Olive Oil by Capillary Electrophoresis: Characterization and Genuineness

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ABSTRACT: Olive oil, obtained from *Olea europaea* L. (Oleaceae) fruits, is an important ingredient in the Mediterranean diet. The purpose of this paper is to review and evaluate olive oil analysis using capillary electrophoresis (CE). This review covers a selection of the literature published on this topic over the past decade. The current state of the art of the topic is evaluated, with special emphasis on separation conditions, analysis purpose, and analytes investigated. CE has been used to characterize or to carry out authenticity studies. Particular attention has been focused on the botanical origin because high-quality monovarietal olive oils have been recently introduced on the markets and their quality control requires the development of new and powerful analytical tools as well as new regulations to avoid fraud. CE represents a good compromise between sample throughput, sample volume, satisfactory characterization, and sustainability for the analysis of target compounds present in olive oils.

KEYWORDS: *olive oil, capillary electrophoresis, characterization, adulterant, classification*

■ INTRODUCTION

Olive (*Olea europaea*) is one of the oldest agricultural tree crops with remarkable cultural and economic importance. Its history goes back almost 8000 years to when the first trees were cultivated. Virgin olive oil (VOO) is obtained from mechanical pressing of ripe olive fruits without any further refining process.[1,2] It represents a typical lipid source of the Mediterranean diet and is frequently associated with a low incidence of cardiovascular diseases as well as with antioxidant properties.[3] These benefits have been associated with its well-balanced fatty acid composition, of which oleic acid is the main componentm and to the presence of minor biomolecules, such as vitamins, carotenoids, tocopherols, phenolic compounds, and other natural antioxidants, which may act, by different mechanisms, as an effective defense against reactive species.[4−6]

Minor components could play a major role in the health effects of olive oil, including the prevention of chronic diseases such as cancer, obesity, diabetes, or coronary diseases. Alternatively, these compounds also affect the sensory properties and oxidative stability of olive oil.[7,8]

According to the definitions of the International Olive Oil Council (IOOC), VOOs are obtained from the fruit of the olive tree exclusively by mechanical or other physical conditions, principally thermal conditions (cold-pressing), which do not lead to alterations in the oil. The fruits cannot undergo any treatment other than washing, decantation, centrifugation, and filtration. However, the IOOC established different types of VOO: (i) "*extra-virgin olive oil*", VOO that has a free acidity, expressed as oleic acid, of not more than 0.8 g per 100 g; (ii) "*virgin olive oil*", VOO that has a free acidity, expressed as oleic acid, of not more than 2 g per 100 g; (iii) "*ordinary virgin olive oil*", VOO that has a free acidity, expressed as oleic acid, of not more than 3.3 g per 100 g.[9]

Actually, there has been an increasing focus on the geographical origin of raw materials and finished products, for several reasons including specific sensory properties.[8] Nevertheless, numerous designations are utilized for quality control and are recognized by European Council Regulation: protected designation of origin (PDO), protected geographical indication (PGI), and traditional specialty guaranteed (TSG). These designations are very effective because the origin and typical area of a food product denote both health and safety.[9,10]

In recent years, there has been a growing interest among consumers in the safety and traceability of food products. To guarantee quality and to protect consumers from commercial fraud, there is an increasing need for analytical tools capable of verifying whether a product, sold with a specific label, is actually compatible with that claim or not.[11] A classical chemical analysis of oil, including fatty acids, sterols, and triterpenic alcohols, followed by chemometric analysis allows the accurate classification of the geographical origin of olive oils.[12] The development of methodologies for the determination of characteristic compounds in VOO (phenolic compounds, fatty acids, etc.) has been discussed by several authors.[10,13,14] Separation and determination of different compounds in extracts obtained from VOO by solid phase extraction (SPE) or liquid−liquid extraction (LLE) have been carried out by high-performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrophoresis (CE), coupled to different detectors, such as UV,[15−17] electrochemical,[18] fluorescence,[19] flame ionization detector (FID),[5] and mass spectrometry (MS).[20−22] Recently, the introduction of rapid-resolution liquid chromatography (RRLC) and ultraperformance liquid chromatography (UPLC) allowed improvement of the chromatographic performance.[23]
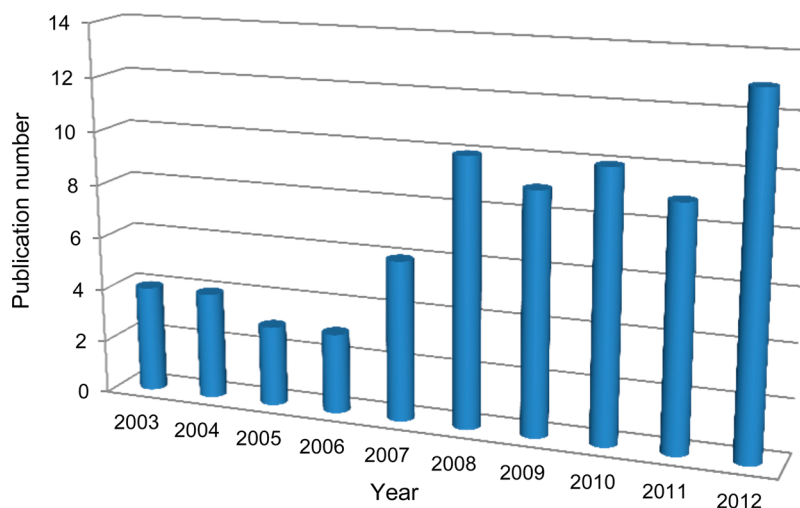
Figure 1. Number of publications appearing annually in the field of olive oil by CE (source: www.scopus.com, using olive oil, capillary electrophoresis, and chemistry as keywords).

On the other hand, spectroscopic techniques, such as infrared and UV−vis spectroscopy, have been successfully used for the analysis of olive oil. Although these techniques give nonselective fingerprints of oil samples, by means of multivariate data analysis, useful information for authentication studies can be achieved.[10] Mid-infrared (MIR),[10,24,25] near-infrared (NIR),[10,26] nuclear magnetic resonance (NMR),[27,28] and UV−vis spectroscopy[10,29] have been successfully applied to determine geographical and varietal origins of olive oil.

Due to recent advances in DNA-based methods, some approaches have been proposed to characterize and authenticate DNA extracted from olive oil with the inherent potential to facilitate assessment of origin and varietal conformity.[30,31] These DNA-based methodologies use molecular markers that can lead to the identification of the variety from which it was extracted.[30] The content of compounds such as fatty acids, triacylglycerols, volatile compounds, and tocopherols (TOHs) is able to differentiate the geographical origin of oils, but it is affected by the environmental conditions of plant growth, the DNA not being environmentally labile.[32−34]

CE is a versatile, simple, rapid, and low-cost technique. Moreover, it usually provides short separation times and high separation efficiency, and it has become one of the major choices for the separation of charged analytes and, using the different CE modes, it is also used for the separation of uncharged analytes.[17,35] CE presents a good compromise between analysis time and satisfactory characterization of compounds in olive oil. The speed, resolution, and simplicity of CE, combined with its low operational cost and small residue generation, make this technique an attractive option for the development of analytical methods for food analysis.[8,36,37]

The aim of the present review is to compile the publications concerning the use of CE methods that have been utilized to determine diverse analytes in olive oil during the past decade, covering a selection of 73 contributions. In this review separation conditions, analysis purpose, and analytes investigated are discussed.

■ ANALYTES STUDIED BY CE IN OLIVE OIL

Over the past 11 years, a number of interesting papers describing the separation of compounds in olive oil by CE have been reported. As can be seen in Figure 1, the analysis of olive oil by capillary electrophoresis represents an area of research of growing interest.

Tables 1 and 2 show the most important analytes studied in olive oil by CE, together with the analysis proposed and principal chemical properties.

