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Phenolic compounds profiling of virgin olive oils from different varieties cultivated in Mendoza, Argentina, by using liquid chromatography-mass spectrometry

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Abstract

The aim of this work was to achieve a preliminary characterization of the profile of the phenolic fraction of virgin olive oils (VOOs) from Maipú (Mendoza, Argentina). Thus, 25 commercial VOO samples from *Arauco, Arbequina, Picual, Frantoio, Changlot, Empeltre, Nevadillo, Manzanilla and Coratina* (both monovarietals and blends) were analyzed using LC-ESI-QTOF MS and LC-ESI-IT MS for identification and quantification purposes, respectively. A rapid LC method (15 min) accomplished quantitative information about a total of 40 phenolic compounds, including secoiridoid derivatives, which have not been evaluated before in samples coming from the sub-region so-called Maipú (Mendoza province, Argentina). The results make evident that olive oils coming from Mendoza can be considered as important sources of phenolic bioactive compounds, exhibiting similar phenolic compounds levels to those shown by oils from other typical world production regions. Moreover, some distinctive features of Arauco variety (Argentinean autochthonous variety) were pointed out; indeed, a correlation between flavonoids content and botanical variety was established herewith.

Keywords: food metabolomics; phenolic compounds; Argentinean olive oil; Arauco olive variety

Introduction

Virgin olive oil (VOO) is a valuable vegetable oil which contains minor biomolecules of outstanding importance, such as vitamins, carotenoids, tocopherols, phenolic compounds, and other natural antioxidants.[1] Among these minor constituents, the relevance of phenolic compounds is irrefutable, since they contribute to the stability of VOO against auto-oxidation, are intimately associated to VOO taste, exhibit anti-inflammatory and antimicrobial activities (among others), and could prevent certain diseases linked with the oxidative damage.[2,3] The just mentioned phenolic fraction is composed by a heterogeneous mixture of analytes (phenolic acids, simple phenolic alcohols, flavonoids, secoiridoids, and lignans),[3,4] what explains the difficulties to achieve their accurate determination. This task has been tackled developing different methodologies.[3,5-7] Separative techniques coupled to different detectors have been used when the individual determination of these compounds is aimed, being liquid chromatography-mass spectrometry (LC-MS) one of the most popular and extensively used couplings nowadays;[8-11] this platform is indeed very appreciated in the field of food metabolomics.

Studies about phenolic compounds present in VOO have been performed pursuing diverse objectives, as for instance, to observe their link with agronomical factors and technological conditions of production[12-15] assessing the influence of climate and soil, olive cultivar, extraction system, processing conditions, etc.[6,16-20] The samples selected in most of this kind of investigations are olive oils coming from the main producing areas of the world (Spain, Italy, Greece, Morocco, among others);[13,17,21,22] however, oils originating from other production regions, such as Argentina, lack this valuable information.

Argentina, located in the South of the American continent, has greatly extended the country olive oil production zones over the last years. Its domestic production has several

remarkable advantages: the strategic location of the country (being able to market fresh oils when Mediterranean producers cannot supply them); the ability to produce increasing volumes of high quality VOOs[23] and the possibility of producing olive oil with remarkable differences on their characteristics (due to the diverse cultivars grown in Argentina and the very heterogeneous soils and microclimate conditions of the producing areas). Within the country, there is a typical production area, central-west located, so-called Mendoza province, which has a long tradition of olive growing characterized for planted trees of about 100 years old. Inside of Mendoza, the sub-region called Maipú is extensively planted with a botanical variety identify as *Arauco*, typically cultivated for producing table olives, mainly due to its good size and high flesh-to-pit ratio.[15,24] However, over the last years, it has been demonstrated that this cultivar has profitable characteristics for commercial production of VOO, since it has relatively high oil content, a well-balanced fatty acid composition and a distinctive profile of minor antioxidants.[15] It is the only cultivar recognized from Argentina in the World Catalogue of Olive Varieties since 1995.[25] Some other varieties grown in Argentina are *Arbequina*, *Manzanilla*, *Picual and Frantoio*, among others.[15,26]

As previously stated, very few reports have been published including information about the phenolic composition of Argentinean olive oils.[7,27,28] For example, one of these studies carried out a characterization of monovarietal Argentinean olive oils from 4 provinces, accomplishing the determination of the phenolic compounds by using a spectrophotometric method (total content) based on Folin-Ciocalteu reactive.[28] Another contribution described the characterization of the phenolic composition of commercial extra-VOOs from different countries (including just few samples from Argentina).[7] Later on, the phenolic compounds and antioxidant capacity of monovarietal olive oils produced in Argentina were evaluated

by capillary zone electrophoresis, but the analytes under study did not include secoiridoids and its derivatives (main group of phenolic compounds from VOO, which represents a high percentage of the total phenolic fraction and is exclusive of plants belonging to the family *Oleaceae*).[27] Finally, another stimulating work focused on physiological aspects and minor antioxidant compounds from *Arauco* cv. during fruit ontogeny should be mentioned, since included very interesting results about the optimum maturity index of this cultivar.[15]

The aim of our work was to undertake a comprehensive characterization of the phenolic fraction of commercial VOOs from different varieties cultivated in the confines of the geographical zone of Mendoza province (Argentina) by LC-MS. A liquid chromatography-electrospray ionization-quadrupole-time of flight mass spectrometry (LC-ESI-QTOF MS) was used to characterize the phenolic profiles and, afterwards, liquid chromatography-electrospray ionization-ion trap mass spectrometry (LC-ESI-IT MS) was used to carry out the quantification. This is the first time that VOOs from this territory have been studied by using this technology, making possible to describe in depth the composition of the phenolic fraction.

Materials and methods

Reagents and materials

All reagents were of analytical grade and were used as received. Methanol and *n*-hexane of HPLC grade were supplied from Panreac (Barcelona, Spain); they were used for the extraction of the phenolic compounds from the olive oil samples. Mobile phases were prepared by using Acetonitrile (ACN) from Lab-Scan (Dublin, Ireland) and acetic acid from Panreac. Doubly deionised water with a conductivity of 18.2 MΩ cm was obtained by using a Milli-Q-system (Millipore, Bedford, MA, USA). Standards of caffeic, *p*-coumaric,

quinic and ferulic acids, as well as hydroxytyrosol (HTY), tyrosol, luteolin, apigenin, and 3,4-dihydroxyphenylacetic acid (DOPAC) (internal standard (IS)) were purchased from Sigma-Aldrich (St. Louis, MO, USA). (+)-Pinoresinol (Pin) was acquired from Arbo Nova (Turku, Finland) and oleuropein (Ole) was purchased from Extrasynthese (Lyon, France). Stock solutions were prepared by dissolving the appropriate amount of the compound in methanol at a concentration of 500 μg mL^{-1} for each phenolic compound. Afterwards, they were serially diluted to working concentrations (within the range 0.5 - 250 μg mL^{-1}). Both the samples and stock solutions were stored in dark flasks at -20 °C and, before being injected into the instrument, they were filtered through a Clarinert™ 0.22 μm nylon syringe filter from Agela Technologies (Wilmington, DE, USA).

Samples

The VOOs studied in this work were commercial samples, acquired from Argentinean companies. The selection included monovarietal olive oils from the following varieties: *Arbequina* (1 sample), *Manzanilla* (3 samples), *Frantoio* (2), *Empeltre* (1), *Nevadillo* (1), *Arauco* (6), *Picual* (1), *Coratina* (2) and *Changlot* (named as Genovesa by some authors in Spain) (1); and different blends (7). Composition of Blends was the following: Blend 2 and 3: 60 % Arbequina, 30 % Frantoio, 3-4 % Arauco, 7-6 % Unknown; Blend 4: 70 % Arauco, 30 %, Arbequina; Blend 5: 70 % Arbequina, 30 % Arauco; and Blends 1, 6 and 7: unknown. The oils were extracted on season 2014 (just one sample was from the end of season 2013 (*Arauco* number 1)) by two phases continuous centrifuge and were obtained from olives with a maturity index of around 3 (ripening index facilitated by the technical department of the factories). All samples were kept refrigerated in appropriate containers until their analysis. Stability tests were applied to different aliquots of the samples as well as to the achieved extracts in order to assure their proper storage until the analysis. These

tests were based on the comparison of the peak areas obtained from the LC-MS analysis of fresh extracts prepared from the properly stored samples with those peak area values of the extracts which had been stored for a certain period of time (max. storage time tested was 4 months), not detecting statistically significant differences.

An important characteristic of this sample-set is that all the different steps of the elaboration process were performed in Maipú (a sub-region of Mendoza province of 617 km^2); the coordinates of the studied zone are 32° 58′ 0″ S, 68° 46′ 0″ W, and their altitudes above the sea level are 804 m (arid temperate and precipitations about 200 mm annual).

Extraction of phenolic compounds

The phenolic compounds were isolated by using a liquid-liquid extraction according to a previously reported procedure,[3] which can be briefly described as follows: 2.0±0.1 g of olive oil were weighed in a test tube with a screw cap. A volume of 0.025 mL of a solution of the compound selected as IS (at a concentration of 500 mg L^{-1}) was added (to have an internal reference within the samples which could give us the chance to assure that the extraction protocol was carried out properly and the system was operating correctly). The solvent of the IS solution (MeOH) was evaporated (using N_2), 1 mL of n-hexane was added and the tube was shaken in a vortex during 30 s. The phenolic compounds under study were extracted three times, by adding 2 mL of methanol/water (60:40, v/v), shaking over 2 minutes and centrifuging at 3500 rpm for 6 minutes (each time). The supernatants were combined and evaporated to dryness using a rotary evaporator. The residue was redissolved in 1 mL of methanol and filtered through a 0.22 μm membrane filter.

LC-MS analysis: chromatographic and MS detection conditions

Two LC-MS platforms were used within this study. One of them was a Waters Acquity UPLC™ H–Class system (Waters, Manchester, UK) coupled to a micrOTOF-Q II™ mass

spectrometer (Bruker Daltonics) by means of an ESI source. The second one was an Agilent 1260-LC system (Agilent Technologies, Waldbronn, Germany), which was coupled to a Bruker Daltonic Esquire 2000™ IT MS (Bruker Daltonics, Bremen, Germany) with an ESI interface. The first platform was used with qualitative purposes and the second one was employed to carry out the quantification experiments.

The separation of the target compounds was performed using a Zorbax Eclipse Plus C_{18} analytical column (4.6 x 150 mm, 1.8 μm particle size) protected by a guard cartridge of the same packing. The temperature of the column oven was set at 35 °C and a flow rate of 1.2 mL min^{-1} was selected. A volume of 10 μL of the olive oil extracts, pure standards and standard mixtures was injected in each case. The mobile phases used were water with acetic acid (0.5 % v/v) (Phase A) and ACN (Phase B), and the solvent changed as follows: 0 to 10 min, 10-50 % B; 10 to 12 min, 50-100 % B; 12 to 13 min, 100-10 % B. Finally, the column was re-equilibrated for 1.5 min.

