Romina Paula Monasterio[1,2]
María de los Angeles Fernández[1]
María Fernanda Silva[1]

[1]Instituto de Biología Agrícola de Mendoza (IBAM-CONICET), Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina
[2]Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa, Santa Rosa, La Pampa Province, Argentina

Research Article

High-throughput determination of phenolic compounds in virgin olive oil using dispersive liquid-liquid microextraction-capillary zone electrophoresis

This article reports a simple methodology using dispersive liquid–liquid microextraction combined with CZE. It has been applied for the simultaneous determination of phenolic compounds such as caffeic, gallic, vanillic, syringic, cinnamic, *p*-coumaric acids and oleuropein, apigenin, luteolin, 3-hydroxytyrosol, and tyrosol, in virgin olive oil (VOO). The optimized extraction conditions for 20 g of VOO were: extractant solvent: 400 μL boric acid 30 mM at pH 9.5; dispersive solvent: 300 μL carbon tetrachloride; vortex: 8 min; centrifugation: 3 min. The composition of the BGE was optimized resulting in the selection of a solution made of 30 mM boric acid at pH 9.5. As a strategy for on-line preconcentration a stacking step was applied, injecting a plug of water before sample injection. The short extraction time, centrifugation and electrophoretic steps allow the selective determination of phenolic compounds in VOO with satisfactory LODs (0.004–0.251 mg/kg), recoveries (89.4–101.0%), and RSD (less than 7.44% in peak area and less than 0.69% in migration time), compatible with the concentration levels present in the samples.

1 Introduction

The beneficial effects of the consumption of virgin olive oils (VOOs) on human health are well known and are related to the characteristic fatty acid composition, and the presence of minor components, such as phenolic compounds (antioxidant properties) [1, 2]. VOO is a juice obtained by exclusively mechanical means (pressing) from the fruit of the olive tree (*Olea europaea L.*). This is one of the few oil types not requiring refining, but merely washing, filtration, decantation, or centrifugation, prior to consumption [3]. Chemically, olive oil consists mainly of triacylglycerols, which accounts for more than 98% of its total weight. In addition, it contains about 2% of other, nearly 250 minor components including aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile, and phenolic compounds [4–6]. Within phenolic compounds present in VOO there are different groups, including derivatives of

benzoic acid (e.g. vanillic acid (VAN)) and cinnamic (e.g. caffeic (CAF) and *p*-coumaric acids (*p*-COU)) acids, phenyl ethyl alcohol such as tyrosol (TY) and hydroxytyrosol (HTY), flavones like apigenin (API) and luteolin (LUT), lignans such as (+)-pinoresinol and (+)-1-acetoxypinoresinol, and secoiridoids including oleuropein (OLE) and ligstroside derivatives [7, 8]. The amounts and composition of these compounds in VOO depends on several factors such as olive cultivar, degree of maturation, and agronomic and technological aspects of production [8, 9]. These compounds extend olive oil's shelf life by delaying oxidation reactions and improve some sensory properties including pungency, astringency, bitterness, and flavor. The content of phenolic compounds is an important factor to be considered when evaluating the quality of VOO [9].

Although HPLC has been used extensively in the analysis of phenolic compounds in different food samples, these methods are hampered mainly by long run times and substantial consumption of mobile phases and hence organic solvents. However, these disadvantages associated with HPLC can be struggled with ultra-high performance liquid chromatography, where flow rates of mobile phases and analysis times are vastly reduced. Another possible alternative to LC is CE. In this sense, CE has proven to be a fast, valid, and reliable tool for food analysis, such as olive oil [10–12]. It is a powerful technique that affords high-resolution separations (10^4–10^6 theoretical plates) while requiring only minute volumes of

Abbreviations: API, apigenin; **CAF**, caffeic acid; **COU**, *p*-coumaric acid; **DLLME**, dispersive liquid–liquid microextraction; **EF**, enrichment factor; **GAL**, gallic acid; **HTY**, hydroxytyrosol; **LUT**, luteolin; **OLE**, oleuropein; **Sy**, siringic acid; **TY**, tyrosol; **VAN**, vanillic acid; **VOO**, virgin olive oil

Colour Online: See the article online to view Fig. 4 in colour.

sample and BGE. Furthermore, a wide range of compounds is amenable to separations by CE.

