Jackson, G.P. & Harrington, P.B. (2010). Differentiation between origins of extra virgin olive oils by GC-C-IRMS using principal component analysis, linear discriminant analysis, and hierarchical cluster analysis. *Spectroscopy,* 25.

Beauchamp, G.K., Keast, R.S.J., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C.H., Smith, A.B. & Breslin, P.A.S. (2005). Ibuprofen-like activity in extra-virgin olive oil. *Nature,* 437, 45-46.

Beltrán, G., Aguilera, M.P., Rio, C.D., Sanchez, S. & Martinez, L. (2005). Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. *Food Chemistry,* 89, 207-215.

Beltrán, G., Del Rio, C., Sanchez, S. & Martinez, L. (2004a). Influence of harvest date and crop yield on the fatty acid composition of virgin olive oils from cv. Picual. *Journal of Agricultural and Food Chemistry,* 52, 3434-3440.

Beltrán, G., Del Rio, C., Sanchez, S. & Martinez, L. (2004b). Seasonal changes in olive fruit characteristics and oil accumulation during ripening process. *Journal of the Science of Food and Agriculture,* 84, 1783-1790.

Beltrán, G., Ruano, M.T., Jiménez, A., Uceda, M. & Aguilera, M.P. (2007). Evaluation of virgin olive oil bitterness by total phenol content analysis. *European Journal of Lipid Science and Technology,* 109, 193-197.

Ben Othman, N., Roblain, D., Thonart, P. & Hamdi, M. (2008). Tunisian table olive phenolic compounds and their antioxidant capacity. *Journal of Food Science,* 73, C235-C240.

Ben Temime, S., Baccouri, B., Taamalli, W., Abaza, L., Daoud, D. & Zarrouk, M. (2006). Location effects on oxidative stability of Chétoui virgin olive oil. *Journal of Food Biochemistry,* 30, 659-670.

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno, A. & Del Rio, J.A. (2000). Antioxidant activity of phenolics extracted from *Olea europaea L*. leaves. *Food Chemistry,* 68, 457-462.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A.M., Segura-Carretero, A., Fernández-Gutiérrez, A. & Lercker, G. (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.

Bendini, A., Cerretani, L., Vecchi, S., Carrasco-Pancorbo, A. & Lercker, G. (2006). Protective effects of extra virgin olive oil phenolics on oxidative stability in the presence or absence of copper ions. *Journal of Agricultural and Food Chemistry,* 54, 4880-4887.

Bianchi, G. (1999). Extraction systems and olive oil. *OCL - Oleagineux Corps gras Lipides,* 6, 49-55.

Bianchi, G. (2003). Lipids and phenols in table olives. *European Journal of Lipid Science and Technology,* 105, 229-242.

Bianco, A., Coccioli, F., Guiso, M. & Marra, C. (2002). The occurrence in olive oil of a new class of phenolic compounds: hydroxy-isochromans. *Food Chemistry,* 77, 405-411.

Blankenship, S.M., Parker, M. & Unrath, C.R. (1997). Use of maturity indices for predicting poststorage firmness of 'Fuji' apples. *HortScience,* 32, 909-910.

Bonoli, M., Bendini, A., Cerretani, L., Lercker, G. & Toschi, T.G. (2004). Qualitative and semiquantitative analysis of phenolic compounds in extra virgin olive oils as a function of the ripening degree of olive fruits by different analytical techniques. *Journal of Agricultural and Food Chemistry,* 52, 7026-7032.

Boselli, E., Di Lecce, G., Strabbioli, R., Pieralisi, G. & Frega, N.G. (2009). Are virgin olive oils obtained below 27°C better than those produced at higher temperatures? *LWT - Food Science and Technology,* 42, 748-757.

Boskou, D. (2006). Characteristics of the Olive Tree and Olive Fruit. In Boskou, D. (Ed.), *Olive Oil Chemistry and Technology*, (pp. 13-17). AOCS Press Champaign, IL USA.

Boskou, D., Blekas, G. & Tsimidou, M. (2005). Phenolic compounds in olive oil and olives. *Current Topics in Nutraceutical Research,* 3, 125-136.

Boskou, D., Blekas, G. & Tsimidou, M. (2006a). Olive Oil Composition. In Boskou, D. (Ed.), *Olive Oil Chemistry and Technology*, (pp. 41-63). AOCS Press Champaign, IL USA.

Boskou, G., Salta, F.N., Chrysostomou, S., Mylona, A., Chiou, A. & Andrikopoulos, N.K. (2006b). Antioxidant capacity and phenolic profile of table olives from the Greek market. *Food Chemistry,* 94, 558-564.

Bouaziz, M., Chamkha, M. & Sayadi, S. (2004). Comparative study on phenolic content and antioxidant activity during maturation of the olive cultivar Chemlali from Tunisia. *Journal of Agricultural and Food Chemistry,* 52, 5476-5481.

Brenes, M., Garcia, A., Garcia, P., Rios, J.J. & Garrido, A. (1999). Phenolic compounds in Spanish olive oils. *Journal of Agricultural and Food Chemistry,* 47, 3535-3540.

, P. & Garrido, A. (2002). Use of 1-acetoxypinoresinol to authenticate Picual olive oils. *International Journal of Food Science and Technology,* 37, 615-625.

, P., Zamora, R. & Garrido, A. (2000). Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. *JAOCS, Journal of the American Oil Chemists' Society,* 77, 715-720.

, A.H. & Garrido, A. (1995). Biochemical changes in phenolic compounds during Spanish-style green olive processing. *Journal of Agricultural and Food Chemistry,* 43, 2702-2706.

Briante, R., Patumi, M., Limongelli, S., Febbraio, F., Vaccaro, C., Di Salle, A., La Cara, F. & Nucci, R. (2002a). Changes in phenolic and enzymatic activities content during fruit ripening in two Italian cultivars of Olea europaea L. *Plant Science,* 162, 791-798.

Briante, R., Patumi, M., Terenziani, S., Bismuto, E., Febbraio, F. & Nucci, R. (2002b). Olea europaea L. leaf extract and derivatives: Antioxidant properties. *Journal of Agricultural and Food Chemistry,* 50, 4934-4940.

Buiarelli, F., Berardino, S.D.I., Coccioli, F., Jasionowska, R. & Russo, M.V. (2004). Determination of phenolic acids in olive oil by capillary electrophoresis. *Annali di Chimica,* 94, 699-705.

Caponio, F. & Gomes, T. (2001). Influence of olive crushing temperature on phenols in olive oils. *European Food Research and Technology,* 212, 156-159.

Caponio, F., Gomes, T. & Pasqualone, A. (2001). Phenolic compounds in virgin olive oils: Influence of the degree of olive ripeness on organoleptic characteristics and shelf-life. *European Food Research and Technology,* 212, 329-333.

Caporale, G., Policastro, S. & Monteleone, E. (2004). Bitterness enhancement induced by cut grass odorant (cis-3-hexen-1-ol) in a model olive oil. *Food Quality and Preference,* 15, 219-227.

Caravita, M., Benincasa, C., De Rose, F., Muzzalupo, I., Parise, A., Pellegrino, M., Perri, E. & Rizzuti, B. (2007). Omega-3 / omega-6 fatty acids ratio in olive oils from Italian olive varieties. *Agro Food Industry Hi-Tech,* 18, 17-18.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Gallina-Toschi, T. & Fernández-Gutiérrez, A. (2005). Analytical determination of polyphenols in olive oils. *Journal of Separation Science,* 28, 837-858.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Gallina-Toschi, T., Lercker, G. & Fernández-Gutiérrez, A. (2006a). Evaluation of individual antioxidant activity of single phenolic compounds on virgin olive oil. *Progress in Nutrition,* 8, 28-39.

- -Caravaca, A.M., Cerretani, L., Bendini, A., Segura-Carretero, A. & Fernández-Gutiérrez, A. (2006b). A simple and rapid electrophoretic method to characterize simple phenols, lignans, complex phenols, phenolic acids, and flavonoids in extra-virgin olive oil. *Journal of Separation Science,* 29, 2221-2233.

Cartoni, G.P., Coccioli, F., Jasionowska, R. & Ramires, D. (2000). HPLC analysis of the benzoic and cinnamic acids in edible vegetable oils. *Italian Journal of Food Science,* 12, 163-173.

Cayuela, J.A. & Camino, M.D.C.P. (2010). Prediction of quality of intact olives by near infrared spectroscopy. *European Journal of Lipid Science and Technology,* 112, 1209-1217.

Cerretani, L., Bendini, A., Del Caro, A., Piga, A., Vacca, V., Caboni, M.F. & Toschi, T.G. (2006). Preliminary characterisation of virgin olive oils obtained from different cultivars in Sardinia. *European Food Research and Technology,* 222, 354-361.

Changying, L., Jiawei, L. & Dan, M. (2011). A novel instrument to delineate varietal and harvest effects on blueberry fruit texture during storage. *Journal of the Science of Food and Agriculture,* 91, 1653–1658.

Cicerale, S., Conlan, X.A., Sinclair, A.J. & Keast, R.S.J. (2009). Chemistry and health of olive oil phenolics. *Critical reviews in food science and nutrition,* 49, 218-236.

Cinquanta, L., Esti, M. & Di Matteo, M. (2001). Oxidative stability of virgin olive oils. *JAOCS, Journal of the American Oil Chemists' Society,* 78, 1197-1202.

Cinquanta, L., Esti, M. & La Notte, E. (1997). Evolution of phenolic compounds in virgin olive oil during storage. *JAOCS, Journal of the American Oil Chemists' Society,* 74, 1259-1264.

Cioffi, G., Pesca, M.S., De Caprariis, P., Braca, A., Severino, L. & De Tommasi, N. (2010). Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. *Food Chemistry,* 121, 105-111.

Clark, C.J., Mcglone, V.A., Requejo, C., White, A. & Woolf, A.B. (2003). Dry matter determination in 'Hass' avocado by NIR spectroscopy. *Postharvest Biology and Technology,* 29, 301-308.

Clark, C.J., White, A., Jordan, R.B. & Woolf, A.B. (2007). Challenges associated with segregation of avocados of differing maturity using density sorting at harvest. *Postharvest Biology and Technology,* 46, 119-127.

Conde, C., Delrot, S. & Gerós, H. (2008). Physiological, biochemical and molecular changes occurring during olive development and ripening. *Journal of Plant Physiology,* 165, 1545-1562.

Conte, L.S., Pizzale, L., Bortolomeazzi, R. & Vichi, S. (2002). Antioxidant activity evaluation of extra virgin olive oils. *Progress in Nutrition,* 4, 17-23.

Cossignani, L., Simonetti, M.S. & Damiani, P. (2001). Structural changes of triacylglycerol and diacylglycerol fractions during olive drupe ripening. *European Food Research and Technology,* 212, 160-164.

Criado, M.N., Morelló, J.R., Motilva, M.J. & Romero, M.P. (2004). Effect of growing area on pigment and phenolic fractions of virgin olive oils of the Arbequina variety in Spain. *JAOCS, Journal of the American Oil Chemists' Society,* 81, 633-640.

Dabbou, S., Chehab, H., Faten, B., Esposto, S., Selvaggini, R., Taticchi, A., Servili, M., Montedoro, G.F. & Hammami, M. (2010a). Effect of three irrigation regimes on Arbequina olive oil produced under Tunisian growing conditions. *Agricultural Water Management,* 97, 763-768.

