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Characterization of Refined Edible Oils Enriched with Phenolic Extracts from Olive Leaves and Pomace

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Supporting Information

ABSTRACT: Refined edible oils (viz., oils from maize, soya, high-oleic sunflower, sunflower, olive, and rapeseed) enriched at two concentration levels (200 and 400 μg/mL total phenolic content) with phenolic extracts isolated from olive pomace and leaves have been characterized and compared with nonenriched oils and extra virgin olive oil (EVOO). Enriched oils were analyzed by LC−TOF/MS to generate representative fingerprints and compared with nonenriched oils and EVOO by unsupervised principal component analysis (PCA). The two raw materials reported enriched oils with profiles which were compared with those provided by EVOOs. Correlation analysis enabled us to establish the enriched oils with a composition more similar to EVOO. Discrimination according to the enrichment level depended on the raw material for extracts, and a global discussion about the enrichment on relevant phenolic compounds present in EVOO has reported quantitative results concerning the enrichment level for those significant compounds with known nutraceutical properties.

KEYWORDS: *refined oils, enrichment, phenolic compounds, olive-tree extracts, extra virgin olive oil*

INTRODUCTION

Extra virgin olive oil (EVOO) is highly appreciated thanks to the organoleptical properties and healthy benefits associated with its balanced composition. Among the different components present in EVOO, the unsaponifiable fraction is composed of a great variety of compounds such as aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds, and antioxidants, most of the latter being carotenes and phenols.[1] Among the different families of compounds forming the unsaponifiable fraction, olive phenols include a major group of secondary metabolites that display a wealth of structural variety and diversity of key activities. The healthy effects of EVOO, mainly due to the presence of these particular phenols, have been widely studied in the last decades.[2,3] Oleuropein, the most abundant phenol in olive leaves and also at high concentrations in olive pomace, has been used in a number of medical treatments since its first reference in the literature.[4] Hydroxytyrosol, an oleuropein derivative, improves cardiac and tumoral diseases with similar effects to those of oleuropein; in addition, it protects against atherosclerosis[5] and is closely related to protection of low-density lipoprotein particles from oxidative damage.[6] Also, the nutraceutical utility of other phenols present in olive leaves and pomace at high amounts such as verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside has been studied.[7−10]

The unsaponificable fraction is almost completely eliminated in refined edible oils from seeds. Refining of edible oils is a common strategy to maximize the amount of oil extracted from seeds by an economical process. The purpose of refining oils is the removal of impurities and natural flavors (compounds that can be easily oxidized) and neutralization of free fatty acids. This is carried out by subjecting oils to heat, solvent extraction, filtering, neutralization, distilling, degumming, bleaching, and high-heat deodorization. As a result of the operational conditions of these steps, polar compounds, including phenolic antioxidants, are completely removed.[11]

Conferring to refined oils the benefits associated with the unsaponifiable fraction can be partially attained by an enrichment process with suited extracts of these components isolated from vegetal raw materials. Particularly, two different raw materials from *Olea europaea* cultivars such as olive pomace and leaves (in fact, the two main sources of olive phenols) have been used for enrichment of refined edible oils with phenolic compounds.[12] Olive pomace is a polluting semisolid residue resulting from the two-phase olive oil extraction method, which is at present the most implemented in this industry. This pomace is a cheap source of natural antioxidants, in concentrations up to 100 times higher than in olive oil,[13] which results from the polar nature of both this residue and olive phenols, but also from the low-polar nature of oil. On the other hand, leaves possess the highest antioxidant and scavenging power between the different parts of the olive tree. As an example, taking oleuropein as a model phenol, its content in olive oil ranges between 0.005 and 0.12%, in pomace up to 0.9%, and in olive leaves between 1 and 14%.[13,14] Attending to the differences in composition of extracts from olive pomace and leaves obtaining tailor-made enriched oils up to the desired level of certain compounds could be perfectly accessible.

The protocol for enrichment of edible oils with phenolic extracts involved two main steps: (1) extraction of the target compounds from the raw material, either leaves or pomace; and (2) enrichment of the oil with the extract. The benefits in terms

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of stability and quality of refined edible oils after enrichment with phenolic extracts have been previously evaluated. [12] However, no qualitative studies have been carried out to compare the composition of enriched oils with that of EVOO. The aim of this research was to compare qualitative fingerprints provided by analysis of EVOOs and enriched refined edible oils with extracts from olive pomace and alperujo. For this purpose, LC−TOF/MS analyses of the target oils were used to obtain representative profiles of their composition.

MATERIALS AND METHODS

Samples. Olive pomace obtained during the 2009/2010 crop season was taken directly from the production line of a mill in Núñez de Prado, C.B. (Baena, Spain) and stored at −20 °C until use. Olive leaves selected for this research were collected in June 2010 (Baena), dried at 35 °C for 60 h, milled by a cyclonic mill up to homogeneous particle size (diameter ≤0.5 mm), and kept at 4 °C until use.

