JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 6687−6693 **6687**

Sensitive Determination of Phenolic Acids in Extra-Virgin Olive Oil by Capillary Zone Electrophoresis

ALEGRÍA CARRASCO PANCORBO, CARMEN CRUCES-BLANCO, ANTONIO SEGURA CARRETERO,* AND ALBERTO FERNÁNDEZ GUTIÉRREZ

Department of Analytical Chemistry, Faculty of Sciences, University of Granada, C/Fuentenueva s/n, E-18071 Granada, Spain

A sensitive, rapid, efficient, and reliable method for the separation and determination of phenolic acids by capillary zone electrophoresis has been carried out. A detailed method optimization was carried out to separate 14 different compounds by studying parameters such as pH, type and concentration of buffer, applied voltage, and injection time. The separation was performed within 16 min, using a 25 mM sodium borate buffer (pH 9.6) at 25 kV with 8 s of hydrodynamic injection. With this method and using a liquid−liquid extraction system, with recovery values around 95%, it has been possible to detect small quantities of phenolic acids in olive oil samples. This is apparently the first paper showing the quantification of this specific family of phenolic compounds in virgin olive oil samples.

KEYWORDS: Phenolic acids; food analysis; capillary zone electrophoresis; antioxidant; olive oil

INTRODUCTION

In recent years, interest in natural antioxidants from vegetable substances has been related to their therapeutic properties (*1*). Among the different vegetable oils, extra-virgin olive oil is unique because it is obtained from the olive fruit (*Olea europaea* L.) solely by mechanical means, without further treatment other than washing, filtration, decantation, or centrifugation. This process retains minor compounds originally present in the olive fruit, which are usually removed from other vegetable oils during various stages of refining.

The cultivars of olive fruit vary considerably in size, shape, oil content, and flavor; for this reason several commercially important olive oil varieties are grown in Spain (*2*).

The increasing popularity of olive oil is mainly attributed to its high content of oleic acid, which may affect the plasma lipid/lipoprotein profiles (*3*), and its richness in phenolic compounds, which act as natural antioxidants (*4*, *5*), and may contribute to the prevention of human disease.

Among the various components of the unsaponifiable fraction of olive oil, phenolic compounds are one of the most important because they are natural antioxidants and a factor to be considered in the evaluation of the quality of an extra-virgin olive oil due to their role in its autoxidation stability, nutritional value, and flavor [bitterness (*6*) and astringency], and organoleptic characteristics in general (*7*).

In discussions of plant metabolites, the term "phenolic acids" refers to a distinct group of organic acids. Phenolic acids are the monomeric phenols in a large class of aromatic plant metabolites named simply phenolics, which exhibit certain sensorial and physiological properties of great interest (*8*).

In recent years, capillary electrophoresis (CE) has proven to be a high-resolution technique and has been applied successfully in the analysis of phenolic compounds of a large variety of samples [honey (*9*), plant extracts (*10*), wine (*11*), oils (*12*, *13*), and olive mill wastewater (*14*)], requiring only small amounts of sample and buffer and short times of analysis. CE offers the analyst a number of key advantages for the analysis of the components of food (*15*, *16*).

Despite these characteristics, the quantitative determination of the special family among the phenolic compounds, the phenolic acids, in olive oil samples has never been done before by capillary zone electrophoresis (CZE) with diode array detection.

For this reason, the aim of the present work has been to demonstrate the potential of the CE technique with ultraviolet (UV) detection for the fast and sensitive simultaneous determination of 14 compounds in extra-virgin olive oils from different varieties.

MATERIALS AND METHODS

Apparatus. Experiments were performed with a Beckman P/ACE System MDQ CE instrument comprising a 0−30 kV high-voltage built-in power supply, a diode array detector, and the Gold software for system control and data handling. All capillary tubing (bare fused silica) was from Beckman Instruments (Fullerton, CA). The temperature was controlled by use of fluorocarbon-based cooling fluid.

Reagents and Stock Solutions. The phenolic acids 4-hydroxyphenylacetic acid, gallic acid (3,4,5-trihydroxybenzoic acid), vanillic acid (4-hydroxy-3-methoxybenzoic acid), protocatechuic acid (3,4-dihy-

* Address correspondence to this author at Research Group FQM-297, Department of Analytical Chemistry, Faculty of Sciences, University of Granada, C/Fuentenueva s/n, E-18071 Granada, Spain (e-mail ansegura@ugr.es; fax +34958249510).

