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IMPACT OF GENETIC AND CULTURAL FACTORS ON EXTRA-VIRGIN OLIVE OIL COMPOSITION

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Introduction

1. Importance of olive industry in the world

The olive tree (*Olea europea L.*) has great cultural and economic importance in the world, especially in the countries located in the Mediterranean basin (Loumou and Giourga 2003). Over time, many olive cultivars have been selected and developed by the humankind. Three of the most commonly used cultivars for oil production in the world are 'Koroneiki', from Greece; 'Frantoio', from Italy; and 'Picual' from Spain. Olive fruits belonging to different cultivars can be different in size, pigments content, oil content and minor compounds composition. Since the olive tree started to be cultivated, more than 1500 olive cultivars were developed and dispersed throughout the Mediterranean area first, and then even further into the American continent, Oceania, Central and Southern Africa and Asia (Kiritsakis and Shahidi 2017). Olives and olive oil production are culturally, socially and economically relevant in the Mediterranean area, where Spain, Italy, Greece, Tunisia and Syria are the five most productive countries in the world (EC 2012). However, in the last decades, not-traditionally producer countries, such as Argentina, Australia and the US, reached a global cultivation area of more than 8.6 million ha (FAOSTAT 2015). Olive oil production has been increasing over the years (International Olive Council, IOC), 2018), although with small fluctuations that are due mostly to environmental factors.

World production of olive oil in the 2016/17 marketing year was 2 586 500 tonnes, with an expected production of 3 311 500 tonnes for the 2017/18 harvest season (IOC 2018). In particular, European producer countries account for 67.7% of the total oil production, with 1 751 500 tonnes of olive oil extracted in 2016/2017, and consume 51.3% of the overall production (IOC 2018). Olive oil consumption has been steadily increasing over the last decades. This trend has been linked to the positive health effects of virgin olive oil intake

and the rise of a new and more informed group of consumers who discovered the excellent gastronomic and nutritional properties of this product. A wide number of scientific studies have revealed the potential of olive oil in cardiovascular disease prevention, cancer risk reduction and delaying the evolution of degenerative diseases (Andreadou et al., 2006; Barbaro et al., 2014; Martín-Peláez et al., 2013). The constant increase of consumption have taken producer countries into the search for new planting and extraction techniques that allow an increase of production without increasing overall cost of production. The successful mechanization of olive trees cultivation, mostly in the form of high-density hedgerow olive groves, has shown to be a good possibility for a rapid expansion of olive cultivation in countries having large tracts of land but limited or costly workforce.

2. Products of the olive industry: olive oil and table olives

Virgin olive oils (VOO) are obtained from the fruit of the olive tree (Olea europaea L.) solely by mechanical means. The extraction process cannot include other operations than washing, malaxation, centrifugation and filtration. In particular, olive oil can be classified as Extra-virgin in accordance to some limits on the quality parameters (e.g. free acidity and peroxide value) described in Table 1.

Olive oil is primarily constituted by triacylglycerols and secondarily from free fatty acids, mono- and diacylglycerols. Minor components such as sterols, pigments, phenols and volatiles, constitute about 1–2% of VOO (Boskou, 2015; M Servili et al., 2004). VOO minor components are responsible for its unique flavour and nutritional properties.

In terms of fatty acids composition, the main compounds find in olive oil are palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids. Myristic (C14:0), heptadecanoic (C17:0) and eicosanoic (C20:0) acids are found in trace amounts. Fatty acid composition changes along fruit maturation, since oleic

acid is the first to be synthetized in the fruit. Moreover, there is a strong antagonistic relationship between oleic and palmitic, palmitoleic and linoleic acids (Ninni 1999).

Along maturity, olive fruit color changes from green to purple and become black in overripe olives. The green color is due to chlorophylls, the purple colour is due to anthocyanins and the black color is formed by the oxidation of phenolic compounds including oleuropein (Ryan et al. 2002). Chlorophylls can be divided in chlorophylls *a* and *b* and their derivatives (pheophytins *a* and *b*, and pheophorbides) (Gallardo-Guerrero et al. 2005). Chlorophylls are found in fresh produced oils. However, under light exposure green pigments degrade (Psomiadou and Tsimidou 2002), and photooxidation occurs (Kiritsakis and Shahidi 2017), leading to a change in oil color along time.

Phenolic compounds concentration of olive fruits can range between 1 and 3% of the fresh pulp weight. The polar phenol classes present in olives are phenolic alcohols such as hydroxytyrosol and tyrosol, phenolic acids, secoiridoids, flavonoids, lignans and anthocyanins. Secoiridoids are the most representative class, with oleuropein being present at levels up to 14% of the dry weight (Amiot et al. 1989). The extraction of the oil from the fruit is the moment when some compounds are formed or their concentrations are modified creating the differences in composition between fruit and oil extracted. Secoiridoids are the main phenolic compounds in VOO. These substances are aglycone derivatives of secoiridoids glucosides contained in the olive fruit, originated by the hydrolysis of oleuropein, demethyloleuropein and ligstroside during the extraction process. These reactions are catalysed by endogenous β-glucosidases (Montedoro et al. 2002). Phenolic alcohols include hydroxytyrosol (3,4-dihydroxyphenyl ethanol; 3,4-DHPEA) and tyrosol (p-hydroxyphenyl ethanol; p-HPEA); their concentration is generally low in fresh oils but increases during oil storage since the hydrolysis of oleupein and ligstroside aglicones that contain 3,4-DHPEA and p-HPEA in their molecular structures (Montedoro et al. 1992).

Phenolic acids in the oil are represented by caffeic, vanillic, syringic, p-coumaric, o-coumaric, ferrulic, p-hydroxybenzoic and gallic acid (Montedoro et al. 1992; Servili et al. 2004a; Boskou et al. 2006). Flavonoids include luteolin and apigenin (Dabbou et al. 2011b). The lignans include (+)-1-acetoxypinoresinol and (+)-1-pinoresinol (Brenes et al. 2000). These compounds migrate from fruit pulp and seed to oil during extraction. Olive phenols are recognized by their nutraceutical potential, having antioxidant, anti-inflammatory, cardiovascular, immune, gastrointestinal, endocrine and respiratory effects. In addition, they intervene in the central nervous system, and present antimicrobial, anticancer and chemo preventive properties (Obied et al., 2012). In particular, the most researched compound in VOO in the last years is oleocanthal. This compound was identified for the first time in 1999 (Servili et al. 1999) as the dialdehydic form of ligstroside aglycon (p-HPEA-EDA) and have a dose dependent anti-inflammatory capacity, similar to ibuprofen (Beauchamp et al. 2005). Besides, phenolic compounds in VOO include antioxidant activity important for healthy and organoleptic properties.

Organoleptic properties of VOO are largely affected by its phenolic composition. The contribution of phenolic fractions from VOO to olive oil bitterness, astringency and pungency has been demonstrated by several studies (Servili et al. 2004b). In this context, numerous authors suggested that secoiridoid, derivatives of oleuropein, derivatives such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA are the main contributors to VOO bitterness and pungency (García et al. 2001; Tovar et al. 2001; Andrewes et al. 2003; Kiritsakis and Shahidi 2017).

The volatile fraction of VOO is mainly constituted by C5 and C6 aldehydes, alcohols and esters produced from polyunsaturated fatty acids throughout the lipoxygenase pathway. The concentration of volatiles in the oil is dependent on the level of activity of each enzyme involved in the lipoxygenase pathway (Aparicio and Morales 1998; Angerosa et al. 1999,

2004). The genetic effect, related to the cultivar, is one of the most important aspects of volatile composition of olive oil. However, climatic and agronomic conditions of olive growing can affect volatile composition of olive oils obtained from the same cultivar (Morales et al. 1996; Servili et al. 2007; Dabbou et al. 2011a)

3. The effect of genetic and environmental factors on olive oil quality

The interaction of genetic and environmental factors are of major importance for olive oil composition and consistence of quality along the years. Ripening stage and area of production were shown to have an effect on fatty acid composition in Spain and Italy (Bianchi 2003; Dabbou et al. 2009). Differences in oil composition and sensorial attributes were also found among several growing areas in Italy, affected by maximum temperature, organic matter in the soil, carbon/nitrogen ratio and the most important of all factors: genetic/cultivar. A correlation between mean temperature in October and total phenol content, tocopherols and volatiles was found in the north of Italy (Tura et al. 2008). Temperature can also affect the ratio between fatty acids. Cumulative temperature during fruit growth were found to positively affected saturated and polyunsaturated fatty acids (Tura et al. 2008). Also, monounsaturated/polyunsaturated ratio decreases with higher temperatures in Argentina and Italy (Mannina et al. 2001). Differences among years due to climatic conditions were reported for fatty acid composition, phenolic compounds and tocopherols (Failla et al. 2002). Those differences were reported to be directly related with any factor that can affect photosynthesis at the beginning of oil accumulation in the fruit (Failla et al. 2002).

4. The importance of olive fruit maturation on oil quality

The amount of oil accumulated, the fatty acid and phenolic profile of the olive oil are important parameters for defining olive oil quality and they are related with the degree of ripening of the fruits along the season.

Olive fruit growth and maturation can be described on a phenological base according to the international standard of the BBCH (Biologische Bundesanstalt, Bundessortenamt, Chemische Industrie) scale (Meier, 2001) modified for olive trees by Sanz-Cortéz et al. (2002) with 8 stages from fruit set to senescence. Fruit growth can also be described by a double sigmoid curve with three distinctive phases. In the first phase, characterized by an intense cell division and enlargement involve mainly the growth and development of the endocarp (seed); the second phase, includes the seed hardening were the cells became sclerified and afterwards the mesocarp development were the fruit increase size rapidly with expansion of preexitsting flesh cells; while in the third phase of growth occurs at a lower rate with changes in epicarp pigments (Hartmann 1949; Lavee 1986). Oil accumulation starts at very early stage of fruit growth, but occurs at highest rates close to the green maturation together with the highest fruit size (Gómez-del-Campo et al. 2014; Bodoira et al. 2015).

The quality of the synthesized oil depends, among other factors, on the composition of triacylglycerols and is influenced by the activity of the enzymes in their biosynthesis during maturation (Sánchez and Harwood 2002). The biosynthesis of the fatty acids occurs within the plastids, and begins with a carboxylation of acetylCoA a malonyl-CoA (Sánchez and Harwood 2002). The cycle proceeds with sequential addition of two carbon atoms to the palmitate which is converted to other fatty acids within the scope of enzyme activity and desaturases (Sakouhi et al. 2008). Among fatty acids, oleic acid increases together with the fruit oil accumulation (Gutiérrez et al. 1999).

Changes in phenolic composition along fruit maturation are important due to the consequences they can have in the resulting health properties of the oils extracted. Phenolic compounds are group of secondary metabolites derived from the Shikimate pathway from L-phenylalanine or L-tyrosine (Morelló et al. 2005a; Cheynier et al. 2013). Phenolic compounds have their origin in phenylpropanoid metabolism, which involves the conversion of L-phenylalanine to various hydroxycinnamic acids in four sequential steps. Enzymes catalyzing the individual steps in this sequence are, respectively, phenylalanine ammonia lyase, cinnamate-4-hydroxylase and 4-coumarate-CoA ligase (Morelló et al. 2005a). The accumulation of phenolic compounds changes with the physiological state of the fruit and is a result of a balance between biosynthesis and catabolism. During fruit development, the most prevalent change is the decrease in oleuropein (Jemai et al., 2009). In advance stages of maturity, oleuropein is replaced with demethyloleuropein and hydroxytyrosol (Morelló et al. 2005b; Obied et al. 2008; Alagna et al. 2012)

Also sensory attributes can change along fruit maturation. Variation in volatile profile were previously attributed to lipoxygenase activity that is reduced in advances stages of fruit ripening (Padilla et al. 2009; Servili et al. 2011).

Oil composition is a decisive parameter for the harvest time, and maturation of olives plays an important role. Maturation of the fruit is mainly measured by a maturity index, an international standard stablished by the International Olive Oil Council based on the changes of the epidermis and endodermis pigments. Is expected that maturity index correspond to certain characteristics of the oil extracted, however not always that was verified (Scamosci et al. 2011; Trapani et al. 2015). Thus, it remains urgent to find a phenotypic parameter that can describe better the changes in fruit composition that are correlated to changes in oil composition and, thus, quality.

4.1. Fruit growth and maturation physiology

The olive is a drupe of green color that passes to violet or black when it matures. It is composed of three well-defined zones: the epicarp or skin, the mesocarp or pulp and the endocarp that surrounds the embryo. It weighs between 1.5 and 12 grams and the pulp represents between 70 and 88% of the fruit. Olive fruit consists mainly of water, which accounts for more than 50% of its weight, and oil, which, depending on the cultivar and the state of ripeness of the fruit, is about 20% by fresh weight (Bianchi 2003). The period of development and growth of the olives is usually long, from 6 to 7 months (Hermoso et al. 1991). In the first 100 days the endocarp develops rapidly and the natural selection of the fruits is made. In the period that follows, from 100 to 110 days, there is a rapid development of the mesocarp and the so-called green maturation, which occurs with a strong reduction of the chlorophyll content. In this phase, with the fruit already fully developed, the pulp represents about 70% to 90%, the endocarp of 9 to 27% and the almond of 2 to 3%. When the olives are still not ripe, the amount of water is greater than that of oil, and this situation is gradually reversed throughout the maturity of the fruit (Bianchi 2003). From growth to maturation, the olive presents variations in its constituents: changes in size, color, texture and flavor. Fruit development and maturation are a biochemical combination and physiological events occurring under strict genetic control and the influence of various environmental conditions. At the optimum harvest time, the mesocarp contains about 60% water and a variable lipid content depending on the cultivar. The remainder corresponds to small amounts of carbohydrates, protein, fiber and minerals. The endocarp contains 10% water, 30% cellulose, 40% other carbohydrates and about 1% lipids. Seed has 30% water, lipids and carbohydrates in equivalent proportions, about 30%, and 10% protein (Connor and Fereres 2005; Conde et al. 2007).

4.2. Changes in olive oil quality and chemical composition during fruit maturation

Oil quality is influenced by several factors, among which the cultivar and fruit maturity are two of the most important (Rotondi et al. 2004a). During maturation, several metabolic processes occur in the olives, with the consequent variation in the profiles of some components. These changes are reflected in the quality of olive oil, oxidative stability and its nutritional value. The effects of aging on the metabolism of olive oil have been reported in the literature (Morelló et al. 2004). Overall, as the fruit matures, the oil becomes less stable an increase in polyunsaturated fatty acids and a decrease in the content of phenolic compounds (Ayton et al. 2007; Dag et al. 2011b), however variations in the content of minority components contribute as a whole to the changes.

4.2.1. Oil quality parameters

Acidity is the result of the presence of free fatty acids produced by hydrolysis and enzymatic lipolysis, expressed as a percentage of oleic acid, the major fatty acid in olive oil. This parameter is considered an indicator of the freshness of the olive oil and the quality of the olives used during the production, being indicative of bad practices of manufacture or use of deteriorated olives. The decline of oil quality is also evaluated by its oxidation, i.e. peroxide value and ultraviolet absorbances at 232 nm and 270 nm. Peroxide value evaluates the formation of hydroperoxides, highly unstable primary oxidation products. The ultraviolet absorbances are a measure of the presence of conjugated dienes and trienes due to the formation of primary and secondary oxidation products, respectively; being a more robust indicator compared to the peroxide value (Vichi et al. 2003). All these parameters are included in international standards with limits that allow the classification or declassification of olive oils and contribute for a distinction in the market. Quality parameters do not usually show significant differences between olive oil obtained from

green olives and olive oil obtained from mature olives. Although previous research shows a slight increase in free acidity and a slight decrease in the peroxide value during maturation, these differences are usually very small. K_{232} decreases slightly, in line with the peroxide value, while K_{270} increases only slightly in olive oils at an advanced stage at maturation. However, significant increases along maturation were founded, for the cultivars 'Barnea', 'Arbequina' and 'Picual' so authors recommend avoiding late harvest on these cultivars (Yousfi et al. 2006; Dag et al. 2011b; Benito et al. 2013).

4.2.2. Chemical composition

Fatty acids changes along the ripening process is related to specific biological and enzymatic activities, together with production zone and olive cultivar (Culeddu et al., 2017; Santona et al., 2018; Deiana et al., 2018). Fatty acids are the main constituents of olive oil and are in part responsible for its nutritional value, mainly because of the high ratio of monounsaturated fatty acids to polyunsaturated fatty acids. Fatty acids composition depends on the production zone, latitude, climate, olive cultivar and their maturation stage (Boskou et al. 2006; Cunha and Oliveira 2006). Oleic, linoleic and palmitic acids are the most abundant fatty acids in olive oil. Several studies reported that saturated fatty acids (palmitic and stearic) decreases with maturation while polyunsaturated fatty acids (PUFA) increase, and oleic acid, the main monounsaturated fatty acid (MUFA), remains constant or shows a slight increase (Servili et al. 2011). Thus, the relationship between monounsaturated and polyunsaturated fatty acids (MUFA/PUFA) also decreases throughout maturation, leading to a compromise of oil oxidative stability (D'Imperio et al. 2010; Dag et al. 2011b; Gharbi et al. 2015).

The maturation course and its effect on the composition and content of phenolic compounds in olives and olive oil have been studied in several countries and olive cultivars. Similar results were observed: phenolic compounds reach a maximum content in the olive

fruits during the cherry phase, decreasing drastically thereafter, when the fruit begins to change color to black (Rotondi et al. 2004a). Oleuropein is the main phenolic compound present in green olives and is responsible for its characteristic bitterness (Andrewes et al. 2003). One of its main derivatives is hydroxytyrosol, which is also one of the most active antioxidants found in olive products. This compound also decreases with maturation and this tendency is presented in several cultivars and environmental conditions. The decrease of hydroxytyrosol in the olives may probably be a consequence of hydrolysis and oxidation processes occurring during the maturation of the fruit (Morelló et al. 2004).

Table 1 Limits for classification of extra virgin olive oil by the International Olive Council (IOOC/T.15/NC No 3/Rev. 11).

Main quality traits	**Limits stablished by IOOC**
Free acidity (%m/m expressed in oleic acid)	≤ 0.8
Peroxide value (mEq O2 kg^{-1})	≤ 20
K_{232}	≤ 2.50
K_{270}	≤ 0.22
ΔK	≤ 0.01
Fatty acid composition as determined by gas chromatography (% m/m methyl esters):	
Myristic acid	< 0.03
Palmitic acid	7.50 - 20.00
Palmitoleic acid	0.30 - 3.50
Heptadecanoic acid	< 0.40
Heptadecenoic acid	< 0.60
Stearic acid	0.50 - 5.00
Oleic acid	55.00 - 83.00
Linoleic acid	2.50 - 21.00
Linolenic acid	< 1
Arachidic acid	< 0.6
Eicosenoic acid	< 0.5
Behenic acid	< 0.20
Lignoceric acid	< 0.2
Organoleptic characteristics:	
Median of the fruity	> 0
Median of defect	0

2. Aim of the thesis

The olive tree (Olea europaea, European) is one of the fruit tree species that, together with citrus fruits, characterize the arboriculture of the Mediterranean area. In regions like Sicily, an island with little space left for new plantations, olive oil production not only needs to reach a fair productivity every year but also to outstand for quality. It is also important that the oil produced count with unique attributes able to distinguish Sicily from other olive oil producing regions. The quality of the oils extracted in the region will be related both to the soil-climatic characteristics and to the important genetic heritage. Genotype and harvest moment demonstrate to be one of the most influential factors in olive oil composition. Besides, there are cultural practices influencing fruit quality variability at harvest that can also have an impact on olive oil. In this sense, the objectives of this thesis are:

- To study quality of olive oils within the best performing Sicilian genotypes in intensive, hedgerow orchards. This research aim at the determination of chemical and sensory traits of EVOOs of 5 minor Sicilian olive cultivars and 10 accessions oils extracted from 5 major Sicilian cultivars and 3 global cultivars adapted to hedgerow high density planting.
- To evaluate the influence of fruit position and planting density on the production and composition of oil obtained from Koroneiki and Cerasuola cultivars. These two variables were chosen because light interception and maturity index of olive drupes are among the main factors contributing to the variability of ripening and quality within harvest seasons. To maximize orchard production, efficiency and oil quality, the optimization of both interception of maximum amount of radiation and radiation distribution within the canopy have paramount importance.

- To investigate the effect of fruit canopy position on fruit maturation and oil composition on Arbequina cultivar in order to determine which factors are determinant on finding the ideal harvest time.

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Experiment 1.

Quantitative evaluation of phenolic profile and sensory traits of monovarietal extra virgin olive oil obtained from Sicilian cultivars/ accessions

Abstract

Studying the composition of monovarietal extra virgin olive oils (EVOO) is important to define and manage their quality and uniqueness. This research aimed at the determination of chemical and sensory traits of EVOOs from 14 minor Sicilian olive cultivars in comparison with oils from 5 major Sicilian cultivars. EVOOs were extracted in 2015 in a two-phase mill from fruit of 23 cultivars/accessions grown in an experimental orchard located in South West Sicily (Italy) at a planting density of 1140 trees ha^{-1} and shaped to free Palmetta. The international cultivars Arbequina, Arbosana and Koroneiki were included as reference. Fatty acid composition, phenols composition, chlorophyll and carotenoid content were analysed along with the most common sensory descriptors, like fruitiness, bitterness, pungency. A panel conducted the sensory evaluation. Acidity, peroxide value and fatty acid composition indicated that all oils fell into the extra virgin category. Chemical composition, oil yield and sensory attributes of olive oils from the 23 cultivars were evaluated by multivariate analysis. Cluster analysis on standardized principal components detected three main groups associating quality traits and genotypes. The first group included the cultivar Arbosana and Koroneiki with three major Sicilian cultivars ('Biancolilla caltabellota', 'Cavalieri' and 'Cerasuola'), and one minor genotype ('Nocellara messinese') along with oleic acid and oil yield. The second group included the minor genotypes and the major cultivar ‘Nocellara Etnea’ along with phenol content and most sensory attributes. Arbequina together with the remaining seven Sicilian cultivars (major and minor) were grouped with saturated and polyunsaturated fatty acids, less

desired for high quality olive oil. These results show that some Sicilian accessions used in this study may represent valid alternatives to produce high quality EVOOs in modern, intensive, hedgerow planting systems.