Phenolic Compounds. Phenolic compounds are secondary plant metabolites, which are important determinants in the sensory and nutritional quality of fruits, vegetables, and other plants. These compounds are very important in olive oil chemical composition. The major phenolic compounds identified and quantified in olive oil belong to five different classes: simple phenols (hydroxytyrosol (HYTY), tyrosol (TY)), secoiridoids (oleuropein (OLE), ligstroside (Lig), and their hydrolysis derivatives), lignans ((+)-pinoresinol (Pin) and (+)-acetoxypinoresinol (Ac Pin)), flavonoids (luteolin (LUT) and apigenin (API)), and phenolic acids (coumaric acid (COU), vanillic acid (VAN), etc.). The phenolic content of VOO is influenced by olive variety, location, environmental conditions, and degree of ripeness and also by the oil extraction procedure utilized.[37,38] Consequently, the content of phenolic compounds is an important factor to be considered when evaluating the quality of VOO. These compounds have been the subject of considerable interest, both because of their chemoprotective effect for human beings and because they are a major factor in the high stability of olive oils. Phenolic compounds also contribute to the organoleptic properties of VOOs (commonly described as bitterness and pungency).[39,40] Phenolic compounds, together with other natural antioxidants (carotenoids and TOHs), have been studied by capillary zone electrophoresis−UV (CZE-UV) with good results.[13,36,41−43] CZE separation is based on different migration of charged solutes (caused by the differences in their charge to mass ratio) in a conductive liquid placed in a capillary under the influence of a high-voltage electric field.[44] The separation of phenolic compounds by CZE is due to ionizable groups present in a high number of phenolic compounds. These groups, according to their pK_a values (Table 1), are predominantly in their ionized form at pH values >7 and then can migrate separately toward the cathode. On the other hand, phenolic compounds are polar, allowing the application of the typical background electrolytes (BGE) used for CZE. As can be seen in Table 1, there is no need to derivatize the phenolic compounds because they are

Table 1. Analytes Studied in Olive Oil by CE

Compound	Chemical structure	Origin	pKa$_1$	pKa$_2$
Phenolic compounds				
Gentisic acid (GEN) (MW: 154.12)		Olive	3.0	11.0
Hydroxyphenil acetic acid (HFA) (MW: 152.14)		Olive	4.5	9.2
Vanillic acid (VAN) (MW: 168.14)		Olive	4.4	9.3
Ferulic acid (FER) (MW: 194.18)		Olive	4.0	10.2
o-Coumaric acid (o-COU) (MW: 164.15)		Olive	3.9	10.6
m-Coumaric acid (m-COU) (MW: 164.15)		Olive	3.8	10.1
p-Coumaric acid (p-COU) (MW: 164.15)		Olive	4.1	10.2
Decarboxymethyl oleuropein aglycon (DOA) (MW: 300.35)		Olive	n.a.[a]	n.a.[a]
Hydroxytyrosol (HYTY) (MW:154.16)		Olive	9.5	-
Tyrosol (TY) (MW: 138.16)		Olive	9.6	-
Pinoresinol (Pin) (MW: 358.38)		Olive	9.54	-

Table 1. continued

Compound	Chemical structure	Origin	pKa$_1$	pKa$_2$
Phenolic compounds				
Acetoxypinoresinol (Ac Pin) (MW: 416.42)		Olive	n.a.[a]	n.a.[a]
Oleuropein aglycon (OA) (MW: 380.38)		Olive	n.a.[a]	n.a.[a]
Oleuropein (OLE) (MW: 540.52)		Olive	9.70	-
Ligstroside aglycon (Lig Agl) (MW:362.37)		Olive	n.a.[a]	n.a.[a]
Elenolic acid (EA) (MW: 242.22)		Olive	n.a.[a]	n.a.[a]
Caffeic acid (CAF) (MW: 180.16)		Olive	4.43	-
Gallic acid (GAL) (MW: 170.12)		Olive	4.5	10

Table 1. continued
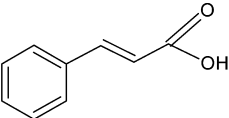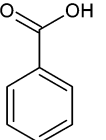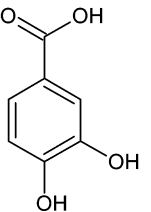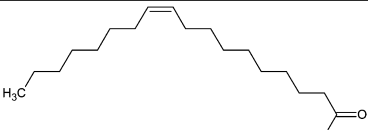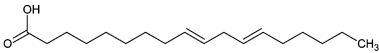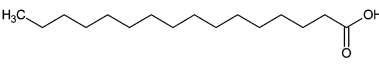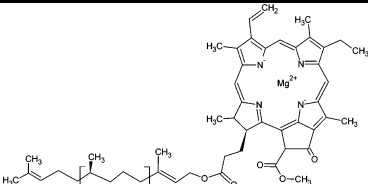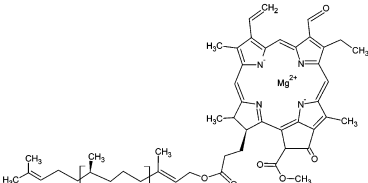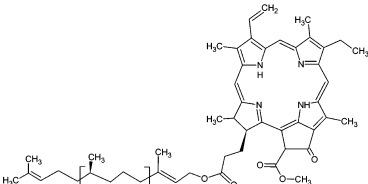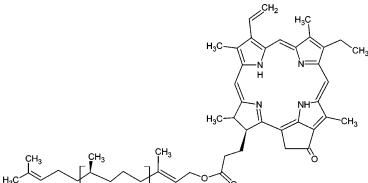
Compound	Chemical structure	Origin	pKa$_1$	pKa$_2$
Phenolic compounds				
Cinnamic acid (CIN) (MW: 148.16)		Olive	4.44	-
Benzoic acid (BEA) (MW: 122.12)		Olive	4.21	-
Protocatequic acid (ProCAA) (MW: 154.12)		Olive	4.48	-
Fatty acid				
Oleic acid (MW: 282.46)		Olive	5.02	-
Linoleic acid (MW: 280.45)		Olive	4.78	-
Palmitic acid (MW: 256.42)		Olive	4.78	-
Chlorophylls				
Chlorophyll a (MW: 893.48)		Olive	4.19	-
Chlorophyll b (MW: 907.47)		Olive	4.19	-
Pheophythin a (MW: 885.18)		Olive	n.a. [a]	-
Pyropheophytin (MW: 811.14)		Olive	4.63	-

Table 1. continued
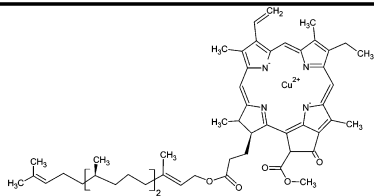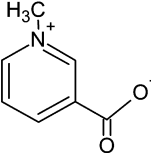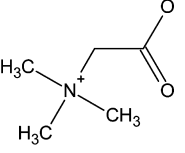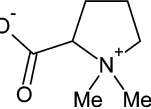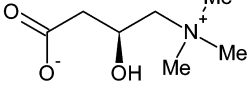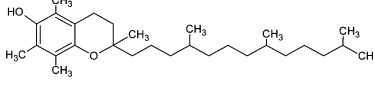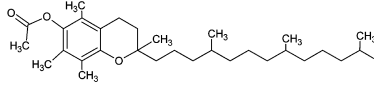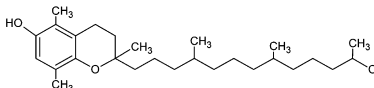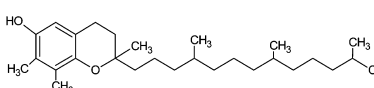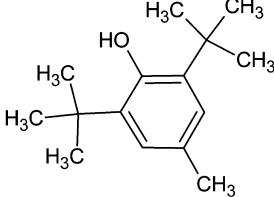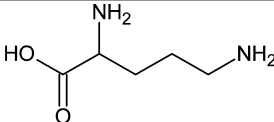
Compound	Chemical structure	Origin	pKa$_1$	pKa$_2$
Chlorophylls				
Copper chlorophyll derivatives (MW: 932.72)		Adulterant	n.a.[a]	-
Betaines				
Trigonelline (MW: 137.13)		Adulterant	1.86	-
Glycine betaine (MW: 118.15)		Adulterant	1.83	-
Proline betaine (MW: 143.19)		Adulterant	2.26	-
Carnitine (MW: 161.19)		Adulterant	3.8	-
Tocopherols and synthetics antioxidant compounds				
α-Tocopherol (α-TOH) (MW: 430.70)		Olive	13.1	-
α-Tocopherol acetate (α-TOH-ac) (MW: 416.67)		Olive	n.a.[a]	n.a.[a]
β-Tocopherol (β-TOH) (MW: 416.67)		Olive	12.8	-
γ-Tocopherol (γ-TOH) (MW: 416.67)		Olive	12.7	-
Butylated hydroxytoluene (BHT) (MW: 220.35)		Adulterant	12.75	-
Proteins and amino acids				
Ornithine (ORN) (MW: 132.16)		Olive	1.94	8.65

Table 1. continued

Compound	Chemical structure	Origin	pKa$_1$	pKa$_2$
Proteins and amino acids				
β-Alanine (β-ALA) (MW: 89.09)		Olive	3.60	10.19
γ-Aminobutyric acid (GABA) (MW: 103.11)		Olive	4.23	10.43
Alloisoleucine (AILEU) (MW: 131.17)		Olive	2.36	9.68
Citrulline (CIT) (MW: 175.18)		Olive	2.43	9.41
Pyroglutamic acid (PGLU) (MW: 129.11)		Olive	3.48	12.76

aromatic and, therefore, they show a strong absorption in the UV region. However, it has to be pointed out that a sample treatment step should be carried out before aqueous CZE. More information concerning these procedures is mentioned under Procedures Employed in Previous CE Separation.

