

Volatile Profile in Greek Grape Marc Spirits with HS-SPME-GC-MS and Chemometrics: Evaluation of Terroir Impact

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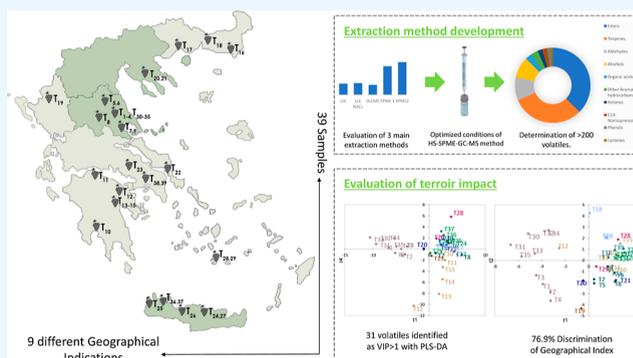
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ABSTRACT: Greek grape marc spirits, although being traditional products, have lately attracted increasing attention as a revisited spirit product category. A headspace solid-phase microextraction coupled to the gas chromatography–mass spectrometry method was optimized in order to determine the volatile composition of 39 samples of grape marc spirits from eight major geographical regions of Greece and 4 protected designations of origin (PDOs). This untargeted analysis approach yielded the identification and quantification of 200 volatiles characterizing those products. Principal component analysis and partial least-squares-discriminant analysis (PLS-DA) combined with the volatile matrix were further employed to identify terroir denomination. Initial results showed a clear separation of the PDO products from Tyrnavos from the rest. Variable importance in projection data pretreatment was further adopted, and with 31 volatiles subsequently used with PLS-DA, products were correctly classified according to region of origin at 76.92% and for 3 out of 4 PDO denominations at 100%.



1. INTRODUCTION

Grape marc distillates are traditional spirit drinks in all European Mediterranean countries, usually consumed after gourmet meals, and are recognized as an important part of their national identities.¹ These spirits have different denominations according to the production country, such as Grappa from Italy, Orujo from Spain, Eau de vie de marc from France, Zivania from Cyprus, Pisco from Chile, Rakija from Slavic countries, etc.^{1–3} In parallel to its winemaking tradition, Greece also has a long tradition in the production of grape marc distillates, named Tsipouro or Tsikoudia, depending on the province of origin. Specifically, Tsikoudia is the grape marc distillate produced on the island of Crete and Tsipouro in continental Greece. Regulation 1576/89 of the EU established the general production procedures for all these traditional alcoholic beverages and fixed common analytical composition limits. Certain production procedures and more restrictive analytical parameters for particular geographical denominations have also been specified.^{4,5} A guideline on general procedures like labeling or geographical indications for all European grape marc distillates has been set by European Union regulation (EU) 2019/78. Presently, in Greece, according to Annex III, Regulation [EC] no. 110/2008, four (4) viticultural regions were recognized as areas for the production of protected designations of origin (PDO) grape marc spirits. Namely, those are Tsipouro of Thessaly, Tsipouro of Macedonia, Tsikoudia of Crete, and Tsipouro of Tyrnavos.

The first three originated from their homonymous geographical areas, while Tyrnavos is a major viticultural area in the periphery of Thessaly.

These are important traditional products, with an increasing value, for the Greek market and with an equally important role in Greece's export value in that sector. First Apostolopoulou et al.⁶ used 10 samples of Tsipouro, five bottled and five traditional (homemade), from the region of Epirus in order to evaluate a gas chromatographic method for the analysis of their principal volatiles. Results of that study showed that the quality and differences between bottled and homemade Tsipouro depended on the initial winemaking process, the applied distillation techniques, and raw material hygiene. Bottled products were found to be more stable and with lower concentrations of toxic compounds (e.g. methanol).⁶ More recently, a study also focusing on the administrative and geographical area of Epirus and the grape variety “Debina” investigated the effect of different distillation techniques on the aroma profile of the final product, measured through gas chromatography–mass spectrometry (GC–MS).⁷ In general,

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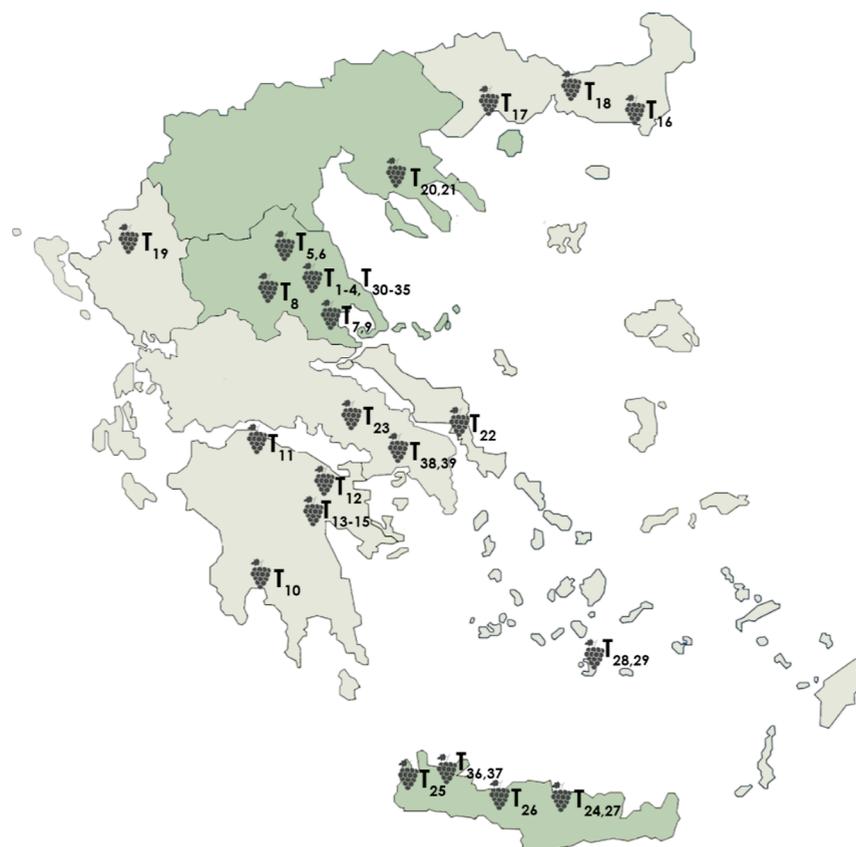


Figure 1. Map of Greece with regions of origin of each spirit sample used in the study. Darker colored areas represent PDO regions for the product category of Greek grape marc spirits.

there are few studies on targeted compounds of those products, such as the one of Geroyiannaki et al.,³ in which 28 samples and a gas chromatographic technique were used for the determination of acetaldehyde and methanol, and the one of Diamantidou et al.,³¹ where they developed a new, fast, high-throughput, accurate, and sensitive ultrahigh performance liquid chromatography-tandem mass spectrometry method for the separation and quantification of 12 phthalate esters in 45 grape marc spirit samples, from Greece and Cyprus. NMR spectroscopy has also been employed, along with multivariate statistical analysis procedures, to classify that product category, using 57 Greek grape marc spirits from different regions (North Greece and Crete Island) and five grape varieties (Romeiko, Malvasia, Xinomavro, Sangiovese, and Nebbiolo).^{2,8} Still, to our knowledge, there is no published systematic study of those products where their full untargeted aroma profile is related to factors such as the region of origin—within Greece—the variety of grapes used, and the distilling methodologies employed.

Similar grape marc distillates from other countries, such as Zivania (Cyprus), Orujo (Spain), and Grappa (Italy), have been studied on the basis of the volatile compounds and their authentication by applying several extraction methods, such as HS-SPME coupled with GC-MS analysis,^{9–11} direct injection with GC-FID or GC-MS,^{12,13} ultrasound-assisted magnetic solid-phase extraction coupled to capillary electrophoresis,¹⁴ liquid-liquid extraction (LLE),^{15,16} multiple neural networks,¹⁸ mid-infrared and near-infrared based spectroscopy¹⁹ and dispersive liquid-liquid microextraction (DLLME).²² Distillate spirits are challenging products due to the complexity

that the ethanol matrix poses: high ethanol concentrations intervene in separation during the extraction, and in combination with the fact that they contain important volatile compounds at low concentrations, the importance of choice of the extraction method for the aroma analysis of those products is crucial.

