JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Headspace Solid-Phase Microextraction Gas Chromatography–Mass Spectrometry Analysis of Volatiles in Orujo Spirits from a Defined Geographical Origin

ROSA M. PEÑA, JULIA BARCIELA, CARLOS HERRERO, AND SAGRARIO GARCÍA-MARTÍN*

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias, Universidad de Santiago de Compostela, Avda. Alfonso X El Sabio s/n, 27002 Lugo, Spain

A headspace solid-phase microextraction (HS-SPME) and gas chromatography−selective ion monitoring/mass spectrometry (GC−SIM/MS) method was optimized for analysis of 22 volatile compounds in orujo spirit samples from the Geographic Denomination "Orujo de Galicia/Augardente de Galicia". HS-SPME experimental conditions, such as fiber coating, extraction temperature, extraction and pre-equilibrium time, sample volume, and the presence of salt, were studied to improve the extraction process. The best results were obtained using a 65 μm Carbowax-divinylbenzene fiber during a headspace extraction at 40 °C with constant magnetic stirring for 15 min and after a 5 min period of pre-equilibrium time. The sample volume was 6 mL of orujo containing 25% of NaCl, placed in 12 mL glass vials equipped with a screw cap and PTFE/silicone septum. Desorption was performed directly in the gas chromatograph injector port for 5 min at 250 °C using the splitless mode. The proposed method is sensible (with detection limits between 0.0045 and 0.2399 mg/L), precise (with coefficients of variation in the range 0.99–8.18%), and linear over more than 1 order of magnitude. The developed method presented recoveries comprised between 76.0 and 112.4%. The applicability of the new method was demonstrated by determining the considered 22 volatile compounds in nine orujo commercial samples with quality and origin brands.

KEYWORDS: Headspace solid-phase microextraction; volatile compounds; orujo spirits; gas chromatography–mass spectrometry

INTRODUCTION

Orujo is a traditional alcoholic distilled beverage produced in Galicia (NW Spain) from vinacce (skins, seeds, and stalks from the grapes) after alcoholic fermentation. In 1989, the European Union established the general regulations concerning the definition, denomination, and production of alcoholic spirits (*1*). This European Regulation included Galicia as the only Spanish region with the possibility of obtaining a geographic denomination for orujo, in the same category as French marcs, Italian grappas, Portuguese bagaceiras, and Greek tsipouros.

In the past few years, since the establishment of Geographic Denomination "Orujo de Galicia/Augardente de Galicia", this beverage has come to be a product of important economic interest for producers and not merely as a complementary activity to wine elaboration (*2*). The Directive Council of this Denomination defined the origin of the raw material and the concentration limits of several volatile compounds present in the distilled product (ethanol, methanol, total content of higher alcohols, ethyl acetate, and acetaldehyde). In this way, the resulting spirits undergo strict quality controls.

Orujo is mainly composed of water and ethanol. However, 300 or more different compounds are present. Several volatile compounds coming from contributions of different production steps characterize the orujo aroma (*3, 4*). The first contribution is due to compounds derived from the grape variety. Most of them are terpenols, nor-isoprenoids, and benzenoids. The second contribution originates from compounds formed by metabolism of yeast and other bacteria during fermentation such as alcohols, methyl and ethyl esters, and acetates. The last contribution is due to compounds formed as a consequence of distillation, by transformation of the above compounds and precursors, favored by high temperature and alcohol concentration. The main ones are nor-isoprenoids and acetals (*5*).

Some of these compounds are present in relatively large amounts and can be determined by direct gas chromatography. Other compounds such as fatty acids and esters are present at much lower concentrations. Therefore, the determination of these constituents often requires the use of a preconcentration step.

* Author to whom correspondence should be addressed: Tel.: 34-982285900. E-mail: sgarciam@lugo.usc.es. Fax: 34-982285872.

10.1021/jf073481f CCC: $40.75
Published on Web 03/25/2008

The sample preparation for the analysis of the flavor constituents in distilled spirits usually involves a concentration of the analytes using the headspace technique, steam distillation and supercritical fluid extraction, trapping over porous polymers, solid–liquid extraction over resins, purge-extraction techniques, simultaneous distillation−extraction, batch, and continuous solvent extraction (*6–11*). These methods have various drawbacks including excessive preparation time and the use of organic solvents. Solid-phase microextraction (SPME) was developed in 1989 by Pawliszyn to facilitate rapid sample preparation (*12*). This technique is a solventless extraction based on the exposure of an immobilized stationary phase into the matrix containing the analytes, which may be liquid, solid, or gaseous, followed by the thermal desorption in the injector of a gas chromatograph. Compared to traditional techniques, especially solid–liquid and liquid–liquid extraction, SPME shows significant advantages: high sensitivity and reproducibility, low cost, solvent-free extraction, the possibility of automatization as well as the fact that prior preparation of samples is not necessary (*13*). It has been shown to be a very suitable technique for the analysis of volatile and semivolatile compounds in alcoholic beverages (*14–19*), as well as in other food matrices such as cheese (*20, 21*).

This paper focuses on the development and optimization of the SPME method for the analysis of 22 selected compounds in spirits samples from the Geographic Denomination "Orujo de Galicia/Augardente de Galicia". The chemical information obtained could be used in the future (using multivariate chemometrical tools such as principal component analysis, cluster analysis, discriminant analysis, artificial neural networks, and others) to classify orujo samples according to their geographical and botanical origins, distillation technique, or quality brand.