The analysis of phenolic compounds in extra-VOO by CZE coupled with electrospray ionization—time of flight—MS (ESI-TOF-MS) has also been reported.[45] In addition, the coupling of CE to MS detection allows structural analysis of newly discovered compounds. Other interesting CE-MS work used semipreparative HPLC as a first dimension of separation to isolate phenolic fractions and CE coupled to TOF-MS as a second dimension, to analyze the composition of the isolated fractions. This method provides enough resolving power to separate hundreds of compounds from highly complex samples.[46] Of all the HPLC and CE detection methods reported to date, MS clearly has the greatest potential. The advantages of MS detection include the capability to both determine molecular weight and provide structural information.

Interestingly, the analyses of phenolic profiles by CE, together with other quality parameters (free acidity, peroxide value, fatty acid composition, water content, oxidative stability, phenols, and antioxidant power of phenolic fraction) have been utilized to show the effect of olive fly attack (*Bactrocera oleae*) over olive oil quality.[47] Therefore, fly attack resulted in the loss of phenols, o-diphenols, and, in particular, some secoiridoid derivatives. Other research involved the analysis of several compounds belonging to four families of phenols (simple phenols, lignans, complex phenols, and phenolic acids) by CZE-UV together with HPLC-UV and HPLC-MS. This approach was applied for the evaluation of the deterioration of extra-VOO during heating. The concentration of phenolic compounds was determined in different extra-VOO samples, as was the concentration of several "unknown" compounds that appeared after the heat treatment.[17]

Phenolic compounds present in oil samples were also separated using nonaqueous capillary electrophoresis (NACE) after SPE.[48] In NACE, an organic solvent or combination of solvents is used, usually methanol and/or acetonitrile (ACN).

The advantages of NACE include solvation of more nonpolar species as well as decreased thermal diffusion and typically decreased electro-osmotic flow (EOF), resulting in enhanced resolution. The use of organic solvents also enables analysis.[49] An inherent advantage of NACE over traditional CZE is the possibility to carry out the determination on phenolic compounds using direct injection of oil. Finally, the methodology allowed the detection of compounds that aqueous CE was not able to see, providing an interesting methodology to determine different groups of phenolic compounds.[48] Recently, another NACE method coupled to UV detection combined with multivariate curve resolution alternating least-squares algorithm was presented. The simultaneous analysis of phenolic acids having overlapped time profiles with the olive oil matrix interferents was achieved.[50]

In conclusion, the phenolic compound determination in VOO can be used for several purposes, such as quality determination, markers of botanical and/or geographical origin, effect of fly attack, deterioration of oil during heating, and evaluation of biotic/abiotic stress responses.

Fatty Acids. Oleic acid is the principal long-chain free fatty acid present in olive oil (C18:1c). Other long-chain free fatty acids such as palmitic acid (C16:0) and linoleic acid (C18:2cc) can also be found but in lower concentrations. The storage control of the olives used as a source of olive oil is an important aspect for oil production, because olives in advanced maturity stage or under enzymatic degradation generate higher long-chain free fatty acid concentrations, imparting an acidity increase to the final product, which affects its overall quality.[51,52]

In the past year, an alternative for the analysis of underivatized long-chain fatty acids by CE has been proposed.[53,54] The principal concerns in analyzing fatty acids by CE have been their relatively their low solubility in aqueous electrolyte systems. To solve this problem, CE separation has been described by using BGEs containing organic solvents, such as methanol,[55] and ACN.[56] In addition, the use of additives to the BGE, such as cyclodextrins[57] or surfactants (sodium dodecyl sulfate (SDS)[58] and polyoxyethylene lauryl

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Table 2. Mode, Analytes, and Analysis Purpose of CE Methodologies for Olive Oil Analysis

mode	analytes	analysis purpose	electrophoretic conditions	analytical performance			ref
				LOD	LOQ	RSD (%)	
			Phenolic Compounds				
CZE-UV	simple, lignans, complex, acids, and flavonoids phenols	compare several Spanish extra-virgin olive oil samples	BGE: 45 mM sodium tetraborate; pH 9.3; time: 10 min; voltage: 28 kV; temperature: 22 °C; capillary: 47 cm (40 cm × 50 μm)	0.311–4.170 μg mL^{-1}	1.037–13.901 μg mL^{-1}	<4.93	42
CZE-UV	CAF, CIN, GEN, HYTY, o-COU, OLE, Que, TY, VAN	compare phenolic profile	BGE: 45 mM sodium tetraborate; pH 9.3; time: 7 min; voltage: 28 kV; temperature: 22 °C; capillary: 47 cm (40 cm × 50 μm)	0.110–0.660 μg mL^{-1}	0.367–2.201 μg mL^{-1}	1.53–3.24	36
CZE-MS	BEA der, GEN, HFA, VAN, CIN der, FER, o-COU, m-COU, p-COU	antioxidant phenolic compounds	BGE: 10 mM ammonium acetate/ammonium hydroxide; pH 10.0; voltage: 25 kV; temperature: 25 °C; capillary: 80 cm (75 μm)	3.5–500 μg L^{-1}	11.6–1666.6 μg L^{-1}	5.2–10.7	37
CE-ESI-MS	BEA der, GEN, HFA, VAN, CIN der, FER, o-COU, m-COU, p-COU	phenolic acids	BGE: 10 mM ammonium/ammonia; pH 10.0; time: 10 min; voltage: 25 kV; temperature: 25 °C; capillary: 80 cm (75 μm)	3.5 μg L^{-1}–0.5 mg L^{-1}	1.5–200 μg L^{-1}	<10	39
NACE-UV	hydroxyaromatic compounds	characteristic profile of the biophenols present in different oil samples	BGE: 40 mM potassium hydroxide, methanol/1-propanol mixture (15.85 v/v); time: 23 min; voltage: −20 kV; temperature: 25 °C; capillary: 33.5 cm (25 cm × 50 μm)	3–5 μg mL^{-1}			64
CZE-UV	BEA, CAF, CIN, o-COU, m-COU, p-COU, 2,4DHyBEA, FER, GAL, p-HyBCA, VAN, Sy, and chlorogenic acid	determination of phenolic acids in vegetable oil samples by in vial LLME	BGE: 25 mM sodium tetraborate; pH 9.15; time: 11 min; voltage: 30 kV; temperature: 25 °C; capillary: 40 cm (50 μm)	0.017–0.16 mg L^{-1}	0.051–0.49 mg L^{-1}	3.72–7.66	71
CZE-DAD	TY, HTY, OLE glycoside, FER, p-COU, CIN, p-HyBEA, GAL, CAF, LUT, API, VAN, and 3,4-DHyBEA	characterization	BGE: 50 mM boric acid; pH 10.2; time: 12 min; voltage: 30 kV; temperature: 25 °C; capillary: nr^a (60 cm × 50 μm)	0.04–0.85 mg L^{-1}	0.013–2.84 mg L^{-1}	2.22–6.96	13
CZE-UV	rosmarinic and CAF	efficiency of different procedure of enrichment of VOO with natural antioxidants contained in various herbs	BGE: 40 mM borate buffer; pH 10; time: 8 min; voltage: 28 kV; temperature: 18 °C; capillary: 49 cm (39 cm × 75 μm)	0.25–0.20 mg L^{-1}	0.67–0.83 mg L^{-1}	1–4	88

