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Aurélia Malapert, Emmanuelle Reboul, Michele Loonis, Olivier Dangles, Valérie Tomao. Direct and Rapid Profiling of Biophenols in Olive Pomace by UHPLC-DAD-MS. Food Analytical Methods, 2017, 11 (4), pp.1-10. 10.1007/s12161-017-1064-2 . hal-01709338

**HAL Id: hal-01709338**

**<https://hal.science/hal-01709338>**

Submitted on 25 May 2018

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# Direct and Rapid Profiling of Biophenols in Olive Pomace by UHPLC-DAD-MS

Aurélia Malapert<sup>1</sup> · Emmanuelle Reboul<sup>2</sup> · Michèle Loonis<sup>1</sup> · Olivier Dangles<sup>1</sup> · Valérie Tomao<sup>1</sup>

**Abstract** Olive mill by-products are effluents generated during olive oil production process. The two-phase centrifugation system produces a semi-solid olive pomace called “alperujo.” This by-product is a combination of liquid and solid wastes derived from the three-phase manufacturing process. A direct and fast analytical method by ultra-high-pressure liquid chromatography-DAD coupled with ESI/MS-MS has been developed for the profiling of phenolic compounds. Thirty-five metabolites belonging to phenyl alcohols, secoiridoids, flavonoids, and iridoids were identified as the main constituents of alperujo in 12 min, including *p*-coumaroyl aldarate and a verbascoside derivative found for the first time in alperujo and a new ligstroside derivative. Six quantitatively significant components were determined at concentrations ranging from 17.7 mg/L for *p*-coumaric acid to 370.7 mg/L for hydroxytyrosol. Our data confirm that alperujo is an interesting source of phenolic compounds that could be extracted for use as nutraceuticals.

**Keywords** Alperujo · Two-phase olive pomace · Olive phenolic compounds · Biophenols · Fast analysis · UHPLC/MS

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## Introduction

The Mediterranean landscape is since ancient time associated with the culture of olive trees. Today, it is estimated that nearly 98% of the global olive production originates in the Mediterranean basin, among which 73% in Southern Europe with Spain, Italy, and Greece as the main producers (Conseil Oléicole International 2013; Ollivier et al. 2014).

The ever-growing olive oil production is correlated with the accumulation of olive mill wastes, and several types of by-products may be generated depending on the process used. Three-phase mills, using large volumes of water to improve the feasibility and the efficiency of oil extraction (Kapellakis et al. 2008; Niaounakis and Halvadakis 2006), generate two by-products: the olive mill wastewater and a solid waste known as olive pomace composed of olive pulp, skins, and stones. The modern two-phase processing technique, requiring no addition of water during oil extraction, produces the wet olive pomace consisting of vegetation water and solid olive particles (Dermeche et al. 2013). This new process is associated with a lower volume of by-products, thus minimizing the associated environmental impact. However, these by-products are still a major source of pollutants from oil mills, largely because of its phenolic compounds labeled as phytotoxic compounds (Albuquerque et al. 2006). On the other hand, alperujo provides a rich source of phenolic compounds (e.g., oleuropein, verbascoside, apigenin-7-glucoside, luteolin-7-glucoside) known to possess important health-promoting properties by different mechanisms (antioxidant activity, modulation of a variety of cell signaling pathways) to protect against degenerative diseases (Giordano et al. 2015; Visioli and Bernardini 2011; Obied et al. 2007a).

The main methods proposed for determination of the phenolic compounds from alperujo include advanced chromatographic techniques coupled to mass spectrometry. Cardoso et al. investigated olive pulp and pomace by-products by electrospray ionization mass spectrometry, allowing the identification of common phenolic compounds and for the first time two oleoside derivatives (Cardoso et al. 2005). Obied et al. achieved high-resolution separation of 52 compounds from alperujo extracts in less than 60 min by chemical screening using reversed phase HPLC-diode array detection (RPLC-DAD) and RPLC-electrospray ionization mass spectrometry (RPLC-ESI-MS) (Obied et al. 2007b). Rubio-Senent et al. identified 26 phenolic compounds in 48 min by HPLC/MS after application of a process based on the hydrothermal treatment of alperujo and subsequent extraction by ethyl acetate (Rubio-Senent et al. 2012). Using the same pretreatment of alperujo, Rubio-Senent et al. identified also a polymeric phenolic fraction at the end of the separation (Rubio-Senent et al. 2013) and were also able to isolate and identify some minor secoiridoids, such as oleuropein derivatives (Rubio-Senent et al. 2015). Besides HPLC-MS, capillary electrophoresis (CE) was also used for alperujo analysis. For instance, after ultrasound-assisted extraction, 20 biophenols were separated and identified in 11 min by CE-DAD (Priego-Capote et al. 2004).

The present study reports a rapid, reliable and efficient analytical method to extract and characterize the wet olive pomace by ultra-high performance liquid chromatography (UHPLC)-DAD coupled with ESI-MS/MS without any preliminary extraction procedure to preserve both the maximal content and integrity of its phenolic compounds.

## Materials and Methods

### Chemicals

Alperujo was collected in October 2014 from *Aglandau* olives processed at a two-phase olive oil mill (Moulin Castelas, Baux-de-Provence, France). Samples were immediately frozen and stored at  $-20\text{ }^{\circ}\text{C}$  before chemical analyses. All solvents were of HPLC grade from VWR International (Darmstadt, Germany). *p*-Coumaric acid, caffeic acid, chlorogenic acid, gallic acid, 3,4-

dihydroxybenzoic acid, hydroxytyrosol, rutin, taxifolin, and vanillin were supplied by Sigma-Aldrich (Deisenhofer, Germany). Quinic acid, (*E*)-cinnamic acid, ferulic acid, homovanillic acid, syringic acid, vanillic acid, apigenin, luteolin, luteolin-7-*O*-glucoside, catechol, oleuropein, tyrosol, and verbascoside were obtained from Extrasynthèse (Genay, France).

### Two-Phase Olive Pomace Preparation

Two-phase olive pomace was pressed (Tompres, France) and filtered through celite on a Buchner funnel, then through a  $0.2\text{-}\mu\text{m}$  PTFE filter (Alltech Associates, Deerfield, IL) prior to UHPLC analyses.

