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Solid-phase extraction and gas chromatographic analysis of phenolic compounds in virgin olive oil

Lolita Liberatore [a], Giuseppe Procida [b], Nicola d'Alessandro [a], Angelo Cichelli [a,*]

[a] *Dipartimento di Scienze, Università G.D'Annunzio di Chieti e Pescara, Viale Pindaro 42, I-65127 Pescara, Italy*

[b] *Dipartimento di Merceologia ed Economia delle Risorse Naturali e della Produzione, Università di Trieste, Via Valerio 6, I-34127 Trieste, Italy*

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Abstract

A careful investigation on the potential application of solid phase extraction (SPE)-gas chromatography procedure in the analysis of phenol compounds in virgin olive oils was carried out. In doped refined olive oil samples a comparison between liquid/liquid and SPE extraction evidenced higher recovery when the C_{18} sorbent phase was employed whereas, in the case of total suppression of residual sylanolic group ($C_{18\ EC}$), only contradictory data was obtained. The same procedures were carried out on 10 genuine samples of virgin olive oil. As observed with the standards, C_{18} sorbent phase gave higher values compared to $C_{18\ EC}$ and the liquid/liquid extraction procedures. Satisfactory results were obtained in the detection of ligstroside aglycon but for the oleuropein aglycon the quantitative is not completely reliable for the overlap of some unknown no-phenol compounds.

Keywords: Virgin olive oil; Simple phenols; Ligstroside aglycon; Oleuropein aglycon; Solid phase extraction

1. Introduction

Phenolic glycosides are present in large amounts in leaves and fruits of olive trees. Among them oleuropein is the most important together with verbascoside and several other glucosidic flavonoids (rutin and luteoline 7-glucoside) which are present in considerable amounts, depending upon variety, agronomic operations, degree of ripeness and fruit storage (Amiot, Fleuriet, & Macheix, 1986; Montedoro & Cantarelli, 1969).

As far as virgin olive oils are concerned, simple phenols and aglycons of glucosydes are present in amounts ranging from 50 to 500 mg/kg (Montedoro, Servili, Baldioli, & Miniati, 1992a; Solinas, 1987; Tsimidou, Papadopoulos, & Boskou, 1992). It is known that phenolic compounds (PCs) play an important role in the antioxidant activity and they are directly linked to both the nutritional value and flavour characteristics of the oil (Angerosa, Mostallino, Basti, & Vito, 2000). Furthermore agronomic phases, cultivars, extraction systems and processing operations differences contribute considerably to the nature and relative amounts of phenols (Di Giovacchino, Solinas, & Miccoli, 1994; Solinas & Cichelli, 1981).

Various analytical methods have been employed to quantify these substances in virgin olive oils. A colorimetric method based on the Folin Ciocalteu reagent was the first recognised methodology employed (Gutfinger, 1981). However it is known that responses of single phenols to the Folin reagent are significantly different from each other, thus making this method unsuitable for the exact measurement of phenolic contents. Liquid/liquid solvent extraction followed by gas chromatography (GC) or high performance liquid chromatography (HPLC) quantitation gives better and objective results. Several examples are reported in the literature, dealing with both neat samples and mixtures of solvents: (1) methanol–water (60:40) on oil dissolved in petroleum ether (Ragazzi & Veronese, 1973) or in hexane (Vazquez Roncero, Janer Del Valle, & Janer Del Valle, 1973, 1976); (2) methanol–water (80:20) again neat oil (Montedoro, 1972; Montedoro & Cantarelli, 1969); (3) methanol–water (80:20) added to Tween 20 on neat oil (Montedoro, Bertuccioli, & Anichini, 1978); (4) methanol on neat oil (Solinas, 1987); and (5) tetrahydrofuran-water (80:20) on neat oil (Cortesi, Azzolini, & Rovellini,

* Corresponding author. Tel.: +39-085-4537509; fax: +39-085-4537545.

E-mail address: cichelli@sci.unich.it (A. Cichelli).

