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Phenolic profile of virgin olive oil from advanced breeding selections

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Abstract

The evaluation of the phenolic composition in advanced selections in breeding programs constitutes the first approach for selecting genotypes with improved olive oil quality. In this work, the influence of genotype and ripening index on the phenolic profile of olive oils from advanced selections in comparison to their genitors was studied. Fruit samples were collected in genotypes from crosses between 'Arbequina' × 'Picual', 'Picual' × 'Arbequina' and 'Frantoio' × 'Picual' at five dates from 1st October to 26th November 2009. Characterization of the phenolic profile was performed by liquid-liquid extraction with 60:40 (v/v) methanol-water and subsequent chromatographic analysis with absorption and fluorescence detection in a sequential configuration. A dual effect of genotype and fruit ripening on the phenolic profile has been observed with more pronounced genetic influence in both total (34.73% and 20.45%, respectively) and individual phenols (16.99% to 49.25% and 1.58% to 23.77%, respectively). A higher degree of variability between genotypes at early ripening stages was also observed ($p < 0.05$). The obtained results allow also the identification of selections with high content of total and individual phenols. These results suggest a strategy based on early harvesting of fruits (at the first three ripening indexes) for better comparison and selection of genotypes in further crosses in olive breeding programs aiming at improving the quality of virgin olive oil.

Additional key words: cross-breeding; genetic variability; *Olea europaea*; phenolic composition; ripening index.

Resumen

Perfil fenólico de aceites de oliva vírgenes obtenidos de selecciones avanzadas en un programa de mejora

La evaluación de la composición fenólica en selecciones avanzadas en programas de mejora constituye el primer paso para la selección de genotipos cuyos aceites son de mejor calidad. Se ha estudiado la influencia del genotipo y del índice de madurez en el perfil fenólico del aceite de oliva de selecciones avanzadas en comparación con sus genitores. Se recogieron muestras de genotipos obtenidos de cruzamientos entre 'Arbequina' × 'Picual', 'Picual' × 'Arbequina' y 'Frantoio' × 'Picual' en cinco fechas entre el 1 de octubre y el 26 noviembre de 2009. Se realizó la caracterización del perfil fenólico por extracción líquido-líquido con metanol-agua al 60:40 (v/v) seguido de un análisis cromatográfico con detección por absorción y fluorescencia en una configuración secuencial. Los resultados obtenidos mostraron un mayor grado de variabilidad entre genotipos en las primeras etapas de maduración de los frutos ($p < 0.05$), así como el efecto de la madurez de los frutos y del genotipo en el perfil fenólico, con una influencia genética más pronunciada tanto para fenoles totales (34,73% and 20,45%, respectivamente) como individuales (entre 16,99% y 49,25% y entre 1,58% y 23,77%, respectivamente). Los resultados obtenidos han permitido la identificación de selecciones con alto contenido en fenoles totales e individuales. Ambos resultados sugieren una estrategia basada en la evaluación de los fenoles totales e individuales en las primeras etapas de la maduración para comparar y seleccionar genotipos en programas de mejora por cruzamiento encaminados a mejorar la calidad del aceite de oliva virgen.

Palabras claves adicionales: composición fenólica; índice de madurez; mejora por cruzamiento; *Olea europaea*; variabilidad genética.

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Abbreviations used: 3,4-DHPEA-EDA (dialdehydic form of elenolic acid linked to hydroxytyrosol); λex (excitation wavelength); λem (emission wavelength); GAE (gallic acid equivalent); PCA (principal component analysis); RI (ripening index); UV (ultraviolet); VOO (virgin olive oil).

Introduction

Virgin olive oil (VOO) has been a main delectable food in the Mediterranean basin for thousands of years. Its consumption is increasing in the United States, Canada, Australia, Japan and other countries (García-González & Aparicio, 2010). This increase is ascribed to the awareness of healthy and organoleptic properties of VOO, which can be consumed in its raw state —without suffering any refining process—, thus conserving the taste, aroma, and healthy properties of olive fruit.

The chemical composition of VOO is characterized by the presence of a group of major compounds (98%) and a group of minor compounds (2%). The first group is constituted mainly by triacylglycerols and small concentrations of diacylgycerols, monoacylglycerols and free fatty acids. However, the second group includes more than 230 compounds, including phospholipids, waxes, hydrocarbons, alcohols, sterols, volatile and phenolic compounds (Boskou, 1996).

Special attention should be paid to phenolic compounds (phenolic acids, phenolic alcohols, hydroxyisochromans, flavonoids, secoiridoids and lignans) because of their antioxidant and healthy properties and their influence on VOO organoleptic characteristics. In fact, phenolic compounds are correlated to VOO stability during storage by virtue of their antioxidant activity that reduces oxidation processes (Gallina-Toschi *et al.,* 2005; Frankel, 2010). Moreover, these compounds may provide a defense mechanism that delays aging and prevents carcinogenesis, atherosclerosis, obesity, liver disorders and inflammations (Tripoli *et al.*, 2005; Bendini *et al.*, 2007; Servili *et al.*, 2009). Also, beneficial effects on breast and colon cancer (Owen *et al.,* 2000), on diabetes accompanied by hypertriacylglycerolaemia and autoimmune diseases such as rheumatoid arthritis (Alarcón de la Lastra *et al.,* 2001) have been attributed to VOO. Finally, phenolic compounds are highly correlated to VOO organoleptic characteristics, mainly bitterness and pungency (Andrewes *et al.*, 2003; Beltrán *et al.*, 2007).

