Food Chemistry 243 (2018) 285–294

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of the composition of extra virgin olive oils on the differentiation and antioxidant capacities of twelve monovarietals

Edwar Fuentes[a], Fiorela Paucar[b], Francisco Tapia[c], Jaime Ortiz[b], Paula Jimenez[d], Nalda Romero[b,*]

[a] Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile
[b] Departamento de Ciencia de los Alimentos y Tecnología Química, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile
[c] Instituto de Investigaciones Agropecuarias (INIA Intihuasi), La Serena, Chile
[d] Departamento de Nutrición, Facultad de Medicina, Universidad de Chile, Santiago, Chile

ARTICLE INFO

Keywords:
Monovarietal virgin olive oil
Composition
Phenols
Classification
Antioxidant capacity
Multivariate analysis

ABSTRACT

The effect of the composition of twelve varieties of extra virgin olive oils (EVOOs) on their differentiation based in agronomic criteria and on the antioxidant capacity was studied. Principal component analysis permitted an overview of the samples and their compositions, showing evidence of grouping and correlation between antioxidant capacity, oleuropein and ligstroside derivatives (OLD) and specific extinction at 270. Oleic and linoleic acids, 3,4-DHPEA-EA and p-HPEA-EDA (OLD), unsaturated/saturated ratio and induction time (IT) allowed the correct classification of samples according to year of harvest, ripening stage and variety. The antioxidant capacity of EVOOs was satisfactory predicted through a partial least square model based on ΔK, hydroxytyrosol, pinoresinol, oleuropein derivate and IT. Validation of the model gave a correlation $R > 0.83$ and an error of 7% for independent samples. This model could be a useful tool for the olive industry to highlight the nutritional quality of EVOOs and improve their marketing.

1. Introduction

Adherence to the Mediterranean diet has been associated with longevity and with a reduced risk of morbidity and mortality. This has been attributed to diverse diet components that are thought to be associated with protective health effects. In this context, extra virgin olive oil (EVOO) plays an important role as the main source of fats in the diet (Servili et al., 2014).

There is ample scientific evidence showing that modulation of dietary fat composition affects blood-lipid concentrations. Regarding oleic acid, the main monounsaturated fatty acid in EVOO, the most noticeable effects have been demonstrated in studies where the substitution of saturated fat with oleic acid was tested. The isocaloric replacement of approximately 5% of the energy from saturated fatty acids by oleic acid has been estimated to reduce coronary heart disease risk by 20–40%, mainly via low-density lipoprotein (LDL)-cholesterol reduction. Other beneficial effects on risk factors for cardiovascular disease, such as factors related to thrombogenesis, in vitro LDL oxidative susceptibility and insulin sensitivity, have also been reported (Lopez-Huertas, 2010).

Several studies carried out in the last year have demonstrated that the beneficial effects should also be attributed to the olive phenols. The study of EVOO phenolic compounds has established that these substances show many health benefits, including the reduction of the risk factors of coronary heart disease, the prevention of several chronic diseases (for example, atherosclerosis), cancer, chronic inflammation, strokes and other degenerative diseases (Casaburi et al., 2013; Cicerale, Lucas, & Keast, 2012; López-Miranda et al., 2010; Servili et al., 2014).

EVOO presents a major fraction of triacylglycerides (oleic acid being the main fatty acid), representing more than 98% of the total weight; a minor fraction (approximately 2% of the weight) is composed of a complex set of compounds, including over 230 chemical compounds (aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds, phenols and pigments) (Barjol, 2013).

The main antioxidants in EVOO are represented by lipophilic and hydrophilic phenols, with the presence of a small amount of carotenoids. Alpha-tocopherol, a lipophilic phenol and primary antioxidant, is the main tocopherol in EVOO, with a wide concentration range (23–751 mg/kg) (Servili et al., 2014). Phenols, secondary plant metabolites, are the main antioxidant in EVOO and constitute a complex matrix of compounds where oleuropein and ligstroside derivatives (OLD) are the most abundant in many varietals. The concentration of

* Corresponding author.
E-mail address: nromero@uchile.cl (N. Romero).

http://dx.doi.org/10.1016/j.foodchem.2017.09.130
Received 24 March 2017; Received in revised form 22 September 2017; Accepted 26 September 2017
Available online 28 September 2017
0308-8146/

lipophilic and hydrophilic phenols is variable in the oils and depends on agronomic and climatic factors (Romero, Saavedra, Tapia, Sepúlveda, & Aparicio, 2015).

Antioxidants present in EVOO delay its autoxidation by inhibiting the formation of free radicals or by interrupting the propagation of free radicals by several mechanisms. The most effective antioxidants are those that interrupt the free radical chain reaction (Augusto, Dillenburg, De Souza, & Teixeira, 2015; Brewer, 2011). Methods commonly used to determine the total antioxidant capacity fall into two major groups: assays based on a single-electron transfer (SET), monitored through a change in colour as the oxidant is reduced (the degree of colour change is correlated with the sample's antioxidant concentrations), and assays based on a hydrogen atom transfer reaction (HAT), where the antioxidant and the substrate (probe) compete for the free radical. Among SET methods are the total phenols assay by Folin−Ciocalteu reagent (FCR) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging capacity assay. The most employed HAT method is the oxygen radical absorbance capacity (ORAC) assay (Augusto et al., 2015; Huang, Ou, & Prior, 2005).

Several studies have related EVOO phenols composition with the EVOO antioxidant capacity and oxidative stability (Angelino et al., 2011; Artajo, Romero, Morello, & Motilva, 2006; Augusto et al., 2015; Baldioli, Servili, Perretti, & Montedoro, 1996; Montaño, Hernández, Garrido, Llerena, & Espinosa, 2016; Paiva-Martins & Gordon, 2005; Ramos-Escudero, Morales, & Asuero, 2015); however, being a complex matrix, other components of the oil may be influencing the measurement of these properties. The objectives of this study were to investigate the influence of the composition of the EVOO on its differentiation based on agronomic variables such as year of harvest, variety and ripening stage and on its antioxidant capacity measured by DPPH· and $ORAC_{FL}$. Several chemometrics tools were used in a multivariate analysis approach to perform the study.

2. Materials and methods

2.1. Reagents

All reagents were either analytical or HPLC grade (Merck, Darmstadt, Germany). AAPH (2,2′-azobis (2-amidinopropane) dihydrochloride), DPPH· (2,2-diphenyl-1-picrylhydrazyl), FAME M RM-1 (methyl arachidate, methyl linoleate, methyl linolenate, methyl oleate, methyl palmitate, and methyl stearate) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The phenol standards (3-hydroxytyrosol, 2-(4-hydroxyphenyl) ethanol (tyrosol), *p*-coumaric acid, vanillic acid, vanillin, luteolin, apigenin, pinoresinol, *p*-hydroxyphenylacetic acid (internal standard 1), *o*-coumaric acid (internal standard 2) and oleuropein) were also obtained from Sigma-Aldrich. Tocopherol standards were purchased from Calbiochem (Merck). All standards had a purity of 98% or higher.

2.2. Plant material

The germplasm bank of the Huasco Experimental Center of the Instituto de Investigaciones Agropecuarias located in the north of Chile (Atacama, III Region; latitude 28° 34′ 45″ S and longitude 70° 47′ 52″ W, at 453 m above sea level), was created in the year 2000, incorporating 36 different varieties of olive trees used commercially. The trees are distributed at distances of 7 × 7 m, with 5 specimens per variety, randomly distributed. Driven to an axis and irrigated by drip system according to the reference evapotranspirative demand, registered in automatic meteorological station.

Rainfall in the periods 2013–2014 and 2014–2015 was 32.7 and 77.7 mm, respectively, concentrated in winter and being the rest of the seasons dry with no rainfall. The maximum and minimum temperatures in these two periods were 21.6 and 8.9 °C and 22.2 and 9.1 °C, respectively. The olive trees were irrigated according to the reference evapotranspirative demand (ETo) with 856 mm/year in the 2013–2014 season and 1143 mm/year in the 2014–2015 season.