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1995b). Recently, solid phase extraction (SPE) (Cortesi et al., 1995b; Favati, Caporale, & Bertuccioli, 1994; Mannino, Cosio, & Bertuccioli, 1993; Papadopoulos & Tsimidou, 1992) has raised much interest. Several solid phases can be employed, depending upon the nature of the analyte, and in particular the family of C_{18} sorbent phase appears as the most used for the purpose.

Two different analytical techniques, GC (Janer Del Valle & Vazquez Roncero, 1980; Solinas, 1987) and HPLC (Akasbi, Shoeman, & Saari Csallany, 1993; Graciani Costante & Vazquez Roncero, 1980; Solinas & Cichelli, 1982), have been employed for the detection and quantitation of the individual PCs. Improvement was obtained with analytical sophisticated techniques such as GC–MS (Angerosa, d'Alessandro, Corana, & Mellerio, 1996), HPLC–MS (Cortesi, Azzolini, Rovellini, & Fedeli, 1995a), and NMR (Montedoro, Servili, Baldioli, Selvaggini, Miniati, & Macchioni, 1993).

A careful assessment of the used methodologies clearly indicates that data is non-homogenous and at the same time contradictory, especially in relation to efficiency of recovery in the extraction with solvents or mixtures of solvents (Angerosa, d'Alessandro, Kostantinou, & Di Giacinto, 1995; Cortesi et al., 1995b; Favati et al., 1994; Montedoro et al., 1992a). The aim of the present study is to compare the efficiency of SPE to liquid-liquid extraction, by determining the recoveries of the most representative simple PCs (Akasbi et al., 1993; Solinas, 1987; Tsimidou et al., 1992; Tsimidou, Lytridou, Boskou, Pappa-Louisi, Kotsifaki, & Petrakis, 1996) and the main aglycons of glucoside by GC. For this purpose two commercial available solid supports, octadecyl (C_{18}) and octadecyl end capped ($C_{18\ EC}$), were taken into account.

2. Materials and methods

2.1. Samples

Ten samples obtained from the 1997 campaign of virgin olive oil from the Abruzzo region (Italy) have been analyzed; they were obtained from Dritta (samples nos. 1–3), Gentile (samples nos. 4–6) and Leccino (samples nos. 7–10) varieties. A sample of refined olive oil was purchased from an industrial plant (Adriaoli, Pescara, Italy).

2.2. Reagents and standards

Chemicals (tyrosol, *p*-hydroxybenzoic acid, vanillic acid, protocatechic acid, syringic acid, *p*-coumaric acid, ferulic acid and caffeic acid) were obtained from FLUKA (Buchs, Switzerland). Resorcinol was purchased from Merck (Darmstadt, Germany). All other chemicals were from Merck (p.a. grade) as well.

2.3. Standard solutions

Each standard compound (62.5 mg) was exactly weighed in a 100-ml volumetric flask and acetone was employed as a solvent. Doped olive oil solutions were obtained by the addition of aliquots (2.4 and 24 ml, respectively) of the standard solutions to 30 g of refined olive oil. The sample was homogenized in an Ultra-turrax T 25 apparatus and the solvent was eliminated in a rotary vacuum evaporator at 40°C. The concentration of 50 and 500 mg/kg of each PC were so obtained.

2.4. Solid-phase extraction

Two commercially available cartridges, octadecyl C_{18} (2 g, 6 ml) and octadecyl $C_{18\ EC}$ (end capped; 2 g, 6 ml) were compared (Int. Sorb. Tech., Hengoed, UK). Diethyl ether resorcinol solution (100 μl; I.S.) was added to 3 g of olive oil (0.5 mg/ml); the mixture was dissolved with 15 ml of hexane and the obtained solution was loaded onto the column previously conditioned with 2×10 ml of methanol and 2×10 ml of hexane. The column was eluted with 4×10 ml of hexane to eliminate the apolar fraction, and the retained polar compounds were recuperated by eluting with 4×10 ml of methanol.

2.5. Liquid–liquid extraction

The polar fraction of virgin olive oil samples was obtained according to Tsimidou et al. (1992). Briefly, 30 g of virgin olive oil, added with to 1 ml of a diethyl ether resorcinol solution (0.5 mg/ml), was dissolved in 50 ml of hexane; the solution was extracted three times with 30 ml of a methanol/water mixture (60:40). The methanol/water layers were separated, washed twice with 50 ml of hexane, evaporated under vacuum at 40°C, re-dissolved with 7 ml of acetone and finally kept for GC analyses.

