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Relationship between environmental features and extra virgin olive oil in north Sardinia

Ph. D. Coordinator:

Chiar.mo Prof. Furio Brighenti

Tutor:

Chiar.mo Prof. Andrea Fabbri

Co-Tutors:

Dott.ssa Annalisa Rotondi

Dott. Tommaso Ganino

Ph.D. Student: Lucia Morrone

Lucia Morrone, 2015

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Thesis Supervisors: Prof. Andrea Fabbri – Department of Food Science, University of Parma

Dr. Annalisa Rotondi – Institute of BIoMETeorology of the National Research Council (IBIMET – CNR), Bologna

Dr. Tommaso Ganino - Department of Food Science, University of Parma

PhD Coordinator: Prof. Furio Brighenti – Department of Food Science, University of Parma

To Maurizio

Preface and Acknowledgements

The agri-food sector is a strategic asset for Italy, representing the 8,7% of GDP. The significance of this sector is not merely economic, even if the agri-food sector is an important item of GDP and it has always a positive mark in export. As a matter of fact, the agri-food sector has both a social and an environmental impact. In this regard, the valorisation of Italian agri food productions, the so-called Made-in-Italy Agri-Food, assumes a crucial importance. The extra virgin olive oil is one of the products that most personify the image of the Made-in-Italy Agri-Food. Notwithstanding a lot of people think at the Italian virgin olive oil like a one and definite product, it is a product having hundreds of chemical and sensory shades. This richness coming from the huge varietal heritage, estimated in almost 42% of word biodiversity, and from the interaction environment-genotype. The environment therefore has a decisive role in the link between extra virgin olive oil production and the origin territory and this role is the object of study of this thesis.

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Table of Contents

1. General introduction

Botanical classification and biodiversity of *Olea europaea L.*

Olive tree (*Olea europaea L.*) belongs to the Oleaceae family, that includes 26 genera, one of which recently extinct (*Hesperelaea*; Green, 2004) and some of economic or aesthetic importance (*Fraxinus, Jasminum, Forsythia, Ligustrum*).

The *Olea* genera consists of 35 species divided into three groups on a geographical basis: Afro-Mediterranean, Indo-Sino-Malaysian and Natalense-Malagasy (Ciferri, 1941), the olive tree being the only species of agricultural relevance. Although controversial opinions remain in the botanical classification of the olive tree, the division into two subspecies within the species *Olea europaea* L. (O. *europaea* L. subs. *sylvestris* Miller, or *Oleaster,* and O. *europaea* subs. *Europaea*, or *sativa*; Hoffm. et Link) is widely accepted. The main difference among these two subspecies is morphological: O. *europaea* subs. *europaea* produces bigger fruits with a higher oil yield compared to O. *europaea* L. subs. *Sylvestris*; for this reason, only the first subspecies is of so the first one is of economical relevance.

Unlike almost all cultivated species that tend to lose their biodiversity as a result of the combined selective breeding process and intensive exploitation, O. *europaea* species has a huge genetic inheritance, estimated at around 1200 cultivar (Bartolini et al., 2005). The cause of such a large expansion of the genetic heritage has to be found in the olive species allogamy, with a high degree of hetero-pollination, leading to high levels of heterozygosity and DNA polymorphism (Angiolillo et al., 1999; Rallo et al., 2000). Moreover, the longevity and the selection of a large number of varieties have contributed to the preservation of the olive tree variability (Rallo et al., 2000) and the ease of propagation of the species has allowed its vast spread (Baldini and Scaramuzzi, 1952). Another factor contributing to the free diffusion of the olive tree cultivar, and thus to preservation and increase in the genetic diversity of the species, has been the lack of an morphologically defined archetype, inasmuch the final product is not the fruit itself but the result of the fruit's milling. Thus a "varietal standard" has never been established for the olive species (Rosselli et al., 1974).

Geographical spread of the species

Domestication of the olive tree has taken place since the fourth millennium BC in the Mediterranean basin in the areas located between Asia Minor and the Middle East (Zohary and Spiegel Roy, 1975; Liphschitz et al., 1991). Much evidences indicates that during the last two millennia, the extension of olive tree cultivated area changed and the climate was the main variable driving this process (Moriondo et al., 2008). In fact, from a reconstruction of the temperatures profile (Fig. 1.1) it is possible to see the seesawing performance, which can be easily correlated to

the crop's expansion. Historical evidences show the spreading of olives and grapes cultures carried out by the Romans to the northern part of Italy (Neumann, 1985). Further expansion of the crop occurred during the warmer, medieval period (950-1200 AC), which was followed by the Little Ice Age (1550-1850 BC) (Holzhauser, 1997; Pfister et al., 1998), causing on the contrary a reducing in the olive trees spread even in the southern Mediterranean regions (Xoplaki et al., 2001), with the only exception of a few protected areas (Toniolo, 1914; Moriondo et al., 2008).

Geographical limits to the spread of the olive between 30° and 45° N are therefore imposed by the climate (Morettini, 1972) due to the plant's sensitivity to low temperature and extreme water stress (fig. 1). In fact in Europe the northern limit coincides roughly with the 4° isotherm in January (Pfister et al., 1998), whereas the southern limit overlaps with the pre-Saharan area (Moriondo et. al., 2008). Nowadays most of the olive production is still concentrated in the Mediterranean basin (Mattingly, 1996), but since the discovery of America in 1492 olive farming spread beyond its Mediterranean confines, to arrive in dry areas of Mexico and subsequently in Peru, California, Chile and Argentina, where one of the plants brought over during the Conquest – the old Arauco olive tree – lives to this day (Wiesman, 2009).

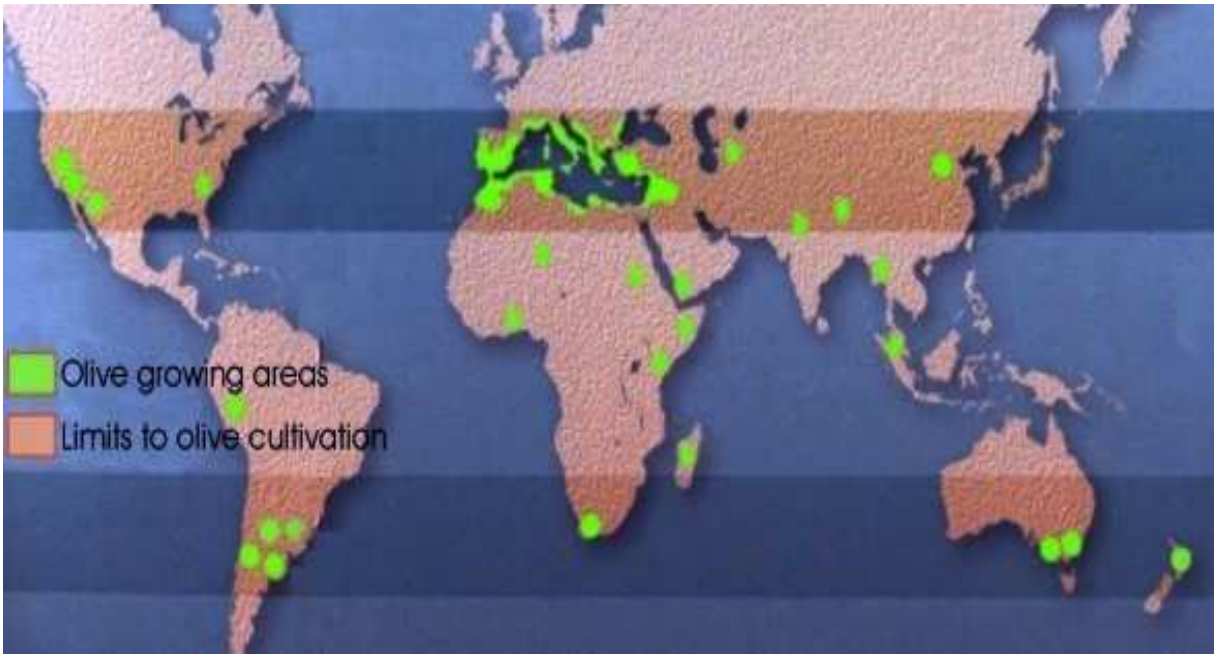


Fig. 1.1 Geographical distribution of olive growing areas.
(From http://www.internationaloliveoil.org/projects/paginas/Section-a.htm)

Economic relevance of olive oil. Focus on Sardinia

Olive growing areas consist of 10 million hectares harvested in 2013, 48% of the surface is in European Union, the main productors being Spain (50%), Italy (11%) and Greece (9%)

(FAOSTAT, 2014). According to Fontanazza & Cipriani (2005) it is possible to distinguish two different types of olive growing areas:

- Suitable olive growing areas
- Marginal olive growing areas

As explained by the name, the suitable olive growing areas, are the ones characterized by optimal conditions, such as climate, water availability and low slope; in these areas is thus possible to obtain higher yields at lower production costs. In Europe these areas are Andalusia, where over the 80% of Spain production is located, Calabria, Apulia, Crete and the Peloponnese.

The marginal olive growing areas are mostly mountainous and areas with specific disadvantages such as slope, leading to unprofitability because of the large amounts of labour required and quite low yields (Fontanazza & Cipriani, 2005). However, it is in these areas that the culture assumes a great importance from a landscape and environmental point of view. In fact the presence of olive trees in these areas prevents soil erosion and landslides, thanks to its wide and relatively superficial root system (Fontanazza & Cipriani, 2005), with the olive trees being a highly distinctive element of the landscape. The proportion of groves located in disadvantaged zones is significant, representing 88% of the total area of Portugal, 71% of Greece, 60% of Spain and 51% of Italy.

In Italy the regions with the larger olive tree cultivations are Apulia and Calabria (Fig. 1.2), with respectively 33.2 and 16.6% of the total area devoted to olive growing. In Sardinia 36471 ha are dedicated to olive groves (ISTAT data, 2010) and the cultivation is widespread in almost all municipalities, as shown by official statistics (Sini, 1996). However, the distribution of olive groves appears patchy and fragmented following the division of the groves due to inheritance (Bandino et al., 2001). This situation caused the progressive drop out of the olive cultivation, mostly in the marginal growing areas, and reached its climax during the sixties while in recent years we are witnessing a revival of the culture (Sini, 1996; Nuvoli & Sini, 1997; Bandino and Sedda 1999). Thanks to EU founds new and modern olive groves were made in flat lands with access to irrigation (Bandino et al., 2001), leading to an increase of the olive oil production. Besides, an overall help to the olive sector came from the rise in interest in the Mediterranean diet; in fact in this diet olive oil represents the 85% of the fat content, a factor that has been linked to longevity, improved life quality and lower incidence of cardiovascular disease, cancer and cognitive deterioration (Pérez-Jiménez et al., 2007).

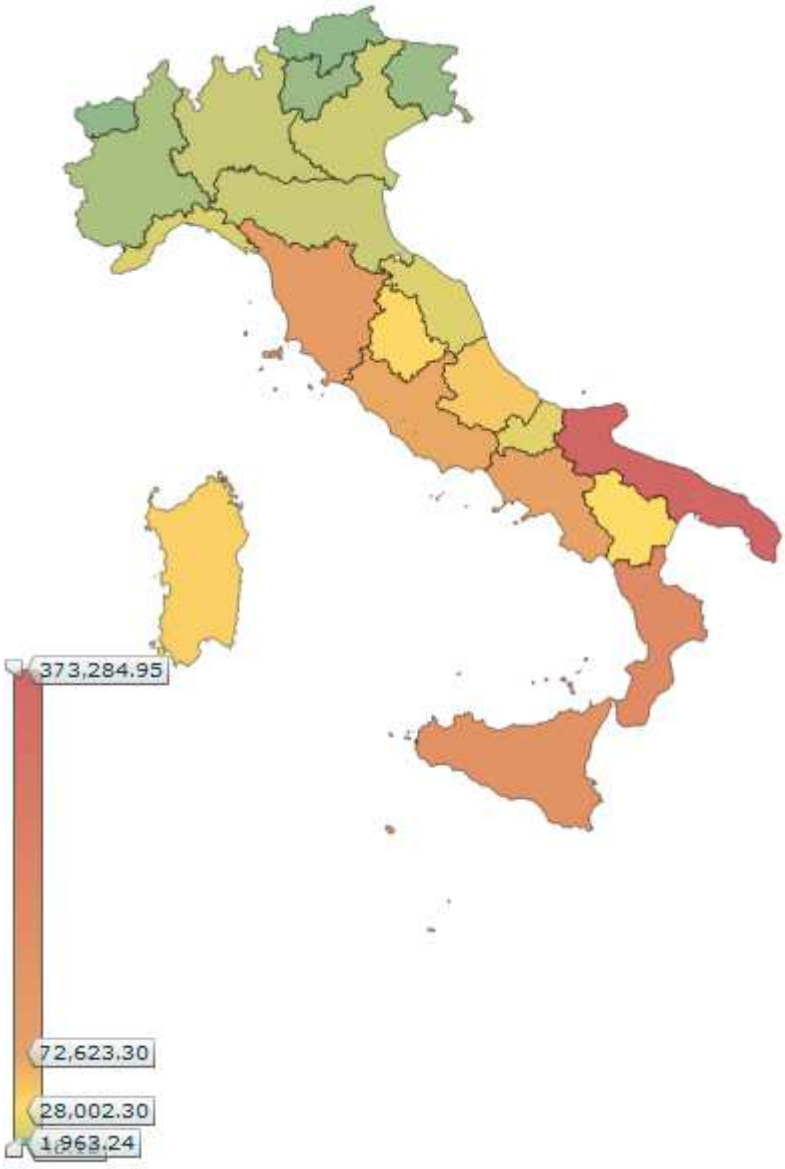


Fig. 1.2 Italian regions classified area devoted to olive tree
(ISTAT 2010 data, From http://censimentoagricoltura.istat.it/explorer/index.html#story=22)

Chemical composition of olive oil

Olive oil is composed for 98-99% from a saponifiable fraction consisting of triglycerides, diglycerides (2-3%) and monoglycerides (0.1-0.2%). While this fraction is qualitatively the same for all the olive oils, it can change quantitatively. The remaining part (1-2%) is constituted by the unsaponifiable fraction that, even if present in small quantities, plays a very important role in the oil quality. This fraction consists of hydrocarbons such as squalene and waxes, tocopherols and tocotrienols, higher aliphatic alcohols, sterols, triterpenic and biterpenic alcohols, pigments such as carotenoids and chlorophylls, and phenols. Conversely, the un-saponifiable fraction is both

qualitatively and quantitatively able to differentiate the olive oils both in organoleptic and nutritional properties.

Saponifiable fraction

Triglycerides and fatty acids

Triglycerides (TGs) are formed by a molecule of glycerol esterified with three fatty acids. Since the very specific regio-selectivity of the enzymatic metabolic pathway (Wan, 1988), fatty acids located in position 2 of triglycerides have been widely used to detect the presence of synthetic TGs obtained by chemical esterification of glycerol with free fatty acids. The analysis of triglycerides may also be useful for the characterization of specific virgin olive oil cultivars grown within a particular geographic region (Vlahov, et al., 1999). Moreover, the analysis of triglycerides is a useful tool to verify the authenticity of olive oil, since frauds could have, beyond commercial relevance, also severe health implications, like the "Spanish toxic syndrome" that caused 400 deaths in 1981 (Tsimidou et al., 1986).

The composition in fatty acids of olive oil varies according to the cultivar, as stated by Uceda and Hermoso (2001), who in a preliminary evaluation of the olive germplasm bank indicated the cultivar as the main source of variability for the major fatty acids. Moreover, the composition in fatty acids is also affected by the olive ripeness and the environmental conditions (Beltrán et al., 2004; Mousa et al., 1996). The fatty acids profile of virgin olive oil has a great relevance for the consumer's health. In the last years the Mediterranean diet was reevaluated, and as previously mentioned olive oil provides some 85% of the total fats, thanks to its high content in monounsaturated fatty acid (MUFA) (Pérez-Jiménez et al., 2007). Several studies have demonstrated the lower levels of low-density lipoprotein (LDL) cholesterol and total cholesterol in diets rich in MUFA (Matson & Grundy, 1985; Mensik & Katan, 1992), and those lower levels are related to the reduction and/or the prevention of cardiovascular diseases (Téres et al., 2008). Oleic acid is the main monounsaturated fatty acid found in olive oil and its content is between 55-83% of the total MUFA (Servili, 2014). The minimum and maximum content in oleic acid are not determined by law (Table 1.1), however it is known that oils richer in oleic acid are produced in cold climates, while oils with an oleic acid content as low as 50% of the total MUFA are the result of the plant-environment interaction in the new areas of the culture expansion such as Argentina.

Table 1.1 Fatty acid composition of virgin olive oil (VOO).

Fatty acid	EEC (Reg.2568/91)
Myristic (C14:0)	<0,05*
Palmitic (C16:0)	7-17
Palmitoleic (C16:1)	0,3-3
Heptadecanoic (C17:0)	<0,05*
Heptadecenoic (C17:1)	<0,05*
Stearic (C18:0)	1,5-4
Oleic (C18:1)	63-83
Linoleic (C18:2)	<13,5*
Linolenic (C18:3)	<0,6*
Arachidic (C20:0)	<0,9*
Eicosenoic (C20:1)	<0,4*
Behenic (C22:0)	<0,2*
Lignoceric (C2:0)	<0,2*

*legal limit
From: Capella, 1997

Unsaponifiable fraction

Tocopherols

Tocopherols are a class of chemical compounds exhibiting vitamin E activity. Because the vitamin activity was first identified in 1936 from a dietary fertility factor in rats, it was given the name "tocopherol" from the Greek words "τόκος" [*tókos*, birth], and "φέρειν", [*phérein*, to bear or carry] the final meaning being "to carry a pregnancy" with the ending "-ol" signifying its status as a chemical alcohol (http://en.wikipedia.org/wiki/Tocopherol). These compounds exhibit varying degrees of antioxidant activity, depending on the site and number of methyl groups and the type of isoprenoids. Eight different compounds can result from the chromanol ring linked to a C16 isoprenic chain: tocopherols are characterized by a saturated isoprenic chain, while in tocotrienols the chain is unsaturated.

In olive oil tocopherols, and the analogues tocotrienols, occur in the 4 different forms α, β, γ and δ, depending the number and position of the methyl group; the configuration at the three chiral centers, 2, 4' and 8', is R. All those compounds and diastereomers have vitamin activity with R,R,R α tocopherol (Fig.1.3) showing the highest activity. The total tocopherols in olive oil are represented mainly by α-tocopherol, with about 90% of total tocopherols, and by minor amounts of β-, γ- and δ-tocopherol. The concentration of tocopherols in the oil, that could range between 23 and 751 mg/kg (Servili, 2014), depends mainly on the stage of fruit ripeness at harvest: Garcia and colleagues (1996) showed that at more advanced maturation corresponds a lower tocopherols concentration. In the olive oil, α-tocopherol is the main chain breaking antioxidant, with its

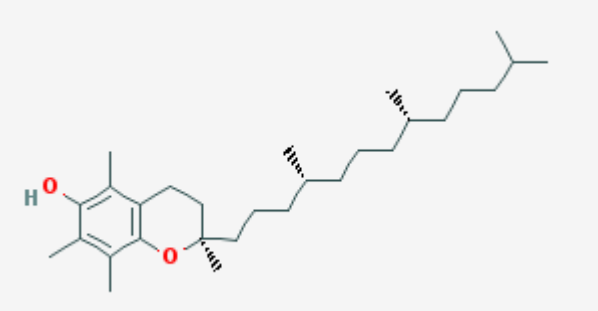


Fig. 1.3 Chemical structure of α-Tocopherol

concentration depending also on pedoclimatic factors such as area of origin (Inglese et al., 2011). In humans, vitamin E is important for the functionality of the reproductive organs and muscles, especially for the myocardium (Lotti, 1985); thanks to its antioxidant properties vitamin E can protect biological tissues from free radicals and reduce the risk of diseases such as coronary heart disease, some cancers and cataracts (Cooper et al., 1999).

Carotenoids and chlorophylls

Carotenoids and chlorophylls are very common pigments in the plant kingdom, playing a key role in the photosynthetic pathway. As the drupe ripeness proceeds, the levels of both chlorophylls and carotenoids decrease progressively (Criado et al., 2004).

Carotenoids are characterized by a long carbon chain; according to the oxygen presence or not in the chain, the carotenoids are divided in the two classes: xantophylls (oxygen in the carbon chain) and carotenes, which are purely hydrocarbons. Carotenoids, namely lutein and β-carotene (Fig 1.4), are pigments with a yellow colouration, acting as quenchers and thus delaying the photooxidation processes (Chen & Liu, 1998). Carotenoids with a β-ionone ring show a provitamin A value (Giuffrida et al., 2011), while several other studies have confirmed the anticancer activity of β-carotene and other carotenoids (Van Poppel & Goldbohm, 1995).

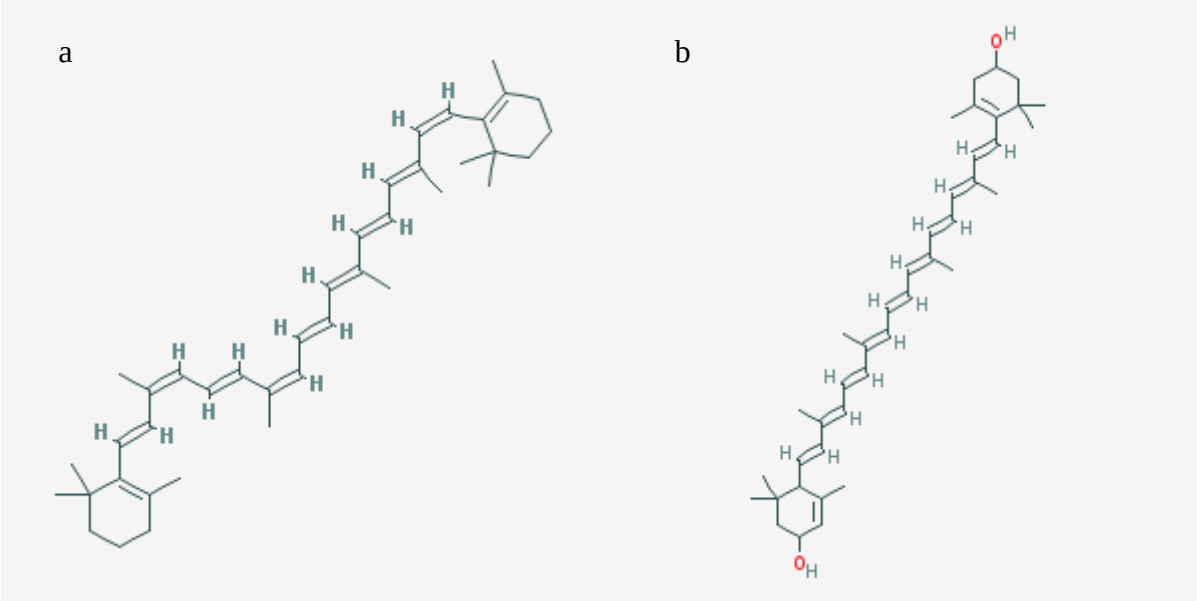


Fig. 1.4 Chemical structure of β -carotene (a) and lutein (b)

The major chlorophyll pigments are chlorophyll *a* and *b*, differing in one of the side chains (chlorophyll *b* has an aldehyde group); in figure 1.5 is shown the structure of chlorophyll *a*. During the production of olive oil, losses of chlorophylls occur due to the structural transformation of the pigments caused by the release of acids, namely the transformation of chlorophylls into pheophytin by removal of the Mg^{2+} ion (Giuffrida et al., 2011). In the oil, chlorophyll pigments in the presence of light catalyse the production of singlet oxygen, which leads to the formation of hydroperoxides triggering the process of rancidity. The oxidizing action of chlorophyll is hampered by β-carotene, therefore a correct balance of chlorophyll and carotenoid pigments is essential for the oil oxidative stability.

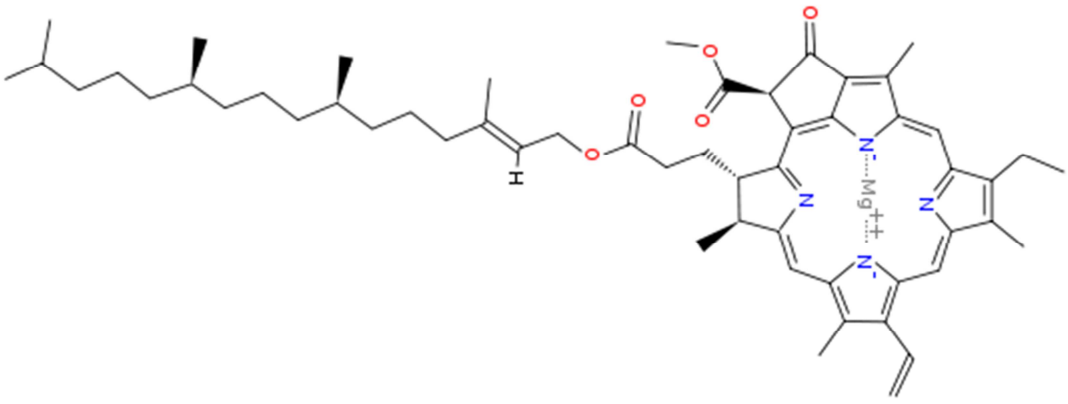


Fig. 1.5 Structure of chlorophyll a

Phenols

The phenolic compounds are secondary metabolites widely distributed in the plant kingdom. They are described by a large variety of chemical structures, sharing as a common feature a benzene ring that can then be attached to one or more hydroxyl groups and other functional groups such as glycosides, esters etc. The occurrence of these hydrophilic molecules in extra virgin olive oil was demonstrated by Cantarelli in 1961, then confirmed by Montedoro and Cantarelli in 1969 (Servili et al., 2004). Since then the phenols have been extensively studied and their antioxidant properties, together with their involvement in the sensory profile and their positive influence on human health, have been highlighted.

In the olive drupe the concentration of phenolic compounds ranges between 1-3% of fresh pulp weight (Garrido et al., 1997), and the main classes of phenols are phenolic acids, phenolic alcohols, flavonoids (flavones glycosides and anthocyanins), lignans and secoiridoids, which are present exclusively in the *Oleaceae* family (Servili et al., 2004). These compounds are hydrophilic, but are present in virgin olive oil (VOO) around water droplets thanks to their amphiphilic characteristics (Lozano-Sanchez et al., 2010). However during the crushing and malaxation steps several enzymes such as esterases and glucosidase act on the phenol substrate, modifying the phenols profile (Romero-Segura et al., 2009). The major phenolic compounds found in VOO are described in figures 1.6 and 1.7.

Phenolic acids are widely spread in the plant kingdom. In VOO there are both (i) benzoic acids, such as vanillic acid, gallic acid, syringic acid, etc., and (ii) cinnamic acids, such as coumaric acid, ferulic acid, caffeic acid, etc. Historically the phenolic acids were the first group of phenols observed in VOO (Servili et al., 2004), however their concentration is lower respect to other phenol classes present in VOO (Montedoro et al., 1992; Mannino et al; 1993; Tsimidou et al., 1996).

Secoiridoids, produced from the secondary metabolism of terpenes, are characterized by the presence of elenoic acid (EA), esterified with a phenyl ethyl alcohol; in detail if EA is esterified with hydroxtyrosol (3,4 DHPEA) oleuropein (3,4-DHPEA-EA) is formed, while if EA is esterified with tyrosol (*p*-HPEA) ligstroside (*p*-HPEA-EA) is formed. Both oleuropein and ligstroside are mainly present in their glycosidic form in fruits while in the aglycon forms in VOO, due to the enzymatic modifications occurring during crashing and malaxation. The aglycon forms can exist in a number of keto-enolic tautomeric equilibria involving the opening of the heterocyclic ring, yielding to compounds of different structures (Angerosa et al., 1996). The most abundant secoiridoids in VOO are the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) or to tyrosol (*p*-HPEA-EDA), and an isomer of the oleuropein aglycon (aldehydic form of oleuropein or ligstroside aglycons) (Servili et al., 2004). The

aforementioned compounds are intermediate structures of the biochemical transformation in the olive fruit of secoiridoids glucosides such as oleuropein, demethyloleuropein and ligstroside in the final aglycon derivatives: 3,4DHPEA-EDA from oleuropein and demethyloleuropein and *p*-HPEA-EDA from ligstroside, respectively (Rovellini & Cortesi, 2002).

Flavonoids are large planar molecules and their general structure is a 15-carbon skeleton which consists of two phenyl rings (A and B) and one heterocyclic ring (C). They can be divided into a variety of classes such as flavones (e.g., flavone, apigenin, and luteolin), flavonols (e.g., quercetin, kaempferol, myricetin, and fisetin), flavanones (e.g., flavanone, hesperetin, and naringenin), flavanonol (e.g. taxifolin), isoflavones (e.g. genistein and daidzein) and flavan-3-ols (e.g. cathechin and epicatechin (Kumar & Pandey, 2013). The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings (Middleton, 1998). In VOO, the phenolic compounds usually recovered were luteolin and apigenin, while taxifolin, a flavanonol, has recently been found in Spanish VOO (Carrasco-Pancorbo et al.,2004).

Lignans are the last group of phenols found in VOO. Lignans are polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols, known as monolignols, to form a dibenzylbutane skeleton (http://en.wikipedia.org/wiki/Lignan). Owen et al. (2000) and Brenes et al. (2000) have recently isolated and characterized (+)-1-acetoxypinoresinol, (+)-pinoresinol, and (+)-1-hydroxypinoresinol as the lignans most frequently present in VOO (Bendini et al., 2007).

Sensory characteristics of VOO

Virgin olive oil is the one of the first and of the few products for which sensory analysis is mandatory; the sensory analysis is carried out together with the evaluation of 26 chemical-physical parameters, in order to classify the oil in its commercial categories (Reg. EC 2568/91, 61/2011, 299/2013). International cooperative studies, supported by the International Olive Oil Council (IOOC or COI) have developed a sensory (methodology for VOOs, known as the “COI Panel test” (Bendini et al., 2012), which was adopted by the European law (EEC Reg. 2568/91). Later, in 2002 the Regulation 796 was adopted and the sensory evaluation sheet modified. The changes involved the reduction of the number of organoleptic descriptors (3 positive and 7 negative) and the adoption of a continuous scale, from 0 to 10 cm, for evaluating the intensity of perception of the different attributes (both positive and negative), instead of a discrete scale.

