ELECTRONIC SUPPLEMENTARY MATERIALS

Deep insight into the minor fraction of virgin olive oil by using LC-MS and GC-MS multi-class methodologies

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Table 1a SM. Main characteristics of diverse methodologies, selected as example, for the specific determination of different minor VOO chemical classes: Single-class methodologies. (The official method, when available, is described in the shaded row).

Sample treatment	Separation conditions	Detection conditions	Number of Analytes	Ref.
Phenolic compounds				
UAE (15 min) 2 g sample + 5 mL MeOH/H_2O (80:20, v/v)	RP-LC C18 (250 × 4.6 mm, 5 µm) Gradient: H_2O (0.2% H_3PO_4)/MeOH/ACN (82 min)	UV 280 nm	27 (total referred to TY)	(International Olive Council, 2009)
LLE 2g sample + 6 mL MeOH/H_2O (60:40, v/v) → 1 mL MeOH	RP-LC C18 (150 × 4.6 mm, 1.8 µm) Gradient: H_2O (0.5% AcH)/ACN (25 min)	MS (ESI-IT/TOF) Negative polarity	20	(Bajoub et al., 2015)
LLE 1 g sample + 2 mL MeOH → 850 µL MeOH/H_2O (80:20, v/v)	RP-LC C18 (100 × 2.1 mm, 1.8 µm) Gradient: H2O (0.1% CO_2H_2)/ACN (0.1% CO_2H_2) (30 min)	MS (ESI-QTOF) Negative polarity	34 (no quantification)	(Capriotti et al., 2014)
SPE (diol-bonded phase cartdridge) 2.5 g sample (10 mL MeOH) → 100 µL derivatization reagent	GC ZB-5MS (30 m × 0.25 mm, 0.25 µm) T: 150-295 ºC (71 min)	MS (EI-IT) Positive polarity	21 (no quantification)	(Ríos, Gil, & Gutiérrez-Rosales, 2005)
SPE (diol-bonded phase cartdridge) 60 g sample (40 mL MeOH) → 2 mL MeOH/H_2O (50:50, v/v)	CE Fused silica capillary (400 × 0.375 mm, 50 µm) Running buffer: (45 mM sodium tetraborate, pH 9.3) (7 min)	DAD 200, 240, 280, 340 nm	16	(Carrasco-Pancorbo et al., 2006)
Triterpenic compounds				
Saponification, TLC 5 g sample → 50 µL silylation reagent/mg analyte	GC SE-52 or SE-54 (20-30 m × 0.25-0.32 mm, 0.10-0.30 µm) T: 260 ºC (30-60 min)	FID	2 (plus 15 sterols)	(European Commission, 2016)
SPE (bonded aminopropyl phase cartdridge) 0.2 g sample (6 mL diethyl ether/acetic acid (98:2, v/v)) → 200 µL silylation reagent	GC SGL-5 (25 m × 0.25 mm, 0.25 µm) T: 260-320 ºC (34 min)	FID	3	(Pérez-Camino & Cert, 1999)
SPE (bonded aminopropyl phase cartdridge) 0.2 g sample (6 mL diethyl ether/acetic acid (98:2, v/v)) → 200 µL silylation reagent	GC Rtx-65TG (30 m × 0.25 mm, 0.10 µm) T: 260-320 ºC	MS (EI-IT MS) Positive polarity	1	(Guinda, Albi, Pérez-Camino, & Lanzón, 2004)
LLE 0.8 g sample (9.6 mL MeOH/EtOH (50:50, v/v)) → 2.4 mL MeOH	RP-LC C18 (250 × 4.6 mm, 5 µm) Isocratic: 8% H_2O (H_3PO_4)/92% MeOH (20 min)	UV 210 nm	2 (plus 2 fatty acids)	(García, Brenes, Dobarganes, Romero, & Ruíz-Méndez, 2008)
UAE 0.2 g sample (10 mL MeOH) → 1 mL MeOH	RP-LC C18 (100 × 4.6 mm, 1.8 µm) Isocratic: 10% ammonium buffer (pH 9.6)/54% ACN/36% MeOH (13 min)	MS (ESI-IT) Negative/positive polarities	6	(Olmo-García, Bajoub, Monasterio, Fernández-Gutiérrez, & Carrasco-Pancorbo, 2018)