CE has been employed to characterize, analyze, and quantify phenolic compounds in olive oil using a wide array of methods and strategies. Some NACE methods coupled with detector such as UV-DAD have been used for phenolic compounds present in olive oil [13–15]. Recently, an interest NACE methodology for determination of phenolic compounds combined with multivariate curve resolution alternating least squares algorithm was presented. The simultaneous analysis of phenolic compounds with overlapped time profiles with the olive oil matrix interferents and between them was achieved [13]. Nevertheless, good resolution between peaks, particularly between the isomers (for example *o-*, *m-*, and *p-* of coumaric acid), which exhibit an identical *m/z* value for their [M−H]− ion (163) is mandatory [16]. However, CZE is the most employed mode for the analysis of phenolic compounds in olive oil.

A number of extraction techniques, involving liquid–liquid extraction and/or SPE have been used for separation and preconcentration of phenolic compounds in VOO. However, some disadvantages arise from the application of these techniques, such as large volumes of toxic and expensive solvents, high amount of wastes, and reduced frequency of analysis. In fact, several microextraction techniques (dispersive liquid–liquid microextraction (DLLME); liquid-phase microextraction, single drop microextraction, etc.) effectively overcome these difficulties by reducing organic solvent consumption as well as allowing sample extraction and preconcentration to be performed in a simple and single step. DLLME, first introduced in 2006, is based on a ternary solvent system in which a dispersive solvent allows for the dispersion of an extraction solvent into the sample [17]. The dispersing solvent must be fully miscible with both the sample and the extraction phase. The extraction solvent must be miscible with the dispersing phase but insoluble in sample and must have a higher density. Extraction equilibrium is quickly achieved, due to the extensive surface contact between the droplets of the extraction solvent and the sample [18, 19]. Within the advantages of this technique are the low cost of common solvents, the use of simple equipments, high recoveries and enrichment factors, fast analysis, simplicity of operation, and low sample volume [17, 20].

Although DLLME, liquid-phase microextraction, and single drop microextraction techniques received favorable responses, the applicability of these techniques for oil-based samples is less explored [21]. DLLME coupled to CZE has been used for determination of phenolic compounds in aqueous cosmetics, using room temperature ionic liquid to extract and back-extracted into the alkaline aqueous phase [20]. Taking into account that phenolic compounds are unstable and highly valuable analytes, the possibility of performing fast, simple, low cost, and robust extractions from complex matrices is of upmost importance. Recently, some interesting approaches have been presented dealing with the extraction of phenolic compounds from olive oil using liquid-liquid microextraction (LLME) [21, 22].

Occasionally, these extraction techniques are combined with other enhancement techniques to obtain satisfactory sensitivity. Using CE, a simple on-line preconcentration procedure based on the differences in velocity of the analytes between the sample zone and the BGE, named stacking is a reliable technique for enhancing sensitivity. It can be carried out by injecting a sample with conductivity lower than BGE or by injecting a plug of a solution with conductivity lower than both the sample and BGE before of sample injection [23, 24]. This conductivity difference will generate a stacking of analytes, improving the resolution and LOD in a simple step [25, 26].

The aim of this work was to develop a rapid CE methodology suitable for detection and quantification of phenolic compounds in VOO. The extraction and preconcentration step was developed by DLLME and, followed with a simple stacking procedure that allows to increase the resolution using a separation with CZE. This DLLME-CZE method allows a simple and highly selective determination of phenolic compounds in VOO. The effect of experimental parameters on DLLME efficiency such as, type and volume of extractant and dispersant solvents, and extraction and centrifugation time were studied and optimized. The proposed method was particularly useful for obtaining information about phenolic profile in monovarietals VOO samples.