Dabbou, S., Issaoui, M., Esposto, S., Sifi, S., Taticchi, A., Servili, M., Montedorob, G.F. & Hammamia, M. (2009a). Cultivar and growing area effects on minor compounds of olive oil from autochthonous and European introduced cultivars in Tunisia. *Journal of the Science of Food and Agriculture,* 89, 1314-1325.

Dabbou, S., Issaoui, M., Servili, M., Taticchi, A., Sifi, S., Montedoro, G.F. & Hammami, M. (2009b). Characterisation of virgin olive oils from European olive cultivars introduced in Tunisia. *European Journal of Lipid Science and Technology,* 111, 392-401.

Dabbou, S., Sifi, S., Rjiba, I., Esposto, S., Taticchi, A., Servili, M., Montedoro, G.F. & Hammami, M. (2010b). Effect of pedoclimatic conditions on the chemical composition of the Sigoise olive cultivar. *Chemistry and Biodiversity,* 7, 898-908.

Dai, J. & Mumper, R.J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules,* 15, 7313-7352.

Damak, N., Bouaziz, M., Ayadi, M., Sayadi, S. & Damak, M. (2008). Effect of the maturation process on the phenolic fractions, fatty acids, and antioxidant activity of the Chétoui olive fruit cultivar. *Journal of Agricultural and Food Chemistry,* 56, 1560-1566.

De La Torre-Carbot, K., - -Sabater, M.C. (2005). Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS. *Journal of Agricultural and Food Chemistry,* 53, 4331-4340.

Di Giovacchino, L., Angerosa, F. & Di Giacinto, L. (1996). Effect of mixing leaves with olives on organoleptic quality of oil obtained by centrifugation. *JAOCS, Journal of the American Oil Chemists' Society,* 73, 371-374.

Di Giovacchino, L., Costantini, N., Ferrante, M.L. & Serraiocco, A. (2002a). Influence of malaxation time of olive paste on oil extraction yields and chemical and organoleptic characteristics of virgin olive oil obtained by a centrifugal decanter at water saving. *Grasas y Aceites,* 53, 179-186.

Di Giovacchino, L., Mucciarella, M.R., Costantini, N., Ferrante, M.L. & Surricchio, G. (2002b). Use of nitrogen to improve stability of virgin olive oil during storage. *JAOCS, Journal of the American Oil Chemists' Society,* 79, 339-344.

Di Giovacchino, L., Sestili, S. & Di Vincenzo, D. (2002c). Influence of olive processing on virgin olive oil quality. *European Journal of Lipid Science and Technology,* 104, 587-601.

Di Giovacchino, L., Solinas, M. & Miccoli, M. (1994). Effect of extraction systems on the quality of virgin olive oil. *Journal of the American Oil Chemists' Society,* 71, 1189-1194.

Diraman, H. & Dibeklioglu, H. (2009). Characterization of Turkish virgin olive oils produced from early harvest olives. *JAOCS, Journal of the American Oil Chemists' Society*, 1-12.

Donaire, J.P., Belver, A., Rodriguez-Garcia, M.I. & Megias, L. (1984). Lipid biosynthesis, oxidative enzyme activities and cellular changes in growing olive fruit. *Revista Espanola de Fisiologia,* 40, 191-203.

Edwards, M. (2006a). *New Zealand olive industry*. Olives New Zealand. Accessed April 27, 2010, from:http://www.olivesnz.org.nz/olive-industry.cfm:

Edwards, M. (2006b). *Olive history*. Olives New Zealand. Accessed April 26, 2010, from:http://www.olivesnz.org.nz/olive-history.cfm:

Edwards, M. (2007). The development of the New Zealand extra virgin olive oil industry. In O'Connor, C.J. (Ed.), *Handbook of Australasian edible oils*, (pp. 38-81). Oils and Fats Specialist Group of the New Zealand Institute of Chemistry, P O Box 25-499, St Heliers, Auckland, New Zealand.

Elmadfa, I. & Kornsteiner, M. (2009). Dietary fat intake - A global perspective. *Annals of Nutrition and Metabolism,* 54, 8-14.

Escrich, E., Ramirez-Tortosa, M.C., Sanchez-Rovira, P., Colomer, R., Solanas, M. & Gaforio, J.J. (2006). Olive oil in cancer prevention and progression. *Nutrition Reviews,* 64, S40-S52.

Esti, M., Cinquante, L. & La Notte, E. (1998). Phenolic compounds in different olive varieties. *Journal of Agricultural and Food Chemistry,* 46, 32-35.

Esti, M., Contini, M., Moneta, E. & Sinesio, F. (2009). Phenolics compounds and temporal perception of bitterness and pungency in extra-virgin olive oils: Changes occurring throughout storage. *Food Chemistry,* 113, 1095-1100.

Farhoosh, R. (2007a). The effect of operational parameters of the Rancimat method on the determination of the oxidative stability measures and shelf-life prediction of soybean oil. *JAOCS, Journal of the American Oil Chemists' Society,* 84, 205-209.

Farhoosh, R. (2007b). Shelf-life prediction of edible fats and oils using Rancimat. *Journal of Lipid Technology,* Vol. 19, 232-234.

Fernandez-Bolanos, J., Rodriguez, R., Guillen, R., Jimenez, A. & Heredia, A. (1995). Activity of cell wall-associated enzyme in ripening olive fruit. *Physiologia Plantarum,* 93, 651-658.

Finotti, E., Bersani, A.M. & Bersani, E. (2007). Total quality indexes for extra-virgin olive oils. *Journal of Food Quality,* 30, 911-931.

Finotti, E., Beye, C., Nardo, N., Quaglia, G.B., Milin, C. & Giacometti, J. (2001). Physico-chemical characteristics of olives and olive oil from two mono-cultivars during various ripening phases. *Die Nahrung,* 45, 350-352.

Flores, G., Ruiz Del Castillo, M.L., Blanch, G.P. & Herraiz, M. (2006). Detection of the adulteration of olive oils by solid phase microextraction and multidimensional gas chromatography. *Food Chemistry,* 97, 336-342.

Franconi, F., Coinu, R., Carta, S., Urgeghe, P.P., Ieri, F., Mulinacci, N. & Romani, A. (2006). Antioxidant effect of two virgin olive oils depends on the concentration and composition of minor polar compounds. *Journal of Agricultural and Food Chemistry,* 54, 3121-3125.

Fritsche, S. & Hrncirik, K. (2007). Quality control of olive oil: Analytical and organoleptic schemes for quality grading and purity control of olive oil, *ACS Symposium Series* (Vol. 952, pp. 110-125).

Galli, C. (2006). Bioactive minor compounds of extra-virgin olive oil: The ibuprofen-like activity of oleocanthal. *European Journal of Lipid Science and Technology,* 108, 95-96.

Gallina-Toschi, T., Cerretani, L., Bendini, A., Bonoli-Carbognin, M. & Lercker, G. (2005). Oxidative stability and phenolic content of virgin olive oil: An analytical approach by traditional and high resolution techniques. *Journal of Separation Science,* 28, 859-870.

Gallina Toschi, T., Biguzzi, B., Cerretani, L., Bendini, A., Rotondi, A. & Lercker, G. (2004). Effect of crushing time and temperature of malaxation on the oxidative stability of a monovarietal extra-virgin olive oil, obtained by different industrial processing systems. *Progress in Nutrition,* 6, 132-138.

Galvano, F., La Fauci, L., Graziani, G., Ferracane, R., Masella, R., Di Giacomo, C., Scacco, A., D'archivio, M., Vanella, L. & Galvano, G. (2007). Phenolic compounds and antioxidant activity of italian extra virgin olive oil Monti Iblei. *Journal of Medicinal Food,* 10, 650-656.

Garcia-Gonzalez, D.L., Aparicio-Ruiz, R. & Aparicio, R. (2008). Virgin olive oil - chemical implications on quality and health. *European Journal of Lipid Science and Technology,* 110, 602-607.

García-Mesa, J.A., Pereira-Caro, G., Fernández-Hernández, A., García-Ortíz Civantos, C. & Mateos, R. (2008). Influence of lipid matrix in the bitterness perception of virgin olive oil. *Food Quality and Preference,* 19, 421-430.

García, J.M., Brenes, M., Martinez, F., Alba, J., Garcia, P. & Garrido, A. (2001a). High-performance liquid chromatography evaluation of phenols in virgin olive oil during extraction at laboratory and industrial scale. *JAOCS, Journal of the American Oil Chemists' Society,* 78, 625-629.

García, J.M., Gutierrez, F., Castellano, J.M., Perdiguero, S., Morilla, A. & Albi, M.A. (1996a). Influence of storage temperature on fruit ripening and olive oil quality. *Journal of Agricultural and Food Chemistry,* 44, 264-267.

Garcia, J.M., Seller, S. & Perez-Camino, M.C. (1996). Influence of Fruit Ripening on Olive Oil Quality. *Journal of Agricultural and Food Chemistry,* 44, 3516-3520.

García, J.M., Seller, S. & Pérez-Camino, M.C. (1996b). Influence of fruit ripening on olive oil quality. *Journal of Agricultural and Food Chemistry,* 44, 3516-3520.

García, J.M., Yousfi, K., Mateos, R., Olmo, M. & Cert, A. (2001b). Reduction of oil bitterness by heating of olive (Olea europaea) fruits. *Journal of Agricultural and Food Chemistry,* 49, 4231-4235.

Gawel, R. & Rogers, D.A.G. (2009). The relationship between total phenol concentration and the perceived style of extra virgin olive oil. *Grasas y Aceites,* 60, 134-138.

Giacometti, J. & Milin, C. (2001). Composition and qualitative characteristics of virgin olive oils produced in northern Adriatic region, Republic of Croatia. *Grasas y Aceites,* 52, 397-402.

Gimeno, E., Castellote, A.I., Lamuela-Raventós, R.M., De La Torre, M.C. & López-Sabater, M.C. (2002). The effects of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherol, and β-carotene) in virgin olive oil *Food Chemistry,* 78, 207-211.

Giugliano, D. & Esposito, K. (2005). Mediterranean diet and cardiovascular health, *Annals of the New York Academy of Sciences* (Vol. 1056, pp. 253-260).

Gomez-Alonso, S., Salvador, M.D. & Fregapane, G. (2002). Phenolic compounds profile of Cornicabra virgin olive oil. *Journal of Agricultural and Food Chemistry,* 50, 6812-6817.

Gomez-Rico, A., Fregapane, G. & Salvador, M.D. (2008). Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. *Food Research International,* 41, 433-440.

Gómez-Rico, A., Salvador, M.D., La Greca, M. & Fregapane, G. (2006). Phenolic and volatile compounds of extra virgin olive oil (Olea europaea L. Cv. Cornicabra) with regard to fruit ripening and irrigation management. *Journal of Agricultural and Food Chemistry,* 54, 7130-7136.

Guerfel, M., Ouni, Y., Taamalli, A., Boujnah, D., Stefanoudaki, E. & Zarrouk, M. (2009). Effect of location on virgin olive oils of the two main Tunisian olive cultivars. *European Journal of Lipid Science and Technology,* 111, 926-932.

Guillaume, C., Ravetti, L. & Gwyn, S. (2009). Characterisation of Phenolic Compounds in Oils Produced from Frosted Olives. *JAOCS, Journal of the American Oil Chemists' Society*, 1-8.