With the aim of avoiding the introduction of humidity into the system and achieving stable electrospray ionization and reproducible results, the flow delivered into the MS detectors from LC was reduced to approx. 0.3 mL min^{-1} using a proper split.

The QTOF MS system was operating in negative and positive mode (to increase the information achieved about the VOO samples) within the range of 50-1200 m/z, at a scan speed of 240 ms. A drying gas (N_2) temperature of 300 ºC and a flow of 9.0 L min^{-1} were selected as optimum. The capillary voltage was set at 4500 V and the end plate offset at -500 V. Internal calibration was performed using sodium formate clusters and using similar strategies to those described in previous works.[3,16]

The IT MS was operated in negative ion mode and the capillary voltage was set at +3200 V. Acquisition was made in full scan mode within the range of 50-1000 m/z. The nebulizer gas was set at 30 psi, dry gas at 9 L min^{-1}, and drying gas (N_2) temperature at 300 ºC.

The data resulting from both MS systems were processed through Data Analysis 4.0 software (Bruker Daltonics). In the case of accurate mass data of the molecular ions, the software provided a list of possible elemental formulas, giving a parameter (Sigma value) which shows the prediction confidence. MS/MS experiments were conducted with the use of AutoMS data acquisition mode, which is based on the fragmentation of the most abundant precursor ions per scan. For certain masses of interest, if the intensity of the *m/z* was low, a second analysis -including the list of the selected precursor ions- was performed in multiple reaction monitoring mode.

Statistical data analysis

The Unscrambler® v9.7 (CAMO software, Inc., Aspen, New Jersey, USA) was the software employed for data treatment. First, we carried out one-way analysis of variance (ANOVA) to determine the significance of the differences among the phenolic compounds concentration levels of the diverse cultivars. Afterwards, principal component analysis (PCA) was performed using the LC-MS data. The PCA matrix was composed by 40 variables (the number of phenolic compounds that were quantified in the VOO samples) and 25 samples (average value of the 4 analyzed replicates). Apart from it, we built a series of 2D plots where the samples were modelled considering the total values of the determined chemical classes (one-to-one).

Results and discussion

Optimization of the chromatographic conditions

One of the objectives of this study was to obtain a rapid and efficient chromatographic method (if possible, shorter than those previously reported), which could allow the separation of the phenolic compounds under study. To achieve the formulated purpose, the work started with the search of the most convenient chromatographic conditions and the optimization was carried out taking into account separation, selectivity, sensitivity, peak shape and analysis time. Different gradients were tested, together with other variables, such as flow rate and column temperature. Figure S1 shows the base peak chromatogram (BPC) obtained by using the optimum conditions; the gradient employed is also illustrated in the figure. It can be observed that good resolution and peak shape were achieved by using a flow rate of 1.2 mL min^{-1} at 35 °C, in particular within the analytical window comprised from 10 to 15 min, where achieving a proper resolution between Pin and Ace Pin, apigenin and diosmetin, as well as some secoiridoids was not trivial (the separation between the mentioned compounds can be properly observed in Figure 1, which is presented in the next section).

Phenolic compounds determination

Peak identification was done bearing in mind the previously reported information,[3-5] retention time (R_t) and ESI-IT MS and ESI QTOF MS and MS/MS information obtained from pure standards and olive oil samples. Figure 1 includes the extracted ion chromatograms (EICs) of the 40 analytes determined. The compounds have been separated into 4 groups to make easier to the reader its visual inspection. As can be seen, phenolic acids are eluted in the time window from 1 to 7 min approximately, needing relatively low percentages of ACN and sharing the analytical window with simple phenolic alcohols. Flavonoids and lignans are at close proximity in the chromatogram; they have been

depicted together with simple phenols. Some of the compounds belonging to secoiridoid class, exhibit lower polarities and, therefore, need higher percentages of ACN. Elenolic and ligstroside derivatives have been represented together, including in the last chromatogram of the figure, the oleuropein derivatives. As mentioned above, 40 compounds could be determined with this method in less than 15 min, demonstrating its great potential for VOO phenolic compounds analysis.

After characterizing the profiles, the analytical parameters of the method were evaluated. The linearity of the detector response was verified with standard solutions at 11 different concentration levels over the range defined from the quantification limit to 250 mg L^{-1} (0.5; 1; 5; 12.5; 25; 35; 50; 100; 150; 200 and 250 mg L^{-1}). Each point of the external calibration curve (no significant matrix effect was observed) was evaluated in triplicate. Calibration curves were built for each standard by plotting the standard concentration as a function of the peak area obtained from LC-ESI-IT MS analyses (using the *m/z* signal considered to quantify). The following equations were obtained: quinic acid ($[M-H]^-$=191; y =43784x-2158; r^2=0.995); HTY ($[M-H]^-$=153; y=33174x+21145; r^2=0.98); tyrosol ($[M-H]^-$=137; y=13415x-4269; r^2=0.98); caffeic acid ($[M-H]^-$=179; y=43411x-19251; r^2=0.98); *p*-coumaric acid ($[M-H]^-$=163; y=21198x-969; r^2=0.994); ferulic acid ($[M-H]^-$=193; y=24317x+714; r^2=0.996); Ole ($[M-H]^-$=539; y=3459x+8238; r^2=0.94); luteolin ($[M-H]^-$=285; y=92191x+30091; r^2=0.98); Pin ($[M-H]^-$=357; y=35311x+147; r^2=0.98); and apigenin ([M-H]=269; y=87233x+157257; r^2=0.98). The compounds which were not available as commercial standards were quantified on the basis of other analytes with similar chemical structures. In particular, lignans hydroxypinoresinol (HPin) and acetoxypinoresinol (AcPin) were quantified in terms of Pin, diosmetin was quantified using the calibration curve of luteolin, and secoiridoids and HTY derivatives were quantified by

comparison with HTY or tyrosol. Specifically, elenolic acid (EA), decarboxymethyl elenolic acid (DEA), hydroxyelenolic acid (HEA), desoxy elenolic acid (DesoxyEA), ligstroside aglycone (Lig Agly) and decarboxymethyl ligstroside aglycone (DLA) were quantified in terms of tyrosol; whilst, oxidized hydroxytyrosol (OxHTY), hydroxytyrosol acetate (AcHTY), decarboxymethyl oleuropein aglycone (DOA), 10-hydroxy oleuropein aglycone (10-H Ole Agly) and oleuropein aglycone (Ole Agly) were quantified by comparison with HTY pure standard. Limits of detection (LOD) and quantification (LOQ) (considering S/N equal to 3 and 10, respectively), as well as repeatability (*intra-day* and *inter-day* in terms of relative standard deviation - %RSD - of peak area and retention time) were calculated; these results are included in Table S1. LODs were found between 6.2 and 72.5 μg L^{-1}; %RSD for *inter-day* repeatability was between 1.92 and 7.52% for peak area, not exceeding 1.24% for retention time. Once that the analytical parameters of the method were established, the next step was the determination of the phenolic compounds in the entire sample set. As already stated, the whole idea behind collecting this sample set (including both monovarietal oils and blends) was to get an overall view of the composition (in terms of phenolic compounds) of the VOOs available in the local market at that time. The main requirement that the samples had to fulfill was that they were cultivated and produced in the sub-region of Maipú, being, logically, suitable for consumption. At this point is possible to say that our contribution had a multiple intention: to explore the potential of several varieties grown in Maipú to obtain high-quality olive oils (information missing so far); to expand the knowledge about the phenolic profile of Argentinean commercial oils; and, to a certain extent, to allow the long-term improvement of their international market positioning.

Table 1 shows the results for the individual phenols, which has been divided in Table 1a and 1b in order to include all the samples and facilitate the visual inspection. Results of ANOVA test revealed that statistically significant differences (95%; $p < 0.05$) were observed for the quantified phenolic compounds according to the cultivar (data not shown to contain the size of Tables 1a and 1b and facilitate its visualization). Figure 2 shows the total phenolic content of each sample, value which has been obtained through the sum of the concentrations of the 40 quantified analytes. In the figure, each bar includes information about the concentration levels of phenolic acids, simple phenolic alcohols, lignans, secoiridoids and flavonoids.

The phenolic profile of all the samples was dominated by the presence of secoiridoid derivatives, being the sample with the highest levels of total phenolic compounds *Arauco* 5 with 404.09 mg kg^{-1}; Blend 7 was, on the contrary, the sample with the lowest concentrations (91.55 mg kg^{-1}). The found levels are comparable with previously published results obtained from commercial samples coming Argentina[7,15,28] and other production areas, such as Spain[29-31], Italy[7] and Morocco[3]. However, remarkable differences can be observed when the comparison is made with other works where the samples were prepared specifically for the study, using pilot scale; in those cases, the found levels are usually higher.[32,33]

Evaluating the quantitative results accordingly to each family, *Arauco 4* was the richest sample in terms of phenolic acids (4.24 mg kg^{-1}), being quinic acid the acid found at highest concentration levels (3.37 mg kg^{-1}).

Other important group of phenolic compounds in olive oil is composed by simple phenolic alcohols; group which is principally form by HTY and tyrosol. In this case, the sample with major levels was *Arauco* 1 (71.85 mg kg^{-1}), which had 40.70 mg kg^{-1} of HTY, 1.90 mg kg^{-1}

of a HTY isomer, and 27.91 mg kg^{-1} of tyrosol, apart from other simple phenol-derivatives (OxHTY and AcHTY). This behaviour is in good agreement with the data previously reported by Brenes et al.,[34] who observed that the main changes in the phenolic compounds were associated with the hydrolysis of the secoiridoid aglycons, increasing the concentration of HTY and tyrosol; *Arauco* 1 is indeed the only sample coming from season 2013.

