Rapid and Complete Extraction of Phenols from Olive Oil and Determination by Means of a Coulometric Electrode Array System

Manuel Brenes,* Aranzazu García, Pedro García, and Antonio Garrido

Food Biotechnology Department, Instituto de la Grasa, CSIC, Avenida Padre García Tejero 4, 41012 Sevilla, Spain

Extraction methods to determine olive oil phenols are not exhaustive. A procedure to test their effectiveness, based on the treatment of the extracted oil with 2 N HCl followed by analysis of phenols in the aqueous phase, has been developed. It was concluded, using this test, that 15−40% of phenols remained unextracted when the liquid/liquid extraction method with 80% methanol was applied. Solid phase extraction (C_{18} cartridge) succeeded in retaining most of the phenols in the cartridge, but the recovery yield from the sorbent material was low. However, a new extraction method, based on the use of *N*,*N*-dimethylformamide (DMF) as an extraction solvent, achieved a complete extraction of phenols from oils. The proposed method requires a lower amount of oil, solvents, energy, and labor than the traditional ones. Because of the low concentration of phenols in the DMF extract, the highly sensitive electrochemical detector (EC) technique was studied. All of the phenols detected by the traditional UV detectors were also detected by EC using a coulometric array system. A rapid and complete analytical methodology of phenols in olive oil has been proposed based on coupling DMF extraction and EC detection.

Keywords: *Olive oil; phenols; analysis; N,N-dimethylformamide; electrochemical detection*

INTRODUCTION

Olive oil contains phenolic compounds that, in vitro, have shown potent biological activities including, but not limited to, antioxidant actions (Visioli and Galli, 1998). The identification and quantification of the phenolic fraction in olive oil are therefore of great importance, and much research has been carried out on this subject.

The extraction procedures utilized for the recovery of phenols from olive oil are mainly based on liquid/liquid partitioning techniques (Vázquez et al., 1973; Montedoro et al., 1992; Caruso et al., 2000) and solid phase extraction (SPE) methodology (Mannino et al., 1993; Favati et al., 1994; Litridou et al., 1997; Pirisi et al., 2000), using in most cases methanol as a solvent. Water at 4 °C (Bianco et al., 1998) and tetrahydrofuran (Cortesi et al., 1995) as extraction solvents have been also proposed.

Liquid/liquid extractions are laborious, and high amounts of solvents are needed. Besides, the effectiveness of this method has always been verified by spiking olive oil with commercial phenolic compounds (Montedoro et al., 1992). However, the recovery of phenols from olive oil using the liquid/liquid extraction methodology depends on the type of phenol and, therefore, a certain amount of these compounds may not be extracted.

A recent comparison between the liquid/liquid and SPE extraction methods showed that the amount of phenol detected was much lower when using SPE compared with liquid/liquid methodology (Servili et al., 1999).

Generally, the quantitative determination of total phenols and *o*-diphenols in olive oil has been performed by spectrophotometric methods (Gutfinger, 1981; Mosca et al., 2000), and, more recently, several biosensors have also been proposed to determine it (Campanella et al., 1999; Dall'Orto et al., 1999). However, these methods are nonspecific, and chromatographic techniques have been developed to elucidate the complex nature of the phenolic fraction in olive oil (Montedoro et al., 1993; Rovellini et al., 1997; Brenes et al., 1999, 2000). Most of these methods employ UV detection, although amperometric detection has also been studied (Akasbi et al., 1993; Mannino et al., 1993; Tsimidou et al., 1996). Electrochemical detection (EC) provides high sensitivity and selectivity, and the new multielectrode array detector allows the analysis of substances that coelute by applying consecutive potentials (Achilli et al., 1993; Guo et al., 1997). Furthermore, coulometric array detection is also gradient fit.

The purpose of this work was to (1) develop a rapid, exhaustive, and low solvent consumption method of extracting phenols from olive oil and (2) study the use of a coulometric array detector to analyze these phenols using gradient reversed-phase HPLC.

MATERIALS AND METHODS

Virgin Olive Oil. Olive fruits of Picual, Picudo, Arbequina, and Hojiblanca cultivars were used to obtain virgin olive oil by the Abencor system (Comercial Abengoa S.A., Seville, Spain), consisting of three basic elements: a mill, a thermobeater, and a pulp centrifuge (Martínez et al., 1975).

Commercial virgin olive oils of Picual, Arbequina, and Hojiblanca cultivars were also obtained from local markets.

Analysis of Phenolic Compounds. *Phenolic Extraction.* The phenolic extracts of virgin olive oils were obtained following the three procedures described in Scheme 1. The liquid/liquid (methanol 80%) and the SPE extraction methods

* Author to whom correspondence should be addressed (fax +34-954691262; e-mail brenes@cica.es).