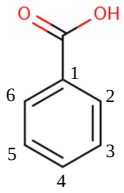
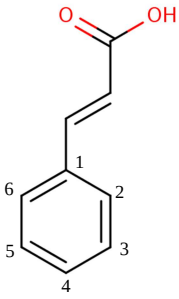
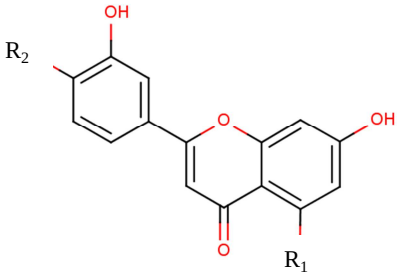
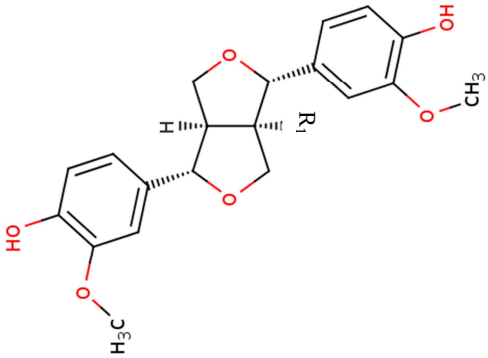
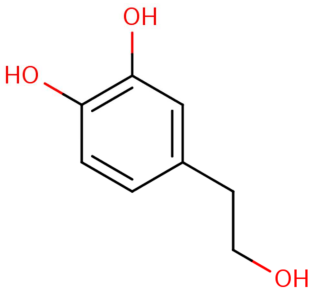
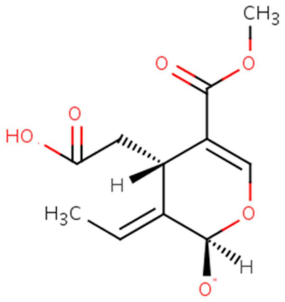
Compound	*Substituent*	*Structure*
3 –Hydroxybenzoic acid	3 – OH	
p- Hydroxybenzoic acid	4 – OH	
3,4 Dhydroxybenzoic acid	3,4 – OH	
Gentistic acid	2,5 – OH	
Vanillica acid	3 – OCH_3, 4 – OH	
Gallic acid	3,4,5 – OH	
Syringic acid	3,5 – OCH_3, 4- OH	
o-Cumaric acid	2 – OH	
p-Cumaric acid	4 – OH	
Caffeic acid	3,4 – OH	
Ferulic acid	3 - OCH_3, 4 – OH	
Sinapinic acid	3,5 - OCH_3, 4 - OH	
Luteolin	R_1 – OH, R_2 OH	
Apigenin	R_1 – OH, R_2 H	
(+) – Pinoresinol	R – H	
(+) –1 - Acetoxypinoresinol	R – $OCOCH_3$	
(+) –1 - Hydroxypinoresinol	R – OH	

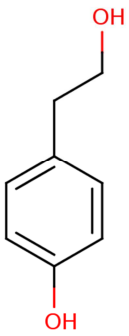
Fig. 1.6 Phenolic acids, flavones and lignans present in VOO



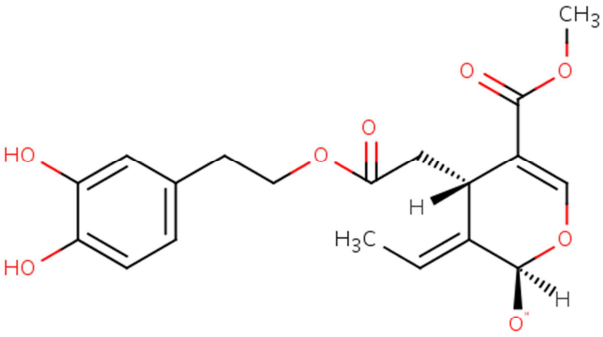
3,4-DHPEA



EA



p-HPEA



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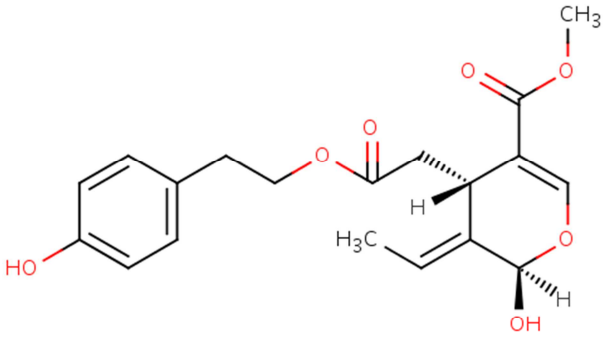

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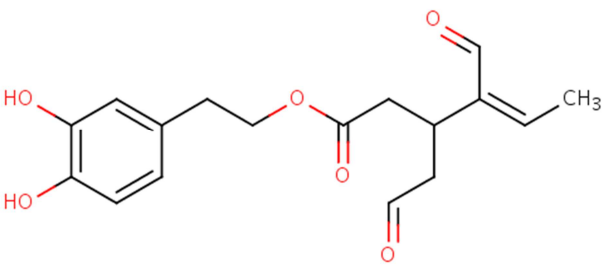

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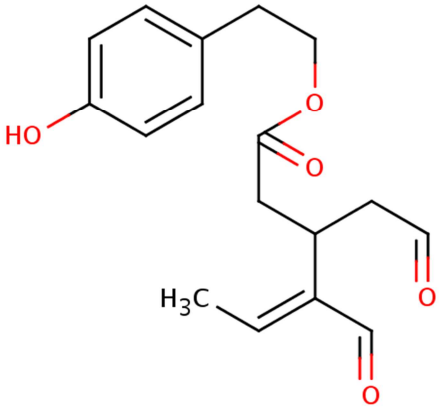

p-HPEA-EDA

Fig. 1.7 Chemical structures of major secoiridoids derivatives

Then, six year later the European Community promulgated the Reg 640/08, in which the sensory vocabulary was updated and the terms and expressions related to the organoleptic characteristics were listed (Cerretani et al., 2008b). Finally in 2013, to ensure the implementation of the most recent international standards established by the IOOC, the regulation No 1348/2013 has been adopted by the European Union. This last regulation listed the specific vocabulary as well (Table 5), but slightly modified compared to the one reported in Reg. 640/08, and it also provided indications for optional labelling.

From Table 1.2 is possible to note that the number of the negative attributes is larger than the one of the positive, because the purpose of the regulation is the oils classification on the basis of sensory characteristics: oils are graded on the median of the fruity attribute and on the median of the defects perceived with the greatest intensity.

However VOO is characterized by a wide range of pleasant flavour attributes which are influenced by cultivar and environmental factors (Rotondi et al., 2010). Since the olive cultivars are very often representative of a territory, the link between cultivar and area of production is very strong, so the sensory characteristics of one oil become distinctive of its production area. This philosophy is the base of the European brands Protected Denomination of Origin (PDO) and Protected Geographical Indication (PGI) In order to protect these labels, the COI has produced a specific regulation (COI/T.20/Doc. no. 22) to assess the characteristic attributes of extra virgin olive oil; the descriptors used for granting designation of origin are listed in Table 1.3.

Sensory attributes mainly depend on the content of minor components like phenolic and volatile compounds (Cerretani et al., 2008b). The correlation between phenolic compound and bitterness was proven by many papers (Gutiérrez et al., 1989; Mateos et al., 2004; Inarejos-Garcia et al., 2009). Depending on the type of phenols present, rather than on the total phenol content, the bitterness intensity of olive oils can be extremely variable (Favati et al., 2013), but few works have been aimed to link a phenolic compound with a given sensory property or intensity (Andrewes et al., 2003; Gutiérrez-Rosales et al., 2003; Mateos et al., 2004). In recent times a few researches have been aimed to define methods to measure bitterness (Gutiérrez-Rosales et al.; 1992; Beltràn et al., 2007) even because sensory analysis is a rather time consuming process that, even if characterized by a certain degree of uncertainty and lack of reproducibility (Angerosa et al., 2000), involves also bureaucracy in the designing, training and work implementation (Inarejos-Garcia et. al., 2009).

The volatile fraction plays an important role in oil flavour. There are many compounds, mainly carbonyl compounds, alcohols, esters and hydrocarbons, in the volatile fraction of virgin olive oil (Flath et al., 1973). They are enzymatically originated by the lipoxygenase (LOX) pathway, their concentrations depending on the level and activity of each enzyme involved in this LOX pathway

(Angerosa et al., 2004). The analytical evaluation of the aroma is not entirely reliable because some compounds present in the oil flavour seem to stimulate at the same time olfactory and gustative receptors, together with the free endings of the trigeminal nerve, thus determining a number of complex interactions and giving rise to some positive or negative synergisms; nevertheless, the application of statistical procedures to the analysis of volatile compounds concentrations and sensory notes intensities, evaluated by means of the official methodology, evidenced relationships between the two (Angerosa et al., 2004).

Technological process of extraction

Virgin Olive Oil (VOO) is obtained from olives only by mechanical or other physical means; it is one of the few vegetable oils that can be consumed without refining so this makes of it a real fruit juice. An Italian saying plays "the olive oil quality born in fields and it have to be preserved during the milling process". That point out the importance of the technological process in virgin olive oil quality. It impacts mainly on the minor components of virgin olive oil that originate during the extraction process (i.e. volatile compounds and phenols), so it's clear how crucial it is for the quality of the product (Romero-Segura, et al., 2009; Servili et al., 2003). The main technological steps that follow one another are crushing, malaxing, oil separation, filtration and each one can affect the final virgin olive oil characteristics.

- Crushing

This operation assent the rupture of both drupe and pit producing the olive paste. In both olive fruit and pit are contained enzymes, such as polyphenoloxidase (PPO) and peroxidase (POD) involved in the oxidation process of phenols, and lipoxygenase (LPO) involved in volatile compounds (C5 and C6 aldehydes, alcohols, and esters) (Servili et al., 2007). Servili and colleagues (2000) reported different concentration of the endogenous enzymes in the constituent parts of olive drupe. By considering this, in order to obtain virgin olive oils with the highest phenols content the technology of de-stoning fruit before crushing had been proposed.

Among the different types of crushers, the stone mill was the first crusher used along history. But starting from the second half of XIX sec. new olive crusher typologies had been developed in order to overcome the main disadvantage of stone mill, namely the inability to feed the continuous systems (Preziuso et al., 2010). The most used crusher are: hammer crusher, blade crusher and toothed disk crusher. All these typologies basically share the characteristic of being placed in a continuous process while they differ in energy released in the crushing chamber, which results in an increase of the olive paste temperature (Caponio & Catalano, 2001), and in the yield and oil

characteristics. Hummer crusher is the strongest crusher and different studies reported a higher phenol content and a more bitter taste in olive oils milled using the hummer crusher (Catalano & Caponio, 1996; Di Giovachino et al., 2002; Inarejos-García et al., 2011) Di Giovacchino et al. (2002) suggest that the higher content in phenolic substances of oils obtained from "violent" crushers is due to complete rupture of the pulp oil, moreover Preziuso et al (2010) suggest a role of the pieces of stone in a quick attainment of the equilibrium of the concentrations of the phenolic substances in the aqueous and in the oily phase and our results agree with those reported by these authors.

- Malaxing

This step aim to promote the aggregation of oil drop in bigger one in order to facilitates the next step of oil separation. But this phase is more than only a physical process, in fact during it the endogenous enzymes of drupes start to act: the enzymes having peroxidase activity (PPO and POD) catalyse the oxidation of phenols during malaxation, while the LPO acting on fatty acids produce volatile compounds (Servili et al., 2007). In addition, the beta-glucosidase plays a role in the production of secoiridoids by hydrolysis of oleuropein and dimetiloleuropein (Clodoveo, 2012). So the technological parameters of time and temperature, as well as the oxygen concentration, are key factors that have to be modulate in order to obtain virgin olive oil with the desiderate characteristics (Angerosa et al., 2001; Boselli et al., 2009; Servili et al., 2003). The importance of temperature during olive oil extraction is underlined by the EC Regulation No. 1019/2002 which introduced the indication ‘cold extraction’ only for VOO or extra-VOO obtained at temperatures below 27 °C by percolation or centrifugation of the olive paste. However a study carried out by Boselli and colleagues (2009), reported no difference in oxidative stability or sensory qualities in virgin olive oils obtained at 27 and 35°C, whereas the oils obtained at 45°C were characterised by ‘heated or burnt’ off-flavour. To an increase of the temperature of the olive paste corresponds a decrease of the phenolic content due to oxidation processes (Servili et al., 1994; Angerosa et al., 2001). Similarly, long time of malaxation, usually done to increase olive yield (Di Giovacchino, 1991) negatively affect the phenol content due to their oxidative degradation, either chemical or enzymatic (Ranalli et al., 2003; Fregapane & Salvador, 2013). To avoid losses in phenol compound, malaxation chambers that replace air with nitrogen were developed, minimizing thus the enzymatic oxidative degradation of phenolic compounds during processing (Servili et al., 2003)

Table 1.2 Specific vocabulary for sensory analysis (Reg. No 1348/2013)

Negative attributes
Fusty/muddy sediment: Characteristic flavour of oil obtained from olives piled or stored in such conditions as to have undergone an advanced stage of anaerobic fermentation, or of oil that settles in underground tanks and vats and which has also undergone a process of anaerobic fermentation which has been left in contact with the sediment
Musty-humid-earthy: Characteristic flavour of oils obtained from fruit in which large numbers of fungi and yeasts have developed as a result of its being stored in humid conditions for several days or of oil obtained from olives that have been collected with earth or mud on them and which have not been washed.
Winey-vinegary-acid-sour: Characteristic flavour of certain oils reminiscent of wine or vinegar. This flavour is mainly due to a process of aerobic fermentation in the olives or in olive paste left on pressing mats which have not been properly cleaned and leads to the formation of acetic acid, ethyl acetate and ethanol.
Rancid: Flavour of oils which have undergone an intense process of oxidation.
Frostbitten olives (wet wood): Characteristic flavour of oils extracted from olives which have been injured by frost while on the tree.
Other negative attributes
Heated or.: Characteristic flavour of oils caused by excessive and/or prolonged
Burnt: Heating during processing, particularly when the paste is thermally mixed, if this is done under unsuitable thermal conditions.
Hay–wood: Characteristic flavour of certain oils produced from olives that have dried out.
Rough: Thick, pasty mouth sensation produced by certain old oils.
Greasy: Flavour of oil reminiscent of that of diesel oil, grease or mineral oil.
Vegetable water: Flavour acquired by the oil as a result of prolonged contact with vegetable water which has undergone fermentation processes.
Brine: Flavour of oil extracted from olives which have been preserved in brine.
Metallic: Flavour that is reminiscent of metals. It is characteristic of oil which has been in prolonged contact with metallic surfaces during crushing, mixing, pressing or storage.
Esparto: Characteristic flavour of oil obtained from olives pressed in new esparto mats. The flavour may differ depending on whether the mats are made of green esparto or dried esparto.
Grubby: Flavour of oil obtained from olives which have been heavily attacked by the grubs of the olive fly (Bactrocera oleae)
Cucumber: Flavour produced when an oil is hermetically packed for too long, particularly in tin containers, and which is attributed to the formation of 2,6 nonadienal.
Positive attributes
Fruity: Set of olfactory sensations characteristic of the oil which depends on the variety and comes from sound, fresh olives, either ripe or unripe. It is perceived directly and/or through the back of the nose.
Bitter: Characteristic primary taste of oil obtained from green olives or olives turning colour. It is perceived in the circumvallate papillae on the "V" region of the tongue.
Pungent: Biting tactile sensation characteristic of oils produced at the start of the crop year, primarily from olives that are still unripe. It can be perceived throughout the whole of the mouth cavity, particularly in the throat.

Table 1.3 List of descriptors for granting designation of origin of EVOO (COI/T.20/Doc. no. 22)

Direct or retronasal aromatic olfactory sensations
Almond: Olfactory sensation reminiscent of fresh almonds
Apple: Olfactory sensation reminiscent of the odour of fresh apples
Artichoke: Olfactory sensation of artichokes
Camomile: Olfactory sensation reminiscent of that of camomile flowers
Citrus fruit: Olfactory sensation reminiscent of that of citrus fruit (lemon, orange, bergamot, mandarin and grapefruit)
Eucalyptus: Olfactory sensation typical of Eucalyptus leaves
Exotic fruit: Olfactory sensation reminiscent of the characteristic odours of exotic fruit (pineapple, banana, passion fruit, mango, papaya, etc.)
Fig leaf: Olfactory sensation typical of fig leaves
Flowers: Complex olfactory sensation generally reminiscent of the odour of flours, also known as floral
Grass: Olfactory sensation typical of freshly mown grass
Green pepper: Olfactory sensation of green peppercorns
Green Complex: olfactory sensation reminiscent of the typical odour of fruit before it ripens
Greenly fruity: Olfactory sensation typical of oils obtained from olives that have been harvested before or during colour change
Herbs: Olfactory sensation reminiscent of that of herbs
Olive leaf: Olfactory sensation reminiscent of the odour of fresh olive leaves
Pear: Olfactory sensation typical of fresh pears
Pine kernel: Olfactory sensation reminiscent of the odour of fresh pine kernels
Ripely fruity: Olfactory sensation typical of oils obtained from olives that have been harvested when fully ripe
Soft fruit: Olfactory sensation typical of soft fruit: blackberries, raspberries, bilberries, blackcurrants and redcurrants
Sweet pepper: Olfactory sensation reminiscent of fresh sweet red or green peppers
Tomato: Olfactory sensation typical of tomato leaves
Vanilla: Olfactory sensation of natural dried vanilla powder or pods, different from the sensation of vanillin
Walnut: Olfactory sensation typical of shelled walnuts
Gustatory sensations
Bitter: Characteristic taste of oil obtained from green olives or olives turning colour; it defines the primary taste associated with aqueous solutions of substances like quinine and caffeine
"Sweet": Complex gustatory-kinaesthetic sensation characteristic of oil obtained from olives that have reached full maturity
Qualitative retronasal sensation
Retronasal persistence: Length of time that retronasal sensations persist after the sip of olive oil is no longer in the mouth
Tactile or kinaesthetic sensations
Fluidity: Kinaesthetic characteristics of the rheological properties of the oil, the set of which are capable of stimulating the mechanical receptors located in the mouth during the test
Pungent: Biting tactile sensation characteristic of oils produced at the start of the crop year, primarily from olives that are still unripe

- Oil separating

During this phase the oily phase is separated from the olive paste. The oldest method to carry out the separating phase is the pressure system. The olive paste is placed on nylon and/or polypropylene filter mats, that are then stacked and pressed by an hydraulic press (Servili et al., 2012). This method is not almost used anymore because it is a discontinuous process having a low working capacity, and also due to issues related to the use of filter mats. In fact, the residues trapped in the filter mats may oxidase during the storage between the different processing steps and contaminate the next oil extracted triggering oxidative processes.

The majority of VOO is currently extracted by centrifugation in Mediterranean countries (Servili et al., 2012). There are three types of centrifugation machines, called decanters, that basically distinguish themselves by the quantity of added water needed. The three phase decanter separates the olive must, vegetation water and solids. To work this machine needs a proper dilution of olive paste (10–30L of added water per 100kg of olive pastes) causing he reduction of phenol content in oil and the production of significant volumes of olive mill waste waters that constitute an important environmental pollution problem (Kalogeropoulos et al., 2014). To avoid these problems two phase decanter were developed. This type of machine do not require the water adding, so the phenolic substances are not washed away as in the three phase decanter (Salvador et al., 2003). Notwithstanding the water saving, its use is not so widespread, mainly due to the high moisture content of the resultant pomace, which hinders the quantitative recovery of pomace oil by solvent extraction (Kalogeropoulos et al., 2014). Finally three-phases water saving decanters have been developed in order to minimise the disadvantages of the others typologies of decanters.

Importance of VOO in relation to health

VOO is obtained from olives by mechanical or other physical means only, it is the only vegetable oil that can be consumed without refining, and those characteristics make it a real olive juice. Thus VOO is different from the other oils present on the marketplace because, besides being a MUFA source, it contains minor quantities of polar compounds, including phenols.

Initially, the public attention was drawn to the Mediterranean diet and to olive oil, and to VOO particularly, by the results of the Seven Country Study and the well-known works of Keys elucidating the effects of MUFA on cholesterol metabolism (Pérez-Jiménez et al., 2007). Then, in the last fifteen year a new paradigm has emerged, demonstrating that the positive effects of Mediterranean diet on human health exceed the benefits on cholesterol and even the lowering of traditional risk factors (Pérez-Jiménez et al., 2007). By showing that phenolic compounds can

reduce the levels of risk for cardiovascular disease, the EUROLIVE (Covas et al., 2006) study provided clear evidence that VOO has benefic effects due to more than just MUFA (López-Miranda et al., 2010). To date several positive effects on health linked to the Mediterranean Diet, of which VOO has been suggested as a key factor for the health benefits (Hu, 2003; Pérez-Jiménez et al., 2007), have been elucidated, as summarized in Table 1.4, published by López-Miranda and colleagues (2010).

Table 1.4 Studies supporting the health effects of the Mediterranean Diet rich in VOO

Level of evidence	Type of effect [reference]
Demonstrated by dietary intervention trials in different populations	1. Beneficial effects on the lipid profile, with a decrease in LDL-cholesterol and higher HDL/total cholesterol ratio versus SFA 2. Reduction of LDL oxidizability 3. Improvement of glucose metabolism in normal subjects and patients with type 2 diabetes. Substitution of MUFA for SFA results in lower insulin requirement and plasma glucose concentrations, and is at least as effective as CHO 4. Improved blood pressure control 5. Improvement of endothelial function 6. Promotion of a less prothrombotic environment compared with SFA-rich diets, influencing different thrombogenic factors: reduction of platelet aggregation, thromboxane B_2 production, von Willebrand factor (vWf), tissue factor, tissue factor pathway inhibitor, PAI-1, Factor VII and Factor XII
Suggested by a few dietary intervention trials, observational studies, or in vitro experiments	1. Favorable effects on obesity 2. Lower NF-$_k$B activation when compared with other types of diet, both in fasting and postprandial state. 3. Reduction in age-related cognitive decline and Alzheimer's disease of increased adherence
vWf, Von Willebrand factor; LDL, low density lipoprotein; HDL, High density lipoprotein; MUFA, Monounsaturated fatty acids; SFA, saturated fatty acids; CHO, carbohydrates; PAI-1, plasminogen activator inhibitor type 1; NF-$_k$B, nuclear factor kappaB	

From: López-Miranda et al., 2010

During the process of understanding the effects of olive oil on human health, one of the first questions needing an answer was the bioavailability of the phenolic compounds from virgin olive oil, that was proved by Cicerale et al. (2010); furthermore studies carried out on hydroxytyrosol and tyrosol had demonstrated that their absorption is dose-dependent (Visioli et al., 2000a; Visioli et al., 2000b; Caruso et al., 2001). This finding, together with the recent authorization of health claim by European Food Safety Authority (EFSA) (EFSA, 2011) related to the protection of LDL from oxidation by hydroxytyrosol, raised the question of phenols content in VOO. The EFSA panel concluded that, as part of a balanced diet (20g of fat/die), 5 mg of hydroxytyrosol are required for

obtaining benefic effects on health, meaning a phenol concentration of 250-300 mg/Kg in VOO, as assert by professor Servili elsewhere.

2. **Aim**

There is a strict relationship between crop cultivation and the site-specificities of the territory in terms of yield, farmer incomes, cost efficiency, economic sustainability and product characteristics (Di Virgilio, 2012). This relationship is defined crop vocation, and its promotion means promoting not only the product but also the territory thus creating positive externalities.

The environment in which the plant grows is the result of the mutual influence of abiotic (soil, temperature, water, light, wind) and biotic (living organisms, animals and plants) factors. All species have, in a more or less accentuated way, a sensibility to these factors. Furthermore, much of the plant productivity, i.e. both in yield and quality, depends on the environmental possibility to support plant requirements, and also it depends on the plant species ability to adapt to environment.

The study was carried out in the north part of Sardinia Island, using cv. Bosana, the most widespread olive variety in the province of Sassari. The studied territory is within the borders of PDO (Protected Designation of Origin) “Sardegna” extra virgin olive oil, which actually includes the whole Island. The production regulations of the PDO “Sardegna” indicates the olive varieties composition of PDO product, which include the Bosana variety and other four autochthonous olive cultivars. Consequently, the PDO “Sardegna” results in an extra virgin olive oil strictly linked to the territory of origin

The aim of this work is the assessment of the characteristics of cv. Bosana virgin olive oil in relation to the environmental features of its native territory, the northern part of Sardinia region. Moreover, keeping in mind the decisive role played by the environmental on plant physiological processes, a trial to understand (i) the environmental effects on ripeness trend and (ii) the effect of ripening stages on chemical and sensory characteristic of Bosana virgin olive oil has been carried out.

3. Pedological, geological and climatic description of the site

Highlight

Orographic, geological and pedological characterization of Sassari province

Subdivision of the Sassari province in three areas in each of which four olive groves had been selected.

Climatic characterization of the province of Sassari with focus on the three areas selected

Characterization of the studied area

The territory of the Sassari province has an extension of about 428489 ha, and is located in the north-west part of Sardinia, bordering with the provinces of Olbia, Nuoro and Oristano (Fig. 3.1). The altitude of the Sassari province ranges between 0 and 1250 m a.s.l. (Fig. 3.2). Most of the territory (45.05%) is characterized by hilly landscape (300-700 m a.s.l.), while 30% of the territory is represented by low hills (100-300 m a.s.l.) and the 19.99% is plain. Only a little part of the territory is classified as low mountain area and mountain area, respectively 3.74% and 1.22% (Fig. 3.2).

The map of land use, distributed by the Sardinia Region mapping service, shows that a large part of the agricultural land is used mainly in meadows, since breeding is an important activity, and then sowable and olive cultivation (Fig. 3.3). The overlay of map of land use with the map of the municipalities of the Province of Sassari, has pointed out the relevance of olive groves in several municipalities (Table 3.1). The cultivation of olive trees is mainly localized in the centre of the province, at a range of altitudes between 100 and 300 m above sea level or even lower areas. Only small portions of olive groves are located in areas between 300 and 700 m above sea level (Fig. 3.3 and Table 3.2). Out of a total of 15478 hectares of olive groves, 7779 ha are located in the low hills and 5436 ha in the hills. A smaller portion of the 5436 ha is located slightly higher up in the hills, ranging from 300 to 700 m above sea, with only 2 ha located above a high of 700 m. Most of the plants are located in the municipalities of Sorso (16.54% of the municipal area), Usini (16.38%), Alghero (11.58%), Uri (10.23%). The most important extensions of olive groves are located in the municipality of Sassari, while 54737 ha are divided between the municipality of Alghero (22524 ha) and Ittiri (11150 ha).

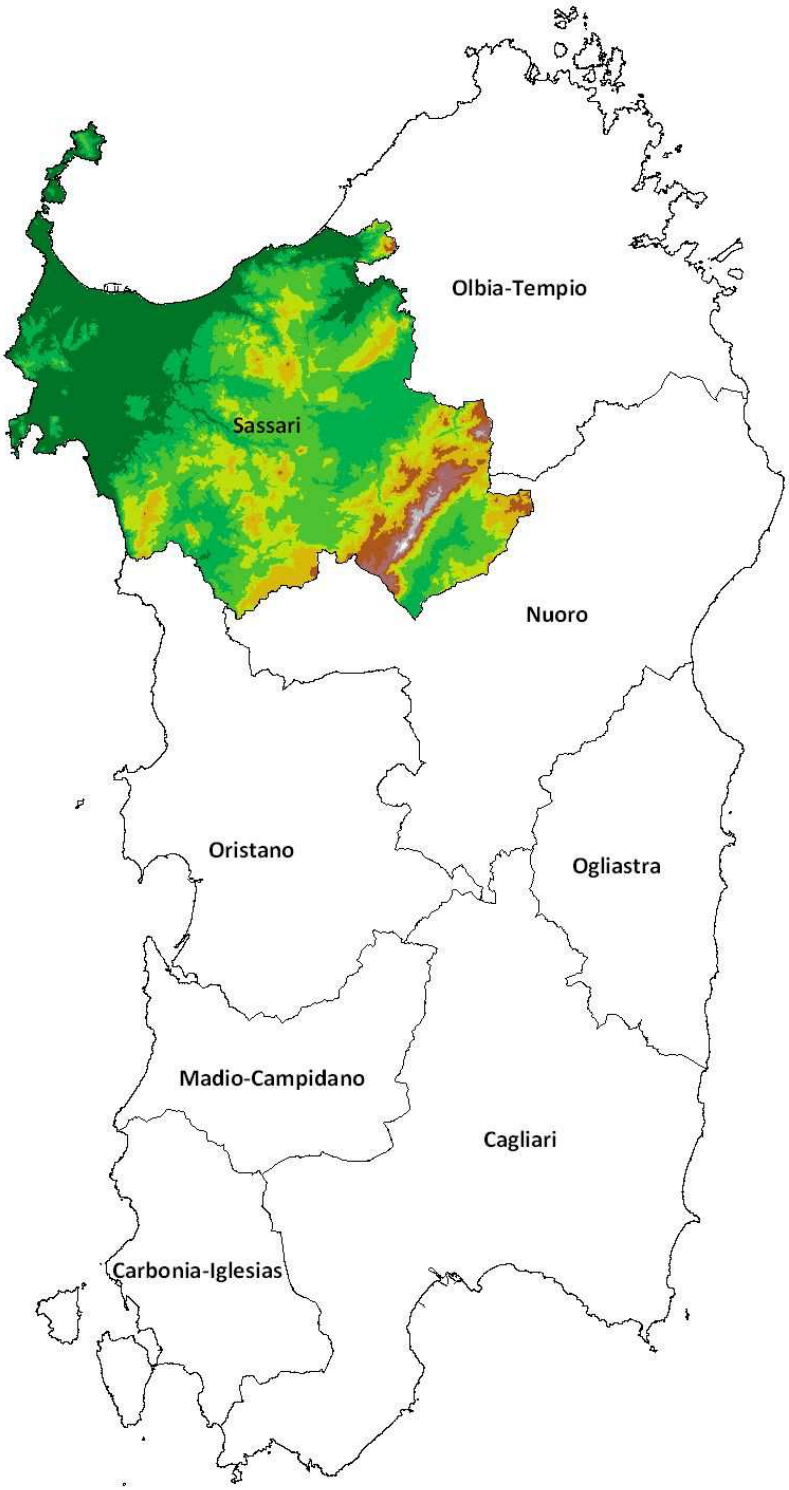

Fig. 3.1 The province of Sassari in the Sardinia region

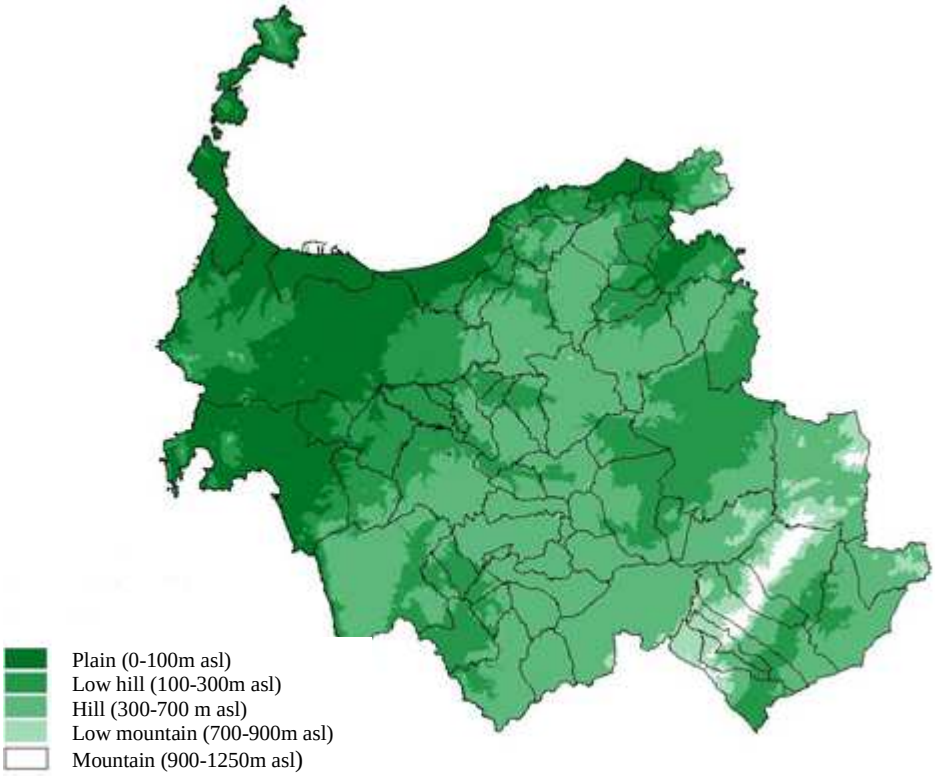

Fig. 3.2 Elevation of the Sassari province

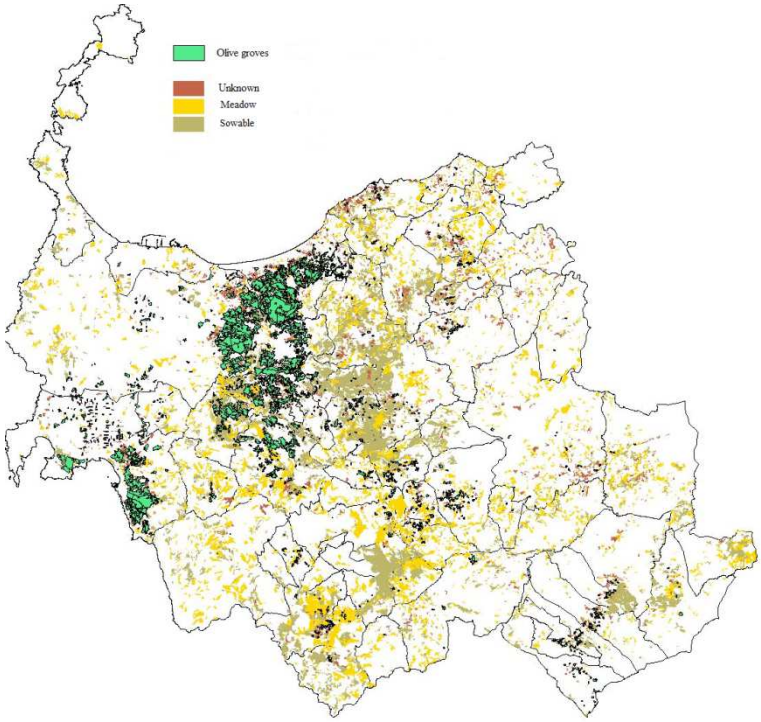

Fig. 3.3 Land use map of the province of Sassari. In green olive groves, in brown unknown use of land, in yellow meadow and in colour bronze sowable

Table 3.1 Municipalities and olive groves extension

Municipality	Olive groves (ha)	Municipality (ha)	%	Municipality	Olive groves (ha)	Municipality (ha)	%
Sorso	1107	6692	16.54	Bulzi	16	2161	0.72
Usini	503	30.7	16.38	Bottida	24	3358	0.71
Alghero	2608	22524	11.58	Osilo	67	9791	0.69
Uri	580	5672	10.23	Cheremule	16	2416	0.64
Sennori	312	3139	9.94	Burgos	11	1796	0.63
Tissi	100	1028	9.73	Thiesi	35	6325	0.56
Ittiri	969	11150	8.69	Tergu	20	3681	0.55
Sassari	4684	54737	8.56	Ploaghe	52	9619	0.54
Ossi	207	3010	6.86	Pozzo Maggiore	40	7969	0.5
Bonnanaro	99	2184	4.55	S.Maria Coghinas	11	14974	0.32
Muros	45	1109	4.1	Bonorva	48	14974	0.32
Banari	76	2130	3.56	Benetutti	27	9452	0.29
Siligo	137	4346	3.16	Ittireddu	6	2369	0.24
Codrongianus	88	3040	2.91	Giave	9	4700	0.19
Romana	57	2169	2.63	Ozieri	44	24596	0.18
Esporlatu	47	1827	2.55	Portotorres	15	10428	0.14
Florinas	84	3612	2.31	Buldei	12	9703	0.12
Mores	216	9490	2.28	Cossoine	5	3902	0.12
Laerru	44	1985	2.19	Viddalba	4	4944	0.07
Torralba	57	3667	1.56	Ardara	2	3810	0.06
Martis	34	2292	1.48	Tula	4	6646	0.06
Illorai	79	5710	1.38	Pattada	9	16464	0.05
Castelsardo	60	4348	1.37	Semestene	2	3968	0.05
Bessude	32	2673	1.19	Perfugas	3	6075	0.04
Padria	51	4823	1.05	Nulvi	2	6751	0.03
Bono	78	7450	1.05	Nughedu S.Nicolò	2	6807	0.02
Putifigari	54	5305	1.02	Villanova Monteleone	2	20228	0.01
Anela	36	3684	0.98	Borutta		473	0
Olmedo	32	3353	0.94	Erula		4564	0
Mara	17	18.63	0.91	Monteleone Rocca Doria		1341	0
Cargeghe	11	1212	0.89	Nule		5209	0
Chiaramonti	83	9868	0.84	Stintino		5870	0
Sedini	31	4100	0.76				
Valledoria	20	2590	0.76	Total	13122	428494	3.06

Table 3.2 Distribution in ha and in percentage of olive groves located in the province of Sassari

Land use	Low hill (100-300m asl)	Low mountain (700-900m asl.)	Hill (300-700m asl)	Mountain (900-1250m asl)	Plain (0 - 100m asl)	Total
Olive groves	7779	2	2261	0	5436	15478
Other	120762	16015	190777	5225	80234	413013
Total	128541	16017	193038	5225	85670	428492
% olive groves	6.05	0.01	1.17	0.00	6.35	3.61

The choosing of experimental orchards

The choose of olive groves in where perform the study was done taking into account the widespread of olive groves (Fig. 3.3) and the agronomic practices since they can modify the oil quality (Servili et al. 2004). In this regard, in collaboration with IBIMET of Sassari, Sardinian Agency Laore (Agenzia regionale per l'attuazione dei programmi in campo agricolo e per lo sviluppo rurale) and Consortium "DOP Sardegna" the data about agronomic practices were collected and 12 olive groves located in 3 macro area (Alghero, Sassari and Ittiri) where chosen (4 experimental sites in each macro-areas) (Fig.3.3). In table 3.3 are shown the agronomic practices adopted in the olive groves chosen for the this research.