Table 2. continued

mode	analytes	analysis purpose	electrophoretic conditions	analytical performance			ref
				LOD	LOQ	RSD (%)	
Phenolic Compounds							
NACE-UV and LIF	TY, HYTY, OA, trans-CIN, HFA, SIA; GEN; LUT	characterization	BGE: 25 mM boric acid; 18 mM potassium hydroxide in a mixture of 74:26 (v/v) 1-propanol/methanol; pH 11.2; **voltage:** 20 kV; **temperature:** 35 °C; **capillary:** 49 cm (nr × 75 µm) with two detection windows	0.51–6.7 µg mL⁻¹	1.7–22 µg mL⁻¹	2.35–9.97	89
Fatty Acids							
CZE-UV (indirect)	myristic, palmitoleic, palmitic, linolenic, linoleic, oleic, and stearic	botanical origins of vegetable oils, including VOO	BGE: 10 mM hydroxybenzoate; 5 mM Tris at pH 8.8; 80 mM Brij 98; 40% ACN; 10% 2-propanol; **time:** 20 min; **voltage:** 25 kV; **temperature:** 45 °C; **capillary:** 80.5 cm (72 cm × 50 µm)	0.020 mM		3.7	54
CE-UV (indirect)	palmitic acid, oleic acid, and linoleic acid	determination of olive oil acidity	BGE: 15 mM phosphate; pH 6.86; 4 mM SDBS; 10 mM Brij 35; 2% v/v 1-octanol and 45% v/v ACN; **time:** 8 min; **voltage:** 20 kV; **temperature:** 25 °C; **capillary:** 48.5 cm (40.0 cm × 75 µm)				51
Chlorophylls							
CZE-LIF	chlorophylls	authenticity and quality of virgin olive oils	BGE: 25 mM borate; pH 8.6; **time:** 10 min; **voltage:** 30 kV; **temperature:** 30 °C; **capillary:** 50 cm (45 cm × 75 µm)	5 µg kg⁻¹		0.55	62
Betaines							
CE-MS²	glycine betaine, trigonelline, proline betaine, and total content of carnitines	adulterations	BGE: 0.1 M formic acid; pH 2.0; **time:** 10 min; **voltage:** 25 kV; **temperature:** 25 °C; **capillary:** 60 cm (55 cm × 50 µm)	0.050–0.075 ng g⁻¹	0.083–0.125 ng g⁻¹	4.8–10.7	35
CZE-UV	trigonelline	adulterations	BGE: 0.1 M formate; pH 2.0; **time:** 15 min; **voltage:** 25 kV; **temperature:** 35 °C; **capillary:** 75 cm (66.5 cm × 50 µm)	0.46 mg L⁻¹	1.15 mg L⁻¹	<5	63
Tocopherols and Synthetic Antioxidant Compounds							
CEC-UV	TOH, δ-TOH; β-TOH; γ-TOH; α-TOH-ac and BHT	adulterations, conservation	BGE: methanol/ACN (50:50 v/v); 0.01% ammonium acetate; **time:** 2.5 min; **voltage:** −25 kV	1.25 µg mL⁻¹	2.50 µg mL⁻¹	2.51	67

Table 2. continued

mode	analytes	analysis purpose	electrophoretic conditions	analytical performance			ref
				LOD	LOQ	RSD (%)	
			Tocopherols and Synthetic Antioxidant Compounds				
NACE-LIF	TOHs	assay of tocopherols in vegetable oils	temperature: 20 °C; capillary: 33 cm (75 μm) partially packed with C$_{18}$ (8.4 and 7 cm effective and packed lengths, respectively); BGE: 12 mM borate buffer (3 mM sodium tetraborate); 60 mM sodium cholate; 12 mM sodium hydroxide in methanol; voltage: 20 kV; temperature: 50 °C; capillary: 40 cm (31.5 cm × 75 μm)	0.18–0.56 μg mL^{-1}	nr	3.6–9.8	72
			Proteins and Amino Acids				
CE-MS^2	ORN, μ-ALA, GABA, AILEU, CIT, and PGLU	markers for the detection of adulterations in olive oils	BGE: 0.1 M formic acid; pH 2.0; time: 15 min; voltage: 25 kV; temperature: 25 °C; capillary: 60 cm (50 μm)	0.04–0.19 ng g^{-1}	0.06–0.31 ng g^{-1}	<7	69

^{a}nr, not reported.

ether (Brij 35)[54] among others), has been described to modify selectivity on analyte separation. The analysis of trans-fatty acids in hydrogenated oils by CZE has been proposed.[59] The separations are usually conducted counter-electro-osmotically, so the longest chain fatty acids elute first and under indirect UV detection. This method has not yet been applied for olive oil; however, it is an interesting subject for future research.

The determination of fatty acids in olive oil by CE has been used with different purposes. Balesteros et al. developed a methodology to determine the free fatty acid present in olive oil, responsible for oil acidity. The methodology is simple, fast, and reliable, and little sample pretreatment is required.[51] On the other hand, the fatty acid profiles observed were used to classify oil samples according to botanical origin using chemometric tools.[54]

In both works the detection is indirect because fatty acids do not possess strong chromophores in their structures (Table 1), which makes difficult their sensitive detection in direct photometric mode. Then, direct UV or fluorescence detection was only employed when a previous derivatization step was performed, through the use of indirect UV and indirect fluorescence detection.

Chlorophylls. Chlorophylls are the pigments responsible for the characteristic green color of the olive drupe as it begins to ripen. About 80% of total chlorophyll is lost during the oil extraction process, meaning that the levels of green pigments are considerably less in the oil and the various byproducts of the extraction than in the fruit itself.[60] The green color of VOOs is due to chlorophylls *a* and *b*, originally found in the fruit, that are irreversibly converted into more stable pigments: pheophytins (the magnesium ion of the porphyrin ring is replaced by two hydrogen ions) and pyropheophytins (products of the removal of the carboxymethyl group) (Table 1). These structural changes in the chromophore group of the chlorophylls affect the color of the oil, which changes from bright green to olive-brown and finally to yellow. The color of olive oil could play a key role as a factor of acceptability among consumers. Moreover, the level of chlorophyll pigments in the oil is a parameter that must be taken into consideration as a predictor of the storage stability of the product, due to the pro-oxidant action of chlorophylls in the presence of light.[60,61]

The natural chlorophyllic pigments are totally absent in refined olive oil. For this reason, occasionally the color of refined olive oil is obtained by synthetic chlorophyll pigments as copper chlorophyll derivatives.[62] In synthetic chlorophyll the magnesium at the center of porphyrin is replaced with a cooper ion. It is more stable when exposed to light and heat and more resistant to oxidative agents in general.[60] The analysis of the pigment responsible for oil color is of great importance, because it can be used to prove the authenticity and quality of VOOs. Del Giovine et al. developed a CZE method with laser-induced fluorescence (LIF) detection to separate copper chlorophyll from other chlorophyll pigments and to determine it in the olive oil.[62] According to the pK_a values observed in Table 1, alkaline pH allows the dissociation of the chlorophylls, which, once dissociated and charged, move differentially toward the detection point according to the atom at the center of the molecule. The choice of the LIF detector granted a sensitivity 1000 times higher than that of a UV detector, and this was possible thanks to the natural fluorescence of chlorophyll.[62] This work is an interesting strategy for detecting adulteration in olive oil, with important analytical performance achieved; however, it is a topic little explored for the CE technique.