Gurdeniz, G. & Ozen, B. (2009). Detection of adulteration of extra-virgin olive oil by chemometric analysis of mid-infrared spectral data. *Food Chemistry,* 116, 519-525.

Gurdeniz, G., Ozen, B. & Tokatli, F. (2008). Classification of Turkish olive oils with respect to cultivar, geographic origin and harvest year, using fatty acid profile and mid-IR spectroscopy. *European Food Research and Technology,* 227, 1275-1281.

Gutfinger, T. (1981). Polyphenols in olive oils. *Journal of the American Oil Chemists Society,* 58, 966-968.

Gutiérrez- Rosales, F., Perdiguero, S., Gutiérrez, R. & Olias, J.M. (1992). Evaluation of the bitter taste in virgin olive oil. *Journal of the American Oil Chemists' Society,* 69, 394-395.

Gutiérrez-Rosales, F., Rios, J.J. & Gomez-Rey, M.L. (2003). Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry,* 51, 6021-6025.

Gutiérrez-Rosales, F., Ríos, J.J. & Gómez-Rey, M.L. (2003). Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry,* 51, 6021-6025.

Gutiérrez, F., Arnaud, T. & Garrido, A. (2001). Contribution of polyphenols to the oxidative stability of virgin olive oil. *Journal of the Science of Food and Agriculture,* 81, 1463-1470.

Gutiérrez, F., Jimenez, B., Ruiz, A. & Albi, M.A. (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. *Journal of Agricultural and Food Chemistry,* 47, 121-127.

Gutiérrez, F., Jímenez, B., Ruíz, A. & Albi, M.A. (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. *Journal of Agricultural and Food Chemistry,* 47, 121-127.

Haddada, F.M. , D., Manai, H., Oueslati, I., Daoud, D. & Zarrouk, M. (2008). Analytical evaluation of six monovarietal virgin olive oils from Northern Tunisia. *European Journal of Lipid Science and Technology,* 110, 905-913.

Haddada, F.M., Manai, H., Oueslati, I., Daoud, D., Sanchez, J., Osorio, E. & Zarrouk, M. (2007). Fatty acid, triacylglycerol, and phytosterol composition in six Tunisian olive varieties. *Journal of Agricultural and Food Chemistry,* 55, 10941-10946.

Hahm, T.S. & Min, D.B. (1995). Analyses of peroxide values and headspace oxygen. In Warmer, K. & Eskin, N.A.M. (Eds.), *Methods to assess quality and stability of oils and fat-containing foods*, (pp. 146-157). AOCS Press Champaign, Illinois.

Hajimahmoodi, M., Sadeghi, N., Jannat, B., Oveisi, M.R., Madani, S., Kiayi, M., Akrami, M.R. & Ranjbar, A.M. (2008). Antioxidant activity, reducing power and total phenolic content of Iranian olive cultivar. *Journal of Biological Sciences,* 8, 779-783.

Hartman, L. (1973). Rapid preparation of fatty acid methyl esters from lipids. *LAB.PRACT.,* 22, 475-476.

Harwood, J. & Aparacio, R. (2000). *Handbook of olive oil*, (Harwood, J. & Aparacio, R., Eds). Gaithersburg, Maryland: Aspen Publication.

Harwood, J.L., Jones, A.L., Perry, H.J., Rutter, A.J., Smith, K.L. & Williams, M. (1994). Changes in plant lipids during temperature adaptation. In Cossins, A.R. (Ed.), *Temperature adaption of biological membranes*, (pp. 107-118). Portland Press: London, U.K.

Harwood, J.L. & Sanchez, J. (2000). Lipid biosynthesis in olives. In Harwood, J.L. & Aparicio, R. (Eds.), *Handbook of olive oil*, (pp. 61–78). Gaithersburg, Maryland: Aspen Publishers.

Hernández, M.L., Guschina, I.A., Martínez-Rivas, J.M., Mancha, M. & Harwood, J.L. (2008). The utilization and desaturation of oleate and linoleate during glycerolipid biosynthesis in olive (Olea europaea L.) callus cultures. *Journal of Experimental Botany,* 59, 2425-2435.

Hopkirk, G., Maindonald, J.H. & White, A. (1996). Comparison of four new devices for measuring kiwifruit firmness. *New Zealand Journal of Crop and Horticultural Science,* 24, 273-286.

Hrncirik, K. & Fritsche, S. (2004). Comparability and reliability of different techniques for the determination of phenolic compounds in virgin olive oil. *European Journal of Lipid Science and Technology,* 106, 540-549.

Huisman, M.M.H., Schols, H.A. & Voragen, A.G.J. (1996). Changes in cell wall polysaccharides from ripening olive fruits. *Carbohydrate Polymers,* 31, 123-133.

Iacumin, P., Bernini, L. & Boschetti, T. (2009). Climatic factors influencing the isotope composition of Italian olive oils and geographic characterisation. *Rapid Communications in Mass Spectrometry,* 23, 448-454.

Ichihara, K. (1999). Low-temperature stress and biosynthesis of plant lipids. *Tanpakushitsu kakusan koso. Protein, nucleic acid, enzyme,* 44, 2158-2164.

Inarejos-Garcia, A.M., Androulaki, A., Salvador, M.D., Fregapane, G. & Tsimidou, M.Z. (2009a). Discussion on the objective evaluation of virgin olive oil bitterness. *Food Research International,* 42, 279-284.

Inarejos-Garcia, A.M., Gomez-Rico, A., Salvador, M.D. & Fregapane, G. (2009b). Influence of malaxation conditions on virgin olive oil yield, overall quality and composition. *European Food Research and Technology,* 228, 671-677.

Inarejos-García, A.M., Santacatterina, M., Salvador, M.D., Fregapane, G. & Gómez-Alonso, S. (2010). PDO virgin olive oil quality-Minor components and organoleptic evaluation. *Food Research International,* 43, 2138-2146.

Indyk, H.E. (1988). Simplified saponification procedure for the routine determination of vitamin E in dairy products, foods and tissues by high-performance liquid chromatography. *Analyst,* 113, 1217-1221.

Trade standard applying to olive oils and olive pomace oils: COI/T.15/NC no. 3/Rev. 2, (2006).

Issaoui, M., Flamini, G., Brahmi, F., Dabbou, S., Hassine, K.B., Taamali, A., Chehab, H., Ellouz, M., Zarrouk, M. & Hammami, M. (2010). Effect of the growing area conditions on differentiation between Chemlali and Chétoui olive oils. *Food Chemistry,* 119, 220-225.

Jiang, L., Yamaguchi, T., Takamura, H. & Matoba, T. (2005). Characteristics of Shodo Island olive oils in Japan: Fatty acid composition and antioxidative compounds. *Food Science and Technology Research,* 11, 254-260.

Jones, P.J.H. & Abumweis, S.S. (2009). Phytosterols as functional food ingredients: Linkages to cardiovascular disease and cancer. *Current Opinion in Clinical Nutrition and Metabolic Care,* 12, 147-151.

Jose Motilva, M., Jess Tovar, M., Paz Romero, M., Alegre, S. & Girona, J. (2000). Influence of regulated deficit irrigation strategies applied to olive trees (Arbequina cultivar) on oil yield and oil composition during the fruit ripening period. *Journal of the Science of Food and Agriculture,* 80, 2037-2043.

Kaiser, C. & Wolstenholme, B.N. (1994). Aspects of delayed harvest of 'Hass' avocado (Persea Americana Mill.) Fruit in a cool subtropical llimate. I. Fruit lipid and fatty acid accumulation *The Journal of Horticultural Science & Biotechnology* 69, 437-446.

Kalua, C.M., Allen, M.S., Bedgood Jr, D.R., Bishop, A.G. & Prenzler, P.D. (2005). Discrimination of olive oils and fruits into cultivars and maturity stages based on phenolic and volatile compounds. *Journal of Agricultural and Food Chemistry,* 53, 8054-8062.

Kalua, C.M., Bedgood Jr, D.R., Bishop, A.G. & Prenzler, P.D. (2006). Changes in volatile and phenolic compounds with malaxation time and temperature during virgin olive oil production. *Journal of Agricultural and Food Chemistry,* 54, 7641-7651.

Keceli, T. & Gordon, M.H. (2001). The antioxidant activity and stability of the phenolic fraction of green olives and extra virgin olive oil. *Journal of the Science of Food and Agriculture,* 81, 1391-1396.

Kiralan, M., Bayrak, A. & Özkaya, M.T. (2008). Oxidation stability of virgin olive oils from some important cultivars in East mediterranean area in Turkey. *JAOCS, Journal of the American Oil Chemists' Society*, 1-6.

Kiritsakis, A.K. (1990). *Olive oil*. Illinois, USA: AOCS Press.

Kiritsakis, A.K. (1998a). Composition of olive oil. In Kiritsakis, A.K., Elizabeth, B.L., Walter, C.W. & Ruben, J.H. (Eds.), *Olive oil from the tree to the table*, (pp. 113-153). Food and Nutrition Press, INC. Trumbull, Connecticut 06611 USA.

Kiritsakis, A.K. (1998b). Factors affecting olive oil quality from the tree to the table. In Kiritsakis, A.K., Elizabeth, B.L., Walter, C.W. & Ruben, J.H. (Eds.), *Olive oil from the tree to the table*, (pp. 227-235). Apostolos K. Kiritsakis: Food and Nutrition Press, INC. Trumbull, Connecticut 06611 USA.

Kiritsakis, A.K. (1998c). Harvest and storage of olive fruit. In Kiritsakis, A.K., Elizabeth, B.L., Walter, C.W. & Ruben, J.H. (Eds.), *Olive oil from the tree to the table*, (pp. 41-52). Food and Nutrition Press, INC. Trumbull, Connecticut 06611 USA.

Kiritsakis, A.K. (1998d). International olive oil council-quality criteria and classification of olive oil. In Kiritsakis, A.K., Elizabeth, B.L., Walter, C.W. & Ruben, J.H. (Eds.), *Olive oil from the tree to the table*, (pp. 237-259). Food and Nutrition Press, INC. Trumbull, Connecticut 06611 USA.

Kiritsakis, A.K. (1998e). Methods of olive oil analysis. In Kiritsakis, A.K., Elizabeth, B.L., Walter, C.W. & Ruben, J.H. (Eds.), *Olive oil from the tree to the table*. Food and Nutrition Press, INC. Trumbull, Connecticut 06611 USA.

Kiritsakis, A.K. (1998f). Olive fruit compostion and growtth. In Kiritsakis, A.K., Elizabeth, B.L., Walter, C.W. & Ruben, J.H. (Eds.), *Olive oil from the tree to the table*, (pp. 25-40). Food and Nutrition Press, INC. Trumbull, Connecticut 06611 USA.

Kiritsakis, A.K. (1998g). Processing of olive fruit. In Kiritsakis, A.K., Elizabeth, B.L., Walter, C.W. & Ruben, J.H. (Eds.), *Olive oil from the tree to the table*, (pp. 53-96). Food and Nutrition Press, INC. Trumbull, Connecticut 06611 USA.

Koprivnjak, O., Škevin, D., Petričević, S., Brkić Bubola, K. & Mokrovčak, Z. (2009). Bitterness, odor properties and volatile compounds of virgin olive oil with phospholipids addition. *LWT - Food Science and Technology,* 42, 50-55.