Introduction

The olive tree (*Olea europaea* L.) is crop with economic and environmental relevance in the Mediterranean area where it has been cultivated since ancient time (Loumou and Giourga 2003). Many olive cultivars/accessions in the Mediterranean area have regional origin (Sarri et al. 2006), due to cross-pollination, climatic differences among sites where the species has been domesticated and the long life span of the olive tree (Lavee and Zohary 2011). In particular, a high pool of genetic variability has been recognized in Sicily (La Mantia et al. 2005). Major olive cultivars used for olive oil production result from farmers selection based on phenotypic and horticultural traits of the trees, particularly on their suitability for extensive cultivation under dry conditions (Belaj et al. 2010). In modern intensive and irrigated orchards, the quality standards have generally declined and some cultivars once abandoned may represent an opportunity to increase the diversity and improve the quality of olive oil produced in Sicily. Currently, in the international scenario only three cultivars (Arbequina, Arbosana and Koroneiki) fit to the modern super intensive orchard design and management, showing good performance also in Sicily (Tous et al. 2008; Godini et al. 2011; Caruso et al. 2012). Nevertheless, the worldwide diffusion of these cultivars/genotypes could lead to a dangerous reduction of biodiversity and to a flattening of the differences in olive oil quality, both from a chemical and an organoleptic point of view. Nowadays, 'Biancolilla', 'Cerasuola', 'Moresca', 'Nocellara del Belice', 'Nocellara Etnea', 'Ogliarola Messinese', 'Santagatese' and 'Tonda Iblea' are the predominate olive oil cultivars in this island (Caruso et al. 2014b). The recovery of Sicilian genotypes and their use in modern intensive orchards may contribute to new opportunities for olive oil production. The genetic richness of Sicilian olive germplasm has been well documented in the recent decades (Besnard et al., 2013; Caruso et al., 2014; Motisi et al., 2006; A. Motisi et al., 2006). In 1985, an ex-situ experimental field with

Sicilian cultivars/accessions was established in order to assess genetic variability, adaptability to high-density systems and differences in olive oil quality in the same environmental and agricultural management conditions.

EVOO is obtained exclusively by mechanical and physical processes. It is composed by a major fraction (more than 98% of the total weight) of mono- and poly-unsaturated fatty acids (mainly triacylglycerides), whereas a minor fraction (approximately 2% of the weight) is composed by minor compounds, which includes over 230 chemical compounds (terpenoids, sterols, pigments, volatile compounds and antioxidants) (Servili et al. 2014). Traditionally, the beneficial effects of virgin olive oil have been attributed to the fatty acid composition and phenolic compounds content. Nowadays, there is a trend to reduce saturated fat and increase the level of unsaturated/polyunsaturated fatty acids and omega 3 fatty acids for health benefits (White 2009) constituting a new challenge for olive oil producers and the emerging of new cultivars. More recently, phenolic compounds became the main reason for olive oil health benefits which have antioxidant, anti-inflammatory, anti-cancer, antimicrobial, antiviral, hypoglycemic, hepatic-, cardiac- and neuro-protective effects (Cicerale et al., 2012; Martín-Peláez et al., 2013; Servili et al., 2014). In general, five major classes of phenolic compounds can be found in olive oils: phenolic acids, phenolic alcohols, flavonoids, lignans and secoiridoids (Mendoza et al. 2013). Secoiridoids are found only within the family of Oleaceae and there are considered the main components (50-70%) of the phenolic fraction of the oil extracted. The most abundant compounds bellowing to this family are the dialdehydic forms of elenoic acid linked either to hydroxytyrosol (3,4-DHPE-EDA) or to tyrosol in oleochantal (p-HPEA-EDA); oleuropein aglycon (3,4-DHPEA-EA) and ligstroside aglycon (p-HPEA-EA). Oleocantal, a secoioridoid derivative with very promising pharmacological properties, has been proposed as an agent to induce apoptosis in colon cancer cells, inhibition of

proliferation in breast cancer and prostate cancer cell lines, stimulating further interest in cancer research (Elnagar et al. 2011; Abuznait et al. 2013). Several animal and in vitro studies have shown that oleocanthal possess important neuroprotective activities against Alzheimer's disease (Abuznait et al. 2013). 3,4-DHPE-EDA as a novel drug aimed to prevent or reduce inflammation of endothelium, plays an important protective role against reactive oxygen species-induced oxidative injury in red blood cells (Paiva-Martins et al. 2009).

The phenolic compounds present in olive oil are also responsible for its unique sensory properties. In this respect, 3,4-DHPEA-EDA and 3,4-DHPEA-EA are considered responsible for "bitter" and ''astringent'' attributes in EVOO (Tovar et al. 2001; Andrewes et al. 2003). Furthermore, Garcia et al. (2001) established a correlation between the bitterness of EVOO and the related chemical compounds behind it, finding that the sum of the two secoiridoids derivatives of hydroxytyrosol, the dialdehydic form of decarboxymethyl oleuropein aglycon and the aldehydic form of oleuropein aglycon represents an objective estimation of the oil organoleptic characteristics.

The phenolic composition of olive oils may depend of agricultural techniques, degree of ripeness of the fruits, soil composition, climate, the processing technique, storage but firstly depends of the olive cultivar (Inglese et al. 2011; Sinesio et al. 2015). Moreover, some Sicilian accessions have been already recognized for a high percentage of oleic acid or high level of phenolic compounds (Motisi et al., 2006), regardless of the year or method of extraction.

According to European Food Safety Authority (EFSA) (EFSA NDA Panel 2011), the high values of mono- and polyunsaturated fatty acids in EVOO helps maintaining a normal blood LDL cholesterol concentration while its phenolic fraction protects LDL particles

from oxidative damage. In this way, EVOO are considered effective in decreasing the risk of cardiovascular disease.

According to the recent new perspectives of health benefits related to high unsaturated fatty acids and polyphenol content in olive oil as described by the EFSA, there is a real opportunity for the Sicilian cultivars and accessions. Thus, the objective of this research is the classification of Sicilian oils obtained both from major and minor cultivars, based on their main chemical and sensory attributes for the individuation of the best performing cultivars in intensive hedgerow orchards.

Twenty-three monovarietal EVOO with high phenol content were analyzed with the aim to evaluate their ability to obtain a health claim from EFSA increasing the chances for the Sicilian olive oils to be introduced in the international market.

Material and methods

Sampling material. Monovarietal EVOO samples were obtained from trees grown in an experimental field located in South West Sicily, (37°53'N, 13°00'E, about 56 m a.s.l.). The orchard was planted in 2006 using one-year-old self-rooted olive plants of 20 Sicilian cultivars and accessions, and 3 international cultivars used in super intensive orchards (Table 2). Sicilian genotypes were classified as major or minor according with their distribution and production in Sicily. Major genotypes are largely diffused in the region and contribute for the distinction of Sicilian olive oils in the market, while minor cultivars are only grown in small farms and contribute to the production of very exclusive olive oils.

Table 2 cultivars and accessions present in this study. Cultivars and accessions are from Sicily (with Major or minor distribution and production in the island) or already recognized internationally for high density planting.

Genotypes	Abbreviation	Geographic origin	Category
Arbequina	AQ	Spain	International
Arbosana	ABS	Spain	International
Koroneiki	KO	Greece	International
Biancolilla	BL	West Sicily	Major
Biancolilla caltabellota	BLC	West Sicily	Major
Cerasuola	CE	West Sicily	Major
Moresca	MO	South-East Sicily	Major
Nocellara del Belice	NB	West Sicily	Major
Nocellara etnea	NE	South-East Sicily	Major
Bottone di gallo	BTTG	West Sicily	Minor
Calatina	CL	Center-South Sicily	Minor
Castriciana rapparina	CAR	North-East Sicily	Minor
Cavaliere	CVL	Center-North Sicily	Minor
Crastu	CRS	North Sicily	Minor
Erbano	EBN	West Sicily	Minor
Giarraffa	GF	North-West Sicily	Minor
Minuta	MNT	North-East Sicily	Minor
Nasitana	NA	North-East Sicily	Minor
Nerba	NR	Center-North Sicily	Minor
Nocellara messinese	NM	North-East Sicily	Minor
Olivo di mandanici	OLM	North-East Sicily	Minor
Piricuddara	PRC	North-West Sicily	Minor
Vaddarica	VDA	North-East Sicily	Minor

A total of 25 plants per genotype, spaced at 2,5 x 3,5 m (about 1140 trees ha^{-1}), were planted in single North-South oriented rows. Trees were trained to hedgerows system (free palmetta shape) to allow partially mechanization of canopy pruning and full mechanization of harvesting. From the 5^{th} year after planting trees were mechanical pruned (topping) to 2.5 m high. Trees were irrigated with an amount of water corresponding to approximately 800 m^3 ha^{-1} $year^{-1}$ on a drip irrigation system. After harvest, maturation index was determined based on fruit skin and pulp color according to Hermoso et al. (Hermoso et al. 1991). Olive fruits, 100 for each sample, were randomly taken, classified into the following ripening categories, and homogenized prior to storage:

0 – olives with intense green or dark green epidermis;

1 – olives with yellow or yellowish green epidermis;

2 – olives with yellowish epidermis but with reddish spots or areas over less than half of the fruit;

3 – olives with reddish or light violet epidermis over more than half of the fruit; 4 – olives with black epidermis and totally white pulp;

5 – olives with black epidermis and less than 50% purple pulp;

6 – olives with black epidermis and violet (more than 50%) or purple pulp;

7 – olives with black epidermis and totally dark pulp.

With the letter *a* to *h* representing the number of fruits observed in each category, maturity index (MI) in calculated by:

$$MI = \frac{a \times 0 + b \times 1 + c \times 2 + d \times 3 + e \times 4 + fx5 + g \times 6 + h \times 7}{100}$$

Olive oil was extracted from olives harvested from October to November 2015 with a two-phase extraction system (Pieralisi Leopard Model 6 DMF Tec Jesi, Italy) at 27ºC with 30 minutes of malaxation time. The resulting oil weight and fruits yield data were used to

calculate yield efficiency and oil yield. The trunk cross-section area was used to calculate yield efficiency (YE = kg of fruit/cm^2). Three samples for each monovarietal EVOO were stored in dark glass bottles and at 10°C until analyses.

Chemicals and standards. Water, methanol, acetonitrile (LC-MS grade) were purchased from Biosolve B.V. (Valkenswaard, The Netherlands).Formic acid (LC-MS grade) used as ionization agent in the chromatographic mobile phase, were purchased from VWR International B.V. (Roden, The Netherlands). Reference phenolic compounds including caffeic acid, cinnamic acid, ferulic acid, gallic acid, p-coumaric acid, p-hydroxybenzoic acid, syringic acid, vanillic acid, apigenin, apigenin 7-glucoside, diosmetin, hydroxytyrosol, tyrosol, luteolin, oleuropein, vanillin and pinoresinol were purchased from Sigma-Aldrich (Steinheim, Germany). All solvents and other chemicals used were of analytical grade purity and were supplied from Merck (Darmstadt, Germany).

Determination of quality traits. Free acidity (% of oleic acid) and peroxide value (mEq O_2 kg^{-1}) were measured according to the European Union standard methods (UE, 1989/2003 modifying the ECC 2568/91). Chlorophyll and carotenoid contents were measured using a Beckman DU 640 UV spectrophotometer at 476 nm and 670 nm, as described by Mineo et al. (Mineo et al. 2007) dissolving 7.5 g of olive oil in 25 ml of cyclohexane. Pigment amounts were calculated using the specific extinction values, E0 = 613 for pheophytin 'a' and E0 = 2000 for lutein. Thus, pigment contents were calculated as follows:

$$[\text{chloropyll}](\text{mg/kg}) = (A_{670}) / (613 \times 100 \times d)$$

$$[\text{carotenoids}](\text{mg/kg}) = (A_{476}) / (2000 \times 100 \times d)$$

Where A is the absorbance and d is the spectrophotometer cell thickness (1 cm). The chlorophyll and carotenoid contents are expressed as mg of chlorophyll ''a'' and b-carotene for kg of oil, respectively. Total polyphenol quantification was carried out

according to the Folin–Ciocalteau colorimetric method and expressed as mg of gallic acid equivalents (GAE)/kg of oil (Montedoro et al. 1992).

Phenolic compounds determination by UHPLC-HESI-MS. Identification and quantification of phenolic compounds was done for each EVOO sample. They were extracted from the monovarietal oils by extraction according to COI procedure and Montedoro et al. 1992, with some modifications. In this method, in a centrifuge tube, 2 g of EVOO were mixed with 5 ml of a solution of (methanol/water 80:20 v/v). The tube was vortexed for 1 min and held in an ultrasonic bath for 15 min, centrifuged at 5000 rpm for 25 min. The surnatant was filtered over PTFE siringe filter 0.45 µm and recoverd at 4°C until UHPLC analysis. Triplicate samples of olive oil were used for each cultivar. Phenolic compounds were identified by ultra high performance liquid chromatography, heated electrospray, and mass spectrometry (UHPLC-HESI-MS). UHPLC analysis was performed using a Dionex Ultimate 3000 System (Dionex Softron GmbH, Germering, Germany) equipped with an autosampler controlled by Chromeleon 7.2 Software (Thermo Fisher Scientific, Bremen, Germany) and coupled to a photodiode array detector (thermo fisher). A UHPLC column (Phenomenex Luna C18(2) 50x1mm, 2.5µ) was set for separation of the selected compounds at 35°C. The mobile phases used were 0.1% formic acid in water (A) and methanol (B). The gradient elution program was: 0-5 min 10% B; 5-50 min linear increase to 99% B, 50-56 min 10% B coming back to the initial conditions until full stabilization. The column temperature was set at 30°C and the injection volume at 1 µl. The flow rate was 50 µl min^{-1}. Phenols were recognized at 280 and 320 nm on the basis of the standards obtained from commercial suppliers. MS detection was performed using a Q-Exactive accurate-mass spectrometer (Thermo Scientific, Bremen, Germany). The HESI parameters were set using negative ion mode with spectra acquired over a mass range from *m/z* 180-2000. The optimum values of HESI-MS parameters were: gas flow

rate at 30 arbitrary units; auxiliary gas unit flow rate at 10 arbitrary units; capillary temperature at 250 °C; auxiliary gas heater temperature at 150 °C; spray voltage at 2.8 kV; and S lens radio frequency level at 50. The automatic gain control was set with a maximum injection time of 200 ms. (-)HESI-MS spectra yield the singly deprotonated ion, $[M-H]^-$, at the same time as the mode FULL-SCAN and t-SIM (targeted Selected Ion Monitoring), to increase sensitivity. The total UHPLC-HESI-MS method runtime was 60 min. Detection was based on calculated exact mass and on retention time of target compounds. The detection was evaluated by Quan/Qual browser Xcalibur 3.0 (Thermo Fisher Scientific, San Jose, CA, USA). Linearity of the MS response was verified with solutions containing all standards at six different concentration levels over the range: 0.250 to 5 ppm. Each point of the calibration graph corresponded to the average of five independent injections. Figure 1, represent the phenols detected on the olive oils from the 23 genotypes studied grouped by their families.

Standard solutions of phenolic compounds. Phenolic standard stock solutions were prepared individually at a concentration of approximately 0.1 mg/mL by dissolving approximately 10 mg of each standard in 20 mL of 80:20 MeOH/H2O (v/v). A STD mix solution at 5 ppm was prepared mixing 1 mL of each individual standard solution in a 100 mL volumetric flask and diluting with methanol up to the mark. The other diluted solutions (at 2.5, 1.0, 0.5, 0.25 ppm) were prepared by dilution of the STD mix. All solutions were corrected for purity and no internal standard was used in this study. Calibration curves were constructed by injecting each standard mix solution at each concentration level in quadruplicate. The peak areas were calculated and plotted against the corresponding concentrations of the standard compounds using linear regression (least squares) to generate standard curves.

Fatty acids determination. Fatty acids of oil samples were determined as methyl esters by gas-chromatography/mass spectrometry. Fatty acid methyl esters were prepared by alkaline trans-methylation. Aliquots of 0.1 g of sample were diluted in 1 ml of n-heptane and manually agitated for 10 s. After was added 0.1 ml of a solution 2N KOH in MeOH and mixed in vortex for 2 min. After the solution turned clear and transparent, 500 µl of upper phase, containing fatty acid methyl esters was decanted and diluted with n-heptane to a final volume of 1 ml and analyzed in GC-MS in a period of 12 hours after preparation. GC-MS analysis were carried out using a Thermo Scientific DSQ II single quadrupole system in EI (Electron Ionization) mode, working in full scan. The temperature of ion source and injector were 260 °C and 270 °C, respectively. The capillary column used was a ZB-WAX (30 m x 0.25 mm i.d., film thickness 0.25 µm, (Phenomenex, Italy). The oven temperature was programmed column temperature started at 165°C for 10 min, increased at 1,5 °C/min to 200 °C, finally increased at 10 °C/min to 250 °C and held for 20 min under isothermal conditions. Helium was used as the carrier gas at a flow rate of 1 mL/min. A sample of 1µl was injected with a split ratio of 1:100. The ion source temperature was 260°C, the MS transfer line temperature was 265°C and injector temperature was 270°C. Ionization voltage was 70eV and the mass range scanned was 35-550 m/z. Peaks areas of 16 fatty acids and their quantification were performed using Thermo Scientific Xcalibur Data system software for Windows. Chemical identification of fatty acid methyl esters was carried out using a mass spectrum libraries (NIST/EPA/NIH mass spectral Library 2.0) and 37-components fatty acid methyl esters mix purchased from Supelco #47885-U, (Sigma Aldrich Milan, Italy). Triplicate samples of olive oil were used for each cultivar (n =3).

Sensory evaluation. Sensory evaluation of the oils was performed according to the panel test method (European Union Commission, 1991) by panelists from the Assessorato

Regionale dell'Agricoltura, dello Sviluppo Rurale e della Pesca Mediterranea, located in Sciacca, Italy. Olive oils have been classified according to the intensity and perception of the positive attributes (fruity, bitter and pungent) as *Intense* (median of at least one attribute is more than 6), *Medium* (median of at least one attribute is between 3 and 6) and *Light* (median of attributes is less than 3).

Data analysis and statistics. Analysis of means (ANOM) was used to establish differences among cultivars/accessions on their total phenolic compounds content; upper and lower decision limits were plotted and used to show differences of cultivar/accession means from grand mean. Principal component analysis (PCA) was carried out with the biplot technique to study the relationship among chemical composition and sensory traits of olive oils from different genotypes; cluster analysis on standardized components was used to separate groups of genotypes based on similar properties.

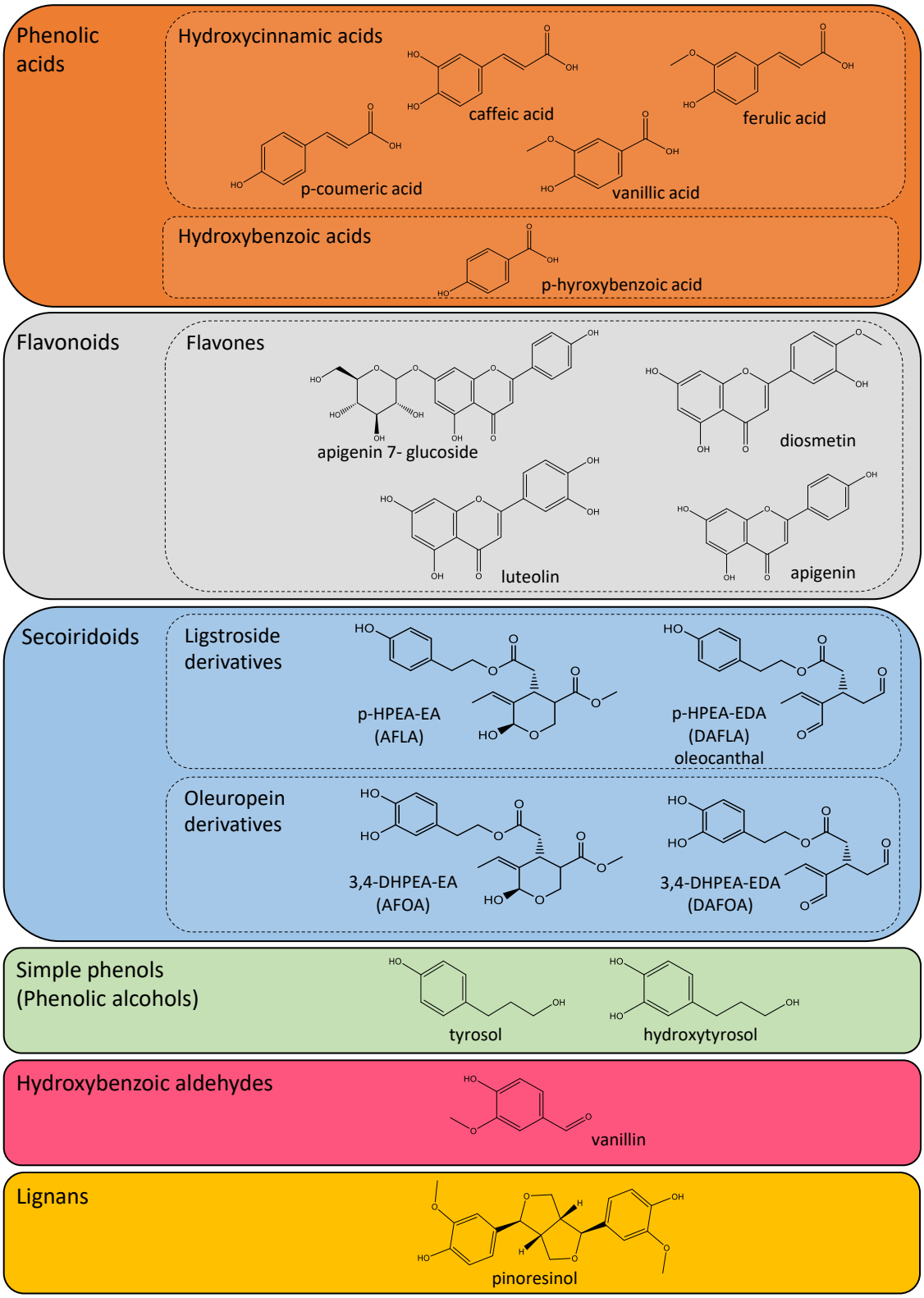

Figure 1 Phenolic compounds, grouped by their families, founded in the 23 genotypes studied.

Results and Discussion

The area in Sicily where this study was conducted was previously proved to be suitable for intensive hedgerow planting systems. The area is characterized by a long growing season that allows a constant (no alternating) bearing (Caruso et al. 2012). The cultivars studied, in order to be considered suitable for high density production must originate heavy production and high quality oils. High levels of monounsaturated fatty acids and phenolic compounds allow EVOO to be consider as a functional food (Stark and Madar 2002). According to the free acidity, peroxide value, fatty acid composition and sensory evaluation limits established by the International Olive Oil Council (IOOC, 2016), all oils studied were classified as EVOOs (Table 3).

Table 3 Olive oils quality traits of all oils studied. Average and standard deviation (SD) of the 23 genotypes (n=69). Limits for classification of extra virgin olive oil by the International Olive Council.