Betaines. Betaines could be included among the variety of substances of different structure that make up the unsaponifiable fraction of oils. They are highly polar zwitterionic molecules possessing a quaternary ammonium group with a permanent positive charge and a carboxylic group.[35] Trigonelline, glycine betaine, proline betaine, and carnitines have been analyzed in olive oil by CE using MS and/or UV as detector.[35,63] Sánchez-Hernández et al. developed a methodology using CE-UV to determine trigonelline in soybean oil, sunflower oil, and olive oil (and in its seeds and olive fruit), for traceability purposes. Interestingly, trigonelline is present in sunflower seeds and soy seeds, and this betaine is expected to be present also in the oils obtained from these seeds, but not in olive oils because it is not present in olive.[63] The same group developed a CE−tandem mass spectrometry (MS^2) methodology enabling the simultaneous determination of betaines (glycine betaine, trigonelline, proline betaine, and total content of carnitines) in vegetable oils. The method was applied for the determination of these betaines in seed oils and extra virgin olive oils. MS^2 experiments provided the fingerprint fragmentation for the betaines identified in vegetable oils. In extra-VOOs, carnitines were not detected, making them feasible novel markers for the detection of adulterations of olive oils.[35]

Tocopherols and Synthetic Antioxidant Compounds. Vegetable oils also contain natural isomers α-, β-, γ-, and δ-TOH and related α-, β-, γ-, and δ-tocotrienols, an important group of biomolecules known as vitamin E, which are characterized by a two-ring structure, which is attached to a branched hydrocarbon side chain with 16 carbon atoms (Table 1). These compounds possess antioxidant properties that protect fats and oils from oxidative rancidity and thus prolong its shelf life. In olive oil α-TOH is the most representative.[64] Exactly for this reason the study of TOH distribution can be used for the discovery of sophisticated adulteration, for example, olive oil with hazelnut oil.[65]

TOHs are homologue compounds with a very close chemical structure differing from each other in the presence of the methyl groups on the aromatic ring (see Table 1). The diverse locations of the methyl groups slightly influence the polarity and the hydrophobicity of these molecules.[64] Due to these physicochemical properties aqueous BGE are not suitable for their separation by CZE. For this reason methodologies using capillary electrochromatographic (CEC) are a good alternative. CEC, a hybrid technique of CE and HPLC, has evolved as a powerful tool in the analysis of complex matrices. It combines the high separation efficiency that CZE offers with the wide range of parameters that can be manipulated in HPLC, particularly the wide range of stationary phases from which to choose.[66] Aturki et al. has reported a CEC-UV to determine TOHs and butylated hydroxytoluene (BHT) in vegetable oils. In this methodology, the electrochromatography column was packed with a RP$_{18}$ stationary phase. The CEC method can be useful as an alternative to the HPLC method to determine TOHs in vegetable oils.[67] Moreover, the CEC method can be used to evaluate the effect of several TOHs on the oxidative stability of vegetable oils or to identify some adulteration of the oil by determining the TOH content.

On the other hand, a NACE mode has been presented by Mendonça et al. for the separation of synthetic antioxidants (3-tert-butyl-4-hydroxyanisole (BHA) and BHT) and E-vitamers and other natural compounds such as phenolic compounds in vegetable oils. In this work, the direct injection of vegetable oil samples in a NACE system, without previous extraction, using

an oil-miscible BGE was developed, achieving the separation and quantification of synthetic antioxidants and TOH.[64]

Moreover, CEC and NACE methods can be used to evaluate the effect of several TOHs and synthetic antioxidants on the oxidative stability of olive oil (and other vegetable oils) or to identify some adulteration of the oil by determining the TOH content.

Proteins and Amino Acids. The proteins present in olives and olive oils have also been studied by CE. The proteins present in the olive mesocarp are not very well-known. Some proteins that are present in the oil bodies of the mesocarp also pass along to the oil during olive oil extraction, contributing to some of the special characteristics of olive oils. The analysis of proteins in VOOs is very attractive for the following reasons: olive oils can be consumed without refining, a process by which proteins may be lost. On the other hand, proteins are minor components that are less influenced by the environmental conditions, fruit ripening, and extraction technology than other markers.[8,68] Taken together, the results can be useful for genuineness evaluation and for varietal classification.

Hidalgo et al. developed in 2001 a procedure that can be applied to the determination of proteins in fats and oils by HPLC. They demonstrated the presence of proteins in all samples under study, suggesting that proteins are normal minor components in these oils.[68] It was in 2010 when the protein profiles using CE were employed for the differentiation of botanical varieties of olive oils, showing an enormous potential as traceability markers for these highly appreciated oils.[8]

An analytical methodology based on CE-MS^2 was presented to enable the identification and determination of six nonprotein amino acids (ornithine (ORN), β-alanine (β-ALA), γ-aminobutyric acid (GABA), alloisoleucine (AILEU), citrulline (CIT), and pyroglutamic acid (PGLU)) in vegetable oils. This methodology proposes these compounds as novel markers for the detection of adulterations in olive oils. The methodology is based on a previous derivatization with butanol and subsequent separation using acidic conditions followed by online coupling to an ion trap analyzer for MS^2 detection established through an electrospray−coaxial sheath flow interface.[69]

■ ANALYTICAL PERFORMANCES ACHIEVED

The objective of this section is to summarize and discuss the analytical performances achieved by different analytes present in VOO using diverse techniques prior to CE analysis. The amounts and composition of the minor components in VOO depend on several factors such as olive cultivar, degree of maturation, and agronomic and technological aspects of production. However, the qualitative and quantitative compositions of VOO are strongly affected by the agronomic and technological conditions of its production.[11]

The methodological developments are basically similar; the first step involves a cleanup/preconcentration depending on the analyte chemical nature. Mainly LLE and SPE have been used. In the next section, more information about sample preparation will be discussed. However, the variety of extraction techniques, separation conditions, and methods of quantification have contributed to differences in reported levels of different compounds studied in VOO, principally for phenolic compounds.

Ideally, under optimized conditions, electrophoretic experiments lead to total analyte separation; that is, each peak belongs to a single compound. The simplest way to characterize the separation of two components is to divide the difference in

migration distance by the average peak width to obtain resolution (R).[50,66] Under optimum conditions R values >5 between peaks have been achieved for the separation of amino acids in VOO by CZE-MS.[69] Nevertheless, complete separation of individual compounds is not required with an ion trap and an ESI-MS interface. The latter is crucial with a view to obtaining quantitative results because the ionization efficiency of the ESI is strongly influenced by changes in the sample matrix. Therefore, good resolution, particularly between isomers (for example, *o*-, *m*-, and *p*-COU), which exhibited identical *m/z* values for their [M − H]⁻ ion (163), is mandatory.[39]

An important advance with respect to resolution power of methodologies is achieved when chemometric tools are coupled to separation techniques. It is interesting to note that, although a complete separation of the peaks could not be performed, second-order data coupled to chemometric tools can be used to achieve selectivity by mathematical means, allowing for resolution and quantitation of overlapped analytes.[50] García-Villalba et al. developed a two-dimension methodology (by coupling HPLC and CE), providing enough resolving power to separate hundreds of compounds compared with tens of compounds from a highly complex sample such as olive oil.[46]

Separation time using CE for analysis of different analytes studied in olive oil is around 15 min (Table 2).[36,42,69] The precision of the methodologies in terms of repeatability generally is evaluated by replicate analysis (for example, *n* = 15) on one of the samples on the same day (intraday precision) and/or on consecutive days (interday precision) of oil-spiked sample with the compounds studied. Then, the relative standard deviations (RSDs) for migration and peak areas are calculated. Generally, if the conditioned procedure prior to runs is appropriated, the RSDs for migration times are <2%, and RSDs for peak areas are between 1.2 and 10%.[35−37,39,63,69] Generally, the highest RSD values for peak area are observed for those analytes with poor sensitivity and nearness to instrumental noise (Table 2).

Direct comparison between analytical performances achieved with, for example, phenolic compounds reported in the literature is difficult, as the reported concentrations often differ greatly (sometimes even by orders of magnitude, as can be seen in Table 2). This could be explained by the fact that there are numerous factors affecting the concentration of different compounds of VOO, as mentioned above. An exhaustive study of all the variables affecting the analytical performance of the proposed methods should be carried out considering the significant matrix effects that have to be faced when oils are involved. Some characteristics of the great majority of methodologies developed are the low throughput and high organic solvent volume used in the sample preparation, as mentioned in the next section.

■ PROCEDURES EMPLOYED IN PREVIOUS CE SEPARATION

Olive oil is composed mainly by triacylglycerols, which account for >98% of its total weight. In addition, it contains about 2% of other minor components (nearly 250) present in a wide concentration range (from mg kg⁻¹ to ng kg⁻¹). Thus, the analysis of trace analytes in such a complex matrix results in an extremely challenging analytical task. Generally, extraction/preconcentration is performed before CE analysis of olive oil analytes. Therefore, this first crucial step has a great influence on the repeatability and accuracy of analysis. Low-concentration sensitivity, due to the small injection volume and short

optical path length, is one of the major limitations of CE. Therefore, many offline or online preconcentration techniques have been developed. LLE,[67,69−71] SPE,[39,72] and combined methods[73,74] were commonly used as offline preconcentration techniques. A variety of organic solvents and extraction cartridges could be chosen to enrich the trace analytes and remove potentially interfering compounds. Anther inconvenience for doing these studies is that the trace analytes of olive oil consist of a heterogeneous mixture of compounds, which are in most cases not commercially available.[73] In this section the procedure employed will be detailed according to analyte separation. The different techniques used for the extraction of different compounds of olive oil can be seen in Table 3.

