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Rapid Quantification of the Phenolic Fraction of Spanish Virgin Olive Oils by Capillary Electrophoresis with UV Detection

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A rapid and reliable capillary zone electrophoresis method was used as a tool to obtain both qualitative and quantitative information about simple phenols, lignans, complex phenols (isomeric forms of secoiridoids), phenolic acids, and flavonoids in the solid-phase separation extracts from different Spanish extra-virgin olive oil in a short time (less than 6 min). Peak identification was done by using commercial and HPLC-isolated standards, studying the information of the electropherograms obtained at several wavelengths and also using the information previously reported. For the quantification of lignans and complex phenols (secoiridoid derivatives), we used a reference compound (oleuropein glucoside) at two different wavelengths (200 and 240 nm) and for the quantification of tyrosol and flavonoids, we used their commercially available standards.

KEYWORDS: Polyphenols; quantification; capillary zone electrophoresis; olive oil; flavonoids; secoiridoids; SPE

INTRODUCTION

The traditional Mediterranean diet is characterized by an abundance of plant foods (fruits, vegetables, cereals, legumes) in which olive oil is the principal source of fat.

Olives (*Olea europaea* L.) and virgin olive oil provide a rich source of natural antioxidants. These include carotenoids, tocopherols, and phenolic compounds, which may act, by different mechanisms, as an effective defense against reactive species (*1*).

The content of phenolic compounds is an important factor to be considered when evaluating the quality of virgin olive oil (*2*), since members of this family of compounds have potent antioxidant activity and contribute significantly to the extraordinary stability of olive oil against oxidation (*3*). Several epidemiological studies have shown that phenolic compounds afford considerable protection against cancer (skin, breast, and colon), coronary heart disease, and aging by inhibiting oxidative stress (*4*−*6*). Also, phenolic compounds are related to the sensory and nutritional qualities of virgin olive oil (*7*−*9*).

Therefore, it would be very interesting to have a simple, rapid, and reliable method in order to quantify these compounds. Traditionally, separation and determination of individual phenolic compounds in the extracts obtained from olive oil have been carried out by high-performance liquid chromatography (HPLC) analysis coupled to different detection systems (*10*, *11*) and gas chromatography (*10*, *11*). Both of them have obtained reliable results, but they show some limitations: the long time necessary for the analysis and the partial separation in cases where components have a complex structure (such as secoiridoid compounds) for HPLC and the problem dealing with the sample derivatization for GC.

On the other hand, capillary electrophoresis (CE) has proven to be a fast, valid, and reliable tool for food analysis (*12*, *13*). It is a powerful technique that affords rapid and high-resolution separations (10^4−10^6 theoretical plates) while requiring only few microliters of sample. Futhermore, a wide range of compounds are amenable to separations by CE.

In particular, this technique has recently been used for the analysis of phenolic compounds of extra-virgin olive oil (*14*−*21*), olive mill wastewater (*22*), and alperujo (*23*).

In almost all the mentioned papers that study the phenolic compounds present in extracts of extra-virgin olive oil the detection system was UV (*14*−*17*, *19*, *21*), only one of them used MS as detection system (*20*). The electrophoretic conditions for the methods which use UV detection are not drastically different regarding the experimental and instrumental variables. However, the differences can be found when the extraction system used and the families under study are observed.

Phenolic acids have been the family of interest in three of these research works (*17*−*19*); in the others (*14*−*16*, *20*, *21*), the aim of the authors was to study the complete polyphenolic fraction of the olive oil, attempting to determine several families of phenols simultaneously.

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Bendini et al. (*14*−*16*) identified several important compounds of the polyphenolic fraction in virgin olive oil samples (tyrosol, hyodroxytyrosol, lignans, and decarboxylated oleuropein zone) by using a fast electrophoretic method. Then, in a published paper by our group (*21*), the number of identified compounds in an electrophoretic profile of an extract of virgin olive oil increased using standards obtained by semipreparative HPLC, although the analysis time in this case was long.

Recently, the characterization of the polar extracts of olive oil has been improved a bit more by using a recent method that is able to determine 20 compounds belonging to simple phenols, lignans, complex phenols (isomeric forms of secoiridoids), phenolic acids, and flavonoids in the solid-phase separation extracts from extra-virgin olive oil in a short time (about 6 min) (*24*).

So, the aim of this work was to demonstrate the usefulness of the latter method (*24*) to compare several Spanish extra-virgin olive oil samples by carrying out the quantitation (individually and in groups taking into account the different categories of phenolic compounds) of 14 phenolic compounds belonging to simple and complex phenols, lignans, and flavonoids. Using multivariate statistics, we also tried to distinguish the oils under study taking into account their phenolic content.

MATERIALS AND METHODS

Apparatus. The CE instrument used was a Beckman 5500 capillary electrophoresis connected to a diode-array detector (belonging to the University of Granada). The system comprises a 0−30 kV high-voltage built in power supply.

All capillaries (fused silica) used were obtained from Beckman instrument, Inc. (Fullerton, CA) and had an inner diameter (i.d.) of 50 μm, a total length of 47 cm, and an effective separation length of 40 cm. The temperature was controlled using a fluorocarbon-based cooling fluid. Data acquisition and processing were carried out with GOLD software (Beckman instrument Inc.) installed on a personal computer.

To carry out the reproducibility studies, the CE instrument used was a Beckman 5500 capillary electrophoresis connected to a diode-array detector (belonging to the University of Bologna) and the capillaries (fused silica) used were obtained from Composite Metal Services (Worcester, England) and had the same inner diameter and length as those described above.