10.1021/jf0497399 CCC: $27.50
Published on Web 10/09/2004

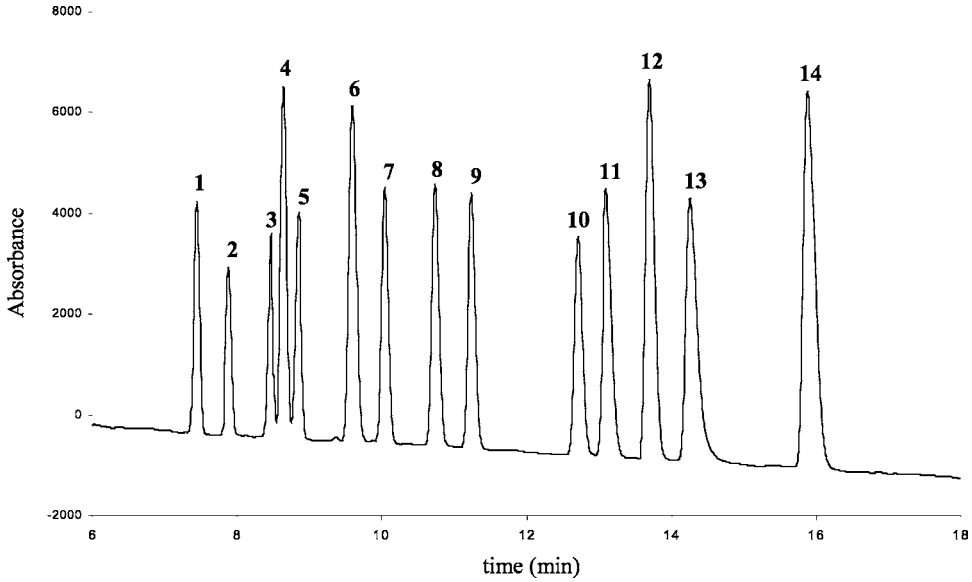

Figure 1. CZE separation of a standard mixture composed of 14 phenolic compounds under optimized conditions. Separation conditions: capillary, 57 cm × 75 μm; applied voltage, 25 kV; applied temperature, 25 °C; buffer, 25 mM sodium borate (pH 9.60); hydrodynamic injection, 0.5 psi for 8 s. Detection was performed at 210 nm. Peaks: 1, *trans*-cinnamic acid; 2, 4-hydroxyphenylacetic acid; 3, sinapinic acid; 4, gentisic acid; 5, (+)-taxifolin; 6, ferulic acid; 7, *o*-coumaric acid; 8, *p*-coumaric acid; 9, vanillic acid; 10, caffeic acid; 11, 4-hydroxybenzoic acid; 12, dopac; 13, gallic acid; 14, protocatechuic acid.

droxybenzoic acid), caffeic acid (3,4-dihydroxycinnamic acid), dopac (3,4-dihydroxyphenylacetic acid), *p*-coumaric acid (4-hydroxycinnamic acid), sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid), *trans*-cinnamic acid, *o*-coumaric acid (2-hydroxycinnamic acid), ferulic acid (4-hydroxy-3-methoxycinnamic acid), and gentisic acid (2,5-dihydroxybenzoic acid) were obtained from Sigma-Aldrich (St. Louis, MO). 4-Hydroxybenzoic acid was acquired from Fluka. The flavanonol (+)-taxifolin was obtained from Extrasynthèse (Genay, France), and all of the analytes were used as received.

The stock solution containing all 14 compounds was prepared in methanol/water (50:50, v/v) at a concentration of 500 μg/mL for each analyte.

Sodium hydroxide was purchased from Merck (Darmstadt, Germany); sodium borate (borax), glycine (aminoacetic acid), and 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO) were obtained from Sigma and sodium carbonate anhydrous and ammonium chloride from Panreac (Barcelona, Spain), which were all used as running buffers at different concentrations and pH values. Methanol and *n*-hexane were acquired from Panreac and were of HPLC grade.

Distilled water was deionized by using a Milli-Q system (Millipore, Bedford, MA).

Electrophoretic Procedure. CE separation was performed on a fused silica capillary (75 μm i.d., 375 μm o.d., total length = 57 cm, effective length = 50 cm). Good repeatability was assured by rinsing the capillary with 0.1 M sodium hydroxide for 5 min followed by 2 min with Milli-Q water at the beginning of each experimental session. The capillary was equilibrated with the running buffer (25 mM sodium borate adjusted to pH 9.6) for 5 min before each sample injection. The optimized running buffer was prepared by dissolving an appropiate amount of solid salt in Milli-Q water and adding a proper amount of 1.0 M NaOH.

Samples were injected hydrodynamically in the anodic end with a low-pressure mode (0.5 psi) for 8 s (1 psi = 6895 Pa),

Each electrophoretic run was carried out at 25 kV with the capillary temperature maintained at 25 °C, resulting in a current of ~95 μA. After each electrophoretic run, the capillary was flushed with Milli-Q water for 5 min. All samples, buffers, and solutions were filtered through a 0.20 μm syringe filter. The running buffer was changed after five runs.

UV detection was performed at 210 and 275 nm simultaneously. Diode array detection was used over the range of 190−600 nm to achieve spectral data. Peak identification was done by comparing both migration time and spectral data obtained from real samples and standards and also with spiked real samples at different concentration levels. Peak areas were used for quantification of the analytes.

Before the first use of a new capillary, it was preconditioned by rinsing with 0.5 M NaOH for 10 min, followed by a 5 min rinse with Milli-Q water.

Analysis of Extra-Virgin Olive Oils. Extra-virgin olive oil samples were obtained using dual phase decanter centrifugation from six Spanish monovarietal olive oils named Picual, Hojiblanca, Cornicabra, Lechín de Granada, Lechín de Sevilla, and Arbequina.

