

Chapter 8

HOW AGRONOMIC FACTORS AFFECTS OLIVE OIL COMPOSITION AND QUALITY

*Ricardo Malheiro^{1,2}, Susana Casal², Paula Baptista¹
and José Alberto Pereira^{1*}*

¹Mountain Research Centre (CIMO), School of Agriculture,
Polytechnic Institute of Bragança, Campus de Santa Apolónia, Bragança, Portugal
²REQUIMTE/Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia,
Universidade do Porto, Rua de Jorge Viterbo Ferreira, Porto, Portugal

ABSTRACT

Olive oil is one of the most popular vegetable oils worldwide but several factors might affect its quality and composition, from the tree to the spoon. Olive oil quality and composition is mainly influenced by olive fruit characteristics, and therefore all aspects that influence their development have a crucial effect on olive products. Those factors include the selection of olive cultivar, its cultivation, degree of crop intensification and production systems, agricultural practices, including irrigation and fertilization, olive pests and diseases management, all these factors clearly defining the composition of olive fruits and the inherent quality and properties of olive products.

In the last decades, huge modifications in olive tree cultivation have been observed, related essentially with two great factors: development of olive cultivations in new producing areas and crop intensification in traditional producing areas. Generally, most agronomic factors, including crop density, farming system, irrigation and fertilization, have no substantial effects on fresh olive oil quality parameters and classification. Nevertheless, a considerable incidence of olive pests and diseases can easily take fresh olive oils to the lampante category. In opposition, all agronomic factors seem to influence olive oil composition. Antioxidants are the main affected components, with a crucial effect on olive oil sensorial attributes, bioactive and nutritional properties, as well as its oxidative stability.

In present chapter the influence of diverse agronomic factors on olive fruits and olive oils production, composition and quality, is reviewed and discussed, giving special

* Email: jpereira@ipb.pt.

importance to olive farming-systems, fertilization and irrigation, as well as the incidence of olive pests and diseases.

Keywords: Olive oil, agronomic factors, composition, quality, olive-farming systems, fertilization, irrigation, olive pests and diseases

INTRODUCTION

Olive oil is a premium vegetable oil that, contrary to the majority of commercial vegetable oils, can be consumed in its crude form, without refining, maintaining and preserving its composition and potentially all associated beneficial properties. Olive oil is the 9th most produced vegetable oil worldwide [1] and it is gaining importance and relevance comparatively to other vegetable oils. Indeed, in the last two decades both production and consumption figures increased considerably (Figure 1).

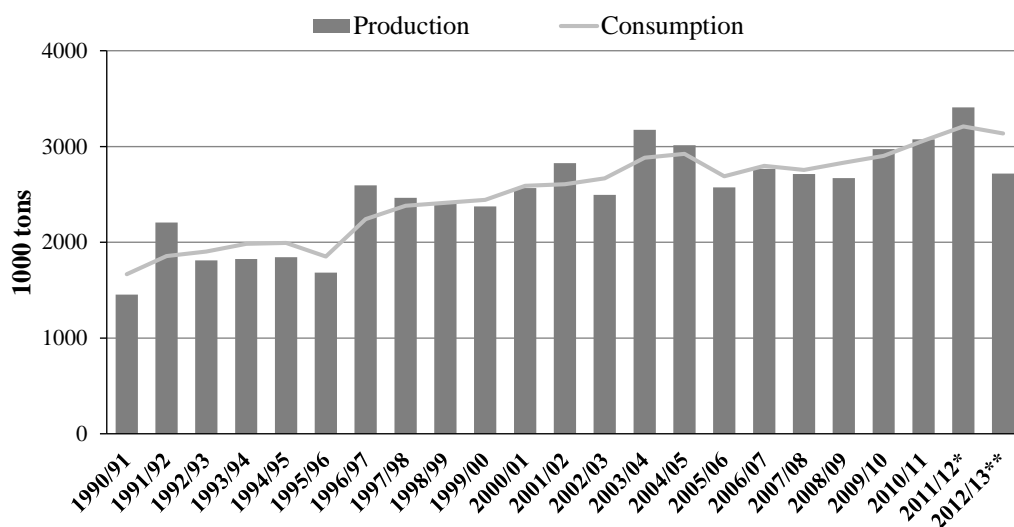


Figure 1. Worldwide olive oil production and consumption data (1000 tons) since 1990/91 (IOC 2012a; IOC 2012b). *Provisional data; **Predicted data.

Since the early 90's, olive oil production increased about 50% while its consumption increased 65% [2, 3]. Such increase is not only associated to global population growth, but mostly with the popularity that this vegetable oil gained over the last decades. Originally restricted almost to the Mediterranean region, olive orchards are now planted in regions where olives were absent or had a small representation. In particular, its cultivation is increasing in America (in the North mainly in California; Central America, mainly in Mexico; and in South America in Argentina, Chile and Brazil), South Africa and Australia. Olive oil is also being introduced in new potential markets, namely in Brazil, China and Russia, which also foment the consumption of this premium vegetable oil.

The inclusion of olive oil and related olive products in the daily diet is associated to innumerable health benefits, a direct consequence of its characteristic chemical composition.

Among those components, monounsaturated fatty acids, pigments (chlorophylls and carotenoids with β -carotene as a very active component), tocopherols (mainly α -tocopherol), sterols (mainly β -sitosterol), and diverse phenolic compounds (hydroxytyrosol; tyrosol; oleuropein and derivatives) are recognized as the most active compounds (Figure 2).

In respect to major components, fatty acids, olive oil is rich in monounsaturated fatty acids (MUFA), mainly oleic acid ($C_{18:1}$), and has reduced amounts of both saturated (SFA) and polyunsaturated fatty acids (PUFA) but provides adequate amounts of essential fatty acids (linoleic and linolenic acids) [4]. This entails a high antiatherosclerotic potential, and a lower risk of cardiovascular diseases [5].

Concerning pigments, β -carotene is one of the most abundant carotenoids in olive oils [6] and, together with lutein, has antioxidant properties [7], inhibiting olive oil photooxidation by acting as singlet oxygen quenchers [8].

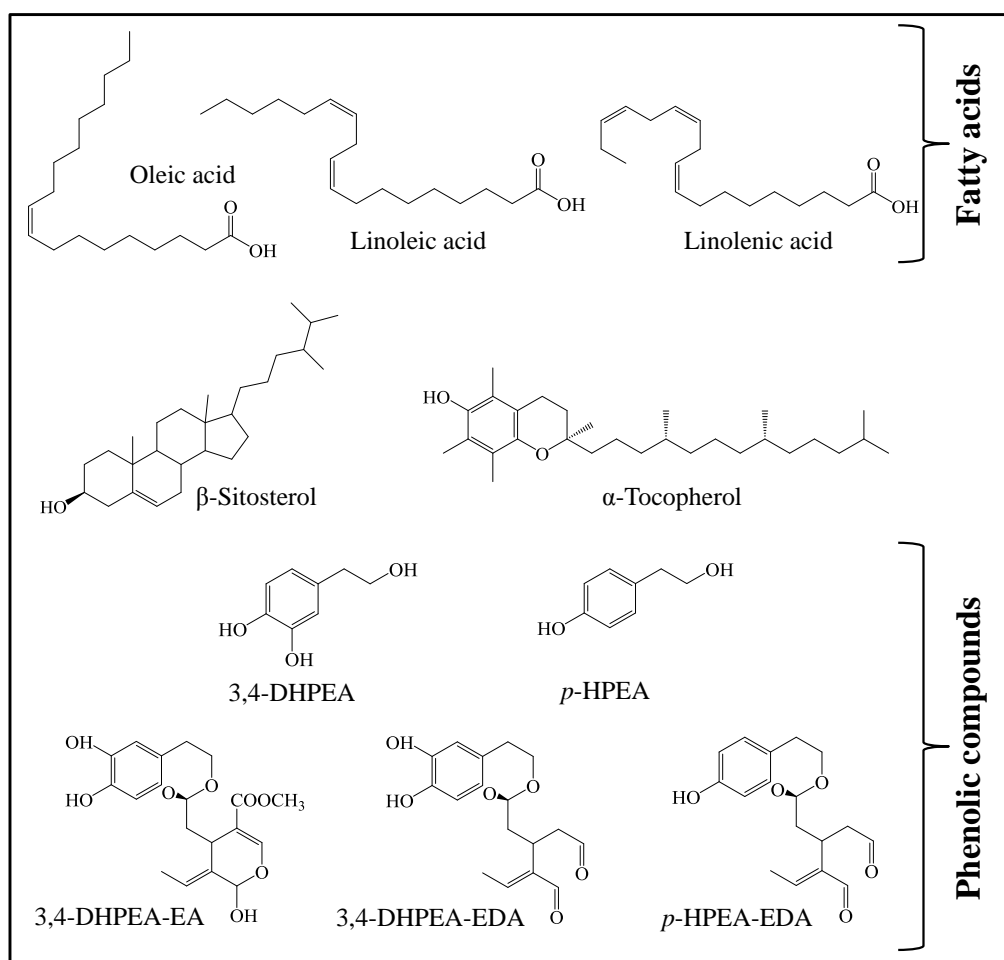


Figure 2. Olive oil components with nutritional and bioactive potential. (3,4-DHPEA – 3,4-dihydroxyphenylethanol (hydroxytyrosol); *p*-HPEA – *p*-hydroxyphenylethanol (tyrosol); 3,4-DHPEA-EA – oleuropein aglycon; 3,4-DHPEA-EDA – Dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA – Dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol).

