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Full Length Research Paper

Compositional quality of virgin olive oils from cultivars introduced in two Tunisian locations

Mokhtar Guerfel[1]*, Mohamed Ben Mansour[1], Youssef ouni[3], Dalenda Boujnah[2] and Mokhtar Zarrouk[3]

[1]Institut Supérieur de Biologie Appliquée, Université de Gabes, B.P. 522, 4100 Medenine, Tunisia.
[2]Institut de l'Olivier, Station de Sousse, Rue Ibn Khaldoun, B.P. 40, 4061 Sousse, Tunisia.
[3]Centre de Biotechnologie de Borj Cédria, B.P. 901, 2050 Hammam-Lif, Tunisia.

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The aim of this study was to evaluate the quality of two European olive cultivars, **'Koroneiki' and 'Arbequina', introduced in South and North of Tunisia. Olives grown in the two locations yielded extra virgin olive oils. Northern olive oils showed a greater amount of oleic acid, phenols and a higher stability, whilst in the southern oils had higher saturated and linoleic acid content. Phenolic compounds were also influenced by the pedoclimatic conditions; hence, oils from the North had the highest level of 3,4-DHPEA-EDA and a higher content in oleuropein aglycon (3,4-DHPEA-EA) than the corresponding olive oil samples obtained from trees cultivated in South. Furthermore, the majority of the studied analytical parameters were greatly influenced by the cultivar–environment interaction. In fact, significant differences between the studied oils were detected.**

Key words: Arbequina cultivar, Koroneiki cultivar, fatty acids, location, phenolic compounds, virgin olive oil.

INTRODUCTION

In the Mediterranean region, olive oil is the main source of fat of the diet mainly because of its use without refining which attribute it distinguishable characteristics such as: aroma, taste, colour and nutritive properties than other vegetable oils (Gutiérrez et al., 1999). Moreover, its healthy, interesting nutritional and sensorial properties have been known for a long time. The sensory quality and health properties of virgin olive oil stem from a prominent and well-balanced chemical composition (Bendini et al., 2007). In fact, the high content of oleic acid in olive oil serves to slow down penetration of fatty acids into arterial walls (Charbonnier, 1982). Oils which are much higher in monounsaturated fatty acids (MUFAs) and lower in saturated fatty acids (SFAs) are preferred because of the proven beneficial effect of MUFAs on serum cholesterol levels. The biological properties of olive oil are also related to the presence of minor components such as antioxidant compounds, particularly phenols (Owen et al., 2000). The most important classes of phenolic compounds in olive fruit include phenolic acids, phenolic alcohols, flavonoids and secoiridoids (Soler-Rivas et al., 2000). The chemical and quality characteristics of a virgin olive oil are determined by a series of factors like the nature of the soil, the climate, variety of the plant, cultivation, oil extraction techniques and storage conditions (Ryan et al., 1998). Moreover, it has long been known that the chemical composition of virgin olive oil (VOO) is influenced by genetic and environmental factors, in that the olive production area is greatly responsible for the specific characteristics of olive oil.

In the last few years, there has been increasing interest in the geographical identification of virgin olive oil, as a reliable criterion for its authentication and quality. Tunisia is the largest African exporter of olive oil and the fourth worldwide after Spain, Italy and Greece. The olive tree is present in practically every region of the country, up to the border of the southern desert. Recently, a major effort has been made to improve the quality of the olive oil produced in Tunisia by planting foreign cultivars.

However, before using new cultivars, their behaviour

*Corresponding author. E-mail: Guerfel_mk@yahoo.fr. Tel: +216 75 633 919. Fax: +216 75 633 918.