10.1021/jf000686e CCC: $19.00
Published on Web 10/06/2000

Scheme 1. Flowchart of the Three Methods Studied To Extract, Separate, and Analyze Phenols from Olive Oil

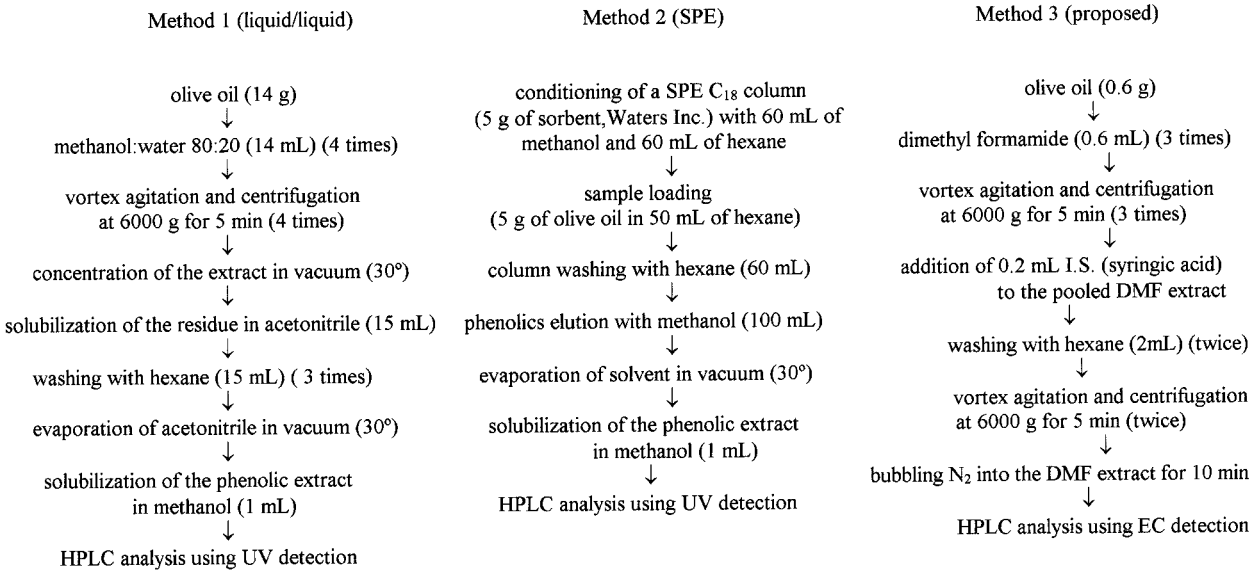

Table 1. Residual Hydroxytyrosol (Hy) and Tyrosol (Ty) Concentrations (Milligrams per Kilogram) in Oils after Extraction of the Phenols by Different Methods[a]

			oil extracted					
	oil without extraction		liquid/liquid		SPE		proposed method	
cultivar	Hy	Ty	Hy	Ty	Hy	Ty	Hy	Ty
Picual	266.1 (5.6)	132.7 (3.1)	62.7 (11.5)	47.3 (1.8)	3.8 (4.5)	17.7 (2.4)	0	0.9 (0.5)
Picudo	381.2 (13.7)	340.8 (7.1)	135.6 (10.7)	144.3 (3.3)	0.2 (0.1)	22.9 (12.4)	0	2.1 (1.3)
Arbequina	113.7 (2.3)	61.7 (6.6)	16.8 (0.8)	17.6 (0.8)	0.1 (0.1)	1.7 (0.1)	0	0.6 (0.1)
Hojiblanca	177.2 (15.6)	91.1 (4.3)	42.4 (5.9)	27.0 (1.4)	7.0 (4.2)	17.0 (8.2)	0	0.7 (0.1)

[a] Extracted oils and 2 N HCl solutions were left in contact for 6 h (with occasional stirring), and phenols were analyzed in the aqueous phase. The extracted oil hydrolyzed using the SPE method was that obtained from the hexane wash step. Assays were run in duplicate. Standard deviations are given in parentheses.

were carried out as described by Montedoro et al. (1992) and Favati et al. (1994), respectively. The proposed new method consisted of extracting the phenolic compounds with *N*,*N*-dimethylformamide (DMF). After centrifugation, the organic solvent was separated from the lipid phase by using a Pasteur pipet. An internal standard (syringic acid) was added to the pooled DMF extract, and the residual oil was eliminated by washing with hexane. Traces of the solvent were removed by flushing nitrogen for 10 min. Finally, the ~2 mL of DMF extract was diluted with 2 mL of methanol and centrifuged, and 20 μL was injected into the chromatograph.

HPLC Analysis of Phenolic Compounds Using UV Detection. The HPLC system consisted of a Waters 717 plus autosampler, a Waters 600 E pump, a Waters column heater module, and a Waters 996 photodiode array detector operated with Millenium 2010 software (Waters Inc., Milford, MA). A Spherisorb ODS-2 (5 μm, 25 cm by 4.6 mm i.d., Technokroma, Barcelona, Spain) column was used. Separation was achieved by elution gradient using an initial composition of 90% water (pH adjusted to 3.1 with 0.2% acetic acid) and 10% methanol. The concentration of the latter solvent was increased to 30% in 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% in 10 min, maintained for 5 min, increased to 50% in 5 min, and maintained for another 5 min. Finally, the methanol percentage was increased to 60, 70, and 100% in 5 min periods. Initial conditions were reached in 15 min. A flux of 1 mL/min and a temperature of 35 °C were also used. Chromatograms were obtained at 280 nm.