Phenolic Compounds. Phenolic compounds are the most important minor analytes studied in olive oil by CE. The phenolic fraction of virgin olive oil consists of a heterogeneous mixture of compounds, which are in most cases not commercially available.

The phenolic fraction of olive oil has been isolated principally using LLE.[17,47,75] These procedures are based on the methodology proposed by Pirisi et al.[76] Basically, this LLE uses 2 g of oil diluted with hexane (1 mL) and a mixture of methanol/water (60:40 v/v) as extraction solvent. The methanol layer is separated and the extraction repeated twice, then washed with hexane, and evaporated to dryness. Considering the physicochemical properties of oil and phenolic compounds, LLE is a good alternative as the extraction technique. The low oil density, together with high nonpolarity, allows the development of LLE using aqueous solutions (methanol/water) as extraction phase. Indeed, the polarity of phenolic compounds improves mass transfer to the extraction phase. Different sample aliquots, hexane volumes, and extraction phase pH values have been used at the extraction step to obtain the best extraction of phenolic compounds. The importance of using a mixture of methanol/water to decrease the coextraction of fatty acid and obtain a stable baseline has to be pointed out.[41,76−78]

More recently, some authors have used SPE to isolate phenolic compounds.[39,73] Gómez-Caravaca et al. compared different extraction systems (C_{18}-SPE, Diol-SPE, Sax-SPE, and LLE) to characterize phenolic profiles. The differences among the phenolic profiles obtained were higher for the quantitative than for the qualitative analysis. The best results were obtained for Diol-SPE; it was appropriate for the extraction of polar fraction from nonpolar matrices. Interestingly, although LLE seems to be slightly more appropriate in terms of intensity of the signals, the authors decided to use Diol-SPE in favor of the shorter extraction time and the ease of using SPE automated workstations.[73]

However, some disadvantages arise from the application of LLE and SPE such as large volumes of toxic and expensive solvents, high amounts of wastes, and reduced frequency of analysis. In fact, several microextraction techniques (liquid phase microextraction (LPME), single-drop microextraction (SDME), dispersive liquid−liquid microextraction (DLLME), ultrasound-assisted dispersive liquid−liquid microextraction (UA-DLLME), 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.[79,80] Although these techniques received favorable responses, the applicability of these techniques for oil-based samples is little explored. Recently, another miniaturized extraction technique, called in-vial LLME, has been proposed.[71]

Table 3. Techniques Used for the Extraction of Different Compounds of Olive Oil

mode	compounds	analytical procedure	preinjection	ref
		Phenolic Compounds		
CZE-DAD	TY; Pin; Ac Pin; DAOA; Lig Agl; HYTY; OA; EA	extraction: SPE-Diol sample mass: 60 g diluent solvent: hexane, 75 mL eluent solvent: methanol, 40 mL	extract dried and redissolved methanol 50%, 2 mL	73
CE–ESI-MS	HFA, GEN, m-COU, o-COU, p-COU, FER, VAN	extraction: SPE-Diol sample mass: 8 g diluent solvent: hexane, 8 mL eluent solvent: methanol 20%, 8 mL	first extract: directly injected second extract: dried and redissolved methanol 20%, 300 μL	39
CZE-DAD	trans-CINHFA, SIA, GEN, TAX, FER, o-COU, p-COU, VAN, CAF, p-HyBCA, DOPAC, GAL, ProCAA	extraction: LLE sample mass: 60 g diluent solvent: hexane, 60 mL eluent solvent: methanol 60%, 80 mL	extract dried and redissolved methanol 50%, 500 μL	70
CE-DAD	CAF,GAL, CIN, FER, CLO, SYR, VAN, BEA, p-HyBCA, m-HyBCA, o-COU, m-COU, p-COU	extraction: in vial LLME sample mass: 20 g diluent solvent: hexane, 2 mL eluent solvent: methanol/5 mM NaOH (60/40 v/v), 600 μL	extract directly injected	71
CE-DAD	OA, TY, HYTY, CIN, LUT, API, FER, CAF, p-COU, VAN, m-HyBCA, GAL, p-HyBCA	extraction: LLE and on line preconcentration (stacking)	injection, 25 s; reverse voltage, −30 kV, 5 s	13
		Fatty Acids		
CE-DAD	myristic acid, palmitoleic acid, palmitic acid, linolenic acid, linoleic acid, oleic acid, stearic acid	separation: saponified with 0.5 M ethanolic sodium hydroxide, 75–80 °C, 25 min diluent solvent: samples were 1:20 diluted with methanol sample mass: 0.4 g	extract directly injected	54
CE-DAD	myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid	separation: ethanol, 60 °C, 10 mL sample mass: 1.5 g	ethanolic phase was directly injected	51
		Chlorophylls		
CZE-LIF	copper and magnesic chlorophylls in their a and b variants	extraction: SPELC-Si sample mass: 1 g diluent solvent: n-hexane, 30 mL eluent solvent: acetone, 18 mL	extract dried and redissolved acetone/water (80:20 v/v), 1 mL	62
		Betaines		
CE-UV	trigonelline	extraction: LLE - methanol/chloroform (2:1, v/v), −20 °C, 160 mL - methanol/chloroform/water (2:1:0.8, v/v/v),100 mL - water 100 mL and chloroform 40 mL sample mass: 40 g	aqueous extract was dried and redissolved in ACN/water (40:60 v/v), 300 μL	63
		Tocopherols		
NACE-LIF	α-TOH, β-TOH, γ-TOH, and δ-TOH	extraction: SPE sample mass: 0.1–0.6 g diluent solvent: hexane 4.5 mL eluent solvent: diethyl ether, 2 mL	extract dried and redissolved in BGE/hexane/ethanol (60:20:20 v/v/v), 1.5 mL	72
CEC-DAD	α-TOH, β-TOH, γ-TOH and δ-TOH and α-TOHs-ac	extraction: LLE	extract dried and redissolved in ethanol 1 mL	67

Table 3. continued

mode	compounds	analytical procedure	preinjection	ref
		Tocopherols		
CE-DAD	peptide bonds, phenylalanine, tyrosine, tryptophan	- methanol 5 mL - methanol/isopropanol (80/20 v/v), 5 mL **sample mass:** 1 g	extract dried and redissolved in ACN 20%, 500 µL	8
		Proteins and Amino Acids		
CE-ESI-MS	CIT, ORN, PGLU, β-ALA, GABA, AILEU	**separation:** cold acetone, −5 °C, 1 h, 400 mL **sample mass:** 400 g **recovery:** THF, 5 mL; dioxane, 5 mL **separation:** multiple LLE extractions - methanol/chloroform (2:1 v/v), −20 °C, overnight, 160 mL - methanol/chloroform/water (2:1:0.8 v/v/v), 100 mL - water (100 mL) and chloroform (40 mL) **sample mass:** 40 g **derivatization:** butanol, 80 °C, 30 min, 1 mL, dried at 80 °C	extract redissolved in ACN 40%, 500 µL	69

The in-vial LLME methodology shows considerable advantages for the extraction of phenolic acids from vegetable oils, especially in terms of the amount of solvents used and the waste generated. Another distinct advantage of the proposed method is that an evaporation step is not required. This not only saves time but, equally importantly, preserves the integrity of the relatively unstable phenolic acidss. Even with the various advances in sample preparation, LLE remains the most popular sample preparation technique for the determination of phenolic compounds.