Quality traits	limits described in IOOC/T.15/NC No 3/Rev. 11	mean ± SD
Free acidity (%m/m expressed in oleic acid)	≤ 0.8	0.35±0.10
Peroxide value (mEq O2 kg^{-1})	≤ 20	3.6±2.1
K_{232}	≤ 2.50	0.96±0.52
K_{270}	≤ 0.22	0.09±0.05
ΔK	≤ 0.01	0.004±0.0003
Fatty acid composition as determined by gas chromatography (% m/m methyl esters):		
Myristic acid	< 0.03	0.01±0.005
Palmitic acid	7.50 - 20.00	13.3±2.4
Palmitoleic acid	0.30 - 3.50	1.6±0.9
Heptadecanoic acid	< 0.40	0.08±0.09
Heptadecenoic acid	< 0.60	0.16±0.14
Stearic acid	0.50 - 5.00	2.3±0.45
Oleic acid	55.00 - 83.00	67.1±5.43
Linoleic acid	2.50 - 21.00	13.4±2.91
Linolenic acid	< 1	0.9±0.18
Arachidic acid	< 0.6	0.5±0.09
Gadoleic acid (eicosenoic)	< 0.5	0.3±0.1
Behenic acid	< 0.20	0.1±0.07
Lignoceric acid	< 0.2	0.1±0.02
Organoleptic characteristics:		Median
Median of the fruity	Me > 0	4.8
Median of defect	Me = 0	0.0

Fruit yield ranged from 3.07 t ha^{-1} for CAR to 12.48 t ha^{-1} for KO. AQ and ABS presented values of 6.39 t ha^{-1} and 9.71 t ha^{-1}, respectively (Table 4). Yield efficiency varied from 0.03 kg cm^{-2} for CAR to 0.19 kg cm^{-2} for CL. AQ Yield efficiency was 0.09 while the values obtained for ABS and KO were 0.18 and 0.17, respectively. Maximum oil yield was 14.8 g kg^{-1} for CE, while the minimum was 6.5 g kg^{-1} for GF. AQ, ABS and KO, respectively, presented values of 8.4, 10.1 and 14.4 g kg^{-1}. Fruit yield, yield efficiency and oil yield were in the same range as previous studies from the same production region, where Koroneiki show to have the highest yield efficiency, oil and fruit yield among the international cultivars (Marino et al. 2017). Harvest occurred from in the months of October and November and fruit maturity index of the 23 cultivars varied from 1.27 (NE) to 3.74 (CRS). No significant correlations were found between maturity indexes and any of the production or quality parameters. Chlorophyll content ranged from 1.3 mg kg^{-1} for CVL to 9.1 mg kg^{-1} for CRS (Table 4). ABS presented a value of 8.1 mg kg^{-1} while 6.3 mg kg^{-1} and 2.2 mg kg^{-1} were measure of chlorophyll content on AQ and KO. Carotenoids presented values between 1.6 mg kg^{-1} and 8.1 mg kg^{-1} for CVL and CAR, respectively. AQ, ABS and KO yielded results of 2.4 mg kg^{-1}, 7.4 mg kg^{-1} and 5.7 mg kg^{-1}, respectively. This study confirms previous research on Sicilian cultivars by Mineo et al., (2007) were CL and MNT were distinguished for their high pigments content. One of the most widely used method for the routine determination of total phenol in olive oil is the colorimetric assay based on the reaction of Folin–Ciocalteu reagent with the functional hydroxyl groups of phenols. Total phenols measured by colorimetric method Folin-Ciocalteu ranged from 148 mg kg^{-1} given by GF to 674 mg kg^{-1} for NM. AQ presented values of 238 mg kg^{-1}, while ABS and KO yielded concentrations of 173 mg kg^{-1} and 208 mg kg^{-1}, respectively. A significant correlation was found with linear regression model (R^2 = 0.79, p = 0.000) between the total phenols with the colorimetric method Folin-Ciocalteu (Table 4) and the

sum of phenols identified by LC (Table 5). Results of both methods were not exactly the same due to a low specificity of Folin-Ciocalteu reagent that, particularly in water–methanol extracts, reacts in presence of other non-phenolic compounds, such as proteins (Lowry et al. 1951).

The secoiridoids identified as the dialdehydic forms of decarboxymethyloleuropein and ligstroside aglycones (3,4-DHPEA-EDA (or DAFOA) and p-HPEA-EDA (or DAFLA), respectively) and the aldehydic forms of oleuropein and ligstroside aglycones (3,4-DHPEA-EA (or AFOA) and p-HPEA-EA (or AFLA),respectively) were the most abundant phenolic compounds found in the samples (Table 5). Among all phenols present in the oil, oleuropein derivatives have the strongest antioxidant activity (Artajo et al. 2006). In this study, the sum of ligstrosides derivatives (p-HPEA-EA and p-HPEA-EDA) ranged from 6.9 mg kg^{-1} for GF to 136 mg kg^{-1} for VDA. While the sum of oleuropein derivatives (3,4-DHPEA-EA and 3,4-DHPEA-EDA) ranged between 85.2 mg kg^{-1}, given by CE, and 280 mg kg^{-1} given by OLM. Thus, the sum of secoiridoids (sum of ligstroside and oleuropein derivatives) ranged between 75.5 mg kg^{-1}, given by GF and 423 mg kg^{-1}, given by EBN. Phenolics acids (ferrulic, caffeic, p-coumeric, vanillic and p-hydroxybenzoic acids) concentrations ranged between 0.6 mg kg^{-1} for PRC to 8.7 mg kg^{-1} for NE. Flavanoids (apigenin, apigenin 7-glucoside, diosmetin and luteolin) ranged between 1.5 mg/Kg for CE to 10.5 mg Kg^{-1} for NM and PRC. Simple phenols, representing the sum of tyrosol and hydroxytyrosol ranged from 3.3 mg kg^{-1} for BL to 281 mg kg^{-1} for MO. Pinoresinol, belonging to the lignan family, ranged from 53.1 mg kg^{-1} for BL to 327.8 mg kg^{-1}. The EC Regulation 432/2012 established a health claim on the phenolic compounds concentration for EVOO. The health claim states that, to provide a protective effect on human health, EVOO should provide at least 5 mg phenols per 20 g oil. This limit, expressed in mg kg^{-1}, is 250 ppm. Phenolic compounds contributing to these values are oleuropein and ligstroside

derivatives, hydroxytyrosol and tyrosol (Tsimidou et al. 2018). The cultivars that fulfil the EU health claim for the minimum phenolic compounds concentration presenting positive health benefits the major genotypes NB and NE and the minor genotypes CRS, MNT, PRC, OLM, NM, CAR, EBN and VDA (Table 4).

Total phenol content (Table 5) varied greatly among cultivars/accessions, ranging from 162 on BL to 828 mg kg^{-1} on VDA. Values for this parameter were higher than those previously found in olive oils from Southern Italy (Barbarisi et al. 2014; Marino et al. 2017). The analysis of means for the total phenol content of the samples is represented in Figure 2. Two major cultivars NB and NE, together with minor genotypes, CL, CAR, CRS, EBN, MNT, NM, OLM, PRC and VDA showed a total polyphenol content above the average (504.2 mg Kg^{-1}). On the other hand, the international cultivars, KO, AS and AQ together with the major Sicilian cultivar BL, BLC, CVL, CE, MO; and other Sicilian minor cultivars GF, NA and NR, had values below the average.

The fatty acid fraction accounts for not less than 98% of the oil components and is characterized by a relative low level of polyunsaturated fatty acids (PUFA) and a high level of monounsaturated fatty acids (MUFA). The fatty acid composition of olive oil samples from this study are shown in Table 6. The major fatty acids were oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) acids. Palmitoleic (C16:1), linolenic (C18:3), stearic (C18:0), arachidic (C20:0), behenic (C22:0), eicosenoic (C20:1), myristic (C14:0), lignoceric (C24:0) and heptadecenoic (C17:0) acids were detected in minor amounts. Moreover, the sum of saturated fatty acids (myristic acid, C14:0; palmitic acid, C16:0; heptadecanoic acid, C17:0; stearic acid, C18:0; arachidonic acid, C20:0; behenic acid, C22:0; and lignoceric acid, C:24:0) ranged from 12.8 % for CE to 19.4% for NA while the sum of unsaturated fatty acids (palmitoleic acid, C16:1; heptadecenoic acid, C17:1; oleic acid, C18:0; linoleic acid, C18:2; linolenic acid, C18:3; and eicosenoic acid, C20:1) ranged from

80.2% for MNT to 87.2% for CE. The ratio UFA:SFA ranged from 4.2 for VDA to 6.9 for CE. The high values of the sum of monounsaturated fatty acids (C16:1, C17:1, C18:1, C20:1) that distinguishes olive oil from other vegetables oils (Inglese et al. 2011) ranged from 61.6% for MO to 76.5% for KO. Additionally, the sum of polyunsaturated fatty acids (C18:2 and C18:3) ranged from 8.5% for NR to 19.9% for MO. MUFA/PUFA ratio changed among genotypes and is also related with the environmental growth conditions. The decrease of MUFA/PUFA ratio is related with advanced fruit development and/or high temperatures during fruit growth (Gutiérrez et al. 1999; Ripa et al. 2008; Inglese et al. 2011; Dag et al. 2014). Salvador et al. (1999), showed that lower MUFA/PUFA takes to a faster deterioration of the oil along storage. In this study, MUFA/PUFA ranged from 3.10 for MO to 8.6 for NR.

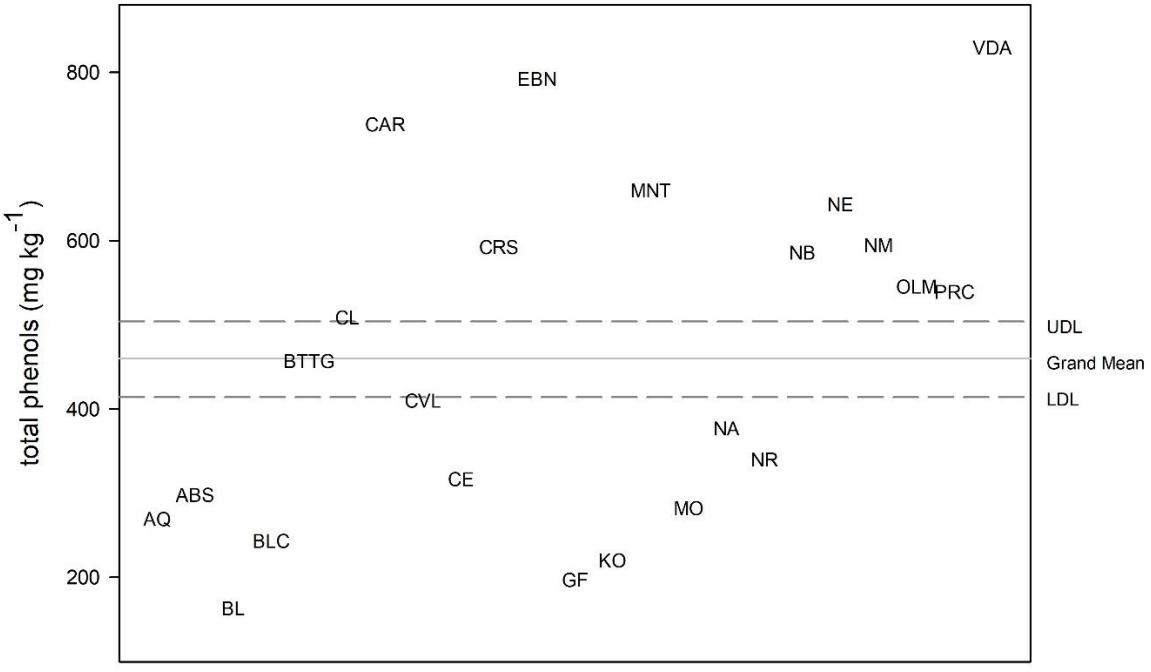

Figure 2 Total polyphenol content (mg kg^{-1}) in olive oils from 23 cultivars (data points are means of three replicates) obtained from HPLC analysis. Solid horizontal line indicate grand mean (460.1 mg kg^{-1}) of polyphenol content (N=69). Dashed horizontal lines indicate upper (504.2 mg kg^{-1}) and lower (414.1 mg kg^{-1}) decision limits from analysis of means.

Table 4 Fruit yield (t ha^{-1}), Yield efficiency (YE, kg cm^{-2}), Oil yield (g.kg^{-1}), maturity index (MI) (of 100 fruits per replicate) and main oil quality traits of the 23 cultivars studied. Free acidity (FA) expressed as percentage of oleic acid, peroxide value (PV) as meq O_2/kg, coefficient of specific extinction at 232 nm (K_{232}), coefficient of specific extinction at 270 nm (K_{270}), chlorophyll and carotenoids content and total phenols are expressed in mg kg^{-1}.

Genotypes	fruit yield	YE	oil yield	MI	FA	PV	k_{232}	k_{270}	ΔK	chlorophyll	carotenoids	∑phenols (folin)
AQ	6.39±2.19	0.09 ± 0.04	8.4	2.5	0.2 ± 0.0	5 ± 0.5	0.98 ± 0.2	0.09 ± 0.03	0.00 ± 0	2.2 ± 0.6	2.4 ± 1.9	238 ± 18.4
ABS	9.71±1.69	0.18 ± 0.03	10.1	2.6	0.3 ± 0.1	5 ± 1.4	1.46 ± 0.4	0.12 ± 0.08	0.00 ± 0	8.1 ± 3.4	7.4 ± 2.1	173 ± 9.8
BL	11.08±3.25	0.17 ± 0.02	10.8	3.1	0.4 ± 0.0	6 ± 2.5	1.73 ± 0.4	0.16 ± 0.07	0.00 ± 0	3.0 ± 1.2	3.4 ± 1.4	176 ± 77.3
BLC	4.15±5.25	0.05 ± 0.04	10.1	2.6	0.5 ± 0.2	4 ± 0.9	1.24 ± 0.1	0.15 ± 0.07	0.00 ± 0	2.4 ± 0.1	3.3 ± 0.2	296 ± 6.3
BTTG	9.2±5.19	0.11 ± 0.05	3.6	2.3	0.2 ± 0.0	6 ± 0.1	1.65 ± 0.1	0.09 ± 0.00	0.00 ± 0	5.3 ± 2.5	5.2 ± 2.4	437 ± 17.9
CL	8.65±2.26	0.19 ± 0.08	10.9	3.5	0.3 ± 0.0	3 ± 0.5	1.21 ± 0.1	0.08 ± 0.01	0.00 ± 0	7.8 ± 1.3	6.3 ± 1.8	424 ± 25.9
CAR	3.07±1.57	0.03 ± 0.02	10.4	2.6	0.4 ± 0.0	1 ± 0.1	0.74 ± 0.2	0.08 ± 0.01	0.01 ± 0	8.8 ± 2.3	8.1 ± 1.3	504 ± 21.4
CVL	9.71±3.76	0.14 ± 0.06	15	3	0.4 ± 0.0	2 ± 0.06	0.36 ± 0.1	0.06 ± 0.00	0.00 ± 0	1.3 ± 0.2	1.6 ± 0.1	416 ± 54.7
CE	4.03±2.19	0.05 ± 0.03	14.8	2	0.3 ± 0.0	2 ± 0.1	0.41 ± 0.2	0.05 ± 0.00	0.00 ± 0	5.6 ± 0.9	5.3 ± 0.7	308 ± 2.9
CRS	4.28±3.44	0.08 ± 0.04	8.5	3.7	0.3 ± 0.0	2 ± 0.5	0.88 ± 0.1	0.13 ± 0.05	0.00 ± 0	9.1 ± 3.6	7.8 ± 2.8	544 ± 26.9
EBN	6.58±4.45	0.05 ± 0.03	1.6	2.4	0.4 ± 0.0	4 ± 0.5	0.64 ± 0.3	0.05 ± 0.06	0.00 ± 0	3.7 ± 1.4	3.5 ± 1.4	630 ± 39.5
GF	3.62±2.39	0.06 ± 0.04	6.5	3	0.3 ± 0.0	5 ± 0.1	1.62 ± 0.5	0.17 ± 0.00	0.00 ± 0	2.9 ± 1.4	3.1 ± 1.5	148 ± 17.6
KO	12.48±1.52	0.17 ± 0.04	14.4	2.7	0.4 ± 0.1	8 ± 0.6	1.57 ± 0.1	0.07 ± 0.01	0.00 ± 0	6.3 ± 3.3	5.7 ± 3.3	208 ± 14.6
MNT	7.73±2.92	0.07 ± 0.05	5.6	3.1	0.3 ± 0.1	5 ± 0.6	1.39 ± 0.2	0.12 ± 0.01	0.00 ± 0	5.9 ± 4.1	5.4 ± 3.2	604 ± 59.8
MO	3.22±1.49	0.04 ± 0.02	4.8	3.5	0.4 ± 0.0	3 ± 0.6	0.74 ± 0.1	0.05 ± 0.01	0.01 ± 0	3.7 ± 1.9	4.3 ± 1.1	171 ± 12.6
NA	6.72±1.73	0.1 ± 0.02	6.1	2.5	0.3 ± 0.0	5 ± 0.1	0.64 ± 0.1	0.05 ± 0.00	0.01 ± 0	3.1 ± 2.3	4.8 ± 2.5	563 ± 23.2
NR	7.77±3.1	0.08 ± 0.04	4	3.4	0.3 ± 0.0	4 ± 0.0	0.16 ± 0.1	0.02 ± 0.00	0.00 ± 0	6.2 ± 0.5	5.7 ± 0.8	254 ± 22.5
NB	3.81±1.62	0.05 ± 0.02	8.1	2.4	0.4 ± 0.0	2 ± 0.3	0.58 ± 0.1	0.09 ± 0.05	0.00 ± 0	4.9 ± 0.6	4.3 ± 0.3	377 ± 28.5
NE	8.97±4.34	0.09 ± 0.04	10.1	1.3	0.3 ± 0.1	2 ± 0.4	0.92 ± 0.4	0.08 ± 0.04	0.00 ± 0	4.9 ± 3.3	4.8 ± 2.9	330 ± 18.2
NM	4.52±1.63	0.06 ± 0.01	13.7	2.1	0.3 ± 0.0	3 ± 0.0	0.53 ± 0.0	0.06 ± 0.00	0.01 ± 0	7.4 ± 2.8	6.5 ± 1.8	674 ± 2.9
OLM	7.64±1.37	0.05 ± 0.02	9.2	3.1	0.5 ± 0.0	7 ± 0.6	1.99 ± 0.0	0.1 ± 0.00	0.00 ± 0	4.3 ± 2.7	3.9 ± 2.1	298 ± 35.3
PRC	9.94±2.43	0.12 ± 0.04	10.5	2.5	0.4 ± 0.0	5 ± 1.0	0.64 ± 0.0	0.08 ± 0.00	0.01 ± 0	7.2 ± 3.8	6.1 ± 2.9	466 ± 51.4
VDA	8.46±3.54	0.06 ± 0.02	8.3	3.4	0.3 ± 0.0	4 ± 0.1	0.62 ± 0.1	0.11 ± 0.06	0.00 ± 0	8.9 ± 1.6	6.9 ± 0.8	522 ± 56
Average	7.±2.75	0.09 ± 0.03	9.07	2.8 ± 0.6	0.4 ± 0.1	3.6 ± 2.1	0.96 ± 0.5	0.09 ± 0.05	0.00 ± 0	5.4 ± 3	4.9 ± 2.3	388 ± 161.8

Table 5 Sum of phenolic acids, flavonoids, ligstroside and oleuropein derivatives, secoiridoids, simple phenols, lignans and total phenols of the 23 cultivars studied. Average (mg kg^{-1}) and standard deviation of 3 replicates of each genotype studied. Health claim represents the sum of secoiridoids and simple phenols.