Reagents, Stock Solutions, and Reference Compounds. 2-(4-Hydroxyphenyl)ethanol (tyrosol (TY)) was acquired from Fluka (Buchs, Swiss) and oleuropein (oleuropein glucoside) was obtained from Extrasynthèse (Genay, France). The stock solution containing these two analytes was prepared in methanol/water (50/50, v/v) at a concentration of 500 μg/mL in the case of tyrosol and 8000 μg/mL for oleuropein glucoside. Tyrosol was used for the quantification of this compound present in the extracts of olive oil, and oleuropein glucoside was used to make the calibration curves for the quantification of lignans and complex phenols.

The flavonoids luteolin and apigenin were obtained from Extrasynthèse and all the analytes were used as received. The standards of luteolin and apigenin were used for the quantification of these two compounds in olive oil; the stock solution containing them was prepared in methanol/water (50:50, v/v) at a concentration of 250 μg/mL in the case of luteolin and 100 μg/mL for apigenin.

Sodium hydroxide was purchased from Merck (Darmstadt, Germany); sodium tetraborate (borax) was obtained from Sigma-Aldrich (St. Louis, MO) and was used as running buffer at different concentrations and pHs.

Methanol, acetonitrile, and *n*-hexane were acquired from Panreac (Montcada I Reixac, Barcelona, Spain) and were HPLC grade.

Doubly deionized water with a conductivity of 18.2 MΩ was obtained by using a Milli-Q system (Millipore, Bedford, MA).

DSC-Diol solid-phase separation cartridges were obtained from Supelco (Bellefonte, PA).

Samples. Spanish extra-virgin olive-oil samples were obtained from a unique variety of olive fruit called picual, hojiblanca, lechín de Sevilla, arbequina, and cornicabra (January 2005). An organic olive oil was obtained in the supermarket, where we also acquired two types of picual olive oil ["suave" (mild) and "intenso" (strong flavor)].

Solid-Phase Extraction (SPE) Procedure and Preparation of Oil Spiked with Phenolic Extract. To isolate the phenolic fraction, we used SPE with DSC-Diol cartridges; the SPE protocol was carried out with the extraction conditions and amounts of oil that are described in a recent paper of our research group (*21*).

Electrophoretic Procedure. CE separation was carried out on a fused silica capillary (50 μm i.d., 375 μm o.d., total length 47 cm; a detection window was created at 40 cm from the capillary inlet, by removing the polyimide coating and using a cartridge with a slide of 100 × 200 μm). Every time a new capillary was used it was preconditioned by rinsing with 0.5 M NaOH for 20 min, followed by a 5 min rinse with Milli-Q water and 15 min with buffer. For the following analyses the capillary was rinsed with 0.1 M NaOH for 2 min, followed by a 2-min flush with Milli-Q water to ensure good repeatability. The capillary was equilibrated with the running buffer (45 mM sodium tetraborate adjusted to pH 9.3) for 3 min before each sample injection. After each analysis, the capillary tubing was rinsed for 2 min with Milli-Q water. All solutions and samples were filtered through a 0.25-μm syringe filter. The running buffer was changed after four runs.

Samples were injected hydrodynamically in the anodic end in low-pressure mode (0.5 psi) for 8 s (1 psi = 6895 Pa). Electrophoretic separations were performed at 28 kV for 10 min, and the temperature was maintained at 22 °C.

UV detection was performed in all cases at 200, 240, 280, and 340 nm, although diode-array detection was used over the range of 190−600 nm to collect spectral data. The quantification of the phenolic compounds determined in this study was carried out at 200 and 240 nm, but the detection at four wavelengths and the complete spectral information were used to facilitate the identification of all the analytes (*24*).

Peak areas and migration velocity (cm/min) were used for the quantification of the analytes versus oleuropein glucoside in some cases (complex phenols and lignans), and peak areas were used for the quantification of tyrosol, luteolin, and apigenin versus the corresponding commercial standards.

The electropherogram obtained for an extract of extra-virgin olive oil of picual variety at optimized conditions is presented in **Figure 1**.

Statistical Analysis. The results reported in this study are the averages of at least three repetitions ($n = 3$), unless otherwise stated. Tukey's honest significant difference (HSD) multiple comparison (one-way ANOVA) and Pearson's linear correlations, both at $p < 0.05$, were evaluated using Statistica 6.0 (2001, StatSoft, Tulsa, OK). Data were also analyzed by multivariate exploratory techniques, in particular, factor analysis and principal components and classification analysis (using Statistica 6.0) to evidence the correlation between the analyses and the differences in the samples. The analytical data were arranged in a matrix with the rows corresponding to the samples (objects) and the columns corresponding to the analytical indices (variables).

RESULTS AND DISCUSSION

Analytical Parameters of the Method Proposed. We carried out a study to check the repeatability and reproducibility of the proposed method, as well as to establish the calibration curves to quantify the compounds under study and calculate the detection, quantification limit, and precision [as relative standard deviation (RSD) of the intermediate concentration value of linear range] of the method.

Repeatability and Reproducibility Study. For carrying out this study extracts from the same extra-virgin olive oil (picual variety) were prepared.