2 Materials and methods

2.1 Reagents and samples

Caffeic (CAF), gallic (GAL), vanillic (VAN) ≥97.0% (Fluka, Buchs, Switzerland), syringic (Sy) ≥95% (Sigma-Aldrich, Milwaukee, WI, USA), *p*-coumaric (COU) ≥98.0% (Sigma-Aldrich), cinnamic (CIN) 99% (Sigma-Aldrich) acids and oleuropein (OLE) ≥80% (Sigma-Aldrich), apigenin (API) ≥95.0% (Sigma-Aldrich), luteolin (LUT) (Fluka), 3-hydroxytyrosol (HTY), and 2-(4-hydrxyphenyl)ethanol (tyrosol, TY), ≥99.5% (Fluka) were used. The phenolic compounds stock solutions were prepared by dissolving an appropriate amount of the compound in methanol (Merck, Darmstadt, Germany). All the stock solutions were kept away from light and stored at 4°C in amber-colored glass bottles. Boric acid (JT Baker, Xalostoc, Mexico) was prepared weighting appropriate amount and the desired pH was set by adding sodium hydroxide (Sigma-Aldrich) and made up to their final concentration. Carbon tetrachloride (Merck) was used as dispersant solvent. All the other chemicals were analytical-reagent grade and they were used without further purification. Ultrapure water (18.3 MΩcm) from a Milli-Q system (Millipore, Paris, France) was used for preparing all solutions.

All the buffers and the samples were submitted to ultrasonic treatment for 5 min and filtered through 0.45 mm before being introduced to the electrophoretic system.

2.2 Instrumentation and conditions

CE analysis of all standards and VOO samples was performed on a Capel 105 system (Lumex, St Petersburg, Russia) equipped with an UV detector and a 0–25 kV high-voltage power supply. The data were collected on a PC configured with Elforun software version 3.2.2. The capillary columns used for separation were bare fused-silica capillaries with 75 μm id and 60.0 cm (55 cm to the detector) from MTC MicroSolv Technology Corporation (Eatontow, USA). Samples were introduced to the capillary by pressure injection at 30 mbar for 3 s. Direct UV detection was performed at 200 nm. All operations were carried out at 30°C.

Before first use, fused-silica capillaries were washed (1000 mbar) with 0.1 M NaOH (10 min), water (5 min), and running buffer (10 min). Capillary conditioning was done every morning rinsing at 1000 mbar with water for 5 min, 0.1 M NaOH for 5 min, and with BGE for 4 min. To achieve a good reproducibility between runs, the following washing protocol was applied (all using 1000 mbar): 3 min with water, 2 min with 0.1 M NaOH, and 2 min with BGE. At the end of the day, 0.1 M NaOH and water were passed through the capillary for 5 min each.

2.3 DLLME procedure

A portion of 20 g of VOO sample was transferred to a graduated centrifuge tube, 300 μL of carbon tetrachloride and 400 μL of boric acid solution 30 mM were added. The mixture was vortexed for 8 min forming a cloudy solution that was centrifuged at 2600 rpm for 3 min. After this process, the aqueous phase was observed at the bottom of conical tube while the upper oil phase was removed. The final aqueous phase ($\sim$300 μL) was directly introduced to the CE unit.

2.4 Sampling and sample preparation

VOO samples were provided for local factories including different monovarietals (Arauco, Nevadillo, Frantoio, Picual, and Arbequina). All samples were kept in their original containers at ambient temperature and they were analyzed within the first week after opening. For the optimization of the method, a pooled sample ($n = 5$) was prepared and homogenized by mixing five VOO in the same proportion of different monovarietals. These samples were left to "equilibrate" for at least for 15 min prior to DLLME extraction.

3   Results and discussion

In this study, a DLLME-CE was developed for the determination of phenolic compounds in VOOs. To improve the extraction efficiency from oils, the effects of different parameters affecting the extraction efficiency, such as type and volume
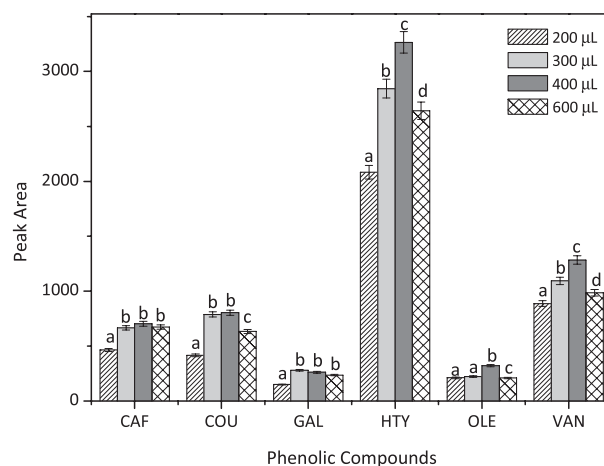

Figure 1. Evaluation of extractant solvent volume on the peak area of six representative phenolic compounds. Full conditions are shown in Section 2.

of extraction solvent, type and volume of dispersant solvent, and extraction time were studied.

