JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Evaluation of the Composition of Vine Shoots and Oak Chips for Oenological Purposes by Superheated Liquid Extraction and High-Resolution Liquid Chromatography–Time-of-Flight/Mass Spectrometry Analysis

M. Pilar Delgado de la Torre, Feliciano Priego-Capote, and María Dolores Luque de Castro*

Department of Analytical Chemistry, Annex Marie Curie Building, Campus of Rabanales, and Institute of Biomedical Research Maimónides (IMIBIC), Reina Sofía Hospital, University of Córdoba, Córdoba, E-14071, Spain

Supporting Information

ABSTRACT: Vine shoots are characterized in this research and compared to oak chips, frequently employed in the aging of wine or spirits. For this purpose, liquid chromatography–diode array detection and liquid chromatography–time-of-flight/mass spectrometry (LC-TOF/MS) analyses of hydroalcoholic extracts from vine shoots pertaining to 18 different vine varieties and from five varieties of oak chips have been carried out. The concentrations of a representative panel of interesting compounds from an oenological point of view have been compared in the extracts, finding similarity patterns for many of them. The analysis by LC-TOF/MS in high accuracy mode has led to the identification of numerous compounds in the hydroalcoholic extracts. The statistical analysis has enabled identification of the vine-shoot varieties providing extracts with more similar composition to that given by extracts from oak chips. Therefore, these vine-shoots varieties are suitable to be presented as an alternative to the use of oak barrels or oak chips in the aging process of wine and spirits.

KEYWORDS: *vine-shoots extracts, oak wood extracts, superheated liquid extraction, chemometric tools, metabolic profile, oenology*

INTRODUCTION

Spain is the country with the largest area in the world dedicated to vineyards, where approximately 1.5 million tons of vine shoots are produced every year. This fact has led to a growing interest on exploitation of this agricultural residue with the aim of turning it into a valuable product. Most research about vine shoots has been focused on the production of paper pulp,[1] ethanol, lactic acid,[2,3] methanol, fuels, biomass, biosurfactants,[2] and activated carbon for wine treatment,[4] extraction of volatile compounds, phenols,[5,6] and ferulic and coumaric acids,[7] among others.

Phenols can be obtained from vine shoots in two compatible ways, namely, (a) by extracting the phenols that are not constituents of lignin and (b) by degrading lignin to obtain low molecular weight phenols. The composition of vine shoots is characterized by a lignin content around 20% (dry weight). As lignin can be hydrolyzed to release aromatic phenolic compounds such as low molecular mass alcohols, aldehydes, ketones, or acids, vine shoots are suitable to be used as a phenols source. In addition, they could play a key role in the oenological or spirits field to improve quality, acting similarly to wine-aging agents either in contact with oak chips or in barrels.[8] In fact, lignin oligomers or intermediate and high molecular mass phenols from lignin are considered the main phenols in old spirits and wine.[6,7]

Traditionally, oak barrels have been used for centuries to store and age wine and other beverages, since the sensory complexity of the substrate subjected to aging is increased as the wood transfers to the liquid phase a series of aromatic compounds.[9] Wine aging involves changes in color and organoleptical properties, which are highly appreciated by the consumers.[10]

Extraction of compounds of interest from oak barrels depends on the quantity of potentially extractable compounds originally present in the barrel and on time-related factors, particularly the contact time between the beverage subjected to aging and wood.[11−13] During barrel aging, wine or spirits are partially enriched with lignin-derivative compounds forming complexes that are hydrolyzed to release aromatic aldehydes, which undergo oxidation reactions generating aromatic acids. Thus, the most important phenolic acids and phenolic aldehydes present in these beverages come exclusively from the barrel wood.

Economic grounds have led to the use of alternatives to oak barrels. This is the case of segments, staves, and, more commonly, oak chips,[12,14−17] which provide results similar to those obtained by barrel aging for several years.[9] The final product is determined not only by the different characteristics of the added oak wood portions such as their origin,[18] size, and toasting process but also by the dosage, period of contact with wine, and possible oxygenation of the aging product.[12]

Superheated liquid extraction (SHLE) can be an attractive industrial alternative for isolation of these compounds as it possesses two fundamental advantages over conventional extraction. The first one is ascribed to the fact that raising the temperature above the boiling point of the extractant (but

Received: December 27, 2011
Revised: March 13, 2012
Accepted: March 14, 2012
Published: March 14, 2012

keeping it under liquid state by increasing the pressure as required) increases the diffusion rate, solubility, and mass transfer of the compounds and decreases the viscosity and surface tension of the extractant. These changes improve the contact of the compounds with the extractant and enhance mass transfer, which can then be achieved more rapidly and with less solvent consumption as compared with conventional industrial extraction methods. Second, the absence of light and air significantly reduces both degradation and oxidation of these compounds during extraction.[19]

At this point, and once the importance of wood in general, and lignin in particular, is discussed in the aging of wine, the comparison of extracts obtained from oak wood and vine shoots would provide information about the possibilities of using vine shoots in the aging process, either as a complement to oak barrels or as a cheaper and available alternative. To this end, the different chemometric tools currently available play a fundamental role in multivariate analysis to treat the large amount of information provided by analytical techniques such as liquid chromatography−time-of-flight/mass spectrometry (LC-TOF/MS). This powerful analytical arrangement actually provides effective validation of the information obtained by other less complex techniques such as LC−diode array detection (DAD), and in addition, it would also provide new information, such as identification, of unknown compounds. On the basis of this background, the objective of this study was to compare the metabolic profile of extracts from vine shoots and oak chips and similarity/dissimilarity patterns among different varieties of vine shoot and oak wood with the final aim of using these vineyard residues in the aging step.

MATERIALS AND METHODS

Samples. Vine shoots from different *Vitis vinifera* cultivars were sampled from Sierra de Segura (Spain). The studied cultivars were as follows: Airén, Baladí, Bobal, Cabernet Franc, Cabernet Sauvignon, Chardonnay, Garnacha Tinta, Garnacha Tintorera, Malbec, Mazuelo, Merlot, Montepila, Moscatel, Pedro Ximénez, Petit Verdot, Sauvignon Blanc, Syrah, and Tempranillo. Five varieties of oak chips were studied, American Blend (toasted), American Fresh, French Sweet (toasted), French Spice, and French Intense (toasted). All species were dried for 72 h at 35 °C and then milled to get a homogeneous 40 mesh particle size (less than 0.42 mm diameter).

Reagents. Ethanol (96% v/v) PA from Panreac (Barcelona, Spain) and distilled water were used to prepare the extractant solution. Methanol (LC-MS grade) and formic acid (MS grade) (both supplied by Panreac) were used to prepare the mobile phases. Deionized water (18 MΩ cm) was obtained from a Millipore (Bedford, MA) Milli-Q plus system, and *n*-hexane (LiChrosolv, Merck, Darmstadt, Germany) was used for liquid−liquid extraction.