### Two-Phase Olive Pomace Characterization in Terms of Phenolic Compounds

#### *Chromatographic and Mass Spectrometric Conditions*

UHPLC-DAD-MS analyses were performed using an Acquity UPLC® system (Waters, Milford, MA, USA) linked to both a diode array detector (DAD 200–800 nm, Waters, Milford, MA, USA) and a Bruker Daltonics HCT Ultra Ion Trap Mass Spectrometer equipped with an Electron Spray Ionization (ESI) source operating in negative ion mode. The separation was performed on an Acquity C18 BEH column ( $1.7\text{ }\mu\text{m}$ ,  $2.1 \times 50\text{ mm}$ ). The solvents were (A) water/formic acid (99.5/0.5) and (B) acetonitrile. The gradient was linear, and the proportions of solvent B used were as follows: 0–10 min 1–20%, 10–12 min 20–30%, and 12–14 min 30–100%. The injection volume was  $1\text{ }\mu\text{L}$ , and the column temperature was kept at  $35\text{ }^{\circ}\text{C}$ . Along the three steps of the gradient, the flow rate was 0.30, 0.35, and  $0.40\text{ mL/min}$ . The spectroscopic detection was performed in the range 200–600 nm with a resolution of 1.2 nm. The concentrations of the main phenolic compounds were estimated from calibration curves (peak area vs. concentration) constructed with hydroxytyrosol, tyrosol, caffeic acid, and *p*-coumaric acid. The quantification of the glucoside forms of hydroxytyrosol were undertaken as millimoles of hydroxytyrosol equivalent per liter. Limits of detection (LOD) and quantification (LOQ) of these four standards were determined by the signal-to-noise

**Table 1** Calibration curves, limits of detection (LOD), and quantification (LOQ) of standard biophenols

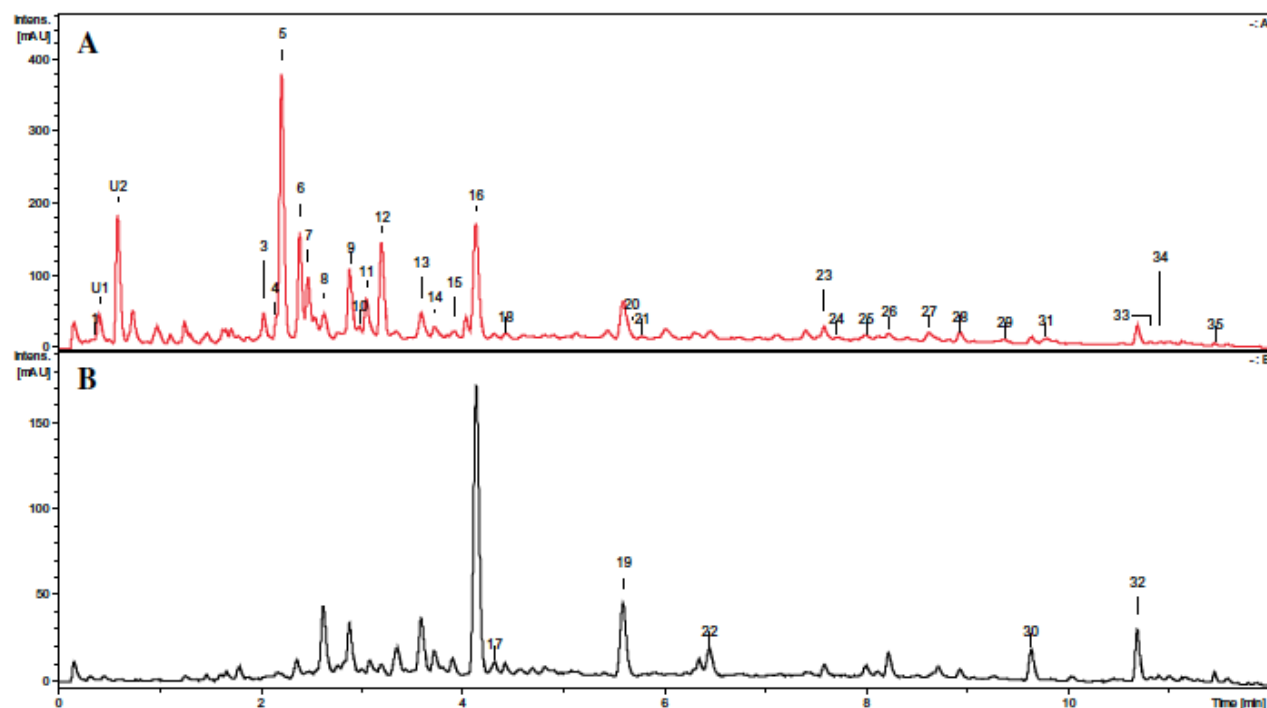
Standard	$t_R$ (min)	$\lambda_{max}$ (nm)	Calibration line	$R^2$	LOD (ng/ $\mu\text{L}$ )	LOQ (ng/ $\mu\text{L}$ )
Hydroxytyrosol	2.28	227, 280	$y = 2.093 \times 10^6 x - 1703.36$	0.9998	0.54	1.79
Tyrosol	3.23	227, 276	$y = 1.907 \times 10^6 x - 2540.69$	0.9996	2.35	7.84
Caffeic acid	4.11	239, 295sh, 324	$y = 2.285 \times 10^6 x - 2128.28$	0.9993	0.23	0.78
<i>p</i> -Coumaric acid	5.50	224, 309	$y = 6.891 \times 10^7 x + 2189.48$	0.9999	0.24	0.80

sh: shoulder, y: peak area, x: mass in milligrams

**Table 2** Retention times ( $t_R$ ) and UV-visible characteristics of standard biophenols

Standard	$t_R$ (min)	$\lambda_{max}$ (nm)	
		Standard	Sample
Gallic acid	1.14	229, 271	nd
3,4-Dihydroxybenzoic acid	2.03	258, 294	nd
Hydroxytyrosol	2.28	227, 280	227, 280
Catechol	2.60	276	nd
Tyrosol	3.23	227, 276	224, 276
Chlorogenic acid	3.91	242, 301, 327	244, 296, 327
Vanillic acid	3.95	261, 293	nd
Caffeic acid	4.11	239, 295sh, 324	234, 295sh, 325
Syringic acid	4.49	227, 275	nd
Homovanillic acid	4.59	235, 280	nd
Vanillin	5.04	242, 280, 309	nd
<i>p</i> -Coumaric acid	5.50	224, 309	227, 309
Ferulic acid	6.41	238, 297sh, 324	237, 296sh, 322
Taxifolin	6.88	289	nd
Rutin	7.35	256, 354	nd
Luteolin-7- <i>O</i> -glucoside	7.76	255, 349	nd
Verbascoside	8.27	235, 290sh, 331	235, 291sh, 330
Oleuropein	10.38	235, 281	nd
( <i>E</i> )-Cinnamic acid	10.96	224, 278	nd
Luteolin	11.13	253, 348	nd