The separation conditions were optimized using fortified samples with a mixture of standards in order to obtain the maximum S/N ratio and resolution between compounds.

3.1 DLLME parameters

3.1.1 Extraction and dispersant solvents

The extraction solvent is an important parameter for DLLME. Typically, in a DLLME, solvents with higher density are preferred because of quick accumulation at the bottom of the tube [17]. According to matrix characteristics and analytes chemical properties, the extraction solvent was chosen within different aqueous solutions. These solutions have higher extraction capacity of phenolic compounds and lower solubility in the oil phase. According to pka values of phenolic compounds, the addition of basic solutions will ionize the compounds, improving the mass transfer to aqueous solutions [18, 27]. Consequently, sodium hydroxide and boric acid solutions in different concentration and pH were studied as extraction solvents. A volume of 400 μL of chlorobencene was used as a generic dispersing solvent and was mixed with 600 μL of different aqueous solutions. The optimal results were achieved when 30 mM boric acid at pH 9.50 was used as extraction solvent. After optimizing the type of extraction solvent, different volumes of buffer solution were evaluated. The volume of extraction solvent is a key parameter that affects the extraction efficiency since lower volumes generally results in high extraction efficiency. On the other hand, insufficient volume of extractant might lead to incomplete recovery of the analytes. Figure 1 shows the responses obtained for four different volumes of boric acid solutions, from 200 to 600 μL. Extractant volumes lower than 400 μL were not suitable due to nonquantitatively extraction. Measured peak areas

decreased when the volume of extractant was 600 μL due to dilution effect. Therefore, 400 μL of boric acid 30 mM pH 9.50 was chosen for all subsequent experiments.

In DLLME, the volume of dispersant must be high enough to provide an emulsion of extractant droplets in the oil phase. Larger volumes result in lower extraction yields due to an increase in the solubility of the analytes. Dispersant solvent should be chosen according to the miscibility properties of the extraction solvent and oil sample [18]. Different solvents, including hexane (C_6H_{14}, solubility in water (g/100 g): 0.014, polarity index (P′): 0.0), 1-butanol ($C_4H_{10}O$, solubility in water (g/100 g): 6.3, P′: 4), chlorobenzene (C_6H_5Cl, solubility in water (g/100g): 0.05, P′: 2.7), and carbon tetrachloride (CCl_4, solubility in water: 0.08, P′: 1.6), were assessed. The volume was also studied within the range 100–400 μL, obtaining the best results when 300 μL of carbon tetrachloride was used as dispersant agent. By using solvents with low polarity, like hexane, the emulsion was not observed. Nevertheless, using solvents with polarities higher than hexane, such as 1-butanol or chlorobenzene, the emulsion was observed but the extraction yield was lower. It could be due for the relatively high polarity of the mentioned solvents, reducing the interaction with oil phase. When the organic solvent has an intermediate polarity, such as carbon tetrachloride, the interaction with both phases improves the extraction of phenolic compounds.

Different VOO sample masses were investigated (5–30 g) with the developed DLLME to enhance the preconcentration factor. The best compromise, between sample mass, extraction, and dispersant solvent volumes and preconcentration factor, was obtained using 20 g of VOO.

3.1.2 Extraction and centrifugation time

The most important characteristic of the DLLME is the formation of microdroplets of the extraction solvent that is finely dispersed in the other phase. Consequently, the large contact surface area between both phases results in fast mass transfer process providing a fast extraction [28]. Thus, vortex time was examined at time intervals between 2.5 and 10 min ($n = 3$). As expected, the extraction time has negligible influence on the peak areas of the analytes and therefore a practical time of 8 min was selected (Fig. 2).

The effect of time centrifugation on the phase separation was examined in the range of 2 at 8 min at 2600 rpm. No noticeable improvements were observed for centrifugation times higher than 3 min. Therefore, a centrifuge time of 3 min at 2600 rpm was chosen.