Calibration curves were constructed for the following phenols: (+)-catechin; 5-hydroxymethylfurfural (5-hydroxymethyl-2-furancarboxaldehyde); C6 phenols: pyrogallol (1,2,3-trihydroxybenzene) and pyrocatechol (1,2-dihydroxybenzene); C6−C1 phenols: acetovanillone [1-(4-hydroxy-3-methoxyphenyl)-ethanone]; vanillin (4-hydroxy-3-methoxybenzaldehyde); guaiacol (2-methoxyphenol); and gallic (3,4,5-trihydroxybenzoic acid); protocatechuic (3,4-dihydroxybenzoic acid); *p*-hydroxybenzoic; vanillic (4-hydroxy-3-methoxybezoic acid) and syringic (4-hydroxy-3,5-dimethoxybenzoic acid) acids; C6−C3 phenols: coniferaldehyde (4-hydroxy-3-methoxycinnamaldehyde); sinapaldehyde (4-hydroxy-3,5-dimethoxycinnamaldehyde) and *p*-coumaric (4-hydroxycinnamic acid); ferulic (4-hydroxy-3-methoxycinnamic acid) and sinapic (4-hydroxy-3,5-dimethoxycinnamic acid) acids; and *p*-cresol (1-hydroxy-4-methylbenzene); used as external standard, were from Sigma-Aldrich (St. Louis, MO).

Apparatus. Vine shoots were milled with a ball grinder (Restch MM301, Haan, Germany). SHLEs were performed by a laboratory-made dynamic extractor,[11] consisting of the following units: (a) an extractant supply; (b) a high-pressure pump (Shimadzu LD-AC10) that propels the extractant through the system; (c) a switching valve placed next to the pump to develop static extractions; (d) a stainless steel cylindrical extraction chamber (550 mm × 10 mm i.d., 4.3 mL internal volume) where the sample is placed. This chamber is closed at both ends with screws whose caps contain cotton-made filters to ensure that the sample is not carried away by the extractant; (e) a restriction valve to maintain the desired pressure in the system; (f) a cooler made of a stainless steel tube (1 m length, 0.4 mm i.d.) and refrigerated with water; and (g) a gas chromatograph oven (Konix, Cromatix KNK-2000) where the extraction chamber is placed and heated.

Shaking and centrifugation of the extracts were carried out by an MS2 minishaker (IKA, Germany) Vortex and a Mixtasel (Selecta, Barcelona, Spain) centrifuge, respectively. An R-220 rotary evaporator from Büchi (Flawil, Switzerland) working with a 50 mL balloon flask was used to concentrate the liquid extracts.

Individual separation of phenolic compounds and carbohydrate derivatives was carried out by an LC consisting of a ProStar 410 autosampler equipped with a 0.5 mL sample loop (Varian, Palo Alto, CA) connected online with an LC pump (Varian, 240 pump). A 330 Varian PDA detector was used to monitor the chromatographic eluate at the optimal wavelength for each analyte. Data processing was carried out using the Star Chromatography Workstation version 5.52 software running on a personal computer.

The Polyview-2000 software (Varian) was used for both characterization of the spectra and assessment of peak purity. This software allows examination and analysis of spectra, including plots of purity parameter, setting of absorbance ratios, and determination of maximum absorbance. Determination of the purity of chromatographic peaks and recalculation of the peak at different wavelengths and integration parameters, which allow exchange signal-to-noise ratio in a diode array data file, are also provided by this software.

Extraction of Phenolic Compounds and Treatment of Extracts. Extracts of vine shoots and oak wood were performed by SHLE as described in ref 15, where the extractant used was 80:20 (v/v) ethanol−water at pH 3, with an extraction time of 1 h and an extraction temperature of 180 °C. The extracts were dried in a rotary evaporator and then reconstituted in 5 mL of methanol. The reconstituted solutions were subjected to liquid−liquid extraction with *n*-hexane (10 mL, 5 min of shaking and 6 min of centrifugation at 855*g*) to remove nonpolar compounds, which could complicate the chromatographic separation. Preconcentration of the methanolic phase was attained by evaporation of 2 mL of it to a final volume of 200 μL. Finally, this fraction was taken up to 650 μL with milli-Q water and filtered using a 0.45 μm pore size filter before injection into the chromatograph.

LC-DAD Analysis. Separation of the analytes was performed on an Inertsil ODS-2 column (250 mm × 4.6 mm i.d., 5 μm particle, Análisis Vínicos, Tomelloso, Ciudad Real, Spain), using an injection volume of 20 μL and a flow rate of 1 mL/min. A mobile phase A consisting of 0.2% (v/v) phosphoric acid aqueous solution and a mobile phase B consisting of methanol were used. The gradient method was as follows: from 96 to 82% mobile phase A in 20 min, held for 20 min, from 82 to 74% mobile phase A in 24 min, and from 74 to 50% mobile phase B in 9 min. The analytes were identified by comparing both their retention times (RTs) and UV spectra with those of the corresponding standards. The absorption wavelengths were set at 260 nm for monitoring ellagic acid; at 280 nm for hydroxybenzoic acids, catechin, and phenolic aldehydes; at 320 nm for hydroxycinnamic acids; and at 360 nm for hydroxycinnamic aldehydes.

LC-TOF/MS Confirmatory Analysis. The analyses, conducted with a view to confirming the identity of the studied compounds, were performed in an Agilent 1200 Series LC system (consisting of a binary pump, a vacuum degasser, an autosampler, and a thermostatted column compartment) interfaced to an Agilent 6540 UHD Accurate-Mass LC-TOF/MS detector (Palo Alto, CA), equipped with an Agilent Jet Stream Technology electrospray ion source operating in the negative and positive ion mode.

Table 1. Concentrations of a Panel of Interesting Compounds in Extracts by SHLE from Oak Wood Chips and the Averaged Concentration Found for Each Compound in the Different Varieties of Vine Shoots, Expressed as μg/g Vine Shoots[a]

	Pg	G.Ac	Hf	Py	P.Ac	H.Ac	C	V.Ac	G	V	S.Ac	Av	C.Ac	F.Ac	Cf	S.Ac	S
French Intense oak	2618	121	126	81	61	66	154	340	ND	259	505	ND	282	52	130	ND	3597
French Sweet oak	5951	275	94	55	82	166	643	298	66	163	316	ND	232	ND	282	ND	7390
American Blend oak	999	45	92	29	79	16	1436	143	D	214	178	ND	236	19	143	ND	4017
American Fresh oak	8489	393	227	77	176	ND	2163	102	8	87	48	ND	41	34	43	4911	953
French Spice oak	6680	309	144	89	38	138	599	58	ND	ND	133	171	83	Nd	202	Nd	2896
vine-shoots media	6756	313	385	121	171	41	3936	78	86	30	45	21	22	269	26	1155	239

[a]D, detected; ND, not detected; Pg, pyrogallol; G.Ac, gallic acid; Hf, hydroxymethylfurfural; Py, pyrocatechol; P.Ac, protocatechuic acid; H.Ac, hydroxybenzoic acid; C, catechin; V.Ac, vanillic acid; G, guaiacol; V, vanillin; S.Ac, syringic acid; Av, acetovanillone; C.Ac, coumaric acid; F.Ac, ferulic acid; Cf, coniferaldehyde; S.Ac, sinapic acid; and S, sinapaldehyde.