Vegetable edible oils were selected for this research according to criteria such as low price (less than 1 euro/kg) and wide variability in the profile of fatty acids. The target oils were refined maize oil (RMO), refined soy oil (RSoO), refined high-oleic sunflower oil (RHSO), refined sunflower oil (RSO), refined olive oil (ROO), refined rapeseed oil (RRO), and extra virgin olive oil (EVOO). All of them were provided by Carbonell (SOS Cuétara S.A., Madrid). Extra virgin olive oils sampled from local mills located at different places in the South of Spain (Jaén, Sevilla, Málaga, Córdoba, Ciudad Real, Badajoz) were provided by Carbonell.

Reagents. The reagents used for characterization of vegetable oils were LC grade methanol, acetonitrile, hexane, and absolute ethanol from Scharlab (Barcelona, Spain). Deionized water (18 MΩ·cm) from a Millipore Milli-Q water purification system (Bedford, MA, USA) was used to prepare mobile chromatographic phases. The most abundant phenolic compounds in olive oil were purchased from Extrasynthese (Genay, France) in the case of hydroxytyrosol, tyrosol, oleuropein, apigenin, and luteolin; meanwhile vanillin, vanillic acid, *p*- and *o*-coumaric acids, and ferulic acid were from Merck. The stock standard solution of each phenol was prepared at 1000 μg/mL by dissolving 10 mg of each phenol in 10 mL of methanol. The multistandard solutions containing 10 phenols were prepared by mixing the appropriate volume of each stock solution and diluting them as required in a 60:40 methanol−water solution. All these solutions were stored in the dark at −20 °C in glass vials until use.

Apparatus and Instruments. Microwave irradiation was applied in the extraction step by means of a MIC-II focused-microwave extraction system of 400 W maximum power (Puebla, Mexico) furnished with a manual power control unit. A Selecta Mixtasel centrifuge (Barcelona, Spain) was used to remove solid particles from the extract. A Büchi R-200 rotary evaporator (Postfach, Switzerland) furnished with a B-490 heating bath was used to concentrate the phenol extracts after microwave-assisted extraction (MAE), and to evaporate traces of ethanol in the enriched oils. A Selecta Vibromatic electrical stirrer (Barcelona, Spain) was used to favor the liquid−liquid extraction of phenols to refined oils. An MS2 minishaker from Ika (Wilmington, NC, USA) was used to favor phenol transfer from oil to methanol for individual quantification of the target compounds.

Profiling analysis for identification of olive metabolites was carried out with an Agilent 1200 series LC system interfaced to an Agilent 6540 UHD Accurate-Mass TOF LC/MS detector (Palo Alto, USA), equipped with an Agilent Jet Stream Technology electrospray ion source operating in the negative ion mode.

Procedure for Extraction of Phenols from Alperujo or Leaves. The procedure was similar to that proposed by Girón et al.[15] and Japón et al.[16] Briefly, 12 g of alperujo or leaves and 100 mL of ethanol were placed into the quartz extraction vessel located in the zone of focused microwave irradiation of the extractor. After extraction (10 min microwave irradiation at 400 W), the suspension was centrifuged at 855*g* for 5 min for phase separation. This process was repeated as many times as required to obtain the necessary extract for the subsequent enrichment step after the extract had been concentrated in a rotary evaporator at 35 °C to reduce ten times its initial volume. The extract thus obtained was reconstituted in 200 mL of ethanol prior to measurement of total phenol concentration by the F−C test. [17]

Enrichment of Edible Vegetable Oils with Phenols Extracts from Alperujo or Leaves. The enrichment was carried out at two concentration levels (200 and 400 μg/mL of phenols according to the F−C test) per oil with extracts from alperujo and from leaves. In all cases, an aliquot of the corresponding ethanolic extract was put into contact with 200 mL of oil, and the ethanol in the two-phase system was evaporated in the rotary evaporator at 30 °C. Then, the mixture was shaken in the electrical stirrer at 700 U/min to favor enrichment. This process was repeated as many times as required until the 200 mL oil portions were enriched in phenols from either of the extracts up to 200 or 400 μg/mL (as determined by the F−C method using caffeic acid as standard). Distinction between the different oils, extract for enrichment, and enrichment degree is as follows: abbreviation as under Samples—uppercase letters—is used for 400 μg/mL enrichment, followed by a decimal point and the initial of the raw material to prepare the extract (A for alperujo and L for leaves). Similar nomenclature, but lowercase letters, is used for oils enriched with 200 μg/mL phenols. For example, RMO.A and rmo.a correspond to refined maize oil enriched with 400 μg/mL and 200 μg/mL, respectively.

LC−TOF/MS Confirmatory Analysis of Olive Phenols. Olive phenol identification was conducted by LC−TOF/MS confirmatory analysis in accurate mode due to the complexity of phenolic extracts from EVOO. The analytical column was a C18 Inerstil ODS-2 (250 × 4.6 mm i.d. 5 μm) from GL Sciences Inc. (Tokyo, Japan). The hydroalcoholic phase was injected into the chromatograph. The mobile phases were A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient program, at 0.9 mL/min constant flow rate, was as follows: initially 96% A and 4% B; 0−44 min, 96−50% A and 4−50% B; 44−54 min, 50−40% A and 50−60% B; 54−74 min, 40−0% A and 60−100% B; 74−85 min, 0% A and 100% B. After analysis, the column was reequilibrated for 15 min.

