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Liquid–liquid and solid-phase extractions of phenols from virgin olive oil and their separation by chromatographic and electrophoretic methods

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Abstract

The high oxidative stability of virgin olive oil is related to its high monounsaturated/polyunsaturated ratio and to the presence of antioxidant compounds, such as tocopherols and phenols. In this paper, the isolation of phenolic compounds from virgin olive oil, by different methods, was tested and discussed. Particularly liquid–liquid and solid-phase extraction methods were compared, assaying, for the latter, three stationary phases (C_8, C_{18} and Diol) and several elution mixtures. Quantification of phenolic and *o*-diphenolic substances in the extracts was performed by the traditional Folin–Ciocalteau method and the sodium molybdate reaction, respectively. Furthermore, the quantification of phenolic compounds in the extracts and in a standard mixture was carried out both with diode array and mass spectrometric detection and capillary zone electrophoresis.

Keywords: Olive oil; Phenols

1. Introduction

In the last years, the interest about natural antioxidants from vegetable substances has been related to their therapeutic properties [1]. Vegetable matrices such as spices (tea, rosemary, sage, oregano), grapes and grape seeds, olive and its products and by-products (olive oil and waste mill waters) has been widely studied in order to verify their antioxidant properties [2–5]. For virgin olive oil several works underlined the role of the phenolic fraction in improving its oxidative stability. In order to determine the real amount of olive oil phenols it is very important to completely extract this fraction from the oil. In literature it is often possible to find references disagreeing about effectiveness of extraction methods based on solid-phase extraction (SPE), or liquid–liquid extraction (LLE) [6–8]. The first aim of this work was to compare and to assess the effectiveness of different extraction methods of phenolic compounds by evaluating their recoveries. For this purpose, a phenolic standard mixture was added to a lipid matrix (made by a refined and purified peanut oil), and then subjected to the different extraction procedures. Moreover, the comparative tests for the quantification of total polyphenols (TP) and *o*-diphenols (*o*-diph) were made also on a real sample (virgin olive oil), extracted by the different methods used. Finally, the phenolic standard mixture and the

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phenolic fraction, extracted from the virgin olive oil, were analysed both by HPLC with diode array (DAD) and mass spectrometric detection (MSD) and capillary zone electrophoresis (CZE). The quantification of tyrosol, hydroxytyrosol and oleuropein derivatives, in the sample, were carried out to evaluate the recoveries by the different extraction procedures while the quantitation of only tyrosol and hydroxytyrosol were carried out to compare the performances of the two analytical techniques. Traditionally, in literature, the determinations of the phenolic profile in olive oil have been reported by HPLC [6,8–16] and by capillary gas chromatography (CGC) [17,18]. Both techniques show some limitations, the former due to the long time necessary for the analysis and to the partial separation of components having a complex structure (secoiridoids), the latter due to problems dealing with the sample derivatization. In this work, the use of capillary electrophoresis as a valid tool able to combine a short analysis time with good separation efficiency for the analysis of olive oil phenols, particularly hydroxytyrosol and tyrosol, is also proposed and discussed [19–21].

2. Experimental

2.1. Reference compounds

The following commercial products were used: protocatechuic acid (PA), 3,4-dihydroxyphenylacetic acid (3,4-DHPAA), tyrosol (Tyr), 4-hydroxybenzoic acid (4-HBA), caffeic acid (CafA), vanillic acid (VA), dihydrocaffeic acid (DHCA), *p*-coumaric acid (*p*-CA), ferulic acid (FA), *o*-coumaric acid (*o*-CA), 3-methoxybenzoic acid (3-MBA) and cinnamic acid (CinA) were from Fluka (Buchs, Switzerland); gallic acid (GalA) and taxifolin (T) were from Sigma (St. Louis, MO, USA); oleuropein (OG) was from Extrasynthèse (Genay, France).