Tocopherols				
Dilution 2 g sample + 25 mL hexane	NP-LC Si60 (250 × 4.0 mm, 5 µm) Isocratic: 0.5% 2-propanol/99.5% hexane (30 min)	FLD λ_{ex} 290 nm, λ_{em} 330 nm UV 290 nm	4	(IUPAC, 1991)
Dilution 2.5 g sample + 5 mL hexane	NP-LC Si60 (250 × 4.6 mm, 5 µm) Isocratic: 1% 2-propanol/98.5% hexane/0.5% EtOH	FLD λ_{ex} 290 nm, λ_{em} 330 nm	4	(Franco, Galeano-Díaz, Sánchez, De Miguel, & Martín-Vertedor, 2014)
LLE 4 g sample + 20 mL MeOH + 10 mL MeOH/2-propanol (80:20 (v/v)) □ 1 mL MeOH	Nano-RP-LC Monolithic C18 (250 × 0.1 mm) Isocratic: 75% ACN/8% MeOH/17% H_2O (0.2% AcH) (20 min)	UV 295 nm	4	(Cerretani, Lerma-García, Herrero-Martínez, Gallina-Toschi, & Simó-Alfonso, 2010)
Dilution (1:10, v/v) with isopropanol	RP-LC C18 (150 × 2.1 mm, 5 µm) Isocratic: 50% MeOH/50% ACN (12 min)	FLD λ_{ex} 290 nm, λ_{em} 330 nm	4	(Bakre, Gadmale, Toche, & Gaikwad, 2015)
Saponification 0.025 g sample → 1 mL MeOH	RP-LC FPF (200 × 4.6 mm, 5 µm) Isocratic: 95% MeOH/5% H_2O (15 min)	MS (APCI/ESI-Q) Negative polarity	4	(Lanina, Toledo, Sampels, Kamal-Eldin, & Jastrebova, 2007)
Sterols				
Saponification, TLC 5 g sample → 50 µL silylation reagent/mg analyte	GC SE-52 or SE-54 (20-30 m × 0.25-0.32 mm, 0.10-0.30 µm) T: 260 ºC (30-60 min)	FID	15 (plus 2 triterpenic alcohols)	(European Commission, 2016)
Saponification, solid supported liquid extraction, SPE (base-activated silica cartdridge) 0.2 g sample (60 mL diethyl ether), (10 mL hexane/diethyl ether (60:40, v/v)) → 250 µL silylation reagent	GC HP-5MS (30 m × 0.25 mm, 0.25 µm) Isothermal T: 260 ºC (55 min)	FID	15 (plus 2 triterpenic alcohols)	(Mathison & Holstege, 2013)
Saponification, TLC 5 g sample → 50 µL silylation reagent/mg analyte	RP-LC C18 (150 × 2.1 mm, 5 µm) Gradient: H_2O (0.01% AcH)/ACN (20 min)	MS (APCI-Q) Positive polarity	5 (plus 2 triterpenic alcohols)	(Cañabate-Díaz et al., 2007)
Saponification, SPE (silica-based octadecyl bonded phase) 1 g sample (15 mL 5% MeOH in chloroform (v/v) → 1 mL MeOH (dilution 1:10, v/v)	Nano-RP-LC C18 (150 × 0.1 mm, sub-2 µm) Isocratic: MeOH (22 min)	DAD 195 nm	5	(Rocco & Fanali, 2009)
Saponification, TLC 5 g sample → 50 µL silylation reagent/mg analyte	CEC Methacrylate ester-based monolithic column (8.5 cm) Mobile phase: 85% ACN/10% 2-propanol/5% aqueous Tris buffer (5 mM, pH 8.0) (8 min)	DAD 210 nm	5	(Lerma-García, Simó-Alfonso, Ramis-Ramos, & Herrero-Martínez, 2008)

Fatty acids				
Trans-esterification 0.1 g → 2 mL heptane	GC Polar polysiloxane column (60 m × 0.20-0.32 mm, 0.10-0.20 µm) T: 165-210 ºC (37 min)	FID	23	(European Commission, 2016)
Saponification, trans-esterification 0.1 g → H_2SO_4 0.5 M in MeOH	GC Supelcowax 10 (30 m × 0.25 mm, 0.25 µm) T: 180- 240 ºC (15 min)	FID	6	(Bodoira et al., 2015)
Trans-esterification 0.1 g → 2 mL hexane (dilution 1:50, v/v)	GC SPTM-2380 (60 m × 0.25 mm, 0.20 µm) T: 120-250 ºC (36 min)	FID	10	(Sánchez de Medina, Calderón-Santiago, El Riachy, Priego-Capote, & Luque de Castro, 2015)
Trans-esterification 0.1 g → 5 mL hexane	GC DB-1 (30 m × 0.25 mm × 0.10 µm) T: 90-250 ºC (9 min)	MS (EI-Q) Positive polarity	18	(Alves et al., 2016)
Dilution (1:20, v/v) with isopropanol	RP-LC C18 (50 × 2.1 mm, 1.9 µm) Gradient: ACN/H_2O (8 min)	MS (ESI-QqQ) Negative polarity	12	(Wabaidur et al., 2016)

Table 1b SM. Main characteristics of different methodologies for simultaneous determination of two (or more) minor VOO chemical classes: Multi-class approaches.