sh: shoulder, nd: not detected



**Fig. 1** UHPLC chromatograms of alperujo juice with detection at 280 nm (a) and 330 nm (b)

**Table 3** Retention times, UV-visible, and MS data of alperujo phenolic compounds and other metabolites

No.	$t_R$ (min)	Compound	$\lambda_{max}$ (nm)	$m/z$	Fragments	References
1	0.46	Quinic acid	–	191	173, 127, 111	D'Antuono et al. (2014)
U <sub>1</sub>	0.52	Unknown 1	232, 267	191	173, 127, 111	
U <sub>2</sub>	0.77	Unknown 2	227, 279	191	173, 127, 111	
2	1.75	Verbascoside—Rha	234, 282, 308	477	459, 367, 161	Cardinali et al. (2012)
3	2.12	Loganic acid glucoside	232, 283	537	375, 331, 179	Peralbo-Molina et al. (2012)
4	2.25	Vanillic acid hexoside	254, 293	329	167, 151, 123, 108	
5	2.31	Hydroxytyrosol (HT)	227, 280	153		Leouifoudi et al. (2014) Rubio-Senent et al. (2013)
6	2.35	Hydroxytyrosol glucoside 1	231, 277	315	153, 123	D'Antuono et al. (2014) Obied et al. (2007b)
7	2.46	Hydroxytyrosol glucoside 2	231, 279	315	153, 123	D'Antuono et al. (2014) Obied et al. (2007b)
8	2.55	<i>p</i> -Coumaroyl aldarate	313	355	337, 209, 191, 147, 129	First report in olive Steingass et al. (2015)
9	2.72	1- $\beta$ -Glucosyl-acyclo-dihydroelenolic acid	236, 294	407	389, 375, 357, 313, 161	Cardoso et al. (2005) D'Antuono et al. (2014) Rubio-Senent et al. (2015)
10	3.05	Hydroxylated DCMEA derivative	236	199	181, 155, 111	Kanakis et al. (2013) Lozano-Sánchez et al. (2014)
11	3.15	Verbascoside—CA	237, 281	461	315, 297, 161, 135	First report in olive Sanz et al. (2012)
12	3.29	Tyrosol	227, 276	137	119	Kanakis et al. (2013) Peralbo-Molina et al. (2012)
13	3.71	Feruloyl-hexoside	286, 320	401	355, 193	
14	3.73	Caffeoyl-hexoside	239, 286sh, 318	341	179, 135	
15	4.02	Chlorogenic acid	244, 296, 327	353	247, 163, 135, 109	Rubio-Senent et al. (2012)
16	4.24	Caffeic acid (CA)	238, 295sh, 325	179	163, 135	Obied et al. (2007b) Suárez et al. (2008)
17	4.43	<i>p</i> -Coumaroyl-hexoside	240, 325	325	265, 163, 119	
18	4.55	Oleoside	236	389	345, 209, 165	Bouaziz et al. (2010) D'Antuono et al. (2014)
19	5.69	<i>p</i> -Coumaric acid	227, 309	163	119	Leouifoudi et al. (2014) Rubio-Senent et al. (2012)
20	5.73	Oleoside deoxyriboside	239, 278	505	389, 345, 121	Peralbo-Molina et al. (2012)
21	5.77	Oleuropein aglycone	245, 282	377	197, 153	Cardoso et al. (2005) D'Antuono et al. (2014) Rubio-Senent et al. (2013)
22	6.60	Ferulic acid	237, 296sh, 322	193	149	Obied et al. (2007b) Peralbo-Molina et al. (2012)
23	7.75	Luteolin- <i>O</i> -rutinoside	246, 346	593	285	Leouifoudi et al. (2014) Rubio-Senent et al. (2012)
24	7.87	Luteolin- <i>O</i> -rutinoside	244, 346	593	285	Leouifoudi et al. (2014) Rubio-Senent et al. (2012)
25	8.18	Elenolic acid	244	241	209, 165, 139, 127, 121, 101	Kanakis et al. (2013) Suárez et al. (2009)
26	8.39	Verbascoside	235, 291sh, 330	623	461, 477, 315	Rubio-Senent et al. (2012) Suárez et al. (2009)
27	8.82	Tetrahydro-oleuropein	246, 279	543	525, 513	Kanakis et al. (2013)
28	9.17	Nüzhenide	246, 282, 334	685	523, 453, 421, 299	Silva et al. (2006, 2010)
29	9.41	Oleuropein glucoside	250, 279	701	539, 377, 307, 275	Cardoso et al. (2005)
30	9.79	Caffeoyl-6'-secologanoside	225, 290, 327	551	507, 389, 385, 341, 281, 251, 221, 179, 161	Obied et al. (2007b)

**Table 3** (continued)

No.	$t_R$ (min)	Compound	$\lambda_{max}$ (nm)	$m/z$	Fragments	References
31	9.91	10-Hydroxy-DCMO aglycone	243, 281	335	199, 155, 111	Rubio-Senent et al. (2012) Lozano-Sánchez et al. (2014) Suárez et al. (2008)
32	10.86	Comselogoside	240, 313	535	491, 389, 345, 307, 265, 163	Obied et al. (2007b) Rubio-Senent et al. (2013)
33	11.02	Oleuroside	244, 282	539	469, 437, 377, 307, 275	Kanakis et al. (2013)
34	11.09	Ligstroside derivative	246, 277	655	517, 361, 291, 259	New compound
35	11.69	Ligstroside isomer	248, 282	523	453, 421, 299	Cardoso et al. (2011)

*sh* shoulder, *DCMEA* decarboxymethyl elenolic acid, *DCMO* decarboxymethyl oleuropein

(*S/N*) evaluations. Low concentrations were injected to attempt a *S/N* equal to 3 and 10 for LOD and LOQ, respectively (Table 1). All analyses were run in triplicate.