The phenolic fraction present in aliquots of EVOO and enriched oils was extracted for subsequent analysis by injecting the extracts into the LC−TOF/MS system without additional pretreatment. The injected extract volume was 20 μL. The operating conditions were as follows: gas temperature, 350 °C; drying gas, nitrogen at 10 L/min; nebulizer pressure, 35 psi; sheath gas temperature, 380 °C; sheath gas flow, nitrogen at 10 L/min; capillary voltage, 3250 V; skimmer, 65 V; octopole radiofrequency voltage, 750 V; focusing voltage, 90 V. Data acquisition (2.5 Hz) in both the centroid and profile modes was governed via the Agilent MassHunter Workstation software. The mass range and detection window were set at m/z 100−1100 and 100 ppm, respectively. Reference mass correction on each sample was performed with a continuous infusion of Agilent TOF biopolymer analysis mixture containing purine (m/z 121.0508) and hexamethoxyphosphazyne (m/z 322.0481) with resolution of 45 000. Analytes were identified by accurate mass detection.

Mass Hunter Workstation software (version 3.01 Qualitative Analysis, Agilent Technologies, Santa Clara, CA, USA) was used for processing raw LC−TOF/MS data. Molecular features were extracted using the molecular feature extraction algorithm (MFE) from raw data files prior to formula generation. Ions with identical elution profiles and related m/z values (representing different adducts, ions generated after specific neutral losses, or isotopic forms from the monoisotopic ions) were extracted as molecular features (MFs) in a matrix characterized by retention time (t_R) and accurate mass and containing intensity in apex of chromatographic peaks as data. The isotope model corresponded to common organic molecules with peak spacing tolerance of m/z 0.0025 ± 7.0 ppm. The MFE algorithm limited extraction to ions exceeding 1000 counts with charge state limited to a maximum of two. The allowed negative ions were deprotonated species and formate adducts. Dehydratation neutral losses were also allowed. The generated raw data files in compound exchange format (.cef files) were created for each sample and exported into the Mass Profiler Professional (MPP) software package (version 2.0, Agilent

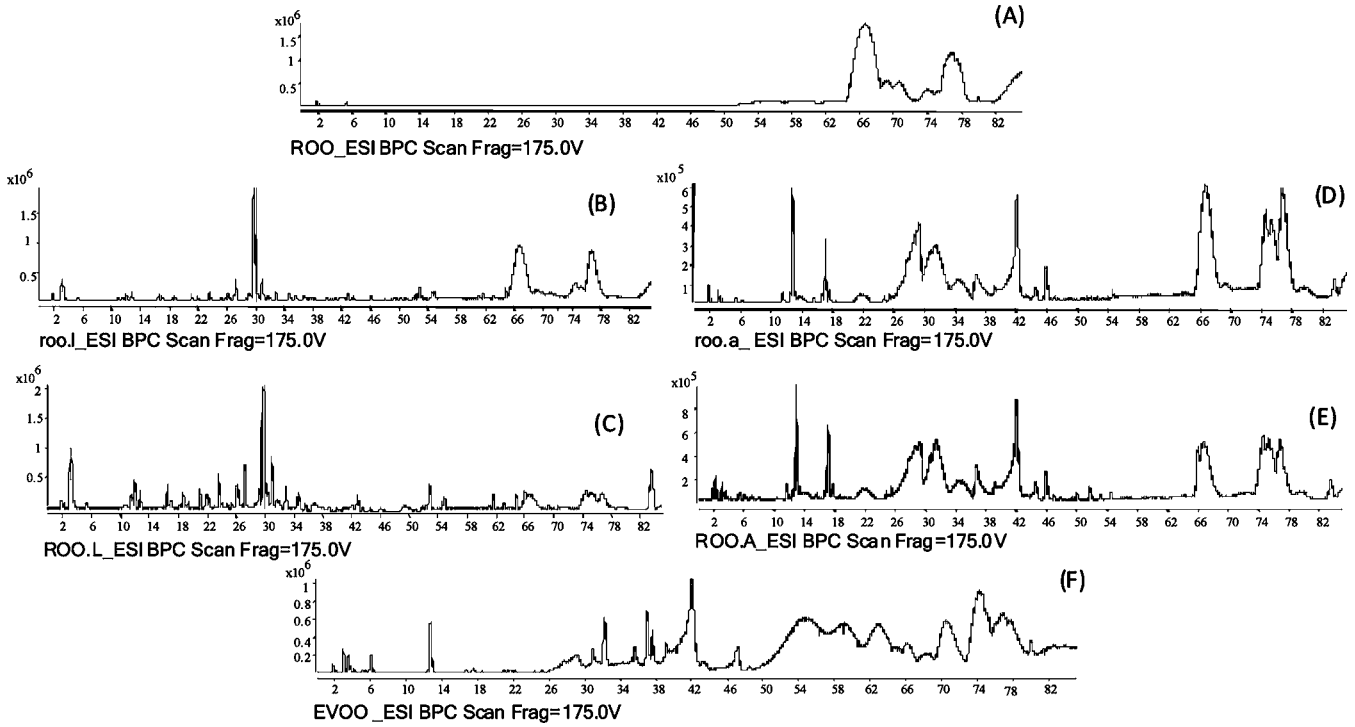

Figure 1. Base peak chromatograms (BPC) obtained by LC−TOF/MS analysis of hydroalcoholic extracts from pure refined olive oil, ROO (A); ROO enriched with leaf extract at 200 μg/mL (B) and 400 μg/mL (C); ROO enriched with extract from olive pomace at 200 μg/mL (D) and 400 μg/mL (E); and extra virgin olive oil (F).