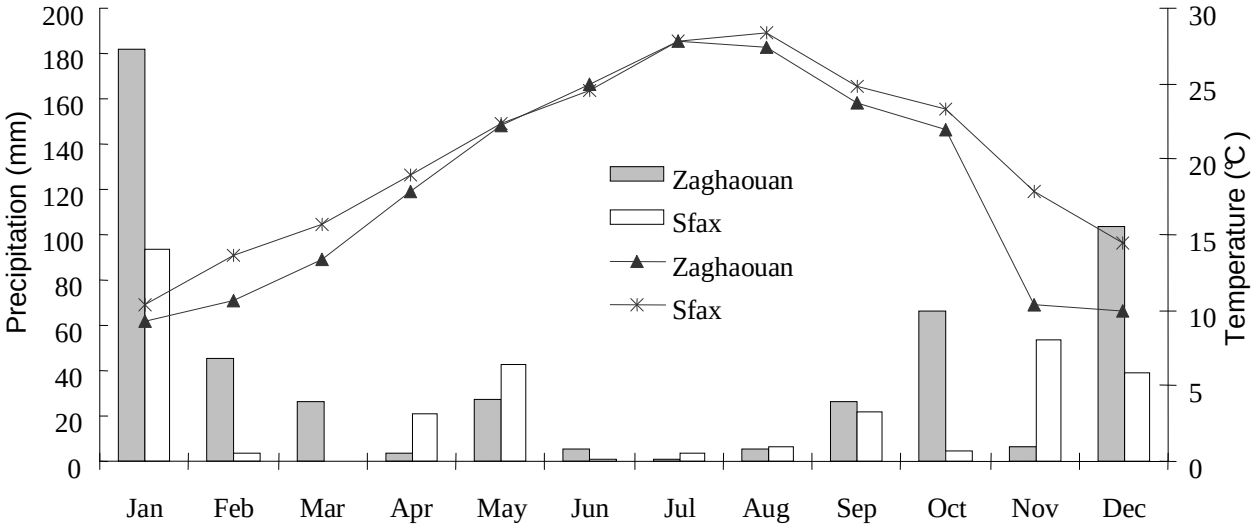

Figure 1. Total precipitation (bars) (mm) and temperatures (lines) recorded in the two locations. The values represent the means of two consecutive crops (2007 to 2008).

under different Tunisian pedoclimatic conditions must be evaluated in order to find the best location where each cultivar could be introduced. We hypothesized that it might be better to introduce European olive cultivars in North of Tunisia where climatic conditions are almost similar to European ones. Therefore, this study sought to examine some characteristics of virgin olive oil samples obtained from two European olive cultivars: 'Arbequina' and 'Koroneiki' when they were planted in north and south of Tunisia.

In particular, the aim of this study was to compare the effects of location on phenolic compounds and oxidative stability of virgin olive oil of these two cultivars.

MATERIALS AND METHODS

Plant material and growing area

Olive oil samples were obtained from homogeneous fruits mixtures of two foreign varieties of olive-trees, 'Koroneiki' (Greece) and 'Arbequina' (Spain), which were picked by hand at the same stage of maturity during two crop seasons (2007 and 2008). Olive samples of these two cultivars were collected from two locations: Sfax (South of Tunisia) and Tunis (North of Tunisia). The climatic characteristics of these two locations are reported in Figure 1. The same laboratory mill was used to prepare the olive oil samples. Only healthy fruits without any visible infection or physical damage were processed. The olives were washed, deleafed and crushed with a hammer crusher. The paste was then mixed at 25℃ for 30 min, centrifuged without addition of warm water and transferred into dark glass bottles.

Fatty acid composition

Fatty acid methyl-esters were prepared at room temperature by vigorous shaking of an olive oil solution in hexane (0.2 g in 3 ml) with 0.4 ml of 2 N methanolic potassium hydroxide solution and analyzed by gas chromatography (GC) on a Hewlett–Packard gas chromatograph (HP 4890 D) equipped with a capillary column (Supelcowax: 30 m × 0.53 mm; 0.25 mm), a split/splitless injector and a flame ionization detection (FID) detector. The carrier gas was nitrogen, with a flow rate of 1 ml/min. The temperatures of the injector, the detector and the oven were held at 220, 250 and 210℃, respectively. The injection volume was 1 µL.