ESI mass spectra were obtained at ionization energies of 50 and 100 eV in negative mode, the capillary voltage was 2 kV, the source temperature was 365 °C, the drying gas was introduced at a flow rate of 10 L/min, and the skimmer voltage was 40 V. Scans were performed in the *m/z* range 100–800. Identification of phenolic compounds in samples was performed after standard injection from analysis of retention times, UV-visible spectra, MS molecular peaks and fragments, and comparison with

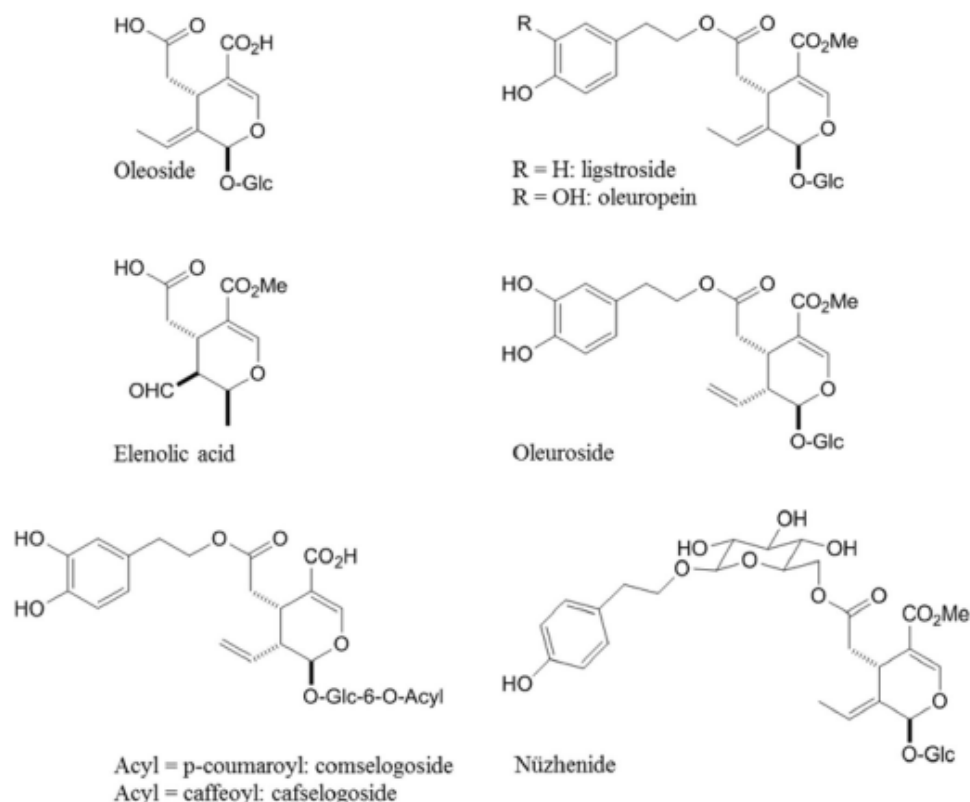
literature data. Tentative identifications of new biophenols were deduced from MS fragmentation.

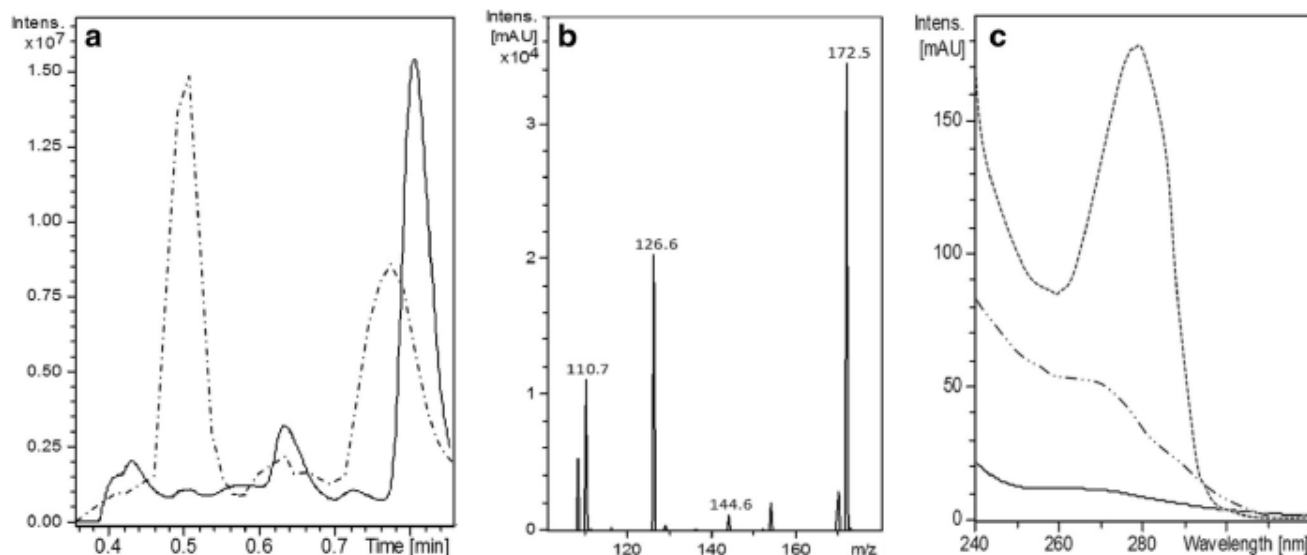
## Results and Discussion

### Phenolic Profiles in Alperujo Juice

In our UHPLC/MS method, LC and mass parameters for native alperujo juice have been optimized, such as injection volume of samples, flow rate of mobile phase, peak resolution, mass collision energy, and fragmentation voltage. The optimized

**Fig. 2** Chemical structures of common secoiridoids in alperujo





**Fig. 3** Extracted ion chromatogram (EIC) at  $m/z = 191$  and chromatogram at 280 nm (a), mass spectrum of quinic acid (b), and UV spectra of compounds at  $m/z = 191$  (c). a Continuous line:

chromatogram (280 nm), discontinuous line: EIC  $m/z = 191$ . c Continuous line: quinic acid, discontinuous line: U1, dotted line: U2

conditions showed appropriate fragmentation with the detection of the parent ion  $[M-H]$  for all compounds over the 12 min runtime. The analysis of injected standards is summarized in Table 2.

