Characterization of new olive fruit derived products obtained by means of a novel processing method involving stone removal and dehydration with zero waste generation

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Abstract

As a result of an innovative olive fruit processing method involving stone removal and dehydration, a new kind of olive oil and olive flour are generated. The main objective of this work was to accomplish the comprehensive characterization of the minor compounds of both products and to evaluate the effect of the dehydration temperature on their composition. To this end, olive oil and flour samples obtained through the novel processing method were analyzed and compared with 'conventional' virgin olive oils (VOO). The applied LC-MS methodology allowed the determination of 57 metabolites belonging to different chemical classes (phenolic compounds, pentacyclic trirterpenes and tocopherols). Both the new oils and flours presented considerable amounts of olive fruit metabolites that are usually absent from VOO. Quantitative differences were found among VOOs and the new oils, probably due to the inhibition of some enzymes caused by the temperature increase or the absence of water during the processing.

Keywords: LC-MS; olive oil; olive-by products; phenolic compounds; secondary metabolites

INTRODUCTION

Since ancient times, virgin olive oil (VOO) production has been traditionally based on the entire olive fruits milling followed by paste pressing and decantation to separate the oil. It was not until the 20th century when some technological developments took place, including the introduction of electric crushers and continuous malaxation and paste centrifugation systems.[1] Nevertheless, VOO is still produced using, essentially, the same principle implemented by Romans, which involves huge simultaneous waste generation (mainly fruit skin, pulp, seeds, pieces of stone and water). There are two main kinds of olive by-products: olive pomace (solid or semi-solid wastes) and mill wastewater (liquid effluents); their amount, composition and environmental impact depend on the extraction system of choice (i.e. two or three-phase systems).[2]

Over the last years, the interest in looking for a cost-efficient, technically feasible and environmentally sound solution for the residues generated from the VOO industry has drastically increased. Different management strategies for the recovery, recycling and upgrading of VOO by-products have been suggested (mainly using them as renewable fuel or fertilizers).[3,4] They have been also recognized as valuable sources of bioactive compounds[5,6] although the scaling up of the extraction processes to the industrial level has not been successfully achieved in many cases. In addition, new approaches involving the separate use of different olive fruit fractions (pulp and stones)[7] or new processing methods pursuing the reduction of waste generation, such as solvent extraction of the oil from dehydrated pulp,[8] have been proposed. The latter does not only avoid the production of pomace and wastewater but also originates a multifunctional ingredient consisting of stoned, dehydrated and defatted olive pulp. A promising alternative to this new methodology, replaces the solvent extraction step by cold pressing with a screw press to obtain olive oil and pulp pellet that can be converted into 'olive flour' by grinding.[9,10]

This powder, which is expected to contain high levels of fiber and bioactive compounds (tocopherols, phenolic compounds and pentacyclic triterpenes, among others),[11,12] could fulfill the criteria to act as a potential ingredient in functional food.[13] Moreover, the olive oil obtained in this way could have higher content of health promoting phytochemicals due to two main facts: on the one hand, stoning of fruits prevents the contact of the oil with seed endogenous peroxidases that may catalyze biomolecules oxidation during the traditional processing;[14,15] and on the other hand, the removal of water from the pulp could avoid the loss of the most hydrophilic metabolites through migration to the vegetation water during malaxation.[16] The other resulting fraction (whole stones) presents many reuse possibilities, such as biofuel, activated carbon precursor, abrasive or plastic filled, as described in previous reports.[17] Thus, the proposed methodology provides a way to achieve the full exploitation of olive fruits, which, at the same time, could overcome the waste generation issue and boost the economic outcomes of the olive grove. Firstly, the resulting olive oil may meet the increasing demand for high-quality oils (with the highest possible content of bioactive compounds);[18] and secondly, the novel and *a priori* highly functional olive flour may represent a very worthwhile new output for the diversification of olive sector.

Carrying out the chemical characterization of both the olive oil and olive flour resulting from applying the described novel olive fruit processing methodology (stone removal, pulp dehydration and cold pressing) is essential to estimate its viability and to check the advantages that it could bring to the VOO industry. Moreover, some technological aspects such as the influence of the dehydration temperature in the obtained products must be evaluated. VOO composition has been extensively investigated and the modulation of its minor compounds has been achieved by studying the influence of different technical aspects related to its conventional production.[19–21] However, as far as we know, the oil

obtained from stoned and dehydrated olive fruits has not been studied so far. In the same way, some stimulating reports have been published with regard to the composition of olive fruit,[22–24] but, to the best of our knowledge, there is no single report including information about olive flour composition. Thus, the main objective of the present work has been to accomplish the comprehensive qualitative and quantitative characterization of the olive oils and the olive flours obtained from 15 different cultivars by applying a novel olive fruit processing method and to evaluate the effect of the dehydration temperature (35, 55, 75 and 100ºC) on the composition of the resulting products. To this end, a total of 75 olive oil samples and 60 olive flour samples have been analyzed by means of a powerful LC-MS method capable of determining a wide number of molecules belonging to three different chemical classes (phenolic compounds, pentacyclic triterpenes and tocopherols).

MATERIALS AND METHODS

Chemicals and standards

Absolute ethanol and LC-MS grade acetonitrile were purchased from Prolabo (Paris, France). Water was daily deionized with a Milli-Q system (Millipore, Bedford, MA, USA). Acetic acid together with pure standards of phenolic compounds (quinic, *p*-coumaric and ferulic acids, vanillin, hydroxytyrosol, tyrosol, oleuropein, rutin, luteolin, luteolin 7-O-glucoside, apigenin and pinoresinol), triterpenic compounds (maslinic, betulinic and oleanolic acids) and tocopherols (α-, β- and γ- tocopherols) were all supplied by Sigma-Aldrich (St. Louis, MO, USA).

Samples

Olive fruit samples from 15 different cultivars were kindly donated by Acer Campestres S.L. (Castillo de Locubín, Jaén, Spain). The varieties under study were: ‘Arbequina’, ‘Brillante’, ‘Chorreao de Montefrío’, ‘Gordal’, ‘Hojiblanca’, ‘Lechín de Granada’,

‘Loaime de Alhama’, ‘Loaime de Tiena’, ‘Lucio’, ‘Manzanilla’, ‘Nevadillo de Alhama’, ‘Ombliguillo’, ‘Picual’, ‘Picual de Huétor Tájar’ and ‘Picudo’. Olive fruits were harvested and processed within six hours from the time they were gathered from the olive trees; ripening indexes were evaluated, and were found, in all the cases, within the range 2.5-4. In a preliminary stage, they were prepared (washing and size-sorting) for the stoning step. Then, homogeneous size fruits were stoned by means of a gauge-adjustable pitting machine (Comainox, Seville, Spain) from the table olive industry. Thereupon, water removal from the pulp was conducted in a lab-scale dehydrator model 100-800 (Memmert, Schwabach, Germany) at four different temperatures (35, 55, 75 and 100ºC) for an average of 50, 18, 9 and 4.5 hours, respectively (until water content was lower than 6%). The average weight loss resulting from the dehydration process was found between 55 and 65%, depending on the variety. Afterwards, dry pulp was pressed with a screw press (oil expeller from Piteba, Scheemda, The Netherlands) to obtain olive oil and defatted pulp separately. Finally, the oils were filtered through a paper filter to remove solid particles and the stoned, dehydrated and defatted pulp was grinded in a AKC-103 (450 W) mincer (Lauson, Barcelona, Spain) for getting ‘olive flour’. Additionally, monovarietal VOOs from each cultivar were obtained in the traditional way (two-phase system). To do this, entire fresh fruits were processed with an Abencor® laboratory oil mill (MC2 Ingeniería y Sistemas, Seville, Spain) equipped with a hammer crusher, malaxer and centrifuge.