Different refined olive oils and commercial mixtures of refined and virgin olive oils were used to compare the amounts of phenolic acids.

Liquid−Liquid Extraction (LLE) of Phenolic Acids from Real Samples. The extraction conditions and amounts of the extraction system were optimized and, finally, phenolic acids were extracted from extra-virgin olive oils as follows: Oil (60 ± 0.001 g) was dissolved in 60 mL of hexane, and the solution was extracted successively with four 20 mL portions of 60% aqueous methanol. The combined extracts of the hydrophilic layer were brought to dryness in a rotary evaporator under reduced pressure and a temperature of 40 °C, and the residue was redissolved in 500 μL of methanol/water (50:50, v/v) and filtered through a 0.20 μm filter before the CE analysis.

RESULTS AND DISCUSSION

Selection of Analytes. To obtain a representative phenolic acid mixture to propose a potent analytical method for the analysis of these compounds in any kind of olive oil, all of the varieties we used had been previously studied in depth to check what compounds were present. With this information and all of the data of compounds previously reported from virgin olive oil, we made a family of phenolic acids and another compound, (+)-taxifolin (flavanonol), very appropriate for real samples. (+)-Taxifolin was included in the family because it was extracted with the LLE system used and appeared in the zone of phenolic acids in the electropherograms of extra-virgin olive oil samples.

Optimization of the separation conditions was achieved through testing of the migration behavior of a standard mixture containing the 14 compounds under study.

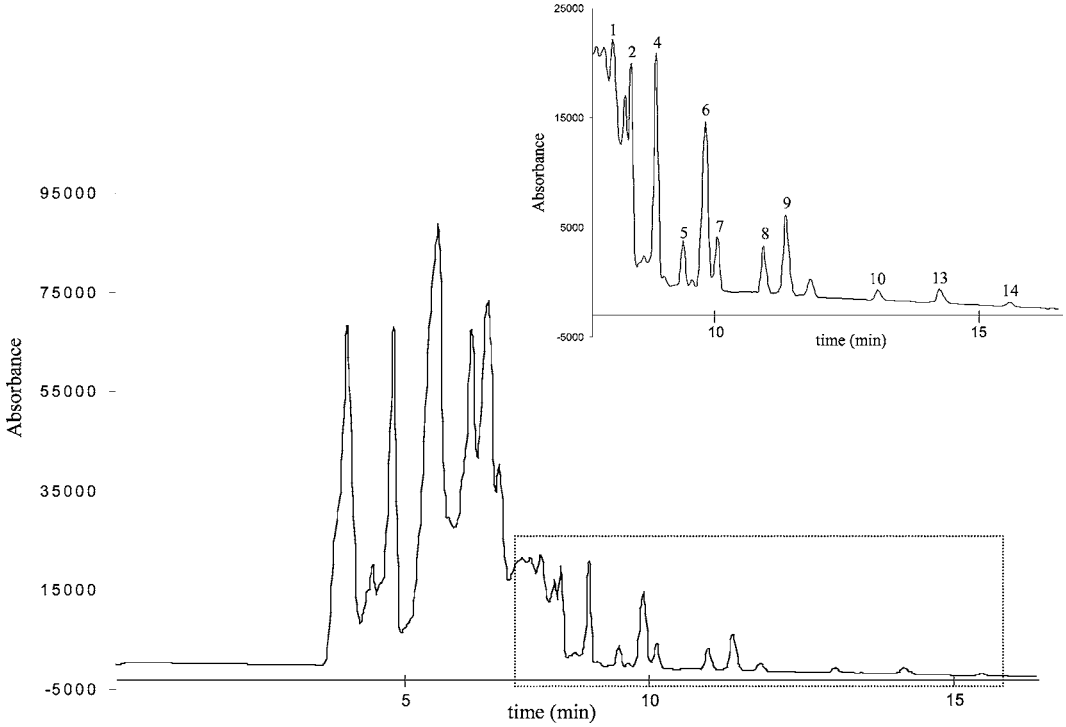

Figure 2. CZE electropherogram of phenolic fraction extracted from extra-virgin olive oil Arbequina by LLE. In the square at the right of the total electropherogram are shown the last ≫8 min of a run. See **Figure 1** for analyte identification numbers. Detection was performed at 210 nm (other conditions as **Figure 1**).

Effect of Experimental Variables. The effect of pH on the peak resolution of the 14 standard compounds was first studied. This effect was studied by adjusting the buffer pH to 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, or 10.5 by adding proper amounts of 1.0 M NaOH or 1.0 M HCl.

It was observed that at high pH values, the separation achieved was better than at lower pH values because the phenolic acids have a double-negative charge and present migration times longer than those of other compounds that can also be extracted with the LLE method employed. A more detailed study between pH 9 and 10 was carried out to obtain the optimum pH value for the correct resolution of the analytes under study by adjusting the buffer pH to 9.2, 9.4, 9.6, or 9.8. A value of pH 9.6 was selected for the rest of the experimental work as providing reasonable analysis times and very good resolution between peaks.

