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Polydimethylsiloxane solid-phase microextraction–gas chromatography method for the analysis of volatile compounds in wines
Its application to the characterization of varietal wines

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Abstract

A study was made of the validity of the solid-phase microextraction method, using a polydimethylsiloxane coated fused-silica fiber, for the extraction–desorption of the minor volatile compounds from wine before their gas chromatographic analysis. The aspects considered were the influence of ethanol on extraction, repeatability, limits of detection, linearity and recovery of compounds. This method, together with the direct injection of the major volatile compounds, was applied to 16 varietal wines. The findings indicate that the method is a highly suitable technique for the analysis of wines and that the volatile composition of wines depends, at least partly, on the grapes with which they have been made.

Keywords: Wine; Food analysis; Solid-phase microextraction; Volatile organic compounds

1. Introduction

In order to ensure the correct labeling of wines it is necessary to have analysis methods that make it possible to know the variety of grape with which a wine has been made. Among the compounds used as variety markers are those that form part of the volatile fraction, most of which are also responsible for the aroma of wines [1–3]. These compounds belong to very heterogeneous groups such as alcohols, aldehydes, ketones, esters, volatile acids, terpenes, etc. The concentration of each of these in wine is highly variable, and ranges from hundreds of milligrams to a few picograms per liter. Therefore, some compounds can be analyzed by gas chromatography (GC) with direct injection, while others need to be extracted and concentrated before their chromatographic analysis.

Several extraction–concentration methods have been used for the analysis of the minor volatile compounds in wines, such as liquid–liquid extraction [4], simultaneous extraction and distillation [5], etc. Solid-phase microextraction (SPME) is a simple, recently-developed method [6] that, thanks to the appearance of fibers of different materials with different polarity ranges, makes it possible to extract and concentrate minor compounds of different substrates.

SPME has been applied to the study of different

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compounds of wine, such as contaminants [7–11] and terpenes [12,13], among others. It has also been used for the study of the non-terpene fraction of the aroma. Vas and co-workers [14,15] used this technique to obtain semi-quantitative data on a large number of compounds of the aroma, with different functional groups. Later, using this same technique, Francioli et al. [16] determined esters and detected vitispirane, a characteristic compound of sparkling wines [17].

While analysis of the major compounds in wines by GC with direct injection is a thoroughly verified technique, the methodology applied until now for SPME analysis of the minor volatile compounds is based on that used in studies of the extraction–desorption of other types of compounds and in other types of samples. Therefore, before addressing the study of the minor compounds in the volatile fraction of wines, it is necessary to verify the validity of the method used. To this end, a study was made on the influence of the matrix effect on extraction, accuracy of the method, limits of detection, linearity, and compound recovery.

Once the method for analyzing the minor compounds was available, a study was made of the major and minor volatile composition of 16 varietal wines, eight white wines of the Malvar and Airén varieties, and eight rosé wines of the Trepat and Monastrell varieties, made from the 1997, 1998 and 1999 harvests. The Malvar white variety was selected because it is the variety preferred for making wines under the Appellation d'Origin *Vinos de Madrid*, and Airén because it is the most common white variety in Madrid. The Trepat variety, native to the area of Catalonia, and the Monastrell variety are used to make quality rosé wines.

2. Experimental

2.1. Samples

The follwoing wines were used: four white wines of the Malvar variety, two of them from the 1998 harvest (Ma98-1 and Ma98-2) and two from the 1999 harvest (Ma99-1 and Ma99-2). Four white wines of the Airén variety, two of them from the 1997 harvest (Ai97-1 and Ai97-2) and two from the 1998 harvest (Ai98-1 and Ai98-2). Four rosé wines of the Trepat variety, two of them from the 1997 harvest (Tr97-1 and Tr97-2) and two from the 1998 harvest (Tr98-1 and Tr98-2). Four rosé wines of the Monastrell variety, two of them from the 1997 harvest (Mo97-1 and Mo97-2) and two from the 1998 harvest (Mo98-1 and Mo98-2). All the analyses were conducted in duplicate.

