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Headspace Solid Phase Microextraction (SPME) Analysis of Flavor Compounds in Wines. Effect of the Matrix Volatile Composition in the Relative Response Factors in a Wine Model

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The application of headspace solid phase microextraction (SPME) for flavor analysis has been studied. Headspace SPME sampling was tested for nine common wine flavor compounds in 10% (v/v) aqueous ethanol: linalool, nerol, geraniol, 3-methyl-1-butanol, hexanol, 2-phenylethanol, ethyl hexanoate, ethyl octanoate, and ethyl decanoate. The chemical groups (monoterpenoids, aliphatic and aromatic alcohols, and esters) showed specific behavior in SPME analysis. SPME sampling parameters were optimized for these components. Relative response factors (RRFs), which establish the relationship between the concentration of the compound in the matrix liquid solution and the GC peak area, were estimated for all compounds. Log_{10}(RRF) varied from 0 (3-methyl-1-butanol) to 3 (ethyl decanoate), according to their molecular weight. Quantification by SPME was shown to be highly dependent on the matrix composition; the compounds with higher RRF were the less affected. As a consequence, the data obtained with this methodology should be used taking into consideration these limitations, as shown in the analysis of four monovarietal Bairrada white wines (Arinto, Bical, Cerceal, and Maria Gomes).

Keywords: *Solid phase microextraction (SPME); headspace; matrix composition; relative response factors; wine*

INTRODUCTION

Solid phase microextraction (SPME) is a sample preparation technique based on sorption (absorption and/or adsorption, depending on the fiber coating), which is useful for extraction and concentration analyses either by submersion in a liquid phase or by exposure to a gaseous phase (*1*). Following exposure of the fiber to the sample, sorbed analytes can be thermally desorbed in a conventional gas chromatography (GC) injection port. SPME has been commercially available since 1993 and now is available with various sorbent materials and various coating thicknesses.

The first application of SPME was to the evaluation of pollutants in water (*2*). Since then, SPME has been used in a range of fields including studies of flavors and taints, especially for quick screening of the volatile composition of a wide range of products. It has been applied to fruits (*3*−*6*), vegetable oils (*3*, *7*), coffee (*3*, *8*), wine (*9*−*16*), cork (*12*, *17*), beer (*18*), meat (*19*), milk (*20*), and biological fluids (*21*−*24*).

SPME provides many advantages over conventional sample preparation techniques. The SPME method is simple to use, takes less than 1 h to complete, is less expensive, does not require solvent extraction and allows characterization of the headspace in contact with the sample (*1*, *25*, *26*).

Conventional methods such as steam distillation or direct solvent extraction produce extracts with a flavor composition that is representative of the liquid matrix and not of the headspace. The molecules present in the headspace are indeed responsible for the smell that is perceived by the olfactory system if they are in concentrations above their sensory detection thresholds. Another drawback of the conventional methods is that the extracts have to be concentrated prior to analysis, resulting in losses of low-boiling volatiles. Also, the solvent required by successive dilutions will mask the first eluting peaks.

The principle of headspace SPME for liquid matrices is the partition process of the flavor compounds between the two phases (*26*). The amount of flavor compounds sorbed on the SPME coating can be determined from the equation $n = C_0V_1V_2K_1K_2/(K_1K_2V_1 + K_2V_3 + V_2)$, where n is the mass of the flavor compound sorbed by the SPME coating; C_0 is the initial concentration of the flavor compound in the liquid matrix; and V_1, V_2, and V_3 are the volumes of the SPME coating, liquid matrix, and headspace, respectively; K_1 is the partition coefficient of the flavor compound between the SPME coating and the headspace; and K_2 is the partition coefficient between the headspace and the liquid matrix. Because the partition coefficient (K) is equal to K_1K_2, it is controlled by both the partition coefficient K_1, between the SPME coating and the headspace gas phase, and the partition coefficient K_2, between the headspace gas phase and liquid matrix (*26*).

The amount of an analyte sorbed on the SPME fiber and the resulting sensitivity are determined both by sorption kinetics and by the distribution coefficient of the compound between the fiber surface and the sample. Unlike conventional solid phase extraction and purge-and-trap sampling techniques, in which a practically quantitative recovery is often achieved, SPME is more sensitive to experimental conditions. Any change of experimental parameters that affects the distribution

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coefficient and the sorption rate will also influence the amount sorbed on the SPME fiber and the corresponding reproducibility and sensitivity (*3*). The partition equilibrium of the flavor compounds between the headspace of the sample and the SPME coating depends on the exposure time, temperature, sample volume and concentration, and type and uniformity of the matrix (*3*, *6*, *9*, *11*, *12*). SPME sampling is a single-batch process, so that quantitative sorption is often very difficult to achieve. Application of this technique to the flavor analysis of food and beverage, namely, wine, still requires knowledge of the fiber affinity for the specific aroma compounds as well as selective studies to improve the reproducibility and sensitivity of the method. Many studies of the wine aroma compounds aim for the characterization/distinction between the different varieties and the following of a specific step of wine-making when variation of the matrix composition occur. However, the extent of this variation in the amount estimated by the SPME technique is usually not considered.

The purpose of this work was to study the behavior of the SPME fiber (polyacrylate) regarding the different chemical classes of the wine aroma compounds (monoterpenoids, aliphatic and aromatic alcohols, and esters). To achieve this objective, the extent of the changes in the concentration of one matrix component in the headspace equilibrium and, consequently, in the SPME sorption of the different matrix components, was analyzed. The SPME sampling conditions of stirring, NaCl addition, temperature, exposure time, and sample volume for the quantitative analysis of nine standard volatile compounds were studied using a wine model. Experiments on four different monovarietal wines were carried out under the optimized conditions of SPME sampling.

MATERIALS AND METHODS

Materials. All standard chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). An SPME fiber coated with 85 μm polyacrylate (PA), which is recommended for polar organic compounds, was purchased from Supelco Inc. (Bellefonte, PA).

PA is an absorbent or liquid-phase coated fiber. The absorptive fibers are indicated to extract semivolatile compounds from the headspace (*27*). Absorptive fibers have greater capacity and linear concentration ranges than adsorptive, because they utilize partitioning for the extraction (*28*, *29*). The SPME fiber was conditioned at 300 °C for 2 h in the GC injector, according to the manufacturer's recommendations. All fibers used were from the same lot.