Cultivar	∑phenolic acids	∑flavonoids	∑ligstroside derivatives	∑oleuropein derivatives	∑secoiridoids	∑simple phenols	pinoresinol	∑phenols	health claim >250 ppm
AQ	2.2 ± 0.8	2.7 ± 0.2	34.4 ± 0.15	160.8 ± 1.2	195.2 ± 1.3	15.2 ± 0.6	53.1 ± 0.3	268.4 ± 1.7	210.4 ± 1.4
ABS	1.1 ± 0.0	6.7 ± 0.5	40.8 ± 0.02	165.6 ± 1.7	206.3 ± 1.7	18.7 ± 0.6	64.2 ± 1.2	297.1 ± 1.8	225.1 ± 1.1
BL	2.4 ± 0.1	2.8 ± 0.1	18.1 ± 0.23	87.5 ± 5.2	105.6 ± 5.8	3.3 ± 0.8	48.2 ± 0.2	162.3 ± 6.5	109.1 ± 6.3
BLC	1.2 ± 0.1	3.0 ± 0.0	15.5 ± 0.58	102.5 ± 5.7	117.9 ± 5.5	8.7 ± 0.5	111.3 ± 5.7	242.3 ± 10.8	126.7 ± 5.5
BTTG	2.7 ± 1.0	4.6 ± 0.1	25.2 ± 0.94	166.4 ± 0.7	191.6 ± 0.3	37.7 ± 0.6	219.5 ± 0.4	456.3 ± 1.5	229.3 ± 0.8
CL	2.8 ± 0.1	4.2 ± 0.0	31.6 ± 0.07	200.8 ± 2.3	232.4 ± 2.3	10.8 ± 0.3	257.6 ± 5.4	508 ± 7.1	243.2 ± 2.6
CAR	1.0 ± 0.1	2.9 ± 0.3	62.5 ± 1.53	315.2 ± 4.9	377.6 ± 4.9	43.3 ± 1.1	312.1 ± 0.6	737.1 ± 5.5	421.0 ± 5.7
CVL	1.8 ± 0.6	4.6 ± 0.2	34.9 ± 0.03	188.8 ± 5.4	223.7 ± 5.4	12.8 ± 0.1	166.0 ± 1.1	409.0 ± 5.5	236.5 ± 5.4
CE	1.1 ± 0.0	1.5 ± 0.1	18.6 ± 0.25	85.2 ± 1.0	103.8 ± 0.8	30.3 ± 0.9	178.9 ± 0.9	315.7 ± 2.6	134.1 ± 1.6
CRS	1.3 ± 0.1	2.4 ± 0.0	58.0 ± 0.07	226.2 ± 4.5	284.3 ± 4.4	25.5 ± 0.6	278.2 ± 0.5	591.7 ± 3.6	309.8 ± 3.8
EBN	2.3 ± 0.1	2.3 ± 0.0	76.7 ± 0.07	346.8 ± 5.9	423.5 ± 5.8	73.4 ± 0.5	289.5 ± 0.3	791.1 ± 6.1	496.9 ± 6.4
GF	5.2 ± 0.7	7.3 ± 0.0	6.9 ± 0.58	68.5 ± 5.7	75.5 ± 5.4	27.2 ± 0.5	81.0 ± 1.0	196.3 ± 4.1	102.7 ± 5.5
KO	0.6 ± 0.1	1.6 ± 0.0	30.8 ± 0.31	91.9 ± 1.5	122.8 ± 1.3	15.7 ± 0.4	78.6 ± 0.6	219.4 ± 0.8	138.5 ± 0.9
MNT	1.8 ± 0.8	5.8 ± 0.3	42.9 ± 0.7	264.7 ± 4.1	307.6 ± 4.7	15.8 ± 0.5	327.8 ± 0.3	658.9 ± 4.1	323.4 ± 5.2
MO	3.7 ± 0.1	8.2 ± 0.6	10.2 ± 0.54	136.4 ± 0.5	146.6 ± 0.9	281.1 ± 0.3	94.6 ± 0.1	281.2 ± 1.4	174.6 ± 0.7
NA	2.0 ± 0.6	9.7 ± 1.1	53.5 ± 1.17	107.5 ± 1.0	161.1 ± 0.1	21.9 ± 0.5	181.3 ± 8.7	376.0 ± 9.6	183.0 ± 0.6
NR	8.2 ± 0.1	7.7 ± 0.7	55.3 ± 0.31	112.4 ± 1.4	167.7 ± 1.1	9.9 ± 0.6	141.9 ± 5.7	338.8 ± 8.2	177.6 ± 1.7
NB	5.3 ± 0.0	5.4 ± 0.3	77.1 ± 0.46	174.9 ± 2.4	251.9 ± 2.3	27.6 ± 0.1	283.3 ± 9.0	585.1 ± 5.1	279.6 ± 1.9
NE	8.7 ± 3.4	8.3 ± 0.1	96.2 ± 4.81	232.1 ± 5.4	328.2 ± 5.1	14.2 ± 0.0	279.8 ± 49.7	642.1 ± 49.8	342.5 ± 4.9
NM	0.6 ± 0.0	10.5 ± 0.2	122.9 ± 0.06	279.6 ± 6.1	402.6 ± 6.1	11.1 ± 0.1	167.3 ± 10.6	593.9 ± 15.3	413.7 ± 5.9
OLM	1.3 ± 0.0	8.3 ± 1.8	76.6 ± 0.31	280.4 ± 7.0	357.1 ± 7.3	14.6 ± 0.1	160.9 ± 0.6	544.5 ± 7.1	371.7 ± 7.2
PRC	0.6 ± 0.1	10.5 ± 0.0	87.5 ± 2.38	180.9 ± 1.5	268.4 ± 3	62.9 ± 0.4	191.9 ± 0.3	538.3 ± 3.0	331.4 ± 2.9
VDA	1.9 ± 1.4	8.4 ± 1.2	136.2 ± 0.03	277.9 ± 5.1	414.1 ± 5.1	139.3 ± 3.4	262.1 ± 2.2	828.1 ± 8.3	553.5 ± 8.4
Average	2.4 ± 2.3	5.8 ± 3.2	54.9 ± 38.83	189.9 ± 95.1	244.8 ± 127.7	30.2 ± 28.1	184.9 ± 85.7	268.4 ± 1.7	275.1 ± 141.4

Table 6 Percentage of saturated fatty acids (SFA), unsaturated fatty acids (UFA), ratio of saturated and unsaturated fatty acids (UFA/SFA), monounsaturated fatty acids (MUFA), poliunsaturated fatty acids (PUFA), ratio of mono and poliunsaturated fatty acids and ratio of linoleico (omega 6) and linolenico (omega 3) of the 23 cultivars studied. Average and standard deviation of 3 replicatesfor each cultivar .

Cultivar	SFA	UFA	UFA/SFA	MUFA	PUFA	MUFA/PUFA
AQ	19.2 ± 0.4	80.7 ± 0.4	4.2 ± 0.1	62.9 ± 0.2	17.8 ± 0.6	3.5 ± 0.1
ABS	14.5 ± 0.1	85.4 ± 1.1	5.8 ± 0.1	73.5 ± 1.1	11.9 ± 0.1	6.1 ± 0.1
BL	15.8 ± 0.0	84.1 ± 0.0	5.3 ± 0.0	67.8 ± 0.1	16.2 ± 0.1	4.1 ± 0.0
BLC	14.4 ± 0.4	85.2 ± 0.1	5.9 ± 0.2	71.9 ± 0.0	13.3 ± 0.1	5.3 ± 0.1
BTTG	18.1 ± 0.9	82.8 ± 0.1	4.5 ± 0.2	63.3 ± 0.1	19.5 ± 0.1	3.2 ± 0.0
CL	17.6 ± 0.3	82.6 ± 0.7	4.6 ± 0.1	67.0 ± 0.6	15.6 ± 0.6	4.3 ± 0.2
CAR	15.9 ± 1.2	83.3 ± 1.1	5.2 ± 0.4	70.5 ± 1.1	12.8 ± 0.1	5.4 ± 0.2
CVL	16.0 ± 1.3	83.3 ± 1.1	5.2 ± 0.5	69.5 ± 1.1	13.8 ± 0.4	5.0 ± 0.2
CE	12.7 ± 1.4	87.1 ± 1.3	6.9 ± 0.8	73.5 ± 0.9	13.6 ± 0.5	5.3 ± 0.2
CRS	17.1 ± 0.9	82.5 ± 0.8	4.8 ± 0.3	68.6 ± 0.8	13.8 ± 0.2	4.9 ± 0.1
EBN	17.1 ± 0.4	82.3 ± 0.1	4.8 ± 0.1	67.4 ± 0.0	14.8 ± 0.07	4.5 ± 0.0
GF	17.2 ± 0.6	82.4 ± 0.9	4.8 ± 0.1	63.9 ± 0.8	18.5 ± 0.08	3.4 ± 0.0
KO	13.4 ± 0.1	86.4 ± 0.8	6.4 ± 0.1	76.5 ± 0.8	9.9 ± 0.03	7.6 ± 0.1
MNT	18.9 ± 0.4	80.1 ± 0.6	4.2 ± 0.1	63.1 ± 0.5	17.1 ± 0.04	3.7 ± 0.0
MO	19.2 ± 0.5	81.5 ± 0.7	4.2 ± 0.1	61.6 ± 0.9	19.8 ± 0.31	3.1 ± 0.1
NA	19.3 ± 0.1	80.8 ± 0.3	4.2 ± 0.0	67.9 ± 0.4	13 ± 0.08	5.2 ± 0.1
NR	17.1 ± 0.6	81.9 ± 1.1	4.8 ± 0.1	73.4 ± 1.1	8.5 ± 0.04	8.6 ± 0.2
NB	16.4 ± 0.5	83.3 ± 0.1	5.1 ± 0.1	68.9 ± 0.1	14.3 ± 0.02	4.8 ± 0.0
NE	17.3 ± 0.6	82.4 ± 0.1	4.7 ± 0.1	66.3 ± 0.4	16.1 ± 0.33	4.1 ± 0.1
NM	14 ± 0.5	86.4 ± 0.9	6.2 ± 0.3	75.2 ± 0.9	11.2 ± 0.07	6.6 ± 0.1
OLM	17.5 ± 0.1	82.4 ± 0.9	4.7 ± 0.1	70.6 ± 0.9	11.7 ± 0.07	6.0 ± 0.1
PRC	15.2 ± 0.1	86.5 ± 1.6	5.6 ± 0.1	73.6 ± 1.5	12.8 ± 0.16	5.7 ± 0.1
VDA	19.3 ± 0.1	80.4 ± 0.2	4.2 ± 0.0	62.8 ± 0.1	17.6 ± 0.03	3.5 ± 0
Average	16.3 ± 2.2	83.5 ± 2.3	5.2 ± 0.9	69.3 ± 4.6	14.3 ± 3	5.1 ± 1.5

From a sensory point of view, all the examined samples belonged to the EVOO category as defined by the International Olive Oil Council (IOOC, 2016). Among the genotypes, EBN and CAR produced “intense” oils, whereas MO and NR produced 'light' oils (Figure 3). It is important to note that EBN and CAR had also lower maturation index when compared to the other cultivars. These results, suggest that an anticipated harvest may improve olive oil aromatic properties of MO and NR genotypes. The three international cultivars together with the remaining 14 Sicilian cultivars produced oils with intermediate intensity of aroma.

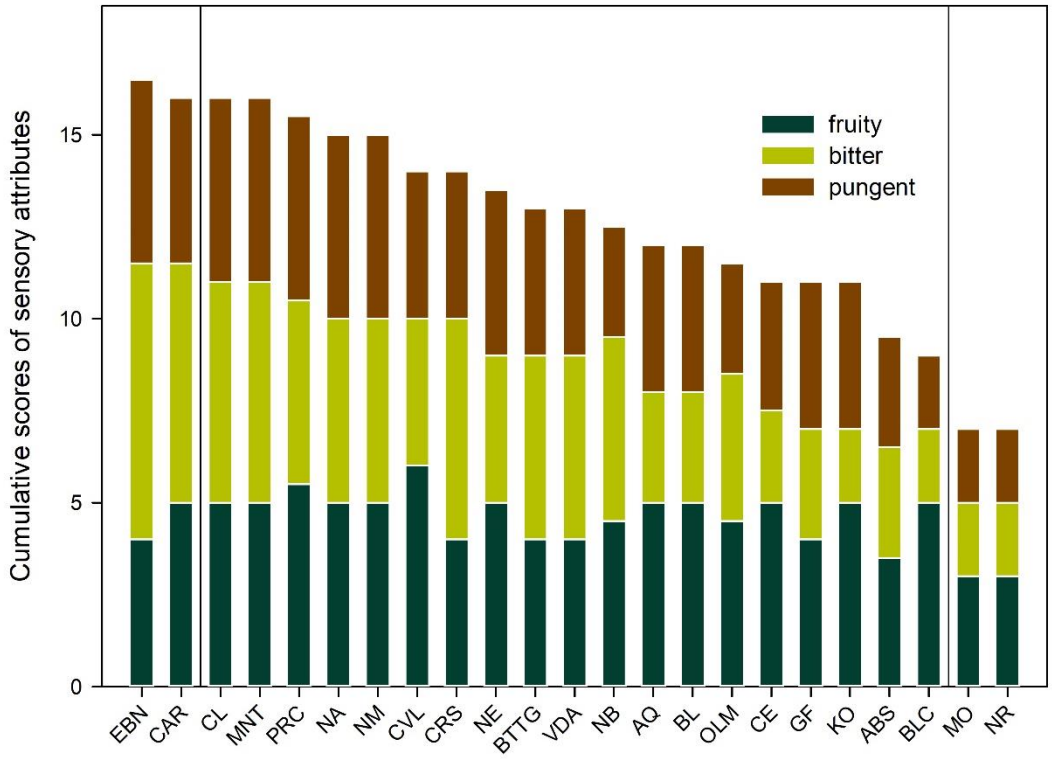

Figure 3 Cumulative scores of sensory attributes 'fruity', 'bitter' and 'pungent' olive oils from 23 cultivars/accessions. Solid horizontal line indicate group of oils classified in 'intense’ (I), 'medium' (M) and ‘light’ (L) olive oils according with their aroma.

When oil yield, chlorophyll content, carotenoids content, sum of phenols, fatty acids composition and organoleptic attributes were considered together, PCA showed that about 79.5% of the variability observed was explained by the first three components. PC1, PC2,

PC3 accounted for 31.7, 27.9, 19.9% of total variability, respectively. Cluster analysis on standardized components allowed for the individuation of three main groups associating the 23 cultivars with specific chemical and organoleptic properties (Table 7). The distribution of those components were plotted on figure 4. In particular, cluster analysis indicated that the cultivars ABS, BLC, CVL, CE, KO and NM were distinguished from the other cultivars by higher oil yield and monounsaturated fatty acids together the sensory descriptor 'fruity'. A second group included CL, CAR, CRS, EBN, MNT, NA, NE, PRC and VDA along with chlorophyll and carotenoids content, sum of phenols and oil sensory properties: density, persistence, pungent and bitter; showing to produce high quality EVOO. A third group included AQ, BL, BTTG, GF, MO, NR, NB and OLM distinguished by their saturated and polyunsaturated fatty acid composition without any particular organoleptic descriptor.

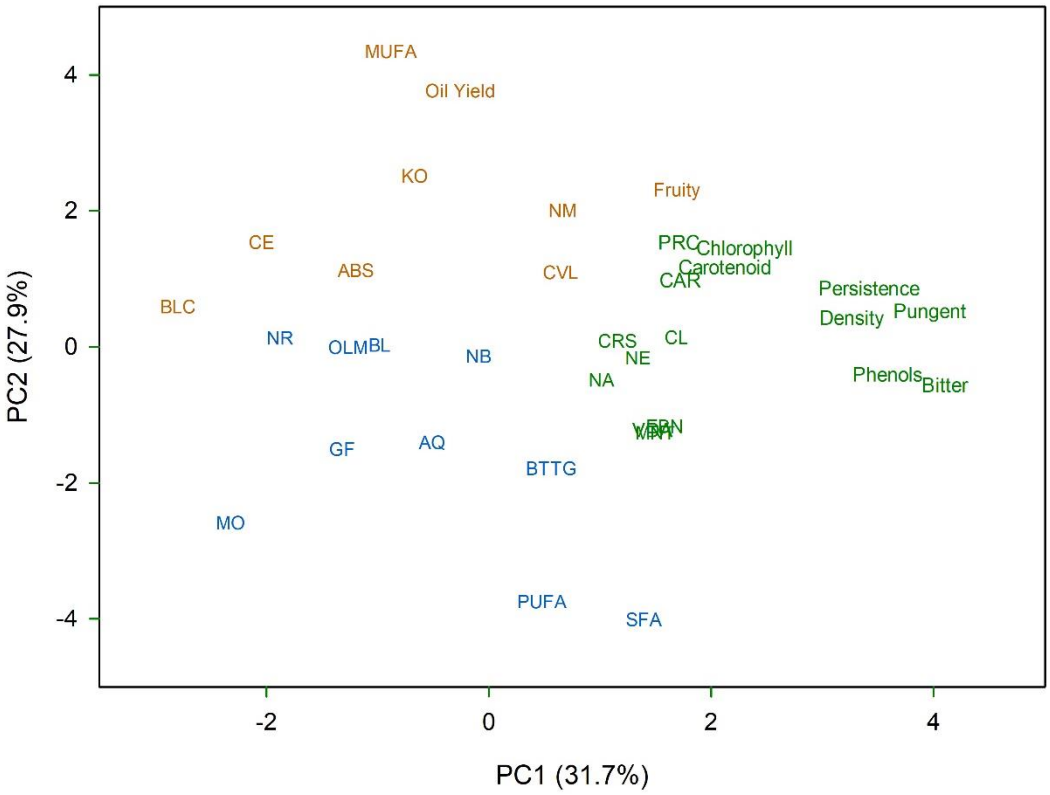

Figure 4 Factor loadings of all olive oil samples for principal components (PC) PC1 and PC2. Diferent colors represent the three groups created by cluster analysis.

Table 7 Standardized component coordinates for olive oils of 23 cultivars, chemical composition, oil yield and sensory traits from biplot analysis and grouping by cluster analysis.

	PC1	PC2	PC3	Cluster
Oil Yield	-0.258	3.769	1.312	1
Chlorophyll	2.301	1.444	-3.492	2
Carotenoid	2.123	1.163	-3.617	2
SFA	1.394	-4.014	-0.044	3
MUFA	-0.881	4.347	-0.733	1
PUFA	0.476	-3.748	1.201	3
Total phenols	3.584	-0.416	-1.697	2
Density	3.267	0.418	1.992	2
Persistence	3.42	0.852	1.991	2
Fruity	1.689	2.312	3.204	1
Bitter	4.101	-0.572	-0.793	2
Pungent	3.966	0.516	1.388	2
AQ	-0.508	-1.407	1.503	3
ABS	-1.196	1.122	-1.525	1
BL	-0.983	0.028	1.142	3
BLC	-2.794	0.589	0.34	1
BTTG	0.563	-1.788	-0.085	3
CL	1.686	0.141	-0.071	2
CAR	1.72	0.97	-1.154	2
CVL	0.644	1.097	1.94	1
CE	-2.045	1.538	0.293	1
CRS	1.158	0.091	-1.414	2
EBN	1.583	-1.172	0.068	2
GF	-1.317	-1.505	0.637	3
KO	-0.664	2.512	0.354	1
MNT	1.5	-1.265	-0.039	2
MO	-2.319	-2.588	-0.545	3
NA	1.015	-0.486	0.844	2
NR	-1.878	0.132	-1.477	3
NB	-0.091	-0.136	-0.114	3
NE	1.343	-0.16	0.784	2
NM	0.663	2.004	-0.191	1
OLM	-1.265	-0.005	-0.429	3
PRC	1.711	1.524	0.304	2
VDA	1.474	-1.237	-1.167	2

Conclusion

Results show that is possible to consider major and minor Sicilian cultivars for high density planting and olive oil production. Olive oils extracted from Sicilian cultivars presented equivalent or higher quality indexes when compared with oils produced from international cultivars Arbequina, Arbosana and Koroneiki. In particular, Sicilian minor cultivars such as CL, CAR, CRS, EBN, MNT, NM, OLM, PRC and VDA revealed a considerable higher phenolic content when compared with other major Sicilian cultivars and the three international cultivars. This study suggests that Sicilian cultivars can be used in high-density planting systems, broadcasting Sicilian high quality standards and biodiversity into the international olive oil market.

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Experiment 2.

Olive oil quality in response to fruit canopy position and tree planting density

Abstract

To maximize orchard production and tree yield efficiency, optimization of both interception of maximum amount of radiation at orchard level and radiation distribution within the canopy at tree level are important strategies. In addition, maturity of olive drupes is one of the major factors contributing to increase oil quality variability within harvest seasons and orchards location. In order to study the influence of planting density and fruit canopy position on oil quality from Cerasuola and Koroneiki, fruits were harvested from the upper layer and lower layer of the canopies of trees at two planting densities: 1000 trees.ha^{-1} (D1) and 500 trees.ha^{-1} (D2). Trees were trained to palmetta, a flat shape that along the row, design a continuously foliage wall. Tree yield efficiency, fruit weight, water and fat content in the fruits were measured together with olive oil quality standard parameters, phenolic and volatile compounds composition. Fruit maturation resulted more advanced in the drupes located in the upper layer of the canopies compared to the lower ones. Neither position in the canopy nor planting density affected fruit weight significantly. Fruits in the upper layers of the canopy always showed higher maturity index, higher fat content and lower water content. However, the proportion between upper and lower layers of the canopy deferred with planting density. Trees at D1 showed the largest differences in maturation, water and fat content between fruit from upper and lower canopy positions, increasing variability of quality and productivity at harvest. Nevertheless, planting density also influences the variability given by fruit canopy position on phenol composition and volatiles. Upper layers of D1 showed the highest phenol content, whereas the lower layers of D2 demonstrated the lowest phenol content. Koroneiki showed more stable oils with higher MUFA/PUFA ratio and phenol content. This study

demonstrate how differently the canopy architecture of hedgerow planting systems and different cultivars can impact olive oil production and quality.

Introduction

Nowadays, due to a global increasing in olive oil consumption more producers are being conducted towards the use of intensive cultivation systems. Yield increasing, maintaining high quality standard, reducing alternate bearing as well as costs and facilitating complete mechanization were taken into account as the main purpose of new planting systems. Sicily represents an important centre of olive oil production in the Mediterranean basin, where 'Cerasuola' is one of the most common olive cultivars (Caruso et al. 2007). The variety adapts well to poor soils, is drought- resistant and provides excellent results in optimal nutritional conditions (Caruso et al. 2007, 2014a). The oil content in the drupes of 'Cerasuola' is considered high (20-25%), and according to olfactory evaluation, generally introduced in the category of medium intensity fruitness along with the taste sensations of bitter, spicy and sweet (Caruso et al. 2007). Recently, the international cultivar 'Koroneiki' has been introduced in Sicily. Koroneiki is a cultivar of medium vigor with an upright growth habit that satisfies requirements for super intensive planting systems. Its fruits are rather small and the olive oil produced by Koroneiki has excellent quality and fragrance, categorized as a very fruity oil with green-apple notes, medium level aroma of leaves and grass, bitter and pungent. It is also astringent with touch of almond, fig and bark (Kandylis et al. 2011).

Due to the nutritional and health promoting effects of olive oil, the consumption of this oil is increasing worldwide, even in countries where it is not produced (Kiritsakis and Shahidi 2017). The nutritional and health promoting effects of olive oil have been associated with the optimal balance between saturated, mono, and polyunsaturated fatty acids as well as to minor components such as chlorophyll, polyphenols and tocopherols (Lazzez et al. 2008). Marketing of high quality olive oils is based on the chemical and sensory attributes; which are strongly affected by genotype, environment, fruit maturity at harvest and the interaction of the above factors (García et al. 1996; Zamora et al. 2001; Rotondi et al. 2004b). Previous studies

evidenced that high quality olive oil requires harvesting olive fruit at the optimum time (Trapani et al. 2015; Zipori et al. 2016). The rate of oil synthesis and the duration of oil accumulation period can be responsible for the final oil content in the olive fruit (Trentacoste et al. 2012).

As aforementioned, high production costs, especially for harvest, have played a key role in the redesigning of olive orchards during the last 30 years (Vossen 2007). For an optimal yield and maximum sun exposure, optimum planting density should be determined (Sibbet and Martin 1994). Besides tree spacing; cultivar, climate, harvest method, tree training system, fertilization, irrigation management and soil conditions should not be forgotten. It was reported that along with the reduction of row spacing (ranging from 8 to 3 m), the management of light interception from the orchards should be taken into consideration. Moreover, increasing the tree planting density leads to greater interception of solar radiation and more appropriate distribution of radiation within the tree canopies during the orchard development (Rom 1991; Lauri et al. 2009; Jackson 2017). This allows for maximum efficiency of using solar radiation for different process including photosynthesis, flower bud formation, growth, and fruit quality. Jackson (2017), indicated that to maximize orchard production and efficiency, both interception of maximum amount of radiation and optimization of the radiation distribution within the canopy are important factors. Tombesi et al. (1999) found that fruits located on the periphery of the canopy exploited more solar radiation with bigger fruits and higher oil contents compared with fruit from internal areas of the tree. In 'Arbequina' hedgerows, maturity and size were more advanced and larger, respectively; in fruits from the upper part of the canopy. Furthermore, oil content increased by nearly 50% from lower to upper layers (Gómez-Del-Campo et al. 2009b). Hence, it can be concluded that intercepted radiation leads to some of these differences, such as fruit size and oil content. In addition, differences in oil quality can result from rapid growth and early maturation in upper

layer of tree canopy. Trentacoste et al. (2016) indicated that irradiance received in different hedgerow positions and orientations influenced fruit development and oil quality in olive. Fruits receiving more radiation showed the highest fruit weight, mesocarp oil content, maturity index, and total polyphenols in virgin olive oil (Trentacoste et al. 2016). Guerfel et al. (2010) demonstrated that olive oil samples obtained from fruits of trees grown at density of 100 trees.ha^{-1} had a higher content of oleic acid, a higher content of chlorophyll and carotenoids, and a higher phenol content than lower planting distances. On the other hand, several studies evidenced that biomass production was directly related to radiation interception in the canopy in other fruit trees such as apple and peach (Mariscal et al. 2000; Willaume et al. 2004; Villalobos et al. 2006; Morandi et al. 2010). Gómez-del-Campo and García (2012) reported that, on cultivar Arbequina, the fatty acid composition of the oils extracted from olives grown in 2.5m to 2.9m hedgerows were significantly affected by the intensity of light intercepted.

