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

Aurélia Malapert¹ · Emmanuelle Reboul² · Michèle Loonis¹ · Olivier Dangles¹ · Valérie Tomao¹

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°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 ($1.7\ \mu\text{m}$, $2.1 \times 50\ \text{mm}$). 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 $1\ \mu\text{L}$, and the column temperature was kept at 35°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) | λ_{max} (nm) | Calibration line | R^2 | LOD (ng/ μL) | LOQ (ng/ μL) |
|-------------------------|----------------------|-----------------------------|-------------------------------------|--------|--------------------------|--------------------------|
| 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) | λ_{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

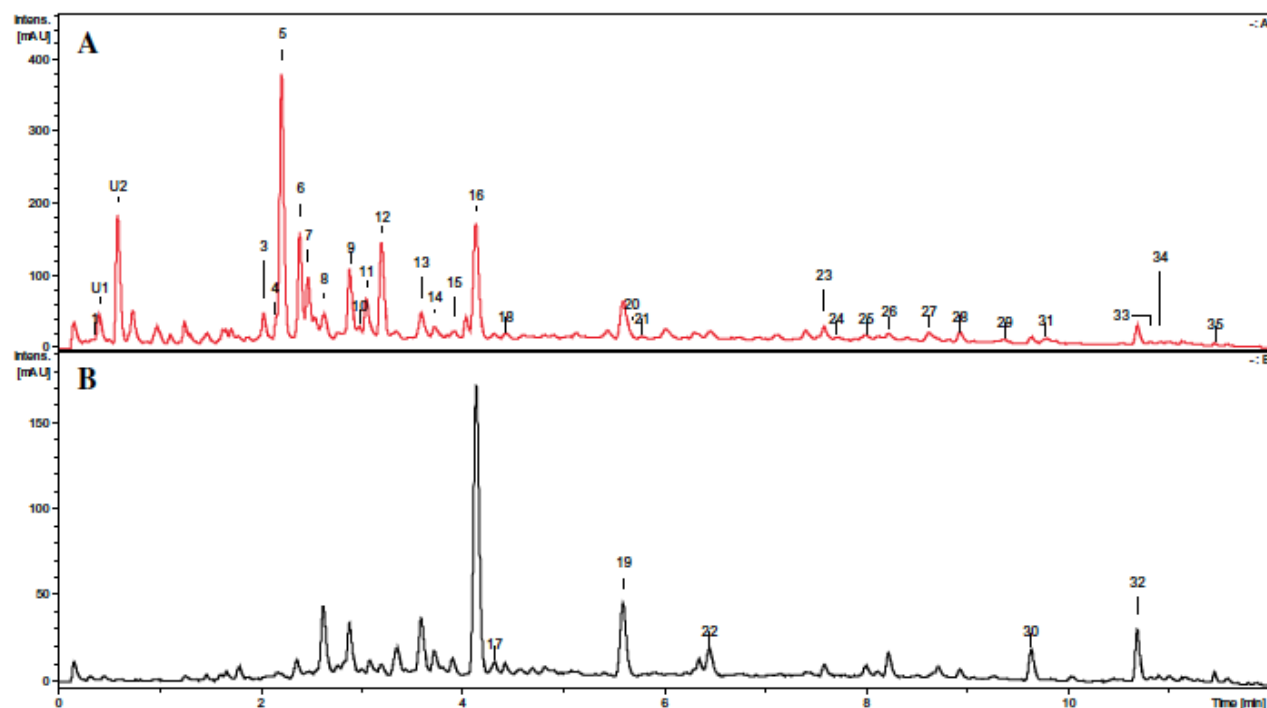


Fig. 1 UHPLC chromatograms of alperujo juice with detection at 280 nm (a) and 330 nm (b)

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

| No. | t_R (min) | Compound | λ_{max} (nm) | m/z | Fragments | References |
|----------------|-------------|--|----------------------|-------|---|--|
| 1 | 0.46 | Quinic acid | – | 191 | 173, 127, 111 | D'Antuono et al. (2014) |
| U ₁ | 0.52 | Unknown 1 | 232, 267 | 191 | 173, 127, 111 | |
| U ₂ | 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- β -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 | λ_{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