With respect to lignans, *Arbequina* and *Picual* were the monovarietal oils with the highest concentrations (6.77 and 7.00 mg kg^{-1}, respectively); however, the most remarkable levels of the whole sample-set were found for Blend 3 and Blend 2. This fact could be understood considering that these blends were prepared containing 60 % of olive oil from *Arbequina* variety. The high concentration of lignans in *Arbequina* oils (or in oils with strong presence of *Arbequina* variety) has been previously observed by other authors.[35,36]

The flavonoids quantified in this work were diosmetin, apigenin and luteolin (all flavones) and their highest levels were found in *Arauco* variety samples (samples *Arauco* 4, 5 and 1 with 14.76, 13.48 and 13.03 mg kg^{-1}, respectively). A remarkable feature of these samples analyzed here is their very high content of flavonoids, if compared to previously reported studies.[3,7,30,33] In some of the samples, the total flavonoid concentration resulted to be three times higher than previously reported values; an hypothesis explaining this fact is the extensive culture and sunny climatic conditions in Maipú department, since these compounds are related to greater exposures to solar radiation.[37]

As described above, to facilitate the evaluation of the results, secoiridoid derivatives have been divided in ligstroside-related compounds (aldehydic derivatives of EA with tyrosol) and oleuropein-related compounds (aldehydic derivatives of EA with HTY). We also include in this chemical class, EA and related compounds. As far as oleuropein-derivatives

313 are concerned, the most important detected compounds were DOA (or oleacein) and Ole
314 Agly. The highest concentration of DOA was observed in *Manzanilla* 3 sample with 46.44
315 mg kg^{-1} (considering the two DOA isomers), whereas the lowest level was detected in
316 *Changlot* sample, with 4.0 mg kg^{-1}. Regarding Ole Agly, Blend 7 showed the lowest value
317 (5.38 mg kg^{-1} (total value combining the amount determined by –the 6 isomers)) and
318 *Arauco* 5 exhibited the highest one (42.96 mg kg^{-1}). When we pay attention to ligstroside-
319 derivatives, it is necessary to say that, in the present work, 5 isomers of Lig Agly and two
320 of DLA (or oleocanthal) were quantified, finding total Lig Agly´s maximum and minimum
321 values in *Arauco* 5 (251.47 mg kg^{-1}) and *Arbequina* (19.73 mg kg^{-1}), respectively. *Arauco* 4
322 (0.80 mg kg^{-1}) and *Frantoio* 2 (38.94 mg kg^{-1}), respectively, defined the extreme values of
323 the found amounts range of DLA isomers. Apart from these analytes, other secoiridoids
324 were identified: 3 isomers of EA, DEA (two isomers), HEA and DesoxyEA; the maximum
325 EA´s concentration was found in *Changlot* sample, with 149.16 mg kg^{-1} (taking into
326 account all the isomers).

327 **Principal Components Analysis and 2D plots**

328 To evaluate the structure of the data, a principal component analysis (PCA) was applied. In
329 Figure 3a, the score and loading plots of PC1 vs. PC2 are shown for the matrix composed
330 by 40 variables and 25 samples. The first two PCs explained 96 % of total variance in raw
331 data; PC1 and PC2 accounted for 92 % and 4 %, respectively. In the figure, it can be
332 observed that the samples *Arauco* 5 and *Changlot* are quite separated from the rest (in
333 particular *Arauco* 5), fact which can be justified having a look at the loading plots and
334 bearing in mind their high concentrations of Lig Agly (isomer designated as principal one
335 in the current study (12.3 min)) and EA (main isomer at 7.7 min). Figure 3a (score plot)

also shows a grouping of *Coratina* 1 and 2, *Arauco* 1 and *Frantoio* 2 samples; this arrangement could be explained because of their levels of HTY isomer, together with their concentrations regarding the principal isomers of Lig Agly and EA.

With the aim of evaluating further a possible discrimination among the samples based on the cultivar, we built a series of 2D plots; the samples were modelled taking into account the total values of the determined chemical classes (one-to-one), trying to establish existing correlations. Figure 3b illustrates the 2D graphic of lignans *vs* flavonoids. Interestingly, the *Arauco* samples are clearly separated from the rest of the olive oils, indicating their very high flavonoids content and the relatively low lignans levels. Two blends (blends 4 and 5) appeared quite close in the graphic to *Arauco* samples; this circumstance can be certainly explained observing that those blends contained 70 and 30 %, respectively, of *Arauco cv*., while the rest of the blends only had 3-4 % of this variety. A greater number of samples are undoubtedly needed to get a more comprehensive insight into the complete phenolic pattern of these varieties and highlight the main differences among them.

Typical Arauco variety´s features

In the introductory section, we made an allusion to the point that *Arauco* variety is the only Argentinean autochthonous cultivar recognized by International Olive Council, for this reason, a brief paragraph trying to delineate its most relevant features seems required. The six *Arauco* samples evaluated in this study possessed important levels of total phenolic compounds, being two of them the richest of the whole sample-set. It is also appealing to note that in the case of Blend 4, which has a 70 % of *Arauco* variety, the levels of total phenolic compounds are markedly higher than in the other blends. In previously reported works, where other methodologies for determining the phenolic compounds were utilized, the high phenolic contents of *Arauco* oils (when compared with other varieties) were

already observed; several authors have attributed this point to a matter of inappropriate adaptation of diverse varieties to the climatic conditions.[28,38] Indeed, Ceci et al.[28] suggested that the national productive sector should recommend the selection of the cultivars which show a best adaptation to the agronomical media, being the analysis and the implementation of the most advisable cultural and processing conditions absolutely necessary.

Identification of phenolic compounds scarcely reported in VOO

As comment above, the identification of phenolic compounds barely reported in this matrix was also intended using high resolution MS (QTOF MS). Besides the accurate MS information, we obviously took into account the previously reported knowledge about the composition of olive oil-related samples (fruits, leaves and by-products of the olive oil industry). A peak with experimental *m/z* 199.0620 and R_t of 3.6 min was found in 21 samples (it was not detected in *Manzanilla* 3, *Picual*, *Coratina* and *Arauco* 5). Its predicted molecular formula ($[M-H]^-$) was $C_9H_{11}H_5$ and their in-source fragments were 155 and 111 *m/z*. These fragments were corroborated by MS/MS experiments. The structure of the compound is included in Figure 4a and it was tentatively assigned to one analyte related to EA, more precisely, the hydroxylated product of the dialdehydic form of DEA. This compound has been already reported in wastes generated during storage of VOO,[39] as well as in drupes and paste.[40] As can be seen in the figure, the fragments of *m/z* 155 and 111 correspond to the molecular formulae $C_8H_{11}O_3$ and $C_7H_{11}O$, respectively, being the first one the loss of a carboxylic group from the original structure. The m/z 111 seems a typical feature of some EA derivatives, as stated by Kanakis et al.[40]

382 Figure 4b shows the MS spectrum of the compound with m/z 213.0771 (with a R_t of 6.4
383 min). This substance was found in 14 samples: *Arauco* 1, 2, 3 and 6, *Nevadillo*, *Frantoio* 1,
384 *Arbequina* and all Blends) and its predicted molecular formula was $C_{10}H_{13}O_5$ ($[M-H]^{-1}$).
385 According to previously reported information, this peak could be identified as another EA
386 derivate, more specifically, the decarboxylated form of hydroxyelenolic acid; compound
387 which has been reported in the wastes generated during the storage of VOO,[39] drupes and
388 paste.[40] In-source fragments were 181, 169 and 111 being 169 and 111 consistently
389 observed when MS/MS analyses were done. The fragment with *m/z* 181 could be attributed
390 to the loss of CH_4O. The fragment of *m/z* 169 corresponded with the loss of a carboxylic
391 group; and the *m/z* 111 could be explained as the consecutive loss of a carboxylic group and
392 the group $COOCH_2$. The possible fragmentation patterns of this compound have been
393 indicated within Figure 4b (supplementary material). These two EA-related compounds
394 have been hardly described in VOO; it could be very interesting including them in future
395 studies and establishing what their usual concentration ranges are.

396 Summing up, this is the first time in which a deep characterization of the phenolic
397 composition of Maipú VOOs is carried out, getting quantitative information about 40
398 phenolic compounds of samples of different botanical varieties. The use of LC-ESI-QTOF
399 MS and LC-ESI-IT MS allowed the accurate and reliable determination of a great number
400 of analytes, including the secoiridoid derivatives (not evaluated before in samples coming
401 from this geographical area). The results make evident that olive oils coming from
402 Mendoza can be considered as important sources of phenolic bioactive compounds,
403 exhibiting similar phenolic compounds levels to those shown by oils from other typical
404 world production regions. Moreover, this study has evinced some peculiarities in the

405 composition of *Arauco* olive oils; indeed, a correlation between flavonoids and botanical
406 variety was established herewith. Even though this contribution could have some
407 limitations related to the relatively low number of samples and the variety of influencing
408 variables, the results could represent a milestone for the producers, enlarging their
409 knowledge about the composition of their oils and making them aware about its
410 commercial value.

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416 **Abbreviations**

417 ACN, acetonitrile; AcPin, acetoxypinoresinol; BPC, base peak chromatogram; DesoxyEA,
418 desoxy elenolic acid; DLA, decarboxymethyl ligstroside aglycone DOA, decarboxymethyl
419 oleuropein aglycon; HPin, hydroxypinoresinol; DOPAC, 3,4-dihydroxyphenylacetic acid;
420 EA, elenolic acid; DEA, decarboxymethyl elenolic acid; EIC, extracted ion chromatogram;
421 10-H Ole Agly, 10-hydroxy oleuropein aglycone; HTY, hydroxytyrosol; AcHTY,
422 hydroxytyrosol acetate; OxHTY, oxidized hydroxytyrosol; IS, internal standard; LC, liquid
423 chromatography; LC-ESI-IT MS, liquid chromatography-electrospray ionization-ion trap
424 mass spectrometry; LC-ESI-QTOF MS, liquid chromatography-electrospray ionization
425 quadrupole-time of flight mass spectrometry; LC-MS, liquid chromatography-mass
426 spectrometry; Lig Agly, ligstroside aglycone; HEA, hydroxyelenolic acid; Ole, oleuropein;

427 Ole Agly, oleuropein aglycone; PCA, principal component analysis; Pin, pinoresinol; R_t,
428 retention time; VOO, virgin olive oil.

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563 **Caption to figures**

564 **Figure 1.** EICs of the 40 analytes quantified in this work. (1) quinic acid; (2) DOPAC; (3)
565 caffeic acid; (4) p-coumaric acid; (5) ferulic acid; (6) OxHTY and isomer; (7) HTY; (8)
566 tyrosol (9) HTY isomer; (10) AcHTY; (11) HPin; (12) luteolin; (13) Pin; (14) AcPin; (15)
567 apigenin; (16) diosmetin; (17) EA and isomers; (18) DEA and isomer; (19) DesoxyEA;
568 (20) HEA; (21) Lig Agly and isomers; (22) DLA; (23) Ole Agly and isomers; (24) DOA
569 and isomers; and (25) 10-H Ole Agly and isomers. The isomers are identified by adding a
570 letter (a, b, c, d, e, f) to the number assigned for the main isomer.

571 **Figure 2.** Total concentration of phenolic compounds found in each sample under study;
572 each bar is indicating the overall concentration (expressed in mg kg^{-1}) of the five main
573 classes determined (phenolic acids, simple phenols, secoiridoids, flavonoids and lignans).

574 **Figure 3.** a) Score and loading plots of PCA considering the concentration of each
575 quantified phenolic compound (average of 4 replicates). b) 2D scatter plot of lignans versus
576 flavonoids. Arb: Arbequina; Arc: Arauco; Ble: blend; Cha: Changlot; Cor: Coratina; Emp:
577 Empeltre; Fra: Frantoio; Man: Manzanilla; Pic: Picual; Nev: Nevadillo.