2.2. Major volatile analysis

A Hewlett-Packard (Palo Alto, CA, USA) 5890 series II gas chromatograph equipped with a flame ionization detection (FID) system and a split/splitless injector was used. Separations were carried out on 15% Carbowax 1500 on a 177–199 μm WNAW column (4 m×0.31 cm I.D.; Supelco, Bellefonte, PA, USA) with helium as carrier gas at a flow-rate of 19 ml/min. The temperature program was as follows: initial temperature 70°C for 10 min and a ramp of 2°C/min to 200°C. A 50-μl volume of 3-pentanol (6 mg/ml 10% ethanol) was added as internal standard to 10 ml of wine. A 2-μl volume of wine with the internal standard was injected in the split mode. Chromatography software from Perkin-Elmer (Norwalk, CT, USA) Nelson Systems (Model 2600, v 5.0) was used for data acquisition from the FID system. The compounds determined by this method were: acetaldehyde, ethyl formate, ethyl acetate, methanol, 1-propanol, isobutanol and 2+3-methyl-1-butanol.

2.3. Minor volatile analysis

2.3.1. Gas chromatographic conditions

A Hewlett-Packard M-5890 series II equipped with an FID system and split/splitless injector was used. Separations were carried out on a Carbowax 20M, fused-silica capillary column (30 m×0.25 mm I.D., Macherey–Nagel, Düren, Germany), coated with a Quadrex stationary phase of 0.25 μm thickness (Supelco). The injector and the detector temperatures were 250°C. The carrier gas was He, 12.5 p.s.i. (1 p.s.i.=6894.76 Pa). The GC temperature program was as follows: initial temperature, 40°C (10 min hold); first ramp, 5°C/min to 200°C (0 min hold); second ramp, 20°C/min to 210°C, (10 min hold). The injector was operated in the splitless

mode, with the purge activation time adjusted to 10 min. A ChemStation data system (HP 3365 series II, v. A.03.21) was used for data processing. Peak identification was accomplished by comparison of the retention times with those of the reference compounds.

2.3.2. SPME headspace analysis

A Supelco SPME fiber holder and a 100 μm polydimethylsiloxane (PDMS) coated fused-silica fiber were used. Prior to the first extraction, the fiber was conditioned in the GC injector port at 250°C for 1 h according to the manufacturer's recommendation.

To facilitate the solubility of the volatile compounds, the standard solution was prepared by dissolving the individual compounds in ethanol absolute. Aliquots of this solution were added to a 12 ml vial containing 5 ml ethanol 10%, 2 g NaCl and 25 μl internal standard (methyl nonanoate, 10 mg/100 ml ethanol). Extraction temperature of headspace and time were 40°C and 10 min, respectively. The samples were agitated during extraction using a magnetic stirrer. For each mixture, the concentrations were analyzed in random order. Thermal desorption was performed in the injector glass liner (78.5×0.75 mm I.D.) at 250°C, for 10 min.

Wine samples were extracted and desorbed in the same conditions as standard solutions.

2.4. Statistical methods

The statistical methods used for analysis were as follows: regression analysis for the calibration curves, principal component analysis to examine the relationships among the variables, stepwise discriminant analysis to select the variables most useful in differentiating the four varietal wines, cluster analysis to confirm the separation between the four groups with the selected variables and one-way analysis of variance (ANOVA) to determine if there are significant differences between varieties. The Statistica program for Windows [18] was used in the calculations. This program was run on a pentium personal computer.

3. Results and discussion

3.1. Validation of the extraction–concentration method for the analysis of the minor volatile compounds in wine

According to most authors [19–22] adsorption of the compounds on the fiber is enhanced with an increase in the ionic strength of the solution. Therefore, in this study the calibrated solutions and the wines received the addition of 2 g/5 ml of NaCl, that is to say, to saturation. The time necessary for complete desorption of the different compounds was evaluated by means of blank injections, with the fiber located in the injector, after injecting the samples. It was observed that the time necessary for complete desorption of the sample was at least 10 min.