Koski, A., Psomiadou, E., Tsimidou, M., Hopia, A., Kefalas, P., Wahala, K. & Heinonen, M. (2002). Oxidative stability and minor constituents of virgin olive oil and cold-pressed rapeseed oil. *European Food Research and Technology,* 214, 294-298.

Krichene, D., Allalout, A., Baccouri, B., Fregapane, G.Q., Salvador, M.D. & Zarrouk, M. (2009). Territorial investigation based on the chemical composition of Chemlali virgin olive oils. *Asian Journal of Biochemistry,* 4, 1-12.

Krichene, D., Taamalli, W., Daoud, D., Salvador, M.D., Fregapane, G. & Zarrouk, M. (2007). Phenolic compounds, tocopherols and other minor components in virgin olive oils of some Tunisian varieties. *Journal of Food Biochemistry,* 31, 179-194.

Lavee, S. & Wodner, M. (2004). The effect of yield, harvest time and fruit size on the oil content in fruits of irrigated olive trees (Olea europaea), cvs. Barnea and Manzanillo. *Scientia Horticulturae,* 99, 267-277.

Lawless, H.T. & Heymann, H. (1998). *Sensory evaluation of food: principles and practices*. New York, NY

Chapman & Hall.

Lazzez, A., Perri, E., Caravita, M.A., Khlif, M. & Cossentini, M. (2008). Influence of olive maturity stage and geographical origin on some minor components in virgin olive oil of the chemlali variety. *Journal of Agricultural and Food Chemistry,* 56, 982-988.

Lee, O.H., Kim, Y.C., Kim, K.J. & Lee, B.Y. (2007). The effects of bioactive compounds and fatty acid compositions on the oxidative stability of extra virgin olive oil varieties. *Food Science and Biotechnology,* 16, 415-420.

Lercker, G., Bendini, A. & Cerretani, L. (2007). Quality, composition and production process of virgin olive oils. *QualitaÌ€, composizione e tecnologia di produzione degli oli vergini di oliva,* 9, 134-148.

Lorgeril, M. & Salen, P. (2008). The mediterranean diet: Rationale and evidence for its benefit. *Current Atherosclerosis Reports,* 10, 518-522.

Lule, S.U. & Xia, W. (2005). Food phenolics, pros and cons: A review. *Food Reviews International,* 21, 367-388.

Lunn, J. & Theobald, H.E. (2006). The health effects of dietary unsaturated fatty acids. *Nutrition Bulletin,* 31, 178-224.

Mailer, R. (2007a). Australian olive oil and the international market. *INFORM - International News on Fats, Oils and Related Materials,* 18, 697-698.

Mailer, R.J. (2005). Variation in oil quality and fatty acid composition in Australian olive oil. *Australian Journal of Experimental Agriculture,* 45, 115-119.

Mailer, R.J. (2007b). Setting quality standards for Australasian olive oils. In O'Connor, C.J. (Ed.), *Handbook of Australasian Edible Oils*, (pp. 61-81). Oils and Fats Specialist Group of the New Zealand Institute of Chemistry, P O Box 25-499, St Heliers, Auckland, New Zealand.

Mailer, R.J., Ayton, J. & Conlan, D. (2002). Comparison and evaluation of the quality of thirty-eight commercial Australian and New Zealand olive oils. *Advances in Horticultural Science,* 16, 259-266.

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

Malik, N.S.A. & Bradford, J.M. (2006). Changes in oleuropein levels during differentiation and development of floral buds in 'Arbequina' olives. *Scientia Horticulturae,* 110, 274-278.

Mateos, R., Cert, A., Carmen Pérez-Camino, M. & García, J.M. (2004). Evaluation of virgin olive oil bitterness by quantification of secoiridoid derivatives. *JAOCS, Journal of the American Oil Chemists' Society,* 81, 71-75.

Mateos, R., Domínguez, M.M., Espartero, J.L. & Cert, A. (2003). Antioxidant effect of phenolic compounds, α-tocopherol, and other minor components in virgin olive Oil. *Journal of Agricultural and Food Chemistry,* 51, 7170-7175.

Mateos, R., Espartero, J.L., Trujillo, M., Riì⍰Os, J.J., Leoì⍰N-Camacho, M., Alcudia, F. & Cert, A. (2001). Determination of phenols, flavones, and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *Journal of Agricultural and Food Chemistry,* 49, 2185-2192.

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

Mcdonald, S., Prenzler, P.D., Antolovich, M. & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry,* 73, 73-84.

Mcglone, V.A. & Kawano, S. (1998). Firmness, dry-matter and soluble-solids assessment of postharvest kiwifruit by NIR spectroscopy. *Postharvest Biology and Technology,* 13, 131-141.

Mcmanus, J.P., Davis, K.G., Beart, J.E., Gaffney, S.H., Lilley, T.H. & Haslam, E. (1985). Polyphenol interactions. Part 1. Introduction; some observations on the reversible complexation of polyphenols with proteins and polysaccharides. *Journal of the Chemical Society, Perkin Transactions 2*, 1429-1438.

Min, J.K., Hae, S.A. & Lee, S.S. (2005). Effects of polyunsaturated/saturated fatty acid ratio and antioxidant supplementation on hepatic TBARS and enzyme activities under the maintenance of dietary peroxidizability index value in young and adult rats. *Annals of Nutrition and Metabolism,* 49, 304-311.

Mitcham, E.J., Murray, C. & Biasi, W.V. (1998). Comparison of devices for measuring cherry fruit firmness. *HortScience,* 33, 723-730.

Montedoro, G., Servili, M., Baldioli, M. & Miniati, E. (1992a). Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricuhural and Food Chemistry,* 40, 1571-1576.

Montedoro, G., Servili, M., Baldioli, M. & Miniati, E. (1992b). Simple and hydrolyzable phenolic compounds in virgin olive oil. 2. Initial characterization of the hydrolyzable fraction. *Journal of Agricuhural and Food Chemistry,* 40, 1577-1580.

Montedoro, G., Servili, M., Baldioli, M., Selvaggini, R., Miniati, E. & Macchioni, A. (1993). Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoid derivatives. *Journal of Agricultural and Food Chemistry,* 41, 2228-2234.

Monteleone, E., Caporale, G., Carlucci, A. & Pagliarini, E. (1998). Optimisation of extra virgin Olive oil quality. *Journal of the Science of Food and Agriculture,* 77, 31-37.

Morales, M.T., Aparicio, R. & Calvente, J.J. (1996). Influence of olive ripeness on the concentration of green aroma compounds in virgin olive oil. *Flavour and Fragrance Journal,* 11, 171-178.

, J.R., Motilva, M.J., Ramo, T. & Romero, M.P. (2003). Effect of freeze injuries in olive fruit on virgin olive oil composition. *Food Chemistry,* 81, 547-553.

Morello, J.R., Romero, M.P. & Motilva, M.J. (2004). Effect of the maturation of the olive fruit on the phenolic fraction of drupes and oils from Arbequina, Farga, and Morrut cultivars. *Journal of Agricultural and Food Chemistry,* 52, 6002-6009.

Morello, J.R., Romero, M.P. & Motilva, M.J. (2006). Influence of seasonal conditions on the composition and quality parameters of monovarietal virgin olive oils. *JAOCS, Journal of the American Oil Chemists' Society,* 83, 683-690.

Morello, J.R., Vuorela, S., Romero, M.P., Motilva, M.J. & Heinonen, M. (2005). Antioxidant activity of olive pulp and olive oil phenolic compounds of the arbequina cultivar. *Journal of Agricultural and Food Chemistry,* 53, 2002-2008.

Mousa, Y.M., Gerasopoulos, D., Metzidakis, I. & Kiritsakis, A. (1996). Effect of altitude on fruit and oil quality characteristics of 'Mastoides' olives. *Journal of the Science of Food and Agriculture,* 71, 345-350.

Mulinacci, N., Giaccherini, C., Ieri, F., Innocenti, M., Romani, A. & Vincieri, F.F. (2006). Evaluation of lignans and free and linked hydroxy-tyrosol and tyrosol in extra virgin olive oil after hydrolysis processes. *Journal of the Science of Food and Agriculture,* 86, 757-764.

Murphy, C. & Cain, W.S. (1980). Taste and olfaction: Independence vs interaction. *Physiology and Behavior,* 24, 601-605.

Murphy, C., Cain, W.S. & Bartoshuk, L.M. (1977). Mutual action of taste and olfaction. *Sensory Processes,* 1, 204-211.

Nam, H.Y. & Lee, K.T. (2007). Analysis of characterization in commercial extra virgin olive oils. *Journal of the Korean Society of Food Science and Nutrition,* 36, 866-873.

Navarro Ruiz, A., Font Perez, G. & Farre Rovira, R. (1990). Fatty acids and sterols composition of olive oil produced in La Montana (Alicante). *Acidos grasos y esteroles de aceites de oliva de la comarca de la montana (alicante),* 56, 407-414.

Nergiz, C. & Engez, Y. (2000). Compositional variation of olive fruit during ripening. *Food Chemistry,* 69, 55-59.

Nergiz, C. & Unal, K. (1991). Determination of phenolic acids in virgin olive oil. *Food Chemistry,* 39, 237-240.

Ninfali, P., Aluigi, G., Bacchiocca, M. & Magnani, M. (2001). Antioxidant capacity of extra-virgin olive oils. *JAOCS, Journal of the American Oil Chemists' Society,* 78, 243-247.

Obied, H.K., Bedgood Jr, D.R., Prenzler, P.D. & Robards, K. (2008a). Effect of processing conditions, prestorage treatment, and storage conditions on the phenol content and antioxidant activity of olive mill waste. *Journal of Agricultural and Food Chemistry,* 56, 3925-3932.

Obied, H.K., Prenzler, P.D., Ryan, D., Servili, M., Taticchi, A., Esposto, S. & Robards, K. (2008b). Biosynthesis and biotransformations of phenol-conjugated oleosidic secoiridoids from Olea europaea L. *Natural product reports,* 25, 1167-1179.

Ocakoglu, D., Tokatli, F., Ozen, B. & Korel, F. (2009). Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. *Food Chemistry,* 113, 401-410.

, J.J. & Sanz, L.C. (1993). Aroma of virgin olive oil: Biogenesis of the "green" odor notes. *Journal of Agricultural and Food Chemistry,* 41, 2368-2373.

Oliveras-Lopez, M.J., Berna, G., Carneiro, E.M., De La Serrana, H.L.G., Martin, F. & Lopez, M.C. (2008). An extra-virgin olive oil rich in polyphenolic compounds has antioxidant effects in Of1 mice. *Journal of Nutrition,* 138, 1074-1078.

Oliveras-Lopez, M.J., Innocenti, M., Giaccherini, C., Ieri, F., Romani, A. & Mulinacci, N. (2007). Study of the phenolic composition of spanish and italian monocultivar extra virgin olive oils: Distribution of lignans, secoiridoidic, simple phenols and flavonoids. *Talanta,* 73, 726-732.

ONZ. (2006). *New Zealand olives and olive oil: Olives New Zealand*. Accessed May 02, 2010, from:http://www.olivesnz.org.nz/:

Oueslati, I., Manai, H., Haddada, F.M., Daoud, D., Sanchez, J., Osorio, E. & Zarrouk, M. (2009). Sterol, triterpenic dialcohol, and triacylglycerol compounds of extra virgin olive oils from some Tunisian varieties grown in the region of tataouine. *Food Science and Technology International,* 15, 5-13.