578 **Figure 4.** Fragmentation patterns of two phenolic compounds scarcely explored in VOO. a)
579 MS spectrum of *m/z* 199.0620 (Rt of 3.6 min). b) MS spectrum of the peak with *m/z*
580 213.0771 (with a Rt of 6.4 min).

581 **Figure S1 (supplementary material):** BPC of some of the phenolic profiles obtained
582 injecting 10 μL of the pool VOO sample using the optimal chromatographic conditions and
583 optimal gradient based on the use of acidic water (acetic acid 0.5 % v/v) as Phase A and
584 ACN as Phase B (see Materials & Methods section).

Table 1 a) Quantitative results expressed in mg kg^{-1}, achieved by using the LC-ESI-IT MS developed method applied of total sample set. The results are given by the mean value (n=4; four independent determinations, including extraction and subsequent injection) ±standard deviation.

Compounds	R_t (min)	m/z	Arauco 1	Arauco 2	Arauco 3	Arauco 4	Arauco 5	Arauco 6	Manzanilla 1	Manzanilla 2	Manzanilla 3	Frantoio 1	Frantoio 2	Coratina 1	Coratina 2
Phenolic acids															
Quinic acid	1.3	191	0.19±0.02	0.07±0.03	n.d.[a]	3.37±0.17	0.05±0.01	0.11±0.02	1.03±0.08	0.03±(<0.01)	0.49±0.05	0.48±0.05	0.11±0.03	0.07±0.03	1.92±0.36
Caffeic acid	4.9	179	0.21±0.08	0.19±0.05	0.08±0.03	n.d.[a]	n.d.[a]	0.09±0.04	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	0.17±0.07
p-Coumaric acid	6.2	163	1.20±0.13	0.77±0.06	0.52±0.01	0.75±0.10	0.49±0.01	0.76±0.05	0.27±0.02	0.32±0.02	0.22±0.02	0.26±0.04	0.13±0.03	0.22±0.05	0.20±0.04
Ferulic acid	6.6	193	0.16±0.02	0.12±0.02	0.13±0.01	0.12±0.02	0.07±0.04	0.18±0.03	0.12±0.02	0.10±0.04	0.09±0.03	0.09±0.02	n.d.[a]	0.06±0.02	0.03±0.01
Simple phenolic alcohols															
OxHTY	1.4	151	0.23±0.05	0.16±0.04	0.25±0.02	0.34±0.03	0.04±0.01	0.17±0.01	0.12±0.03	0.04±0.01	0.04±0.02	0.27±0.01	0.21±0.04	0.17±0.02	0.28±0.02
OxHTY	1.8	151	0.28±0.07	0.17±0.04	0.32±0.05	0.33±0.04	0.05±0.01	0.21±0.02	0.13±0.02	0.02±(<0.01)	0.06±0.02	0.31±0.05	0.25±0.02	0.20±0.03	0.26±0.01
HTY	3.3	153	40.7±8.9	18.4±1.3	7.05±0.85	4.80±0.21	2.50±0.42	21.0±4.8	22.8±2.0	3.92±0.24	4.29±0.28	29.71±0.65	6.83±0.39	5.65±0.21	29.4±3.0
HTY Isomer	5.2	153	1.90±0.63	1.48±0.33	0.74±0.11	n.d.[a]	4.5±1.5	0.41±0.02	1.49±0.06	1.00±0.10	0.98±0.05	0.32±0.09	0.53±0.09	1.36±0.20	0.73±0.04
Tyrosol	4.4	137	27.9±2.4	10.86±0.54	7.50±0.31	11.40±0.40	8.47±0.98	23.9±2.4	8.85±0.50	7.21±0.27	5.28±0.57	18.1±1.7	5.80±0.28	6.80±0.46	19.62±0.59
AcHTY	7.2	195	0.83±0.05	2.80±0.10	1.32±0.20	0.78±0.09	0.27±0.02	1.18±0.14	0.56±0.05	n.d.[a]	0.62±0.06	3.25±0.31	5.67±0.25	0.51±0.05	0.19±0.02
Secoiridoids															
EA I 1	4.7	241	4.42±0.78	0.84±0.22	1.09±0.21	0.78±0.06	1.35±0.31	0.81±0.17	0.74±0.10	1.49±0.33	1.04±0.17	1.68±0.05	2.60±0.27	1.80±0.17	1.84±0.14
EA I 2	7.3	241	8.33±0.18	10.36±0.35	12.6±3.0	16.3±1.4	14.36±0.58	9.21±0.08	14.14±0.99	4.43±0.54	10.7±1.4	22.18±0.41	40.2±1.6	19.35±0.26	21.0±1.2
EA Ppal	7.7	241	78.6±8.6	22.49±0.60	27.5±2.6	55.0±2.4	33.38±0.41	32.0±2.9	27.6±1.1	17.81±0.68	15.04±0.87	48.0±3.6	57.6±2.3	56.9±2.6	64.7±3.1
DEA	4.9	183	0.36±0.07	0.18±0.02	0.14±0.03	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	0.11±0.01	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
DEA Ppal	5.6	183	3.58±0.01	2.95±0.26	0.87±0.05	0.40±0.05	n.d.[a]	6.39±0.55	5.97±0.53	1.52±0.24	n.d.[a]	14.11±0.10	n.d.[a]	0.44±0.05	4.42±0.35
HEA	6.8	257	4.26±0.50	3.54±0.29	1.99±0.25	1.97±0.10	0.37±0.04	1.01±0.10	0.56±0.11	0.51±0.08	0.43±0.04	3.66±0.07	1.56±0.15	0.75±0.11	0.84±0.06
DesoxyEA	6.6	225	5.62±0.61	6.10±0.24	4.28±0.26	8.2±1.0	6.6±1.5	5.48±0.17	7.35±0.74	0.42±0.13	21.3±1.8	3.00±0.20	1.60±0.21	15.56±0.72	19.16±0.94
DOA Ppal	8.8	319	15.21±0.71	24.9±3.2	17.21±0.36	3.67±0.08	11.92±0.55	10.94±0.69	14.7±1.2	4.18±0.27	38.54±0.58	18.3±1.5	26.2±4.7	21.1±5.3	19.8±2.3
DOA	9.2	319	4.57±0.29	8.24±0.66	6.5±1.3	1.34±0.04	0.63±0.04	3.36±0.36	0.87±0.12	3.00±0.01	7.9±8.0	5.68±0.38	3.81±0.35	1.84±0.66	1.25±0.15
Ole Agly I 1	8.1	377	7.70±0.48	1.70±0.17	1.28±0.08	1.87±0.13	2.10±0.27	2.35±0.52	2.43±0.24	0.15±0.03	0.63±0.10	0.44±0.04	0.93±0.06	5.6±1.3	4.76±0.03
Ole Agly I 2	8.5	377	6.35±0.45	3.44±0.46	2.93±0.45	6.51±0.50	4.89±0.19	3.75±0.07	5.55±0.68	0.40±0.05	1.45±0.24	2.89±0.34	3.08±0.30	7.53±0.06	8.9±1.1
Ole Agly I 3	9.3	377	1.44±0.37	1.02±0.18	0.84±0.21	1.93±0.01	1.76±0.52	1.58±0.12	1.16±0.17	0.15±0.04	2.97±0.13	0.46±(<0.01)	1.39±0.20	2.34±0.28	2.34±0.08
Ole Agly I 4	10.0	377	1.90±0.34	1.64±0.72	1.20±0.29	2.20±0.29	2.84±0.14	1.20±0.15	1.34±0.17	0.51±0.07	3.39±0.20	0.38±0.04	3.03±0.02	2.39±0.14	2.37±0.08
Ole Agly I 5	10.4	377	3.59±0.65	3.35±0.25	3.50±0.38	2.22±0.57	4.6±1.2	2.46±0.46	4.22±0.13	1.42±0.31	8.3±3.3	1.15±0.15	6.48±0.29	3.34±0.29	3.23±0.40
Ole Agly Ppal	11.1	377	14.00±0.42	10.99±0.72	9.05±0.51	12.5±4.0	26.77±0.98	14.89±0.96	16.08±0.88	7.3±1.0	21.39±0.91	6.41±0.41	16.37±0.08	9.8±1.0	8.80±0.26
Total Ole Agly			**35.0±1.1**	**22.1±1.2**	**18.80±0.86**	**27.2±4.1**	**43.0±1.7**	**26.2±1.2**	**30.8±1.2**	**10.0±1.1**	**38.1±3.5**	**11.73±0.56**	**31.28±0.47**	**31.0±1.7**	**30.4±1.2**
10-H Ole Agly I 2	9.5	393	0.61±0.08	0.60±0.15	0.24±0.01	0.18±0.02	0.09±0.02	0.19±0.03	0.12±0.03	0.15±0.06	0.09±0.01	0.33±0.02	0.14±0.01	1.26±0.08	n.d.[a]
10-H Ole Agly I 3	9.7	393	7.02±0.12	4.80±0.50	2.69±0.36	2.69±0.44	1.43±0.16	2.42±0.14	1.13±0.08	0.08±0.06	0.45±0.05	1.97±0.14	2.56±0.07	0.23±0.05	2.16±0.14
10-H Ole Agly Ppal	9.9	393	1.28±0.25	1.40±0.29	0.63±0.08	0.80±0.05	0.19±0.02	0.38±0.04	0.27±0.04	0.17±0.03	0.19±0.07	0.70±0.06	0.44±0.03	0.26±0.02	0.27±0.03
Lig Agly I 1	9.4	361	16.0±1.0	10.7±1.7	8.3±1.8	23.11±0.74	30.5±1.2	7.4±1.9	3.20±0.36	0.74±0.11	3.23±0.85	3.61±0.05	11.9±3.6	24.04±0.29	21.11±0.15
Lig Agly I 2	9.9	361	23.0±1.9	25.0±2.8	25.0±1.6	21.8±0.5	35.1±3.2	11.54±0.04	8.15±0.54	2.19±0.09	10.76±0.77	7.70±0.09	18.05±0.60	30.7±1.5	25.2±0.7
Lig Agly I 3	11.2	361	4.08±0.27	2.09±0.05	2.69±0.18	1.72±0.09	9.03±0.44	1.75±0.17	0.92±0.05	0.62±0.12	1.57±0.26	1.33±0.08	4.41±0.30	2.47±0.88	3.51±0.20
Lig Agly I 4	11.6	361	4.55±0.13	2.18±0.60	5.02±0.78	1.49±0.59	27.5±2.7	3.77±0.61	2.61±0.02	1.78±0.29	4.53±0.75	1.21±0.26	10.2±2.7	5.40±0.44	6.0±3.5
Lig Agly Ppal	12.3	361	67.6±1.5	53.6±13.7	49.6±4.5	28.2±1.9	149.5±5.3	47.3±5.0	32.9±1.2	24.4±1.5	42.3±7.2	24.4±2.2	78.6±9.2	81.8±1.3	86.1±3.2
Total Lig Agly			**115.3±2.6**	**94±14**	**90.6±5.2**	**76.4±2.2**	**251.5±6.9**	**71.8±5.4**	**47.8±1.4**	**29.8±1.6**	**62.3±7.4**	**38.3±2.2**	**123.1±10.3**	**144.4±18.0**	**141.9±4.8**
DLA	10.3	303	7.13±0.14	28.0±1.5	18.07±0.93	0.80±0.06	8.68±0.72	10.3±1.5	20.5±1.7	11.96±0.48	33.7±1.8	11.35±0.75	38.9±2.7	18.53±0.07	9.56±0.55
Lignans															
HPin	7.7	373	0.03±0.01	0.03±0.01	0.04±0.02	0.03±0.01	0.03±0.02	0.02±0.01	0.07±0.01	0.28±0.05	0.15±0.02	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
Pin	9.5	357	0.19±0.04	0.53±0.04	0.91±0.10	0.47±0.04	0.26±0.05	0.44±0.02	1.63±0.23	2.53±0.18	1.49±0.16	1.08±0.10	0.56±(<0.01)	0.44±0.01	0.47±0.04
AcPin	9.8	415	0.10±0.07	0.71±0.12	1.51±0.03	0.20±0.01	n.d.[a]	0.15±0.03	1.23±0.11	1.60±0.14	1.12±0.03	3.99±0.27	2.67±0.28	0.78±0.16	0.63±0.05
Flavonoids															
Luteolin	9.0	285	8.42±0.74	6.94±0.57	6.45±0.21	9.52±0.44	7.20±0.22	6.92±0.23	4.11±0.46	3.12±0.04	4.64±0.15	5.27±0.35	5.84±0.20	4.44±0.15	5.11±0.10
Apigenin	10.2	269	3.99±0.19	2.85±0.08	2.07±0.12	4.25±0.05	4.44±0.36	3.57±0.23	0.89±0.17	1.48±0.22	1.07±0.07	1.48±0.04	0.66±0.07	0.96±0.02	1.04±0.13
Diosmetin	10.5	299	0.61±0.02	0.98±0.10	1.13±0.15	0.98±0.07	1.41±0.06	0.74±0.05	0.39±0.02	0.73±0.09	1.02±0.12	0.72±0.05	0.91±0.14	0.32±0.02	0.27±0.04