MATERIALS AND METHODS

Orujo Samples. Nine samples of commercial orujo spirits, from the Geographic Denomination "Orujo de Galicia/Augardente de Galicia", were used for this study. All samples were orujo monovarietal spirits (2004 vintage), obtained from the distillation of grape pomace from Albariño variety grapes grown in the Rias Baixas restricted geographical area. The spirits studied were distilled with an "alembic" (the distillation traditional device used in Galicia): the still is heated directly by fire, a water bath or steam, and the distillates were obtained by a Charentais-type system. All the samples used were provided by Geographic Denomination Directive Council so their geographical origin and authenticity are guaranteed. Samples were collected in 400 mL glass bottles and stored at 4 °C before analysis.

Apparatus. (a) Gas Chromatographic system: An Agilent 6890 gas chromatograph equipped with a mass spectrometric detector (MSD) model 5973N was employed (Agilent Technologies Deutschland Gmbh, Waldbronn, Germany). The capillary column used was an HP-Innowax (30 m × 0.25 mm id, film thickness 0.25 μm) from Agilent Technologies.

(b) Data acquisition: The chromatographic data were analyzed on an HP-Chemstation version D.00.00.38 (Agilent Technologies).

SPME Fibers. The SPME manual fiber holder and fibers were obtained from Supelco (Bellefonte, PA, USA). Due to the wide range of polarities of the studied compounds, two fibers were tested: polydimethylsiloxane PDMS (nonpolar fiber preferred for the extraction of nonpolar analytes) with a film thickness of 100 μm and carbowax-divinylbenzene CW-DVB (a mixed coated fiber mainly used for the extraction of volatile low-molecular-mass and polar analytes) with a film thickness of 65 μm. Using other common fibers such as polyacrilate (PA), it has been confirmed that the extraction of alcohols interferes in the determination of other volatile compounds. In all cases, the fibers were conditioned before use by inserting them into the GC injector port for 1 h at 250 °C (PDMS) and for 30 min at 220 °C (CW-DVB). Between injections, the fibers were desorbed for 10 min at 250 °C in split mode to prevent any contamination.

Reagents. All volatile compound standards such as alcohols (1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-phenylethanol, 3-octanol (used as internal standard)), esters (ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl succinate, 2-phenyl ethyl acetate, ethyl dodecanoate, ethyl tetradecanoate), acids (acetic, hexanoic, octanoic, decanoic), aldehydes (benzaldehyde), and terpenes (linalool, α-terpineol, citronellol, nerol, geraniol) were supplied by Aldrich Flavor and Fragrances (Alcobendas, Madrid, Spain). Sodium chloride, used to control the ionic strength, was supplied by Panreac (Barcelona, Spain). Absolute ethanol (Panreac, Barcelona, Spain) and ultrapure Milli-Q water (Millipore Co., Bedford, USA) were used as solvents. All solvents and reagents used were analytical grade.

Stock standard solutions of 10^3 or 10^4 mg/L of each component were prepared by dissolving the pure standards in 40% (v/v) ethanol. Then they were stored at 4 °C. Working standard solutions of each compound were prepared daily by mixing an aliquot of each individual solution and diluting with ultrapure water to obtain a final ethanol content of 10% (v/v).

Solid-Phase Microextraction Procedure. An amount of 6 mL of the orujo sample or standard solution, containing 10 mg/L in 10% ethanol of each compound and 25% of NaCl, was placed in 12 mL PTFE-coated septum-closed vials. SPME extractions were performed by inserting the fiber in the headspace for 15 min at 40 °C using continuous magnetic stirring of the liquid phase at 1100 rpm. Before the extraction, the sample with 25% (w/v) of NaCl was maintained at 40 °C for 5 min to establish equilibrium between headspace and sample. After each extraction, the fiber was inserted into the GC injector port using a 0.75 mm i.d. liner (to improve the GC resolution). The chromatographic analysis was performed under the conditions described in the GC−MS Conditions section. Desorption time and temperature were 5 min and 250 °C, respectively. All experiments and sample measurements were carried out in triplicate, and the average values were calculated.

GC−MS Conditions. The gas chromatographic operation conditions were as follows. The injector temperature was 250 °C, and the carrier gas employed was Helium (purity 99.9995%) at a constant flow rate of 1 mL/min. The oven temperature program was 5 min at 40 °C, then 1.5 °C/min up to 80 °C, and finally 5 °C/min up to 200 °C. This final temperature was maintained for 0.5 min. The injection was made in splitless mode for 2 min at a temperature of 250 °C and using a SPME inlet guide and predrilled Thermogreen LB-2 septa from Supelco (Bellefonte, PA, USA).

The mass spectrometer was operated in electron impact mode with the following conditions. The source temperature was 230 °C; the quadrupole temperature selected was 150 °C; and the relative electron multiplier voltage (EM) applied was 400 V with a resulting voltage of 1553 V. To improve the detection limits, the selected ion monitoring (SIM) mode was used. Compounds were identified using the NIST98 version 2.0 mass spectra library. Each compound was further confirmed by comparing its mass spectra, linear retention index (LRI), and retention times with those obtained for standards. Linear retention indices were determined by injection of a solution containing homologous series of normal alkanes (C_{11}−C_{20}) in a temperature-programmed run, as described above. The values obtained were compared with those reported in previous literature (*22–24*).

Quantitative Analysis of Volatile Compounds by GC−SIM/MS. Mass spectrometry covers the mass range of m/z 20–200. The selected ions for each compound are listed in **Table 1**. The areas of ion peaks used for quantification were normalized by the area of the internal standard (3-octanol) ion peak. The relative areas were interpolated in the calibration graphs built by the analysis of different concentration standard solutions using the SPME procedure described above.