The stock solutions were prepared by dissolving about 1 mg of each standard in 1 ml of HPLC-grade methanol. Appropriate dilutions (six points approximately from 0.05 to 0.0015 mg/ml) for the calibration curves were prepared. Hydroxytyrosol (Htyr) was prepared by chemical reduction of 3,4-dihydroxyphenylacetic acid, according to Baraldi et al. [22].

A solution of oleuropein standard was partially hydrolyzed by the method of Ciafardini and Zullo to verify the presence of its hydrolysis derivatives [23].

2.2. Samples and sample preparation

For the evaluation of the HPLC phenolic compounds recoveries, a refined and purified peanut oil (passed through an Al_2O_3 column), spiked with the standard mixture constituted by the 15 following phenolic compounds: GalA, PA, 3,4-DHPAA, Tyr, 4-HBA, CafA, VA, DHCA, *p*-CA, FA, T, *o*-CA, OG, 3-MBA, CinA, was employed.

2.3. Extractions of the phenolic fraction

The following extraction methods were used for the phenolic compounds recoveries from virgin olive oil.

Method A (C_8-SPE): under the conditions described by Pirisi et al. [8] (500 mg/3 ml, Isolute IST, Hengoed, UK).

Method B (C_8mod.-SPE): an octil-bonded phase cartridge (500 mg/3 ml, Isolute IST) was placed in a vacuum elution apparatus, washed with 6 ml of *n*-hexane and then conditioned with 6 ml of acetonitrile. One gram of oil, dissolved in 6 ml of *n*-hexane, was charged onto the column and washed with 6 ml of *n*-hexane in order to remove the non-polar fraction of the oil. The polar fraction was consecutively eluted with 6 ml of acetonitrile, 6 ml of methanol and then 6 ml of CH_3OH–water (1:1, v/v). The fractions were combined and evaporated. After extraction, the residues were dissolved in 1.0 ml of CH_3OH–water (1:1, v/v) and filtered through a 0.45-μm nylon filter.

Method C (C_{18}-SPE): under the conditions described by Servili et al. [6] (2 g/25 ml, Isolute IST).

Method D (Diol-SPE): according to the method reported by Mateos et al. [7] (500 mg/3 ml, Isolute IST).

Method E (LLE): the procedure was carried out following the method described by Pirisi et al. [8].

2.4. Colorimetric determination of total phenols

The total phenols content of the extracts was

determined by the Folin–Ciocalteau spectrophotometric method at 750 nm [24], using a gallic acid calibration curve. The spectrophotometric analysis was repeated three times for each type of extract.

2.5. Colorimetric determination of o-diphenols

According to Mateos et al. [7], 0.5 ml of phenolic extract obtained from olive oil by LLE and SPE methods was dissolved in 5 ml of CH_3OH–water (1:1, v/v); a mixture of 4 ml of the solution and 1 ml of a 5% solution of sodium molybdate dihydrate in CH_3CH_2OH–water (1:1, v/v) was shaken vigorously. After 15 min, the absorbance at 370 nm was measured using gallic acid for the calibration curve. The spectrophotometric analysis was repeated three times for each type of extract.