Sample treatment	Separation conditions	Detection conditions	Number of Analytes	Ref
LLE 10 g sample + 50 mL MeOH + 25 mL MeOH/2-propanol (80:20 (v/v)) → 5 mL MeOH/2-propanol/hexane (1:3:1, v/v/v)	RP-LC C18 (250 × 4 mm, 5 µm) Gradient: H_2O (2% AcH)/MeOH/ACN/2-propanol (70 min)	DAD 280 nm	10 phenolic compounds, 2 tocopherols	(Tasioula-Margari & Okogeri, 2001)
LLE 1 g sample + 15 mL MeOH → 1 mL MeOH	GC HP-5 MS (30 m × 0.25 mm, 0.25 µm) T: 70-300 ºC (60.5 min)	MS (EI-Q) Positive polarity	13 phenolic compounds, 3 triterpenic compounds	(Kalogeropoulos, Kaliora, Artemiou, & Giogios, 2014)
Saponification 5 g sample → 0.5 mL ACN	RP-LC C18 (250 × 3 mm, 5 µm) Gradient: H_2O (0.01% AcH)/ACN (65 min)	MS (APCI-IT) Positive polarity	9 sterols, 3 tocopherols, 2 triterpenic dialcohols	(Zarrouk, Carrasco-Pancorbo, Zarrouk, Segura-Carretero, & Fernández-Gutiérrez, 2009)
Saponification 1 g sample → 130 µL silylation reagent/mg analyte	GC SPB-5 (30 m × 0.53 mm, 0.5 µm) T: 180-270 ºC (76 min)	FID	4 sterols, α-tocopherol, squalene, 7 aliphatic alcohols	(Giacometti, 2001)
Dilution 0.5 g sample → 10 mL 2-propanol/ hexane (1.5:98.5, v/v)	NP-LC Si60 (250 × 4.6 mm, 5 µm) Isocratic: 1.5% 2-propanol/98.5% hexane (30 min)	DAD 409, 430, 433, 452 nm FLD λ_{ex} 295 nm, λ_{em} 330 nm	3 tocopherols, 3 tocotrienols, 2 chlorophylls, 2 pheophytins, β-carotene	(Seppanen, Rahmani, & Csallany, 2003)

The symbol "→" means evaporation and reconstitution.
For solid supported liquid extraction and SPE, only the extraction or elution solvent (not considering cleaning steps) is indicated between brackets.

Abbreviations (used in Tables 1a and b SM)

methanol (MeOH), ethanol (EtOH), liquid-liquid extraction (LLE), solid phase extraction (SPE), ultrasounds assisted extraction (UAE), liquid/gas chromatography (LC/GC), capillary electrophoresis (CE), diode array detector (DAD), fluorescence detector (FLD), flame ionization detector (FID), mass spectrometry (MS), electron impact (EI), electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), ion trap (IT), time of light (TOF), quadrupole (Q), triple quadrupole (QqQ).