Repeatability was studied by performing a series of separations using the optimized method on one of the samples on the same day (intraday precision, $n = 12$) and on three consecutive days (interday precision, $n = 36$). The relative standard deviations (RSDs) of peak areas/migration time and migration times were determined considering five of the compounds present in

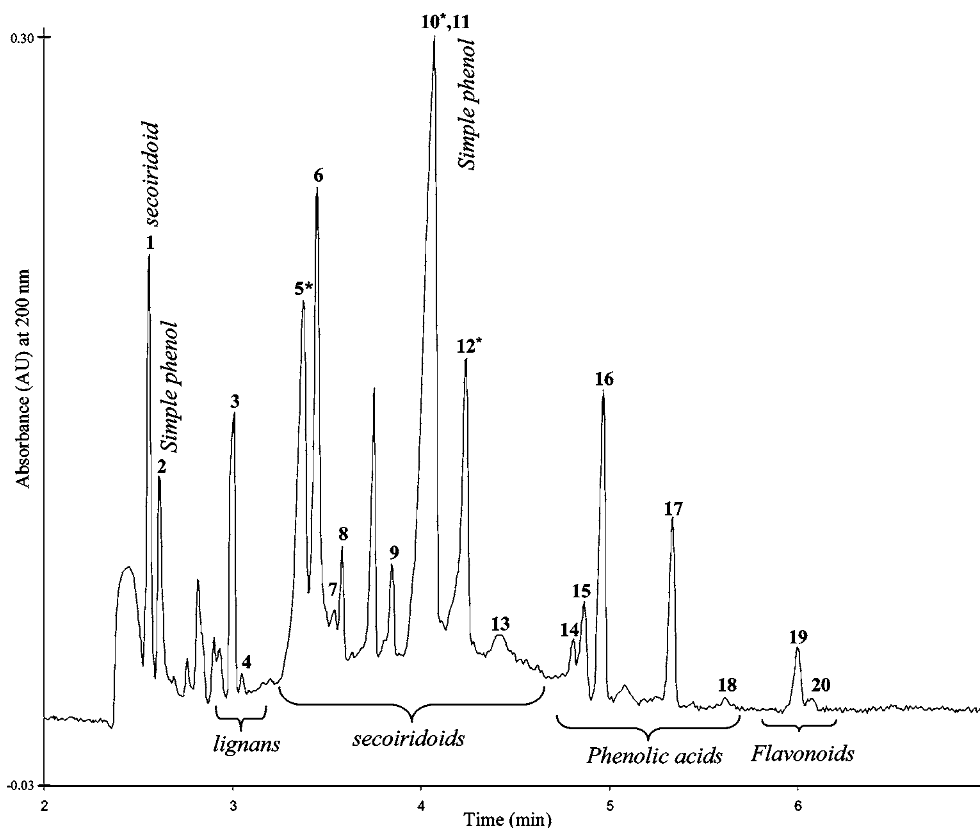

Figure 1. CZE of extra-virgin olive oil (picual variety) sample under optimized conditions. Detection was performed at 200 nm. Peak identification numbers: 1, Lig Agl (a); 2, TY; 3, Pin; 4, Ac Pin; 5, Ol Agl (a) + DOA (a); 6, DOA (b); 7, Lig Agl (b); 8, Ol Agl (b); 9, EA (a); 10, Ol Agl (c) + Lig Agl (c) + DOA (c) + EA (b,c); 11, HYTY; 12, DOA (d) + EA (d); 13, EA (e); 14, *trans*-cinnamic acid; 15, 4-hydroxyphenylacetic acid; 16, sinapinic acid; 17, gentisic acid; 18, *o*-coumaric acid; 19, luteolin; and 20, apigenin. *Peaks overlapped. The letters (a), (b), (c), (d), and (e) are used to distinguish the different isomeric forms of secoiridoids.

Table 1. RSD Values of the Peak Area and Migration Time Obtained in the Study of Repeatability and Reproducibility

	repeatability					
	intraday ($n = 12$)		interday ($n = 36$)		reproducibility ($n = 20$)	
compound	peak area/migration time	migration time	peak area/migration time	migration time	peak area/migration time	migration time
tyrosol	2.32	0.48	4.99	0.89	6.29	0.91
(+)-pinoresinol	1.26	0.49	2.87	0.82	3.03	0.95
DOA	2.03	0.62	3.25	0.91	3.35	1.25
4-HFA	1.32	0.51	2.32	0.81	2.45	0.87
luteolin	1.25	0.91	2.41	1.61	2.93	2.57

the extracts [tyrosol, (+)-pinoresinol, decarboxylated oleuropein aglycon (peak b), 4-hydroxyphenylacetic acid, and luteolin].

All the data obtained in this study are summarized in **Table 1**. Both the intraday and the interday repeatabilities calculated on the migration time for these five analytes (expressed as RSD) and the intraday and interday repeatability values on the total peak area/migration time (expressed as RSD) were acceptable.

The reproducibility was studied by performing 20 separations of another extract, under the same conditions but with a different operator, different capillary, and different CE instrument (belonging to the University of Bologna). The results obtained for the migration times and for the total peak area/migration time (expressed as RSD) considering the five analytes previously mentioned were satisfactory.

Calibration Curves. Standard calibration graphs for lignans and complex phenols were prepared using oleuropein (oleuropein glucoside) at two wavelengths (200 and 240 nm). An external standard methodology (with oleuropein glucoside) was used for the quantification of lignans and complex phenols (as there are no commercially available standards of these compounds).

Using this methodology, time-corrected area counts are necessary for quantitative CE. Since separation is dependent on analytes migrating at different rates, the separated analytes will pass the detector at different rates. Time-corrected area counts are the product of the area counts and the velocity for each peak in the electropherogram.

