DIRECT CHEMICAL PROFILING OF OLIVE (*Olea europaea*) FRUIT EPICUTICULAR WAXES BY DIRECT ELECTROSPRAY-ULTRAHIGH RESOLUTION MASS SPECTROMETRY

Stefania Vichi[a,*], Nuria Cortés-Francisco[a], Agustí Romero[b], Josep Caixach[c]

[a] Food Science and Nutrition Department, XaRTA (Catalonian Reference Network on Food Technology), University of Barcelona, Food and Nutrition Torribera Campus, Av. Prat de la Riba, 171. 08921, S.ta Coloma de Gramenet, Spain.

[b] Olive Production, Oil Processing and Nut Trees, IRTA, Mas de Bover Crta. de Reus, El Morell. 43120, Constantí (Tarragona), Spain.

[c] Mass Spectrometry Laboratory/Organic Pollutants, IDAEA-CSIC. Jordi Girona, 18-26. 08034, Barcelona, Spain.

*Corresponding author

Phone: +34 93 4033794; Fax: +34 93 4035931; e-mail: stefaniavichi@ub.edu

ABSTRACT

In the present paper, an ESI-Orbitrap method is proposed for the direct chemical profiling of epicuticular wax (EW) from *Olea europaea* fruit. It constitutes a rapid and efficient tool suitable for a wide-ranging screening of a large number of samples. In a few minutes, the method provides a comprehensive characterization of total EW extracts, based on the molecular formula of their components. Accurate mass measurements are obtained by UHRMS, and compositional restrictions are set on the basis of the information available from previous studies of olive EW. By alternating positive and negative ESI modes within the same analysis, complementary results are obtained and a wide range of chemical species is covered. This provides a detailed compositional overview that otherwise would only be available by applying multiple analytical techniques.

Keywords: ultra high resolution mass spectrometry (UHRMS), direct injection, profiling, epicuticular waxes, olive.

Short title: Olive epicuticular waxes by direct ESI-UHRMS

INTRODUCTION

Epicuticular wax (EW) is the term used to define the external hydrophobic layer produced by plants as a barrier against the biotic and abiotic environment; such as water loss, herbivorous insects and fungal pathogens. It covers a fatty acid (FA)-based polyester structural backbone: cutin.[1] The highly hydrophobic compounds that compose the EW are deposited both within the cuticular matrix and on its surface as an unstructured film.[2,3] The morphology, as well as the composition of EW varies widely between species or cultivars[4-6] and is also affected by plant age and several environmental factors, such as heat, humidity and irradiance levels.[7,8] Surface wax has been also shown to play a role in various plant–insect interactions. [9]

Genetic and metabolite profiling has been carried out on a number of plant species, but very few studies have characterized olive fruit EW. Those studies evidenced the presence of alkanes, alcohols, aldehydes, alkyl and phenyl esters, triacylglycerols, triterpenols, FAs and triterpenic acids, among others. [7,10-12]

The most common methods for profiling plant EW are based on solvent extraction, purification and derivatization followed by gas chromatographic analysis.[1,5,13] Such EW profiling procedures are generally time-consuming and may require a relatively large sample. Advances in MS instrumentation have led to novel approaches for lipid analysis, in accordance with the increasing demand for high-throughput, fast and reliable analytical methods that are suitable for comprehensive screening.[14,15] Shotgun profiling of total lipid extracts allows a broad speciation without any chromatographic separation, thus enabling fast analysis without any (or only minimal) sample preparation. This direct chemical profiling of total lipid extracts can be performed via direct-infusion high- or ultrahigh-resolution mass spectrometry (UHRMS). This approach identifies lipids by accurately determined masses. It enables a vast number of data to be collected in a single run and shows great promise as a screening tool.

In the case of olive, rapid and broad screening of EW metabolites is desirable to extend knowledge of plant–insect interactions and to study the role of EW in resistance to pathogens and various environmental factors, as quickly and economically as possible. Such screening could then be used to prevent decreases in the quality of olive fruit and oil, and the resulting economic losses. In addition, EW are partially diluted in virgin oil during the extraction process in the mills, and these diluted waxes are considered as authenticity markers by EU and international regulations. EW can vary between cultivars, maturity and growing areas, resulting in a different level of potential incorporation in the virgin oil. There is a lack of studies on these topics due to the complexity of actual analytical methods for waxes, which could be solved by a rapid and reliable method.

In this work, we present an ESI-Orbitrap method for direct chemical profiling of EW from *Olea europaea* fruit. UHRMS is used to obtain accurate mass measurements from total EW extracts; compositional restrictions are set on the basis of the information available from previous studies of olive EW. Complementary mass spectral profiles and relative abundance information were successfully obtained from both positive and negative ESI, and various chemical families of compounds were identified on the basis of their elemental formulae. Olive samples from six different varieties were analyzed to assess the abundance of different EW components, expressed as relative intensity.

MATERIAL AND METHODS

Chemicals. Reagents were of mass spectrometry grade. Dichloromethane, methanol (MS SupraSolv®) and chloroform were purchased from Merck (Darmstadt, Germany). Ammonium formate was from by Sigma-Aldrich (St. Louis, MO, USA). Nitrogen (Alphagaz, purity 99.999%, Air Liquide) was used in the Orbitrap-Exactive as the nebulization gas.

Samples.

Olive fruits were hand-picked in November 2010 from the collection of IRTA-Mas de Bover (Constantí, Spain), and were of six varieties: Arbequina, Picual, Morrut, Sevillenca, Canetí and Gordal Sevillana. A further Sevillenca olive sample was obtained in August 2011.

For the extraction of EW, each olive fruit was placed into a screw-cap tube, covered with 3 mL of chloroform and gently stirred for 1 min. Before analysis, the extract was diluted 1:100 with dichloromethane:methanol (70:30). Each extract was injected in duplicate.

Direct Electrospray–Ultra-high resolution mass spectrometry (ESI-UHRMS)

Flow injection analysis (FIA) of 5 µL of the samples was carried out with an Orbitrap-Exactive HCD (Thermo Fisher Scientific, Bremen, Germany) equipped with an electrospray source (H-ESI II). The LC system consisted of a Surveyor MS Plus pump (Thermo Fisher Scientific, San Jose, CA, USA). The mobile phase was methanol:dichloromethane (80:20) with ammonium formate (20 mM) at a flow rate of 50 µL/min.

Optimization was carried out using a real EW extract prepared in methanol:dichloromethane (70:30) and analyzed by infusion in the Orbitrap-Exactive in full scan mode. The parameters optimized were spray voltage, capillary voltage, skimmer voltage, tube lens voltage, sheath gas flow rate, auxiliary gas flow rate, capillary temperature, and heater temperature. Finally, mass spectra were acquired in full scan positive and negative ionization modes. Optimized conditions in positive mode were: spray voltage 3.00 kV, capillary voltage 35 V, skimmer voltage 18 V, tube lens voltage 90 V. Optimized conditions in negative ionization mode were: spray voltage -3.00 kV, capillary voltage -35 V, skimmer voltage -18 V, tube lens voltage -90 V. In both cases, the sheath gas flow rate was set at 20 au (arbitrary units) and the auxiliary gas flow rate was 2 au. Capillary and heater temperatures were fixed at 275ºC and 30ºC, respectively. The mass range was set to *m/z* 120-1200 and the total analysis time was 6 minutes, including blanks.

The automatic gain control was used to fill the C-trap and gain accuracy in mass measurements (ultimate mass accuracy mode, $5x10^5$ ions). Ultrahigh resolution defined as R: 100,000 (*m/z* 200, FWHM) was set.

The mass peaks considered were single charged ions with relative intensities ≥0.1% and absolute intensity $>10^3$. These peaks were exported to peak lists and from these lists feasible elemental formulae were generated. In order to obtain a limited list of possible candidate formulae from a mass measurement, heuristic criteria based on accuracy in mass measurement, the number of ring plus double bond equivalents (RDBE), the charge, the adducts formed and the elements in use was applied. Different restrictive criteria were set to generate reliable elemental formulae: C ≤ 150, H ≤ 400, O ≤ 10, N ≤ 1 and RDBE ≥ -1.5. The molecular formula calculation was performed with Xcalibur 2.1 (Thermo Fisher Scientific, Bremen, Germany) and the data was later analyzed using excel files. Mass accuracies better than 2 ppm were achieved.