Major components characterization

In order to determine the major composition of the prepared samples together with the quality parameters of the obtained oils, blend samples of each category were prepared by mixing equivalent amounts of the samples coming from every variety. In that way, 4 olive flour blend samples (mixtures of all the flour samples obtained with each evaluated

dehydration temperature) and 5 oil blend samples (a mix of all the VOOs obtained in the traditional way and blends of the oils prepared at 35, 55, 75 and 100 ºC, respectively) were subjected to further analysis. The aim of this stage of the project was to determine the olive oil quality parameters and the major components of a representative group of oils and olive flours, respectively.

Olive oil quality parameters, including free acidity (expressed as the percentage of oleic acid), peroxide value and UV spectrophotometric examination ($K_{270,}$ K_{232} and delta K), as well as fatty acids and sterols profiles were determined according to the European Commission Regulation 2568/91 and subsequent amendments.[25] Major components of the olive flours were determined according to AOAC guidelines: moisture (925.10), fat content (922.06), dietary fiber (total, 985.29; soluble and insoluble, 991.43), proteins (992.23) and ashes (923.03).[26] Sugars were determined by HPLC-RID.

Minor compounds analysis

- Minor fraction extraction

The isolation of the minor compounds from the oils was achieved by applying the liquid-liquid extraction protocol described in a recent publication.[27] Briefly, 1.00 (± 0.01) g of olive oil were extracted three times with ethanol/water mixtures by vortex shaking followed by centrifugation to separate the aqueous phase from the oil. The first extraction step was done with 6 mL of ethanol/water (60:40, v/v) and the next two steps with 6 mL of ethanol/water (80:20, v/v). Olive flours were subjected to a homologous solid-liquid extraction procedure, using ultrasounds to assist the release of the targeted metabolites from the fruit tissues. Therefore, after sieving the olive flours through a 0.5 mm metal sieve, 0.25 (± 0.01) g of sample were extracted in an ultrasonic bath for 30 min in three consecutive steps with 10 mL of the same ethanol/water mixtures used for the oils. For both kinds of samples, the three supernatant phases were collected together and after

158 solvent evaporation, the residue was redissolved in the adequate volume of ethanol/water
159 (80:20, v/v) (1 mL for the olive oils and 5 mL for the flour samples). The prepared extracts
160 were filtered through 0.22 μm nylon syringe filters from Agela Technologies
161 (Wilmington, DE, USA) before their analysis.

162 ***- LC-MS analysis***

163 Olive oil and olive flour extracts were analyzed according to a previously reported LC-
164 MS methodology[28] on an Agilent 1260 LC system (Agilent Technologies, Waldbronn,
165 Germany) coupled to a Bruker Daltonics Esquire 2000™ ion trap mass spectrometer
166 (Bruker Daltonik, Bremen, Germany) through an electrospray ionization source. A
167 Zorbax Extend C18 column (4.6 × 100 mm, 1.8 μm particle size) (Agilent Technologies)
168 was used for compound separation. The elution of the analytes was carried out at 40 °C
169 with a mobile phase gradient of acidified water and acetonitrile (both of them containing
170 0.5% acetic acid (v/v)) and a flow rate of 1 mL/min (increasing it at 1.5 mL/min from
171 min 23 to 29.5). MS spectra were acquired in full scan (50-1000 *m/z*), in negative ion
172 mode from the beginning to min 22.5 and in positive polarity from that point until the end
173 of the run (total run time of 31 min).

174 ***- Data treatment and statistics***

175 The quantification of the analytes under study was carried out by using external
176 calibration curves, which were prepared by dissolving the appropriate amount of the
177 commercially available pure standards in ethanol/water (80:20, v/v). Then, the stock
178 solution containing all the standards was serially diluted to working concentrations over
179 the range 0.1-500 mg/L.

180 Two replicate extractions were conducted for each sample, followed by two LC-MS
181 measurements (n=4). Data were expressed as mean values and relative standard
182 deviations (RSD) were calculated. Compass DataAnalysis 4.4 (Bruker Daltonik) and

Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA) were used for data processing and statistical analysis.

RESULTS AND DISCUSSION

Quality parameters and major composition of oils and flours obtained from stoned and dehydrated olive fruits

Table 1a includes the main quality parameters, fatty acids and sterols profiles of the selected representative oils (blends prepared from monovarietal samples belonging to each category). All the evaluated oil samples presented percentages of free acidity, peroxide values and UV specific extinction coefficients (K_{232}, K_{272} and delta K) within the limits established by the European Union for the extra virgin olive oil (EVOO) category.[25,29] That fact indicates that the proposed novel processing method (involving stoning and pulp dehydration) did not critically affect the olive oil quality (oxidation status). Equally, it did not seem to affect the fatty acids and sterols profile, which also met the purity criteria covered by the current European legislation.[25,29] Therefore, all the tested representative blends fulfilled the chemical requirements to be classified as EVOOs. The sensory evaluation of the oils was not carried out, since the obtained products presented a characteristic flavor that differs from the typical organoleptic standards of the conventional VOOs and thus, there were not any trained panel test able to reliably perform such kind of analysis. The purpose of carrying out these determinations was just to demonstrate that the new process does not alter major composition of the resulting products; any assumption regarding the commercial category that the new products would potentially have in the olive oil market was not intended.

Table 1b shows the main components determined in the flour representative blends. The obtained results could not be compared with previously published data, since similar studies are missing in literature. Nevertheless, having in mind the reduction in water and

fat content that takes place during the dehydration and defatting steps, our findings are in good agreement with the global ranges reported by Ryan and Robards for olive pulp composition.[30] As inferred from the table, the new production system leads to olive flours with a very similar profile of major components regardless of the used dehydration temperature.

Qualitative characterization of the minor fraction of oils and flours obtained from stoned and dehydrated olive fruits

The qualitative characterization of the minor compounds of the samples under study was addressed in the next stage of this work. The applied LC-MS methodology allowed the determination of 57 metabolites belonging to three different chemical classes (phenolic compounds, pentacyclic triterpenes and tocopherols). Figure 1 shows some examples of the chromatograms acquired for VOO, the olive oil obtained from stoned and dehydrated olives and its homologous olive flour. 100ºC was the used dehydration temperature for the Manzanilla samples shown in Figure 1; it was selected to be included in the illustration since it led to chromatograms exhibiting the highest content of a wide number of the compounds under study.

The determined peaks are listed in Table 2, which includes retention time (Rt), *m/z* of the *pseudo*-molecular ion, molecular formula of the assigned compound, name, chemical family and analytical standard used for its quantification. Peak identification was achieved by comparing relative Rt and *m/z* of the available pure standards, as well as using information from previous reports.[22,27,31,32] The last column of Table 2 indicates the type of matrix where each substance was detected. 45 and 37 compounds were determined in oils and flour samples, respectively. Just 25 out of the 57 total determined metabolites were found in both kind of matrices: quinic acid, hydroxytyrosol glucoside, acyclodihydroelenolic acid hexoside, a compound with *m/z* 389 (Rt 2.4 min) which could

correspond to either oleoside or secologanoside, elenolic acid and its glucoside, comselogoside, oleuropein, ligstroside, some isomers of oleuropein and ligstroside aglycones, decarboxymethyl oleuropein aglycone (also designated as oleacein), luteolin and luteolin 7-O-glucoside, four triterpenic compounds (maslinic, betulinic and oleanolic acids and a monohydroxylated derivative of maslinic acid), three tocopherols (α, β and γ-tocopherols) and two unknown compounds with *m/z* 363 (Rt 6.6 min) and 421 (Rt 9.7 min). Peak assignment could not be achieved for these two compounds, although the latter one had been already found by our research team in several 'Picudo' olive tree derived matrices (leaves, stems, seed, fruit skin and pulp).[27] Its reported molecular formula (calculated from the exact mass measured with a QTOF MS analyzer) was $C_{21}H_{26}O_9$.