Figure 1 shows a typical chromatographic analysis with detection at 280 nm (total phenols) and 330 nm (hydroxycinnamic acids, flavones, and flavonols). Table 3 summarizes the phenolic compounds identified in the alperujo juice and their UHPLC-DAD-MS characteristics (peak numbering according to elution order).

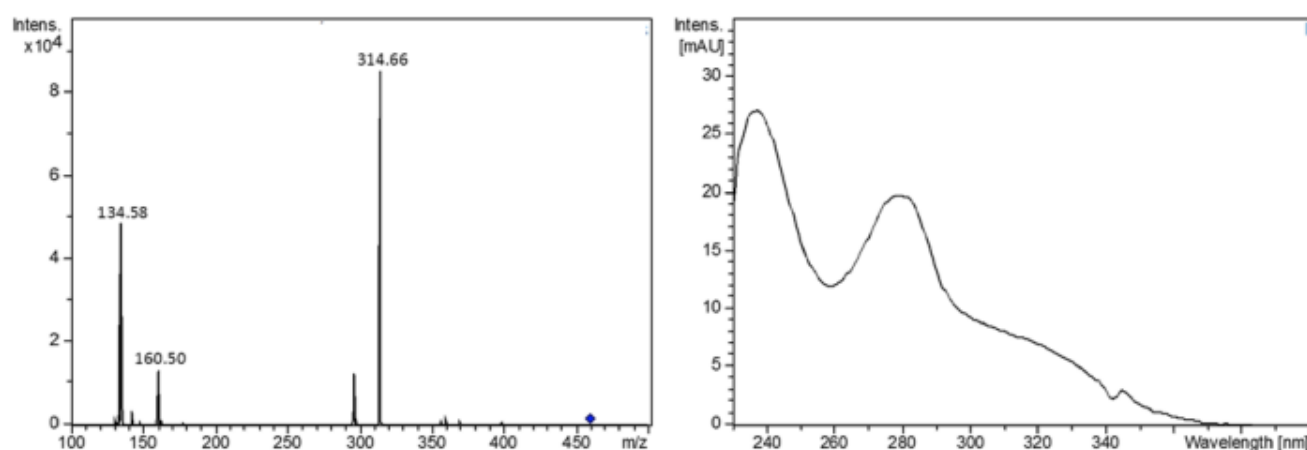
The large number of peaks confirmed the complexity of alperujo juice in terms of its phenolic composition. Figure 2 shows some chemical structures of common secoiridoids identified in alperujo.

### Quinic Acid and Isomers at $m/z = 191$

Compound 1 assigned to quinic acid was not detected by DAD at 280 nm, but its identity was confirmed by injection of the authentic standard. Two peaks at  $m/z = 191$  and retention times of 0.52 and 0.77 min show the same parent ion and molecular fragments as quinic acid. However, they are detectable by DAD and slightly differ by their UV spectra (Fig. 3). These data did not permit their identification.

### Verbascoside and Derivatives

Peak 2 displays a molecular ion at  $m/z = 477$  consistent with hydrolysis of the rhamnose residue from verbascoside



**Fig. 4** MS/MS and UV absorption spectra of verbascoside—CA identified in alperujo (compound 11)

**Table 4** UHPLC-DAD quantification of major phenolic compounds in alperujo juice (on fresh basis)

Phenolic compound	Content (mg/L)	Content (mM)
Hydroxytyrosol	370.7 ± 5.1	2.40 ± 0.03
Hydroxytyrosol glucoside 1	165.2 ± 1.2	1.07 ± 0.01
Hydroxytyrosol glucoside 2	88.3 ± 1.3	0.57 ± 0.01
Tyrosol	148.4 ± 1.0	1.08 ± 0.01
Caffeic acid	68.0 ± 1.0	0.38 ± 0.01
<i>p</i> -Coumaric acid	17.7 ± 0.5	0.11 ± 0.01

Expressed as hydroxytyrosol equivalent. Values are mean ± standard deviation from triplicate analyses

( $m/z = 623$ ) (Cardinali et al. 2012). The ion fragments produced at 459 and 367 correspond to a loss of water and catechol respectively. Peak 11 ( $m/z = 461$ ) may be identified as the verbascoside derivative formed after hydrolysis of the caffeic acid residue. According to literature, it has never been found in olive pomace but among others in ash (gender: *Fraxinus*) (Fig. 4) (Sanz et al. 2012). Its fragments at  $m/z = 315$  and 297 correspond to the successive losses of Rha and H<sub>2</sub>O.

### Phenolic Alcohols and Derivatives

In alperujo juice, phenolic alcohols are the main compounds, such as hydroxytyrosol (peak 5) at  $m/z = 153$  and its glucosides (peaks 6 and 7) at  $m/z = 315$ . HT glucosides are commonly identified in olive mill wastes (Leouifoudi et al. 2014; Suárez et al. 2009) but are generally eluted before HT (El-Abbassi et al. 2012; Obied et al. 2005). However, sometimes, the elution order can be reversed (Aranda et al. 2007; Bouaziz et al. 2010; Kanakis et al. 2013; Savarese et al. 2007). This phenomenon may be due to differences in chromatographic phase, particle shape, and particle porosity among the different studies. Peak 12 was identified as tyrosol by standard injection, which was confirmed by its molecular ion at  $m/z = 137$ . Hydroxytyrosol generated the most intense UV

signal. The high concentrations of tyrosol, hydroxytyrosol, and its glucosides confirm the interest of alperujo juice for applications in the food and cosmetic industries (Table 4) (Kalogerakis et al. 2013; Obied et al. 2005).