2.6. Chromatographic analysis of the standard phenolic mixture and the virgin olive oil's phenolic fraction

HPLC analysis were carried out on an HP 1100 Series (Agilent Technologies, Palo Alto, CA, USA), equipped with a binary pump delivery system, a degasser, an autosampler, a HP diode-array UV–Vis detector and a HP mass spectrometer. A C_{18} Luna column 5-μm particle size, 25 cm×3.00 mm I.D. (Phenomenex, Torrance, CA, USA), with a Rheodyne precolumn filter was used. All solvents were filtered trough a 0.45-μm filter disk (Millipore). A gradient elution was carried out using the following solvent system: mobile phase A, water–acetic acid (98:2, v/v); mobile phase B, methanol–acetonitrile (1:1, v/v). The linear gradient elution system was: from 0 to 25 min, 95 to 70% A; from 25 to 35 min, 70 to 60% A; from 35 to 40 min, 60 to 52% A; from 40 to 50 min, 52 to 30% A; from 50 to 55 min, 30 to 0% A; from 55 to 65 min, 0 to 95% A; from 65 to 70 min, 95% A, as post-run. All solvent used were of HPLC grade. The flow-rate was 0.5 ml/min. The quantification of polyphenols by DAD was performed at 280 nm. The injection volumes were 10 and 5 μl, respectively, for the samples and for the standard solutions. All the analyses were carried out at room temperature. For the MS analyses, atmospheric pressure chemical ionization (APCI) was applied operating in negative mode, under the following conditions: drying gas flow, 9 l/min; nebulizer pressure, 50 p.s.i. (1 p.s.i=6894.76 Pa); gas drying temperature, 350 °C; vaporizer temperature, 450 °C; capillary voltage, 4000 V; corona current, 4 μA.

2.7. Electrophoretic analysis of the standard phenolic mixture and the virgin olive oil phenolic fraction

Electrophoretic analyses were performed with a Beckman P/ACE 5000 model equipped with UV–Vis detector (Beckman Instruments, Fullerton, CA, USA). The capillary cartridge contained not deactivated fused-silica tubing (50 μm I.D.×375 μm O.D.), supplied by Beckman. Total capillary length was 47 cm, whereas effective length was 40 cm. The running buffer was 45 m*M* sodium tetraborate (pH 9.6), prepared by dissolving an appropriate amount of solid salt in HPLC-grade water. The buffer was sonicated and filtered trough a 0.2-μm syringe filter. Samples were injected hydrodynamically in the anodic end at low-pressure mode (0.5 p.s.i.), for 3 s. Each electrophoretic run was carried out at 27 kV maintaining the capillary temperature at 30 °C, resulting in a current of approximately 110 μA. After each electrophoretic run, the capillary was rinsed with HPCE-grade water for 2 min, then between each run the capillary was rinsed at high pressure (20 p.s.i.) consecutively with a 0.1 *M* HCl solution for 2 min, HPCE-grade water for 2 min and re-equilibrated with running buffer, for 2 min. All steps of washing were performed at 30 °C. The running buffer was changed after two runs. UV detection was performed at 200 nm; rise time was set at 0.17 s and data rate was 10 Hz.

2.8. Statistical analysis

The analytical results was evaluated by the software Statistica (StatSoft, 1999) by the analysis of variance (ANOVA) one-way with Tukey's HSD multiple comparison to determine differences significant at the 5% level.

3. Results and discussion

3.1. Standard phenolic mixture recoveries by SPE and LLE methods

The recoveries of phenolic compounds were determined and compared in both SPE methods and LLE method. SPE extractions were carried out, as reported in the experimental section, using cartridge having three different stationary phases, namely C_8, C_{18} and Diol. Particularly, two mobile phases were tested to elute the phenolic compounds from C_8 cartridge; they were denominated Method A (C_8-SPE) and Method B (C_8mod.-SPE). The recoveries were calculated using a standard mixture composed of 15 phenolic compounds, commercially available, and being representative of the phenolic fraction of olive oil [25]. This mixture contained simple phenols, such as derivatives of benzoic acid (GalA, PA, 4-HBA, VA, 3-MBA), phenylacetic acid (3,4-DHPAA), cinnamic acid (CafA, DHCA, *p*-CA, FA, *o*-CA, CinA), phenylethyl alcohol (Tyr) and complex phenols as taxifolin (flavononols) and oleuropein (secoiridoid). The standard mixture was added to a refined peanut oil, and then subjected to each extraction. Every extracts were analyzed by HPLC 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. Table 1 shows that the highest recovery (92.1%) was obtained by Method E (LLE), followed by Method C (C_{18}-SPE) (74.0%), Method D (Diol-SPE) (72.2%), Method B (C_8mod.-SPE) (64.5%) and by Method A (C_8-SPE) (51.6%). The recoveries of the standard mixture, by the different procedures, were statistically different. Regarding Method E, it should be underlined that, except for tyrosol (89.9%), cinnamic acid (73.6%) and taxifolin (62.0%), the recoveries of the standard phenolic compounds were higher than 90%. Moreover, Method E permitted the higher recovery of oleuropein (96%).