Chromatographic separation was performed using an Inertsil ODS-2 column (250 mm × 4.6 mm i.d., 5 μm particle, Análisis Vínicos, Tomelloso, Ciudad Real, Spain), kept at a temperature of 25 °C. Mobile phases were water (phase A) and acetonitrile (phase B), both LC-MS/MS grade and with 0.1% formic acid as the ionization agent. The LC pump was programmed with a flow rate of 1 mL/min, and the following gradient elution was carried out: from 4 to 18% mobile phase B in 20 min, held for 20 min, from 18 to 26% mobile phase B in 44 min, from 26 to 50% mobile phase B in 26 min, and from 50 to 100% phase B in 30 min. The injection volume was 10 μL, and the injector needle was rinsed five times with 70% methanol. Furthermore, the needle seat back was flushed for 12 s at a flow rate of 4 mL/min with 70% methanol to clean it.

The operating conditions of the mass spectrometer 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; and focusing voltage, 90 V. Data acquisition (2.5 Hz) in both the centroid and the profile modes was governed via the Agilent MassHunter Workstation software. The instrument was operated in MS-high accuracy mode. The mass range and detection window were set at m/z 100−1100 and 100 ppm, respectively. The instrument was calibrated and tuned according to procedures recommended by the manufacturer. To ensure the desired mass accuracy of recorded ions, continuous internal calibration was performed during analyses with the use of signals at m/z 121.0509 (protonated purine) and m/z 922.0098 [protonated hexakis (1H, 1H, 3H-tetrafluoropropoxy)phosphazine or HP-921] in positive ion mode; in negative ion mode, ions with m/z 119.0362 (proton abstracted purine) and m/z 966.000725 (formate adduct of HP-921) were used.

Data Processing and Statistical Analysis. MassHunter Workstation software (version 3.01 Qualitative Analysis, Agilent Technologies, Santa Clara, CA) was used for processing all data obtained with LC-TOF/MS in full single MS mode. The feature extraction algorithm took into account all ions exceeding 5000 counts with a charge state equal or above to one, and a feature had to be composed of two or more ions to be valid (e.g., two ions in the isotope cluster). Within the algorithm employed for full single MS data, ions with identical elution profiles and related m/z values (representing different adducts or isotopes of the same compound) were extracted as molecular features (MFs) or entities characterized by RT, intensity in apex of chromatographic peak, and accurate mass. Various intensity threshold settings, ranging from 3000 to 15000 counts per second (cps), were tested for the MFs extraction in the whole RT range. 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 Technologies) for further processing. In the next step, alignment of RT and m/z values was carried out across the sample set using a tolerance window of 0.2 min in RT and 5 ppm mass accuracy. Baseline correction eliminated the contribution of background noise. Stepwise reduction of MFs number was performed based on frequency of occurrence, abundance of the respective MFs in classes and PCA results of the data. MPP software also enabled one-way analysis of variance (ANOVA). The data were mean centered as a data pretreatment to lower relatively large differences among the respective MFs abundances.

RESULTS AND DISCUSSION

Composition of Extracts from Different Varieties of Vine Shoot and Oak Wood by LC-DAD Analysis. Eighteen vine-shoot varieties and five types of oak chips were subjected to SHLE under optimum operation conditions to evaluate the content of interesting compounds from an oenological point of view. Oak samples could be grouped as follows: intense oak, sweet oak, and spice oak for the French variety and blend oak and fresh oak for the American variety. Also, three of them were toasted oak chips, particularly American Blend, French Sweet, and French Intense. Table 1 lists the concentrations of a panel of representative compounds in extracts from vine shoots, which are in common with those in oak wood chips used in oenology.

First, hydroxymethylfurfural, a degradation product from hexoses, was found in all extracts, which reported similar levels of this furanic aldehyde responsible for light creamy toast and toffee flavors in vine shoots and oak chips, as can be seen in Table 1. Taking into account the content of cellulose and hemicellulose in vine shoots, furanic compounds are a family of compounds to be taken into account. The extraction temperature, 180 °C, foreseeably enhances the degradation of sugars released from vine-shoots wood and promotes the formation of furans.[20] There is a controversy about the interest of these compounds. The contribution of two furanic compounds such as furfural and hydroxymethylfurfural to flavoring in processed food by heating is well-known.[21] However, several international organisms, such as the Food and Drug Administration in United States (U.S. FDA), have examined furans, not only as flavor compounds but also as novel harmful substances in foods that undergo a thermal treatment. The European Food Safety Authority (EFSA) has articulated that furan is obviously carcinogenic in rats and mice, probably due to the combination of a genotoxic mechanism[22,23] and hepatotoxicity.[24] From a safety perspective and for food quality assurance, the EC Regulation No. 1493/99 sets up a maximum limit for hydroxymethylfurfural of 25 mg/kg in concentrated rectified grape must. With these premises, furans contents in extracts should be controlled for a proper exploitation of the latter. Anyway, attending to the concentrations of the extracts (Table 1), an enrichment on hydroxymethylfurfural that surpasses legal established limits in spirits or wine would not be a shortcoming.