Five different aqueous buffers were tested: glycine, CAPSO, ammonium chloride, sodium carbonate, and sodium borate, all adjusted to range in pH between 9 and 10 (9.2, 9.4, 9.6, and 9.8).

Among the different buffers tested, sodium borate gave the best separation plus a satisfactory analysis time. The influence of sodium borate concentration on migration times and resolution was investigated using concentrations between 5 and 75 mM. It was concluded that migration and resolution increased when the concentration of sodium borate also increased. However, at concentrations >40 mM an unstable baseline and badly shaped peaks began to appear in the electropherograms of the mixture compounds. For this reason, a 25 mM concentration of sodium borate buffer (pH 9.6) was selected for the rest of the experimental work in order to reduce the analysis time and maintain good resolution.

Effect of Instrumental Variables. The effect of the applied voltage on the resolution of the 14 compounds was studied using the optimized buffer composition. When the applied voltage was increased from 10 to 30 kV, shorter analysis times and higher separation efficiencies were obtained. As the voltage is increased, there is more joule heat generated in the capillary, which can be detrimental to the separation because it causes broader peaks, the possibility of sample decomposition, or the formation of bubbles in the capillary. The better the heat is dissipated by the equipment, the higher the voltage that can be used. In the present work, it was possible to work at 25 kV without losing resolution and reducing the duration of the run analysis.

Among the different injection modes, hydrodynamic injection was selected. It is observed that as the sample plug length is increased, efficiency decreases and peaks broaden. An injection time of 8 s was finally selected.

Table 1. Recoveries Calculated by Analyzing, Three Times, Physically and Chemically Refined and Purified Oil Spiked with a Mixture of 14 Standard Phenolic Compounds

	phenolic compounds recovery (%) ($n = 3$)					
	20 μg/mL		50 μg/mL		200 μg/mL	
analyte	av	SD[a]	av	SD	av	SD
trans-cinnamic acid	99.2	5.53	98.9	6.32	98.20	4.25
4-hydroxyphenyl-acetic acid	125.11	4.28	113.10	4.78	102.35	5.78
sinapinic acid	91.83	6.23	91.74	5.89	89.10	6.23
gentisic acid	92.32	7.54	91.98	7.98	89.74	6.18
(+)-taxifolin	82.10	5.29	80.10	6.29	78.74	7.03
ferulic acid	91.31	6.44	89.31	5.99	86.10	6.12
o-coumaric acid	90.03	9.05	91.03	8.53	89.98	9.54
p-coumaric acid	89.95	10.01	90.10	9.03	88.73	10.25
vanillic acid	95.47	9.98	95.30	10.02	94.31	9.80
caffeic acid	92.26	8.23	92.01	7.63	87.05	9.78
4-hydroxybenzoic acid	95.17	7.57	97.12	7.98	96.12	5.25
dopac	98.97	6.75	97.97	8.51	95.03	4.78
gallic acid	94.20	8.25	92.10	4.28	89.16	6.82
protocatechuic acid	98.80	5.15	97.90	5.12	94.02	7.25

[a] Standard deviation.

