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Journal of the American Oil Chemists' Society

ISSN 0003-021X
Volume 91
Number 12

J Am Oil Chem Soc (2014) 91:2021-2033
DOI 10.1007/s11746-014-2558-3

Springer

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ORIGINAL PAPER

Phenolic Compounds and Antioxidant Capacity of Monovarietal Olive Oils Produced in Argentina

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Received: 13 June 2014 / Revised: 16 September 2014 / Accepted: 11 October 2014 / Published online: 11 November 2014

Abstract Virgin olive oil has high levels of phenolic compounds that are highly bioavailable; these compounds are receiving considerable attention for their antioxidant activity, closely related to the prevention of non-communicable chronic diseases. The aim of this work was to characterize the phenolic profile and antioxidant capacity of monovarietal olive oils cvs. Arauco, Arbequina, Farga and Empeltre produced in Argentina. This study focused on the relationship between the single molecules or classes of molecules quantified by SPE-CZE, the corresponding Folin-Ciocalteu results, and antioxidant capacity using three different tests. Fifteen compounds were simultaneously determined: tyrosol, vinylphenol, oleuropein, hydroxytyrosol, rutin, catechin, naringenin, cinnamic acid, chlorogenic acid, syringic acid, luteolin, apigenin, vanillin acid, quercetin, and caffeic acid. The phenolic contents of the monovarietal olive oils show significant differences between different varieties ($p < 0.05$), with positive and significant Pearson's correlation found between Folin–Ciocalteu and CZE. Besides, the correlation between the content of total polyphenols and antioxidant capacity was high for all the antioxidant assays performed. When analyzing the correlation coefficients of the different families of phenolic compounds studied, simple phenols and cinnamic acid derivatives show a higher correlation with antioxidant capacity. Thus, findings obtained in this study demonstrated that Arauco olive oil, autochthonous for Argentina, possesses the highest antioxidant/free-radical scavenging properties, which are very likely due to the presence of high contents of phenolic compounds.

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Keywords Phenolic compounds · Virgin olive oil · Solid phase extraction (SPE) · Capillary zone electrophoresis (CZE) · Argentina · Folin–Ciocalteu method · Antioxidant capacity · 1,1 diphenyl-2-picrylhydrazyl (DPPH) · 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) · Ferric reducing antioxidant power (FRAP)

Introduction

Virgin olive oil (VOO) is a juice obtained by exclusively mechanical means (pressing) from the fruit of the olive tree (*Olea europaea* L.) [1]. In recent years, VOO consumption in a balanced diet has been promoted on a global scale due to its well-known nutritional and sensory proprieties [2, 3]. These benefits have been associated with its well-balanced fatty acids composition, of which oleic acid is the main component, and to the presence of minor biomolecules, such as vitamins and natural antioxidants [4]. The main antioxidants in VOO are carotenes, and phenolic compounds including fat-soluble and water-soluble phenols. While the fat-soluble phenols (tocopherols) of VOO can be found in other vegetable oils, some of its water-soluble phenols are rarely present in other oils or fats [1]. Olive oils are known to contain different classes of phenolic compounds such as simple phenols (hydroxytyrosol and tyrosol), cinnamic (caffeic acid and p-coumaric acid) and benzoic (vanillic acid) acids and derivatives, flavones (apigenin and luteolin), and secoiridoids (oleuropein and ligtroside derivatives) [2]. Such compounds are important markers for evaluating virgin oil quality [5]. Phenolic components affect the taste, in particular, the positive bitter and pungent sensory attributes [3]. On the other hand, its antioxidant properties are responsible for the shelf life of oils [2, 6]. At least 36 phenolic compounds have been identified in extra

virgin olive oil (EVOO) to date, and there is much variation in the composition and concentration of these phenolic compounds. Variation may be caused by numerous factors including: agricultural techniques used to cultivate the olive, maturity of the olive fruit at harvest, processing, variety and region in which the olive is grown [7].

The Mediterranean region is the major olive oil producer, and there are several studies about the chemical and analytical properties of Mediterranean olive oils, whereas oils produced in other olive-growing regions have not frequently been addressed [8–11].

Among the emerging producer countries, Argentina is considered as a new EVOO exporter. The growth of its olive oil production in the last years has propelled the country to become the world's largest exporter of olive oil outside of the Mediterranean region. Argentina exports more than 70 % of its production, and principal export markets in 2012 were Brazil (45% of total exports by volume), the US (32 %), and Spain (10 %) [12]. Argentina's olive growing areas are mainly concentrated in a series of valleys at the foot of the Andes Mountains in the western part of the country. The most important cultivars grown in Argentina are Arbequina, Coratina, Picual, Frantoio, Manzanilla, Changlot Real, Barnea, Arauco, Farga, Nevadillo and Empeltre [13]. Arauco is Argentina's flagship variety and the country has the largest Arauco acreage in the world. Today, Argentina's olive oil processing sector has an increased focus on quality. Because of the modernization efforts, more than 90 % of Argentine olive oil is now reported to be extra virgin [14]. The location of Argentina's olive oil industry presents some advantages. On the one hand, Argentina's location in the Southern Hemisphere can provide export markets with fresh olive oil with a unique flavor profile during the months when producers around the Mediterranean have limited supplies and are using olive oil that has been stored since the previous harvest. Additionally, because Argentine growers can plant olives in a variety of different soils and microclimates, the country can produce oils with a wide range of sensory attributes.

The present study was motivated by a need to explore the potential of varieties grown in Argentina to obtain quality olive oil. Characterizing their oils could be seen as a first step for producers to be aware of their commercial value. The aim of this work was to characterize the phenolic profile and the antioxidant capacity of the monovarietal olive oils obtained from cvs. Arauco, Arbequina, Farga and Empeltre that are the most representative cultivars produced in the Midwest region from Argentina. The phenolic compounds selected were chosen considering their potentiality as traceability markers to the botanical origin in olive oils [15–19].This study focused on the relationship between the single molecules or classes of molecules quantified by SPE-CZE, the corresponding Folin-Ciocalteu results, and antioxidant capacity using three different tests (Ferric ions reducing antioxidant power assay (FRAP), and radical scavenging activity by DPPH and ABTS). Antioxidant activities of olive oil were determined and associated with their phenolic content previously reported by other researchers [20, 21]. However, to the best of our knowledge, there is no report about antioxidant capacity of olive oils produced in Argentina.

Materials and methods

Reagents and solutions

Ultrapure water (resistivity 18.3 MΩcm) was obtained from a RiO/Elix3-Sinergy185 purification system (Millipore, Sao Pablo, Brazil) and was used to prepare solutions including the background electrolyte. Methanol of chromatographic grade was purchased from Merck (Darmstadt, Germany).

Luteolin, tyrosol, hydroxytyrosol, vinylphenol, catechin, naringenin, rutin, cinnamic acid, syringic acid, chlorogenic acid, apigenin, vanillic acid, quercetin, luteolin, and caffeic acid were purchased from Sigma (St. Louis, MO).