Owen, R.W., Haubner, R., Mier, W., Giacosa, A., Hull, W.E., Spiegelhalder, B. & Bartsch, H. (2003). Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food and Chemical Toxicology,* 41, 703-717.

Owen, R.W., Mier, W., Giacosa, A., Hull, W.E., Spiegelhalder, B. & Bartsch, H. (2000a). Identification of lignans as major components in the phenolic fraction of olive oil. *Clinical Chemistry,* 46, 976-988.

Owen, R.W., Mier, W., Giacosa, A., Hull, W.E., Spiegelhalder, B. & Bartsch, H. (2000b). Phenolic compounds and squalene in olive oils: The concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignansand squalene. *Food and Chemical Toxicology,* 38, 647-659.

Paganuzzi, V. & Leoni, E. (1979). On the composition of Iranian olive oil. *Journal of the American Oil Chemists' Society,* 56, 925-930.

Paiva-Martins, F. & Pinto, M. (2008). Isolation and characterization of a new hydroxytyrosol derivative from olive (Olea europaea) leaves. *Journal of Agricultural and Food Chemistry,* 56, 5582-5588.

Pajuelo, B. (2004). The system of the International Olive Oil Council. *Accreditation and Quality Assurance,* 9, 576-577.

Pancorbo, A.C., Cruces-Blanco, C., Carretero, A.S. & GutieìRrez, A.F. (2004). Sensitive determination of phenolic acids in extra-virgin olive oil by capillary zone electrophoresis. *Journal of Agricultural and Food Chemistry,* 52, 6687-6693.

Papadopoulos, G. & Boskou, D. (1991). Antioxidant effect of natural phenols on olive oil. *Journal of the American Oil Chemists Society,* 68, 669-671.

Parenti, A., Spugnoli, P., Masella, P. & Calamai, L. (2008). The effect of malaxation temperature on the virgin olive oil phenolic profile under laboratory-scale conditions. *European Journal of Lipid Science and Technology,* 110, 735-741.

Pauwels, E.K.J. & Covas, M.I. (2009). The mediterranean diet, part I: The anticancer effect of olive oil. *Drugs of the Future,* 34, 307-313.

Peng, Y. & Lu, R. (2007). Prediction of apple fruit firmness and soluble solids content using characteristics of multispectral scattering images. *Journal of Food Engineering,* 82, 142-152.

Pérez-Jiménez, F., Lista, J.D., Pérez-Martínez, P., López-Segura, F., Fuentes, F., Cortés, B., Lozano, A. & López-Miranda, J. (2006). Olive oil and haemostasis: a review on its healthy effects. *Public health nutrition,* 9, 1083-1088.

Petrakis, C. (2006). Olive oil extraction. In Boskou, D. (Ed.), *Olive oil chemistry and technology*, (pp. 191-218). AOCS Press Champaign, IL USA.

Pike, O.A. (1998). Fat characterisation. In Nielsen, S.S. (Ed.), *Food analysis*, (pp. 217-226). Aspen Publishers, Inc., Gaithersburg, Maryland.

Pirisi, F.M., Cabras, P., Cao, C.F., Migliorini, M. & Muggelli, M. (2000). Phenolic compounds in virgin olive oil. 2. Reappraisal of the extraction, HPLC separation, and quantification procedures. *Journal of Agricultural and Food Chemistry,* 48, 1191-1196.

Poerio, A., Bendini, A., Cerretani, L., Bonoli-Carbognin, M. & Lercker, G. (2008). Effect of olive fruit freezing on oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology,* 110, 368-372.

Pokorn´Y, J. (2005). *Volumetric Analysis of Oxidized Lipids*.

, J. (2005). *Volumetric analysis of oxidized lipids*.

Prescott, J. (1999). Flavour as a psychological construct: Implications for perceiving and measuring the sensory qualities of foods. *Food Quality and Preference,* 10, 349-356.

Psomiadou, E., Karakostas, K.X., Blekas, G., Tsimidou, M.Z. & Boskou, D. (2003). Proposed parameters for monitoring quality of virgin olive oil (Koroneiki cv). *European Journal of Lipid Science and Technology,* 105, 403-408.

Psomiadou, E. & Tsimidou, M. (2002). Stability of virgin olive oil. 2. Photo-oxidation studies. *Journal of Agricultural and Food Chemistry,* 50, 722-727.

Psomiadou, E., Tsimidou, M. & Boskou, D. (2000). Alphatocopherol content of Greek virgin olive oils. *Journal of Agricultural and Food Chemistry,* 48, 1770-1775.

Ranalli, A., De Mattia, G. & Ferrante, M.L. (1997). Comparative evaluation of the olive oil given by a new processing system. *International Journal of Food Science and Technology,* 32, 289-297.

Ranalli, A., De Mattia, G., Patumi, M. & Proietti, P. (1999). Quality of virgin olive oil as influenced by origin area. *Grasas y Aceites,* 50, 249-259.

Ranalli, A., Malfatti, A., Lucera, L., Contento, S. & Sotiriou, E. (2005). Effects of processing techniques on the natural colourings and the other functional constituents in virgin olive oil. *Food Research International,* 38, 873-878.

Ranalli, A., Modesti, G., Patumi, M. & Fontanazza, G. (2000). The compositional quality and sensory properties of virgin olive oil from a new olive cultivar - I-77. *Food Chemistry,* 69, 37-46.

Ranalli, A., Pollastri, L., Contento, S., Iannucci, E. & Lucera, L. (2003). Effect of olive paste kneading process time on the overall quality of virgin olive oil. *European Journal of Lipid Science and Technology,* 105, 57-67.

Ravetti, L. (2009). Influence of agronomic aspects Paper presented at the *Olive oil processing course olives New Zealand*, The Olive Place: Alfa Laval New Zealand (3-5April).

Requejo-Jackman, C., Farrel, M., Olsson, S., Beresford, M., Haker, R., Wohlers, M., Mcghie, T., Cimeskey, D., Woolf, A., Wang, Y. & Wong, M. (2009). *Effect of harvest maturity on extra virgin olive oil Year 1– SFF project 07/103*). Auckland, New Zealand: The New Zealand Institute for Plant and Food Research Ltd.

Requejo-Tapia, L.C. (1999). *International trends in fresh avocado and avocado oil production and seasonal variation of fatty acids in New Zealand-grown cv. Hass.* Masters thesis, Agribusiness Massey University, Palmerston North.

Ribarova, F., Zanev, R., Shishkov, S. & Rizov, N. (2003). alpha-tocopherol, fatty acids and their correlations in Bulgarian foodstuffs. *Journal of Food Composition and Analysis,* 16, 659-667.

Ripa, V., De Rose, F., Caravita, M.A., Parise, M.R., Perri, E., Rosati, A., Pandolfi, S., Paoletti, A., Pannelli, G., Padula, G., Giordani, E., Bellini, E., Buccoliero, A. & Mennone, C. (2008). Qualitative evaluation of olive oils from new olive selections and effects of genotype and environment on oil quality. *Advances in Horticultural Science,* 22, 95-103.

Robards, K. & Antolovich, M. (1997). Analytical chemistry of fruit bioflavonoids. A review. *Analyst,* 122, 11R-34R.

Romani, A., Lapucci, C., Cantini, C., Ieri, F., Mulinacci, N. & Visioli, F. (2007). Evolution of minor polar compounds and antioxidant capacity during storage of bottled extra virgin olive oil. *Journal of Agricultural and Food Chemistry,* 55, 1315-1320.

Romani, A., Mulinacci, N., Pinelli, P., Vincieri, F.F. & Cimato, A. (1999). Polyphenolic content in five tuscany cultivars of Olea europaea L. *Journal of Agricultural and Food Chemistry,* 47, 964-967.

Romani, A., Pinelli, P., Mulinacci, N., Galardi, C., Vincieri, F.F., Liberatore, L. & Cichelli, A. (2001). HPLC and HRGC analyses of polyphenols and secoiridoid in olive oil. *Chromatographia,* 53, 279-284.

Romero, C., Brenes, M., Garcia, P., Garcia, A. & Garrido, A. (2004a). Polyphenol Changes during Fermentation of Naturally Black Olives. *Journal of Agricultural and Food Chemistry,* 52, 1973-1979.

Romero, C., Brenes, M., Garcia, P. & Garrido, A. (2002a). Hydroxytyrosol 4-β-D-glucoside, an important phenolic compound in olive fruits and derived products. *Journal of Agricultural and Food Chemistry,* 50, 3835-3839.

Romero, C., Brenes, M., Yousfi, K., Garcia, P., Garcia, A. & Garrido, A. (2004b). Effect of cultivar and processing method on the contents of polyphenols in table olives. *Journal of Agricultural and Food Chemistry,* 52, 479-484.

Romero, C., Garcia, P., Brenes, M., Garcia, A. & Garrido, A. (2002b). Phenolic compounds in natural black Spanish olive varieties. *European Food Research and Technology,* 215, 489-496.

Romero, M.P., Tovar, M.J., Girona, J. & Motilva, M.J. (2002c). Changes in the HPLC phenolic profile of virgin olive oil from young trees (Olea europaea L. Cv. Arbequina) grown under different deficit irrigation strategies. *Journal of Agricultural and Food Chemistry,* 50, 5349-5354.

Romero, M.P., Tovar, M.J., Ramo, T. & Motilva, M.J. (2003). Effect of crop season on the composition of virgin olive oil with protected designation of origin "Les Garrigues". *JAOCS, Journal of the American Oil Chemists' Society,* 80, 423-430.

Ros, E. (2008). Mediterranean diet and cardiovascular disease. *Dieta mediterrÃ¡nea y enfermedad cardiovascular,* 25, 9-15.

Rotondi, A., Bendini, A., Cerretani, L., Mari, M., Lercker, G. & Toschi, T.G. (2004). Effect of Olive Ripening Degree on the Oxidative Stability and Organoleptic

Properties of Cv. Nostrana di Brisighella Extra Virgin Olive Oil. *Journal of Agricultural and Food Chemistry,* 52, 3649-3654.

Rotondi, A., Fabbri, A. & Ganino, T. (2008). Sensory and chemical properties of extra virgin olive oils produced in two different Italian regions: Tuscany and Emilia-Romagna. *Journal of Food, Agriculture and Environment,* 6, 71-77.

Ruiz-Canela, M. & Martínez-González, M.A. (2011). Olive oil in the primary prevention of cardiovascular disease. *Maturitas,* 68, 245-250.

Ryan, D., Antolovich, M., Herlt, T., Prenzler, P.D., Lavee, S. & Robards, K. (2002a). Identification of phenolic compounds in tissues of the novel olive cultivar Hardy's Mammoth. *Journal of Agricultural and Food Chemistry,* 50, 6716-6724.

Ryan, D., Antolovich, M., Prenzler, P., Robards, K. & Lavee, S. (2002b). Biotransformations of phenolic compounds in Olea europaea L. *Scientia Horticulturae,* 92, 147-176.

Ryan, D., Prenzler, P.D., Lavee, S., Antolovich, M. & Robards, K. (2003). Quantitative changes in phenolic content during physiological development of the olive (Olea europaea) cultivar Hardy's Mammoth. *Journal of Agricultural and Food Chemistry,* 51, 2532-2538.

Ryan, D. & Robards, K. (1998). Phenolic compounds in olives- critical review. *Analyst,* 123, 31-44.