One of the most characteristic phenolic compounds found in extracts from all varieties of vine shoots was gallic acid, a final product from the hydrolysis of ellagitannins, responsible for the

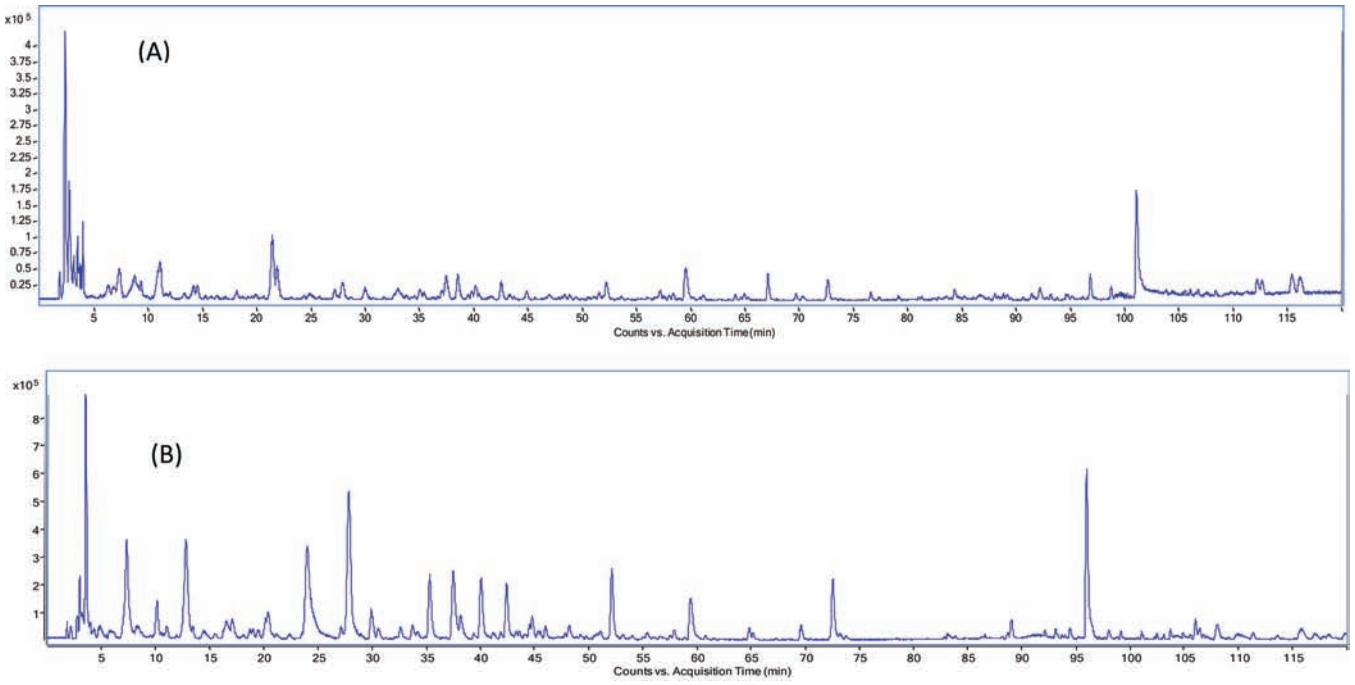

Figure 1. BPCs in positive (A) and negative (B) ionization modes by LC-TOF/MS analysis of an extract by SHLE from Garnacha Tintorera vine shoot.

astringency character of wines, and was found in a concentration similar to that in extracts from oak wood. Pyrogallol, formed from gallic acid decarboxylation, was detected in all extracts with similar concentrations. There was a similarity between concentrations of pyrogallol and gallic acid among vine-shoots varieties, demonstrating that both compounds are connected through a biochemical pathway. The same behavior was not found in the case of protocatechuic acid and pyrocatechol (decarboxylated products of protocatechuic acid). The pyrocatechol/protocatechuic acid pair was more concentrated in extracts from vine shoots than in those from any oak wood variety except for the American Fresh oak variety. The same situation was found for the guaiacol/vanillic acid pair. The concentration of vanillic acid in oak extracts was higher than that found in any vine-shoot variety, except for the French Spice oak class. Guaiacol was exclusively detected in two oak varieties: French Sweet oak and American Fresh oak. The same behavior as guaiacol was found for vanillin, which was slightly more concentrated in oak wood (except French Spice oak) than in extracts from vine shoots (Table 1). This compound is of special interest because of its contribution to flavor.

Catechin, the building block for tannins synthesis, was found at significant concentrations, which is indicative of an important effect of the extraction process on the hydrolysis of tannins. The maximum concentration of catechin in extracts from oak wood was found in the American class, with 1.4 and 2.1 mg/g for fresh oak and blend oak woods, respectively.

The rest of phenolic acids reported similar concentrations in extracts from vine shoots and those from oak wood; therefore, no discrimination was possible attending to the concentration of these compounds. Acetovanillone was detected in all varieties of vine shoots, while this compound—with a high flavor contribution—was only detected in extract from French Spice oak chips. Other compounds with organoleptical incidence were coniferaldehyde and sinapaldehyde, which were detected at lower concentrations in extracts from vine shoots, particularly in the case of sinapaldehyde, as can be seen in Table 1.

LC-TOF/MS Analysis of Vine-Shoot Extracts. The LC-TOF/MS analysis provided a global profile of polar or midpolar compounds present in extracts by SHLE that represent a characteristic fingerprinting of each variety. Comparison between them enables one to evaluate the similarity/dissimilarity patterns among cultivars considering the complete data set representative of the extracts composition. Additionally, the identification of characteristic compounds for each variety is allowed, which is not accessible by LC-DAD. This information could lead to the selection of a panel of compounds with interest from the oenological point of view, which would enable one to select the cultivar varieties for collection of vine shoots to extract the target compounds. In this study, nontargeted analysis of extracts was performed. The reverse-phase chromatographic method selected for this research enabled separation of a wide range of compounds present in the extracts. A representative example is given in Figure 1 that illustrates the base peak chromatograms (BPCs) corresponding to the analysis in positive and negative ionization modes of extract isolated from Garnacha Tintorera vine shoots.

As described in the Materials and Methods, MFs or entities were extracted to compare vine-shoot cultivars in both ionization modes. The range of MFs extracted for the different varieties encompasses from 90 to 422 for positive ionization mode and from 310 to 1138 for negative ionization mode. Identification of MFs in both ionization modes was supported on online searching of monoisotopic masses corresponding to molecular entities found in the analyses to PlantCyc database. Monoisotopic masses included in this database were searched in the raw data files obtained by the analysis of extracts from vine-shoot varieties. Search parameters were mass accuracy cutoff below 10 ppm, minimum peaks height of 2500 counts on