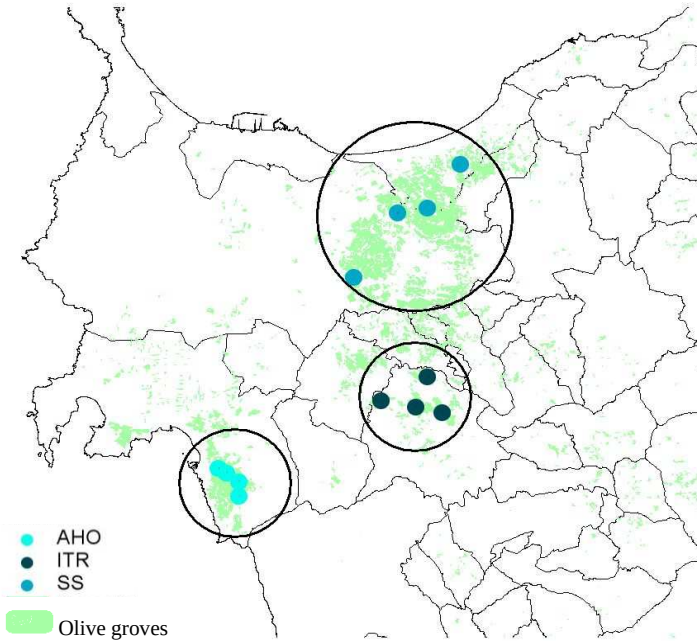

Fig. 3.3 Geographical location of the 12 olive orchards selected for the study. AHO Alghero, ITR Ittiri and SS Sassari

Table 3.3 Agronomical practices used in the olive grove selected

	Olive grove size (ha)	Plantation spacing (m)	Age of trees	Training System	Frequency of pruning	Soil management	Irrigation	Fertilisers
AHO 1	0.8	9 x 9	centuries-old	vase	biennial	ploughing, harrowing	no	NPK 20 – 10 - 10
AHO 2	44	9 x 9	centuries-old	vase	biennial	ploughing, harrowing	no	NPK 20 – 10 - 10
AHO 3	4	9 x 9	centuries-old	vase	biennial	ploughing, harrowing	no	NPK 20-10-10 and urea
AHO 4	0.75	10 x 10	centuries-old	vase	biennial	ploughing, harrowing	no	NPK 20 - 10 - 10
ITR 1	2.5	10 x 10	centuries-old	vase - multiple cones	biennial	grassing	no	organic and NPK 20-20-20
ITR 2	2	10 x 10	centuries-old	vase - multiple cones	biennial/triennial	chopping 2/3 times per year	no	NPK 20-20-20
ITR 3	1	10 x 8	50 years old	vase - multiple cones	triennial	chopping	no	NPK 20-20-20
ITR 4	0.5	8 x 8	55 years old	vase - multiple cones	4/5 years	chopping and weeding	no	NPK 20-20-20
SS 1	13	8 x 8 / 10 x 10	centuries-old	vase	biennial	ploughing	no	no
SS 2	1.7	8 x 10	>50 years old	vase	4/5 years	chopping 2 times per year	no	manure
SS 3	27	8 x 8	>50 years old	vase	annual	chopping and ploughing	no	olive pomace
SS 4	16	8 x 8	centuries-old	vase - multiple cones	triennial	chopping 3 times per year	no	olive pomace

Morpho-pedological characterization of the studied territory

A Digital Elevation Model (DEM) (Fig. 3.4) of the area of interest was built using elevation information as digital isolines and points (the last mainly for flatter areas) with a resolution of 5 m by the spatial analyst tool of Arcview 3.2 (ESRI). The DEM represents the continuous variation of over space reliefs produced by interpolating known elevation values from isolines and points. By DEM, it is possible to produce continuous thematic maps of altitude, slope and terrain aspects through the use of GIS analysis tools.

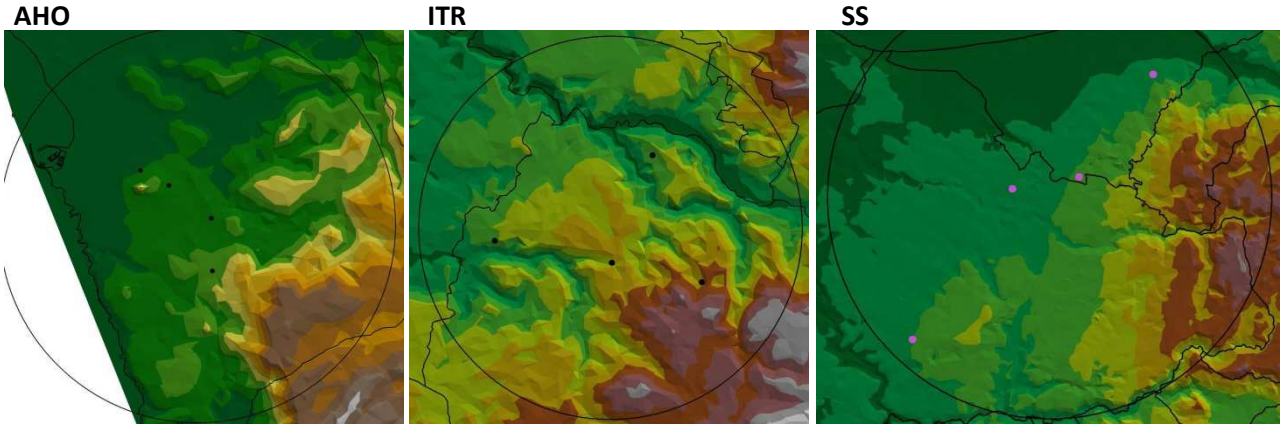

Fig. 3.4 Digital Elevation Model. Enlargement of the three areas studied: Alghero (AHO), Ittiri (ITR) and Sassari (SS).

The overall morphological information of the selected olive groves are shown in Table 3.4. These data are derived by the geo-localization obtained with DEM combined with the known maps of altitude, slope and exposure.

The olive groves located in the Alghero area are characterized by an altitude ranging between 50m and 110 m a.s.l. with a mean value of 78.14 m a.s.l.. Olive orchards of Sassari area are all located above 100 m a.s.l., while those of Ittiri, the most inland zone, are located at an altitude significantly higher, ranging from 191.31 to 331.79 m a.s.l., with an averaged 253.80 m. The slope is quite variable both among the three macro zones and among the different groves as well. The Alghero and Sassari areas are on average flat, with values ranging between 1.51° and 0.45° and 7.99° and 6.1° respectively; the Ittiri zone is characterized by a greater slope (11.33° on average) the greatest slope (18.78°) being in the olive grove codified as ITR 4. Moreover, Ittiri orchards are also the most distant from the sea (about 21 km on average), while those of Alghero are the closest (about 3 km on average). Thus the orchards located in the Sassari area are positioned halfway both for altitude and distance from the sea (6.42 km).

Table 3.4 Morphological informations of the olive grove selected for study

	Altitude (m)	Slope (°)	Exposure (°)	Exposure	Distance from the sea (Km)
AHO 1	110.38	2.21	318.24	NO-N	2.96
AHO 2	77.01	1.51	282.42	O-NO	3.26
AHO 3	75.02	2.55	96.29	E-SE	2.32
AHO 4	50.14	7.99	317.91	NO-N	1.97
Mean	**78.14**	**3.57**	**253.72**	**-**	**2.63**
ITR 1	275.73	8.85	231.90	S-SO	19.98
ITR 2	331.79	13.16	257.63	S-SO	22.12
ITR 3	191.31	4.54	234.31	S-SO	20.90
ITR 4	216.38	18.78	183.51	S-SO	16.69
Mean	**253.80**	**11.33**	**226.84**	**-**	**19.92**
SS 1	133.11	4.15	268.16	SO-O	11.22
SS 2	108.53	6.01	346.99	NO-N	2.90
SS 3	145.67	0.45	330.66	NO-N	5.79
SS 4	108.95	1.60	197.42	S-SO	5.77
Mean	**124.07**	**3.05**	**285.81**	**-**	**6.42**

Geological characterization

Consulting the geological map provided by the Sardinia region it became clear that the whole region is characterized by a quite complex geological history (Fig. 3.5). However the olive groves we selected are located in areas rather homogeneous from a geological point of view (Fig 3.5).

Olive groves of the Alghero area are all located in the same geological formation called “PVMb”, pleistocene deposits of continental area, mainly made up of *wind*-deposited *sands* are *arenites*. Three olive groves of the Sassari area (SS 2, SS3 and SS 4), and three of the Ittiri area (ITR 1, ITR 2 and ITR3) are located in the formation called “RTU”, oligo-miocene sedimentary layers of “Logudoro- sassarese”, manly composed of marlstone and limestone-marl. The other two groves (SS1 and ITR4) are located in the formation called “RESa”, oligo-miocene sedimentary layers charachterized by the presence of calcarenite and bioclastic limestones and with gastropods, *Ostreidae* and *Echinide.* It is however important to underline the variability of the geology of the Ittiri area, representing a transitional zone between the two different geological formations. In Table 3.5 are summarised the geological characteristics of the soils of the 12 olive groves selected.

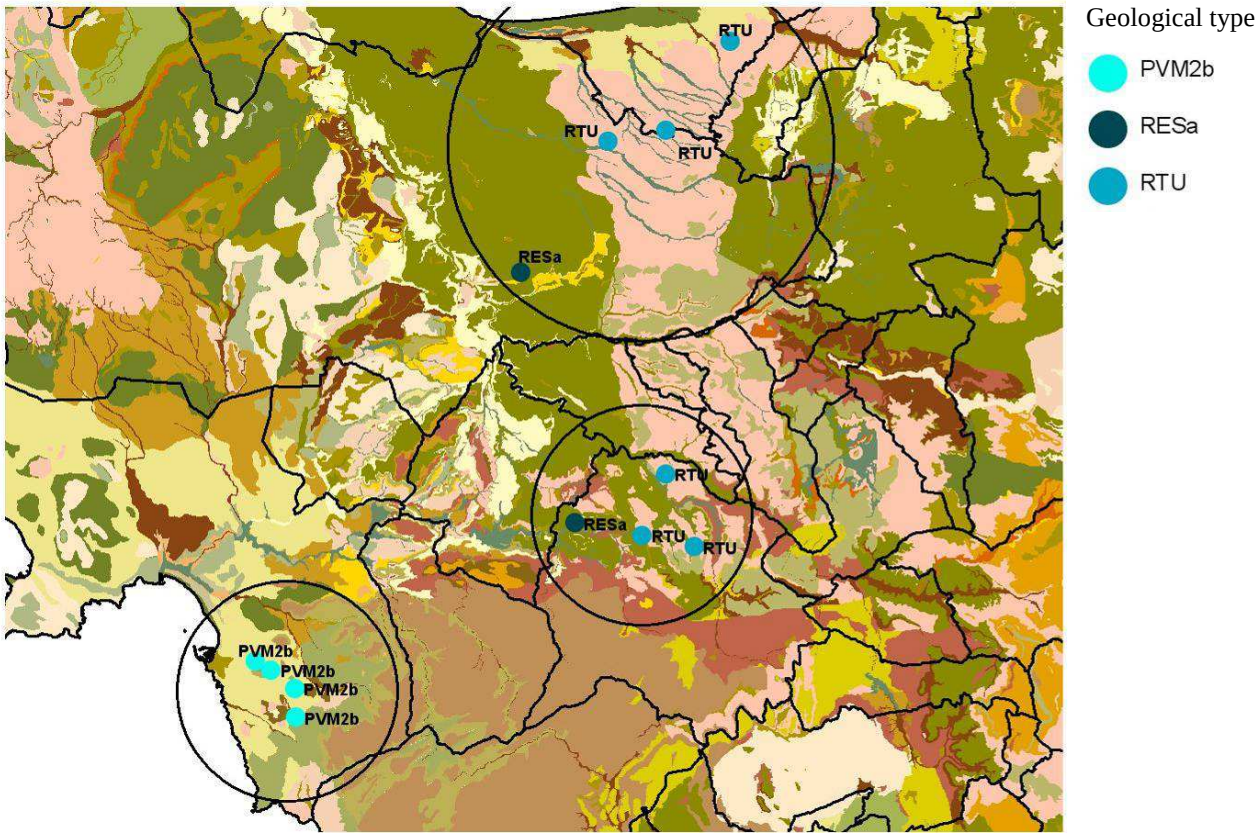

Fig. 3.5 Geological map of the areas under study

Soils characterization

The soil typologies of north Sardinia are reported in figure 3.6. The territory is classified in "soil regions" according to the criteria of the Manual of Procedures for the Georeferenced soil database of Europe, Version 1.0 (European Commission, 1998). In the studied territory there are two soil region typologies: the "59.1" and "59.8". the "59.1" (Fig. 3.6). All the olive groves from Sassari and Ittiri belong to the 59.1 typology, characterized by several sedimentary rocks from Triassic to Miocene (marl, limestones, sandstones), while the ones from Alghero belong to the 59.8 typology, characterized by acid igneous and effusive (Tertiary basalts and trachyte) rocks, and in part by metamorphic and sedimentary rocks.

The soil map is shown on a more detailed scale in figure 3.7. From the figure it is possible to deduct further information on the different soils on which the olive trees are cultured, as well as to list some interesting agronomic parameters, such as the soil depth, reaction and texture. On the base of the soil map three of the four Alghero olive groves (AHO 2, AHO 3 and AHO 4) belong to the "I1" soil typology. This soil typology is characterized by a sub-acid and acid reaction, from permeable to low permeability, with a moderate surface soil erodibility and depth more than 1 m.

Table 3.5 Geological characteristics of the twelve olive groves selected

	SOIL REG	SR_NAME	SR_PMAS	SR_MATHI [°C]	SR _MAPLO [mm]	SR _MAPHI [mm]	SR _HIPREC	SR _DROUG	SR _ALTHI [m. asl]
AHO 1	59.8	Cambisol - Leptolsol region with Vertisols and Andosol of north-west Sardinia	Acid igneous and effusive (Tertiary basalts and trachite) rocks; partly metamorphic and sedimentary rocks	20	600	1200	NOV, DEC	Jun - Sep	1000
AHO 2	59.8	Cambisol - Leptolsol region with Vertisols and Andosol of north-west Sardinia	Acid igneous and effusive (Tertiary basalts and trachite) rocks; partly metamorphic and sedimentary rocks	20	600	1200	NOV, DEC	Jun - Sep	1000
AHO 3	59.8	Cambisol - Leptolsol region with Vertisols and Andosol of north-west Sardinia	Acid igneous and effusive (Tertiary basalts and trachite) rocks; partly metamorphic and sedimentary rocks	20	600	1200	NOV, DEC	Jun - Sep	1000
AHO 4	59.8	Cambisol - Leptolsol region with Vertisols and Andosol of north-west Sardinia	Acid igneous and effusive (Tertiary basalts and trachite) rocks; partly metamorphic and sedimentary rocks	20	600	1200	NOV, DEC	Jun - Sep	1000
ITR 1	59.1	Cambisol - Leptolsol region of Sardinia with Vertisols, Arenosol and Fluvisol	Very different sedimentary rocks of Triassic to Miocene (marl, limestones, sandstones)	18	600	1200	DEC, JAN	Jun - Aug	1000
ITR 2	59.1	Cambisol - Leptolsol region of Sardinia with Vertisols, Arenosol and Fluvisol	Very different sedimentary rocks of Triassic to Miocene (marl, limestones, sandstones)	18	600	1200	DEC, JAN	Jun - Aug	1000
ITR 3	59.1	Cambisol - Leptolsol region of Sardinia with Vertisols, Arenosol and Fluvisol	Very different sedimentary rocks of Triassic to Miocene (marl, limestones, sandstones)	18	600	1200	DEC, JAN	Jun - Aug	1000
ITR 4	59.1	Cambisol - Leptolsol region of Sardinia with Vertisols, Arenosol and Fluvisol	Very different sedimentary rocks of Triassic to Miocene (marl, limestones, sandstones)	18	600	1200	DEC, JAN	Jun - Aug	1000
SS 1	59.1	Cambisol - Leptolsol region of Sardinia with Vertisols, Arenosol and Fluvisol	Very different sedimentary rocks of Triassic to Miocene (marl, limestones, sandstones)	18	600	1200	DEC, JAN	Jun - Aug	1000
SS 2	59.1	Cambisol - Leptolsol region of Sardinia with Vertisols, Arenosol and Fluvisol	Very different sedimentary rocks of Triassic to Miocene (marl, limestones, sandstones)	18	600	1200	DEC, JAN	Jun - Aug	1000
SS 3	59.1	Cambisol - Leptolsol region of Sardinia with Vertisols, Arenosol and Fluvisol	Very different sedimentary rocks of Triassic to Miocene (marl, limestones, sandstones)	18	600	1200	DEC, JAN	Jun - Aug	1000
SS 4	59.1	Cambisol - Leptolsol region of Sardinia with Vertisols, Arenosol and Fluvisol	Very different sedimentary rocks of Triassic to Miocene (marl, limestones, sandstones)	18	600	1200	DEC, JAN	Jun - Aug	1000

Soil reg, Number Soil Region; SR_NAME, Climate, parent material and regional code. Description of soil region with dominant soil types and regional name; SR_MATHI [°C], Mean annual temperature (higher value, °C); SR _MAPLO mm, Mean annual precipitation (lower value, mm); SR _MAPHI mm, Mean annual precipitation (higher value, mm); SR _HIPREC, Months with high precipitation;; SR _DROUG, Months with drought.

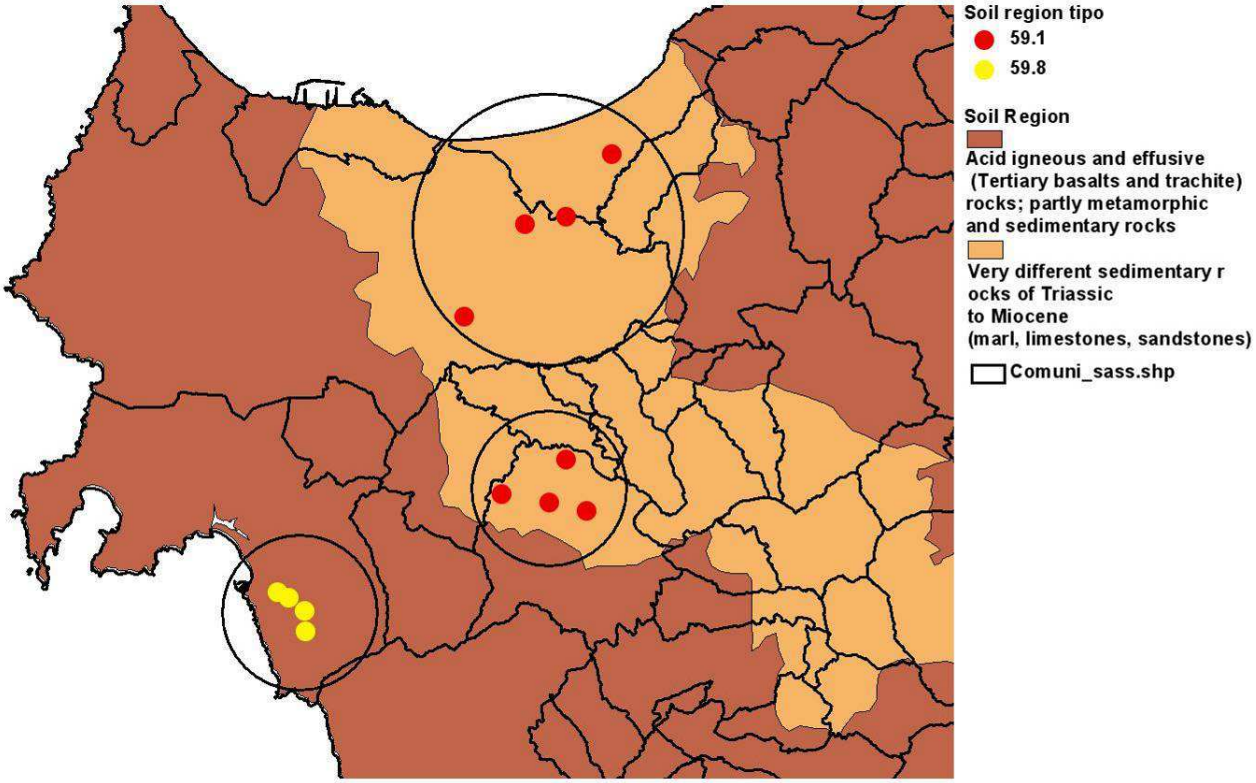

Fig 3.6 Soil typologies of north Sardinia

The AHO 1 olive grove is located on “D4” soil typology, characterized by a neutral reaction, from permeable to medium permeability, with high soil erodibility and with a depth from shallow to moderate (Table 3.6). The Sassari olive groves belong to “F” typology; SS 3 and SS 4 are characterized by soil typology “F1”, SS 1 and SS 2 by soil typology “F2”. The typologies “F1” and “F2” are quite similar, having a neutral reaction, permeable, with high soil erodibility and moderate depth, but differ for the outcrop (Table 3.6). Olive groves of the Ittiri area are all located in the “F1” soil typology, (Table 3.6).

Concluding, Alghero olive groves are located in a different soil typology compared to Ittiri and Sassari. The Alghero typology is characterized by soil with an acid reaction, and with an higher depth and a lower erodibility than the other typologies; moreover the “I1” typology has a greater sandy component on the surface and a dial clay at more depth. The Alghero groves are also the closest to the sea and the lowest in altitude of the zones under study, being located at an average 78.14 m above sea level (Table 3.6).

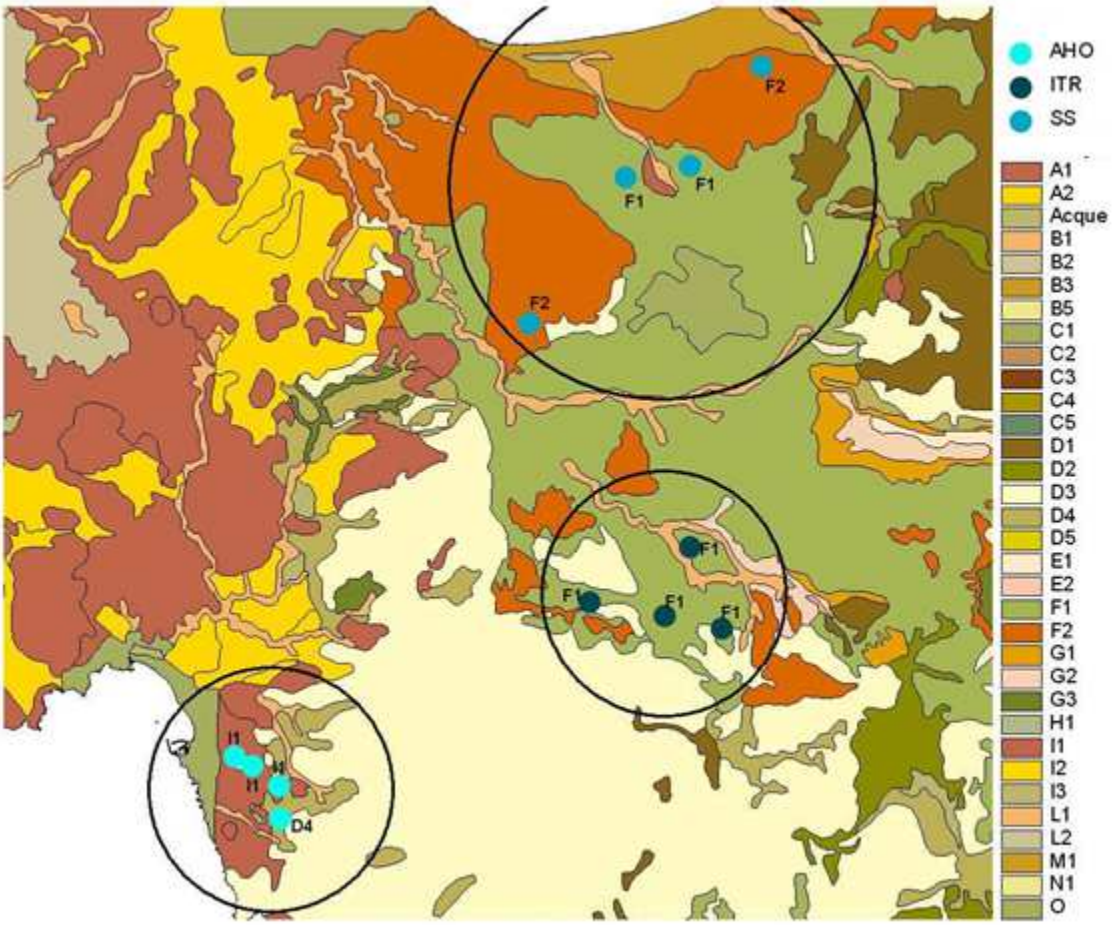

Fig. 3.7 Soil map on detailed scale of Sardinia focused on the understudied territory

Table 3.6 Characteristics of the oil typologies of the selected olive groves

	Soil typology	Soil horizon	Reaction	Permeability	Erodibility	Soil texture	Depth
AHO 1	D4	Layers A-Bw-C, A-C and sub. outcrop,	neutral	from permeable to medium permeable	high	from sandy loam to sandy clay	shallow to moderately deep
AHO 2	I1	Layers A-Bt-C, A-Btg-Cg and below A-C	sub-acid and acid reaction	from permeable to low permeable	moderate	from sandy loam to sandy clay loam on surface, from sandy clay loam to clay in depths	depth more than 1 m
AHO 3	I1	Layers A-Bt-C, A-Btg-Cg and below A-C	sub-acid and acid reaction	from permeable to low permeable	moderate	from sandy loam to sandy clay loam on surface, from sandy clay loam to clay in depths	depth more than 1 m
AHO 4	I1	Layers A-Bt-C, A-Btg-Cg and below A-C	sub-acid and acid reaction	from permeable to low permeable	moderate	from sandy loam to sandy clay loam on surface, from sandy clay loam to clay in depths	depth more than 1 m
ITR 1	F1	Outcrop, Layers A-C and A-Bt-C,	neutral	permeable	high	from sandy clay loam to clay	shallow to moderately deep
ITR 2	F1	Outcrop, Layers A-C and A-Bt-C,	neutral	permeable	high	from sandy clay loam to clay	shallow to moderately deep
ITR 3	F1	Outcrop, Layers A-C and A-Bt-C,	neutral	permeable	high	from sandy clay loam to clay	shallow to moderately deep
ITR 4	F1	Outcrop, Layers A-C and A-Bt-C,	neutral	permeable	high	from sandy clay loam to clay	shallow to moderately deep
SS 1	F2	Layers A-C, A-Bw-C, A-Bt-C and sub. Outcrop	neutral	permeable	high	from sandy clay loam to clay	shallow to moderately deep
SS 2	F2	Layers A-C, A-Bw-C, A-Bt-C and sub. Outcrop	neutral	permeable	high	from sandy clay loam to clay	shallow to moderately deep
SS 3	F1	Outcrop, Layers A-C and A-Bt-C,	neutral	permeable	high	from sandy clay loam to clay	shallow to moderately deep
SS 4	F1	Outcrop, Layers A-C and A-Bt-C,	neutral	permeable	high	from sandy clay loam to clay	shallow to moderately deep

Mesoclimatic survey

Studies of land suitability use the mesoclimatic characterization to underline the climate factors that can be limiting for growing a crop. However in this study the mesoclimatic characterization was used to understand the differences in the quality of the different productions. In fact climate variables such as temperature and rainfall can strongly influence the composition of virgin olive oils.

The climate of Sardinia is classified as Mediterranean (Chessa & Delitala, 1997), respecting the limit fixed by Koeppen (70% of the total precipitation taking place in wintertime) (Mariani, 2002). Sardinia is characterized by a dry summer (from May to September), the climate being influenced by the Azores Anticyclone that strongly reduces the penetration of Atlantic disturbances or the formation of local disturbances in the region. In contrast, winter in Sardinia, from October to April, can be very wet (Delitala et al., 2000).

A spatial temperature technique was used in order to describe the temperature graphically. Interpolation (or spatialization) is used to generate maps indicating how a certain variable behaves in space, in order to obtain the variable values at points where no measures are available. Several methods, such as distance weighting, polynomial interpolation, multiple and polynomial regression, kriging and its various forms (ordinary, universal, co-kriging splines and neural networks) are commonly used for spatialization (Attorre et al. 2007). For our purpose a regressive model with altitude as the only independent variable was used, since altitude is characterized by a strong co-variation with topographic characteristics (Dobesch et al. 2010).

The temperature and precipitation data recorded from 1961 to 1990 were obtained from the regional meteorological services of Sardinia (SAR). Since only six weather stations are located in the province of Sassari the data from stations located near the province's borders were used as well in order to increase the data representativeness. The geographic position of the weather station used is shown in figure 3.8.

By analysing the temperature and the rainfall values of the macro-areas under study it was possible to reach the following conclusions. The coldest month in Alghero is January, with a mean temperature of 9.8°C, while the hottest month is August, with a mean temperature of 23.4°C. Considering extreme events, in the period between 1961 and 1990 the temperature went below 0°C 89 times (on average 3 times per year), and the absolute minimum temperature recorded was of -4.8°C in January 1981 (with an average annual absolute minimum of -1.1°C). The absolute maximum was reached instead in July 1983, with values of +41.8° C. The average days of rainfall in Alghero are 69 per year, having considered only events with an intensity greater than 1 mm of

rainfall per day; July and August are the driest months while October, November and December are the wettest (Fig. 3.9).

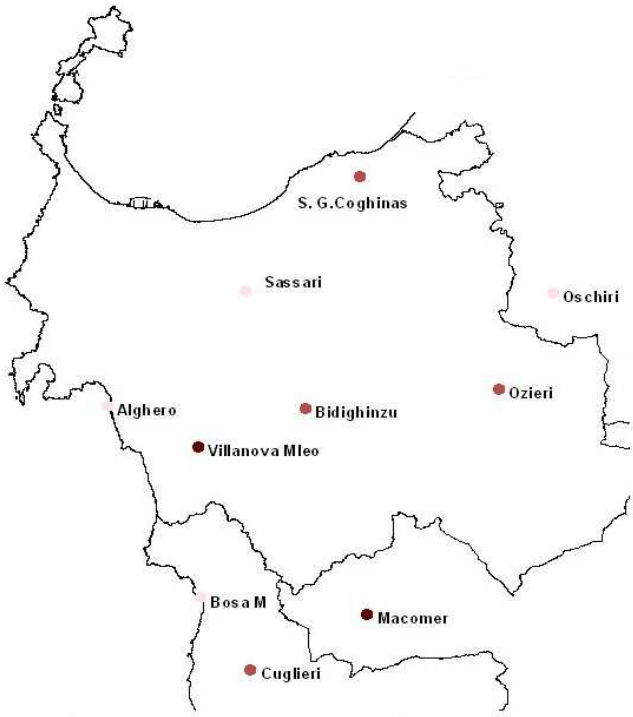

Fig. 3.8.Geographical location of the weather stations used in this study

In Sassari, January is the coldest and August the hottest month, with average temperatures of 9.7°C and 23.7°C respectively. The lowest temperature recorded was in January 1979 (-3°C) while in July 1983 the maximum absolute temperature (43°C) was registered. The average of rainy days per year is 70.9, and rainfall occurs mainly in October, November and December (Fig. 3.10).