Twelve monovarietals (Arbequina, Arbequina I18, Ascolana de Huasco, Coratina, Kalamata, Koroneiki, Leccino, Manzanilla Chilena, Nocellara del Bélice, Oliva di Cerignola, Picual and Sevillana) from the germplasm bank were sampled during the 2014 and 2015 harvests. From the 2014 harvest, one sample of each variety, all in ripening stage 3-4, were obtained. From the 2015 harvest, three samples by variety in ripening stages 2-3 for 'Coratina', 'Koroneiki' and 'Sevillana'; 4-5 for 'Kalamata'; and 3-4 for the remaining varieties. Only two samples of 'Manzanilla Chilena' were obtained in the 2015 harvest, and no samples from 'Leccino' or 'Ascolana de Huasco' were available. The ripening stage of the fruits harvested was defined based on the coloration of the skin of the fruit, determined visually, according to methodology of the Index of Ferreira, that goes from class 0 (fruit of green skin), to class 7, in that the fruit has black skin and flesh (Uceda & Hermoso, 2001).

At each harvest, 10 kg of olives (experimental unit) was handpicked from the middle portions of three randomly selected trees; the olives were mixed prior to extracting the oil. Only healthy fruits, without any sign of infection or physical damage, were used. In total, 41 samples of EVOO were processed.

2.3. Olive oil extraction

Olive oils were collected at olive mills where olives were processed using Frantoino model Monoblock extraction equipment (Toscana Enologica Mori, Florence, Italy) with a two-phase centrifugation system. The fresh olives (10 kg) were crushed and then slowly mixed for 30 min at 26 ± 2 °C. The resulting paste was centrifuged at 1027*g* for 5 min to separate the oil. All samples were subsequently filtered through hydrophilic cotton, placed in amber glass bottles and stored in the dark at −23 °C until analysis (within 1 month). The samples were analysed in triplicate using the chemical analytical methods described below. All of the olive oils were extra virgin according to official analytical methods and limits (free acidity ≤0.8% in oleic acid, $K_{232} \leq 2.50$, $K_{270} \leq 0.22$, $\Delta K \leq 0.01$; IOOC, 2015).

2.4. Quality parameters

Free fatty acids (Ca 5a-40), peroxide value (Cd 8-53), and specific extinctions of oils (K_{232}, K_{270}, ΔK) (Ch 5-91) were determined according to American Oil Chemists's Society (1993).

2.5. Fatty acid composition

Fatty acids were transformed into methyl esters using potassium hydroxide in methanol, according to International Olive Council (IOOC, 2001), and analysed by gas chromatography (GLC) using an HP 5890 chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with an FID detector. A BPX70 fused silica capillary column (50 m, 0.25 μm film; SGE, Incorporated, Austin, TX, USA) was used. The temperature was programmed between 160 °C and 230 °C at 2 °C/min, and 0.5 μL samples were run with hydrogen as the carrier gas. The injection was carried out in split mode. Standard fatty acid methyl esters (FAME) from Sigma-Aldrich Co. (St. Louis, MO, USA) were used for identification purposes.

2.6. Determination of phenolic compounds

A standard solution (0.5 mL) of *p*-hydroxyphenylacetic (0.12 mg/mL) and *o*-coumaric (0.01 mg/mL) acids in methanol was added to the EVOO (2.5 g). The phenolic compounds were isolated by solid-phase extraction using a Waters diol-bonded phase cartridge (Milford, MA, USA) and analysed by reverse phase HPLC using a Waters HPLC system equipped with a binary pump (model 1525), a diode array UV detector (model 2998), an autosampler (model 2707) and a Waters Spherisorb

ODS RP-18 column (4.6 mm i.d. × 250 mm; 5 μm particle size). Phenols were identified using Sigma standards and an Agilent 1100 HPLC-MS (Agilent Technologies Inc., California, USA) system coupled to an electrospray Esquire 4000 ion trap ESI-IT mass spectrometer (Bruker Daltonik GmbH, Germany) and comparing the acquired absorbance spectra to those in the literature (IOOC, 2009; Mateos et al., 2001). The quantification of phenolic compounds other than flavones and ferulic acid was carried out at 280 nm using *p*-hydroxyphenylacetic acid as an internal standard, while flavones (luteolin and apigenin) and ferulic acid were quantified at 335 nm using *o*-coumaric acid as an internal standard. The recovery and response factors were obtained from a previous study (Mateos et al., 2001). The results were expressed in mg/kg.

2.7. Folin-Ciocalteau's reducing capacity

Phenolic compounds were extracted according to IOOC (2009). EVOO (2.5 g) was weighed into a 10 mL screw-cap tube, and 5 mL of a methanol/water (80/20) mixture was added. The mixture was vortexed for 30 s and sonicated for 15 min, and then, it was centrifuged at 1027*g* for 25 min. The methanol/water extract containing the phenolic compounds was separated. The total phenolic content was determined spectrophotometrically in methanol/water following the Folin–Ciocalteau colourimetric method, as modified by Chun, Vattem, Lin, and Shetty (2005). The optical density of the resulting blue solution was measured at 725 nm using an ATI Unicam model UV3-200 UV/vis spectrophotometer (UNICAM, Cambridge, UK), and the results were expressed as micrograms of caffeic acid equivalents (μg CAE) per g of oil. The calibration curve was constructed using standard solutions of caffeic acid (Sigma Chemicals Co.) from 50 to 500 μg/mL (R^2 = 0.9964).

2.8. Hydrophilic orac assay (H-ORAC$_{FL}$)

H-ORAC$_{FL}$ assays were performed according to those described by Prior et al. (2003) with some modification. A stock fluorescein (FL) solution (Stock #1) was prepared by dissolving 0.0220 g of FL in 50 mL of 0.075 M phosphate buffer (pH 7.0). A second stock solution was prepared by diluting 50 μL of stock solution #1 in 10 mL of phosphate buffer. A 2 mL portion of solution #2 was added to 100 mL of phosphate buffer (solution #3).

A stock standard of Trolox (2000 μM) was aliquoted into small vials for storage at −70 °C until use. In the standard assay, 25 μL Trolox calibration solutions (12.5, 25, 50, and 100 μM) in phosphate buffer (0.075 M, pH 7.0) were pipetted into appropriate wells. A new set of stock Trolox vials was removed from the freezer daily for use. Studies were completed using a 96-well black plate in which excitation (485 nm)/emission (528) was from the top of the plate. Phosphate buffer (pH 7) was used as the blank to dissolve the Trolox and to prepare the samples (dilution).

To conduct the H-ORAC$_{FL}$ assay, 25 μL of the diluted methanol/water extract containing phenolic compounds and 150 μL of FL solution #3 were added to the 96-well black plate. The microplate was equilibrated (30 min, 37 °C), and then, the reaction was initiated by the addition of 25 μL (150 mM) of AAPH (2,2′-azobis (2-amidino-propane) dihydrochloride); readings were obtained immediately in an FLx800-TBID fluorescence reader (Biotek, Winooski, VT, USA). The antioxidant capacity was expressed as μmol Trolox equivalent (TE)/*g* oil.

2.9. DPPH· assay

The antiradical capacity of the extract containing the phenolic compounds was measured by the DPPH· (2,2-diphenyl-1-picrylhydrazyl) assay in agreement with Brand-Williams, Cuvelier, and Berset (1995). Measurements were carried out at 517 nm using an ATI Unicam model UV3-200 UV/vis spectrophotometer, and the results were expressed as the effective quantity of oil that neutralized the DPPH· radical at 50% (IC_{50}).

2.10. Tocopherol content

Tocopherols were determined by high-performance liquid chromatography (HPLC) according to the AOCS standard method Ce 8-89 (1993). A LiChro-CART Superspher Si 60 column (25 cm × 4 mm id, 5 μm particle size; Merck, Darmstadt, Germany) was used. The mobile phase was propan-2-ol in hexane (0.5/99.5, v/v) at a flow rate of 1 mL/min. The HPLC system consisted of a Merck–Hitachi L-6200A pump (Merck, Darmstadt, Germany), a Rheodyne 7725i injector with 20-μl sample loop, a Hitachi Chromaster 5440 fluorescence detector and a Merck–Hitachi D-2500 chromato-integrator. Peaks were detected at 290 nm and 330 nm, the excitation and emission wavelengths, respectively. Tocols were identified and quantified using Calbiochem tocopherols and tocotrienols (Merck, Darmstadt, Germany) as external standards.

2.11. Oxidative stability

The induction time (IT) was determined using a Rancimat Oxidative Stability Instrument (Metrohm Ltd, Herisan, Switzerland) at 110 °C and an air flow of 20 L/h according to the AOCS standard method Cd 12b-92 (1993).