Rancimat assay

Oxidative stability was evaluated by the Rancimat method (Gutiérrez, 1989). Stability was expressed as the oxidation induction time (h), measured with the Rancimat 743 apparatus (Metrohm, Herisau Switzerland), using an oil sample of 3.6 g. The oil temperature was 101.6℃ and the air flow was 10 L/h.

Extraction of phenolic compounds from olive oil

The extraction of phenolic compounds was carried out as previously described (Montedoro et al., 1992). 30 ml of methanol/water (80:20) was added to 30 g of olive oil and mixed with an Ultra-Turrax T25 at 15,000 g for 1 min, then centrifuged at 5,000 × g for 10 min. The extraction was repeated twice. The combined methanolic extracts were concentrated in a vacuum rotary evaporator at 35℃ until they reached a syrupy co nsistency. 10 ml of acetonitrile was added to the syrup, which was then washed twice with 20 ml of hexane. The acetonitrile phase was brought to dryness using a rotary evaporator. Finally, the sample was dissolved in gradient grade methanol and analyzed colorimetrically for total phenols and by HPLC for the fractionation of phenolic compounds.

HPLC analysis of the phenolic compounds

The HPLC system consisted of a Hewlett Packard quartenary pump Series 1100 (Hewelett Packard, Palo Alto, CA) coupled with a UV detector (Jasco UV 970) and HP Chemstation software for

Table 1. Fatty acids composition (%) of virgin olive oils of 'Koroneiki' and 'Arbequina' cultivars from two locations in Tunisia.

Variable	'Koroneiki'		'Arbequina'	
	Southern	Northern	Southern	Northern
Palmitic acid (16:0)	12.90[a]	11.55[a]	17.52[b]	16.13[b]
Palmitoleic acid (16:1)	1.23[a]	1.90[a]	2.40[b]	2.16[b]
Stearic acid (18:0)	2.47[b]	2.20[b]	1.87[a]	1.91[a]
Oleic acid (18:1)	73.44[c]	74.82[c]	57.82[a]	61.04[b]
Linoleic acid (18:2)	7.22[a]	8.53[a]	12.81[b]	14.05[c]
Linolenic acid (18:3)	0.86[a]	0.52[a]	0.60[a]	0.63[a]
Arachidic acid (20:0)	0.48[a]	0.34[a]	0.40[a]	0.41[a]
Saturated fatty acids (SFAs)	15.85[b]	14.09[a]	19.79[c]	18.45[c]
Unsaturated fatty acids (UFAs)	82.75[c]	85.77[c]	73.63[a]	77.88[b]
Monounsaturated fatty acids (MUFAs)	74.67[c]	76.72[d]	60.22[a]	63.25[b]
Polyunsaturated fatty acids (PUFAs)	8.08[a]	9.05[a]	13.41[b]	14.68[b]

Significant differences within the same row are shown by different letters ($P < 0.01$).

processing the acquired data. Injection was by means of a Rheodyne injection valve (Model 7125) with a 10 µl fixed loop (Rheodyne, CA, USA). Analytical separation was achieved on a Lichrosphere 100 RP-18.5 µm column (250 × 4 mm i.d.) fitted with a guard column (40 mm) of the same phase (Merck, Darmstadt, Germany). Eluates were detected at 278 nm. The mobile phase consisted of 0.2% acetic acid in water (solvent A) and methanol (solvent B) at a flow rate of 1 ml min^{-1}. Phenolic compounds were tentatively identified on the basis of their retention times compared to those of the standard compounds. The quantitative determination was performed using standards.