TOTAL LEVELS	378.2±13.1	278±15	233.2±6.9	233.1±5.5	404.1±7.7	240.4±8.5	216.22±4.0	108.0±2.5	251.4±34.2	246.3±5.1	360.2±12.1	335.5±19.2	377.6±7.3

[a]n.d.: non-detectable

Table 1 b) Quantitative results expressed in mg kg^{-1}, achieved by using the LC-ESI-IT MS developed method applied of total sample set. The results are given by the mean value (n=4) ±standard deviation.

Compounds	R_t (min)	m/z	Arbequina	Picual	Empeltre	Changlot	Nevadillo	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5	Blend 6	Blend 7
Phenolic acids														
Quinic acid	1.3	191	0.75±0.10	0.43±0.08	0.17±0.01	0.11±0.02	0.21±0.03	0.04±(<0.01)	0.17±0.04	0.16±0.02	n.d.[a]	0.08±0.03	0.15±0.02	0.07±0.03
Caffeic acid	4.9	179	0.10±0.01	n.d.[a]	n.d.[a]	n.d.[a]	0.15±0.06	0.09±	n.d.[a]	0.10±0.01	0.12±(<0.01)	0.15±0.04	0.21±(<0.01)	n.d.[a]
p-Coumaric acid	6.2	163	0.25±0.03	0.27±0.05	0.11±0.03	0.32±0.01	0.14±0.03	0.22±0.05	0.27±0.05	0.30±0.05	0.56±0.02	0.57±0.09	0.30±0.05	0.25±0.01
Ferulic acid	6.6	193	0.08±0.03	0.08±0.03	0.03±0.03	0.13±0.02	0.04±0.03	0.09±0.02	0.11±0.04	0.13±0.02	0.12±0.02	0.18±0.01	0.05±0.02	0.10±0.02
Simple phenolic alcohols														
OxHTY	1.4	151	0.35±0.08	0.05±0.02	0.07±0.02	0.08±0.01	0.28±0.07	0.08±0.02	0.21±0.05	0.18±0.05	0.34±0.08	0.25±0.05	0.18±0.01	0.11±0.02
OxHTY	1.8	151	0.37±0.03	0.05±0.02	0.08±0.01	0.09±0.01	0.32±0.07	0.11±0.03	0.22±0.03	0.23±0.03	0.35±0.08	0.31±0.08	0.22±0.02	0.16±0.03
HTY	3.3	153	10.89±0.04	2.61±0.50	0.51±0.03	4.44±0.29	18.5±7.5	6.5±1.1	9.6±1.2	8.94±0.34	15.0±1.0	8.91±0.61	16.9±1.5	7.0±1.9
HTY isomer	5.2	153	0.44±0.01	4.20±0.33	0.31±0.03	1.42±0.11	0.62±0.38	0.84±0.74	0.81±0.26	0.47±0.01	0.81±0.03	0.45±0.10	0.87±0.64	0.32±0.01
Tyrosol	4.4	137	4.30±0.50	5.85±0.66	1.93±0.04	5.73±0.21	12.56±0.74	5.59±0.31	7.48±0.79	7.79±0.68	8.70±0.70	6.72±0.47	12.95±0.34	4.52±0.26
AcHTY	7.2	195	3.54±0.11	1.76±0.03	0.44±0.03	n.d.[a]	0.21±0.01	0.67±0.05	2.41±0.31	2.26±0.18	0.71±0.09	0.89±0.07	0.31±0.04	1.27±0.04
Secoiridoids														
EA I 1	4.7	241	0.74±0.04	0.26±0.02	0.67±0.01	3.34±0.22	0.20±0.08	0.44±0.31	0.13±(<0.01)	1.12±0.16	2.59±0.21	1.99±0.11	0.84±0.17	0.14±0.04
EA I 2	7.3	241	2.36±0.29	5.74±0.14	10.19±0.09	46.7±4.1	3.0±1.2	2.42±0.40	3.75±0.02	4.14±0.66	11.3±1.0	8.43±0.86	8.34±0.95	3.45±0.65
EA Ppal	7.7	241	10.2±1.7	14.51±0.28	26.3±1.2	96.3±6.5	10.40±0.47	6.88±0.91	12.42±0.47	14.67±0.47	29.06±0.34	21.7±1.1	23.89±0.77	9.70±0.11
DEA	4.9	183	0.13±0.03	n.d.[a]	n.d.[a]	n.d.[a]	0.13±0.02	0.17±0.02	0.15±0.03	n.d.[a]	0.15±0.01	0.17±0.02	0.12±0.02	0.13±0.02
DEA Ppal	5.6	183	3.56±0.10	n.d.[a]	n.d.[a]	n.d.[a]	10.9±1.1	0.79±0.18	9.59±0.10	16.38±0.32	1.05±0.08	3.29±0.19	9.33±0.33	0.59±0.04
HEA	6.8	257	2.54±0.19	0.57±0.04	2.37±0.06	1.59±0.32	1.33±0.24	2.14±0.36	2.53±0.22	1.53±0.32	3.39±0.29	3.38±0.32	1.43±0.10	1.17±0.12
DesoxyEA	6.6	225	1.25±0.01	2.45±0.28	1.23±0.02	1.30±0.27	0.73±0.09	0.77±0.16	1.38±0.07	1.09±0.18	4.49±0.42	4.19±0.38	1.52±0.12	0.65±0.01
DOA Ppal	8.8	319	9.00±0.06	4.81±0.33	6.37±0.09	3.66±0.21	11.3±1.2	8.64±0.98	10.11±0.14	9.64±0.46	19.38±0.65	11.8±1.0	8.62±0.26	9.50±0.03
DOA	9.2	319	5.44±0.07	0.25±0.02	2.75±0.20	0.34±0.02	6.15±0.40	8.28±0.34	4.77±0.25	3.35±0.24	5.94±0.51	6.81±0.93	4.25±0.59	5.27±0.24
Ole Agly I 1	8.1	377	n.d.[a]	2.17±0.19	n.d.[a]	1.50±0.20	0.13±0.04	n.d.[a]	n.d.[a]	n.d.[a]	0.73±0.06	0.23±0.03	1.44±0.07	n.d.[a]
Ole Agly I 2	8.5	377	0.26±0.04	4.67±0.58	0.18±0.04	4.36±0.89	0.41±0.11	0.17±0.04	0.22±0.04	0.23±0.04	2.35±0.08	0.73±0.11	3.54±0.18	0.29±0.06
Ole Agly I 3	9.3	377	0.14±0.02	1.39±0.14	0.22±0.08	1.12±0.09	0.26±0.05	0.12±0.02	0.25±0.02	0.19±0.03	0.54±0.09	0.28±0.02	0.42±0.09	n.d.[a]
Ole Agly I 4	10.0	377	0.18±0.05	1.84±0.20	0.14±0.05	2.14±0.15	0.43±0.04	0.45±0.02	0.15±(<0.01)	0.30±0.08	1.37±0.20	0.47±0.02	0.74±0.10	0.31±0.06
Ole Agly I 5	10.4	377	0.72±0.16	3.06±0.26	0.69±0.03	3.13±0.11	1.08±0.21	0.70±0.22	1.56±0.02	1.05±0.19	2.71±0.39	1.88±0.39	2.26±0.24	0.45±0.02
Ole Agly Ppal	11.1	377	5.37±0.84	20.7±1.9	5.16±0.21	16.81±0.57	4.80±0.40	6.03±0.92	6.49±0.77	5.76±0.28	20.0±4.9	9.42±0.60	10.65±0.51	4.34±0.37
	Total Ole Agly		**6.67±0.86**	**33.8±2.0**	**6.40±0.31**	**29.1±1.1**	**7.11±0.47**	**7.61±0.95**	**8.67±0.78**	**7.53±0.72**	**27.7±4.9**	**12.99±0.72**	**19.03±0.61**	**5.38±0.38**
10-H Ole Agly I 2	9.5	393	0.23±0.04	0.25±0.16	0.06±0.02	0.14±0.09	0.25±0.05	0.26±0.07	0.27±0.06	0.24±0.04	0.31±0.03	0.27±0.01	0.16±0.04	0.24±0.05
10-H Ole Agly I 3	9.7	393	0.56±0.03	0.72±0.06	0.16±0.01	1.36±0.21	0.57±0.05	0.84±0.16	0.64±0.04	0.62±0.22	3.91±0.57	1.73±0.29	1.66±0.23	0.65±0.14
10-H Ole Agly Ppal	9.9	393	0.38±0.03	0.91±0.41	0.05±0.04	1.06±0.04	0.30±0.08	0.39±0.04	0.29±0.01	0.36±0.08	0.67±0.09	0.66±0.10	0.31±(<0.01)	0.18±0.01
Lig Agly I 1	9.4	361	1.20±0.06	7.35±0.22	3.95±0.14	6.38±0.43	1.22±0.22	0.59±0.12	1.08±0.06	1.38±0.28	7.16±0.33	4.65±0.76	7.27±0.03	0.73±0.15
Lig Agly I 2	9.9	361	3.57±0.13	10.0±1.2	1.78±0.81	25.6±3.4	3.27±0.02	2.58±0.27	2.36±0.34	3.44±0.29	22.91±0.78	12.3±2.5	9.79±0.86	2.54±0.37
Lig Agly I 3	11.2	361	0.82±0.24	2.54±0.88	0.88±0.20	3.37±0.60	0.57±0.06	1.22±0.12	1.85±0.30	0.91±0.21	4.30±0.35	1.57±0.17	1.31±(<0.01)	0.83±0.03
Lig Agly I 4	11.6	361	0.81±0.20	7.6±1.2	2.4±1.2	8.17±0.44	0.67±0.06	1.38±0.31	1.51±0.62	1.62±0.46	5.87±0.21	3.38±0.29	1.47±0.41	1.96±0.09
Lig Agly Ppal	12.3	361	13.3±2.4	56.8±9.5	23.2±2.1	60.08±0.62	18.57±0.17	22.75±0.86	17.4±2.2	21.1±3.4	78.0±6.8	33.0±1.7	46.84±0.30	14.88±0.19
	Total Lig Agly		**19.7±2.4**	**84.2±9.7**	**32.2±5.1**	**103.6±3.6**	**24.30±0.29**	**28.52±0.97**	**24.2±2.4**	**28.4±5.4**	**118.2±6.9**	**54.9±3.1**	**66.7±1.0**	**20.94±0.45**
DLA	10.3	303	9.36±0.70	3.17±0.94	21.44±0.70	2.03±0.78	20.16±0.16	23.6±4.2	15.7±1.1	18.6±1.6	17.0±2.5	16.15±0.53	9.16±0.80	11.3±1.9
Lignans														
HPin	7.7	373	0.14±0.01	0.09±0.02	n.d.[a]	n.d.[a]	0.18±0.02	0.15±0.02	0.19±0.04	0.17±0.02	0.03±0.01	0.07±0.02	0.09±0.03	0.09±0.02
Pin	9.5	357	2.35±0.13	6.67±0.46	1.86±0.01	1.61±0.26	2.51±0.24	1.77±0.15	1.59±0.18	1.74±0.18	0.69±0.03	1.25±0.09	1.54±0.21	1.54±0.20
AcePin	9.8	415	4.28±0.32	0.24±0.04	2.52±0.17	2.74±0.12	0.55±0.07	2.78±0.11	4.57±0.21	5.65±0.76	1.65±0.26	2.95±0.19	0.29±0.07	2.64±0.02