Solutions with mixtures containing nine standards (Sigma-Aldrich, St. Louis, MO), 3-methyl-1-butanol, ethyl hexanoate, hexanol, ethyl octanoate, linalool, ethyl decanoate, nerol, geraniol, and 2-phenylethanol, were prepared in ethanol. The standards were chosen to represent compounds known to be present in Portuguese white wines and their possible concentrations in the wines from Demarcated Bairrada Region, in Portugal.

The four white monovaritetal wines used, Arinto, Bical, Cerceal, and Maria Gomes, 1999 harvest, 10% ethanol, were provided by Estação Vitivinícola da Bairrada, Portugal.

General Conditions for the Headspace Volatile Compound Extraction by SPME. A 20 μL aliquot of solution containing the standards was transferred into a 120 mL bottle containing 40 mL of 10% (v/v) aqueous ethanol with 8 g of NaCl and a 6 mm stirring bar (1000 rpm). The pH was adjusted to 3.2 with acetic acid. A Teflon septum and an aluminum cap sealed the bottles. The SPME fiber was manually inserted into the headspace of the sample bottle. The sample was placed inside the flask always for a period of 60 min, in which the SPME fiber was kept for 45 min.

Optimization of SPME Parameters. The amounts of each standard in the model solution and the experimental conditions used are shown in Figures 1−5. The model solutions were used to optimize the following parameters.

Stirring Effect. SPME was carried out with (1000 rpm) and without stirring. The concentrations used were as follows: 3-methyl-1-butanol, 10.1 mg/L; ethyl hexanoate, 1.8 mg/L; hexanol, 5.1 mg/L; ethyl octanoate, 0.60 mg/L; linalool, 0.60 mg/L; ethyl decanoate, 0.10 mg/L; nerol, 0.70 mg/L; geraniol, 0.90 mg/L; and 2-phenylethanol, 13 mg/L. The analyses were carried out under the following conditions: 40 mL of model solution, addition of 8 g of NaCl, at 25 °C. The sample was placed inside the flask always for a period of 60 min, in which the SPME fiber was kept for 45 min.

NaCl Addition Effect. To determine the effect of salt addition, 4, 8, 12, or 16 g of NaCl was added to the model solutions. SPME was also studied without salt addition. The concentrations used were as follows: 3-methyl-1-butanol, 6.1 mg/L; ethyl hexanoate, 0.30 mg/L; hexanol, 3.0 mg/L; ethyl octanoate, 0.30 mg/L; linalool, 0.30 mg/L; ethyl decanoate, 0.32 mg/L; nerol, 0.30 mg/L; geraniol, 0.30 mg/L; and 2-phenylethanol, 7.7 mg/L. The analyses were carried out under the following conditions: 40 mL of model solution, at 40 °C, with stirring (1000 rpm). The sample was placed inside the flask always for a period of 60 min, in which the SPME fiber was kept for 45 min.

Temperature Effect. Model solutions were submitted to three temperatures (25, 30, or 40 °C). The concentrations used were as follows: 3-methyl-1-butanol, 4.0 mg/L; ethyl hexanoate, 0.20 mg/L; hexanol, 2.0 mg/L; ethyl octanoate, 0.20 mg/L; linalool, 0.20 mg/L; ethyl decanoate, 0.040 mg/L; nerol, 0.22 mg/L; geraniol, 0.22 mg/L; and 2-phenylethanol, 5.1 mg/L. The analyses were carried out under the following conditions: 40 mL of model solution, addition of 8 g of NaCl, with stirring (1000 rpm). The sample was placed inside the flask always for a period of 60 min, in which the SPME fiber was kept for 45 min.

Exposure Time Effect. The sample was placed inside the flask for a period of 60 min, in which the SPME fiber was kept for 60, 45, 30, or 15 min. The concentrations used were as follows: 3-methyl-1-butanol, 10.1 mg/L; ethyl hexanoate, 1.8 mg/L; hexanol, 5.1 mg/L; ethyl octanoate, 0.60 mg/L; linalool, 0.60 mg/L; ethyl decanoate, 0.10 mg/L; nerol, 0.70 mg/L; geraniol, 0.90 mg/L; and 2-phenylethanol, 13 mg/L. The analyses were carried out under the following conditions: 40 mL of model solution, addition of 8 g of NaCl, at 25 °C, with stirring (1000 rpm).

Sample Volume. Model solutions (20, 30, or 40 mL) were held in 120 mL sealed glass bottles. The concentrations used were as follows: 3-methyl-1-butanol, 6.1 mg/L; ethyl hexanoate, 1.1 mg/L; hexanol, 3.0 mg/L; ethyl octanoate, 0.30 mg/L; linalool, 0.30 mg/L; ethyl decanoate, 0.30 mg/L; nerol, 0.30 mg/L; geraniol, 0.30 mg/L; and 2-phenylethanol, 7.7 mg/L. The analyses were carried out under the following conditions: addition of 8 g of NaCl, at 40 °C, with stirring (1000 rpm). The sample was placed inside the flask always for a period of 60 min, in which the SPME fiber was kept for 45 min.

Estimation of the Relative Response Factor (RRF) for Each Chemical Standard in the Model Matrix. A base solution containing all nine standard volatile compounds was used (Table 1). The concentration of each of the nine compounds was varied two or three times (shown by the number of columns in Table 3) using an experimental matrix of 23 standard solutions. The range of concentrations used was as follows: 3-methyl-1-butanol, 10.1−131 mg/L; ethyl hexanoate, 1.00−3.19 mg/L; hexanol, 2.04−14.2 mg/L; ethyl octanoate, 0.22−1.16 mg/L; linalool, 0.30−4.22 mg/L; ethyl decanoate, 0.11−0.50 mg/L; nerol, 0.20−1.51 mg/L; geraniol; 0.20−2.42 mg/L; and 2-phenylethanol, 5.01−67.4 mg/L. The analyses were carried out under the following conditions: stirring (1000 rpm), 30 mL of model solution, addition of 8 g of NaCl, at 25 °C. The sample was placed inside the flask always for a period of 60 min, in which the SPME fiber was kept for 45 min. A