Tocopherols, especially α -tocopherol, display a dual activity since they are antioxidant compounds and exert a vitaminic action (vitamin E) contributing considerably to olive oils stability [9, 10] and consumer's health. Additionally, ingestion of α -tocopherol appears to have a preventive effect on the some of the most important diseases of the modern society, as cancer and Alzheimer [11, 12].

Olive oil phenolic compounds, also with recognized antioxidant capacity, are associated to innumerable healthy properties and benefits to consumers' health. There are several reports and literature revisions that highlight the *in vitro* [13-15] and *in vivo* [16-18] activities of these compounds and their pharmacological properties [19]. Phenolic compounds also have a marked influence on the olive oil sensorial attributes, particularly the spicy, astringent and pungent sensations, as well as on the olive oil global quality and stability.

Olive oil composition, however, is the results of a diversified array of agronomic and technological factors. On this chapter, the impact of the application of fertilizers in olive orchards, the new planting systems linked to irrigation methods, together with the incidence of olive pests and diseases on the production, on the olive oil composition and quality will be reviewed.

PLANTING SYSTEMS AND IRRIGATION

Olive trees are cultivated in three main olive-farming systems: i) traditional/low density olive groves (Figure 3A); ii) intensive olive groves (Figure 3B); iii) and high density or super intensive olive groves, also known as hedgerow olive orchards (Figure 3C) [20].

Traditional olive groves have usually 100 to 200 trees ha^{-1} (Figure 3A), are rain fed and, in some cases, harvest is still manual. These groves are usually composed by large and old trees, with several decades and, in some cases, even centenary olive trees, making mechanical harvest difficult. In some regions this kind of olive-farming system is being gradually abandoned or replaced by the two other more modern olive-farming systems. Intensive plantations are mainly irrigated, have between 200 and 550 olive trees ha^{-1} , and harvest is usually made mechanically (Figure 3B). In hedgerow olive orchards olives are exclusively collected by mechanical means to turn harvest as profitable as possible, being the olive trees irrigated following deficit irrigation programmes. These kind of olive orchards contain more than 1500 olive tree ha^{-1} (Figure 3C) [21]. Additionally, olive groves can be classified according to their production system: conventional olive groves and organic olive groves.



Figure 3. Different olive-farming systems. A – Traditional/low density olive grove; B – intensive olive grove; C – super intensive or hedgerow olive grove.

Planting System and Olive Oils Quality and Composition

Studies comparing the influence of different olive-farming systems on the olive oil composition and quality are scarce. These studies are difficult to implement since many variables interfere in the outline, starting from olive varieties, irrigation regimes, soil composition and the standardization of olive trees density. Therefore, most studies on olive-farming systems focus mainly in trials to test the adaptation of different olive cultivars to high density olive orchards, irrigation and fertilization optimization, as well as to observe production yields and the quality and composition of the obtained olive oil from specific olive cultivars.

By studying low density plantations (from 51 to 156 trees ha⁻¹) in traditional olive groves of cv. Chemlali, Guerfel et al., [22] verified that planting density is a key aspect to be considered for the quality and composition of olive oils in arid areas. These authors verified that tree densities from 100 to 156 trees ha⁻¹ result in olive oils with higher oxidative stability, as observed in Figure 4. Authors verified higher content of chlorophylls and carotenoids, oleic acid and total phenols in densities of 100 trees per ha, contributing to the observed effects, but the specific coefficients of extinction at 232 and 270 nm (K_{232} and K_{270} respectively) increased, eventually threatening extra-virgin olive oil classification.

Regarding intensive olive groves, adaptation of cv. Arbequina in different planting densities (from 179 to 385 trees ha⁻¹) was studied by Tous et al., [23]. These authors verified that, at full production, an average of more than 5000 kg of olives per ha were obtained, with the highest economic return with 312 trees ha⁻¹. The same group also studied adaptation of different olive cultivars (Arbequina-i 18, Arbosana, Canetera, Joanenca, Koroneiki, and Fs-17) to super intensive olive orchards with a plantation density near 2500 trees ha⁻¹ [24]. The results clearly demonstrated that Arbequina-i 18 is the most adapted to this olive-farming system, producing nearly 24000 kg of olives per ha, followed by Arbosana and Canetera [24]. According to these authors, an increased plant density is mainly affected by light competition: being cultivated closely light competition is high, reducing crop yield [23].

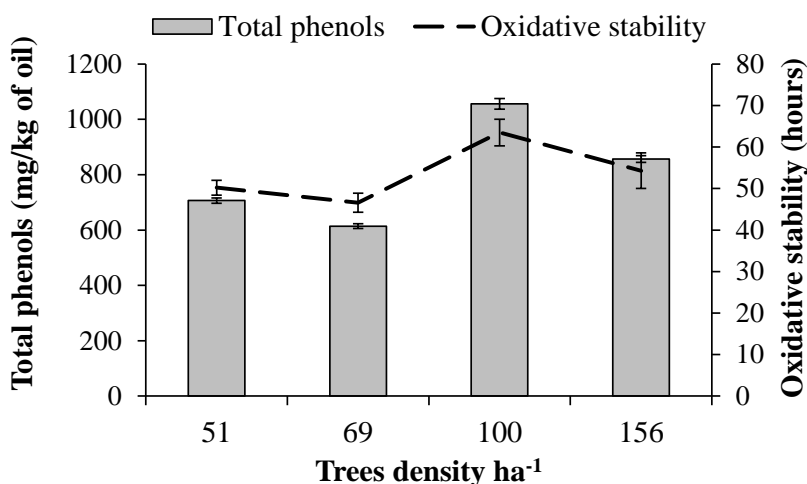


Figure 4. Oxidative stability (hours) and total phenols content of olive oils from cv. Chemlali cultivated in traditional olive groves under different olive tree densities (data updated from Guerfel et al., 2010).

In high density olive orchards (1250 trees ha⁻¹) Allalout et al., [25] studied olive oil quality and composition of several olive cultivars (Arbequina, Arbequina-i 18, Arbosana, and Koroneiki). The authors verified that Arbequina was the cultivar with lower oil content, while in the extreme opposite was Koroneiki. Nevertheless, all cultivars reported good quality indices, being classified as extra-virgin olive oils.

Plant density seems to have a crucial effect on olive oil yield but it appears to have no influence in the olive oils quality. Meanwhile, some aspects need to be taken in consideration because if plant densities are extremely high it can affect plants physiological development. The competition for soil mineral nutrients as well as for light exposure can influence negatively the production of olive fruits, affecting yield, and possibly having repercussions at quality and composition of the obtained olive products. Furthermore high density olive-farming plantations are irrigated, which also influence productivity, quality and composition of olive oil, as we discuss ahead in this chapter.

Organic vs. Conventional Olive Oil: Quality and Composition

Organic agriculture is increasing in all crops worldwide, mainly due to consumers' awareness about pesticides residues in food products. Indeed, conventional agriculture, due to the application of phytosanitary products, leads to pesticide residues in olives [26], passing them to olive products [27, 28]. In 2010, nearly half a million ha were already dedicated to organic olives production [29]. A consumers' satisfaction study in Greece demonstrated that 78% of consumers were satisfied with organic olive oil, describing its beneficial health aspects as the most competitive advantage [30].

Comparing productivities of conventional and organic olive groves, higher productions are usually observed in conventional groves. Ninfali et al., [31] verified that both Leccino and Frantoio olive varieties produce more in conventional groves (5514 and 4721 kg/ha, respectively) than in organic ones (4125 and 3494 kg/ha). The same authors also verified higher pest incidence in organic olive groves (10%) than in conventional ones (4%).

In which respect to olive oil quality from organic and conventional olive groves, data reported in literature is sometimes contradictory. Gutiérrez et al., [32] reported that organic olive oils possess higher quality when compared to conventional ones, while Ninfali et al., [31] were unable to found the same trend during a 3-year consecutive study. In the study performed by Gutiérrez et al., [32] the olive oil from cv. Picual displayed better results in all quality indices evaluated (free acidity, peroxide value and K₂₃₂). The differences found between both cultural practices were more pronounced with olives maturation, with higher degradation in conventional olive oils. Sensorial evaluation was also better scored in organic olive oil than in conventional ones, in particular the positive attributes green fruit, mature fruit, bitter and spicy [32]. In which respect to Frantoio and Leccino olive cultivars [31], the same quality parameters were not consistent since in some years conventional olive oils display significant higher quality, and in other years the same trend is not observed. In the sensorial evaluation, no marked differences were observed between both agricultural practices in cv. Frantoio. However, conventional olive oils from cv. Leccino were fruitier, pungent, bitter, and exhaled higher cut grass sensations than olive oils extracted from organic olive fruits [31].

Regarding olive oils composition, and according to Gutiérrez et al., [32], organic olive oils are richer in antioxidant compounds, such as α -tocopherol, *o*-diphenols and phenolic compounds, while Ninfali et al., [31] did not found significant differences in the same components when the three years of study were compared. The same authors' found no differences in the antioxidant activity displayed by organic and conventional olive oils while organic cv. Picual olive oils were reported as having significantly higher oxidative resistance than conventional olive oils, partially attributed by the high content of antioxidant compounds, by Gutiérrez et al., [32]. Sterolic fractions of both organic and conventional olive oils were similar, while the fatty acids profiles differ mainly in oleic and linoleic acids contents. Oleic acid content was higher in organic olive oils while linoleic acid content was superior in conventional olive oils [32, 33].