The aim of this study was to evaluate the influence of fruit position and planting density on the production and the qualitative and quantitative attributes of oils from Koroneiki and Cerasuola cultivars. Koroneiki was included as comparison since it is a cultivar already implemented in high density planting systems while Cerasuola is a major Sicilian cultivar usually grown in traditional planting systems.

Material and methods

Plant material and experimental design. Fruits were obtained from trees grown in an experimental field located southwest of Sicily, (37°31ˈN, 13°03ˈE, about 120 m a.s.l.). The orchard was planted in March 2012 using one-year-old self-rooted olive plants of the Sicilian cultivar 'Cerasuola' and 'Koroneiki'. The 28 plants per cultivar were planted in a single north-south row at two planting densities: 2×5 m (1000 trees/ha), and 4×5 m (500 trees/ha). The trees were pruned lightly during the first 5 years after planting to shape them to a palmetta training system to facilitate mechanical harvesting. Five self-compensating in-line drippers per plant spaced at 50 cm intervals, which delivered 16 l/h, delivered weekly irrigation from July through mid-September. The total seasonal application rate was 800 m^3/ha/year. Climatic data were acquired from the nearest public weather station (SIAS – Servizio Informativo Agrometeorologico Siciliano), positioned 13km from the experimental site (37°26'19.312" N, 13°15'59.177" E; altitude 30 m. a.s.l.

Olive harvest. In November 15th (for the cultivar Cerasuola) and November 17th (for the cultivar Koroneiki), of 2017, 20 trees for each planting density were selected from three different blocks based on similarities for fruit load, number of branches and light distribution in canopy. To compare the fruit position in the canopy the fruits were chosen to be above two meters from the ground (upper) or below two meters from the ground (lower), since the average height of the canopy was 3.5meters starting at 0.1 meters from the ground. The fruits were hand harvested and placed in one-ton bins for processing. Fruits were weighed and processed with a commercial two-phase mill (Toscana Enologica Mori-TEM) with a capacity to process 200 kg of olives/time. The oil was subsequently weighed, and subsamples taken for chemical analyses.

Fruit measurements. Fruits from upper and lower canopy of each planting density were assessed by fresh weight of 100 fruits (g) and maturity index based on skin and pulp

pigmentation according to Uceda and Frias (Uceda and Frias 1975). Fruit moisture content (% of fresh weight) and fat content (% of fresh weight) were determined by near infrared (NIR) analysis using Olivia instrument (FOSS, Hillerød, Denmark).

Determination of quality traits. Free acidity (% of oleic acid) and peroxide value (mEq O_2 kg^{-1}) were measured according to the European Union standard methods (UE, 1989/2003 modifying the ECC 2568/91). Chlorophyll and carotenoid contents were measured colorimetrically using a Beckman DU 640 UV spectrophotometer at 476 nm and 670 nm, as described by Mineo et al. (2007) . The chlorophyll fraction at 670 nm and the carotenoid fraction at 476 nm were evaluated from the absorption spectrum of each virgin olive oil sample (5 g) dissolved in hexane (25 mL). The chlorophyll and carotenoid contents are expressed as mg of chlorophyll ‘‘a’’ and b-carotene for kg of oil, respectively. Total polyphenol quantification was carried out according to the Folin–Ciocalteau colorimetric method and expressed as mg of gallic acid equivalents (GAE)/kg of oil (Montedoro et al. 1992).

Phenolic compounds profile. Identification and quantification of phenolic compounds was performed for each olive oil sample. They were extracted from the monovarietal oils according to IOC procedure and Montedoro et al. 1992, with some modifications. In this method, in a centrifuge tube, 2 g of EVOO were mixed with 5 ml of a solution of (methanol/water 80:20 v/v). The tube was vortexed for 1 min and held in an ultrasonic bath for 15 min, centrifuged at 5000 rpm for 25 min. The surnatant was filtered over PTFE siringe filter 0.45 μm and recoverd at 4°C until UHPLC analysis. Triplicate samples of olive oil were used for each cultivar. Phenolic compounds were identified by ultra high performance liquid chromatography, heated electrospray, and mass spectrometry (UHPLC-HESI-MS). UHPLC analysis was performed using a Dionex Ultimate 3000 System (Dionex Softron GmbH, Germering, Germany) equipped with an autosampler controlled by Chromeleon 7.2 Software

(Thermo Fisher Scientific, Bremen, Germany) and coupled to a photodiode array detector (thermo fisher). A UHPLC column (Phenomenex Luna C18(2) 50x1mm, 2.5µ) was set for separation of the selected compounds at 35°C. The mobile phases used were 0.1% formic acid in water (A) and methanol (B). The gradient elution program was: 0-5 min 10% B; 5-50 min linear increase to 99% B, 50-56 min 10% B coming back to the initial conditions until full stabilization. The column temperature was set at 30°C and the injection volume at 1 µl. The flow rate was 50 µl min^{-1}. Phenols were recognized at 280 and 320 nm on the basis of the standards obtained from commercial suppliers. MS detection was performed using a Q-Exactive accurate-mass spectrometer (Thermo Scientific, Bremen, Germany). The total UHPLC-HESI-MS method runtime was 60 min. Detection was based on calculated exact mass and on retention time of target compounds, and data were evaluated by Quan/Qual browser Xcalibur 3.0 (Thermo Fisher Scientific, San Jose, CA, USA). Linearity of the MS response was verified with solutions containing all standards at six different concentration levels over the range from 0.250 to 5 ppm. Each point of the calibration graph corresponded to the average of five independent injections.

Fatty acid profile. Fatty acids of oil samples were determined as methyl esters by gas chromatography using the method described by the International Olive Oil Council (IOOC/T20 doc N33). Quantification was carried out using a Focus GC equipped with a MEGA 10 column (50 m × 0.32 mm × 0.25 µm, Agilent Technologies, USA) and helium as carrier gas. Data were expressed as percentage of total area of the picks from each chromatogram.

Sensory evaluation. Sensory evaluation of the oils was performed according to the panel test method (IOC/T.20/DOC.15/Rev.10) by panelists from the Assessorato Regionale dell'Agricoltura, dello Sviluppo Rurale e della Pesca Mediterranea (Sciacca, Italy). This method is only applicable to VOOs and is based on the intensity of attributes perceived by a

group of tasters trained and monitored as a panel. The main positive attributes are fruity, bitter and pungent. Oils were tasted two times at different days and results are expressed in median of the sensory attribute.

Volatile profile. Sample (1.0 ± 0.1 g), spiked with 4-methyl-2-pentanol as internal standard (2.5 mg/kg), was weighed into a 20 mL glass vial and sealed with a PTFE/silicon septum. After 15 min at 45 °C, a solid-phase micro extraction (SPME) fiber (DVB/CAR/PDMS, Sigma-Aldrich, St. Louis, MO) was exposed to the sample headspace for 45 min for volatile extraction. The volatile compounds analysis was performed on a Varian 450-GC equipped with a Varian 220-MS ion trap (Agilent Technologies). A Supelcowax 10 (30 m x 0.25 mm x 0.25 μm, Sigma-Aldrich, St. Louis) was used for compounds separation. After sampling, the fiber was thermally desorbed in the GC injector for 10 min at 260 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. GC oven temperature started at 40 °C and ramped at 3 °C/min after 10 min to the final temperature of 200 °C. Ionization energy of 70 eV was adopted and the ions were analysed in the m/z range from 40 to 400. The data were recorded and analysed using MS Workstation v6.9.3. Volatile compounds were identified by their mass spectra.

Statistical analysis. The data were analysed using Systat (13) to perform analysis of variance (ANOVA) with $p \leq 0.05$ to identify significant differences among all parameters analysed in the olive oils.

Results and discussion

The year of 2016 was characterized by high comulated precipitation in the months of June, and July, with 212.08 and 210.82 mm respectively. The coolest month was January (average 12.6ºC), while the hottest month was August (average 25 ºC).

The influence of cultivar, planting density and fruit canopy position on production and fruit characteristics is presented in Table 8. Fruit yield ranged between 4.9 kg/tree and 8.2 kg/tree. D2 trees showed higher fruit yield for both cultivars. The lower yield was obtained for Koroneiki at D1. The same trend was observed for yield efficiency with D2 giving the highest values (0.2 kg cm^{-2}). Fruit yield expressed in $t.ha^{-1}$ indicated that lower fruit yield per tree from D1 are compensated from a bigger number of trees per hectare for Cerasuola cultivar. However, Koroneiki did not show significant differences between D1 and D2. Results on the percentage of fruit yield harvested in the upper or lower layer of the canopy demonstrated that the majority of the production occurred on the upper layers. Cerasuola presented the biggest differences in fruit canopy distribution with the highest percentage of fruit harvested on upper layers of D1 and D2, 60.7% and 61.6%, respectively; and the lowest percentage of fruit harvest in the lower layers (39.3% and 38.4%). Upper layer of the canopy presented the highest oil yield for both cultivars and planting densities. However, oil yield did not follow the same pattern registered for fruit fat content; confirming that other factors, such as the differences of fruit characteristics among treatments (upper and lower; D1 and D2), can influence the oil yield even when the same extraction conditions are used. Percentage of fruit production in the lower layers of the canopy was higher than previously described by Castillo-Ruiz et al., (2015) for Arbequina in the south of Spain (17%), demonstrating that cultivars and cultural practices have a great influence on fruit distribution within the canopy.

During maturation, most of chlorophylls are degraded and replaced by anthocyanins, producing a change in color from green to black in the olive fruits. Maturity index ranged

between 2.2 to 3.3 (Table 8). Differences between upper and lower layers of the canopy were larger at planting density D1. These differences might be due to a less homogeneous distribution of the light intercepted from the canopy when the plants grow within short distances (Trentacoste et al. 2015c). Cerasuola presented higher fruit weight compared with Koroneiki. In terms of fruit weight, Cerasuola always showed to have significantly higher fruit weight than Koroneiki. Fruit weight was not affected by planting density or canopy position and the differences might be diminished as the fruits can attract assimilates from other areas of the canopy during development (Proietti et al. 2006).

Planting density and canopy position did not affect moisture content of Koroneiki. Cerasuola showed the highest (at lower canopy position) and the lower values for moisture content in this study at D1.

Cerasuola showed higher values of fat content at the upper layers of the canopy independently of planting density. Koroneiki showed higher values at upper layers only at D1. This study confirms previous studies showing that the fruits from lower canopy positions represented approximately a third of tree fruit production (26.%) and oil content (25.2%) (Castillo-Ruiz et al. 2015). Fruits from the upper canopy position were also characterized by the highest weight and ripening index values, as well as oil and phenolic compounds content (Gómez-Del-Campo et al. 2009a; Gómez-del-Campo and García 2012).

Table 8 Interaction of the independent variables cultivar, planting density and fruit canopy-layer position on production and fruit characteristics. Production described as fruit yield (kg tree^{-1}), yield efficiency (kg cm^{-2}), yield (t ha^{-1}), percentage of total fruit harvest distributed on the upper and lower layers of the canopy (g 100g-1 fresh weight) and oil yield (kg oil 100kg^{-1} fruit). Fruit conditions at harvest described by maturity index, fresh weight (g), moisture and fat content (g 100g^{-1} fresh weight). Values with the same letters are not significantly different by Tukey's test at $p<0.05$ (n=20).

Cultivar	Cerasuola				Koroneiki			
Planting density	D1		D2		D1		D2	
Canopy layer	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Fruit yield	6.8 ab		8 a		4.9 b		8.2 a	
Yield efficiency	0.2 ab		0.2 a		0.1 b		0.2 a	
Yield	6.8 a		4.0 b		4.9 b		4.1 b	
Fruit production	60.7 a	39.3 c	61.6 a	38.4 c	58.4 ab	41.6 bc	52.5 abc	47.5 abc
Oil yield	15.7	15.4	18.5	14.9	21.2	15.6	17.3	13.1
Maturity index	2.6 c	2.2 d	3.1 ab	2.8 bc	3.3 a	2.8 bc	2.7 bc	2.5 cd
Fruit Weight	1.3 a	1.3 a	1.4 a	1.4 a	0.9 b	0.8 b	0.8 b	0.8 b
Moisture content	49.5 c	53.7 a	51.4 bc	52.5 ab	49.5 c	50.5 bc	49.9 c	51.7 abc
Fat content	27.4 a	24.8 d	27.1 a	25.5 cd	27.6 a	25.8 bcd	26.9 ab	26.4 abc

According to the limits established by the International Olive Oil Council (IOOC, 2016) for free acidity, peroxide value and organoleptic characteristic, all oils studied were classified as extra virgin olive oils (EVOOs) (Table 9).

Table 9 Main olive oil quality traits. Average and standard deviation (SD) of all olive oil samples (n=24). Limits for classification of extra-virgin olive oil by the International Olive Council. Values represent means ± standard deviation of 24 replicates.

Main quality traits	limits described in IOOC/T.15/NC No 3/Rev. 11	mean ± SD
Free acidity (%m/m expressed in oleic acid)	≤ 0.8	0.4 ±0.18
Peroxide value (in milleq. oxygen per kg/oil)	≤ 20	7 ±3.8
K_{232}	≤ 2.50	1.35 ±0.18
K_{270}	≤ 0.22	0.10 ±0.02
ΔK	≤ 0.01	0.001 ±0.0013

The main effect of cultivar, planting density and fruit canopy position on chlorophyll, carotenoids, fatty acids, phenolic compounds and volatile compounds are presented in Table 10.

Chlorophylls are mostly located in the skin of the olive fruit, where the highest photosynthetic activity is observed (Roca and Mínguez-Mosquera 2001). Due to their liposolubility, chlorophylls migrate to the oil phase during the extraction process (Roca and Mínguez-Mosquera 2001). The final concentration in the oil is affected by the initial concentration in the fruit, but also by the extraction variables (Giuliani et al. 2011). Cultivar and planting density showed no effect on chlorophyll and carotenoids content. Differently, the lower layer presented significantly higher values for these pigments compared to the upper layer.

Identified fatty acids were myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1n9), margaric (C17:0), stearic (C18:0), oleic (C18:1n9), linoleic (C18:2n6), linolenic (C18:3n3), arachidic (C20:0), gadoleic (C20:1n9), behenic (C22:0) and lignoceric (C24:0) acids. In order to calculate the ratios presented on Table 10 and Table 11, fatty acids were grouped according to the number of insaturations in saturated fatty acids (SFA; myristic, palmitic, margaric, stearic, arachidic, behenic and lignoceric acids), unsaturated fatty acids (UFA; palmitoleic, oleic, linoleic, linolenic and gadoleic), monounsaturated fatty acids (MUFA; palmitoleic, oleic and gadoleic) and polyunsaturated fatty acids (PUFA; linoleic and linolenic). MUFAs are the predominant fatty acids in olive oil, with oleic acid being the most abundant (55-83%) (Al-Bachir and Sahloul 2017). Monounsaturated profile of fatty acids is one of the factors that contribute to explain the healthy benefits of olive oil in the Mediterranean Diet (Pérez-Rodrigo and Aranceta 2015; Piroddi et al. 2016). In all the samples, the oleic acid was always the predominant fatty acid. Cultivar showed a main effect on fatty acid ratios. Cerasuola had significantly higher UFA/SFA than Koroneiki but lower than Koroneiki on MUFA/PUFA. Fruit canopy layer position also affected fatty acid ratios. Lower layer showed significantly higher values for UFA/SFA, while upper layer was significantly higher in MUFA/PUFA. Higher MUFA/PUFA in the upper layers is not related with an increase in oleic acid, but instead to an increase of linoleic acid. The increase of linoleic acid was previously reported

in Arbequina cultivar and justified by a higher maturity of the olives harvested on higher layers (Gómez-del-Campo and García 2012).

Oleuropein and ligstroside are the most relevant phenolic compounds identified in olive fruit (Servili et al., 2004). These substances are hydrolyzed after crushing by the enzyme β-glucosidase, leading to the formation of oleuropein (3,4-DHPEA-EDA, 3,4-DHPEA-EA) and ligstroside (p-HPEA-EDA, p-HPEA-EA) aglycones; which exhibit a higher lipophilicity and constitute the most abundant phenolic compounds in virgin olive oil (Romero-Segura et al. 2012). In addition to β-glucosidase, polyphenol oxidase and peroxidase degrade phenolic compounds during the extraction, also shaping the phenolic profile in the oil (Hachicha Hbaieb et al. 2015). Cultivar significantly affected phenolic composition of the oils (Table 10). Cerasuola presented significantly lower phenol composition compared to Koroneiki, with the exception of 3,4-DHPEA-EDA, which presented higher values for the Italian cultivar. p-HPEA-EDA showed no difference between cultivars. Planting density D1 was significantly higher than D2 in phenol content, except for hydroxytyrosol that showed higher values for the lowest planting density. Tyrosol was not affected by planting density. Fruit position in the canopy significantly affected phenol content on the oil. Upper layers showed the highest total phenol concentration with higher 3,4-DHPEA-EA, 3,4-DHPEA-EDA and p-HPEA-EA; except for hydroxytyrosol where the lower layer presented the higher values for these phenolic compound. This results are in accordance with previous studies on Arbequina, where canopy position was a determinant factor for phenol concentration, increasing with height. Tyrosol and p-HPEA-EDA were not affect by fruit position on the canopy.

Volatile compounds are responsible of the fruity and green aroma of fresh olive oil (Aparicio and Morales 1998). These compounds are synthetized during processing from free polyunsaturated fatty acids, through an enzymatic pathway known as lipoxygenase (LOX) pathway. The two main enzymes involved in the LOX pathway are lipoxygenase (LPO) and

hydroperoxide lyase (HPL). LPO catalyzes the oxygenation of polyunsaturated fatty acids (linoleic and linolenic) to produce their corresponding hydroperoxides. HPL catalyzes the cleavage of fatty acid hydroperoxides at the bond situated between the carbon atom carrying the hydroperoxide group and the adjacent trans double bond, yielding C5 and C6 aldehydes, the main compounds identified in olive oil (Clodoveo et al. 2014). C5 and C6 volatile compounds were only affected by cultivar and fruit canopy position. Both families of volatile compounds demonstrated to be higher for Cerasuola and upper layers of the canopy.

Table 10 Main effect of cultivar, planting density and fruit canopy layer position on oil chlorophyll and carotenoid content, ratios of unsaturated and saturated (UFA/SFA); and monounsaturated and polyunsaturated (MUFA/PUFA) fatty acids, main phenols and C5 and C6 volatiles. Oil content in chlorophyll, carotenoids, 3,4-DHPEA-EA, 3,4-DHPEA-EDA, p-HPEA-EDA, p-HPEA-EA, hydroxytyrosol, tyrosol, sum of total phenols, sum of C5 volatiles and sum of C6 volatiles represented in $mg.kg^{-1}$. Values with different letters represent significant differences between each independent variable by Tukey's test at $p<0.05$ (n=12).

	Cultivar		Planting density		Canopy layer	
	Cerasuola	Koroneiki	D1	D2	Upper	Lower
Chlorophyll	15 a	13.8 a	13.7 a	15.1 a	11.4 b	17.3 a
Carotenoids	11.2 a	10.4 a	10.3 a	11.3 a	9 b	12.7 a
UFA/SFA	5.7 a	5.3 b	5.5 a	5.4 a	5.1 b	5.8 a
MUFA/PUFA	5.4 b	7.6 a	6.5 a	6.5 a	6.7 a	6.2 b
3,4 DHPEA-EA	243.1 b	366.1 a	343 a	266.2 b	378.1 a	231.1 b
3,4 DHPEA-EDA	67.8 a	20.1 b	47 a	41 b	47.4 a	40.6 b
p-HPEA-EDA	23.7 a	23.7 a	23.8 a	23.6 b	23.7 a	23.7 a
p-HPEA-EA	123.1 b	129.4 a	133.8 a	118.6 b	138.9 a	113.6 b
Hydroxytyrosol	10.5 b	18.4 a	12 b	16.9 a	11.9 b	17 a
Tyrosol	25.9 b	34 a	30.7 a	29.2 a	29.6 a	30.3 a
∑ Phenols	607.7 b	691.1 a	694.8 a	604 b	738 a	560.8 b
∑ C5	0.5 a	0.3 b	0.4 a	0.4 a	0.5 a	0.3 b
∑ C6	22.6 a	8 b	13.2 a	17.5 a	21.3 a	9.3 b

Interaction between cultivar, planting density and fruit canopy position were significant for fatty acid ratios, phenolic and volatile composition of the oils (Table 11). The upper layer presented the lowest values of UFA/SFA and those values are influenced by the planting density. Longer distances among trees (D2) originated the biggest differences of UFA/SFA

between upper and lower canopy layers. Cerasuola always presented the highest values of UFA/SFA except for the upper layer at D2, were Koroneiki showed the highest values. For the ratio MUFA/PUFA Cerasuola evidenced the lowest values and showed to not be affected by planting distance nor fruit position in the canopy. Differently, Koroneiki showed the highest values on the upper layers of the canopy and the lowest values at the lower layer of the canopy at density D2. These results demonstrate that less dense planting systems allow an increase of monounsaturated fatty acids at the upper layers of the canopy for the cultivar Koroneiki. Previous reports relate lower fruit temperatures along oil accumulation to an increase of oleic acid (Di Vaio et al. 2013; Bodoira et al. 2016). In this study, longer distance between trees may consent further vegetative growth, where fruits may grow in inner sides of the canopy and less exposed to direct irradiation with higher temperatures, increasing the concentration of oleic acid. Koroneiki showed significantly higher phenolic content and both cultivars were equally influenced by planting density and fruit canopy position (Table 11). Upper layers of D1 show the highest phenol content, followed by upper layers of D2 and lower layers of D1. The lower layers of D2 demonstrate the lowest phenol content. Volatiles C5 of cultivar Cerasuola were significantly affected by planting density and fruit canopy position, with the highest values at the D2 and upper layer of D1. No significant interaction between planting density and canopy layer were found for C5 volatiles on Koroneiki. The C6 volatiles show to be highest in the upper layers of Cerasuola cultivar and lowest values were found in the lower canopy layers at D2 of the cultivar Koroneiki.