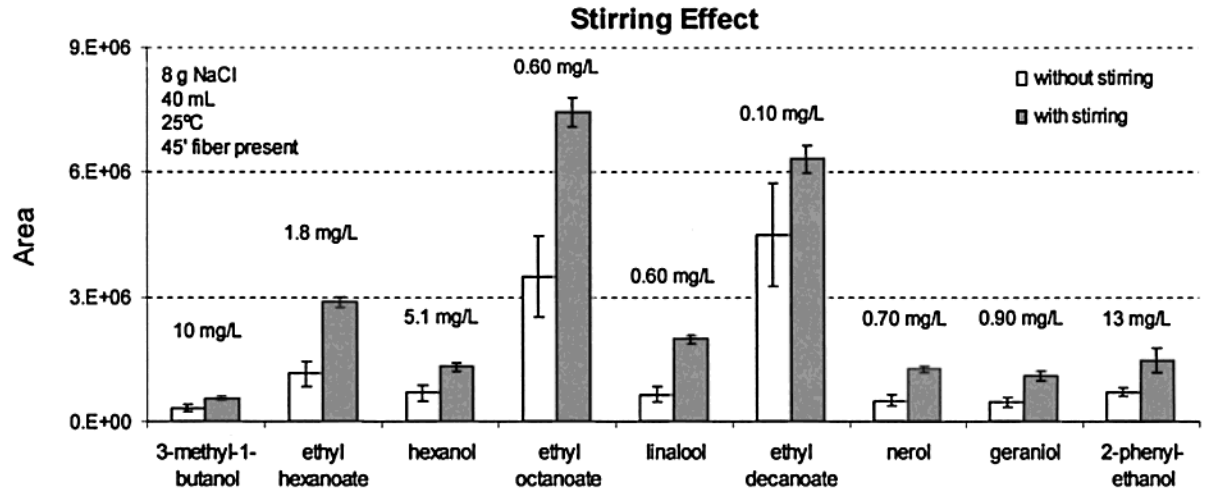

Figure 1. Stirring effect on SPME headspace sampling (the values in the figure are the amounts of each standard added to the model solution).

calibration line with four or five points, including the blank solution, was obtained for each compound by plotting the GC peak area versus the different concentrations of each standard compound.

Estimation of the Concentration of the Standard Compounds in White Wines. According to the GC area of the peaks of the nine compounds present in the four wines used, calibration curves were established: 3-methyl-1-butanol, 72.8−376 mg/L; ethyl hexanoate, 0.17−0.94 mg/L; hexanol, 0.41−2.85 mg/L; ethyl octanoate, 0.40−1.45 mg/L; linalool, 0.017−0.10 mg/L; ethyl decanoate, 0.086−0.62 mg/L; nerol, 0.009−0.053 mg/L; geraniol, 0.15−0.43 mg/L; and 2-phenylethanol, 3.58−34.8 mg/L. The analyses of the standard solutions and wines were carried out under the conditions described for the estimation of the RRF for each chemical standard in the model matrix. The calibration curves were used for the estimation of the concentration of the standard compounds in the wines.

Headspace Volatile Compound Analysis by GC. The SPME coating containing the headspace flavor compounds was introduced into the GC injection port at 250 °C and kept for 5 min for the desorption. The injection port was lined with a 0.75 mm i.d. splitless glass liner. The desorbed flavor compounds were separated in a Hewlett-Packard 5890, equipped with a 30 m × 0.32 mm (i.d.) DB-FFAP fused silica capillary column and a flame ionization detector (250 °C). The oven temperature was programmed from 35 to 220 °C at 2 °C/min. Carrier gas was hydrogen at a 35 cm/s linear velocity. The injection purge on the GC was off for the initial 5 min. All measurements were made with, at least, five replicates, and the reproducibility is expressed as the coefficient of variation (CV) or as error bars in the figures. Blanks were run between each triplicate set.

Principal Component Analysis (PCA). A PCA was applied to the normalized areas of the seven standard compounds identified by SPME-GC-MS (Arinto, Bical, Cerceal, and Maria Gomes varieties, with three to four extraction replicates, giving a total of 14 measures). As an exploratory technique, PCA allows one to study the main sources of variability present in the data sets, to detect clustering formation, and to establish relationships between samples (objects) and compounds (variables) (*30*). The data were normalized by sample and autoscaled by variable in order to give the same weight to all components.

RESULTS AND DISCUSSION

Optimization of SPME Parameters. *Stirring Effect.* Figure 1 shows the effect of stirring on the amount of compounds retained by the fiber. Stirring raises the overall detector signal by a factor of 1.4 (ethyl decanoate) to 3.1 (linalool), facilitating the release of volatile compounds by increasing the surface of the liquid−vapor interface. High increases were also observed for nerol, geraniol, and ethyl hexanoate (2.5). The sample should be stirred to increase the amount of the standards absorbed.

NaCl Addition Effect. The behavior of selected flavor compounds in the presence of various salt concentrations in SPME sorption was described by Yang and Peppard (*3*). Generally, the presence of electrolyte in a sorption system can influence the sorption in two ways: changing the properties of the phase boundary and decreasing the solubility of hydrophobic compounds in the aqueous phase. This effect of "salting out" is widely used to increase the sensitivity of analytical methods (*3*). As shown in Figure 2, for all analyzed standard compounds, peak areas increased with increasing salt concentration until 8 g of NaCl, by a factor of 1.4 (ethyl decanoate) to 6.8 (linalool). The increase observed for linalool, as well as the increase observed for nerol (3.8) and geraniol (3.1), indicates that salt addition may be helpful in running samples in which monoterpenes occur in trace quantities. The monoterpenoids are responsible for the typical character and quality of the wine aroma and exist in trace amounts in a few neutral grape varieties (*31*−*33*) such as the white grape varieties from the specific Demarcated Portuguese Bairrada Region (*34*, *35*). Higher concentrations of NaCl (12 or 16 g) affect in different ways the compounds: the sensitivity for the esters decreases, and the sensitivity for the alcohols increases; a slight increase is also observed for nerol and geraniol. Furthermore, the reproducibility of the analysis with 12 g of NaCl is lower than all of the others possibly because, at this concentration, the NaCl in the system water−ethanol begins to reach the saturation point. The addition of 8 g of NaCl should be used as a compromise for the small CV and the highest amount of volatile compounds absorbed.