RESULTS AND DISCUSSION

Sample Preparation. The high ethanol content of the orujo spirits (40% v/v) required dilution to 10% ethanol before solid-phase microextraction. This dilution minimized the competition

Table 1. Chemical Standards and MS Fragments Used for Quantitative Analysis

compound	ions	slope	intercept	r^2	range (mg/mL)
1-propanol	31,42,60	2.16E−02	4.01E−04	0.9979	0–20
2-methyl-1-propanol	31,43	2.79E−02	2.57E−05	0.9994	0–20
3-methyl butanol	41,55,70	6.90E−02	−3.17E−03	0.9909	0–30
ethyl hexanoate	43,60,88,99	8.29E−01	1.29E−02	0.9957	0–10
ethyl lactate	29,45	2.26E−02	−1.20E−03	0.9916	0–30
ethyl octanoate	57,88,101,127	9.07E−01	2.42E−02	0.9965	0–30
acetic acid	42,43,45,60	2.04E−02	−1.02E−03	0.9919	0–30
benzaldehyde	51,77,106	5.98E−01	2.39E−03	0.9954	0–2.5
linalool	41,55,71,93	9.09E−01	2.62E−03	0.9962	0–2.5
ethyl decanoate	43,73,88,101	3.17E−01	1.37E−02	0.9947	0–30
diethyl succinate	29,101,129	2.00E−01	2.29E−03	0.9996	0–20
α-terpineol	55,93,121,136	6.39E−01	2.36E−03	0.9922	0–2.5
citronellol	41,67,69,81	8.19E−01	2.39E−03	0.9954	0–2.5
nerol	41,68,69,93	1.15E+00	6.26E−03	0.9900	0–2.5
2-phenyl ethyl acetate	43,91,104,105	3.44E+00	3.97E−03	0.9910	0–0.6
ethyl dodecanoate	43,55,88,101	8.56E−02	3.93E−03	0.9941	0–23
geraniol	41,68,69,93	9.61E−01	4.77E−03	0.9913	0–2.5
hexanoic acid	41,43,60,73	1.42E−01	−6.66E−03	0.9933	0–30
2-phenyl ethanol	65,91,92,122	1.89E−01	3.41E−04	0.9998	0–13
ethyl tetradecanoate	43,55,88,101	8.72E−02	−8.50E−04	0.9933	0–7.5
octanoic acid	41,43,60,73	1.61E−01	−9.94E−03	0.9900	0–30
decanoic acid	43,60,73,129	1.47E−01	−6.30E−03	0.9934	0–30

between ethanol and other volatile components in the extraction process and decreased interferences in chromatography produced by the large ethanol peak. Some authors have found that an increase in the ethanol content decreases the extraction efficiency (*25–28*). However, dilution to less than 10% ethanol resulted in a loss of sensitivity for most volatiles determined by SPME (*29, 30*). Therefore, working standard solutions and samples were diluted to obtain a final ethanol concentration of 10%.

An aliquot of 6 mL of this solution (or the orujo sample) was placed in a 12 mL PTFE coated septum-closed vial. An amount of 1.5 g of sodium chloride and 20 mg/L of the internal standard (3-octanol) were added. SPME extractions were performed using the procedure described in the Solid-Phase Microextraction Procedure section.

HS-SPME Parameter Optimization. To optimize the SPME method, some parameters controlling the performance of the extraction were taken into account. The standard solution employed contained 10 mg/L of each compound studied and had a final ethanol content of 10% (v/v).

SPME Fiber Selection. Two fiber coatings, PDMS and CW-DVB, were used to determine which coating was the more appropriate for the determination of flavor volatiles present in orujo spirits. The data obtained for the analyzed compounds (grouped into five classes: alcohols, esters, terpenes, acids, and aldehydes) showed that the best choice was the use of 65 μm of CW-DVB fiber. In all cases, the amount extracted with this fiber, expressed as peak area, was greater than those extracted with 100 μm of PDMS fiber except for terpenes where the performance of both fibers was similar. Thus, the CW-DVB fiber was therefore chosen for the remaining optimization studies.

Influence of Sample Volume. The amount of volatile compounds adsorbed on the SPME fiber may be dependent on the sample volume. When the sample volume changes from 6 to 10 mL, the extent of SPME adsorption was slightly lower for alcohols, acids, and aldehydes and much lower for terpenes. A significant increase was observed only for esters when the volume was increased from 6 to 10 mL. This nonlinear behavior was also detected and explained by other authors (*31*). The sample volume of 6 mL was maintained for posterior analysis with a volume ratio of liquid phase and headspace of 1:1.