2.12. Statistical analysis

The results are presented as mean ± standard deviation. The data were statistically analyzed using an unpaired Student's *t*-test and one-way ANOVA to compare the means and a Mann-Whitney test to compare the medians. In all the test p values lower than 0.05 were considered significant. The analyses were performed using the Statgraphic XV software (Rockville, MD, USA). The multivariate general characterization of the samples, considering all the physicochemical parameters determined, was performed by principal component analysis (PCA) using The Unscrambler software (CAMO PROCESS AS, Oslo, Norway). The discrimination by year of harvest, ripening stage and variety was performed by linear discriminant analysis (LDA) with forward selection of parameters using Statgraphics Centurion XV software (StatPoint Inc., Rockville, Maryland, USA). Additionally, partial least squares (PLS) was selected to build a model using The Unscrambler software to predict the antioxidant activity (H-ORAC$_{FL}$, μmol·TE/g oil) based on the physicochemical parameters. The optimum number of factors to be used within the PLS regression was determined through a full cross-validation procedure, which consists of systematically removing one of the training samples, in turn, and using only the remaining ones for construction of the latent factors and/or regression coefficients. All data were previously centred and standardized.

3. Results and discussion

3.1. Chemical characterization of the varieties

Different chemical compounds were determined in EVOO to identify which of these compounds influence on the differentiation of oils by agronomic variables, such as year of harvest, variety and ripening stage and how the composition of EVOO is related with its antioxidant capacity.

Table 1 shows the composition of fatty acids of the twelve monovarietals of EVOO at harvest. The main fatty acids in EVOO are palmitic, oleic and linoleic acids. Palmitic acid, a saturated fatty acid, presented values between 8.6 and 14.5% in 2014 harvest and between 8.7 and 15.6% in 2015 harvest, with 'Kalamata' being the variety with the highest level at this harvest. All the oils were majority monounsaturated with high values of oleic acid ranging between 68.9 and

Table 1
Fatty acid compositions of twelve varieties of EVOO for two harvest seasons.

Fatty acid	Harvest	Arbequina	Arbequina	Ascolana	Coratina	Kalamata	Koroneiki	Leccino	Manzanilla	Nocellara	Oliva di	Picual	Sevillana
			I-18	Huasco					Chilena	del Belice	Cerignola		
(% methyl esters)													
Palmitic	2014	13.1	12.1	14.3	8.6	14.5	10.4	12.6	13.2	9.3	10.9	11.3	14.3
(C16:0)	2015	13.9 ± 0.1^{e}	13.7 ± 0.6^{e}	–	8.70 ± 0.02^{a}	15.6 ± 0.1^{f}	10.60 ± 0.04^{b}	–	11.9 ± 0.2^{cd}	8.50 ± 0.04^{a}	12.2 ± 0.1^{d}	11.50 ± 0.03^{c}	14.2 ± 0.4^{e}
Palmitoleic	2014	0.9	0.8	1.0	0.2	1.7	0.6	1.0	1.1	0.5	0.6	0.9	1.4
(C16:1ω9)	2015	1.20 ± 0.01^{f}	1.1 ± 0.1^{e}		0.30 ± 0.05^{a}	2.20 ± 0.06^{g}	0.60 ± 0.03^{c}		1.20 ± 0.04^{ef}	0.40 ± 0.03^{b}	0.70 ± 0.01^{d}	0.70 ± 0.03^{d}	1.10 ± 0.03^{ef}
Margaric	2014	0.1	0.1	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND
(C17:0)	2015	0.09 ± 0.03^{ab}	0.10 ± 0.01^{ab}	–	0.10 ± 0.05^{ab}	ND^{ab}	ND^{a}	–	ND^{a}	ND^{a}	0.20 ± 0.05^{b}	ND^{a}	0.1 ± 0.1^{ab}
Margaroleic	2014	0.2	0.2	0.1	0.1	ND	ND	0.1	0.1	0.1	0.2	0.1	0
(C17:1ω8)	2015	0.20 ± 0.03^{b}	0.20 ± 0.01^{b}		0.10 ± 0.03^{a}	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}		0.10 ± 0.04^{a}	0.09 ± 0.01^{a}	0.20 ± 0.03^{b}	0.10 ± 0.03^{a}	0.10 ± 0.01^{a}
Stearic	2014	2.1	2.1	2.3	2.4	2.5	2.6	2.2	2.2	2.2	3.3	2.8	3.0
(C18:0)	2015	2.0 ± 0.1^{a}	2.0 ± 0.1^{a}	–	2.50 ± 0.03^{c}	2.2 ± 0.1^{b}	2.50 ± 0.03^{c}	–	2.30 ± 0.04^{b}	2.50 ± 0.01^{c}	3.00 ± 0.03^{d}	3.00 ± 0.03^{d}	3.6 ± 0.1^{e}
Oleic	2014	75.3	76.7	74.1	82.2	74.8	80.8	78.6	78.0	81.4	73.4	80.8	68.9
(C18:1 ω9 + ω7)	2015	71.2 ± 0.1^{d}	70.9 ± 0.5^{d}		80.9 ± 0.1^{h}	68.7 ± 0.1^{b}	79.2 ± 0.1^{g}		77.7 ± 0.1^{f}	76.90 ± 0.03^{e}	67.9 ± 0.1^{a}	79.00 ± 0.01^{g}	69.6 ± 0.1^{c}
Linoleic	2014	7.1	6.6	6.8	4.9	5.2	4.1	4.5	4.3	5.2	10.2	3.2	10.9
(C18:2ω6)	2015	10.3 ± 0.1^{d}	10.8 ± 0.1^{e}	–	5.70 ± 0.03^{b}	10.0 ± 0.1^{cd}	5.30 ± 0.01^{b}		5.60 ± 0.01^{b}	10.20 ± 0.04^{d}	14.2 ± 0.1^{f}	4.20 ± 0.01^{a}	9.6 ± 0.4^{c}
Linolenic	2014	0.3	0.3	0.3	0.4	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2
(C18:3ω3)	2015	0.20 ± 0.01^{b}	0.20 ± 0.03^{b}		ND^{a}	0.10 ± 0.06^{b}	ND^{a}		ND^{a}	ND ^{a}	0.02 ± 0.05^{b}	ND^{a}	0.20 ± 0.01^{b}
Arachidic	2014	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3
(C20:0)	2015	0.40 ± 0.01^{abc}	0.40 ± 0.03^{bc}	–	0.40 ± 0.03^{c}	0.30 ± 0.03^{ab}	0.40 ± 0.03^{c}	–	0.30 ± 0.01^{ab}	0.30 ± 0.01^{a}	0.40 ± 0.03^{c}	0.40 ± 0.01^{c}	0.60 ± 0.06^{d}
Gadoleic	2014	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
(C20:1ω9)	2015	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}		0.10 ± 0.01^{a}	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}		0.10 ± 0.07^{a}	0.09 ± 0.01^{a}	0.10 ± 0.03^{a}	0.10 ± 0.01^{a}	0.20 ± 0.03^{b}
Behenic	2014	0.5	0.6	0.7	0.7	0.6	0.6	0.5	0.5	0.6	0.7	0.5	0.6
(C22:0)	2015	0.40 ± 0.01^{a}	0.40 ± 0.01^{a}	–	1.10 ± 0.06^{f}	0.50 ± 0.03^{b}	0.90 ± 0.01^{e}	–	0.80 ± 0.01^{d}	0.80 ± 0.01^{d}	0.80 ± 0.01^{d}	0.90 ± 0.01^{e}	0.70 ± 0.05^{c}
UNSFA/SATFA	2014	5.2	5.6	4.7	7.3	4.6	6.1	5.4	5.2	7.1	5.5	5.8	4.4
	2015	5.00 ± 0.01^{b}	5.0 ± 0.2^{b}	–	6.80 ± 0.05^{e}	4.40 ± 0.01^{a}	5.90 ± 0.02^{d}	0.02	5.5 ± 0.1^{c}	7.20 ± 0.02^{f}	5.0 ± 0.1^{b}	5.30 ± 0.01^{c}	4.2 ± 0.2^{a}

2015 values are expressed as mean ± SD, (n = 3). Different letters mean significant differences between varieties ($p < 0.05$). UNSFA: unsaturated fatty acids; SATFA: saturated fatty acids. ND: Non detected.

Table 2
Concentrations of total phenols and their principal families (mg/kg oil) quantified in twelve varieties of EVOO.