Thus, peak areas and migration velocity (cm/min) were used to calculate the time-corrected area counts to compensate the differences in migration velocities among oleuropein glucoside, lignans, and complex phenols.

Lig Agl (a), Pin, Ac Pin, Ol Agl (a) + DOA (a), DOA (b), Lig Agl (b), Ol Agl (b), EA (a), and DOA (d) + EA (d) were quantified with the data obtained for the calibration curve of oleuropein obtained at 200 nm. However, for the quantification of Ol Agl (c) + Lig Agl (c) + DOA (c) + EA (b+c) and Lig Agl (d) + Ol Agl (d) + EA (e) the curve of oleuropein at 240 nm was used. Although oleuropein aglycon [peak c and other secoiridoid forms overlapped, Lig Agl (c) + EA (b + c)] and hydroxytyrosol have the same retention time (overlap), it was possible to calculate (approximately) the concentration of secoiridoids with this migration time ($t_{mig} = 4.07$ min) in the virgin olive oil extracts using the curve of calibration of oleuropein at 240 nm, since hydroxytyrosol has a minimum of absorbance at this wavelength (approximately) (*24*).

Tyrosol was quantified using the calibration curve of its commercial standard at 200 nm, and flavonoids were quantified

Table 2. Analytical Parameters of Proposed Method

analyte	RSD[a] (%) (intermediate value)	detection limit (μg/mL)	quantification limit (μg/mL)	calibration range (μg/mL)	calibration equations	r^2
tyrosol[b]	2.53	0.836	2.787	2.787–500	$y = 1.88{\cdot}10^{-5}x + 1.37{\cdot}10^{-5}$	0.994
oleuropein glucoside[b] $\lambda = 200$ nm	2.21	2.085	6.950	6.950–6500	$y = 1{\cdot}10^{-5}x + 1{\cdot}10^{-4}$	0.996
oleuropein glucoside[c] $\lambda = 200$ nm	2.01	0.104	0.348	0.348–6500	$y = 2{\cdot}10^{-4}x + 9{\cdot}10^{-4}$	0.997
oleuropein glucoside[b] $\lambda = 240$ nm	4.93	4.170	13.901	13.901–2000	$y = 5{\cdot}10^{-6}x + 6{\cdot}10^{-6}$	0.998
oleuropein glucoside[c] $\lambda = 240$ nm	1.73	0.348	1.158	1.158–2000	$y = 6{\cdot}10^{-5}x - 2{\cdot}10^{-4}$	0.995
luteolin[b]	3.06	0.355	1.183	1.183–200	$y = 5.88{\cdot}10^{-5}x + 5.67{\cdot}10^{-5}$	0.992
apigenin[b]	2.23	0.311	1.037	1.037–200	$y = 6.7{\cdot}10^{-5}x + 4.8{\cdot}10^{-5}$	0.995

[a] RSD: relative standard deviation. LD = $3\sigma_b/b$ ($\sigma_b = 6.95 \times 10^{-6}$, calculated using 100 data); LQ = $10\sigma_b/b$. Data obtained using the software Microcal Origin, Microcal Software, Inc. [b] $y = bx \pm a$, where y is the peak area (AU), x is the concentration (μg/mL), a is the y intercept, and r^2 is the correlation coefficient. [c] $y = bx \pm a$, where y is the [time corrected area counts = peak area (AU) × migration velocity (cm/min)].

using the calibration curves obtained at 200 nm for commercial standards of luteolin and apigenin respectively. Peak areas were used for the quantification of these three compounds versus the corresponding commercial standards.

The determination of the phenolic acids in olive oil has been carried out by other research groups and by our own group (*17*−*19*) (for this reason we do not show the data corresponding to the calibration curves and quantification results for this family of compounds). However, it is the first time in which flavonoids (luteolin and apigenin) and several secoiridoid compounds from olive oil are identified and quantified using capillary zone electrophoresis (CZE). We have also obtained the quantification in terms of families of phenolic compounds (simple phenols, complex phenols, lignans, and flavonoids) present in olive oil.

The detection, quantification limit, and precision (as relative standard deviation of the intermediate concentration value of the linear range) of this method were calculated for the studied analytes using the method proposed by Curie (*25*). Three replicates of each analyte at different concentrations were done in order to set up the calibration.

All calibration curves showed good linearity in the studied range of concentrations. The calibration plots indicate good correlation between peak areas and analyte concentrations in the case of tyrosol and flavonoids, and between time-corrected area counts and analyte concentrations for the secoiridoids and lignans; regression coefficients were higher than 0.994 for tyrosol and oleuropein (at the two wavelengths), and were higher than 0.992 for luteolin and apigenin. All the features of the proposed method are summarized in **Table 2**.

Recovery of Secoiridoid Compounds and Flavonoids. As far as the recoveries of these compounds are concerned using solid-phase extraction, it is important to explain that we have used the protocol described by Gómez-Caravaca et al. (*21*), and we have spiked a refined sunflower oil with an exactly specified dose of a phenolic extract previously obtained. The results are summarized in **Table 3**.