Although the just mentioned metabolites were found in both oils and flours, some of them were absent from specific samples, depending on the cultivar and processing conditions. For example, oleuropein, ligstroside and luteolin 7-O-glucoside were detected at very low concentrations in all the analyzed VOOs. This finding was in agreement with previous reports describing the presence of an endogenous enzyme so-called β-glucosidase in the olive fruit, which catalyzes the enzymatic hydrolysis of glucosidic bounds during the conventional oil extraction procedure.[21,22] As a result, glucosilated phenolic compounds (mainly secoiridoids and flavonoids), which usually appear in olive leaves and fruits, are just found in aglycone forms in VOO. However, these glucosidic forms were found in relative abundance in the oils obtained with the new olive fruit processing method that includes the dehydration step. This may be caused by the absence of water during the oil extraction, which could hinder β-glucosidase action to a certain extent. Nevertheless, the general trend was that aglycone forms were found at higher relative concentrations in all the oil samples. This fact could be due to the residual activity of the just mentioned

enzyme or to the thermal hydrolysis of the glucosidic bonds (physical mechanism) previously reported for phenolic compounds conjugated forms.[33,34]

Apart from the previously mentioned secoiridoids, which were found in both kind of matrices, great differences were found between the rest of the members of this chemical family. As already exposed, VOO mainly presented aglycone forms: desoxy and hydroxy elenoic acid, hydroxytyrosol acyclodihydroelenolate, 10-hydroxy oleuropein aglycone, hydroxy decarboxymethyl oleuropein aglycone, decarboxymethyl ligstroside aglycone (also known as oleocanthal) and two extra ligstroside aglycone isomers. Conversely, olive flours were dominated by glycosylated secoiridoids. Those solely detected in defatted and grinded pulp samples were tentatively identified as neo-nuzhenide, hydroxyoleuropein, verbascoside, oleuropein glucoside, caffeoyl 6-oleoside, caffeoyl 6-secologanoside, 6-O-[(2E)-2,6-dimethyl-8-hydroxy-2-octenoyloxy] secologanoside and lucidumoside C. Regarding flavonoids, besides luteolin and luteolin 7-O-glucoside, three glycosilated flavonoids (rutin and two luteolin glucoside isomers) were detected in dehydrated and defatted pulp, whilst two additional non-glycosilated flavonoids (apigenin and methyl luteolin) were found in the oils. As far as simple phenols are concerned, while tyrosol, hydroxytyrosol and three derivatives (the oxidized, the glucosilated and the acetylated forms) were detected in the oils, only the glucosidic form of hydroxytyrosol was found in olive flours. Two phenolic acids (*p*-coumaric and ferulic) and one aldehyde (vanillin), as well as three lignans (syringaresinol, pinoresinol and acetoxypinoresinol) were determined in the oils, but none of them was found in olive flours. Furthermore, another unknown peak with relatively high intensity was detected in olive flours at Rt 3.1 min (*m/z* 377). This analyte had been also reported in a previous publication, where $C_{16}H_{26}O_{10}$ was assigned as its calculated molecular formula.[27]

Quantitative analysis of minor compounds in the new olive derived matrices

The quantitative evaluation of the obtained chromatograms was carried out in another stage of the project. All the metabolites described in the previous section were quantified in terms of their pure standard or on the basis of a compound presenting a related chemical structure, as indicated in Table 2. Quantification of unknown peaks was carried out in terms of oleuropein. It is important to bear in mind that the obtained quantitative data for the compounds lacking their corresponding pure standard are just an estimation of the real concentration, even though they are perfectly valid to compare the occurrence of those metabolites in the studied matrices.

Table 1 SI (Supporting Information) and Table 2 SI present the results of the quantitative analysis of 75 oil samples (VOO and oils obtained from stoned and dehydrated fruits at 35, 55, 75 and 100 ºC) and 60 olive flours (dehydrated at the same four temperatures), respectively. Apart from the clear differences found between samples obtained using different processing conditions, most of the analytes were found in very wide ranges of concentration even in samples processed at the same dehydration temperature. That points out a strong compositional dependence with the olive cultivar from which they were produced. Table 3 provides an overview of the concentration ranges found for each chemical class in every kind of matrix (VOO, oils and flours obtained using different dehydration temperatures). The given concentration values are the sum of all the metabolites belonging to each chemical family of compounds. Cultivars presenting the concentrations at the lower and upper ends of the range are also displayed below in the table. As clearly seen, not all the varieties were proportionally affected by the dehydration temperature (i.e. the cultivar presenting the highest concentration of a family of compounds in an oil obtained at a given temperature may not be the richest one at a different temperature or in the homologous flour). Nevertheless, some general trends can be inferred from the table. As far as “acids and derivatives” class is concerned, great

307 variability was found in oil samples; Arbequina presented the lowest content of quinic
308 acid in the flours obtained with three dehydration temperatures, and Picual de Huétor was
309 the richest variety regardless of the processing conditions. In respect of simple phenols,
310 Picudo presented the lowest content in oil samples at two dehydration temperatures while
311 Picual de Huétor had the highest concentrations at 35, 55 and 100 ºC; in the flours,
312 Ombliguillo was the poorest variety at every tested temperature, whilst Lucio presented
313 the highest content of simple phenols at 35, 75 and 100ºC. Arbequina stood out for its
314 low content in terms of secoiridoids in oils and flours obtained at the four different tested
315 dehydration temperatures, whereas Picual de Huétor and Ombliguillo (in oils) and Lechín
316 and Gordal (in flours) were the richest cultivars (each one at two different temperatures).
317 Concerning flavonoids, Loaime de Alhama and Manzanilla were among the poorest
318 varieties in oils and flours, respectively; Lechín and Hojiblanca presented the highest
319 flavonoids content in flour matrices. With regard to lignans, which were just determined
320 in oil samples, a typical feature of Lechín variety was its low content; while in contrast,
321 Arbequina presented the highest concentrations at three diverse temperatures. With
322 respect to triterpenic compounds, it is worth mentioning that the lowest average content
323 was found in oils from Hojiblanca (at 35ºC), Arbequina (obtained at 55 and 100ºC) and
324 Picudo (75ºC) varieties and Picual and Picual de Huétor flours obtained at two
325 temperatures each; in contrast, Nevadillo and Ombliguillo were pointed out among the
326 richest cultivars in three oils and two flours, respectively. Manzanilla and Lucio stood out
327 for their low and high tocopherols content in oils, apiece; Chorreao and Picudo were the
328 poorest varieties in terms of tocopherols in the flours, whilst Picual was one of the richest
329 cultivars. In general terms, Arbequina presented reduced amounts of the unknowns peaks
330 in both kind of matrices (oils and flours), whereas Ombliguillo and Brillante could be
331 underlined among the richest cultivars.

Figure 1 SI illustrates the described differences among samples obtained from different olive varieties using the same processing conditions. Sum concentrations of all the metabolites belonging to each chemical class are displayed at the same scale in the Y axis to facilitate the visual comparison of the bars.

Evaluation of the impact of the dehydration temperature in the metabolite profiles of the obtained olive oils and flours

Once the characterization of the previously unexplored matrices obtained by the new olive fruit processing method was carried out, the influence of the dehydration temperature on the metabolite profiles of the new products was thoroughly evaluated. The high number of analytes determined in the 135 evaluated samples made difficult the visualization and trends assessment in the obtained quantitative data. Thus, average concentrations for the determined compounds in each kind of matrix (VOO extracted in the conventional way, as well as oils and flours produced through the novel methodology, using four different dehydration temperatures) were calculated in order to facilitate the inspection of the data and the finding of common tendencies in all the samples obtained in the same way. Nevertheless, these mean values should be taken cautiously, bearing in mind the differences among cultivars and the wide concentration ranges for each chemical family established in Table 3.

Table 4 includes the calculated mean values for each metabolite in the 15 tested cultivars, together with the sum concentrations (global concentration levels) of the analytes belonging to each chemical family, and Figure 2 depicts the general trends followed by each family of compounds in oils and flours as a function of the dehydration temperature (including VOO obtained in the traditional way).