The elemental compositions assigned were searched for in lists of compounds or lipid classes previously identified in olive EW.

RESULTS AND DISCUSSION

Despite the compositional complexity of olive fruit EW, comprehensive analysis was performed by direct ESI-UHRMS. First of all, as we previously reported [16] the mobile phase constituted by methanol:dichloromethane 80:20 prevented the carry over generally observed in the analysis of hydrophobic substances when using conventional LC-MS solvents, and enabled a good sensitivity. At these conditions two blanks were analysed after each sample in order to ensure that no cross-contamination occurred.

Moreover, although other ionization sources, such as APCI and APPI, have been described as the most suitable for apolar compounds,[15,16,29] the present results confirmed the versatility of

the widely used ESI source and the good performances in the ionization of lipid compounds, as previously observed for triacylglycerols. [16]

The strategy was intended to provide rapid and comprehensive characterization of EW composition and was based on accurately determined masses of EW constituents. A large number of heterogeneous EW constituents, comprising several isobaric species that cannot be distinguished by conventional low resolution MS, were distinguished and directly analyzed in total extracts. Using this approach, once the accurate mass of the ions was measured, additional information was needed to derive appropriate elemental compositions. Restrictions on the number of elements, isotopic pattern, RDBE, nitrogen rule, mass tolerance and charge were set to obtain a suitable list of possible candidates, according to Cortés-Francisco and Caixach.[17] Compositional restrictions were set on the basis of previous information on the sample, i.e. olive EW constituents or classes of compounds previously reported in olive fruit [7,10-11] or in olive oil by conventional or non-conventional analysis. [18-21]

As chemical species containing heteroatoms other than oxygen have never been reported in EW, the treatment of the data was greatly simplified by assuming that EW classes only comprised species composed of C, H and O, including N for NH_4^+ adducts in positive ESI. The principal EW classes, whose general structures are shown in **Figure 1**, could then be clustered into several groups according to the number of oxygen atoms, RDBE and carbon number (**Table 1**). These three variables were set on the basis of the natural occurrence of each molecular species. Predominant FAs in olive fruit, and in plants in general, have an even number of carbon atoms and an unsaturation number between 0 and 3; and olive fruit EW can contain FAs of up to 34 C atoms, with a predominance of C18 species.[7,11] Consequently, identification of FA derivatives such as acylglycerols and wax esters was carried out assuming these parameters and taking into account the number of oxygen atoms and the RDBE involved in each ester bond. Compositional restrictions, together with mass tolerance ≤2 ppm, allowed

us to obtain only one possible candidate for each accurate mass measured. **Figure 2** shows the spectra of olive EW obtained by direct ESI-UHRMS of olive EW, for both positive and negative ionization, and summarizes the compositional information obtained. Typical clusters of triacylglycerols (TAGs), diacylglycerols (DAGs) and wax esters were present in the ESI+ spectra, as were triterpenic acids, as major signals. These molecular classes were easily distinguished in the spectrum according to the *m/z* ratio. In particular, the expanded ESI+ spectrum (**Figure 3**) shows the complexity of the clusters corresponding to wax esters in olive EW. A much less intricate spectrum was obtained in negative ESI (**Figure 2**), where FAs, triterpenic acids and some phenolic compounds can be distinguished.

Although cross-class comparison is not suitable, due to suppression phenomena and differences in the ionization capacity of each lipid class, differences in the abundance of EW classes or single compounds may be used for comparative analysis of samples, providing a detailed compositional view otherwise available only by applying multiple analytical techniques.

Tables 2 and **3** list the detailed molecular composition of olive fruit EW provided by this rapid and comprehensive method. These tables are limited to the compounds which could be tentatively identified on the basis of their elemental composition and previous information on olive EW. In positive mode (**Table 2**), the spectra of organic extracts showed elemental compositions that could be attributed to esters, FAs, hydroxylated and dicarboxylic FAs, mono-, di- and triacylglycerols, triterpenic acids, and hydrocarbons; all them detected as NH_4^+ adducts. Most of these molecular formulae matched with acylglycerols and wax esters species (**Table 2**). More than thirty elemental formulae were attributable to wax esters from C31 to C44. Among them, benzyl esters of FAs from C22 to C30 were detected, in agreement with Bianchi et al., [18-21] with C26 and C28 being the most abundant, in agreement with Biedermann et al.[18] Moreover, diterpene esters such as geranylgeranyl and phytyl esters[7,12,19] were

detected for FAs from C16 to C22. Finally, formulae matching with aliphatic waxes from C34 to C44 were found. [7,19-21] Moreover, the elemental formulae of eight monoacylglycerols (MAGs), fifteen DAGs and more than thirty TAGs were identified in the positive ESI spectrum. FAs from C16 to very-long-chain FAs such as C32, as reported by Bianchi et al.,[7] were tentatively identified in the extract using positive ESI mode. Moreover, for the first time, series of compounds matching mono- and dihydroxylated FAs, and dioic FAs, from C16 to C28 were tentatively identified in olive fruit EW (**Table 2**). They could originate from the cutin polymer matrix, which consists mainly of esterified hydroxy, polyhydroxy, epoxy and dioic FAs.[22-24] These compounds had not previously been reported in the EW of olive fruit, but they have been reported in other fruit [25] and plants.[26] Finally, among the most abundant constituents of olive EW triterpenic acids such as oleanolic, maslinic and ursolic acids, were identified (**Table 1**).

UHRMS spectra in negative ionization mode showed a smaller number of compounds, mainly FAs, hydroxylated and dicarboxylic FAs, triterpenic acids and phenolic compounds, with a predominance of triterpenic acids and in some cases of phenolic compounds (**Table 3**). The presence of secoiridoid derivatives and simple phenols in the extract may be due to the partial rupture of the cuticule structure, although the presence of phenols embedded in the cutine matrix has been reported in other plants.[23,24] It is worth mentioning the detection of the whole series of FAs from C7 to C30 in negative mode, including saturated, and mono-, di- and tri-unsaturated species, some of them showing important relative abundances. Moreover, the molecular attribution of hydroxylated and dicarboxylic FAs in positive mode was confirmed in negative mode; thus corroborating this identification. In the same way, triterpenic acids were identified in both positive and negative ESI, and in both cases presented the highest intensities. Although positive UHRMS spectra included most of the compounds identified in negative ionization, the latter provided more intense signals and more complete information

on FAs and their derivatives. We can thus state that the polarity switching performed within analysis of the same sample allowed us to obtain complementary results and cover a wide range of chemical species.

In conclusion, the direct ESI-UHRMS method proposed here for the direct chemical profiling of EW from *Olea europaea* fruits provided a rapid and detailed characterization of a large number of heterogeneous EW constituents present in total extracts. It provided a detailed compositional view otherwise available only by applying multiple analytical techniques. Complementary mass spectral profiles and relative abundance information were successfully obtained by alternating positive and negative ESI, and compounds from various chemical families were identified on the basis of their elemental formulae, obtained from the accurately determined masses of EW constituents and after compositional restrictions set according to data in the literature. This method enables large numbers of samples to be analyzed for wide-ranging screening and it could be applied or adapted to study other plant species.

Acknowledgements

This study was supported by the Spanish *Ministerio de Economia y Competitividad* through an INIA Research Project (RTA-2010-00013-C02-C2) and through the Ramón y Cajal program (RYC-2010-07228).

References

[1] R. Jetter, L. Kunst, L. Samuels. In: *Annual Plant Reviews, Biology of the Plant Cuticle*, (Eds: M. Riederer, C. Muller) Wiley & Sons, **2008**.

[2] Fahn A. *Plant anatomy*. Pergamon Press, Oxford **1990**.

[3] L. Kunst, A.L. Samuels. Biosynthesis and secretion of plant cuticular wax. *Progr. Lipid Res.* **2003***, 42,* 51.

[4] H.M. Hull, H.L. Morton, J.R. Wharrie. Environmental infuences on cuticle development and resultant foliar penetration. *The Botanical Rev*. **1975**, *41*, 421.

[5] G. Bianchi, G. Vlahov, C. Anglani, C. Murelli. Epicuticular wax of olive leaves. *Phytochem.* **1993***, 32*, 49.