The different extraction procedures were then tested on a virgin olive oil sample; Table 2 shows the spectrophotometric values of total phenols and *o*-diphenols; from the data elaboration, a significant difference resulted only between Method E and

Table 1
Recoveries (%) calculated by analyzing, three times, refined peanut oil spiked with a mixture of 15 standard phenolic compounds

	Phenolic compounds recovery (%)									
	Method A		Method B		Method C		Method D		Method E	
	Average[a]	SD	Average	SD	Average	SD	Average	SD	Average	SD
GalA	18.31	1.77	25.55	1.36	42.02	0.95	25.27	2.35	91.80	9.05
PA	55.51	5.23	60.61	5.49	78.61	2.54	71.78	3.04	97.29	6.48
3,4-DHPAA	33.82	1.12	40.31	0.86	53.04	1.30	42.07	3.90	95.26	6.68
Tyr	73.93	3.55	83.38	8.20	93.03	0.50	92.97	7.13	89.90	7.99
4-HBA	69.14	6.72	80.48	7.12	93.16	4.62	87.91	6.61	98.33	6.12
CafA	40.79	3.36	46.62	3.55	61.74	0.92	60.81	4.49	92.32	7.74
VA	65.11	5.14	84.43	7.63	94.45	4.27	90.31	7.89	99.27	6.18
DHCA	71.37	6.29	80.42	6.99	90.84	2.36	94.51	1.14	99.61	6.44
p-CA	60.53	5.53	66.67	5.41	84.88	0.51	74.96	7.35	96.47	6.35
FA	65.42	6.29	76.36	7.21	85.49	1.40	89.90	8.77	97.85	5.29
T	37.14	3.43	21.77	2.15	33.15	3.24	61.12	5.82	62.02	6.04
o-CA	60.53	5.31	71.25	7.02	84.25	0.55	74.65	1.71	96.11	6.80
OG	17.88	1.67	n.d.	–	39.47	3.58	n.d.	–	96.01	7.01
3-MBA	41.14	3.82	85.01	8.32	93.85	8.70	68.94	6.80	95.63	6.32
CinA	63.83	5.56	80.35	7.86	81.35	1.69	75.52	7.41	73.57	1.79
Average	51.63	4.32	64.51	5.65	73.95	2.47	72.19	5.31	92.10	6.42

GalA, gallic acid; PA, protocatechuic acid; 3,4-DHPAA, 3,4-dihydroxyphenylacetic acid; Tyr, tyrosol; 4-HBA, 4-hydroxybenzoic acid; CafA, caffeic acid; VA, vanillic acid; DHCA, dihydrocaffeic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; T, taxifolin; *o*-CA, *o*-coumaric acid; OG, oleuropein; 3-MBA, 3-methoxybenzoic acid; CinA, cinnamic acid.

[a] $n=3$.

Table 2
Recoveries of the phenolic fraction extracted from a virgin olive oil, determined by spectrophotometric determinations of total polyphenols (TP) and *o*-diphenols (*o*-diph)

	TP		*o*-diph	
	Average (n=3)	RSD (%)	Average (n=3)	RSD (%)
Method A	233.3[a]	9.8	66.7[b]	9.9
Method B	231.7[a]	9.0	82.0[a]	4.9
Method C	245.7[a]	5.2	73.7[a]	9.6
Method D	274.5[a]	9.7	82.0[a]	6.5
Method E	286.7[a]	6.5	90.0[a]	10.7

Values are expressed as mg of gallic acid per kg of oil. Different letters in the row indicate significantly different values ($P<0.05$).

Method A recoveries, with regard to *o*-diphenols quantification.