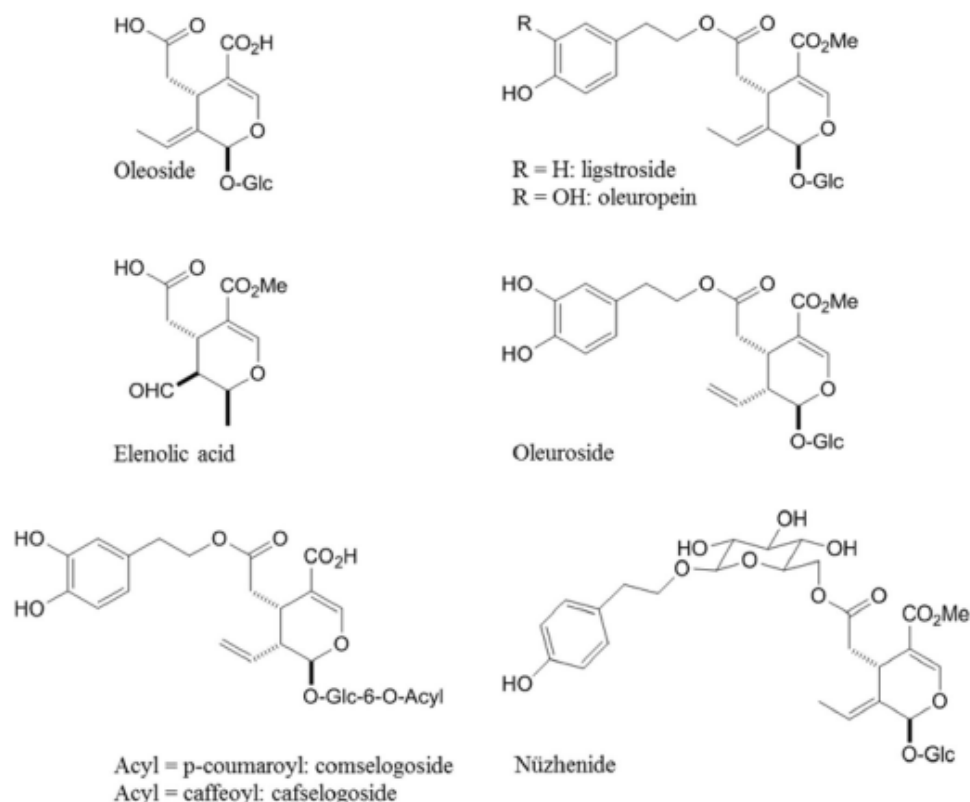
literature data. Tentative identifications of new biophenols were deduced from MS fragmentation.

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



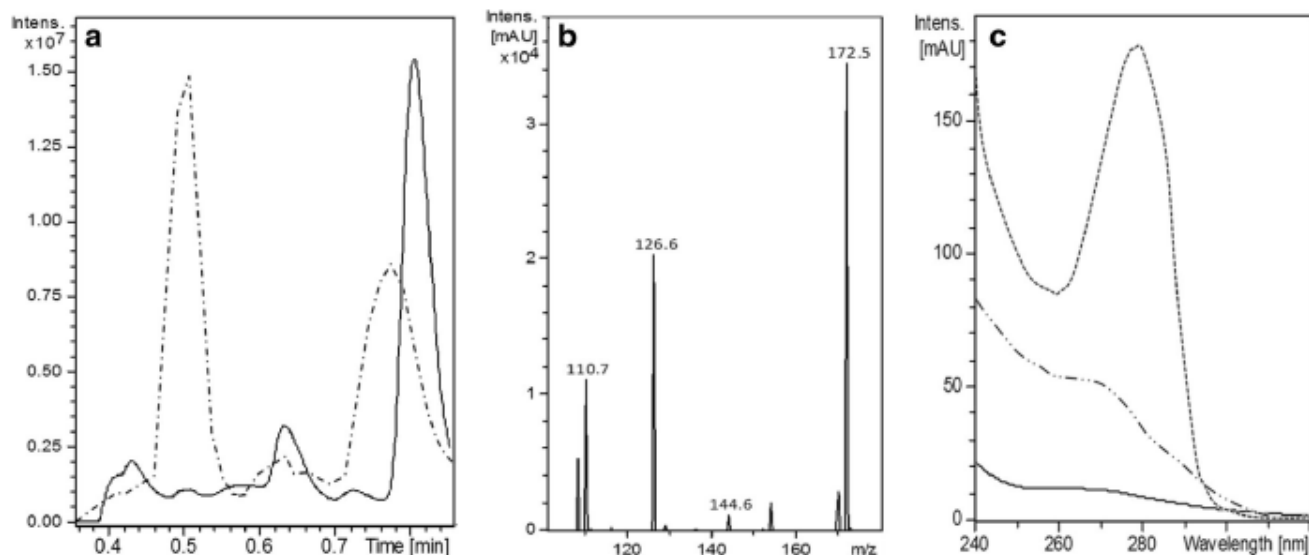


Fig. 3 Extracted ion chromatogram (EIC) at $m/z = 191$ and chromatogram at 280 nm (a), mass spectrum of quinic acid (b), and UV spectra of compounds at $m/z = 191$ (c). a Continuous line:

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

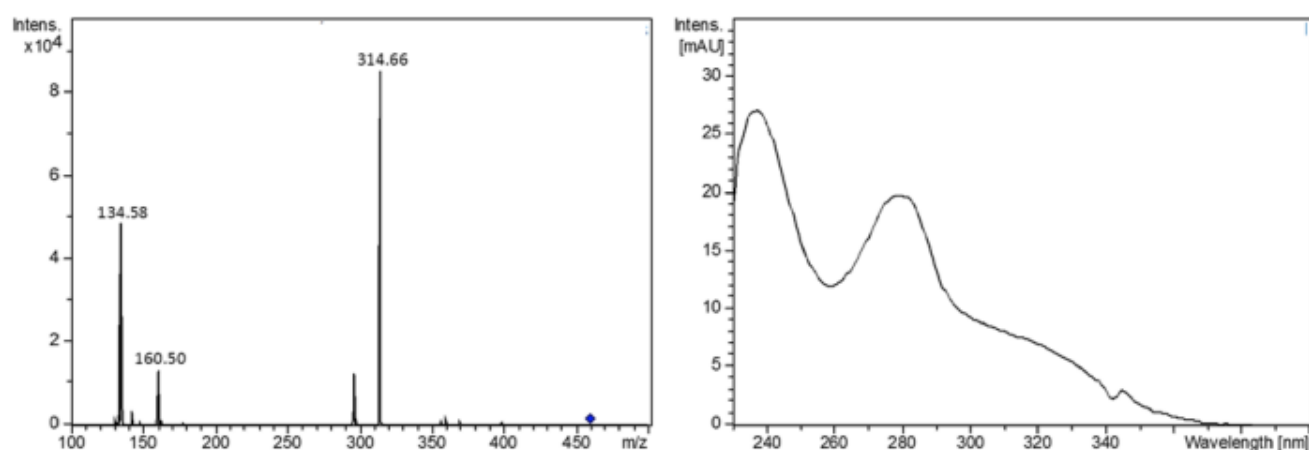


Fig. 4 MS/MS and UV absorption spectra of verbascoside—CA identified in alperujo (compound 11)

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 H₂O.

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).

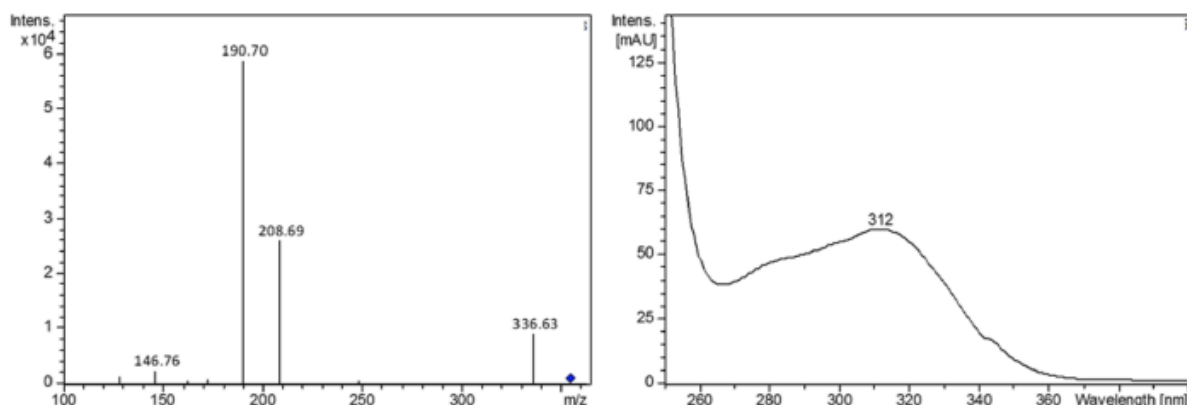
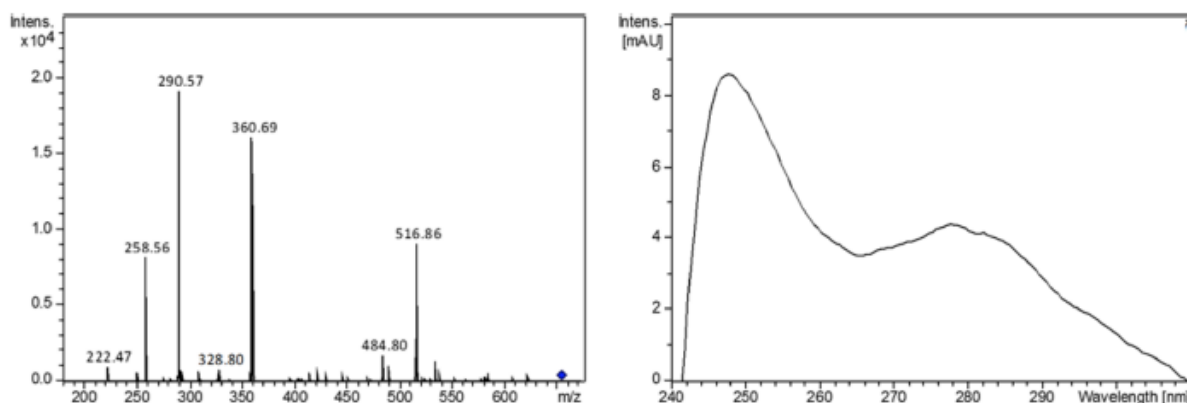
Phenolic Acids and Derivatives

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 HCO₂H 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 $m/z = 507$ (−CO₂), 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- β -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.

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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.

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