Determination of chlorophyll and carotenoid compounds

Chlorophyll and carotenoid contents were determined colorimetrically as previously described (Minguez-Mosquera et al., 1991). The maximum absorption at 670 nm is related to the chlorophyll fraction, while the maximum absorption at 470 nm is related to the carotenoid fraction. The values of the coefficients of specific extinction applied were $E_0 = 613$ for pheophytin, a major component in the chlorophyll fraction, and $E_0 = 2{,}000$ for lutein, a major component in the carotenoid fraction. Thus, the pigment contents were calculated as follows:

$$\text{Chlorophyll (mg/kg)} = (A_{670} \times 10^6)/(613 \times 100 \times d),$$

$$\text{Carotenoid (mg/kg)} = (A_{470} \times 10^6)/(2{,}000 \times 100 \times d),$$

Where *A* is the absorbance and d is the spectrophotometer cell thickness (1 cm).

Determination of oil quality parameters

Free acidity, expressed as percent of oleic acid (%18:1); peroxide value, given as milliequivalents of active oxygen per kilogram of oil ($meqO_2/kg$); and UV absorption characteristics (K_{232} and K_{270}) were determined according to the analytical methods described in the European Union Commission Regulations EEC/2568/91 and EEC/1429/92.

Statistical analysis

The results are shown as mean values with standard deviations. Significant differences among cultivars and locations were determined by an analysis of variance, which applied a Duncan's test. Differences were considered statistically significant when the probability was greater than 99% ($P < 0.01$). The statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc., 2004).

RESULTS AND DISCUSSION

Fatty acid composition

The mean fatty acid composition of the oils of both cultivars, from each location is shown in Table 1. The palmitic acid mean value for the 'Arbequina' cultivar was 17.5% in the south and 16.1% in the north. Compared to virgin olive oil of this variety when it is cultivated in Spain, 'Arbequina' variety grown in the two location in Tunisia produced oils with higher level of palmitic acid and lower level of oleic one (Allalout et al., 2009). The olive cultivars showed different mean values for oleic acid, with 'Koroneiki' North having the highest value (74.8 %) and 'Arbequina' South the lowest value (57.8%). When the oils from the different locations were compared, a trend showing greater oleic content for northern oils, and significant differences between cultivars ($p < 0.01$) was evident (Table 1). Both varieties of oil also contained low amounts of arachidic acid (20:0) and palmitoleic acid (16:1) (Table 1). Another characteristic of their oils is their low linolenic acid content (Table 1). 'Arbequina' olive oil samples obtained from trees cultivated in South and North were found to be rich in total saturated fatty acids (more than 18 %), essentially due to their high content of palmitic acid. 'Koroneiki' olive oil samples obtained from trees cultivated in North were found to show the higher content in total monounsaturated fatty acids (about 77%), due to their high percentage in oleic acid. Confirming the previous study by Allalout et al. (2009), the 'Koroneiki' Greek variety maintains the same composition of major fatty acids in Tunisia as well as in its original growing

Table 2. Mean values of analytical parameters of virgin olive oils of 'Koroneiki' and 'Arbequina' cultivars from two locations in Tunisia.

Variable	'Koroneiki'		'Arbequina'	
	Southern	Northern	Southern	Northern
Acidity (%C18 :1)*	0.27^{b}	0.50^{c}	0.19^{a}	0.30^{b}
PV ($MeqO_2/K$)	5.2^{c}	2.87^{a}	4.2^{b}	3.52^{a}
K_{232}	1.70^{a}	1.00^{a}	1.21^{a}	1.45^{a}
K_{270}	0.15^{a}	0.20^{a}	0.11^{a}	0.09^{a}
Chlorophylls (mg kg^{-1})	4.42^{a}	6.45^{b}	3.24^{a}	4.2^{a}
Carotenoids (mg kg^{-1})	2.03^{a}	3.72^{b}	1.82^{a}	2.55^{a}
Oxidative stability (h)	40.12^{c}	57.20^{d}	9.27^{a}	15.52^{b}

Significant differences within the same row are shown by different letters ($P < 0.001$). PV: peroxide value; K_{232} and K_{270}: values of specific extinction given as absorbance at 232 and 270 nm, respectively.

area. 'Arbequina' olive oil samples obtained from trees cultivated in Sfax were found to have the higher percentage in polyunsaturated fatty acids (about 15%) due to their high content in linoleic acid.