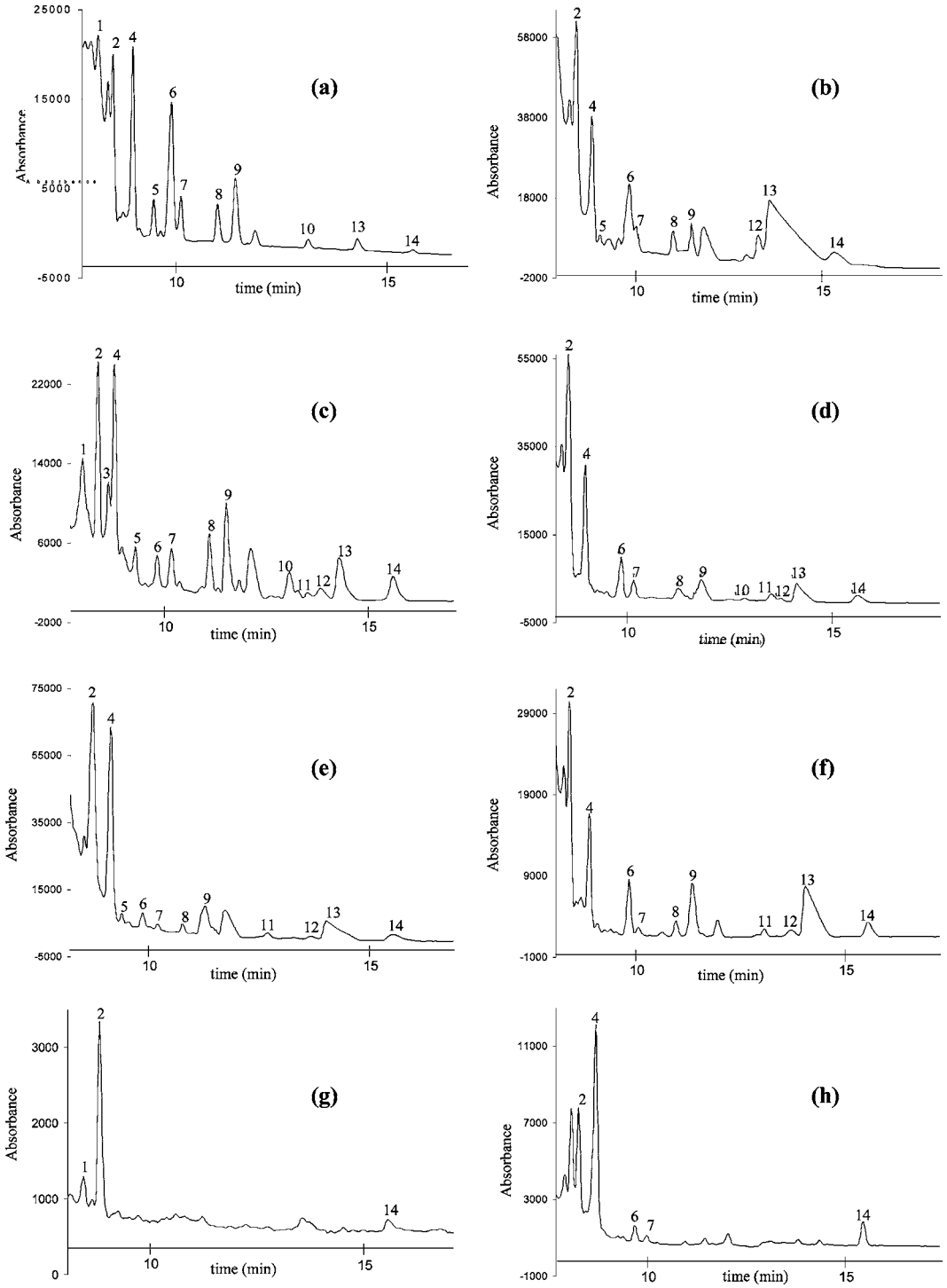

Figure 3. CZE electropherogram of phenolic fraction extracted from real olive oil sample by LLE: (**a**) Arbequina; (**b**) Lechín de Sevilla; (**c**) Picual; (**d**) Hojiblanca; (**e**) Lechín de Granada; (**f**) Cornicabra; (**g**) refined olive oil; (**h**) mixture of refined and virgin olive oils. For identification of the compounds see **Figure 1**. Detection was performed at 210 nm (instrumental and experimental parameters as **Figure 1**).

At the optimized conditions, the optimum electropherogram of the mixture of the 13 phenolic acids and the flavanonol (+)-taxifolin is presented in **Figure 1**, where the elution order observed is as follows: *trans*-cinnamic acid, 4-hydroxyphenylacetic acid, sinapinic acid, gentisic acid, (+)-taxifolin, ferulic acid, *o*-coumaric acid, *p*-coumaric acid, vanillic acid, caffeic acid, 4-hydroxybenzoic acid, dopac, gallic acid, and protocatechuic acid. As can be seen, the peaks were completely separated in only 16 min.

Repeatability Study. Repeatability was assessed for each standard compound at two concentration levels (20 and 100 μg/mL). The two solutions containing all of the analytes were prepared and analyzed 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 and the RSDs of migration times were determined for each analyte in the two standard mixtures.

The intraday repeatabilities on the migration time (expressed as RSD) were 0.45 and 0.89% for the 20 and 100 μg/mL concentrations, respectively, whereas the interday repeatabilities on the migration time were within 0.51 and 1.41% for the 20 and 100 μg/mL concentrations, respectively.

Table 2. Results of the Analysis of Real Samples ($n = 7$)[a]

	μg/L = μg of analyte/L of olive oil = ppb							
analyte	Arbequina	Picual	Hojiblanca	Lechín de Sevilla	Lechín de Granada	Cornicabra	refined oil	mixture of oils
trans-cinnamic acid	nq	620.80 ± 30.01	nd	nd	nd	nd	nd	nq
4-hydroxyphenyl-acetic acid	849.71 ± 45.48	1069.75 ± 53.45	3563.34 ± 178.15	2648.35 ± 165.36	3493.21 ± 132.31	1333.58 ± 66.31	132.42 ± 3.99	202.25 ± 10.10
sinapinic acid	nd	387.23 ± 17.21	nd	nd	nd	nd	nd	nd
gentisic acid	423.73 ± 19.31	499.38 ± 20.20	581.29 ± 27.03	545.32 ± 26.05	1486.34 ± 64.28	327.66 ± 15.25	nd	272.06 ± 13.62
(+)-taxifolin	129.42 ± 5.47	107.69 ± 4.89	nd	nq	nq	nd	nd	nd
ferulic acid	412.61 ± 17.63	116.45 ± 5.32	337.50 ± 16.88	351.30 ± 16.25	100.21 ± 4.28	174.31 ± 7.11	nd	nd
o-coumaric acid	103.85 ± 3.89	186.70 ± 7.25	137.73 ± 7.02	155.69 ± 7.75	nq	nq	nd	nd
p-coumaric acid	125.25 ± 5.05	429.99 ± 18.75	142.67 ± 7.15	136.56 ± 6.28	114.97 ± 5.53	nq	nd	nd
vanillic acid	156.24 ± 5.98	347.06 ± 16.99	236.90 ± 12.10	213.07 ± 10.25	318.21 ± 14.91	235.94 ± 9.87	nd	nd
caffeic acid	nq	145.06 ± 5.28	nq	nd	nq	nq	nd	nd
4-hydroxybenzoic acid	nd	nq	nq	nd	nd	nd	nd	nd
dopac	nd	nq	nd	72.58 ± 3.61	nd	nd	nd	nd
gallic acid	124.91 ± 5.12	181.71 ± 6.87	251.42 ± 11.23	1376.83 ± 65.01	411.06 ± 25.55	382.61 ± 17.25	nd	nd
protocatechuic acid	37.02 ± 1.63	110.24 ± 5.11	56.76 ± 2.63	107.29 ± 4.98	80.47 ± 3.59	56.26 ± 2.16	nd	33.91 ± 1.28