Standard stock solutions of the analytes were prepared by dissolving an appropriate amount of each pure substance in 10 mL of a HPLC-grade methanol to obtain a final concentration of 1,000 mg L^{-1}. The resulting solutions were stored at 4 °C in amber glasses. Working standard solutions of different concentrations were prepared daily by diluting the aliquots appropriate stock solutions in methanol. Before use, all solutions were filtered through 0.22 μm nylon filters.

The 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma–Aldrich (St. Louis, MO).

Olive oil samples

Four monovarietal virgin olive oils from Mendoza have been studied. The four oils were Arauco, Arbequina, Empeltre, and Farga. The olive samples were obtained from the same orchard located in the "East Area" of Mendoza (Rivadavia). The olive trees were cultivated under an identical irrigation system and the same growing conditions. A randomized complete block (RCB) design was used. The selection of blocks was carried out as a function of soil texture. The alluvial origin of the soils causes variability of edaphic conditions. Three soil profiles were found and used as a blocking factor. Within each soil profile, all cultivars were sampled. The olives were obtained from the four directions of the tree. Fifty kilograms of olives per sample

were handpicked at the same stage of maturity (maturity index around four). The maturity index was calculated according to the method proposed by the International Olive Oil Council, based on evaluation of the olive skin and pulp colors [22]. Olive oil extractions were performed on an industrial scale using an OLIOMIO monoblock (Toscana Enológica Mori Snc, Italy). The malaxation was carried out at 25 °C for 40 min, and the oil separation was obtained using a three-phase decanter. The VOO samples were decanted, filtered, and stored at room temperature in amber glass bottles without headspace until the analysis.

The oil quality was assessed by the acidity value, the peroxide number, and absorbance at 270 and 232 nm according to the International Olive Oil Council regulations [22].

Extraction phenolic compounds

The extraction of phenolic compounds in olive oil samples was performed by a solid phase extraction (SPE) using a homemade column packed with suitable filtering material. Diol cartridges (50 mg) were made in 1 mL syringes using 25 mg of glass wool as frits. These cartridges were placed in a vacuum elution apparatus (Varian Vac Elut 20 manifold and a Vacuubrand vacuum pump ME 2C) and preconditioned passing 5 mL of methanol and 5 mL of hexane. The olive oil samples (0.8 g) were thoroughly mixed with 0.5 mL of hexane until complete homogenization and carefully loaded onto the preconditioned column, leaving the sample in the solid phase under vacuum. Then, the column was washed with 3 mL of hexane. The whole phenolic fraction was eluted with methanol (1 mL).

Total phenolic content

The amount of total phenolic was determined using the Folin–Ciocalteu (FC) method described by [23] with modifications. In a test tube, 50 μL of phenolic extracts previously obtained, were mixed with the Folin–Ciocalteu reagent (200 μL) and, after 5 min, with an aqueous solution of Na_2CO_3 (1,250 μL, 5 % w/v). Then, ultrapure water was used to bring the final volume to 5,000 μL. The mixture was incubated for 60 min in the dark, at room temperature, and the total phenol content was determined colorimetrically at 750 nm. Calibration curves were prepared for working solutions of methanolic extracts of caffeic acid in the concentration range. The final result, expressed in micrograms of caffeic acid (CA) per gram of oil, was obtained through a calibration curve with a range from 0–1,000 μg/mL ($R^2 = 0.9946$).

SPE-CZE phenolic profile

The CZE analysis of the phenolic extracts was carried out using a CapelTM 105 M apparatus equipped with a 67 cm full length, 50 cm effective length, and 75 μm ID and 375-μm OD fused silica capillary. The capillary tube was conditioned prior to its daily use by flushing with water (5 min), 0.1 mol L^{-1} NaOH for 5 min, followed by water for another 2 min, and finally with the buffer for 5 min. The running buffer was boric acid 30 mmol L^{-1}, pH 9.5, that was prepared by weighing the required amount of boric acid and adjusting its pH with a few drops of sodium hydroxide. All solutions and buffers were degassed by sonication for 5 min before use in order to avoid changes during ionization and ensure acceptable reproducibility. The pH measurements were made with an Altronix model TPX-I pH meter furnished with a combined glass electrode.

The separation voltage was 25 kV, and the capillary temperature was 25 °C. Samples were injected by hydrodynamic injection at 30 mbar for 2 s. Electropherograms were recorded at 240 nm. Between runs, the capillary was flushed with water (2 min), 0.1 mol L^{-1} NaOH (2 min), water (2 min), and fresh buffer (2 min). The capillary tube was rinsed with 0.1 mol L^{-1} NaOH for 10 min, then with water for 10 min every day after use.

The identification of phenolic compounds was carried out comparing the peak migration times with those obtained by injection of pure standards and by a method of standard addition. Also, peak purity was checked by the software contrasts facilities. The quantification was performed by constructing calibration curves for all analyzed compounds.

Antioxidant capacity

There are several considerations regarding the methodology to evaluate the antioxidant capacity. Due to the complex nature of olive oils extracts, and taking in consideration the different mechanisms of antioxidant activity, more than one method for antioxidant activity measurement should be employed in order to estimate the total antioxidative effects [20, 24–26]. In this study, three antioxidant capacity tests, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothia-zoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) were used, in order to obtain reliable data.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure described by [27] with some modifications. The method is based on the reduction of the ferric tripyridyltriazine (TPTZ-Fe^3) complex to its stable ferrous form (TPTZ-Fe^2) complex at low pH. This reduction was monitored by measuring the absorption change at 593 nm. The FRAP reagent was prepared at the time of use and was composed of 10 mL of a 10 mmol L^{-1} TPTZ solution in 40 mmol L^{-1}

HCl, 10 mL of 20 mmol L^{-1} $FeCl_3$,100 mL of 0.1 mol L^{-1} acetate buffer (pH 3.6), and incubated at 37 °C for 10 min. Briefly, 3.6 mL of FRAP reagent was mixed with 400 µL of diluted phenolic extract (1:2), subsequently the mixture was incubated 10 min at 37 °C. The absorbance of extracts (A_E) was measured against a reagent blank (3.6 mL of FRAP reagent with 400 µL of water) (A_0). The reducing capacity of the phenolic extracts was calculated taking a reaction signal given by a fresh working solution of $FeSO_4{\cdot}7H_2O$ (1 mM) (A_{REF}). All determinations were performed in triplicate. FRAP was expressed in percentage using the formula:

$$\text{FRAP}\ \% = \frac{A_E - A_0}{A_{REF} - A_0} \times 100.$$

2,2′-diphenyl-1-picrylhydrazyl (DPPH) assay

The radical scavenging activity was measured in the phenolic extracts, following the methodology described by [28], using a discoloration curve of the stable radical, 2,2′-diphenyl-1-picrylhydrazyl. A methanolic solution 3.5 ml of DPPH (45 mg mL^{-1}) was rapidly mixed with 250 mL of phenolic extracts. The absorbance at 515 nm was measured after 5 min (A_E). The initial absorbance of the DPPH solution was 1.3. The decline in radical concentration indicated the radical scavenging activity of the sample. Samples were measured against methanol and methanol with a DPPH blank (A_B). The experiment was carried out three times, and the absorbance sample (A_0) was considered. Pyrogallol solution (1 mM) was used as a reference (A_{REF}). Radical scavenging activity was calculated in percentage of inhibition (I %) as follows:

$$\text{DPPH} - \text{I}\ \% = \left[\frac{\frac{A_B-(A_E-A_0)}{A_B}}{\frac{A_B-A_{REF}}{A_B}}\right] \times 100.$$