Table 11 Interaction of the independent variables cultivar, planting density and fruit canopy layer position on oil chlorophyll and carotenoid content, ratios of unsaturated and saturated (UFA/SFA); and monounsaturated and polyunsaturated (MUFA/PUFA) fatty acids and main phenols. Chlorophyll, carotenoids, 3,4-DHPEA-EA, 3,4-DHPEA-EDA, p-HPEA-EDA, p-HPEA-EA, hydroxytyrosol, tyrosol, sum of total phenols, sum of C5 volatiles and sum of C6 volatiles represented in $mg.kg^{-1}$. Values with the same letters are not significantly different by Tukey's test at $p<0.05$ (n=3).

Cultivar	Cerasuola				Koroneiki			
Planting density	D1		D2		D1		D2	
Canopy layer	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Chlorophyll	13.1 a	17.3 a	9.5 a	20 a	10.2 a	14.1 a	13 a	18 a
Carotenoids	10.2 a	12.5 a	8 a	14.2 a	7.8 a	10.7 a	9.9 a	13.2 a
UFA/SFA	5.7 b	6.3 a	4.7 d	6.2 a	5 cd	5.2 c	5.2 c	5.7 b
MUFA/PUFA	5.4d	5.4 d	5.2 d	5.6 d	8 ab	7.1 bc	8.3 a	6.7 c
3,4 DHPEA-EA	368 b	187 d	248 c	169.2 d	434 a	383.2 b	462.4 a	185.1 d
3,4 DHPEA-EDA	75.1 a	70.3 b	66.9 b	59 c	21.6 e	20.8 e	25.9 d	12.3 f
p-HPEA-EDA	23.8 a	23.8 a	23.5 a	23.6 a	23.8 a	23.7 a	23.7 a	23.7 a
p-HPEA-EA	115.7 d	137.5 bc	126.7 cd	112.5 d	165.2 a	117 d	147.8 b	87.4 e
Hydroxytyrosol	9.7 e	9.8 e	15 d	7.5 f	6.7 g	21.7 b	16.1 c	29.2 a
Tyrosol	30.5 b	25.2 bc	22.3 c	25.8 c	27. bc	39.9 a	38.2 a	30.5 b
Σ phenols	733.8 c	564 e	626.1 d	507 f	776.3 b	705.2 c	816 a	467 g
Σ C5	0.6 a	0.2 b	0.6 a	0.7 a	0.3 b	0.3 b	0.3 b	0.1 b
Σ C6	21.2 b	13.5 bc	43.1 a	12.8 bc	10.4 bc	7.9 bc	10.7 bc	3.1 c

The quantitative descriptive sensory profiles for the oils extracted for this study are described in Table 12. From a sensory point of view, all the samples are classified as extra virgin olive oil with medium intensity of the positive attributes. The attributes artichoke, almond and tomato very commonly found in Sicilian olive oils characterized the oils studied. In particular, Cerasuola oils are characterized by higher artichoke, almond, tomato and oregano then Koroneiki. While Koroneiki oils were distinguished by low fruity and banana attributes. Bitter and pungent sensations were previously positively related to the amount of secoiridoid compounds. In particular, p-HPEA-EDA is known to be responsible for pungency in the oil (Andrewes et al. 2003). Moreover, most C5 volatile compounds in addition to the C6 ones contribute to almond (mainly trans-2-hexenal), tomato (mainly trans-2-hexen-1-ol) and banana (mainly cis3-hexenyl acetate) odour notes (Angerosa et al. 2000). In our study, values of p-HPEA-EDA were not affected by cultivar, planting density or canopy layer even if the oils extracted show slightly increase in pungency on upper layers and D1. The contribution

of volatile compounds to the overall aroma of virgin olive oil depends not only on their concentration but also on their sensory threshold values (Angerosa et al. 2004; Kalua et al. 2007). Consequently, high concentration of volatile compounds are not necessarily related to a major contribution to the oil aromas (Angerosa et al. 2004; Essid et al. 2016).

Table 12 Quantitative descriptive profile for sensory evaluation of Cerasuola and Koroneiki oils obtained from two planting densities and two fruit canopy position. Numbers represent the median of the attributes by eight panellists according to the standard stablished by the international olive council (COI/T.20/DOC.15/Rev.10). The letters 'nd' represent descriptors not detected by the panellists.

Cultivar	Cerasuola				Koroneiki			
Planting density	D1		D2		D1		D2	
Canopy layer	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Fruity	5	6	5	5.5	3	3	3	2.8
Bitter	5	6	5.5	5	5	5	5	4
Pungent	6	5.5	6	5	5	4	4	3
Artichoke	3	3.5	3	3.5	2	nd	2	nd
Almond	2	2	2.5	nd	1	nd	1	nd
Grass	2.5	3	nd	3	nd	nd	nd	nd
Green tomato	2.5	3	nd	3.5	nd	1	nd	nd
Banana	nd	nd	nd	nd	2	nd	1	1
Oregano	nd	1	nd	2	nd	nd	nd	nd
Chicory	nd	1	1	1	nd	nd	nd	nd

Conclusions

Given the relevance of harvest costs on the overall cost for olive oil production, tree density in newly planted olive orchards has been increasing steadily as part of the intensification of the olive tree cultivation. Data obtained in this study show that higher planting density does not affect production in the studied cultivars. The Sicilian cultivar Cerasuola proved to adapt to higher density orchards, without losing the quality and particular organoleptic attributes. Our results also demonstrate that higher planting densities increase Cerasuola productivity but had no effect on Koroneiki. Upper layers of the canopy were characterized by higher crop load and more mature fruits with higher fat content. All studied variables had an influence on fatty acid, phenols and volatiles composition. Fruit canopy position showed to be the variable that influenced the majority of the factors studied. The differences in production, fruit maturity and fat content between upper and lower layers increased for shorter distances between trees; while differences in oil quality between treatments did not followed the same trend for both cultivars, showing that genetic factors interact with environmental conditions to influence oil quality. Taken together, the impact of the interactions of cultivar, planting density and fruit canopy position on oil quality and sensory properties established in this study provides a new insight into the relationships between yield and structure in moders high density cultivation systems.

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Experiment 3.

Influence of fruit canopy position and maturity on chemical composition of virgin olive oil

Abstract

Maturity and light environment are relevant variables determining olive fruit physiology. However, the interaction between canopy position and maturity has not been studied yet. Olive fruits from upper and lower layers of the canopy were harvested from September to January. Fruit maturity, weight, detachment force, size, moisture and fat content were measured together with minor components of the oil extracted, with the aim of determine the impact of the fruit position in the canopy and their stage of ripening on the decision factors for an optimal harvest time.

Differences in light interception between upper and lower layers of the canopy resulted in differences in the quantity of fruits and oil extracted. Upper layer presented 60% of the overall production; fruit had one unit more of maturity index, 3% less moisture and 5% more fat content and oil extracted had less oleic acid than lower layer of the canopy. Oil extracted from the upper layers presented higher concentration of secoiridoids derivatives, hydroxytyrosol and tyrosol. Fruits from upper layers at maturity index 2 showed 20% more fat together with higher total phenol content on the oil extracted when compared with fruits from lower layer with the same maturity index.

Harvest time and light environment influenced the morphometric and chemical characteristics of fruits and oil extracted. Differences between layers were not due to a maturity delay; instead, fruit position is a determinant factor for physiological processes related to fruit growth and maturity. Fruit fat content demonstrated to have a higher potential as harvest indicator rather than maturity index.

Introduction

Virgin olive oil is distinguishable from other oils due to its particular fatty acid composition and the presence of minor components such as phenolic compounds, which contribute to its unique flavour and important biological properties with nutritional value. The concentration and profile of these compounds have a wide range of variability as a result of genotypes, agronomic and environmental influences (Inglese et al. 2011; Bodoira et al. 2016). The most studied factors include cultivar, fruit maturation, irrigation and pruning. Previous studies have demonstrated that fruit growth and composition is significantly modified by the canopy position in vase-shaped and hedgerows olive canopies (Acebedo et al. 2000; Gómez-del-Campo et al. 2009; Trentacoste et al. 2015a). The effect of canopy position on fruit yield, size, maturity and oil content in Arbequina hedgerows was studied and the greater values of these parameters were observed on the upper layers of the canopy (Trentacoste et al. 2015b). Canopy position can also alter the commercial and nutritional value of the olive oil, modifying fatty acid composition and total phenol content (Gómez-del-Campo and García 2012; Caruso et al. 2017). A detailed study by Gomez-del-Campo et al. (Gómez-del-Campo and García 2012) demonstrated that fruits from higher layers of the canopy (with high light interception) yielded oil of higher stability against oxidation than fruits from lower layers of the canopy. This observation was explained by the higher composition of saturated fats and phenols in the oil extracted from the fruits in upper layers. Differences observed in fruits and oils from different canopy positions and orientations have shown to be related to the light intercepted on each area of the canopy (Gómez-Del-Campo et al. 2009b; Connor et al. 2012; Cherbiy-Hoffmann et al. 2015; Caruso et al. 2017). Cherbiy-Hoffmann et al. (Cherbiy-Hoffmann et al. 2015) demonstrated that shading of the fruit and leaves during maturation have consequences on oil accumulation; Trentacoste et al. (Trentacoste et al. 2017) showed that greater irradiance

increases fruit number due to more and longer fruiting shoots that result in higher number of flower sites.

A variety of methods has been proposed to determine the ideal harvest time in relation with the quantity and quality of olive fruits and oil. One of the most common method, by the International Olive Council (IOC), is the measurement of fruit external and internal color, allowing the calculation of the so-called maturity index (International Olive Council 2011). However, changes in the fruit color do not necessarily match oil composition (Scamosci et al. 2011; Trapani et al. 2015). Therefore, other methods have been developed using organic acids such the malic and citric acid ratio (Donaire et al. 1975), sugars such mannitol (Marsilio et al. 2001), degree brix (Migliorini et al. 2011), dry matter (Mickelbart and James 2003) or fruit respiration (Ranalli et al. 1998). Studies have also found that there are connections between fruit maturation and specific sterols and fatty acids present in the fruit pulp (Mendoza et al. 2013). Additionally non-destructive methods have been developed by measuring anthocyanin content in the peel (Agati et al. 2005) or using fruit external colour and texture (Garcia and Yousfi 2005). Olive oil quality parameters, such free fatty acidity, peroxide value, specific UV absorbance and sensory attributes have also been shown to change along fruit maturation (Dag et al. 2011a; Bengana et al. 2013).

Previous studies were performed to study how temperature (García-Inza et al. 2014), incident light (Cherbiy-Hoffmann et al. 2013; Connor et al. 2016), maturation or canopy position (Gómez-del-Campo and García 2012; Caruso et al. 2017) in the fruit influence productivity and composition on the oils extracted. However, there is a lack of information on how canopy management can influence fruit maturation along harvest season in order to understand if there are possible interactions between these factors. Therefore, the aim of this research is to investigate the effect of fruit canopy position on fruit maturation, oil composition and to determine which parameters are determinant on finding the ideal harvest time.

Material and methods

Plant material and experimental design. The study was carried out at the Wolfskill Experimental Orchard Field Station from University of California, Davis (Winters, California) (38°30'10.5"N, 121°58'37.6"W). Trees of Arbequina cultivar with 10 years old, managed under sprinkler irrigation were used in this experiment. The trees were trained to vase system (5m x 6m) with a single trunk with two main branches, oriented along the row, starting by 0.5 m height above ground. Two more couples of main branches were inserted at 150 and 230 cm above ground respectively. The average height of the trees was 3 m and the average canopy volume was 14 m3. Trees have been pruned every two-years according to standard procedure and were not pruned along the period of study. In September, 100 days after full bloom, 15 trees distributed in three different blocks in the field were selected based on similarities for fruit load, number of branches and light distribution in the canopy. To compare the fruit position in the canopy, the fruit itself was chosen to be above two meters from the ground (upper) or below two meters from the ground (lower). From middle of September 2017 to beginning of January 2018 (14/9; 28/9; 12/10; 26/10; 9/11; 23/11; 7/12; 21/12; 5/1), approximately 1kg of fruits from upper and lower parts of the canopies were sampled every 15 days, for a total of nine harvests and following morphological and chemical determinations performed together with oil extraction. Oils were stored at -5 ºC until quality measurements were performed. All the determinations followed described were carried out in duplicate for each block of trees (N= 6 for each canopy position).

The morphometric changes. Fruits from upper and lower canopy along maturation were assessed by fresh weight of 100 fruits (g), pulp, and pit; flesh/pit ratio and maturity index based on the degree of skin and pulp pigmentation according to Uceda and Frias (Uceda and Frias 1975). Fruit length and width were measured using a digital caliper (Neiko, model 01407A). Maturity index (MI) was calculated using two replicates of 100 fruits per canopy

layer of each block (n=12), weight (FWg), pulp/stone ratio, fruit detachment force (FDF), length (fruitL), width (fruitW) was calculated using 10 fruits per replicate (n=12).

Light interception. The ceptometer (model SF 80; Decagon Devices, Pullman, Washington) was used as a sensor that integrated readings of 80 light sensors placed at 1-cm intervals along an 80-cm-long probe. A microprocessor recorded an average value of all sensors along the probe at each reading, giving the value of photosynthetically active radiation (PAR) (µmol photon m^{-2} s^{-1}). Measurements were done on July 20 and 21, the period of the year with highest incident PAR. Ten below-canopy readings, ten half-canopy readings ($_{transmitted}$PAR), and one above-canopy ($_{incident}$PAR), open-sky reading were taken for each tested tree. A total of nine trees were used distributed in 3 blocks of 3 trees each in the field. A bubble level on the ceptometer and an extra bubble level tool was attached to the probe end were used to hand-position the probe horizontally to the orchard floor. All readings were taken from five hours before and five hours after solar noon on sunny, clear days. Intercepted light by canopy (*i*PAR) was calculated for each canopy position along daytime:

$$iPAR = {}_{incident}PAR - {}_{transmitted}PAR$$

Fruit detachment force (FDF). was measured using an Imada DPA-11 digital force gauge (Imada, Northbrook, IL). Two branches from both canopy positions of each block were removed and brought to the laboratory. Ten olive fruit of each branch were inserted into the gauge and the pedicel pulled parallel to the fruit axis until it separated from the fruit. The force necessary to remove fruit from the pedicel was measured in grams of force.

Moisture content. Olive paste (60 ± 0.1 g) or olive pomace (100 ± 0.1 g) were weighed in a 600 ml beaker and placed in the oven at 105 ºC until constant weight. The beaker was transferred to a desiccator and the weight of the dry paste registered at room temperature.

Fat content. Previously dried sample (paste or pomace) from moisture analysis (20 ± 0.1 g) was weighed in a cellulose extraction thimble, placed in the Soxhlet extractor, and extracted using *n*-hexane for 6 h. Once the extraction finished, solvent was distilled in a rotary evaporator and residual solvent was eliminated from the oil by placing it in an oven at 105 ºC for 3 h. Fat content (Fc) was expressed as wet basis and calculated according to: $Fc = Fc_{drybasis}/[1-(Mc/100)]$ where Mc is the moisture content of paste/pomace and $Fc_{dry\ basis}$ the fat content of paste/pomace expressed in dry basis.

Olive oil extraction. Olive oil was extracted using a laboratory scale extraction system (Abencor analyzer, MC2 Ingenieria y Sistemas S.L., Seville, Spain). Olives were crushed in a hammer mill and 700 g of paste with 20 g of talc were malaxed for 45 min. After malaxation, olive paste was centrifuged for two min in the basket centrifuge. Obtained oil was separated by decantation. Samples were later centrifuged at 4000 rpm for 10 min and stored at -20ºC in dark containers using plastic bottles without head space.

Efficiency. Extraction efficiency was calculated as follow:

$$\text{Extraction efficiency (\%)} = (0.915 \times V_{oil})/(m_{olives} \times Fc_{olives}) \times 100$$

Where V_{oil} is the volume of oil extracted, m_{olives} is the mass of olives in grams and Fc_{olives} is the fat content of the same olives.

Quality parameters. Free fatty acids (FFA), peroxide value (PV), and UV absorbances (K_{232}, K_{270}) were determined according to AOCS standard methods Ca 5a-40 (09), Cd 8b-90(09) and Ch 5-91(09) (American Oil Chemist's Society, 1998), respectively.

Chlorophylls. Chlorophylls were determined measuring the absorbance at 670 nm, correcting the results for background absorption at 630 and 710 nm, according to AOCS method Cc 13i -96 (09) (American Oil Chemist's Society, 1998).

Fatty acid profile. Oil sample (0.010 ± 0.001 g) was weighed in a 12 mL amber vial and dissolved in toluene (0.4 mL). Methanol (3 mL) and methanol/HCl (0.6 mL, 80:20, v/v) were added. The sample was then kept at 80 °C for 1 hr in a heat stock and hexane (1.5 mL) and nanopure water (1 mL) were then added and vortexed. The upper layer containing the methyl esters was decanted into a 1.5 mL Eppendorf tube with the aid of a glass transfer pipet. Anhydrous sodium sulfate was added to dry out water residue. The clear solution was then transferred into GC vials for further injection. GC analysis was conducted on a Varian 450-GC (Agilent Technologies, Santa Clara, CA, USA) equipped with a Flame Ionization Detector (FID). Helium was used as carrier gas at a flow rate of 1.5 mL/min. Fatty acids were separated on a 60 m x 0.25 mm x 0.25 μm DB-23 capillary column (Agilent Technologies, Santa Clara, CA, USA) with the injector held at 270 °C at a split ratio of 1:100. The GC oven program was initially held at 100 °C for 8 min, ramped at 6.5 °C/min to 160 °C, ramped at 2.7 °C/min to 215°C and held for 12 min, ramped at 15 °C/min to 230 °C and held for 10 min. FID temperature was 280 °C. The injection volume was 1 μL. Quantification was achieved by area normalization.

Phenolic profile composition. For extraction of phenolic compounds a sample (2.0 ± 0.1 g) of olive oil was dissolved in 5 mL of methanol/water (80/20, v/v), mixed for 2 minutes and centrifuged for 20 min at 5000 rpm, supernatant was collected and extraction repeated two times. Samples were filtered through a syringe filter of 0.45 μm and capped at -20°C until analysis. The analysis condition was adopted from Daskalaki et al. (Daskalaki et al. 2009). The sample injection was 20 μL and the flow rate was 1 mL.min-1. A 5 μm, 250 mm x 4.6mm C18 column (agilent Technologies, Santa Clara, CA, USA) was used for a HPLC with a diode array detector (DAD). In this analysis, the mobile phase A was water/acetic acid (97:3, v/v) and B was methanol/acetonitrile (1:1, v/v). The solvent gradient changed according to the following conditions: from 0 to 25 min, 95%A - 5%B to 70%A – 30%B; from 25 min to 50

min, 65%A – 35%B; from 50 min to 65 min, 30%A-70%B in 5 min. The diode array detector was performed at 280 nm and 340 nm. The pick identification was done by comparison with commercial standards while the quantification was determined by using relative concentration to the internal (syringic acid) and external standards (tyrosol and syringic acid).

Volatile profile. Sample (1.0 ± 0.1 g), spiked with 4-methyl-2-pentanol as internal standard (2.5 mg/kg), was weighed into a 20 mL glass vial (Agilent Technologies, Santa Clara, CA) and sealed with a PTFE/silicon septum (Supelco, Bellefonte, PA). After 10 min at 40 °C, a solid-phase micro extraction (SPME) fiber (DVB/CAR/PDMS, Sigma-Aldrich, St. Louis, MO) was exposed to the sample headspace for 40 min for volatile extraction. The volatile compounds analysis was performed on a Varian 450-GC equipped with a Varian 220-MS ion trap (Agilent Technologies, Santa Clara, CA). A Supelcowax 10 (30 m x 0.25 mm x 0.25 μm, Sigma-Aldrich, St. Louis) was used for compounds separation. After sampling, the fiber was thermally desorbed in the GC injector for 5 min at 260 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. GC oven temperature started at 40 °C and ramped at 3 °C/min after 10 min to the final temperature of 200 °C. Ionization energy of 70 eV was adopted and the ions were analysed in the m/z range from 40 to 400. The data were recorded and analysed using MS Workstation v6.9.3. Volatile compounds were identified by their mass spectra and using Kovatz retention index (KI).

Statistical analysis. The data were analysed using Systat (13) to perform analysis of variance (ANOVA) with $p \leq 0.05$ to identify significant differences among all parameters analysed in the olive oils from different harvest dates.

Results and Discussion

The year of 2017 was characterized by high comulated precipitation in the months of January and February, with 423.7 and 324.87 mm respectively. The coolest month was January (average 15ºC), while the hottest month was July (average 36.9 ºC). During the period of study temperatures range from 40 ºC in September to -3 ºC in December (Figure 5). Cumulative rainfall from September 1 to January 5 was 63.2 mm. The first rainfall was registered in November 19 and the last on January 5. The higher amount of rainfall within harvest dates (23.4 mm) occurred before the harvest on November 23.

Diurnal profile of light interception (Figure 6) showed that the upper layer of the canopy intercepted in average 80.5 % of incident PAR while the lower layer intercepted 9.5% of incident PAR along the day. Diurnal courses of PAR also showed that light availability to the lower layer of the canopy was constant throughout the day with the maximum interception at noon and 6 pm, but not at solar noon as observed with the upper layer. The reduced PAR level in the lower canopy layer that can take to changes in environmental conditions for the fruits such as temperature and relative humidity, influenced the fruit growth causing some morphological and biochemical changes in comparison with fruits exposed to higher irradiance.