The composition of the headspace of the vial in which the sample is deposited depends on the relative proportions of the different volatile compounds to be determined. Therefore, it was considered necessary to verify the influence of the presence of ethanol on the adsorption of the other compounds on the fiber. Fig. 1 shows the relationship of the relative areas to the internal standard, versus the concentration of two of the compounds analyzed,

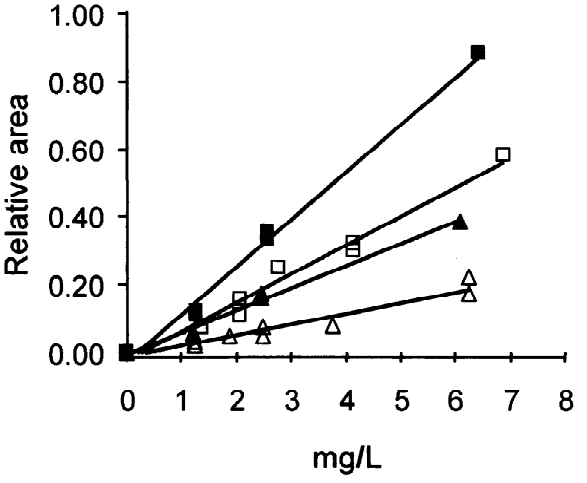

Fig. 1. Relative area to the internal standard versus concentration of: butyl acetate dissolved in water (■); butyl acetate dissolved in 10% ethanol (□); 1-hexanol dissolved in water (▲); 1-hexanol dissolved in 10% ethanol (△).

one ester, butyl acetate, and one alcohol, 1-hexanol. It can be seen that the relative area of each of these two compounds is greater when they are dissolved in water (solid symbols) than when they are dissolved in a solution with 10% ethanol (empty symbols). Therefore, the calibrated solutions were prepared with 10% ethanol, which is the usual alcoholic strength of wines.

The repeatability of the method was estimated by the relative standard deviation (RSD) of the areas relative to the internal standard for four consecutive headspace injections of a 10% ethanol standard solution. Table 1 shows the values obtained for this parameter, which range from 1.38% for γ-butyrolactone, to 12.83% for butyl acetate, with a mean value of 6.9%, which is considered acceptable for this type of analysis. These values coincide with those found by Vas et al. [15]. Due to the possible lack of reproducibility from one fiber to another, it is advisable to make up a new calibrate each time the fiber is changed.

Limits of detection were estimated from the area corresponding to three times the system noise [23]. The system noise was calculated as the mean of the area of the noise of seven blank injections. The values obtained (Table 1) range from 0.002 mg/l for ethyl decanoate to 1.390 mg/l for γ-butyrolactone. Generally speaking, the values of the limits of detection are lower for the esters (from 0.002 to 0.084 mg/l) than for the alcohols (0.152 and 0.490 mg/l) and the acids (from 0.091 to 0.577 mg/l).

Table 1
Repeatability and limits of detection of the SPME–GC method using PDMS for the analysis of minor volatile compounds from wines

Compound	Repeatability, RSD (%)	Limit of detection (mg/l)
Esters		
Isobutyl acetate	7.63	0.049
Ethyl butyrate	6.14	0.033
Butyl acetate	12.83	0.055
Isopentyl acetate	9.89	0.017
Ethyl hexanoate	11.39	0.007
Hexyl acetate	12.71	0.006
Ethyl octanoate	4.53	0.003
Ethyl decanoate	2.33	0.002
Diethyl succinate	6.36	0.084
Alcohols		
1-Hexanol	8.73	0.152
cis-3-Hexen-1-ol	10.41	0.490
Fatty acids		
Hexanoic acid	3.14	0.577
Octanoic acid	6.73	0.095
Decanoic acid	7.39	0.091
Other compounds		
γ-Butyrolactone	1.38	1.390

Linear regression analysis of relative areas versus concentration of volatile compounds in the standard solution, using two replicates at five points, was used. In order to judging the adequacy of the models, the F-ratio for lack of fit was calculated [24], and when significant results were obtained, a second-degree polynomial regression was used (Table 2). The values of the coefficients of determination (R^2 in Table 2) were higher than 0.900 and indicated that the fits were acceptable. The 95% confidence interval (CI) for the slope, for those compounds with a first-order model and the calibration range, are also shown in Table 2.