Typically, the objectives of a nontargeted analysis involve identifying, discovering, and/or quantifying analytes of interest that are largely unknown for the given products, and there are two principal classifications in it: unsupervised and supervised. Currently, pattern recognition and classification methods are being extensively used in various scientific fields such as pharmaceutical and food chemistry.²³ Unsupervised pattern recognition or clustering approaches do not involve any knowledge regarding the membership of the objects, and principal component analysis (PCA) is a crucial unsupervised and exploratory tool for data analysis for reducing the number of dimensions in a data set, allowing the visualization of patterns and insights gathering for multivariate data sets.²⁴ In contrast, classification methods involve establishing a connection between a set of qualitative variables that define membership to one or more predefined classes. Briefly, they typically involve a mathematical model that relates a series of descriptive variables derived from chemical measurements to a set of qualitative variables that define the membership in multiple predetermined classes. Partial least-squares analysis is a common supervised approach for nontargeted analysis that can establish a correlation between chemical variables, such as chromatographic peak areas, and categorical variables that indicate membership of a particular class.²⁴

Solid-phase microextraction (SPME) is a simple, reusable, and solvent-free extraction method which requires zero or minimum amount of solvents, characterizing it as a “green” technique. Enables sampling and sampling preparation into one step, a factor that is very attractive for analysts as a simplified methodology. It is the most applied method for the analysis of volatile and semivolatile compounds in distillate products.^{16,22} The extraction efficiency of the method is affected by several parameters, such as the fiber coating, time and mode of extraction, temperature, and agitation speed.^{11,12,25} In addition, the optimal ethanol–water ratio for SPME might affect it.⁹ LLE, on the other side, is also an efficient extraction method that has been widely used in distillate spirits due to the ability to separate the substances through different solubility, despite the matrix phenomenon.²⁶

This study had two objectives: (1) To evaluate and optimize an extraction method, which coupled to GC–MS can be tailored to the analyses of Greek Grape mark spirits, using as criteria maximum volatile detection and minimum sample preparation/manipulation and cost, and (2) To analyze a set of thirty-nine (39) Tsipouro/Tsikoudia spirit products coming from eight (8) major geographical regions of Greece, resulting from the use of 12 different grape varieties and produced by various distillation techniques, through an untargeted approach for their volatile aroma using the method developed in the first objective. Furthermore, the “terroir” denomination and the aroma–flavor profile of each product were used as classification factors in multivariate analyses.

2. MATERIALS AND METHODS

2.1. Materials. The fibers (DVB/C-WR/PDMS 50/30 μm and DVB/PDMS 65 μm) and a SPME holder were purchased from Supelco. Diethyl ether (95%), pentane (95%), anhydrous sodium sulfate, acetonitrile (99.9%), chloroform (99.8%) ethyl decanoate, isoamyl alcohol (99.9%), phenyl ethyl alcohol (99.9%), linalool (97%), and undecane-*n* (99%) [internal standard (IS)] were purchased from Chem Lab, Athens, Greece. Limonene (97%) and Ethyl decanoate (99%) were purchased from Sigma-Aldrich (USA). All reagents were of analytical quality.

Thirty-nine (39) grape marc spirits were collected from all over Greece, representing all major Greek PDOs (Figure 1). The samples were either provided by the producers themselves or purchased in retail markets. Information regarding the grape variety used and production techniques was obtained in parallel, either from the producers themselves or through product labels in the case of the samples bought from retailers. Among those products, 29 were produced by local distilleries of the regions, and the remaining 10 were homemade (traditional grape marc spirits). Twenty (20) Tsipouro/Tsikoudia spirits were monovarietal, and 19 (19) were plurivarietal obtained by the distillation of grape marc. In Figure 1, a map is presented with the exact accommodation of each spirit with a code name (T1–T39). The geographical denominations with darker colors represent the PDOs for each region (Figure 1).

2.2. Method Development—Optimization for Sample Preparation. Three different existing methods were evaluated for optimal extraction of the volatiles and semivolatiles of this product category: LLE, including a variation of the method with sodium chloride (NaCl) addition, DLLME, and SPME. For SPME, the additional factors of fiber used, headspace

adsorption time, and adsorption temperature were further evaluated.

2.2.1. Liquid–Liquid Extraction. The procedure of LLE was adapted from the work of Tsapou et al.²⁶ Each spirit was diluted with deionized water, up to 10% (v/v) ethanol. A certain amount of the solution (50 mL) was extracted two times with a mixture (50 mL) of pentane and diethyl ether (1:1 v/v). The organic layer was dried over anhydrous sodium sulfate and spiked with 10 μL of *n*-undecane solution (2.5 g/L). The solvent was removed by heating in a water bath at 50 °C with a pear flask fitted with a Vigreux column 20–25 cm long until a volume of 1 mL.

The same procedure was carried out, with the only difference being the addition of NaCl. The salting out effect by adding NaCl to samples is used to promote the extraction efficiency of volatile compounds.²⁵

2.2.2. Dispersive Liquid–Liquid Microextraction. DLLME was performed according to the work of Fontana, Rodriguez, and Cela.²² In a glass tube, with a conical-shaped bottom, were added 2.5 mL of aliquot spirit, 6.5 mL of ultrapure water, and 1 g of NaCl. The mixture was spiked with 0.1 mL of 10 g/mL *n*-undecane in an acetone solution. Then, 0.4 mL of acetonitrile and 0.1 mL of CHCl_3 were added. Tubes were shaken for 1 min and centrifuged for 5 min at 3500 rpm and room temperature. Subsequently, the CHCl_3 extract was recovered and transferred to a vial for injection in a GC–MS system.

2.2.3. Solid-Phase Microextraction. Fibers were pre-conditioned for 1 h at 250 °C in the inlet of the GC prior to sampling, as instructed by the manufacturer. Two different material SPME fibers were evaluated, a 50/30 μm DVB/C-WR/PDMS (Divinylbenzene/Carbon Wide Range/Polydimethylsiloxane) and a 65 μm PDMS/DVB (Supelco, Bellefonte, PA, U.S.A.). Sensitivity analysis was measured by calculating the normalized value of the cumulative peak areas according to the methodology of Zhang et al.¹⁶

In the fiber with the largest standardized value, two more phases of the process were examined: extraction time and temperature. Those have a strong influence on the extraction efficiency of SPME, and in this work, the temperatures of 25, 35, and 45 °C and the times of 15, 25, and 35 min were tested, respectively. An *n*-undecane solution (1 g L⁻¹) was prepared in 40% (v/v) ethanol and then diluted with deionized water to obtain a final ethanol content of 10% (v/v), to be used as an IS. All samples were diluted in 10% final ABV with distilled water (HPLC).

Under optimized conditions, the following process was followed: 7 mL of diluted sample was spiked with the IS solution at 5 mg/L and transferred into a 20 mL autosampler glass vial containing 1 g of NaCl to control the ionic strength. The vial was sealed with a PTFE/silicone septum and aluminum crimp cap (Macherey-Nagel, Bethlehem, PA, USA).

Fibers were exposed for 10 min at 260 °C in the inlet of the GC prior to every sampling to avoid the memory effect, while the samples were maintained for 5 min at 35 °C (optimized temperature), under agitation, to establish equilibrium between headspace and sample. After the incubation time, the fiber was exposed to the headspace for 15 min (optimized time) at the same temperature, and the sample was kept under agitation at 750 rpm. After the extraction time, the fiber was immediately desorbed in the GC injector for thermal desorption of the volatile fraction at 260 °C for 5 min. The injection was in splitless mode, and the transfer line was at 260 °C.

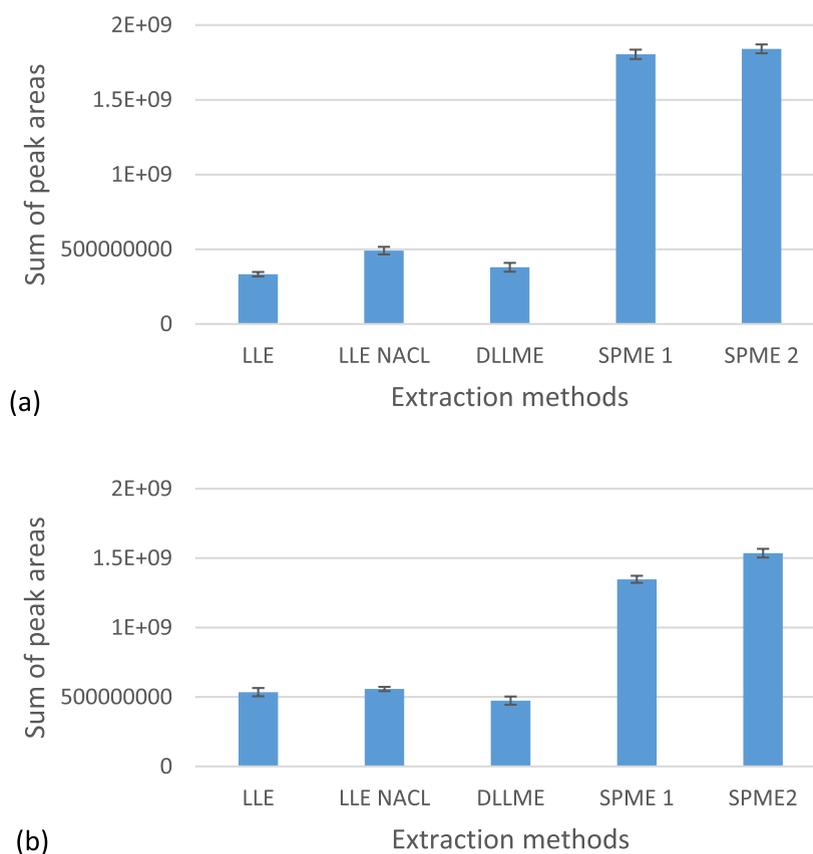


Figure 2. Summarized values of peak areas for the (a) T1 sample and (b) T2 sample in all evaluated methods for extraction. SPME 1 is with DVB/CWR/PDMS, and SPME 2 is with DVB/PDMS fibers, respectively.