3.2. HPLC–DAD–MS analysis of the standard phenolic mixture

In this study two binary solvent systems, both having acidified water as eluent A [16], were tested. The first one was composed by A_1 water–acetic acid (99.8:0.2, v/v) (pH 3.51) and B methanol–acetonitrile (50:50, v/v) and the second one was composed by A_2 water–acetic acid (98:2, v/v) (pH 2.75) and B. The second system brought about the best separation, particularly of the OG/3-MBA critical pair. Thus, the A_2 mobile phase was used for the following HPLC–DAD–MS analysis. Fig. 1 shows the separation of the standard phenolic mixture. Concerning the MS detection, the negative APCI mode [15,26] was chosen after some preliminary comparison trials between this and a positive atmospheric pressure ionization (API)-electrospray ionization (ESI) mode. The negative APCI mode showed a higher abundance of the pseudomolecular ion, here found as $[M-1]^-$, rather than the $[M+Na]^+$, for the positive API–ES interface.

3.3. CZE analysis of standard phenolic mixture

Many analytical parameters were studied to realize the best CZE separation of the standard phenolic compounds; particularly, the choice of the background electrolyte, its concentration, the pH, the applied voltage and the temperature. The standard mixture of 15 phenolic compounds was analysed, using different conditions to achieve the best separation, particularly for the critical pair 3,4-DHPAA/GalA, in the shorter analytical time.

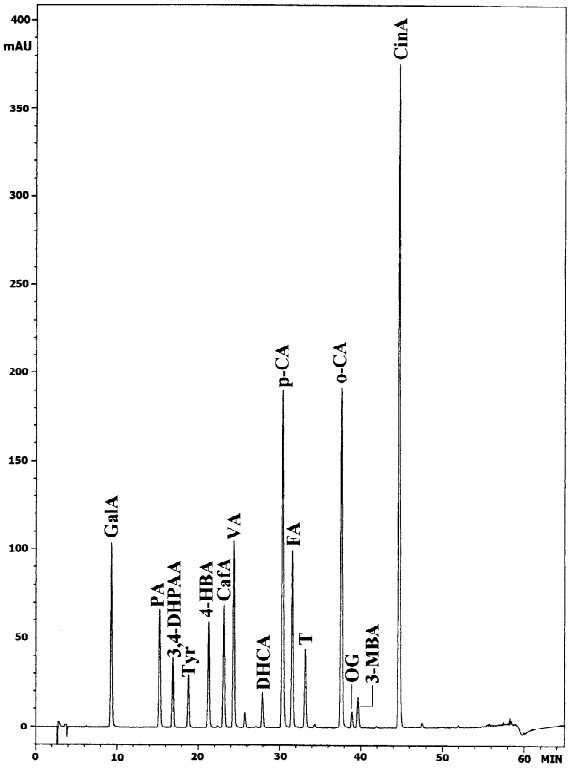

Fig. 1. HPLC trace of the standard phenolic mixture constituted by the following phenolic compounds: GalA, gallic acid; PA, protocatechuic acid; 3,4-DHPAA, 3,4-dihydroxyphenylacetic acid; Tyr, tyrosol; 4-HBA, 4-hydroxybenzoic acid; CafA, caffeic acid; VA, vanillic acid; DHCA, dihydrocaffeic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; T, taxifolin; *o*-CA, *o*-coumaric acid; OG, oleuropein; 3-MBA, 3-methoxybenzoic acid; CinA, cinnamic acid. Detection was performed at 280 nm (for the other analysis conditions see Section 2).

The best result was obtained by using a 45 m*M* tetraborate buffer (pH 9.60) as background electrolyte, an applied voltage of 27 kV and a temperature of 30 °C. The resulting electropherogram is reproduced in Fig. 2. Clearly, the peaks were completely separated at the baseline and the last peak, corresponding to protocatechuic acid, was detected at about 9 min. Therefore, the total analysis time, including rinse steps, was about 18 min.