Phenolic composition of VOO is the result of a very complex multivariate interaction between genotype and agronomic, environmental and technological factors (Montedoro & Garofolo, 1984; Lavee & Wodner, 1991). In fact, drastic variability between cultivars was registered (Baccouri O. *et al.*, 2007; Montedoro *et al.*, 2007; Tura *et al.*, 2007; Baccouri *et al.*, 2008; Issaoui *et al.*, 2010). In addition, harvest time, determined by sequential physiological and biochemical changes and influenced by several environmental and cultural practices (Conde *et al.*, 2008), has a considerable effect on the VOO phenolic profile (Yousfi *et al.*, 2006; Baccouri B. *et al.*, 2007; Damak *et al.*, 2008; Gómez-Rico *et al.*, 2008). Moreover, water availability, cultivation zone and sanitary status of the olive fruits (mainly infestation by *Bactrocera olea*) have a large effect on the phenolic profile of VOO (Morelló *et al.*, 2005; Gómez-Rico *et al.*, 2007; Servili *et al.*, 2007; Gómez-Caravaca *et al.*, 2008; Ripa *et al.*, 2008; Tura *et al.*, 2008). On the other hand, the extraction process, mainly crushing and malaxation steps, highly affects the phenolic composition of VOO due to the activity of endogenous enzymes of olive fruits, which are released in the paste during these steps (Servili *et al.*, 1998; Bianco *et al.*, 2001; Ranalli *et al.*, 2001; Gómez-Rico *et al.*, 2009).

The influence of agronomic practices on VOO phenols has been widely studied in the principal cultivars of producing countries. Nevertheless, few studies have been published on segregating populations and on advanced selections from breeding programs developed in these countries. As an example, García-González *et al.* (2010) described the evolution along ripening of phenolic and volatile compounds of the new olive cultivar 'Sikitita' obtained by a crossing of 'Picual' and 'Arbequina' cultivars in the olive breeding program established in Córdoba (Spain). The objective of this work was to study the variation of phenolic compounds along fruit ripening and among advanced selections from the olive breeding program carried out in Córdoba (Spain) and their genitors. The influence of two relevant factors, genetic variability and ripening index, on the phenolic profile of VOOs was studied. The final aim of the research here reported was to provide a strategy for selection of potential genotypes in future crosses in breeding programs.

Material and methods

Plant material and fruit samples

Selections evaluated in this work come from crosses between 'Arbequina', 'Frantoio' and 'Picual' cultivars, carried out in the olive cross-breeding program of Córdoba in 1991-1992. Several genotypes were selected in the initial seedling population after three consecutive harvest seasons mainly on the basis of their early bearing (short juvenile period) and high oil con-

tent. The selected genotypes were propagated by softwood cuttings and planted, in 2001, in a comparative field trial together with the three genitors as control at 6 × 5 m spacing. Trees were trained as single-trunk vase, with three to four main branches, and minimal pruning was carried out to allow early bearing. Standard cultural practices were followed, including irrigation supply by in-line drips to avoid water stress of plants. Results of their agronomic evaluation at the initial seedling stage (León *et al.*, 2004) and comparative field trials (León *et al.*, 2007; De la Rosa *et al.*, 2008) have been reported so far.

Olive fruit samples were collected from 12 genotypes including the three genitors ('Arbequina', 'Picual' and 'Frantoio'), and three advanced selections from each cross: 'Arbequina' × 'Picual' ('UC-I 6-9', 'UC-I 7-8', 'UC-I 9-67'), 'Picual' × 'Arbequina' ('UC-I 2-68', 'UC-I 5-44', 'UC-I 7-34') and 'Frantoio' × 'Picual' ('UC-I 4-62', 'UC-I 7-60', 'UC-I 10-30'). The samples were collected at five dates from 1st October to 26th November 2009 (every two weeks) and two trees-per-genotype were sampled each date. No samples were available from 'Picual' at the first date (1st October).

Ripening index of fruit samples was recorded as described by Frías *et al.* (1991). Briefly, an aliquot of 100 randomly selected fruits was taken from each fruit sample, and fruits were classified into the following categories: 0 = the skin is a deep or dark green color; 1 = the skin is yellow or yellowish-green color; 2 = the skin is a yellowish color with reddish spots; 3 = the skin is a reddish or light violet color; 4 = the skin is black.

The total number of olives in each category (n_0, n_1,…, n_4) was recorded, and, then, the following equation was applied to determine the ripening index (RI):

$$RI = \frac{[(0 \times n_0) + (1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4)]}{100}$$

Then, RI was categorized as following: RI ≤ 0.5 (RI_0); RI = 0.5-1.5 (RI_1); RI = 1.5-2.5 (RI_2); RI = 2.5-3.5 (RI_3) and finally RI ≥ 3.5 (RI_4).

Olive oil extraction

Olive fruit samples were processed using an Abencor system (MC2 Ingenierías y Sistemas, Sevilla, Spain), which consists of three essential elements: the mill, the thermo-malaxer and the olive paste centrifuge. VOO was extracted according to the manufacturer protocol; briefly, olives were crushed with the hammer mill; the olive paste thus obtained was malaxated for 30 min with the water bath set at 28±1°C. Then, olive oil was separated from the paste by centrifugation for 2 min. Subsequently, the oil was separated from the wastewater by decantation and collected in dark brown glass bottles and stored at –18°C until analysis.

Reagents

Methanol, *n*-hexane, *o*-phosphoric acid, Folin-Ciocalteu (F-C) reagent and anhydrous sodium carbonate were purchased from Panreac (Barcelona, Spain). Hydroxytyrosol, tyrosol, vanillic acid, syringic acid, *p*-coumaric acid, *o*-coumaric acid, oleuropein, luteolin and apigenin were acquired from Extrasynthese (Genay, France) and Sigma Aldrich (Madrid, Spain). High quality water (Millipore Milli-Q) was used to prepare 60:40 (v/v) methanol–water extractant, the chromatographic mobile phase and analytical samples.

Phenols extraction

A solution of syringic acid (internal standard) was prepared by dissolving 15 mg of syringic acid in 10 mL of 60:40 (v/v) methanol–water. Then, 1 mL of this solution was diluted in a 25 mL volumetric flask with 60:40 (v/v) methanol–water. Syringic acid was used as internal due to its similarity to phenolic compounds present in VOO and to its absence in this product.

An aliquot of 3 g of olive oil was dissolved in 2 mL of *n*-hexane and homogenized by stirring for 15 s. Subsequently, 250 µL of syringic acid solution and 1.75 mL of 60:40 (v/v) methanol-water mixture were added, and the solution was subject to stirring for 2 min. Then, the extract was separated from the oily solution to which 2 mL of methanol-water mixture was added, followed by stirring for 2 min for a second extraction. The collected extracts from both separations were mixed and stored under refrigeration at – 18°C for further determinations.