Technologies, Santa Clara, CA, USA) to create the data matrix for further processing. Stepwise protocols for reduction of MFs number based on frequency filters and fold-change analysis were followed. Principal component analysis was used to find clustering of samples attending to level of enrichment and material used for enrichment.

Identification of the compounds proceeded by generation of candidate formulas with a mass accuracy limit of 5 ppm. The contribution to mass accuracy, isotope abundance, and isotope spacing scores was 100.00, 60.00, and 50.00, respectively. Retention times, formulas, experimental and theoretical masses, and errors, in ppm, obtained by accurate mass measurements of a panel of phenolic compounds were considered in the identification step. This confirmatory analysis enabled to predefine the cutoff value for accuracy in the study.

■ RESULTS AND DISCUSSION

Qualitative Fingerprinting of Refined Oils after Enrichment. As a result of the operational conditions of the steps involved in refining oils, polar compounds, including phenolic antioxidants, are completely removed. The enrichment process was planned to supply the phenolic fraction isolated from raw materials in refined oils. The mass transfer occurring in the enrichment process was checked by analysis of the hydroalcoholic extracts from both the refined oils and the same oils after enrichment with the two types of raw materials. LC−TOF/MS chromatograms can be used as representative fingerprints of the content in polar and midpolar compounds. The results of the enrichment step with the two extracts can be seen in Figure 1. This figure illustrates the base peak chromatograms (BPC) obtained by LC−TOF/MS analysis of hydroalcoholic extracts from refined olive oil (ROO, Figure 1A) and EVOO (Figure 1F) and those corresponding to enriched ROO with extracts from olive pomace (Figure 1D,E) and leaves at both enrichment levels (Figure 1B,C). As above exposed, TOF chromatograms reveal the absence of phenolic compounds in pure ROO owing to the refining process. In fact, practically none of these compounds are detected in this analysis. On the opposite side, EVOO analysis reports a representative profile that could be considered like a fingerprint of the polar and midpolarity fraction. In between, the contribution of the enrichment process with both extracts, with clear differences associated with their composition, can be observed. Thus, the enrichment with extract from olive leaves is particularly significant in the first part of the chromatogram (up to 30 min elution time), where the polar phenolic fraction is eluted. On the other hand, the enrichment with extract from olive pomace is more pronounced in the elution window from 26 to 50 min, which could be directly related to secoiridoids. Additionally, two characteristic chromatographic peaks are detected at 13 and 17 min that could be identified as hydroxytyrosol and tyrosol (confirmed in subsequent sections). An additional effect can be visualized in the BPC corresponding to enriched ROO, different depending on the concentration level, 200 and 400 μg/mL. Thus, this effect can be clearly visualized if the *y*-axis scale is compared for BPC obtained from ROO enriched with extracts from the same raw material. These preliminary analyses open a discussion to the possibility of preparing tailor-made enriched oils by optimum selection of the raw material (single or a mixture) to be extracted and the enrichment concentration. Thus, oils can be prepared either with the desired organoleptical and healthy properties to attain the EVOO benefits associated with these olive phenols (OPs) or with other modified characteristics depending on the final aim.

Mass Transfer of Phenols to Refined Oils as a Function of the Extract. Distinction among the different oils, different degree of enrichment and different extracts used for enrichment are discussed using the abbreviations under in Materials and Methods. The discussions exposed below

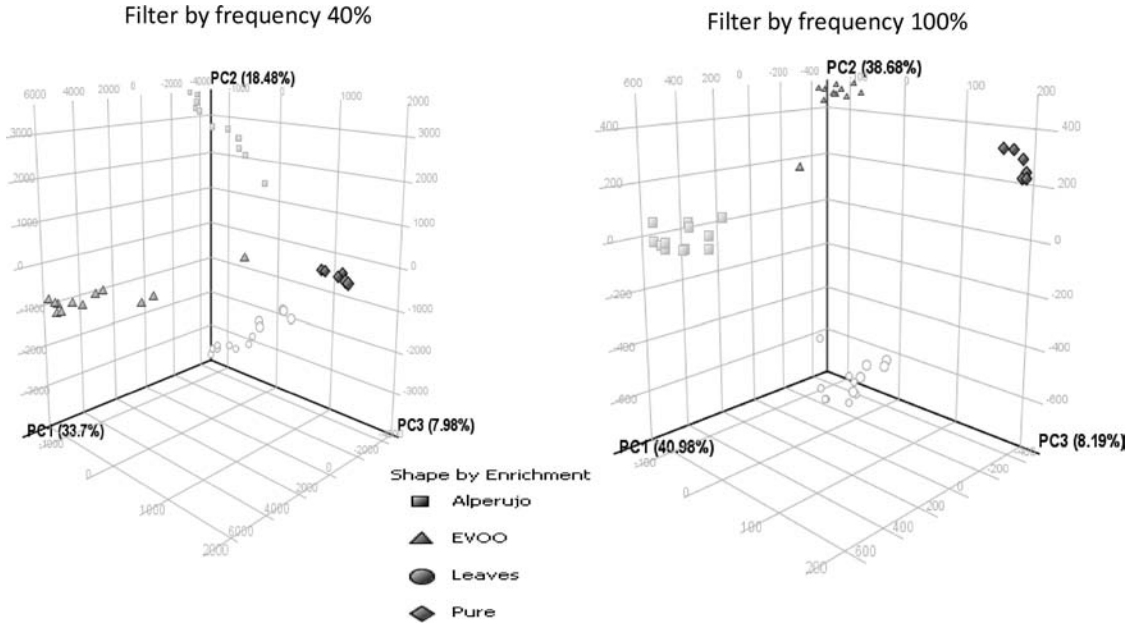

Figure 2. Scores plots for PCA of oils enriched with the two types of extracts and pure oils depending on the filter used for reduction of molecular features.