Irrigation and Olive Oils Quality and Composition

Irrigation is already a common practice in intensive, super intensive or hedgerow olive orchards and, in minor proportion, in traditional olive groves. It is clear that irrigation practices increment considerably olive fruits as well as olive oil production yields [34-41]. But do irrigation practices influence olive oils quality and composition? One approach to context this question could be given in Figure 5, which schematizes the general effect of olive trees irrigation on quality, composition, stability and bioactivity of olive oils.

As illustrated in Figure 5, olive trees irrigation considerably affects olive oils composition, sensorial component and, in minor extension, olive oils quality. Starting from quality, several studies highlight slight increases in free acidity and higher peroxide formation in olive oils extracted from irrigated olives, but without statistical meaning [37, 41-43]. Regarding specific extinction coefficients at 232 and 270 nm (K_{232} and K_{270} , respectively) some studies report a slight decrease in these quality parameters while others report no significant differences with different irrigation regimes. However, olive oil classification is not endangered by irrigation practices since all olive oils continue within the legal limits of extra-virgin olive oils [44]. Regarding sensorial characteristics, olive oils extracted from irrigated olives are less fruity, pungent, and bitter, and the higher the quantity of water applied to olive trees, the lower the score of those positive attributes [41-45]. By contrast, the same studies report that olive oil becomes sweeter due to the lower grade of bitterness and pungency of the oils obtained from irrigated trees. The bitterness index (K_{225}) is in accordance to these observations, being negatively correlated with the amount of water applied to olive trees, a fact also linked to the phenolic composition of olive oils. Almost all studies which conducted trials with different irrigation regimes determined the phenolic content of the extracted olive oils. The results are clear and concise: irrigation reduces drastically the phenolic compounds in olive oils [35, 39, 41-45]. These results were also corroborated by Romero et al., [46] and Servili et al., [47] that verified richer qualitative and quantitative profiles of phenolic compounds in olives from different regulated deficit irrigation programs. Besides phenolic compounds, chlorophylls, carotenoids, tocopherols [43], sterolic fraction [41], and volatile composition [41, 47] are reduced in olive oils extracted from irrigated olives. Changes in the fatty acids profile were also observed, generally with higher contents of oleic acid being reported in olive oils from irrigated trees and higher linoleic content in olive oils from non-irrigated olive orchards [43, 47].

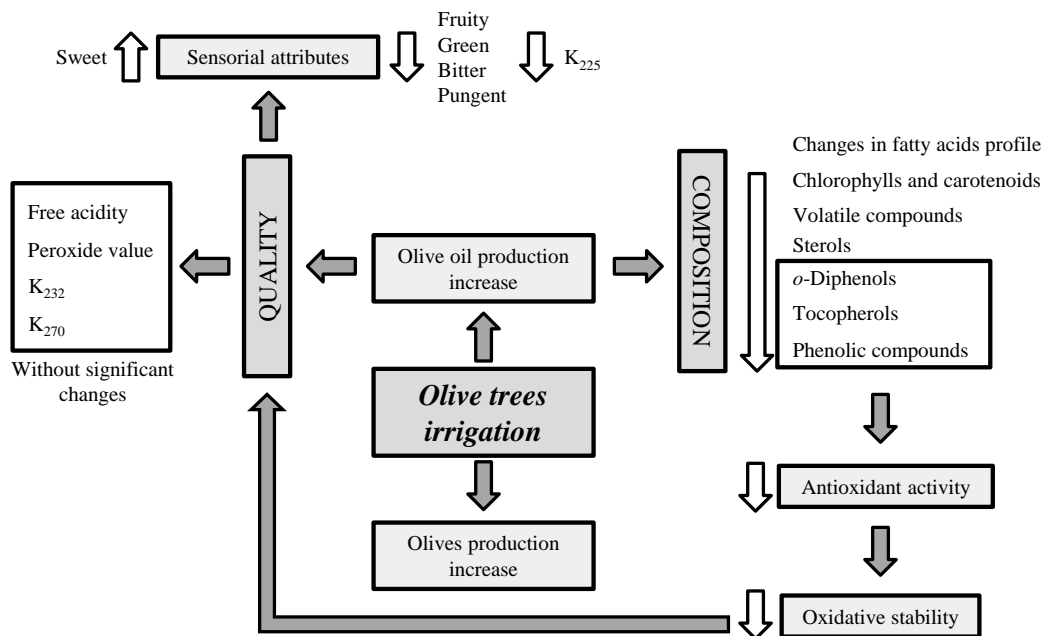


Figure 5. Global impact of irrigation practices on olives and olive oil productivity, quality and composition.

The changes inflicted by irrigation practices in the composition of olive oils interfere with their bioactivity, reducing the antioxidant potential, since olive oils from irrigated olives lose important antioxidant compounds, including phenolics, *o*-diphenols and tocopherols. This loss of antioxidant potential by olive oils is critical, affecting their stability and resistance to oxidation.

Indeed, oxidative stability loss is directly correlated with the amount of water applied in the irrigation regimes, with lower oxidation resistance as water increases. Berenguer et al., [42] reported oxidative stability losses between 10% and 71% in Arbequina olive oils with an irrigation treatment of 140% and 104% of ET_c (olive tree evapotranspiration), respectively. Other authors reported, for the same olive cultivar, losses between 17% [43] and 27% [46] in studies of linear irrigation strategies and deficit irrigation strategies, respectively. Gómez-Rico et al., [44] also reported oxidative stability losses between 4% and 36% in Cornicabra olive oils.

Overall, olive oils extracted from irrigated olive fruits display good quality after extraction, allowing them to be classified as extra-virgin olive oils. However, since olive oil composition is severely affected, particularly being depleted of antioxidant compounds, their storage stability could be reduced. This is a very important aspect since, once stored and bottled, olive oils from irrigated olives may pass through preservation deficiencies and may arrive to consumers already degraded and rancid due to autoxidation [48]. Furthermore, these aspects may raise authentication concerns, since labelled extra-virgin olive oils may arrive to consumers with sensorial defects, being the product inconsistent with the denomination labelled.

FERTILIZATION

One of the goals of olive trees fertilization is improving and increasing olives production and yields. In each campaign, substantial amounts of mineral nutrients are lost in olive groves due to soil lixiviation and erosion, irrigation, tillage practices, fruits removal, branches removal by pruning practices, and natural drop of olive leaves. Therefore, it is essential to provide to the crop the necessary mineral nutrients in order to assure adequate growth and yields in the following years. The most modern olive-farming systems with intensive production foment an increased consumption of mineral nutrients, needed to ensure a proper production. According to Rodrigues et al., [49], olive tree responds markedly to the application of nitrogen (N), mainly in low fertility soils. Also, the application of potassium (K) leads to an increase in olive trees growth and olives yield in fertilized olive groves. Indeed, 60% of K is located in olive fruits [50], which are annually removed with the harvest, being its reposition essential. Phosphorous (P) is another mineral nutrient applied to olive trees, being essential to root growth and plant tissues. Boron (B) and magnesium (Mg) are other two mineral nutrients applied to olive trees [51, 52].

Nevertheless the application of fertilizers is not free of consequences to olive production and the quality and composition of the obtained olive oils. The most related cases are nitrogen over-fertilization [53], as excessive doses reduce flowering, flower quality and ovule longevity [54] as well as fruit set, reducing fruit load [50] and consequently also olive oil production. Excess of nitrogen has also influence in olive oils quality and composition [55, 56] as described in the next section of the present chapter. Therefore, a proper diagnostic of olive tree mineral status is essential, including foliar diagnosis [57], as well as an optimization in the application of fertilizers according to the olive tree needs [58].

Fertilization and Olive Oil Quality and Composition

Most studies regarding fertilization effects in olive oils quality seem to be in agreement as to no major interferences are observed. A good example is that the application of different doses of N-P-K (4N-1P-3K complex fertilizer) in olive orchards submitted to irrigation did not cause quality changes in olive oils from cv. Manzanilla de Sevilla [59]. Quality parameters (free acidity, peroxide value, K_{232} and K_{270}) remained low with the increasing dose of fertilizers. However, these observations do not extendable to the olive oil composition, with differences observed at the molecular level. The main components affected are the phenolic compounds [55, 56, 59, 60]. According to the different treatments applied to olive trees in different years, cv. Manzanilla olive oils lost between 16 and 32% of phenolic compounds comparatively to control treatments without application of fertilizers [59, 60]. Regarding conditions of N over-fertilization, olive oils from cv. Picual lost between 16 and 51% of phenolic compounds [55, 56]. With a considerable decrease in phenolic compounds, several aspects of olive oil are expectedly affected: being strong antioxidant compounds, olive oil becomes less protected from oxidative agents. This hypothesis was confirmed in the above mentioned studies, since the oxidative stability in both olive oils from cvs. Manzanilla and Picual was considerably reduced [55, 56, 59, 60]. Another aspect directly related with the reduction of phenolic compounds is the olive oil natural bitterness (measured by K_{225}). K_{225}

parameter reduces with fertilization, therefore the sensorial score is compromised, with positive attributes like bitterness, spicy and pungent sensations being considerably reduced in olive oils due to phenolics loss.