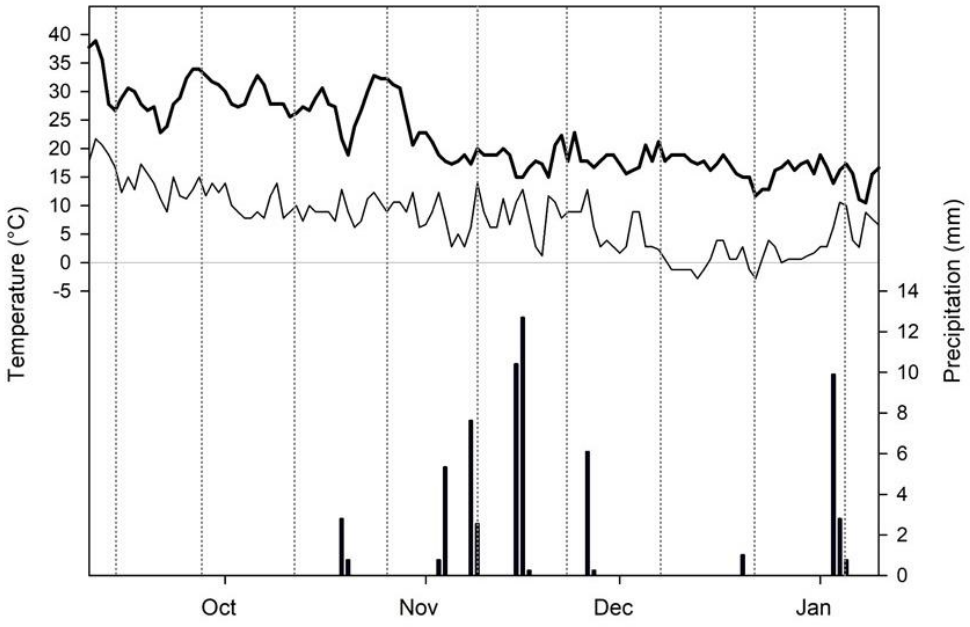

Figure 5 Average daily maximum (bold line) and minimum temperature (thin line) and cumulative daily rainfall along the period of study. The vertical dotted lines represent harvest dates (09/14; 09/28; 10/12;10/26t; 11/9; 11/23; 12/7; 12/21; 01/05).

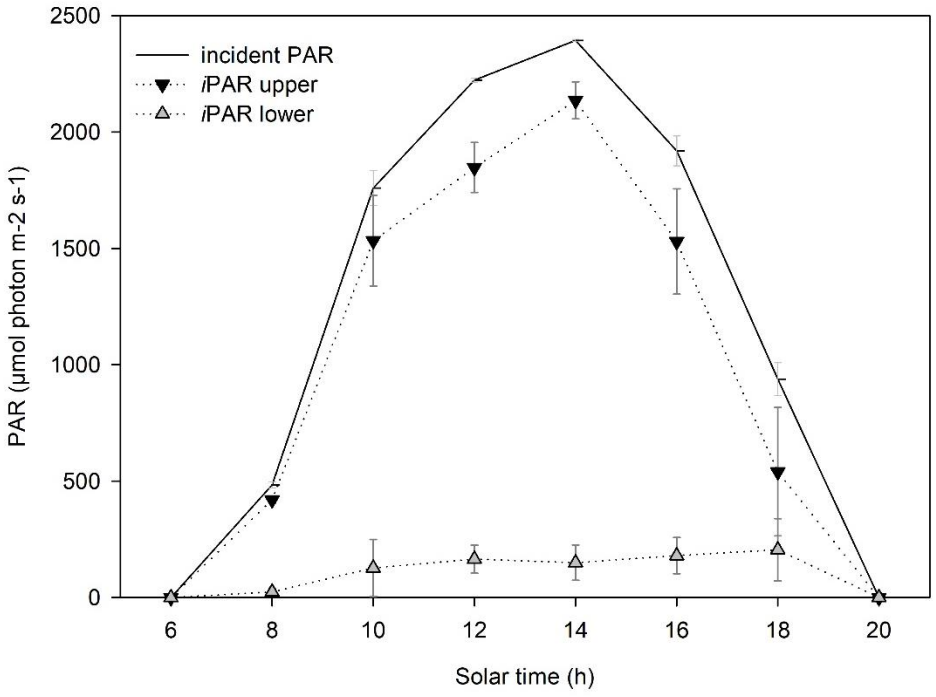

Figure 6 Diurnal courses of incident photosynthetically active radiation (incident PAR) and intercepted PAR (iPAR) at upper and lower canopy positions.

At November 9, two trees per block were harvested to calculate total yield on upper and lower layers of the canopy. Lower area of the canopy produced 7.6 (±2.9) kg of fruits while the upper side of the canopy presented a statistically higher production (ρ=0.017) with an average of 11.4 (±4.1) kg of fruits per tree.

Maturity index (Table 13) was consistently higher at the upper layer of the canopy (p=0.000), where it ranged from 0 to 2.7. The range for the lower layer was 0 to 1.6. Cherbiy-Hoffman et al., (Cherbiy-Hoffmann et al., 2013) studied the effect of different percentages of PAR intercepted by canopy of Arbequina cultivar and reported that maturity index was delayed by one unit value when only 3% PAR to 70%PAR was intercepted, which is the same range of values measured in our study for the lower layer of the canopy.

Fruit weight increased more drastically until the end of October, from 0.5g to 1.3g (Table 1). After this date, no significant increment in fruit weight was detected. Regarding the difference between the upper and lower layers of the canopy, the upper layer presented fruit with 30% higher fruit fresh weight (p=0.000). These results are in accordance with previous studies performed in high density orchards of Frantoio and Arbequina cultivars (Caruso et al., 2017; Cherbiy-Hoffmann et al., 2015, 2013; Connor et al., 2016).

As a general trend, pulp to stone ratio increased with harvest time of the olive fruit. A slight decrease in this parameter was observed between the second week of December and beginning of January. A possible explanation for this decrease is the low temperatures in this period of the year (Figure 5) that cause chilling injury to the fruits. There were no differences in pulp to stone ratio between upper and lower layers of the canopy. Our results demonstrate that cell division at younger stages of fruit development is a determinant factor for the fruit grown and production at harvest. In fact, Rapoport et al., (2004) reported that fruit size in the cultivar Arbequina is largely determined by cell number.

Fruit detachment force (FDF) remained mostly unaffected by harvest time or canopy position. In a previous study by Farinelli et al., (2012) on Arbequina cultivar in Italy, FDF/fruit weight ratio demonstrated a linear relationship with fruit yield using mechanical harvest. Contrary, our results show no differences along maturation in FDF and fruit weight after October, make evident that at the conditions on this study, FDF/fruit weight should not be consider for Arbequina as a determinant factor for increasing productivity and quality at harvest date. Fruit length and width increased until 9/11, decreasing afterwards until January. In respect to canopy position, the fruits located in the upper layer presented larger values for both length and width.

Moisture content of the fruits decreased with harvest time except for 9/11 and 5/1, when it increased. This observation is in accordance with the precipitations occurred before those two harvests. In respect to canopy position, fruit located in the lower layer presented higher moisture content (57.1%) than fruits from upper layer (54.3%) with $\rho < 0.000$.

Fat content increased with harvest time. As reported in literature (Migliorini et al., 2011) the increment was more pronounced at the beginning of harvest season, reaching a plateau by early December. Fruit located at the upper layer of the canopy showed a significant higher fat content than the lower layer. A higher oil content in fruits exposed to more incident light was previously reported by Bartolini et al., (2014) and explained by a higher number of oil bodies inside the mesocarp since the beginning of oil accumulation. Our results also demonstrated that canopy position and subsequent reduced intercepted PAR had a negative effect on fat content in the fruit. In the upper layer of the canopy where PAR intercepted was from 0% to 80%, the fat content was 40.7%; and in lower side of the canopy where the range for PAR intercepted was from 0 to 9.5%, the fat content was 33.5%. This is in resonance with previous research (Cherbiy-Hoffmann et al., 2013; Connor et al., 2016; Gómez-del-Campo and García,

2012; Trentacoste et al., 2016); Cherbiy-Hoffman et al., (2013) showed that oil accumulation in the fruit increased linearly with PAR intercepted by the canopy up to 40% of incident PAR. Extraction efficiency was not significantly affected within the canopy position ($p=0.123$), 51.3% using the fruits from the lower layer and 55.7% using the fruits from the upper layer positions. However, the two harvest dates with higher extraction efficiency were 26/10 and 21/11 for both upper (63.8% and 78%) and lower (71.3% and 80.1%) layers of the canopy, possibly due to the combination of high fat content and low moisture content.

Results for quality parameters are reported in Table 13. Quality parameters were unaffected by canopy position of the fruit. Regarding the impact of harvest time, values for FFA, PV, K_{232}, K_{270} and ΔK are consistent with extra virgin category according to International Olive Council standard. These results are expected since the fruit was cautiously harvested by hand and the oil was extracted within 3 hours after the harvest.

Chlorophylls are responsible for the green color of olive drupes and are mainly located in the skin of the fruit, where the higher photosynthetic activity is observed (Roca and Mínguez-Mosquera, 2001). Results for chlorophyll content are detailed in table 13. Chlorophylls concentration was not affected by canopy position of the olive fruit. In accordance with previously reported experiments (Roca and Mínguez-Mosquera, 2001; Salvador et al., 2001), chlorophylls content decreased considerably with harvest time from 72.1 ppm at the beginning of the experiment down to 30.1 ppm on 26/10. After that date, values remain constant until January. The decrease of chlorophyll content was previously found and explained by the transformation of chlorophyll (a) and chlorophyll (b) into pheophytin (a) and pheophytin (b) changing the olive oil from green to yellow coloration (Bengana et al., 2013; Benito et al., 2013).

Total phenols values are reported on Table 13. As a general trend, total phenols concentration increased along harvest season with minimum of 70 ppm to a maximum of 123 ppm. Since

the maximum maturity index reached in our study was 3, our results are in accordance with Kalogeropoulos and Kaliora (2015) that described at the stage between the green and purple skin, the olives have the highest phenolic compound content. Regarding canopy location of the fruit, the upper layer had higher values of total phenols (107 ppm) compared to the lower layer (85 ppm). This also agrees with previous studies done in Arbequina comparing different canopy fruit position (Gómez-del-Campo and García, 2012; Kalogeropoulos and Kaliora, 2015).

The main fatty acids identified in the samples were palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0) and gadoleic (C20:1) acids. Palmitic, oleic and linoleic acids were the main fatty acids identified in all the samples, being oleic acid the most abundant compound (Table 14). The upper layer of the canopy presents higher values for oleic acid than the lower layer. Oleic acid increased with sampling time from 66.2% in 14/9 to 71.3% in 21/12. Saturated fatty acids (SFA) and unsaturated fatty acids (UFA) showed no differences between upper and lower layers of the canopy. SFA decreased along maturation, apart from the last sample, in January, when an increment was observed. UFA increased with sampling time, with an exception for the last sample, in January, where a decrease in its value was observed. Mono-unsaturated fatty acids (MUFA) showed differences between canopy position ($p=0.011$) with the lower layer having the highest values. Along sampling time, an increment was observed, except for the last sample, collected in January, where a decrease was observed. PUFA was affected by canopy position ($p=0.000$); the upper layer had the highest values. The lower layer showed higher MUFA/PUFA ratio when compared to the upper layer ($p=0.000$). It was previously reported for cultivar Arbequina that oleic acid remained constant and MUFA/PUFA ratio decreased, along maturation, due to an increase of linoleic acid by the action of the enzyme oleate desaturase that actively transformed oleic acid into linoleic (Castillo-Ruiz et al., 2015), though

in our study there are no significant changes along maturation for this parameters. Regarding canopy position, our results are in agreement with previous results in the cultivars Arbequina (Gómez-del-Campo and García, 2012) and Frantoio (Caruso et al., 2017) were significant lower values of oleic acid together with higher values of linoleic and linolenic acids in lower layers of the canopy. A linear correlation was previously found between temperature increase and decrease of oleic acid and increase of palmitoleic, linoleic and linoleic acids (García-Inza et al., 2014). While temperature was not measured in this study, we can assume that temperature range is higher in the upper layer of the canopy and temperature variations influenced fatty acid composition among canopy positions.

VOO phenolic fraction is mainly constituted by oleuropein and ligstrosides derivatives that are partitioned into the olive oil during the extraction process (Romero-Segura et al., 2012). Table 15 shows the concentrations of the main phenolic compounds expressed as mg per kg of VOO samples obtained at the upper and lower layers of the canopy at different sampling date of the olive fruit. The main secoiridoid compound presented in the olive oil was dialdehydic form of oleuropein aglycone (DAFOA), followed by dialdehydic form of ligstroside aglycone (DAFLA), aldehydic form of oleuropein aglycone (AFOA) and finally aldehydic form of ligstroside aglycone (AFLA). Both oleuropein and ligstroside derivatives presented higher concentration at the upper layer of the canopy ($p<0.04$). Regarding sampling data, DAFOA, AFOA and AFLA showed an increment up to 21/12, decreasing by around 15% for the last harvest, on 5/1. DAFLA showed an opposite trend, decreasing with harvest time. Pinoresinol was the most concentrated phenolic compounds after DAFOA. No differences on its concentration were detected regarding canopy position. Regarding sampling time, concentration peaked on 26/10 at 26.1 mg kg^{-1}. Flavonoid Luteolin concentration was significantly higher on the upper layer of the canopy, where the mean concentration was 14.3 mg kg^{-1} compared to 6.2 mg kg^{-1} at the lower layer. Regarding harvest time, luteolin

concentration increased from 4.9 mg/kg to 15 mg/kg by 21/12, slightly decreasing for the last harvest in January. Following the same result observed for luteolin, flavonoid apigenin concentration was significantly higher at the upper layer of the canopy. Starting at 2.5 mg kg^{-1}, apigenin concentration peaked on 23/11 at 3.4 mg kg^{-1} and slightly decreased by the beginning of January at 3.2 mg kg^{-1}. Phenolic alcohols presented very low concentration in accordance to previously reported results (Monasterio et al., 2016). Statistically differences ($p=0.000$) were found for the upper and lower layer of the canopy, were in both cases the upper layer presented slightly higher values. Regarding sampling time, hydroxytyrosol (H-tyr) peaked on 26/11 decreasing on 7/12 and remaining constant up to the beginning of January. Tyrosol (Tyr) show no differences along harvest season except for a slightly decrease until 0.8 mg/kg on the beginning of January. In our study simple phenols in the oil extracted do not demonstrate a particular trend along maturation remaining unchanged or change slightly from September to January as found in previous research (Romero-Segura et al., 2012). Vanillic, *p*-coumaric, *o*-coumaric and ferulic acids were the main phenolic acids detected in the samples. For vanillic and *o*-coumaric acids, there was no differences between the upper and lower layers of the canopy. Differently, ferulic and p-coumaric acid concentrations were higher on the upper layer of the canopy ($p<0.009$). The concentration of the four compounds was affected by harvest time ($p=0.000$), all of them presenting a concentration peak on 26/10.

C5 and C6 volatile are responsible for the positive green sensory attributes of VOO (Aparicio and Morales, 1998). These compounds are generated during crushing and malaxation through a cascade of enzymatic reactions known as the LOX pathway (Clodoveo et al., 2014). The concentrations of C5 and C6 volatile compounds (expressed as mg of 4-methyl-2-pentanol/kg) in oils extracted from olives located at the upper and lower layer of the canopy at different harvest times are detailed in Table 16.

As observed before in Arbequina cultivar (Breton et al., 2009; Clodoveo et al., 2014), C6 volatiles presented the highest concentration in all the samples, with (E)-2-hexenal being the major volatile compound with a concentration range between 13.4 and 44.7 mg kg^{-1}. The highest concentration of (E)-2-hexenal occurred in 14/9 and the lowest occurred in 7/12. No differences were observed for the upper and lower layer of the canopy for both groups of volatile compounds. Regarding the impact of harvest time, C5 and C6 compounds decreased drastically (aprox. 50%) between 28/9 and 26/10 and continued to decrease more slowly until the beginning of January. This is in agreement with the study by Aparicio and Morales (Aparicio and Morales, 1998), who found the decreasing of volatiles along maturation in mainly due to a decrease of the (E)-2-hexenal, the volatile found in higher concentration.

The impact of both harvest time and canopy position on maturity index, weight, moisture and fat content in the fruit and total phenols in the oil are presented in Figure 7. No differences on maturity index were observed until 12/10. After that, fruit located on the lower layer of the canopy showed a significantly lower maturity index when compared with the upper layer of the canopy ($p=0.000$). Fruit weight followed the same trend as maturity index. However, differences between canopy positions were significant only after 26/10 ($p= 0.000$). Moisture and fat content followed similar though opposite trends regarding sampling time and canopy position of the fruit. Nevertheless, moisture content showed a slightly increase in both sampling in November due to more precipitations during that month. Fat content was not affected by rainfall, which is in agreement with previous studies suggesting that oil accumulation is mostly independent of climatic variations, and oil yield depend on the initial slope of oil accumulation in the fruit at more young maturity stages (Bartolini et al., 2014; Breton et al., 2009).

Differences on total phenols between upper and lower layers of the canopy were statistically significant after 26/10: the total phenols content of the oils extracted from fruits located in the

upper layer of the canopy increased at a higher rate compared to those extracted from the lower layer of the canopy.

Fruits from upper and lower canopy have differences morphologically and biochemically along maturation at each sampling date. Moreover, maturity index is widely accepted as a predictor of olive oil quality. To further understand the differences observed in fruit and oil extracted from different canopy positions, samples were grouped and analysed according to their maturity index (0 to 2) regardless of the harvest date (Figure 8). This allows us to see if the changes along maturity were due to different exposure to environmental conditions (e.g. incident PAR) that change the fruit biochemical and oil chemical characteristics or due to a delay in the fruit maturity itself. Samples classified with maturity index 0 and 1 showed no significant differences between upper and lower layer of the canopy for fruit weight, moisture content, fat content, total phenols, H-tyr, Tyr, secoiridoids and lignans in the oil. Therefore, differences among sampling dates at the beginning of the season were due to a delay on maturity. However, when samples were classified with maturity index 2, their fruit weight, fat content, total phenols and secoiridoids, presented significant differences according to canopy position ($p<0.032$). According to these results, olive fruits with the same maturity index can have different compositions, determined by its position in the olive tree canopy. Our results suggest that weight and fat content of the fruit reflect better the fruit and oil biochemical characteristics than fruit maturity index that is based on skin and flesh color changes.

Table 13 Maturity index (MI) (of 100 fruits per replicate), weight (FWg), pulp/stone ratio, fruit detachment force (FDF), length (fL), width (fW) of 10 fruits per replicate. Moisture content (%moisture), fat content (%fat at dry basis) of the fruit. Oil quality parameters on upper (n=6) and lower (n=6) layers of the canopy at nine harvest dates from September to January. Coefficient of specific extinction at 232 nm (K232), coefficient of specific extinction at 268 nm (K268), Free fatty acids (FFA) expressed as percentage of oleic acid, peroxide value (PV) as meq O2/kg, chlorophyll content (CHL) and total phenols are expressed in ppm.

Parameter	Upper	Lower	14/09	28/09	12/10	26/10	09/11	23/11	07/12	21/12	05/01
MI	1.4 ±1	0.8 ±0.8	0 ±0	0.3 ±0.3	0.6 ±0.4	0.8 ±0.6	1 ±0.4	1.3 ±0.6	1.4 ±0.6	2.3 ±0.7	2.5 ±0.5
FWg	1.3 ±0.4	1 ±0.3	0.5 ±0.2	1.1 ±0.2	1.1 ±0.2	1.3 ±0.3	1.3 ±0.2	1.3 ±0.2	1.3 ±0.2	1.3 ±0.2	1.4 ±0.2
Pulp/stone	2.9 ±0.6	2.6 ±0.5	2.2 ±0.2	2.3 ±0.4	2.3 ±0.2	2.4 ±0.4	2.8 ±0.3	2.8 ±0.4	3.2 ±0.5	3 ±0.6	3.4 ±0.4
FDF	0.4 ±0.2	0.4 ±0.2	0.5 ±0.1	0.5 ±0.1	0.4 ±0.2	0.4 ±0.2	0.4 ±0.2	0.4 ±0.1	0.4 ±0.2	0.4 ±0.2	0.4 ±0.1
fL	14.6 ±1.1	13.6 ±1.0	13.6 ±0.8	13.8 ±1.2	13.6 ±1.1	14.2 ±1.1	14.9 ±1.2	14.8 ±1.2	14.5 ±1.3	14 ±1.3	14 ±1.1
fW	12.5 ±1.0	11.5 ±0.9	11.4 ±0.8	11.6 ±1	11.5 ±1	12.1 ±0.9	12.7 ±1.1	12.7 ±1.1	12.4 ±1.2	12 ±1.1	12 ±0.9
%moisture	54.3 ±4.3	57.1 ±3.7	62 ±2.3	60.6 ±2.4	58.3 ±1.9	55.8 ±2	56.8 ±2.7	55.8 ±2.5	51.3 ±3.1	50.5 ±2.1	53.4 ±2.4
%fat (DB)	40.3 ±7	33.5 ±6.4	21.5 ±4.4	29.9 ±4.8	33.8 ±2.9	35.3 ±5	38.2 ±4.3	38.6 ±3.9	44.1 ±5.5	41.9 ±7.5	41.0 ±5.2
FFA	0.16 ±0.1	0.15 ±0.1	0.15 ±0.1	0.15 ±0.1	0.15 ±0.1	0.14 ±0.1	0.25 ±0.2	0.13 ±0.1	0.13 ±0.1	0.15 ±0.1	0.15 ±0.1
PV	6.1 ±4.3	6.19 ±3.4	6.19 ±3.4	3.33 ±1.4	5.5 ±0.6	7.05 ±1.9	4.95 ±1.5	3.87 ±0.9	6.82 ±2.8	9.07 ±3.2	5.26 ±1.4
K_{232}	1.25 ±0.11	1.25 ±0.09	1.25 ±0.09	1.31 ±0.1	1.21 ±0.04	1.31 ±0.04	1.28 ±0.02	1.26 ±0.05	1.24 ±0.09	1.26 ±0.11	1.11 ±0.11
K_{268}	0.13 ±0.08	0.13 ±0.09	0.13 ±0.09	0.31 ±0.12	0.11 ±0.01	0.11 ±0.02	0.11 ±0.01	0.1 ±0.02	0.12 ±0.03	0.1 ±0.01	0.09 ±0.02
CLH	35 ±16.6	39 ±14.4	72.1 ±3.3	71.4 ±6.5	43.1 ±3.5	30.1 ±7.6	28.7 ±8.2	31 ±8.7	33.9 ±6.1	28.8 ±8.7	26.6 ±5.9
Phenols	107 ±22	85 ±16	70 ±14	75 ±12.9	88 ±10	95 ±16	93 ±16	109 ±19	107 ±17	123 ±19	105 ±20

Table 14 Selected fatty acid composition (%) in the oil extracted from olives on upper (n=6) and lower (n=6) layers of the canopy at nine harvest dates from September to January. SFA, saturated fatty acids; UFA: unsaturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids.