Influence of Extraction Temperature. Temperature is an important parameter for the SPME extraction process. The

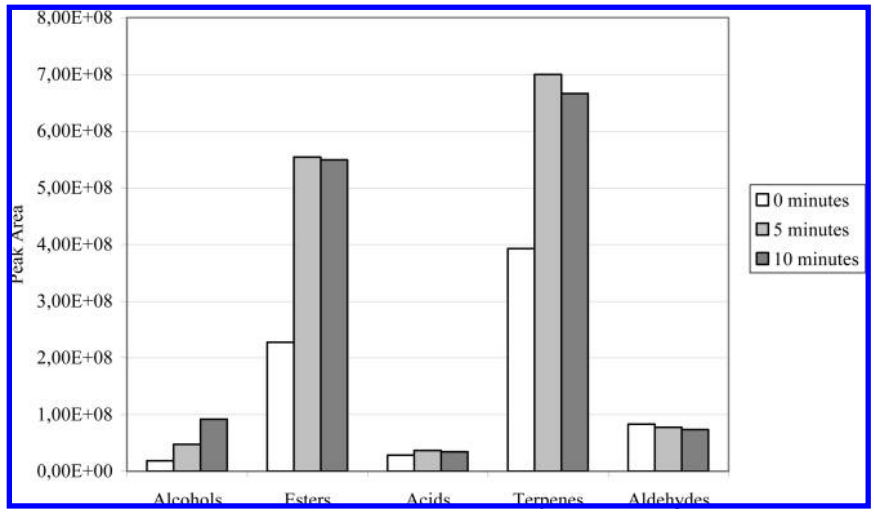

Figure 1. Influence of equilibrium time on GC−MS peak areas for selected volatile compounds.

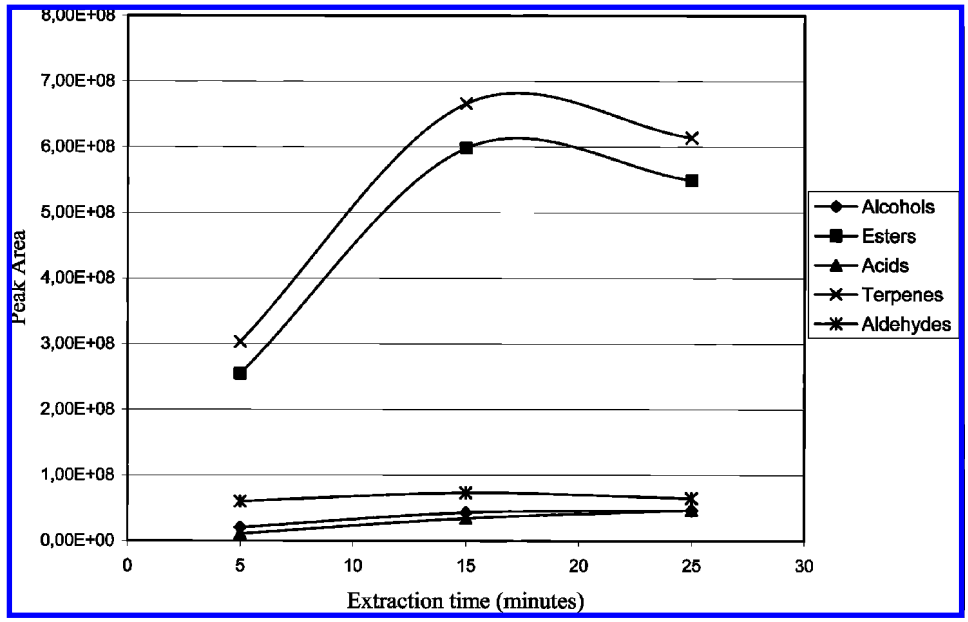

Figure 2. Influence of extraction time on GC−MS peak areas for selected volatile compounds.

temperature increased the concentration of the analytes in the headspace. Different extraction temperatures of 30, 40, and 50 °C were tested to determine coating fiber concentration efficiency. The results confirmed that the concentration in the headspace increased as the temperature rose from 30 to 40 °C. When the temperature was set at 50 °C, the concentration in the headspace only increased in the case of alcohols and terpenes while it decreased for esters, acids, and aldehydes. Hence, for posterior analysis, an extraction temperature of 40 °C was selected.

Influence of Ionic Strength. In SPME methods, the amount of analyte adsorbed onto the fiber can be affected by the sample composition. Adding 25–30% (w/v) of sodium chloride to the sample or adjusting the sample pH before extraction increases the ionic strength of the solution and, in turn, reduces the solubility of some analytes. The addition of salt to a sample greatly increases the extraction efficiency for many analytes, particularly polar and volatile compounds.

The influence of sodium chloride concentration in the solution was studied using different amounts of NaCl ranging between 0 and 25%. It is observed that peak areas rise with increased salt concentration until saturation is reached. For posterior analyses, the sample solution was added with NaCl to saturation (25%) to improve the extraction.

Sample Vial Equilibrium. Prior to exposure of the fiber to headspace, the sample vials (containing 6 mL of sample and 25% (w/v) of NaCl) were held at 40 °C in a thermostatic bath with magnetic stirring for different lengths of time (from 0 to 10 min) to establish an equilibrium between headspace and sample. Significant differences were observed. Higher equilibrium times corresponded to higher peak areas for most of the volatile compounds studied (**Figure 1**). In the case of alcohols, a continuous increment in the peak areas was observed from 0 to 10 min of equilibrium time. For the other kinds of compounds, the amount extracted augmented from 0 to 5 min but decreased or did not increase when the equilibrium time lasted 10 min. Therefore, an equilibrium time of 5 min was employed in posterior analysis.

Influence of Extraction Time. The influence of the extraction time on the yield of microextraction is presented in **Figure 2**. This parameter was evaluated by exposing the fiber into the headspace between 5 and 25 min. In all cases, 6 mL of the standard solution satured with 25% of NaCl was used. For all the compounds studied, the kinetic curves show that equilibrium between sample and fiber was essentially achieved within 15 min. This exposure time was enough to obtain a quantitative extraction with good reproducibility.