Total phenols determination

An aliquot of 20 µL of methanol–water extract was introduced in an Eppendorf vial; then, 1.58 mL of deionized water, 300 µL of 20% Na_2CO_3 and 100 µL

of F-C reagent were added to the extract. The reaction was accelerated by putting the Eppendorfs in an oven at 50°C for 5 min and then set to rest for 30 min at room temperature. A blank and calibration solutions using gallic acid as standard were prepared similarly to the samples. The absorption at 765 nm was measured by a Spectronic Helios Gamma UV-Vis spectrophotometer from Thermo Fisher Scientific (Waltham, MA, USA) equipped with a tungsten–halogen lamp. The results were expressed as mg equivalents of gallic acid (GAE) kg^{-1} of oil.

Separation, identification and quantification of phenols

Sample extracts were analyzed using a Varian ProStar 230 solvent delivery module equipped with a reciprocating single piston pump. The chromatographic column was a Microsorb-MV 100 – 8 C18 (250 × 4.6 × 6.35 mm). The elution solvents were 0.2% *o*-phosporic acid in water (phase A) and pure methanol (phase B). The samples were eluted according to the following elution gradient: at 0 min: 96% of A and 4% of B; 0-40 min: 96-50% of A and 4-50% of B; 40-45 min: 50-40% of A and 50-60% of B; 45-60 min: 40-0% of A and 60-100% of B; 60-70 min: 0% of A and 100% of B and finally 70-77 min: 0-96% of A and 100-4% of B, and a constant flow rate of 1 mL min^{-1} was maintained during all the chromatographic step.

Detection was made by a Varian ProStar 335 ultraviolet diode-array detector and a Varian ProStar 363 fluorescence detector (λex = 250 nm, λem = 350 nm) in a sequential configuration. Hydroxytyrosol, tyrosol, vanillic acid and the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA; the most abundant secoiridoid form) were identified by their fluorescence emission spectra and retention times; while, *p*-coumaric and *o*-coumaric acids, luteolin and apigenin were identified by their absorption spectra (maximum absorbance at 280 nm) and their retention times. The results were elaborated by the Varian workstation. Quantification of phenolic compounds was performed relatively to the internal standard (syringic acid) and based on calibration curves of the eight commercially available standards. Accordingly, the results were expressed as mg of the target analyte kg^{-1} of oil for all individual phenols, except for 3,4-DHPEA-EDA, expressed as mg of oleuropein kg^{-1} of oil.

Statistical analyses

HPLC analyses were carried out in triplicate, while the values of total phenols were the result of a single analysis. The data were subjected to analysis of variance to test the effect of genotype, RI and interaction between both factors, in order to determine the relative importance of each factor. Means comparison was obtained using the Duncan test at a significance level of 5%. Statistical analysis was carried out using the Statistix (Analytical Software, Tallahassee, FL, USA) and SAS (SAS Institute Inc., Cary, NC, USA) statistical packages.

Multivariate analysis based on principal component analysis (PCA) was applied using the Unscrambler software (CAMO A/S, Trodheim, Norway).

Results

Harvesting time and ripening index (RI)

Significant differences were observed in ripening index between genotypes ($p < 0.0001$) and sampling dates ($p < 0.0001$). Besides, the interaction genotype × date was also significant ($p = 0.01$), indicating that the ripening progress in the different genotypes was different. The evolution of the ripening process showed that 'Frantoio', 'Picual', UC-I 10-30, UC-I 7-34 and UC-I 7-8 ripened earlier than genotypes 'Arbequina', UC-I 4-62, UC-I 5-44, UC-I 6-9 and UC-I 7-60, which ripened earlier than UC-I 2-68 and UC-I 9-67 (Fig. 1). For instance, by the fourth sampling date (November 12) an average ripening index

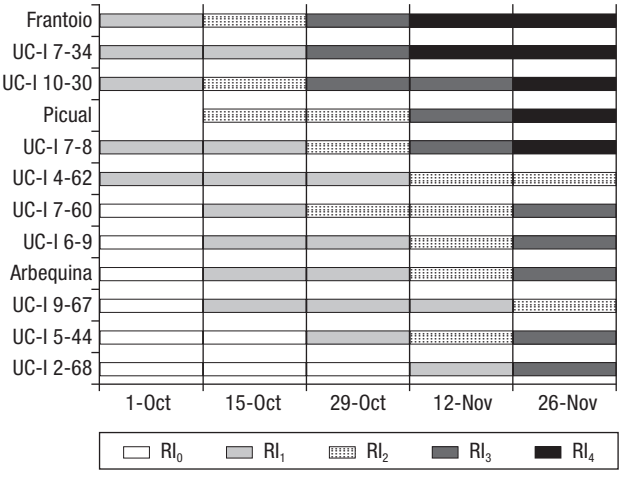

Figure 1. Average ripening index (RI) of genitors and advanced selections along the five harvesting dates. Genotypes are presented in descending order of RI.

3 or 4 was observed in genotypes of the first group, while this value was 2 and 1 on the second and third group, respectively. To avoid the difference in ripening among the studied genotypes, the categorization of RI previously described in the materials and methods section — *viz.* RI ≤ 0.5 (RI_0); RI = 0.5-1.5 (RI_1); RI = 1.5-2.5 (RI_2); RI = 2.5-3.5 (RI_3) and finally RI ≥ 3.5 (RI_4)— was used for comparison between the genotypes.

Phenols variability

Genotype variance was the main contributor to total variance for total phenols content, accounting for 35% of total sum of squares, whereas 20% and 10% accounted for RI and interaction between both factors, respectively (Table 1).

Significant differences for total phenols content were obtained between genotypes and RI. Total phenols, determined by the F-C method, showed high variability between genotypes from 494 ('UC-I 5-44') to 131 ('UC-I 7-8') mg GAE kg^{-1} of oil (Table 2). Significant differences between genotypes were more clearly observed at the initial stages of ripening (RI_0, RI_1 and RI_2) and smooth away at the end of the ripening process (Table 3).