[6] T. Shepherd, G.V. Robertson, D.W. Griths, A.N.E. Birch, G. Duncan. Effects of environment on the composition of epicuticular wax from kale and swede. *Phytochem.* **1995***, 40*, 407.

[7] G. Bianchi, C. Murelli, G. Vlahov. Surface waxes from olive fruits. *Phytochem.* **1992**, *31*,3503.

[8] M. Riederer , C. Markstaedter. Plant cuticles. Oxford: Bios Scientific Publishers, **1996**.

[9] S.D. Eigenbrode. In: Plant cuticles. (Ed: G. Kerstiens) Oxford: Bios Scientific Publishers, **1996**, pp. 201-221.

[10] G. Bianchi, N. Pozzi, G. Vlahov. Pentacyclic triterpene acids in olives. *Phytochem.* **1994**, *37*, 205.

[11] G. Bianchi, G. Vlahov. Composition of Lipid Classes in the Morphologically Different Parts of the Olive Fruit, cv. Coratina (*Olea europaea Linn*.). *Lipid/Fett.* **1994***, 96*, 72.

[12] G. Vlahov, G. Rinaldi, P. Del Re, A.A. Giuliani. ^{13}C nuclear magnetic resonance spectroscopy for determining the different components of epicuticular waxes of olive fruit (*Olea europaea*) Dritta cultivar. *Anal. Chim. Acta*, **2008**, *624*, 184.

[13] M.C. Suh, A. L. Samuels, R. Jetter, L. Kunst, M. Pollard, J. Ohlrogge, F. Beisson. Cuticular lipid composition, surface structure, and gene expression in arabidopsis stem epidermis, *Plant Physiol.* **2005**, *139*, 1649.

[14] X. Han, R.W. Gross. Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. *Mass Spectrom. Rev.* **2004***, 24,* 367.

[15] R.W. Gross, X. Han. Shotgun lipidomics of neutral lipids as an enabling technology for elucidation of lipid-related diseases. *Am. J. Physiol. Endocrinol Metab.* **2009***, 297*, 297.

[16] S. Vichi, N. Cortés-Francisco, J. Caixach. Ultrahigh resolution mass spectrometry and accurate mass measurements for high-throughput food lipids profiling. *J. Mass Spectrom.* ***2012****, 47,* 1177.

[17] N. Cortés-Francisco, J. Caixach. in Chromatographic Analysis of the Environment: Spectrometry Based Approaches, 4th Edition. (Eds: M.L. Leo Nollet , D. Lambropoulou) CRC Press Boca Raton, Florida, USA*, in press.*

[18] M. Biedermann, A. Bongartz, C. Mariani, K. Grob. Fatty acid methyl and ethyl esters as well as wax esters for evaluating the quality of olive oils. *Eur. Food Res. Technol*., **2008**, *228*, 65.

[19] B. Reiter, E. Lorbeer. Analysis of the Wax Ester Fraction of Olive Oil and Sunflower Oil by Gas Chromatography and Gas Chromatography–Mass Spectrometry. *JAOCS,* **2001***, 78*, 881.

[20] M.C. Pérez-Camino, W. Moreda, R. Mateos, A. Cert. Simultaneous determination of long-chain aliphatic aldehydes and waxes in olive oil. *J. Chromatogr. A.* **2003***, 983*, 283.

[21] A.M. Giuffré. Wax ester variation in olive oils produced in Calabria (Southern Italy) during olive ripening. *JAOCS*, **2014**, *91*, 1355.

[22] S. Peschel, R. Franke, L. Schreiber, M. Knoche. Composition of the cuticle of developing sweet cherry fruit. *Phytochem.,* **2007***, 68*,1017.

[23] M. Pollard, F. Beisson, Y. Li, J.B. Ohlrogge. Building lipid barriers: biosynthesis of cutin and suberin. *Trends Plant Sci.,* **2008***, 13*, 236.

[24] L. Schreiber. Transport barriers made of cutin, suberin and associated waxes. *Trends Plant Sci.*, **2010**, *15*, 546.

[25] G. Eglinton, D. H. Hunneman. Gas chromatographic-mass spectrometric studies of long chain hydroxy acids-I. The constituent cutin acids of apple cuticle. *Phytochem.,* **1968***, 7,* 31.

[26] P.J. Holloway, A.H.B. Deas. Occurrence of positional isomers of dihydroxyhexadecanoic acid in plant cutins and suberins. *Phytochem.,* **1971***, 10*, 2781.

Figure captions:

Figure 1. General structure of some of the chemical families detected in olive fruit EW by direct ESI-UHRMS. 1: benzyl esters; 2: alkyl esters; 3: phytyl esters; 4: geranylgeranyl esters; 5: hydroxyl FAs; 6: dioic FAs. R: $-CH_2-(CH_2)_n-CH_3$.

Figure 2. Positive and negative ESI-UHRMS spectra of Morrut olive EW extracts showing the main classes of EW components. Elemental formulae, RDBE and mass error are shown. R: 100,000 (*m/z* 200, FWHM).

Figure 3. Expanded ESI+ spectrum of Morrut olive EW extracts corresponding to the main wax ester clusters. Elemental formulae, RDBE and mass error are shown. R: 100,000 (*m/z* 200, FWHM).

Table 1. Compositional restrictions applied for the assignment of EW classes' molecular formulae.

Compound classes[a]	O[b]	C[c]	N[d] (ESI+)	Odd/even C[e]	RDBE[f] ESI+[g]	RDBE[f] ESI-
FA	2	≤32	≤1	Mainly even	-0.5-2.5	1.5-4.5
MAG	4	≤35	≤1	Mainly odd	-0.5-2.5	-
DAG	5	≤70	≤1	Mainly odd	0.5-6.5	-
TAG	6	≤100	≤1	Mainly odd	1.5-10.5	-
Benzyl esters	2	≤40	≤1	Mainly odd	3.5	-
Phytyl esters	2	≤52	≤1	Mainly even	0.5-2.5	-
Geranylgeranyl esters	2	≤52	≤1	Mainly even	3.5	-
Alkyl esters	2	≤46	≤1	Mainly even	-0.5-2.5	-
Hydrocarbons	0		≤1			-
OH FA	3	≤32	≤1	Mainly even	-0.5-2.5	1.5-4.5
di-OH FA	4	≤32	≤1	Mainly even	-0.5-2.5	1.5-4.5
Dicarboxylic FA	4	≤32	≤1	Mainly even	0.5-2.5	2.5-5.5
Triterpenic acids	3-4	30	≤1		5.5	7.5

[a]: EW classes of compounds as reported in previous studies[7,10-11,14-17]; [b]: number of oxygen atoms; [c]: number of carbon atoms; [d]: number of nitrogen atoms due to the formation of the NH_4^+ adduct in positive ESI; [e]: predominance of odd/even carbon number in agreement with the natural occurrence of compounds; [f]: rings and double bonds equivalents calculated as RDBE= C-1/2(H)+1/2(N)+1; [g]: corresponding to NH_4^+ adducts.

Table 2. EW metabolites of *Olea europaea* fruits detected in positive mode by direct-ESI-UHRMS. Mass accuracy and tentative identification according to their molecular formula are also shown, as well as relative intensity ranges within six olive varieties.