Flavonoids														
Luteolin	9.0	285	4.99±0.34	3.37±0.56	3.17±0.15	3.17±0.06	2.30±0.12	2.24±0.27	2.61±0.31	3.86±0.05	6.34±0.75	7.25±0.19	3.11±0.21	2.23±0.06
Apigenin	10.2	269	1.47±0.09	0.64±0.13	0.98±0.02	0.95±0.03	0.99±0.17	1.24±0.13	1.11±0.45	1.32±0.11	2.53±0.24	2.41±0.37	1.53±0.05	0.91±0.11
Diosmetin	10.5	299	1.52±0.11	0.46±0.09	0.82±0.10	0.37±0.02	0.55±0.09	1.26±0.12	1.11±0.19	1.10±0.11	1.16±0.10	1.77±0.20	0.55±0.07	1.01±0.10
TOTAL LEVELS			**108.0±3.3**	**178.4±10.0**	**123.2±9.2**	**311.7±8.6**	**136.9±8.1**	**115.4±5.1**	**127.1±3.3**	**142.1±4.2**	**284.2±9.1**	**182.6±4.4**	**194.6±2.7**	**91.6±2.8**

[a]n.d.: non-detectable

Table 1S. Analytical parameters related to detection and quantification limits of the described method and *intra-day* and *inter-day* repeatability.

Compound	LOD (μg L^{-1})	LOQ (μg L^{-1})	*Intra-day* Repeatability (% RSD)[a]		*Inter-day* Repeatability (% RSD)[b]	
			Area	R_t	*Area*	R_t
Quinic acid	13.6	45.2	2.17	<0.01	2.70	<0.01
Hydroxytytrosol	6.2	20.6	3.12	0.04	5.71	1.24
Tyrosol	41.1	137.0	3.66	0.04	5.79	0.93
Caffeic acid	11.3	37.8	4.35	<0.01	2.83	<0.01
Homovanillic acid	11.5	38.3	1.44	<0.01	3.64	<0.01
p-Coumaric acid	72.5	241.5	1.03	0.05	7.01	0.84
Ferulic acid	6.3	21.1	1.62	<0.01	4.73	<0.01
Oleuropein	17.3	57.7	3.13	0.05	7.52	0.68
Luteolin	51.4	171.2	3.17	0.04	2.97	0.45
Pinoresinol	9.2	30.6	1.20	<0.01	1.92	<0.01
Apigenin	10.0	33.3	3.13	0.04	4.79	0.40

[a] RSD values (%) for peak areas and retention times (expressed in min) of the analytes under study measured from 5 injections carried out within the same day.

[b] RSD values (%) for peak areas and retention times (expressed in min) of the analytes under study measured from 5 injections carried out in five different days.

Figure 1

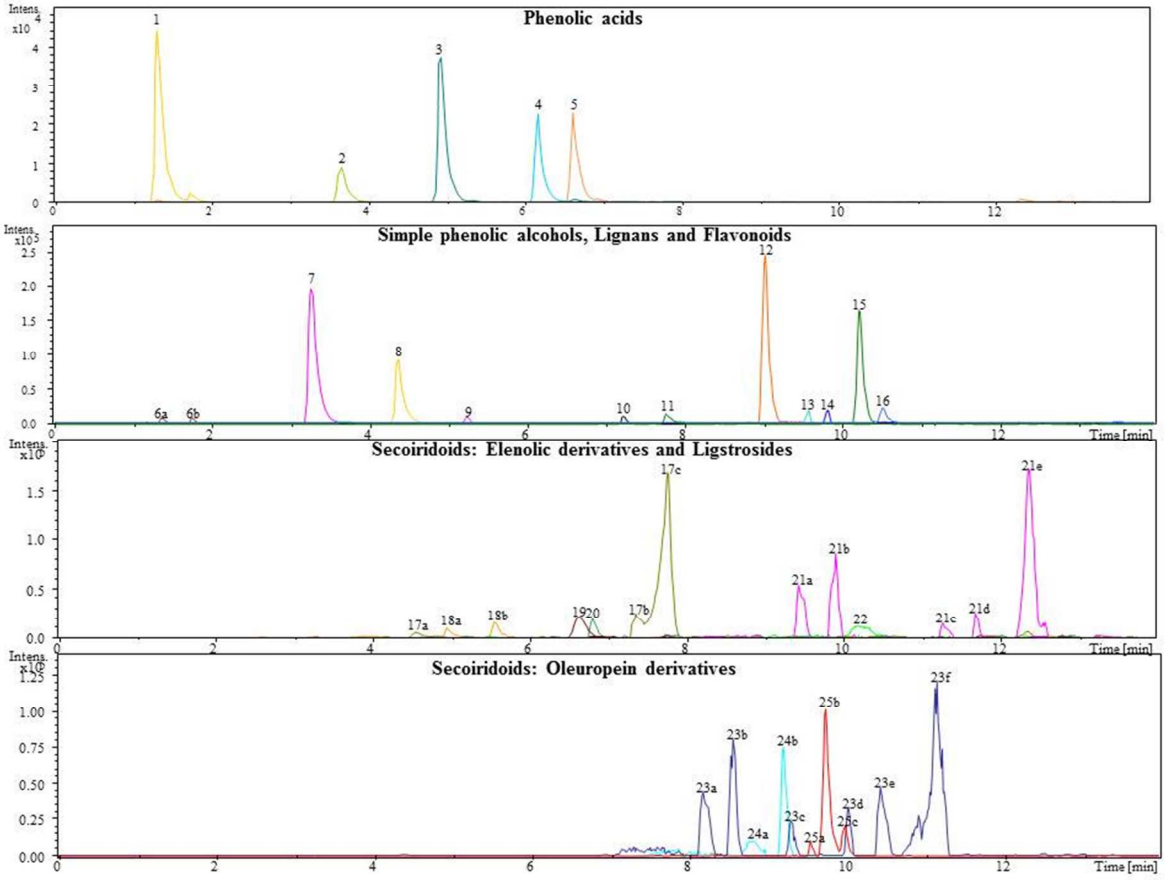

Phenolic acids
Simple phenolic alcohols, Lignans and Flavonoids
Secoiridoids: Elenolic derivatives and Ligstrosides
Secoiridoids: Oleuropein derivatives
Time [min]

Figure 2

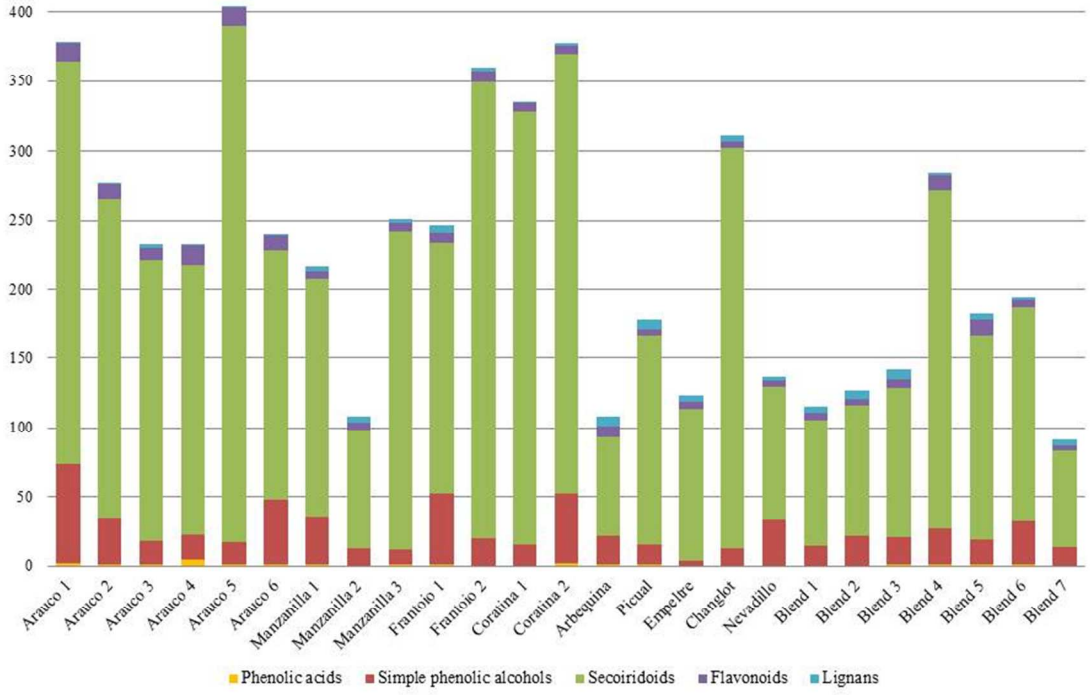

400
350
300
250
200
150
100
50
0
Arauco 1
Arauco 2
Arauco 3
Arauco 4
Arauco 5
Arauco 6
Manzanilla 1
Manzanilla 2
Manzanilla 3
Frantoio 1
Frantoio 2
Coratina 1
Coratina 2
Arbequina
Picual
Empeltre
Changlot
Nevadillo
Blend 1
Blend 2
Blend 3
Blend 4
Blend 5
Blend 6
Blend 7
Phenolic acids
Simple phenolic alcohols
Secoiridoids
Flavonoids
Lignans