Tocopherols (mainly α -tocopherol) and pigments (chlorophylls and carotenoids) appear to have an opposite tendency. Nitrogen application significantly increases the amounts of α -tocopherol in olive oils, while for β - and γ -tocopherols no significant changes are observed [56]. Fernández-Escobar et al., [56] also verified an increment in chlorophylls and carotenoids with increasing N doses, but without statistical meaning. Regarding fatty acids composition of olive oils, the changes observed due to N application appears to be related to olive cultivar as only merely small changes were observed in cv. Picual [56] but some important modifications were reported in cv. Manzanilla olive oils with different fertilization treatments [59]. The PUFA fraction increased significantly with the application of higher doses of N-P-K, mainly due to the increase in linoleic acid, while MUFA decreased significantly due to the reduction in oleic acid representation. These observations were accompanied during two consecutive years, which corroborate the influence of fertilization in the fatty acids profile of olive oils.

In a long term, just as discussed in the section “Irrigation and olive oils quality and composition” of the present chapter, the changes inflicted by fertilization in olive oils composition may be critical for its preservation. Oxidative stability is reduced due to antioxidant compounds loss, and the unsaturation degree increases, which turn them more prone to oxidation.

OLIVE PESTS AND DISEASES

Olive pests and diseases are responsible for serious crop losses and quality degradation of olive products, including olive oil. According to Bueno and Jones [61] 15% of olives production is lost each year due to pest incidence. Nowadays, due to the increase of olives cultivation and their expansion in new areas of the globe, olive pests spread across olive producing areas, raising damages. On this chapter we will focus on two of the most important olive pests: the olive fruit fly (*Bactrocera oleae* (Rossi); Diptera: Tephritidae), and the olive moth (*Prays oleae* Bern.; Lepidoptera, Plutellidae), and in two of the main diseases that affect olive tree: the olive anthracnose (*Colletotrichum* sp.) and verticillium wilt (*Verticillium dahliae* Kleb.) (Figure 6).

Olives Production Losses Caused by Olive Pests and Diseases

Olive moth has important economic effects on olives production, affecting therefore olive oil quality and amounts. Olive moth cause high levels of fruit drop due to burrowing into fruits in the early stages of development. The larvae of antrophagous generation feed on the seed inside olive stone and then exit the fruit, increasing fruit drop, mainly in the month of September, but also during fruit maturation. In a long-term study, Ramos et al., [62] verified the consequences of different levels of incidence of olive moth in southern Spanish olive groves. These authors observed that high levels of olive moth attack (more than 40% of fruit

drop) occurs approximately every three years and cause an average loss of 131792 tons of olives. These losses entailed an economic damage of about 79.5 million € at that time. With moderate levels of attack (average of 29% of fruit drop), 50332 tons of olives were lost, with a damage of 30.4 million €. With low level of attack (average of 10% of fruit drop) 18623 tons of olives were lost with an economic prejudice of 11.2 million € [62]. These results highlight the importance and threat that this olive pest represents to olive crop.

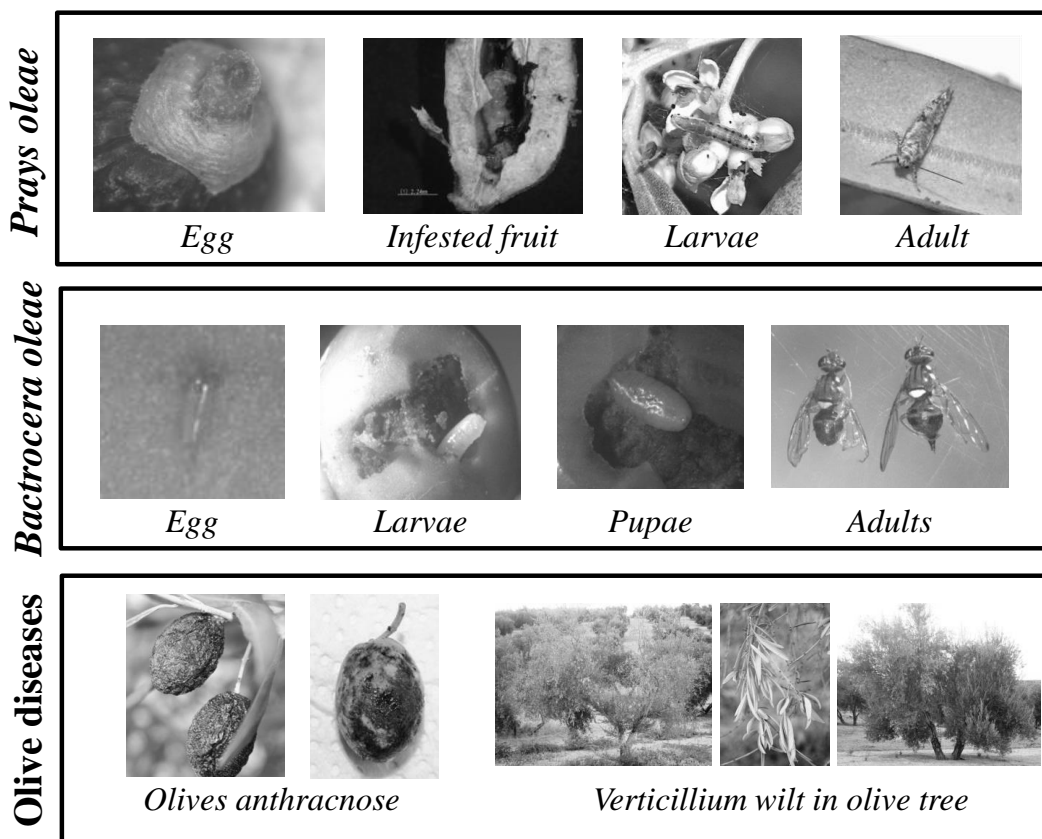


Figure 6. Main olive pests and diseases that affect *Olea europaea* L..

Even regarding olives production losses, after olive moth attack, olives are susceptible to olive fly infestation. The adult female of this dipteran lay her eggs on olive fruits, and the larvae feed on olive pulp, consuming from 3 to 20% of pulp according to the olive variety [63, 64], and creating galleries inside the fruit. When the larvae is ready to pupate, that can occur inside the fruit or in the soil, larvae opens an exit hole in fruit for larvae or adults leave. Similarly to what happens with olive moth, olive fly infestation also causes fruit drop from the tree but in lower amounts comparatively to olive moth, as witnessed by Bento et al., [65]. In no chemical sprayed olive groves, these authors report a fruit drop of about 19% of olives per tree. The most critical data was checked when authors assessed the infestation level of olives at the harvest moment, and verified that 84% of the fruits were infested.

Concerning olive diseases, anthracnose is considered the most destructive disease worldwide in olive crop. Firstly reported in Portugal [66] this disease rapidly spread to all

olive producing areas in the globe. Olive anthracnose is caused by several causal agents, including *Colletotrichum acutatum*, more prevalent and aggressive than other species, as example *C. gloeosporioides* [67, 68]. Anthracnose causes fruits dehydration, fruit rot and mummification [69] as observed in Figure 6. This pest also aids in the spread and entrance of the causal agent in olive fruits. In years when *B. oleae* populations and infestation levels are high, olive anthracnose can cause up to 100% of fruit production losses [70, 71]. According to Moral et al., [72], only in Spain, about 70 million € are lost each year due to anthracnose prevalence.

Other disease with global importance is verticillium wilt. This is a vascular disease caused by the soil-borne fungus *Verticillium dahliae* Kleb. [73]. Similarly to olive anthracnose, verticillium wilt can be found in almost all olive producing regions in the world (Table 1).

Table 1. Verticillium wilt incidence in olive groves and disease incidence in olive trees cultivated in different olive producing countries

Country	Incidence in olive orchards	Disease incidence in olive trees	References
Algeria	90%	12%	[74]
Greece	-	2-3%	[75]
Israel	-	12-50%	[76]
Italy	6.2-35.8%	-	[77]
Morocco	60%	10-30%	[78]
Spain	39.5%	-	[79]
Syria	-	0.85-4.5%	[80]
Turkey	35%	3.1%	[81]

This is a very dangerous disease since it can attack partially the trees or it can kill the entire olive tree. Another worrying aspect about this disease is the high infestation levels recorded at nurseries (about 50%), as witnessed in Italian olive nurseries by Nigro et al., [77]. Its symptoms are partial leave defoliations, inflorescences necrosis and branches dieback. This disease influence considerably olive trees development and growth, as well as fruit production affecting considerably olive oil production.

Olive Pests and Diseases on Olive Oil Quality

Studies regarding the effect of olive moth on olive oil quality are inexistent, once attacked fruits drop to the soil and this fruits are not recommended to be used for oil extraction. However, literature highlights accordingly the nefarious effect of olive fruit fly infestation in olive oil quality. Olive oils quality degradation is directly proportional to the amount of olive fruits infested by olive fly and mainly olive fruits with exit holes [82-85]. Two main chemical processes occur in infested olives, hydrolysis and oxidation. During its development, olive fly larvae consume considerable amounts of olive pulp, creating galleries in the fruit. This consumption and tissue destruction lead to enzymatic reactions between lipases and triglycerides, increasing the amounts of free fatty acids in the pulp and therefore

olive oils free acidity (FA). By other hand olive pulp become oxidized, by the entrance of exogenous elements as air, cold/heat, water and several types of microorganisms, mainly bacteria and fungi that provoke fruit rot, through the hole created in the fruits. Olive oils extracted from infested olives report higher peroxide value (PV), due to the compounds formed during primary oxidation, mainly hydroperoxides [48] when compared with olive oils extracted with healthy fruits. Others quality parameters that allow monitoring oxidation occurrence also report higher values in olive oils extracted from infested fruits: K_{232} and K_{270} (which measure respectively the presence of primary and secondary products of oxidation).