2,2′-azinobis(3-ethylbenzothiaziline-6- sulfonic acid (ABTS) assay

Antioxidant activities were also measured using the modified method proposed by Re et al. [29], using 2,2′-azinobis(3-ethylbenzothia-zoline-6-sulfonic acid) diammonium salt (ABTS). ABTS was dissolved in water to a concentration of 7 mM and radical cations (ABTS*) were produced by reacting this stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. ABTS* solution was diluted with methanol, to an absorbance of 0.70 (± 0.02) at 734 nm. Then, 80 µL of phenolic extract were added at methanolic ABTS* solution (3,920 µL). The absorbance of the extracts (A_E) was measured against methanol and methanol with an ABTS* blank (A_B). All the determinations were carried out three times, and it was necessary to correct for the sample absorbance (A_0). A gallic acid solution (1 mM) was used as reference (A_{REF}) and results were calculated according to the formula shown below.

$$\text{ABTS–I}\ \% = \left[\frac{\frac{A_B-(A_E-A_0)}{A_B}}{\frac{A_B-A_{REF}}{A_B}}\right] \times 100.$$

Statistical analysis

Data obtained were analyzed using the Statgraphics Plus Version 5.0 program (Manugistic Inc., Rockville, MD, USA). VOO phenolic compounds were expressed as µg per g (ppm). All data was reported as the mean ± SD for three replications. Comparison of the means was achieved by analysis of variance (ANOVA). The Pearson correlation coefficient and its corresponding significance were calculated between every single phenolic compound determinate and the antioxidant capacity of monovarietal olive oil. The results were significant at $p < 0.05$ unless specified otherwise.

Results and discussion

For all variables studied, the ANOVA showed that the block factor was not significant statistically (data not shown). Therefore, the analysis with a completely randomized design was used in order to increase the power of the analysis.

Classical quality parameter values of olive oils

The acidity, expressed as oleic acid, of all analyzed samples was below 0.8. Peroxide values ranged from 4.12 to 5.21 meq O_2/kg of oil, and extinction coefficients at 232 and 270 nm, respectively, were less than 2.50 and 0.25. The methods used for the determination of these quality parameters were those proposed in the International Olive Oil Council regulations [22]. The values obtained for these quality parameters correspond to those specified for the category of extra virgin olive oil.

Total phenolic content

The phenolic contents of the monovarietal olive oils analyzed by the Folin–Ciocalteu method (TPC) showed significant differences between different varieties ($p < 0.05$). The highest contents were detected in oils cv. Arauco (514.15 ± 5.15 µg of caffeic acid (CA) per g of olive oil). Farga samples showed a mean value of 278.58 ± 18.78 µg/g^{-1}. The lowest amounts were recorded in oils of cvs. Arbequina and Empeltre (170.93 ± 7.55 and 206.52 ± 6.6 µg/g^{-1}, respectively), and among them no significant differences were observed. The concentrations of phenols in

the studied olive oils ranged from 171 and 514 μg of CA g^{-1}. These results were comparable to those reported by different authors in Mediterranean olive oils: 126–347 μg/g^{-1} [30], 139–340 μg/g^{-1} [31], 160–1,203 μg/g^{-1} [32], 133–328 μg/g^{-1} [33], and 253–1,400 μg/g^{-1} [34]. On the basis of total polyphenols contents, according to the classification established by [35], the oils obtained of the Arbequina cultivar were within the category "low content in polyphenols" because they showed a concentration lower than 200 μg/g^{-1} (ppm). The latter is in agreement with those reported by other authors [6, 32, 36]. Empeltre and Farga belonged to the category "intermediate content in polyphenols" because the presented concentration was between 200 and 350 μg/g^{-1}. The olive oils of Arauco cultivar were placed in the "high content in polyphenols" category because they presented a concentration between 350 and 550 μg/g^{-1} or even higher.

Folin–Ciocalteu is a simple and highly efficient procedure, but it is limited by a low specificity. This reagent has been considered by some authors as nonspecific, because it can be reduced by nonphenolic compounds [37]. Consistent information about Folin–Ciocalteu validation by reliable analytical protocols is currently lacking for olive oil quality assessment. The International Olive Oil Council (IOC) gives a provisional approval to a method for the colorimetric determination of *o*-diphenols, based on sodium molibdate dehydrate and 370 nm absorbance, thus, confirming the need of simple analytical approaches.

SPE-CZE phenolic profile

With the aim to propose a robust analytical tool to characterize olive oils, the following phenolic compounds were chosen for the method development: luteolin, tyrosol, hydroxytyrosol, vinylphenol, catechin, naringenin, rutin, cinnamic acid, syringic acid, chlorogenic acid, apigenin, vanillic acid, quercetin, luteolin, and caffeic acid. Although oleuropein and ligstroside derivatives and lignans are the most concentrated phenols in VOOs, they were not analyzed in the present study because high-quality pure standards are not commercially available. The latter is crucial if UV detection is used. On the other hand, phenolic compounds present at minor concentrations in olive oils have proved to be adequate traceability markers of the botanical origin [15–19].

Analytical quality parameters were evaluated in order to assess the performance of the SPE-CZE procedure with the selected conditions. Repeatability of the CZE method was determined by four replicates of a standard mixture solution spiked at levels of 2, 5, and 10 μg/mL^{-1}. The linearity was evaluated by plotting the area of each analyte (at five levels between 2 and 10 μg/mL^{-1}) against concentration. The peak area and concentration of each analyte were subjected to regression analysis to obtain the calibration equations and correlation coefficients. LOD and LOQ were calculated as LOD = 3 and LOQ = 10 s/m, respectively, where s is the standard deviation of the baseline noise,

Table 1 Resulting performance characteristics

Phenolic compound	tm	Repeatability RSD (%) $n = 4$			Linearity R^2 (2–10 μg/mL^{-1})	LOD[a] (μg/mL^{-1})	LOQ[b] (μg/mL^{-1})
		2 μg/mL^{-1}	5 μg/mL^{-1}	10 μg/mL^{-1}			
Tyrosol	3.415	3.734	5.552	9.359	0.989	0.136	0.762
Vinylphenol	3.757	0.797	0.817	3.273	0.981	0.477	0.721
Oleuropein	3.868	1.626	0.867	3.058	0.973	0.161	0.619
Hydroxytyrosol	4.653	1.484	2.263	2.426	0.991	0.915	1.542
Rutin	4.885	2.154	5.244	7.229	0.998	0.548	1.508
Catechin	5.018	7.357	7.516	0.898	0.987	0.712	0.826
Naringenin	5.160	0.610	1.706	3.844	0.990	0.136	0.175
Cinnamic acid	5.240	5.160	6.166	5.117	0.998	0.301	0.518
Chlorogenic acid	5.612	2.062	5.403	8.679	0.997	1.094	1.413
Syringic acid	6.220	4.708	3.790	4.554	0.998	0.287	0.302
Luteolin	6.278	8.319	6.071	3.297	0.996	0.078	0.153
Apigenin	6.358	10.354	4.823	2.881	0.998	0.241	0.334
Vanillic acid	6.718	10.687	4.959	4.977	0.998	0.590	0.689
Quercetin	6.873	8.571	7.118	9.610	0.998	0.456	0.565
Caffeic acid	7.228	9.143	1.429	3.770	0.996	0.494	0.551

tm: mean of migration time

[a] Limit of detection in μg/mL^{-1}

[b] Limit of quantification in μg/mL^{-1}

and m is the slope of the calibration curve. The resulting performance characteristics are summarized in Table 1. The relative standard deviations (RSDs) of the peak area for standards analyzed were between 0.6 % and 10.7 %. Linearity of the analysis of standards showed that within the concentration range there was an excellent correlation between the peak area and the concentration of each analyte (always>0.97).