concern first the degree of individual enrichment of the most important phenols detected in EVOO as a function of the extracts. These results were supported by LC−TOF/MS analyses in accurate mode for the tested oils. Supplementary Figure 1 in the Supporting Information illustrates extracted ion chromatograms for representative compounds of different families after analysis of the hydroalcoholic extracts of enriched ROO and EVOO. The trends discussed here were found in the resting enriched oils. The behavior of the different OPs is exposed as follows.

Hydroxytyrosol and Tyrosol. Oils were preferentially enriched with these simple phenols by extract from olive pomace. In fact, a clear difference in the concentration of hydroxytyrosol was found in the case of ROO enriched with OPs from olive pomace at 400 μg/mL with respect to all enriched oils and EVOO, while the enrichment at 200 μg/mL reported a level of hydroxytyrosol similar to that of EVOO (Supplementary Figure 1A in the Supporting Information). In the case of leaf extracts, lower hydroxytyrosol concentrations than in EVOO were found for all enriched oils. A similar behavior was found for tyrosol, except for ROO enriched with extract from olive leaves in which tyrosol was below the detection limit.

Secoiridoids. A different behavior was found as a function of the monitored compounds (viz., decarboxymethyl aglycon or EDA derivatives, aglycons or EA forms, and oleuropein). Thus, oils were substantially enriched with decarboxymethyl oleuropein aglycon (3,4-DHEPA-EDA) by extracts from olive pomace and leaves at 400 μg/mL (superior for extracts from olive pomace). Intermediate enrichment was attained at 200 μg/mL as compared to EVOO, which did not provide detectable levels of this secoiridoid despite its organoleptical and healthy contribution (Supplementary Figure 1B in the Supporting Information). The decarboxymethyl ligstroside aglycon (*p*-HPEA-EDA) was detected at trace level for all ROO enrichments (Supplementary Figure 1C in the Supporting Information); therefore, the concentration of this secoiridoid was significantly lower in enriched oils than in the EVOO used as reference. The concentration of oleuropein aglycon (in equilibrium with its aldehydic form) in ROO enriched with extract from olive pomace was considerably higher than in ROO enriched with leaf extract and EVOO, with also a clear difference between the two enrichment degrees (Supplementary Figure 1D in the Supporting Information). Ligstroside aglycon (in equilibrium with the aldehydic form) was only detected in ROO enriched with extracts from olive pomace but at low concentrations as compared with those in EVOO (Supplementary Figure 1E in the Supporting Information). Finally, it is worth mentioning the presence of oleuropein in ROO enriched with extract from olive leaves (Supplementary Figure 1F in the Supporting Information). Additional research is here demanded for guarantying the null contribution of oleuropein to food taste if this oil is employed for frying.

Flavonoids. The two main flavonoids detected in EVOO, luteolin and apigenin, were also detected in enriched ROO. In the case of luteolin, the enrichment was higher when it was performed with extract from olive pomace. In fact, practically the same enrichment degree was found for ROO enriched with extract from olive pomace at low concentration than with extract from olive leaves at the higher enrichment concentration (Supplementary Figure 1G in the Supporting Information). The situation was different for apigenin, which was found at higher concentration in ROO enriched with extract from leaves (Supplementary Figure 1H in the Supporting Information).

Pinoresinols. The enrichment process was studied for this family of compounds with nutraceutical interest. 1-Hydroxypinoresinol was detected in ROO enriched with both raw materials and at the two enrichment concentrations. Similar concentrations were found in enriched ROO at low concentration and EVOO, being at higher concentration for ROO prepared at the superior OP concentration (Supplementary Figure 1I in the Supporting Information).

Other Compounds. Two triterpenes such as oleanolic and ursolic acids were monitored as representative of enrichment of