2.6. Gaschromatographic analysis

One millilitre of acetone solution deriving from both SPE and liquid–liquid extraction were derivatized with 150 μl of bis(trimethylsilyl)trifluoroacetamide. GC analysis was carried out with a Mega Series 5300 gaschromatograph (C. Erba, Milan, Italy) equipped with a cold on-column injector and a FID detector. A SPB-5 fused-silica capillary column, 30 m×0.32 i.d., 0.10 μm film thickness (Supelco, Inc. Bellefonte, PA) was employed. The oven temperature was programmed from 70 to 135°C at 2°C/min, held for 10 min at 135°C, then from 135 to 220°C at 4°C/min, held for 10 min at 220°C, then from 220 to 270°C at 4°C/min and maintained for 20 min; detector temperature was 290°C; carrier gas (helium) flow rate 2.0 ml/min.

GC–MS was performed with a HP Model 5890, equipped with an on column injection system and a

mass selective detector Model HP 5971, on a HPInnovax silica capillary column (30 m×0.32 i.d., 0.25 μm film thickness). The carrier gas was He, and the carrier pressure on the head of the column was 10 kPa. The oven temperature program was the same as the one used for GC determination; the transfer line temperature was held at 300°C.

3. Results and discussion

Recovery efficiency of SPE technique has been compared to the previously reported liquid/liquid extraction procedure. Eight PCs (tyrosol, *p*-hydroxybenzoic acid, vanillic acid, protocatechic acid, syringic acid, *p*-coumaric acid, ferulic acid and caffeic acid), that are considered the most representative of the simple phenolic fraction present in virgin olive oils (Akasbi et al., 1993; Janer Del Valle & Vazquez Roncero, 1980; Montedoro et al., 1992a; Nergiz & Unal, 1991; Tsimidou et al., 1992), have been added to a refined olive oil to obtain final concentrations of 50 and 500 mg/kg. A careful analysis of literature data gives us some evidence regarding the use of the mixture methanol/water 60/40 as the extraction solvent. After the extraction procedure, the PCs were evaluated by GC. For each sample five analyses were performed (Table 1). Quantitative recoveries of tyrosol were obtained with both cartridges, in agreement to previous literature data (Favati et al., 1994; Papadopoulos & Tsimidou, 1992). Recoveries of the other examined PCs were not always quantitative. Unsatisfactory recoveries were obtained in particular, with $C_{18\ EC}$, especially at low PCs contents (50 mg/kg), whereas C_{18} cartridges resulted in the practically quantitative recovery of all the examined compounds, particularly at high PC contents, with the only exception of *p*-coumaric, ferulic and caffeic acids. Low recoveries of ferulic and caffeic acids were already reported by other researchers (Angerosa et al., 1995). The different behaviour between C_{18} and $C_{18\ EC}$ might be due to the different interactions existing between the sorbing material and the analyte: indeed, both C_{18} and $C_{18\ EC}$ cartridges are reverse phase apolar materials and very strong interactions between the stationary phase and the analyte are expected. However, the mechanism of release of the analyte due to the eluent probably depends on a secondary interaction between the residual Si-OH groups located on the surface of the silica and the adsorbed compounds. In the case of $C_{18\ EC}$ the residual polar groups are suppressed and this feature apparently makes the mechanism of release of the analyte worse.