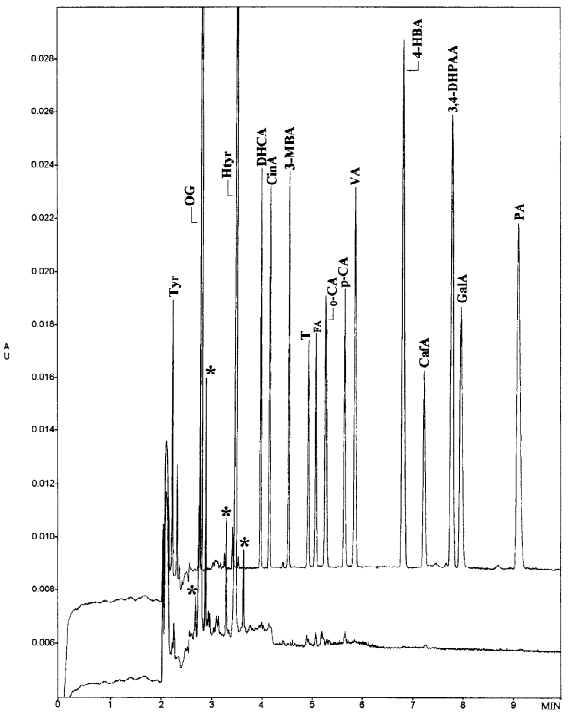

Fig. 2. In the upper trace, CZE separation of a standard mixture composed by 15 phenolic compounds: Tyr, tyrosol; OG, oleuropein; DHCA, dihydrocaffeic acid; CinA, cinnamic acid; 3-MBA, 3-methoxybenzoic acid; T, taxifolin; FA, ferulic acid; *o*-CA, *o*-coumaric acid; *p*-CA, *p*-coumaric acid; VA, vanillic acid; 4-HBA, 4-hydroxybenzoic acid; CafA, caffeic acid; 3,4-DHPAA, 3,4-dihydroxyphenylacetic acid; GalA, gallic acid; and PA, protocatechuic acid. At the bottom trace, CZE separation of derivative compounds obtained by partially enzymatic hydrolysis of oleuropein, is shown. Detection was performed at 200 nm (for the other analysis conditions see Section 2).

3.4. HPLC–DAD–MS and CZE analysis of the virgin olive oil's phenolic fraction

Table 3 reports the amounts of the two major simple phenols, hydroxytyrosol (Htyr), considered one of the most active antioxidant of the phenolic fraction [13,14,27], and tyrosol (Tyr). A 95% pure standard of hydroxytyrosol, which is not commercially available, but is necessary for the calibration curve, was prepared by chemical reduction of 3,4-dihydroxyphenylacetic acid. The HPLC determination of hydroxytyrosol by Methods E, D and B resulted not significantly different and higher than the Method A and Method C; concerning tyrosol, Method C demonstrated the worst recovery.

Htyr and Tyr were used also to compare HPLC and CZE quantitative performances. In general, significant differences regarding the quantification of the two phenols, by the different procedures, emerged only for Tyr especially by Method C. The results rather appeared in good agreement considering that HPLC and CZE operate by different principles and that the analyses were carried out at two different wavelength of detection (280 and 200 nm, respectively).

The limit of detection for Htyr and Tyr, analyzed by HPLC, were 1.0 and 0.7 μg/ml, respectively, while by CZE were both equal to 0.001 μg/ml. In general, the CZE method in comparison to the HPLC one, depending on the standard phenolic compounds, showed higher sensitivity of a variable factor from 100 to 1000.