In order to evaluate selectivity and matrix effect, a recovery test was carried out. The whole methodology (SPE-CZE) was applied to six portions of olive oil (0.8 g), and the average concentrations determined for each compound were taken as a base value. Then, known quantities of the analytes were added to other four aliquots, and the phenolic compounds were determined following the recommended procedure. The recovery studies showed satisfactory robustness leading to recoveries higher than 79 % and lower than 103 % for the analytes under study. Cinnamic acid was the analyte with the poorest recovery (79 %) of the 15 compounds. Similar results (73.6 %) have previously been reported by other authors [38]. A typical electropherogram for the standard mixture solution under the optimum conditions is shown in Fig. 1. Baseline separation for all analytes can be achieved within 8 min. The electropherogram for olive oil extract spiked with the 15 phenolic compound standards is shown in Fig. 2, which also shows the selectivity of the method in a polar extract.

The SPE-CZE method was then applied to determine the phenolic profile of olive oils produced in Argentina. Representative electropherograms corresponding to four analyzed monovarietal olive oils are shown in Fig. 3. A markedly qualitative and quantitative analytical difference in the phenolic profile of olive oils was observed (Table 2). Taking into account that all extra virgin olive oil under study

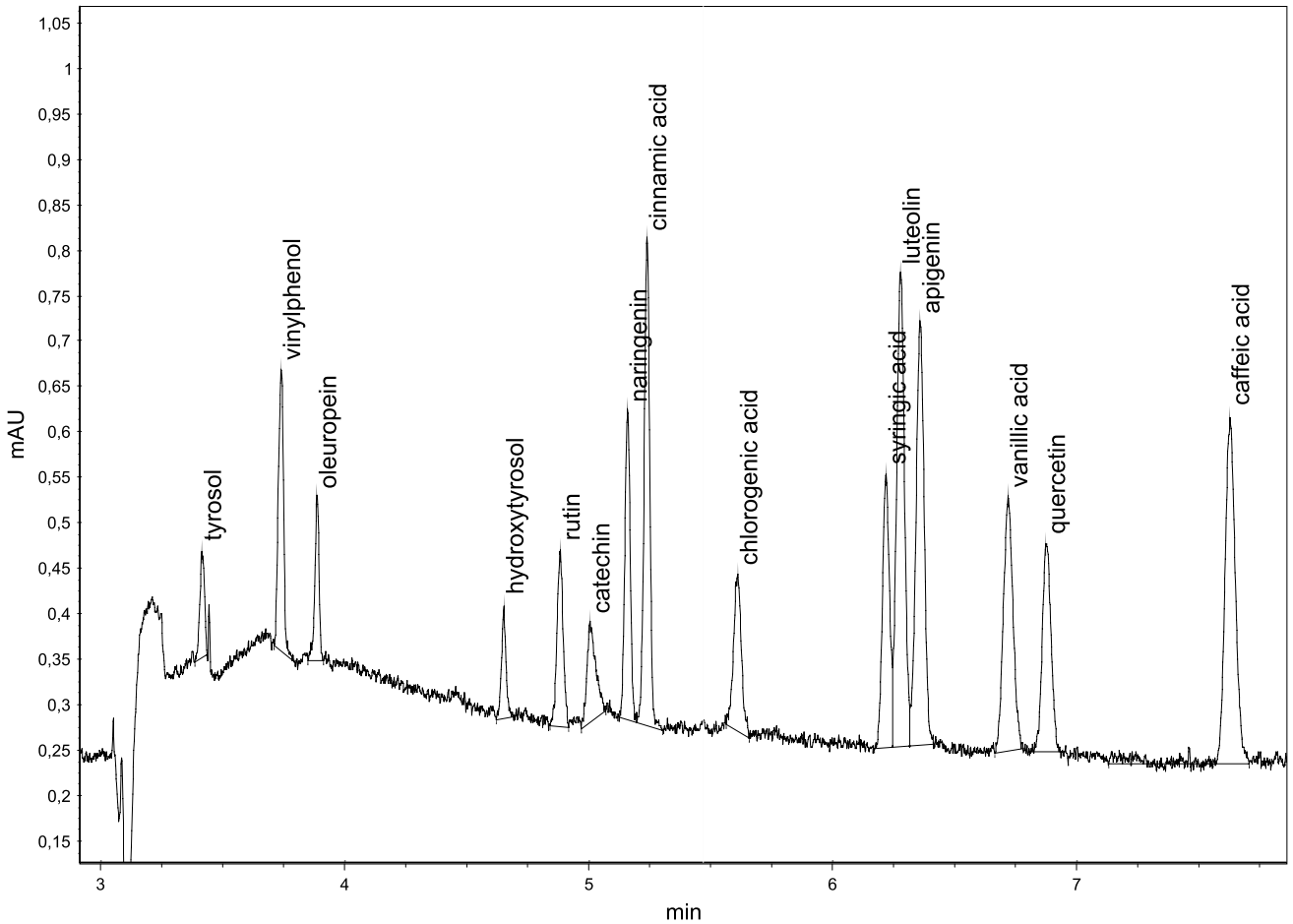

Fig. 1 Electropherogram of a phenolic standard mixture solution (10 μg mL^{-1}). *Peaks 1* tyrosol, *2* vinylphenol, *3* oleuropein, *4* hydroxytyrosol, *5* rutin, *6*, catechin, *7*, naringenin, *8* cinnamic acid, *9* chlorogenic acid, *10* syringic acid, *11* luteolin, *12* apigenin, *13* vanillic acid, *14* quercetin, *15* caffeic acid. Conditions: 30 mM boric acid buffer, pH 9.5; capillary 67 cm full length, 50 cm effective length, 75 m ID, 375 m OD; samples were injected by hydrodynamic injection at 30 mbar for 2 s; 25 kV constant voltage and the capillary temperature was 25 °C; detection by UV absorbance at 240 nm