HPLC Analysis of Phenolic Compounds Using EC Detection. Pump, injector, heater module, column, flux, and temperature were the same as used for UV detection. The mobile phases were a 30 mM $LiClO_4$ solution (pH adjusted to 3.1 with $HClO_4$) and methanol containing 30 mM $LiClO_4$. The elution profile was the same as used for UV detection. The detector was an ESA coulometric system (ESA Inc., Chelmsford, MA), which consisted of one cell containing four electrodes in series.

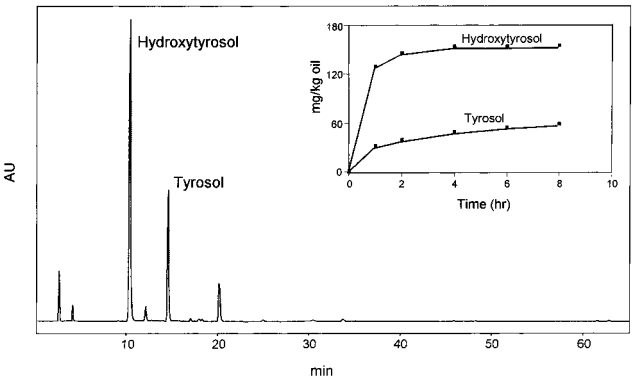

Figure 1. HPLC chromatogram of the 2 N HCl solution used to hydrolyze an olive oil of Picual cultivar. Before hydrolysis, phenols were extracted from the oil using the liquid/liquid (80% methanol) method. (Inset) Evolution of hydroxytyrosol and tyrosol concentrations in the acid solution during the hydrolysis of an olive oil of the Picual cultivar with 2 N HCl.

Reference Compounds. These were obtained as described elsewhere (Brenes et al., 1999).

Test To Check the Effectiveness of the Extraction Methods. Oil (0.5 mL) was put in contact with 0.5 mL of 2 N HCl for hydrolyzing aglycons. The resulting polar phenols can thus diffuse into the acidic medium. The mixture was occasionally stirred for 6 h, and the aqueous phase was centrifuged at 13000*g* for 5 min. Finally, phenols in the aqueous phase were determined as mentioned above using UV detection and 100 μL injected into the chromatograph.