Table 3. Recovery (%) of Phenolic Compounds (Flavonoids and Secoiridoids) Isolated by Solid-Phase Extraction from Reference Sunflower Oil Samples ($n = 3$)

	method 1[a]	SD[b]	method 2[c]	SD
		flavonoids		
luteolin	89.34	2.43	89.56	2.29
apigenin	91.01	1.98	92.12	2.45
		secoiridoids		
Lig Agl (a)[d]	71.80	2.57	72.07	1.66
Ol Agl (a) + DOA (a)[d]	72.34	1.45	72.45	1.56
Ol Agl (b)[d]	87.41	2.31	88.54	2.00
EA (a)[e]	65.67	1.67	66.99	3.01
EA (d) + DOA (d)[e]	93.33	3.12	93.66	2.48
EA (d)[f]	92.43	2.43	92.12	1.12

[a] Refined sunflower oil spiked with 1 mL of extract of virgin olive oil phenols. [b] SD = standard deviation [c] Refined sunflower oil spiked with 0.5 mL of extract of virgin olive oil phenols. [d] Calculated using the information at 200 and 240 nm. [e] Calculated using the information at 200 nm. [f] Calculated using the information at 240 nm.

Analysis of Several Extra-Virgin Olive Oil Samples. Extracts of eight extra-virgin olive oils were analized. Five of them were of monovarietal extra-virgin olive oils: picual, hojiblanca, lechín de Sevilla, arbequina, and cornicabra (January 2005). An organic olive oil and two types of picual olive oil ("suave" and "intenso") were acquired from a supermarket. All samples were injected in the CE instrument seven times ($n = 7$).

Using the described SPE system and CZE method, all the virgin olive oils were analyzed. The polyphenolic profiles are shown in **Figure 2**. The absorbance scales in the electropherograms are not exactly the same, in order to show each electrophrogram at its maximum of absorbance and see with clarity and ease all the peaks. The quantitative results are presented in **Table 4**.

TY was found in the range of 6781.0−11457.0 μg of analyte/kg of olive oil in this study, although for lechín de Sevilla it was possible to see the highest peak of this compound.

(+)-1-Acetoxypinoresinol was very abundant in arbequina and hojiblanca oils, while in the picual variety and commercial oils its quantity was low. In terms of (+)-pinoresinol, picual and cornicabra were the richest varieties.

As far as the amount of complex phenols is concerned, it is important to highlight that Lig Agl (peak a) was abundant in cornicabra and lechín de Sevilla olive oils, while Lig Agl (peak b) was found at high concentrations in the commercial and cornicabra oils. In terms of Ol Agl (c) + DOA (a), cornicabra was the richest with twice the amount of these compounds as in organic, picual suave, arbequina, and hojiblanca oils. This oil was the richest in terms of DOA (b) as well; however one of the commercial olive oils (picual intenso) and the oil obtained from olives of picual variety had the highest quantities of DOA (d) + EA (d). Ol Agl (b) was not found in all the olive oils analized and EA (a) were in the range of 2607.3−20521.5 μg of analyte/kg of olive oil.

Keeping in mind the two compounds which are quantified at 240 nm [(Ol Agl (c) + Lig Agl (c) + DOA (c) + EA (b,c) and