Unfortunately there isn't a weather station in the Ittiri area, so the data collected at the closest weather station, Villanovamonteleone, have been used instead to describe its climate. We however checked for and found a consistency between the data of Villanovamonteleone weather station and those provided by climate-data.org. In Ittiri the coldest month is January and the hottest July, with average values of 6.56 and 22.89 °C respectively. In this territory a greater number of days below 0°C (8.8 days for year on average) occur than in Alghero and Sassari. In the Ittiri area there are 80 days of rainfall per year, with the rainiest months being November and December (Fig. 3.11).

Thus, the climate in the three macro areas is different (Table 3.7). The monthly mean of maximum temperatures is higher in Alghero and Sassari than in Ittiri. Moreover during winter the difference between the means of maximum temperatures increases. The same trend can be established also for the monthly mean of minimum temperatures, with Ittiri being characterized by lower values than

the other zones, and being thus the coldest of the areas under study. The rainfall trend is the same in the three areas under study, with the average number of rainy days being quite similar, even if Ittiri is characterized by a higher amount of rain mainly during winter. Finally the days with temperatures at or below 0 are concentrated in the months of December, January, February and Mach; Alghero and Sassari show similar values, while a higher number of days at temperatures below 0°C is recorded in Ittiri.

Table 3.7 Means of maximum temperature (Tmax), minimum temperature (Tmin), rainfall and number of rainy days of the three areas under study.

	Tmax (°C)			Tmin (°C)			Rainfall (mm)			Days T°<0°C			Days of rainfall		
	AHO	ITR	SS	AHO	ITR	SS	AHO	ITR	SS	AHO	ITR	SS	AHO	ITR	SS
January	13.40	8.95	13.36	6.22	4.18	6.16	63.38	100.59	51.84	1.4	2.7	1.1	8.7	10.3	8.5
February	13.64	9.64	13.78	6.35	4.28	6.28	66.05	104.40	51.60	0.8	3.1	0.8	8.6	9.8	8.5
March	14.91	12.01	15.26	7.03	5.33	6.94	51.07	76.18	48.79	0.4	1.3	0.4	7.3	8.7	7.8
April	17.46	15.21	17.91	8.88	7.49	8.76	45.32	74.70	40.54	0.0	0.0	0.0	6.5	8.5	6.6
May	21.32	19.84	22.16	11.65	11.08	11.63	26.74	46.19	31.22	0.0	0.0	0.0	4.3	5.3	4.5
June	25.42	23.90	26.19	15.21	14.63	14.72	11.59	20.15	14.27	0.0	0.0	0.0	1.9	2.7	2.4
July	28.90	28.02	29.72	17.76	17.78	17.26	4.93	6.53	5.26	0.0	0.0	0.0	0.8	0.8	0.8
August	28.93	27.41	29.81	18.06	17.83	17.75	11.91	11.19	16.38	0.0	0.0	0.0	1.2	1.4	1.7
September	26.35	23.63	26.84	16.29	15.12	15.95	37.82	46.02	37.01	0.0	0.0	0.0	3.9	4.5	4.5
October	22.43	18.93	22.85	13.21	11.77	13.29	77.34	111.77	70.10	0.0	0.0	0.0	6.9	7.6	7.0
November	17.62	13.56	17.65	9.78	7.96	9.48	104.92	147.86	92.68	0.0	0.2	0.1	10.0	10.5	9.9
December	14.44	9.92	14.15	7.33	5.04	7.03	87.07	121.28	69.99	0.3	1.6	0.4	9.0	10.4	8.5

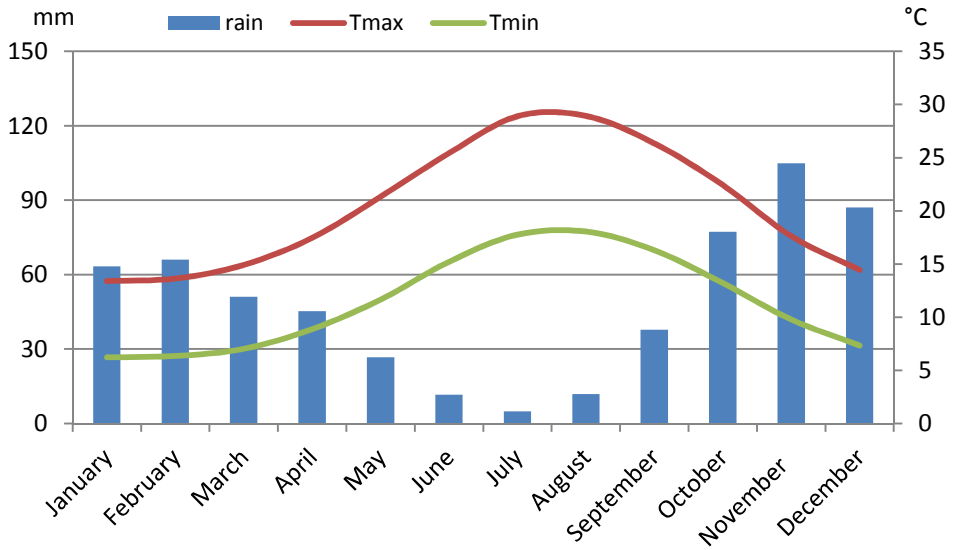

Fig. 3.9 Means of minimum and maximum temperature (°C) and rainfall recorded in Alghero during the period 1961-1990

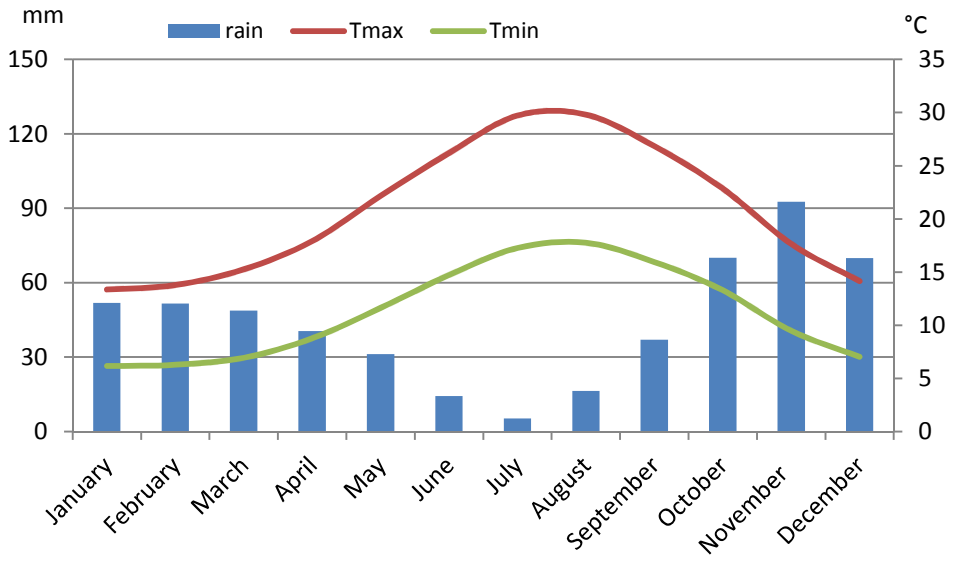

Fig. 3.10 Means of minimum and maximum temperature (°C) and rainfall recorded in Sassari during the period 1961-1990

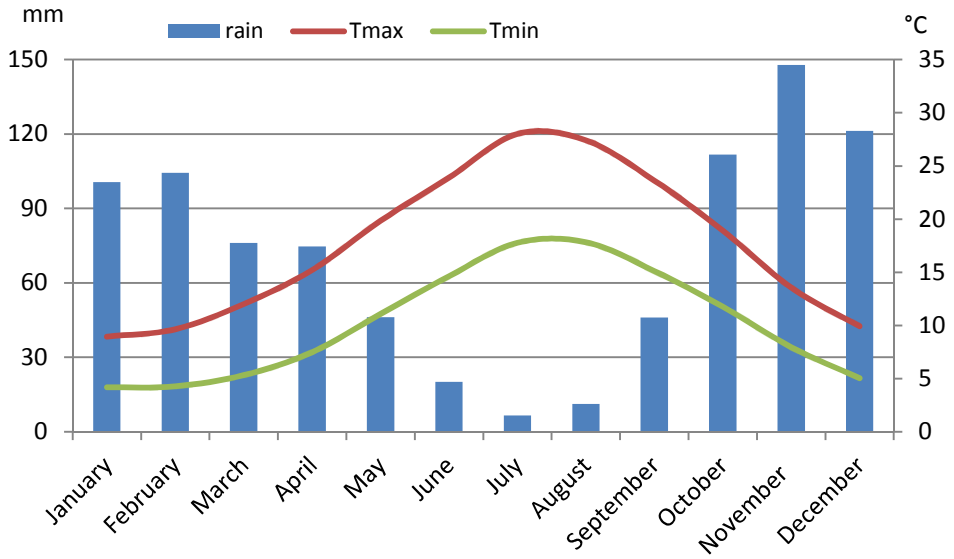

Fig. 3.11. Means of minimum and maximum temperature (°C) and rainfall recorded in Ittiri during three consecutive crop seasons in the period 1961-1990

The mean temperature data showed a good correlation with elevation (Fig. 3.12), in agreement with De Marco (2006). By using GIS tools, intercept and slope were applied in each DEM pixel of the territory of Sassari province (Fig. 3.13).

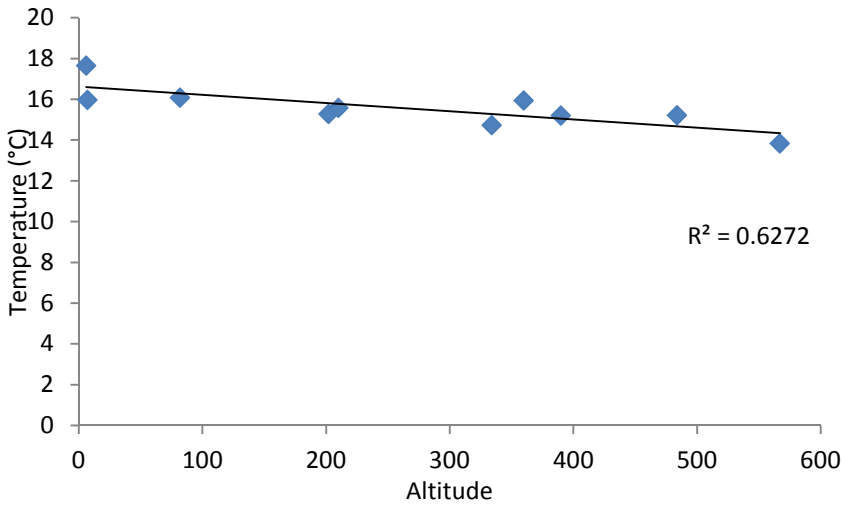

Fig. 3.12 Linear regression between altitude and mean temperatures recorded at the weather stations

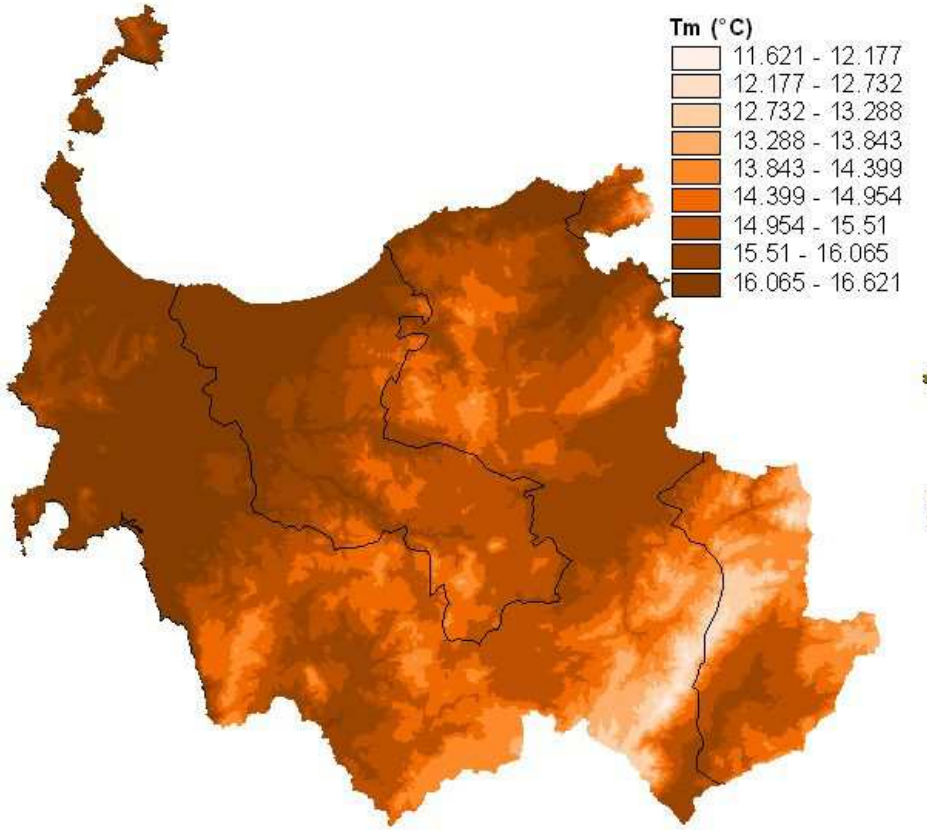

Fig. 3.13 Map of mean temperature layers for the Sassari province obtained by spatialization mean temperature data

The spatialisation of rainfall was more complex to achieve since precipitation is the result of a combination of variables, including thermodynamic, dynamic and cloud microphysical processing, that are characterized by a complicated interaction with topographical features (acting over a wide range of temporal and spatial scales (Mestre-Barceló). To produce a thematic map of rainfall two methods were used: Kriging (Krige, 1984) and Inverse Distance Weighted (IDW). Briefly, Kriging is an interpolation method that allows to interpolate a variable in space, minimizing the mean square error, while IDW assumes that on each point there is a local influence decreasing with the distance (Di Virgilio et al., 2007). An appropriate model could not be built using the Kriging method; in fact considering the Root Mean Square Error (RMSE) we verified the lack of a clear spatial pattern, probably due to the small number of weather stations. Among the several models available for the semivariograms, the linear model with sill was characterized by the lower RMSE values, and was thus chosen to estimate rainfall value; the map of rainfall obtained is shown in figure 3.14. The IDW model was used as well, in order to have a comparison with the Kriging method. In figure 3.14 is shown the rainfall maps obtained using the Kriging method and in figure 3.15 the one obtained by IDW method.

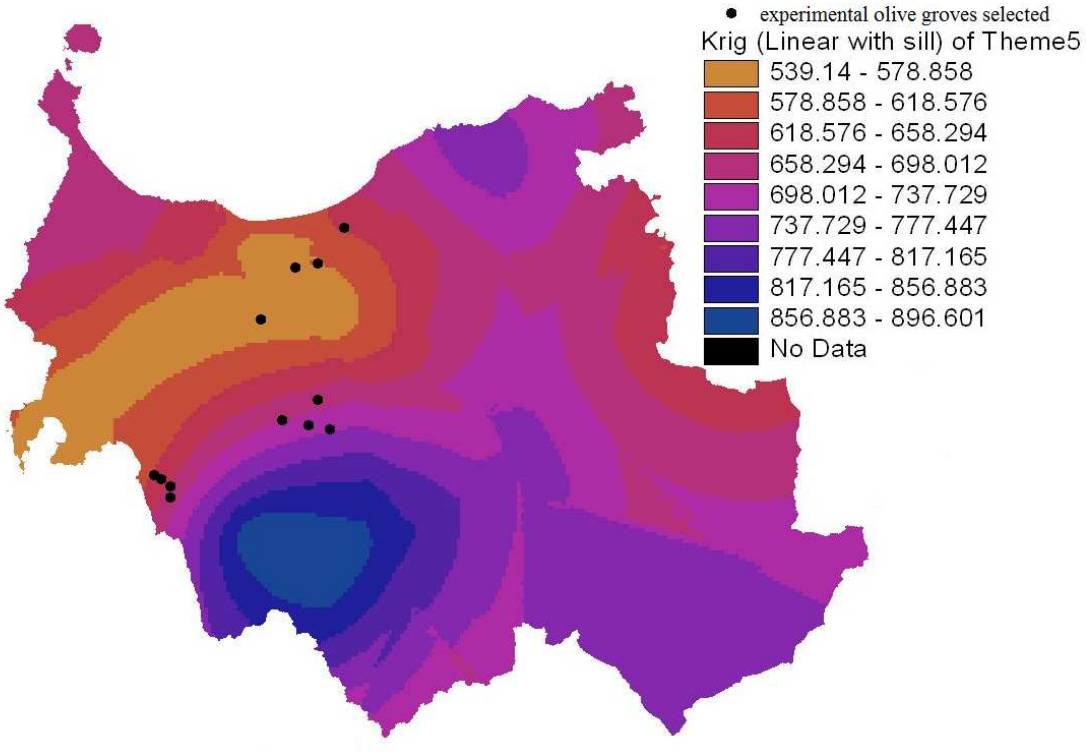

Fig. 3.14. Rainfall maps obtained using the Kriging method.

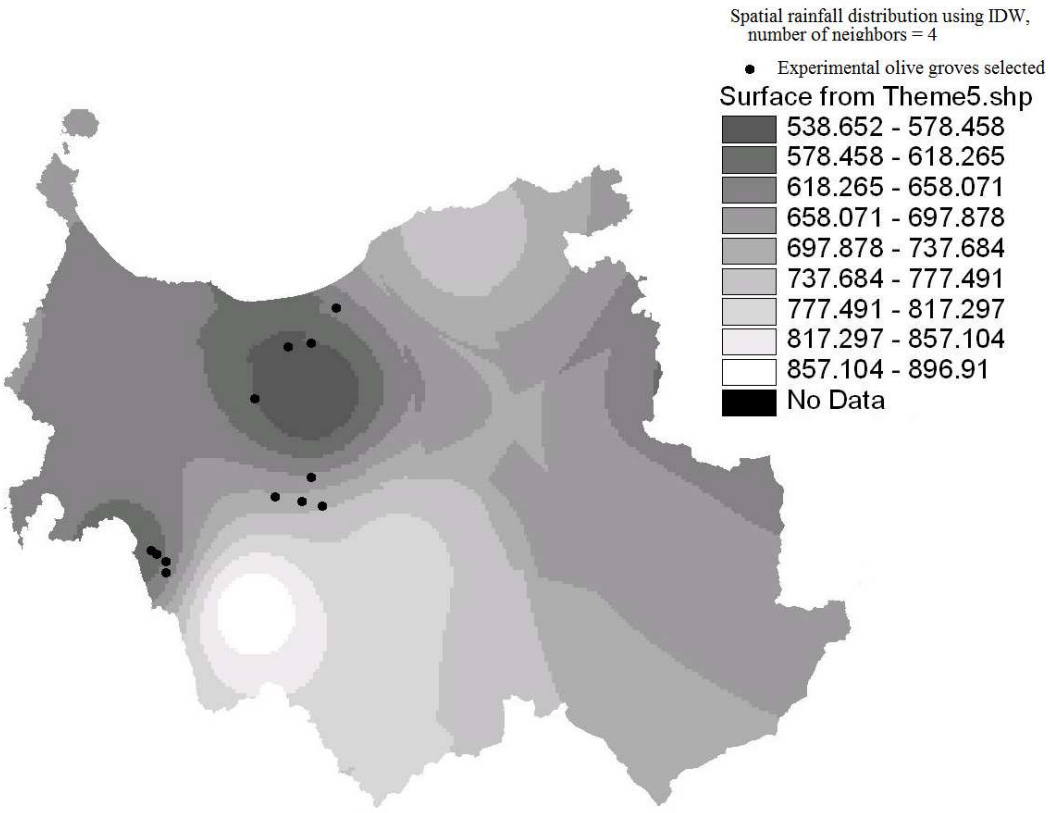

Fig 3.15. Rainfall maps obtained using the IDW method.

It is possible to notice that values are quite similar for the two methods by extracting the rainfall values of the olive groves under study (Table 3.8). Ittiri is the area characterized by most rainy, while the Sassari area is the least rainy. Considering the temperature model instead, the annual mean temperature in the province of Sassari ranged between 15.30 and 16.04°C. The Ittiri area was the coldest of the zones under study.

In order to have a complete information on the climatic characteristics of the areas, during the three-year period of the study, the data of temperatures and rainfalls were collected from the Environmental Protection Agency of Sardinia (ARPAS). The weather stations from which the data were collected are described in Table 3.9, while their location in the Sassari province is shown in figure 3.16.

Table 3.8. Values of annual rainfall (Kriging and IDW methods) and mean temperature (mean temperature map) for the olive groves selected.

	Rainfall KRIG (mm)	Rainfall IDW (mm)	Mean temperature (°C)
AHO 1	682.05	638.42	16.19
AHO 2	668.29	627.44	16.32
AHO 3	639.87	604.28	16.34
AHO 4	629.66	599.47	16.41
ITR 1	717.78	718.27	15.46
ITR 2	737.73	729.68	15.30
ITR 3	683.61	666.54	15.87
ITR 4	696.88	710.82	15.76
SS 1	556.73	580.49	16.08
SS 2	621.58	631.84	16.19
SS 3	580.69	576.37	16.04
SS 4	548.56	565.23	16.20

Table 3.9. Details of the weather stations from which the data of the three year period under study (2011-2013) were collected.

Name	District	Latitude	Longitude	Altitude (a.s.l.)	Distance from sea
Olmedo	*Bonassai*	40° 39' 43" N	08° 21' 44" E	32 m	9397 m
Putifigari	*Minalzu*	40° 32' 49" N	08° 27' 37" E	423 m	9472 m
Sassari S.A.R.	*Viale Porto Torres, 119*	40° 44' 25" N	08° 32' 19" E	150 m	9478 m
Sorso	*Scala d'Otteri*	40° 49' 51" N	08° 36' 35" E	57 m	1972 m
Usini Mobile	*Piras Peglias*	40° 39' 26" N	08° 31' 16" E	201 m	18372 m

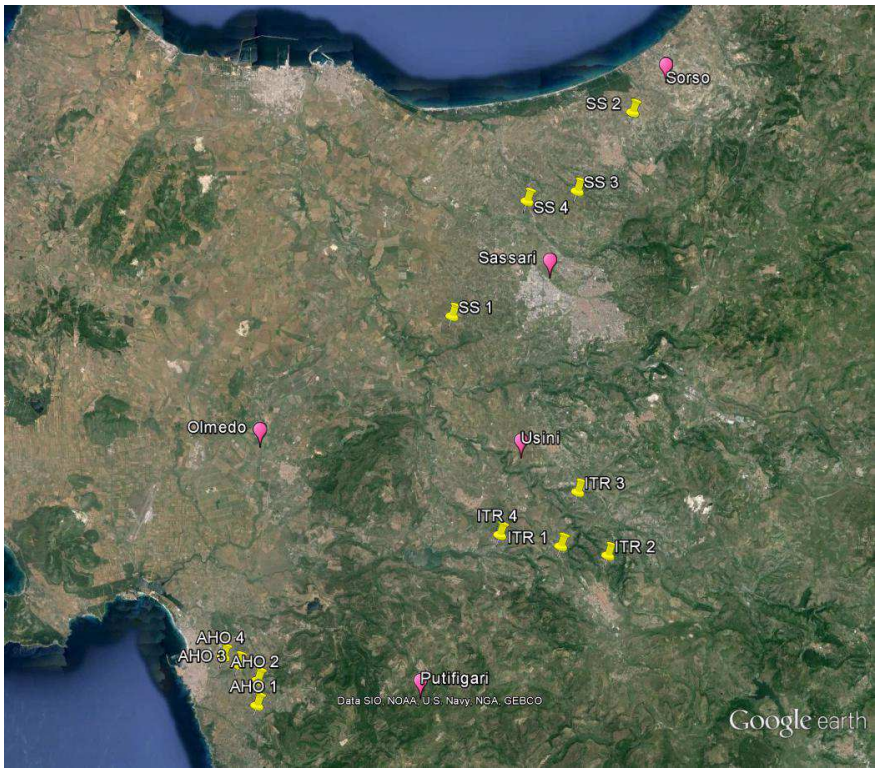

Fig. 3.16 Location of the weather stations (in pink) and of the experimental olive groves (in yellow) in the Sassari province.

The data coming from the weather stations of Sassari province allowed us to observe the variables size of rainfall and number of rainy days behaved differently in the three years of study. The third year of study, 2013, was the most rainy; particularly the firsts months of the year were more rainy than the same months in 2011 and 2012 (Fig. 3.16, 3.17, 3.18, 3.19 and 3.20). The year 2012 was characterized by heavy rains fallen in May, while the values and trend of rainfall for the year 2011 were closer to the ones recorded in in the period 1961-1990, as detailed above.

Comparing the minimum temperatures recorded it is possible to see the variability occurring in the Sassari province. The lowest minimum temperature (0.67°C) detected during the three years of study was recorded in Olmedo, while the highest maximum temperatures were recorded by the weather stations located in Sassari and Usini. The highest maximum temperature was recorded by the climatic station of Usini in August, and the maximum temperatures never went under 8.6°C recorded in Putifigari. No significative difference in temperatures has been recorded among the years under study.

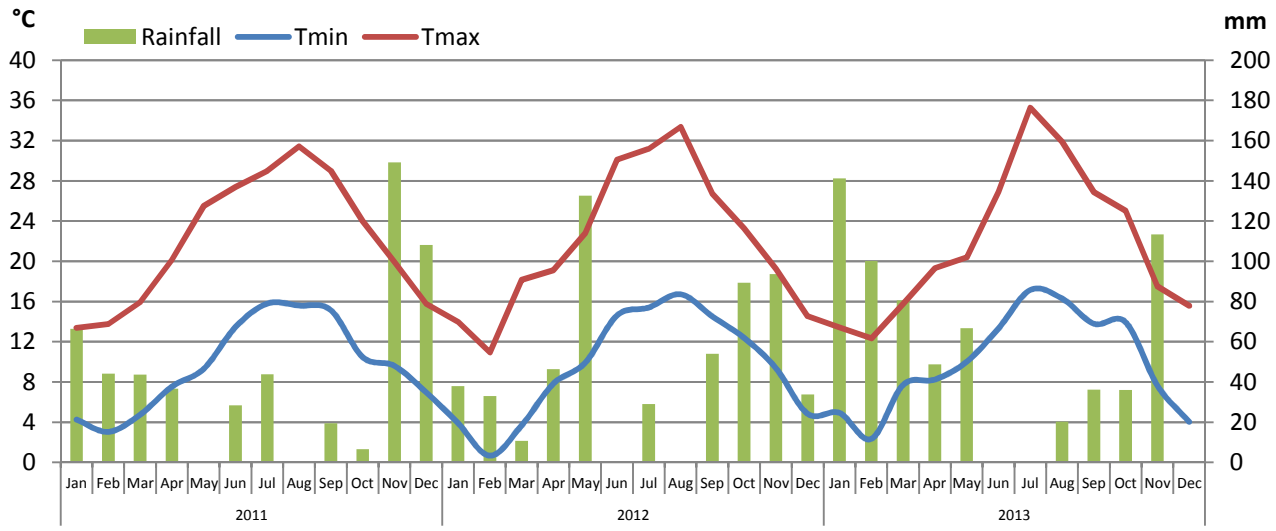

Fig. 3.16 Values of temperature and rainfall in Olmedo for the years 2011, 2012 and 2013

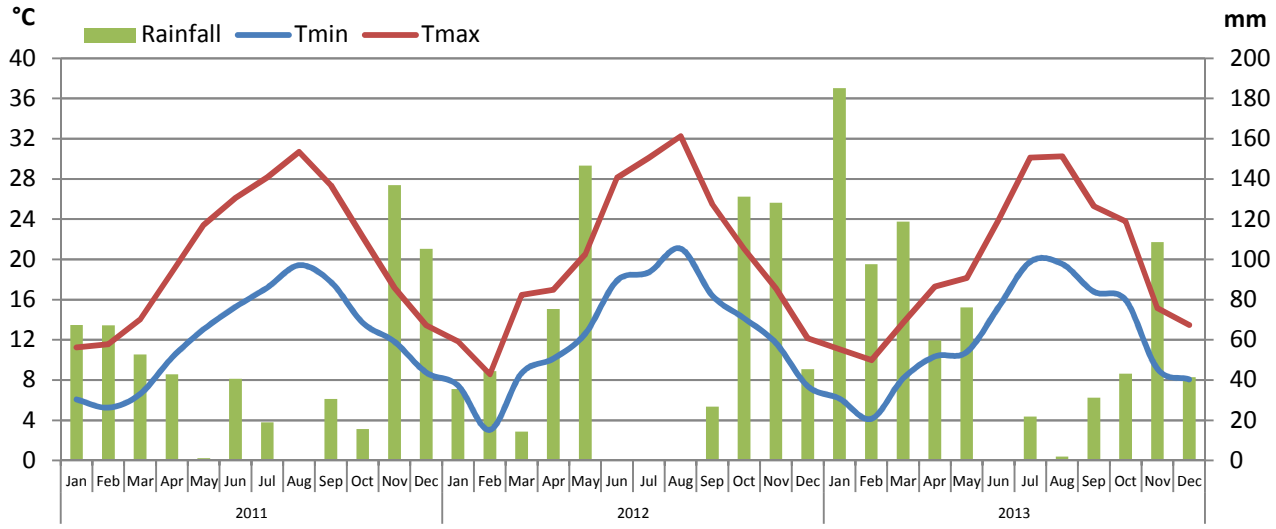

Fig. 3.17 Values of temperature and rainfall in, Putifigari for the years 2011, 2012 and 2013

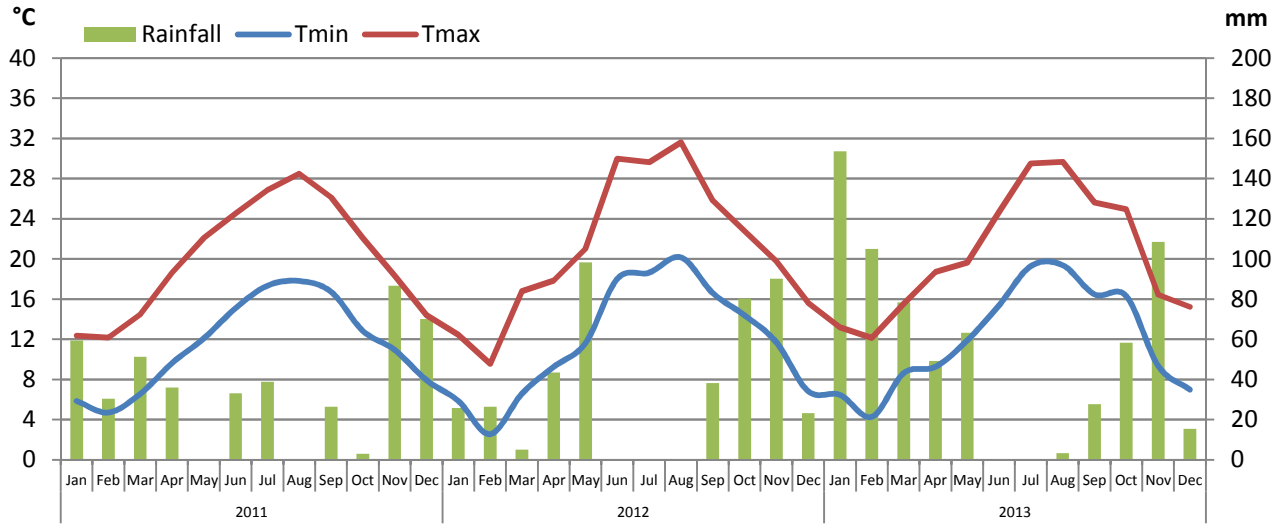

Fig. 3.18 Values of temperature and rainfall in Sassari for the years 2011, 2012 and 2013

Fig. 3.19 Values of temperature and rainfall in Sorso for the years 2011, 2012 and 2013

Fig. 3.20. Values of temperature and rainfall in Sorso and Usini for the years 2011, 2012 and 2013

By aggregating the mean temperatures in growing degrees day (GDD) it was possible to see the non-homogeneity of the year's differences. In Olmedo there was no big differences in GDD between the three years; in Putifigari and in Sorso the lowest GDD, of 2198 and 2074 respectively, was obtained for the year 2013, while in Sassari and Usini the lower GDD accumulation was found during 2011. Unfortunately the temperatures data for 2013 at the weather station of Usini were not available (Fig. 3.21).