On the other hand, to increase sensitivity as well as reduce solvent consumption, online preconcentrations (inside the capillary) based on stacking have been evaluated. It can be easily accomplished by exploiting the ionic strength differences between the sample matrix and separation buffer.[66] Stacking results from the fact that sample ions have an enhanced electrophoretic mobility in a lower conductivity environment. Stacking can be utilized with either hydrostatic or electrokinetic injection and can typically yield a 10-fold enhancement in sample concentration and, thus, sensitivity. Knowing the optimum separation conditions, ionic strength mediated stacking, large-volume sample stacking (LVSS), and reverse electrode polarity stacking modes (REPSM) have been proposed, by means of dissolving the standards in methanol/water (low conductivity sample), enlarging the sample injection volume, and applying a reverse voltage without instrument modification. Using this methodology, when the extract obtained from 2 g of olive oil was injected with reverse voltage, it was possible to obtain a higher detectability than when the extract obtained from 15 g was injected during 5 s. Thus, it was possible to use 7.5 times smaller quantities of sample, methanol, and hexane by simply increasing the injection time and applying reverse voltage for a short time.[13]

Fatty Acids. Traditionally, analysis of fatty acids has been performed chromatographically and spectroscopically. The chromatographic technique most widely applied to determine fatty acid profiles of lipids is GC, in which long-chain fatty acids are analyzed as methyl or trimethylsilyl esters in polar columns.[51]

As stated under the previous Fatty Acids paragraph, the determination of fatty acids in olive oil by CE has been used to determine the acidity of olive oil. In this methodology, a saponification is not included; the samples are simply treated with ethanolic solutions (at 60 °C) extracting oil long-chain free fatty acids, and extracts obtained were directly injected into the CE. This makes possible the determination of the acidity of olive oil with a simple step of extraction. In this case the derivatization is not used because this procedure includes both free and glycerol-bound fatty acids, undesirable interfering side products.[51] For this reason, CE has been proposed as an interesting alternative for the analysis of underivatized long-chain fatty acids.[51,54]

On the other hand, the fatty acid profiles observed were used to classify oil sample according to botanical origin using chemometric tools. In this case the saponification prior to CE separation was performed by refluxing at 75−80 °C for 25 min with ethanolic sodium hydroxide solution. After saponification, samples were diluted with methanol and directly injected.[54]

Chlorophylls. Chlorophyll pigments can be extracted from the oil samples by SPE. CZE, in conjunction with a LIF detector, has been applied for the copper chlorophyll separation from other natural chlorophylls. An extraction technique was used to produce fat-free pigments; oil was directly passed onto

a SPE-LC-Si cartridge and totally absorbed by connecting the cartridge to a vacuum system and then washed with hexane; finally, the pheophytins were desorbed from silica with acetone. The acetone solution was evaporated until dryness at ambient temperature in a nitrogen current, and the residue was recovered with acetone/water solution.[62]

Betaines. Sanchez-Hernandez et al. developed a sensitive CZE-UV method without derivatization enabling the determination of trigonelline in edible oils, that is, olive oil. In this work a LLE was used for the extraction of betaines, using multiple extractions with a mixture of methanol/chloroform. Finally, it was evaporated to dryness and reconstituted with ACN/water.[63]

Recently, the same LLE was chosen for the determination of betaines in oil, but in this case Sánchez-Hernández et al. determined four types of betaines in oil and after butyl ester derivatization. The derivatization of compounds that contain mono- and dicarboxylic acid groups using butanol as the derivatizing agent not only greatly improves ionization efficiencies, and hence analytical sensitivity, but also improves the mass differentiation among the analytes, increasing the selectivity. As a consequence, butanol was employed in this work as derivatizing agent for betaines to develop a CE-MS^2 methodology, enabling their sensitive determination in vegetable oils.[35]

Tocopherols and Synthetic Antioxidant Compounds. Relatively few procedures have been developed using CE separation for the determination of TOHs. TOHs were extracted from the samples by LLE using methanol, and then these methanolic extracts were combined and extracted again twice with methanol/isopropanol. The extracts were combined and evaporated to dryness using a rotary pump in a water bath. The residue was dissolved in ethanol and separated by CEC.[67]

Galeano-Diaz et al. developed a NACE with LIF detection methodology to separate the different TOHs. This work employed a SPE (Sep-Pak Plus silica cartridge), and TOHs eluted finally with diethyl ether. The eluate was evaporated to dryness under N$_2$, and the residue was treated with a mixture of BGE/hexane/ethanol (BGE: sodium tetraborate, sodium cholate, and sodium hydroxide in methanol).[72]

Proteins and Amino Acids. Protein separation from olive oils by CE was achieved for the first time by Montealegre et al. in 2010. In that work the extraction procedure is based on the precipitation of proteins in cold acetone and their isolation in a hydro-organic medium. However, this extraction procedure presented poor sensitivity and inadequate reproducibility when extracts were analyzed by CZE-UV; other alternatives were investigated without satisfactory results.[8]

On the other hand, for determination of nonprotein amino acids (ORN, β-ALA, GABA, AILEU, CIT, and PGLU), vegetable oil samples were extracted using the same LLE as used for betaines (methanol/chloroform). Subsequently, a derivatization with butanol was carried out before injection in the CE-MS^2 system with acidic migration conditions.[69] The butylation of the carboxylic fraction of the amino acids improves ionization efficiency and detection sensitivity. In addition, it increases the mass of the esters and provides better mass selectivity.[69]

CLASSIFICATION OF OLIVE OILS ACCORDING TO ORIGIN AND CULTIVAR

The quality characteristics and taste in VOO are largely related to their origin, both geographical and genetic factors (cultivar),

as well as to the climate, agronomic techniques, harvesting, transport, and storage systems of olives, ripening degree of drupes, and extraction procedures used.[1,24,28,36] This has resulted in the appearance on the market of oils with specific olive variety composition, that is, coupage or monovarietal olive oils or with a denomination of origin. To protect and preserve the specificity of many traditional foods, which owe their peculiar characteristics to the area of origin or to the local techniques of production, there have been established designations such as PDO and PGI. PDO status indicates a food whose production, processing, and preparation take place in a particular geographical area, and these foods are characterized by a recognized and certified know-how. PGI, on the other hand, indicates a product of some repute, for which the link with the territory is present in at least one of the stages of production, processing, or preparation of the finished product.[28] To be able to boast of such quality status, a food must undergo a strict production regulation, restricting the borders of the geographical area, raw materials, and processing techniques used.[24]

Analytical methods are necessary to guarantee the authenticity and traceability of olive oils. CE has proved to be a fast technique for the analysis of olive oil components that allows varietal and geographical origin classification.[8,36,42,81]

Phenolic compounds have been used as markers of origin. In 2004 Carrasco-Pancorbo et al. demonstrated the potential of the CE technique for the fast and sensitive simultaneous determination of 14 compounds in extra-VOO from different varieties with results very promising for the use of phenolic compounds as markers.[70] Later, five monovarietal extra-VOO samples, Picual, Hojiblanca, Lechín de Sevilla, Arbequina, and Cornicabra, and an organic olive oil and two types of Picual olive oil ("suave" and "intenso") were analyzed. In this work CE joined to statistical analysis permits discrimination among different olive oils.[42] Finally, by using an electrophoretic method that detects and quantifies simultaneously 18 phenolic compounds, coupled to statistical analysis, the discrimination of olive oils belonging to two different PDOs was possible: one of Italy (Chianti Classico) and the other one of Spain (Sierra de Segura).[36]

Indeed, Lerma-García et al. classified extra-VOO samples belonging to three different geographical origins (Croatia, Italy, and Spain) with an excellent resolution among all categories.[81]

Among the different components of olive oils, proteins are the compounds less influenced by environmental conditions, fruit ripening, and extraction technology. The CE protein profiles obtained from three monovarietal oils (Hojiblanca, Picual, and Arbequina) were compared. Preliminary results demonstrate the possibility of differentiating monovarietal olive oils according to their botanical origin.[8]

However, the latest trends in organic compound analysis have been to take advantage of ultrahigh-resolution mass spectrometry, which allows qualitative and quantitative analysis for target, nontarget, and unknown compounds. Nontarget analysis is a modern tool for classification of botanical and biological samples. Nevertheless, to our knowledge, this methodology has been employed only partially by some authors. More studies of this type should be performed in the near future.[36,82]

CE VERSUS OTHER TECHNIQUES

A large number of works are available on the development of methods for the determination of different analytes in olive oil.

The qualitative and quantitative determination of numerous compounds in olive oil samples is very important, and several analytical methodologies have been reported. The phenolic compounds have received more attention. In early years, nonspecific analytical methods, such as UV spectroscopy (Folin),[83] were applied for the analysis of polyphenols with limited success. Afterward, these traditional methods were replaced by other more specific methods, such as HPLC,[23,84] GC,[85,86] and CE,[78] given the need to profile and identify the individual analytes in olive oil samples, both major and minor analytes.