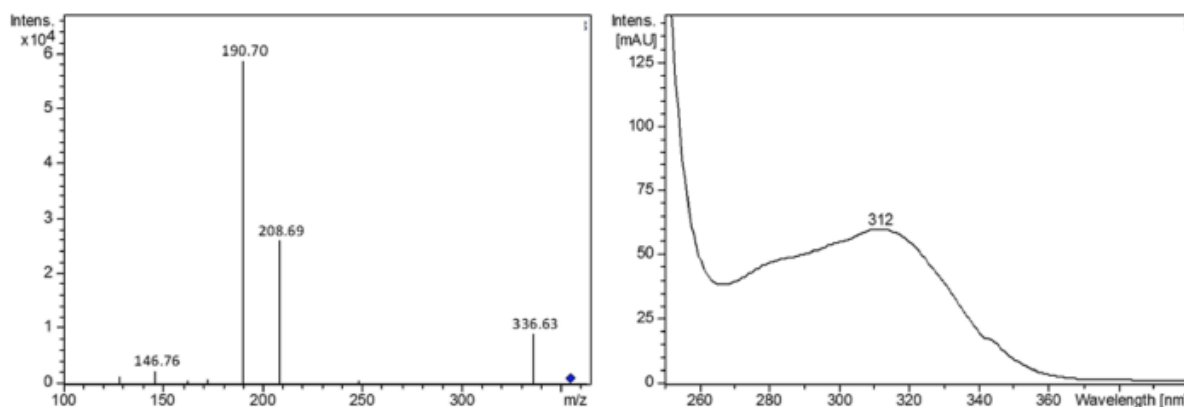
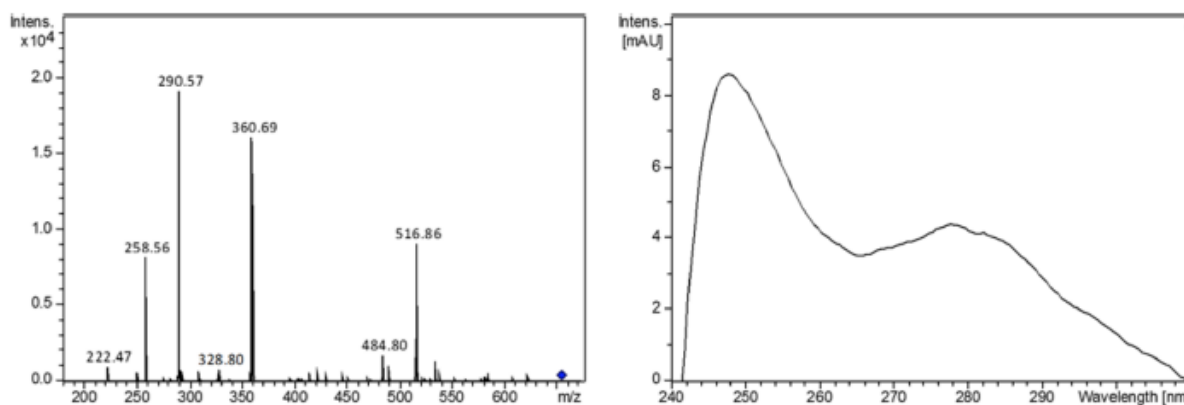
### Phenolic Acids and Derivatives

Peak 4 at  $m/z = 329$  is consistent with a vanillic acid hexoside in agreement with its UV spectrum with absorption maxima at 254 and 285 nm and its fragment at  $m/z = 167$  (vanillic acid). Additional fragment ions result from demethylation (− 14 Da) and decarboxylation (− 44 Da), which is characteristic of vanillic acid fragmentation.

Caffeic acid (peak 16) and *p*-coumaric acid (peak 19) were two of the main phenolic acids identified in alperujo (Obied et al. 2007b). Some of their hexosides (peaks 14 and 17) were also detected with their typical fragment ions featuring hexose loss and subsequent decarboxylation. The presence of chlorogenic acid (peak 15) and ferulic acid (peak 22) was confirmed by injection of internal standards.

A ferulic acid hexoside (peak 13) was detected as its formic acid ester at  $m/z = 401$  (an artifact due to the use of HCO<sub>2</sub>H for elution) and its typical fragment ions at  $m/z = 355$  and 193 featuring the successive losses of formic acid and hexose. Another *p*-coumaric acid derivative was detected at  $m/z = 355$  (peak 8) with its typical UV spectrum and MS fragments at  $m/z = 337$  (loss of water), 209, and 191 (loss of *p*-coumaric acid with or without water addition) (Fig. 5).

A compound with the same characteristics was already found in pineapple and assigned to *p*-coumaroyl aldarate (Steingass et al. 2015). In this work, it is evidenced for the first time in olive. Peak 30 ( $m/z = 551$ ), which displays major fragments at  $m/z = 507$  (−CO<sub>2</sub>), 389 (loss of a hexose unit), and 179 (caffeic acid) unit, was identified to caffeoyl-6'-secologanoside. Finally, peak 32 was identified as conselogoside, the analog of peak 30 with a *p*-coumaric acid unit (Rubio-Senent et al. 2013).

**Fig. 5** MS/MS and UV absorption spectra of *p*-coumaroyl aldarate identified in alperujo (compound 8)**Fig. 6** MS/MS and UV absorption spectra of ligstroside derivative identified in alperujo (compound 34)



## Flavonoids

With their major fragment at  $m/z = 285$  and their UV absorption maxima at 245 and 345 nm, peaks 23 and 24 ( $m/z = 593$ ) were identified as luteolin derivatives. The loss of 308 Da corresponds to the cleavage of a rutosyl moiety. Thus, both peaks are proposed to be luteolin-*O*-rutoside isomers (Leouifoudi et al. 2014).

## Iridoids and Derivatives

Iridoids are monoterpenes characterized by a cyclopentane ring. Ring opening leads to the secoiridoid class. Peaks 3, 9, 10, 18, 20, 21, 25, 27, 28, 29, 31, 33, 34, and 35 were identified as iridoid and secoiridoid derivatives. For instance, peak 3 ( $m/z = 537$ ) is loganic acid glucoside. Its fragments at  $m/z = 375$  and 331 feature hexose loss and subsequent decarboxylation. Peak 25 ( $m/z = 241$ ) was identified as elenolic acid in agreement with the literature (Lozano-Sánchez et al. 2014). Peak 9 ( $m/z = 407$ , fragments at  $m/z = 389, 375, 357, 313$ , and 161) was previously described by several authors (Cardoso et al. 2005; D'Antuono et al. 2014) and recently identified as 1- $\beta$ -glucopyranosyl acyclodihydroelenolic acid (Rubio-Senent et al. 2015). Peak 10 displays a molecular ion at  $m/z = 199$  and fragment ions at  $m/z = 181$  and 155 that are typical of water loss and decarboxylation. It may be ascribed to a hydroxylated derivative of decarboxymethyl elenolic acid. Peak 18 could be assigned to oleoside with its molecular ion at  $m/z = 389$  and fragment ions at  $m/z = 345, 209$ , and 165, respectively, due to decarboxylation, glucose elimination, and the combination of both. An oleoside deoxyriboside ( $m/z = 505$ , peak 20) was also detected (Peralbo-Molina et al. 2012). Peak 21 was identified as the oleuropein aglycone ( $m/z = 377$ , fragment ions at  $m/z = 197$  and 153 generated by glucose elimination and additional decarboxylation) (Cardoso et al. 2005; D'Antuono et al. 2014; Rubio-Senent et al. 2013). Tetrahydro-oleuropein (peak 27) was detected