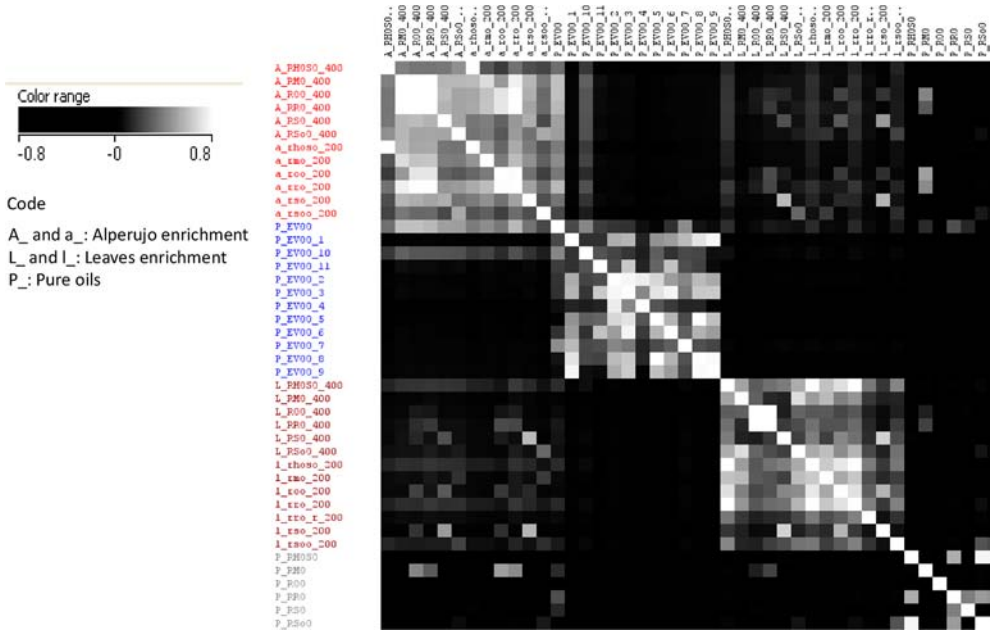

Figure 3. Heat map based on correlation analysis between refined oils, EVOOs, and enriched oils.

ROO in this family. In fact, EVOO did not report detectable levels of both acids, which appeared at significant concentration in the oil when enriched with extract from olive leaves (Supplementary Figure 1J in the Supporting Information).

Statistical Analysis Based on LC−TOF/MS Data. The purpose of the enrichment of refined edible oils with compounds present in EVOO was to enhance oils' stability and improve their healthy properties. The composition of the oils enriched with extract from the two raw materials has been tested and compared in this study. For this purpose, statistical analysis was carried out using as data set that composed by molecular features extracted from LC−TOF/MS analysis. Prior to analysis, the possibility of filtering molecular features in the original data set according to frequency in the variability of samples included in the study should be discussed. For this purpose, the samples were classified in four classes: pure refined oils, oils enriched with extract from olive pomace, oils enriched with extract from olive leaves, and EVOOs. Attending to this preclassification, two different filters were tested, namely: (i) restrictive analysis, which means a filtration of the data matrix to include only those molecular features present in all samples belonging to one of the four classes, and (ii) nonrestrictive analysis, which involves a filtration of the data matrix to include those molecular features present in at least 40% of all samples belonging to one of the four classes. One of the characteristic aspects of EVOO is the significant influence on its composition of factors such as maturation of olive fruits and genetic variability (cultivars). Additionally, there is another group of factors encompassing agropedoclimatic conditions that contribute considerably to the composition of EVOO.[18,19] The purpose of the first filter was to reduce the data matrix only to those molecular features which are representative of the four predefined classes. On the opposite side, the nonrestrictive filtration was focused on removing those molecular features present in a reduced number of samples of each class. After both operations, PCA was carried out resulting in the score graphs in Figure 2. As can be seen, similar results are obtained with both the restrictive and the nonrestrictive analysis since a clear discrimination is observed in the four groups formed by (i) pure oils, (ii) oils enriched with extract from olive pomace, (iii) oils enriched with extract from olive leaves, and (iv) extra virgin olive oils. The grouping of the four classes is perfect attending to the symbols used to label each class. Also, the samples representing EVOOs are separated from the group of pure oils being closer to both groups of enriched oils.

The similarity between samples integrating each class can also be detected with the heat map shown in Figure 3, based on correlations between each pair of samples. Apart from the diagonal line exposing the evident maximum correlation between equal samples, two main areas can be discriminated. The first area corresponds to the pure oils that are not practically correlated except for (i) RHOSO, RSoO, and RMO that are highly correlated; (ii) RRO that shows an intermediate positive (established in the scale shown in the figure) correlation to RSO and EVOOs; and (iii) RSO that is softly correlated to EVOOs. The heat diagram also reveals interesting associations between EVOOs and enriched oils. Thus, there is a low correlation between EVOOs and oils enriched with extract from olive leaves. By contrast, oils enriched with extract from olive pomace showed a high correlation to EVOO. Therefore, the enrichment of refined oils with extracts from olive pomace is the suited strategy to obtain oils with similar composition of phenolic compounds to EVOO. Apart from these results, this study confirms a different profile for oils enriched with both types of extracts.

Evaluation of Significant Molecular Features for Each Enrichment. After these statistical tests, a stepwise strategy was followed to assess the influence of molecular features on the PCA analysis, and also to identify those molecular features in which the enrichment process was more significant in relative terms. Thus, a fold change analysis was applied to molecular features included in the data set. This algorithm enabled isolation of those ions with more impact to explain the variability observed in previous PCA tests. Table 1 summarizes the number of molecular features after the different operations both in the restrictive and nonrestrictive analysis. As can be seen, the number of molecular features was considerably reduced for higher fold change ratios. Additionally, Figure 4

Table 1. Number of Molecular Features along Statistical Tests in Both the Restrictive and Nonrestrictive Analysis

	original data set	freq filter	fold change anal. (cutoff 4.0)
restrictive[a]	12336	178	113
nonrestrictive[b]		1785	119