were obtained from the same region with identical climatic characteristics, from the fruit with same state of maturity, and the same elaboration process, the differences could be only attributed to the difference between cultivars. Of the 15 compounds tested, only 12 were found in the samples. As can be seen in Fig. 3, several peaks have not been identified. The total amount of determined phenolic compounds (PC) ranged between 91 and 874 $\mu g/g^{-1}$ for the Arbequina and Arauco samples, respectively. Others studies carried out by our research group demonstrated that the sum of phenolic compounds concentration also was the highest in Arauco samples when analyzed by DLLME- CZE [39]. Farga and Empeltre oils showed intermediate contents of 119 and 125 $\mu g/g^{-1}$, respectively. Tyrosol, hydroxytyrosol, cinnamic acid, syringic acid, luteolin, and caffeic acid were found in all samples. Arauco presented significantly higher concentrations of hydroxytyrosol and tyrosol and also of naringenin, caffeic acid and cinnamic acid compared to olive oils of the other cultivars. Arbequina olive oils presented significantly higher concentrations of luteolin. Catechin, apigenin, and vanillic acid were detected only in the samples of this cultivar. In Farga samples, the phenolic compound found in the highest concentration was rutin. This compound was detected only in the oils from this cultivar. The concentration of syringic acid was also high in relation to others cultivars. Empeltre showed the highest concentration of oleuropein, this being the main compound found.

The characterization of phenolic profile of Argentina monovarietal oils shows some compounds that are not generally analyzed in VOO such as catechin and naringenin. However, these compounds have been previously found in olive fruit and olive oil mill wastes, so its presence in olive oil is expected [40–42]. Vinyl phenol has also been found in olive oils [43]; though also it was not detected in any of the analyzed samples (Table 2).

In order to display more clearly the differences between cultivars, phenolic compounds detected were grouped

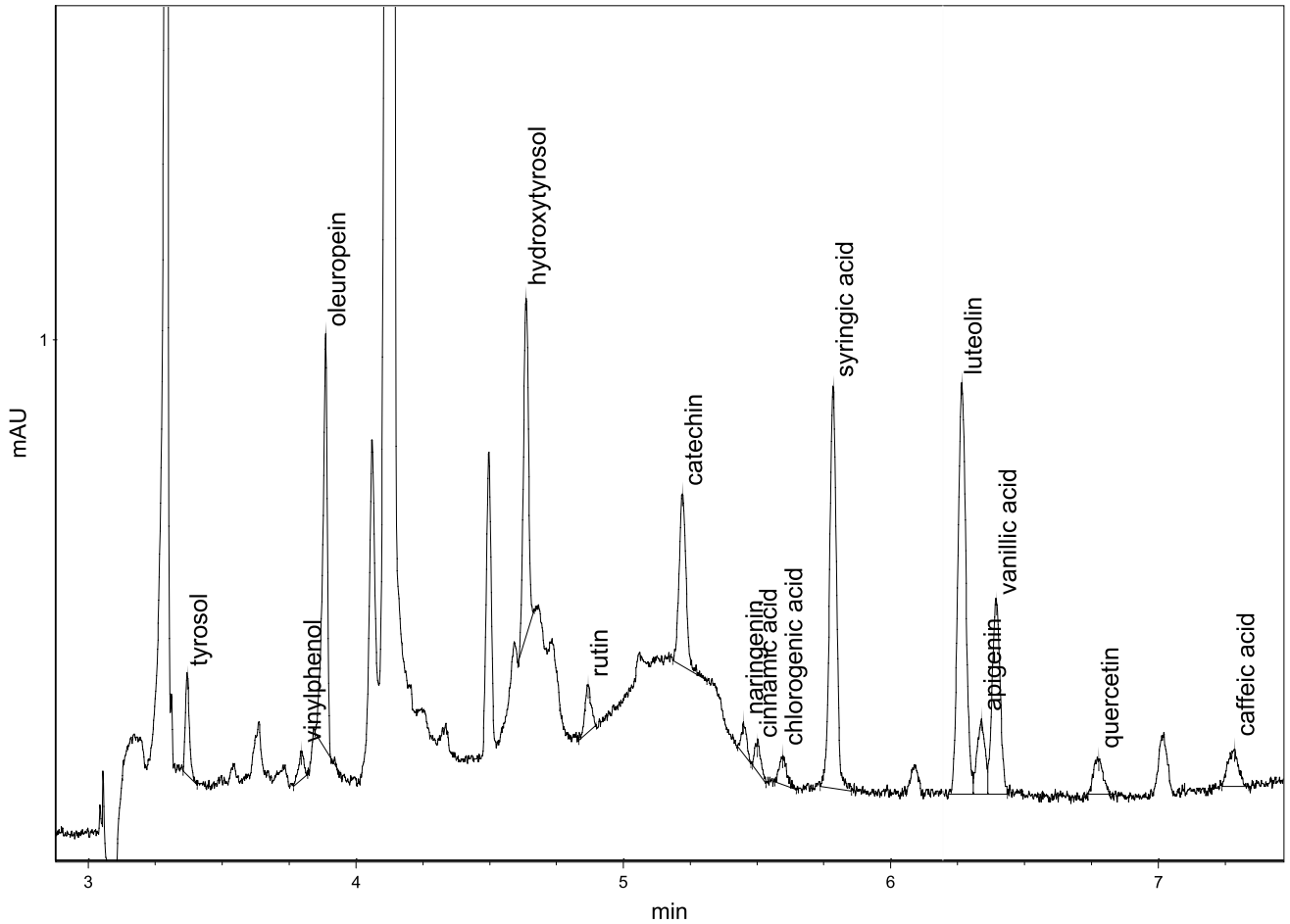

Fig. 2 Electropherogram of the extract of a sample olive oil spiked with the 15 phenolic compound standards. Conditions: 30 mM boric acid buffer, pH 9.5; capillary 67 cm full length, 50 cm effective length, 75 m ID, 375 m OD; samples were injected by hydrodynamic injection at 30 mbar for 2 s; 25 kV constant voltage and the capillary temperature was 25 °C; detection by UV absorbance at 240 nm