Figs. 3 and 4 show, respectively, the HPLC and a CZE plots of the phenolic fraction from the virgin olive oil, extracted by Method E. A partial qualitative analysis of the phenolic complex fraction eluting, in HPLC, between 30 and 50 min, was realized by MS; in this region the oleuropein aglycon (m/z 377) and other compounds, chemically related to the oleuropein aglycon structure, such as the ligstroside aglycon (m/z 361) and the decarboxymethyl oleuropein aglycon (m/z 319), were tentatively identified. In Table 4, the quantification, expressed as peak areas at 280 nm (mAUs), of the oleuropein derivatives is shown; generally, the recoveries of oleuropein aglycon, ligstroside aglycon and decarboxymethyl oleuropein aglycon by Method E and Method D were higher than those obtained by other extraction procedures. Method E was not significantly different from Method D even if the standard deviation values of the latter were higher.

The CZE trace showed a group of peaks in the region from 2 to 4 min (see Fig. 4). The peaks marked with an asterisk are supposed to be secoiridoid compounds and correspond to the substances of the complex fraction eluting, by HPLC, from 30 to 50 min. This hypothesis is supported by the analysis of enzymatic hydrolysis products of oleuropein, as shown in the electropherogram at the bottom of Fig. 2.

From the reported chromatographic traces it is

Table 3
Hydroxytyrosol (Htyr) and tyrosol (Tyr) amounts, determined by HPLC and CZE in the virgin olive oil's phenolic fraction obtained by different extraction procedures

	Phenolic compound (mg/kg of oil)							
	HPLC				HPCE			
	Htyr		Tyr		Htyr		Tyr	
	Average (n=3)	RSD (%)	Average (n=3)	RSD (%)	Average (n=3)	RSD (%)	Average (n=3)	RSD (%)
Method A	44.1[b]	10.0	36.1[a]	6.1	47.0[b]	2.9	40.1[b]	11.4
Method B	59.7[a]	7.5	41.3[a]	3.0	54.2[b]	9.6	40.4[b]	10.3
Method C	38.2[b]	8.6	30.7[b]	7.0	45.7[b]	11.1	49.5[b]	3.2
Method D	58.0[a]	18.0	38.4[a]	19.0	65.4[a]	21.2	59.8[a]	21.0
Method E	61.8[a]	7.5	39.3[a]	9.2	62.8[a]	5.3	49.2[b]	10.5

Values are expressed as mg of phenolic compound per kg of oil. Different letters in the row indicate significantly different values ($P<0.05$).

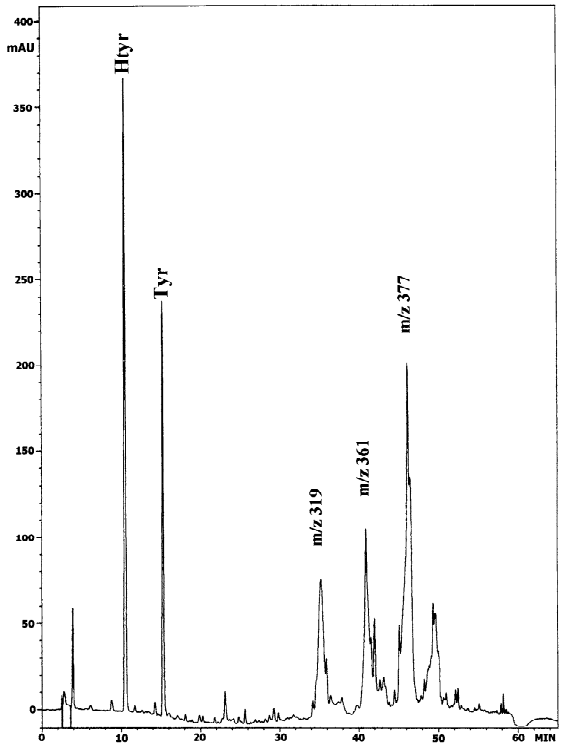

Fig. 3. HPLC trace of the phenolic fraction extracted from virgin olive oil by LLE. Detection was performed at 280 nm (for the other analysis conditions see Section 2). Tyr, tyrosol; Htyr, hydroxytyrosol; m/z 377, oleuropein aglycon; m/z 361, ligstroside aglycon; m/z 319, decarboxymethyl oleuropein aglycon.