Theoretical mass [a]	Δ (ppm)	RDBE [b]	Formula $[M+NH4]^+$	Relative intensity [c] MIN	Relative intensity [c] MAX	Tentative identifcation
Benzyl esters						
476.4462	0.8	3.5	$C_{31}H_{58}O_2N$	0.0	2.4	C24-benzyl ester
490.4619	-0.5	3.5	$C_{32}H_{60}O_2N$	0.0	0.3	C25-benzyl ester
504.4775	0.8	3.5	$C_{33}H_{62}O_2N$	5.8	38.9	C26-benzyl ester
518.4932	0.6	3.5	$C_{34}H_{64}O_2N$	0.4	2.2	C27-benzyl ester
532.5088	0.8	3.5	$C_{35}H_{66}O_2N$	1.5	10.7	C28-benzyl ester
560.5401	0.5	3.5	$C_{37}H_{70}O_2N$	0.0	0.9	C30-benzyl ester
Phytyl and geranyl esters						
546.5245	0.1	3.5	$C_{36}H_{68}O_2N$	0.0	0.4	Geranylgeranyl-C16:0
550.5558	0.8	1.5	$C_{36}H_{72}O_2N$	1.7	20.8	phytyl-C16:1/C18:1-C18:1/C18:0-C18:2
552.5714	0.7	0.5	$C_{36}H_{74}O_2N$	0.2	1.0	phytyl-C16:0/C18:0-C18:1
576.5714	0.5	2.5	$C_{38}H_{74}O_2N$	0.0	1.5	phytyl-C18:2/C18:1-C20:0/C16:1-C22:2
578.5871	0.9	1.5	$C_{38}H_{76}O_2N$	1.1	4.5	phytyl-C18:1/C18:2-C20:0/C16:1-C22:1
580.6027	0.9	0.5	$C_{38}H_{78}O_2N$	1.7	2.5	Phytyl-C18:0/C18:1-C20:0/C16:1-C22:0
602.5871	0.9	3.5	$C_{40}H_{76}O_2N$	0.0	0.5	geranylgeranyl-C20:0
608.6340	0.9	0.5	$C_{40}H_{82}O_2N$	12.1	32.1	phytyl-C20:0/C18:1-C22:0/C20:1-C20:1
622.6497	1.1	0.5	$C_{41}H_{84}O_2N$	0.6	1.5	Phytyl-C23:0
630.6184	1.0	3.5	$C_{42}H_{80}O_2N$	0.0	0.7	geranylgeranyl-C22:0
634.6497	0.9	1.5	$C_{42}H_{84}O_2N$	7.1	36.9	phytyl-C22:1/C20:1-C22:1
636.6653	0.8	0.5	$C_{42}H_{86}O_2N$	8.5	17.4	Phytyl-C22:0
664.6966	1.2	0.5	$C_{44}H_{90}O_2N$	0.2	1.4	phytyl-C24:0/C22:1-C22:0/C18:1-C24:0
Alkyl esters						
522.5245	0.6	1.5	$C_{34}H_{68}O_2N$	0.0	0.4	C34:2 (C16:1-C18:1/C16:0-C18:2)
524.5401	0.3	0.5	$C_{34}H_{70}O_2N$	0.0	0.9	C34:1 (C16:0-C18:1)
536.5401	1.6	1.5	$C35H_{70}O_2N$	0.0	0.4	C35:2 (C17:1:C18:1/C17:0-C18:2)
538.5558	0.9	0.5	$C_{35}H_{72}O_2N$	0.0	0.4	C35:1 (C17:0:C18:1/C17:1-C18:0)
548.5401	0.7	2.5	$C_{36}H_{70}O_2N$	0.0	1.4	C36:3 (C18:1-C18:2/C18:0-C18:3)
582.6184	0.2	-0.5	$C_{38}H_{80}O_2N$	0.2	2.8	C38:0 (C18:0-C20:0/C16:0-C22:0)
594.6184	0.8	0.5	$C_{39}H_{80}O_2N$	0.2	0.7	C39:1
604.6027	0.9	2.5	$C_{40}H_{78}O_2N$	1.9	9.3	C40:3 (C18:3-C22:0)
606.6184	0.8	1.5	$C_{40}H_{80}O_2N$	10.3	47.3	C40:2 (C18:2-C22:0)
632.6340	1.0	2.5	$C_{42}H_{82}O_2N$	1.3	9.1	C42:3 (C18:2-C22:1/C18:3-C22:0)
660.6653	1.7	2.5	$C_{44}H_{86}O_2N$	0.0	0.7	C44:3 (C18:3-C24:0)
Triacylglycerols (TAG)						
754.6919	1.2	1.5	$C_{46}H_{92}O_6N$	0.0	1.0	C43:0
766.6919	1.2	2.5	$C_{47}H_{92}O_6N$	0.0	0.6	C44:1
768.7076	1.1	1.5	$C_{47}H_{94}O_6N$	0.0	0.7	C44:0
780.7076	1.5	2.5	$C_{48}H_{94}O_6N$	0.0	0.9	C45:1
782.7232	1.6	1.5	$C_{48}H_{96}O_6N$	0.0	1.2	C45:0
792.7076	1.8	3.5	$C_{49}H_{94}O_6N$	0.4	1.0	C46:2
794.7232	1.5	2.5	$C_{49}H_{96}O_6N$	0.5	1.4	C46:1
796.7389	1.6	1.5	$C_{49}H_{98}O_6N$	0.0	1.4	C46:0
806.7232	1.6	3.5	$C_{50}H_{96}O_6N$	0.3	1.0	C47:2
808.7389	1.9	2.5	$C_{50}H_{98}O_6N$	0.4	1.5	C47:1
818.7232	1.7	4.5	$C_{51}H_{96}O_6N$	0.0	0.7	C48:3
820.7389	1.6	3.5	$C_{51}H_{98}O_6N$	0.5	1.8	C48:2
822.7545	1.2	2.5	$C_{51}H_{100}O_6N$	0.0	1.8	C48:1
834.7545	1.3	3.5	$C_{52}H_{100}O_6N$	0.0	1.2	C49:2
846.7545	1.6	4.5	$C_{53}H_{100}O_6N$	0.3	1.0	C50:3
848.7702	1.6	3.5	$C_{53}H_{102}O_6N$	0.9	2.4	C50:2
850.7858	1.8	2.5	$C_{53}H_{104}O_6N$	0.0	3.2	C50:1
860.7702	1.5	4.5	$C_{54}H_{102}O_6N$	0.0	0.2	C51:3
862.7858	1.6	3.5	$C_{54}H_{104}O_6N$	0.0	0.7	C51:2
872.7702	0.7	5.5	$C_{55}H_{102}O_6N$	0.3	3.2	C52:4
874.7858	1.5	4.5	$C_{55}H_{104}O_6N$	1.1	8.0	C52:3
876.8015	1.4	3.5	$C_{55}H_{106}O_6N$	2.9	23.4	C52:2
888.8015	1.4	4.5	$C_{56}H_{106}O_6N$	0.0	0.3	C53:3
890.8171	1.6	3.5	$C_{56}H_{108}O_6N$	0.0	0.1	C53:2
896.7702	1.2	7.5	$C_{57}H_{102}O_6N$	0.0	1.9	C54:6
898.7858	1.7	6.5	$C_{57}H_{104}O_6N$	0.0	5.5	C54:5
900.8015	1.4	5.5	$C_{57}H_{106}O_6N$	1.1	19.2	C54:4
902.8171	1.3	4.5	$C_{57}H_{108}O_6N$	5.1	72.6	C54:3
930.8484	0.7	4.5	$C_{59}H_{112}O_6N$	0.0	1.6	C56:3
932.8641	1.9	3.5	$C_{59}H_{114}O_6N$	0.0	0.3	C56:2
934.8797	1.4	2.5	$C_{59}H_{116}O_6N$	0.0	0.6	C56:1
Fatty acids (FA)						