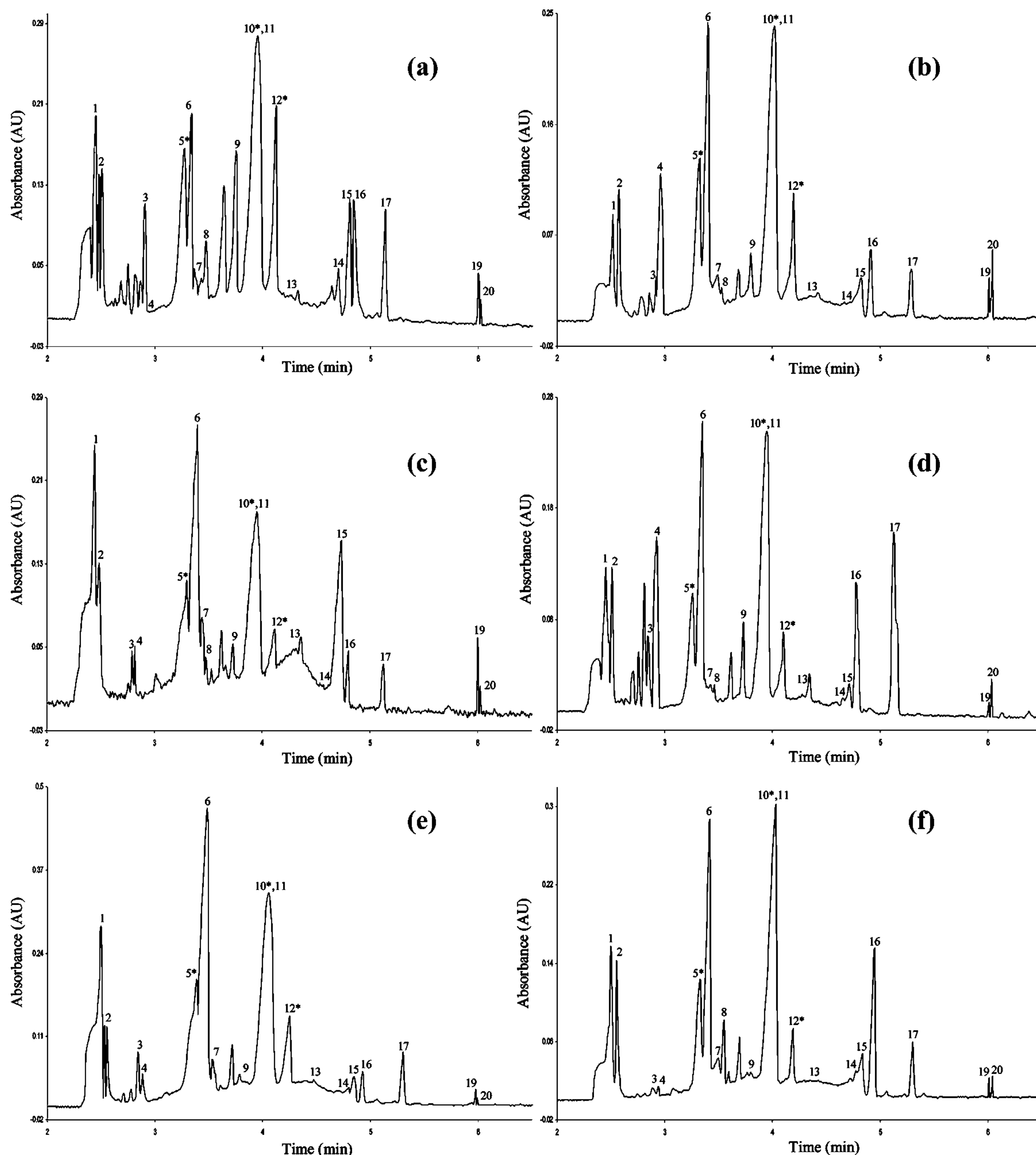

Figure 2. CZE electropherograms of phenolic fraction extracted from extra-virgin olive oil samples by solid-phase extraction: (**a**) picual, (**b**) hojiblanca, (**c**) lechín de Sevilla, (**d**) arbequina, (**e**) cornicabra, and (**f**) organic oil. For identification of the compounds, see **Figure 1**. Detection was performed at 200 nm. The absorbance scales (AU) in the electropherograms are different (Instrumental and experimental parameters as in **Figure 1**).

EA(e) + Lig Agl (d) + Ol Agl (d)], they were significantly more abundant in cornicabra, lechín de Sevilla, and picual intenso, which had very similar quantities.

Flavonoids were found in the range of 657.9−7598.9 μg of analyte/kg of olive oil for the luteolin, and 365.8−2203.8 μg of analyte/kg of olive oil for the apigenin. In hojiblanca extra-virgin olive oil, the quantity of apigenin was greater than that of luteolin.

Considering the families of phenolic compounds that are present in olive oil and doing the quantification in terms of simple phenols, lignans, complex phenols, and flavonoids, the results are those which are shown in **Table 5**.

In our opinion, it is also interesting to observe in detail the differences between picual suave and intenso. As mentioned before, polyphenols are an important functional minor components of virgin olive oils that are responsible for the key sensory characteristics of bitterness, pungency, and astringency (*7*). The intensity of the bitterness of virgin olive oil has been related to the presence of phenolic compounds derived from the hydrolysis of the oleuropein. It is known that oleuropein is the major bitter compound found in olives; however, it is not found at significant concentrations in olive oils, but oleuropein aglycon and isomers of oleuropein are. In several papers it has been concluded that the greater the intensity of bitterness for an olive oil, the greater the content of dialdehydic and aldehydic forms of decarboxymethyl oleuropein aglycon and the dialdehydic form of decarboxymethyl ligstroside aglycon (*8*, *26*, *27*).

We have observed that the quantities found in picual suave and intenso are very similar in terms of simple phenols (TY). Lignans were present in a bigger quantity in picual suave. However, in our opinion the most valuable information can be obtained by taking into account the concentrations of the

Table 4. Results (μg of analyte/kg of olive oil) of the Analysis of Real Samples; Quantification of the Individual Components (n = 7) (±SD)