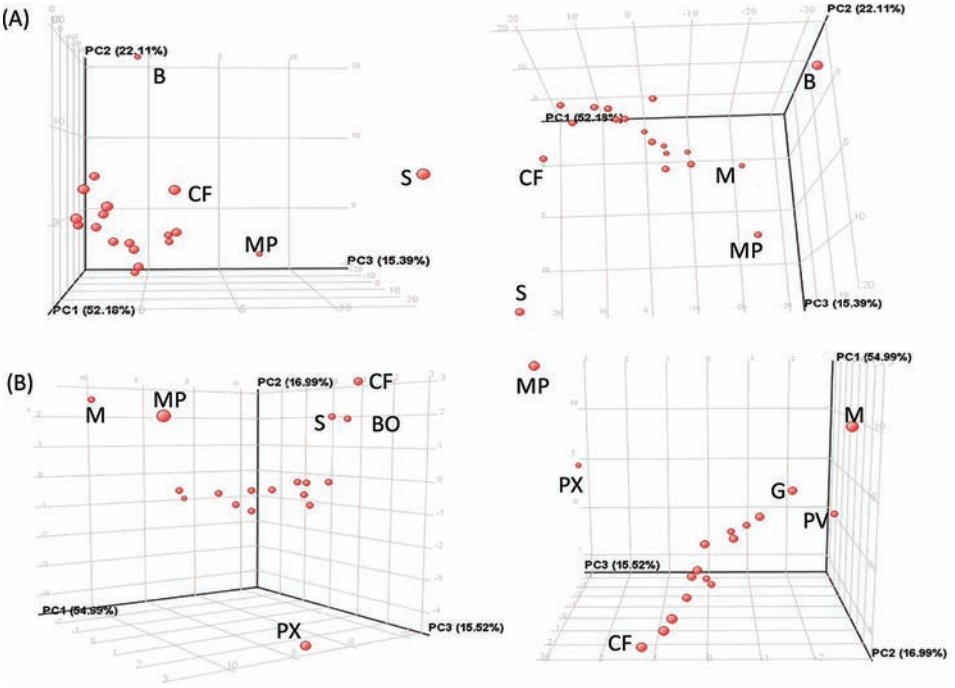

Figure 2. PCA scores plot of the metabolite profiles obtained for different vine-shoot varieties in positive (A) and negative (B) ionization modes. The original data set was filtered by frequency eliminating MFs not detected in at least 80% of the samples pertaining to each class. Baladí, Cabernet Franc, Montepila, Syrah, Merlot, Bobal, Pedro-Ximénez, Garnacha Tintorera, and Petit Verdot (B, CF, MP, S, M, BO, PX, G, and PV).

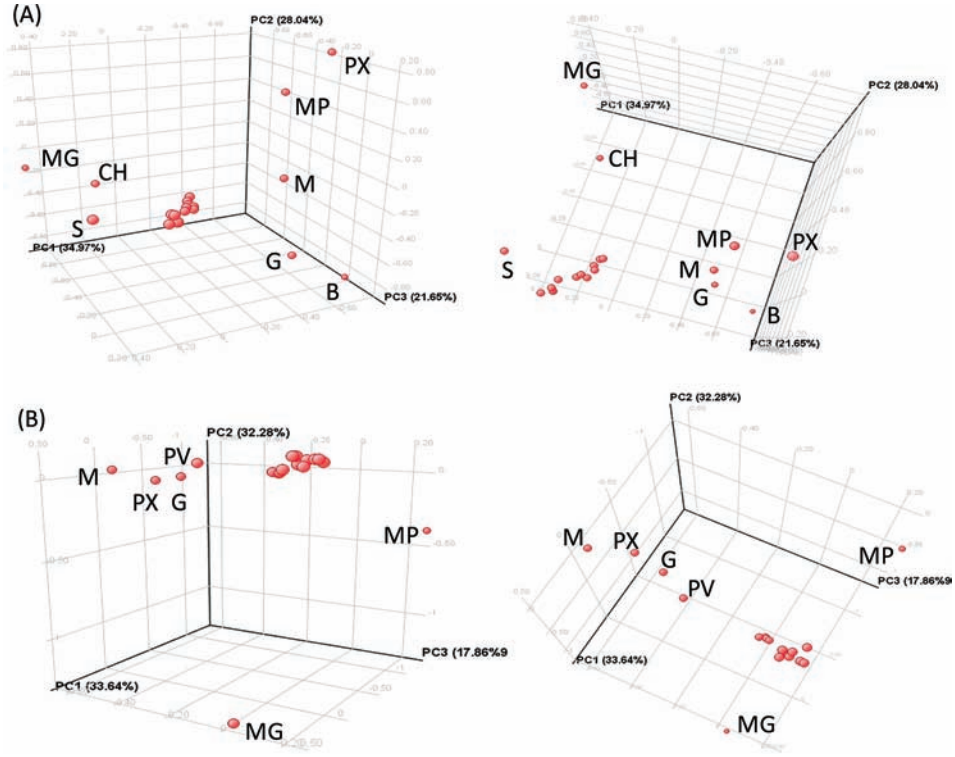

Figure 3. PCA scores plot resulting from the data set obtained in negative ionization mode after one-way ANOVA with (A) 98.5 and (B) 99% confidence level. Moscatel, Chardonnay, Syrah, Pedro-Ximénez, Merlot, Garnacha Tintorera, Baladí, and Petit Verdot (MG, CH, S, PX, MP, M, G, B, and PV).

the profile and centroid spectra, and a peak spacing tolerance of 0.0025 m/z plus 7 ppm. Table 1 in the Supporting Information lists the compounds identified in the extracts from Garnacha Tintorera, Merlot, and Syrah vine-shoot cultivars, thus demonstrating their different composition.

Qualitative Comparison of LC-TOF/MS Fingerprints Corresponding to Vine-Shoot Extracts. Attending to the complexity of the obtained data sets, a strategy was designed to simplify the dimensionality of the multivariate matrix. The first algorithm employed for this purpose was the application of a