The total phenol content significantly decreased during fruit ripening, with average values 2.5 times higher for RI_0 than for RI_4 (Table 4).

All individual phenols were identified in all genotypes at all ripening indexes, but with quantitative differences. As for the total phenols content, genotype variance was the main contributor to total variance for hydroxytyrosol, vanillic acid, 3,4-DHPEA-EDA, *p*- and *o*-coumaric acids, accounting for 31-49% of total sum of squares (Table 1). Genotype × RI interaction was the main contributor to total variance for tyrosol, luteolin and apigenin.

Similarly to total phenols, high variability between genotypes was observed in all individual phenols determined by HPLC. Significant differences between genotypes were obtained for all phenols and the Duncan multiple comparison test provided 2–7 subsets for the different phenols evaluated (Table 2). The highest differences were obtained for vanillic acid, with values from 0.21 ('UC-I 4-62') to 1.33 ('Arbequina') mg kg^{-1} of oil and 3,4 DHPEA-EDA with values from 85.30 ('Picual') to 334.74 ('UC-I 5-44') mg of oleuropein kg^{-1} of oil. Significant differences between genotypes were more clearly observed at ripening indexes RI_1 or RI_2. The pattern evolution for hydroxytyrosol and 3,4-DHPEA-EDA are presented in Table 5, and similar results were obtained for all other individual phenols evaluated (data not shown).

Significant differences between RI were obtained for tyrosol, vanillic acid and 3,4-DHPEA-EDA, decreasing the values for these phenols during fruit ripening (Table 4). Significant genotype × RI interaction was also obtained for tyrosol, vanillic acid and apigenin (Table 1). This significant interaction was mainly attributable to more pronounced decrease in tyrosol content for UC-I 7-60 and UC-I 9-67, in vanillic acid for 'Arbequina' and UC-I 7-34, and no clear trends between genotypes for apigenin content (Table 5).

Correlation between traits

Significant correlations were obtained between some of the evaluated characters (Table 6). These correlations confirm the inverse relationships between RI and total

Table 1. Relative importance of genotypes and ripening index (RI) expressed as percentages of total sum of squares and significance in the ANOVA for different compounds under study. Means are in mg gallic acid equivalent (GAE) kg^{-1} of oil for total phenols, mg kg^{-1} of oil for all individual phenols, except dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) that was expressed as mg of oleuropein kg^{-1} of oil

Parameter	Total phenols	Hydroxy-tyrosol	Tyrosol	Vanillic acid	3,4-DHPEA-EDA	*p*-Coumaric acid	*o*-Coumaric acid	Luteolin	Apigenin
Genotype	34.73***	49.25***	21.88***	31.01***	42.91***	34.5***	36.11***	24.83***	16.99*
RI	20.45***	2.49	21.31***	23.22***	23.77***	1.80	1.58	2.31	4.71
Interaction	9.66	13.23	29.73***	21.46**	11.07	20.32	15.52	28.94	31.93*
Error	35.16	35.03	27.08	24.31	22.25	43.38	46.79	43.92	46.38
CV (%)	53.35	28.52	49.78	64.25	37.58	45.75	50.15	35.09	37.63
Mean	277.61	4.74	1.79	0.62	168.70	2.14	8.52	3.52	1.85
SE	93.95	0.96	0.58	0.26	38.69	0.80	2.95	0.93	0.55

CV, coefficient of variation; SE, standard error; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 2. Overall evaluation of genitors and advanced selections for total phenols content (mg gallic acid equivalent (GAE) kg^{-1} of oil), and individual phenols content (mg kg^{-1} of oil), except dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA, mg of oleuropein kg^{-1} of oil)

Genotype	Total phenols	Hydroxytyrosol	Tyrosol	Vanillic acid	3,4-DHPEA-EDA
'Arbequina'	154.78 e (11)	4.18 bc (8)	1.45 bc (8)	1.33 a (1)	176.47 cd (5)
'Frantoio'	378.46 abc (3)	3.95 bc (10)	1.93 bc (3)	0.33 d (9)	139.12 cdefg (8)
'Picual'	216.05 de (7)	4.91b (3)	1.36 bc (10)	0.25 d (11)	85.30 g (12)
UC-I 2-68	178.99 de (10)	3.88 bc (11)	1.37 bc (9)	0.48 d (7)	187.63 c (3)
UC-I 4-62	249.87 cde (6)	3.99 bc (9)	1.12 c (12)	0.21 d (12)	104.37 efg (10)
UC-I 5-44	494.37 a (1)	7.23a (2)	1.90 bc (4)	1.03 ab (3)	334.74 a (1)
UC-I 6-9	210.81 de (8)	4.29 bc (6)	1.68 bc (7)	1.07 ab (2)	120.17 defg (9)
UC-I 7-8	131.19 e (12)	3.28c (12)	1.14 c (11)	0.45 d (8)	157.14 cdef (7)
UC-I 7-34	310.80 bcd (5)	7.46 a (1)	1.78 bc (6)	0.90 bc (4)	265.58 b (2)
UC-I 7-60	370.98 abc (4)	4.82 b (4)	3.54 a (1)	0.49 d (6)	177.42 cd (4)
UC-I 9-67	184.39 de (9)	4.22 bc (7)	2.24 b (2)	0.57 cd (5)	163.65 cde (6)
UC-I 10-30	438.26 ab (2)	4.66 bc (5)	1.85 bc (5)	0.32 d (10)	96.15 fg (11)