As shown in Figure 2 (I-V), in general, the higher the selected dehydration temperature, the greater the phenolic compounds content in both the oils and flours obtained through

357 the novel methodology. This finding suggest that the degradative enzyme
358 polyphenoloxidase may be inhibited by the temperature increase, as described in
359 literature.[35] However, trend variations were interestingly found among some of the
360 evaluated phenolic subfamilies. Moreover, the comparison of the new oils with the VOO
361 obtained in the traditional way, also revealed different responses to the new process
362 depending on the type of phenolic compound.
363 In order to reduce the number of graphs in Figure 2 and despite the chemical disparity,
364 the sole representative of organic acids (quinic acid) was grouped together with phenolic
365 acids and aldehydes (in oil samples; they were not found in flours) in the "acids and
366 derivatives" family (the same as in Table 3). The sum concentrations of this
367 "miscellaneous category" showed an upward trend with increasing dehydration
368 temperature in the oils obtained through the novel methodology, and the same trend was
369 observed for quinic acid in the flours. VOOs presented higher mean contents of "acids
370 and derivatives" than the oils obtained from fruits dehydrated at 35, 55 and 75 ºC. The
371 richest oils in terms of this family of compounds were those obtained using 100ºC as
372 dehydration temperature (this result can be explained considering the much higher
373 relative concentration of quinic acid at this temperature). Nevertheless, *p*-coumaric and
374 ferulic acids, as well as vanillin concentrations were generally higher in VOOs.
375 A similar general trend was found for simple phenols in both kind of matrices. The
376 concentration of hydroxytyrosol glucoside grew with increasing temperatures in the
377 flours, following the same trend as the five simple phenols determined in the oils.
378 Moreover, for all the members of this chemical class except for hydroxytyrosol acetate,
379 the concentrations found in VOOs were higher than in the new oils produced using 35,
380 55 and 75 ºC as dehydration temperatures. However, when the temperature was set at
381 100ºC, similar or even higher concentrations than in VOOs were achieved. Compared

382 with VOOs, the oils obtained from stoned and dehydrated olive fruits (especially at
383 100ºC) stood out for their notably high content of hydroxytyrosol acetate. This fact is
384 very remarkable, since this simple phenol has an antioxidant capacity similar to that of
385 hydroxytyrosol but presents higher lipophilicity, which may facilitate membrane crossing
386 and cell uptake, and thus, it may exhibit enhanced bioavailability.[36,37]
387 As far as secoiridoids are concerned, the general ascending trend with increasing
388 dehydration temperature was more severe in the oils. The concentration in VOOs of seven
389 secoiridoids (oleoside/secologanoside, comselogoside, elenolic acid and its glucoside,
390 oleuropein, ligstroside and oleacein) was always lower than in the oils obtained by means
391 of the novel methodology. VOOs average content of the other ten secoiridoids
392 (acyclodihydroelenolic acid hexoside, oleacein, oleocanthal, 10-hydroxy oleuropein
393 aglycone, and the sum of all the oleuropein and ligstroside aglycone isomers (3 isomeric
394 forms in each case)) was slightly higher than the concentration levels of the new oils
395 obtained from fruits dehydrated at low and moderate temperatures, but lower than in the
396 oils resulting from fruits dehydrated at 100ºC. Just three secoiridoids (desoxy elenolic
397 acid, hydroxytyrosol acyclodihydroelenolate and hydroxy oleacein) were always more
398 concentrated in VOOs than in the new oils. In olive flour samples, the highest average
399 total secoiridoids content was found when using 75ºC as dehydration temperature,
400 followed by 100ºC and 55ºC, respectively. This trend was mostly influenced by the high
401 relative content of oleuropein (around 12.3, 10.5 and 6.2 mg/g, at 75, 100 and 50ºC,
402 correspondingly). Great variability was found for the less abundant secoiridoids; for
403 example, 35ºC was the most favorable temperature to obtain flours rich in lucidumoside
404 C, 55ºC for elenolic acid glucoside, and 100ºC for oleuropein aglycone.
405 Flavonoids, which were among the scarcest determined families, were the most adversely
406 affected by the new processing method. Their content drastically decreased in the oils

obtained from stoned and dehydrated fruits (in around a 75%) compared to the VOOs, excluding luteolin 7-O-glucoside, which was almost absent from VOOs and increased its levels with the dehydration temperature. The minimum total flavonoids average concentration in the oils was found for those produced using 55ºC as dehydration temperature. In the case of the flours, flavonoids were the second less abundant family (after tocopherols) and followed a slight downward trend with increasing temperatures. The observed low thermal stability of flavonoids had been already reported by Allouche et al.[38]

The opposite trend to the one observed for flavonoids was monitored for lignans. They were systematically found at higher concentrations in the oils produced through the novel methodology and their content augmented as the dehydration temperature increased. This family of compounds had also been found among the lesser affected by thermal treatments by other authors.[38,39] As already discussed, they were not found in olive flours.

Triterpenic substances represented one of the most abundant chemical families in both the novel olive oils and flours. They were found at higher concentrations in oils obtained from stoned and dehydrated fruits than in VOOs, and their content grew with temperature increments. Their concentration in the flours remained almost unaffected by the temperature, although the highest average concentration of the two main triterpenic acids (maslinic and oleanolic) was found in flours obtained at 100ºC. This finding was in agreement with previous reports describing the high thermal stability of this chemical family.[38,40]

Tocopherols also showed an ascending tendency in oils, what suggests an improved transfer from the olive cells to the oil at higher temperatures. Although, according to previous reports, the concentration of tocopherols in VOO generally decreases with temperature, some authors have linked tocopherols thermal behaviour to the olive

variety[38] or the presence of phenolic compounds which are able to reduce their oxidized forms.[41] Thus, the higher amount of tocopherols found in the oils obtained through the new procedure could be explained by the protective effect of other coexisting antioxidants. Tocopherols presence in flours was very low and their content did not follow any clear pattern depending on the dehydration conditions. The lowest average tocopherols concentration was found in flours obtained at 100 ºC, although the value (0.16 mg/g) was quite similar to the concentration found at 55ºC (0.18 mg/g).

Lastly, the unknown compounds, generally decreased in oils and flours produced at higher temperatures of dehydration. As a matter of fact, the richest oils in terms of those compounds achieved by applying the new procedure were the ones obtained from olive fruits dehydrated at 35ºC (with concentration levels of 1.68 mg/kg).

Over the last years, the public environmental concern has encouraged researchers to look for industrial processes that follow the "zero waste" philosophy. In this context, a novel methodology for olive fruit processing, has been applied as an alternative to the traditional VOO extraction systems and the two new generated products have been comprehensively characterized by LC-MS. Concentration ranges for the determined metabolites were established for the first time in the previously unexplored matrices and the effect of the dehydration temperature in the composition of the resulting products was studied in depth. In general terms, all the evaluated chemical families were found at higher concentration levels in samples produced from fruits dehydrated at 100ºC. The oils obtained in these conditions were also richer than the conventional VOO in terms of most of the determined metabolites except for phenolic acids and aldehydes, three minor secoiridoids and the aglycone flavonoids.

Abbreviations: EVOO, extra virgin olive oil; VOO, virgin olive oil; RSD, relative standard deviation; Rt, retention time

Acknowledgement

Authors would like to thank M Sagrario García Castillo and Pilar Anaïs Nzobouh Fossi for their assistance with sample preparation and sample treatment, respectively.

Funding sources

This project received financial support from the Spanish Government (Ministerio de Educación, Cultura y Deporte) by means of a predoctoral fellowship (FPU13/06438), from the Mobility Program for young CONICET researchers (Argentina), and from the Andalusian Regional Government (Young Research Program "Programa Operativo de Empleo Juvenil").

Supporting Information description

Figure 1 SI. Sum concentrations of all the metabolites belonging to each chemical family in every evaluated kind of sample, from the 15 evaluated cultivars.