Subsequently we tried to extract 10 samples of virgin olive oil with both extraction procedures (an example of a C_{18} SPE chromatogram is reported in Fig. 1). We evaluated the content of the previously investigated phenols (Table 2) and, in addition, we studied the part of chromatogram relative to complex phenols in detail, namely aglycons of the glucosides present in olive fruits (Table 3). For each sample five analyses were performed for each extraction procedure, whereas with SPE, with each sorbent phase. The quantitation was done by the addition of a known amount of resorcinol (internal standard) before the extraction procedure. The results reported in Table 2 indicate that there is no significant difference between SPE and liquid/liquid extraction procedure. The amounts detected by employing C_{18} cartridges are sometimes slightly higher than those of $C_{18\ EC}$, as seen in the above-mentioned recovery trials. However, for the protocatechuic acid (samples 1, 4, 6, and 9), syringic acid (samples 2, 3, 6, and 7), ferulic acid (samples 4, 5, and 7) and caffeic acid (samples 3, 6, 8 and 9) no statistically significant difference was observed. Data obtained for solvent extraction showed that in most cases, i.e. ferulic and caffeic acid, slightly lower values than those reported for SPE. This evidence is likely attributable to the very poor recovery of the above compounds, also shown in previous literature data (Angerosa et al., 1995; Montedoro et al., 1992a; Montedoro, Servili, Baldioli, & Miniati, 1992b).

As reported in previous papers, the most abundant aglycon present in the phenolic fraction are tyrosol and hydroxytyrosol derivatives. In particular we detected

Table 1
Recovery of eight standards in two doped refined olive oil (50 and 500 ppm)

	C_{18}		$C_{18\ EC}$		Liquid extraction	
	50 ppm	500 ppm	50 ppm	500 ppm	50 ppm	500 ppm
Tyrosol	> 95%	> 95%	>95%	95%	>95%	>95%
p-Hydroxybenzoic acid	>95%	>95%	73%±7.2	65%±5.8	>95%	>95%
Vanillic acid	>95%	>95%	63%±6.8	65.4%±14.1	>95%	>95%
Protocatechic acid	83%±6.1	>95%	48%±7.1	51%±9.4	75%±5.5	>95%
Syringic acid	78%±8.9	90.1%±10.1	33.3%±10.3	43%±8.6	35%±6.8	79%±5.8
p-Coumaric acid	60%±6.1	82%±5.0	34%±6.4	24%±3.4	40%±4.6	48%±6.2
Ferulic acid	70%±5.7	55%±5.4	20%±3.7	18%±3.4	25%±5.0	35%±4.2
Caffeic acid	65%±5.9	58%±5.1	21%±3.8	19%±3.0	28%±4.4	36%±5.5

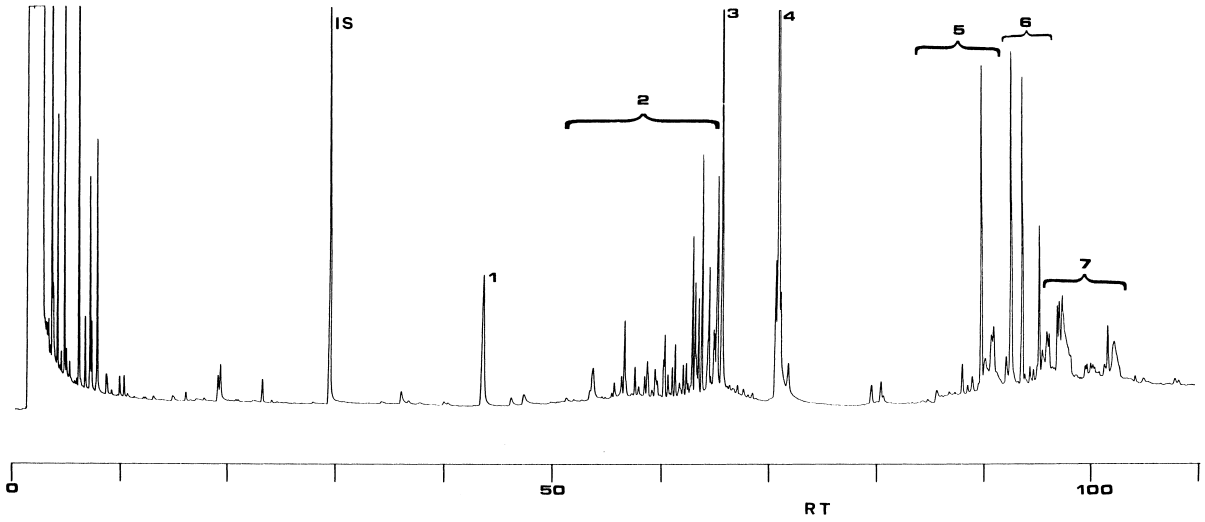

Fig. 1. An example of chromatogram deriving from the C_{18} SPE procedure (Oil sample No. 1). Legend; I.S.: resorcinol (internal standard); 1, tyrosol; 2, hydroxytyrosol and phenolic acids zone; 3, $C_{16:0}$ fatty acid TMS derivative; 4, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ fatty acids TMS derivatives; 5, ligstroside aglycons zone; 6, monoglycerid TMS derivatives zone; 7, oleuropein aglycons zone.