with its molecular ion at  $m/z = 543$  and its fragment at  $m/z = 525$  (water loss). Peak 28 was ascribed to nüzhenide, an ester of elenolic acid glucoside and tyrosol glucoside commonly found in olive seeds. Its molecular peak ( $m/z = 731$ ) actually corresponds to the formate ester with fragments at  $m/z = 685$  (nüzhenide), 523 (loss of glucose), and 299 (tyrosol glucoside after loss of elenolic acid glucoside) (Silva et al. 2006, 2010). The identification of peak 29 ( $m/z = 701$ ) as oleuropein glucoside was consistent with the literature (Cardoso et al. 2005; Herrero et al. 2011). The hydroxy-decarboxymethyl oleuropein aglycone (peak 31) was also identified by comparison of its UV and mass spectra with literature data (Lozano-Sánchez et al. 2014; Suárez et al. 2008). Peak 34 ( $m/z = 655$ ) and peak 35 ( $m/z = 523$ ) were also detected at the end of the elution. Peak 34 may be a ligstroside derivative as the molecular ion at  $m/z = 655$  generated fragments at  $m/z = 517$  (loss of tyrosol), 361 (ligstroside after loss of glucose), 291 (ligstroside after loss of glucose and a  $C_3H_2O_2$  moiety), and 259 (ligstroside after loss of  $C_3H_2O_2$  and  $CH_3OH$  moieties) (Fig. 6). According to our knowledge, this potential ligstroside derivative has never been identified in alperujo juice. Peak 35 displays a molecular peak consistent with ligstroside although its fragments at  $m/z = 453, 421$ , and 299 rather suggest that it is an isomer (Cardoso et al. 2011). Finally, peak 33 ( $m/z = 539$ ) was identified as oleuroside, an oleuropein isomer, with a fragment ion at  $m/z = 377$  consistent with a hexose loss (Cardoso et al. 2011; Kanakis et al. 2013).

## Conclusion

Alperujo juice was analyzed by UHPLC-DAD-MS/MS after a direct injection to preserve the maximal integrity of phenolic compounds and other olive metabolites. Despite the great structural diversity and complexity of alperujo juice, 35 olive metabolites have been separated and identified in only 12 min, including a newly discovered compound (probably a ligstroside

derivative) and two compounds so far not reported in olive (*p*-coumaroyl aldarate and a verbascoside derivative). This analytical method confirms the high content of hydroxytyrosol and its glucosides in alperujo juice with a total concentration estimated at about 4 mM. This method is simple, fast, and effective and can thus be routinely applied in the lab and in the industry to the metabolic profiling of olive juice for the selection of samples according to their biophenol content.

**Acknowledgements** This work was supported by the SFR TERSYS, University of Avignon. We thank Mr. Jean-Benoît Hugues and his team (Moulin Castelas, Baux-de-Provence, France) for providing us the two-phase olive pomace.

**Funding Information** This study was funded by the SFR TERSYS, University of Avignon.

#### **Compliance with Ethical Standards**

**Conflict of Interest** Aurélie Malapert declares that she has no conflict of interest. Emmanuelle Reboul declares that she has no conflict of interest. Michèle Ioonis declares that she has no conflict of interest. Olivier Dangles declares that he has no conflict of interest. Valérie Tomao declares that she has no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed Consent** Not applicable.

## **References**

- Albuquerque JA, González J, García D, Cegarra J (2006) Measuring detoxification and maturity in compost made from “alperujo”, the solid by-product of extracting olive oil by the two-phase centrifugation system. *Chemosphere* 64:470–477
- Aranda E, García-Romera I, Ocampo JA, Carbone V, Mari A, Malorni A, Sannino F, De Martino A, Capasso R (2007) Chemical characterization and effects on *Lepidium sativum* of the native and bioremediated components of dry olive mill residue. *Chemosphere* 69:229–239
- Bouaziz M, Jemai H, Khabou W, Sayadi S (2010) Oil content, phenolic profiling and antioxidant potential of Tunisian olive drupes. *J Sci Food Agric* 90:1750–1758
- Cardinali A, Pati S, Minervini F, D’Antuono I, Linsalata V, Lattanzio V (2012) Verbascoside, isoverbascoside, and their derivatives recovered from olive mill wastewater as possible food antioxidants. *J Agric Food Chem* 60:1822–1829
- Cardoso SM, Guyot S, Marnet N, Lopes-da-Silva JA, Renard CM, Coimbra MA (2005) Characterisation of phenolic extracts from olive pulp and olive pomace by electrospray mass spectrometry. *J Sci Food Agric* 85:21–32
- Cardoso SM, Falcão SI, Peres AM, Domingues MRM (2011) Oleuropein/ligstroside isomers and their derivatives in Portuguese olive mill wastewaters. *Food Chem* 129:291–296
- Conseil Oléicole International (2013) *ECONOMIE: 1,5 milliards d’oliviers dans le monde—Olive Info*
- D’Antuono I, Kontogianni VG, Kotsiou K, Linsalata V, Logrieco AF, Tasioula-Margari M, Cardinali A (2014) Polyphenolic

characterization of olive mill wastewaters, coming from Italian and Greek olive cultivars, after membrane technology. *Food Res Int* 65:301–310