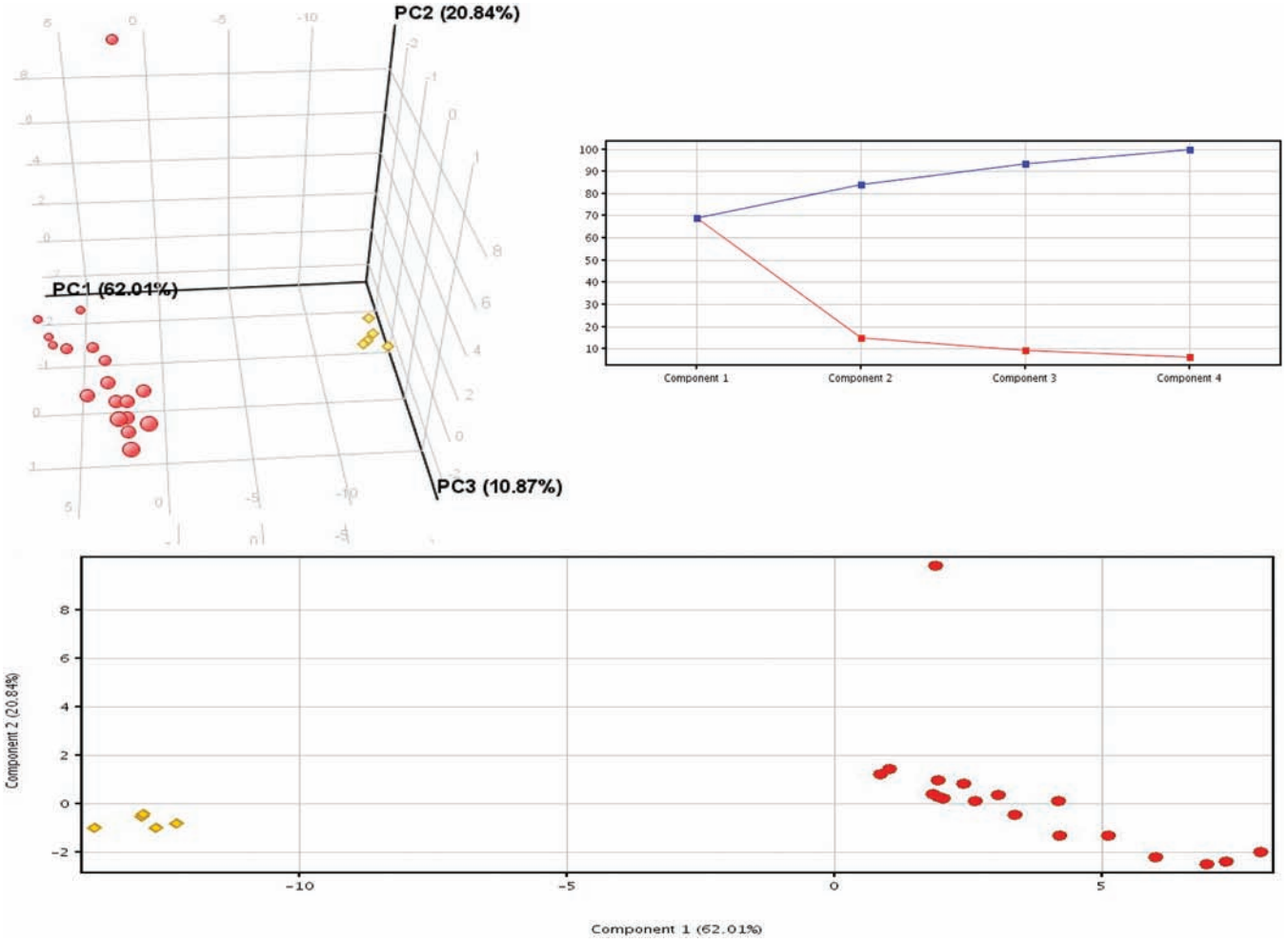

Figure 4. PCA scores plot for positive ionization mode using the original data set after filtration of those MFs not present in all samples belonging to each class: vine shoots or oak wood. As can be seen, there is a clear discrimination between extracts from oak wood (diamonds) and vine shoots (circles).

frequency filter, which involved the elimination of those MFs absent in at least 20% of the samples. In this way, comparability of vine-shoot extracts is based on two fractions: one of them common to all extracts and another one that could be characteristic of each variety. This data mining involved the reduction of the MFs to 68 in the negative mode and 190 in the positive mode as the pretreated data set. After this pretreatment, the data matrix was mean-centered prior to statistical analysis, which was initiated by nonsupervised analysis using PCA both to evaluate the distribution of the samples and to detect clusters in the new space defined by principal components. Figure 2 shows the scores plots obtained for data matrices acquired in the positive and negative ionization modes. The variability explained by PC1, PC2, and PC3 was 89.68 and 87.50% in positive and negative ionization modes, respectively. As can be seen, there is a random distribution of the vine-shoot varieties, but some trends can be observed. There is a main cluster grouping most of the varieties, but also, some extracts present differences in their composition. Thus, in the positive ionization mode, extracts from Bobal, Montepila, Syrah, Merlot, and Cabernet Franc (B, MP, S, M, and CF) provided significant composition differences, whereas the negative ionization mode revealed differences in the composition of extracts from Montepila, Merlot, Syrah, Bobal, and Pedro-Ximénez (MP, M, S, BO, and PX) vine shoots. The BPCs obtained from these extracts (and their replicates) do not reveal anomalous behaviors that could justify their labeling as laboratory outliers, and for this reason, the discrimination should be linked to their composition.

After this preliminary statistical analysis, the next objective was to apply an ANOVA test to identify MFs with the highest contribution to explain the variability observed in PCA graphs. The Mann−Whitney test against zero was employed for estimation, the p value computation was asymptotic, and no multiple testing correction was performed. A key parameter to be defined was the confidence level to set the number of features. Thus, the higher the confidence level, the lower number of MFs. A 95% confidence level involved a reduction from 68 and 190 to 47 and 27 MFs for the positive and negative ionization modes, respectively. A 98.5% confidence level filtered MFs to 10 and 11 in the negative and positive ionization modes (Figure 3A), while a 99% confidence level reduced them to 8 in global terms (Figure 3B).

This test allowed labeling of the most significant features contributing to explain the variability visualized for composition of extracts from vine-shoot varieties. Figure 3 shows the PCA scores plots resulting from the data set obtained after ANOVA with a 98.5% confidence level, which limited the number of MFs to 10. Attending to this panel of compounds, a main cluster was observed with other two additional groupings: one