2.3. GC–MS Analysis. A gas chromatograph (Shimadzu, Nexis GC-2030 combined with an autosampler/injector (Shimadzu, AOC 20i Plus) and a mass spectrometer (Shimadzu, GCMS QP2020 NX) were used. In the case of the SPME method, the injection system contained a glass liner of 2 mm i.d., 2.75° d., 120 mm, and the autosampler/injector was removed. In the case of the LLE and DLLME, the injector was kept at 250 °C with a split/splitless mode 1/100 the MEGA-WAX ms capillary column was 30 m × 0.25 mm. i.d. X 0.25 μm film thickness with polyethylene glycol as the stationary phase (Agilent Technologies, Santa Clara, CA, USA). The injector was kept at 250 °C, and the GC oven temperature was programmed to 50 °C for 2.5 min and then ramped at 2.5 °C/min to 100 °C, 4 °C/min to 165 and 7 °C/min to 250 °C, remaining at this temperature for 2 min. The gas carrier was helium at a constant flow rate of 1.5 mL/min. The mass spectrometer was operated in electron impact mode at 70 eV in the mass range of m/z 50–550 amu, with the ion source and transfer line kept at 230 °C. All analyses were performed in duplicate. According to the metabolomics standards initiative,²⁸ the qualitative analysis without the use of reference standards should be reported as “putative compounds identifications”. Concentrations of volatiles were calculated with IS (*n*-undecane) as reported in Section 2.2.3 paragraph. Identification of compounds was performed by comparing their mass spectra to those available in the NIST library. Identification was considered valid only for compounds with a minimum match factor of 80%.

2.4. Validation of the HS-SPME-GC-MS Method. The selectivity and linearity were obtained according to the

following procedures: the selectivity of the method was carried out through the injection of individual and mixed solutions spiked with 200 mg L⁻¹ of 5 compounds (isoamyl alcohol, phenyl ethyl alcohol, ethyl decanoate, limonene, and linalool) in ethanol: water solution (40:60 v/v) and injection of ethanolic solution without the addition of the analytes.²⁹ Calibration curves were obtained in order to evaluate linearity of the compounds with each level injected in triplicate in the range of 50–500 mg L⁻¹ for ethyl decanoate, 5–100 mg L⁻¹ for isoamyl alcohol, linalool, and 1–10 mg L⁻¹ for limonene and phenylethyl alcohol, spiked in the solvent (ethanol: water solution, 40:60 v/v). *n*-Undecane was used as an IS at 20 mg L⁻¹. Recovery, accuracy, precision, limit of detection, and limit of quantification data are presented in the Supporting Information Table S2.

2.5. Statistical Treatment of the Data. After identification and partial quantification, the acquired gas chromatographic data were sorted in Excel to match the compounds between different samples and replicates. Subsequently, the matrix was subjected to a multivariate statistical analysis. All statistical procedures, including PCA and partial least-squares-discriminant analysis (PLS-DA), were performed using XLSTAT Addinsoft statistical and data analysis solutions (New York, USA).

3. RESULTS AND DISCUSSION

3.1. Sample Preparation Optimization Suited for GC–MS Analyses of Greek Grape Marc Spirits. **3.1.1. Comparison between LLE, DLLME, and SPME.** Initially, a “qualification” of the chromatographs resulting from the

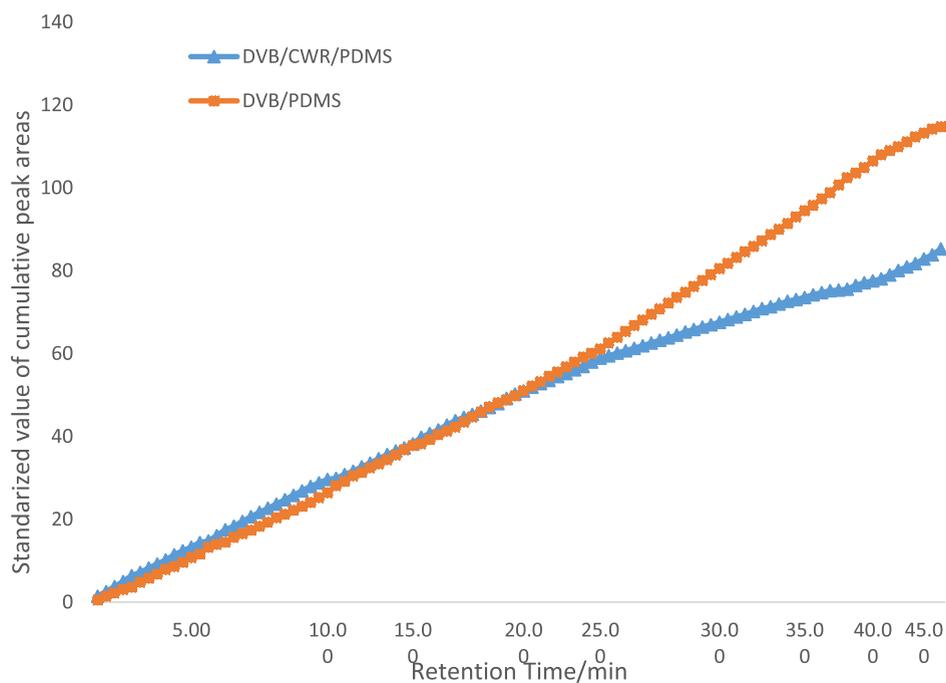


Figure 3. Standardized value of the cumulative peak areas of flavor components obtained by two different SPME fibers combined with GC–MS for Greek grape marc spirits.

different extraction methods took place. The “qualification” of chromatographs is a semiautomated process, in which standard parameters, such as peak width, are compared between different runs, in order to compare the quality of the chromatograms from different sample preparations on the resulting volatile profile of the spirits. The sums of peak areas for the five preparation methods in two different samples are shown in Figure 2. According to that figure, the peak areas of the qualified volatile and semivolatile compounds were approximately 3–4 times higher with the SPME preparation method for both fibers compared to the LLE one, for both samples. LLE preparation with NaCl gave an increase of 32.2 and 4.08% for T1 and T2 samples, respectively, compared with the LLE without salt addition, confirming the importance of the salting effect.^{10,25,27} DLLME gave quality characteristics in the same order of magnitude as the LLE combined with NaCl in both samples, an important observation considering this method as a “greener” one due to the smaller amount of solvents and the fastest extraction method (Figure 2). Taking all parameters into account, SPME was the method of choice for further optimization of the sample preparation suited to the particular type of spirits. Similarly, this has been the preferred method of sample preparation in the study of Kokoti et al.⁷ and several studies related to grape marc distillate drinks.^{10,11,25,30}

Distillate drinks compose a complex matrix due to their high ethanol content and the presence of several hundreds of volatile and semivolatile compounds, posing challenges from a chromatographic point of view.^{9,31} HS-SPME is the dominant technique used for sample preparation prior to GC–MS analyses for distilled spirits, such as whisky, Grappa, gin, and Orujo,^{9,25,30} and the ideal extraction conditions can vary per product but remain important for optimal extraction and volatile profiling. The dilution of a high ethanol content of spirits (40% v/v) before SPME extraction is a parameter that has been extensively studied. During the extraction, competition is created between ethanol and other volatile compounds,

while the large peak area of ethanol alters the signal of the other compounds in chromatographs. There are studies showing that higher ethanol content decreases the extraction efficiency,^{16,32} while dilution to less than 10% ethanol decreases the sensitivity of the extraction.^{25,33}

3.1.2. Fiber Coating. The choice of the SPME fiber coating is very important for the extraction efficiency of the spirit samples. Two different fiber coatings: PDMS/DVB and DVB/CAR/PDMS were evaluated for their extraction efficiency of volatile and semivolatile components in this product category (Greek grape marc spirits). The performance of each fiber was evaluated based on the standardized areas of identified flavor compounds. The normalized value of the cumulative peak areas for the evaluation of the sensitivity was calculated according to the method of Zhang et al.¹⁶ Figure 3 shows that for identical extraction and detection conditions, the fiber DVB/PDMS gave the largest value of peak areas for the Greek grape marc spirit samples. While in the first part of the run (up to 25 min approximately), the two fibers appear to have the same sensitivity, later in the chromatographic analyses, starting at temperatures above 110 °C-, the DVB/PDMS fiber demonstrated an important rise of the standardized value (Figure 3). In those temperatures (110–165 °C), most of the terpenes were identified, representing a very important flavor category in general and in particular for the classification of those products. Hamm et al.¹⁷ came to the same conclusion in 2003, when evaluating five different SPME fibers on their extraction efficiency for terpenoids in olibanum (resin), for which the DVB/PDMS coating gave also the highest quantities.