Temperature Effect. The effect of temperature of the sample on the analysis of headspace flavor compounds with SPME-GC is shown in Figure 3. The increase in the temperature increased slightly the absorption of geraniol and nerol; however, no significant changes in absorption were observed for linalool and other alcohols. Tthe esters showed a statistically significant decrease. The absorption is an exothermic process: more mol-

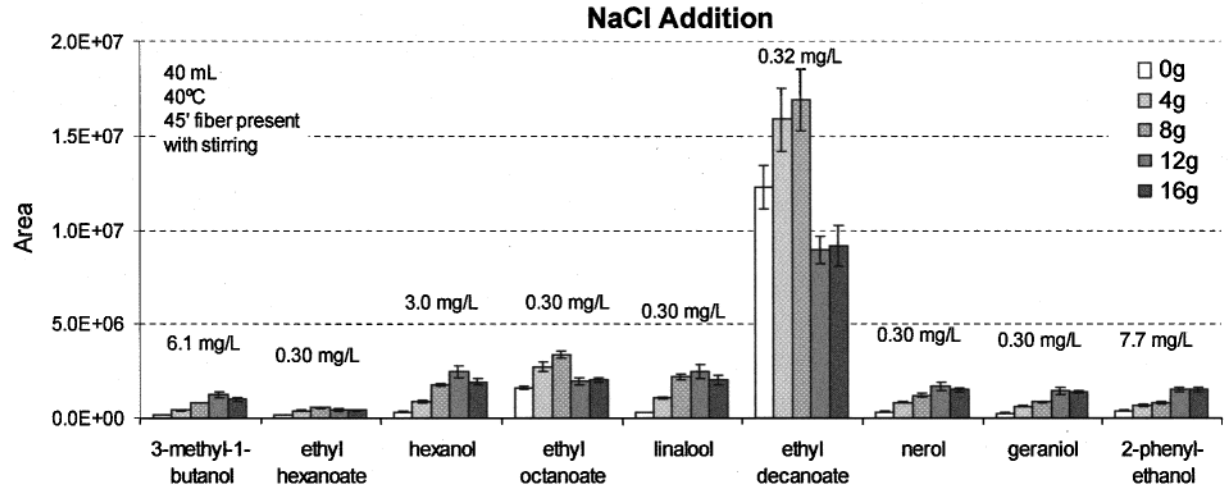

Figure 2. NaCl addition effect on SPME headspace sampling (the values referenced in the figure are the amounts of each standard added to the model solution).

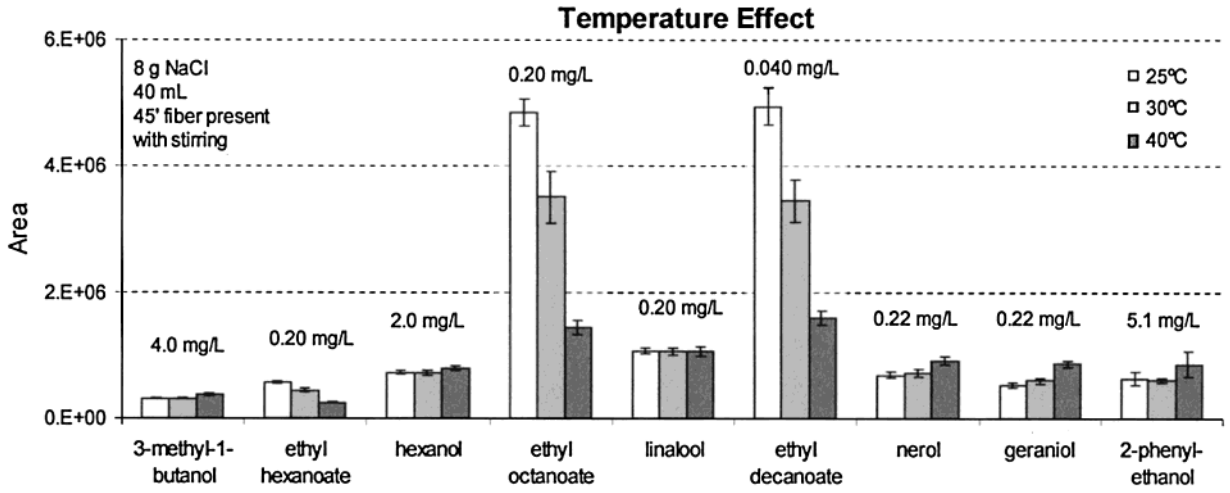

Figure 3. Temperature effect on SPME headspace sampling (the values referenced in the figure are the amounts of each standard added to the model solution).

ecules absorbed on the stationary phase of the SPME fiber diffused to gas phase with increasing temperature, which inversely decreased the partition coefficient K_1. The temperature effect on the amount of volatiles absorbed seems also to be the result of a compromise between the solubility and volatility of the compounds (K_2). The compounds insoluble in water, such as the esters (data in Figure 7), showed a decrease in the amount absorbed with the increase in temperature, suggesting an increase of their solubility in the matrix. However, the compounds relatively soluble in water, such as the terpenoids, and the compounds soluble in water, such as the alcohols used, showed a slight increase in the amount absorbed with the increase in temperature. For these compounds, the effect of the increase of temperature was more effective on the increase of their volatility than on the increase in their solubility. The preheating step was effective for volatiles with lower boiling points, such as 3-methyl-1-butanol and 1-hexanol but not effective for the esters (*23*).

For the conditions assayed, a temperature of 25 °C should be used.

Exposure Time Effect. To have good reproducibility for the quantitative analysis of headspace volatile compounds, the partition coefficient should reach an equilibrium state. Figure 4 shows that the amount of compounds absorbed to the fiber reached a maximum at 45 min. Longer exposure time does not increase the total amount of volatile compounds absorbed but changes the relative amount of the esters: the absorption of ethyl decanoate increased, replacing the short-chain esters. The effect of exposure time was less effective for the more volatile compounds (3-methyl-1-butanol, ethyl hexanoate, and hexanol). These data suggest that less volatile compounds need a longer exposure time to achieve the equilibrium, as previously observed by Matich et al. (*36*) and Ruiz et al. (*19*). Zhang and Pawliszyn (*37*) reported that the sensitivity of the SPME coating to less volatile compounds was high, but low partition coefficients for these compounds between the sample and the headspace resulted in long equilibration times, which explains the esters' behavior. The time of 15 min equilibrium promotion between the matrix and the headspace plus the 45 min of time absorption were considered the more adequate.

Sample Volume Effect. The amount of volatile compounds absorbed on the SPME fiber may be dependent on the sample volume (*3*). Figure 5 shows that, when the sample volume changes from 20 to 30 mL and to 40 mL, the extent of SPME absorption was similar for the terpenoids and other alcohols. A significant increase was observed only for esters when the volume was increased from 20 to 30 mL. The sample volume of 30 mL should be used.