As already mentioned, exit holes created by olive fly are an infectious window, since many pathogenic agents enter by those sites. *Camarosporium dalmaticum* (Thüm.) Zachos & Tzav.-Klon, and *Botryosphaeria dothidea* are some examples of pathogenic agents that cause fruit rot and that are correlated with olive fly infestation [86, 87]. *C. acutatum* incidence increase in years of high olive fly populations and infestation levels. Olive oil extracted from olives with anthracnose reports lower quality compared to healthy olives and lower quality than olives infested by olive fly [88]. Furthermore, an increase in bacteria, yeasts and moulds is observed in olives infested by pests leading to an increase in the free acidity of olive oils especially if olives are stored prior to oil's extraction [89].

Sensorial characterization is a very important component of olive oils quality and classification. Regarding sensorial component, olive oils from infested fruits by pests and diseases have lower fruity, green, bitter and pungent sensorial attributes and increased defects are noted, mainly fusty, musty, winy, grubby and many times rancid [83, 84, 90]. Many defects arise from degradation and fermentative processes of olives. The perceived sensorial component is mainly affected by the changes observed in the volatile compounds released by the olive oil. In fact, olive oils from fruits infested by pests and diseases report lower green and cut-grass sensations due to loss in (*E*)-2-hexenal, one of the main volatiles responsible for those notes [91, 92], and one of the most abundant volatile compounds in olive oils [93]. The increase in fusty and musty defects is due to microbial contamination of the olive fruits and winy defect due to fermentative processes that release high amounts of alcohols (methanol, ethanol and isoamylic) [94] and acetic acid [95].

The conjunction of chemical degradation due to pests and diseases incidence together with the sensorial component gives us an idea of the changes inflicted in olive oils and the overall quality that they display. Olive oil quality is severely affected and fresh olive oils cannot be classified as extra-virgin olive oils, being considered virgin, or even lampante olive oils [96, 97] due to both quality and sensorial defects.

Olive Pests and Diseases on Olive Oil Composition, Stability and Bioactivity

Olive oil minor components are directly implicated in the olive oil quality and its properties as well. A very important factor that enhances the deleterious effects of pests and diseases in olive oils composition is the maturation process. Several authors studied the composition of olive oils extracted from infested fruits (mainly olive fly) and they highlight the fact that mature olives are characterized by a poorer composition in several olive oil components than greener ones and the intrinsically related quality is also severely affected [83, 84, 95]. Some of the most affected compounds include the fatty acids, mainly unsaturated

fatty acids, sterols (β -sitosterol), tocopherols (α -tocopherol) and, in great extent, phenolic compounds (Figure 2).

In this section we will focus mainly in olive fly due to the scarce and many times inexistent information about the impact of olive moth, anthracnose and verticillium wilt in the composition of olive oils. Therefore, and starting by olive oil pigments, both chlorophylls and carotenoids are severely affected by olive fly infestation. Olive oils extracted from green cv. Chemlali olive fruits lost about 73.8% and 39.2% of chlorophylls and carotenoids, respectively, when 100% of the fruits are infested [84]. Similar remarks were observed by Tamendjari et al., [95] when studying Algerian olive cultivars. This is an aspect that not only influences the composition of olive oil, but can easily have repercussions at the consumers' preference, since olive oils become less green and more yellow, an aspect which isn't appreciated by regular consumers.

Regarding fatty acids profile, the information collected from literature is not consistent. Some authors [84, 85, 95] studied the effect of olive fly in fatty acids of olive oils without noticeable t changes in the profile. However, by studying three Algerian olive cultivars (cvs. Chemlal, Azzeradj and Bouchouk), Tamendjari et al., [97] established negative correlations between olive infestation level and unsaturated fatty acids, mainly oleic and linoleic acids. The same authors report a positive correlation with saturated fatty acids according to the infestation level of olive fly. These data highlights the oxidative processes suffered by the olive fruits during infestation, which leads to a lower amount of unsaturated fatty acids in the olive oil. Furthermore, unsaturated fatty acids are proner to oxidative processes [98] than saturated ones.

Sterolic fraction (mainly β -sitosterol) and the triterpenic alcohol uvaol are affected by olive fly infestation [99]. These compounds have their content reduced with olive fly infestation level.

Besides being important minor components of olive oil, tocopherols are scarcely studied regarding the effect of pests and diseases incidence on their contents in olive oil. When studying Portuguese olive cultivars (cvs. Cobrançosa, Madural and Verdeal Transmontana), Pereira et al., [85] verified that the β -tocopherol and γ -tocopherol content in the olive oils was not affected by the infestation level. However, α -tocopherol content decreased with the olive fly infestation, with a characteristic response according to the olive cultivar, as witnessed in Figure 7.

Cv. Cobrançosa was the one that lost lower amounts of α -tocopherol, only 6% at 100% infestation level. Meanwhile cvs. Madural and Verdeal Transmontana reported losses of about 22 and 38%, respectively, a lost that clearly compromises the composition and quality of the olive oils obtained from these olive cultivars. The trend observed in Figure 7 for α -tocopherol is the same observed for total tocopherols, since α -tocopherol is the most abundant tocopherol in olive oils. Therefore, olive fly influences the amount of a powerful antioxidant compound with impact on the olive oil stability and therefore, as already mentioned, in the quality of olive oils.

In which concerns to phenolic compounds, literature is consistent: olive oils extracted from olive fly infested fruits are poor in phenolic compounds [82-84, 97]. Gucci et al., [82] verified that all phenolic compounds represented in Figure 2, namely hydroxytyrosol, tyrosol, 3,4-DHPEA-EA, 3,4-DHPEA-EDA, and p -HPEA-EDA, are negatively correlated with the percentage of olive fruits with exit holes. Only (+)-pinosresinol and (+)-1-acetoxypinosresinol maintain their contents with the increasing percentage of exit holes [82].

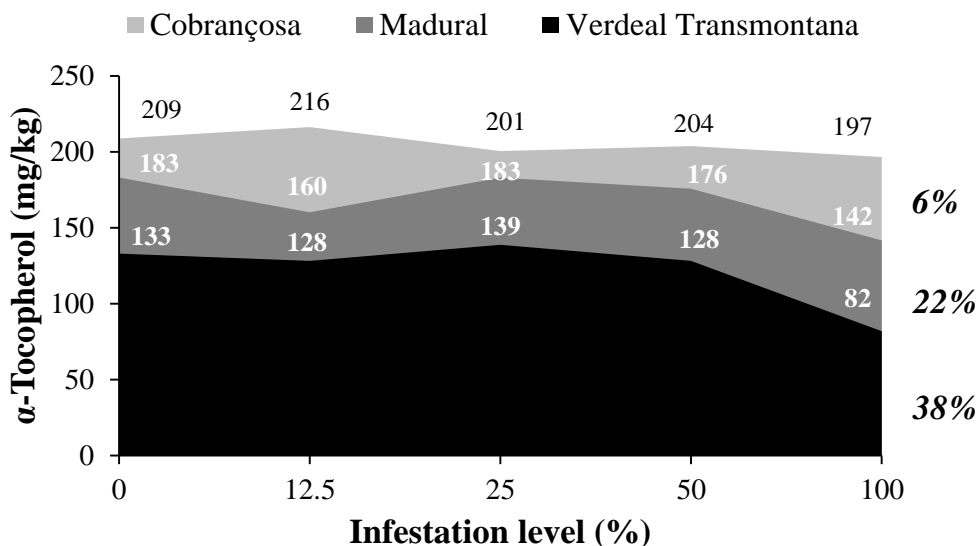


Figure 7. Changes recorded in α -tocopherol amounts in olive oils from cvs. Cobrançosa, Madural and Verdeal Transmontana extracted from olive fruits with different infestation levels (in bold and italic is presented the percentage of loss of α -tocopherol between 0 and 100% infestation level). Data from Pereira et al., (2004).

Regarding total phenols content, Gucci et al., [82] report a loss of about 75% of phenolic compounds when comparing cv. Frantoio olive oils from healthy olives and those extracted from olive fruits with 100% exit holes. Similar results were verified in other olive varieties: 21% and 50% respectively in Croatian olive cultivars Istarska bjelica and Buža [83]; 83% in Tunisian olive cultivar Chemlali [84]; and between 60-68% in Algerian olive cultivars Azzeradj, Chemlal and Bouchouk [97]. The loss of phenolic compounds is induced by the olive fly larvae. Part of the phenolic compounds lost are ingested by the larvae during its development and pulp consumption, while other part of phenols are oxidized in the olive fruit and degraded by microorganisms and enzymes present in the fruit or that contaminate the fruit after adult emergence.

With all the described changes suffered by olive fruits components due to olive fly infestation, the oxidative stability of olive oils as well as its bioactivity are compromised. According to Gómez-Caravaca et al., [100] and Mraïcha et al., [84], olive oil antioxidant properties are reduced with olive fly infestation, as reported in Figure 8A.