Table 2
Amounts of the eight considered simple phenols by the two extraction procedures (for SPE C_{18} and $C_{18\ EC}$) expressed as ppm of resorcin in 10 samples of virgin olive oils (five replicated for each analysis)

Sample		Phenols							
	Extraction technique	Tyrosol	*p*-OH-benzoic acid	Vanillic acid	Protocatechic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Caffeic acid
Oil No. 1	C_{18}	14.70±1.51	0.96±0.08	1.43±0.23	0.96±0.10	2.07±0.31	1.09±0.15	0.62±0.08	1.19±0.25
	$C_{18\ EC}$	14.47 ±1.59	0.60±0.11	1.07±0.22	0.96±0.16	1.98±0.18	1.11±0.28	0.67±0.11	1.04±0.23
	L-L	14.70±2.42	0.73±0.08	1.46±0.06	0.70±0.07	1.71±0.09	0.66±0.06	0.29±0.09	0.53±0.10
Oil No. 2	C_{18}	13.40±1.91	0.77±0.13	1.83±0.18	1.31±0.06	1.62±0.13	1.65±0.16	0.73±0.05	1.13±0.08
	$C_{18\ EC}$	11.30±1.71	0.71±0.08	1.81±0.20	1.07±0.05	1.61±0.14	1.46±0.23	0.65±0.06	0.95±0.11
	L-L	12.92±1.68	0.52±0.07	1.40±0.16	1.33±0.21	1.45±0.06	1.18±0.21	0.84±0.16	0.40±0.07
Oil No. 3	C_{18}	12.46±1.72	0.56±0.04	0.76±0.06	0.81±0.07	1.15±0.11	1.36±0.09	0.61±0.07	0.56±0.09
	$C_{18\ EC}$	10.30±1.61	0.60±0.15	0.67±0.08	0.78±0.06	1.14±0.06	1.09±0.11	0.57±0.04	0.58±0.08
	L-L	12.33±1.68	0.38±0.08	0.52±0.08	0.80±0.13	1.05±0.07	0.38±0.09	0.57±0.10	0.42±0.07
Oil No. 4	C_{18}	27.37±2.42	0.42±0.10	0.98±0.21	0.90±0.16	1.14±0.10	1.77±0.24	0.86±0.06	1.13±0.12
	$C_{18\ EC}$	26.63±3.66	0.37±0.08	0.94±0.27	0.90±0.18	1.21±0.14	1.55±0.14	0.85±0.08	0.72±0.07
	L-L	24.54±1.77	0.49±0.05	0.45±0.09	0.91±0.10	1.02±0.21	1.09±0.20	0.75±0.08	0.25±0.06
Oil No. 5	C_{18}	13.34±1.28	0.33±0.02	0.52±0.04	1.24±0.14	1.64±0.30	1.70±0.38	0.45±0.04	0.55±0.10
	$C_{18\ EC}$	11.87±1.51	0.28±0.04	0.58±0.04	0.86±0.08	1.45±0.12	1.18±0.19	0.45±0.04	0.48±0.03
	L-L	11.47±0.45	0.34±0.09	0.56 ±0.09	0.98±0.17	1.16±0.16	1.13±0.19	0.40±0.08	0.27±0.06
Oil No. 6	C_{18}	13.85±1.45	0.15±0.01	0.32±0.03	0.60±0.06	1.10±0.09	1.07±0.07	0.56±0.03	0.14±0.01
	$C_{18\ EC}$	12.47±1.28	0.15±0.01	0.37±0.04	0.55±0.03	1.05±0.08	0.69±0.04	0.44±0.04	0.14±0.01
	L-L	10.74±0.95	0.13±0.02	0.17±0.03	0.49±0.05	0.72±0.04	1.09±0.16	0.36±0.06	0.15±0.02
Oil No. 7	C_{18}	11.61±1.64	0.23±0.01	0.37±0.06	0.66±0.08	0.80±0.09	0.79±0.14	0.53±0.05	0.30±0.02
	$C_{18\ EC}$	10.57±0.83	0.18±0.02	0.21±0.03	0.83±0.17	0.80±0.07	0.63±0.04	0.52±0.12	0.23±0.04
	L-L	9.06±0.76	0.31±0.05	0.15±0.02	0.67±0.06	0.57±0.05	0.56±0.08	0.37±0.06	0.22±0.02
Oil No. 8	C_{18}	13.96±1.28	0.56±0.16	1.73±0.24	1.68±0.28	1.50±0.18	0.90±0.08	0.65±0.13	0.32±0.02
	$C_{18\ EC}$	11.35±1.35	0.57±0.12	1.71±0.26	1.43±0.29	1.25±0.18	0.88±0.07	0.55±0.05	0.32±0.03
	L-L	12.82±1.15	0.42±0.07	1.83±0.27	0.56±0.05	0.84±0.05	0.72±0.11	0.41±0.06	0.24±0.03
Oil No. 9	C_{18}	9.19±0.57	0.54±0.06	0.58±0.08	0.88±0.11	1.25±0.14	1.07±0.14	0.51±0.05	025±0.02
	$C_{18\ EC}$	7.45±0.41	0.49±0.04	0.51±0.07	0.86±0.18	1.07±0.11	0.90±0.14	0.53±0.15	0.21±0.03
	L-L	5.55±0.41	0.46±0.08	0.40±0.06	0.70±0.07	0.59±0.04	0.46±0.07	0.34±0.05	0.17±0.02
Oil No. 10	C_{18}	2.58±026	0.25±0.04	1.67±0.25	1.75±0.22	1.75±0.10	1.34±0.17	0.67±0.10	n.d.
	$C_{18\ EC}$	2.18±0.17	0.17±0.01	1.58±0.25	1.51±0.22	1.48±0.08	1.02±0.10	0.59±0.09	n.d.
	L-L	1.91±0.07	0.12±0.02	1.27±0.04	1.08±0.04	1.05±0.10	0.72±0.02	0.40±0.07	n.d.