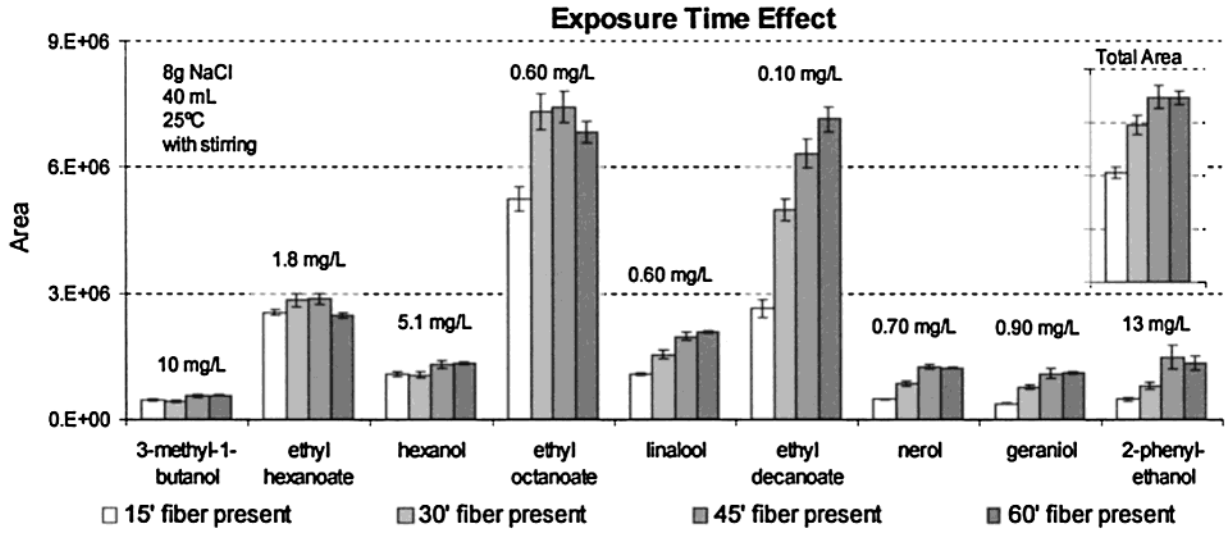

Figure 4. Exposure time effect on SPME headspace sampling (the values referenced in the figure are the amounts of each standard added to the model solution).

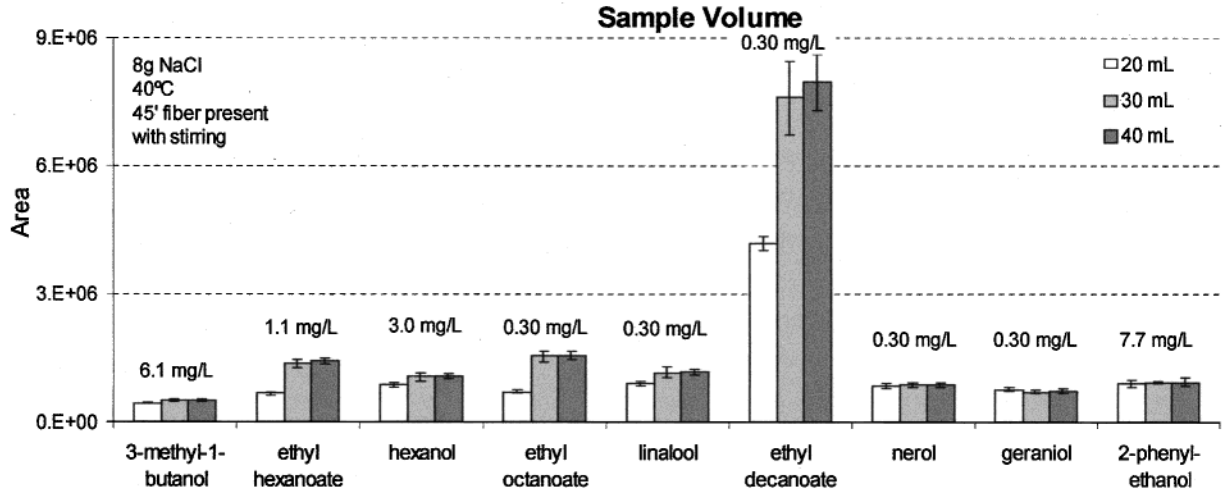

Figure 5. Sample volume effect on SPME headspace sampling (the values referenced in figure are the amounts of each standard added to the model solution).

Table 1. Concentration of the Standards under Study, Statistical Parameters, and Relative Response Factors (RRFs) Resultant of 23 Independent Assays with, at Least, 5 Replicates Each

peak no.	standard	concn of base solution (mg/L)	concn range (mg/L)	R^2	RRF	CV (%) mean	CV (%) range
1	3-methyl-1-butanol	10.1	10.1−131	0.9841	1	4.5	1.5−7.3
2	ethyl hexanoate	1.00	1.00−3.19	0.9931	45	5.5	2.7−10
3	hexanol	2.04	2.04−14.2	0.9984	5	4.4	1.6−7.6
4	ethyl octanoate	0.50	0.22−1.16	0.9999	391	6.8	2.7−12
5	linalool	0.30	0.30−4.22	0.9954	87	4.9	1.6−8.4
6	ethyl decanoate	0.50	0.11−0.50	0.9945	1473	7.0	4.0−11
7	nerol	0.20	0.20−1.51	0.9900	47	5.9	1.8−9.7
8	geraniol	0.20	0.20−2.42	0.9912	56	7.2	3.9−11
9	2-phenylethanol	5.01	5.01−67.4	0.9936	2	16	1.7−40

Reproducibility of Volatile Compound Analysis by SPME-GC. The CVs of the GC peak areas of all nine standard volatile compounds in 23 model solutions are shown in Table 1. The means of the CVs for each compound ranged from 4.4 for hexanol and 4.5 for 3-methyl-1-butanol to 16 for 2-phenylethanol. This variation may be associated with two factors: volatility of the compounds and their concentration in the matrix solution. Figure 6 shows that a global tendency was observed for the variation of the mean CV with the variation of the boiling point and molecular weight of the compounds, with the exception of 2-phenylethanol. This observation may be due to the required higher time for establishing the liquid−gas equilibrium of the less volatile compounds. Furthermore, for higher concentrations of each compound, the CVs were lower than the mean CV. The results observed for 2-phenylethanol suggest that this may be associated with its aromatic structure, which is not present in any of the other compounds used.