662.6810	1.1	1.5	$C_{44}H_{88}O_2N$	0.4	3.9	C44:2 (C22:1-C22:1/C18:2-C24:0)
Hydrocarbons						
200.2373	1.07	-0.5	$C_{13}H_{30}N$	0.0	0.0	C13:1
214.2529	1.2	-0.5	$C_{14}H_{32}N$	0.0	1.4	C14:1
242.2842	1.27	-0.5	$C_{16}H_{36}N$	0.0	0.0	C16:1
270.3155	1.27	-0.5	$C_{18}H_{40}N$	0.0	0.4	C18:1
284.3312	0.5	-0.5	$C_{19}H_{42}N$	0.0	1.5	C19:1
298.3468	0.97	-0.5	$C_{20}H_{44}N$	0.0	0.0	C20:1
312.3625	0.74	-0.5	$C_{21}H_{46}N$	0.2	1.5	C21:1
326.3781	0.91	-0.5	$C_{22}H_{48}N$	3.0	25.4	C22:1
340.3938	0.76	-0.5	$C_{23}H_{50}N$	0.4	3.2	C23:1
368.4251	0.85	-0.5	$C_{25}H_{54}N$	1.9	19.6	C25:1
466.5346	1.53	-0.5	$C_{32}H_{68}N$	0.0	0.4	C32:1
494.5659	1.33	-0.5	$C_{34}H_{72}N$	0.3	1.8	C34:1
522.5972	0.5	-0.5	$C_{36}H_{76}N$	0.7	4.6	C36:1
536.6129	0.88	-0.5	$C_{37}H_{78}N$	0.0	0.0	C37:1
550.6285	0.62	-0.5	$C_{38}H_{80}N$	0.8	4.5	C38:1
Monoacylglycerols (MAG)						
370.2952	0.74	2.5	$C_{21}H_{40}O_4N$	0.0	0.3	C18:3
372.3108	1.19	1.5	$C_{21}H_{42}O_4N$	0.0	0.5	C18:2
374.3265	0.91	0.5	$C_{21}H_{44}O_4N$	0.4	1.1	C18:1
376.3421	0.92	-0.5	$C_{21}H_{46}O_4N$	0.9	2.4	C18:0
402.3578	0.67	0.5	$C_{23}H_{48}O_4N$	0.6	6.7	C20:1
418.3891	0.94	-0.5	$C_{24}H_{52}O_4N$	0.1	1.0	C24:0
430.3891	0.9	0.5	$C_{25}H_{52}O_4N$	0.5	2.6	C21:1
458.4204	1.25	0.5	$C_{27}H_{56}O_4N$	0.7	6.8	C24:0
Diacylglycerols (DAG)						
584.5249	0.71	1.5	$C_{35}H_{70}O_5N$	0.2	0.6	C32:1 (C16:0-C16:1)
586.5405	0.45	0.5	$C_{35}H_{72}O_5N$	0.0	0.6	C32:0 (C16:0-C16:0)
610.5405	0.78	2.5	$C_{37}H_{72}O_5N$	0.0	7.9	C34:2 (C16:0-C18:2)
612.5562	0.71	1.5	$C_{37}H_{74}O_5N$	1.6	13.4	C34:1 (C16:0-C18:1)
626.5718	1.19	1.5	$C_{38}H_{76}O_5N$	0.3	1.1	C35:1(C17:0-C18:1)
628.5875	0.38	0.5	$C_{38}H_{78}O_5N$	0.0	3.2	C35:0 (C17:0-C18:0)
636.5562	1.18	3.5	$C_{39}H_{74}O_5N$	1.2	9.9	C36:3 (C18:1-C18:2)
638.5718	0.74	2.5	$C_{39}H_{76}O_5N$	2.4	18.4	C36:2 (C18:1-C18:1)
640.5875	0.82	1.5	$C_{39}H_{78}O_5N$	0.0	4.6	C36:1 (C18:0-C18:1)
642.6031	-0.08	0.5	$C_{39}H_{80}O_5N$	0.0	0.7	C36:0 (C16:0-C20:0)
666.6031	-0.32	2.5	$C_{41}H_{80}O_5N$	0.3	1.0	C38:2 (C18:1-C20:1/C18:2-C20:0)
668.6188	0.79	1.5	$C_{41}H_{82}O_5N$	1.0	6.7	C38:1 (C18:1-C20:0)
272.2584	1.2	0.5	$C_{16}H_{34}O_2N$	0.0	0.6	C16:1
286.2741	0.5	0.5	$C_{17}H_{36}O_2N$	0.0	0.5	C17:1
296.2584	0.7	2.5	$C_{18}H_{34}O_2N$	0.0	0.4	C18:3
298.2741	0.8	1.5	$C_{18}H_{36}O_2N$	0.2	1.0	C18:2
300.2897	0.8	0.5	$C_{18}H_{38}O_2N$	0.0	0.8	C18:1
328.321	0.8	0.5	$C_{20}H_{42}O_2N$	0.0	0.1	C20:1
356.3523	1.0	0.5	$C_{22}H_{46}O_2N$	0.0	0.1	C22:1
358.368	1.0	-0.5	$C_{22}H_{48}O_2N$	0.0	0.4	C22:0
412.4149	1.2	0.5	$C_{26}H_{54}O_2N$	0.0	0.6	C26:1
414.4306	0.5	-0.5	$C_{26}H_{56}O_2N$	0.5	1.2	C26:0
440.4462	0.6	0.5	$C_{28}H_{58}O_2N$	0.0	0.5	C28:1
442.4619	0.8	-0.5	$C_{28}H_{60}O_2N$	0.0	3.6	C28:0
496.5088	1.0	0.5	$C_{32}H_{66}O_2N$	0.0	31	C32:1
510.5245	0.8	0.5	$C_{33}H_{68}O_2N$	0.0	0.4	C32:0
Hydroxy fatty acids (OH FA)						
288.2533	1.1	0.5	$C_{16}H_{34}O_3N$	0.0	1.0	OH-C16:1
290.269	1.2	-0.5	$C_{16}H_{36}O_3N$	0.0	0.6	OH-C16:0
312.2533	1.4	2.5	$C_{18}H_{34}O_3N$	0.0	0.2	OH-C18:3
314.269	0.8	1.5	$C_{18}H_{36}O_3N$	0.2	0.7	OH-C18:2
316.2846	0.7	0.5	$C_{18}H_{38}O_3N$	0.9	2.2	OH-C18:1
344.3159	1.1	0.5	$C_{20}H_{42}O_3N$	0.0	0.4	OH-C20:1
372.3472	0.6	0.5	$C_{22}H46O_3N$	0.0	1.0	OH-C22:1
386.3629	0.4	0.5	$C_{23}H_{48}O_3N$	0.0	0.0	OH-C23:1
400.3785	0.8	0.5	$C_{24}H_{50}O_3N$	0.2	1.3	OH-C24:1
428.4098	0.4	0.5	$C_{26}H_{54}O_3N$	0.2	7.4	OH-C26:1
456.4411	0.6	0.5	$C_{28}H_{58}O_3N$	0.0	0.6	OH-C28:1
Dihydroxy fatty acids (di-OH FA)						
306.2639	0.4	-0.5	$C_{16}H_{36}O_4N$	0.0	0.3	2OH-C16:0
334.2952	1.0	-0.5	$C_{18}H_{40}O_4N$	0.2	0.8	2OH-C18:0
362.3265	0.6	-0.5	$C_{20}H_{44}O_4N$	0.0	0.2	2OH-C20:0
390.3578	0.4	-0.5	$C_{22}H_{48}O_4N$	0.2	0.6	2OH-C22:0
446.4204	1.4	-0.5	$C_{26}H_{56}O_4N$	0.2	0.8	2OH-C26:0
Dicarboxylic fatty acids						
302.2326	1.2	1.5	$C_{16}H_{32}O_4N$	0.0	0.0	C16:1-dioic acid
304.2482	0.9	0.5	$C_{16}H_{34}O_4N$	0.0	5.5	C16:0-dioic acid
328.2482	0.8	2.5	$C_{18}H_{34}O_4N$	0.0	0.2	C18:2-dioic acid
330.2639	0.6	1.5	$C_{18}H_{36}O_4N$	0.3	0.6	C18:1-dioic acid
332.2795	0.7	0.5	$C_{18}H_{38}O_4N$	0.0	2.2	C18:0-dioic acid
360.3108	1.2	0.5	$C_{20}H_{42}O_4N$	0.0	0.0	C20:0-dioic acid

670.6344	0.53	0.5	$C_{41}H_{84}O_5N$	0.0	0.8	C38:0 (C18:0-C20:0)	416.3734	0.9	0.5	$C_{24}H_{50}O_4N$	0.1	1.0	C24:0-dioic acid
694.6344	1.44	2.5	$C_{43}H_{84}O_5N$	0.0	0.9	C40:2 (C20:1-C20:1/C22:1-C18:1)	444.4047	0.9	0.5	$C_{26}H_{54}O_4N$	0.2	1.3	C26:0-dioic acid
696.6501	0.87	1.5	$C_{43}H_{86}O_5N$	0.5	3.8	C40:1 (C20:0-C20:1/C22:0-C18:1)	472.436	0.7	0.5	$C_{28}H_{58}O_4N$	0.0	16.8	C28:0-dioic acid
							Triterpenic acids						
							474.3942	0.8	5.5	$C_{30}H_{52}O_3N$	0.1	14.8	oleanolic+ursolic acid
							490.3891	0.9	5.5	$C_{30}H_{52}O_4N$	100	100	maslinic acid

[a]: *m/z* values of $NH4^+$ adduct ions; [b]:rings and double bonds equivalents; [c]: relative intensity ranges within six olive varieties.