Table 2. Analytical Figures of Merit

compound	RSD [$n = 6$] (%)	recovery (%)	LOD (mg/L)	LOQ (mg/L)
1-propanol	3.97	102.3	0.0727	0.2424
2-methyl-1-propanol	4.80	98.6	0.0378	0.1260
3-methyl butanol	4.97	99.8	0.2271	0.7570
ethyl hexanoate	7.57	76.0	0.0538	0.1795
ethyl lactate	5.05	101.7	0.2186	0.7287
ethyl octanoate	5.23	94.3	0.1402	0.4674
acetic acid	7.44	99.1	0.2142	0.7141
benzaldehyde	5.84	102.0	0.0139	0.0462
linalool	0.99	100.7	0.0126	0.0421
ethyl decanoate	1.85	100.9	0.1727	0.5757
diethyl succinate	2.22	103.1	0.0348	0.1162
α-terpineol	2.47	107.6	0.0181	0.0602
citronellol	3.72	112.4	0.0138	0.0461
nerol	3.31	105.9	0.0204	0.0682
2-phenyl ethyl acetate	5.27	85.1	0.0045	0.0151
ethyl dodecanoate	1.15	101.8	0.1447	0.4824
geraniol	4.41	103.6	0.0191	0.0636
hexanoic acid	6.30	82.8	0.1950	0.6498
2-phenyl ethanol	3.82	97.0	0.0168	0.0559
ethyl tetradecanoate	7.91	98.7	0.0485	0.1615
octanoic acid	8.18	87.3	0.2399	0.7996
decanoic acid	5.85	90.7	0.1932	0.6441

Optimization of Desorption Conditions. The optimization of thermal desorption has an important influence on precision and sensitivity. Hence, parameters such as desorption time and injection port temperature must be also optimized for the analytes involved. Some experiments using different desorption times (1, 2.5, and 5 min) were performed. The results showed that desorption from the fiber incremented slightly with longer times. For posterior analysis, we considered that thermal desorption of the analytes was best completed using the splitless mode and 5 min of desorption time.

The study of the influence of the injector port temperature was performed. Three different temperatures (150, 200, and 250

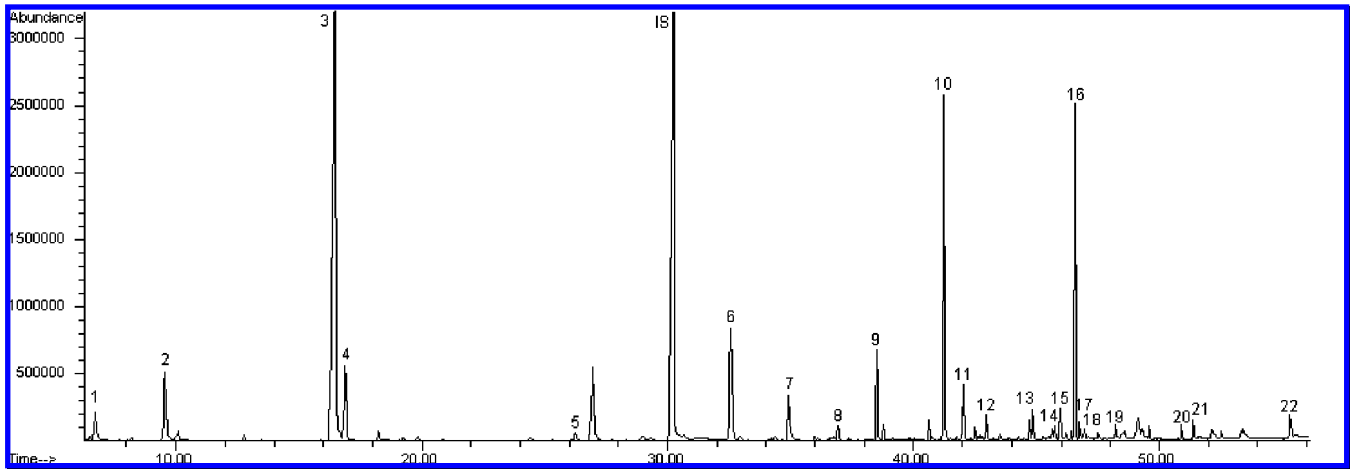

Figure 3. Typical HS−SPME−GC−MS of orujo spirits. Identified analytes are listed in **Table 3**.

Table 3. Chemical Composition of Orujo Samples[a]