[a] Values are given as $X \pm$ SD. nd, nondetectable. nq, nonquantified.

Table 3. Analytical Parameters of Proposed Method

analyte	linearity (%)	RSD (%) (mean value)	detection limit (mg/mL)	quantification limit (mg/mL)	calibration range (mg/mL)	calibration eqs	r^2
trans-cinnamic acid	98.56	3.86	3.22	10.75	5–200	$y = 1720.3x - 1802.5$	0.998
4-hydroxyphenylacetic acid	98.59	1.61	1.32	4.40	5–500	$y = 893.6x + 2069.3$	0.997
sinapinic acid	96.79	6.51	5.35	17.83	5–200	$y = 1232.3x - 2820.2$	0.994
gentisic acid	98.79	3.62	2.99	9.78	5–300	$y = 2427.6x - 3854.6$	0.997
(+)-taxifolin	98.68	2.63	2.16	7.22	5–100	$y = 1573.8x + 2200.8$	0.996
ferulic acid	97.67	5.28	3.05	10.51	5–200	$y = 2407.2x - 2052.6$	0.996
o-coumaric acid	98.74	3.15	3.04	10.16	5–100	$y = 1672.5x - 4413.0$	0.996
p-coumaric acid	97.52	11.10	3.50	12.25	5–200	$y = 1638.2x - 5950.3$	0.995
vanillic acid	98.94	3.15	2.86	9.21	5–300	$y = 2180.5x - 3266.5$	0.995
caffeic acid	96.98	5.25	3.02	11.15	5–100	$y = 1923.2x - 4475.2$	0.997
4-hydroxybenzoic acid	97.17	3.09	2.53	8.01	5–100	$y = 2559.9x - 1432.2$	0.997
dopac	97.47	2.99	2.37	7.90	5–100	$y = 3309.0x + 1129.6$	0.997
gallic acid	98.31	2.00	1.58	5.28	5–300	$y = 2995.6x - 3840.8$	0.998
protocatechuic acid	98.97	1.22	0.96	3.21	5–200	$y = 4323.4x - 7998.5$	0.998

The intraday repeatabilities on the total peak area (expressed as RSD) were 1.3 and 2.5% for the 20 and 100 μg/mL concentrations, respectively, whereas the interday repeatabilities on total peak area were 2.9 and 5.6% for the 20 and 100 μg/mL concentrations, respectively. For (+)-taxifolin only, the precision obtained (expressed as RSD) was ~9%, which could be due to the closeness to the gentisic acid peak.

Despite the intraday precision being higher than the interday precision, as could be expected, good overall repeatability has been obtained.

Identification and Quantification of Extra-Virgin Olive Oil Components. After the optimization of the conditions of the CZE for the separation of the mixture of 14 standard compounds, its usefulness was further checked by analyzing real samples.

The present method has been applied to extra-virgin olive oil samples of different geographical origins and different varieties of one type of olive fruit named Picual, Hojiblanca, Cornicabra, Lechín de Granada, Lechín de Sevilla, and Arbequina. Also, refined olive oils and commercial mixtures of refined and virgin olive oils were analyzed to compare the amounts of phenolic acids present. All samples were injected in the CE instrument seven times ($n = 7$).

In **Figure 2** is shown a typical electropherogram of a virgin olive oil extract. The compounds were identified by comparing UV spectra of unknown peaks with those of a standard, by comparing migration times, and by spiking the samples with standard compounds at several concentration levels to clarify their identifications.

To determine the real amount of olive oil phenolic acids, it is very important to completely extract this fraction from the extra-virgin olive oil. In the literature it is often possible to find references disagreeing about effectiveness of extraction methods based on solid-phase extraction (SPE) or LLE (*12*, *18*−*24*). The effectiveness of different extraction methods of phenolic acids was compared by evaluating the percentage recoveries for each analyte under study and the number of analytes that were extracted.

The results obtained indicated that the LLE procedure permitted the extraction of a higher number of phenolic acids, which give a greater potential to the present methodology for the analysis of these compounds in any kind of olive oil. The SPE procedures do not allow a sufficient amount of oil to be passed across the cartridge to produce more diluted extracts and, consequently, it is more difficult to detect compounds at subparts per million levels with diode array detection.