3.2 Separation and BGE optimization

In this work, the electrophoretic separation was carried out using a phase extraction fortified with phenolic standards because matrix effects were observed. According to the pkas of different phenolic compounds studied (4.1 to about 10) a BGE, pH around 9 was studied. For this reason a boric acid
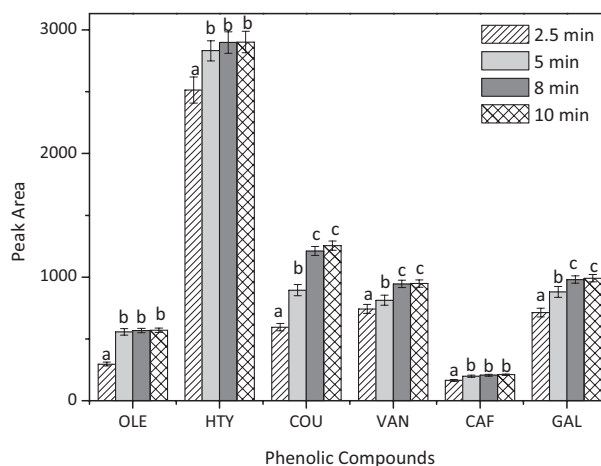

Figure 2. Evaluation of extraction time on the peak area.

solution at pH 9.5 was chosen as the BGE. At this pH, the analytes are in anionic form and are almost fully dissociated. Other BGE tested was a sodium tetraborate buffer, but it did not showed satisfactory resolution. The boric acid buffer concentration was studied within the range: 10–50 mM. The optimized BGE was composed of 30 mM boric acid buffer, exhibiting a linear relationship between current and applied voltage (0–25 kV) according to Ohm's law [23]. Using buffer concentrations above 30 mM the current is increased a 60%, but EOF decreased, increasing the analysis time, peak dispersion, and affecting the reproducibility. This BGE presented satisfactory results in relation to the analysis time, peak shape, resolution, and electric current, suitable for the separation of phenolic compounds.

Following DLLME, a simple stacking prior to separation by CZE was evaluated. In a first step, a diluted boric acid solution was tested. In this opportunity the extraction decreased when the solvent extraction was diluted. For this reason, the stacking was performed by injecting a water plug prior to sample injection (pressure injection at 30 mbar). Using this water plug improvements in resolution (Fig. 3) and theoretical plates were observed, principally for TY and OLE. Although significant increases in enhancement factors were not observed, considering the simplicity of the procedure, it is a good alternative to achieve TY determination. Different times of water plug injection were studied obtaining the best results with 7 s of injection. Figure 3 shows the electropherograms of a VOO sample, with and without applying the stacking procedure. In fact, the electropherogram shows some peaks with a little of fronting or tailing. However, considering the complexity of samples and the simplified extraction technique, the methodology could be considered as a good alternative to evaluate phenolic compounds in olive oil.

3.3 Analytical performance

The efficiency of DLLME is usually evaluated on the basis of achieved enrichment factors (EFs = Ce/Cs, being Ce and
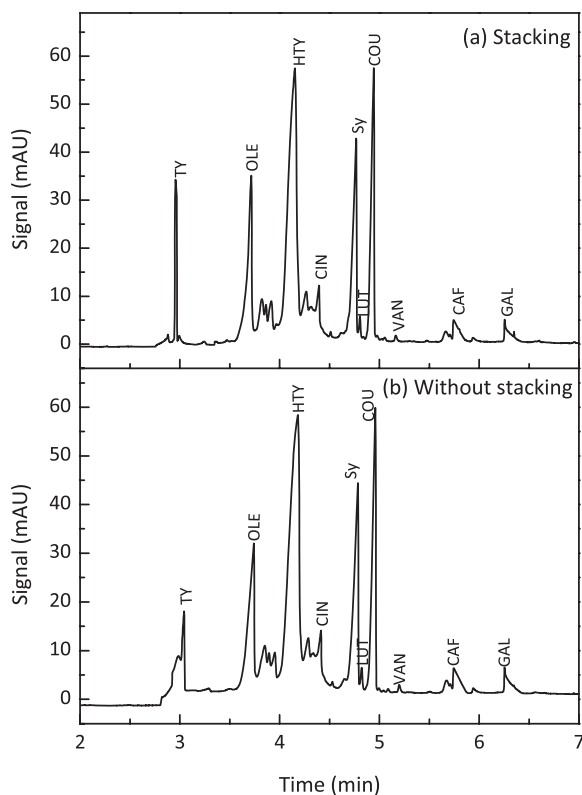
Figure 3. Electropherograms profile of VOO spiked samples (2.5 mg/kg), (A) applied stacking procedure and (B) without stacking procedure.