[a]Frequency filter 100% samples of one condition. [b]Frequency filter at least 40% samples of one condition.

represents the PCA tests applied to the data matrix generated after fold change analysis setting ratio at 4.0 in both the restrictive and nonrestrictive analysis. This cutoff value was set according to the fold change ratio obtained for two representative compounds such as hydroxytyrosol and 3,4-DHPEA-EDA, known because of their antioxidant properties.[20] In both cases, there is a clear discrimination between oils enriched with extract from olive pomace and those enriched with extract from olive leaves, but also from pure oils. The only difference between restrictive and nonrestrictive analysis is the dispersion within groups. Thus, samples pertaining to a specific group are more dispersed in the case of nonrestrictive analysis. In fact, the samples corresponding to EVOO are close to enriched oils and practically separated from the rest of pure oils that are quite grouped between them.

A more advanced level of information can be attained by including in the PCA the enrichment concentration. The inclusion of this variable in the PCA analysis reveals an additional discrimination based on concentration of enrichment, apart from that observed for type of raw material for enrichment. Thus, Figure 5 represents the graphs of scores corresponding to PCAs for nonrestrictive and restrictive analysis. As can be seen, in both cases, a gradual discrimination between oils enriched at low and high concentration of phenols can be observed. This graduation is perfectly visualized in both types of enrichment in the restrictive analysis including just those molecular features only detected in all samples belonging to a specific group. The discrimination in terms of concentration enrichment is less perceptible in the case of nonrestrictive analysis for oils enriched with extract from olive leaves. In fact, two oils enriched at high concentration such as RHOSO and RSO remain overlapped with oils enriched at low concentration. The number of molecular features considered in each study were 1785 and 178 for the nonrestrictive and restrictive analysis.

Table 2 lists the fold change ratios for characteristic compounds in olive oil that confirms the results exposed in Supplementary Figure 1 in the Supporting Information. As can be seen, the relative concentration of hydroxytyrosol in enriched oils was affected as a consequence of the raw material used with a higher increase for those oils enriched with olive pomace. A more critical effect was observed in the case of tyrosol, which was only enriched in oils prepared with extract from olive pomace. These results were also found for secoiridoids (3,4-DHPEA-EDA, 3,4-DHPEA-EA, and *p*-HPEA-EA) with higher relative concentrations in refined oils enriched with olive pomace extract. Particularly, the secoiridoid aglycon forms were not detected or slightly detected in oils enriched with extracts from leaves. Transfer to oils of one simple phenol such as *p*-coumaric acid was similar with extracts from both vegetal materials, while vanillic acid and elenolic enriched preferentially oils prepared with extract from olive pomace. On the other hand, flavonoids enriched preferentially oils prepared with extract from olive leaves, except for apigenin that was similarly distributed in oils independently of the raw material from which the extract was obtained. A similar behavior to apigenin was found with a lignan derivative such as 1-hydroxypinoresinol. Finally, two terpenic compounds such as oleanolic and maslinic acids were significantly more enriched in oils prepared with extracts isolated from olive leaves. Attending to these results, the relevance of the vegetal raw material used for enrichment is critical in the composition of prepared oils. The elevated antioxidant potential of secoiridoids and two

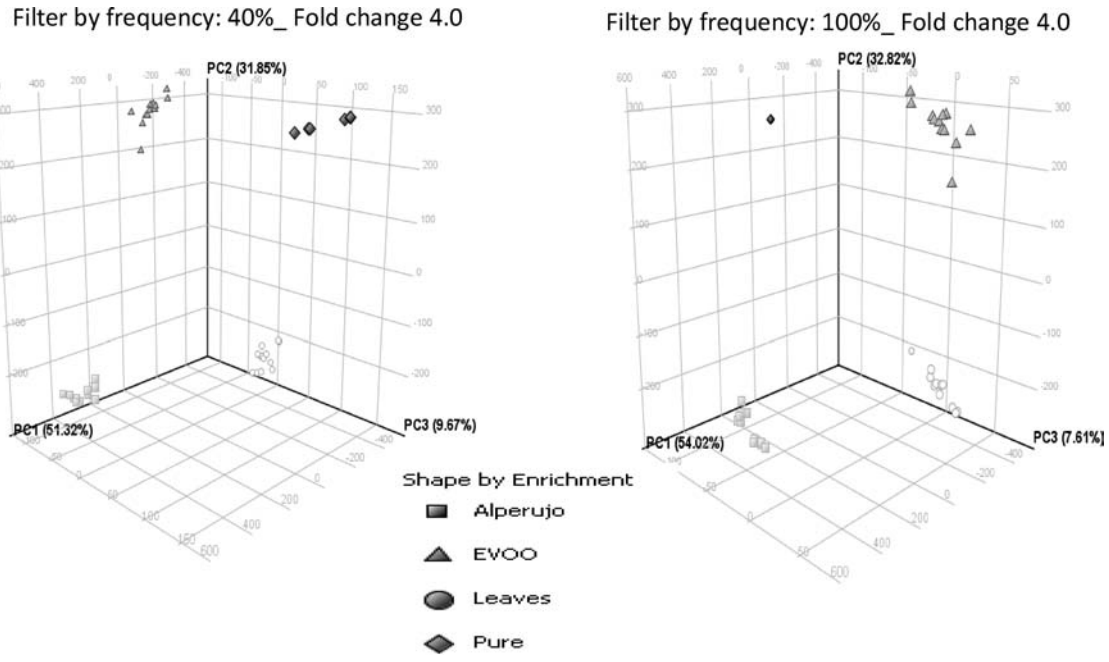

Figure 4. Scores plots for PCA tests applied to the data matrix generated after fold change analysis setting ratio at 4.0 both in the restrictive and nonrestrictive analysis.