Table 1 SI. Quantitative data obtained for the oils evaluated in this study (VOO and oils produced from stoned and dehydrated olive fruits).

Table 2 SI. Quantitative data obtained for the flours evaluated in this study (produced from stoned, dehydrated and defatted olive fruits).

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606 **Figure captions**

607 **Figure 1.** Extracted ion chromatograms of samples from 'Manzanilla' variety: (A) VOO
608 obtained by the conventional two-phase system; and (B) olive oil and (C) flour obtained
609 from stoned and dehydrated (at 100 ºC) olive fruits. Peak identification numbers as in
610 Table 2. In order to facilitate the visual comparison of samples, chromatograms are shown
611 at two different scales: 0-5.2×10^5 intensity units (white background), 0-2.0×10^6 intensity
612 units (shaded background).

613 **Figure 2.** Average concentrations for each family of compounds in every kind of matrix
614 (from the 15 evaluated varieties). Green lines (left axis) correspond to the VOO samples
615 and the oils obtained at each tested dehydration temperature (sum concentrations
616 expressed in mg/kg). Purple lines (right axis) correspond to the four kind of olive flours
617 produced by the novel methodology (sum concentrations expressed in mg/g).

618 **Table 1a.** Quality parameters, fatty acids and sterols profiles of the QC oil samples
619 prepared by using each processing conditions.

Quality Parameter	**VOO**	**35 ºC**	**55 ºC**	**75 ºC**	**100 ºC**
Acidity (% oleic acid)	0.23	0.36	0.27	0.31	0.42
Peroxide value (meq/Kg)	8.13	11.73	12.23	12.43	9.87
K_{270}	0.12	0.12	0.11	0.14	0.22
K_{232}	1.62	1.30	1.25	1.34	1.59
Delta K	<0.01	<0.01	<0.01	<0.01	<0.01
Fatty acids profile					
Miristic (%)	<0.1	<0.1	<0.1	<0.1	<0.1
Palmitic (%)	10.5	10.6	10.7	10.6	10.7
Palmitoleic (%)	0.7	0.6	0.6	0.7	0.7
Heptadecanoic (%)	0.2	0.2	0.2	0.2	0.2
Heptadecenoic (%)	0.2	0.2	0.2	0.2	0.2
Stearic (%)	2.5	2.5	2.5	2.5	2.4
Oleic (%)	75.8	75.7	75.6	75.5	75.6
Linoleic (%)	8.9	8.7	8.8	8.9	8.8
Linolenic (%)	0.6	0.5	0.6	0.6	0.6
Arachidic (%)	0.4	0.4	0.4	0.4	0.3
Eicosenoic (%)	0.4	0.4	0.4	0.4	0.4
Behenic (%)	0.1	0.1	0.1	0.1	0.1
Lignoceric (%)	<0.1	<0.1	<0.1	<0.1	<0.1
Total sterols (mg/Kg)	1415	1450	1446	1433	1398
Cholesterol (%)	0.1	0.1	0.1	0.1	0.1
Brassicasterol (%)	<0.1	<0.1	<0.1	<0.1	<0.1
Campesterol (%)	2.8	3.0	3.1	2.9	2.9
Stigmasterol (%)	0.9	0.9	0.8	0.8	0.9
β-Sitosterol (%)	94.7	94.4	94.7	94.8	94.6
Δ-7-Stigmastenol(%)	0.4	0.4	0.3	0.3	0.4
Erythrodiol+Uvaol (%)	1.9	2.0	1.8	1.7	2.0

620 **Table 1b.** Major composition of the olive flours QC samples obtained with each
621 dehydration temperature.

Component (g/100g)	**35 ºC**	**55 ºC**	**75 ºC**	**100 ºC**
Moisture	9.5	9.8	10.3	9.4
Fat	19.7	21.1	19.7	18.7
Total sugars	13.8	14.1	15.0	15.5
Fructose	2.0	2.1	2.4	2.3
Glucose	11.8	12.0	12.6	13.2
Lactose	<0.1	<0.1	<0.1	<0.1
Maltose	<0.1	<0.1	<0.1	<0.1
Sacarose	<0.1	<0.1	<0.1	<0.1
Dietary fiber	20.9	19.3	20.8	21.8
Insoluble fiber	19.2	17.9	19.2	20.3
Soluble fiber	1.7	1.4	1.6	1.5
Proteins	5.4	5.1	6.1	5.7
Ashes	21.0	21.2	24.0	24.8

622

623 **Table 2.** Metabolites found in olive oil and olive flour samples obtained by the novel fruit processing method involving stone removal, dehydration

624 and cold pressing. (Analyses conducted by LC-MS).

Peak number	Rt (min)	*m/z*	Molecular formula	Name	Chemical family	Standard for quantification	Matrix	
							Olive oil	*Olive flour*
1	0.9	191	$C_7H_{12}O_6$	Quinic acid	Organic acids	Quinic acid	x	x
2	1.0	151	$C_8H_8O_3$	Oxidized hydroxytyrosol	Simple phenols	Hydroxytyrosol	x	
3	1.4	315	$C_{14}H_{20}O_8$	Hydroxytyrosol glucoside	Simple phenols	Hydroxytyrosol	x	x
4	1.7	407	$C_{17}H_{28}O_{11}$	Acyclodihydroelenolic acid hexoside	Secoiridoids	Oleuropein	x	x
5	1.9	153	$C_8H_{10}O_3$	Hydroxytyrosol	Simple phenols	Hydroxytyrosol	x	
6	2.4	389	$C_{16}H_{22}O_{11}$	Oleoside/Secologanoside	Secoiridoids	Oleuropein	x	x
7	2.7	137	$C_8H_{10}O_2$	Tyrosol	Simple phenols	Tyrosol	x	
8	3.0	403	$C_{17}H_{24}O_{11}$	Elenolic acid glucoside	Secoiridoids	Oleuropein	x	x
9	3.1	377	$C_{16}H_{26}O_{10}$	Unknown 1	Unknown	Oleuropein		x
10	3.5	609	$C_{27}H_{30}O_{16}$	Rutin	Flavonoids	Rutin		x
11	3.6	701	$C_{31}H_{42}O_{18}$	Neo-nuzhenide	Secoiridoids	Oleuropein		x
12	3.7	163	$C_9H_8O_3$	*p*-coumaric acid	Phenolic acids and derivatives	*p*-coumaric acid	x	
13	3.7	555	$C_{25}H_{32}O_{14}$	Hydroxyoleuropein	Secoiridoids	Oleuropein		x
14	3.8	447	$C_{21}H_{20}O_{11}$	Luteolin 7-O-glucoside	Flavonoids	Luteolin 7-O-glucoside	x	x
15	3.9	151	$C_8H_8O_3$	Vanilllin	Phenolic acids and derivatives	Vanilllin	x	
16	4.0	193	$C_{10}H_{10}O_4$	Ferulic acid	Phenolic acids and derivatives	Ferulic acid	x	
17	4.0	623	$C_{29}H_{36}O_{15}$	Verbascoside	Secoiridoids	Oleuropein		x
18	4.1	701	$C_{31}H_{42}O_{18}$	Oleuropein glucoside	Secoiridoids	Oleuropein		x
19	4.1	551	$C_{25}H_{28}O_{14}$	Caffeoyl 6-oleoside	Secoiridoids	Oleuropein		x
20	4.2	447	$C_{21}H_{20}O_{11}$	Luteolin glucoside (isomer I)	Flavonoids	Luteolin 7-O-glucoside		x
21	4.3	225	$C_{11}H_{14}O_5$	Desoxy elenoic acid	Secoiridoids	Oleuropein	x	
22	4.3	257	$C_{11}H_{14}O_7$	Hydroxy elenolic acid	Secoiridoids	Oleuropein	x	
23	4.4	551	$C_{25}H_{28}O_{14}$	Caffeoyl 6-secologanoside	Secoiridoids	Oleuropein		x
24	4.4	447	$C_{21}H_{20}O_{11}$	Luteolin glucoside (isomer II)	Flavonoids	Luteolin 7-O-glucoside		x
25	4.6	535	$C_{25}H_{28}O_{13}$	Comselogoside	Secoiridoids	Oleuropein	x	x
26	4.6	195	$C_{10}H_{12}O_4$	Hydroxytyrosol acetate	Secoiridoids	Hydroxytyrosol	x	
27	4.7	381	$C_{19}H_{26}O_8$	Hydroxytyrosol acyclodihydroelenolate	Secoiridoids	Oleuropein	x	