analyte[a]	picual	hojiblanca	lechín de Sevilla	arbequina	cornicabra	organic oil	picual suave	picual intenso
Lig Agl (a)[c]	26200.8 ± 786.8	11946.8 ± 298.6	41286.9 ± 1238.6	22691.6 ± 907.6	44434.4 ± 1692.7	24102.5 ± 990.6	15311.3 ± 566.5	23632.2 ± 562.1
TY[b]	9492.2 ± 456.6	7278.4 ± 348.5	11457.0 ± 373.4	7726.1 ± 191.6	6781.0 ± 128.1	9417.5 ± 277.9	8148.8 ± 300.7	8173.8 ± 404.6
Pin[c]	8110.5 ± 324.4	3027.2 ± 75.7	2289.9 ± 45.8	4944.9 ± 199.8	6223.3 ± 242.9	1372.8 ± 38.9	2111.3 ± 65.1	473.7 ± 22.6
Ac Pin[c]	299.2 ± 14.0	11972.6 ± 359.2	2630.7 ± 92.1	15694.3 ± 627.8	3504.9 ± 136.3	485.5 ± 26.1	370.1 ± 6.7	335.2 ± 16.0
Ol Agl (a) + DOA (a)[c]	31390.6 ± 1176.2	20949.2 ± 733.2	25349.9 ± 1069.7	17149.8 ± 524.9	45604.1 ± 1582.5	20074.5 ± 850.3	17887.8 ± 734.5	29477.3 ± 1146.7
DOA (b)[c]	19089.9 ± 572.7	25638.9 ± 1261.9	42233.2 ± 1123.4	26547.9 ± 753.4	80246.1 ± 2383.3	29111.7 ± 753.9	15151.1 ± 424.1	39762.7 ± 1500.6
Lig Agl (b)[c]	4496.5 ± 89.9	5086.0 ± 203.4	5566.3 ± 192.0	2706.2 ± 121.3	7400.3 ± 273.1	6548.8 ± 218.3	6767.1 ± 163.2	7443.9 ± 322.0
Ol Agl (b)[c]	5540.8 ± 110.8	1675.7 ± 67.0	1718.5 ± 79.0	783.7 ± 31.3	nd[f]	5143.4 ± 231.4	1670.6 ± 44.3	2486.9 ± 71.0
EA (a)[c]	20521.5 ± 820.8	5464.3 ± 56.6	6545.4 ± 327.3	5541.6 ± 257.7	6236.5 ± 217.0	2607.3 ± 49.5	5155.5 ± 219.9	13726.5 ± 508.6
Ol Agl (c) + Lig Agl (c) + DOA (c) + EA(b,c)[d]	87397.1 ± 3295.9	132407.5 ± 5296.3	207546.4 ± 10149.0	118984.6 ± 2260.7	265177.2 ± 7341.5	117671.5 ± 4585.6	96880.7 ± 2718.3	204555.5 ± 4394.6
DOA (d) + EA (d)[c]	17073.9 ± 341.5	9182.6 ± 367.3	7430.8 ± 221.4	5612.3 ± 224.5	17691.2 ± 577.6	4944.9 ± 107.7	8532.0 ± 315.64	16189.70 ± 634.6
EA (e) + Lig Agl (d) + Ol Agl (d)[d]	57821.7 ± 2023.7	88259.6 ± 3530.4	233478.9 ± 9689.3	89602.4 ± 4659.3	208604.3 ± 6953.5	137049.7 ± 5402.9	145426.5 ± 2668.4	246587.7 ± 9343.2
Lut[e]	5255.8 ± 223.8	657.9 ± 31.1	7598.9 ± 187.5	2454.8 ± 88.0	2213.5 ± 77.6	699.9 ± 23.6	5123.4 ± 156.7	4879.0 ± 123.5
Apig[f]	879.0 ± 43.8	2203.8 ± 109.5	897.7 ± 34.7	1256.3 ± 46.8	365.8 ± 12.0	609.9 ± 19.9	765.7 ± 33.3	658.0 ± 30.8

[a] TY, tyrosol; Pin, (+)-pinoresinol; Ac Pin, 1-(+)-acetoxypinoresinol; DOA, decarboxylated oleuropein aglycon; Lig Agl, ligstroside aglycon; Ol Agl, oleuropein aglycon; EA, elenolic acid; Lut, luteolin; Apig, apigenin. [b] Quantified with a calibration curve of tyrosol at $\lambda = 200$ nm. [c] Quantified with a calibration curve of oleuropein glucoside at $\lambda = 200$ nm (time corrected area counts vs concentration). [d] Quantified with a calibration curve of oleuropein glucoside at $\lambda = 240$ nm (time corrected area counts vs concentration). [e] Quantified with a calibration curve of luteolin at $\lambda = 200$ nm. [f] Quantified with a calibration curve of apigenin at $\lambda = 200$ nm. [g] nd: not detected.

Table 5. Results (mg of analyte/kg of olive oil) of the Analysis of Extra-Virgin Olive Oil Samples; Quantification in Terms of Families of Phenolic Compounds (n = 7) (±SD)

family of compounds[a]	picual	hojiblanca	lechín de Sevilla	arbequina	cornicabra	organic oil	picual suave	picual intenso
simple phenols	9.49 ± 0.39	7.28 ± 0.28	11.46 ± 0.44	7.73 ± 0.25	6.78 ± 0.12	9.42 ± 0.31	8.15 ± 0.19	8.17 ± 0.31
complex phenols (A)	124.31 ± 3.03	799.43 ± 35.64	130.13 ± 4.15	81.03 ± 2.56	201.61 ± 5.01	92.53 ± 4.57	70.48 ± 3.15	132.72 ± 4.21
complex phenols (B)	145.22 ± 4.21	220.77 ± 6.29	441.03 ± 11.21	208.59 ± 5.55	473.78 ± 10.09	254.72 ± 6.21	242.31 ± 5.12	451.14 ± 18.04
lignans	8.41 ± 0.42	15.00 ± 0.28	4.92 ± 0.13	20.64 ± 0.30	9.73 ± 0.23	1.86 ± 0.11	2.48 ± 0.04	0.81 ± 0.03
flavonoids	6.27 ± 0.29	3.16 ± 0.12	8.61 ± 0.38	4.05 ± 0.11	2.63 ± 0.09	1.39 ± 0.05	5.99 ± 0.28	5.62 ± 0.20

[a] Simple phenols = tyrosol (quantified with a calibration curve of tyrosol at $\lambda = 200$ nm). Complex Phenols = (A) secoiridoid forms quantified with the calibration curve of oleuropein glucoside at $\lambda = 200$ nm; (B) secoiridoid forms quantified with the calibration curve of oleuropein glucoside at $\lambda = 240$ nm. Lignans = 1-(+)-acetoxypinoresinol + (+)-pinoresinol (quantified with a calibration curve of oleuropein glucoside at $\lambda = 200$ nm). Flavonoids = luteolin + apigenin (quantified with a calibration curve of luteolin at $\lambda = 200$ nm).

secoiridoid compounds. When we express the results using the quantification in terms of families of phenolic compounds, picual intenso is richer than picual suave in complex phenols (both those complex phenols quantified with the calibration curve of oleuropein glucoside at $\lambda = 200$ nm and those quantified with the calibration curve of oleuropein glucoside at $\lambda = 240$ nm). Regarding individual secoiridoid compounds, in all the cases the concentrations of these compounds present in picual intenso were greater than those of the picual suave. These results demonstrate the essential role played by the compounds mentioned before in the bitter taste of virgin olive oil. As far as the flavonoids luteolin and apigenin are concerned, the quantities found in these two virgin olive oils were very similar. The differences between these two commercial olive oils can be seen clearly in **Tables 4** and **5**.