Genotype	*p*-Coumaric acid	*o*-Coumaric acid	Luteolin	Apigenin
'Arbequina'	2.79 ab (3)	10.25 b (3)	3.36 b (7)	1.88 abc (8)
'Frantoio'	1.69 cde (9)	8.66 bc (7)	3.43 b (6)	1.97 abc (4)
'Picual'	2.57 abc (5)	5.19 cd (10)	3.55 b (4)	1.98 abc (3)
UC-I 2-68	2.11 bcd (6)	8.90 b (6)	2.63 b (11)	1.25 c (12)
UC-I 4-62	1.30 de (11)	4.22 d (12)	3.19 b (8)	1.57 bc (10)
UC-I 5-44	2.01 bcd (7)	9.78 b (5)	5.01 a (1)	1.92 abc (7)
UC-I 6-9	3.10 a (2)	10.77 b (2)	3.91 ab (3)	1.92 abc (6)
UC-I 7-8	3.13 a (1)	6.90 bcd (8)	4.83 a (2)	2.46 a (1)
UC-I 7-34	1.57 de (10)	15.23 a (1)	3.18 b (9)	1.47 c (11)
UC-I 7-60	1.99 bcd (8)	6.87 bcd (9)	3.53 b (5)	2.30 ab (2)
UC-I 9-67	2.60 abc (4)	9.79 b (4)	3.00 b (10)	1.59 bc (9)
UC-I 10-30	0.91 e (12)	5.02 cd (11)	2.62 b (12)	1.97 abc (5)

Different letters in a column indicate significant differences (Duncan test, 0.05). Numbers in brackets indicate the ranking of each genotype for each component. 'Arbequina' × 'Picual': UC-I 6-9; UC-I 7-8; UC-I 9-67. 'Picual' × 'Arbequina': UC-I 2-68; UC-I 5-44; UC-I 7-34. 'Frantoio' × 'Picual': UC-I 4-62; UC-I 7-60; UC-I 10-30.

Table 3. Evolution along ripening of total phenols content (mg gallic acid equivalent (GAE) kg^{-1} of oil) in the advanced selections and genitors

Genotype	Total phenols				
	RI_0	RI_1	RI_2	RI_3	RI_4
'Arbequina'	182.96	197.38	132.84	95.61	–
'Frantoio'	–	744.47	305.31	318.57	198.76
'Picual'	–	–	279.18	193.69	167.83
UC–I 10–30	–	660.51	361.27	431.41	182.02
UC–I 2–68	220.12	160.64	113.96	152.94	–
UC–I 4–62	449.88	271.59	202.75	151.43	–
UC–I 5–44	633.51	491.14	396.36	380.73	–
UC–I 6–9	362.94	185.25	103.37	128.38	–
UC–I 7–34	–	425.74	553.61	204.34	104.96
UC–I 7–60	430.57	491.72	324.69	283.27	–
UC–I 7–8	–	246.82	109.44	60.07	62.91
UC–I 9–67	306.18	166.24	86.807	–	–
p	*	**	*	NS	NS

RI_0-RI_4, ripening index categories. Significant differences between genotypes in a common ripening category (column) are indicated (NS, non significant;*, $p < 0.05$; **, $p < 0.01$).

Table 4. Mean total phenols (mg gallic acid equivalent (GAE) kg^{-1} of oil), and mean tyrosol and vanillic acid contents (mg kg^{-1} of oil), and dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) contents (mg of oleuropein kg^{-1} of oil) by ripening indexes

Ripening index	Total phenols	Tyrosol	Vanillic acid	3,4-DHPEA-EDA
RI_0	359.18 a	2.86 a	1.29 a	259.7 a
RI_1	328.07 ab	2.05 b	0.76 b	208.24 b
RI_2	244.50 b	1.44 c	0.47 c	142.59 c
RI_3	247.15 b	1.45 c	0.34 cd	116.51 cd
RI_4	146.08 c	0.95 c	0.20 d	91.08 d

Different letters in a column indicate significant differences (Duncan test, 0.05); RI0-RI4, ripening index categories.

Table 5. Example of evolution of some individual phenols contents of different VOOs during fruit ripening

Genotype	Hydroxytyrosol					3,4-DHPEA-EDA				
	RI_0	RI_1	RI_2	RI_3	RI_4	RI_0	RI_1	RI_2	RI_3	RI_4
'Arbequina'	3.11	5.07	4.31	3.74	–	245.43	187.88	162.00	112.07	–
'Frantoio'	–	3.83	4.33	4.08	3.53	–	182.52	128.00	138.45	102.93
'Picual'	–	–	5.74	3.73	4.86	–	–	107.17	70.23	73.46
UC-I 10-30	–	3.77	4.75	4.46	7.25	–	133.47	104.03	82.95	71.75
UC-I 2-68	3.80	3.88	4.21	3.90	–	255.12	157.22	117.22	109.73	–
UC-I 4-62	3.52	4.01	4.11	3.95	–	168.64	108.13	87.41	92.92	–
UC-I 5-44	7.12	7.75	7.19	6.09	–	422.39	328.00	269.90	286.55	–
UC-I 6-9	4.36	4.14	3.89	4.82	–	205.88	106.96	63.25	68.35	–
UC-I 7-34		8.83	9.07	6.74	5.50	–	470.28	277.69	195.48	99.56
UC-I 7-60	6.14	4.38	4.60	4.39	–	232.35	277.92	121.98	132.87	–
UC-I 7-8	–	3.99	3.28	2.68	2.97	–	241.72	152.38	91.67	114.16
UC-I 9-67	5.00	4.47	3.11	–	–	215.05	154.53	124.39	–	–
p	*	*	***	NS	NS	NS	***	**	**	NS

RI_0-RI_4, ripening index categories. Significant differences between genotypes in a common ripening category (column) are indicated (NS, non significant;*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Table 6. Pearson correlations between all studied traits

	RI	Total phenols	Hydroxy-tyrosol	Tyrosol	Vanillic acid	3,4-DHPEA-EDA	*p*-Coumaric acid	*o*-Coumaric acid	Luteolin
RI	1								
Total phenols	–0.31***	1							
Hydroxytyrosol	NS	0.47***	1						
Tyrosol	–0.39***	0.44**	0.45***	1					
Vanillic acid	–0.50***	NS	0.31***	0.50***	1				
3,4-DHPEA-EDA	–0.53***	0.53***	0.55***	0.44	0.60***	1			
p-Coumaric acid	NS	NS	NS	NS	NS	NS	1		
o-Coumaric acid	NS	0.27**	0.52***	NS	0.25**	0.40***	0.24**	1	
Luteolin	NS	NS	NS	NS	NS	0.34***	0.57***	NS	1
Apigenin	NS	NS	NS	NS	NS	NS	0.54***	NS	0.59***

RI, ripening index; **, $p < 0.01$; ***, $p < 0.001$.

phenols content, tyrosol, vanillic acid and 3,4-DHPEA-EDA. Significant correlation coefficients were also found between hydroxytyrosol and total phenols, between hydroxytyrosol and tyrosol, and between tyrosol and vanillic acid.