3.1.3. Extraction Temperature. Extraction temperature and extraction time were evaluated next for the DVB/PDMS fiber by comparing the total amount of identified volatile compounds (in duplicate) between the different conditions. In all cases, the same quantity of sample was used, coupled with the same quantity of NaCl. Three different temperatures,

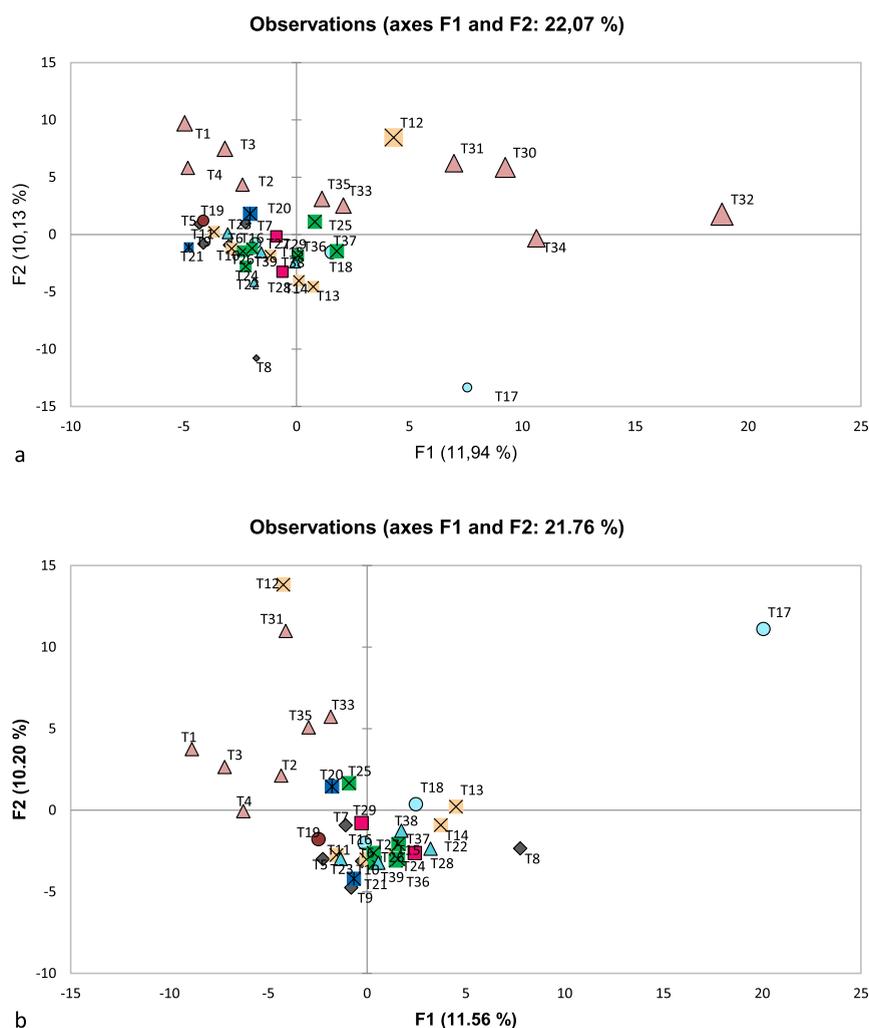


Figure 4. PCA of thirty-nine (39) Greek grape marc spirits based on two hundred (200) volatiles: (a) all thirty-nine (39) spirits and (b) all spirits except T30, T32, and T34 and reduced matrix of 190 volatiles.

namely: 25, 35, and 45 °C, were employed and compared for their adsorption efficiency. With the initial temperature rise, the adsorption of volatile compounds increased as well, demonstrating the highest efficiency at 35 °C. Nevertheless, for temperatures above 35 °C, the adsorption efficiency of the fiber decreased (Figure S1a). Hence, for posterior analysis, an extraction temperature of 35 °C was selected.

3.1.4. Extraction Time. Generally, a shorter extraction time is more attractive for analysts and more suitable for volatile compound extractions. In this study, three extraction times were tested: 15, 25, and 30 min at the same temperature (35 °C). In the literature, HS-SPME methods for distilled products usually suggest an extraction time in the range of 20–50 min. For example, in rum and brandy, a better efficiency showed in extraction time of 30 min^{16,33} while in grape marc distillates, shorter extraction times were used, such as 15³⁰ or 20 min⁹ for Orujo and Grappa, respectively. In this study, results showed that an extraction time of 15 min gave better adsorption of volatile compounds compared with the longer times evaluated (25 and 30 min, Figure S1b).

As a result, the chosen extraction method for GC–MS analyses of the Greek grape marc spirit samples was an HS-SPME technique with a DVB/PDMS fiber, with an extraction time of 15 min at a temperature of 35 °C.

3.2. HS-SPME-GC-MS Analyses of the Spirits. Grape marc spirits are complex drinks containing some hundreds of flavor compounds in a water–ethanol matrix. Their flavor components can originate from different sources. Those components can be derived from grape variety, such as terpenes and nor-isoprenoids, by yeast or bacteria metabolism during fermentation, such as alcohols, esters, and organic acids. Last but not least, an important contribution to the flavor is a consequence of the distillation process, by possible transformation of the compounds and precursors, favored by high temperature and alcohol concentration, or due to wood compounds during spirit aging.⁴¹ Our optimized HS-SPME-GC-MS method, when applied to thirty-nine (39) Greek grape marc samples, led to the identification and quantification of more than 300 volatiles and semivolatiles.

Initially, certain minor peaks were eliminated as absent from one replicate or present in few products. That resulted in a reduced matrix of 200 volatiles, which could be further divided into specific chemical categories, as follows: seventy-five (75) esters, 18 (18) alcohols, six (6) organic acids, 20 (20) aldehydes, four (4) ketones, sixty-two (62) terpenes (monoterpenes and sesquiterpenes), four (4) C13-norisoprenoids, two (2) lactones, three (3) phenols, and six (6) other

aromatic hydrocarbons (furans, ethers, etc.) (Supporting Information Table).

In the work of Apostolopoulou et al.,⁶ when studying five commercial and five homemade Tsipouro products from the Epirus region, they were able to detect around ten (10) volatiles in total for which the products varied. Similarly, in the study of Tsachaki et al.,³⁴ in which three Tsipouro samples were analyzed on their volatile content and compared with six Ouzo products, they mention “tens of volatiles” identified but reported about nine (9) volatiles that were used for product differentiation in support to sensory data. Last, in the most recent GC–MS study of Tsipouro products,⁷ the authors were able to identify forty-four (44) volatiles in total, in samples from different parts of the distillation process for the production of Tsipouro from the region of Epirus. In the work of Cortes et al.,¹³ in which 29 grape marc spirits (Orujo and Grappa) were compared for their flavor profile, around 30 (30) major volatiles were identified. In comparison to those studies, after the optimization of sample preparation that we applied, we were able to get an improved, rich-in-flavor component profile for the product category of grape marc spirits represented by around two hundred (200) volatiles (Supporting Information Table). Our results are comparable in terms of the number and type of identified volatiles to the ones in the work of Lukic et al.,¹⁵ in which the flavor profile of a set of Croatian grape marc spirits was studied.