Figure 3

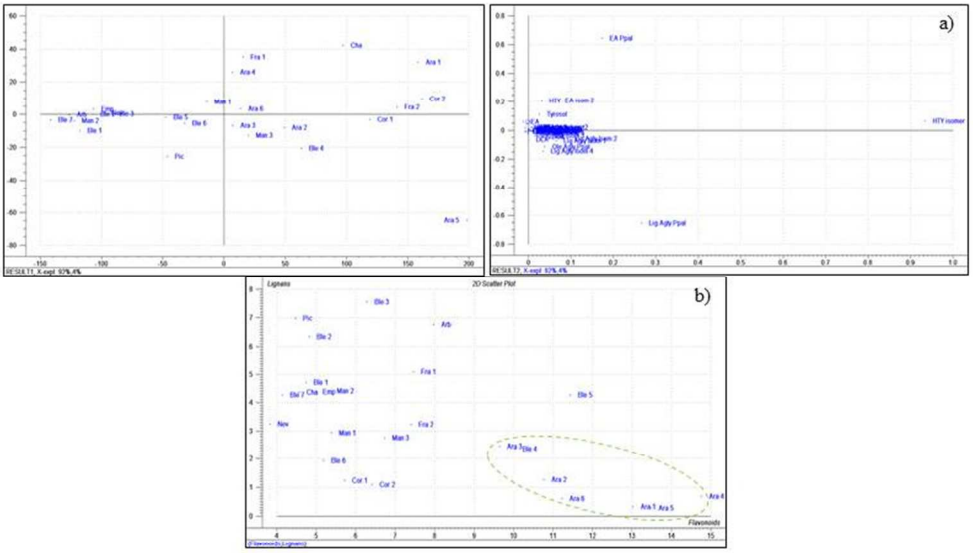


Figure 4

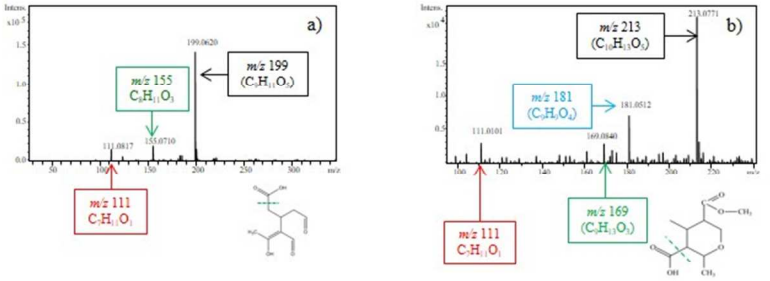
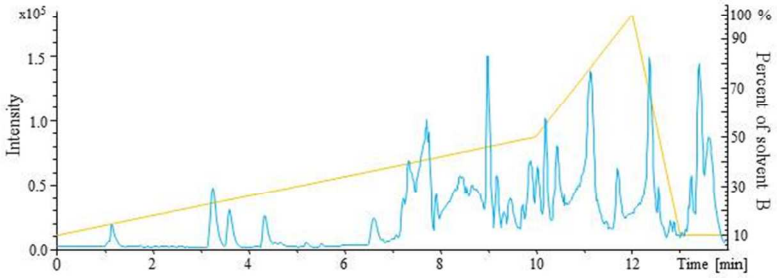
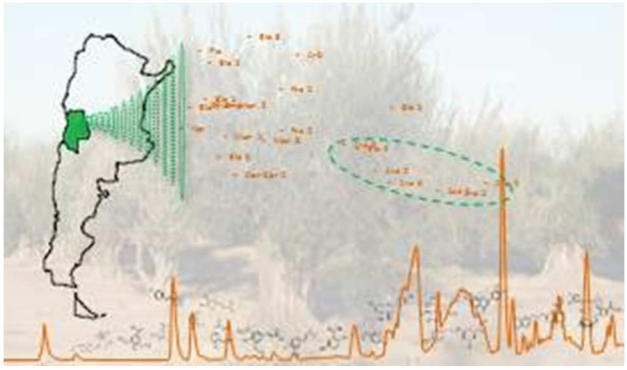

a)
m/z 199
(C9H11O5)
m/z 155
C7H7O3
m/z 111
C5H3O2
b)
m/z 213
(C10H13O6)
m/z 181
(C9H9O5)
m/z 169
(C8H9O5)
m/z 111
C5H3O2

Figure S1. (Supplementary data)



TOC



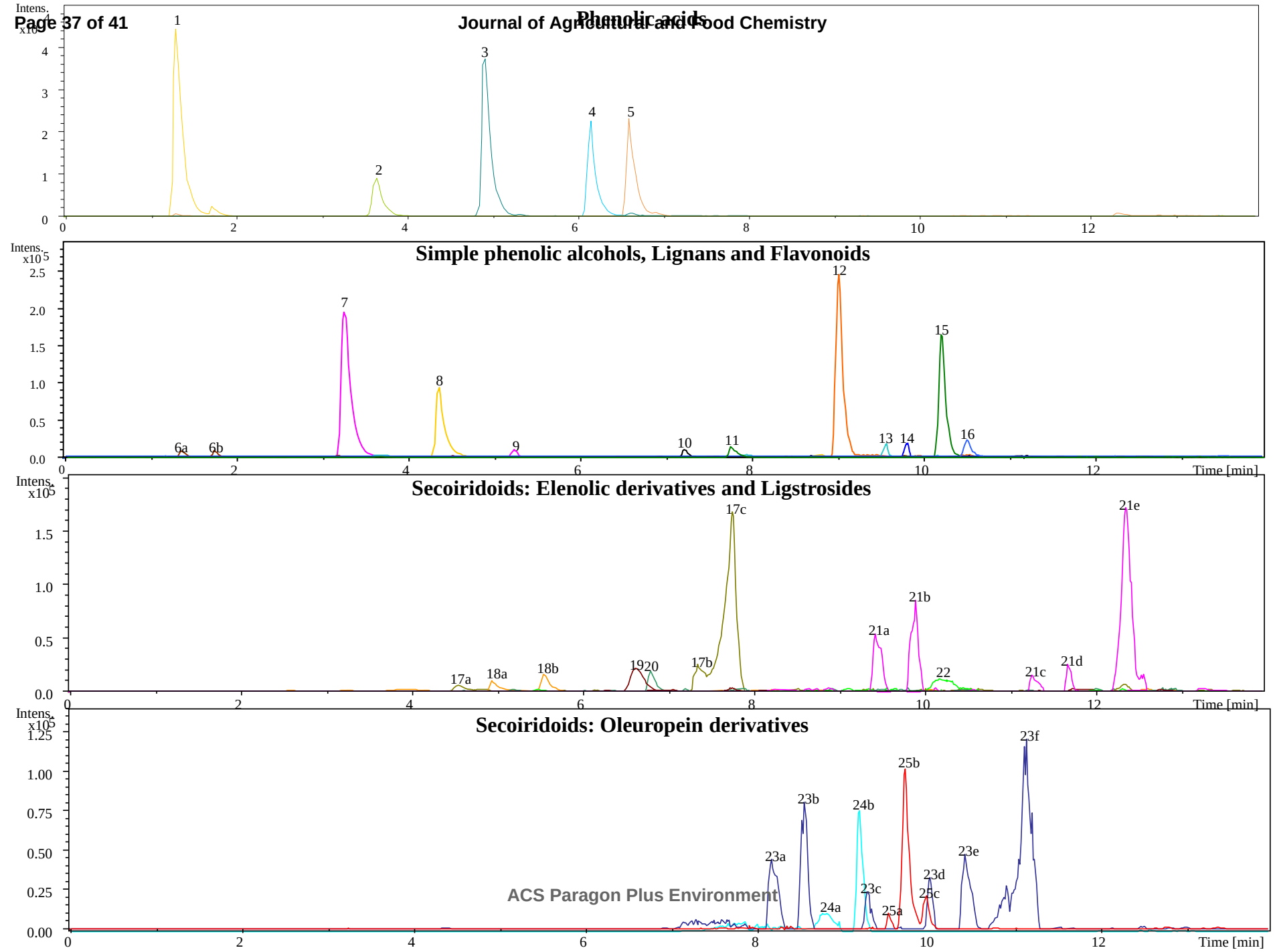

Phenolic acids
Intens.
x10 5
1
2
3
4
5
Simple phenolic alcohols, Lignans and Flavonoids
6a
6b
7
8
9
10
11
12
13
14
15
16
Time [min]
Secoiridoids: Elenolic derivatives and Ligstrosides
17a
17b
17c
18a
18b
19
20
21a
21b
21c
21d
21e
22
Time [min]
Secoiridoids: Oleuropein derivatives
23a
23b
23c
23d
23e
23f
24a
24b
25a
25b
25c
Time [min]
ACS Paragon Plus Environment