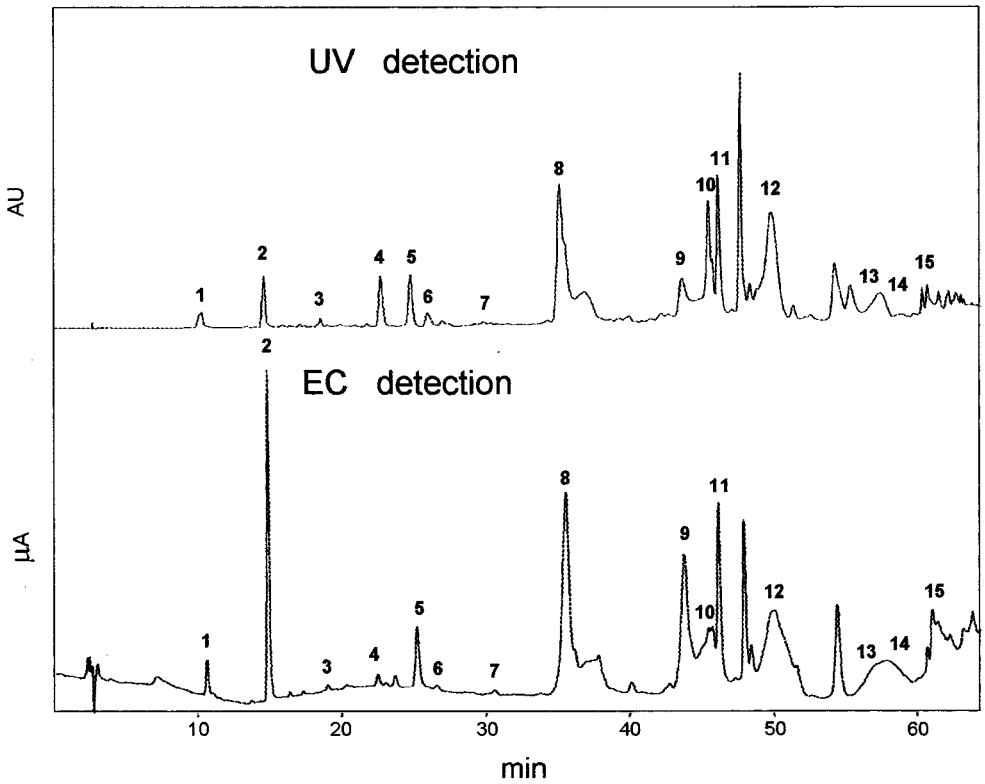

Figure 2. HPLC chromatogram of phenolic compounds obtained from an olive oil of the Picual cultivar using the liquid/liquid (80% methanol) extraction method. UV detection was carried out at 280 nm and EC detection at 1000 mv. Both chromatograms were obtained with the same sample, although a dilution of 1:100 with methanol was needed when using EC detection. Peaks: (1) hydroxytyrosol; (2) tyrosol; (3) vanillic acid; (4) vanillin; (5) 4-(acetoxyethyl)-1,2-dihydroxybenzene (3,4-DHPEA-AC); (6) *p*-coumaric acid; (7) ferulic acid; (8) dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA); (9) dialdehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EDA); (10) 1-acetoxypinoresinol; (11) pinoresinol; (12) oleuropein aglycon (3,4-DHPEA-EA); (13) luteolin; (14) ligstroside aglycon (*p*-HPEA-EA); (15) apigenin.

Table 2. Chromatographic and Electrochemical Characteristics of Phenolic Compounds Found in Virgin Olive Oils

peak	name	relative retention time[a]	dominant potential (mV)	peak area ratio[b]
1	hydroxytyrosol	0.51	250	15.5
2	tyrosol	0.71	750	3.0
3	vanillic acid	0.91	500	1.2
4	syringic acid	1.00	500	39.7
5	vanillin	1.08	750	4.6
6	3,4-DHPEA-AC	1.19	250	13.0
7	*p*-coumaric acid	1.24	750	2.9
8	ferulic acid	1.47	500	1.4
9	3,4-DHPEA-EDA	1.73	250	16.5
10	*p*-HPEA-EDA	2.15	750	2.6
11	1-acetoxypinoresinol	2.20	500	6.1
12	pinoresinol	2.26	500	3.0
13	3,4-DHPEA-EA	2.43	250	25.9
14	luteolin[c]	2.76	250/1000	
15	*p*-HPEA-EA[c]	2.79	750	
16	apigenin	2.95	750	2.2

[a] Using internal standard, syringic acid, as reference. [b] Ratio of the peak area of the dominant potential and postdominant potential. [c] Luteolin showed two dominant potentials at 250 and 1000 mV and coeluted with *p*-HPEA-EA.

RESULTS AND DISCUSSION

No methodology is available to check the effectiveness of the different extraction procedures used to extract phenols from olive oil. In most of these procedures oils were spiked with commercial phenols (Montedoro et al., 1992; Favati et al., 1994) and the recovery percentage was tested. However, certain aglycons found in olive oil may be difficult to extract with the conventional methods. A simple test was used to check if the exhaustiveness of the extraction procedures was developed. This consisted of treating the extracted oil with HCl during a certain length of time and analyzing the phenols in the aqueous phase. Figure 1 shows a chromatogram of the HCl solution used to hydrolyze an olive oil from which phenols had previously been extracted by the liquid/liquid method. Peaks corresponding to hydroxytyrosol and tyrosol were the most representative in the chromatogram, although a peak running at 20 min was also significant. It should be noticed that after acid hydrolysis, new liquid/liquid extraction steps with 80% methanol were applied to the hydrolyzed oil and no phenolic compounds were detected in the liquid/liquid extract. Thus, the acidic hydrolysis gave rise to water soluble phenolic compounds that completely diffused into the aqueous phase. The evolution of hydroxytyrosol and tyrosol concentrations in the HCl solution with time of hydrolysis is also shown in Figure 1. It seems that hydrolysis and diffusion were almost complete after 2−4 h for hydroxytyrosol, taking longer in the case of tyrosol. Six hours of hydrolysis was chosen to verify the effectiveness of the different extraction methods.

From all of the systems described in the literature on the subject of extraction of phenols from olive oil, we chose to test the liquid/liquid and SPE methods, these being the most representative, and a new proposed method (Scheme 1). Table 1 shows the concentrations of hydroxytyrosol and tyrosol in oils of four cultivars hydrolyzed with HCl before and after extraction of phenols. The liquid/liquid extraction method, which is the most common method used, was not successful in extracting all of the phenols from oils. From 15 to 40%