3.3. Projection Methods and the Role of Region of Origin in the Separation of the Greek Grape Marc Spirits.

3.3.1. Principal Component Analyses. The reduced matrix of two hundred (200) quantified volatiles in the total sample set (Supporting Information Table) was used to observe relationships between the products and associations between products and volatiles and types of volatiles through multivariate statistical methods. First, the data were subjected to PCA after having been pretreated to obtain the mean value of each flavor volatile for each product (each product was analyzed in duplicate). A total of 37.82% of the total variance in the data was explained in four principal components (PC1:11.94%, PC2:10.13%, PC3:8.10%, and PC4:7.65%). In the study of Cortes et al.,¹³ the authors report a total amount of 54.59% variance explained by the first two principal components, when applied to just 13 (13) volatiles and twenty-nine (29) grape marc distillates (a mixture of Orujo and Grappa). In a similar study of headspace congeners in blended whiskies from different product categories though, Lee et al.³⁵ could get a total of 60% variance explained in six principal components by analyzing 53 volatiles in a set of 40 (40) products in the initial product space. Considering the large variable (200) and sample (39) data set, the amount of explained variance in our work was considered a satisfactory model for understanding product clustering, separation, and positioning, especially since this is the variation of products belonging to the same category, being Greek grape marc distillates. A graphical representation of the results is shown in Figure 4a. In that figure where PC1 is plotted against PC2, the main separation of the products is driven by some of the distillates from Tyrnavos, which were clearly differentiated from other terroir samples with very high scores in PC1. Those were the samples T30–32 and T34, with the spirit T32 showing the highest PC1 scores and clearly separated from all of the rest. Products T30–32 and T34 were all traditional homemade Tsipouro samples from the PDO of Tyrnavos, but so were also products T33 and T35, which were largely

clustered in the first and fourth quadrants, all with positive PC1 values but at the same time with variation in their PC1 scores. On the other hand, samples T1–4 were clustered in the second quadrant and represent the samples from local distillers from Tyrnavos (so not homemade products). Therefore, in the initial PCA product space, with all detected volatiles as variables, the PDO of Tyrnavos was clearly differentiated from other PDOs, and a clear distinction was made between samples from official local distillers and ones from traditional homemade producers, as well as between the local home-making Tsipouro producers of Tyrnavos PDO (Figure 4a).

As the initial PCA model was heavily driven by the homemade Tsipouro products T32, T34, and T30, the analyses were rerun without those products, yielding the space shown in Figure 4b. There, we saw that Tyrnavos terroir samples were differentiated again but this time in both PC1 and PC2. Specifically, those were the products T1–T4 and T31, T33, and T35, with T31 spirit showing the highest PC2 score among them and clustered in the second quadrant. Product T12, from the Peloponnese terroir, had the highest scores in PC2 and was clustered with Tyrnavos spirits. On the other side in the first quadrant, sample T17, belonging to Thrace terroir, with the highest scores in PC1, was clearly differentiated alone from all other samples. In this PCA model with 190 volatiles as variables (removing products T32, T34, and T30 resulted also in removing some volatiles not relevant for the remaining samples), the PDO of Tyrnavos was clustered again and separated from other PDOs (Figure 4b).

For both PCA product spaces, the variables plot was very busy (200 and 190 variables, respectively), and it is therefore not shown at this point, as it does not help in understanding the principal variable–sample associations.

3.3.2. Partial Least-Squares Discriminant Analysis. PLS-DA is one of the most widely used classification techniques in chemometrics. The main difference between that method and PCA is that PLS-DA is a supervised classification method where extra information about grouping of the samples is provided. PLS-DA is more suitable for identifying species with differences between products.³⁶ This method can overcome problems related to ill-conditioned variance/covariance matrices, coming from the higher number of variables when compared to the number of analyzed samples, which is common when handling instrumental data matrices.^{9,34} Volatile organic compounds are the main components that cause the formation of food aromas, and the most common pretreatment of volatiles in food and beverages is HS-SPME, combined with a chemometric model.³⁶ PLS-DA was the most suitable supervised method used as tool in classification of geographical origin of products such as tea for two different origins³⁷ and fruit distillates for three different origins,³⁸ with an accuracy of 100 and 91.2%, respectively in those studies.

Therefore, the same data matrix used for running the initial PCA was used to run a PLS-DA using as extra information the general region of origin of the samples. Those were eight regions (Central Greece, Crete, Cyclades, Epirus, Macedonia, Peloponnese, Thessaly, and Thrace) and the PDO of Tyrnavos, which is at the same time a subregion of the Thessaly (Figure 1). The outcome was a product space with clear clusters according to the region of origin–terroir of the spirits, even though other factors such as grape variety used or distillation techniques were not considered separately. A well-defined cluster spreading along the first and fourth quadrants is formed by samples from the Tyrnavos PDO, while in the fourth and

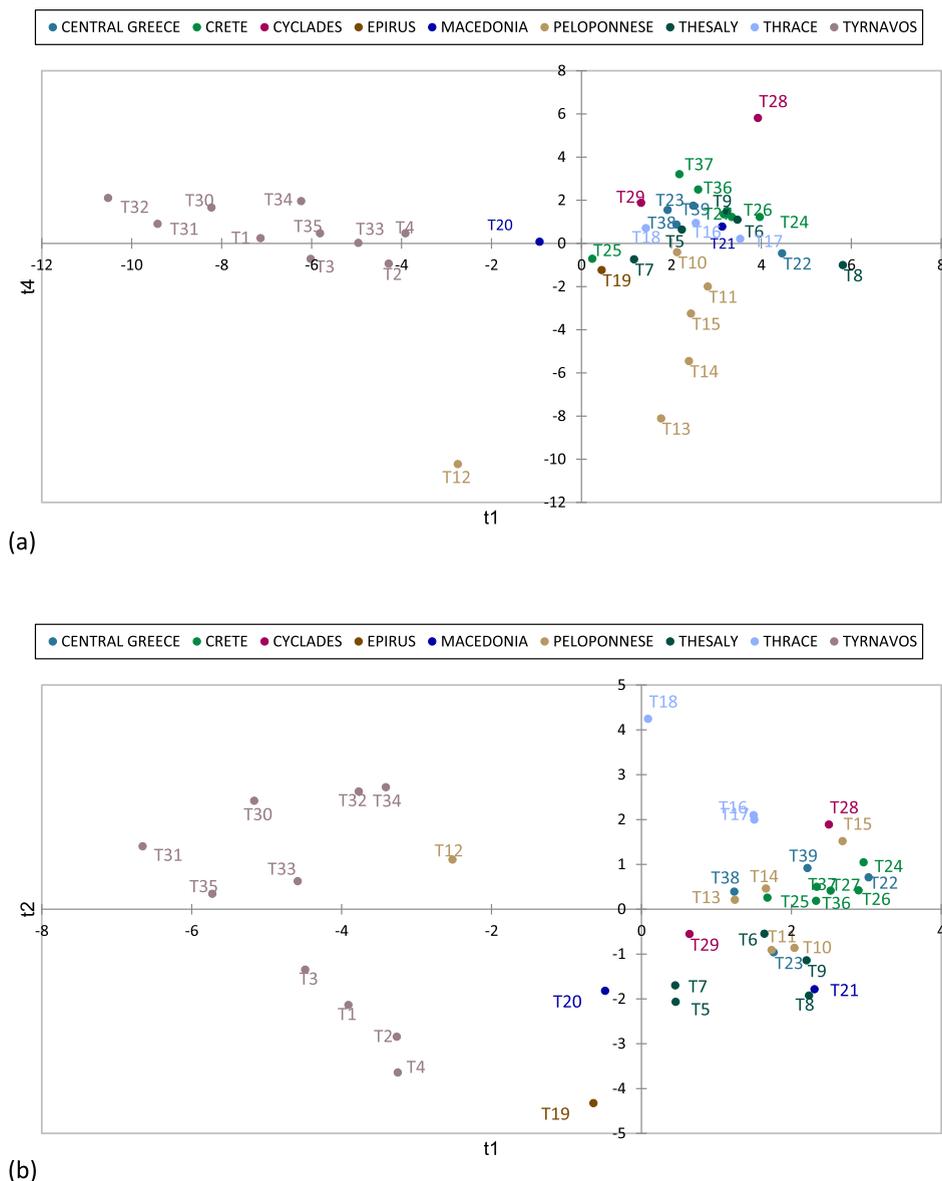


Figure 5. (a) PLS-DA on 39 Greek grape marc spirits and two hundred (200) volatiles, using region of origin as classification parameter. (b) PLS-DA with thirty-one (31) VIP variables for discrimination of Greek grape marc spirits according to their region of origin.

fifth, there is a cluster of samples from Peloponnese. A smaller cluster of Tsikoudia spirits from Crete was formed in the first quadrant, beside T25. Cyclade samples were found in the same quadrant with Cretan Tsikoudia spirits and created a bigger cluster that could relate to the wider Aegean region. Thessalian, Thrace, and Central Greece spirits seemed to create a big cluster without a large separation between them in the first and fifth quadrants. From both modeling approaches, namely, PCA and PLS-DA, when using the initial two hundred (200) volatiles as variables and looking at the effect of terroir only, the intense diversity of Tyrnavos terroir could be easily observed when compared with the other geographical regions of origin of the spirits (Figures 4 and 5).