				concentration (mg/L)										
				sample										
peak	t_R(min)	LRI_{calc}[b]	compd	1	2	3	4	5	6	7	8	9	mean	Id[c]
1	6.77	1030	1-propanol	139.3	147.4	168.1	124.5	185.3	128.7	225.2	209.5	126.8	161.6	MS,t_R,LRI
2	9.55	1107	2-methyl-1-propanol	168.2	189.7	189.8	145.1	188.9	105.0	167.5	223.0	150.6	169.7	MS,t_R,LRI
3	16.05	1195	3-methyl butanol	368.7	420.1	424.4	362.3	474.0	396.8	375.9	442.2	478.8	415.9	MS,t_R,LRI
4	17.23	1225	ethyl hexanoate	3.155	6.844	3.327	4.507	2.849	1.155	5.051	4.742	5.429	4.118	MS,t_R,LRI
5	26.10	1347	ethyl lactate	40.47	44.73	59.10	53.89	58.48	76.09	84.88	87.91	107.0	68.06	MS,t_R,LRI
6	32.82	1404	ethyl octanoate	5.544	20.38	4.221	25.55	2.016	1.401	27.65	7.135	29.08	13.67	MS,t_R,LRI
7	34.98	1469	acetic acid	109.4	157.7	198.6	132.1	61.06	174.7	70.22	152.1	156.3	134.7	MS,t_R,LRI
8	36.96	1506	benzaldehyde	0.595	0.770	1.240	0.644	0.632	0.479	1.110	1.038	1.112	0.847	MS,t_R,LRI
9	38.55	1546	linalool	1.875	1.331	2.199	2.149	1.612	1.522	2.936	2.049	2.694	2.041	MS,t_R,LRI
10	41.32	1614	ethyl decanoate	27.15	84.30	16.75	77.49	1.604	3.265	108.8	13.41	178.8	56.84	MS,t_R,LRI
11	42.53	1675	diethyl succinate	1.285	0.920	2.574	2.675	2.605	2.011	1.195	2.449	2.589	2.034	MS,t_R,LRI
12	42.99	1692	α-terpineol	0.409	0.426	0.856	0.632	0.436	0.347	0.674	0.778	0.658	0.580	MS,t_R,LRI
13	44.87	1761	citronellol	0.183	0.110	0.083	0.205	0.024	0.136	0.160	0.090	0.089	0.120	MS,t_R,LRI
14	45.67	1794	nerol	0.020	ND[d]	0.043	0.111	0.057	0.064	0.180	0.032	0.043	0.069	MS,t_R,LRI
15	45.99	1806	2-phenyl ethyl acetate	0.191	0.125	0.289	0.258	0.256	0.606	0.318	0.220	0.191	0.273	MS,t_R,LRI
16	46.57	1830	ethyl dodecanoate	85.58	154.8	167.0	42.12	6.582	36.71	55.86	109.7	211.2	96.62	MS,t_R,LRI
17	46.78	1844	geraniol	0.302	1.286	1.388	0.289	0.414	0.268	0.409	0.397	0.435	0.576	MS,t_R,LRI
18	46.97	1857	hexanoic acid	2.421	2.712	3.935	4.217	1.968	6.613	2.289	3.129	4.258	3.505	MS,t_R,LRI
19	48.22	1917	2-phenyl ethanol	2.248	1.234	3.212	3.705	2.347	7.032	2.491	3.058	3.626	3.217	MS,t_R,LRI
20	50.92	2041	ethyl tetradecanoate	4.523	20.86	10.16	5.750	4.469	12.05	4.710	16.81	5.274	9.401	MS,t_R,LRI
21	51.41	2067	octanoic acid	3.919	3.194	6.551	6.477	3.582	4.225	3.735	6.213	7.898	5.088	MS,t_R,LRI
22	55.35	2253	decanoic acid	4.291	4.479	4.510	5.733	4.105	3.377	3.430	8.630	8.431	5.221	MS,t_R,LRI

[a] Results are expressed in mg/L; each data is the mean of two determinations. [b] LRI_{calc}, linear retention index calculated. [c] Id Identification: (t_R) identification by comparison retention time with authentic reference compounds recorded under the same conditions. (MS) identification by comparison with the mass spectrum stored in the NIST library. (LRI) identification by comparison with data from previous literature (*22–24*). [d] ND: not detected.

°C) were investigated. The amount of compounds desorbed from the fiber increased with temperature, so 250 °C was set as the optimum temperature. To evaluate the grade of desorption, a blank run was performed before each run. The results show that all the compounds were completely desorbed from the fiber at 250 °C.

Performance Evaluation of the SPME Method. The linearity of the method was evaluated using various solutions with different concentrations for all the compounds studied. These solutions were extracted using the optimized SPME method. The CW-DVB fiber exhibited a directly proportional relationship between the extracted amount and its initial concentration in the sample as can be seen in **Table 1**. The calibration lines were obtained using the internal standard (IS) method. Plotting relative ion peak area vs the concentration of the test compounds related to the IS concentration, the calibration line obtained presented correlation coefficients (r^2) in the range of 0.9900 to 0.9998.

The precision of the experimental procedure was also evaluated. A series of six consecutive SPME gave a relative standard deviation (RSD) ranging from 0.99 to 8.18% as can be seen in the results summarized in **Table 2**. The detection limits (signal-to-noise ratio = 3) calculated in mg/L are presented in **Table 2**. Recovery of the proposed method was studied using an orujo sample spiked with different quantities of the compounds under analysis. The recoveries, shown in **Table 2**, were satisfactory, ranging from 112.4 ± 76.0%.

Analysis of Real Samples. The applicability of the SPME technique was evaluated using nine monovarietal Albariño orujo samples from the Geographic Denomination "Orujo de Galicia/Augardente de Galicia". Distillation equipment most frequently used in Portugal, Spain, and Italy are: (1) alquitara and alembic; (2) a steam-distillation unit, and (3) a vacuum system distillation unit. Orujo samples analyzed in this work were produced with the "alembic" distillation technique. **Figure 3** shows representa-

tive total ion chromatograms for one of the samples analyzed. The results obtained from quantification are summarized in **Table 3**.

Alcohols constitute the group of compounds with the highest concentration in distilled beverages and are responsible for their flavoring aromas. The level of these compounds depends on the grape variety, fermentation conditions, and distillation technique. The predominant alcohol in orujo spirits was 3-methyl-1-butanol (isoamyl alcohol) with a mean value of 415.9 mg/L. This alcohol together with 2-methyl-1-butanol quantitatively constitutes the majority of higher alcohols, and they are considered predictors of sensory character in distilled products. The results were compared to those obtained by other authors. It was found that the content of 3-methyl-1-butanol in orujo spirits is lower than in Portuguese bagaceira with an average content of 915.5 mg/L but higher than that in Italian grappa, 243.5 mg/L (*32*). Other alcohols like 2-methyl-1-propanol and 1-propanol are present in orujo spirits at high mean concentrations (169.7 and 161.3 mg/L, respectively). Another alcohol studied was 2-phenyl ethanol, which is an aromatic alcohol that introduces a pleasant roselike aroma to distillates. The mean value found in orujo samples was 3.217 mg/L less than those found in bagaceira and grappa. The distillation technique plays an important role in the content of this alcohol.