To study the recoveries of each extraction, a phenolic standard mixture was added to a physically and chemically refined and purified oil after it was checked that this olive oil did not contain

any amount of the compounds under study; only a small amount of 4-hydroxyphenylacetic acid was detected.

All of the olive oil extracts were analyzed by CE, and the amount of each standard was compared to that of the standard mixture not subjected to any extraction procedure. Tests were performed in triplicate ($n = 3$) and for three different concentration levels (20, 50, and 200 μg/mL) to simulate the extraction system possibilities at real concentration levels found in olive oils.

All of the recoveries of the standard mixtures of the 14 compounds at the three concentrations levels shown in **Table 1** were ~95% for all of the analytes except for (+)-taxifolin. For 4-hydroxyphenylacetic acid, recoveries of >100% were obtained due to the small contribution of the physically and chemically refined and purified oil used for this study. If this contribution is eliminated, the real recovery for this analyte (at 50 μg/mL) was 95.35%. Naturally, the recoveries were worse when the concentration of spiked refined oil was higher, because the 500 mL methanol/water mixture (50:50, v/v) was not sufficient to carry away and redissolve all of the phenols in the flask after evaporation in the rotary evaporator.

The differences in the phenolic acid profiles shown in the electropherograms of the different varieties of olive oil extracts are very clear (see **Figure 3**). The differences observed in these electropherograms are completely logical because the phenolic content of an olive oil is the result of a complex interaction among several factors, including cultivar, location, type of crushing machine, degree of ripeness, and storage conditions of olive oil fruits as well as the type of oil extraction procedure used and olive oil storage conditions (*25*, *26*).

It is an important factor to bear in mind, in order to compare the analyses of the samples correctly, that the absorbance scales of the different electropherograms for each variety of olive oil are different. When refined olive oil and mixtures of refined and virgin olive oils were analysed, the amounts of phenolic compounds obtained were smaller than for the other types of olive oils.

The differences among the varieties of olive oils were greater when the relative quantities of these compounds were determined (see **Table 2**) than when the profiles of the electropherograms were studied by checking what analytes were in each olive oil.

Nevertheless, there are compounds such as *trans*-cinnamic acid, sinapinic acid, (+)-taxifolin, caffeic acid, and dopac that appeared in only several olive oils; therefore, these compounds could be considered as potential markers for the geographical origin or the olive fruit variety in the future.

Finally, the detection limit (DL), quantification limit (QL), and precision (as RSD of the intermediate concentration value of the linear range) were calculated for the studied analytes using the method proposed by Curie (*27*). Three replicates of each analyte at different concentrations were made to set up the calibration.

All of the features of the proposed method are summarized in **Table 3**.

Conclusions. A CZE method for the quantification of phenolic acids in extra-virgin olive oil extracts at sub-parts per million levels has been developed and optimized. Separation and identification of 14 compounds in olive oils of different geographical origins and different varieties of olive fruit were performed after LLE with good repeatability and short analysis times.

The results obtained are very promising, but more detailed studies are necessary to confirm the phenolic acid pattern that is typical for each type of extra-virgin olive oil and its relationship with the different factors that affect these profiles.

ACKNOWLEDGMENT

We thank Enrique Álvarez-Manzaneda and Blas Molina Molina of the Department of Organic Chemistry.

LITERATURE CITED

(1) Hollman, P. C. H.; Hertog, M. G. L.; Katan, M. B. Analysis and health effects of flavonoids. *Food Chem.* **1996**, *57* (1), 43−46.

(2) Hidalgo, F.; Navas, M. A.; Guinda, A.; Ruiz, A.; León, M.; Lazón, A.; Maestro, R.; Janer, M. L.; Pérez, M. C.; Cert, A.; Alba, J.; Gutiérrez, F.; Dobarganes, M. C.; Graciani, E. *Grasas Aceites* **1993**, *44*, 10−17.

(3) Delplanque, B.; Jusselin, I.; Le Roy, B.; Motta, C. The nutritional importance of olive oil. *OCL-Olèagineux Corps Gras Lipides* **1999**, *6* (1), 86−93.

(4) Fito, M.; Covas, M. I.; Lamuela-Raventos, R. M.; Vila, J.; Orrents, J.; De la Torre, C.; Marrugat, J. Protective effect of olive oil and its phenolic compounds against low-density lipoprotein oxidation. *Lipids* **2000**, *35* (6), 633−638.

(5) Galli, C.; Visioli, F. Antioxidant and other activities of phenolics in olives olive oil, typical components of the Mediterranean diet. *Lipids* **1999**, *34*, S23−S26.

(6) Gutiérrez Rosales, F.; Ríos, J. J.; Gómez Rey, M. L. Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 6021−6025.

(7) Ryan, D.; Robards, K.. Phenolic compounds in olives. *Analyst* **1998**, *123*, 31R−44R.

(8) Harborne, J. B. *The Flavonoids: Advances in Research since 1986*; Chapman and Hall: London, U.K., 1994.