Variable importance in the projection (VIP) approach is mainly used in a PLS-based context, where the influence of each instrumental variable in defining the variable space is determined. According to this approach, when a variable has a VIP index higher than 1, it is considered relevant for the classification.¹⁹ By using the above threshold in VIP, we ended

with a selection of thirty-one (31) important volatiles for the classification of our Greek grape marc spirits according to their region of origin, which were thereafter used for further classification. Among them, there were three alcohols, 12 esters, 10 terpenes, two C-13 norisoprenoids, two aldehydes, one phenol, and one furan (Table 1). When using the VIP data pretreatment, the resulting clusters were created according to the region of origin at 76.92% correct classification (accuracy). The sensitivity and selectivity of classification of the PLS-DA method were calculated at 65% and 97%, respectively. VIP values for the first two components of the resulting PLS-DA model are given in the Supporting Information as Figure S2. Crete, Thessaly, Tyrnavos (PDO geographical denominations), Thrace, and Epirus had 100% correct classification, while Peloponnese had 83.33%, and Central Greece, Cyclades and Macedonia did not discriminate at all. Cyclades and Macedonia had a much smaller representation in the sample set, though, contributing with just two products per region, respectively. This could probably be the main reason for the

Table 1. List of Thirty-One VIP Variables (Volatiles), Used in PLS-DA, for the Classification of Greek Grape Marc Spirits According to the Origin Region

number	name of compound	odor type
119	ethyl pentadecanoate	sweet
17	β -ocimene (cis and trans)	floral
60	linalool	floral
122	ethyl butyrate	fruity
1	acetaldehyde butyl ethyl acetal	winey
48	geranyl ethyl ether	fruity
124	1-butanol	fermented
66	hotrienol	tropical
86	geraniol (cis and trans)	floral
106	trans nerolidol	floral
19	linalool oxide	floral ^a
132	ethyl 9-hexadecenoate	fruity ^b
53	geranyl methyl ether	floral
147	2,4-decadienal, (<i>E,Z</i>)-	fried
23	hexyl acetate	fruity
126	furan, 2-pentyl-	fruity
44	nerol oxide	green
138	hexanedioic acid, diisobutyl ester	estery
31	<i>cis</i> -rose oxide	floral
57	2-nonanol	waxy
16	isoamyl alcohol	fermented
105	4-ethylguaiaicol	spicy
134	ethyl hexadecanoate	waxy, milky ^{a,c}
159	ethyl (<i>E</i>)-4-decenoate	green
139	(<i>Z</i>)-ethyl heptadec-9-enoate	milky ^c
163	2-decenal, (<i>E</i>)-	fatty, citrus
129	ethyl linoleate	
104	glutaric acid, di(isobutyl) ester	
98	.alpha.-ionone	floral
73	butanedioic acid, diethyl ester	fruity
95	β -damascenone	floral

^aThe good scents company. ^bBleicher et al., 2022.²⁰ ^cLiang et al., 2023.²¹

inadequate region of origin classification for those two regions when compared with the others. As for the PDO of Tyrnavos, the volatiles that contributed the most to the separation of its products from other regions were mainly terpenes such as linalool, geraniol *cis*- and *trans*- β -ocimene, geranyl methyl and ethyl ether, nerol, and linalool oxide. Spirits from Thrace were mainly separated due to higher levels of *trans*-nerolidol and hexyl acetate. As far as Crete terroir was concerned, its “Tsikoudia” spirits were characterized by higher values in acetaldehyde, butyl ethyl ether, 4-ethyl guaiacol, *trans*-nerolidol, and geraniol (Table 1 and Figure 6). All in all, it was interesting to observe a good classification for both the Tyrnavos PDO and the Peloponnese region of origin even before reducing the number of variables in the model through the Variables Importance in Projection approach (200 variables to run the model; Figure 5a). For that particular region (Peloponnese), when we reduced the variables through VIP, the classification did not improve but remained at the 83.33% level of correct classification. This could be because some of the removed variables might have been important for the distinction of that area from the rest or that other factors (such as grape variety used in the raw materials) were more important for the flavor of the products compared to the origin from that specific area. On the contrary, correct classification

values for the regions of Crete, Thrace, and Epirus improved significantly, as expected, reaching a 100% correct classification level after rerunning the model with VIP. Those regions had the initial classification values of 83.33, 66.67, and 0%, respectively, in the initial model of the 200 volatiles (Figure 5a,b)

Related to the type of compounds identified as VIP variables, amyl alcohols are produced by deamination and decarboxylation from isoleucine and leucine during fermentation.^{39,40} An increased concentration of them can negatively affect the aroma of the spirit,⁴¹ even though they are very important alcohols for the total flavor. Those compounds (active amyl and isoamyl alcohol) are usually correlated to the nitrogen available to the yeast in the matrix, according to Rapp and Versini.⁴² Isoamyl alcohol is frequently related to the fermentation process. The main source of nitrogen in fermentation is amino acids; therefore, volatiles that are immediately related to amino acids could also be an index of terroir and not only fermentation conditions. There are some factors, such as geographical location and grape variety, that affect the amino acid composition of grapes, wines, and, subsequently, grape marc spirits.^{43,44} In previous studies on the volatile profile of Greek grape marc spirits with gas chromatography, Apostolopoulou et al.⁶ studied spirits from Epirus using direct injection in a GC-FID and an LLE technique with a GC-MS, Fluoros et al.¹ studied the effect of different storage conditions on resulting volatiles and Kokoti et al.⁷ studied again products from the Epirus region, but this time focusing on the distillation techniques applied. Amyl alcohols in the first study were one of the ten (10) major volatiles, in a concentration of 133.1–325.0 mg/mL p.a. in the study of Fluoros et al.¹ they were found in an average of 1027.5–1076.9 mg/L. Soufleros et al.⁴⁴ reported values for isoamyl alcohol at 86.51–264.44 mg/L in Tsipouro and 81.94–387.54 mg/L in Tsikoudia samples and in the latest study of Kokoti et al.⁷ they were found in a range of 91.85–143.55 mg/L. Cortes et al.¹³ reported range concentration values for 3-methyl-1-butanol at 693–1049 mg/L for Grappa and 254–847 mg/L for Orujo samples. All the above values are not very different from our results in which isoamyl alcohol was in the range of 36.59–394.02 mg/L for the studied spirits (Tables S1 and Table 1).

In studies of grape marc spirits from other regions, such as Italy and Spain, terpenes like nerol, linalool, and geraniol were also used for a successful classification of the samples according to their geographical denominations.^{10,30} Diethyl succinate (Butanedioic acid, diethyl ester) was also a flavor compound identified in Orujo and Grappa spirits for the same classification.^{9,10,30} Finally, when looking at the classification goodness of model fit, a 76.92% correct classification was achieved with PLS-DA of thirty-three (33) variables with VIP >1. In the research of Giannetti et al.,⁹ in which a set of eighty-two (82) different spirits (60 Grappa and 22 other distillates) were analyzed with HS-SPME, the same procedure of multivariate analyses (PLS-DA/VIP) for the classification of Grappa and other spirits (fruit and cereals distillates) was used, and it provided a correct classification rate of 94.3% (100.0% for Grappa and 87.5% for Others), when using 12 (12) variables with VIP >1. In that study, correct classification was at a clearly higher rate; nevertheless, in our research, the analyzed spirits all belonged to the same drink category, being Greek grape marc distillates. The fact that the analyzed spirits were from the same raw material could also be the reason for