Antioxidant potential is reduced (Figure 8A) due to the loss of antioxidant compounds as it is the case of tocopherols, namely α -tocopherol (Figure 7), and, in higher extent, in the content of phenolic compounds as already witnessed and reported in Figure 8B. Olive oils extracted from olives with an infestation level below 30% report nearly three times more phenolic compounds than olive oils extracted from olives above the mentioned infestation level [100]. This scenario leads, in a final stage, to loss of oxidative stability by olive oils. Higher infestation levels are related with lower resistance to oxidation (Figure 8C) since lower amounts of antioxidants are available to protect fatty acids from oxidative agents, specially unsaturated ones, therefore the quality of the obtained olive oils is reduced. Below 30% of olive fly infestation, the oxidative stability is comparatively 10 hours higher than from olive oils extracted from olives with an infestation level superior than 30% [100].

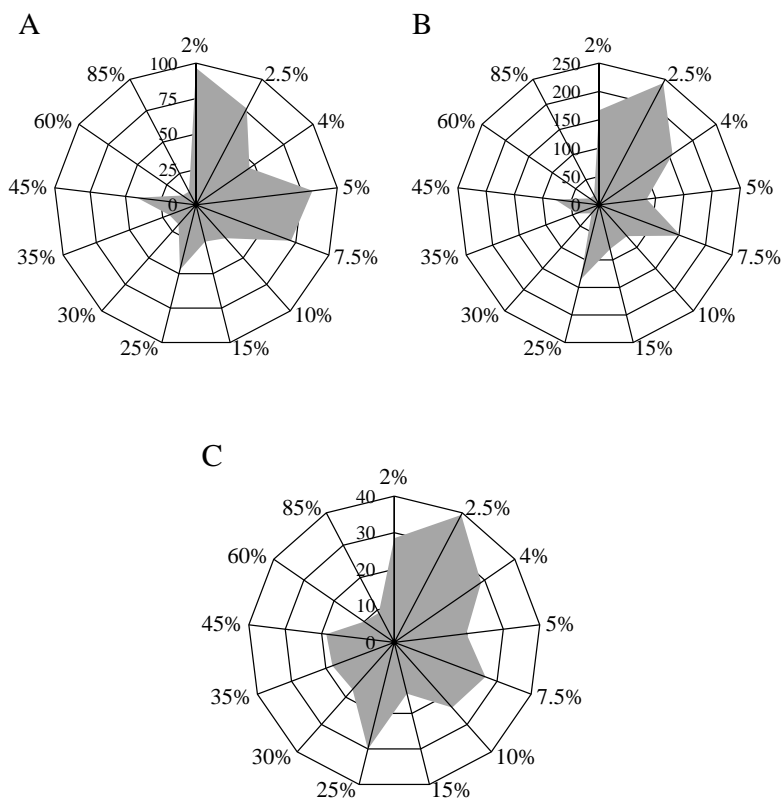


Figure 8. Antioxidant activity (Figure 8A) (μg quercetin/mL of extract), phenolic content (sum of simple phenols, lignans and secoiridoids; Figure 8B) (mg/kg of oil) and oxidative stability (Figure 8C) (hours), of olive oils extracted from olive fruits with different infestation levels. Data updated from Gómez-Caravaca et al., (2008).

CONCLUSION

Agronomic factors influence considerably olive oils quality and composition. While irrigation and fertilization applied in proper amounts, according to the plant needs, does not affect olive oils quality significantly, it considerably affects olive oil composition. For both factors this entails important issues regarding olive oils stability and preservation during storage, reducing significantly olive oils shelf-life. Furthermore, a diversified array of functions and health properties are lost in olive oils from over-irrigated and over-fertilized olives, due to the considerable reduction in antioxidant compounds (mainly phenols, *o*-diphenols, α -tocopherol).

Regarding olive pests and diseases, olive oil is primarily affected on the economic field, since significant losses are entailed each year in olive fruits production. Olive oils quality and composition is significantly changed by olive fly, a fact that leads to reduced quality, and stability. Olive fly actions are so severe that olive oils are frequently classified as lampante olive oils.

Therefore, proper optimization of irrigation and fertilization programs need to be carried out according to the olive varieties, the geographical region, climate conditions, soil properties and most of all, plant status and needs. Regarding olive pests diseases, effective control programs and phytosanitary programs need to be implemented to ensure olive oils quality, composition, stability and properties.

REFERENCES

- [1] FAOSTAT (2013). Available at <http://faostat.fao.org/site/636/default.aspx#ancor>; Accessed 24th October 2013.
- [2] International Olive Council (IOC) (2012a) World Olive Oil Figures – Production. Available at: <http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures>; Accessed 24th October 2013.
- [3] International Olive Council (IOC) (2012b) World Olive Oil Figures – Consumption. Available at: <http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures>; Accessed 24th October 2013.
- [4] Simopoulos AP (2002) Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition*, 21, 495-505.
- [5] Huang CL, & Sumpio BE (2008) Olive oil, the Mediterranean diet, and cardiovascular health. *Journal of the American College of Surgeons*, 207, 407-416.
- [6] Gandul-Rojas B, & Minguéz-Mosquera MI (1996) Chlorophyll and carotenoid composition in virgin olive oils from various Spanish olive varieties. *Journal of the Science of Food and Agriculture*, 72, 31-39.
- [7] Sies H, & Stahl W (1995) Vitamin E and C, beta-carotene, and other carotenoids as antioxidants. *American Journal of Clinical Nutrition*, 62, 1315S-1321S.
- [8] Velasco J, & Dobarganes C (2002) Oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology*, 104, 661-676.
- [9] Blekas G, Tsimidou M, & Boskou D (1995) Contribution of α -tocopherol to olive oil stability. *Food Chemistry*, 52, 289-294.
- [10] Aparicio R, Roda L, Albi MA, & Gutiérrez F (1999) Effect of various compounds on virgin olive oil stability measured by Rancimat. *Journal of Agricultural and Food Chemistry*, 47, 4150-4155.
- [11] Albanes D, Malila N, Taylor PR, Huttunen JK, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Barrett MJ, Pietinen P, Hartman TJ, Sipponen P, Lewin K, Teerenhovi L, Hietanen P, Tangrea JA, Virtanen M, & Heinonen OP (2000) Effects of supplemental α -tocopherol and β -carotene on colorectal cancer: results from a controlled trial (Finland). *Cancer Causes & Control* 11, 197-205.
- [12] Dysken MW, Sano M, Asthana S, Vertrees JE, Pallaki M, Llorente M, Love S, Schellenberg GD, McCarten R, Malphurs J, Prieto S, Chen P, Loreck DJ, Trapp G, Bakshi RS, Mintzer JE, Heidebrink JL, Vidal-Cardona A, Arroyo LM, Cruz AR, Zachariah S, Kowall NW, Chopra MP, Craft S, Thielke S, Turvey CL, Woodman C, Monnel KA, Gordon K, Tomaska J, Segal Y, Peduzzi PN, & Guarino PD (2014) Effect of vitamin E and memantine on functional decline in Alzheimer disease. The TEAM-AD

- VA Cooperative Randomized Trial. *The Journal of the American Medical Association*, 311, 33-44.
- [13] Andrikopoulos NK, Kaliora AC, Assimopoulou AN, & Papageorgiou VP (2002) Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil against in vitro low-density lipoprotein oxidation. *Journal of Medicinal Food*, 5, 1-7.
- [14] Malheiro R, Sousa A, Casal S, Bento A, & Pereira JA (2011) Cultivar effect on the phenolic composition and antioxidant potential of stoned table olives. *Food and Chemical Toxicology*, 49, 450-457.
- [15] Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalter B, & Bartsch H (2000) The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *European Journal of Cancer*, 36, 1235-1247.
- [16] Covas MI, Ruiz-Gutiérrez V, de la Torre R, Kafatos A, Lamuela-Raventós RM, Osada J, Owen RW, & Visioli F (2006a) Minor components of olive oil: evidence to date of health benefits in humans. *Nutrition Reviews*, 64, S20-S30.
- [17] Covas MI, Nyyssönen K, Poulsen HE, Kaikkonen J, Zunft H-JF, Kiesewetter H, Gaddi A, de la Torre R, Mursu J, Bäumler H, Nascetti S, Salonen JT, Fitó M, Virtanen J, & Marrugat J (2006b) The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Annals of Internal Medicine*, 145, 333-341.
- [18] Konstantinidou V, Covas MI, Muñoz-Aguayo D, Khymenets O, de la Torre R, Saez G, Tormos MC, Toledo E, Marti A, Ruiz-Gutiérrez V, Mendez MVR, & Fito M (2010) *In vivo* nutrigenomics effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. *The FASEB Journal*, 24, 2546-2557.
- [19] Obied HK, Prenzler PD, Omar SH, Ismael R, Servili M, Esposto S, Taticchi A, Selvaggini R, & Urbani S (2012) Pharmacology of olive biophenols. In: Fishbein JC, & Heilman JM (Eds.) *Advances in Molecular Toxicology*, 6, 195-242.
- [20] Loumou A, & Giourga C (2003) Olive groves: "The life and identity of the Mediterranean". *Agriculture and Human Values*, 20, 87-95.
- [21] Rallo L (2009) Iberian olive growing in a time of change. *Chronica Horticulturae*, 49, 15-17.
- [22] Guerfel M, Zaghdoud C, Jebahi K, Boujnah D, & Zarrouk M (2010) Effects of the planting density on virgin olive oil quality of "Chemlali" olive trees (*Olea europaea* L.). *Journal of Agricultural and Food Chemistry*, 58, 12469-12472.
- [23] Tous J, Romero A, Plana J, & Baiges F (1999) Planting density trial with 'Arbequina' olive cultivar in Catalonia (Spain). *Acta Horticulturae*, 474, 177-180.
- [24] Tous J, Romero A, Plana J, & Hermoso JF (2008) Olive oil cultivars suitable for very-high density planting conditions. *Acta Horticulturae*, 791, 403-408.
- [25] Allalout A, Krichène D, Methenni K, Taamalli A, Oueslati I, Daoud D, & Zarrouk M (2009) Characterization of virgin olive oil from super intensive Spanish and Greek varieties grown in northern Tunisia. *Scientia Horticulturae*, 120, 77-83.
- [26] Cunha SC, Fernandes JO, & Oliveira MBPP (2007a) Determination of phosmet and its metabolites in olives by matrix solid-phase dispersion and gas chromatography-mass spectrometry. *Talanta*, 73, 514-522.
- [27] Cunha SC, Fernandes JO, & Oliveira MBPP (2007b) Comparison of matrix solid-phase dispersion and liquid-liquid extraction for the chromatographic determination of