Fatty acid esters are probably the group of compounds that contribute the most to the distillate's aroma and quality. Ethyl esters are produced during the fermentation. They gain access to the spirits and increase during aging. Grape variety is an important factor in determining the concentration of ethyl esters in these beverages (*33*). The results obtained for these esters in the analyzed samples showed values between 4.118 mg/L for ethyl hexanoate and 96.62 mg/L for ethyl dodecanoate. The results obtained are similar to those provided by other authors for bagaceira and grappa in the case of ethyl hexanoate and octanoate. For ethyl decanoate and ethyl dodecanoate, the concentrations obtained for orujo spirits (56.84 and 96.62 mg/L, respectively) are much higher than in bagaceira (10.7 and 2.3 mg/L) and grappa (39.6 and 16.2 mg/L).

The acetic acid esters like 2-phenyl ethyl acetate, isoamyl acetate, and hexyl acetate are responsible for the flowery and fruity aroma of distillates, while ethyl acetate, ethyl lactate, and diethyl succinate derive mainly from bacterial spoilage of the distillate marc. The concentration obtained for 2-phenyl ethyl acetate, ethyl lactate, and diethyl succinate was 0.273, 68.06, and 2.034 mg/L, respectively. The value of the two last esters is lower than the content found in bagaceira and grappa. This means that the maintenance of the pomace was satisfactory, and no undesirable lactic and acetic fermentations took place.

Long chain fatty acids such us hexanoic, octanoic, and decanoic are a group of compounds that effect flavor in the distillates to a lesser degree. The concentration obtained for these acids ranged between 3.505 mg/L for hexanoic acid and 5.088 and 5.221 mg/L for octanoic and decanoic acids, respectively. Other volatile carboxylic acids, such as acetic, showed a mean concentration of 134.7 mg/L.

Benzaldehyde was also analyzed because its formation is associated with microbial development during the ensilage of the grape pomace. The mean concentration obtained for the samples measured was 0.847 mg/L.

Terpenes are another very important chemical group, which has an effect on flavor and aroma. These compounds are present in the grapes and can be used for varietal differentiation of wines and other related products. Five terpenic alcohols were identified in orujo samples: linalool, α-terpineol, citronellol, nerol, and geraniol. The mean concentration ranged between 2.041 and 0.069 mg/L for linalool and nerol, respectively. There was one sample in which nerol was not detected; thus, it is obvious that the spirits produced from Albariño grape pomace are characterized for their aromatic condition.

The results obtained indicated that the proposed method is a powerful alternative for the quantitative analysis of volatile compounds in orujo spirits. However, further studies are necessary to evaluate if the volatile profile might be an important tool for the characterization and control of the geographical origin (grape variety) of these distillates, as well as the production technique employed in their production (alembic or still). Volatile profiles could be processed using multivariate chemometric classification procedures (such as linear discriminant analysis, K nearest neighbors, soft independent modeling of class analogy, and artificial neural networks) with the aim of classifying orujo samples by either the distillation technique used to make them or by the zone in which they were produced.

LITERATURE CITED

(1) Council Regulation (EEC) No. 1576/89, Official Journal of the European Communities L. 1989, *160*, 1–17.

(2) Orde de 5 de mayo de 1989. Diario Oficial de Galicia. 1989, *97*, 22/05/1989.

(3) Silva, M. L.; Malcata, F. X.; De Revel, G. Volatile contents of grape marcs in Portugal. *J. Food Compos. Anal.* **1996**, *9*, 72–80.

(4) Silva, M. L.; Malcata, F. X. Relationships between storage conditions of grape pomace and volatile composition of spirits obtained therefrom. *Am. J. Enol. Vitic.* **1998**, *49*, 56–64.

(5) Flamini, R. Some advances in the knowledge of grape, wine and distillates chemistry as achieved by mass spectrometry. *J. Mass Spectrom.* **2005**, *40*, 705–713.

(6) Cortés Diéguez, S.; Gil de la Peña, M. L.; Fernández Gómez, E. Approaches to spirit aroma: Contribution of some aromatic compounds to the primary aroma in samples of orujo spirits. *J. Agric. Food Chem.* **2003**, *51*, 7385–7390.

(7) Versaria, A.; Natali, N.; Russo, M. T.; Antonelli, A. Analysis of Some Italian Lemon Liquors (Limoncello). *J. Agric. Food Chem.* **2003**, *51*, 4978–4983.

(8) Guichard, H.; Lemesle, S.; Ledauphin, J.; Barillier, D.; Picoche, B. Chemical and Sensorial Aroma Characterization of Freshly Distilled Calvados. 1. Evaluation of Quality and Defects on the Basis of Key Odorants by Olfactometry and Sensory Analysis. *J. Agric. Food Chem.* **2003**, *51*, 424–432.

(9) Lablanquie, O.; Snakkers, G.; Cantagrel, R.; Ferrari, G. Characterisation of young cognac spirit aromatic quality. *Anal. Chim. Acta* **2002**, *458*, 191–196.

(10) 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*, 625–630.