Variations in fatty acid content observed in olive oil samples obtained from both cultivars were probably related to both genetic factors and environmental conditions during fruit development and maturity (Lavee and Wodner, 1995). These results are in agreement with the findings of other authors (Lanza et al., 1998; Morello et al., 2004). They reported that several agronomic parameters could modify the fatty acid composition of olive oil. The most studied aspects include cultivar and origin, fruit ripening, harvest period and pedo-climatic conditions.

Oil quality parameters

Table 2 shows the physicochemical quality parameters of olive oils from the studied cultivars. All the analyzed oils showed very low values for the regulated physicochemical parameters evaluated, with all of them falling within the ranges established for ''extra virgin olive oil'' category, as required by Regulation EC/1989/2003 (EEC, 2003).

Our results show that the location had no significant influence on these analytical parameters, which are affected by factors causing damage to the fruits (for example, olive fly attacks or improper systems of harvesting, transport and storage of olives) (Kiritsakis et al., 1998). However, some significant differences ($p < 0.01$) in the values of free acidity and peroxide value according to location were found.

Pigment contents

Chlorophyll and carotenoids contents of the studied oils are shown in Table 2. The growing area conditions had a significant effect on 'Koroneiki' olive oils; olive oils from the north had the highest level of chlorophylls (6.4 mg kg^{-1}) (Table 2). The same phenomenon was observed with carotenoids. 'Arbequina' olive oils from the south contained the lowest levels (1.8 mg/kg) (Table 2).

Changes in the oxidative stability and total phenols

The amount of phenolic compounds is an important factor when evaluating the quality of virgin olive oil because of their involvement in resistance to oxidation and the sharp bitter taste of the oil. The research conducted on olive oil chemical composition highlights that the polyphenols are remarkably variable according to the variety, the agronomic conditions, the state of ripeness and the technology of conservation (Gutierrez et al., 2001; Krichene et al., 2007).

As shown in Table 3, the amounts of total phenols and *o*-diphenols in the analyzed oils show significant differences ($p < 0.01$) according to growing area. It was noticeable that olive oils samples obtained from trees cultivated in North were found to have the higher content in total phenols (230 and 108 mg kg^{-1}, for 'Koroneiki' and 'Arbequina', respectively) (Table 3). Therefore, different responses to the geographic growing area conditions were observed for each variety. Differences were also found in *o*-diphenol content (Table 3). Their variation was parallel to that of total phenols ($p < 0.01$).

It is important to note that northern cultivars had the highest concentration of *o*-diphenols. Aguilera et al. (2005) reported that the amount of total phenols is depending on various factors such as cultivar, climate, location, degree of maturation, type of crushing machine and oil extraction procedures among others. The oxidative stability of the virgin olive oils was measured with Rancimat equipment. For both cultivars, more stable oil being obtained in North (57 and 15 h, for 'Koroneiki' and 'Arbequina', respectively) (Table 2). The differences observed in the oxidative stability of studied oils from different locations can be explained by their antioxidant

Table 3. Phenolic composition of the examined olive oil samples (levels in mg/kg as syringic acid).

Variable	'Koroneiki'		'Arbequina'	
	Southern	Northern	Southern	Northern
Hydroxytyrosol	10.31[b]	11.37 [b]	2.52[a]	3.38[a]
Tyrosol	12.52[c]	14.61[d]	5.62[a]	7.72 [b]
Vanillic acid	0.80[a]	0.82[a]	0.73[a]	0.86[a]
Caffeic acid	0.59 [b]	0.89[c]	0.15[a]	0.16[a]
Syringic acid	0.73 [b]	0.16 [a]	0.15 [a]	0.14[a]
p-coumaric acid	0.15[a]	0.17[a]	0.27[b]	0.33[b]
Ferulic acid	16.71[b]	16.81[b]	1.82[a]	2.71[a]
3.4 -DHPEA-EDA	57.81[b]	69.82[c]	8.51[a]	9.31[a]
o-Coumaric acid	0.68 [a]	1.43 [a]	0.22 [a]	0.34 [a]
3.4 -DHPEA-EA	35.12[b]	37.10[b]	7.65[a]	8.71[a]
Total phenols	186.85[c]	230.48[d]	83.12[a]	108.27[b]
o-diphenols	50.41[b]	76.91[c]	28.51[a]	40.41[c]

Table 4. Correlations (r) between oxidative stability and some oil chemical characteristics.