28	4.8	539	$C_{25}H_{32}O_{13}$	Oleuropein	Secoiridoids	Oleuropein	x	x
29	5.0	241	$C_{11}H_{14}O_6$	Elenolic acid	Secoiridoids	Oleuropein	x	x
30	5.1	335	$C_{17}H_{20}O_7$	Hydroxydecarboxymethyl oleuropein aglycone	Secoiridoids	Oleuropein	x	
31	5.4	583	$C_{27}H_{36}O_{14}$	Lucidumoside C	Secoiridoids	Oleuropein		x
32	5.6	523	$C_{25}H_{32}O_{12}$	Ligstroside	Secoiridoids	Oleuropein	x	x
33	5.7	319	$C_{17}H_{20}O_6$	Decarboxymethyl oleuropein aglycone (oleacein)	Secoiridoids	Oleuropein	x	x
34	5.8	285	$C_{15}H_{10}O_6$	Luteolin	Flavonoids	Luteolin	x	x
35	6.0	557	$C_{26}H_{38}O_{13}$	6-O-[(2E)-2,6-Dimethyl-8-hydroxy-2-octenoyloxy] secologanoside	Secoiridoids	Oleuropein		x
36	6.3	417	$C_{22}H_{26}O_8$	Syringaresinol	Lignans	Pinoresinol	x	
37	6.6	363	-	Unknown 2	Unknown	Oleuropein	x	x
38	6.7	357	$C_{20}H_{22}O_6$	Pinoresinol	Lignans	Pinoresinol	x	
39	6.8	393	$C_{19}H_{22}O_9$	10-hydroxy oleuropein aglycone	Secoiridoids	Oleuropein	x	
40	7.0	415	$C_{22}H_{24}O_8$	Acetoxypinoresinol	Lignans	Pinoresinol	x	
41	7.2	269	$C_{15}H_{10}O_5$	Apigenin	Flavonoids	Apigenin	x	
42	7.3	303	$C_{17}H_{20}O_5$	Decarboxymethyl ligstroside aglycone (oleocanthal)	Secoiridoids	Oleuropein	x	
43	7.6	299	$C_{16}H_{12}O_6$	Methyl luteolin	Flavonoids	Luteolin	x	
44	8.5	361	$C_{19}H_{22}O_7$	Ligstroside aglycone (isomer I)	Secoiridoids	Oleuropein	x	
45	8.6	377	$C_{19}H_{22}O_8$	Oleuropein aglycone (isomer I)	Secoiridoids	Oleuropein	x	x
46	9.2	377	$C_{19}H_{22}O_8$	Oleuropein aglycone isomer (isomer II)	Secoiridoids	Oleuropein	x	x
47	9.7	421	$C_{21}H_{26}O_9$	Unknown 3	Unknown	Oleuropein	x	x
48	10.5	361	$C_{19}H_{22}O_7$	Ligstroside aglycone (isomer II)	Secoiridoids	Oleuropein	x	x
49	11.1	361	$C_{19}H_{22}O_7$	Ligstroside aglycone (isomer III)	Secoiridoids	Oleuropein	x	
50	11.4	377	$C_{19}H_{22}O_8$	Oleuropein aglycone(isomer III)	Secoiridoids	Oleuropein	x	x
51	12.8	487	$C_{30}H_{48}O_5$	Monohydroxylated derivative of maslinic acid	Triterpenic compounds	Maslinic acid	x	x
52	18.6	471	$C_{30}H_{48}O_4$	Maslinic acid	Triterpenic compounds	Maslinic acid	x	x
53	21.3	455	$C_{30}H_{48}O_3$	Betulinic acid	Triterpenic compounds	Betulinic acid	x	x
54	21.5	455	$C_{30}H_{48}O_3$	Oleanolic acid	Triterpenic compounds	Oleanolic acid	x	x
55-56	27.4[a]	415[b]	$C_{28}H_{48}O_2$	β– and γ–tocopherols	Tocopherols	β– and γ–tocopherols	x	x
57	28.3	429[b]	$C_{29}H_{50}O_2$	α– tocopherols	Tocopherols	α– tocopherol	x	x

[a] Analytes coeluting in reverse-phase LC

[b] Analytes detected in positive polarity; *m/z* corresponding to $[M\text{-}H_2+H]^+$

627 **Table 3.** Establishment of the found concentration ranges of each chemical family in all the evaluated kinds of sample, from the 15 selected
628 cultivars. For each type of sample, minimum and maximum levels are given together with the name of the variety presenting these values.

	Concentration ranges (mg/kg) in olive oils					Concentration ranges (mg/g) in olive flours			
Dehydration T (ºC)	**VOO**	**35**	**55**	**75**	**100**	**35**	**55**	**75**	**100**
Acids and derivatives	1.05 - 6.04 OM/PD	0.36 - 2.52 LA/OM	0.35 - 2.27 LE/BR	0.35 - 5.49 LE/HO	0.52 - 19.06 PI/LE	4.07 - 23.88 GO/PH	3.15 - 23.01 AR/PH	3.95 -22.98 AR/PH	6.48 - 26.19 AR/PH
Simple phenols	3.40 - 25.70 AR/PI	1.81 - 22.05 LA/PH	2.40 - 29.64 OM/PH	8.68 - 56.37 PD/AR	21.61 - 103.88 PD/PH	0.10 - 1.63 OM/LU	0.07 - 2.05 OM/HO	0.10 -3.06 OM/LU	0.12 - 3.19 OM/LU
Secoiridoids	18.72 - 203.80 AR/CH	23.10 - 257.03 PD/PH	8.91 - 306.92 GO/PH	18.44 - 672.80 PI/OM	45.23 - 1574.96 AR/OM	2.66 - 20.01 LT/LE	2.56 - 25.75 AR/LE	2.83 -33.12 AR/GO	3.08 - 27.91 PD/GO
Flavonoids	0.88 - 4.10 LA/LE	0.03 - 3.39 LA/HO	0.10 - 0.72 LA/NE	0.05 - 1.45 CH/HO	0.09 - 3.57 LU/LE	0.13 - 0.93 MA/LE	0.10 - 1.02 MA/HO	0.12 -1.01 MA/HO	0.12 - 0.78 MA/LE
Lignans	0.59 - 8.70 LE/MA	0.67 - 8.97 LE/AR	0.58 - 13.73 LE/AR	0.92 - 12.79 LE/PI	1.12 - 13.10 PD/AR	n.d.	n.d.	n.d.	n.d.
Triterpenic compounds	9.92 - 98.97 MA/HO	53.13 - 159.24 HO/PD	92.29 - 149.87 AR/NE	97.15 - 159.79 PD/NE	106.42 - 161.49 AR/NE	8.42 – 20.41 PI/OM	3.45 - 18.99 PH/OM	3.95 -20.51 PH/LA	5.45 - 20.38 PI/BR
Tocopherols	83.61 - 447.30 AR/LU	77.54 - 419.45 MA/LU	118.69 - 461.54 MA/LU	125.59 - 439.95 MA/PH	162.29 - 573.01 MA/LU	0.02 - 0.57 CH/PI	0.02 - 0.59 CH/PI	0.01 -0.75 PD/PI	0.05 - 0.41 PD/PH
Unknowns	0.22 - 2.63 LE/CH	0.20 - 5.39 AR/BR	<0.01 - 1.77 AR/OM	<0.01 - 1.21 PI/OM	0.10 - 1.29 AR/BR	0.62 - 2.84 PH/BR	0.52 - 1.95 AR/OM	0.35 -1.44 AR/LA	0.38 - 1.51 LT/GO

629 AR, Arbequina; BR, Brillante; CH, Chorreao; GO, Gordal; HO, Hojiblanca; LE, Lechín; LA, Loaime de Alhama; LT, Loaime de Tiena; LU, Lucio;
630 MA, Manzanilla; NE, Nevadillo; OM, Ombliguillo; PI, Picual; PH, Picual de Huétor; PD, Picudo; n.d., non detected.
631 The upper and lower limits of the defined concentration ranges are the mean value of two independent replicates injected twice (n=4). RSD (%)
632 values were lower than 9.8% for the concentrations found in olive oil samples and lower than 8.7% for the concentrations found in olive flours.