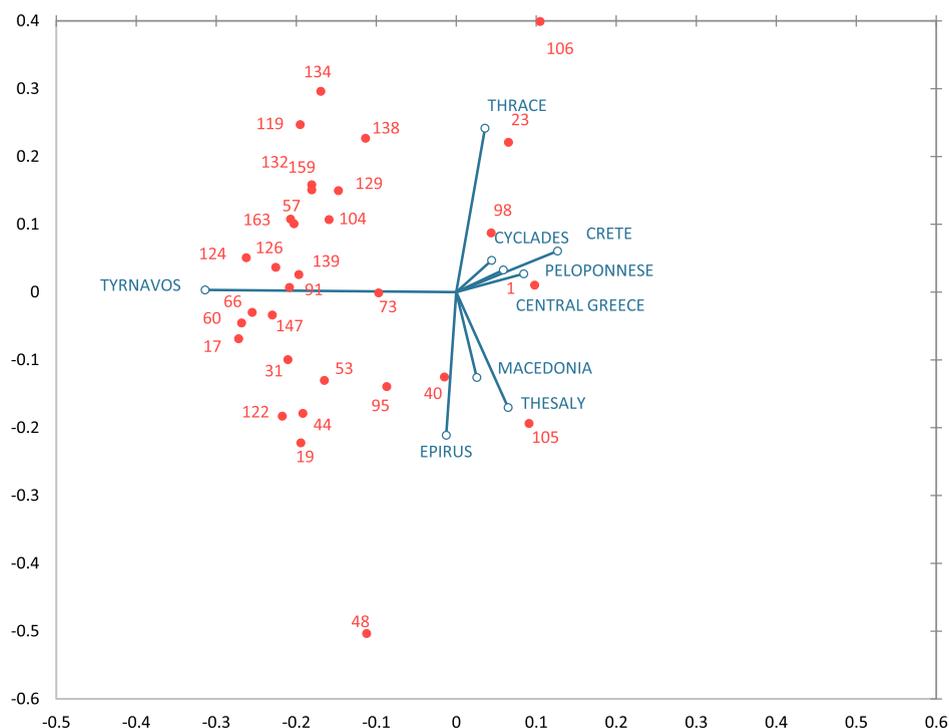


Figure 6. Biplot of PLS-DA with thirty-one (31) VIP variables for discrimination of Greek grape marc spirits according to their region of origin.

the higher number of important variables (33) compared with the work of Giannetti et al.⁹ (12 VIP variables) (Tables S1 and Table 1; Figure 6).

Last, in the work of Lee et al.,³⁸ 40 (40) blended Scotch whiskeys from four (4) different product categories (deluxe, standard, retailer, and West Highland) were also analyzed with the method of HS-SPME-GC-MS, and the acquired GC-MS data were also analyzed by discriminant partial least-squares. In that research, product clustering was explained in three valid factors with a total of 61% variance explained for the X-variables and 47% of variance explained for the Y variables (64% total variance explained for the Deluxe category, 49% for the standard, 42% for the West Highland and 36% for the retailer blends). These results showed a good classification, and the values are close to the ones of our study. Additionally, in that study, different raw materials, terroir, and production methods were included in the sample set, just like in our sample set.

4. CONCLUSIONS

This study had the double objective of a) optimizing an extraction method tailored for GC-MS analyses of Greek Grape must spirits and 2) analyzing a set of thirty-nine (39) Tsipouro/Tsikoudia spirit products coming from eight (8) major geographical regions of Greece on their flavor volatiles. Initially, it was clear that an HS-SPME method was preferred to LLE and DLLME. Results showed that optimal conditions for the extraction of Tsipouro/Tsikoudia were with a DVB/PDMS fiber for a total of 20 min (5 min incubation) at 35 °C, with the addition of NaCl and a decrease of ethanol to 10% v/v. This optimized method was subsequently applied to thirty-nine (39) Greek grape marc spirits, and over 200 volatile compounds were identified and partially quantified. Multivariate statistical projective methods demonstrated product spaces where the samples could be clearly separated and

clustered based on their flavor profile according to their region of origin and PDO representation based on all two hundred volatiles. The most profound separation though was that of the spirits coming from Tyrnavos PDO and the rest of the products. Using the VIP approach combined with PLS-DA helped to identify thirty-one (31) important variables/compounds for which when we rerun the PLS-DA a 76.92% correct classification was achieved for the spirits based on their terroir region of origin. Again, the biggest separation was for the products of Tyrnavos PDO and the rest of the products and less between other regions. Nevertheless, Crete, Thessaly, Tyrnavos (PDO geographical denominations), Thrace, and Epirus demonstrated 100% correct classification using the above approach for the 31 volatiles identified as VIP. HS-SPME-GC-MS analysis combined with PLS-DA proved to be an interesting tool for the flavor profile characterization of the geographical denominations of Greek grape marc spirits (Tsipouro/Tsikoudia), giving valid associations between products and flavor congeners.

Additionally, the thirty-one (31) volatiles identified as variables important in projection for that product set could be further validated to be used in the future as authentication markers for the Greek grape marc spirits. Last but not least, other factors affecting the flavor of those spirits, namely, grape variety used as raw material and distillation techniques employed, remain to be further explored for their contribution effect on the resulting volatiles of the final products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c05686>.

Retention time, Kovats Index, and concentrations in mg L⁻¹ for compounds identified in thirty-nine (39) Greek

grape marc spirits, obtained by HS-SPME-GC/MS (XLSX)

Recovery, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision for the validation of the HS-SPME-GC/MS method; influence of the extraction temperature and the extraction time on the extraction efficiency of the SPME fiber chosen in the method; variable important in projection (VIP) values for thirty-one (31) volatile compounds for the first component and the second component of the PLS-DA model, shown in Figure Sb(PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Flouros, A. I.; Apostolopoulou, A. A.; Demertzis, P. G.; Akrida-Demertzi, K. Influence of the Packaging Material on the Major Volatile Compounds of Tsipouro, A Traditional Greek Distillate. *Food Sci. Tech Int.* **2003**, *9* (5), 371–378.
- (2) Fotakis, C.; Christodouleas, D.; Kokkotou, K.; Zervou, M.; Zoumpoulakis, P.; Moulos, P.; Liouni, M.; Calokerinos, A. NMR metabolite profiling of Greek grape marc spirits. *Food Chem.* **2013**, *138*, 1837–1846.
- (3) Geroyiannaki, M.; Komaitis, M. E.; Stavrakas, D. E.; Polysiou, M.; Athanasopoulos, P. E.; Spanos, M. Evaluation of acetaldehyde and methanol in greek traditional alcoholic beverages from varietal fermented grape pomaces (*Vitis vinifera* L.). *Food Control* **2007**, *18*, 988–995.
- (4) Versini, G. Problematrice della qualita 'della grappa e punti critici del processo. In *Grappa & Vino—CertiWcazione Della Qualita*; Centro Studi e Formazione Assaggiatori: Brescia, Italy, 1995; pp 33–41.
- (5) EEC. Council Regulation 1576/89 on the deWnition, description and presentation of spirit drinks; Oficial Journal of European Community, 1989; Vol. L160, pp 1–17.
- (6) Apostolopoulou, A. A.; Flouros, A. I.; Demertzis, P. G.; Akrida-Demertzi, K. Differences in concentration of principal volatile constituents in traditional Greek distillates. *Food Control* **2005**, *16*, 157–164.
- (7) Kokoti, K.; Kosma, I. S.; Tataridis, P.; Badeka, A. V.; Kontominas, M. G. Volatile aroma compounds of distilled "tsipouro" spirits: effect of distillation technique. *Eur. Food Res. Technol.* **2023**, *249*, 1173–1185.
- (8) Fotakis, C.; Zervou, M. NMR metabolic fingerprinting and chemometrics driven authentication of Greek grape marc spirits. *Food Chem.* **2016**, *196*, 760–768.
- (9) Giannetti, V.; Mariani, M. B.; Marini, F.; Torrelli, P.; Biancolillo, A. Flavour fingerprint for the differentiation of Grappa from other Italian distillates by GC-MS and chemometrics. *Food Control* **2019**, *105*, 123–130.
- (10) Garcia-Martin, S.; Herrero, C.; Pena, R. M.; Barciela, J. Solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC–MS) determination of volatile compounds in orujo spirits: Multivariate chemometric characterisation. *Food Chem.* **2010**, *118*, 456–461.
- (11) Galano, E.; Imbelloni, M.; Chambery, A.; Malorni, A.; Amoresano, A. Molecular fingerprint of the alcoholic Grappa beverage by mass spectrometry techniques. *Food Res. Int.* **2015**, *72*, 106–114.
- (12) Lopez-Vazquez, C.; Herminia Bollaín, M.; Berstsch, K.; Orriols, I. Fast determination of principal volatile compounds in distilled spirits. *Food Control* **2010**, *21*, 1436–1441.
- (13) Cortes, S.; Rodriguez, R.; Salgado, J. M.; Dominguez, J. M. Comparative study between Italian and Spanish grape marc spirits in terms of major volatile compounds. *Food Control* **2011**, *22*, 673–680.
- (14) Peña Crecente, R. M.; Lovera, C. G.; García, J. B.; Latorre, C. H.; Martín, S. G. Ultrasound-assisted magnetic solid-phase extraction for the determination of some transition metals in Orujo spirit samples by capillary electrophoresis. *Food Chem.* **2016**, *190*, 263–269.
- (15) Lukic, I.; Milicevic, B.; Banovic, M.; Tomas, S.; Radeka, S.; Persuric, D. Characterization and Differentiation of Monovarietal Grape Marc Distillates on the Basis of Varietal Aroma Compound Composition. *J. Agric. Food Chem.* **2010**, *58*, 7351–7360.
- (16) Zhang, M.; Chen, Y.; Liu, J.; Li, K.; Li, J. Comparison of LLE and SPME Methods for Screening the Aroma Compounds in Rum. *J. Am. Soc. Brew. Chem.* **2022**, *80*, 136–145.
- (17) Hamm, S.; Lesellier, E.; Bleton, J.; Tchaplá, A. Optimization of headspace solid phase microextraction for gas chromatography/mass spectrometry analysis of widely different volatility and polarity terpenoids in olibanum. *J. Chromatogr. A* **2003**, *1018* (1), 73–83.
- (18) Nassirpour, S.; Chang, P.; Henning, A. MultiNet PyGRAPPA: Multiple neural networks for reconstructing variable density GRAPPA (a 1H FID MRSI study). *NeuroImage* **2018**, *183*, 336–345.
- (19) Schiavone, S.; Marchionni, B.; Bucci, R.; Marini, F.; Biancolillo, A. Authentication of Grappa (Italian grape marc spirit) by Mid and Near Infrared spectroscopies coupled with chemometrics. *Vib. Spectrosc.* **2020**, *107*, 103040.
- (20) Bleicher, J.; Ebner, E. E.; Bak, K. H. Formation and analysis of volatile and odor compounds in meat—a review. *Molecules* **2022**, *27* (19), 6703.
- (21) Sam, F. E.; Ma, T.; Wang, J.; Liang, Y.; Sheng, W.; Li, J.; Jiang, Y.; Zhang, B. Aroma improvement of dealcoholized Merlot red wine using edible flowers. *Food Chem.* **2023**, *404*, 134711.
- (22) Fontana, A.; Rodriguez, I.; Cela, R. Dispersive liquid–liquid microextraction and gas chromatography accurate mass spectrometry for extraction and non-targeted profiling of volatile and semi-volatile compounds in grape marc distillates. *J. Chromatogr., A* **2018**, *1546*, 36–45.
- (23) Trinklein, T. J.; Cain, C. N.; Ochoa, G. S.; Schöneich, S.; Mikaliunaitė, L.; Synovec, R. E. Recent advances in GC× GC and chemometrics to address emerging challenges in nontargeted analysis. *Anal. Chem.* **2023**, *95* (1), 264–286.
- (24) Yousefinejad, S.; Bahram, M.; Baheri, T. Classification of methamphetamine seized in different regions of Iran using GC–MS and chemometrics. *J. Iran. Chem. Soc.* **2018**, *15* (1), 163–170.
- (25) Barnes, Q.; Vial, J.; Thiébaud, D.; De Saint Jores, C.; Steyer, D.; Contamin, M.-A.; Papaiconomou, N.; Fernandez, X. Characterization