Principal component analysis (PCA) showed that 62% of the total variance was associated to the first two principal components: the first component accounted for 36% of total variance and was positively correlated to hydroxytyrosol, tyrosol, vanillic acid, 3,4-DHPEA-

EDA and *o*-coumaric acid; the second component accounted for 26% of total variance and was mainly positively associated with apigenin, *p*-coumaric acid and luteolin. However, in spite of the relationship between RI and some of the analyzed components, no special grouping was found in the scores biplot of the PC1 and PC2 according to the RI of samples (data not shown).

Discussion

The comparison of phenolic profile between genotypes in an olive breeding program focused on improving VOO quality is of great importance, not only at initial selection but also in case of comparison between advanced selections, in order to attain a correct and effective selection of the genotypes with higher content in total and individual phenols. The results obtained in this study reveal that comparison of phenolic profile between genotypes across harvesting dates could be highly influenced by their different fruit ripening patterns. A previous study underlined the need for using objective criteria (such as olive fruits skin color) different from progressive sampling dates for a good estimation of fruit maturity, facilitating in this way the monitoring of the effect of olive ripening on the changes of the phenolic compounds in the VOO (Yousfi *et al.*, 2006).

On the other hand, the influence of the genotype seems to be greater than that of the ripening index and interaction on both total phenols content and individual phenols content, except for tyrosol, luteolin and apigenin where the interaction genotype × RI was the main contributor. This information constitutes an important aspect for determining the selection protocols in the olive breeding programs. These results are in agreement with other works showing that genetic variability is the main factor affecting phenolic composition (Montedoro & Garofolo, 1984; Brenes *et al.*, 1999). Conversely, Skevin *et al.* (2003) showed that ripeness of olive fruits exerts greater effect than cultivars on the total phenols content of olive oils. The differences among the selections evaluated in this work were significant only at the initial stages of ripening, when the phenolic content was higher; then, these differences smooth away at the end of the ripening process. As a consequence, for an effective selection of genotypes with an improved VOO phenolic composition in olive breeding programs, fruits must be harvested at the initial stages of ripening. The obtained values for total phenols content and for individual phenols content are comparable to those provided for the corresponding compounds by Brenes *et al.* (1999), Krichene *et al.* (2007), Tura *et al.* (2007), Baccouri *et al.* (2008), and Gómez-Rico *et al.* (2008) with some discrepancies probably originated from differences in the analytical methods or from genetic and other agronomic factors such as irrigation, pedo-climatic conditions, sanitary status of the fruits and/or alternate bearing. Also, the high variability among genotypes for phenolic composition was obtained in other studies (Gallina-Toschi *et al.,* 2005; Torres & Maestri, 2006; Krichene *et al.*, 2007). The overall evaluation of the advanced selections presented in Table 2 showed the particularly high content of total phenols and of the main individual phenols of the advanced selection 'UC-I 5-44'.

On the other hand, a significant decrease of total phenols content during ripening was previously reported (Skevin *et al.*, 2003; Rotondi *et al.*, 2004; Baccouri B. *et al.*, 2007). This decrease was most likely correlated with the increased activity of hydrolytic enzymes observed during ripening (Amiot *et al.*, 1989). Similar results have been obtained in this work, with a significant decrease in total phenols content during the ripening period (Table 4). However, other studies have reported an increase in total phenols content to a maximum level at the 'reddish' and 'black' pigmentation, the content decreasing drastically as ripening progressed (Salvador *et al.*, 2001; Conde *et al.*, 2008). A similar pattern of total phenols evolution during ripening was observed in some of the genotypes evaluated in this study ('UC-I 7-34', 'UC-I 7-60' and 'Arbequina'), in which a slight, not significant increase in total phenols content was observed at the initial stages of the ripening process (Table 3). Therefore, early harvesting should be recommended to obtain high content of phenolic compounds, although the evolution of other parameters such as total oil content, extractability, sensory properties and fruit detachment force should be also taken into account to determine the optimal harvesting period (García *et al.*, 1996; Beltrán *et al.*, 2004; Dag *et al.*, 2011).

The evolution of individual phenols throughout the ripening period of olive fruits has been studied by several authors with contradictory results. Results similar to those obtained in this work have been reported for 3,4-DHPEA-EDA (Brenes *et al.*, 1999; Bonoli *et al.*, 2004; Gallina-Toschi *et al.*, 2005; Yousfi *et al.*, 2006), *p*- and *o*-coumaric acids (Brenes *et al.*,

1999; Bonoli *et al.*, 2004), luteolin and apigenin content (Yousfi *et al.*, 2006). However, different patterns have also been reported on evolution of these compounds during ripening, namely: (a) Gómez-Rico *et al.* (2008) found a significant decrease of tyrosol content in 'Picolimon' cultivar, but a significant increase in the case of 'Picual' and 'Morisca'; (b) several works reported no changes in vanillic acid content during ripening progress (Brenes *et al.*, 1999; Bonoli *et al.*, 2004; Yousfi *et al.*, 2006); and (c) an increase in *o*-coumaric acid and luteolin during ripening has also been reported (Brenes *et al.*, 1999; Baccouri *et al.*, 2008).