Cs the concentrations of the analytes in the extractant phase and those added to the sample, respectively) and/or extraction efficiencies (EEs = EFs × (Ve/Vs) × 100, with Ve and Vs as the volumes of extract and sample) [17, 18]. Abovementioned approaches require determining the values of Ce and to measure Ve. In this case, phenolic compounds are not measured directly in the matrix (this procedure require other experimental conditions). For this reason EF was not calculated. Nevertheless, signal-enhancement factors for the stacking procedure was calculated, obtaining values between 2.26 and 1.20.

The optimized method was characterized in terms of linear response range, precision, and accuracy. Linearity was

investigated with a sample pool (Arauco, Arbequina, Frantoio, Nevadillo, Manzanilla, and Picual in the same proportion). Aliquots (20 g) of this pool samples were spiked at eight different levels from 0.1 to 15 mg/kg. Within this interval, the plots of responses (peak areas) versus concentration fitted a linear model, with determination coefficients (r^2) comprised between 0.9874 and 0.9955. The RSD resulting from the analysis of six replicates of 20 g of sample pool containing 2.5 mg/kg phenolic compounds was less than 7.44% in peak area and less than 0.69% in migration time. The LODs of the proposed methodology, defined for a S/N of 3, were estimated from S/N values of target species in low level (0.1 mg/kg) spiked samples, were between 0.004–0.251 mg/kg. The noise was evaluated in the electropherogram regions (ca. 0.5 min) before and after each peak. The LOQs of the method, defined for a S/N of 10, were comprised between 0.013 and 0.837 mg/g (Table 1). The calibration graphs and correlation coefficients showed satisfactory values for the different studied phenolic compounds (see Table 1). Recovery values obtained at two concentration levels (0.5 and 2.5 µg/kg) of each phenolic compound varied between 89.4 and 101.0% (see Table 2). Furthermore, the throughput was at least 5.4 samples per hour. Finally, a comparative study on analytical performance allows us to show the strengths of the proposed methodology with respect to others already reported in the literature (Table 1). The proposed method presents lower LOD than those methods developed for phenolic compounds in olive oil samples with the least consume of organic solvents and a high throughput.

3.4 Application to monovarietal VOO samples

The optimized method was used to investigate the levels of phenolic compounds in VOOs (Fig. 4). Table 3 summarizes the concentrations of phenolic compounds measured in each VOO samples (monovarietals). TY, OLE, HTY, LUT, COU, and GAL were found in all samples, with maximum values above 11 mg/kg (11.789 mg//kg to OLE in Frantoio sample). As can be seen in Fig. 4, HTY is not completely separated in some of the analyzed samples. However, quantification of this compound was possible because recovery studies validated its determination even for Arauco, Nevadillo, and Picual

Table 1. Performance data of the proposed method compared to others previously reported for phenolic compounds determination in olive oil

Method	Throughput (h^{-1})	Organic solvent[a] (mL)	Sample consumption (g)	Analyte number	LOD (mg/kg)	RSD (%)	Calibration range (mg/kg)	Reference
SPE-CZE	n.r.[b]	100	60	7	0.032 – 0.824	3.37 – 4.93	0.108 – 6500	[29]
In-vial LLME-CZE	5	2.6	20	14	0.017 – 0.16	3.72 – 7.66	0.1 – 30	[21]
SPE-NACE-ESI-TOF MS	n.r.[b]	115	60	16	n.r.[b]	1.01 – 2.03	n.r.[b]	[30]
DLLME-CZE	5.45	0.3	20	11	0.004 – 0.251	1.42 – 7.44	0.1 – 15	This methodology

LLME, liquid-liquid microextraction.
a) Organic solvent consumption for each sample.
b) Non reported.