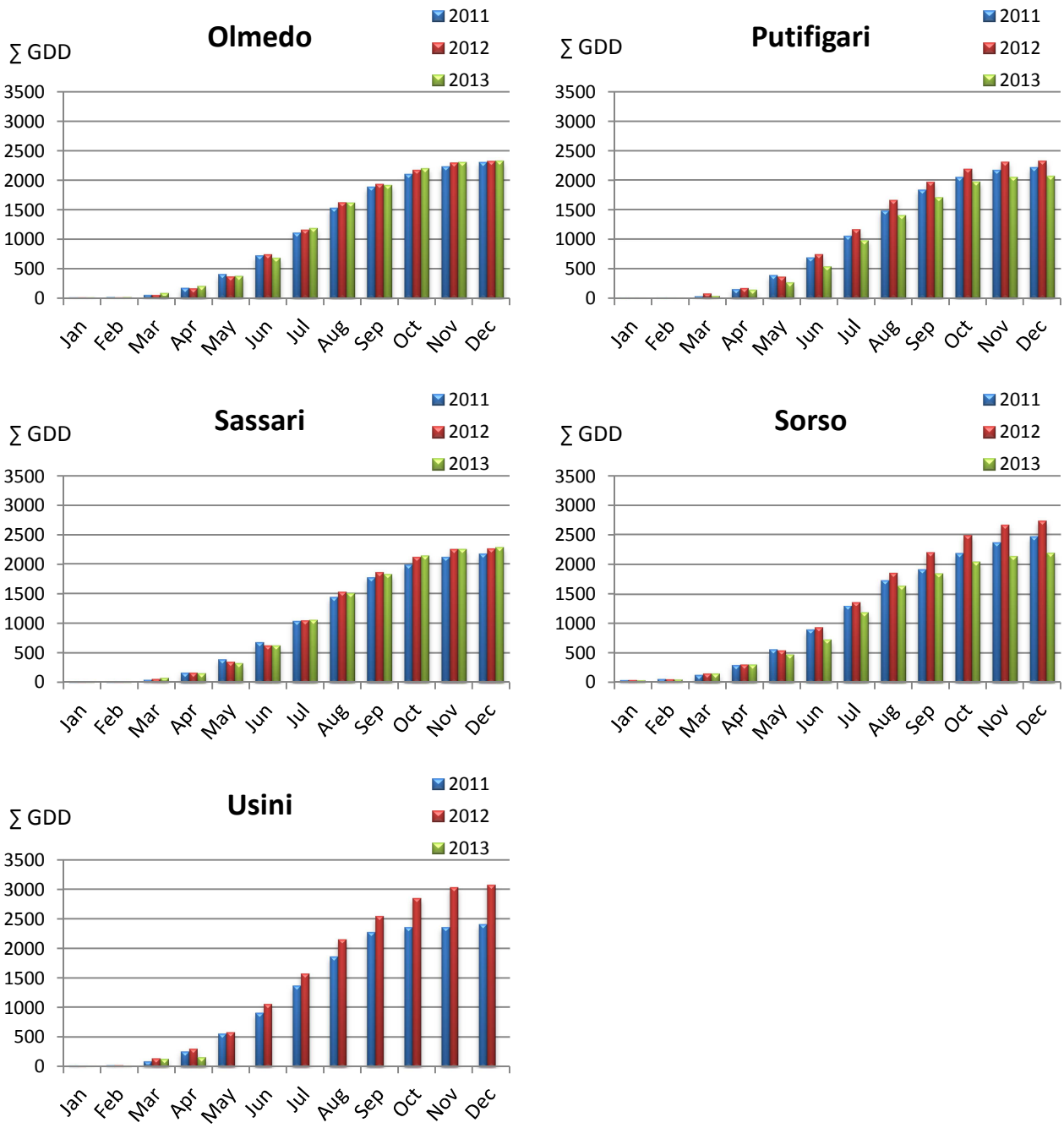

Fig. 3.21. Accumulation of growing degree-days (GDD) at different weather stations in the three years of study.

4. Materials and methods

Plant materials

The study was carried out during three consecutive crop seasons, 2011/2012; 2012/2013 and 2013/2014, on cv Bosana olive plants cultivated in olive groves sited in the Sassari province. In the four olive groves in each of the three macro areas (Alghero, Ittiri and Sassari) (Fig. 4.1), chosen as described in chapter 3, olive fruits were manually harvested from five trees and 30 kg were taken to form the sample from which will extracted the oil.

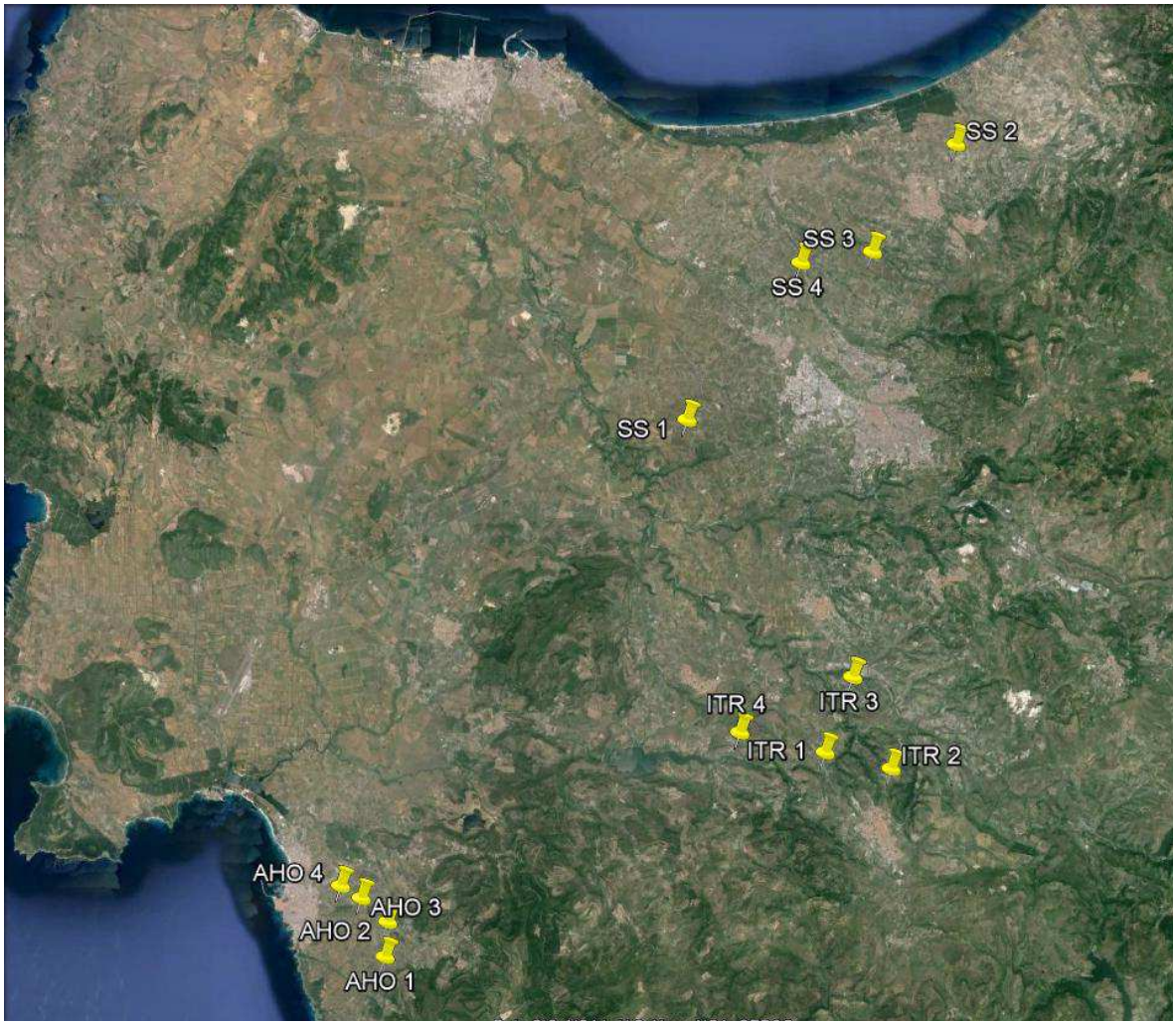

Fig. 4.1 Geographical location of the olive groves from which the olive productions were collected. AHO, Alghero; ITR, Ittiri; SS, Sassari.

Fruits analysis

The ripening Index was calculated for each sample according to the method developed by the Agronomic Station of Jaén defining the RI as function of fruit colour in both skin and pulp (Uceda and Hermoso, 1998). It includes the following eight classes: intense green (0), yellowish-green (1),

green with reddish spots (2), reddish brown (3), black with white flesh (4), black with < 50% purple flesh (5), black with 50% purple flesh (6) and black with 100% purple flesh (7).
Fruit moisture was determined by desiccation in stove at 110°C for 12 h until constant weight. The crude fat was determined in triplicate by extracting 20g of grinded olive sample with diethyl ether, using a Soxhlet apparatus.

Olive oil analysis

Olive processing and oils storage

A low scale continuous mill (Oliomio®; Toscana Enologica Mori, Firenze, Italy) equipped with an horizontal malaxator and two phase decanter was used. Olive samples (25 kg) were processed within 24 hours of collection. During mechanical extraction the olive paste temperature was always below 27°C, the time of malaxation was 20 minutes and a minimum addition of water during the transport of olive paste from malaxator to centrifuge (2 l/h) (Cantini et al., 2012). For each sample the processing parameters were standardized (temperature and time of malaxation, speed of centrifuge, flux of water in the separator) in order to minimize the variability due to the extraction procedures. Oils samples were filtered through cotton filters and poured in dark glass bottles keeping the head space to a minimum. Bottles were stored in a cooled incubator set at 13°C until the analysis.

Chemical analysis

Analytical Indices

Free acidity, peroxide value, UV-spectrophotometric indices (K232, K270, ΔK) were evaluated according to the official methods described in Regulation EC 2568/91, 61/2011, 299/2013 of the Commission of the European Union . All parameters were determined in triplicate for each sample.

Analysis of the phenolic fraction

The phenolic fraction was extracted in triplicate according to Pirisi et al. (2000) with some modifications: 8 g of the oil sample were added to 4 mL of n-hexane and 8 mL of a methanol:water (60:40, v:v) solution; after vigorous shaking, the hydro-alcoholic phase was collected and the extraction was repeated twice. The combined extracts were evaporated to dryness and re-suspended in 0.5 mL of a methanol:water (50:50, v:v) solution and filtered through a 0.2 μm RC (Whatman Inc., Clifton, NJ, USA) before the spectrophotometric and chromatographic analysis.

Spectrophotometric Determination of Total Phenols

Total phenol content of the phenolic extracts was determined by the Folin–Ciocalteau spectrophotometric method at 750 nm. (Cerretani et al., 2003) using a Jasco Spectrophometer (V-500, Tokyo, Japan). Results were expressed as mg gallic acid/Kg oil. The spectrophotometric analysis was repeated three times for each extract.

HPLC analysis of the phenolic fraction

HPLC analysis was carried out using a Schimadzu LC-10ADvp equipped with a low pressure gradient unit, FCV-10Alvp (Schimadzu), degasser Flow154, (Gastorr), and a column oven CTO-10A (Schimadzu). Analytes were separated on a Kinetex 5µ C18 150×4.6mm (Phenomenex) column and identified using a Diode-Array UV-VIS Detector (UV 6000 ThermoQuest). The mobile phase flow rate was 1 mL $min^{-1}$and the gradient elution (Table 4.1) was carried out using water/formic acid (99.5: 0.5, v/v) as mobile phase A and acetonitrile as mobile phase B of the solvent system, in accordance with Rotondi et al. (2004a). The wavelengths were set at 280 nm for phenolic alcohols and secoiridoids, and at 330 nm for flavonoids and phenolic acids. Identification of phenolic compounds was carried out by the comparison with the retention time and spectra of the standard compounds and with data literature. Hydroxytyrosol was quantified using the tyrosol calibration curve; derivatives of oleuropein and ligstroside were quantified using an oleuropein calibration curve; tyrosol, vanillin, vanillic acid, o-cumaric acid, luteolin and apigenin were quantified using the calibration curve of the relative standard.

Pigments analysis

For quantitative analysis of tocopherols, lutein, β-carotene and xantophylls the method reported by Rotondi et al., (2004b) was used. In detail, 2 mL of virgin oil were filtered through a PTFE membrane filter with 0.2 µm pore size (GyroDisc 25 mm, Orange Scientific). These samples were injected into a liquid chromatograph (LC-10ADvp, Schimadzu) equipment with a degasser (Flow 154, Gastorr), a low pressure gradient unit (FCV-10ALvp, Shimadzu) and a column oven (CTO-10ASvp, Shimadzu). Analytes were separated on a c18 column, 150mm x 4.6mm (Inertsil ODS-2 5U, Alltech), and identified and quantified by a photodiode array detector (UV6000, Thermo-Quest). The flow rate was 1ml min^{-1}, the injection volume 20 µl and the column temperature 25°C. The eluents used were solution A methanol:water (80:20, v:v), and solution B methanol: tetrahydrofuran (20:80, v: v). Analytes were eluted using the following gradient scheme: initially 80 % of A and 20 % of B modified by a linear rate for 40 min until reaching a final concentration of 0 % of A and 100 % of B, and maintaining this isocratic rate for 5 min. Identification and quantification of analytes was based on comparison of retention time and adsorption spectra with

ones of standard compounds. Tocopherols quantification was carried out at 280 nm, carotenoids at 450 nm and chlorophyll pigments at 410nm.

Table 4.1 HPLC gradient composition for the phenolic analysis

Time (min.)	A (%)	B (%)
0	95	5
5	93	7
10	91	9
15	88	12
18	85	15
20	84	16
30	82	18
32	80	20
33	78	22
35	75	25
38	72	28
40	70	30
42	69	31
45	68	32
48	66	34
50	65	35
55	60	40
60	50	50
70	5	95
75	95	5
80	Post-run	

A, water/formic acid (99.5: 0.5, v/v); B, acetonitrile

Sensory analysis

Sensory analysis was performed by the "ASSAM – Marche panel", a fully-trained taste panel recognized by the International Olive Oil Council (IOOC) of Madrid, Spain, and by the Ministry for Agriculture, Food, and Forestry Policy. Since the main objective of the sensory IOOC method T20/Doc. n.15/Rev (2000) is to give a commercial classification of the oils, a profile sheet IOOC method T20 modified by ASSAM standard was used, in order to obtain a complete description of the organoleptic properties of the oils sampled. In this sheet twelve attributes were evaluated: nine during the olfactory phase (olive fruity, olive fresh leaf, grass, fresh almond, artichoke, tomato, apple, berries and aromatic herbs) and three during the gustatory phase (bitter, pungent and the fluidity). Attributes were assessed on an oriented 10 cm line scale and quantified measuring the location of the mark from the origin (Rotondi et al., 2010). The choice of using the 'ASSAM – Marche Panel' sensory sheet was done in order to be able to compare our sensory results to the ones

of Bosana oils coming out from the Italian National Database of Monovarietal Extra Virgin Olive Oils (Rotondi et al., 2013).

5. Influence of the growing area

Highlights

The major fatty acids are significantly influenced by the growing area

High variability related to crop season was observed for secondary metabolites

LDA grouped Bosana virgin olive oils according to the growing area

Introduction

Phenotypic plasticity is the ability of an organism to change its phenotype in response to changes in the environment (Price et al., 2003). This ability is a characteristic of all organisms, since individuals that show a plastic response have higher fitness than those that do not (Price et al., 2003), but it is of particular importance in plants, whose sessile lifestyle requires them to deal with ambient conditions (Schlichting, 1986). The concept of phenotypic plasticity is involved in the idea of PDO products, defined as "products and foodstuffs which are produced, processed and prepared in a given geographical area using recognised know-how". The link between geographical area and product can characterize the product itself, thus the product becomes identifiable with the territory: this link could positively increase the prospects of local agriculture in the global market (Costantini & Buccelli, 2008). PDO products, and more in general the EU labels of geographical indications and traditional specialities, answer to the emerging demand of consumers towards regional agri-food products, 're-localization' of an increasing part of food production and shift the attention of consumers towards food products that can be traced to particular people and places (Moschini et al., 2008).

The link between olive cultivation and territory of origin is tight, because olive culturing preserves the landscapes of marginal agricultural areas and delivers a product with both nutritional and peculiar organoleptic characteristics.

Olive oil composition is directly related to production area. This trait is firstly ascribable to the grove cultivar, because thanks to the huge olive genetic variability often only a few or even just one genotypes are distinctive of the area. Then the effect of the growth environment is crucial in expressing the characteristics and quality typical of a given olive cultivar (Di Vaio et al. 2013), but the oil composition is also influenced by other factors, such as agronomic and technological practices. Furthermore all these factors interact with each other, resulting in a complex multivariate matrix (Montedoro & Garofalo, 1984; Lavee & Wodner, 1991; Inglese et al. 2011).

The dependence of the fatty acid fraction of olive oil on latitude was discovered a long time ago, in 1934, by Frezzotti (Inglese et al., 2011). Since then many researchers showed the dependence of the composition in fatty acids on factors such as production area and crop year, namely thus on temperatures. As a general rule, cold climate, and so higher altitude, lead to higher content in oleic acid, and an associated lower content of palmitic and linoleic acids (Lombardo et al., 2008; Ripa et al., 2008). However, genotypes behaviour may differ, according to the general concept of phenotype that is the result of genotype interaction with environment. Furthermore, Lombardo and colleagues (2008) noted that cultivars typical of northern areas of Italy are more subject to

phenotypic plasticity (considering in this case changes in the fatty acid profile) than the cultivars from south of Italy, ascribing the phenomenon to a lack of "selective pressure". Also Mannina and collaborators (2001) described a lower influence of pedoclimatic conditions on two southern cultivar, Coratina and Cerasuola , during a research aiming to find the Mediterranean olive cultivar that could adapt best to the extreme climatic conditions of Catamarca region (Argentina), producing at the same time a good quality olive oil. The abovementioned researches (Mannina et al., 2001; Lombardo et al., 2008) underlined the issue of uprooting olive cultivars to different places without a proper evaluation of the new environment's possible influences on the plants first. The same conclusion was reached by Ceci & Carelli (2007) in a study pointing out that Argentinian olive oil doesn't comply to the standards given by the EU and the International Olive Council on the content in fatty acids, probably due to the aforementioned issue.

Several studies have been carried out on the phenol fraction to understand and correctly attribute their source of variability. Many authors pointed out the influence of the cultivar on phenolic fraction, mostly influencing the phenols quantity (Servili et al., 2004; Cerretani et al., 2005), although demethyl-oleuropein and verbascoside has been proposes as marker of genetic origin (Amiot et al., 1986). Olive ripening and agronomic practices influence as well the phenolic fraction (Servili et al., 2004); for instance irrigation has been widely studied and an inverse correlation between water availability and phenol content has been concluded (Patumi et al., 2002). In fact, as reported by Pannelli et al. (1994), oil obtained in years characterized by a high percentage of rainfall has a lower phenolic content. However there are only few studies trying to relate seasonal climate and phenolic content and the results are ambiguous. Ripa et al. (2008) reported an inverse correlation between phenolic content and degree-day accumulation from fruits set to harvest, while Tura et al. (2008) in the same year reported a positive correlation between heat summation and phenols content and an interaction between cultivar and environmental factors. Di Vaio et al. (2013), comparing oils of the same cultivar grown at different altitude, found more phenols in oils from olive grown at a higher altitude, thus characterized by a lower accumulation of growing degree-day. Other researcher (Aguilera et al. 2005) did not find any clear. Other studies have been carried out on lipophilic phenols such as tocopherols, confirming their dependence on different environmental factors (Ranalli et al., 1999; Salvador et al., 2003; Tura et al., 2007; Arslan et al., 2013). Tocopherols are in fact involved in the plant's tolerance to stress, maintaining an adequate redox state in chloroplasts (Munne´-Bosch, 2005), which can explain the impact of environmental conditions and seasonality on the content of tocopherols of olive oil.

The knowledge of the effects of the growth environment on the chemical and sensorial attributes of olive oil is crucial to have tools to guarantee authenticity and to endorse the link between product

and territory, thus promoting the territory itself. The aim of this study is to clarify if any consistent difference in the chemical and sensorial properties of oils is noticeable between oils differing from production areas, in particular oils produced in the three macro areas of Alghero, Ittiri and Sassari,.

Experimental design

Experiments were carried out in the selected macro areas (Fig. 4.1) over three years. In the first year the productions of four olive groves per each macro area were collected (n=12), while in the second and third year productions of three groves were collected in Alghero and Ittiri ,while in Sassari it was possible to collect only from two groves due to a heavy olive fly attack in the third orchard. The sampling plan is summarized in Table 5.1.

Table 5.1 Sampling plan adopted during the three years of study

	AHO	ITR	SS	Total
1st year	4	4	4	12
2nd year	3	3	2	8
3rd year	3	3	2	8
Total	10	10	8	28

Chemical analysis

On the olive fruit samples collected from the three growing areas the ripening index, the water and oil content were analysed by using the methodologies described in chapter 4.

The olive oil production was carried out using a low scale mill as described in chapter 4. On the resulting virgin olive oil free acidity, peroxide number, UV spectrophotometric indices (at 232 and 270 nm), total phenol content, fatty acid profile, HPLC pigment, tocopherol and phenolic fractions were analysed, as well as the sensory analysis performed by a professional panel test. All analysis were carried out using the methods reported in chapter 4.

Statistical analysis

The significance of differences at a 5% level between the averages of three area (Alghero, Ittiri and Sassari) was determined by one-way ANOVA using Tukey's test by means of Microsoft® Excel 2007/XLSTAT© (Version 2009.3.02, Addinsoft, Inc., Brooklyn, NY, USA).

Stepwise Linear Discriminant Analysis (SLDA) was carried out by the Systat 11 software (Systat Software Inc. Richmond, CA, USA) to discriminate between growing area and to define which variables are able to discriminate groups.

Results and discussion

The research has been carried out for 3 years on 28 olive oil productions. It is important to underline that a standardization of ripening index, harvest methods and technological features of milling has been pursued in order to ascribe the variability only to the area of production.

The ripening indexes distribution of olive samples collected in the three years under study, divided by growing area, is shown in figure 5.1. In Sassari's box plot the wideness of distribution is smaller than in the other growing areas, moreover Sassari is the only area where an outlier is present. In contrast the widest distribution of ripening indexes was found in the Alghero area, while the highest mean and median values were recorded in Ittiri.

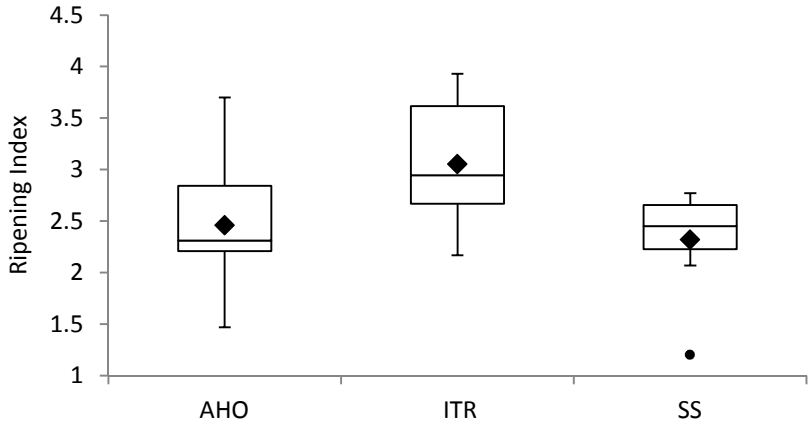

Fig. 5.1 Box plots of ripening index of the samples collected in Alghero (AHO), Ittiri (ITR) and Sassari (SS) during the three years of study. The boundary of the box indicates the 25th and 75th (top and bottom) percentiles. The line within the box marks the median and the symbol ♦ indicate the mean; the box plot outliers are designated a ●.

The analytical indices free acidity, peroxide value and spectrophotometric constants, indicated by the EU reg. 2568/91 and subsequent amendments for the classification of olive oils, did not statistically differ among the three production areas studied (Table 5.2). Several authors described the dependence of analytical indices by the dupes phytosanitary state and by the technological features of extraction process (Kandylis et al., 2011; Abu-Reidah et al., 2013), although some authors found differences in these analytical indices even among different production areas (Salvador et al., 2003; Issaoui et al., 2010). The total phenol content of the oils ranged between 350 mg/kg of Alghero and 322 mg/kg of Sassari. It has been previously reported that phenolic content is influenced by the growing area (Salvador et al., 2003; Issaoui et al., 2010), however in our study we found an high within-group variability for the total phenol content which didn't allow to notice

differences related to the production areas (Table A). The mean values of total phenol content found in this study for Bosana virgin olive oils were slightly lower than the values reported in the database of Italian National Review of Monovarietal olive oils (Retrieved from: http://www.olimonovarietali.it/database/monovarietale?id=BOSANA).

Table 5.2 Analytical indices and fatty acids composition of the three production areas Alghero (AHO), Ittiri (ITR), and Sassari (SS). The data are presented as mean, minimum and maximum of values of the three years for each production area (AHO 10 samples, ITR 10 samples and SS 8 samples).

	P value	Growing area					
		AHO		ITR		SS	
		Mean	Range	Mean	Range	Mean	Range
Olive moisture	0.255	48.94a	45.65-56.25	51.16a	46.19-56.9	49.17a	46.46-55.79
% oil[1]	0.158	39.32a	31.2-46.5	36.24a	29.5-41.95	35.16a	28.05-42.5
Free acidity[2]	0.945	0.38a	0.3-0.51	0.38a	0.33-0.49	0.38a	0.33-0.49
PV[3]	0.782	11.11a	7.26-16.72	10.69a	6.22-17.2	11.94a	6.32-19.14
K232	0.758	2.02a	1.74-2.23	1.98a	1.81-2.09	2.00a	1.74-2.19
K270	0.654	0.14a	0.08-0.21	0.14a	0.1-0.18	0.15a	0.13-0.18
ΔK	0.374	-0.01a	-0.01-0	-0.01a	-0.01-0	-0.01a	-0.01-0
TP[4]	0.750	350.41a	243.17-445.82	336.71a	185.56-412.28	322.66a	230.57-423.98

[1] on dry matter; [2] g Oleic acid in 100g oil; [3] POV, Peroxide value, mEq O_2 kg^{-1} of oil; [4] TP, total phenols, mg of gallic acid kg^{-1} of oil. Different letters in the same row show the membership to different groups by Tukey HSD (P<5%)

The composition in fatty acid of Bosana olive oils from the different growing areas are shown in Table B. Significant differences ascribable to the areas of production were found for the three main fatty acids (palmitic, oleic and linoleic acid), as well as the sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Similar findings have been reported for Turkish oils (Arslan et al., 2013), for Tunisian (Issaoui et al., 2010) and Greek oils (Tsimidou and Karakostas, 1993) and for Italian oils (Lanza at al., 1998). In particular, focusing on the results of Tukey's test it is clear that the two groups of oils grown in Alghero and Ittiri differed significantly, while the group from Sassari was not significantly different from the other two, with values somewhere in the middle (Table 5.3). Oils produced in the Alghero area had lower oleic acid content and higher palmitic acid content than the ones produced in Ittiri and Sassari. This difference in the fatty acid profile is ascribable to the warmer temperature (Fig. 3.9) characterizing the Alghero area, in agreement with previously reports by Lombardo et al. (2008) and Ripa et al. (2008).

Table 5.3 Fatty acids composition of oils from the three production areas Alghero (AHO), Ittiri (ITR), and Sassari (SS). The data are presented as mean, minimum and maximum of values of the three years for each production area (AHO 10 samples, ITR 10 samples and SS 8 samples).

	P value	Growing area					
		AHO		ITR		SS	
		Mean	Range	Mean	Range	Mean	Range
C 16	**0.004**	13.2a	12.19-14.14	11.92b	10.97-13.59	12.58a,b	11.97-13.4
C16:1	0.649	0.73a	0.55-0.95	0.7a	0.49-0.93	0.76a	0.6-0.84
C17	0.286	0.04a	0.03-0.05	0.03a	0.02-0.05	0.03a	0.02-0.05
C17:1	0.683	0.07a	0.05-0.08	0.07a	0.06-0.1	0.07a	0.06-0.08
C18	0.199	2.74a	2.16-3.44	2.48a	2.09-3.37	2.43a	1.99-2.91
C18:1	**0.003**	69.36b	65.21-72.64	72.82a	70.2-74.81	71.68a,b	69.09-74.49
C18:2	**0.049**	12.15a	8.87-15.63	10.29b	8.98-12.31	10.76a,b	8.82-13.39
C18:3	0.753	0.66a	0.57-0.8	0.64a	0.53-0.71	0.65a	0.62-0.7
C20	0.978	0.53a	0.41-0.67	0.52a	0.39-0.65	0.52a	0.39-0.7
C20:1	0.747	0.36a	0.28-0.46	0.37a	0.29-0.44	0.38a	0.29-0.45
∑SFA[1]	**<0.001**	16.51a	15.45-17.42	14.96b	14.08-17.14	15.56b	14.83-16.27
∑MUFA[2]	**0.002**	70.52b	66.59-73.63	73.96a	71.34-75.98	72.88a	70.38-75.69
∑PUFA[3]	**0.051**	12.80a	9.46-16.29	10.92b	9.51-13	11.41a,b	9.48-14.08
MUFAs/PUFAs	**0.041**	5.67b	4.09-7.78	6.86a	5.57-7.98	6.51a,b	5-7.99
C18:1/C18:2	**0.042**	5.89b	4.17-8.19	7.17a	5.79-8.33	6.81a,b	5.16-8.41

[1] Sum of saturated fatty acids; [2] sum of monounsaturated fatty acids, [3] sum of polyunsaturated fatty acids.
Different letters in the same row show the membership to different groups by Tukey HSD (P<5%)

The chlorophyll, carotenoid and tocopherol contents of monovarietal virgin olive oils from the Bosana variety are shown in Table C. The ratio chlorophylls/carotenoids was the only parameter among pigments that showed significative differences. Gandul-Rojas & Minguez-Mosquera (1996) reported that, independently from the content in pigments, the ratio chlorophylls/carotenoids is constant with a value close to unity, meaning that the green and yellow fractions are in balance. In this study we found that values of the chlorophylls/carotenoids ratio ranged between 0.41 in Ittiri and 0.57 in Alghero, meaning that the carotenoid content is on average twice the size of the chlorophyll content. The values of pigments and tocopherols were characterized by a wide variability (Table 5.4). By plotting the data of the sum of chlorophylls and carotenoids as well as the α tocopherol contents is possible to see a variability among crop years and within a group (Fig. 5.2, 5.3 and 5.4). The oils from Alghero showed the biggest within group variability in all the years under study for both chlorophylls and carotenoids contents, while in Ittiri and Sassari the variability seems mostly due to the crop year (Fig. 5.2 and 5.3). In particular, the oils from Ittiri differ considerably among different years in the carotenoids and chlorophylls contents. The lowest values

of carotenoids and chlorophylls for the oils from Ittiri have been recorded in 2012, while in the other two production areas the values were homogenous.

Table 5.4 Pigments content of oils from the three production areas Alghero (AHO), Ittiri (ITR), and Sassari (SS). The data are presented as mean, minimum and maximum of values of the three years for each production area (AHO 10 samples, ITR 10 samples and SS 8 samples).

		Growing area					
	P value	AHO		ITR		SS	
		Mean	Range	Mean	Range	Mean	Range
Neoxanthin	0.915	0.24a	0.05-0.38	0.22a	0.04-0.31	0.24a	0.05-0.4
Violaxanthin	0.746	0.87a	0.15-1.88	0.8a	0.14-1.45	0.99a	0.36-2.52
Antheraxanthin	0.592	0.26a	0.08-0.43	0.49a	0.12-2.96	0.29a	0.16-0.63
Lutein	0.905	2.40a	1.34-3.74	2.36a	1.36-3.62	2.24a	1.50-3.50
Chlorophyll b	0.921	0.12a	0.01-0.27	0.10a	0.01-0.24	0.11a	0.01-0.24
Chlorophyll a	0.691	0.15a	0.02-1.03	0.07a	0.01-0.31	0.09a	0.02-0.4
Pheophytin b	0.96	0.08a	0.02-0.31	0.07a	0.01-0.3	0.07a	0.02-0.24
Pheophytin a	0.267	2.78a	1.13-5.41	2.00a	0.43-3.68	2.49a	1.49-3.12
β_carotene	0.373	1.85a	0.65-2.83	1.48a	0.33-2.4	1.93a	1.05-3.46
∑ chlorophylls	0.215	3.12a	1.32-5.48	2.24a	0.49-3.93	2.76a	1.63-3.76
∑carotenoids	0.937	5.61a	2.34-8.68	5.34a	2.09-9.47	5.69a	3.97-10.42
∑chloro/∑carot.	**0.054**	0.57a	0.36-0.86	0.41b	0.22-0.59	0.50a,b	0.36-0.62

Values are expressed as mg of relative standard compound per kg of oil
Different letters in the same row show the membership to different groups by Tukey HSD (P<5%)

The lower content in pigments in the oils collected from Ittiri in 2012 could be related to the more advanced ripening stage of the samples that year respect to the other years, as reported by Criado and colleagues (2008). In 2013 carotenoids contents higher than the ones of the other years of the study were recorded in all the production areas (Fig. 5.2). The variability of chlorophylls values ascribable to the crop year was lower (Fig. 5.3). As in our study, Arslan et al. (2013) didn't find significant differences in the chlorophyll content between oils from three locations in the south of Turkey, while they found differences in the carotenoids content due to an exposure of the fruits to lower temperatures, exposure that could have led to a deterioration of the olive fruits and a degradation of the pigments. Romero and co-authors (2003) found as well differences in the chlorophyll and carotenoids contents of oils from four different crop years, and related them to the minimum air temperature recorded during the harvest period (November–December), and to the rainfall regime as a secondary effect. In the present study the minimum temperatures didn't differ considerably between the three zones investigated, although differences in GDD have been recorded. During 2013 there was in fact a lower accumulation of GDD (2319 °C in 2011, 2547 °C in 2012 and 2223°C in 2013), and this factor could be the explanation for the higher content of carotenoids that we recorded in all the growing areas.