633 **Table 4.** Average concentrations for the oils and flours obtained at each temperature

634 (or processing method, in the case of VOO) from the 15 evaluated olive varieties.

	Olive oils mean values (mg/kg)					Olive flours mean values (mg/g)			
Dehydration T (ºC)	**VOO**	**35**	**55**	**75**	**100**	**35**	**55**	**75**	**100**
Quinic acid	0.99	0.40	0.36	0.60	2.74	13.06	13.13	13.99	15.30
p-coumaric acid	0.22	0.11	0.18	0.24	0.19				
Vanillin	0.41	0.29	0.29	0.36	0.37				
Ferulic acid	0.28	0.13	0.14	0.14	0.13				
Total acids and derivatives[a]	***1.91***	***0.93***	***0.97***	***1.34***	***3.42***	***13.06***	***13.13***	***13.99***	***15.30***
Oxidized HTY	0.15	0.11	0.09	0.13	0.14				
HTY glucoside	0.36	0.20	0.24	0.60	1.82	0.69	0.85	1.02	1.08
HTY	6.31	1.87	2.16	3.74	6.34				
Tyrosol	6.58	3.07	5.08	6.05	8.60				
HTY acetate	1.21	1.47	4.48	10.17	25.93				
Total simple phenols	***14.59***	***6.72***	***12.05***	***20.69***	***42.83***	***0.69***	***0.85***	***1.02***	***1.08***
Acyclodihydro EA hexoside	0.07	0.04	0.03	0.04	0.33	0.37	0.41	0.37	0.32
Oleoside/secologanoside	0.15	0.66	0.62	0.41	1.25	0.62	0.67	0.72	0.68
EA glucoside	0.14	0.20	0.30	0.41	0.65	0.79	0.84	0.75	0.45
Neo-nuzhenide						0.02	0.02	0.03	0.02
Hydroxyoleuropein						0.16	0.30	0.14	0.08
Verbascoside						0.04	0.06	0.10	0.13
Oleuropein glucoside						0.06	0.06	0.07	0.07
Caffeoyl 6-oleoside						0.16	0.18	0.20	0.16
Desoxy EA	0.66	0.05	0.01	0.22	0.36				
Caffeoyl 6-secologanoside						0.03	0.03	0.05	0.03
Comselogoside	0.01	0.01	0.02	0.03	0.09	0.19	0.18	0.20	0.20
HTY acyclodihydroelenolate	1.33	0.32	0.36	0.57	0.27				
Oleuropein	0.02	0.37	0.59	1.87	9.37	4.89	6.18	12.28	10.50
EA	5.11	8.38	12.12	19.20	46.92	0.09	0.09	0.15	0.30
Hydroxy EA	0.03	0.05	0.03	0.03	0.06				
Hydroxy oleacein	1.45	0.61	0.61	1.02	1.38				
Lucidumoside C						0.70	0.61	0.12	0.06
Ligstroside	<0.01	0.04	0.06	0.10	0.26	0.16	0.29	0.40	0.32
Oleacein	19.01	11.76	14.10	29.53	37.77	0.11	0.14	0.32	0.39
6-O-[…] secologanoside						0.06	0.05	0.06	0.06
10-hydroxy OleAgly	1.16	0.59	0.42	0.49	1.72				
Oleocanthal	1.84	5.25	5.81	8.16	7.10				
OleAgly (isomers I+II+III)	36.71	23.36	32.77	60.21	226.35	0.19	0.15	0.32	0.74
LigAgly (isomers I+II+III)	52.13	33.72	35.03	44.56	132.76	0.03	0.04	0.04	0.09
Total secoiridoids	***119.82***	***85.39***	***102.86***	***166.85***	***466.65***	***8.66***	***10.30***	***16.33***	***14.63***
Rutin						0.24	0.21	0.23	0.21
Luteolin 7-O-glucoside	0.01	0.01	0.02	0.10	0.25	0.18	0.18	0.19	0.19
Luteolin glucoside (isomer I)						0.03	0.04	0.03	0.03
Luteolin glucoside (isomer II)						0.01	0.01	0.01	0.01
Luteolin	1.50	0.38	0.17	0.20	0.14	0.06	0.06	0.06	0.06
Apigenin	0.44	0.11	0.07	0.09	0.11				
Methyl luteolin	0.17	0.07	0.04	0.06	0.06				
Total flavonoids	***2.12***	***0.58***	***0.31***	***0.45***	***0.56***	***0.53***	***0.51***	***0.51***	***0.49***
Syringaresinol	0.42	0.50	0.53	0.70	1.00				
Pinoresinol	0.43	0.66	0.66	0.75	1.04				
Acetoxypinoresinol	1.99	2.15	2.25	2.10	2.48				