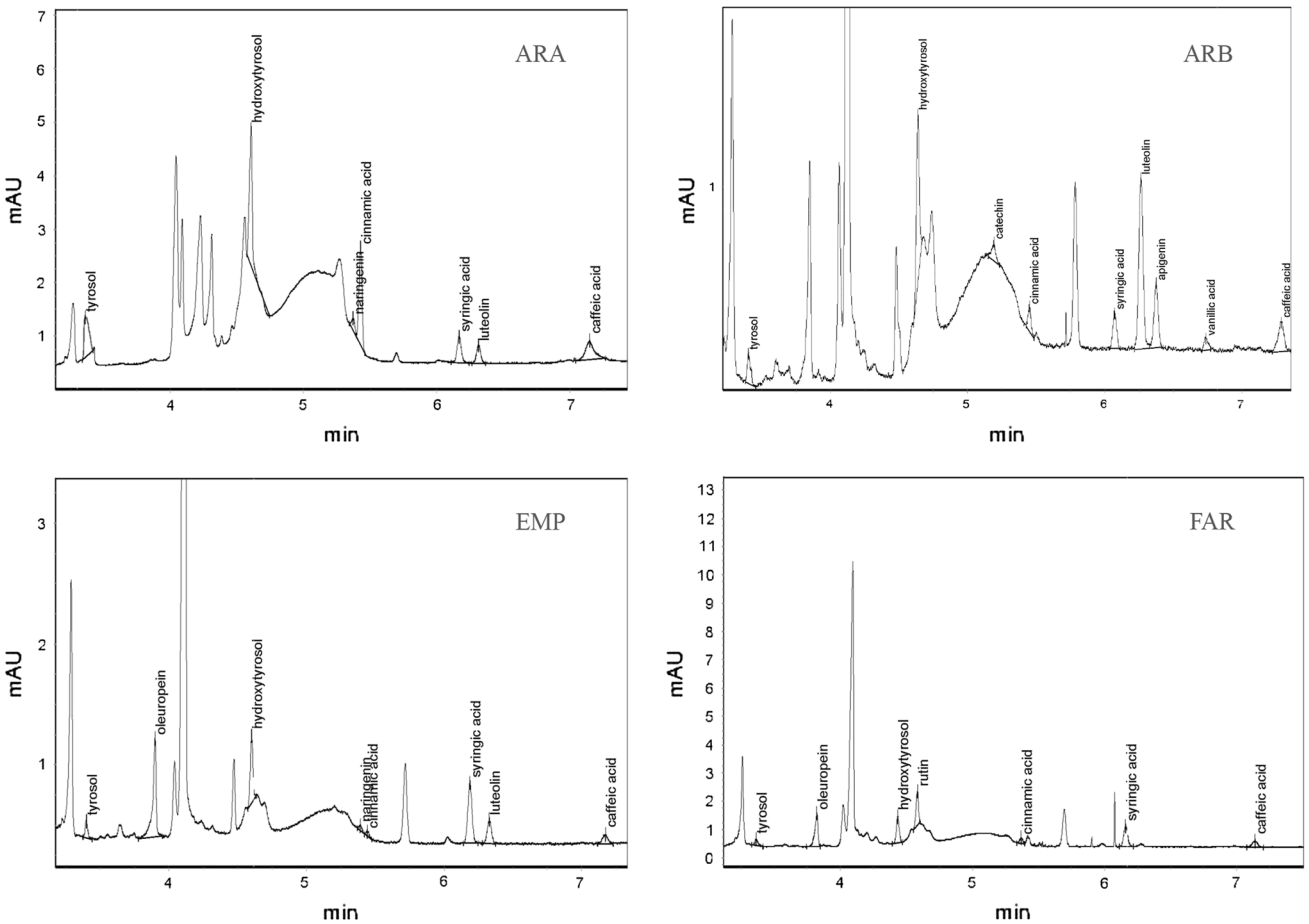

Fig. 3 CZE profile of phenolic compounds from different monovarietal VOO (*ARA* Arauco, *ARB* Arbequina, *EMP* Empeltre, *FAR* Farga). The absorbance scales (mAU) in the electropherograms are different. Conditions: 30 mM boric acid buffer, pH 9.5; capillary 67 cm full length, 50 cm effective length, 75 m ID, 375 m OD; samples were injected by hydrodynamic injection at 30 mbar for 2 s; 25 kV constant voltage and the capillary temperature was 25 °C; detection by UV absorbance at 240 nm

into different classes characterized by different functional groups: simple phenols (tyrosol, hydroxytyrosol); flavonoids (luteolin, catechin, naringenin, rutin, apigenin, and quercetin); cinnamic acid and derivate (cinnamic acid, chlorogenic acid, caffeic acid); benzoic acid and derivate (syringic acid, vanillic acid) and secoiridoids (oleuropein). Principal component analysis (PCA) was used to highlight the data structure and to find the relationships between phenolic compounds and olive oil variety (Fig. 4). PCA permitted a reduction of phenolic compounds found in olive oil to three principal components (with eigenvalues>1). These three PCs were extracted explaining 99.6 % of the total variance of EVOO samples. The first principal component (PC 1) represented about 49.6 %, and the next PCs, 33.3, and 16.6 %, respectively. Arauco was generally represented with high concentrations of simple phenols, cinnamic acid and its derivatives, and PT. In contrast, Arbequina, Farga, and Empeltre presented low concentrations of these compounds. The benzoic acid derivate concentration was higher in Farga and Empeltre cultivars. The samples of Empeltre were characterized by high concentrations of secoiridoids. Flavonoids were the most important compounds of Farga olive oils.

Antioxidant capacity

In general, the methods for determining the antioxidant capacity of food components can deactivate radicals by two major mechanisms and were divided into two major groups: assays based on the SET (single electron transfer) reaction and assays based on a HAT (hydrogen atom transfer). SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound. HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation [44]. The DPPH and FRAP tests performed in this work are SET-based methods. The ABTS methods reported used both HAT and mechanisms SET [45].

Table 2 Concentration of phenolic compounds (μg/g^{-1}) determined by SPE/CZE in monovarietal olive oil samples from Mendoza

Phenolic compound	ARA	ARB	FAR	EMP
Tyrosol	127.16 ± 0.09 a	7.97 ± 0.12 d	16.31 ± 0.32 b	9.33 ± 0.17 c
Oleuropein	nd	nd	1.65 ± 0.03 b	72.65 ± 0.81 a
Hydroxytyrosol	670.94 ± 13.91 a	49.37 ± 0.63 b	30.01 ± 1.48 b	24.7 ± 0.24 b
Rutin	nd	nd	74.81 ± 1.54 a	nd
Catechin	nd	3.35 ± 0.35 a	nd	nd
Naringenin	5.62 ± 0.16 a	nd	nd	1.24 ± 0.06 b
Cinnamic acid	40.3 ± 1.28 a	1.97 ± 0.02 bc	4.82 ± 0.33 b	1.21 ± 0.01 c
Syringic acid	8.82 ± 0.22 b	2.14 ± 0.05 c	12.6 ± 0.6 a	9.5 ± 0.24 b
Luteolin	7.06 ± 0.19 b	17.31 ± 0.42 a	1.98 ± 0.15 d	4.2 ± 0.01 c
Apigenin	nd	4.19 ± 0.11 a	nd	nd
Vanillic acid	nd	1.65 ± 0.04 a	nd	nd
Caffeic acid	13.89 ± 0.04 a	2.97 ± 0.02 c	4.96 ± 0.28 b	2.22 ± 0.06 d
Simple phenols	798.11 ± 13.82 a	57.34 ± 0.67 b	46.32 ± 1.34 b	34.03 ± 0.41 b
Flavonoids	12.68 ± 0.04 c	24.85 ± 0.27 b	76.78 ± 1.45 a	5.44 ± 0.06 d
Phenolic acid (CD)	54.18 ± 1.24 a	4.94 ± 0.04 c	9.77 ± 0.25 b	3.42 ± 0.08 c
Phenolic acid (BD)	8.82 ± 0.22 b	3.78 ± 0.08 c	12.6 ± 0.6 a	9.5 ± 0.24 b
Secoiridoids	nd	nd	1.65 ± 0.03 b	72.65 ± 0.81 a
PT	873.79 ± 15.3 a	90.92 ± 0.64 c	147.12 ± 0.41 b	125.04 ± 1.1 bc