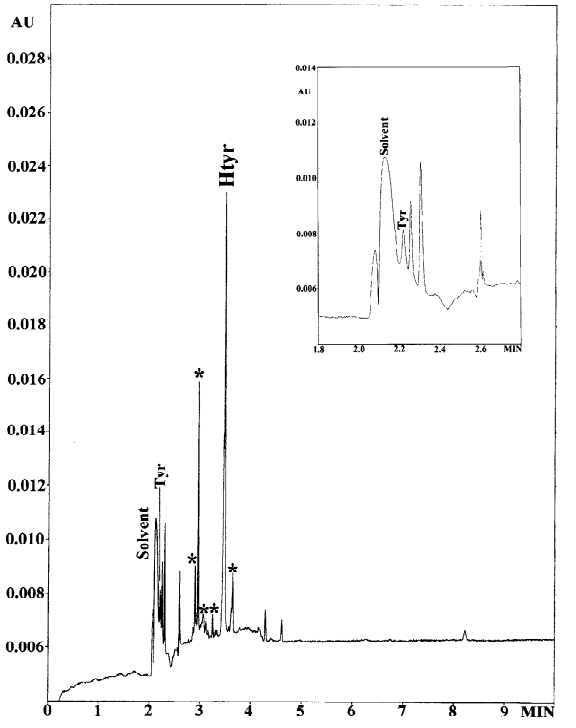

Fig. 4. CZE electropherogram of the phenolic fraction extracted from virgin olive oil by LLE; Tyr, tyrosol; Htyr, hydroxytyrosol. Detection was performed at 200 nm (for the other analysis conditions see Section 2). In the square at the right of the total electropherogram is shown an enlargement of first 3 min of the run. The peaks marked with an asterisk are probably secoiridoid compounds (see Fig. 2).

Table 4
Peak areas at 280 nm of oleuropein aglycon (m/z 377), ligstroside aglycon (m/z 361) and decarboxymethyl oleuropein aglycon (m/z 319), recovered by different extraction procedures

	Peak areas at 280 nm (mAUs)					
	m/z 319		m/z 361		m/z 377	
	Average (n=3)	RSD (%)	Average (n=3)	RSD (%)	Average (n=3)	RSD (%)
Method A	288.8[b]	9.6	371.6[a]	14.7	625.0[a]	3.9
Method B	118.2[c]	34.4	168.7[b]	26.6	644.2[a]	22.4
Method C	244.8[b]	7.8	378.2[a]	11.2	768.2[a]	11.8
Method D	465.2[a]	18.1	537.1[a]	22.0	852.7[a]	30.3
Method E	388.6[a]	14.0	465.2[a]	11.0	788.6[a]	9.1

Different letters in the row indicate significantly different values ($P<0.05$).

possible to appreciate the substantial difference, in terms of time of analysis, between the two analytical techniques. The HPLC analysis required about 60 min against about 10 min required by CZE. Moreover, the fraction defined as complex, eluting by HPLC between 30 and 50 min and comparable to the fraction eluting in CZE between 2 and 4 min, presented, in the HPLC trace, several phenolic compounds overlapping. The same complex fraction seems to be more resolved by CZE.

4. Conclusions

Method E gave the best results in terms of recovery of the phenolic standard mixture composed of 15 compounds, added to the refined peanut oil. In general, for virgin olive oil sample, Method E and Method D showed higher recoveries of total phenols, *o*-diphenols, tyrosol, hydroxytyrosol and secoiridoids than the other extraction procedures. The performances of these two methods, beyond the cost of material and the time of extraction, which are advantageous for Method E, can be considered equivalent. The comparison between HPLC and CZE analysis of the phenolic standard mixtures and of the phenolic fraction, extracted from a virgin olive oil, demonstrated that CZE techniques could be considered a useful tool, particularly considering the higher sensitivity, the rapidity of the analysis and the low solvent consumption.

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