Table 2. Relative recoveries (%) for spiked VOO samples, $n = 3$ replicates

Sample	Added concentration (mg/kg)	Recovery (%)[a]										
		TY	OLE	API	HTY	CIN	Sy	LUT	COU	VAN	CAF	GAL
Arbequina	0.5	91.6	93.6	93.6	91.6	92.5	102	95.7	96.3	97.5	99.6	89.4
	2.5	99.7	101.0	94.6	98.5	97.2	99.9	98.1	96.2	98.4	100.4	99.2
Picual	0.5	99.3	89.9	99.5	94.6	89.5	94.6	100.1	99.1	93.6	99.1	94.7
	2.5	100.1	98.5	100.5	95.2	96.7	100.1	95.3	100.3	99.4	99.9	99.6
Frantoio	0.5	92.4	97.6	95.3	98.2	93.5	90.6	94.5	93.8	94.7	99.8	95.3
	2.5	100.4	96.4	98.8	99.4	92.4	99.6	93.0	95.9	95.9	100.4	96.6
Arauco	0.5	88.8	93.7	96.5	99.8	89.9	100.4	98.6	97.2	95.6	98.7	96.9
	2.5	99.4	95.1	98.7	100.5	101.5	99.9	100.9	99.8	100.9	96.2	90.8
Nevadillo	0.5	96.4	89.9	96.3	99.4	95.3	95.7	96.8	100.5	94.3	99.5	90.5
	2.5	99.3	95.3	99.6	100.3	99.6	100.4	98.5	99.7	97.5	100.1	98.5

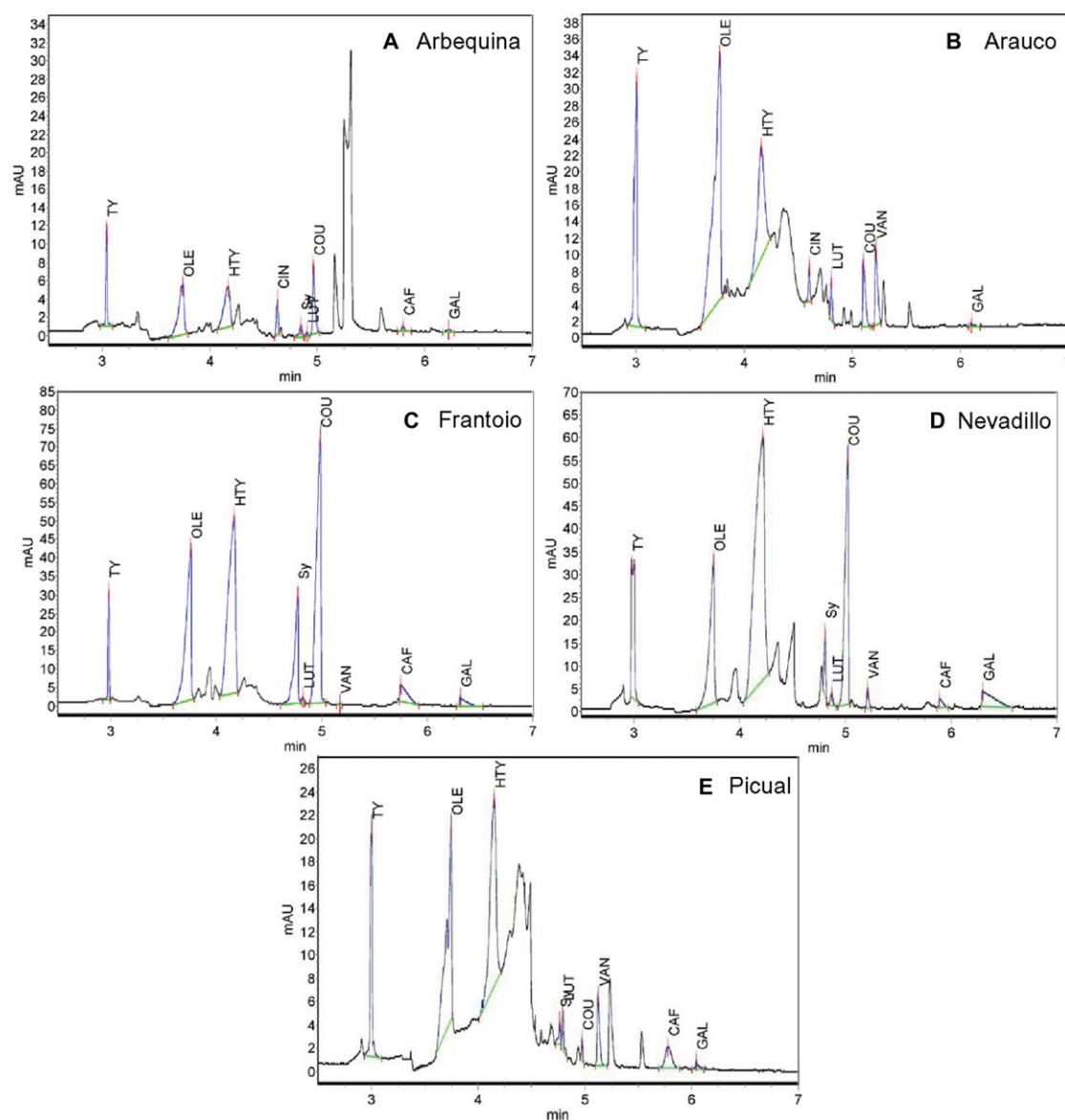a) Recovery (%) = 100 × [(found − initial)/added].