Data represent average of triplicate measurements with indicated standard error for each cultivar in μg/g^{-1} (ppm)

Different letters represent significant differences between samples ($p < 0.05$)

PT total amount by CZE quantified phenolic compounds

Nd not detected, Argentine *ARA* Arauco, *ARB* Arbequina, *FAR* Farga, *EMP* Empeltre

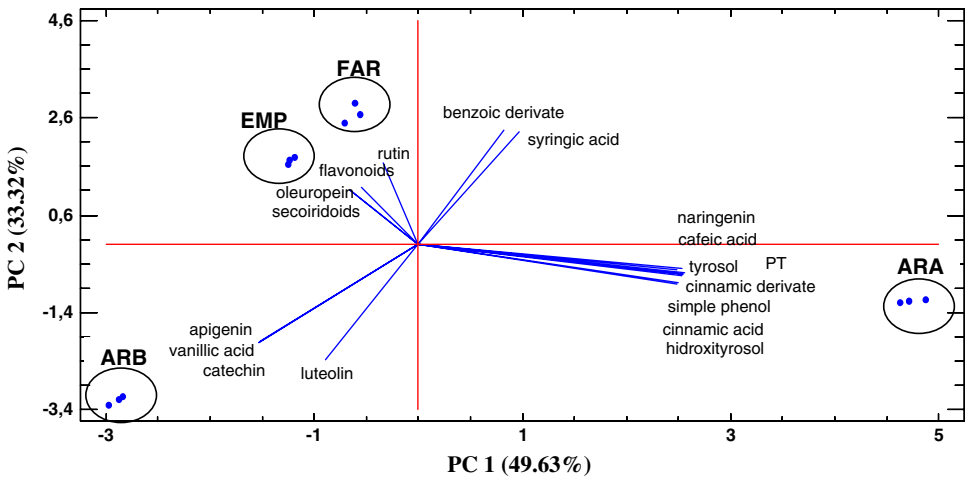

Fig. 4 Principal Component Analysis of phenolic content (μg/g^{-1}) from different monovarietal VOOs (*ARA* Arauco, *ARB* Arbequina, *EMP* Empeltre, *FAR* Farga) analyzed by CZE

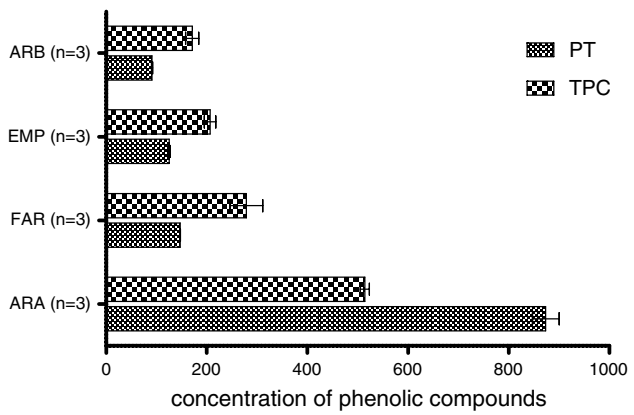

Fig. 5 Comparison of total phenolic compounds (μg/g^{-1}) analyzed by Folin-Ciocalteu (TPC) and by CZE (PT). Experimental conditions as shown in the Materials and Methods section

The data for antioxidant capacities of the Argentine olive oils studied are summarized in Table 3. The results obtained are similar in the three assays performed. As can be seen, the Arauco olive oil shows the highest antioxidant capacity in all tests, following in descending order the oils obtained from the Farga, Empeltre, and Arbequina cultivars.

Relationships between CZE phenolic profile, TPC, and antioxidant capacity

Comparison of the mean concentrations of phenolic compounds determined by CZE and Folin–Ciocalteu showed that the order of the cultivars considering their phenolic content was: Arauco>Farga>Empeltre>Arbequina for both methods (Fig. 5). The positive Pearson's correlation found between the TPC and PT-CZE ($r^2 = 0.97$, $p < 0.001$)

Table 3 Antioxidant capacity of monovarietal olive oils

Mean ± standard error of the inhibition percentage for each varietal VOO different letters represent significant differences

Antioxidant test	ARA	ARB	FAR	EMP
ABTS	77.01 ± 1.23 a	24.98 ± 0.82 d	38.13 ± 0.64 b	30.93 ± 0.8 c
DPPH	97.12 ± 0.82 a	30.78 ± 0.36 d	56.85 ± 0.58 b	42.11 ± 0.42 c
FRAP	98.23 ± 1.87 a	30.34 ± 0.67 d	52.02 ± 0.94 b	38.52 ± 1.28 c

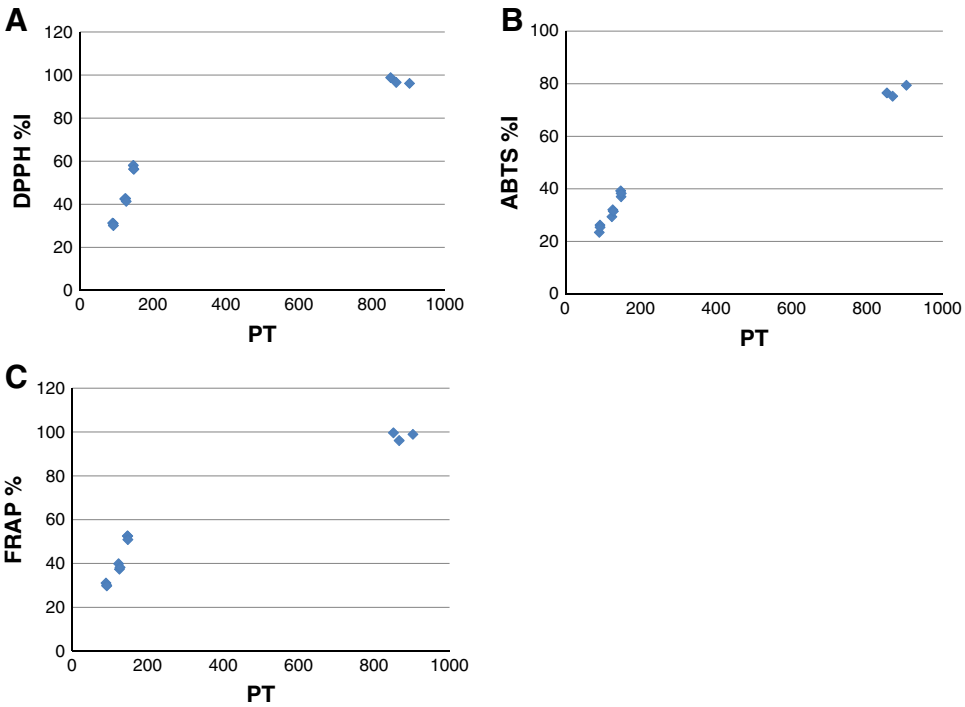

Fig. 6 Correlation between total phenolic compounds analyzed by CZE (PT, $\mu g/g^{-1}$) and antioxidant capacity of monovarietal VOO (A: DPPH % I; B: ABTS % I and C: FRAP %)