- fenthion and its metabolites in olives and olive oils. *Food Additives and Contaminants*, 24, 156-164.
- [28] Cunha SC, Lehotay SJ, Mastovska K, Fernandes JO, & Oliveira MBPP (2007c) Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olives. *Journal of Separation Science*, 30, 620-632.
- [29] Willer H, & Lukas K (2012) The World of Organic Agriculture – Statistics and Emerging Trends 2012.
- [30] Sandalidou E, Baourakis G, & Siskos Y (2002) Customers' perspectives on the quality of organic olive oil in Greece: a satisfaction evaluation approach. *British Food Journal*, 104, 391-406.
- [31] Ninfali P, Bacchiocca M, Biagiotti E, Esposto S, Servili M, Rosati A, & Montedoro G (2008) A 3-year study on quality, nutritional and organoleptic evaluation of organic and conventional extra-virgin olive oils. *Journal of the American Oil Chemists' Society*, 85, 151-158.
- [32] Gutiérrez F, Arnaud T, & Albi MA (1999) Influence of ecological cultivation on virgin olive oil quality. *Journal of the American Oil Chemists' Society*, 76, 617-621.
- [33] Samman S, Chow JWY, Foster MJ, Ahmad ZI, Phuyal JL, & Petocz P (2008) Fatty acid composition of edible oils derived from certified organic and conventional agricultural methods. *Food Chemistry*, 109, 670-674.
- [34] Dabbou S, Chehab H, Faten B, Dabbou S, Esposto S, Selvaggini R, Taticchi A, Servili M, Montedoro GF, & Hammami M (2010) Effect of three irrigation regimes on Arbequina olive oil produced under Tunisian growing conditions. *Agricultural Water Management*, 97, 763-768.
- [35] d'Andria R, Morelli G, Giorgio P, Patumi M, Vergari G, & Fontanazza G (1999) Yield and oil quality of young olive trees grown under different irrigation regimes. *Acta Horticulturae*, 474, 185-188.
- [36] Inglese P, Barone E, & Gullo G (1996) The effect of complementary irrigation on fruit growth, ripening pattern and oil characteristics of olive (*Olea europaea* L.) cv. Carolea. *Journal of Horticultural Science*, 71, 257-263.
- [37] Ismail AS, Stavroulakis G, & Metzidakis J (1999) Effect of irrigation on the quality characteristics of organic olive oil. *Acta Horticulturae*, 474, 687-690.
- [38] Palese AM, Nuzzo V, Favati F, Pietrafesa A, Celano G, & Xiloyannis C (2010) Effects of water deficit on the vegetative response, yield and oil quality of olive trees (*Olea europaea* L., cv Coratina) grown under intensive cultivation. *Scientia Horticulturae*, 125, 222-229.
- [39] Patumi M, d'Andria R, Fontanazza G, Moreli G, Giorgio P, & Sorrentino G (1999) Yield and oil quality of intensively trained trees of three cultivars of olive (*Olea europaea* L.) under different irrigation regimes. *Journal of Horticultural Science and Biotechnology*, 74, 729-737.
- [40] Patumi M, d'Andria R, Marsilio V, Fontanazza G, Moreli G, & Lanza B (2002) Olive and olive oil quality after intensive monocone olive growing (*Olea europaea*, cv. Kalamata) in different irrigation regimes. *Food Chemistry*, 77, 27-34.
- [41] Stefanoudaki E, Williams M, Chartzoulakis K, & Harwood J (2009) Effect of irrigation on quality attributes of olive oil. *Journal of Agricultural and Food Chemistry*, 57, 7048-7055.

- [42] Berenguer MJ, Vossen PM, Grattan SR, Connell JH, & Polito VS (2006) Tree irrigation levels for optimum chemical and sensory properties of olive oil. *HortScience*, 41, 427-432.
- [43] Tovar MJ, Motilva MJ, Luna M, Girona J, & Romero MP (2001a) Analytical characteristics of virgin olive oil from young trees (Arbequina cultivar) growing linear irrigation strategies. *Journal of the American Oil Chemists' Society*, 78, 843-849.
- [44] Gómez-Rico A, Salvador MD, Moriana A, Pérez D, Olmedilla N, Ribas F, & Fregapane G (2007) Influence of different irrigation strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. *Food Chemistry*, 100, 568-578.
- [45] Tovar MJ, Motilva MJ, & Romero M (2001b) Changes in the phenolic composition of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under linear irrigation strategies. *Journal of Agricultural and Food Chemistry*, 49, 5502-5508.
- [46] Romero MP, Tovar MJ, Girona J, & Motilva MJ (2002) Changes in the HPLC phenolic profile of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under different deficit irrigation strategies. *Journal of Agricultural and Food Chemistry*, 50, 5349-5354.
- [47] Servili M, Esposto S, Lodolini E, Selvaggini R, Taticchi A, Urbani S, Montedoro G, Serravalle M, & Gucci R (2007) Irrigation effects on quality, phenolic composition, and selected volatiles of virgin olive oils cv. Leccino. *Journal of Agricultural and Food Chemistry*, 55, 6609-6618.
- [48] Laguerre M, Lecomte J, & Villeneuve P (2007) Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Progress in Lipid Research*, 46, 244-282.
- [49] Rodrigues MA, Ferreira IQ, Claro AM, & Arrobas M (2012) Fertilizer recommendations for olive based upon nutrients removed in crop and pruning. *Scientia Horticulturae*, 142, 205-211.
- [50] Erel R, Dag A, Ben-Gal A, Schwartz A, & Yermiyahu U (2008) Flowering and fruit set of olive trees in response to nitrogen, phosphorous and potassium. *Journal of the American Society for Horticultural Science*, 133, 639-647.
- [51] Marcelo ME, Jordão PV, Matias H, & Rogado B (2010) Influence of nitrogen and magnesium fertilization of olive tree 'Picual' on yield and olive oil quality. *Acta Horticulturae*, 868, 445-450.
- [52] Rodrigues MA, Pavão F, Lopes JI, Gomes V, Arrobas M, Moutinho-Pereira J, Ruivo S, Cabanas JE, & Correia CM (2011) Olive yields and tree nutritional status during a four-year period without nitrogen and boron fertilization. *Communications in Soil Science and Plant Analysis*, 42, 803-814.
- [53] Fernández-Escobar R (2011) Use and abuse of nitrogen in olive fertilization. *Acta Horticulturae*, 888, 249-258.
- [54] Fernández-Escobar R, Ortiz-Urquiza A, Prado M, & Rapoport HF (2008) Nitrogen status influence on olive tree flower quality and ovule longevity. *Environmental and Experimental Botany*, 64, 113-119.
- [55] Fernández-Escobar R, Sánchez-Zamora MA, Uceda M, & Beltrán G (2002) The effect of nitrogen overfertilization on olive tree growth and oil quality. *Acta Horticulturae*, 586, 429-431.