of Flavor Compounds in Distilled Spirits: Developing a Versatile Analytical Method Suitable for Micro-Distilleries. *Foods* **2022**, *11*, 3358.

(26) Tsapou, E. A.; Drosou, F.; Koussissi, E.; Dimopoulou, M.; Dourtoglou, T.; Dourtoglou, V. Addition of yogurt to wort for the production of spirits: Evaluation of the spirit aroma over a two-year period. *J. Food Sci.* **2020**, *85* (7), 2069–2079.

(27) Endo, S.; Pfennigsdorff, A.; Goss, K. U. Salting-out effect in aqueous NaCl solutions: trends with size and polarity of solute molecules. *Environ. Sci. Technol.* **2012**, *46* (3), 1496–1503. 46

(28) Sumner, L. W.; Amberg, A.; Barrett, D.; Beale, M. H.; Beger, R.; Daykin, C. A.; Fan, T. W. M.; Fiehn, O.; Goodacre, R.; Griffin, J. L.; et al. Proposed minimum reporting standards for chemical analysis. *Metabolomics* **2007**, *3*, 211–221.

(29) Danzer, K.; Currie, L. A. Guidelines for calibration in analytical chemistry. Part I. Fundamentals and single component calibration (IUPAC Recommendations 1998). *Pure Appl. Chem.* **1998**, *70* (4), 993–1014.

(30) Peña, R. M.; Barciela, J.; Herrero, C.; García-Martín, S. Headspace Solid-Phase microextraction gas chromatography–mass spectrometry analysis of volatiles in orujo spirits from a defined geographical origin. *J. Agric. Food Chem.* **2008**, *56* (8), 2788–2794.

(31) Diamantidou, D.; Begou, O.; Theodoridis, G.; Gika, H.; Tsochatzis, E.; Kalogiannis, S.; Kataifsi, N.; Soufleros, E.; Zotou, A. Development and validation of an ultrahigh-performance liquid chromatography-tandem mass spectrometry method for the determination of phthalate esters in Greek grape marc spirits. *J. Chromatogr., A* **2019**, *1603*, 165–178.

(32) Conner, J. M.; Birkmyre, L.; Paterson, A.; Piggott, J. R. Headspace Concentrations of Ethyl Esters at Different Alcoholic Strengths. *J. Sci. Food Agric.* **1998**, *77*, 121–126.

(33) Ebeler, S. E.; Terrien, M. B.; Butzke, C. E. Analysis of brandy aroma by solid-phase microextraction and liquid–liquid extraction. *J. Sci. Food Agric.* **2000**, *80* (5), 625–630.

(34) Tsachaki, M.; Arnaoutopoulou, A. P.; Margomenou, L.; Roubedakis, S. C.; Zabetakis, I. Development of a suitable lexicon for sensory studies of the anise-flavoured spirits ouzo and tsipouro. *Flavour Fragrance J.* **2010**, *25*, 468–474.

(35) Lee, K. Y. M.; Paterson, A.; Birkmyre, L.; Piggott, J. R. Headspace congeners of blended Scotch whiskies of different product categories from SPME analysis. *J. Inst. Brew.* **2001**, *107* (5), 315–332.

(36) Dou, X.; Zhang, L.; Yang, R.; Wang, X.; Yu, L.; Yue, X.; Ma, F.; Mao, J.; Wang, X.; Zhang, W.; et al. Mass spectrometry in food authentication and origin traceability. *Mass Spectrom. Rev.* **2023**, *42* (5), 1772–1807.

(37) Raj, D. R. K.; da Silva Ferreira, M. V.; Braunger, M. L.; Riul, A.; Thomas, J.; Barbin, D. F. Exploration of an impedimetric electronic tongue and chemometrics for characterization of black tea from different origins. *J. Food Compos. Anal.* **2023**, *123*, 105535.

(38) Dehelean, A.; Cristea, G.; Feher, I.; Hategan, A. R.; Magdas, D. A. Differentiation of Transylvanian fruit distillates using supervised statistical tools based on isotopic and elemental fingerprint. *J. Sci. Food Agric.* **2023**, *103* (3), 1454–1463.

(39) Boulton, R.; Singleton, V. L.; Bisson, L. F.; Kunkee, R. E. Preparation of musts and juice. *Principles and Practices of Winemaking*; Springer, 1996; pp 65–101.

(40) Falqué, E.; Fernández, E.; Dubourdieu, D. Differentiation of white wines by their aromatic index. *Talanta* **2001**, *54* (2), 271–281.

(41) Rapp, A.; Versini, G. Influence of Nitrogen Compounds in Grapes on Aroma Compounds of Wines. *Dev. Food Sci.* **1995**, *37*, 1659–1694.

(42) Mirás-Avalos, J. M.; Bouzas-Cid, Y.; Trigo-Córdoba, E.; Orriols, I.; Falqué, E. Amino Acid Profiles to Differentiate White Wines from Three Autochthonous Galician Varieties. *Foods* **2020**, *21* (2), 114. 9

(43) Soufleros, E. H.; Bouloumpasi, E.; Tsarchopoulos, C.; Biliaderis, C. G. Primary amino acid profiles of Greek white wines and their use in classification according to variety, origin and vintage. *Food Chem.* **2003**, *80* (2), 261–273.

(44) Soufleros, E. H.; Natskoulis, P.; Mygdalia, A. S. Discrimination and risk assessment due to the volatile compounds and the inorganic elements present in the Greek marc distillates Tsipouro and Tsikoudia. *J. Int. Sci. Vigne Vin* **2005**, *39*, 31–45.