Table 4 Correlation (r^2) between antioxidant capacity determined for ABTS test and the phenolic compounds analyzed (individual compounds and families of compounds)

Variable 2	*n*	*r*	*p* value
Tyrosol	12	0.98	<0.0001
Oleuropein	12	−0.34	0.2784
Hydroxytyrosol	12	0.97	<0.0001
Rutin	12	−0.13	0.6859
Catechin	12	−0.5	0.0961
Naringenin	12	0.94	<0.0001
Cinnamic acid	12	0.98	<0.0001
Syringic acid	12	0.3	0.3469
Luteolin	12	−0.26	0.4149
Apigenin	12	−0.5	0.0953
Vanillic acid	12	−0.51	0.0938
Caffeic acid	12	0.98	<0.0001
Simple phenol	12	0.97	<0.0001
Flavonoids	12	−0.19	0.5601
Phenolic acid (CD)	12	0.99	<0.0001
PHENOLIC acid (BD)	12	0.25	0.4424
Secoiridoids	12	−0.34	0.2784

confirmed a good agreement in the quantification of the main phenolic compounds of olive oil achieved by the two techniques. The results indicate that the TPC and CZE estimations of total phenols content are reliably correlated, regardless for the absolute contents, and are independent of the relative composition of the phenolic fraction. Similar results are shown when comparing total phenolic composition data obtained by HPLC–DAD and Folin [46]. This suggests caution about interpretation of Folin results for olive oils characterized by very different phenolic profiles.

On the other hand it can be seen that increases in the total phenolic content also increases the antioxidant capacity of oils (Fig. 6a, b, c). The latter is in agreement with results obtained by other researchers for other cultivars [32, 47]. The correlation between the content of total polyphenols and antioxidant capacity is high for the three antioxidant assays performed ($r^2 > 0.95$, $p < 0.001$), however the determination of antioxidant capacity by ABTS is shown to have a greater coefficient ($r^2 = 0.98$, $p < 0.001$). This result is consistent with previous papers determining the antioxidant activity of olive oil by different methods [9, 21]. For this reason, this test was used to assess the relationship

between the antioxidant capacity and phenolic composition of the olive oils under study (Table 4). Tyrosol, hydroxytyrosol, naringenin, caffeic acid, and cinnamic acid showed a highly significant and strong positive correlation ($r > 0.90$) with the antioxidant capacity of oils. When analyzing the correlation coefficients of the different families of phenolic compounds studied, it was found that simple phenols and cinnamic acid derivatives showed a higher correlation with antioxidant capacity (Table 4). The antioxidant activity of these groups of compounds had been previously studied [15, 48]. Carrasco-Pancorbo, et al. (2005) determined that the presence of different substituents in the phenol backbone structures modulates their antioxidant properties, in particular their hydrogen-donating capacities. The presence of an *o*-diphenol enhances the ability of the phenolic compounds to act as antioxidants. This gives hydroxytyrosol a strong antioxidant activity in comparison with tyrosol, which has shown to have a lower antioxidant power. These results are similar to the reports by Gomez-Alonso et al. (2002). On the other hand, the derivatives of cinnamic acid are more active antioxidants than the derivatives of benzoic acid derivatives. The presence of the CH2=CH–COOH group in cinnamic acids ensures greater antioxidant capacity than the COOH group in benzoic acid [47]. On the other hand, although it is possible that the radical scavenging activity of EVOOs could be mediated by individual phenolic acids, the overall antioxidant potential of EVOOs is likely exhibited by the synergistic effect of the combinations of phenolic acids and other antioxidant components, considering the wide mixture of phenolic antioxidants present in olive oils [5].

The content of phenolic compounds is an important parameter in the evaluation of VOO quality because phenols largely contribute to oil flavor and aroma and protect the free fatty acid fraction from oxidation. The recognized nutritional value of extra VOO is a direct expression of its antioxidant power, namely its ability to inhibit oxidative reactions that are involved in the beginning and progression of many human diseases [49]. Thus, the findings obtained in this study demonstrate that Arauco olive oil, autochthonous of Argentina, possesses antioxidant/free-radical scavenging properties, which are very likely due to the presence of high contents of phenolic compounds, particularly higher concentrations of hydroxytyrosol, tyrosol, and cinnamic acid derivatives compared with oils of other cultivars' studies. In the same way Arauco shows the highest content of total phenols. Studies carried out by various investigators have shown that the organoleptic properties of EVOO are largely affected by their phenolic composition. The use of total phenol concentration as a bitter predictor is not recommended. In fact, it is assumed that the stimuli responsible for bitterness in virgin olive oils are tyrosol, hydroxytyrosol, and their derivatives [50]. It is found that, among the phenolic compounds contained in olive oil, the antioxidant effect is, in a decreasing order: hydroxytyrosol>oleuropein>tyrosol [51]. According to Papadopoulos and Boskou [52] and Tsimidou et al. [53], hydroxytyrosol (but not tyrosol) concentration was closely correlated with the stability of the oil.

Considering the complexity of phenolic composition in olive oil, the current, single Folin assay may not be the best choice to characterize an oil sample. Choosing an appropriate phenolic assay depends on what kind of information is required. For example, the total phenol content estimated by the Folin assay may be useful to determine the approximate actual content of phenolics, but this amount may not correspond directly to the organoleptic property of bitterness, or to specific health properties such as the total antioxidant power.

Conclusions

The phenolic composition represents a useful contribution to the biochemical characterization of Argentinean olive oil cultivars. Oleuropein, hydroxytyrosol, tyrosol, and flavonoids, vary quantitatively according to the variety; in fact, the cultivar affects quantitative phenolic fractions of these EVOOS, demonstrating a unique and characteristic phenolic profile. These phenolic fractions also influenced and allowed differentiation of the total antioxidant observed in the cultivars.

The chemical characterization of Argentinean monovarietal olive oils is mandatory for selection of varieties that can produce virgin olive oil with good quality characteristics and adapted to the environmental conditions, especially the arid climate of midwest Argentina. The analysis of extra virgin olive oils from four varieties demonstrates that excellent olive oils in terms of quality indices, phenolic contents, and antioxidant capacity can be produced in this region. Also, Arauco olive oil shows higher mean values of total phenols and antioxidant capacity. This variety can be used to improve the shelf life of other Argentine varieties (Arbequina) by blending oils. On the other hand, this Argentine olive oil may constitute a good source of healthy compounds in the diet, especially phenolic compounds, suggesting that their consumption could be useful in the prevention of diseases in which free radicals are implicated. As far as we know, this is the first report considering the antioxidant potential of a phenolic fraction in EVOO from Argentina.

Acknowledgments This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo (Mendoza, Argentina). We are particularly grateful to Enrique Tittarelli for providing the equipment for the extraction of olive oil and assistance in the technical aspects of olive tree cultivation.

Conflict of interest María de los Angeles Fernandez declares that she has no conflict of interest. Verónica Carolina Soto Vargas declares that she has no conflict of interest. María Fernanda Silva declares that she has no conflict of interest. This article does not contain any studies with human or animal subjects.

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