Parameter	Upper	Lower	14/09	28/09	12/10	26/10	09/11	23/11	07/12	21/12	05/01
C16:0	16 ±1.8	15.6 ±1.7	17.4 ±0.5	17.1 ±0.5	17.2 ±0.4	16.7 ±0.7	16.2 ±1.5	15 ±0.9	13.9 ±1.6	13.2 ±0.9	15.5 ±1.4
C16:1	1.5 ±0.2	1.4 ±0.2	1.6 ±0.2	1.6 ±0.2	1.6 ±0.1	1.6 ±0.2	1.5 ±0.2	1.4 ±0.2	1.3 ±0.2	1.3 ±0.2	1.2 ±0.2
C18:0	1.8 ±0.1	1.8 ±0.1	1.8 ±0.1	1.8 ±0.1	1.9 ±0.1	1.9 ±0.1	1.9 ±0.1	1.9 ±0.1	1.7 ±0.2	1.8 ±0.1	1.8 ±0.1
C18:1	67.6 ±2	69.1 ±2.5	66.2 ±1.1	67 ±1.4	66.4 ±1.4	67.3 ±1.7	67.6 ±1.6	69.2 ±2.1	70.9 ±1.7	71.3 ±1.9	69.4 ±1.6
C18:2	11.5 ±1	10.5 ±0.8	10.8 ±0.7	10.6 ±0.8	11.1 ±1	11 ±1	11.5 ±1.2	11.2 ±1.3	10.8 ±1.2	11 ±1.4	10.6 ±1.1
C18:3	0.8 ±0.2	0.8 ±0.2	1.1 ±0.1	0.9 ±0.1	0.8 ±0.1	0.7 ±0.1	0.7 ±0.1	0.7 ±0.1	0.7 ±0.1	0.6 ±0.1	0.6 ±0.1
C20:0	0.4 ±0.1	0.4 ±0.1	0.5 ±0.1	0.5 ±0.1	0.5 ±0.1	0.4 ±0.1	0.4 ±0.1	0.4 ±0.1	0.4 ±0.1	0.4 ±0.1	0.4 ±0.1
C21:0	0.3 ±0.1	0.3 ±0.1	0.4 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.2 ±0.1	0.2 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1
SFA	18.7 ±1.9	18.3 ±1.8	20.3 ±0.5	19.9 ±0.5	20 ±0.4	19.5 ±0.6	18.8 ±1.5	17.5 ±0.8	16.4 ±1.6	15.8 ±0.9	18.2 ±1.3
UFA	81.4 ±1.9	81.8 ±1.8	79.8 ±0.5	80.2 ±0.5	80.1 ±0.4	80.6 ±0.6	81.3 ±1.5	82.6 ±0.8	83.8 ±1.5	84.3 ±0.9	81.9 ±1.3
MUFA	69.3 ±1.9	70.7 ±2.3	68 ±1	68.8 ±1.3	68.2 ±1.3	69 ±1.6	69.2 ±1.5	70.7 ±1.9	72.4 ±1.7	72.7 ±1.8	70.7 ±1.5
PUFA	12.2 ±1	11.2 ±0.9	11.8 ±0.6	11.4 ±0.8	11.9 ±1	11.7 ±1	12.2 ±1.3	11.9 ±1.3	11.5 ±1.2	11.6 ±1.4	11.2 ±1.2
SFA/UFA	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.2 ±0.1	0.2 ±0.1	0.3 ±0.1
MUFA/PUFA	5.8 ±0.5	6.5 ±0.7	5.8 ±0.4	6.1 ±0.5	5.8 ±0.6	6 ±0.7	5.8 ±0.7	6.1 ±0.9	6.5 ±0.8	6.4 ±0.9	6.4 ±0.8

Table 15 Phenolic composition (mg/kg) of oil extracted from olives on upper (n=6) and lower (n=6) layers of the canopy at nine harvest dates from September to January. DAFOA, dialdehydic form of oleuropein aglycone; AFOA, aldehydic form of oleuropein aglycone; DAFLA, dialdehydic form of ligstroside aglycone; AFLA, aldehydic for of ligstroside aglycone.

Parameter	Upper	Lower	14/09	28/09	12/10	26/10	09/11	23/11	07/12	21/12	05/01
DAFOA	30.8±15.7	25 ±13.6	9.7 ±7.9	12.2 ±3.9	15.2 ±3.9	23.5 ±5.8	27.9 ±9.1	38.2 ±7.4	38.5 ±7.3	46.8 ±7.6	39.4±12.9
AFOA	8.4 ±3	4.6 ±1.7	2.5 ±1.3	3.8 ±1.5	6.1 ±2.3	5.9 ±2.1	7.2 ±2	8 ±3.4	7.5 ±2.5	9.7 ±3.2	7.9 ±1.9
DAFLA	13.8 ±3.8	11.9 ±2.7	15.9 ±3.1	14.4 ±4.8	15.8 ±3.6	11.6 ±1.7	10.9 ±2	12.6 ±1.7	12.6 ±2.1	12.9 ±1.7	8.9 ±1.7
AFLA	2.2 ±0.6	1.8 ±0.6	1.3 ±0.3	1.4 ±0.4	1.8 ±0.3	1.7 ±0.5	2.3 ±0.6	2.3 ±0.6	2.2 ±0.5	2.6 ±0.7	2.1 ±0.3
Pinoresinol	25.3 ±2.2	24.6 ±2	25 ±0.9	25.5 ±2.3	26.1 ±1.6	27.1 ±1.8	24.3 ±2	24.3 ±1.4	24 ±1.9	25 ±1.6	23.6 ±2.9
Luteolin	14.3 ±5.6	6.2 ±2.4	4.9 ±2.1	5.9 ±2.6	9.8 ±4.1	10.2 ±4.6	9.6 ±3.7	12.4 ±6.9	11.4 ±5.9	15 ±8.1	13.3 ±5.8
Apigenin	3.2 ±0.6	2.7 ±0.5	2.5 ±0.5	2.3 ±0.4	3 ±0.5	3 ±0.4	3.1 ±0.5	3.4 ±0.6	3 ±0.6	3.3 ±0.6	3.2 ±0.5
hydroxytyrosol	0.7 ±0.3	0.6 ±0.2	0.3 ±0.2	0.6 ±0.1	0.7 ±0.3	0.9 ±0.2	0.7 ±0.3	0.9 ±0.2	0.7 ±0.3	0.7 ±0.2	0.7 ±0.2
Tyrosol	1 ±0.2	0.9 ±0.2	0.7 ±0.3	0.9 ±0.3	1.1 ±0.2	1.1 ±0.2	1 ±0.2	0.9 ±0.2	1 ±0.2	1 ±0.2	0.8 ±0.2
vanillic acid	2.4 ±0.6	2.4 ±0.5	2.3 ±0.5	2 ±0.3	2.6 ±0.4	3.1 ±0.5	2.7 ±0.2	2.5 ±0.2	2.2 ±0.4	2.3 ±0.3	1.7 ±0.3
ρ-coumeric acid	0.7 ±0.2	0.8 ±0.2	0.7 ±0.2	0.8 ±0.3	0.8 ±0.2	0.8 ±0.2	0.7 ±0.2	0.7 ±0.2	0.6 ±0.2	0.6 ±0.1	0.6 ±0.2
ferrulic acid	2.5 ±1.2	1.4 ±1	1.3 ±0.7	2.2 ±1	2.9 ±1	3.8 ±1.2	1.9 ±0.9	1.2 ±0.6	1.3 ±0.6	1.6 ±0.8	1 ±0.6
o-coumeric acid	0.4 ±0.2	0.4 ±0.2	0.4 ±0.2	0.4 ±0.3	0.4 ±0.2	0.6 ±0.3	0.3 ±0.2	0.4 ±0.1	0.4 ±0.1	0.4 ±0.1	0.4 ±0.1

Table 16 Volatile composition (mg/kg) of the oil extracted from olives on upper (n=6) and lower (n=6) layers of the canopy at nine harvest dates from September to January.

Parameter	Upper	Lower	14/09	28/09	12/10	26/10	09/11	23/11	07/12	21/12	05/01
1,5-hexadiene-(3,4)-diethyl	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.3 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1
meso-1,5-hexadiene-3,4-diethyl	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.3 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1
Pentanal	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1
3-pentanone	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1
3-ethyl-1,5-octadiene (I)	0.6 ±0.3	0.6 ±0.3	0.9 ±0.2	0.6 ±0.5	0.5 ±0.2	0.6 ±0.1	0.5 ±0.1	0.7 ±0.2	0.5 ±0.1	0.7 ±0.2	0.5 ±0.1
1-penten-3-one	1.2 ±0.5	1 ±0.5	1.3 ±0.4	2 ±0.6	1.2 ±0.4	1.1 ±0.1	1.1 ±0.3	1 ±0.2	0.7 ±0.3	0.8 ±0.2	0.7 ±0.2
3-ethyl-1,5-octadiene (II)	0.7 ±0.3	0.6 ±0.2	1 ±0.2	0.9 ±0.3	0.5 ±0.2	0.6 ±0.2	0.5 ±0.2	0.7 ±0.2	0.5 ±0.1	0.6 ±0.2	0.5 ±0.1
3,7-decadiene (I)	0.5 ±0.3	0.5 ±0.4	1.1 ±0.2	0.9 ±0.5	0.4 ±0.1	0.4 ±0.1	0.4 ±0.2	0.4 ±0.2	0.3 ±0.1	0.4 ±0.1	0.3 ±0.1
(Z)-2-penten-1-ol	0.6 ±0.4	0.5 ±0.3	0.6 ±0.2	1.1 ±0.4	0.6 ±0.2	0.5 ±0.1	0.4 ±0.1	0.4 ±0.1	0.4 ±0.1	0.3 ±0.1	0.3 ±0.1
3,7-decadiene (III)	1.6 ±1.3	1.3 ±1	4.1 ±0.8	2 ±0.5	2 ±0.5	1.3 ±0.1	0.9 ±0.2	0.9 ±0.3	1.1 ±0.2	0.7 ±0.1	0.4 ±0.1
3,7-decadiene (II)	1.5 ±1.6	0.6 ±0.4	2.7 ±2.3	2 ±1.5	1.2 ±0.5	0.3 ±0.1	0.9 ±0.2	0.8 ±0.3	0.5 ±0.3	0.7 ±0.2	0.4 ±0.1
3,7-decadiene (IV)	0.4 ±0.3	0.3 ±0.2	0.6 ±0.2	0.4 ±0.3	0.5 ±0.5	0.3 ±0.1	0.3 ±0.2	0.4 ±0.2	0.2 ±0.2	0.3 ±0.1	0.2 ±0.2
(E)-2-pentenal	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.2 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1
1-penten-3-ol	0.3 ±0.2	0.3 ±0.2	0.3 ±0.1	0.6 ±0.2	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1
Sum of C5	7.4 ±4	5.6 ±2.4	12.6 ±3.7	10.8 ±2.1	7.1 ±1.4	5.2 ±0.4	5.3 ±1	5.4 ±1.2	4.3 ±0.9	4.7 ±0.8	3.2 ±0.6
Hexanal	0.9 ±0.5	1.3 ±1.3	2.4 ±2	1.5 ±1.1	1.7 ±0.7	1.1 ±0.3	0.8 ±0.2	0.8 ±0.3	0.5 ±0.2	0.7 ±0.2	0.4 ±0.2
(E)-2-Hexenal	25.2 ±10.6	23.9 ±11.5	43.8 ±2.6	44.4 ±2.4	20.8 ±1.5	23.5 ±0.6	18.4 ±1.4	19 ±2.3	13 ±0.5	20.6 ±2.8	17.4 ±2.1
Acetic acid hexyl ester	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-hexen-1-ol acetate	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1
2-hexen-1-ol acetate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Hexanol	0.1 ±0.1	0.1 ±0.1	0 ±0	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1
3-hexen-1-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(E)-2-hexenol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sum of C6	26 ±10.6	25 ±12.5	46.1 ±3	45.8 ±2.8	22.4 ±1.5	24.5 ±0.6	19.1 ±1.5	19.8 ±2.4	13.5 ±0.6	21.3 ±2.9	17.9 ±2.2

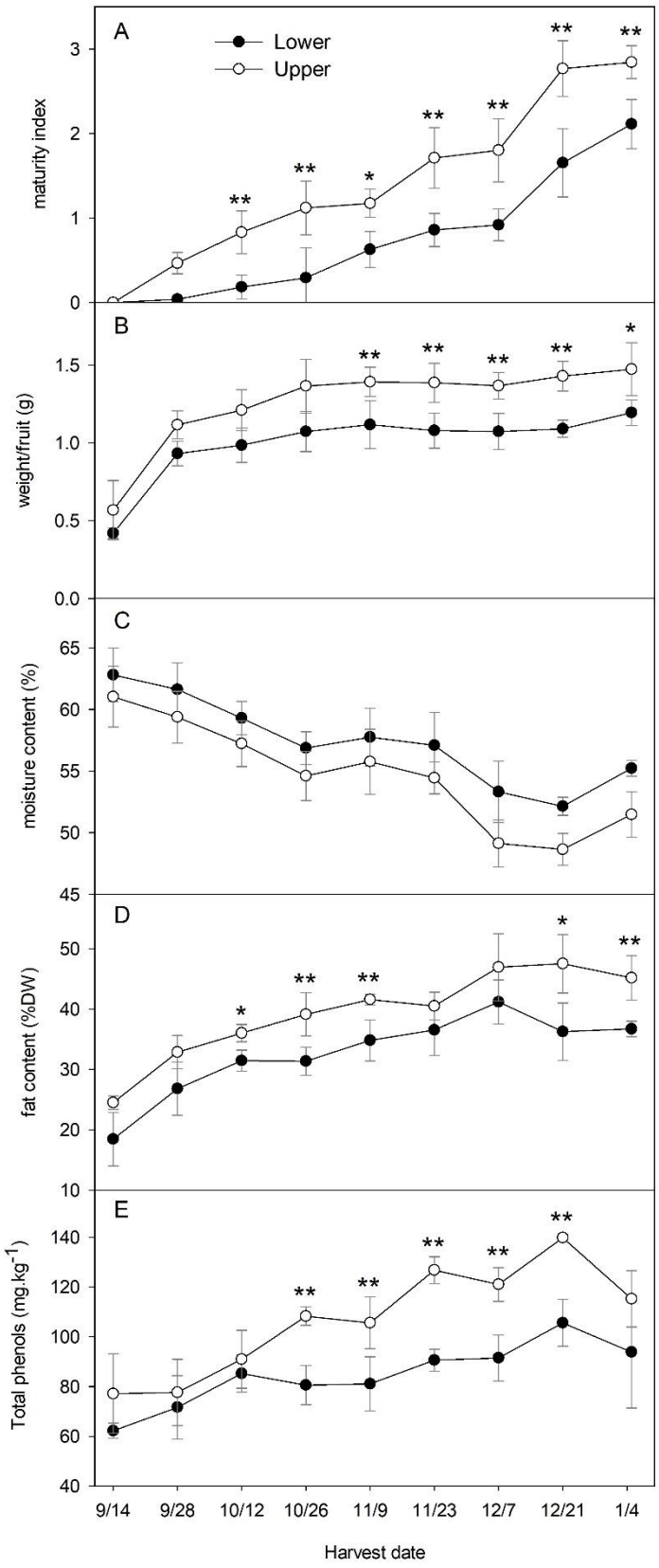

Figure 7 Maturity index (A), fruit weight in grams (B), percentage of fruit moisture (C) and fat content on dry basis (D); and total phenols in oil (E) at nine harvest dates on upper (n=6) and lower (n=6) sides of the canopy from September to January. For each variable the asterisk (*) represents significant differences with $\rho<0.05$ and (**) represents significant differences with $\rho<0.01$ between upper and lower positions by Tukey's test at each harvest date.

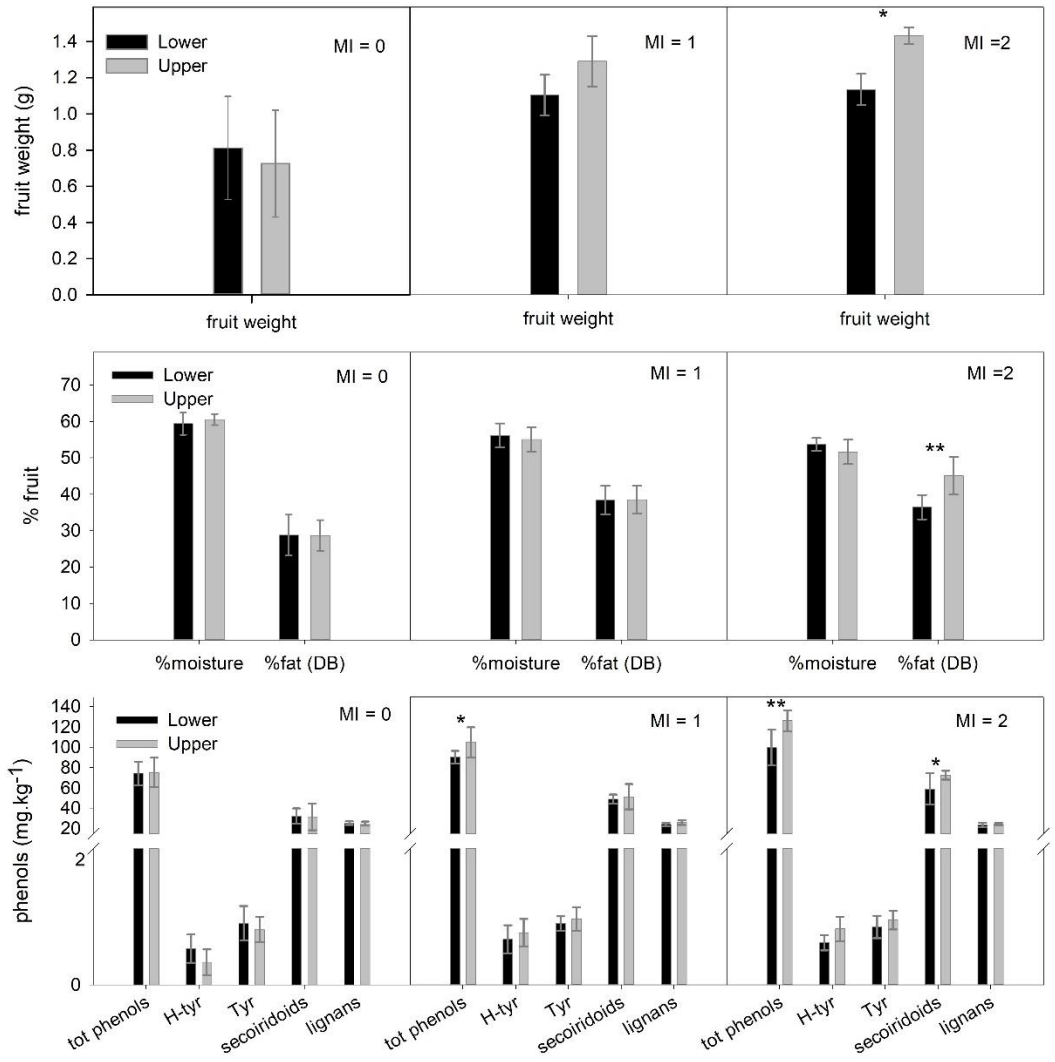

Figure 8 Fruit weight (g), moisture content (%), fat content (% dry weight), total phenols, hydroxytyrosol (H-yr), tyrosol (Tyr), secoiridoids and lignans in the oil extracted from olives on upper and lower layers of the canopy grouped within the same range maturity index 0, 1 or 2 (sd= ± 0.5) of the fruits, along the period from September to January. For each variable the asterisk (*) represents significant differences with $\rho<0.05$ and (**) represents significant differences with $\rho<0.01$ between upper and lower positions by Tukey's test at each maturity index group.

Conclusions

The impact of fruit canopy position and harvest time on maturity and production of olive fruits and quality and composition of the extracted olive oil was evaluated for Arbequina variety cultivated in California. This study confirms that harvest time and light conditions influenced the morphometric and chemical characteristics of fruits; and that fruit position can be a determinant factor for some physiological processes related to fruit growth and maturity. Lower light interception at the bottom canopy positions (9.5% of incident light) allow differences in the fruits and oil extracted between lower and upper canopy layers. Fruit on the upper layer presented 60% of the overall production, one unit more of maturity index, 3% less moisture and 5% more fat content and less oleic acid than lower layer of the canopy. In terms of phenolic composition, upper layers of the canopy presented higher oleuropein and ligstroside derivatives together with hydroxytyrosol and tyrosol than lower layers. Fruit canopy influence in maturity index, fruit fat accumulation and weight and oil phenol composition was significant after the end of October. These differences remain along harvest season showing that environmental conditions determined by fruit position in the canopy (e.g. temperature, light) are the major factor for oil accumulation and phenol composition in the fruit. The differences founded from the different layers of the canopy are independent for the external appearance of the fruit, suggesting that maturity index might not be the consistent indicator of oil quality. Instead, other parameters such as fat content on dry basis and fruit weight showed potential as harvest indicators.

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Conclusions

In the last decades, the increase of olive oil consumption have driven olive growers to increase production adopting new planting systems. High and super-high density planting systems are rapidily expanding throughout the world due to the reduction of production costs as consecuence of the harvest mechanization.

However, few cultivars have horticultural traits suitable for more dense planting systems, particularly when canopy of trees are flattered, as those trained to free palmetta shape. Other aspects to be taken into considerations are the implications of this new planting system on olive oil quality.

The results obtained in this thesis show the potential of some minor Sicilian cultivars to produce in hedgerow planting system, maintaining high quality of the olive oils extracted. Minor cultivars showed to produce EVOOs with high antioxidant content satisfying the requirements of the health claims established by EFSA. Thus, local cultivars may contribute for the enrichment of the diversity of oil available in the market and the valorisation Sicilian biodiversity.

Moreover, this study evidenced the consequences of the hedgerow planting systems on the homogeneity of the olive fruits within the canopy. Particularly, high-density hedgerow systems allow higher yield per hectare and have a direct effect on fruit characteristics. The differences on the fruit along the canopy also have implications on oil quality, where upper layer of the canopy showed the highest phenol content. Since higher density planting produce the majority of the fruits at upper layers of the canopy, hedgerow systems contribute to a faster ripening of the fruits, more oil extracted and higher phenol content. The different responses of Cerasuola and Koroneiki to the treatments demonstrate the effect of the planting systems of fruit ripening along the season and consequences for olive oil quality on different cultivars.

This thesis also contributes to the understanding of which ripening trait can be used by producers to detect the best harvest time. The exocarp colour of Arbequina did not change in accordance with the biochemical variations in the fruit. In Arbequina grown in California, maturity index might not be a consistent indicator of oil quality at harvest. Instead, other parameters such as fat content on dry basis and fruit weight showed potential as harvest indicators.

The results obtained in this thesis are of great importance for the olive oil industry interested in introducing new cultivars and high-density planting systems; furthermore, evidenced the importance of treating each cultivar separately to maximize quality and yield. Overall, more knowledge on the effects of cultural practices and harvest time are required to improve quality of the olive oil during the harvest season.

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