Ryan, D., Robards, K. & Lavee, S. (1999). Changes in phenolic content of olive during maturation. *International Journal of Food Science and Technology,* 34, 265-274.

Sakouhi, F., Harrabi, S., Absalon, C., Sbei, K., Boukhchina, S. & Kallel, H. (2008). α-tocopherol and fatty acids contents of some Tunisian table olives (Olea europea L.): Changes in their composition during ripening and processing. *Food Chemistry,* 108, 833-839.

Salvador, M.D., Aranda, F. & Fregapane, G. (1998). Chemical composition of commercial cornicabra virgin olive oil from 1995/96 and 1996/97 crops. *JAOCS, Journal of the American Oil Chemists' Society,* 75, 1305-1311.

Salvador, M.D., Aranda, F. & Fregapane, G. (2001a). Influence of fruit ripening on 'Cornicabra' virgin olive oil quality: A study of four successive crop seasons. *Food Chemistry,* 73, 45-53.

Salvador, M.D., Aranda, F., Gómez-Alonso, S. & Fregapane, G. (2001b). Cornicabra virgin olive oil: A study of five crop seasons. Composition, quality and oxidative stability. *Food Chemistry,* 74, 267-274.

Salvador, M.D., Aranda, F., Gómez-Alonso, S. & Fregapane, G. (2003). Influence of extraction system, production year and area on Cornicabra virgin olive oil: A study of five crop seasons. *Food Chemistry,* 80, 359-366.

Schijlen, E.G.W.M., Ric De Vos, C.H., Van Tunen, A.J. & Bovy, A.G. (2004). Modification of flavonoid biosynthesis in crop plants. *Phytochemistry,* 65, 2631-2648.

Sciancalepore, V. & Longone, V. (1984). Polyphenol oxidase activity and browning in green olives. *Journal of Agricultural and Food Chemistry,* 32, 320-321.

Selvaggini, R., Servili, M., Urbani, S., Esposto, S., Taticchi, A. & Montedoro, G. (2006). Evaluation of phenolic compounds in virgin olive oil by direct injection in high-performance liquid chromatography with fluorometric detection. *Journal of Agricultural and Food Chemistry,* 54, 2832-2838.

Servili, M., Baldioli, M., Selvaggini, R., Macchioni, A. & Montedoro, G. (1999a). Phenolic compounds of olive fruit: One- and two-dimensional nuclear magnetic resonance characterization of nuzhenide and its distribution in the constitutive parts of fruit. *Journal of Agricultural and Food Chemistry,* 47, 12-18.

Servili, M., Baldioli, M., Selvaggini, R., Miniati, E., Macchioni, A. & Montedoro, G. (1999b). High-performance liquid chromatography evaluation of phenols in olive fruit, virgin olive oil, vegetation waters, and pomace and 1D- and 2D-nuclear magnetic resonance characterization. *JAOCS, Journal of the American Oil Chemists' Society,* 76, 873-882.

Servili, M., Esposto, S., Fabiani, R., Urbani, S., Taticchi, A., Mariucci, F., Selvaggini, R. & Montedoro, G.F. (2009). Phenolic compounds in olive oil: Antioxidant, health and organoleptic activities according to their chemical structure. *Inflammopharmacology,* 17, 76-84.

Servili, M., Esposto, S., Lodolini, E., Selvaggini, R., Taticchi, A., Urbani, S., Montedoro, G., Serravalle, M. & Gucci, R. (2007). Irrigation effects on quality, phenolic composition, and selected volatiles of virgin olive oils cv. leccino. *Journal of Agricultural and Food Chemistry,* 55, 6609-6618.

Servili, M. & Montedoro, G. (2002). Contribution of phenolic compounds to virgin olive oil quality. *European Journal of Lipid Science and Technology,* 104, 602-613.

Servili, M., Piacquadio, P., De Stefano, G., Taticchi, A. & Sciancalepore, V. (2002). Influence of a new crushing technique on the composition of the volatile compounds and related sensory quality of virgin olive oil. *European Journal of Lipid Science and Technology,* 104, 483-489.

Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G. & Morozzi, G. (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil. *Journal of Chromatography A,* 1054, 113-127.

Servili, M., Taticchi, A., Esposto, S., Urbani, S., Selvaggini, R. & Montedoro, G. (2008). Influence of the decrease in oxygen during malaxation of olive paste on the composition of volatiles and phenolic compounds in virgin olive oil. *Journal of Agricultural and Food Chemistry,* 56, 10048-10055.

Shahar, D.R. & Grotto, I. (2006). Mediterranean diet and longevity. *Current Nutrition and Food Science,* 2, 337-342.

Shahtahmasebi, S. (2003). A case report of possible health benefits of extra virgin olive oil. *TheScientificWorldJournal [electronic resource],* 3, 1265-1271.

Shibasaki, H. (2005). Influence of fruit ripening on chemical properties of "mission" variety olive oil in Japan. *Food Science and Technology Research,* 11, 9-12.

Silva, S., Gomes, L., Leitã£O, F., Coelho, A.V. & Boas, L.V. (2006). Phenolic compounds and antioxidant activity of Olea europaea L. Fruits and leaves. *Food Science and Technology International,* 12, 385-396.

Sinesio, F., Moneta, E. & Esti, M. (2005). The dynamic sensory evaluation of bitterness and pungency in virgin olive oil. *Food Quality and Preference,* 16, 557-564.

Singleton, V.L., Orthofer, R. & Lamuela-Raventós, R.M. (1998). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, *Methods in Enzymology* (Vol. 299, pp. 152-178).

Sivakumar, G., Briccoli Bati, C. & Uccella, N. (2005). HPLC-MS screening of the antioxidant profile of Italian olive cultivars. *Chemistry of Natural Compounds,* 41, 588-591.

Skevin, D., Rade, D., Strucelj, D., Mokrovcak, Z., Nederal, S. & Bencic, D. (2003). The influence of variety and harvest time on the bitterness and phenolic compounds of olive oil. *European Journal of Lipid Science and Technology,* 105, 536-541.

Slover, H.T., Thompson, R.H. & Merola, G.V. (1983). Determination of tocopherols and sterols by capillary gas chromatography. *Journal of the American Oil Chemists' Society,* 60, 1524-1528.

Soler-Rivas, C., Espiå„, J.C. & Wichers, H.J. (2000). Oleuropein and related compounds. *Journal of the Science of Food and Agriculture,* 80, 1013-1023.

Stefanoudaki, E., Kotsifaki, F. & Koutsaftakis, A. (2000). Sensory and chemical profiles of three European olive varieties (Olea europea L); an approach for the characterisation and authentication of the extracted oils. *Journal of the Science of Food and Agriculture,* 80, 381-389.

Taber, A. (2005). *A buyer's guide to New Zealand olive oil*, (Fredricsen, J., Ed. Auckland: Renaissance Publishing, Northcote, Auckland.

Tasioula-Margari, M. & Okogeri, O. (2001a). Isolation and characterization of virgin olive oil phenolic compounds by HPLC/UV and GC-MS. *Journal of Food Science,* 66, 530-534.

Tasioula-Margari, M. & Okogeri, O. (2001b). Simultaneous determination of phenolic compounds and tocopherols in virgin olive oil using HPLC and UV detection. *Food Chemistry,* 74, 377-383.

Therios, I. (2009a). Climatic and soil conditions. In Atherton, J. (Ed.), *Olives*, (pp. 51-80). Reading, UK: MPG Books Group.

Therios, I. (2009b). Hectareage, number of trees and production of olive oil and table olivs. In Atherton, J. (Ed.), *Olives*, (pp. 33-55). Reading, UK: MPG Books Group.

Therios, I. (2009c). Oleuropein, olive leaf extract, olive oil and the benefits of the Mediterranean diet to human health. In Atherton, J. (Ed.), *Olives*, (pp. 303-318). Reading, Uk: MPG Books Group.

Therios, I. (2009d). Olive oil. In Atherton, J. (Ed.), *Olives*, (pp. 279-294). Reading, Uk: MPG Books Group.

Therios, I. (2009e). Olive ripening. In Atherton, J. (Ed.), *Olives*, (pp. 229-244). Reading, UK: MPG Books Group.

Therios, I. (2009f). Structure and composition of the olive fruit. In Atherton, J. (Ed.), *Olives*, (pp. 24-32). Reading, UK: MPG Books Groups.

Tous, J., Romero, A., Plana, J., Guerrero, L., Diaz, I. & Hermoso, J.F. (1997). Chemical and sensory characteristics of Arbequina olive oil obtained in different growing areas of Spain. *Caracteristicas quimico-sensoriales de los aceites de oliva Arbequina obtenidos en distintas zonas de Espana,* 48, 415-424.

Tovar, M.J., Motilva, M.J. & Romero, M.P. (2001). Changes in the phenolic composition of virgin olive oil from young trees (Olea europaea L. cv. Arbequina) grown under linear irrigation strategies. *Journal of Agricultural and Food Chemistry,* 49, 5502-5508.

Tovar, M.J., Paz Romero, M., Girona, J. & Jos Motilva, M. (2002). L-phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (Olea europaea L. cv Arbequina) fruit grown under different irrigation regimes. *Journal of the Science of Food and Agriculture,* 82, 892-898.

Tripoli, E., Giammanco, M., Tabacchi, G., Di Majo, D., Giammanco, S. & La Guardia, M. (2005). The phenolic compounds of olive oil: Structure, biological activity and beneficial effects on human health. *Nutrition Research Reviews,* 18, 98-112.

Tsimidou, M. (1998). Polyphenols and quality of virgin olive oil in retrospect. *Italian Journal of Food Science,* 10, 99-116.

Tsimidou, M., Lylridou, M., Boskou, D., Pappa-Louisi, A., Kotsifaki, F. & Petrakis, C. (1996). On the determination of minor phenolic acids of virgin olive oil by RP-HPLC. *Grasas y Aceites,* 47, 151-157.

Tsimidou, M., Papadopoulos, G. & Boskou, D. (1992). Determination of phenolic compounds in virgin olive oil by reversed-phase HPLC with emphasis on UV detection. *Food Chemistry,* 44, 53-60.

Tsimidou, M.Z. (2006). Olive oil quality. In Boskou, D. (Ed.), *Olive oil chemistry and technology*, Vol. 2, (pp. 93-105). AOCS Press Champaign, IL USA.

Tura, D., Failla, O., Bassi, D., Pedã², S. & Serraiocco, A. (2009). Environmental and seasonal influence on virgin olive (Olea europaea L.) oil volatiles in northern Italy. *Scientia Horticulturae,* 122, 385-392.

Valero, C., Crisosto, C.H. & Slaughter, D. (2007). Relationship between nondestructive firmness measurements and commercially important ripening fruit stages for peaches, nectarines and plums. *Postharvest Biology and Technology,* 44, 248-253.

Van Der Klaauw, N.J. & Frank, R.A. (1996). Scaling component intensities of complex stimuli: The influence of response alternatives. *Environment International,* 22, 21-31.

Veillet, S., Tomao, V., Bornard, I., Ruiz, K. & Chemat, F. (2009). Chemical changes in virgin olive oils as a function of crushing systems: Stone mill and hammer crusher. *Comptes Rendus Chimie.*

Velasco, J. & Dobarganes, C. (2002). Oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology,* 104, 661-676.