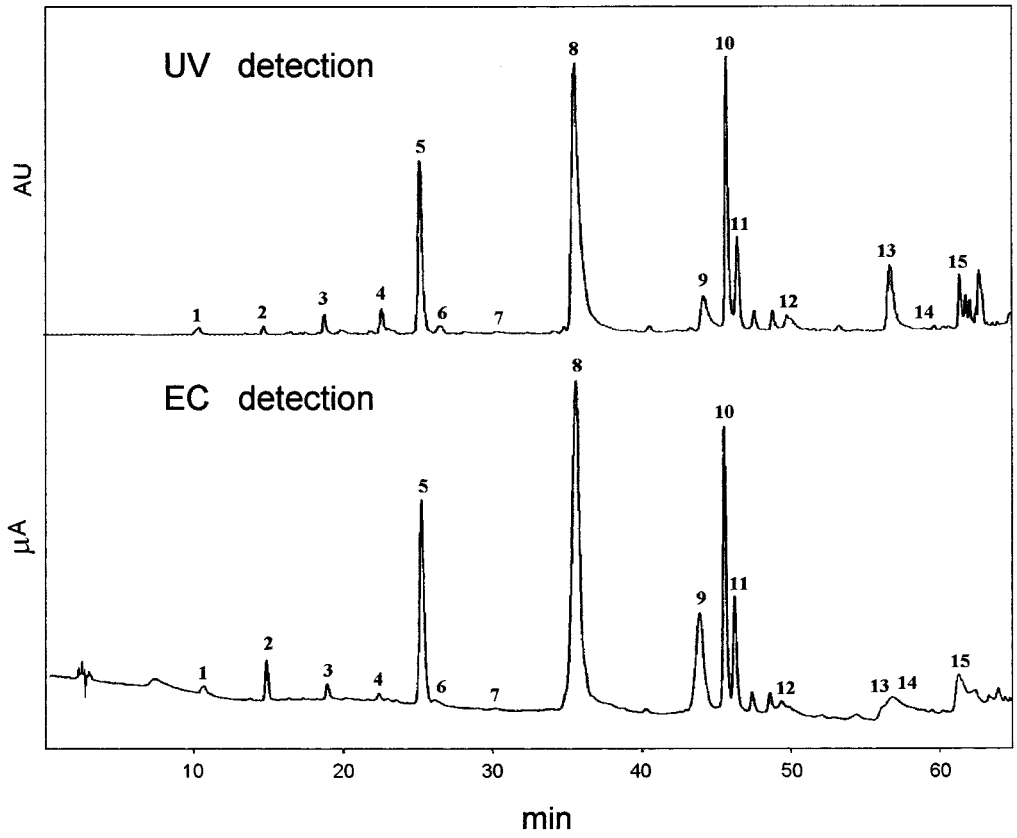

Figure 3. HPLC chromatogram of phenolic compounds obtained from an olive oil of the Arbequina cultivar using the liquid/liquid (80% methanol) extraction method and both UV and EC detections. More details are given in the caption of Figure 2.

of the initial content in both hydroxytyrosol and tyrosol in oils remained unextracted. In contrast, phenols detected in the oil obtained after evaporation of the washing hexane used for the SPE method represented only 1−18% of the initial content in oil.

Looking for a more efficient solvent than 80% methanol, we studied the use of 80% ethanol, 50% THF, acetonitrile, water at 5 °C, and *N*,*N*-dimethylformamide (DMF). None of the solvents assayed were more efficient than 80% methanol except DMF. The latter solvent is already used to extract chloroplast pigments from oils (Minguez et al., 1991), and no work has been done on using it for extracting phenols from oils. As can be seen in Table 1, the use of three extraction steps with DMF succeeded in extracting almost all of the phenolic compounds in oils. A certain amount of oil was solubilized in the solvent, and two washing steps with hexane were necessary to remove the lipids. It must be stressed that the amount of oil required to extract phenols using the DMF method was much lower than the liquid/liquid and SPE methods. Furthermore, low amounts of solvents, energy, and labor were also needed. DMF solvent, however, has a high boiling point and does not allow the sample to be concentrated. New extraction steps of phenols from the DMF extract should thus be needed, as is the case for chloroplastic pigments if UV detection is employed (Minguez et al., 1991).

An alternative process assayed was the use of coularray electrochemical (EC) detection. This type of detector is much more sensitive than the UV detectors and allows only the analysis of electroactive compounds. In previous studies (Akasbi et al., 1993; Mannino et al., 1993; Tsimidou et al., 1996), amperometric detection has been used, although this was only for the analysis of simple phenols under isocratic conditions.

Figures 2 and 3 show the chromatograms obtained using UV and EC detection of two virgin olive oils of Picual and Arbequina cultivars. Phenols were extracted from the oil using the liquid/liquid methodology, and a dilution of 1:100 of the methanol extract before injection into the chromatograph was needed when using EC detection. It is obvious from Figures 2 and 3 that all phenols were detected by using both UV and EC detection. These EC chromatograms were obtained at 1000 mV, but the coularray electrochemical detector allows chromatograms in different channels to be obtained, depending on the applied voltage. The chromatograms at 250, 500, 750, and 1000 mV of a phenolic extract obtained using the DMF method are depicted in Figure 4. *o*-Diphenol compounds oxidized at lower potentials than monophenols, as has been previously reported (Mannino et al., 1999). Thus, hydroxytyrosol and oleuropein aglycons oxidized mostly at 250 mV, whereas tyrosol and ligstroside aglycons did so at 750 mV. In most cases analytes responded over three adjacent electrodes, termed the lower (preceding), dominant, and upper (following) sensors. The response of an analyte across these three channels is a characteristic of that analyte and is independent of the analyte's concentration within a certain concentration range. The analyte's response ratio on the lower to dominant channel is a ratio accuracy for that analyte.