Variety	Harvest	Simple phenols	OLD	Lignans	Flavonoids	Total phenols[A]	Total polyphenols[B] (CAE)
Arbequina	2	62.0	8.5	0.01	14.1	85	75
Arbequina I18	0	62.2	26.1	7.16	7.99	104	101
Ascolana Huasco	1	63.3	27.1	1.54	6.67	99	121
Coratina	4	62.2	40.0	6.91	5.06	116	158
Kalamata		102.6	216.7	14.4	32.1	371	251
Koroneiki		97.0	87.0	9.08	16.6	210	190
Leccino		42.8	66.5	3.05	12.8	125	97
Manzanilla Chilena		45.9	154.8	3.59	10.3	215	138
Nocellara del Belice		81.9	47.1	2.93	5.34	140	138
Oliva di Cerignola		34.7	138.9	20.5	5.57	200	146
Picual		64.0	156.4	5.93	13.4	240	214
Sevillana		80.7	483.9	0.0	33.4	598	383
Arbequina	2	$60^{bc} \pm 8$	$179^{a} \pm 7$	$19^{f} \pm 4$	$29^{d} \pm 3$	$288^{a} \pm 3$	$182^{ab} \pm 22$
Arbequina I18	0	$42^{ab} \pm 25$	$78^{a} \pm 8$	$12^{de} \pm 5$	$16^{bcd} \pm 14$	$149^{a} \pm 68$	$155^{a} \pm 16$
Coratina	1	$52^{abc} \pm 6$	$192^{a} \pm 7$	$13^{def} \pm 2$	$9^{abc} \pm 1$	$266^{a} \pm 34$	$251^{bc} \pm 10$
Kalamata	5	$96^{d} \pm 6$	$570^{b} \pm 92$	$11^{cde} \pm 1$	$16^{abcd} \pm 3$	$694^{b} \pm 139$	$368^{d} \pm 54$
Koroneiki		$36^{ab} \pm 1$	$146^{a} \pm 2$	$8.40^{bcd} \pm 0.04$	$13.4^{abc} \pm 0.1$	$204^{a} \pm 4$	$244^{abc} \pm 15$
Manzanilla Chilena		$38^{ab} \pm 4$	$252^{a} \pm 2$	$9.2^{bcd} \pm 0.2$	$17.3^{abcd} \pm 0.4$	$317^{a} \pm 10$	$216^{abc} \pm 16$
Nocellara del Belice		$71^{cd} \pm 2$	$64.0^{a} \pm 0.1$	$4.20^{ab} \pm 0.04$	$2.00^{a} \pm 0.01$	$142^{a} \pm 1$	$206^{ab} \pm 9$
Oliva di Cerignola		$30.6^{a} \pm 0.3$	$158.5^{a} \pm 0.2$	$17.5^{ef} \pm 0.4$	$3.90^{ab} \pm 0.04$	$211^{a} \pm 4$	$229^{abc} \pm 20$
Picual		$48.8^{abc} \pm 0.3$	$249^{a} \pm 3$	$5.10^{abc} \pm 0.02$	$13.50^{abc} \pm 0.02$	$317^{a} \pm 9$	$321^{cd} \pm 12$
Sevillana		$69^{c} \pm 5$	$953^{b} \pm 30$	ND	$19^{cd} \pm 4$	$1041^{b} \pm 197$	$588^{e} \pm 70$

Notes:
[A] Sum of phenols determined by HPLC method.
[B] Determined by Folin-Ciocalteau's reducing capacity. CAE: Caffeic acid equivalents. OLD: Oleuropein ligstroside derivatives. 2015 values are expressed as mean ± SD, (n = 3). Different letters mean significant differences between varieties ($p < 0.05$). ND: non detected.

82.2% in 2014 harvest and between 67.9 and 80.9% in 2015 harvest, with 'Coratina' being the variety with the highest concentration in last harvest. Linoleic acid presented low concentrations in most varieties, especially in the 2014 harvest, with range between 3.2 and 10.9%. In 2015 harvest, the range was between 4.2 and 14.2%, with Picual and Oliva di Cerignola being the varieties with the lowest and the highest values for this fatty acid, respectively. The variation of concentration of linoleic acid between harvests will be discussed later. In 2015 harvest, the varieties presented significant differences for the different fatty acids ($p < 0.05$). The ratio unsaturated fatty acids/saturated fatty acids (UNSFA/SATFA) presented ranges of 4.4 to 7.3 and 4.2 to 7.2 for 2014 and 2015 harvests, respectively, with Nocellara del Belice being the variety with the highest value for this parameter in 2015 harvest.

Table 2 presents the composition of the principal families of phenolic compounds in the twelve varieties of EVOO by harvest. Simple phenols and OLD were the major compounds in all varieties. The results also indicated a broad range of phenolic contents. Simple phenols presented ranges between 42.8 and 102.6 mg/kg of oil in 2014 harvest and between 30.6 and 95.8 mg/kg of oil in 2015 harvest, with Oliva di Cerignola and Kalamata being the varieties with the lowest and the highest values of simple phenols, in the last harvest.'Sevillana' and 'Kalamata' were the varieties with the highest values of OLD and total phenols in 2015 harvest, showing 953 and 570 mg/kg of oil of OLD and 1041 and 694 mg/kg of oil of total phenols, respectively. The 2014 harvest exhibited ranges of 8.5 to 483.9 mg/kg of oil of OLD and ranges of 85 to 598 mg/kg of oil of total phenols. In 2015 harvest, 'Arbequina' showed a value of lignans of 19.3 mg/kg of oil presenting significant differences ($p < 0.05$) with the most of the varieties, with the exception of Coratina and Oliva di Cerignola. Flavonoids presented ranges between 5.3 and 33.4 mg/kg of oil in 2014 harvest and between 2.0 and 28.9 mg/kg of oil in 2015 harvest. Significant differences were observed between varieties for all phenol families ($p < 0.05$). A colorimetric assay based on the reaction of the Folin-Ciocalteu reagent with the functional hydroxy groups of the phenolics was included in this study for the possibility of having an easy and fast method for the quantification of total phenols and so that they could be correlated with the antioxidant capacity. The differences in the values of total phenols determined by HPLC methods and colorimetric method could be explained by the different form of expression of the results (Hrncirik & Fritsche, 2004). The data generated by the HPLC method are given by the sum of each individual phenol, whereas the results of the colourimetric method are expressed in caffeic acid equivalents. In addition, the Folin-Ciocalteau reagent reacts in different manners with the different phenols of EVOO depending on their composition and number of reactive hydroxy groups (Singleton, Orthofer, & Lamuela-Raventós, 1999). Thus, samples with comparable amounts of total phenols, but considerably varying phenolic compositions, will give different responses in the colourimetric method (Hrncirik & Fritsche, 2004). A comparison between the HPLC method and the traditional colourimetric assay (Folin-Ciocalteu method) in 23 samples of virgin olive oils showed a strong correlation between both methods ($R = 0.97$), suggesting that the colourimetric assay is reasonably valid for a rough prediction of the total phenolic content (Hrncirik & Fritsche, 2004).

The results presented in Table 3 complement the results discussed above, showing the type of phenols present in EVOO. Table 3 highlights the levels of the oleuropein derivatives 3,4-DHPEA-EDA-DOA (dialdehydic form of oleuropein aglycone), 3,4-DHPEA-EDA (dialdehydic form of decarboxymethyl oleuropein aglycon) and 3,4-DHPEA-EA (oleuropein aglycon) and the ligstroside derivative p-HPEA-EDA (dialdehydic form of decarboxymethyl ligstroside aglycon) as well as elenolic acid in both harvests, distinguishing the higher values of p-coumaric acid, pinoresinol, 3,4-DHPEA-EDA and total phenols in the 2015 harvest ($p < 0.05$).

Table 4 shows the alpha-tocopherol content and the antioxidant capacity measured by H-ORAC$_{FL}$, DPPH· and IT in the twelve monovarieties of EVOO. The varieties presented a range of alpha-tocopherol from 68 to 192 mg/kg (2014) and 37 to 135 mg/kg (2015), with 'Kalamata', 'Manzanilla Chilena', 'Picual', 'Arbequina' and 'Coratina' having the highest concentrations in 2015 harvest. In terms of antioxidant capacity, 'Picual' and 'Sevillana' had the highest values of H-ORAC$_{FL}$ in 2015 harvests, with values of 18.3 and 18.1 μmol TE/g oil, respectively. For DPPH·, 'Sevillana' 'Kalamata' and 'Picual' showed higher antioxidant activity than 'Arbequina I18', 'Nocellara del Belice',

Table 3
Concentration of phenolic compounds (mg/kg) quantified in EVOOs in two harvest seasons, regardless of cultivar.