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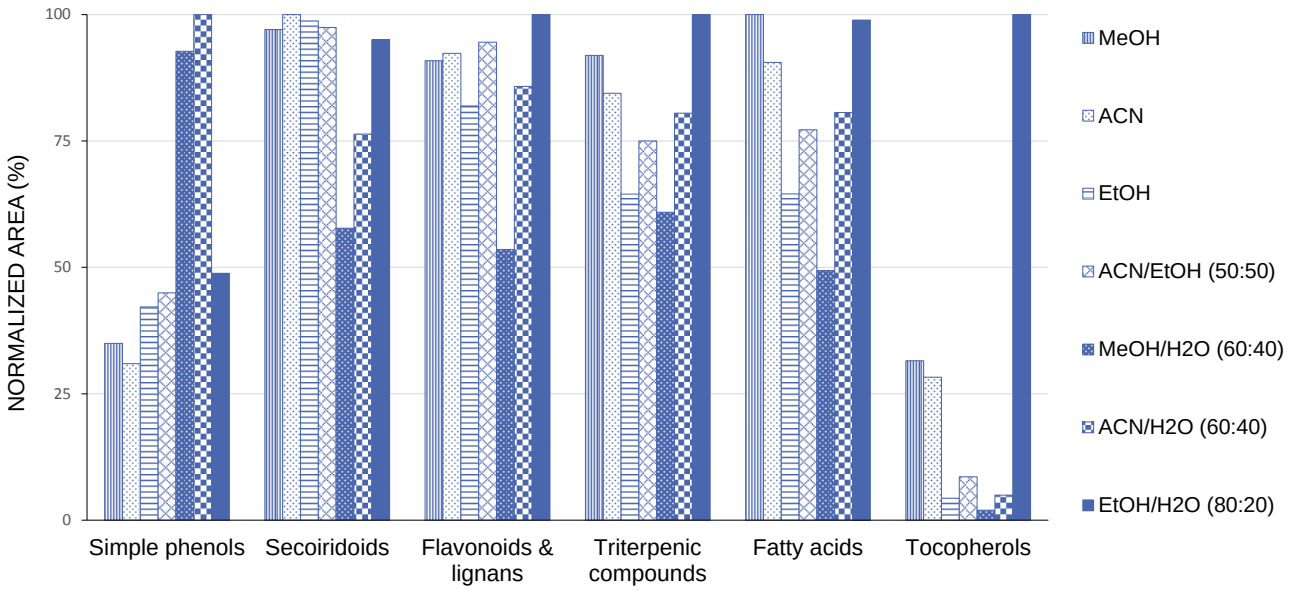

Fig. 1 SM. Total (normalized) peak areas of the different VOO minor chemical classes (simple phenols, secoiridoids, flavonoids and lignans, triterpenic compounds, fatty acids, and tocopherols) found in the extracts obtained with the seven extractant agents selected in the first stage of the sample treatment optimization (MeOH, ACN, EtOH, ACN/EtOH (50:50, v/v), MeOH/H_2O (60:40, v/v), ACN/H_2O (60:40, v/v) and EtOH/H_2O (80:20, v/v)). Analyses were made in the LC-IT MS system. Normalization was made considering as 100% the total area value of the most effective extractant agent (for each chemical class) and referring the rest to that value. The compounds considered as members of each chemical class are those included in Table 2 SM.

Table 2 SM. Evaluation of the performance (efficiency) of the extraction protocol considering the percentage of different target compounds obtained in each successive extraction stage as well as the extraction repeatability.

Chemical class	Compound	1st%	2nd%	3rd%	Repeatability (RSD)	
Simple phenols	quinic acid	99.5	0.5	-	9.7	*LC-MS*
	hydroxytyrosol	75.6	24.1	0.3	3.6	
	tyrosol	93.6	6.4	-	6.4	
	p-coumaric acid	100.0	-	-	1.1	
	vanillin	100.0	-	-	0.7	
	ferulic acid	100.0	-	-	8.8	
Secoiridoids	desoxy elenolic acid	99.9	0.1	-	0.6	
	elenolic acid	93.7	5.2	1.1	3.3	
	decarboxymethyl oleuropein aglycone	76.8	14.8	8.4	7.8	
	oleuropein aglycone	84.7	11.9	3.4	7.9	
	decarboxymethyl ligstroside aglycone	77.9	11.2	10.9	3.0	
	ligstroside aglycone	89.7	5.5	4.7	9.8	
Flavonoids	luteolin	99.8	0.2	-	0.9	
	apigenin	99.2	0.8	-	6.5	
Lignans	pinoresinol	98.7	1.3	-	5.0	
	acetoxy pinoresinol	97.9	2.1	-	5.4	
Triterpenic compounds	maslinic acid	96.4	2.8	0.7	2.7	
	betulinic acid	99.8	0.2	-	4.3	
	oleanolic acid	95.7	2.4	2.0	7.4	
	erythrodiol	75.5	24.5	-	8.0	
Fatty Acids	linolenic acid	97.5	2.3	0.2	2.8	*GC-MS*
	linoleic acid	98.4	1.5	0.2	3.5	
	oleic acid	94.5	5.0	0.5	5.9	
	palmitic acid	95.2	3.7	1.1	1.5	
	palmitoleic acid	98.9	1.1	0.0	1.7	
	stearic acid	88.3	7.5	4.1	1.5	
Tocopherols	*α*-tocopherol	78.1	15.9	5.9	1.3	
	β-tocopherol	86.6	10.3	3.0	2.5	
	γ-tocopherol	82.9	13.1	4.0	0.1	
	δ-tocopherol	90.5	7.3	2.2	4.0	
Sterols	*β*-sitosterol	70.6	22.4	7.0	4.4	
	campesterol	78.6	17.2	4.2	5.4	
	stigmasterol	80.7	15.4	3.9	0.7	
	Δ^5-avenasterol	76.1	18.6	5.2	7.2	
	cycloartenol	75.3	19.2	5.6	0.5	
	methylencycloartanol	70.6	22.7	6.6	5.0	