Figure 2

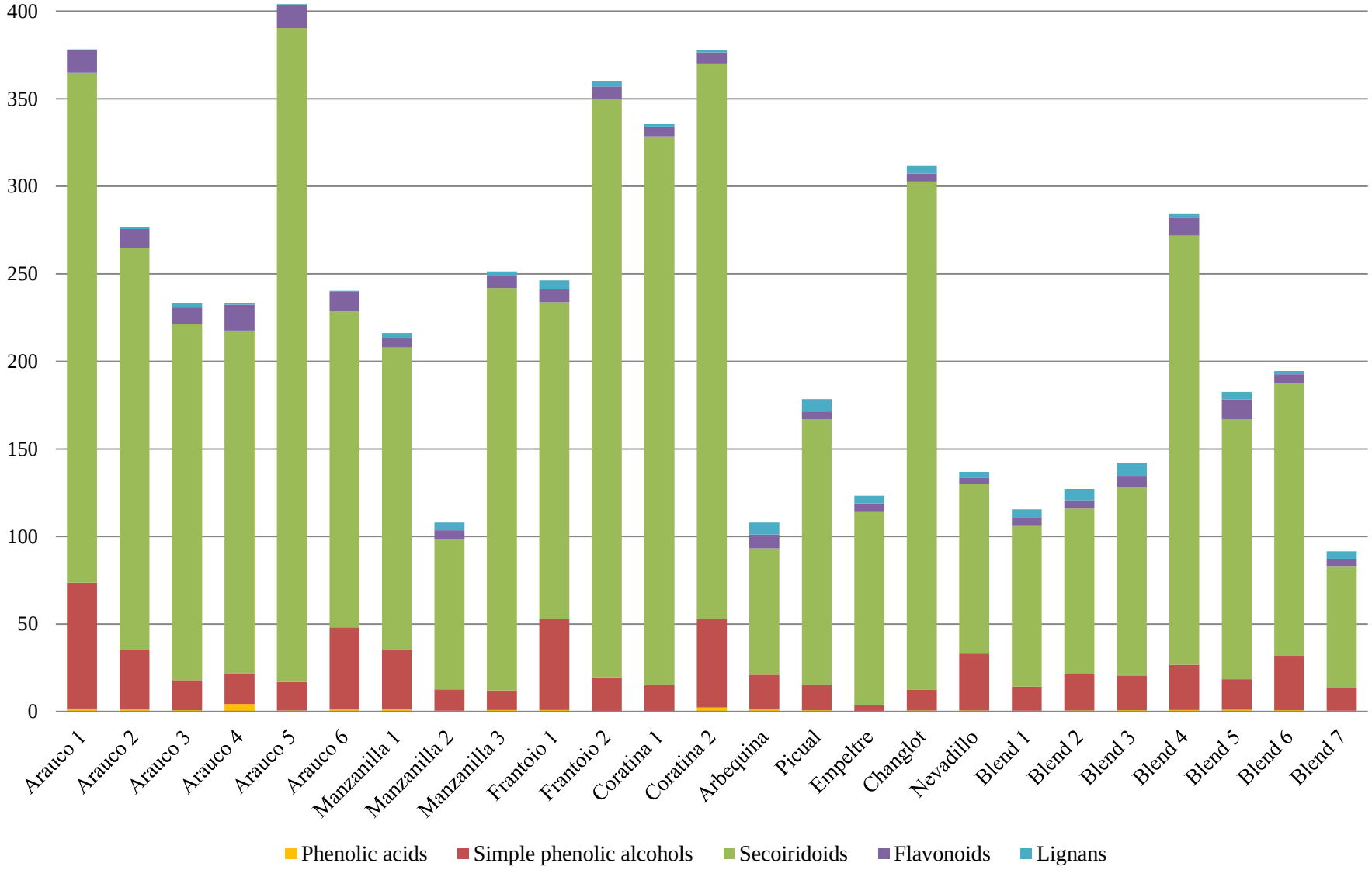

400
350
300
250
200
150
100
50
0
Arauco 1
Arauco 2
Arauco 3
Arauco 4
Arauco 5
Arauco 6
Manzanilla 1
Manzanilla 2
Manzanilla 3
Frantoio 1
Frantoio 2
Coratina 1
Coratina 2
Arbequina
Picual
Empeltre
Changlot
Nevadillo
Blend 1
Blend 2
Blend 3
Blend 4
Blend 5
Blend 6
Blend 7
Phenolic acids
Simple phenolic alcohols
Secoiridoids
Flavonoids
Lignans

Figure 3

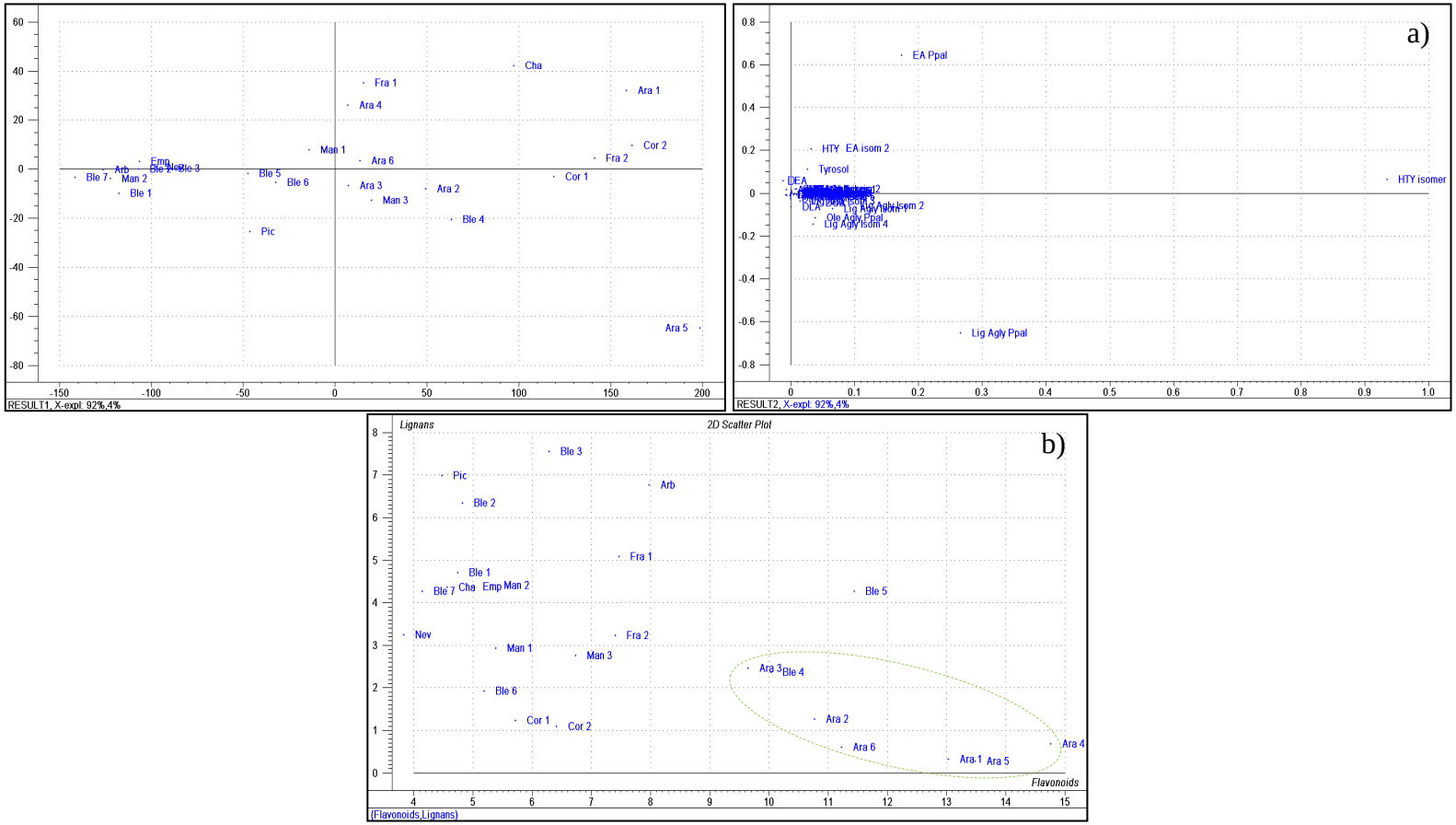
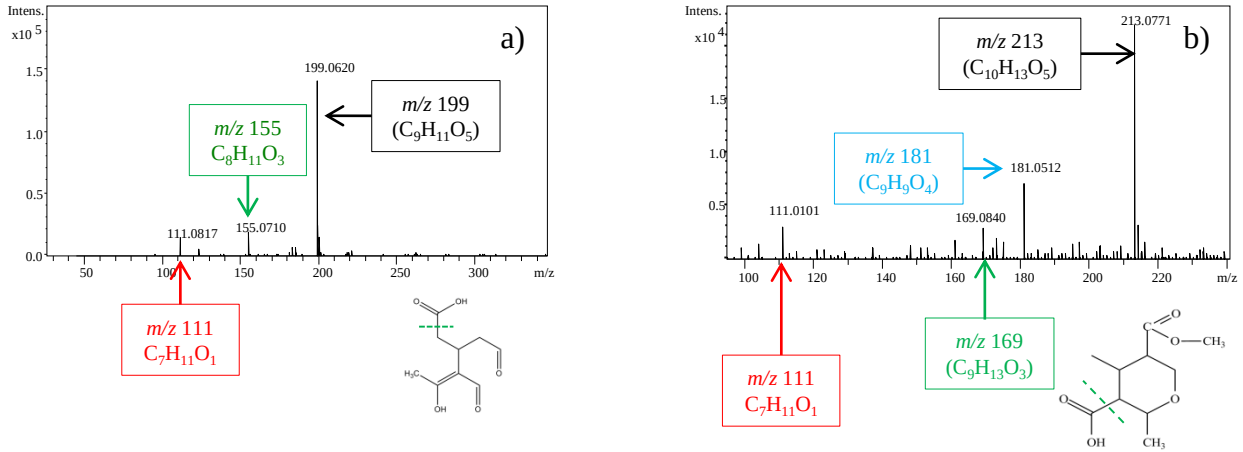

Cha
Fra 1
Ara 1
Ara 4
Cor 2
Man 1
Fra 2
Ara 6
Emp
Nev
Ble 3
Arb
Ble 7
Man 2
Ble 5
Ble 6
Ble 1
Ara 3
Ara 2
Cor 1
Man 3
Ble 4
Pic
Ara 5
RESULT1, X-expl: 92%,4%
a)
EA Ppal
HTY EA isom 2
Tyrosol
HTY isomer
DEA
DLA
Lig Agly Ppal
RESULT2, X-expl: 92%,4%
Lignans
2D Scatter Plot
b)
Ble 3
Pic
Arb
Ble 2
Fra 1
Ble 1
Ble 7
Cha
Emp
Man 2
Ble 5
Nev
Fra 2
Man 1
Man 3
Ara 3
Ble 4
Ble 6
Cor 1
Cor 2
Ara 2
Ara 6
Ara 4
Ara 1
Ara 5
Flavonoids
(Flavonoids,Lignans)

Figure 4


a)
Intens.
x10 5
1.5
1.0
0.5
0.0
199.0620
m/z 199
(C9H11O5)
m/z 155
C8H11O3
111.0817
155.0710
50
100
150
200
250
300
m/z
m/z 111
C7H11O1
b)
Intens.
x10 4
1.5
1.0
0.5
213.0771
m/z 213
(C10H13O5)
m/z 181
(C9H9O4)
181.0512
111.0101
169.0840
100
120
140
160
180
200
220
m/z
m/z 111
C7H11O1
m/z 169
(C9H13O3)
OH
CH3

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