The decrease in 3,4-DHPEA-EDA is generally accompanied by an increase in hydroxytyrosol content with ripening, probably due to gradual hydrolysis of oleuropein first to the aglycon form and then, to hydroxytyrosol (Brenes *et al.*, 1999; Baccouri *et al.*, 2008; Jemai *et al.*, 2009). However, other studies reported that both 3,4-DHPEA-EDA and hydroxytyrosol decreased along ripening (Morelló *et al.*, 2004; Damak *et al.*, 2008). This could be due to the fact that oleuropein, in irrigated conditions such as in this experiment, may disappear in the fruits and be transformed into phenolic oligomers, as described by Cardoso *et al.* (2006). These authors reported that the polymerization of oleuropein explained the decrease of its concentration with the formation of phenolic oligomers and, thus, the decrease in the hydroxytyrosol content during ripening. In this work, no significant differences in hydroxytyrosol content was found through the ripening period, although the content of 3,4-DHPEA-EDA decreases with ripening.

The results confirm the inverse relationships between RI and total phenols content, tyrosol, vanillic acid and 3,4-DHPEA-EDA. In addition, the relationships among phenolic compounds may be due to the same biosynthetic pathway of these compounds. Some of the correlations showed in this study were also found by Tura *et al.* (2007). The principal component analysis reflects the main correlation between the characters above mentioned. However, no special grouping either for genotypes or ripening index could be identified. This reflects the high variability for these components in any of the RI evaluated.

In summary, high differences in fruit ripening patterns have been observed between genotypes, which underlined the need for using objective criteria (*e.g.* RI instead of sampling dates) to compare phenolic profiles between genotypes. The results obtained indicate that genotype variance was the main contributor to total variance both for total phenols content and the main individual phenols studied, although the RI effect was significant in some cases. Also significant differences between genotypes were obtained for all the characters evaluated and clear pattern evolution was observed for some of them throughout the ripening process. Some selections with higher content of all examined phenolic compounds than the genitors were observed, which confirm the possibility of obtaining new cultivars with enhanced phenol concentration by cross breeding. It should be noted that, for all the evaluated characters, significant differences between genotypes were more clearly observed at the initial stages of ripening (RI_1 and RI_2, corresponding to 'yellowish' to 'reddish spots' peel color). These results improve our knowledge about the variation of phenolic compounds along fruit ripening and suggest a strategy based on early harvesting of fruits (at the first three ripening indexes) for better comparison and selection of genotypes in further crosses in olive breeding programs aiming at improving the quality of virgin olive oil.

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References

Alarcón de la Lastra C, Barranco MD, Motilova V, Herrerías JM, 2001. Mediterranean diet and health: biological importance of olive oil. Curr Pharm Design 7: 933-950.

Amiot MJ, Fleuriet A, Macheix JJ, 1989. Accumulation of oleuropein derivatives during maturation. Phytochemistry 28: 67-69.

Andrewes P, Busch JLHC, De Joode T, Groenewegen A, Alexandre H, 2003. Sensory properties of virgin olive oil polyphenols: identification of deacetoxy-ligstroside aglycons as a key contributor to pungency. J Agric Food Chem 51: 1415-1420.

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

Baccouri O, Cerretani L, Bendini A, Caboni MF, Zarrouk M, Pirrone L, Ben Miled DD, 2007. Preliminary chemical characterization of Tunisian monovarietal virgin olive oils and comparison with Sicilian ones. Eur J Lipid Sci Technol 109: 1208-1217.

Baccouri O, Guerfel M, Baccouri B, Cerretani L, Bendini A, Lercker G, Zarrouk M, Ben Miled DD, 2008. Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. Food Chem 109: 743-754.

Beltrán G, Del Rio C, Sánchez S, Martínez L, 2004. Seasonal changes in olive fruit characteristics and oil accumulation during ripening process. J Sci Food Agric 84: 131783-131790.

Beltrán G, Ruano MT, Jiménez A, Uceda M, Aguilera MP, 2007. Evaluation of virgin olive oil bitterness by total phenol content analysis. Eur J Lipid Sci Technol 108: 193-197.

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

Bianco A, Coccioli F, Guiso M, Marra C, 2001. The occurrence in olive oil of a new class of phenolic compounds: hydroxyl-isochromans. Food Chem 77: 405-411.

Bonoli M, Bendini A, Cerretani L, Lercker G, Gallina-Toschi T, 2004. Qualitative and semiquantitative analysis of phenolic compounds in extra virgin olive oils as a function of the ripening degree of olive fruits by different analytical techniques. J Agric Food Chem 52: 7026-7032.

Boskou D, 1996. Olive oil composition. In: Olive oil: Chemistry and technology; AOCS Press: Champaign, IL (USA). pp: 67-103.

Brenes M, García A, García P, Ríos JJ, Garrido A, 1999. Phenolic compounds in Spanish olive oil. J Agric Food Chem 47: 3535-3540.

Cardoso SM, Guyot S, Marnet N, Lopes-Da-Silva JA, Silva MSA, Renard CMGC, Coimbra MA, 2006. Identification of oleuropein oligomers in olive pulp and pomace. J Sci Food Agric 86: 1495-1502.

Conde C, Delrot S, Gerós H, 2008. Physiological, biochemical and molecular changes occurring during olive development and ripening. J Plant Physiol 165: 1545-1562.

Dag A, Kerem Z, Yogev N, Zipori I, Lavee S, Ben-David E, 2011. Influence of time of harvest and maturity index on olive oil yield and quality. Sci Horticult 127: 358-366.

Damak N, Bouaziz M, Ayadi M, Sayadi S, Damak M, 2008. Effect of the maturation process on the phenolic fractions, fatty acids, and antioxidant activity of the Chétoui olive fruit cultivar. J Agric Food Chem 56: 1560-1566.

De La Rosa R, León L, Moreno I, Barranco D, Rallo L, 2008. Ripening time and fruit characteristics of advanced olive selections for oil production. Austral J Agr Res 59: 46-51.

Frankel EN, 2010. Chemistry of extra virgin olive oil: adulteration, oxidative stability, and antioxidants. J Agric Food Chem 58: 5991-6006.

Frías L, García-Ortiz A, Hermoso M, Jiménez A, Llavero Del Pozo MP, Morales J *et al.*, 1991. Analistas de laboratorio de almazara. Junta de Andalucía, Sevilla. [In Spanish].

Gallina-Toschi T, Cerretani L, Bendini A, Bonoli-Carbognin M, Lercker G, 2005. Oxidative stability and phenolic content of virgin olive oil: an analytical approach by traditional and high resolution techniques. J Sep Sci 28 : 859-870.