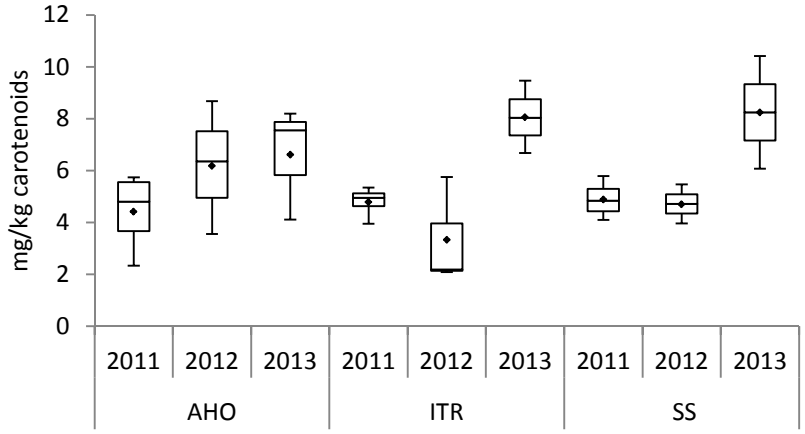

Fig. 5.2 Carotenoids content in oils from the three growing areas, Alghero (AHO), Ittiri (ITR) and Sassari (SS), during the three years of study (2011, 2012 and 2013). The boundaries of the box indicate the 25th and 75th (top and bottom) percentiles. The line within the box marks the median.

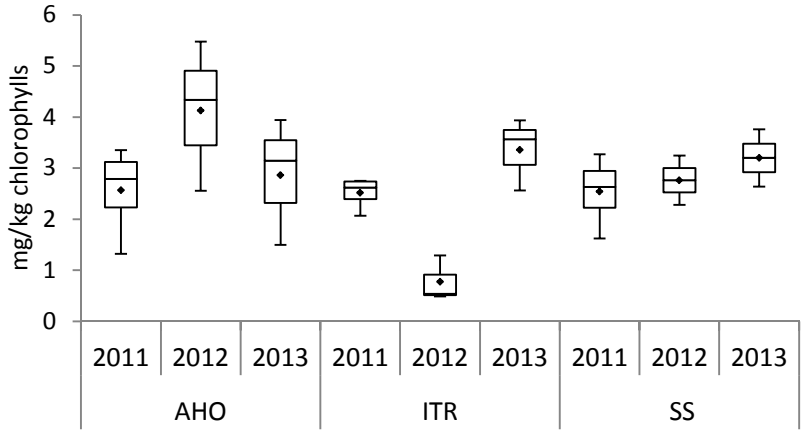

Fig. 5.3 Chlorophylls contents in oils from the three growing areas, Alghero (AHO), Ittiri (ITR) and Sassari (SS), during the three years of study (2011, 2012 and 2013). The boundaries of the box indicate the 25th and 75th (top and bottom) percentiles. The line within the box marks the median.

Regarding the tocopherols fraction, 4 isomers have been described, α, β, γ and δ tocopherol, though, due to the lack of chromatographic resolution, the β and γ isomers were quantified together (Table 5.5). A-tocopherol is the most abundant tocopherol in virgin olive oil. The values of α-tocopherol found in this study ranged between 129.28 and 304.13 mg/kg oil (Table 5.5). Those values are slightly lower than the ones found by Cerretani and colleagues (2005) for Bosana virgin olive oils, probably due to the influence of crop year on the α-tocopherol content, as stated by Salvador et al. (2003). It is interesting to note that both the minimum and maximum values of α-tocopherol were recorded in oils from Ittiri (Table 5.5). This result shows the high variability existing within oils

produced in the same growing areas. The variability in α-tocopherol is ascribable to the different crop year, as shown in figure 5.4, where the α-tocopherol content is plotted by crop year and by production areas. In 2011, the first year of the study, the oils from all the three zones were characterized by the highest α-tocopherol content, while the lowest content for all the three zones was recorded in 2012 (Fig. 5.4). Thus in this study it is possible to state the non-dependence of the α-tocopherol content from the production area, but a connection with the crop year is detectable. Some works found differences in the tocopherol content among different growing areas (Romero et al., 2003; Arslan et al., 2013), while other authors described the altitude influence on tocopherol content (Mohamed Mousa et al. 1996; Aguilera et al. 2005). The influence of temperature during seed maturation on tocopherol content has been reported for canola, soybean, sunflower, oats, flax and shea butter (Almonor et al 1998; Dolde et al 1999; Britz & Kremer, 2002; Maranz & Wiesman 2004). In our study however no connection between the hottest year, 2012, and a higher α-tocopherol content has been recorded, while on the contrary in that year the oils showed the lowest α-tocopherol content.

Table 5.5 Tocopherols and phenols content in oils from the three production areas Alghero (AHO), Ittiri (ITR), and Sassari (SS). The data are presented as mean, minimum and maximum of values of the three years for each production area (AHO 10 samples, ITR 10 samples and SS 8 samples).

	P value	Growing area					
		AHO		ITR		SS	
		Mean	Range	Mean	Range	Mean	Range
δ tocopherol	0.81	0.51a	0.11-1.24	0.44a	0.13-0.78	0.52a	0.13-0.88
β+γ tocopherol	0.732	5.49a	2.05-7.99	6.01a	2.98-9.34	6.38a	3.23-9.33
α tocopherol	0.934	207.89a	158.79-250.65	214.71a	129.28-304.13	207.62a	130.1-251.4
Hydroxytyrosol	0.108	9.97a	2.91-39.4	3.73a	1.72-10.01	4.50a	1.43-8.96
Tyrosol	0.117	5.74a	1.65-12.4	3.27a	1.68-5.89	4.53a	1.19-6.77
Vanillic acid	0.973	0.45a	0-1.21	0.49a	0-1.36	0.48a	0.12-0.92
Vanillin	0.446	0.15a	0-0.31	0.20a	0.09-0.31	0.21a	0.07-0.4
DAOA	0.803	192.32a	75.51-440.7	186.30a	37.59-302.21	163.57a	45.16-301.46
Pinoresinol	0.130	8.56a	3.95-17.09	12.05a	5.65-18.55	12.15a	6.1-18.4
Luteolin	0.789	2.71a	0.3-6.81	3.21a	1.88-5.7	3.07a	1.24-7.09
Apigenin	0.406	2.82a	0.44-5.33	3.22a	0.55-6.17	4.19a	0.84-8.84
∑SIDs[1]	0.895	312.97a	185.72-576.57	310.35a	118.24-462.44	290.46a	141.18-463.78

[1] sum of secoiridoids

Hydroxytyrosol is expressed as mg of tyrosol per kg oil; DAOA, deacetoxy oleuropein aglycon, is expressed as mg of oleuropein per kg oil; the other compounds are expressed as mg of relative standard compound per kg of oil

Different letters in the same row show the membership to different groups by Tukey HSD (P<5%)

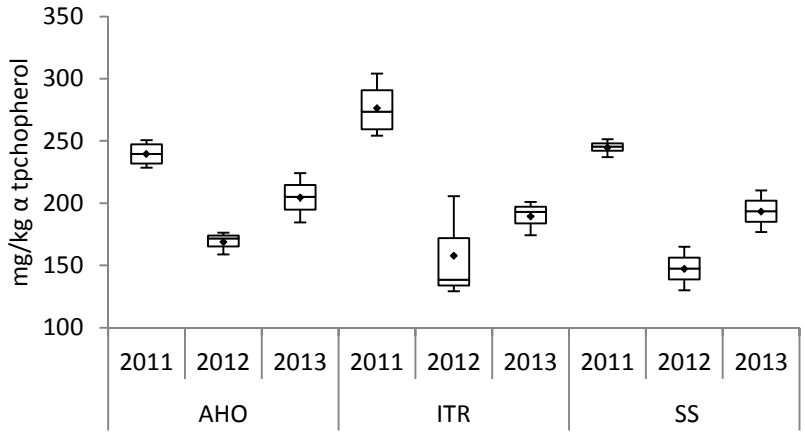

Fig. 5.4 A-tocopherol contents in oils from the three growing areas, Alghero (AHO), Ittiri (ITR) and Sassari (SS), during the three years of study (2011, 2012 and 2013). The boundaries of the box indicates the 25th and 75th (top and bottom) percentiles. The line within the box marks the median and the symbol ♦ indicate the mean The line within the box marks the median.

An UV chromatogram of the phenolic extract from cv. Bosana is show in figure 5.6. Bosana virgin olive oils were characterized by a similar content in the phenolic alcohols hydroxytyrosol and tyrosol (Table 5.5) in all of the growing areas except for Alghero, where the mean value was made higher by a single very high value recorded in 2012. The phenolic alcohols occur in virgin olive oil due to the lysis of secoiridoid compounds (Montedoro et al., 1992), so their concentrations are related to several factors affecting the secoiridoids concentration, such as technological features of the extraction process (Fregapane and Salvador, 2013) and oxidative damage. In fact, hydroxytyrosol especially possess

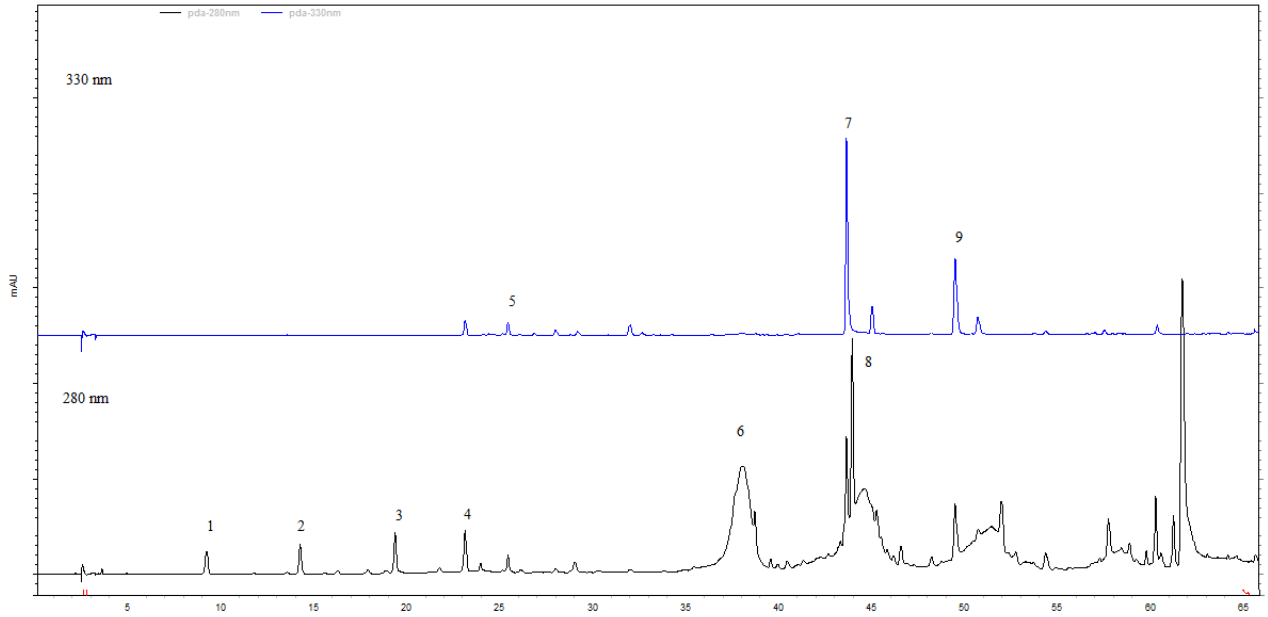

Fig. 5.6 Representative HPLC chromatogram of cv. Bosana virgin olive oil sample. (1) tyrosol, (2) hydroxytyrosol, (3) vanillic acid, (4) vanillin, (5) cumaric acid, (6) deacetoxy oleuropein aglycon, (7) luteolin, (8) pinoresinol, (9) apigenin

a great antioxidant power (Carrasco-Pancorbo et al., 2005), hence its concentration decreases after reacting with oxidants. Deacetoxy oleuropein aglycon (DAOA) is the most represented secoiridoid, ranging between 32 and 76% of the sum of secoiridoids. The DAOA contents found in this study are similar to the ones found by Cerretani et al. (2005) for Bosana oils. Pinoresinol, belonging to the lignans compounds, has been detected and quantified between 3.95 and 18.55 mg/kg (Table 5.5). Owen et al. (2000) described lignans as the major components of the olive seed, therefore their occurrence in virgin olive oil is due to the breaking of the pit when olives are crushed. The contents of pinoresinol reported in literature are quite variable, ranging between 0.4 -1.6 mg/kg for cv. Chemlali (Taamalli et al., 2012) and 15–44 mg/kg for cvv. Koroneiki, Chemlali and Picual (Dabbou et al., 2011). Finally, the flavones luteolin and apigenin were identified, with concentrations ranging between 0.3 and 7.09 mg/kg, and between 0.44 and 8.84 mg/kg respectively (Table 5.5). Our values are higher than the ones reported by Arslan et al. (2013) for Turkish Sariulak variety and by Bakhouche et al. (2013) for Arbequina, but are consistent with the ones reported by García et al. (2002) for Picual variety.

The phenolic content didn't vary significantly according to the growing locations (Table 5.5). Several authors described the influence of the geographical origin on the phenolic fraction for different cultivars (Bakhouche et al., 2013; Ouni et al., 2011; Taamalli et al., 2012); our result indicates that the phenolic content in the cultivar Bosana is less affected by the production area than in the cultivars studied in the abovementioned researches.

The virgin olive oil from cv. Bosana is characterized by a medium olive fruity, grassy with prevalent scent of thistle and artichoke and hints of almond and tomato, and has medium intensity of bitter and pungent notes (Fig. 5.7). The sensory profile of virgin olive oil from cv. Bosana found in this study matches perfectly the profile described in the Italian National Database of Monovarietal Extra Virgin Olive Oils, a dynamic database including a large number of observations for each monovarietal virgin olive oil, undergoing updates every year, and thus providing accurate chemical and sensory average data for the virgin olive oils (Rotondi et al., 2013).

The oils of cv. Bosana olives collected from the three different production areas showed very similar sensory profiles, even if small differences were observed in the intensities of the scents artichoke, bitter and pungent (Fig. 5.7). However, by applying the analysis of variance is possible to state that the production area didn't influence the sensory profiles, since the oils have statistically the same sensory profile.

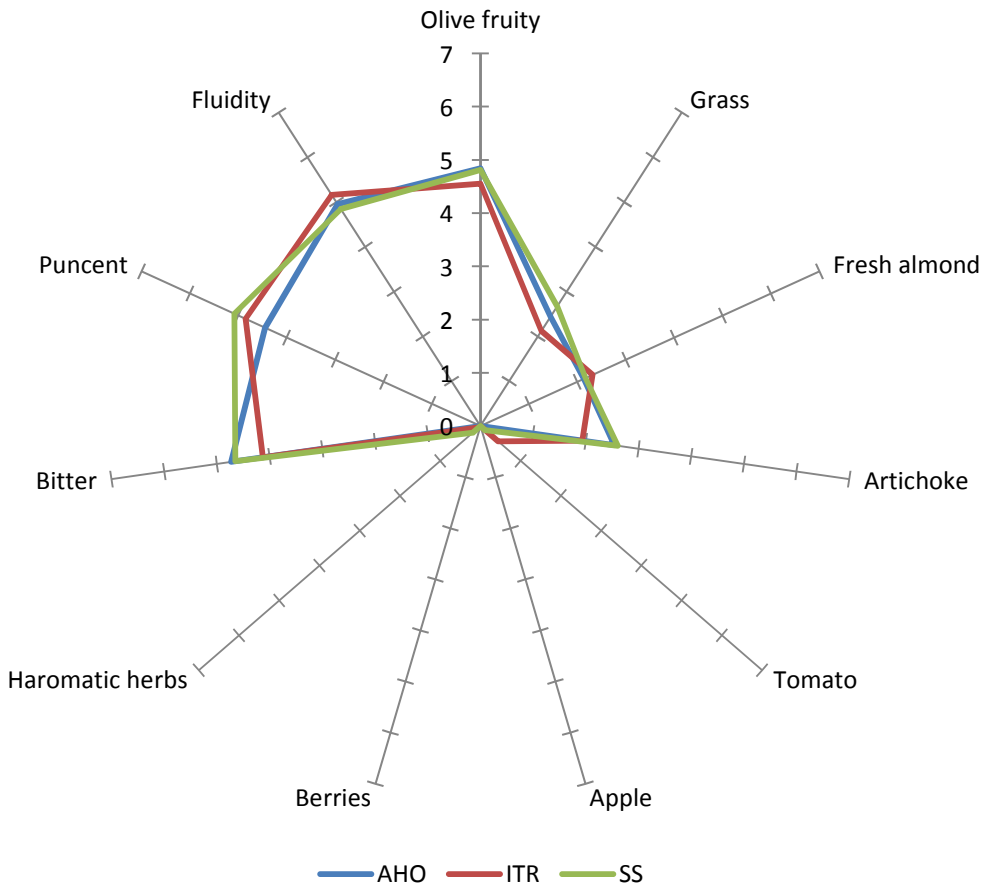

Fig. 5.7 Sensory profile of Bosana virgin olive oils from the three growing area, Alghero (AHO), Ittiri (ITR) and Sassari (SS)

Classification of virgin olive oils according to the production area

In order to discriminate and group cv. Bosana virgin olive oils by production area stepwise Linear Discriminant Analysis (LDA) was used on the standardized chemical data. The scatter plot obtained by discriminant analysis is shown in Figure E; in where the x-axis plots the values of discriminant function 1, the y-axis plots the values of discriminant function 2 and the z-axis plots the values of discriminant function 3. A good separation was obtained mostly for oils from the Alghero area (AHO), while the groups from Ittiri (ITR) and Sassari (SS) were close (Fig. 5.8).

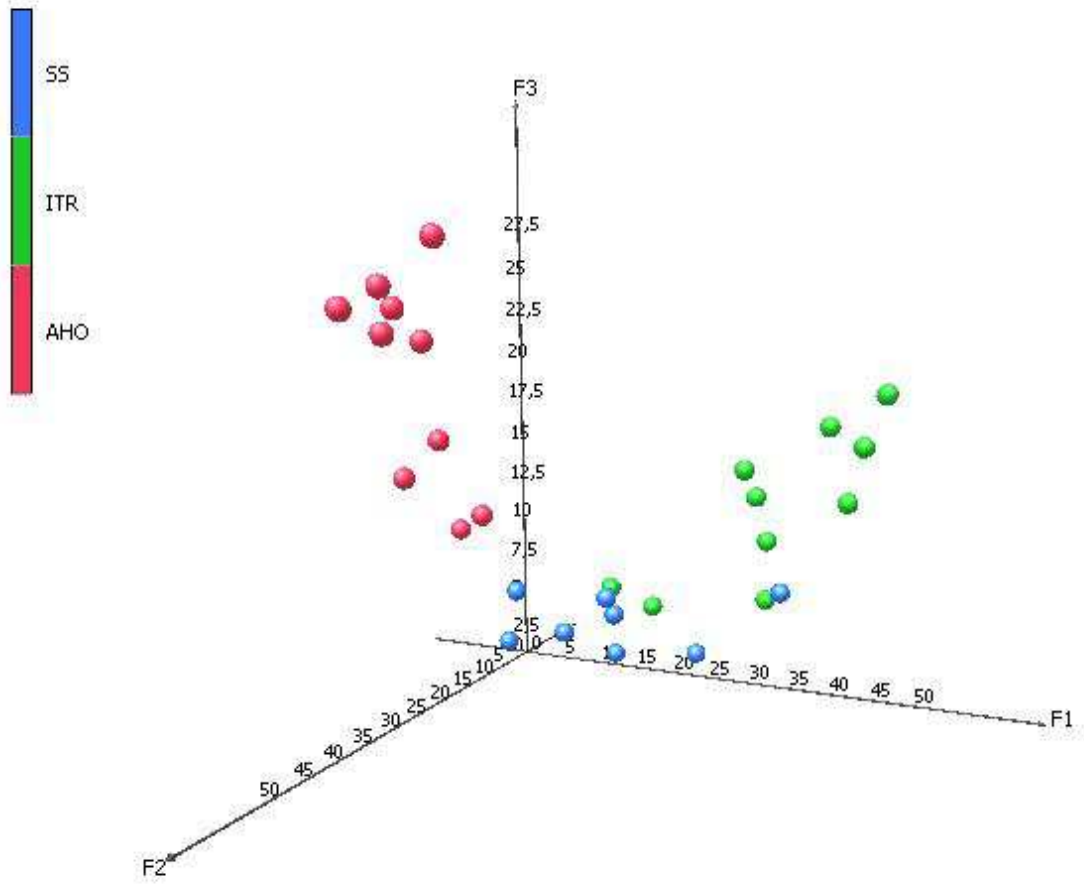

Fig. 5.8 Score plot of three discriminant functions of LDA model build using all chemical parameters analysed in this study of cv. Bosana virgin olive oils from the three growing areas, Alghero (AHO), Ittiri (ITR) and Sassari (SS). The total variance explained by the three functions is 57%.

The cumulative percentage variance explained for the three functions in the discrimination of growing areas in this study is 57%.

The analysis of the factor loadings allows us to identify the variables with the highest discriminant power: peroxide number, linoleic acid and β+γ tocopherol were the most remarkable variables on the discriminant function 1; palmitic acid, violaxanthin, pheophytin A and ratio chlorophylls carotenoids were the most important variables on discriminant function 2, the discriminant function that mostly contributes to separate Alghero group from the other ones; heptadecanoic acid, neoxanthin and sum of chlorophylls were the variable most remarkable on discriminant function 3.

The soil influence on virgin olive oil is difficult to establish since is quite difficult to have a balanced experimental plan, or rather to have olive samples having soil typologies as the only non-standardised factor. In the wine researches some scientists stated the influence of soils on the wine aromatic composition (Sabon et al., 2002; Gómez-Míguez et al., 2007). Huggett (2005) in her review on the relationship between geology and wines reported the soil influence on the sensory

notes of wines, mainly in the saltiness ones while Jackson (1994) reported that there is no evidence supporting the common belief that grapes derive specific flavours from the soil in which they grow, as implied by the terms “flinty”, “chalky” or “goût de terroir.”

As previously reported in chapter 3, only one olive grove of Alghero area (AHO1) is located on “D4” soil typology, characterized by a neutral reaction, from permeable to medium permeability, with high soil erodibility and with a depth from shallow to moderate, while the other three olive groves of the Alghero area are located on “I1” soil typology characterized by a sub-acid and acid reaction, from permeable to low permeability, with a moderate surface soil erodibility and depth more than 1 m. Both Sassari and Ittiri olive groves have soils belonging to “F” typology. Olive groves named SS 3 and SS 4 and all from Ittiri area are characterized by soil typology “F1” while SS 1 and SS 2 are located on “F2” soil typology. The typologies “F1” and “F2” are quite similar, having a neutral reaction, permeable, with high soil erodibility and moderate depth, but differ for the outcrop in “F1” typology.

In order to explore the hypothesis that the chemical variables of virgin olive oils could discriminate the soil typologies, and thus verify if there is a relationship between virgin olive oil chemical profile and soil typology, stepwise LDA were performed. The variance explained by the three functions in the discrimination of soil typologies accounts for 73.84%. In figure 5.9 is shown the stepwise linear discriminant analyse score plots of cv. Bosana virgin olive oils according to soil typologies. The “D4” typology is better clustered than the other soil typology (Fig. 5.9). The variables that allow the “D4”discrimination are the ones included in function 1 (Table 5.6). In order to identify which compounds cause the discrimination, the factor loadings were analysed showing that the bigger contribution is due by free acidity, palmitic and palmitoleic acids. Function 2 is the most discriminated by apigenin and heptadecanoic acid; it is these two axes to allow better discrimination. Thus, soil typologies seems to have an influence on chemical characteristics of Bosana virgin olive oil, mostly linked to free acidity and fatty acid composition. Caruso and colleagues (2014) reported the non-dependence of fatty acid composition by soil moisture, whereas the phenolic compounds are the most affected by water availability in agreement with other studies (Tovar et al., 2002; Servili et al., 2007). However the permeability is the only characteristic that differentiates “D4” typology form the other ones. Thus our data suggest that soil permeability could influence the chemical characteristics of the oil produced.

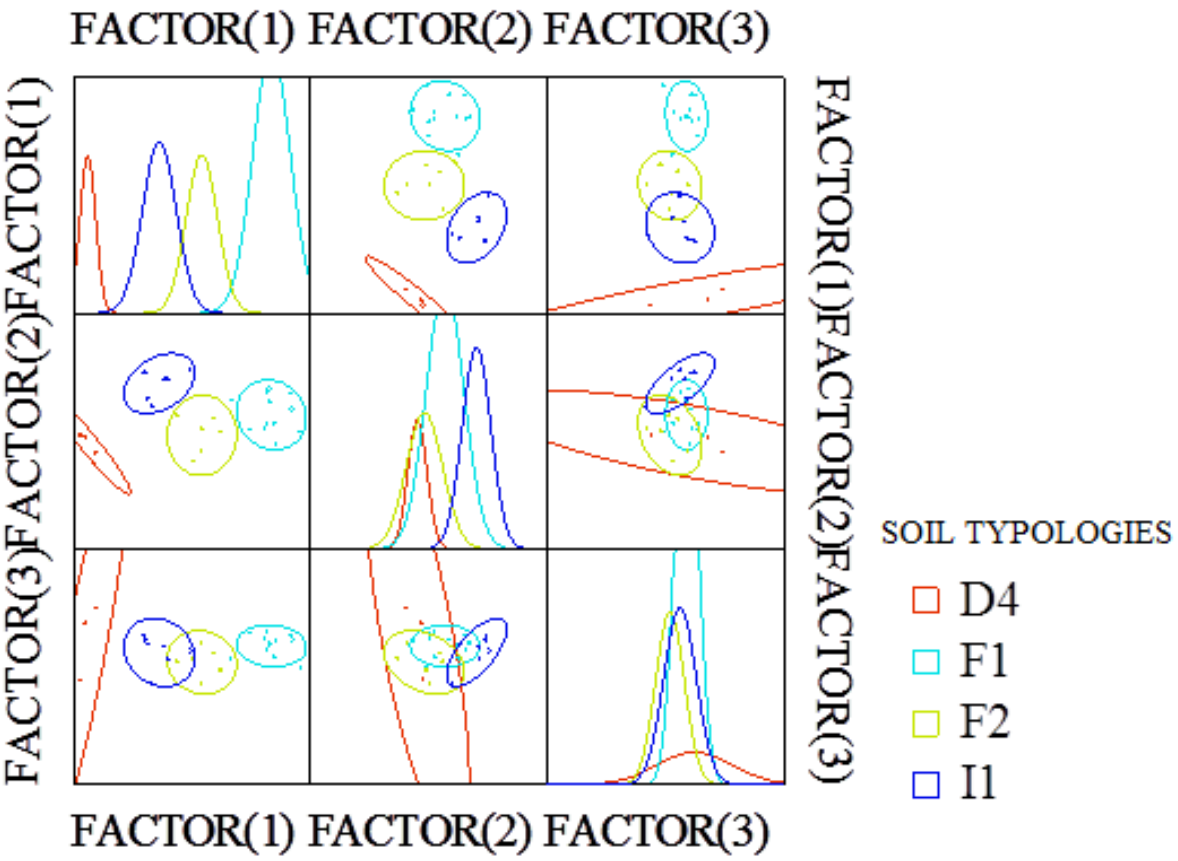

Fig. 5.9 Score plots of cv. Bosana virgin olive oils according to soil typologies

Table 5.6 Standardized discriminant function coefficients defined for discrimination between soil typologies

	Functions		
Variables	1	2	3
Free acidity	0.893	-0.057	0.055
C16:1	0.750	-0.400	0.218
C16	0.657	-0.109	-0.395
C17:1	0.621	0.641	0.216
C18:3	0.562	0.109	-0.326
C18:2	0.506	-0.510	-0.592
C18	-0.504	0.221	-0.795
C17	0.210	0.808	-0.297
Apigenin	0.468	0.744	0.107
ΔK	0.262	-0.703	0.029

Conclusion

Univariate analyses of variance and discriminant analysis were carried out on chemical dataset collected in three years. The results of the analysis of variance showed the significative differences in palmitic, oleic and linoleic acids (the most important fatty acids in virgin olive oil), as well as in

the nutritional categories of fatty acids (SFA, MUFA and PUFA) and the ratio chlorophyll/carotenoids had difference contents mostly between Alghero and Ittiri areas. We than used stepwise linear discriminant analysis to cluster virgin olive oil samples of cv. Bosana on the basis of their geographical origin; as a result, the samples grouped together from the Alghero area were discriminated mostly by palmitic acid, violaxanthin, pheophytin A and the ratio chlorophylls/carotenoids, whereas the samples from Sassari and Ittiri were closely grouped together, demonstrating the similarity of those two growing areas. By applying the same statistical procedure the hypothesis of a soil influence on chemical characteristics was tested. The results showed a definite cluster of “D4” soil typology, the soil typology having a medium permeability, leading to conclude that the soil permeability has an influence on chemical characteristics of virgin olive oil.

6. Influence of fruit ripening

Highlights

No differences in the ripening trend in the three areas

Oils produced at three different ripeness stages showed chemical differences

No detected sensory differences in oils

Introduction

The quality of virgin olive oil is a variable influenced by all factors intervening during the entire production process. These factors have been divided in “principal” and “secondary” by. D’Imperio et al., (2010), on the basis that the first cannot be governed while the second could. The “principal” factors are the cultivar and the pedoclimatic conditions, and their influence on the VOO quality has been underlined by several authors (Vinha et al., 2005; Ceci & Carelli, 2007; Tura et al., 2008; Rotondi et al., 2010). The “secondary” factors, also widely studied, include agronomic practices, technological features of the milling process and oil storage conditions (Inglese et al. 2011; Fregapane & Salvador, 2013). Among the secondary factors the ripeness degree is one of the most studied due to its interdependence with the other factors; ripeness is in fact directly related both to genetic matrix and environmental conditions. Olive varieties are classified as early or late on the basis of their ripeness timing, which is genetically determined factor; however the ripeness trend is affected by the climatic conditions of the olive grove, namely temperature, sunlight and bioavailability of water and nutrients. For example, Di Vaio and colleagues (2012) noted that olives of the Ortice cultivar grown at 50 a.s.l. ripened approximately 10 to 15 days before olives of the same cultivar grown at 100 m a.s.l..

The most common tools available to determine olive ripeness are currently visual methods for colour measurement (Cherubini et al., 2008). In particular the Jaén Index is one of the most effective methods currently in use for olive growers to determine the real ripening level of olives. The index is based on the degree of skin and pulp pigmentation according to the method developed by the Agronomic Station of Jaén defining the Ripening Index (RI) (Uceda and Hermoso, 1998). The characteristic colour change from green to purple for both skin and pulp identify the onset of ripening. During this period severe changes take place in fruits: changes in weight, pulp/stone ratio and colour, as well as changes in chemical composition, enzyme activity and oil accumulation (Beltrán et al., 2004). The oil amount in the fruit is an important parameter for a grower given its direct impact on the cost of production. It has been reported that the oil yield is genetically controlled (Lavee & Wodner, 1991), and it is affected by the environmental condition (Mailer et al., 2007) and fruit load (Gucci et al., 2007). Since olives should be harvested when the oil content is at its highest and the best oil quality can be obtained (Tombesi & Tombesi, 2007), in order to choose the correct harvesting time several factors should be taken into account: (i) the increasing weight rate of fruit, (ii) the trend of oil content, (iii) the fruit number on the tree or the number of fruit dropped and (iv) the olive oil quality parameters (Tombesi & Gucci, 2011).

The chemical and sensorial properties of olive oil are deeply affected by the ripening degree at which olives are processed, so the identification of the correct harvesting time is crucial to ensure a high oil quality and to please the consumers. During ripeness the chemical composition of olive fruit changes due to different metabolic activities (Brkić Bubola et al. 2012); hence, oils produced using olives at different ripening degrees will present different chemical and sensorial characteristics. Several authors studied the relationships between pigment composition of olive oils and fruit ripeness (Roca & Mínguez-Mosquera, 2001; Dufossé et al., 2005; Beltrán et al. 2005), since pigments are responsible for virgin olive oil final colour and other important parameters that influence consumers choice. The pigments present in virgin olive oil include chlorophylls a and b, lutein, β-carotene, violaxanthin, neoxanthin, antheraxanthin, and β-cryptoxanthin, deriving from the olive fruit, and pheophytins a and b, luteoxanthin, auroxanthin, neochrome, and mutatoxanthin, that are instead formed during the extraction process (Mínguez-Mosquera et al. 1990, 1992; Gandul-Rojas and Mínguez- Mosquera 1996). As ripening progresses and the fruit chloroplasts are transformed into chromoplasts (Gandul-Rojas et al. 2013) there is a concomitant decrease in photosynthetic activity and both chlorophylls and carotenoids concentrations (Roca & Mínguez-Mosquera, 2001; Beltrán et al. 2005; Baccouri et al. 2008); furthermore the tocopherol content decreases during ripening, even if the observed rate of decrease varied according to the year (Gutiérrez et al., 1999; Beltran et al., 2005).