Compounds	Harvest		p-values *
	2014	2015	
Hydroxityrosol	2 ± 1	1.8 ± 0.9	0.100
Tyrosol	3 ± 4	2 ± 1	0.105
Vanillic acid	0.2 ± 0.1	0.2 ± 0.1	0.841
p-coumaric acid	0.2 ± 0.4	0.5 ± 0.4	0.001
Elenolic acid	61 ± 19	50 ± 19	0.132
Pinoresinol[a]	6 ± 6	10 ± 6	0.048
3,4-DHPEA-EDA[b]	24 ± 25	64 ± 72	0.009
3,4-DHPEA-EDA-DOA[c]	46 ± 62	100 ± 135	0.132
p-HPEA-EDA[d]	25 ± 25	77 ± 131	0.148
p-HPEA-EDA-DLA[e]	4 ± 8	17 ± 32	0.314
Luteoline	7 ± 5	9 ± 5	0.519
3,4-DHPEA-EA[f]	22 ± 34	44 ± 79	0.405
Apigenine	6 ± 4	4 ± 2	0.807
Methyl luteoline	0.8 ± 0.7	1 ± 1	0.414
Total phenols	209 ± 147	383 ± 319	0.020

Values are mean ± SD (n = 41).
[a] mixed with 1-acetoxy-pinoresinol.
[b] 3,4-DHPEA-EDA: dialdehydic form of decarboxymethyl oleuropein aglycon.
[c] 3,4-DHPEA-EDA-DOA: dialdehydic form of oleouropein aglycon.
[d] p-HPEA-EDA: dialdehydic form of decarboxymethyl ligstroside aglycon.
[e] p-HPEA-EDA-DLA: dialdehydic form of ligstroside aglycon.
[f] 3,4-DHPEA-EA: Oleuropein aglycon. ND: Non detected.
* Mann-Whitney test to compare the medians of the two harvest period.

and 'Coratina'. These results indicate that polar compounds from EVOO, mostly phenolic compounds, can react by hydrogen atom transfer (HAT) or single-electron transfer (SET) mechanisms, measured by the $ORAC_{FL}$ and DPPH· methods, respectively (Augusto et al., 2015).

The IT, which measures the resistance of the oil to forced oxidation, showed 'Picual' to be the variety with the highest resistance and therefore the most stable EVOO, with values close to 60 h at 110 °C. This resistance would be due to a high value of oleic acid, low values of linoleic and linolenic acids, moderate levels of phenols and alpha-tocopherol with a high antioxidant capacity. Similar results were reported by Montaño et al. (2016). The oil's stability is provided by the interaction of several variables, including the oil chemical quality, the fatty acid composition and the type and the quantity of antioxidants.

3.2. Multivariate general characterization of samples by PCA

A matrix array (samples in rows and parameters in columns) was built to perform a multivariate characterization of samples by PCA and to investigate possible correlations between the composition and the antioxidant activity of EVOOs. According to cross-validation, 10 components were necessary to explain the variability of the data. However, the first three components accounted for 56% of the variability. Fig. 1A shows the loading plot with the relationships between the oil components and the antioxidant activity indicators. Total phenols (determined by HPLC) appear closely related to their individual components such as OLD and HT (hydroxytyrosol) (lower left quadrant). The same quadrant also shows the antioxidant activity by the H-$ORAC_{FL}$ method (μmol TE/g oil) linked to individual phenolic compounds such as p-HPEA-EDA-DLA (dialdehydic form of ligstroside aglycon), 3,4-DHPEA-EDA-DOA, 3,4-DHPEA-EA and HT. In the diagonally opposite quadrant (right upper), DPPH· (IC_{50} mg oil) indicates the inverse relationship that exists between the two methods to determine the antioxidant activity. However, the oxidative stability measured by induction time (hr) is related to the antioxidant activity, some fatty acids such as stearic acid (C18:0) and gadoleic acid (C20:1), and the K_{270} parameter of chemical quality.

The graphics of the scores labelled by year of harvest, variety and ripening stage for the first three components obtained from this analysis are presented in Fig. 1B, C and D, respectively. In the case of year of harvest, grouping into the two campaigns was defined mainly by the first and third components. These components are represented predominantly by the oleic acid content in the oils, which was the differentiating factor between crop years. For the ripening, a grouping associated with the three stages was observed, but with a significant dispersion in the quadrants of the score plot. This indicates that there is a set of components in the olives that vary simultaneously during ripening; this is reflected in the composition of the oil and the distribution of the samples. For the varieties, despite the multiple varieties included in the analysis, a relatively homogeneous group for each one was described in space by the first three components. The exceptions were 'Arbequina' and 'Arbequina I18', which appear as a unique group. These results showed that the chemical composition of oils allowed a differentiation by year of harvest, ripening stage and variety, in addition to the possibility of modelling and predicting the antioxidant activity of EVOO as a function of certain physicochemical parameters.

Similar behaviour has also been observed in previous studies, where the compositions of virgin olive oils allowed discrimination by the year of harvest, ripening stage and geographical origin (Romero et al., 2015).

Table 4
Alpha-tocopherol content, antioxidant capacity and oxidative stability in twelve varieties of EVOO in two harvest seasons.

Variety	Alpha-tocopherol		H-$ORAC_{FL}$		DPPH		Induction Time	
	mg/kg oil		μmol TE/g oil		IC_{50} (mg oil)		hours	
	2014	2015	2014	2015	2014	2015	2014	2015
Arbequina	129	111 ± 13^{cde}	8.3	8 ± 2^{a}	385	101 ± 2^{bcd}	10.8	21.7 ± 0.2^{bc}
Arbequina I18	159	100 ± 1^{cd}	10.1	9 ± 1^{a}	346	135 ± 37^{d}	17.9	19 ± 1^{ab}
Ascolana Huasco	171	–	4.9	–	146	–	25.0	–
Coratina	129	103 ± 16^{cde}	6.4	6.3 ± 0.9^{a}	250	122 ± 32^{cd}	23.8	27 ± 2^{cd}
Kalamata	188	135 ± 12^{e}	7.7	9 ± 2^{ab}	80	32 ± 3^{a}	40.2	31 ± 1de
Koroneiki	125	90 ± 3^{bc}	8.9	13 ± 1^{b}	125	68 ± 3^{ab}	38.2	34 ± 3^{e}
Leccino	172	–	7.5	–	218	–	30.1	–
Manzanilla Chilena	192	129 ± 3de	7.0	8.20 ± 0.02^{a}	161	75 ± 3^{abc}	39.4	35.5 ± 0.3^{e}
Nocellara del Belice	75	37 ± 4^{a}	6.0	5.5 ± 0.7^{a}	208	135 ± 5^{d}	26.5	14.2 ± 0.2^{a}
Oliva di Cerignola	68	59 ± 10^{ab}	5.3	7 ± 1^{a}	123	60 ± 9^{ab}	23.7	20.8 ± 0.1^{abc}
Picual	98	116 ± 6^{cde}	11.0	18.3 ± 0.3^{c}	98	43 ± 7^{a}	59.9	57.4 ± 0.6^{f}
Sevillana	145	95 ± 21^{cd}	28.5	18 ± 1^{c}	38	22 ± 1^{a}	34.5	38 ± 5^{e}

2015 values are expressed as mean ± SD, (n = 3). Different letters mean significant differences between varieties ($p < 0.05$).
H-$ORAC_{FL}$: Hydrophilic orac assay.