Figure 4. Phenolic compounds analysis in VOO sample studied with DLLME-CZE: (A) Arbequina sample; (B) Arauco sample; (C) Frantoio sample; (D) Nevadillo sample; (E) Picual sample.

Table 3. Determination of phenolic compounds in monovarietal VOO samples (95% confidence interval; $n = 5$)

Phenolic compounds	LOD (mg/kg)	LOQ (mg/kg)	Samples (mg/kg)				
			Arbequina	Picual	Frantoio	Arauco	Nevadillo
TY	0.004	0.013	0.407 ± 0.004	1.185 ± 0.005	1.076 ± 0.030	2.275 ± 0.043	2.874 ± 0.003
OLE	0.012	0.041	1.651 ± 0.073	5.147 ± 0.582	11.789 ± 0.275	10.632 ± 0.128	8.256 ± 0.214
API	0.004	0.015	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
HTY	0.006	0.022	0.136 ± 0.003	0.456 ± 0.002	1.568 ± 0.038	0.406 ± 0.017	2.355 ± 0.044
CIN	0.253	0.843	1.625 ± 0.112	n.d.[a]	n.d.[a]	10.593 ± 0.505	n.d.[a]
Sy	0.009	0.032	n.q.[b]	n.q.[b]	0.743 ± 0.013	n.d.[a]	0.150 ± 0.001
LUT	0.251	0.837	0.869 ± 0.237	4.939 ± 0.492	3.381 ± 0.998	6.821 ± 0.086	6.106 ± 0.128
COU	0.005	0.018	0.171 ± 0.023	0.023 ± 0.001	2.932 ± 0.079	0.175 ± 0.004	1.682 ± 0.033
VAN	0.029	0.096	n.d.[a]	1.038 ± 0.051	n.q.[b]	1.844 ± 0.048	0.695 ± 0.021
CAF	0.005	0.017	0.019 ± 0.005	0.114 ± 0.006	0.299 ± 0.004	n.d.[a]	0.079 ± 0.002
GAL	0.015	0.050	n.q.[b]	n.q.[b]	0.294 ± 0.018	n.q.[b]	0.839 ± 0.052

a) Not detected.
b) Under quantification limit.

samples (see Table 2). On the other hand, the sum of phenolic compounds concentration was the highest in Arauco samples (32.746 mg/kg). API was not detected in any sample, while Sy, VAN, and CAF were detected in four samples (Sy and CAF: Nevadillo, Frantoio, Arbequina, and Picual; VAN: Nevadillo, Frantoio, Arauco, and Picual) and cinnamic acid only was detected in two samples (Arbequina and Arauco). However, some of the reported concentrations are between LODs and LOQs, for example Arbequina and Picual for Sy and GAL (more details in Table 3). Nine of the eleven studied phenolic compounds were found (Nevadillo, Picual, Frantoio, and Nevadillo) in four of the five analyzed samples.

4   Concluding remarks

In the present work, a DLLME-CZE methodology for the determination of 11 phenolic compounds in the complex matrix of VOO was developed for the first time. The proposed DLLME offers significant advantages principally, the high throughput compared with current methods that use large volumes of organic solvents, tedious clean-up, large evaporation step (that may affect the stability of the analytes). The methodology allows the selective determination of phenolic compounds in VOOs with satisfactory sensitivities, recoveries, and RSDs, compatible with levels present in olive oil samples. The developed method allows a fast analysis, low consumption of reagents, minimum generation of residues, and, consequently, reduced costs, without causing negative environmental impacts. The new approach may help for the classification of VOOs according to varietal origin, studying an important number of samples and using chemometric tools.

The authors have declared no conflict of interest.

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