Vinha, A.F., Ferreres, F., Silva, B.M., Valentão, P., Gonçalves, A., Pereira, J.A., Oliveira, M.B., Seabra, R.M. & Andrade, P.B. (2005). Phenolic profiles of Portuguese olive fruits (Olea europaea L.): Influences of cultivar and geographical origin. *Food Chemistry,* 89, 561-568.

Vinson, J.A., Zubik, L., Bose, P., Samman, N. & Proch, J. (2005). Dried fruits: Excellent in vitro and in vivo antioxidants. *Journal of the American College of Nutrition,* 24, 44-50.

Visioli, F. & Galli, C. (1998). Olive Oil Phenols and Their Potential Effects on Human Health. *Journal of Agricultural and Food Chemistry,* 46, 4292-4296.

Welsh, F.W. & Williams, R.E. (1989). The use of vegetable oils to recover compounds from aqueous solutions. *Journal of Chemical Technology and Biotechnology,* 46, 169-178.

Wesley, I.J., Barnes, R.J. & Mcgill, A.E.J. (1995). Measurement of adulteration of olive oils by near-infrared spectroscopy. *Journal of the American Oil Chemists' Society,* 72, 289-292.

Wesley, I.J., Pacheco, F. & Mcgill, A.E.J. (1996). Identification of adulterants in olive oils. *JAOCS, Journal of the American Oil Chemists' Society,* 73, 515-518.

Wong, M., Farrel, M., Olsson, S., Beresford, M., Beresford, M., Harker, F.R., Wang, Y., Wohlers, M., Mcghie, T., Woolf, A. & Requejo-Jackman, C. (2009). *Effect of olive maturity at harvest on quality of extra virgin olive oil in New Zealand*. Paper presented at the World Congress on Oils and Fats & 28th ISF Congress.

Woyengo, T.A., Ramprasath, V.R. & Jones, P.J.H. (2009). Anticancer effects of phytosterols. *European Journal of Clinical Nutrition,* 63, 813-820.

Yousfi, K., Cayuela, J.A. & García, J.M. (2008). Reduction of virgin olive oil bitterness by fruit cold storage. *Journal of Agricultural and Food Chemistry,* 56, 10085-10091.

Yousfi, K., Cert, R.M. & García, J.M. (2006). Changes in quality and phenolic compounds of virgin olive oils during objectively described fruit maturation. *European Food Research and Technology,* 223, 117-124.

Youssef, N.B., Zarrouk, W., Carrasco-Pancorbo, A., Ouni, Y., Segura-Carretero, A., Fernández-Gutiérrez, A., Daoud, D. & Zarrouk, M. (2010). Effect of olive ripeness on chemical properties and phenolic composition of Chétoui virgin olive oil. *Journal of the Science of Food and Agriculture,* 90, 199-204.

Zarrouk, W., Haddada, F.M., Baccouri, B., Oueslati, I., Taamalli, W., Fernandez, X., Lizzani-Cuvelier, L., Daoud, D. & Zarrouk, M. (2008). Characterization of virgin olive oil from Southern Tunisia. *European Journal of Lipid Science and Technology,* 110, 81-88.

Chapter Eight: Appendices

Appendix 1: International Olive Council (IOC) profile sheet for rating VOO positive attributes and defects in olive oil.

PERCEPTION OF DEFECTS

Fusty/ I ⇨
Muddy Sediment
Musty I ⇨

Winey/Vinegar I ⇨
Acid-Sour
Rancid I ⇨

Metallic I ⇨

Other (Specify)I ⇨

PERCEPTION OF POSITIVE ATTRIBUTES

Fruity I ⇨

Greenly ☐ Ripley ☐
Bitter I ⇨

Pungent I ⇨

Name of Taster Sample Code Date
Comments

Appendix 2: Fatty acid composition (%) evaluated in ‘Frantoio’ olive oil samples from Hawke’s Bay, Bombay and Waiheke. The oils were extracted by solvent at different maturities. Values are means of duplicate GC injections (±standard error of the mean).

Orchard/full bloom date	C16:0 (±SEM)	C16:1 (±SEM)	C17:0 (±SEM)	C17:1 (±SEM)	C18:0 (±SEM)	C18:1 (±SEM)	C18:2 (±SEM)	C18:3 (±SEM)	C20:0 (±SEM)	C20:1 (±SEM)	C22:0 (±SEM)
Hawke’s Bay											
139	9.90(0.28)	0.46(0.27)	0.02(0.00)	0.07(0.00)	1.06(0.01)	79.74(0.11)	7.36(0.12)	0.90(0.01)	0.19(0.01)	0.25(0.01)	0.06(0.01)
151	9.76(0.00)	0.15(0.01)	0.01(0.00)	0.06(0.00)	1.08(0.02)	79.34(0.04)	8.00(0.04)	1.07(0.01)	0.21(0.00)	0.26(0.00)	0.06(0.01)
164	9.54(0.01)	0.16(0.00)	0.01(0.00)	0.07(0.00)	0.88(0.00)	79.75(0.02)	8.71(0.04)	0.47(0.00)	0.10(0.07)	0.22(0.00)	0.08(0.01)
172	9.76(0.01)	0.21(0.00)	0.01(0.00)	0.07(0.00)	0.99(0.01)	78.94(0.02)	9.06(0.00)	0.47(0.00)	0.17(0.00)	0.21(0.00)	0.06(0.01)
181	9.46(0.02)	0.17(0.00)	0.02(0.00)	0.07(0.00)	0.91(0.01)	79.63(0.01)	8.79(0.02)	0.52(0.00)	0.17(0.00)	0.24(0.00)	0.05(0.01)
193	10.03(0.00)	0.11(0.00)	0.01(0.00)	0.06(0.00)	1.16(0.01)	80.11(0.04)	7.77(0.01)	0.36(0.01)	0.18(0.00)	0.21(0.00)	0.05(0.01)
201	8.72(0.00)	0.09(0.01)	0.01(0.00)	0.06(0.00)	1.13(0.02)	80.52(0.03)	8.58(0.03)	0.46(0.00)	0.18(0.01)	0.20(0.00)	0.06(0.01)
Bombay											
105	9.87(0.01)	0.10(0.00)	0.02(0.00)	0.09(0.00)	0.92(0.01)	80.03(0.03)	7.14(0.00)	1.32(0.00)	0.18(0.00)	0.26(0.00)	0.08(0.00)
119	9.19(0.00)	0.08(0.00)	0.02(0.00)	0.08(0.00)	1.00(0.02)	80.73(0.05)	7.25(0.03)	1.19(0.00)	0.14(0.06)	0.25(0.00)	0.07(0.01)
133	9.10(0.01)	0.08(0.01)	0.02(0.00)	0.08(0.00)	0.98(0.00)	80.43(0.00)	7.80(0.01)	1.02(0.00)	0.20(0.00)	0.24(0.00)	0.07(0.02)
147	8.38(0.01)	0.03(0.00)	0.02(0.00)	0.08(0.00)	0.96(0.01)	81.77(0.02)	7.30(0.01)	1.03(0.00)	0.15(0.04)	0.25(0.00)	0.03(0.02)
161	8.37(0.00)	0.03(0.00)	0.02(0.00)	0.07(0.00)	0.95(0.00)	81.12(0.02)	7.90(0.01)	1.07(0.00)	0.15(0.03)	0.27(0.00)	0.06(0.01)
171	8.33(0.02)	0.03(0.00)	0.02(0.00)	0.07(0.00)	0.56(0.37)	81.63(0.37)	7.74(0.01)	1.06(0.00)	0.20(0.00)	0.27(0.00)	0.09(0.02)
190	8.20(0.01)	0.02(0.00)	0.02(0.00)	0.07(0.00)	0.89(0.00)	81.06(0.02)	8.16(0.00)	1.09(0.00)	0.14(0.01)	0.28(0.00)	0.07(0.01)
204	8.19(0.01)	0.02(0.00)	0.02(0.00)	0.07(0.00)	0.89(0.01)	80.98(0.02)	8.25(0.04)	1.09(0.01)	0.16(0.01)	0.27(0.00)	0.06(0.04)
Waiheke											
162	10.74(0.25)	0.47(0.28)	0.01(0.00)	0.05(0.00)	1.72(0.00)	77.41(0.08)	8.65(0.08)	0.48(0.00)	0.24(0.01)	0.18(0.00)	0.04(0.01)
176	10.77(0.01)	0.18(0.00)	0.00(0.00)	0.04(0.00)	1.78(0.00)	77.20(0.05)	9.11(0.04)	0.45(0.00)	0.24(0.00)	0.18(0.00)	0.04(0.00)
190	10.26(0.04)	0.18(0.00)	0.01(0.00)	0.04(0.00)	1.80(0.03)	77.42(0.01)	9.42(0.07)	0.43(0.00)	0.22(0.01)	0.18(0.00)	0.05(0.01)
204	9.90(0.01)	0.14(0.01)	0.00(0.00)	0.05(0.00)	1.73(0.01)	77.86(0.05)	9.43(0.04)	0.42(0.00)	0.22(0.00)	0.18(0.00)	0.06(0.01)
215	9.17(0.01)	0.11(0.00)	0.01(0.00)	0.06(0.00)	0.14(0.01)	81.18(0.01)	7.95(0.01)	0.94(0.00)	0.19(0.00)	0.21(0.00)	0.04(0.00)

Appendix 3: Fatty acid composition (mg/g) evaluated in ‘Frantoio’ olive oil samples from Hawke’s Bay, Bombay and Waiheke. The oils were extracted by solvent at different maturity stages. Values are means of duplicate GC injections (±standard error of the mean).