Estimation of the RRF for Each Chemical Standard in the Model Matrix. Linear relationships between GC peak areas and the concentrations of the nine standard compounds were in the range of $R^2 =$ 0.9841−0.9999 (Table 1). These high correlations indicate that the ethanol/water solution was capable of

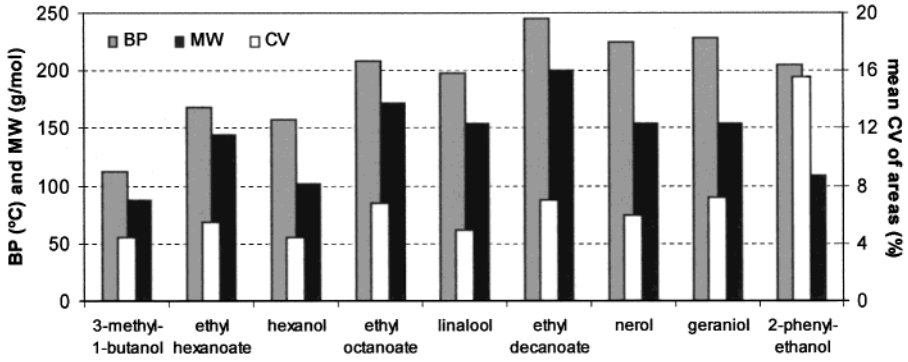

Figure 6. Comparison of the molecular weight (MW), boiling point (BP), and mean coefficient of variation (CV) of the GC peak areas of each standard compound.

Table 2. Effect of the Variation of Concentration of One Compound in the Areas of the Other Standard Compounds

compound	3-methyl-1-butanol (4.00)[a]	ethyl hexanoate (2.10)	hexanol (3.99)	ethyl octanoate (2.32)	linalool (4.27)	ethyl decanoate (0.44)	nerol (4.25)	geraniol (3.80)	2-phenylethanol (4.11)
3-methyl-1-butanol	3.75	0.75	0.77	0.77	0.87	0.99	0.82	0.83	0.93
ethyl hexanoate	0.95[b]	2.12	0.87	0.89	0.87	1.02	0.96	0.83	0.94
hexanol	0.99	0.77	3.37	0.80	0.87	1.01	0.86	0.84	0.93
ethyl octanoate	0.94	0.87	0.91	2.40	0.90	1.03	1.06	0.84	0.94
linalool	1.01	0.86	0.87	0.90	4.76	1.00	0.94	0.89	0.96
ethyl decanoate	0.92	0.90	0.92	0.99	0.92	0.46	1.03	0.83	0.93
nerol	0.97	0.88	0.87	0.89	0.94	1.07	4.62	0.94	0.93
geraniol	0.95	0.83	0.91	0.83	0.89	1.08	0.91	4.88	0.91
2-phenylethanol	0.88	0.62	0.69	0.64	0.84	0.91	0.67	0.77	3.34

[a] Factor of multiplication of the concentration of the standard compound in relation to its concentration in the base solution. [b] Ratio (A_x/A_0) of the GC peak areas of ethyl hexanoate when the concentration of 3-methyl-1-butanol was increased 4.00 times. A_0 is the area of ethyl hexanoate in the base solution, and A_x is the area of ethyl hexanoate in a solution that contains only one of the compounds (3-methyl-1-butanol) in higher concentration.

forming a stable matrix with standard compounds to produce a reproducible equilibrium between the SPME coating and the headspace of ethanolic solution. The RRFs of the standard compounds to the fiber shown in Table 1 were calculated by the ratio $RRF_i = (m_i/m_a)$, in which m_i is the slope of the GC peak area of compound i versus its concentration and m_a is the lower slope found (3-methyl-1-butanol). The RRF is considered to result from the characteristics of each compound, such as its molecular weight, boiling point, molecular structure, solubility in the liquid matrix, affinity to be absorbed to the fiber coating, and FID response (*29*). According to the RRF values, three groups can be defined (Figure 7): group A (3-methyl-1-butanol, 2-phenylethanol, and hexanol; RRF = 1, 2, and 5, respectively), group B (ethyl hexanoate, nerol, geraniol, and linalool; RRF = 45, 47, 56, and 87, respectively), and group C (ethyl octanoate and ethyl decanoate; RRF = 391 and 1473, respectively). Group A, $Log_{10}(RRF) = 0$, contains the shorter molecules (C_5, C_6, and C_7); group B, $Log_{10}(RRF) = 1$, contains C_8 and C_{10} molecules; and group C, $Log_{10}(RRF) = 2$ and 3, contains C_{10} and C_{12} molecules. This shows that the RRF may be related to the molecular weight of the volatiles [$Log_{10}(RRF) = 0.0284 \times MW - 2.501$], possibly because small molecules had the small contact surface with the fiber area. The RRF values seem to be determined by the equilibrium of the headspace and the fiber (K_1). This may explain the lower RRF values for the small and branched molecule of 3-methyl-1-butanol, the aromatic structure of 2-phenylethanol, and the short linear chain of hexanol. This was also verified for the molecules of groups B and C. Linalool showed an RRF higher than that of geraniol and nerol, which may be explained by its higher volatility, as was shown by its higher increase with the stirring effect (Figure 1) and early stages of the exposure time effect (Figure 4). The increase of RRF with the increase of the chain length of the esters suggests that the affinity to the fiber was ruled by hydrophobic interactions. As shown in Figure 4, an increase of the exposure time resulted in the displacement of ethyl hexanoate and ethyl octanoate by ethyl decanoate. Because this compound exhibits lower volatility and was less concentrated than the others, this showed its higher affinity to the fiber.

Effect of the Matrix Relative Volatile Composition in the RRFs. To clarify the extent of the changes in the concentration of one matrix component on the headspace equilibrium and, consequently, in the SPME absorption of the other liquid matrix components, variations in the concentration of each standard were analyzed. Table 2 shows the effect of the variation of concentration of one compound in the areas of the other standard compounds, for factors of multiplication of the concentration of the standard compounds between 0.44 and 4.25. Each value of Table 2 was the result of the following expression: A_x/A_0, in which A_0 is the area of compound i in the base solution and A_x is the area of the same compound i in a solution that contains only one of the other compounds in a different concentration. Table 2 shows that, in general, the increase of the concentration of one compound results in a decrease of the absorption of all the others and, as a consequence, their RRFs. To verify the statistical relevance of this effect, a Student's t test was applied to all 23 liquid matrices. Table 3 shows the effect of the variation of concentration of one compound in the areas of the other standard compounds, for factors of multiplication of the concentration of the standard compounds from 0.2 to 14. In general, the change in the concentration of one matrix component affects the GC peak areas of all the others. This effect becomes statistically significant when