The content in fatty acids is also affected by the ripening stage. As the ripeness proceeds, a decreasing trend for palmitic and linoleic acid and an increasing trend for the oleic acid were found by Fuentes de Mendoza et al. (2013) and Baccouri et al (2008), while Beltrán et al.(2004) described a rise in oleic acid content, in agreement with Cimato et al.(1991), who observed the same trend analysing oils produced from olives at different ripeness. The oil stability during storage can be influenced by these changes (Rotondi et., 2004), since the ratio Mono Unsaturated Fatty Acids (MUFA) and Poly unsaturated Fatty acids (PUFA) as well as ratio oleic/linoleic acid are correlated to the oil oxidative stability. The phenol fraction of olive oils is correlated to the oxidative stability as well, and its concentration in virgin olive oil is affected by ripeness (Rotondi et al., 2004a). In fact, a decrease of oleuropein content and an increase of demethyloleuropein during the ripeness process has been reported (Amiot, et al., 1989). In virgin olive oil a decrease in the phenolic fraction, especially in the secoiridoid compounds, as the maturation proceeds has been reported by several authors (Trovar et al., 2002b; Morellò et al., 2004); this process could be related to the decrease of the content in phenolic precursors in the olive and to the enzymatic activities occurring during the fruit ripening (Briante et al., 2002; Gómez-Rico et al., 2008; Fregapane & Salvador, 2013).

Oils produced from olives with an high ripening degree have lower intensity of olive fruitiness, bitterness and pungency, as pointed out by several researches on different olive varieties (Salvador et al., 2001; Rotondi et al., 2004a; Brkić Bubola et al. 2012). The bitter and pungent tastes in oils are due to the presence of secoiridoid compounds (Gutiérrez et al., 1989), thus the decline of the secoiridoid content during ripeness is reflected in the decreasing trend of those flavour characteristics. Thus, by identifying the optimal ripeness stage it is possible to produce virgin olive oils with a high content of antioxidants and with pleasant flavours, such as the "sweet" typology favoured by the consumers (Gutiérrez-Rosales et al., 1992Predieri et al., 2013).

Experimental design

The study was conducted during the crop years 2012-2013 and 2013-2014. Three harvests were carried out at different ripening stages (15th of November, 14th of December and 11st of January) in the 3 macro areas (Alghero, Ittiri and Sassari).

Chemical analysis

On the olive fruit samples collected from the three growing areas the ripening index, the water and oil content were analysed by using the methodologies described in chapter 4.

The olive oil production was carried out using a low scale mill as described in chapter 4. On the resulting virgin olive oil free acidity, peroxide number, UV spectrophotometric indices (at 232 and 270 nm), total phenol content, fatty acid profile, HPLC pigment, tocopherol and phenolic fractions were analysed, as well as the sensory analysis performed by a professional panel test, by using the methods reported in chapter 4.

Statistical analysis

The data collected from the chemical analyses were elaborated using Microsoft® Excel 2007/XLSTAT© (Version 2009.3.02, Addinsoft, Inc., Brooklyn, NY, USA). The significance of differences among means at a 5% level was determined by two-way ANOVA, in order to examine treatment interdependences (harvest date and growing area), followed by a Tukey's Honestly Significant Difference (HSD) test. Sensory data were submitted to the ANOVA procedure using SAS software 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Result and discussion

The data obtained by the analysis of olive fruits during the two years period are reported in Table 6.1. In 2012 olives the crop from Ittiri was characterized by the lowest RI at all of the harvesting times showing thus a late trend of ripeness. Maturation trends of olive cultivated in Alghero and Sassari showed the same trend during all harvest dates. In the second year the RI trend of Alghero was similar to the former year, with values ranged 1.2 and 4.5. Contrarily, olives cultivated in Ittiri were undergoing a fast ripening process respect to the previous crop season reaching in the last harvest date t RI=4,5. In Sassari the olive ripening trend was more gradual respect the previous year, in fact at the last harvest RI value was 3,6 respect to the RI of 4,7 collected in 2013.Sassari reached in the last harvest date the lowest RI (3.9). Moisture content determined in olive fruits cultivated in Ittiri showed a decreasing trend in the first year while in the second year moisture values were constant at all harvest dates. Also Sassari olives had a constant moisture values during ripeness in the first year while in the second an higher value was recorded at the last date. Environmental conditions of Alghero area differently influenced the moisture content: in the first crop season an increase of water content was observed, in the second year the olive have maintained the same moisture content at all dates. The crude fat content (Table 6.1) in samples did not differ statistically with both production zone and harvesting date and was characterized by a clear increasing trend in agree with Jiménez et al., (2013) and Di Vaio et al., (2013). The latter author also recorded an higher oil content in olive grown at higher altitude, data not supported by our findings: in fact in the data here presented the altitude effect is not detectable since the crops from Ittiri (placed at the higher altitude) presented the lower oil content.

Table 6.1 Harvest data, ripening index, moisture (g/100g,) and crude fat content (g/100g of dry weight) of samples collected during two consecutive years in three areas of Sardinia, Alghero (AHO), Ittiri (ITR) and Sassari (SS).

		AHO			ITR			SS		
	Harvesting date	Ripening Index	Moisture [g/100g]	Crude fat [g/100g]	Ripening Index	Moisture [g/100g]	Crude fat [g/100g]	Ripening Index	Moisture [g/100g]	Crude fat [g/100g]
Crop year 2012/2013	16/11	1.7	54.4	39.6	1.2	54.9	31.2	1.5	55.3	39.0
	14/12	3.1	56.3	40.8	2.1	53.0	41.0	2.7	55.8	42.5
	11/1	4.6	58.6	42.3	3.8	51.7	35.4	4.7	55.3	42.8
Crop year 2013/2014	15/11	1.4	50.5	33.1	1.3	49.1	39.3	1.2	48.0	34.9
	14/12	2.6	49.6	35.2	2.6	48.7	37.9	2.1	46.5	28.1
	11/1	4.5	48.9	46.6	4.5	48.9	46.6	3.6	51.7	48.6

The analytical parameters of free acidity, peroxide value, and UV spectrophotometric indices of all the samples of cv. Bosana olive oil were within the limits established by of Reg. 2569/91 and following amendments, so the oils could be labelled as extra virgin according to EU rules. The significance of the chemical parameters analysed is shown in Table 6.2 An increasing trend was of free acidity was observed with the proceeding of the olive ripening s (Fig. 6.1), due to the action of fruit lipase (Yousfi et al., 2008). It is interesting to note that in both years the highest acidity was reached in oils from Alghero (0.62 and 0.55% respectively), while the maximum level of acidity for samples both from Ittiri and Sassari was 0.4%. (Fig. 6.1). However poor information is available on the presence of the lipase in olive fruits albeit many papers concern the oil palm lipase (Morcillo et al., 2013). Panzanaro and colleagues (2010) reported the dependence of lipase activity on the fruit stage: they observed an increase in enzymatic activity during ripening process with the maximum lipase activity at spotted II stage and a lower value thereafter. This finding is in contrast with other reports (Pannelli et al. 1990; Ripa et al., 2008) that describe no changes in free fatty acid content if olives are healthy and processed within 24h. But Panzanaro himself explains that this conflicting data may be related to olives soften during fruit ripening, then the ripe fruits are more susceptible to mechanical damages.

Table 6.2 Analytical indices of virgin olive oils from Bosana cv. at three ripening stages (I, II and III) and for the three production areas Alghero (AHO), Ittiri (ITR), and Sassari (SS). The data are presented as means ± standard deviation

		Free acidity[1]	POV[2]	K_{232}	K_{270}	TP[3]
Ripeness	I	0.36b±0.01	7.67a±1.35	2.06a±0.10	0.15a±0.02	458.2a±134.1
	II	0.4a,b±0.07	8.4a±2.19	2.01a±0.16	0.15a±0.02	333.7a±64.6
	III	0.44b±0.12	8.31a±2.07	2.02a±0.08	0.15a±0.02	322.0a±107.2
	P-value	***0.027***	*0.765*	*0.756*	*0.883*	*0.051*
Production area	AHO	0.47a±0.11	9.58a±1.78	2.04a±0.09	0.14a±0.01	282.9b±83.9
	ITR	0.36b±0.04	7.75a±1.31	2.03a±0.08	0.15a±0.02	398.6a,b±99.8
	SS	0.36b±0.03	7.05a±1.52	2.02a±0.17	0.16a±0.02	432.6a±124.7
	P-value	***0.002***	*0.102*	*0.984*	*0.229*	***0.034***
Ripeness* Production area	***P-value***	***0.032***	*0.921*	*0.632*	*0.889*	*0.239*

[1] g Oleic acid in 100g oil
[2] POV, Peroxide value, mEq O_2 kg^{-1} of oil.
[4] TP, total phenols, mg of gallic acid kg^{-1} of oil

No significant differences both for production zone and harvesting date were found in the number of peroxide and the spectrophotometric indices K_{232} and K_{270} (Table 6.2); these data are in

agreement with other reports (Rotondi at al., 2004a; Jiménez et al., 2013) since these quality indices are mostly correlated to sanitary state of olive (Servili et al., 2012). Although no significant differences were found in total phenol content among samples from olives differing for the ripening index (Table 6.2), a decreasing trend was detectable as the ripening progressed in samples collected at all the locations (Fig. 6.2), in accordance with other works (Rotondi et al., 2004a; Beltran, et al., 2005; Fuentes de Mendoza et al., 2013). Oils from Alghero were characterized by the lowest phenolic content at the first harvesting date, both in 2012 and 2013 (Fig. 6.2). Samples from Sassari were characterized by the highest phenol content in both years, particularly the highest content (647 mg kg^{-1} of gallic acid) was recorded at the first harvesting date in 2013. The difference in the total content of phenols has therefore proved significant for the production area (Table 6.3), with 46.52% of variability explained by the production area factor discriminating. Several report described the phenol content variability according to the production area (Di Vaio et al., 2013; Abu-Reidah et al., 2013).

Fatty acids

The fatty acid composition is an important parameter for the evaluation of oil quality due to its influence on the oxidative processes (Rotondi et al., 2004a). Among the fatty acids identified in olive oil obtained from cv. Bosana, oleic, palmitic, stearic and linoleic were the most abundant, with more than 95% of the total fat content (Table 6.4). Palmitic acid, the saturate fatty acid mostly represented in olive oil, showed a significant decreasing trend in agreement with other authors (Gutiérrez et al, 1999; Beltrán et al., 2004; Fuentes de Mendoza et al, 2013). In fact the variability of palmitic acid, expressed as percent of the total sum of the squares, was mostly due to the harvesting date (Table 6.5). However, Gutiérrez et al. (1999) stated that the decrease in palmitic acid could be due to a dilution effect, in its turn due to the increase in oleic acid content by the active triglyceride biosynthesis. The content of stearic acid showed a slightly decreasing trend during the maturation process (Table 6.4, Fig. 6.3 A) and its variability was mainly related to the harvesting date (62.57%) (Table 6.5). There is no agreement in the literature about the behaviour of stearic acid during ripeness: Salas and colleagues (2000) found no stearic acid accumulation during maturation, while both a growing and a decreasing trend have been revealed by other works (Beltràn et al., 2004; Damak et al., 2008). Oils obtained by the cv. Bosana are characterized by a medium content in oleic acid (≈72%), in agreement with what reported in the database of the Italian monovarietal olive oils (http://www.olimonovarietali.it/). The oleic acid content did not vary significantly according to ripeness (P =0.193) (Table 6.4), in agreement with Cimato et al., (1991) and Bengana et al. (2013), who reported no accumulation during ripeness, conversely to other

authors that reported both a decrease (Salvador et al., 2001; Desouky et al., 2009) or an increasing trend during ripening (Beltran et al., 2004; Fuentes de Mendoza et al., 2013). However, oleic acid varied significantly according to the production area (Table 6.4). In fact the oils produced in the Alghero area showed a lower content of oleic acid (Fig 6.3 B) in both the years of study. The dependence of oleic acid content by the crop year was described by Beltran and colleagues (2004) and is due mainly to rainfall; in fact oils characterized by a low oleic acid content are related to high rainfalls during summer (Romero et al. 2003). No significant differences related to ripeness were found for the content in linolenic acid (P=0.450) (Table 6.4), even if a slightly increasing trend was detectable (Fig. 6.3 A), while the linolenic acid content was significantly affected by the production area (Table 6.4); in fact the oils produced in the Alghero area showed an higher content respect to the ones produced in the Sassari and Ittiri areas (Fig. 6.3 A). This difference in the fatty acid composition, mainly for oleic and linoleic acids, of oils from Alghero is probably due to the warmer temperature of the area. The parameters related with the fatty acid composition, namely SFA, MUFA, PUFA, the ratio MUFAs/PUFAs and the oleic/linoleic ratio, have great importance due to the nutritional implications and the oxidative stability of olive oil. The SFA were the fatty acids mainly affected by ripeness stage, since this class is composed by palmitic acid and stearic acid. Both MUFA and PUFA, as well as their ratio and the ratio oleic/linoleic acid were affected by the production area (Table 6.4), with oils from olives produced in Alghero statistically different from oils deriving from drupes produced in Sassari and Ittiri. Thus, the production area of the crop influenced the fatty acid profiles; this finding was in agreement with Ranalli et al. (1997) and Abu-Reidah et al. (2013).

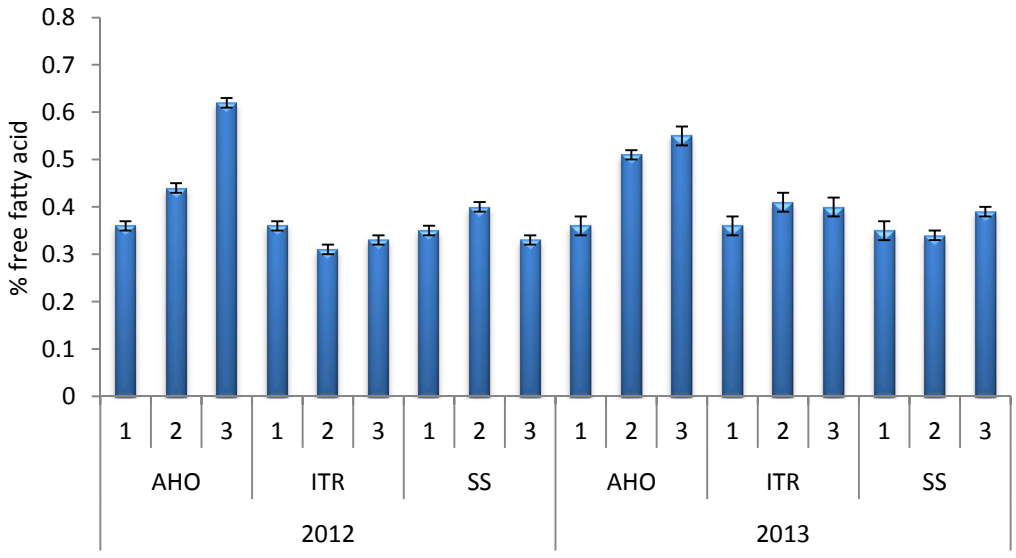

Fig. 6.1 Free acidity trend as the olive ripening proceeds (1, 2 and 3)in the three production areas, AHO, Alghero, ITR, Ittiri, SS, Sassari for the two crop years

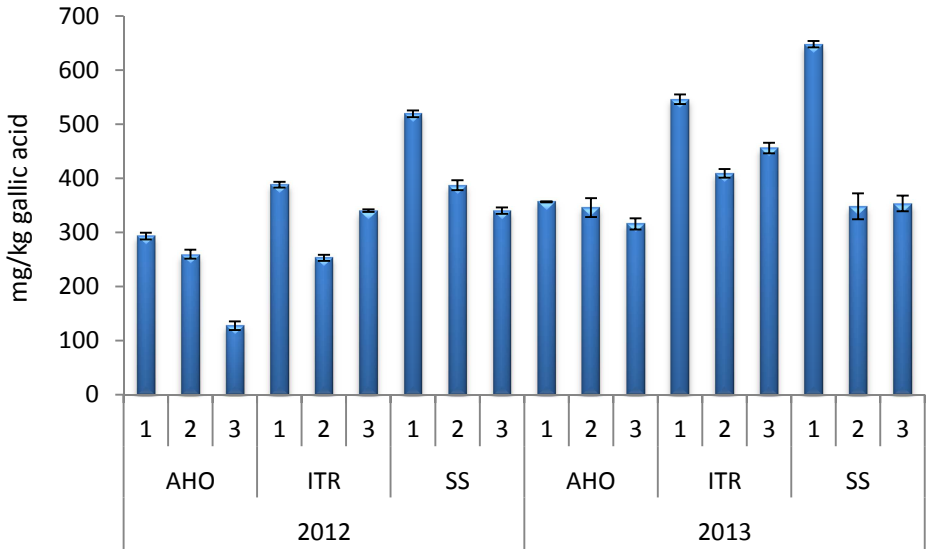

Fig. 6.2 Phenol content as the olive ripening proceeds (1, 2 and 3) in the three production areas Alghero (AHO), Ittiri (ITR) and Sassari (SS), for the two years under analysis.

Table 6.3 Variability expressed as percent of the total sum of the squares for analytical indices of virgin olive oils from Bosana cv

	Harvest date	Production area	Production area * harvest date
Free acidity	20.23*	48.57**	31.2*
POV	7.5ns	80.62ns	11.88ns
K232	17.67ns	0.99ns	81.34ns
K270	5.23ns	72.29ns	22.48ns
TP	38.68ns	46.52*	14.81ns

Significance level at **, P=0.001 and ***P< 0.001. POV, peroxide value; TP, total phenol

Table 6.4 Fatty acid profiles of the oils obtained at three stage of ripeness (I, II and III) in the three production areas of, Alghero(AHO), Ittiri (ITR) and Sassari (SS). Means ± SD.

		C 16	C16:1	C17	C17:1	C18	C18:1	C18:2	C18:3	C20	C20:1	∑SFA	∑MUFA	∑PUFA	MUFAs/ PUFAs	C18:1/ C18:2
Ripeness	I	13.45a± 0.61	0.83a± 0.08	0.04a± 0.01	0.07b± 0.00	2.62a± 0.17	70.76a± 2.11	10.36a± 1.80	0.72a± 0.04	0.52a± 0.13	0.35a± 0.06	16.63a± 0.53	72.01a± 2.11	11.08a± 1.82	6.67a± 1.27	7.03a± 1.41
	II	13.07a,b ±0.7	0.75a± 0.11	0.04a± 0.01	0.08a,b ±0.01	2.62a± 0.37	70.39a± 2.43	11.09a± 1.99	0.72a± 0.07	0.55a± 0.13	0.39a± 0.08	16.28a± 0.69	71.60a± 2.39	11.81a± 2.02	6.24a± 1.2	6.55a± 1.32
	III	12.08b± 0.66	0.73a± 0.05	0.04a± 0.01	0.08a± 0.01	2.15b± 0.25	72.00a± 2.37	11.12a± 1.91	0.66a± 0.06	0.47a± 0.11	0.38a± 0.09	14.75b± 0.72	73.20a± 2.43	11.78a± 1.92	6.39a± 1.26	6.67a± 1.38
P-value		***0.028***	*0.237*	*0.817*	***0.042***	***0.019***	*0.193*	*0.450*	*0.122*	*0.690*	*0.809*	***0.003***	*0.216*	*0.464*	*0.639*	*0.633*
Production area	AHO	13.26α± 0.85	0.77α± 0.11	0.04α± 0.01	0.08α± 0.01	2.4α±0 .36	68.62β± 1.27	12.87α ±1.01	0.74α± 0.05	0.53α± 0.14	0.40α± 0.07	16.23α± 1.04	69.87β± 1.23	13.61α± 1.04	5.17β± 0.51	5.37β± 0.55
	ITR	12.63α± 0.86	0.72α± 0.08	0.04α± 0.01	0.08α± 0.01	2.67α± 0.35	71.60α± 1.81	10.41β± 1.2.	0.68α± 0.08	0.52α± 0.12	0.36α± 0.08	15.87α± 1.09	72.76α± 1.86	11.09β± 1.16	6.64α± 0.85	6.97α± 0.95
	SS	12.71α± 0.86	0.81α± 0.07	0.04α± 0.01	0.08α± 0.01	2.32α± 0.26	72.94α± 1.04	9.29β± 0.89	0.68α± 0.02	0.49α± 0.1	0.36α± 0.09	15.56β± 1.07	74.19α± 1.06	9.97β±0 .88	7.49α± 0.7	7.91α± 0.79
P-value		*0.329*	*0.350*	*0.431*	*0.444*	*0.102*	***0.002***	***0.001***	*0.111*	*0.861*	*0.824*	*0.328*	***0.002***	***0.001***	***0.002***	***0.003***
Ripeness* Production area																
P-value		*0.992*	*0.913*	*0.974*	*0.369*	*0.785*	*0.910*	*0.715*	*0.448*	*0.992*	*0.998*	*0.980*	*0.924*	*0.661*	*0.792*	*0.825*

Different letters (a, b, c) within a column indicate significant difference at 5% level for the ripeness factor while greek letters (α, β, γ) within a column indicate significant difference at 5% level for the production area factor.

Table 6.5 Variability expressed as percent of the total sum of the squares for fatty acids and related parameters of virgin olive oils from Bosana cv

	Harvest date	Growing area	Growing area * harvest date
C16	79.73*	18.51ns	1.76ns
C16:1	50.71ns	35.42ns	13.87ns
C17	15.15ns	67.95ns	16.9ns
C17:1	58.1*	11.22ns	30.68ns
C18	62.57*	29.04ns	8.39ns
C18:1	12.34ns	84.69**	2.97ns
C18:2	4.9ns	89.12**	5.98ns
C18:3	35.52ns	37.54ns	26.94ns
C20	58.71ns	23.1ns	18.19ns
C20:1	46.07ns	41.93ns	12ns
∑SFA	88.62**	9.86ns	1.52ns
∑MUFA	12.11ns	85.05**	2.84ns
∑PUFA	4.37ns	89.18***	6.45ns
MUFAs/PUFAs	3.25ns	90.97**	5.78ns
C18:1/C18:2	3.54ns	91.04**	5.42ns

Significance level at **, P=0.001 and ***P< 0.001. POV, peroxide value; TP, total phenol

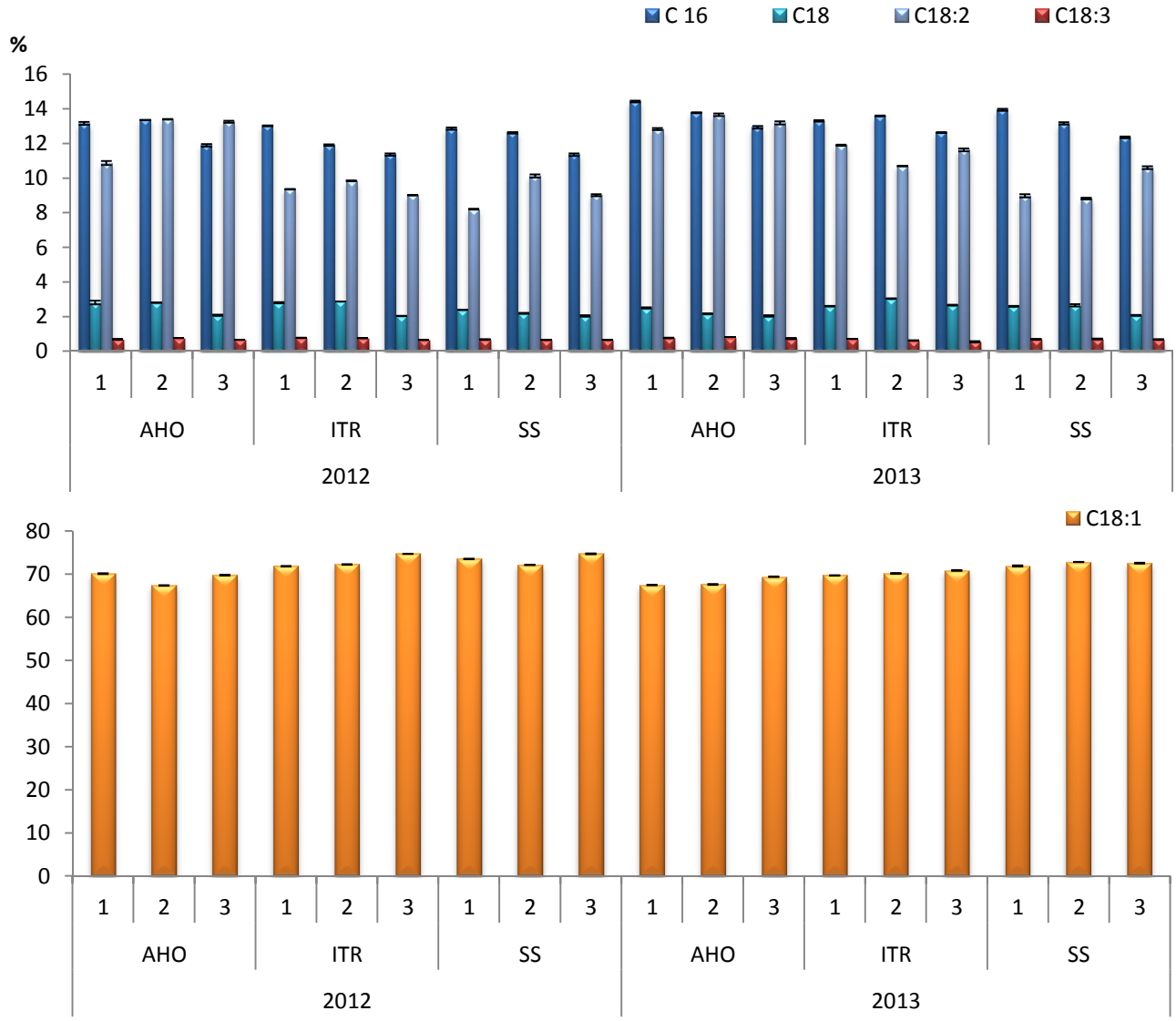

Fig. 6.3 Fatty acid profiles of oils obtained from olives grown in the three production areas Alghero(AHO), Ittiri (ITR) and Sassari (SS,), and collected at different ripening stages (1, 2 and 3)

Tocopherols

The tocopherols content found in the Bosana oil analysed is reported in Table 6.6. The content of β and γ tocopherol isomers are reported as sum of the two isomers since under the chromatographic condition used there was a coelution. Beltrán et al. (2005) described a decreasing trend for α and β tocopherol during ripeness, a decreasing trend was instead detectable from our data (Fig 6.5 F), but the analysis of variance didn't indicate significance (Table 6.6). An influence of the production area on the β+γ tocopherols content was however clear, since oils produced in Alghero presented a significantly higher content than oils from Ittiri and Sassari (Table 6.6); the influence of the crop production area on tocopherols content was also described by Tura et al. (2007) and by Ranalli et al. (1999).

Table 6.6 Tocopherols content of the oils at three stages of ripeness (I, II and III) and for the three production areas (AHO, Alghero, ITR, Ittiri, SS, Sassari). Means ± SD

		δ tocopherol	β+γ tocopherols	α tocopherol
Ripeness	I	0.4a±0.32	5.14a±1.87	203.68a±16.02
	II	0.44a±0.4	4.75a±1.79	193.53a±19.99
	III	0.45a±0.41	6.05a±2.3	177.4a±11.98
P-value		0.976	0.220	0.102
Production area	AHO	0.73a±0.51	7.52a±1.52	187.46a±19.82
	ITR	0.28a±0.05	4.25b±0.97	197.16a±16.92
	SS	0.27a±0.07	4.18b±1.04	189.99a±21.95
P-value		0.106	**0.001**	0.664
Ripeness* area				
P-value		0.998	0.727	0.829

Pigment content is expressed as mg of relative standard compound per kg of oil Different letters (a, b, c) within a column indicate significant different at 5% level for ripeness factor while greek letters (α, β, γ) within a column indicate significant different at 5% level for production area factor.

Pigment profile

Colour is an important attribute for evaluating the quality of olive oils and depends on the different pigments concentration (Pizarro et al. 2013). The pigments concentrations of Bosana monovarietal oils during the two years of study and their variability respect to ripeness and production area are shown in Table 6.7. According to the results of two ways ANOVA both the quality and the quantity of pigments present in olive oil are not influenced by the production area. This finding is in agreement with Cerretani et al. (2008a) who reported no differences in pigment compositions in virgin olive oil deriving from different regions of Sicily; the authors as well didn't find a clear effect of the ripening stage on the concentration of chlorophylls and carotenoids, supposedly due to the procedures used for evaluating the RI. However our results showed a clear influence of the RI on the chlorophylls and carotenoids content, as it is possible to see in table 6.8 where are reported the variability expressed as percentage of the total sum of square. Among the carotenoids fraction, neoxanthin, violaxanthin and β carotene decreased significantly with the progress of maturation (Fig. 6.4 A and B). These results match the ones obtained by Roca and Minguez-Mosquera (2001) in a study on drupes. It is interesting to note that the pigment content varies greatly according to the production year. In fact in 2013 the oils were richer in violaxanthin and chlorophyll b (Fig. 6.4 A and C), and thus in the total pigment content (Fig. 6.5 B); the content of lutein, β carotene and pheophytin A was however similar between the two years analysed. (Fig. 6.4 B and 6.5 A).

Table 6.7 Pigment profiles of the oils at three stage of ripeness (I, II and III) and for the three production area (AHO, Alghero, ITR, Ittiri, SS, Sassari). Means ± SD.

		Neoxan-thin	Violaxa-nthin	Anthera-xanthin	Lutein	β Carotene	Chlorophyll b	Chlorophyll a	Pheophytin b	Pheophytin a	∑ Chlorophylls	∑Carotenoids	∑Chloro/∑ Carot
Ripeness	I	0.45a± 0.25	1.7a± 0.64	0.33a± 0.19	2.83a± 0.64	4.01a± 0.85	0.22a± 0.26	0.19a± 0.15	0.07± 0.03	7.16a± 2.78	7.63a± 3.08	9.31a± 2.44	0.81a± 0.15
	II	0.2a,b± 0.12	1.15a,b± 0.81	0.34a± 0.18	2.89a± 0.64	2.18b± 0.95	0.08a± 0.09	0.15a± 0.16	0.11± 0.1	2.8b± 0.45	3.14b± 0.7	6.75a,b± 2.6	0.5b± 0.13
	III	0.08b± 0.06	0.31b± 0.19	0.21a± 0.09	2.05a± 0.45	0.72c± 0.36	0.03a± 0.02	0.05a± 0.03	0.03± 0.03	0.91b± 0.45	1.02b± 0.5	3.37b± 1.05	0.29c± 0.07
P-value		**0.024**	**0.019**	0.460	0.072	**0.001**	0.255	0.317	0.252	**0.001**	**0.001**	**0.006**	**0.001**
Production areα	AHO	0.17α± 0.16	0.73α± 0.57	0.22α± 0.12	2.17α± 0.67	1.86α± 1.3	0.09α± 0.1	0.1α± 0.08	0.06± 0.06	2.97α± 1.94	3.22α± 2.1	5.15α± 2.67	0.58α± 0.23
	ITR	0.29α± 0.26	1.18α± 0.84	0.29α± 0.13	2.86α± 0.57	2.41α± 1.6	0.1α± 0.15	0.15α± 0.18	0.07± 0.06	3.49α± 2.88	3.8α± 3.1	7.02α± 3.14	0.46α± 0.22
	SS	0.28α± 0.26	1.25α± 1.02	0.37α± 0.21	2.74α± 0.68	2.63α± 1.89	0.15α± 0.25	0.14α± 0.15	0.08± 0.08	4.39α± 4.41	4.76α± 4.7	7.26α± 3.86	0.56α± 0.31
P-value		0.568	0.392	0.434	0.166	0.285	0.823	0.839	0.947	0.322	0.381	0.287	0.264
Ripeness* area ***P-value***		0.921	0.937	0.998	0.987	0.801	0.890	0.929	0.965	0.323	0.458	0.969	0.562

Pigment content is expressed as mg of relative standard compound per kg of oil Different letters (a, b, c) within a column indicate significant different at 5% level for the ripeness factor while greek letters (α, β, γ) within a column indicate significant different at 5% level for production area factor.