Table 3. EW metabolites of *Olea europaea* fruits detected in negative mode by direct-ESI-UHRMS. Mass accuracy and tentative identification according to their molecular formula are also shown, as well as relative intensity ranges within six olive varieties

Theoretical mass	Δ (ppm)	RDBE[a]	Formula [M-H]⁻	Relative intensity[b] MIN	Relative intensity[b] MAX	Tentative identifcation[a]
Fatty acids (FA)						
129.0921	-1.2	1.5	$C_7H_{13}O_2$	<0.1	0.34	C7:0
143.1078	-0.8	1.5	$C_8H_{15}O_2$	<0.1	2.6	C8:0
157.1234	-1.7	1.5	$C_9H_{29}O_3$	<0.1	0.23	C9:0
171.1389	-0.9	1.5	$C_{10}H_{19}O_2$	<0.1	0.17	C10:0
199.1704	0.3	1.5	$C_{12}H_{23}O_2$	<0.1	0.12	C12:0
227.2017	-0.1	1.5	$C_{14}H_{27}O_2$	0.11	0.38	C14:0
241.2173	0.4	1.5	$C_{15}H_{29}O_2$	0.11	0.46	C15:0
253.2173	0.8	2.5	$C_{16}H_{29}O_2$	0.53	1.42	C16:1
255.2330	0.7	1.5	$C_{16}H_{31}O_2$	1.5	4.7	C16:0
267.2330	0.7	2.5	$C_{17}H_{31}O_2$	<0.1	0.32	C17:1
269.2486	0.7	1.5	$C_{17}H_{33}O_2$	0.15	0.5	C17:0
277.2173	-0.5	4.5	$C_{18}H_{29}O_2$	<0.1	0.11	C18:3
279.2330	0.4	3.5	$C_{18}H_{31}O_2$	0.67	1.21	C18:2
281.2486	0.7	2.5	$C_{18}H_{33}O_2$	4.26	10.13	C18:1
283.2643	0.6	1.5	$C_{18}H_{35}O_2$	1.18	4.08	C18:0
309.2799	-0.3	2.5	$C_{20}H_{37}O_2$	0.1	0.29	C20:1
311.2956	0.4	1.5	$C_{20}H_{39}O_2$	<0.1	0.26	C20:0
337.3112	0.5	2.5	$C_{22}H_{41}O_2$	0.15	2.44	C22:1
339.3269	0.7	1.5	$C_{22}H_{43}O_2$	1.62	7.54	C22:0
353.3425	0.7	1.5	$C_{23}H_{45}O_2$	0.17	0.5	C23:0
365.3425	-0.5	2.5	$C_{24}H_{45}O_2$	<0.1	0.53	C24:1
367.3582	0.9	1.5	$C_{24}H_{47}O_2$	9.61	18.36	C24:0
381.3738	0.0	1.5	$C_{25}H_{49}O_2$	0.68	1.15	C25:0
393.3738	-0.8	2.5	$C_{26}H_{49}O_2$	<0.1	0.27	C26:1
395.3895	1.0	1.5	$C_{26}H_{51}O_2$	13.3	22.89	C26:0
409.4051	-0.6	1.5	$C_{27}H_{53}O_2$	0.25	0.61	C27:0
423.4208	0.1	1.5	$C_{28}H_{55}O_2$	1.61	9.17	C28:0
451.4521	-0.3	1.5	$C_{30}H_{59}O_2$	0.16	0.71	C30:0
Dicarboxylic fatty acids						
171.1027	0.1	2.5	$C_9H_{15}O_3$	0.37	0.81	C9:0-dioic acid
201.1132	1.7	2.5	$C_{10}H_{17}O_4$	<0.1	0.34	C10-dioic acid
327.2541	0.1	2.5	$C_{19}H_{35}O_4$	0.2	0.4	C19:0-dioic acid/ di-OH C19:1
Hydroxy fatty acids (OH FA)						
269.2122	0.5	2.5	$C_{16}H_{29}O_3$	0.12	0.4	OH-C16:0
271.2279	0.6	1.5	$C_{16}H_{31}O_3$	0.11	0.36	OH-C16:1
295.2279	0.3	3.5	$C_{18}H_{31}O_3$	0.41	0.79	OH-C18:0
297.2435	0.4	2.5	$C_{18}H_{33}O_3$	1.32	3.01	OH-C18:1
299.2592	0.3	1.5	$C_{18}H_{35}O_3$	0.1	0.23	OH-C18:0
355.3218	-0.2	1.5	$C_{22}H_{43}O3$	0.15	0.43	OH-C22:0
381.3374	-0.7	2.5	$C_{24}H_{45}O_3$	<0.1	0.1	OH-C24:1
383.3531	-0.5	1.5	$C_{24}H_{47}O_3$	0.13	0.38	OH-C24:0
409.3687	-0.1	2.5	$C_{26}H_{49}O_3$	<0.1	0.2	OH-C26:1
411.3844	-0.2	1.5	$C_{26}H_{51}O_3$	<0.1	0.17	OH-C26:0
437.4000	-1.5	2.5	$C_{28}H_{53}O_3$	0.15	0.29	OH-C28:1
439.4157	-1.2	1.5	$C_{28}H_{55}O_3$	<0.1	0.12	OH-C28:0
159.1027	0.1	1.5	$C_8H_{15}O_3$	0.15	0.35	OH-C8:0
173.1183	0.3	1.5	$C_9H_{17}O_3$	0.16	0.42	OH-C9:0
Dihydroxy fatty acids (di-OH FA)						
231.1602	1.7	1.5	$C_{12}H_{23}O_4$	<0.1	0.16	di-OH C16:0
287.2228	0.1	1.5	$C_{16}H_{31}O_4$	0.12	0.34	di-OH C16:0
301.2384	-0.4	1.5	$C_{17}H_{33}O_4$	0.12	0.34	di-OH C17:0
315.2541	0.2	1.5	$C_{18}H_{35}O_4$	0.31	0.54	di-OH C18:0
329.2697	0.2	1.5	$C_{19}H_{37}O_4$	<0.1	0.3	di-OH C19:0
385.3323	-0.4	1.5	$C_{23}H_{45}O_4$	0.11	0.47	di-OH C23:0
413.3636	-0.3	1.5	$C_{25}H_{49}O_4$	0.71	1.42	di-OH C25:0
427.3793	-1.2	1.5	$C_{26}H_{51}O_4$	<0.1	0.11	di-OH C26:0
441.3949	-0.1	1.5	$C_{27}H_{53}O_4$	0.97	1.89	di-OH C27:0
469.4262	-1.5	1.5	$C_{29}H_{57}O_4$	0.18	0.82	di-OH C29:0
Triterpenic						
471.3480	1.4	7.5	$C_{30}H_{47}O_4$	100	100	maslinic acid
455.3531	1.5	7.5	$C_{30}H_{47}O_3$	54.4	82.29	ursolic/oleanolic acid
471.3480	1.4	7.5	$C_{30}H_{47}O_4$	100	100	maslinic acid
Phenolics						
151.0401	-1.7	5.5	$C_8H_7O_3$	0.11	0.4	vanillin/hydroxyphenylacetic acid
153.0557	-0.6	4.5	$C_8H_9O_3$	0.15	2.67	vanillyl alcohol

311.2228	0.4	3.5	$C_{18}H_{31}O_4$	0.15	0.28	C18:1-dioic acid/ di-OH C18:2	195.0663	1.9	5.5	$C_{10}H_{11}O_4$	0.1	7.2	Hydroxytyrosyl acetate
313.2384	0.1	2.5	$C_{18}H_{33}O_4$	0.38	0.7	C18:0-dioic acid/ di-OH C18:1	241.0718	0.5	5.5	$C_{11}H_{13}O_6$	<0.1	19.14	Elenolic acid aglycone
369.3010	-1.1	2.5	$C_{22}H_{41}O_4$	<0.1	0.12	C23:0-dioic acid/ di-OH C22:1	319.1187	0.0	8.5	$C_{17}H_{19}O_6$	<0.1	12.22	Decarboxymethyl oleuropein aglycone
383.3167	0.54	2.5	$C_{23}H_{43}O_4$	<0.1	0.2	C23:0-dioic acid/ di-OH C23:1							
523.4732	-0.86	2.5	$C_{33}H_{63}O_4$	<0.1	2.51	C33:0-dioic acid/ di-OH C33:1							
551.5045	-0.63	2.5	$C_{35}H_{67}O_4$	<0.1	0.35	C35:0-dioic acid/ di-OH C35:1							

[a]: rings and double bonds equivalents; [b]: relative intensity ranges within six olive varieties.