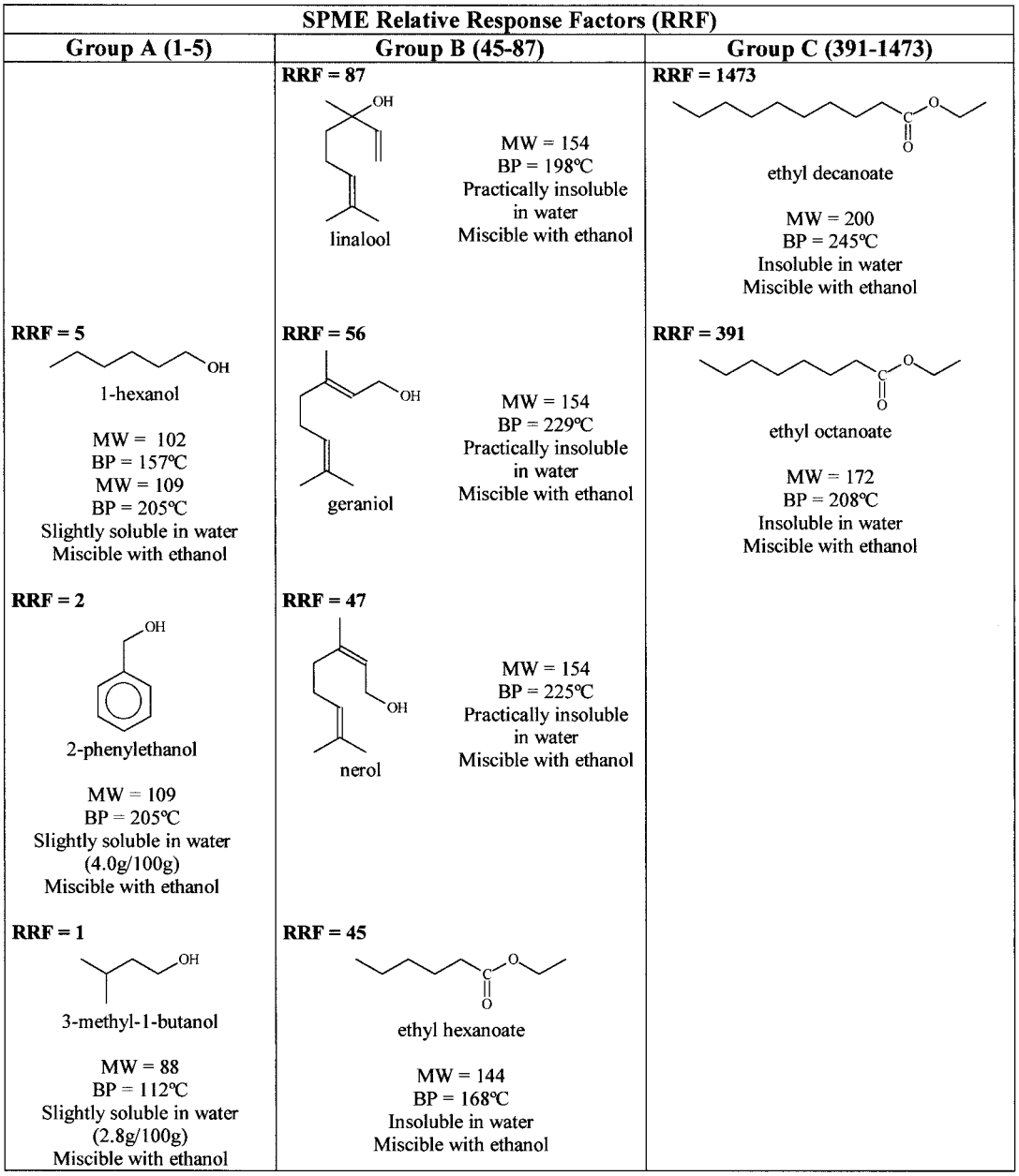

Figure 7. Characterization of the chemical standards used: SPME relative response factor (RRF), chemical structure, molecular weight (MW), boiling point (BP), and solubility in water and ethanol.

Table 3. Effect of the Different Concentrations of One Compound on the GC Peak Areas of the Other Standard Compounds

	3-methyl-1-butanol			ethyl hexanoate		hexanol		ethyl octanoate		linalool			ethyl decanoate		nerol		geraniol			2-phenyl-ethanol		
compound	4[a]	7	13	2	3	4	7	0.4	2	4	8	14	0.2	0.4	4	8	4	7	12	4	7	14
3-methyl-1-butanol				0.75[b]	0.78	0.78	0.77	0.83	0.77	0.87	0.85	0.75			0.82	0.79	0.83	0.81	0.79		0.88	0.74
ethyl hexanoate		0.93	0.87			0.90	0.86		0.89	0.87	0.80	0.80				0.91	0.83	0.81	0.85		0.85	0.81
hexanol		0.93	0.76	0.77	0.80			0.83	0.80	0.87	0.85	0.76			0.86	0.82	0.84	0.81	0.74		0.87	0.74
ethyl octanoate			0.90	0.87						0.90	0.84	0.87	1.16				0.84	0.82	0.93		0.85	0.83
linalool			0.93	0.86	0.89	0.89	0.87	0.88	0.90							0.89	0.89	0.89	0.89			0.79
ethyl decanoate												0.88					0.83	0.86				0.84
nerol		0.89	0.82	0.88		0.88	0.85				0.90	0.82							1.13			0.76
geraniol				0.83	0.83			0.79	0.83		0.87					0.88						0.83
2-phenylethanol		0.66	0.68	0.62		0.65		0.74	0.64		0.77	0.63			0.67	0.73	0.77	0.75	0.72			

[a] Factor of multiplication of the concentration of the standard compound in relation to its concentration in the base solution. [b] Ratio (A_x/A_0) of the GC peak areas of 3-methyl-1-butanol when the concentration of ethyl hexanoate was increased 2 times. A_0 is the area of 3-methyl-1-butanol in the base solution, and A_x is the area of 3-methyl-1-butanol in a solution that contains only one of the compounds (ethyl hexanoate) in higher concentration. This ratio is only shown for GC peak areas that were significantly different, at 99% level (Student's t test), to the peak area of the base solution.