(by GC–MS) in our extra virgin olive oil samples the aglycon of ligstroside, a tyrosol derivative, and the aglycon of oleuropein, an hydroxytyrosol derivative together with their derivatized isomeric structures (Angerosa et al., 1995, 1996). An authentic oleuropein aglycon sample was obtained by β-glucosidase hydrolysis of a commercial oleuropein and it was used as reference for the retention time. The quantitation was achieved by means of the internal standard, as performed previously for the simple phenols.

Larger values are obtained with the SPE procedures and, compared to the simple phenols data, in this case the difference between C_{18} and $C_{18\ EC}$ are noteworthy and therefore only the results obtained by C_{18} cartridge are reported (Table 3). In the case of ligstroside aglycon a comparison between the C_{18} and the liquid/liquid extraction procedures shows that the values are still comparable and the difference is likely attributable to different recovery factors. Exploration of the ligstroside zone of chromatogram by GC–MS gives no evidence for other unknown compounds, thus indicating that SPE recovery of tyrosol derivatives is much more effective. Regarding the oleuropein aglycon, on the contrary, we observed completely different amounts for the two extraction procedures in three oil samples. In fact, by analyzing the cited chromatograms obtained from the SPE procedure by GC–MS, it is possible to note that other peaks are present, besides those of the oleuropein aglycon, which cannot be attributed to phenol derivatives. This result obviously causes an error in the quantitation by GC methodology and explains why we obtained much higher values than expected. In conclusion, recovery of aglycons are much more satisfactory for SPE procedure, but attention must be payed in the evaluation of hydroxytyrosol derivatives because of the presence of some unknown compound with the same retention times.