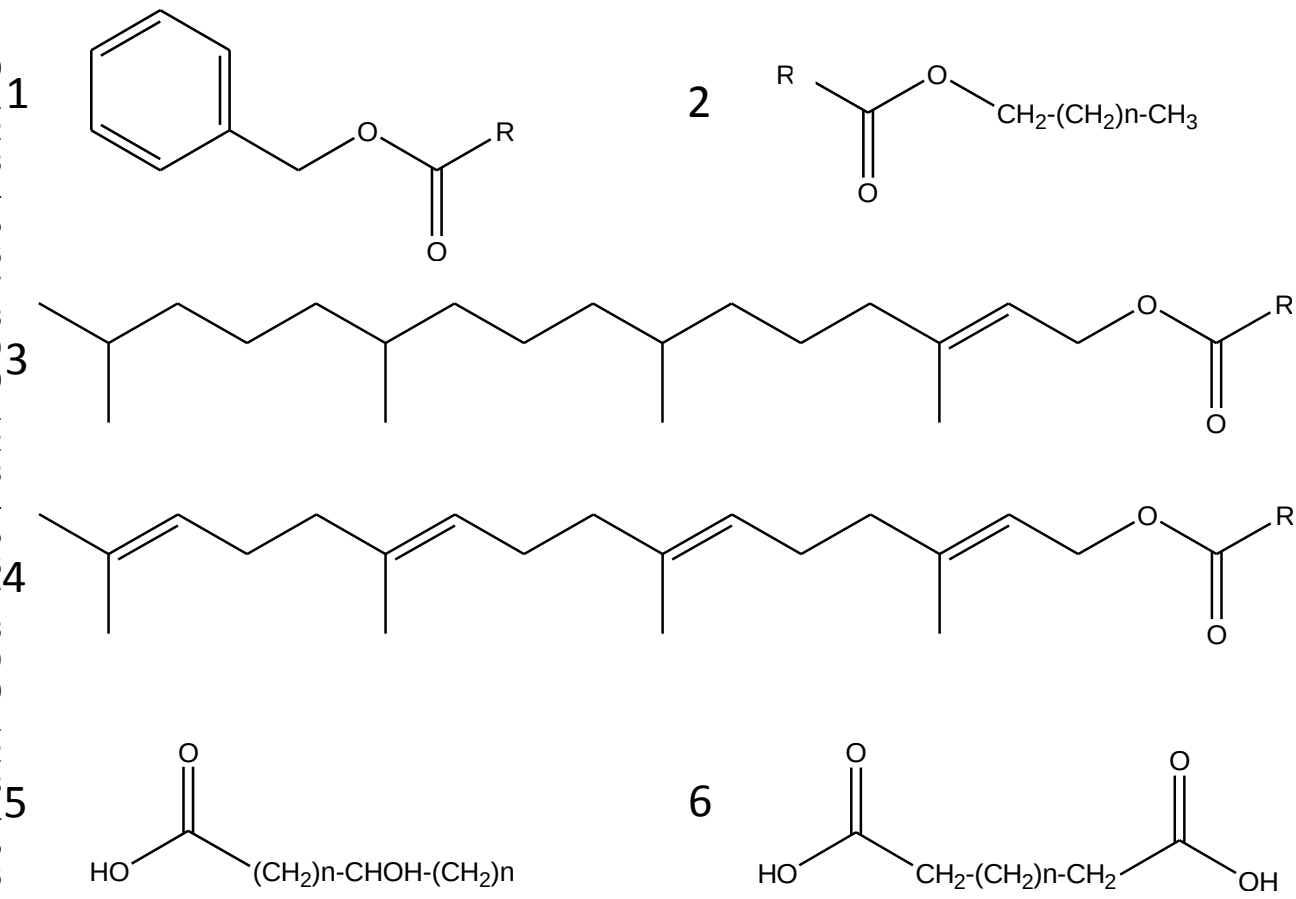
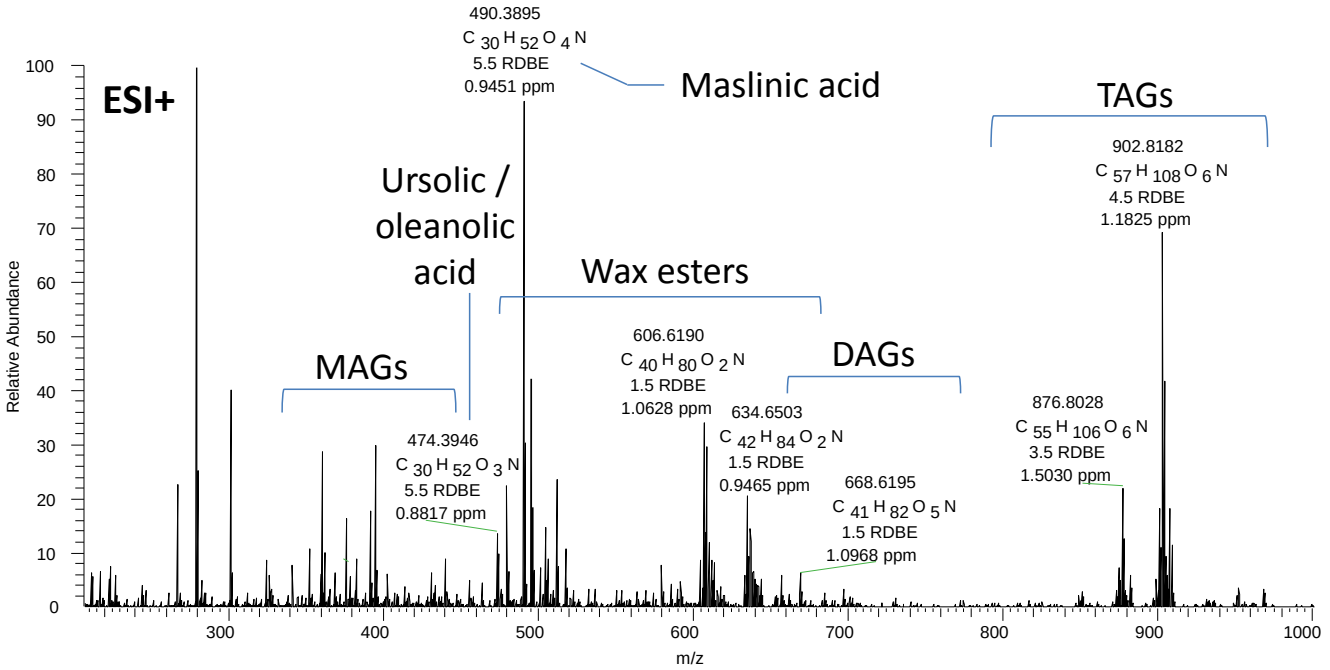

1
O
R
O
2
R
O
CH2-(CH2)n-CH3
O
3
O
R
O
4
O
R
O
5
O
HO
(CH2)n-CHOH-(CH2)n
6
O
O
HO
CH2-(CH2)n-CH2
OH

Figure 1


ESI+
Relative Abundance
m/z
490.3895
C 30 H 52 O 4 N
5.5 RDBE
0.9451 ppm
Maslinic acid
Ursolic / oleanolic acid
Wax esters
TAGs
902.8182
C 57 H 108 O 6 N
4.5 RDBE
1.1825 ppm
MAGs
606.6190
C 40 H 80 O 2 N
1.5 RDBE
1.0628 ppm
DAGs
634.6503
C 42 H 84 O 2 N
1.5 RDBE
0.9465 ppm
876.8028
C 55 H 106 O 6 N
3.5 RDBE
1.5030 ppm
474.3946
C 30 H 52 O 3 N
5.5 RDBE
0.8817 ppm
668.6195
C 41 H 82 O 5 N
1.5 RDBE
1.0968 ppm