dx.doi.org/10.1021/jf205337a | J. Agric. Food Chem. 2012, 60, 3409−3417

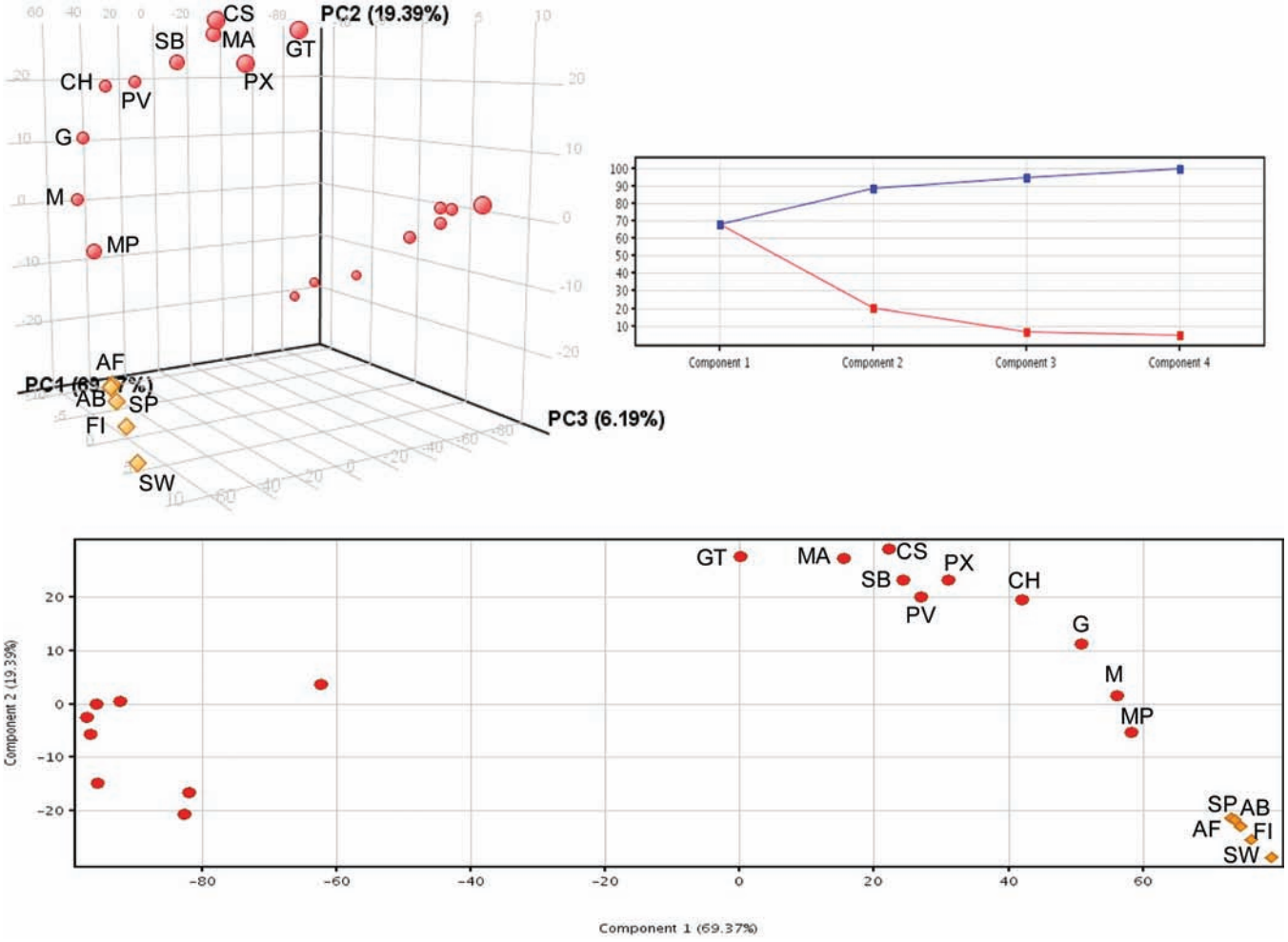

Figure 5. PCA scores plot for negative ionization mode using the original data set after filtration of MFs not present in 40% of samples belonging to each class: vine shoots or oak wood. Garnacha Tinta, Malbec, Sauvignon Blanc, Petit Verdot, Cabernet Sauvignon, Pedro-Ximénez, Chardonnay, Garnacha Tintorera, Merlot, Montepila, American Fresh oak, American Blend oak, French Spice oak, French Intense oak, and French Sweet oak (GT, MA, SB, PV, CS, PX, CH, G, M, MP, AF, AB, SP, FI, and SW).

formed by Pedro-Ximénez, Montepila, Merlot, Garnacha Tintorera, and Bobal (PX, MP, M, G, and B) and a second one formed by Moscatel, Chardonnay, and Syrah (MG, CH, and S).

This study can be completed with the PCA corresponding to 99% of the confidence level. By analogy to the positive ionization modes, differences in composition were found for extracts from Montepila and Moscatel (MP and MG) varieties and, to a lesser extent, Petit Verdot, Pedro-Ximénez, Garnacha Tintorera, and Merlot (PV, PX, G, and M) varieties, as can be seen in Figure 3. The identification of these MFs with significant contribution explains the observed variability reported interesting compounds such as 5-hydroxymethylfurfural, pyrogallol, acetovanillone, coniferyl alcohol, acetoveratrone, homovanillic acid, syringaldehyde, desapidinol A, or ethyl protocatechuate.

Comparison of SHL Extracts from Vine Shoots and Oak Wood Attending to Their LC-TOF/MS Profiles. After comparison of global composition profiles of extracts from different varieties of vine shoots, the following step was to compare the similarity of these extracts with those obtained from oak wood chips. This study could provide information about those vine-shoot varieties with a more similar composition to that of oak wood as a preliminary test to evaluate the applicability of vine shoots or their extracts with oenological purposes. Thus, the first statistical analysis with this aim was to filter by frequency the original data set composed by MFs obtained from TOF analysis from both types of extracts. The restriction for this type of filter is crucial to ensure comparability. Thus, if the filter limits MFs to those existing in all extracts of each type of sample, vine shoots or oak wood chips, the representativeness of each type of sample is ensured. This filter reduced the data set to 69 and 37 MFs in positive and negative ionization modes, respectively, and provided PCAs such as that illustrated in Figure 4 for the positive ionization mode. As can be seen, there is a clear discrimination between extracts from oak wood and extracts from vine shoots.

Taking into account that extracts from two different types of samples are being compared, a less restrictive filter should be employed to estimate the similarity level between both raw materials. Thus, an additional analysis was carried out by filtering the original data set to eliminate those MFs not present in at least 40% of the extracts from vine shoots or from oak wood chips. In this case, the original data set was reduced to 647 MFs in positive and negative ionization modes. Table 2 in the Supporting Information lists the compounds identified in the extracts from American Fresh, American Blend, and French

dx.doi.org/10.1021/jf205337a | J. Agric. Food Chem. 2012, 60, 3409−3417

Intense oak varieties, thus demonstrating the varied composition.

The scores graph obtained by principal component analysis of this new data set, shown in Figure 5, illustrates a cluster formed by extracts from different vine-shoot varieties with a high similarity to those from oak wood that remain grouped. The varieties were Garnacha Tinta, Malbec, Cabernet Sauvignon, Sauvignon Blanc, Petit Verdot, Pedro-Ximénez, Chardonnay, Garnacha Tintorera, Merlot, and Montepila (GT, MA, CS, SB, PV, PX, CH, G, M, and MP). According to sample distribution, two red grape varieties such as Garnacha Tintorera and Merlot and a white grape variety such as Montepila reported extracts with the most similar composition to that from oak chips. Therefore, the vine-shoot type in terms of grape color seems not to play a relevant role to yield extracts with similar composition to those provided by oak chips. As can be seen, PC1, PC2, and PC3 explained more than 90% of the variability observed in the scores graph.

This study is the basis to support the valorization of this agricultural residue produced in vineyards. The extracts from this residue have been characterized in this research resulting in some vine cultivars in a composition similar to that of extracts from oak wood chips, which are used with oenological applications.

■ ASSOCIATED CONTENT

Supporting Information

Tables containing a list of compounds identified in the extracts from Garnacha Tintorera, Merlot, and Syrah vine-shoot varieties as well as in the extracts from American Blend, American Fresh, and French Intense oak varieties. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +34957218615. E-mail: qa1lucam@uco.es.