(9) Andrade, P.; Ferreres, F.; Gil, M. I.; Tomás Barberán, F. A. Determination of phenolic compounds in honeys with different floral origin by capillary zone electrophoresis. *Food Chem.* **1997**, *60*, 79−84.

(10) Seitz, U.; Oefner, P.; Nathakarnkitkool, S.; Bonn, G. K. Capillary electrophoretic analysis of flavonoids. *Electrophoresis* **1992**, *13*, 35−38.

(11) Pazourek, J.; González, G.; Revilla, A. L.; Havel, J. Separation of polyphenols in Canary Island wine by capillary zone electrophoresis without preconcentration. *J. Chromatogr. A* **2000**, *874*, 111−119.

(12) Bendini, A.; Bonoli, M.; Cerretani, L.; Biguzzi. B.; Lercker, G.; Gallina Toschi, T. Liquid−liquid and solid-phase extractions of phenols from virgin olive oil and their separation by chromatographic and electrophoretic methods. *J. Chromatogr. A* **2003**, *985*, 425−433.

(13) Bonoli, M.; Montanucci, M.; Gallina Toschi, T.; Lercker, G. Fast separation and determination of tyrosol, hydroxytyrosol and other phenolic compounds in extra-virgin olive oil by capillary zone electrophoresis with ultraviolet-diode array detection. *J. Chromatogr. A* **2003**, *1011*, 163−172.

(14) Lafont, F.; Aramendia, M. A.; García, I.; Borau, V.; Jiménez, C.; Marinas, J. M.; Urbano, F. J. Analyses of phenolic compounds by capillary electrophoresis electrospray mass spectrometry. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 562−567.

(15) Frazier, R. A.; Ames, J. M.; Nursten, H. E. The development and application of capillary electrophoresis methods for food analysis. *Electrophoresis* **1999**, *20*, 3156−3180.

(16) Boyce, M. C. Determination of additives in food by capillary electrophoresis. *Electrophoresis* **2001**, *22*, 1447−1459.

(17) Cifuentes, A.; Bartolomé, B.; Gómez Cordobés, C. Fast determination of procyanidins and other phenolic compounds in food samples by micellar electrokinetic chromatography using acidic buffers. *Electrophoresis* **2001**, *22*, 1561−1567.

(18) Pirisi, F. M.; Cabras, P.; Falqui Cao, C.; Migliorini, M.; Muggelli. J. Phenolic compounds in virgin olive oil. 2. Reappraisal of the extraction, HPLC separation, and quantification procedures. *J. Agric. Food Chem.* **2000**, *48*, 1191−1196.

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

(20) Montedoro, G.; Servili, M.; Baldioli, R.; Miniati, E. Simple and hydrolyzable phenolic compounds in virgin olive oil. 2. Initial characterization of the hydrolyzable fraction. *J. Agric. Food Chem.* **1992**, *40*, 1577−1580.

(21) Montedoro, G.; Servili, M.; Baldioli, R.; Selvagini, R.; Miniati, E.; Macchioni, A. Simple and hydrolyzable phenolic compounds in virgin olive oil. Spectroscopic characterization of the seco-iridoid derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228−2234.

(22) Angerosa, F.; D'Alessandro, N.; Corana, F.; Mellerio, G. Characterization of phenolic and secoiridoid aglycons present in virgin olive oil by gas chromatography-chemical ionization mass spectrometry. *J. Chromatogr. A* **1996**, *736*, 195−203.

(23) Pirisi, F. M.; Angioni, A.; Cabras, P.; Garau, V. L.; Sanjust de Teulada, M. T.; Dos Santos, M. K.; Bandiono, G. Phenolic compounds in virgin olive oils. I. Low-wavelength quantitative determination of complex phenols by high-performance liquid chromatography under isocratic elution. *J. Chromatogr. A* **1997**, *768*, 207−213.

(24) Mateos, R.; Espartero, J. L.; Trujillo, M.; Ríos, J. J.; León Camacho, M.; Alcudia, F.; Cert, A. Determination of phenols, flavones, and lignans in virgin olive oil by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *J. Agric. Food Chem.* **2001**, *49*, 2185−2192.

(25) Esti, M.; Cinquanta, L.; La Notte, E. Phenolic compounds in different olive varieties. *J. Agric. Food Chem.* **1998**, *46*, 32−35.

(26) Cinquanta, L.; Esti, M.; La Notte, E. Evolution of phenolic compounds virgin olive oil during storage. *J. Am. Oil Chem. Soc.* **1997**, *74* (10), 1259−1264.

(27) Curie, L. A. Nomenclature in evaluation of analytical methods including detection and quantification capabilities. *Pure Appl. Chem.* **1995**, *67* (10), 1699−1723.

Received for review February 17, 2004. Revised manuscript received June 8, 2004. Accepted August 31, 2004. We gratefully acknowledge the financial support of the Andalusian Autonomous government (Consejería de Agricultura y Pesca). We express our sincere gratitude to the research group FQM 297 of the Junta of Andalucía and the Ministry of Science and Technology (Project BQU 2002-03418) and for a grant from the Ministry of Education, Culture and Sport (Ref. AP2002-1043).

JF0497399