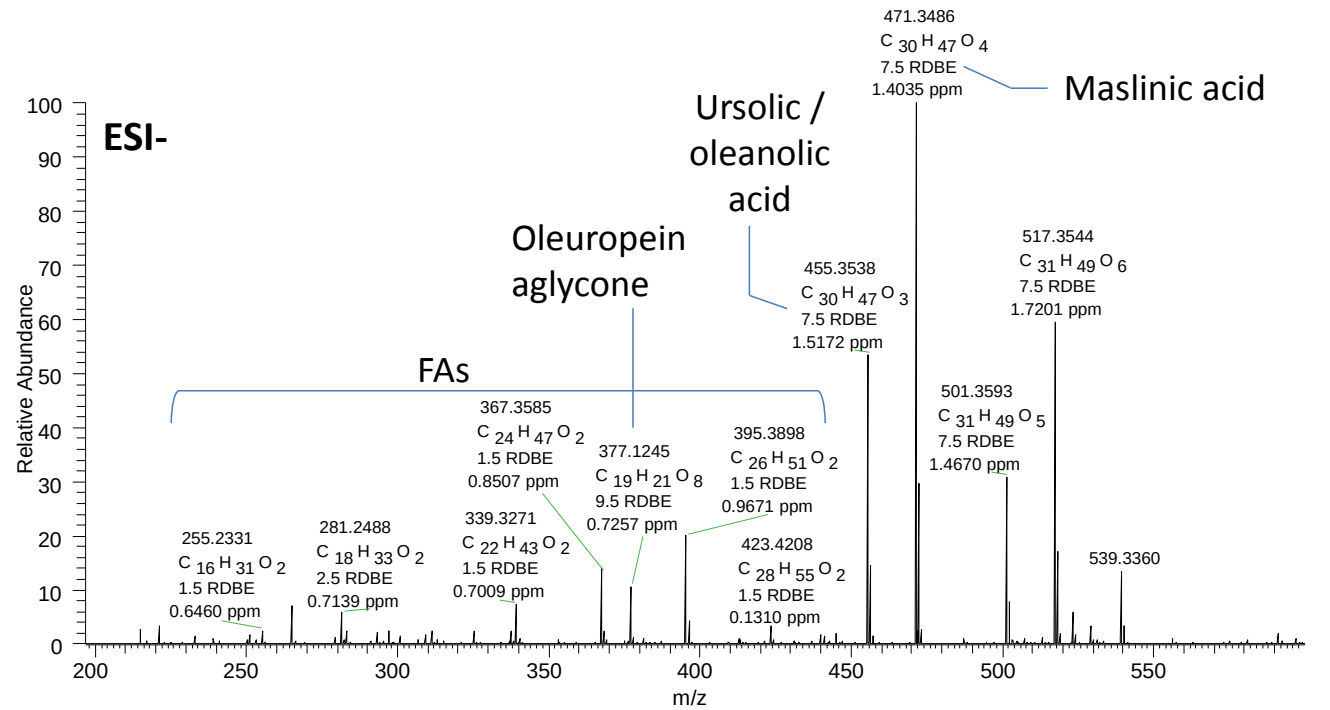

ESI-
Relative Abundance
m/z
471.3486
C 30 H 47 O 4
7.5 RDBE
1.4035 ppm
Maslinic acid
Ursolic / oleanolic acid
Oleuropein aglycone
455.3538
C 30 H 47 O 3
7.5 RDBE
1.5172 ppm
517.3544
C 31 H 49 O 6
7.5 RDBE
1.7201 ppm
FAs
501.3593
C 31 H 49 O 5
7.5 RDBE
1.4670 ppm
367.3585
C 24 H 47 O 2
1.5 RDBE
0.8507 ppm
377.1245
C 19 H 21 O 8
9.5 RDBE
0.7257 ppm
395.3898
C 26 H 51 O 2
1.5 RDBE
0.9671 ppm
255.2331
C 16 H 31 O 2
1.5 RDBE
0.6460 ppm
281.2488
C 18 H 33 O 2
2.5 RDBE
0.7139 ppm
339.3271
C 22 H 43 O 2
1.5 RDBE
0.7009 ppm
423.4208
C 28 H 55 O 2
1.5 RDBE
0.1310 ppm
539.3360

Figure 2

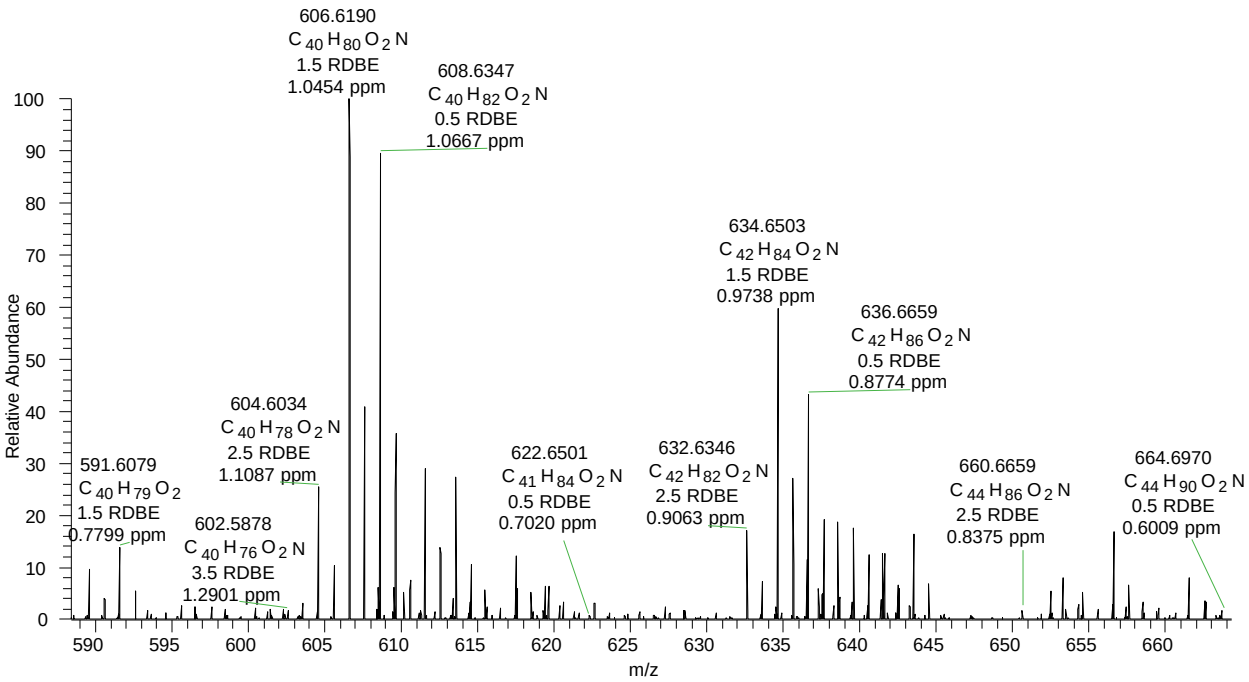

606.6190
C 40 H 80 O 2 N
1.5 RDBE
1.0454 ppm
608.6347
C 40 H 82 O 2 N
0.5 RDBE
1.0667 ppm
634.6503
C 42 H 84 O 2 N
1.5 RDBE
0.9738 ppm
636.6659
C 42 H 86 O 2 N
0.5 RDBE
0.8774 ppm
604.6034
C 40 H 78 O 2 N
2.5 RDBE
1.1087 ppm
622.6501
C 41 H 84 O 2 N
0.5 RDBE
0.7020 ppm
632.6346
C 42 H 82 O 2 N
2.5 RDBE
0.9063 ppm
660.6659
C 44 H 86 O 2 N
2.5 RDBE
0.8375 ppm
664.6970
C 44 H 90 O 2 N
0.5 RDBE
0.6009 ppm
591.6079
C 40 H 79 O 2
1.5 RDBE
0.7799 ppm
602.5878
C 40 H 76 O 2 N
3.5 RDBE
1.2901 ppm
Relative Abundance
m/z

Figure 3