GC approaches with various detection techniques including MS and NMR have been used to separate and quantify several analytes in olive oil, principally. GC-MS is superior for fatty acids and lipids. However, the GC technique is less widely used for polar analytes, such as phenolic compounds, because these are unstable at high temperatures and require a derivatization reaction for their determination. The limited volatility of many analytes has restricted the use of GC for their separation, so HPLC and CE currently represent reliable techniques for the analysis of olive oil.

Actually, HPLC and CE are currently used in the resolution of macromolecules of interest in biotechnology, pharmaceutical, biological, and biochemistry industries. In recent years, CE has become one of the major choices for the separation of charged analytes and a solid alternative to HPLC. HPLC coupled to MS detectors has been used for the characterization and analysis of olive oil samples. Nevertheless, HPLC analyses generally require longer analysis times and, moreover, the reagent consumption and waste generation are higher compared with CE. CE has gained increasing acceptance because it presents a good compromise between analysis time and satisfactory characterization for several compounds in olive oil. The speed, resolution, and simplicity of CE, combined with its low operational cost and small residue generation, make this technique an attractive option for the development of analytical methods for food analysis.[5,87] MS detection has reached increasing acceptance as a supplement or replacement for conventional detectors in CE. CE-MS is a highly attractive analytical tool combining the high efficiency and resolution power of CE with the high selectivity inherent in MS.[69] A resume of the principal characteristics of CZE compared with HPLC can be seen in Figure 2. As can be seen, the separation principle is totally different. Those differences are clearly shown by Carrasco-Pancorbo et al.; differences can be found when the results for the secoiridoids are studied. The cause of these apparent discrepancies could be that CE is able to separate several isoforms of the secoiridoids. Therefore, the secoiridoids that appear in the HPLC profile as one peak can be separated into several peaks with the CE method. With respect to analysis time, the CE method was able to detect and determine several phenolic acids that appear within 7 min of analysis. The type of flow with a plane profile allows narrow peaks and improves the *R*. The short time of analysis and little waste generated using CE are other important advantages in high-throughput and environmentally friendly methodologies.[17]

With regard to detectors, although optical detectors are most common for this purpose, MS is a powerful detector taking into account that it provides lower LODs than UV in most cases, with the additional benefit that it does not require a derivatization procedure with chromophore or fluorophore. Chromatographic methods coupled with MS are powerful techniques that provide resolving power and structural
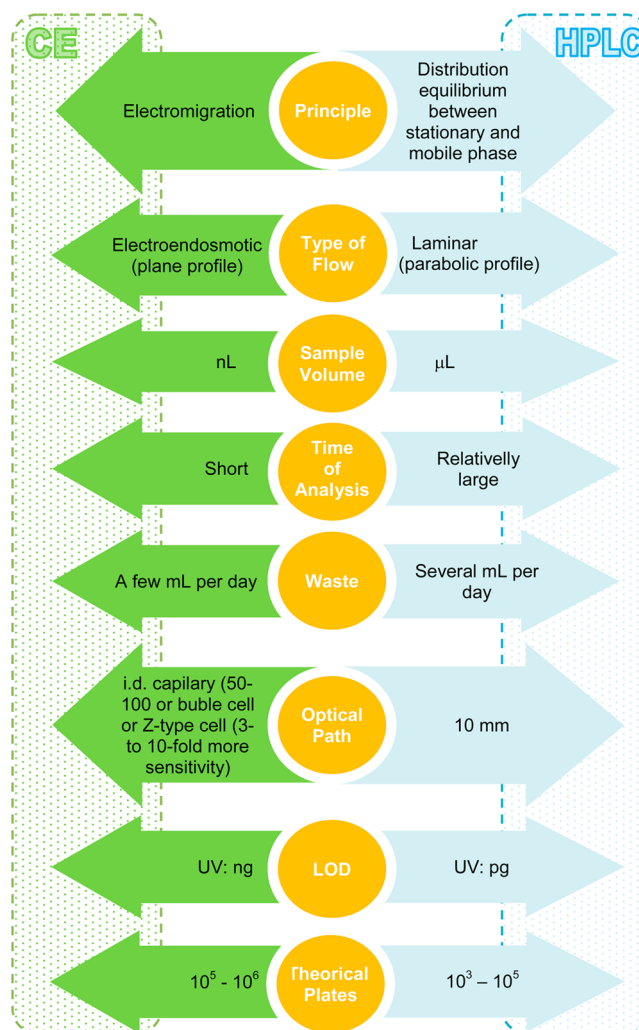

Figure 2. Principal characteristics of CE compared with HPLC.

information to unequivocal confirmation of the presence of particular species. In addition, tandem MS[2] affords structural elucidation and enhanced selectivity with a view to reducing chemical noise through increased sensitivity.[39,69] Additionally, MS may greatly facilitate the identification and quantification of newly discovered analytes of health, nutritional, or toxic interest in olive oil.

■ FUTURE PERSPECTIVES

The major objective of this review was to evaluate several analytical methods used to analyze diverse compounds in VOO by CE. The CE methods have the advantages of high speed, high resolution, low operational cost, low consumption of chemicals, and robustness. In recent years, the coupling of CE with MS as detectors allowed structural identification of the analytes to be obtained, information very important in samples with high complexity such as olive oil. However, it is necessary to continue developing preconcentration methodologies, online and offline, to improve sensitivity. These methodologies would be simple, quick, and environmentally friendly. It is important to highlight that simplicity in this step would be one of the main goals of the methodologies to allow use by less experienced operators for routine quality control of VOO.

It has to be pointed out that the combination of fingerprinting separation techniques with chemometric tools

represents a great alternative to identify the origin or identification of fraud.

On the other hand, the use of proteins as markers for traceability studies is a very promising area of research. Nevertheless, the long path to elucidate their role in classification has begun. Robust and reliable analytical methods are of outstanding importance in this matter, the bottleneck being the extraction of proteins from oil.

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Notes

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■ ABBREVIATIONS USED

ACN, acetonitrile; Ac Pin, acetoxypinoresinol; β-ALA, β-alanine; AILEU, alloisoleucine; API, apigenin; GABA, γ-aminobutyric acid; BEA, benzoic acid; BHA, 3-*tert*-butyl-4-hydroxyanisole; BHT, butylated hydroxytoluene; CAF, caffeic acid; CAT, catechin; CE, capillary electrophoresis; CEC, capillary electrochromatographic; CIN, cinnamic acid; CIT, citrulline; *o*-COU, *o*-coumaric acid; *m*-COU, *m*-coumaric acid; *p*-COU, *p*-coumaric acid; 3,4DHyBEA, 3,4-dihydroxybenzoic acid; 3,4DiMBEA, 3,4-dimethoxybenzoic acid; DAOA, deacetoxy oleuropein aglycon; DLLME, dispersive liquid—liquid microextraction; DOA, decarboxymethyl oleuropein aglycon; EA, elenolic acid; EOF, electro-osmotic flow; ESI-TOF-MS, electrospray ionization—time of flight—mass spectrometry; FER, ferulic acid; FID, flame ionization detector; GAL, gallic acid; GC, gas chromatography; GEN, gentisic acid; *p*-HyBCA, *p*-hydroxybenzoic acid; HFA, hydroxyphenylacetic acid; HPLC, high-performance liquid chromatography; HYTY, hydroxytyrosol; LIF, laser-induced fluorescence; Lig Agl, ligstroside aglycon; LLE, liquid—liquid extraction; LPME, liquid phase microextraction; LUT, luteolin; LVSS, large-volume sample stacking; MIR, mid-infrared; MS, mass spectrometry; NACE, nonaqueous capillary electrophoresis; NIR, near-infrared; NMR, nuclear magnetic resonance; OA, oleuropein aglycon; OLE, oleuropein; ORN, ornithine; PGLU, pyroglutamic acid; Pin, pinoresinol; ProCAA, protocatechuic acid; Que, quercetin; REPSM, reverse electrode polarity stacking modes; RRLC, rapid-resolution liquid chromatography; RSD, relative standard deviation; SDME, single-drop microextraction; SDS, sodium dodecyl sulfate; SIA, sinapic acid; SPE, solid phase extraction; SyAl, syringaldehyde; TAA, tannic acid; TOH, tocopherol; α-TOH-ac, α-tocopherol acetate; TY, tyrosol; UPLC, ultra-performance liquid chromatography; UA-DLLME, ultrasound-assisted dispersive liquid—liquid microextraction; VAN, vanillic acid; VOO, virgin olive oil

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