Table 3
Amounts of aglycons (ligstroside and oleuropein) obtained by internal standard methodology (as ppm of resorcin)

Sample	Ligstroside aglycon		Oleuropein aglycon	
	Liquid extraction	SPE	Liquid extraction	SPE
Oil No. 1	12±8.4	18±3.5	4±1.1	8±1.8
Oil No. 2	30±6.6	51±4.9	8±2.6	15±2.0
Oil No. 3	18±2.3	26±2.1	3±1.5	3±0.6
Oil No. 4	28±1.1	47±3.2	15±3.3	114±12.5
Oil No. 5	37±2.2	39±5.4	3±2.1	40±11.1
Oil No. 6	17±4.6	20±2.2	4±1.1	27±5.1
Oil No. 7	30±3.7	48±4.4	10±1.3	15±2.4
Oil No. 8	23±2.0	14±1.7	4±1.2	7.6±1.3
Oil No. 9	14±3.2	49±4.5	13±1.5	24±1.9
Oil No. 10	30±5.8	51±4.6	11±2.2	15±1.7

4. Conclusions

The results presented in this paper show that the SPE technique might be successfully used for the extraction and quantitation of PCs in virgin olive oils. We obtained sufficient results to demonstrate that both the trapping first and the release of analyte later from C_{18} solid phases is highly competitive with the liquid/liquid extraction procedure. The discrimination between C_{18} and $C_{18\ EC}$ solid phases is not so vast as expected since the two stationary phases differ only for the presence of free silanolic groups; however their presence (C_{18}) seems to improve the release mechanism increasing the recovery.

It is difficult to obtain pure samples of each aglycon for the evaluation of the canonical recovery. So we demonstrate in another way that the values obtained by SPE are more reliable than the liquid/liquid extraction. Oleuropeyn aglycon is the special case due to the presence, sometimes, of other no-phenolic compounds. With this substance, for the quantitation by SPE procedure, it is important previously to analyse the sample by GC–MS to ascertain if other no-phenol compounds could be present.

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References

Amiot, M. J., Fleuriet, A., & Macheix, J. J. (1986). Importance and evolution of phenolic compounds in olive during growth and maturation. *Journal of Agriculture and Food Chemistry, 34*, 823–826.

Angerosa, F., d'Alessandro, N., Konstantinou, P., & Di Giacinto, L. (1995). GC- Ms evaluation of phenolic compounds in virgin olive oil. *Journal of Agriculture and Food Chemistry, 43*, 1802–1807.

Angerosa, F., d'Alessandro, N., Corana, F., & Mellerio, G. (1996). Characterization of phenolic and secoiridoid aglycons present in virgin olive oil by gas chromatography-chemical ionization mass spectrometry. *Journal of Chromatography A, 736*, 195–203.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2000). Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chemistry, 68*, 283–287.

Akasbi, M., Shoeman, D. W., & Saari Csallany, A. (1993). High-Performance Liquid Chromatography of Selected Phenolic Compounds in Olive Oils. *Journal of the American Oil Chemist's Society, 70*, 367–370.

Cortesi, N., Azzolini, M., Rovellini, P., & Fedeli, E. (1995a). I componenti minori polari degli oli vergini di oliva: ipotesi di struttura mediante LC-MS. *La Rivista Italiana delle Sostanze Grasse, 72*, 241–251.

Cortesi, N., Azzolini, M., & Rovellini, P. (1995b). Dosaggio dei componenti minori polari (CMP) in oli vergini di oliva. *La Rivista Italiana delle Sostanze Grasse, 72*, 333–337.

Di Giovacchino, L., Solinas, M., & Miccoli, M. (1994). Effect of extractions systems on the quality of virgin olive oil. *Journal of the American Oil Chemist's Society, 71*, 1189–1193.

Favati, F., Caporale, G., & Bertuccioli, M. (1994). Rapid determination of phenol content in extra virgin olive oil. *Grasas y Aceites, 45*, 68–70.

Graciani Costante, E., & Vazquez Roncero, A. (1980). Estudio de los componentes polares del Aceite de oliva por cromatografia liquida de alta eficacia (HPLC). *Grasas y Aceites, 31*, 237–243.