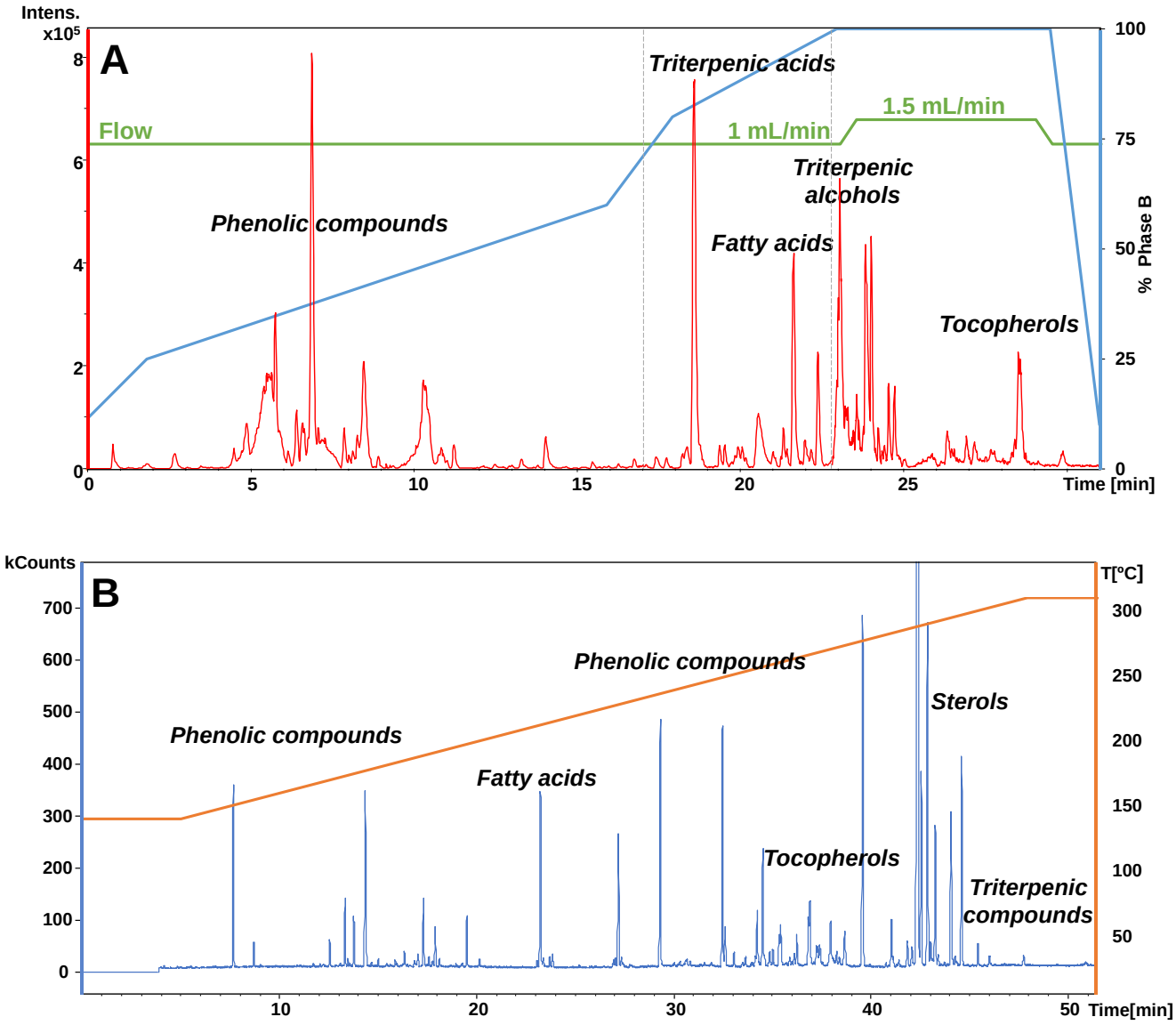

Fig. 2 SM. A) LC-MS Base Peak Chromatogram (BPC) of a VOO extract of Cayon variety obtained under the optimum conditions. The optimized gradient composition of mobile phases and flow are also shown in the figure, as well as the elution areas of each one of the determined families. B) GC-MS BPC of the same VOO extract together with the optimized temperature ramp gradient and elution areas of each chemical class.