Variable	Oxidative stability
Total phenols	0.83
o-diphenols	0.79
Hydroxytyrosol	0.75
Tyrosol	0.65
3,4-DHPE-EDA	0.60
3,4-DHPEA-EA	0.73

profiles.

Oils produced from the north had an important amount of phenols and a high level of oleic acid. As described by many authors (Aparacio et al., 1999; Aguilera et al., 2005), the last two parameters are the main factors responsible for the oxidative stability of olive oils. Oxidative stability was positively correlated with total phenols (r = 0. 83) and with *o*-diphenols (r = 0. 79) (Table 4).

Phenolic compounds

Identification of the phenolic compounds was based on comparisons of the chromatographic retention time and UV absorbance spectra of compounds in olive extracts with those of authentic standards. Phenolic compounds have a strong antioxidant and a free radical scavenging ability (Visioli et al., 1998). Moreover, their presence in olive oil contributes to the sensory characteristics, like its bitter, astringent and pungent taste (Gutiérrez-Rosales et al., 2003). As shown in Table 3, significant differences between locations ($p < 0.01$) were observed in phenolic contents. The 'Koroneiki' olive oil samples obtained from trees cultivated in North were found to contain 3,4-DHPEA-EDA at a concentration of about 69.8 mg kg^{-1} and a higher content in oleuropein aglycon (3,4-DHPEA-EA) than the corresponding olive oil samples obtained from trees cultivated in the South and olive oil samples of 'Arbequina' cultivar in the two locations (Table 3). Therefore, different responses to the environmental conditions were observed for the oils obtained from fruits of each olive cultivar. As shown in Table 3, the same variation was observed for the content in the examined olive oil samples in the main 2-phenylethanol derivatives. Olive oil samples obtained from trees cultivated in North were found to contain hydroxytyrosol and tyrosol at concentrations of about 11.3 and 14.6 mg kg^{-1}, respectively; while northern oil samples obtained of 'Arbequina' cultivar were found to contain these phenols at concentration of about 3.3 and 7.5 mg kg^{-1}, respectively. Other simple phenols such as vanillin, vanillic acid, *o*-coumaric acid, caffeic acid, syringic acid and ferulic acid were found in very low concentrations.

In the case of these compounds, however, differences were not significant. Oxidative stability was positively correlated to hydroxytyrosol (r = 0.75), tyrosol (r = 0.65), 3,4-DHPEA-EDA (r = 0.60) and 3,4-DHPEA-EA (r = 0.73) (Table 4). As previous investigations showed, the main determinants of virgin olive oil antioxidant activity are phenolic compounds that share o-diphenolic structures such as hydroxytyrosol and its derivates (Lavelli, 2002;

Guerfel et al., 2009).

Conclusion

Chemical characterization of virgin olive oil from 'Koroneiki' and 'Arbequina' grown in Tunisia showed that all quality parameters fell within the limits established for the extra virgin olive oil category. However, and taking into consideration the effect of the growing area conditions, it is important to note that both varieties grown in North of Tunisia have good quality characteristics in terms of natural antioxidants, oxidative stability and fatty acids compared to southern ones probably due to high temperature and low rainfall of the South. Further analyses, such as sterol composition and sensory analysis are necessary for a complete evaluation of these two cultivars in the two locations.

REFERENCES

Aguilera MP, Beltran G, Ortega D, Fernandez A, Jimenez A, Uceda M (2005). Characterisation of virgin olive oil of Italian olive cultivars: Frantoio and Leccino, grown in Andalousia. Food Chemistry, 89: 387–391.