(11) García-Jares, C. M.; García-Martín, M. S.; Cela, R. Analysis of Some Highly Volatile Compounds of Wine by Means of Purge and Cold Trapping Injector Capillary Gas Chromatography. Application to the Differentiation of Rias Baixas Spanish White Wines. *J. Agric. Food Chem.* **1995**, *43*, 764–768.

(12) Pawliszyn, J. B. *Solid-Phase Microextraction. Theory and Practice*; Wiley-VCH: New York, 1997.

(13) Arthur, L.; Killam, L.; Buchholz, K.; Pawliszyn, J. Automation and optimization of solid-phase microextraction. *Anal. Chem.* **1992**, *64*, 1960–1966.

(14) Peña, R. M.; Barciela, J.; Herrero, C.; García-Martín, S. Optimization of solid-phase microextraction methods for GC-MS determination of terpenes in wine. *J. Sci. Food Agric.* **2005**, *85*, 1227–1234.

(15) Peña-Alvarez, A.; Capella, S.; Juárez, R.; Labastida, C. Determination of terpenes in tequila by solid phase microextraction-gas chromatography-mass spectrometry. *J. Chromatogr. A* **2006**, *1134*, 291–297.

(16) Câmara, J. S.; Marquez, J. C.; Perestrelo, R. M.; Rodrigues, F.; Oliveira, L.; Andrade, P.; Caldeira, M. Comparative study of the whisky aroma profile based on headspace solid phase microextraction using different fiber coatings. *J. Chromatogr. A* **2007**, *1150*, 198–207.

(17) Pino, J. A. Characterization of rum using solid-phase microextraction with gas chromatography-mass spectrometry. *Food Chem.* **2007**, *104*, 421–428.

(18) Jurado, J. M.; Ballesteros, O.; Alcázar, A.; Pablos, F.; Martín, M. J.; Vílchez, J. L.; Navalón, A. Characterization of aniseed-flavored spirit drinks by headspace solid-phase microextraction gas chromatography-mass spectrometry and chemometrics. *Talanta* **2007**, *72*, 506–511.

(19) Vichi, S.; Riu-Aumatell, M.; Mora-Pons, M.; Buxaderas, S.; López-Tamames, E. Characterization of Volatiles in Different Dry Gins. *J. Agric. Food Chem.* **2005**, *53*, 10154–10160.

(20) Dahl, S.; Tavaria, F. K.; Malcata, F. X. Relationships between flavour and microbiological profiles in Serra da Estrela cheese throughout ripening. *Int. Dairy J.* **2000**, *10*, 255–262.

(21) Tavaria, F. K.; Ferreira, A. C.; Silva, M. L.; Malcata, F. X. Volatile free fatty acids as ripening indicators for serra da estrela cheese. *J. Dairy Sci.* **2004**, *87*, 4064–4072.

(22) Flavor database. University of Florida, Citrus Research and Education Centre. http://www.crec.ifas.ufl.edu/rouseff/.

(23) Acree, T.; Arn, H. *Flavornet. Gas chromatography-olfatometry (GCO) of natural products.* http://www.flavornet.org.

(24) *LRI & Odour database on the web*. http://www.odour.org.uk.

(25) Urruty, L.; Montury, M. Influence of Ethanol on Pesticide Extraction in Aqueous Solutions by Solid-Phase Microextraction. *J. Agric. Food Chem.* **1996**, *44*, 3871–3877.

(26) Fischer, C.; Fischer, U. Analysis of Cork Taint in Wine and Cork Material at Olfactory Subthreshold Levels by Solid Phase Microextraction. *J. Agric. Food Chem.* **1997**, *45*, 1995–1997.

(27) Mestres, M.; Busto, O.; Guasch, J. Headspace solid-phase microextraction analysis of volatile sulphides and disulfides in wine aroma. *J. Chromatogr. A* **1998**, *808*, 211–218.

(28) Mestres, M.; Sala, C.; Martí, M. P.; Busto, O.; Guasch, J. Headspace solid-phase microextraction of sulphides and disulphides using Carboxen-polydimethylsiloxane fibers in the analysis of wine aroma. *J. Chromatogr. A* **1999**, *835*, 137–144.

(29) De la Calle García, D.; Magnaghi, S.; Reinchenbaecher, M.; Danzar, K. Systematic optimization of the analysis of wine bouquet components by solid-phase microextraction. *J. High Res. Chromatogr.* **1996**, *19*, 257–262.

(30) Pino, J.; Marti, M. P.; Mestres, M.; Pérez, J.; Busto, O.; Guasch, J. Headspace solid-phase microextraction of higher fatty acid ethyl esters in white rum aroma. *J. Chromatogr. A* **2002**, *954*, 51–57.

(31) Rocha, S.; Ramalheira, V.; Barros, A.; Delgadillo, I.; Coimbra, M. Headspace solid phase microextraction (SPME) analysis of flavor compounds in wines. Effects of the matrix volatile composition in the relative response factors in a wine model. *J. Agric. Food Chem.* **2001**, *49*, 5142–5151.

(32) Silva, M. L.; Macedo, A. C.; Malcata, F. X. Review: Steam distilled spirits from fermented grape. *Food Sci. Technol. Int.* **2000**, *6*, 285–300.

(33) Milicevic, B.; Banovic, M.; Kovacevic-Ganic, K.; Gracin, L. Impact of grape varieties on wine distillates flavor. *Food Technol. Biotechnol.* **2002**, *40*, 227–232.

Received for review November 29, 2007. Revised manuscript received February 6, 2008. Accepted February 9, 2008.

JF073481F