Table 6.8 Variability expressed as percentage of the total sum of the squares for pigments and tocopherols of virgin olive oils from Bosana cv

	Harvest date	Growing area	Growing area * harvest date
Neoxanthin:	84.82*	8.78ns	6.4ns
Violaxanthin:	81.64*	13.41ns	4.95ns
Antheraxanthin:	46.5ns	50.31ns	3.19ns
Lutein:	60.21ns	37.17ns	2.62ns
Chlorophyll b	68.35ns	8.53ns	23.12ns
Chlorophyll a	68.89ns	9.41ns	21.71ns
Pheophytin b	83.1ns	2.81ns	14.09ns
Pheophytin a	86.51***	4.35ns	9.14ns
β carotene	91.59***	5.39ns	3.02ns
∑ chlorophylls	86.86***	4.62ns	8.52ns
∑ carotenoids	84.94**	12.82ns	2.25ns
∑ chloro/∑ carot	89.54***	5.19ns	5.27ns
δ tocopherol	0.81ns	97.04ns	2.15ns
β+γ tocopherols	10.23ns	83.93**	5.84ns
α tocopherol	72.06ns	10.37ns	17.57ns

Significance level at **, P=0.001 and ***P< 0.001. POV, peroxide value; TP, total phenol

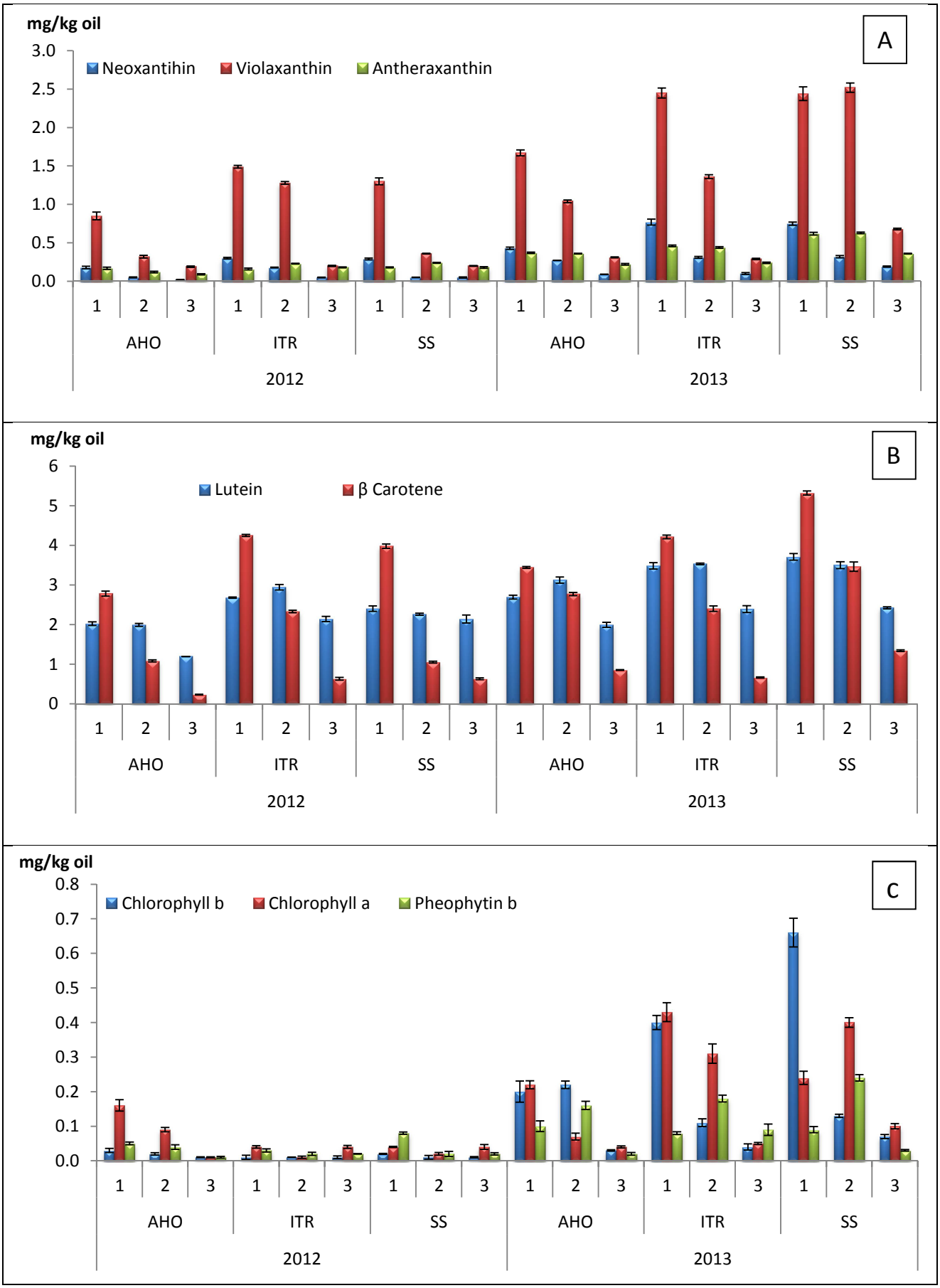

Fig. 6.4 A, minor xanthophylls content; B, lutein and β carotene content; C in the oil samples analysed coming from three production areas Alghero (AHO), Ittiri (ITR) and Sassari (SS) and at different ripening stages

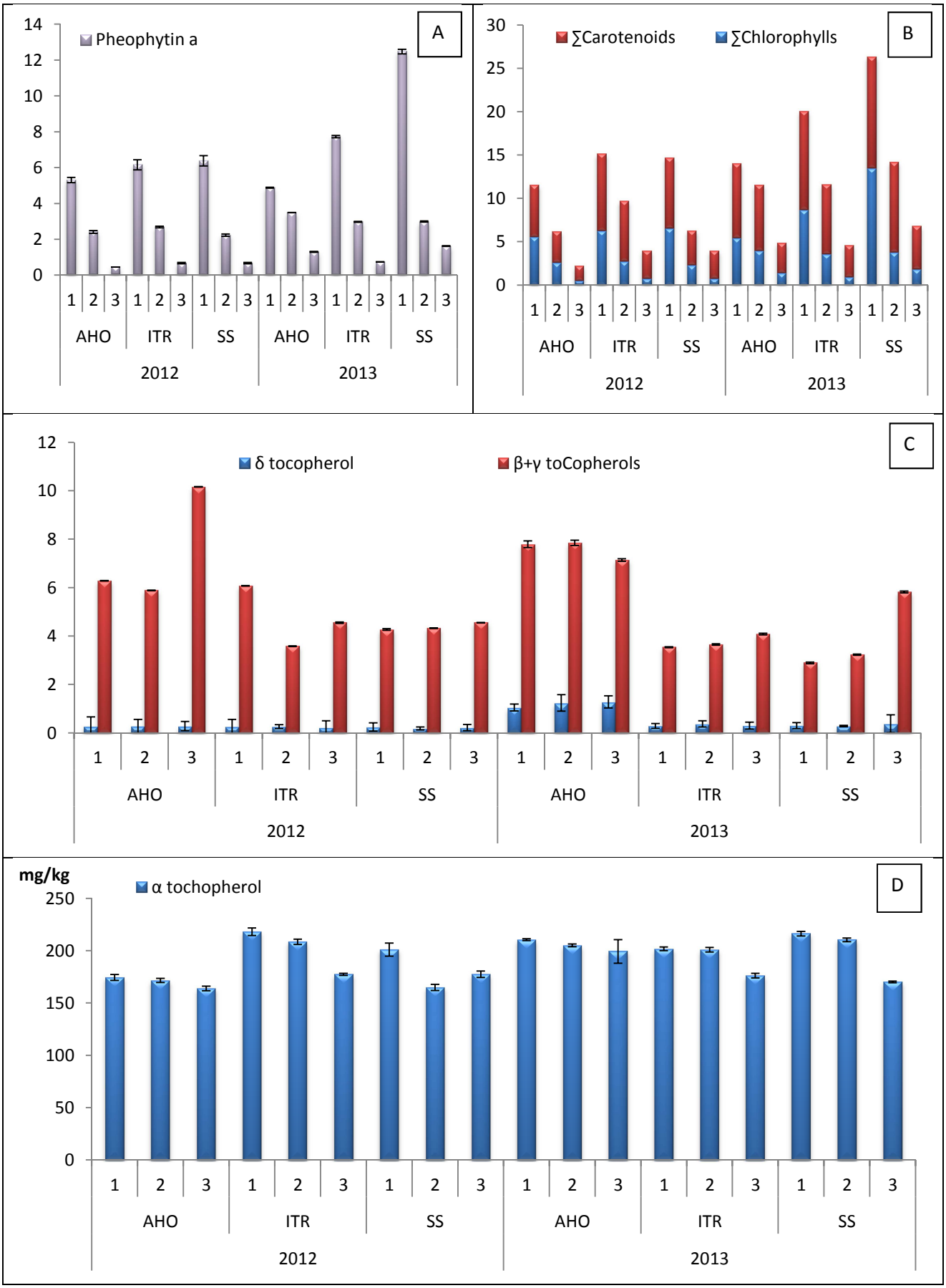

Fig.6.5 Pigments and tocopherols content in the oil samples analysed coming from three production areas Alghero (AHO), Ittiri (ITR) and Sassari (SS) and at different ripening stages

Phenolic content

The phenolic fraction of the oils analysed is reported in Table 6.9; the results were processed with ANOVA. This class of compounds has been widely studied due to phenols antioxidant properties and sensory influences on virgin olive oils, as well as their positive effects on human health. Several works studied the phenolic fraction during ripeness and an inverse relation between phenol content and the progress of maturation has been established, in particular for secoiridoids (Amiot et al., 1996; Servili et al., 1999; Rotondi et al., 2004a; Gòmez-Rico et al., 2008; Jiménez et al., 2013; Bengana et al., 2013). The main phenolic compound found in our study on monovarietal oils obtained from cv. Bosana olives was deacetoxy oleuropein aglycon (DAOA), in agreement with reports for the same cultivar (Cerretani et al. 2006). The variability for DAOA depended mainly on fruit ripeness (73.45%) while the production area is responsible for 16.23% of variability (Table 6.10), although in the last case the null hypothesis cannot be rejected (p-value = 0.064) (Table 6.9). The DAOA presented a decreasing trend during ripening and only at the first harvesting date it showed a statistically higher content respect to the other dates. The same trend was observed for the total of secoiridoids compounds since the DAOA is the most represented secoiridoid (Fig. 6.6). The content of the simple phenols hydroxytyrosol and tyrosol was on average 5.47 and 3.69 mg/kg respectively (Fig c, B), in agreement with values reported in bibliography (Jiménez et al., 2013; Bengana et al., 2013). A decreasing trend for phenolic alcohols during the progress of maturation has been reported by Morelló et al. (2004) for drupes, but no such trend was confirmed in our study (Fig. 6.7). However, as it possible to see in figure 6.7, B, a very high hydroxytyrosol content (39.40 mg/kg) was found at the second harvesting date in Alghero in 2012. This result is quite difficult to explain since it is possible to exclude oleuropein hydrolysis because the DAOA content is in average with the content of the other samples, but it could be related to problems during the extraction process or oil storage. The area of production seemed to affect only the vanillic acid content (Tables 6.9 and 6.10), being the cause of 85,96% of the content fluctuations. The content of vanillic acid was also higher in 2012 than 2013 (Fig. 6.8), in detail oils from Alghero were characterized by the highest content in both years while oils from Ittiri by the lowest. Several authors Gomez-Rico et al. 2006; Marsilio et al. 2006 ; Romero et al. 2002 reported an increase in vanillic acid and vanillin in virgin olive oils in irrigated olive trees, this is the cause of the difference in this acid content in the two years of study.

As far as flavones concentration are concerned, the flavones concentration was not influenced significantly by the two factors under study, even if both luteolin and apigenin contents increased during the maturation process (Fig 6.9) in accordance with other studies (Jiménez et al., 2013)

Table 6.9 Phenolic content of the oils at three stages of ripeness (I, II and III) and for the three production areas of Alghero (AHO), Ittiri (ITR) and Sassari (SS). The data are expressed as means ± standard deviation.

		OhTy	TY	Vanillic acid	Vanillin	DAOA	(+)-pinoresinol	Luteolin	Apigenin	∑SIDs
Ripeness	I	5.09a± 2.25	3.08a± 0.78	0.55a± 0.44	0.3a± 0.11	393.23a± 104.03	9.94a± 4.12	2.94a± 1.2	2.17a±1.97	512.35a± 121.87
	II	9.32a± 14.83	4.51a± 4.19	0.76a± 0.35	0.22a± 0.07	227.8b± 40.09	9.39a± 3.07	4.43a± 1.99	3.32a±3.02	347.53b± 61.78
	III	3.35a± 0.98	3.6a± 1.89	0.71a± 0.38	0.2a± 0.05	200.29b± 62.63	9.82a± 5.54	6.55a± 2.73	3.76a±3.09	317.53b± 71.89
P-value		0.495	0.648	0.558	0.125	**0.001**	0.979	0.079	0.703	**0.006**
Production area	AHO	9.56α± 14.64	5.39α± 3.75	1.03α± 0.24	0.28α± 0.12	226.61α± 94.41	9.21α± 3.35	5.28α± 3.35	2.39α± 1.17	332.91α± 96.83
	ITR	4.27α± 2.43	2.7α± 0.88	0.45β±0.34	0.22α± 0.06	270.13α± 82.69	8.72α± 4.16	3.66α± 2.00	2.76± 2.77	395.74α± 87.48
	SS	3.93α± 2.04	3.09α± 1.76	0.53α,β ±0.28	0.21α± 0.06	324.57α± 144.77	11.22α± 4.96	4.97α ±1.94	4.1α± 3.63	448.76α± 158.57
P-value		0.475	0.215	**0.035**	0.307	0.064	0.662	0.493	0.657	0.102
Ripeness* area										
P-value		0.391	0.579	0.982	0.611	0.374	0.759	0.962	0.988	0.642

OhTy, Hydroxytyrosol, is expressed as mg/kg tyrosol; TY, tyrosol, is expressed as mg/kg of tyrosol; DAOA, deacetoxy oleuropein aglycon, and SIDs, sum of secoiridoids, are expressed as mg/kg of oleuropein, while the other compounds are expressed as mg/kg of relative standard. Different letters (a, b, c) within a column indicate significant difference at 5% level for the ripeness factor while greek letters (α, β, γ) within a column indicate significant difference at 5% level for the production area factor.

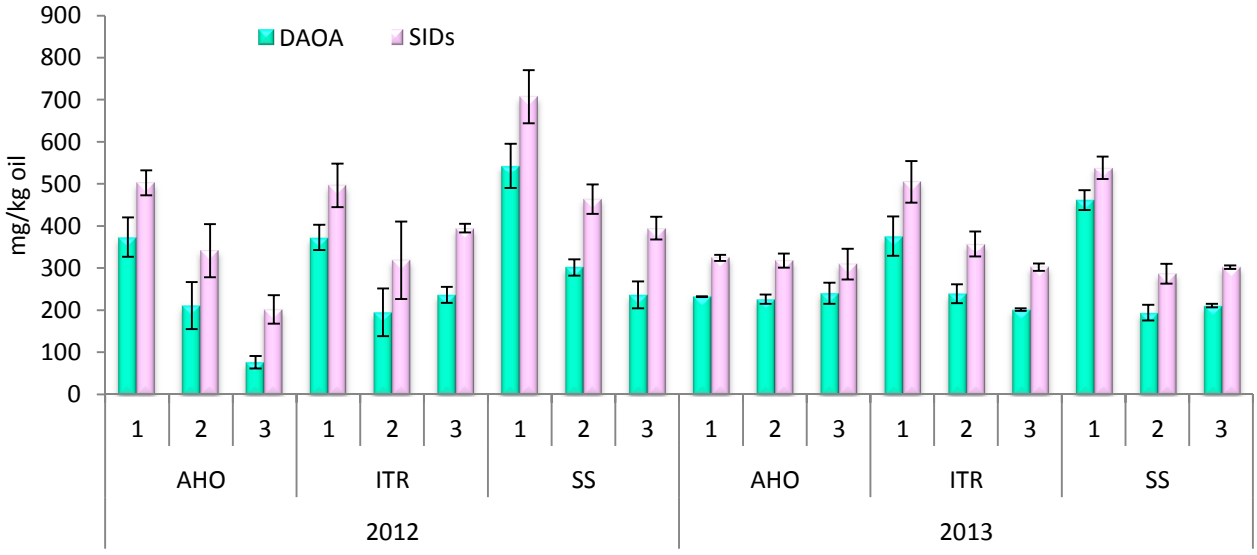

Fig.6.6 Deacetoxy oleuropein aglycon (DAOA) content and sum of secoiridoids (SIDs) in oil samples from olives coming from the three areas Alghero (AHO), Ittiri (ITR) and Sassari (SS), and collected at different ripening stages (1, 2 and 3).

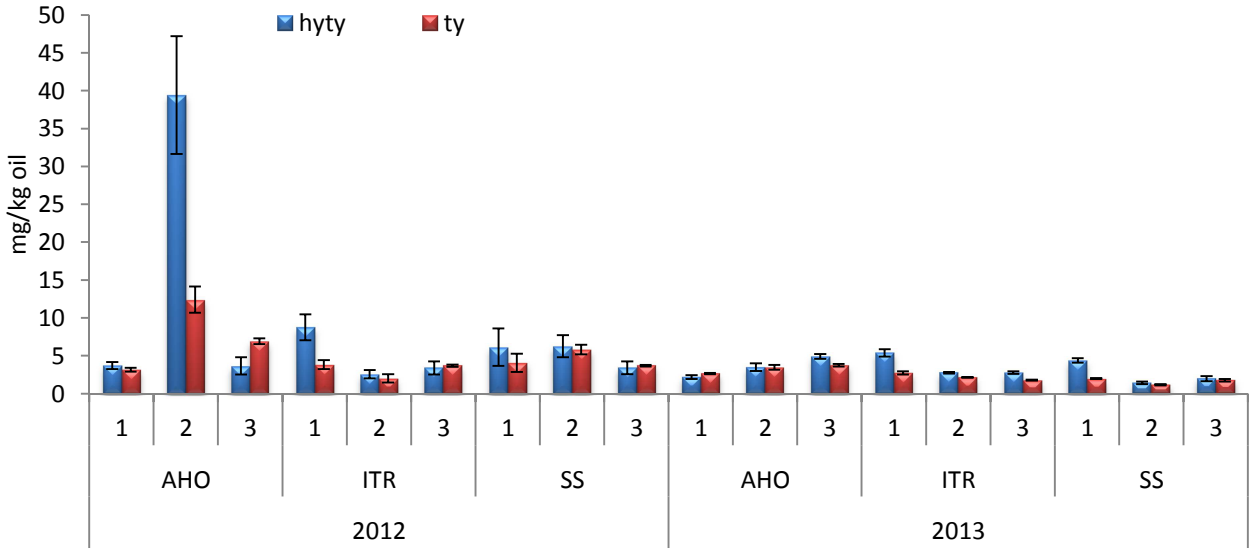

Fig.6.7 Hydroxytyrosol (hyty) and tyrosol (ty) content in oil samples from olives coming from the three areas Alghero (AHO), Ittiri (ITR) and Sassari (SS), and collected at different ripening stages (1, 2 and 3).

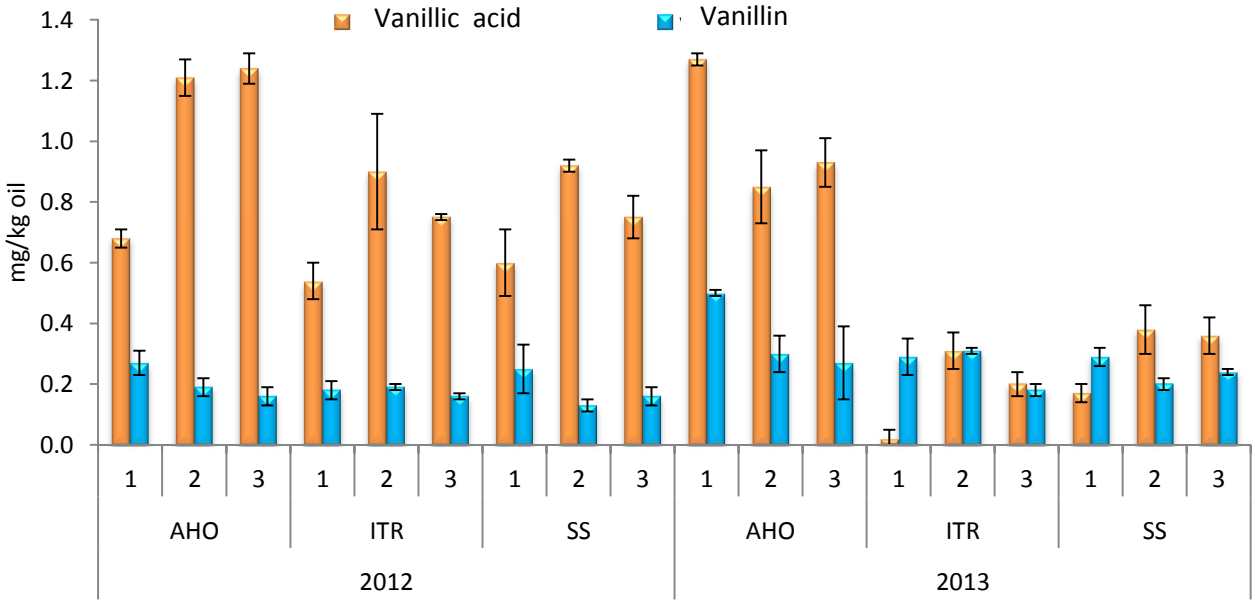

Fig.6.8 Vanillic acid and Vanillin content in oil samples from olives coming from the three areas Alghero (AHO), Ittiri (ITR) and Sassari (SS), and collected at different ripening stages (1, 2 and 3).

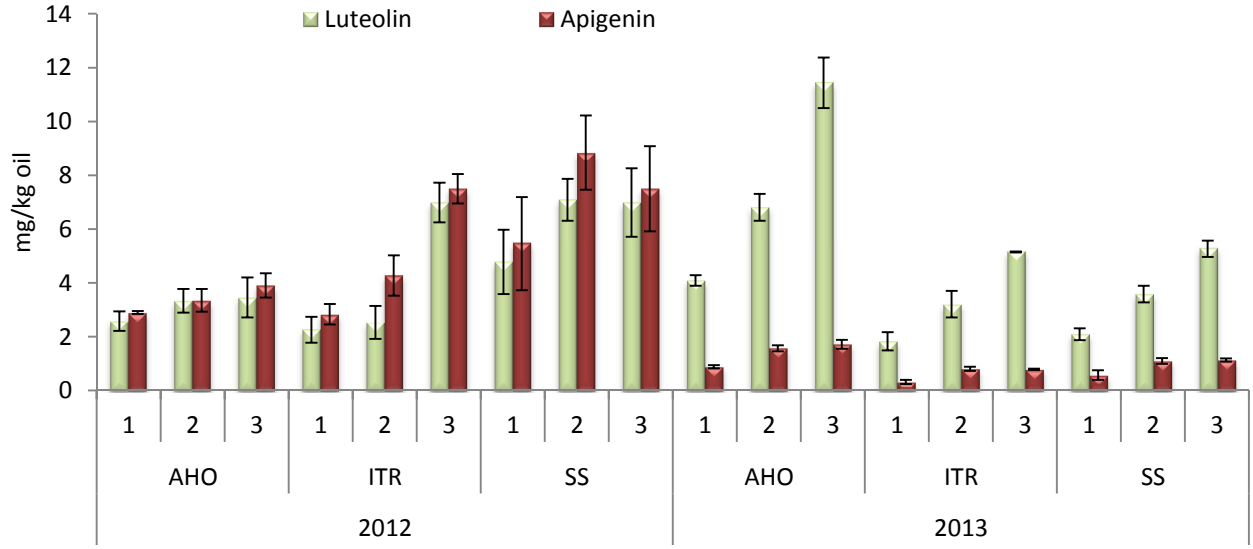

Fig.6.9 Luteolin and apigenin content in oil samples from olives coming from the three areas Alghero (AHO), Ittiri (ITR) and Sassari (SS), and collected at different ripening stages (1, 2 and 3).

Table 6.10 Variability expressed as percentage of the total sum of the squares phenols of virgin olive oils from Bosana cv

	Harvest date	Growing area	Growing area * harvest date
OhTY	19.61ns	20.82ns	59.57ns
TY	11.99ns	48.17ns	39.84ns
Vanillic acid	10.77ns	85.97*	3.26ns
Vanillin	48.95ns	25.05ns	26ns
DAOA	73.42***	16.24ns	10.34ns
Pinoresinol	1.51ns	31.17ns	67.32ns
Luteolin	76.4ns	17.21ns	6.39ns
Apigenin	38.28ns	45.81ns	15.91ns
SIDs	69.48**	21.24ns	9.28ns

Significance level at **, P=0.001 and ***P< 0.001. POV, peroxide value; TP, total phenol

Sensory analysis

The results of the sensory analysis of cv Bosana oils are shown in Table 6.11 and in figure 6.10. The sensory profile of monovarietal Bosana oil is described as medium olive fruity, grassy with prevalent scents of thistle and artichoke and hints of almond and tomato, with a medium intensity of bitter and pungent notes (Rotondi et al., 2013). The results here presented match the sensory

description given above. A number of authors reported that olive ripeness has a strong impact on the sensory characteristics of virgin olive oils (Rotondi et al., 2004a; Bouaziz et al., 2005; Jiménez et al., 2013). However, in our study none of the sensory descriptors was affected by the ripening degree (table x). This finding suggests that harvesting the crops in November or in January doesn't have any effect on the oil sensory profile. Conversely, the area of production influenced significantly both the artichoke and the pungent scents.

Table 6.11. Sensory intensities of the oils. The oils differed for stages of ripeness (I, II and III) and production areas (AHO, Alghero, ITR, Ittiri, SS, Sassari).

		Olive fruity	Grass	Fresh almond	Artichoke	Bitter	Pungent
Ripeness	I	4.98 α	2.60 α	2.35 α	2.62 α	5.16 α	5.08 α
	II	4.95 α	2.55 α	2.38 α	2.45 α	4.62 α	4.85 α
	III	4.40 α	2.09 α	2.20 α	2.09 α	4.17 α	4.36 α
P value		*0.7054*	*0.4807*	*0.8897*	*0.236*	*0.0657*	*0.181*
Production area	AHO	4.36 α	2.25 α	2.16 α	1.77α	3.98α	4.07α
	ITR	4.88 α	2.37 α	2.37 α	2.74β	4.83 α	5.06α,β
	SS	5.10 α	2.61 α	2.39 α	2.66α,β	5.14 α	5.15 α,β
P value		*0.5299*	*0.2831*	*0.8928*	***0.032***	*0.085*	***0.0215***
Ripeness* production area ***P-value***		*0.5841*	*0.815*	*0.8324*	*0.2676*	*0.357*	*0.8496*

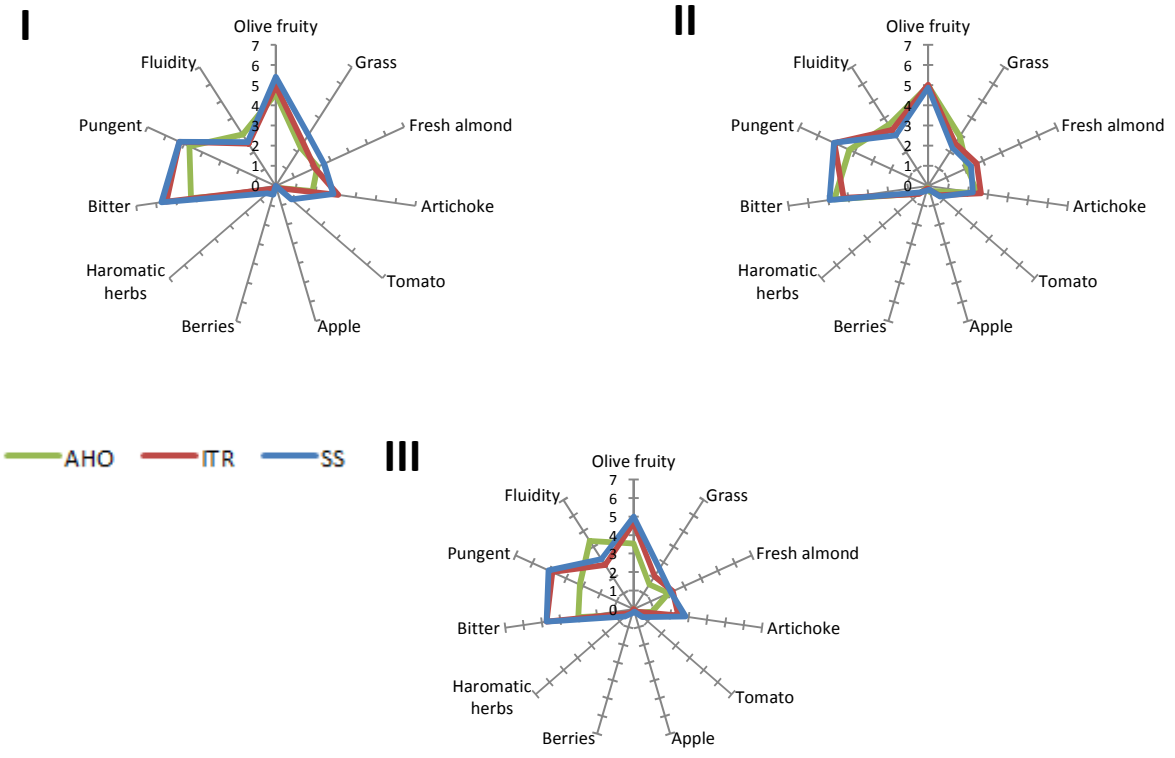

Fig. 6.10 Sensory profiles at different ripening stages (I, II, III).

The sensory analysis was repeated after six months of storage in order to detect if took place different evolution pattern. This hypothesis was not confirmed by data since slightly decrease (Table 6.12) in scents took place but in a homogenous way.

Table 6.12 Sensory intensities of the oils recorded six months after production. The oils differed for stages of ripeness (I, II and III) and production areas (AHO, Alghero, ITR, Ittiri, SS, Sassari).

		Olive fruity	Grass	Fresh almond	Artichoke	Bitter	Pungent
Ripeness	I	4.57	2.33	1.99	2.27	5.11	5.08
	II	4.59	2.55	2.65	2.54	4.88	4.72
	III	3.95	2.18	2.15	1.78	4.48	4.02
P value		*0.7884*	*0.9385*	*0.6427*	*0.3931*	*0.6088*	*0.1841*
Production area	AHO	4.10	2.13	2.35	1.94	3.99	4.09
	ITR	4.43	2.34	2.25	2.28	5.18	4.69
	SS	4.57	2.59	2.19	2.36	5.30	5.04
P value		*0.585*	*0.3022*	*0.848*	*0.422*	***0.0163***	*0.0646*
Ripeness*production area ***P-value***		*0.9329*	*0.8738*	*0.9406*	*0.394*	*0.457*	*0.9907*

Conclusion

In this study a chemical and sensory characterization of Bosana virgin olive oils was carried out in a wide time window (from November to January). Ripeness significantly influenced free acidity, palmitic and stearic acid content and thus the total content of saturated fatty acid, as well as heptadecenoic acid, the content of pigments neoxanthin, violaxanthin, pheophytin s and β-carotene, the ratio chlorophylls/carotenoids, the content of DAOA and finally the sum of secoiridoid compounds. The production area significantly affected the free acidity, the content of oleic and linoleic acid as well as their ratio, the MUFA, PUFA and their ratio, the sum of β and γ tocopherol and the content of vanillic acid.

It is interesting to note that, except in the case of free acidity, the interaction between the factors ripeness stage and production area was never significant. Thus the two factors under study can be considered as totally independent from each other. Finally, our data suggest that harvesting fruits of cv Bosana not at an early stage of ripeness is more suitable for the producer. In fact, even though in virgin olive oils from olives collected at early stages of ripeness the pigment content is higher, thus causing a brighter colour that positively influences the consumer's choice, while the secoiridoids content is lower, the bitterness and pungency intensities remain constant during ripeness. So from

this study it is possible to state that for Bosana virgin olive oil no loss of quality can be reported as the ripeness of the fruits progresses.

7. **Concluding remarks**

The environmental features influencing the chemical and sensory characteristics of virgin olive oil are a complex matrix of biotic and abiotic factors including morphologic characteristics of the territory, climate and soil typologies. The decomposition of this matrix in order to attribute a particular characteristic of virgin olive oil to a single factor is a challenging project; however the results obtained in this study show the influence of the territory of origin and its environmental characteristics on virgin olive oil obtained from fruits of the cv. Bosana.

The climate in Sardinia consists of a succession of dry summers, from May to September, and rainy winters, from October to April. The three areas chosen as representative of the olive cultivation of the provinces of Sassari, Alghero, Ittiri and Sassari, had different mesoclimatic characteristics. The Ittiri area is the coldest of the three, as it became clear by the analysis of temperature time series, with temperatures in the winter months lower by a few degrees than the other areas. Rainfall shows the same trend in the three areas studied, with the average number of rainy days being quite similar, even if a higher amount of rain is recorded in Ittiri, mainly during winter.

A chemical and sensory characterization of virgin olive oil from cv. Bosana was carried out in this study. The sensory characteristics of virgin olive oil from this particular cultivar are medium olive fruity, grassy with a prevalent scent of thistle and artichoke and hints of almond, and medium intensity of bitter and pungent notes. The chemical properties of virgin olive oil from cv. Bosana include an average content of phenolic compounds and a fatty acid profile with a balanced content of oleic acid and a good oleic/linoleic acid ratio. The fatty acid fraction was affected by the production area, in particular the content in fatty acid was considerably different between the oils from Alghero and the ones from Ittiri. Moreover, the fatty acid content of Bosana virgin olive oil also had a pivotal role in clustering the samples according to the soil typologies. The content of antioxidant molecules such as tocopherols and phenols was on average within the values reported in the literature; the year of production had however a marked influence on the content of those antioxidants, as ascribable to their role of secondary metabolites.

Significant differences between the three areas under study were not found for the trend of maturation; thus it was possible to conclude that the mesoclimatic differences of the three macro area were not strong enough to influence the ripening trend. Noticeable were the lateness of the Bosana cv; in fact no qualitative decay was observed in any of the oils, not even those produced in January.

In conclusion our results indicate the existence of a relationship between virgin olive oil and its territory of origin. For this reason the results of this work can be used to better characterize the production of Bosana POD Sardinia extra virgin olive oil. The study showed that it is possible to

differentiate, within the area of the POD Bosana extra virgin olive oils characterized by unique chemical and sensory attributes. This aspect could be instrumental in promoting the production area, since it is possible to differentiate the product according to its provenance. On the other hand, the oil producer could use the results of our study using the width of cv. Bosana harvesting period in order to shape the virgin olive oil characteristics and thus producing different virgin olive oils to meet different types of consumers' taste.

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