The retention time, dominant potential, and peak area ratio between channels of phenolic compounds in olive oil are presented in Table 2. This dominant potential was lower for *o*-diphenols than monophenols. Other simple phenols such as vanillic acid, vanillin, syringic acid, and *p*-coumaric acids showed dominant potentials between 500 and 750 mV. It should be stressed that the dominant potential depends on the

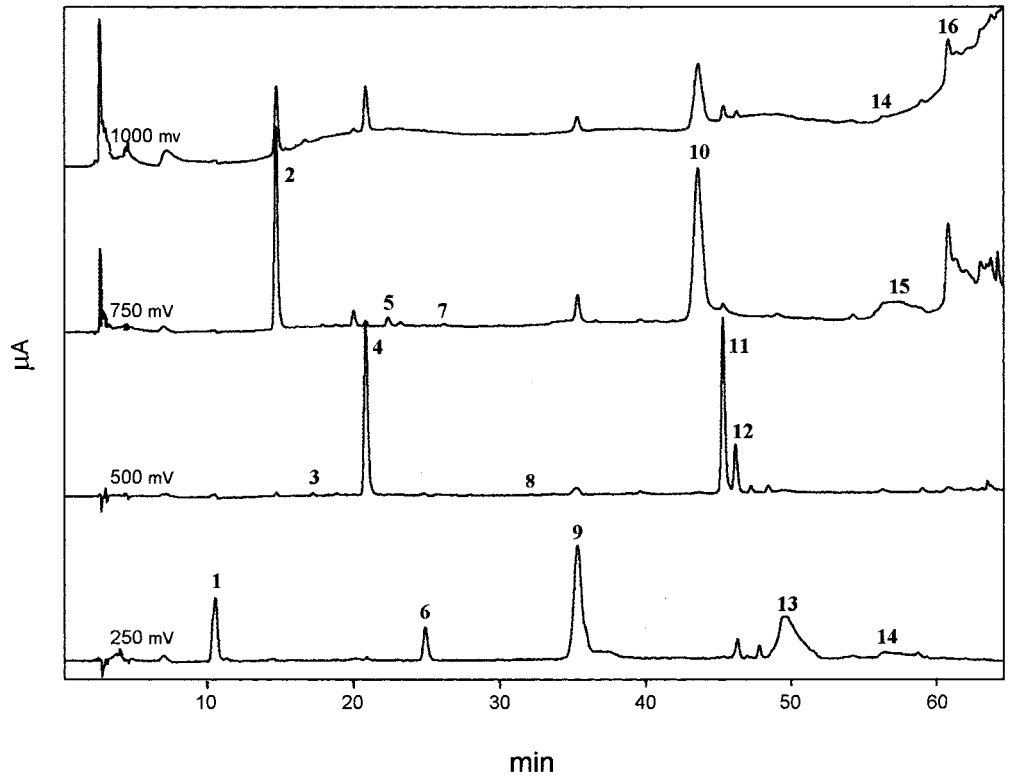

Figure 4. HPLC chromatogram of phenolic compounds in virgin olive oil of Hojiblanca cultivar obtained using the proposed extraction method. Chromatograms were recorded at 250, 500, 750, and 1000 mV. More details are given in the caption of Figure 2.

Table 3. Concentration of Phenolic Compounds in Commercial Olive Oils Analyzed Using Two Different Methods[a]

	cultivar					
	Picual		Arbequina		Hojiblanca	
compound	liquid/liquid	proposed	liquid/liquid	proposed	liquid/liquid	proposed
hydroxytyrosol	2.7 (0.1)	3.0 (0.1)	5.8 (0.5)	5.8 (0.1)	5.2 (0.4)	5.8 (0.3)
tyrosol	7.3 (0.3)	7.3 (0.2)	5.9 (0.1)	5.5 (0.1)	6.0 (0.4)	6.0 (0.1)
vanillic acid	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	0.2 (0.1)	0.3 (0.1)	0.2 (0.1)
vanillin	0.5 (0.1)	0.6 (0.1)	0.2 (0.1)	0.4 (0.1)	0.8 (0.2)	0.7 (0.1)
3,4-DHPEA-AC	6.3 (0.5)	5.3 (0.1)	7.3 (0.1)	7.4 (0.1)	12.0 (0.5)	10.1 (0.1)
p-coumaric acid	0.6 (0.1)	0.1 (0.1)	0.3 (0.1)	0.4 (0.1)	0.6 (0.2)	0.4 (0.3)
3,4-DHPEA-EDA	27.7 (1.3)	32.4 (1.0)	86.6 (6.3)	115.0 (0.3)	90.9 (3.7)	132.0 (1.2)
p-HPEA-EDA	19.5 (0.8)	19.6 (0.8)	14.7 (0.9)	20.5 (0.8)	22.4 (1.3)	27.1 (1.6)
1-acetoxypinoresinol	4.4 (0.4)	5.9 (0.3)	33.5 (0.8)	31.9 (0.6)	3.9 (0.5)	3.7 (1.1)
pinoresinol	25.3 (1.8)	23.8 (0.2)	28.4 (0.4)	26.4 (0.3)	31.8 (2.4)	30.8 (0.4)
3,4-DHPEA-EA	88.8 (5.9)	95.4 (7.3)	43.5 (1.9)	53.0 (1.6)	132.8 (12.1)	134.9 (0.9)
luteolin	1.3 (0.1)	1.4 (0.1)	2.6 (0.5)	2.0 (0.5)		
p-HPEA-EA	15.2 (1.1)	15.2 (0.7)	3.1 (0.9)	4.0 (0.5)	14.9 (1.8)	15.5 (0.2)
apigenin	0.5 (0.1)	0.5 (0.1)	0.7 (0.3)	0.5 (0.1)	0.1 (0.1)	0.2 (0.1)
total	200.2 (6.4)[b]	210.6 (7.5)	233.8 (6.8)	273.0 (2.1)	321.7 (13.1)	367.4 (2.5)