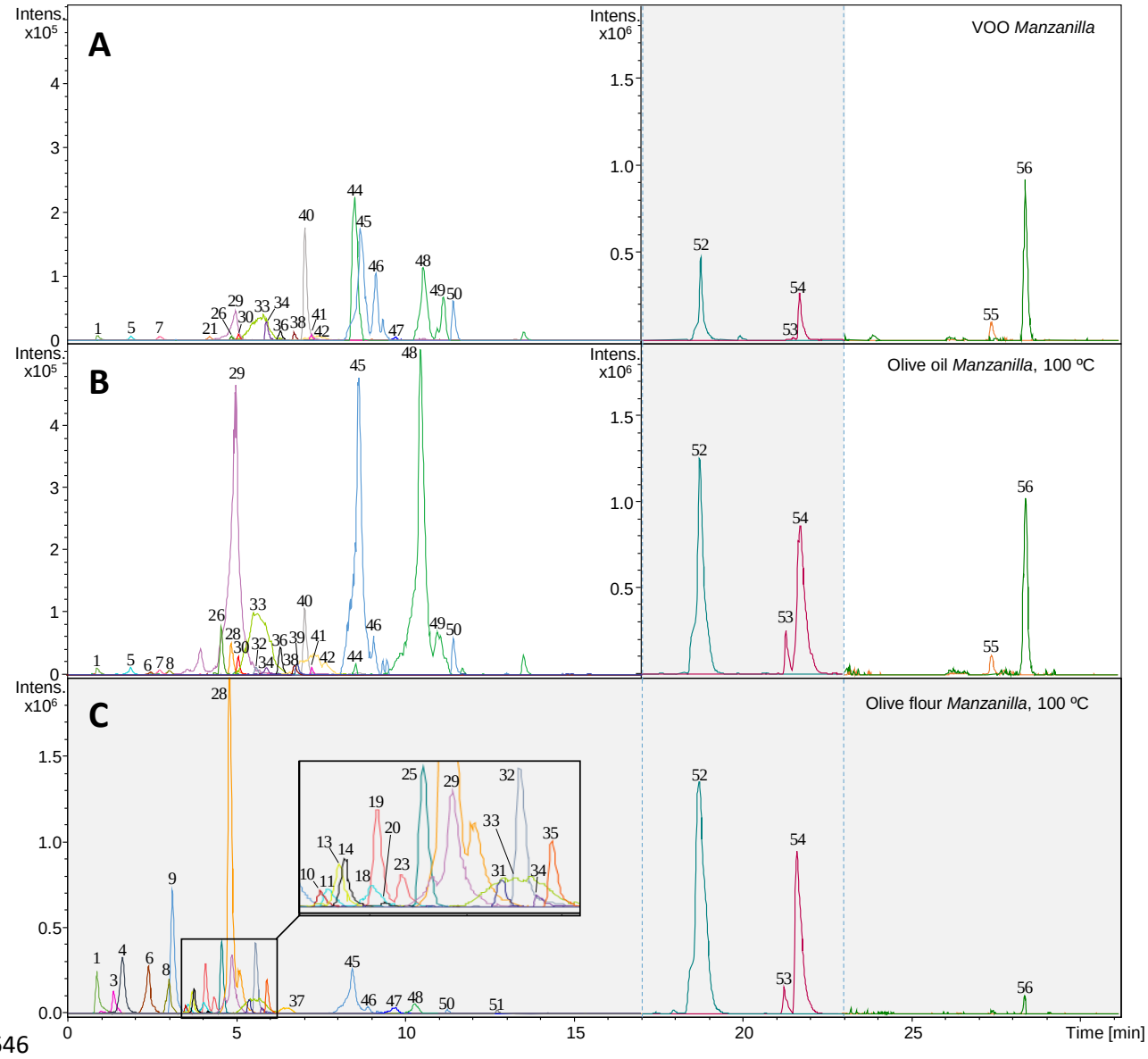
Total lignans	***2.84***	***3.30***	***3.44***	***3.56***	***4.53***	***n.d.***	***n.d.***	***n.d.***	***n.d.***
Hydroxy maslinic acid	0.09	0.24	0.26	0.25	0.26	0.02	0.02	0.02	0.02
Maslinic acid	15.14	64.88	71.03	72.69	75.01	9.77	9.79	9.82	10.69
Betulinic acid	0.81	1.11	1.59	2.65	3.26	0.04	0.03	0.04	0.04
Oleanolic acid	9.99	38.06	48.18	56.80	62.98	3.83	3.74	3.68	3.92
Total triterpenic acids	***26.03***	***104.29***	***121.07***	***132.39***	***141.51***	***13.66***	***13.58***	***13.55***	***14.67***
β+γ-tocopherols	21.42	23.07	24.64	25.04	25.71	0.01	0.01	0.01	<0.01
α-tocopherol	214.44	228.39	242.54	287.83	323.37	0.19	0.17	0.20	0.16
Total tocopherols	***235.86***	***251.46***	***267.18***	***312.87***	***349.08***	***0.20***	***0.18***	***0.21***	***0.16***
Unknown 1						0.49	0.54	0.62	0.64
Unknown 2	0.42	0.82	0.51	0.42	0.43	0.71	0.28	0.11	0.05
Unknown 3	1.11	0.87	0.33	0.22	0.18	0.49	0.26	0.12	0.06
Total unknown	***1.53***	***1.68***	***0.83***	***0.63***	***0.61***	***1.68***	***1.08***	***0.85***	***0.75***
Overall concentration	***404.7***	***454.3***	***508.7***	***638.8***	***1009.2***	***38.47***	***39.63***	***46.45***	***47.08***

[a]"acids and derivatives" class includes organic acids, phenolic acids and aldehydes in oil samples.

Abbreviations: HTY: hydroxytyrosol; EA: elenolic acid; OleAgly: oleuropein aglycone; LigAgly: ligstroside aglycone; 6-O-[…] secologanoside: 6-O-[(2E)-2,6-Dimethyl-8-hydroxy-2-octenoyloxy] secologanoside; n.d, non detected.

To calculate the average values for each kind of matrix (and dehydration temperature), the considered concentration values are the mean value of two independent replicates injected twice within the LC-MS system (n=4). RSD (%) values were lower than 9.8% for the concentrations found in olive oil samples and lower than 8.7% for the concentrations found in olive flours.

645 **Figure 1**



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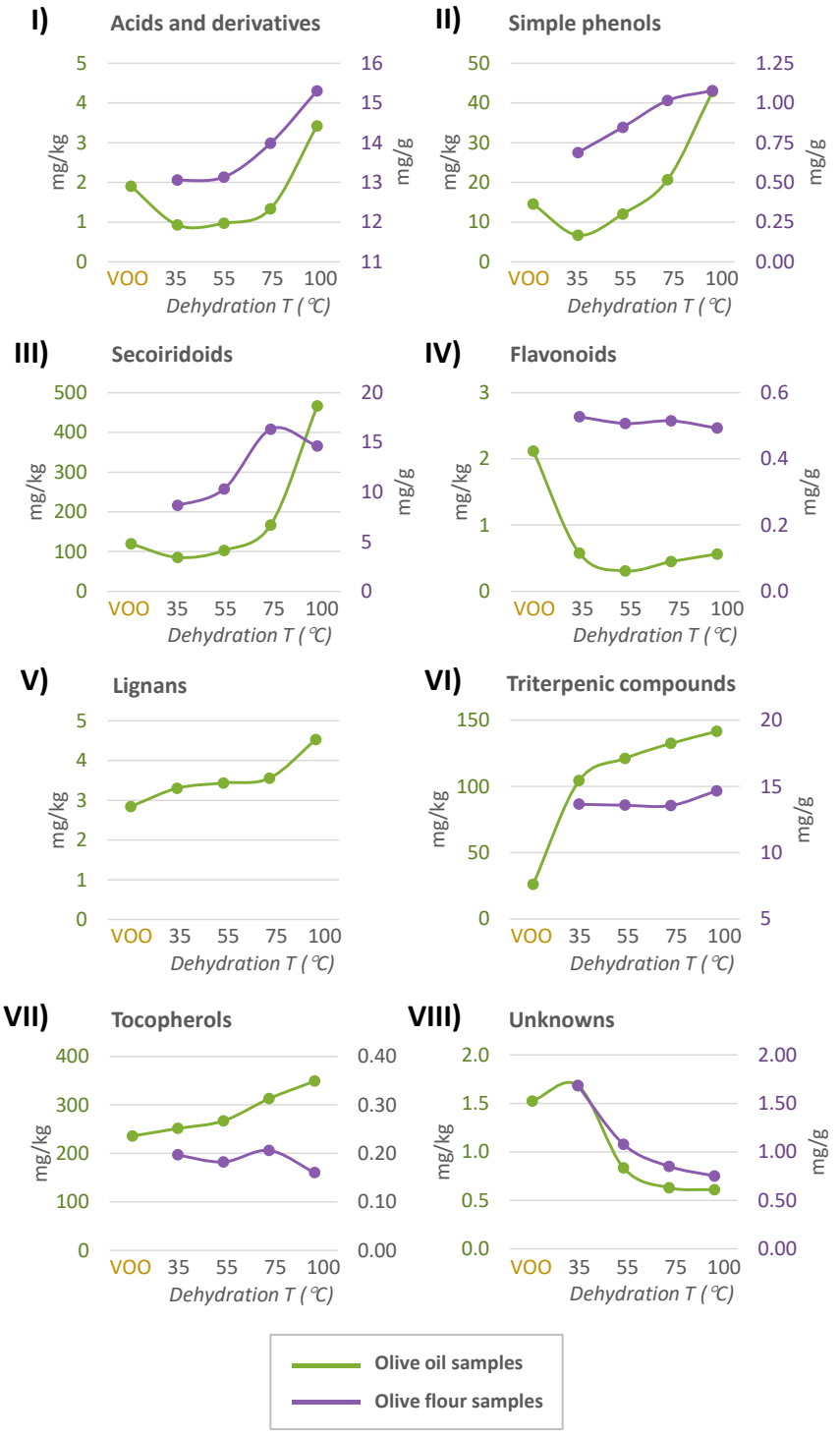
647 **Figure 2**



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Graphic for the table of contents