It is possible to say that, in general, the results obtained by this method are in good agreement with those previously reported obtained by using other techniques (*5*, *28*−*31*), although the comparison can often be hard because the results are not expressed in identical units (in terms of the same reference standard).

Statistical Analysis. To evaluate the possibility of differentiating the samples, taking into account the phenolic compounds identified and quantified by this electrophoretic method, we

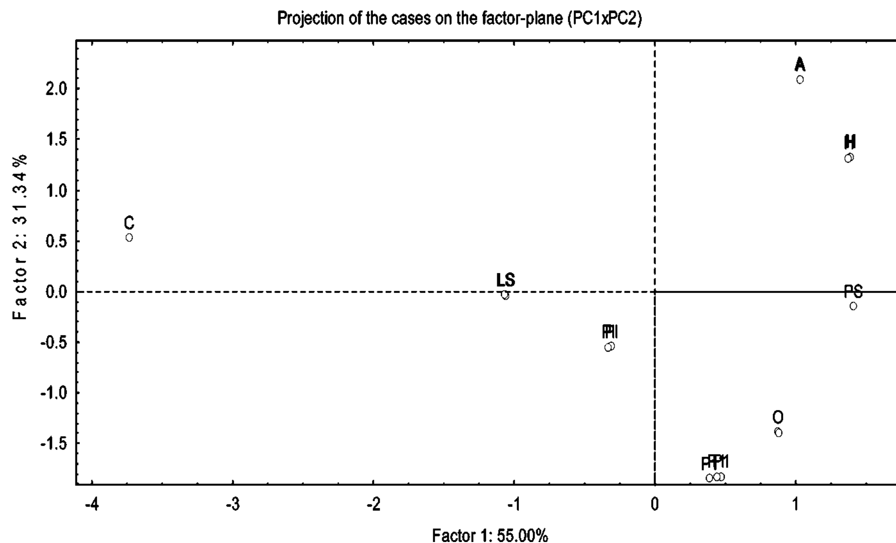

Figure 3. Score plot for the two principal components: picual, P1; hojiblanca, H; lechín de Sevilla, LS; arbequina, A; cornicabra, C; organic oil, O; picual suave, PS; picual intenso, PI.

chose a multivariate statistical approach. This statistical analysis, applied to a limited number of samples, did not have the purpose of distinguishing monovarietal olive oils obtained from different cultivars but to check the discrimination capacity achieved by using the electrophoretic variables considered; that means our aim was to prove that we could differentiate the samples considering the phenolic fraction quantified by CE. All the phenolic compounds quantified were considered to identify the two principal factors. Five variables [Ac Pin, DOA (b), Lig Agl (a), Ol Agl (a) + DOA (a), Ol Agl (b)] that had factor loading higher than 0.7 (evaluated by factors extraction test) were selected for the PCA and the explained variance was higher than 80%.

A map of samples (score plot) for the two principal components is shown in **Figure 3**. Extra-virgin olive oils made from cornicabra olives are quite different from the others, since these samples lie far from those olive oils obtained from olives of other cultivars or those commercial olive oil samples. This fact can be explained by taking into account the amount of Ol Agl (a) + DOA (a) and DOA (b) present in the cornicabra extra-virgin olive oils under study. Picual intenso and lechín de Sevilla contain high concentrations of Ol Agl (a) + DOA (a) and DOA (b) as well, but not as high as cornicabra olive oil; thus, they can be found in the third section of the plot.

Arbequina and hojiblanca oils, however, are very close in the score plot shown (in the first section or quadrant) because of their high Ac Pin content. Picual, picual suave, and the organic olive oil are located in the fourth section. Picual and organic olive oil have a similar Ol Agl (b) content, and picual suave is situated in this fourth quadrant very near the *X* axis because of its low content of DOA (b), Lig Agl (a), and Ol Agl (a) + DOA (a).

It is important to highlight the discriminant capacity of the function of the selected variables to distinguish the samples under study.

This is the first time in which an electrophoretic method was demonstrated to have the ability to detect and quantify simultaneously members of five different families of phenolic compounds present in olive oil. Its usefulness has been demonstrated by analyzing five monovarietal extra-virgin olive oils (January 2005), an organic olive oil, and two types of picual olive oils with different commercial names related to their sensorial properties, and very interesting results were found. CE joined to statistical analysis permits the discrimination among different olive oils. In our opinion, to carry out a wider study taking into account the phenolic composition of olive oils obtained from the main varieties of Spanish, Italian, and Greek olives would be really interesting. This topic will be the next step in our research. The relative standard deviations (RSD) obtained in the study of repeatability were lower than 0.91% for the migration times and 2.32% for the peak areas/migration time (intraday study).

ABBREVIATIONS USED

TY, tyrosol; Pin, (+)-pinoresinol; Ac Pin, 1-(+)-acetoxypinoresinol; DOA, decarboxylated oleuropein aglycon; Lig Agl, ligstroside aglycon; Ol Agl, oleuropein aglycon; EA, elenolic acid; HYTY, hydroxytyrosol; Apig, apigenin; and Lut, luteolin.

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