[a] Assays were run in duplicate. Standard deviations are given in parenthese. [b] Pooled standard deviation (Box et al., 1978).

number of electrodes used in the detection and the potentials applied (Achilli et al., 1993; Guo et al., 1997).

The only difference between UV and EC detection was the coelution of luteolin and *p*-HPEA-EA when using EC detection. However, this problem could be solved by analyzing these two compounds at different potentials. Luteolin could be analyzed at 250 mV and *p*-HPEA-EA at 750 mV without interference.

Not only were the extraction methods studied tested by hydrolyzing the extracted oils with 2 N HCl, but a quantification of the phenolic extract was also made using EC detection. DMF extraction gave rise to 6−36% more phenols than the liquid/liquid (methanol 80%) extraction methodology (Figure 5). This difference must be related to the higher amount of unextracted phenols found when using the latter methodology (Table 1). SPE methodology was very efficient in leaving a low amount of phenols in the extracted oils (Table 1) and, therefore, a high amount of phenols in the methanol extract should be expected. However, the amount of phenols detected in the methanol extract when using SPE was very low (Figure 5), which is in agreement with recent studies (Servili et al., 1999). It seems that phenols were retained in the cartridge but were not eluted by the methanol solvent. THF, ethyl acetate, and DMF were also tested to recover phenols from the adsorbent cartridge, but none of them improved the recovery yield. SPE cartridges of C_8, diol, and NH_2 as sorbent materials were also studied, but no increase in phenol recovery was found (data not shown).

A comparison of the concentration of each phenolic compound in commercial virgin olive oils of Picual, Arbequina, and Hojiblanca cultivars using the liquid/liquid (80% methanol) and the DMF extraction methods

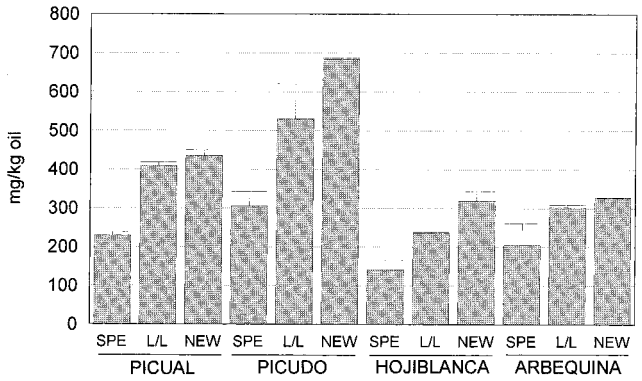

Figure 5. Total phenolic concentration in olive oils of different cultivars obtained using different extraction methods. Phenols were analyzed by HPLC, and the detection was carried out with the coulometric array detector. Assays were run in duplicate.

is reported in Table 3. The amounts of phenyl acids, phenyl alcohol, lignans, and flavonoids were found to be very similar for both methods. In contrast, secoiridoid derivatives such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA, which are often the main phenolic compounds in olive oils (Brenes et al., 2000), were found in a higher amount in oils extracted using the DMF method. It seems that these secoiridoid derivatives were the phenols that remained in oil when employing the liquid/liquid (80% methanol) extraction methodology.

The combined use of DMF extraction and EC detection represents a reliable alternative to the traditional techniques used to analyze phenols in olive oil. Besides, this method requires low amounts of solvents, labor, and energy and allows an accurate quantification of all the phenols present in the olive oil.

LITERATURE CITED

Achilli, G.; Cellerino, G. P.; Hamache, P. H. Identification and determination of phenolic constituents in natural beverages and plant extracts by means of a coulometric electrode array system. *J. Chromatogr.* **1993**, *632*, 111−117.

Akasbi, M.; Shoeman, D. W.; Csallany, A. S. High-performance liquid chromatography of selected phenolic compounds in olive oils. *J. Am. Oil Chem. Soc.* **1993**, *70*, 367−370.

Bianco, A.; Mazzei, R. A.; Melchioni, C.; Romero, G.; Scarpati, M. L.; Soriero, A.; Uccella, N. Microcomponents of olive oil—III. Glucosides of 2(3,4-dihydroxy-phenyl)ethanol. *Food Chem.* **1998**, *63*, 461−464.

Box, G. E. P.; Hunter, W. G.; Hunter, J. S. Experiments to compare *k* treatment means. In S*tatistics for Experimenters*; Wiley: New York, 1978; Chapter 6.