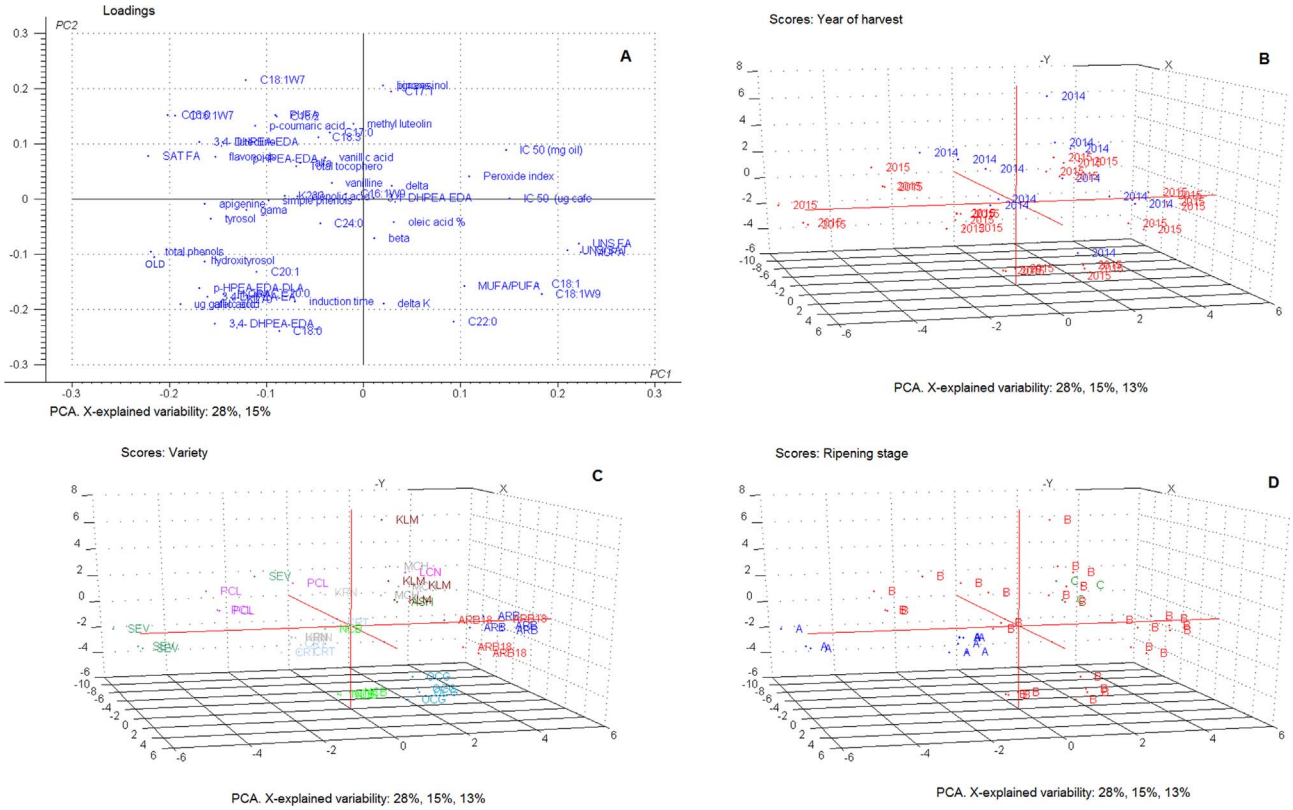

Fig. 1. Multivariate general characterization of oil samples by PCA. Plots of coefficient (A), and score labeled by year of harvest (B), variety (C) and ripening stages (D). Letters in D plot show ripening stages: A, 2-3; B, 3-4; C, 4-5. Abbreviations: ARB, Arbequina; ARB18, Arbequina I18; ASH, Ascolana Huasco; CRT, Coratina; KLM, Kalamata; KRN, Koroneiki; LCN, Lechino; MCH, Manzanilla Chilena; NCB, Nocellara del Belice; OCG, Oliva di Cerignola; PCL, Picual; SEV, Sevillana.

3.3. Statistical grouping by LDA

Linear discriminant analysis was used to construct models of classification of samples according to three criteria (year of harvest, ripening stage and variety) and was initially based on all the descriptive variables (quality parameters, fatty acids, antioxidant compounds, antioxidant activity and oxidative stability). LDA with two levels of selection by year of harvest had 83% and 100% of the 2014 and 2015 samples, respectively, correctly classified (in total, 90% of the samples were correctly classified). According to forward selection (F to enter = 8), only oleic and linoleic acid were determined to be statistically significant for discrimination ($p < 0.05$). Samples from the 2015 harvest showed a higher content of linoleic acid and a lower content of oleic acid than samples harvested in 2014 ($p < 0.05$).

During the biosynthesis of olive oil, saturated fatty acids are formed in the early stages of fruit development, and the chain is later elongated and desaturated to supply the full range of saturated and unsaturated fatty acids (Conde, Delrotb, & Gerós, 2008; Hernández, Padilla, Sicardo, Mancha, & Martínez-Rivas, 2011). The equilibrium between the different fatty acids of olive oil will depend on the olive variety and the climatic conditions, such as thermal accumulation and light, during the period of fruit development (Hernández et al., 2011; Uceda & Hermoso, 2001).

The increase in linoleic acid in the 2015 harvest could be explained by the increase in thermal accumulation, given by accumulated degree-days, registered in the locality of Huasco during the development of the olive fruit mesocarp in this period (See Supplementary data, Table S1) which was higher than that registered in the 2014 harvest. The increase in temperature would increase photorespiration, which would affect the photosynthetic carbon cycle (Taiz & Zieger, 2002a, chap. 8; Taiz & Zieger, 2002b). García-Inza, Castro, Hall, and Rousseaux (2016) reported an increase in linoleic acid and a decrease in oleic acid in olive fruit mesocarps when the fruit mean growth temperature increased. The same authors reported a similar behaviour in these fatty acids with temperature in experiments on the Arauco variety in La Rioja, Argentina (García-Inza, Castro, Hall, & Rousseaux, 2014).

Vanillin, syringic acid, peroxide value and a colour parameter were the most influential variables to distinguish six Turkish monovarietal EVOOs by year of harvest. This same study found that the harvest year was the most effective factor to discriminate the olive oil samples regardless of their botanical origin (Ocakoglu, Tokatli, Ozen, & Korel, 2009).

In the classification of samples by ripening stage, all samples were correctly classified. The forward selection (F to enter = 4) of the relevant components for discrimination included p-HPEA-EDA, 3,4-DHPEA-EA, behenic acid (C22:0), stearic acid (C18:0), K_{270} and gadoleic acid (C20:1) ($p < 0.05$). However, among these, the p-HPEA-EDA content was the predominant factor for the discrimination according to ripening stage ($F = 147$), increasing markedly for ripening stage 4-5. Sample with ripening stage 4-5 showed a higher content of p-HPEA-EDA than samples with ripening stages 2-3 and 3-4 ($p < 0.05$). The differentiation by ripening would be facilitated by the varietal effect, due to 'Kalamata' having the highest contents of p-HPEA-EDA.

The discrimination was complemented by the 3,4-DHPEA-EA content, which decreased significantly from ripening stage 2-3 to 3-4 and 4-5 ($p < 0.05$). Studies of changes in the phenolic profile and contents related to maturation have largely been focused on the olive fruit and have indicated that the concentration of total phenols during olive ripening progressively increases to a maximum level at the "cherry" stage and then decreases sharply as ripening progresses (Conde et al. 2008). This behaviour was observed in VOOs from seven varieties cultured in an experimental olive cultivar in Badajoz (Franco et al., 2014). The rapid decline of phenolic content that occurs during the black maturation phase is most likely correlated with the increased activity of

hydrolytic enzymes observed during this period (Conde et al., 2008). Romero et al. (2015) reported reductions of 70% and 65% in 3,4-DHPEA-EA and p-HPEA-EDA with ripening, respectively. Gómez-Rico, Fregapane, and Salvador (2008) reported a significant decrease in 3,4-DHPEA-EA and p-HPEA-EDA in six varieties of Spanish virgin olive oils as the fruit ripened.

The LDA for varieties showed that seven parameters allowed 100% of samples to be correctly classified into the twelve varieties of EVOO. These results suggest that the evaluated varieties are adapted to this area, since they have maintained the genetic expressions differentiated between them. For classification a forward selection (F to enter = 10) was used. The factors were the UNSFA/SATFA ratio, IT, saturated fatty acids (SATFA), octadecenoic acid (C18:1ω7), tyrosol, 3,4-DHPEA-EDA-DOA and polyunsaturated fatty acids (PUFA) ($p < 0.05$). In this case, the UNSFA/SATFA ratio was the predominant factor for the discrimination according to variety (F = 82). 'Sevillana' and 'Kalamata' showed the lowest values with 4.2 and 4.4, respectively and 'Nocellara del Belice' showed the highest value (mean value 7.2). The discrimination was complemented by the IT as the second factor, being 'Nocellara del Belice' one of the varieties with the lowest values of IT and 'Picual' the variety with the highest value. The low values of alpha-tocopherol and total phenols and the highest ratio of UNSFA/SATFA present in 'Noccellara del Belice' from the 2015 harvest could explain the low oxidative stability in this variety demonstrated by the IT parameter. The 'Picual' and 'Cornicabra' oils presented the greatest values of oxidative stability in a study performed by Montaño et al. (2016).