Table 4. Concentrations of the Volatile Components in the Maria Gomes, Cerceal, Bical, and Arinto Wines

	Maria Gomes[a]	CV (%)	Cerceal[a]	CV (%)	Bical[a]	CV (%)	Arinto[a]	CV (%)	RRF[b]
3-methyl-1-butanol	319.18	3.3	172.71	2.2	277.28	1.6	298.07	2.7	1
ethyl hexanoate	0.32	6.9	0.54	2.0	0.62	0.7	0.36	2.4	36
hexanol	1.85	3.9	1.06	1.9	1.22	0.5	1.18	2.8	6
ethyl octanoate	0.59	5.3	1.03	2.5	1.26	2.8	0.70	5.1	236
linalool	0.08	2.9	0.04	2.3	0.03	4.3			118
ethyl decanoate	0.16	5.7	0.28	1.6	0.35	5.8	0.19	3.6	821
nerol									58
geraniol									51
2-phenylethanol	20.02	5.5	6.05	5.1	16.16	10.5	22.21	7.8	2

[a] Concentration in milligrams per liter. [b] Estimated from the standards used for wine quantification.

the variations in concentration increased [e.g., when the concentration of 3-methyl-1-butanol increased 4 times relative to the base matrix, no significant reduction of the GC peak areas was observed in all other compounds; when the concentration increased 7 times, a reduction of the GC peak areas of four of the eight compounds was observed; when the concentration of 3-methyl-1-butanol increased 13 times relative to the base matrix, a reduction of the GC peak areas of six of the eight compounds was observed (Table 3)]. Also, a tendency was observed for the decrease of the ratio A_x/A_0 with the increase of concentration of the compounds. The exception was the influence of the increase in concentration of ethyl hexanoate. Also, the decrease in concentration of ethyl octanoate showed unexpected A_x/A_0 values <1. No plausible explanation can be yet given for these cases. It was observed that the decrease of ethyl decanoate concentration did not influence the GC peak areas of the other compounds; the exception was ethyl octanoate. This may be due to the fact that ethyl decanoate was the compound present in the lowest concentration (0.11−15 mg/L, due to the high RRF of this compound), possibly not sufficient to influence the headspace equilibrium.

The most influenced compounds by the variation of the relative matrix composition were 3-methyl-1-butanol and hexanol, both with lower RRF values. On the contrary, ethyl decanoate was not influenced by the variations in the concentration of the other compounds, which may be explained by its high affinity to the fiber, reflected in the higher RRF value. These results showed that the compounds with higher RRFs were less influenced by the matrix composition. The quantification by SPME was shown to be highly dependent on the matrix composition. As a consequence, it can be concluded that the relative composition of the liquid matrix should be considered when the values obtained with this methodology are used.

Analysis of the Wines by SPME-GC-MS. The monovarietal white wines analyzed had seven of the nine standard compounds used (Table 4), as nerol and geraniol were not detected. As expected, the alcohols were the major compounds. The amount of 3-methyl-1-butanol was very much higher (17−32 times) than that present previously in the base solution used for the calculation of the RRF shown in Table 1. Previous works carried out with these varieties in our laboratory using liquid−liquid extraction did not allow the quantification of this compound due to its high volatility and coelution with the solvent.

According to the results of the effect of the concentration of one compound on the GC peak areas of the other compounds (Table 3), it is expected that, in the wines, the concentration estimated for all other compounds could decrease due to the presence of the higher amounts of 3-methyl-1-butanol. However, due to its low RRF, it did not significantly influence the amount absorbed of the other compounds present. In fact, the estimation of the new RRF (Table 4) showed values of the same order of magnitude for all compounds.

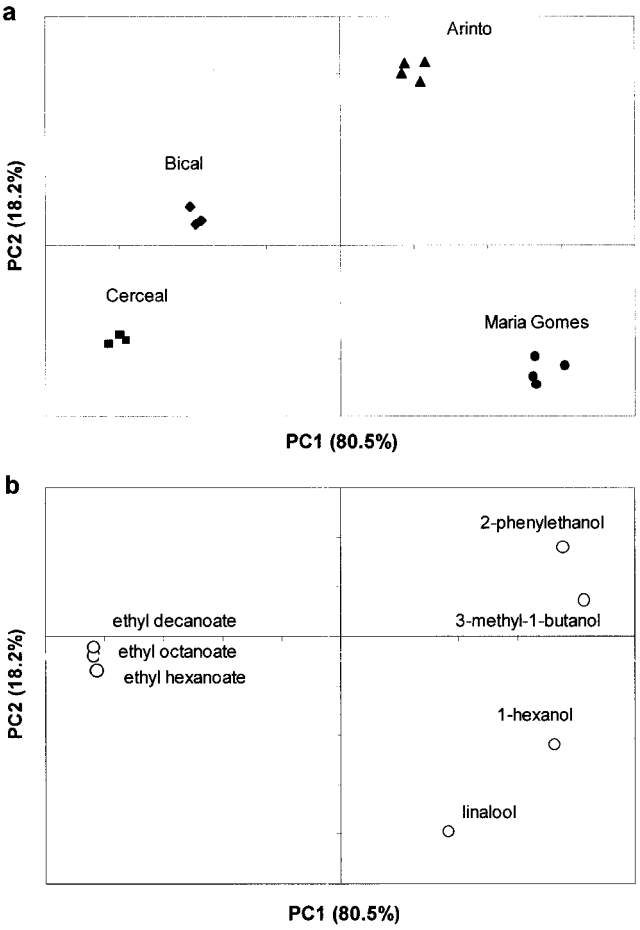

Figure 8. PC1 × PC2 scatter plots of the main sources of variability between the Arinto, Bical, Cerceal, and Maria Gomes varieties: (a) distinction among the monovarietal wines (scores); (b) relationship among the seven volatile components (loadings).

The application of a PCA to the normalized areas of the seven standard compounds identified by SPME-GC-MS (Arinto, Bical, Cerceal, and Maria Gomes varieties) allows the distinction of the four wine varieties. Figure 8a shows the scores (projections of the samples onto the most important principal components) scatter plot in which the two first principal components account for 98.7% of the total variability present in the data set. From this plot one can see that the four wine varieties can be separated. On the one hand, PC1, which explains 80.5% of the total variability, characterizes the separation between Maria Gomes + Arinto and Cerceal + Bical wine varieties. On the other hand, the PC2 axis, which explains 18.2% of the total variability, is related to the

distinction between Arinto + Bical and Cerceal + Maria Gomes wine varieties.

This scores plot can be related to the loadings plot (contributions of the GC peak areas of the volatile compounds to the principal components, in Figure 8b) which reveals the relationship between wine components and wine varieties. The relationship between each loadings plot region and the wine varieties shows that the Maria Gomes variety is characterized by linalool and 1-hexanol and that 2-phenylethanol and 3-methyl-1-butanol characterize mainly the Arinto variety. The Cerceal wine variety is related to the ester components, and the Bical wine variety is anti-correlated to the linalool and 1-hexanol variability amounts. The occurrence of linalool in higher amounts in Maria Gomes, near the sensorial perception limits, is in accordance with the results previously reported for the occurrence of this terpenoid in the musts (*35*).

These results showed that SPME can be used for the analysis of the wine volatile compounds provided the effect of its relative composition is taken in consideration, as shown in the analysis of the four monovarietal Bairrada white wines.

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