Gutfinger, T. (1981). Polyphenols in Olive Oils. *Journal of the American Oil Chemist's Society, 58*, 966–968.

Janer Del Valle, C., & Vazquez Roncero, A. (1980). Estudio de los componentes polares del aceite de oliva por cromatografia gaseosa. *Grasas y Aceites, 5*, 309–316.

Mannino, S., Cosio, M. S., & Bertuccioli, M. (1993). High performance liquid chromatography of phenolic compounds in virgin olive oils using amperometric detection. *Italian Journal of Food Science, 4*, 363–370.

Montedoro, G., & Cantarelli, C. (1969). Indagini sulle sostanze fenoliche presenti negli oli di oliva. *La Rivista Italiana delle Sostanze Grasse, 46*, 115–124.

Montedoro, G. (1972). Costituenti fenolici presenti negli oli vergini di oliva. Nota 1: Identificazione di alcuni acidi fenolici e loro potere antiossidante. *Scienze e Tecnologie Alimentari II*, 177–186.

Montedoro, G., Bertuccioli, M., & Anichini, F. (1978). Aroma analysis of virgin olive oil by head space (volatiles) and extraction (polyphenols) techniques. In Charolambus, & Inglett, *Flavor of foods and beverages: chemistry and technology* (pp. 247–279). Academic Press Inc., NY.

Montedoro, G., Servili, M., Baldioli, M., & Miniati, E. (1992a). Simple and hydrolyzable phenolic compounds in virgin olive oil 1. Their extraction, separation, and quantitatice and semiquantitative evaluation by HPLC. *Journal of Agricolture and Food Chemistry, 40*, 1571–1576.

Montedoro, G., Servili, M., Baldioli, M., & Miniati, E. (1992b). Simple and hydrolyzable phenolic compounds in virgin olive oil. 2. Initial characterization of the hydrolyzable fraction. *Journal of Agricolture and Food Chemistry, 40*, 1577–1580.

Montedoro, G., Servili, M., Baldioli, M., Selvaggini, R., Miniati, E., & Macchioni, A. (1993). Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoid derivates. *Journal of Agricolture and Food Chemistry, 41*, 2228–2234.

Nergiz, C., & Unal, K. (1991). Determination of phenolic acids in virgin olive oil. *Food Chemistry, 39*, 237–240.

Papadopoulos, G., & Tsimidou, M. (1992). Rapid method for the isolation of phenolic compounds from virgin olive oil using solid phase extraction. In *XV Intern. Conference of Group Polyphenols, Proceedings* II, pp. 192–196, INRA ed., Paris

Ragazzi, E., & Veronese, G. (1973). Quantitative analysis of phenolic compounds after thin layer chromatographic separation. *Journal of Chromatography A, 77*, 369–375.

Solinas, M., & Cichelli, A. (1981). Sulla determinazione dei composti fenolici degli oli d'oliva. *La Rivista della Societa' Italiana di Scienza dell'Alimentazione, 3*, 159–164.

Solinas, M., & Cichelli, A. (1982). Il dosaggio per GLC e HPLC delle sostanze fenoliche dell'olio di oliva: ruolo ipotetico del tirosolo nell'accertamento della quantità di olio vergine nelle miscele con i rettificati. *La Rivista della Societa' Italiana di Scienza dell'Alimentazione, 4*, 223–230.

Solinas, M. (1987). HRGC analysis of phenolic components in virgin olive oils in relation to the ripening and the variety of olives. *La Rivista Italiana delle Sostanze Grasse, 64*, 255–262.

Tsimidou, M., Papadopoulos, G., & Boskou, D. (1992). Determination of phenolic compounds in virgin olive oil by reversed-phase HPLC with emphasis on UV detection. *Food Chemistry, 45*, 141–144.

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

Vazquez Roncero, A., Janer Del Valle, C., & Janer Del Valle, L. (1973). Determinaciòn de los polifenoles totales del aceite de oliva. *Grasas y Aceites, 24*, 350–355.

Vazquez Roncero, A., Janer Del Valle, C., & Janer Del Valle, L. (1976). Componentes fenòlicos de la aceituna.III Polifenoles del aceite. *Grasas y Aceites, 27*, 185–191.