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.

Aparicio R, Roda L, Albi MA, Gutiérrez F (1999). Effect of various compounds on virgin olive oil stability measured by rancimat. J. Agric. Food Chem., 47: 4150 – 4155.

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

Charbonnier A (1982). Main conclusion dawn from the International Symposium on the recent medical researches on the value of the olive oil to health (pp. 1–4) Paris, 17 November.

EEC (2003). Characteristics of olive and olive pomace oils and their analytical methods. EEC Regulation1989/2003. Offic. J. Eur. Commun., 295: 57–66.

Guerfel M, Ouni Y, Taamalli A, Boujnah D, Stefanoudaki E, Zarrouk M. (2009). Effect of location on virgin olive oils of the two main Tunisian olive cultivars. Europ, J, Lipid Sci, Technolo., 111: 926–932.

Gutiérrez F (1989). Determinación de la estabilidad oxidativa de aceites de oliva vírgens. Comparación entre el método del oxígeno activo (AOM) y el método Rancimat (Determinación of the oxidative stability of virgin olive oils. Comparison between AOM and Rancimat methods). Grasas Aceites, 40: 1–4.

Gutiérrez F, Arnaud T, Albi AM (1999). Influence of ecological cultivation on virgin olive oil quality. J. Amer. Oil Chem. Soc., 76(5): 617–621.

Gutiérrez F, Arnaud T, Garrido A (2001). Contribution of polyphenols to the oxidative stability of virgin olive oil. J. Sci. Food Agric., 81: 1–8.

Gutiérrez-Rosales F, Rios JJ, Gomez-Rey MAL (2003). Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. J. Agric. Food Chem., 51: 6021–6025.

Kiritsakis AK, Nauos GD, Polymenoupoulos Z, Thomai T, Sfakiotakis EY (1998). Effect of fruit storage conditions on olive oil quality. J. Amer. Oil Chem. Soc., 75: 721–724.

Krichene D, Taamalli W, Daoud, D, Salvador MD, Fregapane GD, Zarrouk M (2007). Phenolic compounds, tocopherols and other minor components in virgin olive oils of some Tunisian varieties. J. Food biochem., 31**:** 194-197.

Lanza CM, Russo C, Tomaselli F (1998). Relationship between geographical origin and fatty acid composition of extravirgin olive oils produced in three areas of eastern Sicily. Italian J. Food Sci., 4**:** 359–366.

Lavee S, Wodner M (1995). The effect of growing region, maturation and fruit handling on oil quality of cv. "Nabali" olives in West Bank Mountains. Agric. Med., *125*: 395–403.

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

Minguez-Mosquera MI, Rejano-Navarro L, Gandulrojas B, Sanchez GAH, Garrido-Fernandez J (1991). Color-pigment correlation in virgin olive oil. J. Amer. Oil Chem. Soc., 86: 332–336.

Montedoro G, Servili M, Baldioli M, Miniati E (1992). Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. J. Agric. Food Chem., 40: 1571–1576.

Morello JR, Motilva MJ, Tovar MJ, Romero MP (2004). Changes in commercial virgin olive oil (cv. Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry, 85: 357–364.

Owen, RW, Giacosa A, Hull WE, Haubner B, Spiegelhalder B, Bartsch, H (2000). Identification of lignans as major components in the phenolic fraction of olive oil. Clinical Chemistry, 46: 976–988.

Ryan D, Robards K, Lavee S (1998). Evolution de la qualité de l'huile d'olive. Olivae, *72*: 23–41.

Soler-Rivas C, Espĩn JC, Wichers HJ (2000). Oleuropein and related compounds. J. Sci. Food Agric., 80: 1013–1023.

Visioli F, Bellomo G, Galli C (1998). Free radical-scavenging properties of olive oil polyphenols. Biochem. Biophys. Res. Communic., 247: 60–64.