Funding

The Spanish Ministerio de Ciencia e Innovación (MICINN) and FEDER program are acknowledged for financial support through project CTQ2009-07430. F.P.-C. is grateful to MICINN for a Ramón y Cajal contract (RYC-2009-03921).

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Jiménez, L.; Rodríguez, A.; Pérez, A.; Moral, A.; Serrano, L. Alternative raw materials and pulping process using clean technologies. *Ind. Crop Prod.* **2008**, *28*, 11−16.

(2) Moldes, A. B.; Torrado, A. M.; Barral, M. T.; Domínguez, J. M. Evaluation of biosurfactant production from various agricultural residues by lactobacillus pentosus. *J. Agric. Food Chem.* **2007**, *55*, 4481−4486.

(3) Moldes, A. B.; Torrado, A.; Converti, A.; Domínguez, J. M. Complete bioconversion of hemicellulosic sugars from agricultural residues into lactic acid by lactobacillus pentosus. *Appl. Biochem. Biotechnol.* **2006**, *135*, 219−227.

(4) Valente Nabais, J. M.; Laginhas, C.; Carrott, P. J. M.; Ribeiro Carrott, M. M. L. Thermal conversion of a novel biomass agricultural residue (vine shoots) into activated carbon using activation with CO_2. *J. Anal. Appl. Pyrolysis* **2010**, *87*, 8−13.

(5) Luque-Rodríguez, J. M.; Pérez-Juan, P.; Luque de Castro, M. D. Extraction of polyphenols from vine shoots of *Vitis vinifera* by superheated ethanol−water mixtures. *J. Agric. Food Chem.* **2006**, *54*, 8775−8781.

(6) Max, B.; Salgado, J. M.; Corte, S.; Domínguez, J. M. Extraction of phenolic acids by alkaline hydrolysis from the solid residue obtained after prehydrolysis of trimming vine shoots. *J. Agric. Food Chem.* **2010**, *58*, 1909−1917.

(7) Pan, G. X.; Bolton, J. L.; Leary, G. J. Determination of ferulic and *p*-coumaric acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment. *J. Agric. Food Chem.* **1998**, *46*, 5283−5288.

(8) Luque de Castro, M. D.; Luque-Rodríguez, J. M.; Japón-Luján, R. Exploitation of residues from vineyards, olive groves, and wine and oil production to obtain phenolic compound of high-added value. In *Methods of Analysis for Functional Foods and Nutraceuticals*; Hurst, J., Ed.; Hershey Foods Techhnical Center: Hershey, PA, **2007**; pp 205−244.

(9) Morales, M. L.; Benítez, B.; Troncoso, A. M. Accelerated aging of wine vinegars with oak chips: evaluation of wood flavour compounds. *Food Chem.* **2004**, *88*, 305−315.

(10) Tesfaye, W.; Morales, M. L.; García-Parrilla, M. C.; Troncoso, A. M. Evolution of phenolic compounds during an experimental aging in wood of Sherry vinegar. *J. Agric. Food Chem.* **2002**, *50*, 7053−7061.

(11) Bautista-Ortín, A. B.; Lencina, A. G.; Cano-López, M.; Párdo-Minguez, F.; López-Roca, J. M.; Gómez-Plaza, E. The use of oak chips during the aging of a red wine in stainless steel tanks or used barrels: Effect of the contact time and size of the oak chips on aroma compounds. *Aust. J. Grape Wine Res.* **2008**, *14*, 63−70.

(12) Álamo del, M.; Nevares, I.; Gallego, L.; Martín, C.; Merino, S. Aging markers from bottled red wine aged with chips, staves and barrels. *Anal. Chim. Acta* **2008**, *621*, 86−99.

(13) Garde Cerdán, T.; Rodríguez Mozaz, S.; Ancín Azpilicueta, C. Volatile composition of aged wine in used barrels of French oak and American oak. *Food Res. Int.* **2002**, *35*, 603−610.

(14) Arapitsas, P.; Antonopoulos, A.; Stefanou, E.; Dourtoglou, V. G. Artificial aging of wines using oak chips. *Food Chem.* **2004**, *86*, 563−570.

(15) Fernández de Simón, B.; Cadahía, E.; Álamo del, M.; Nevares, I. Effect of size, seasoning and toasting in the volatile compounds in toasted oak wood and in a red wine treated with them. *Anal. Chim. Acta* **2010**, *660*, 211−220.

(16) Tesfaye, W.; Morales, M. L.; Benítez, B.; García-Parrilla, M. C.; Troncoso, A. M. Evolution of wine vinegar composition during accelerated aging with oak chips. *Anal. Chim. Acta* **2004**, *513*, 239−245.

(17) Karvela, E.; Makris, D. P.; Kelafas, P.; Moutounet, M. Extraction of phenolics in liquid model matrices containing oak chips: Kinetics, liquid chromatography-mass spectroscopy characterization and association with in vitro antiradical activity. *Food Chem.* **2008**, *110*, 263−272.

(18) Ortega-Heras, M.; Pérez-Magariño, S.; Cano-Mozo, E.; González-San José, M. L. Differences in the phenolic composition and sensory profile between red wines aged in oak barrels and wines aged with oak chips. *LWT Food Sci. Technol.* **2010**, *43*, 1533−1541.

(19) Luque-Rodríguez, J. M.; Luque de Castro, M. D.; Pérez-Juan, P. Dynamic superheated liquid extraction of anthocyanins and other phenolics from red grape skins of winemaking residues. *Bioresour. Technol.* **2007**, *98*, 2705−2713.

(20) Eskin, N. A. M. Biochemistry of food processing: Browning reactions in foods. In *Biochemistry of Foods*, 2nd ed.; Eskin, N. A. M., Ed.; Academic Press: London, 1990; pp 239−296.

(21) Pereira, V.; Albuquerque, F. M.; Ferreira, A. C.; Cacho, J.; Marqués, J. C. Evolution of 5-hydroxymethylfurfural (HMF) and furfural (F) in fortified wines submitted to overheating conditions. *Food Res. Int.* **2011**, *44*, 71−76.

(22) Report of the scientific papel on contaminants in the food chain on provisional findings of furan in food. *EFSA J.* 2004, *137*, 1−20; Available at http:// http://www.efsa.europa.eu/en/scdocs/doc/137.pdf.

(23) Jun, H. J.; Lee, K. G.; Lee, Y. K.; Woo, G. J.; Park, Y. S.; Lee, S. J. Correlation of urinary furan with plasma gamma-glutamyltranspepti-

dase levels in healthy men and women. *Food Chem. Toxicol.* **2008**, *46*, 1753−1759.
(24) Falcone, P. M.; Tagliazucchi, D.; Verzelloni, E.; Giudici, P. Sugar conversion induced by the application of heat to grape must. *J. Agric. Food Chem.* **2010**, *58*, 8680−8691.