Orchard/full bloom date	C16:0 (±SEM)	C16:1 (±SEM)	C17:0 (±SEM)	C17:1 (±SEM)	C18:0 (±SEM)	C18:1 (±SEM)	C18:2 (±SEM)	C18:3 (±SEM)	C20:0 (±SEM)	C20:1 (±SEM)	C22:0 (±SEM)
Hawke’s Bay											
139	376.72(16.5)	18.36(8.95)	< 0.01	1.63(0.01)	38.67(0.19)	2742.62(37.7)	284.15(8.84)	37.64(1.02)	5.93(0.22)	9.16(0.04)	1.28(0.21)
151	325.77(1.20)	7.30(0.14)	< 0.01	1.20(0.01)	34.02(0.08)	2395.90(8.26)	270.95(2.29)	39.24(0.55)	5.48(0.01)	8.75(0.10)	1.08(0.40)
164	404.06(2.59)	8.74(0.11)	< 0.01	2.01(0.06)	35.24(0.17)	3053.26(17.66)	374.36(3.91)	21.23(0.09)	3.23(2.63)	9.31(0.11)	2.23(0.28)
172	351.16(0.96)	9.56(0.13)	< 0.01	1.43(0.04)	33.58(0.44)	2571.80(5.56)	331.33(0.67)	17.67(0.03)	4.92(0.08)	8.00(0.08)	1.10(0.02)
181	350.91(2.41)	8.42(0.07)	< 0.01	1.60(0.03)	31.93(0.23)	2673.27(11.72)	331.04(2.22)	20.32(0.12)	4.98(0.11)	9.19(0.00)	1.04(0.19)
193	239.63(0.48)	5.34(0.07)	< 0.01	0.52(0.06)	25.57(0.23)	1733.34(5.1)	188.06(0.15)	7.79(0.18)	3.16(0.07)	5.96(0.01)	1.18(0.01)
201	363.50(0.32)	6.26(0.17)	< 0.01	1.36(0.05)	44.99(0.51)	3036.67(17.25)	363.11(3.43)	20.06(0.10)	5.98(0.29)	8.77(0.02)	1.50(0.54)
Bombay											
105	428.67(1.00)	6.82(0.06)	0.05(0.01)	2.60(0.07)	37.97(0.57)	3144.15(2.24)	314.67(0.50)	63.99(0.06)	6.38(10.93)	10.93(0.03)	2.18(0.05)
119	378.01(2.35)	5.96(0.11)	< 0.01	2.25(0.05)	39.11(0.89)	3002.93(20.04)	302.40(0.74)	54.09(0.29)	4.47(2.10)	10.13(0.05)	1.74(0.47)
133	374.71(1.11)	5.85(0.55)	< 0.01	2.01(0.03)	38.32(0.14)	2996.45(4.78)	326.07(1.02)	46.51(0.05)	6.50(0.06)	9.72(0.05)	1.94(0.72)
147	329.09(0.66)	3.86(0.02)	0.07(0.02)	2.04(0.04)	35.71(0.28)	2904.06(8.88)	291.03(1.18)	44.73(0.08)	4.61(1.29)	9.60(0.05)	0.46(0.80)
161	333.02(0.89)	3.80(0.14)	0.01(0.02)	1.88(0.00)	35.96(0.01)	2921.40(7.10)	319.53(1.08)	47.00(0.03)	4.67(0.99)	10.50(0.01)	1.42(0.21)
171	417.07(1.96)	4.03(0.13)	0.04(0.03)	2.59(0.05)	25.90(18.24)	3696.62(26.86)	393.50(0.55)	59.10(0.45)	8.51(0.13)	12.76(0.03)	3.12(0.83)
190	388.31(2.67)	3.89(0.06)	0.06(0.02)	2.23(0.00)	40.21(0.50)	3472.81(28.87)	392.43(3.10)	57.10(0.62)	5.05(0.28)	12.57(0.09)	2.37(0.44)
204	328.83(2.80)	3.32(0.12)	< 0.01	1.84(0.05)	33.98(0.18)	2941.13(22.56)	336.48(3.94)	48.48(0.05)	5.01(0.15)	10.53(0.05)	1.32(1.35)
Waiheke											
162	360.72(0.61)	17.27(9.02)	< 0.01	0.71(0.03)	55.94(1.34)	2352.42(57.04)	295.13(10.23)	16.54(0.34)	6.56(0.09)	6.78(0.04)	0.57(0.32)
176	378.41(4.18)	8.39(0.07)	< 0.01	0.66(0.04)	60.51(0.75)	2451.82(22.85)	325.00(4.83)	16.23(0.10)	6.89(6.83)	6.83(0.04)	0.63(0.06)
190	350.02(3.60)	8.09(0.08)	< 0.01	0.57(0.01)	59.39(0.11)	2388.19(32.45)	326.39(6.79)	15.06(0.19)	5.92(6.62)	6.62(0.04)	0.68(0.34)
204	454.63(4.10)	9.00(0.17)	< 0.01	1.05(0.02)	77.41(1.29)	3232.79(22.17)	439.96(5.08)	20.28(0.07)	8.21(8.59)	8.59(0.04)	1.80(0.37)
215	419.59(3.07)	7.18(0.01)	< 0.01	1.68(0.04)	4.61(0.62)	3360.11(27.59)	369.61(2.52)	47.67(0.03)	6.97(0.06)	9.40(0.04)	1.01(0.01)

Appendix 4: The annual climatic data obtained from nearest weather stations to the orchards studied.

Orchard	Month 2010	Total Rainfall (mm)	Max* air Temp. ^{o}C	Min* air Temp. ^{o}C	Total GDD*	Relative Humidity*
Hawke's Bay	Jan	331	31.4	4.7	234.3	78.3
	Feb	103	29.7	6.3	225.1	87.8
	Mar	18	28.8	3.2	183.9	76.4
	Apr	33	24.8	-1.8	2.7	80.8
	May	244	21.4	-3.3	0	88.6
	Jun	179	19.1	-3.8	0	87.8
	Jul	124	16.9	-3.4	0	89.5
	Aug	106	14.8	-2.8	0	87.5
	Sep	106	24.2	-0.7	64.5	75.8
	Oct	166	23.5	0.2	61.1	80.6
	Nov	28	29.6	2.1	140.2	80.4
	Dec	32	33.4	3.9	256.5	78
	Annual	**1470**	**297.6**	**4.6**	**1168.3**	**82.6**
Bombay	Jan	92.2	22.5	13	239.7	78.2
	Feb	11.6	24	14.9	264.9	83.4
	Mar	13.6	21.9	12.3	221.2	79.8
	Apr	51	20	10.8	162.4	80.9
	May	128	16.6	8.8	85.7	88.2
	Jun	230	14.3	6.9	30.8	92.6
	Jul	103.2	13.5	4.6	7.9	91
	Aug	213.6	14.2	7.2	33.1	92.9
	Sep	149.6	15.3	8.4	59.4	84.8
	Oct	41.2	17.4	8.1	85.8	81.1
	Nov	38.4	20.1	10.9	164.8	78.6
	Dec	91	22.7	14.5	267.7	80.8
	Annual	**1163.4**	**18.6**	**10**	**1623.1**	**84.4**
Waiheke	Jan	37.6	24.3	13.7	278.5	85.6
	Feb	14	26.3	16	313.3	83.8
	Mar	15.5	24	13.8	274.7	83
	Apr	21.5	21.7	11.1	193	84.1
	May	226	18.5	10.2	134	86
	Jun	200	16.2	8.3	69.5	86.3
	Jul	76.9	15.1	5.7	30.6	82.3
	Aug	218.5	16	8.3	71.9	91.1
	Sep	125.5	17	9	90.5	84.3
	Oct	37	18.3	8.2	100.9	83.4
	Nov	36	21	10.6	173.9	83.6
	Dec	122.5	23.4	15.4	293.3	85.7
	Annual	**1131**	**20.1**	**10.8**	**2023.9**	**84.9**

Source: The National Climatic Data Base-NIWA. *Mean daily maximum and minimum air temperature; Mean of 9 am relative humidity; Total Growing Degree Days (base-line temperature, 10°C). Data were obtained from stations nearest to the orchard: Waiheke orchard (NIWA Waiheke Island, Awaroa Valley), Bombay orchard (NIWA Pukekohe) and Hawke's Bay orchard (Pernod Ricard, http://waterwatch.net.nz).

Appendix 5: Individual tocopherols in ‘Frantoio’ olive oils obtained at different stages of maturity. The oils were extracted by solvent and cold press. The values reported are the means of duplicate HPLC injections (±standard error of the mean).

Orchard	DAFB	Tocopherol			
		Alpha α	Beta β	Gamma γ	Delta δ
Solvent extraction					
Hawke’s Bay	139	258.69(2.15)	2.998(0.10)	8.71(0.16)	ND
	151	210.90(4.14)	2.678(0.20)	5.23(0.04)	ND
	164	182.47(0.02)	2.294(0.19)	6.41(0.24)	ND
	172	171.59(0.62)	2.366(0.22)	6.34(0.06)	ND
	181	174.91(0.27)	2.116(0.12)	5.84(0.16)	ND
	193	166.25(3.89)	2.177(0.04)	5.00(0.24)	ND
	201*	168.84(0.29)	2.009(0.08)	5.22(0.19)	ND
	215**	131.49(1.38)	1.694(0.03)	3.49(0.12)	ND
Bombay	105	455.32(1.50)	4.292(0.16)	6.85(0.12)	ND
	119	310.26(1.86)	3.223(0.31)	5.96(0.01)	ND
	133	248.46(0.19)	1.735(0.09)	5.24(0.52)	ND
	147	223.49(0.35)	1.406(0.12)	4.41(0.10)	ND
	161	208.26(1.99)	1.231(0.50)	4.27(0.01)	ND
	171	187.39(2.49)	1.535(0.46)	4.19(0.27)	ND
	190*	175.59(0.44)	1.930(0.04)	3.02(0.09)	ND
	204**	175.92(2.09)	1.156(0.43)	3.37(0.11)	ND
Waiheke	162	185.44(0.34)	1.767(0.43)	5.15(0.14)	ND
	176	173.46(0.12)	1.125(0.22)	4.99(0.36)	ND
	190*	166.48(1.86)	0.893(0.31)	4.81(0.45)	ND
	204**	158.41(0.91)	1.019(0.21)	3.85(0.30)	ND
Cold press extraction					
Hawke’s Bay CH	201	146.22(0.75)	2.919(0.41)	6.91(0.29)	ND
Hawke’s Bay LH	215	77.09(2.97)	2.037(0.09)	2.07(0.39)	ND
Bombay CH	190	137.24(0.12)	1.041(0.01)	3.26(0.45)	ND
Bombay LH	204	126.04(2.23)	1.341(0.18)	3.49(0.07)	ND
Waiheke CH	190	156.57(2.56)	0.984(0.31)	5.15(0.68)	ND
Waiheke LH	204	145.79(0.36)	0.686(0.01)	3.13(0.22)	ND

DAFB days after full bloom; * commercial harvest (CH); ** late harvest (LH)

Appendix 6: Total phenolics, oleuropein bitter index (OBI), K225 and intensity of bitterness (IB) in 'Frantoio' olive oils obtained at different stages of maturity. The oils were extracted by solvent. The values reported are the means of duplicate HPLC injections (±standard error of the mean).

Sample	Total phenolics (±SEM) (mg/kg caffeic acid eq.)	OBI (±SEM) (mg/kg oleuropein eq.)	K_{225}	IB
1	620.21(22.04)	2872.06(2.65)	0.68	8.24
2	492.41(2.27)	2357.79(1.46)	0.56	6.68
3	122.09(0.72)	1144.02(2.69)	0.27	2.78
4	178.67(3.702)	1111.87(4.91)	0.26	2.68
5	514.81(24.52)	1735.79(2.30)	0.41	4.65
6	123.35(3.25)	995.36(2.58)	0.24	2.35
7	1220.62(9.33)	7377.15(7.95)	1.76	22.65
8	908.26(27.89)	4850.60(5.95)	1.17	14.70
9	818.39(18.74)	4516.75(9.13)	1.07	13.45
10	713.62(384)	4198.84(6.30)	0.99	12.37
11	496.56(14.29)	3178.16(4.90)	0.75	9.19
12	754.29(36.73)	3661.59(2.68)	0.44	5.03
13	814.39(9.52)	3560.45(7.45)	0.42	4.82
14	654.12(19.67)	3257.47(2.29)	0.78	9.50
15	455.52(29.03)	3124.03(2.22)	0.75	9.18
16	382.79(26.61)	2339.10(0.49)	0.56	6.58
17	426.67(5.51)	2360.09(0.91)	0.55	6.54
18	413.50(14.44)	2293.76(2.07)	0.54	6.41
19	1412.05(1.45)	4648.07(5.84)	1.09	13.63
20	817.62(1.02)	6696.39(2.12)	1.60	20.56
21	1008.33(42.80)	5197.96(11.47)	1.24	15.73
22	723.11(21.61)	3177.26(6.99)	0.76	9.26
23	535.10(26.34)	3173.96(1.99)	0.76	9.26
24	704.36(36.87)	3411.56(1.53)	0.81	9.98
25	667.99(19.45)	3906.45(2.78)	0.93	11.58
26	270.97(16.75)	2171.23(2.43)	0.51	5.99