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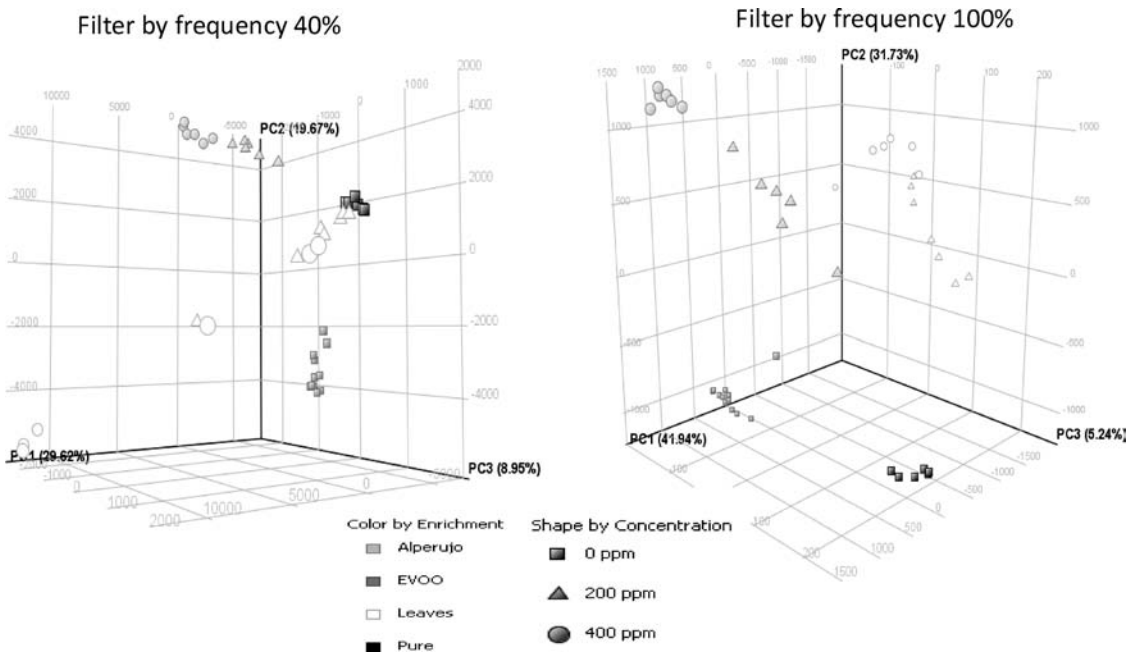

Figure 5. Scores plots for PCA tests corresponding to nonrestrictive and restrictive analysis including enrichment concentration.

Table 2. Fold Change Ratio Found for a Panel of Representative Compounds

compound	fold change ratio [Alperujo] vs [pure]	fold change ratio [leaves] vs [pure]	adduct	mass (database)
hydroxytyrosol	4.11	4.00	M − H [1−]	154.063
tyrosol		4.41	M−H20 − H [1−]	138.0681
3,4-DHPEA-EDA	4.00	2.53	M + FA − H [1−]	320.126
3,4-DHPEA-EA	3.90	2.75	M − H [1−]	378.1315
p-HPEA-EA	3.48	3.01	M − H [1−]	362.1366
apigenin	4.08	4.02	M − H [1−]	270.0528
rutin	2.09	2.48	M − H [1−]	610.1534
quercetin		2.61	M − H [1−]	302.0427
diosmetin	2.05	4.21	M − H [1−]	300.0634
taxifolin	3.20	4.29	M − H [1−]	304.0583
catechin	2.15	3.27	M − H20 − H [1−]	290.079
apigenin-7-glucoside		4.42	M − H [1−]	432.1056
luteolin-7-glucoside	3.11	3.97	M − H [1−]	448.1006
vanillic acid	4.01		M − H20 − H [1−]	168.0423
p-coumaric acid	3.60		M − H [1−]	164.0463
elenolic acid	3.34		M − H [1−]	242.079
1-hydroxypinoresinol	3.09		M − H [1−]	374.1366
maslinic acid		2.71	M + FA − H [1−]	472.3553
oleanolic acid	2.37	3.10	M − H [1−]	456.3603

simple phenols such as hydroxytyrosol and tyrosol is highly responsible for the higher correlation between oils enriched with olive pomace extract and extra virgin olive oil.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary Figure 1 depicting extracted ion chromatograms for representative compounds of different families after analysis of the hydroalcoholic extracts of enriched ROO, namely, hydroxytyrosol, 3,4-DHEPA-EDA, *p*-HPEA-EDA, 3,4-DHEPA-EA, *p*-HPEA-EA, oleuropein, luteolin, apigenin, 1-hydroxypinoresinol, and oleanolic and ursolic acids. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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