Four phenolic compounds (oleuropein, apigenin 7-O-glucoside, hydroxytyrosol and cyanidin 3-O-rutinoside) have been shown to allow correct classification according to variety in olive fruits (Gómez-Rico et al. (2008)).

Partial least square discriminate analysis (PLS-DA) was also evaluated with the aim of grouping the samples under the three criteria considered. Bajoub et al. (2017) reported that this algorithm has permitted a correct classification and varietal authentication of olive oils using chromatographic fingerprints data matrices either individually or joined. In this case, each sample was assigned to a class according to a threshold between 0 (does not belong) and 1 (belongs) on the basis of the Bayes theorem; where the class threshold is selected minimizing false positives and false negatives (Barker & Rayens, 2003). In the classification of samples by ripening stage and year of harvest all samples were correctly classified, while for the classification by varieties this value was 80%. Moreover, the relevant parameters for each classification using this algorithm were similar to those obtained by LDA. Then, in general terms, the efficiency in the classification of the samples was similar between both methods.

3.4. Modelling of the antioxidant activity by PLS

PLS regression modelling was the algorithm selected to develop the regression. Although modelling the antioxidant activity was initially evaluated using the DPPH· and H-ORAC$_{FL}$ methods, only the latter case granted a satisfactory predictive model for self-prediction. Therefore, the validation was continued only for the H-ORAC$_{FL}$ values. At first, the 41 samples and 59 physicochemical parameters were used in the regression. Subsequently, with the aim of simplifying the predictive model, the five parameters that had the highest values in their regression coefficients were selected; they included ΔK, HT, pinoresinol, 3,4-DHPEA-EDA-DOA and IT. Moreover, six samples with the highest values of leverage and residue were discarded. For this model, one PLS factor was selected by cross-validation that accounted for 79% of the variance in the antioxidant activity (H-ORAC$_{FL}$; μmol TE/g oil). The correlation coefficient value was 0.88 (slope = 0.74; offset = 2.36; relative error of prediction 20%) and demonstrated a linear relationship between the measured antioxidant capacity and the predicted values obtained by the multivariate calibration with the model in Eq. (1):

$$\mu\text{mol TE/goil} = 0.346 + 0.270\Delta\,\text{K} + 0.201\text{HT} - 0.172\text{Pi} + 0.328\text{Od} + 0.356\text{IT} \quad (1)$$

where

- ΔK: $k_{270} - (k_{266} + k_{274})/2$
- HT: hydroxytyrosol
- Pi: pinoresinol
- Od: 3,4-DHPEA-EDA-DOA
- IT: Induction time

As an alternative model, a multi-lineal regression (MLR) was performed using the five variables described above obtaining similar results (r = 0.87; slope = 0.80; offset = 1.83 and a relative error of prediction 20%).

The high and positive values of the regression coefficients of HT and 3,4-DHPEA-EDA-DOA in the modelling of antioxidant capacity by H-ORAC$_{FL}$ assay, which works by the HAT mechanism, show that the phenolic compounds from EVOO are able to donate hydrogen and act as scavengers of free radicals in EVOO.

Ramos-Escudero et al. (2015) reported that the antioxidant capacity measured by different assays, including the H-ORAC$_{FL}$ assay, was highly influenced by the phenolic content, especially the dialdehydic form of elenolic acid linked to tyrosol and hydroxytyrosol. Montaño et al. (2016) found a significant relationship ($p < 0.01$) between 3,4-DHPEA-EDA, *o*-diphenols and oxidative stability in the Arbequina variety. Furthermore, 3,4-DHPEA-EDA and 3,4-DHPEA-EA have been associated with a high capacity to chelate trace metals (Paiva-Martins & Gordon, 2005). Other authors have reported a significant positive correlation between the total phenolic content and the ORAC results (Augusto et al., 2015).

The antioxidant power of lignans is controversial. Owen et al. (2000) correlated the radical scavenging ability of the EVOO phenolic extract with the concentration of lignans, although other authors did not note the observed antioxidant activity of these phenolic compounds (Servili et al., 2014). Condelli et al. (2015) predicted satisfactorily the IC_{50} oil antioxidant activity by using K_{225} values.

3.5. Validation of the model

To determine the ability of the model to predict future samples correctly, it should be validated through a test set of samples not used to construct the model. Thus, 10 samples (approximately 30% of the samples) were randomly excluded to develop successive given models, with the remaining 26 samples constituting the corresponding training set. Then, their antioxidant capacity was predicted by using the respective model with five parameters. The statistics in Table 5 summarize the results obtained for nine representative regression models. The correlations were equal to or higher than 0.83 with one or two PLS

Table 5
Statistics of the validation step of the PLS model to predict the antioxidant activity of EVOO with 10 samples for each test set.

	Test Set								
	1	2	3	4	5	6	7	8	9
PLS factors	1	2	1	1	2	2	2	2	1
Slope	0.84	0.88	0.93	1.02	0.74	1.10	0.73	0.74	1.00
Offset	1.94	0.98	0.29	−0.14	3.04	−1.90	1.81	2.10	−0.41
R	0.96	0.93	0.89	0.87	0.87	0.96	0.92	0.83	0.91
RMSEP (μmol TE/g oil)	1.43	1.79	2.34	1.75	2.08	1.57	2.24	2.19	2.07
REP (%)	14	18	23	18	21	16	22	22	21
Variance Y (%)	76	80	80	83	84	81	81	86	79

RMSEP: The root mean square error of prediction. REP: relative error of prediction.

factors, corroborating the linear relationship between the antioxidant capacity predicted by the model and the values measured by the H-ORAC$_{FL}$ method. The root mean square error of prediction (RMSEP) and relative error of prediction (REP) values ranged from 1.43 to 2.34 μmol TE/g oil and from 14 to 23%, respectively; and were similar to those obtained in the full cross-validation with all samples.

The prediction model was evaluated with a set comprising two commercial packed samples of Chilean EVOO purchased in local supermarket in March 2016 ('Arbequina' and 'Picual'), and nine EVOO samples collected in two olive mills in Molina (El Maule, VII region; latitude 35° 07′ S, longitude 71° 16′ W). The antioxidant capacity (H-ORAC$_{FL}$) and five parameters (ΔK, HT, pinoresinol, 3,4-DHPEA-EDA-DOA and IT) were determined in the eleven samples. The determined and predicted H-ORAC$_{FL}$ values are summarized in Table S2 (supplementary data). The antioxidant capacities predicted by means of multivariate calibration ranged from 6.9 to 22.9 μmol TE/g oil. There were no significant differences (p-value 0.489 in paired *t*-test, 95% confidence level) between the results predicted by the model and those determined by the standard method. RMSEP and REP were 0.83 μmol TE/g oil and 7%, respectively. This error is considered acceptable given that real samples were tested.

It should be noted that the prediction of the H-ORAC$_{FL}$ value was adequate only if the oil samples had HT values less than or equal to 10 mg/kg. Higher values resulted in an overestimation of the H-ORAC$_{FL}$.

4. Conclusions

Principal components analysis allowed an overview of the samples and variables, showing evidence of their grouping according to variety, harvest year and ripening stage based on oil composition (56% of the variability of the data was explained by the first three components).

Linear discriminant analysis for the variety showed that the fatty acids and phenol composition allowed the correct classification of all samples for all varieties of olive oils, with a total of 7 variables relevant to the correct discrimination, being UNSFA/SATFA ratio and IT the most relevant factors. The classification of the samples by ripening stage was also 100% with 5 relevant variables, although the p-HPEA-EDA and 3,4-DHPEA-EA content had particular importance on the classification. For the year of harvest, 90% were correctly classified based only on oleic and linoleic acid content, evidencing the importance of these components and their variation in the fruits due to the prevailing climatic conditions during development.

A partial least squares predictive model was established for the antioxidant capacity of olive oil (H-ORAC$_{FL}$) based on the following five parameters: ΔK, HT, pinoresinol, 3,4-DHPEA-EDA-DOA and IT. With this model, a correlation equal or higher than 0.83 for the validation and an error of 7% for a group of independent samples were obtained.

The use of the model obtained in this research to predict the value of ORAC$_{FL}$ in the oil, from parameters that are normally requested by the olive industry, could help improve the product. The nutritional quality of EVOO is valued, and its marketing could thus be improved. This can be considered an advantage given the current expectations of consumers, who are demanding more healthy products for purchase.