- [56] Fernández-Escobar R, Beltrán G, Sánchez-Zamora MA, García-Novelo J, Aguilera MP, & Uceda M (2006) Olive oil quality decreases with nitrogen over-fertilization. *HortScience*, 41, 215-219.
- [57] Fernández-Escobar R, Parra MA, Navarro C, & Arquero O (2009) Foliar diagnosis as a guide to olive fertilization. *Spanish Journal of Agricultural Research*, 7, 212-223.
- [58] Marín L, & Fernández-Escobar R (1997) Optimization of nitrogen fertilization in olive orchards. *Acta Horticulturae*, 448, 411-414.
- [59] Morales-Sillero A, Jiménez R, Fernández JE, Troncoso A, & Beltrán G (2007) Influence of fertigation in 'Manzanilla de Sevilla' olive oil quality. *HortScience*, 42, 1157-1162.
- [60] Tognetti R, Morales-Sillero A, d'Andria R, Fernández JE, Lavini A, Sebastiani L, & Troncoso A, (2008) Deficit irrigation and fertigation practices in olive growing: convergences and divergences in two case studies. *Plant Biosystems*, 142, 138-148.
- [61] Bueno AM, & Jones O (2002) Alternative methods for controlling the olive fly, *Bactrocera oleae*, involving semiochemicals, *IOBC wprs Bulletin*, 25, 1-11.
- [62] Ramos P, Campos M, & Campos JM (1998) Long-term study on the evaluation of yield and economic losses caused by *Prays oleae* Bern. in the olive crop of Granada (southern Spain). *Crop Protection*, 17, 645-647.
- [63] Neuenschwander P, & Michelakis S (1978) The infestation of *Dacus oleae* (Gmel.) (Diptera, Tephritidae) at harvest time and its influence on yield and quality of olive oil in Crete. *Journal of Applied Entomology*, 86, 420-433.
- [64] Neuenschwander P, & Michelakis S (1981) Olive fruit drop caused by *Dacus oleae* (Gmel.) (Dipt. Tephritidae). *Journal of Applied Entomology*, 91, 193-205.
- [65] Bento A, Torres L, & Sismeiro JLR (1999) A contribution to the knowledge of *Bactrocera oleae* (Gmel.) in Trás-os-Montes region (Northeastern Portugal): phenology, losses and control. *Acta Horticulturae*, 474, 541-544.
- [66] de Almeida MJV (1899) La gaffa des olives en Portugal. *Bulletin de la Société Mycologique de France*, 15, 90-94.
- [67] Talhinhos P, Neves-Martins J, Oliveira H, & Sreenivasaprasad S (2009) The distinctive population structure of *Colletotrichum* species associated with olive anthracnose in the Algarve region of Portugal reflects a host-pathogen diversity hot spot. *FEMS Microbiology Letters*, 296, 31-38.
- [68] Talhinhos P, Mota-Capitão C, Martins S, Ramos AP, Neves-Martins J, Guerra-Guimarães L, Várzea V, Silva MC, Sreenivasaprasad S, & Oliveira H (2011) Epidemiology, histopathology and aetiology of olive anthracnose caused by *Colletotrichum acutatum* and *C. gloeosporioides* in Portugal. *Plant Pathology*, 60, 483-495.
- [69] Cacciola SO, Faedda R, Sinatra F, Agosteo GE, Schena L, Frisullo S, & San Lio GM (2012) Olive anthracnose. *Journal of Plant Pathology*, 94, 29-44.
- [70] Moral J, Bouhmidi K, & Trapero A (2008) Influence of fruit maturity, cultivar susceptibility, and inoculation method on infection of olive fruit by *Colletotrichum acutatum*. *Plant Disease*, 92, 1421-1426.
- [71] Moral J, & Trapero A (2009) Assessing the susceptibility of olive cultivars to anthracnose caused by *Colletotrichum acutatum*. *Plant Disease*, 93, 1028-1036.
- [72] Moral J, Oliveira R, & Trapero A (2009) Elucidation of disease cycle of olive anthracnose caused by *Colletotrichum acutatum*. *Phytopathology*, 99, 548-556.

- [73] López-Escudero FJ, & Mercado-Blanco J (2011) Verticillium wilt of olive: a case study to implement an integrated strategy to control a soil-borne pathogen. *Plant Soil*, 344, 1-50.
- [74] Bellahcene M, Fortas Z, Geiger JP, Matallah A, & Henni D (2000) Verticillium wilt in olive in Algeria: geographical distribution and extent of the disease. *Olivae*, 82, 41-43.
- [75] Thanassouloupoulos CC, Biris DA, & Tjamos EC (1979) Survey of Verticillium wilt of olive trees in Greece. *Plant Disease Report*, 63, 936-940.
- [76] Levin AG, Lavee S, & Tsrur L (2003) Epidemiology of *Verticillium dahliae* on olive (cv. Picual) and its effect on yield under saline conditions. *Plant Pathology*, 52, 212-218.
- [77] Nigro F, Gallone P, Romanazzi G, Schena L, Ippolito A, & Salemo MG (2005) Incidence of Verticillium wilt on olive in Apulia and genetic diversity of *Verticillium dahliae* isolates from infected trees. *Journal of Plant Pathology*, 87, 13-23.
- [78] Serrhini MN, & Zeroual A (1995) Verticillium wilt in Morocco. *Olivae*, 58, 58-61.
- [79] Sánchez-Hernández ME, Ruiz-Dávila A, Pére de Algaba A, Blanco-López MA, & Trapero-Casas A (1998) Occurrence and aetiology of death of young olive trees in Southern Spain. *European Journal of Plant Pathology*, 104, 347-357.
- [80] Al-Ahmad MA, & Mosli MN (1993) Verticillium wilt of olive in Syria. *Bulletin OEPP/EPPO Bulletin*, 23, 521-529.
- [81] Dervis S, Mercado-Blanco J, Erten L, Valverde-Corredor A, & Pérez-Artés E (2010) Verticillium wilt of olive in Turkey: a survey on disease importance, pathogen diversity and susceptibility of relevant olive cultivars. *European Journal of Plant Pathology*, 127, 287-301.
- [82] Gucci R, Caruso G, Canale A, Loni A, Raspi A, Urbani S, Taticchi A, Esposito S, & Servili M, (2012) Qualitative changes of olive oils obtained from fruits damaged by *Bactrocera oleae* (Rossi). *HortScience*, 47, 301-306.
- [83] Koprivnjak O, Dminić I, Kosić U, Majetić V, Godena S, & Valenčič V (2010) Dynamics of oil quality parameters changes related to olive fruit fly attack. *European Journal of Lipid Science and Technology*, 112, 1033-1040.
- [84] Mraicha F, Ksantini M, Zouch O, Ayadi M, & Sayadi S (2010) Effect of olive fruit fly infestation on the quality of olive oil from Chemlali cultivar during ripening. *Food and Chemical Toxicology*, 48, 3235-3241.
- [85] Pereira JA, Alves MR, Casal S, & Oliveira MBPP (2004) Effect of olive fruit fly infestation on the quality of olive oil from cultivars Cobrançosa, Madural, and Verdeal Transmontana. *Italian Journal of Food Science*, 16, 355-365.
- [86] Iannotta N, Noce ME, Ripa V, Scalercio S, & Vizzarri V (2007) Assessment of susceptibility of olive cultivars to the *Bactrocera oleae* (Gmelin, 1790) and *Camarosporium dalmaticum* (Thüm.) Zachos & Tzav.-Klon. attacks in Calabria (Southern Italy). *Journal of Environmental Science and Health Part B*, 42, 789-793.
- [87] Latinović J, Mazzaglia A, Latinović N, Ivanović M, & Gleason ML (2013) Resistance of olive cultivars to *Botryosphaeria dothidea*, causal agent of olive fruit rot in Montenegro. *Crop Protection*, 48, 35-40.
- [88] Sousa A, Pereira JA, Casal S, Oliveira B, & Bento A (2005) Effect of the olive fruit fly and the olive antrachnose on oil quality of some Portuguese cultivars. 2nd Meeting of the IOBC/WPRS WG Integrated Protection of Olive Crops. Florence, Italy, book of abstracts: 35pp.

-
- [89] Torres-Vila LM, Rodríguez-Molina MC, & Martínez JA (2003) Olive fly damage and olive storage effects on paste microflora and virgin olive oil acidity. *Grasas y Aceites*, 54, 285-294.
- [90] Angerosa F, Di Giacinto L, & Solinas M (1992) Influence of *Dacus oleae* infestation on flavor of oils, extracted from attacked olive fruits, by HPLC and HRGC analyses of volatile compounds. *Grasas y Aceites*, 43, 134-142.
- [91] Angerosa F, Mostallino R, Basti C, & Vito R (2000) Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chemistry*, 283-287.
- [92] Angerosa F, Servili M, Selvaggini R, Taticchi A, Esposto S, & Montedoro G (2004) Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. *Journal of Chromatography A*, 1054, 17-31.
- [93] Kalua CM, Allen MS, Bedgood DR, Bishop AG, Prenzler PD, & Robards K (2007) Olive oil volatile compounds, flavor development and quality: A critical review. *Food Chemistry*, 100, 273-286.
- [94] Bendini A, Cerretani L, Cichelli A, & Lercker G (2008) Come l'infestazione da *Bactrocera oleae* può causare variazioni nel profilo aromatico di oli vergini da olive. *Rivista Italiana di Sostanze Grasse*, 86, 167-177.
- [95] Tamendjari A, Angerosa F, & Bellal MM (2004) Influence of *Bactrocera oleae* infestation on olive oil quality during ripening of Chemlal olives. *Italian Journal of Food Science*, 16, 343-354.
- [96] Tamendjari A, Laribi R, & Bellal MM (2011) Effect of attack of *Bactrocera oleae* on olive oil by the quality of the volatile fraction of oil from two varieties Algerian. *Rivista Italiana di Sostanze Grasse*, 88, 114-122.
- [97] Tamendjari A, Sahnoune M, Mettouchi S, & Angerosa F (2009) Effect of *Bactrocera oleae* infestation on the olive oil quality of three Algerian varieties: Chemlal, Azzeradj and Bouchouk. *Rivista Italiana di Sostanze Grasse*, 86, 103-111.
- [98] Kamal-Eldin A (2006) Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. *European Journal of Lipid Science and Technology* 58, 1051-1061.
- [99] Parlati MV, Petruccioli G, & Pandolfi S (1990) Effects of the *Dacus* infestation on the oil quality. *Acta Horticulturae*, 286, 387-390.
- [100] Gómez-Caravaca AM, Cerretani L, Bendini A, Segura-Carretero A, Fernández-Gutiérrez A, Del Carlo M, Compagnone D, & Cichelli A (2008) Effects of fly attack (*Bactrocera oleae*) on the phenolic profile and selected chemical parameters of olive oil. *Journal of Agricultural and Food Chemistry*, 56, 4577-4583.