- Demeche S, Nadour M, Larroche C, Moulti-Mati F, Michaud P (2013) Olive mill wastes: biochemical characterizations and valorization strategies. *Process Biochem* 48:1532–1552
- El-Abbassi A, Kiai H, Hafidi A (2012) Phenolic profile and antioxidant activities of olive mill wastewater. *Food Chem* 132:406–412
- Giordano E, Dangles O, Rakotomanomana N, Baracchini S, Visioli F (2015) 3-O-Hydroxytyrosol glucuronide and 4-O-hydroxytyrosol glucuronide reduce endoplasmic reticulum stress in vitro. *Food Funct* 6:3275–3281
- Herrero M, Temirzoda TN, Segura-Carretero A, Quirantes R, Plaza M, Ibañez E (2011) New possibilities for the valorization of olive oil by-products. *J Chromatogr A* 1218:7511–7520
- Kalogerakis N, Politi M, Foteinis S, Chatzisyneon E, Mantzavinos D (2013) Recovery of antioxidants from olive mill wastewaters: a viable solution that promotes their overall sustainable management. *J Environ Manag* 128:749–758
- Kanakakis P, Termentzi A, Michel T, Gikas E, Halabalaki M, Skaltsounis A-L (2013) From olive drupes to olive oil. An HPLC-Orbitrap-based qualitative and quantitative exploration of olive key metabolites. *Planta Med* 79:1576–1587
- Kapellakis IE, Tsagarakis KP, Crowther JC (2008) Olive oil history, production and by-product management. *Rev Environ Sci Biotechnol* 7:1–26
- Leouifoudi I, Ziyad A, Amechrouq A, Oukerrou MA, Mouse HA, Mbarki M (2014) Identification and characterisation of phenolic compounds extracted from Moroccan olive mill wastewater. *Food Sci Technol Camp* 34:249–257
- Lozano-Sánchez J, Castro-Puyana M, Mendiola J, Segura-Carretero A, Cifuentes A, Ibañez E (2014) Recovering bioactive compounds from olive oil filter cake by advanced extraction techniques. *Int J Mol Sci* 15:16270–16283
- Niaounakis M, Halvadakis CP (2006) Olive processing waste management literature review and patent survey. Elsevier, Amsterdam; London
- Obied HK, Allen MS, Bedgood DR, Prenzler PD, Robards K (2005) Investigation of Australian olive mill waste for recovery of biophenols. *J Agric Food Chem* 53:9911–9920
- Obied HK, Bedgood DR, Prenzler PD, Robards K (2007a) Bioscreening of Australian olive mill waste extracts: biophenol content, antioxidant, antimicrobial and molluscicidal activities. *Food Chem Toxicol* 45:1238–1248
- Obied HK, Bedgood DR, Prenzler PD, Robards K (2007b) Chemical screening of olive biophenol extracts by hyphenated liquid chromatography. *Anal Chim Acta* 603:176–189
- Ollivier D, Pinatel C, Ollivier V, Artaud J (2014) Composition en acides gras et en triglycérides d'huiles d'olive vierges de 34 variétés et 8 Appellations d'Origine Françaises et de 2 variétés étrangères implantées en France: Constitution d'une banque de données (1ère partie). *Olivae* 36–48
- Peralbo-Molina Á, Priego-Capote F, Luque de Castro MD (2012) Tentative identification of phenolic compounds in olive pomace extracts using liquid chromatography–tandem mass spectrometry with a quadrupole–quadrupole–time-of-flight mass detector. *J Agric Food Chem* 60:11542–11550
- Priego-Capote F, Ruiz-Jiménez J, Luque de Castro M (2004) Fast separation and determination of phenolic compounds by capillary electrophoresis–diode array detection. *J Chromatogr A* 1045:239–246
- Rubio-Senent F, Rodríguez-Gutierrez G, Lama-Muñoz A, Fernández-Bolaños J (2012) New phenolic compounds

- hydrothermally extracted from the olive oil byproduct alperujo and their antioxidative activities. *J Agric Food Chem* 60:1175–1186
- Rubio-Senent F, Rodríguez-Gutiérrez G, Lama-Muñoz A, Fernández-Bolaños J (2013) Phenolic extract obtained from steam-treated olive oil waste: characterization and antioxidant activity. *LWT—Food Sci Technol* 54:114–124
- Rubio-Senent F, Martos S, Lama-Muñoz A, Fernández-Bolaños JG, Rodríguez-Gutiérrez G, Fernández-Bolaños J (2015) Isolation and identification of minor secoiridoids and phenolic components from thermally treated olive oil by-products. *Food Chem* 187:166–173
- Sanz M, Simón BF, Cadahía E, Estenuelas E, Muñoz AM, Hernández T, Estrella I, Pinto E (2012) LC-DAD/ESI-MS/MS study of phenolic compounds in ash (*Fraxinus excelsior* L. and *F. americana* L.) heartwood. Effect of toasting intensity at cooperage: LC-DAD/ESI-MS/MS of ash wood phenolic compounds. *J Mass Spectrom* 47:905–918
- Savaresi M, Demarco E, Sacchi R (2007) Characterization of phenolic extracts from olives (*Olea europaea* cv. *Pisciottana*) by electrospray ionization mass spectrometry. *Food Chem* 105:761–770
- Silva S, Gomes L, Leitao F, Coelho AV, Boas LV (2006) Phenolic compounds and antioxidant activity of *Olea europaea* L. fruits and leaves. *Food Sci Technol Int* 12:385–395
- Silva S, Gomes L, Leitão F, Bronze M, Coelho AV, Boas LV (2010) Secoiridoids in olive seed: characterization of nūzhenide and 11-methyl oleosides by liquid chromatography with diode array and mass spectrometry. *Grasas Aceites* 61:157–164
- Steingass CB, Glock MP, Schweiggert RM, Carle R (2015) Studies into the phenolic patterns of different tissues of pineapple (*Ananas comosus* [L.] Merr.) infructescence by HPLC-DAD-ESI-MS n and GC-MS analysis. *Anal Bioanal Chem* 407:6463–6479
- Suárez M, Macià A, Romero M-P, Motilva M-J (2008) Improved liquid chromatography tandem mass spectrometry method for the determination of phenolic compounds in virgin olive oil. *J Chromatogr A* 1214:90–99
- Suárez M, Romero M-P, Ramo T, Macià A, Motilva M-J (2009) Methods for preparing phenolic extracts from olive cake for potential application as food antioxidants. *J Agric Food Chem* 57:1463–1472
- Visioli F, Bernardini E (2011) Extra virgin olive oil's polyphenols: biological activities. *Curr Pharm Des* 17:786–804