Acknowledgement

This study was supported by the INNOVA project (11BPC-10039).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.09.130.

References

American Oil Chemists's Society (1993). *Official methods and recommended practices of the American oil chemists' society*. Champaign, IL: AOCS Press.

Angelino, D., Gennari, L., Blasa, M., Selvaggini, R., Urbani, S., Esposto, S., ... Ninfali, P. (2011). Chemical and cellular antioxidant activity of phytochemicals purified from olive mill waste waters. *Journal of Agricultural and Food Chemistry, 59*, 2011–2018.

Artajo, L., Romero, M., Morello, J., & Motilva, M. (2006). Enrichment of refined olive oil with phenolic compounds: Evaluation of their antioxidant activity and their effect on the bitter index. *Journal of Agricultural and Food Chemistry, 54*, 6079–6088.

Augusto, C., Dillenburg, A., De Souza, F., & Teixeira, H. (2015). Total phenolics of virgin olive oils highly correlate with the hydrogen atom transfer mechanism of antioxidant capacity. *Journal American Oil Chemist Society, 92*, 843–851.

Bajoub, A., Medina-Rodríguez, S., Gómez-Romero, M., Ajal, E. A., Bagur-González, M. G., Fernández-Gutiérrez, A., & Carrasco-Pancorbo, A. (2017). Assessing the varietal origin of extra-virgin olive oil using liquid chromatography fingerprints of phenolic compound, data fusion and chemometrics. *Food Chemistry, 215*, 245–255.

Baldioli, M., Servili, M., Perretti, G., & Montedoro, G. F. (1996). Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *Journal of the American Oil Chemists Society, 73*, 1589–1593.

Barjol, J. L. (2013). Introduction. In R. Aparicio, & J. Harwood (Eds.). *Handbook of olive oil, analysis and properties* (pp. 1–17). New York: Springer.

Barker, M., & Rayens, W. (2003). Partial least squares for discrimination. *Journal of Chemometrics, 17*(3), 166–173.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technology, 28*, 25–30.

Brewer, M. S. (2011). Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety, 10*, 221–247.

Casaburi, I., Puoci, F., Chimento, A., Sirianni, R., Ruggiero, C., Avena, P., & Pezzi, V. (2013). Potential of olive oil phenols as chemopreventive and therapeutic agents against cancer: A review of in vitro studies. *Molecular Nutrition & Food Research, 57*, 71–83.

Chun, S., Vattem, D., Lin, Y., & Shetty, K. (2005). Phenolic antioxidant from clonal oregano (Origanum vulgare) with antimicrobial activity against Helicobacter pylori. *Process Biochemistry, 40*, 809–816.

Cicerale, S., Lucas, L. J., & Keast, R. S. J. (2012). Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. *Current Opinion in Biotechnology, 23*, 129–135.

Conde, C., Delrotb, S., & Gerós, H. (2008). Physiological biochemical and molecular changes occurring during olive development and ripening. *Journal of Plant Physiology, 165*, 1545–1562.

Condelli, N., Caruso, M. C., Galgano, F., Russo, D., Milella, L., & Favati, F. (2015). Prediction of the antioxidant activity of extra virgin olive oils produced in the mediterranean area. *Food Chemistry, 177*, 233–239.

Franco, M. A., Galeano-Díaz, T., López, O., Fernández-Bolaños, J. G., Sánchez, J., De Miguel, C., ... Martín-Vertedor, D. (2014). Phenolic compounds and antioxidant capacity of virgin olive oil. 2014. *Food Chemistry, 163*, 289–298.

García-Inza, G., Castro, D., Hall, A., & Rousseaux, M. (2014). Responses to temperature of fruit dry weight oil concentration and oil fatty acid composition in olive (Olea europaea L. var. 'Arauco'). *European Journal of Agronomy, 54*, 107–115.

García-Inza, G. P., Castro, D. N., Hall, A. J., & Rousseaux, M. C. (2016). Opposite oleic acid responses to temperature in oils from the seed and mesocarp of the olive fruit. *European Journal of Agronomy, 76*, 138–147.

Gómez-Rico, A., Fregapane, G., & Salvador, M. D. (2008). Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. *Food Research International, 41*, 433–440.

Hernández, M. L., Padilla, M. L., Sicardo, M. D., Mancha, M., & Martínez-Rivas, J. M. (2011). Effect of different environmental stresses on the expression of oleate desaturase genes and fatty acid composition in olive fruit. *Phytochemistry, 72*, 178–187.

Hrncirik, K., & Fritsche, S. (2004). Comparability and reliability of different techniques for the determination of phenolic compounds in virgin olive oil. *European Journal of Lipid Science and Technology, 106*, 540–549.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841–1856.

IOOC (2001). International Olive Oil Council. Preparation of the fatty acids methyl esthers from olive oil and olive-pomace oil. COI/T.20/Doc n24/ 2001. http://www.internationaloliveoil.org/. [Online, accessed 14-May-2013].

IOOC (2009). International Olive Council (IOC) Determination of biophenols in olive oils by HPLC. COI/T.20/Doc No 29 November 2009. http://www.internationaloliveoil.org/ [Online, accessed 20-January-2015].

IOOC (2015). International Olive Oil Council. Trade standard applying to olive oil and olive pomace oils COI/T.15/NC No 3/Rev. 8/2015. http://www.internationaloliveoil.org/. [Online, accessed 12- May-2015].

Lopez-Huertas, E. (2010). Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. *Pharmacological Research, 61*, 200–207.

López-Miranda, J., Pérez-Jiménez, F., Ros, E., de Caterina, R., Badimón, L., Covas, M. I., ... Abiá, R. (2010). Olive oil and health: Summary of the II international conference on olive oil and health consensus report, Jaén and Córdoba (Spain) 2008. *Nutrition Metabolism and Cardiovascular Diseases, 20*, 284–294.

Mateos, R., Espartero, J. L., Trujillo, M., Ríos, J. J., Camacho, L. M., Alcudia, F., & Cert, A. (2001). Determination of phenols flavones and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *Journal of Agricultural and Food Chemistry, 49*, 2185–2192.

Montaño, A., Hernández, M., Garrido, I., Llerena, J. L., & Espinosa, F. (2016). Fatty acid and phenolic compound concentrations in eight different monovarietal virgin olive oils from extremadura and the relationship with oxidative stability. *International Journal of Molecular Science, 17*(11), 1960.

Ocakoglu, D., Tokatli, F., Ozen, B., & Korel, F. (2009). Distribution of simple phenols phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. *Food Chemistry, 113*, 401–410.

Owen, R., Mier, W., Giacosa, A., Hull, W. E., Spiegelhalder, B., & Bartsch, H. (2000). Olive-oil consumption and health: The possible role of antioxidants. *Food Chemical Toxicology, 38*, 647–659.

Paiva-Martins, F., & Gordon, M. H. (2005). Interactions of ferric ions with olive oil phenolic compounds. *Journal of Agricultural and Food Chemistry, 53*, 2704–2709.

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., ... Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry, 51*, 3273–3279.

Ramos-Escudero, F., Morales, M. T., & Asuero, A. G. (2015). Characterization of bioactive compounds from monovarietal virgin olive oils: Relationship between phenolic compounds-antioxidant capacities. *International Journal of Food Properties, 18*, 348–358.

Romero, N., Saavedra, J., Tapia, F., Sepúlveda, B., & Aparicio, R. (2015). Influence of agroclimatic parameters on phenolic and volatile compounds of Chilean virgin olive oils and characterization based on geographical origin cultivar and ripening stage. *Journal of the Science of Food and Agriculture, 96*, 583–592.

Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Di Maio, I., ... Taticchi, A. (2014). Biological activities of phenolic compounds of extra virgin olive oil. *Antioxidants, 3*, 1–23.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Taiz, L., & Zeiger, E. (2002a). *Plant physiology* (3rd ed.). Sunderland: Sinauer Associates.

Taiz, L., & Zeiger, E. (2002b). Photosynthesis: Carbon reactions. *Plant physiology* (pp. 145–170). (3rd ed.). Sunderland: Sinauer Associates.

Uceda, M., & Hermoso, M. (2001). La calidad del aceite de oliva. In D. Barranco, R. Fernández-Escobar, & L. Rallo (Eds.). *El Cultivo del Olivo* (pp. 153–166). Madrid: Mundi Prensa.