Brenes, M.; García, A.; García, P.; Rios, J. J.; Garrido, A. Phenolic compounds in Spanish olive oils. *J. Agric. Food Chem.* **1999**, *47*, 3535−3540.

Brenes, M.; Hidalgo, F. J.; García, A.; Rios, J. J.; García, P.; Zamora, R.; Garrido, A. Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. *J. Am. Oil Chem.* Soc. **2000**, *77*, 715−720.

Campanella, L.; Favero, G.; Pastorino, M.; Tomassetti, M. Monitoring the rancidification in olive oils using a biosensor operating in organic solvents. *Biosens. Bioelectron.* **1999**, *14*, 179−186.

Caruso, D.; Colombo, R.; Patelli, R.; Giavarini, F.; Galli, G. Rapid evaluation of phenolic component profile and analysis of oleuropein aglycon in olive oil by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS). *J. Agric. Food Chem.* **2000**, *48*, 1182−1185.

Cortesi, N.; Azzolini, M.; Rovellini, P. Dosaggio dei componenti minori polari (CMP) in oli vergini di oliva. *Riv. Ital. Sostanze Grasse* **1995**, *73*, 333−337.

Dall'Orto, V. C.; Danilowicz, C.; Mascini, M. Comparison between three amperometic sensors for phenol determination in virgin oil samples. *Anal. Lett.* **1999**, *32*, 1981−1990.

Favati, F.; Caporale, G.; Bertuccioli, M. Rapid determination of phenol content in extra virgin olive oil. *Grasas Aceites* **1994**, *45*, 68−70.

Guo, C.; Cao, G.; Sofic, M.; Prior, R. High-performance liquid chromatography coupled with coulometric array detection of electroactive components in fruits and vegetables: relationship to oxygen radical absorbance capacity. *J. Agric. Food Chem.* **1997**, *45*, 1787−1796.

Gutfinger, T. Polyphenols in olive oils. *J. Am. Oil Chem. Soc.* **1981**, *11*, 966−968.

Litridou, M.; Linssen, J.; Schols, H.; Bergmans, M.; Posthumus, M.; Tsimidou, M.; Boskou, M. Phenolic compounds in virgin olive oils: fractionation by solid-phase extraction and antioxidant activity assessment. *J. Sci. Food Agric.* **1997**, *74*, 169−174.

Mannino, S.; Cosio, M. S.; Bertuccioli, M. High performance liquid chromatography of phenolic compounds in virgin olive oils using amperometric detection. *Ital. J. Food Sci.* **1993**, *4*, 363−370.

Mannino, S.; Buratti, S.; Cosio, M. S.; Pellegrini, N. Evaluation of the "antioxidant power" of olive oils based on a FIA system with amperometric detection. *Analyst* **1999**, *124*, 1115−1118.

Martínez, J. M.; Muñoz, E.; Alba, J.; Lanzón, A. Informe sobre la utilización del analizador Abencor. *Grasas Aceites* **1975**, *26*, 379−385.

Minguez, M. I.; Jarén, M.; Hornero, D.; Garrido, J.; Gallardo, L.; Gandul, B. Decoloration of vegetable oils and oleoresins with recovery of unaltered pigments. *J. Am. Oil Chem. Soc.* **1991**, *68*, 809−813.

Montedoro, G. F.; Servili, M.; Baldioli, M.; 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.

Montedoro, G. F.; Servili, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macchioni, A. Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterization of the secoiridoid derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228−2234.

Mosca, L.; DeMarco, C.; Visioli, F.; Cannella, C. Enzymatic assay for the determination of olive oil polyphenol content: Assay conditions and validation of the method. *J. Agric. Food Chem.* **2000**, *48*, 297−301.

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

Rovellini, P.; Cortesi, N.; Fedeli, E. Analysis of flavonoids from *Olea europaea* by HPLC-UV and HPLC-electrospray-MS. *Riv. Ital. Sostanze Grasse* **1997**, *74*, 273−279.

Servili, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macchioni, A.; Montedoro, G. F. High-performance liquid chromatography evaluation of phenols in olive fruit, virgin olive oil, vegetation waters, and pomace and 1D-and 2D-nuclear magnetic resonance characterization. *J. Am. Oil Chem. Soc.* **1999**, *76*, 873−882.

Tsimidou, M.; Lytridou, M.; Boskou, D.; Pappa-Louisi, A.; Kotsifaki, F.; Petrakis, C. On the determination of minor phenolic acids of virgin olive oil by RP-HPLC. *Grasas Aceites* **1996**, *47*, 151−157.

Visioli, F.; Galli, C. Olive oil phenols and their potential effects on human health. *J. Agric. Food Chem.* **1998**, *46*, 4292−4296.

Received for review June 2, 2000. Revised manuscript received August 8, 2000. Accepted August 8, 2000. We are indebted to Project 1FD-1116 (CICYT, Spanish Government) and AIR3-CT94-1355 (European Union) for the financial support.

JF000686E