García JM, Seller S, Pérez Camino MC, 1996. Influence of fruit ripening on olive oil quality. J Agric Food Chem 44: 113516-113520.

García-González DL, Aparicio R, 2010. Research in olive oil: challenges for the near future. J Agric Food Chem 58: 12569-12577.

García-González DL, Tena N, Aparicio R, 2010. Quality characterization of the new virgin olive oil var. 'Sikitita' by phenols and volatile compounds. J Agric Food Chem 58: 8357-8364.

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. J Agric Food Chem 56: 4577-4583.

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 Chem 100: 568-578.

Gómez-Rico A, Fregapane G, Salvador MD, 2008. Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. Food Res Int 41: 433-440.

Gómez-Rico A, Inarejos-García AM, Salvador MD, Fregapane G, 2009. Effect of malaxation conditions on phenol and volatile profiles in olive paste and the corresponding virgin olive oils (*Olea europaea* L. Cv. Cornicabra). J Agric Food Chem 57: 3587-3595.

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

Jemai H, Bouaziz M, Sayadi S, 2009. Phenolic composition, sugar contents and antioxidant activity of Tunisian sweet olive cultivar with regard to fruit ripening. J Agric Food Chem 57: 2961-2968.

Krichene D, Taamalli W, Daoud D, Salvador MD, Fregapane G, Zarrouk M, 2007. Phenolic compounds, tocopherols and other minor components in virgin olive oils of some Tunisian varieties. J Food Biochem 31: 179-194.

Lavee S, Wodner M, 1991. Factors affecting the nature of oil accumulation in fruit of olive. J Hort Sci 66: 583-591.

León L, Martín LM, Rallo L, 2004. Phenotypic correlations among agronomic traits in olive progenies. J Am Soc Hort Sci 129: 271-276.

León L, De La Rosa R, Barranco D, Rallo L, 2007. Breeding for early bearing in olive. HortScience 42: 499-502.

Montedoro GF, Garofolo L, 1984. Caratteristiche qualitative degli oli vergini di oliva. Influenza di alcune variabili: varietà, ambiente, conservazione, estrazione, condizionamento del prodotto finito. Riv It Sost Grasse LXI: 3-11. [In Italian].

Montedoro GF, Taticchi A, Esposto S, Selvaggini R, Urbani S, Servili M, 2007. Antioxidants in virgin olive oil (Review). Olea 26: 5-13.

Morelló JR, Romero MP, Motilva MJ, 2004. Effect of the maturation process of the olive fruit on the phenolic fraction of drupes and oils from Arbequina, Farga and Morrut cultivars. J Agric Food Chem 52: 6002-6009.

Morelló JR, Romero MP, Ramo T, Motilva MJ, 2005. Evaluation of L-phenyllanine ammonia-lyase activity and phenolic profile in olive drupe from fruit setting period to harvesting time. Plant Sci 168: 65-72.

Owen RW, Giacosa A, Hull WE, Haubner R, Wurtele G, Spiegelhalder B, Bartsch H, 2000. Olive-oil consumption and health: the possible role of antioxidants. Lancet Oncology 1: 107-112.

Ranalli A, Contento S, Schiavone C, Simone N, 2001. Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. Eur J Lipid Sci Technol 103: 228-238.

Ripa V, De Rose F, Caravita ML, Parise MR, Perri E, Rosati A, Pandolfi S, Paoletti A, Pannelli G, Padula G, Giordani E, Bellini E, Buccoliero A, Mennone C, 2008. Qualitative evaluation of olive oils from new olive selections and effects of genotype and environment on oil quality. Adv Hort Sci 22: 95-103.

Rotondi A, Bendini A, Cerretani L, Mari M, Lercker G, Gallina- Toschi T, 2004. Effect of olive ripening degree on the oxidative stability and organoleptic properties of cv. Nostrana di Brisighella extra virgin olive oil. J Agric Food Chem 52: 3649-3654.

Salvador MD, Aranda F, Fregapane G, 2001. Influence of fruit ripening on 'Cornicabra' virgin olive oil quality. A study of four successive crop seasons. Food Chem 73: 45-53.

Servili M, Baldioli M, Selvaggini R, Mariotti F, Federicci E, Montedoro GF, 1998. Effect of malaxation under N_2 flush on phenolic and volatile compounds of virgin olive oil. Proc. 13th Int Symp on Plant Lipids, Sevilla, Spain. pp: 307-310.

Servili M, Esposto S, Lodolini E, Selvaggini R, Taticchi A, Urbani S, Montedoro GF, Serravalle M, 2007. Irrigation effects on quality, phenolic composition, and selected volatiles of virgin olive oils Cv. Leccino. J Agric Food Chem 55: 6609-6618.

Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci F, Selvaggini R, Montedoro GF, 2009. Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure. Inflammopharmacology 17: 1-9.

Skevin D, Rade D, Strucelj D, Mokrovcak Z, Nederal S, Bencic D, 2003. The influence of variety and harvest time on the bitterness and phenolic compounds of olive oil. Eur J Lipid Sci Technol 105: 536-541.

Torres MM, Maestri DM, 2006. The effects of genotype and extraction methods on chemical composition of virgin olive oils from Traslasierra Valley (Córdoba, Argentina). Food Chem 96: 507-511.

Tripoli E, Giammanco M, Tabacchi G, Di Majo D, Giammanco S, De La Guardia M, 2005. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nut Res Rev 18: 98-112.

Tura D, Gigliotti C, Pedo S, Failla O, Bassi D, Serraiocco A, 2007. Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (*Olea europea* L.) and correlations with oxidative stability. Sci Horticult 112: 108-119.

Tura D, Failla O, Pedò S, Gigliotti C, Bassi D, Serraiocco A, 2008. Effects of seasonal weather variabilità on olive oil composition in northern Italy. Acta Hort 791: 769-776.

Yousfi K, Cert RM, García JM, 2006. Changes in quality and phenolic compounds of virgin olive oils during objectively described fruit maturation. Eur J Food Res Technol 223: 117-124.