In order to evaluate the occurrence of proportional systematic errors, which are caused by the fact that the calibration line obtained with standards does not have the same slope as the functional relationship between the measurement result and the concentration in the sample, new recovery experiments were carried out [24]. Five different concentrations of the standard solution were added to a wine, and two injections were carried out at each point, in the range indicated in Table 3. The calculation of regression equations resulted in the findings shown in Table 3. The slopes of the straight lines (coefficients b) are within the confidence interval of the values of the slopes of the 10% ethanol standard solutions (Table 2) for ethyl hexanoate, hexyl acetate, ethyl octanoate, ethyl decanoate, diethyl succinate, 1-hexanol, *cis*-3-hexen-1-ol, hexanoic acid and γ-butyrolactone (D=no in Table 3). That is to say, it can be accepted that the slopes of the regression line obtained in the recovery experiments and of the calibration line obtained with pure standards, are equal.

Table 3 also shows the mean values of the recovery results. Recovery has been estimated as (the amount found in the spiked sample−the amount found in the sample)·100/the amount added [24]. The mean values correspond to the individual values obtained from the recovery experiments and also to

Table 2
Polynomial regression ($y=ax^2+bx+c$) for relative area versus concentration of the standard solution

Compound	Range (mg/l)	*a*	*b*	*c*	R^2	Number of data points	CI
Esters							
Isobutyl acetate	0–7.5	0.0032	0.0467	−0.0081	0.967	10	–
Ethyl butyrate	0–6.9	0.0037	0.0513	−0.0054	0.958	10	–
Butyl acetate	0–6.9	ns	0.0842	−0.0214	0.979	10	0.074–0.094
Isopentyl acetate	0–5.8	ns	0.2692	−0.0329	0.992	10	0.250–0.288
Ethyl hexanoate	0–13.2	ns	0.7163	0.1818	0.959	10	0.595–0.837
Hexyl acetate	0–8.2	ns	0.7403	0.1467	0.963	10	0.621–0.859
Ethyl octanoate	0–13.4	ns	2.6742	−0.3174	0.993	10	2.493–2.855
Ethyl decanoate	0–8.7	ns	2.3123	1.4499	0.938	9	1.783–2.842
Diethyl succinate	0–10.7	ns	0.0121	−0.0083	0.900	10	0.009–0.015
Alcohols							
1-Hexanol	0–3.8	ns	0.0219	0.0021	0.926	9	0.016–0.027
cis-3-Hexen-1-ol	0–15.1	ns	0.0072	0.0015	0.945	9	0.006–0.009
Fatty acids							
Hexanoic acid	0–18.0	ns	0.0047	0.0006	0.936	10	0.004–0.006
Octanoic acid	0–5.3	ns	0.0300	0.0053	0.934	10	0.023–0.036
Decanoic acid	0–3.2	0.0377	0.0034	0.0152	0.966	10	–
Other compounds							
γ-Butyrolactone	0–65.8	ns	0.0060	−0.0022	0.961	10	0.005–0.007

R^2, coefficient of determination; CI, 95% confidence interval for the slope of first-order model; ns, non significantly different from zero ($p>0.05$).

Table 3
Polynomial regression ($y=ax^2+bx+c$) for relative area versus concentration of a wine added with standard solution, comparison of the slopes of linear models and recovery of the extraction method

Compound	Range (mg/l)	*a*	*b*	*c*	R^2	Number of data points	D	Recovery (%)
Esters								
Isobutyl acetate	0–7.5	−0.0018	0.0429	−0.0065	0.945	8	–	90
Ethyl butyrate	0–6.9	0.0111	0.0247	−0.0032	0.986	10	–	93
Butyl acetate	0–6.9	ns	0.0618	−0.0056	0.965	10	Yes	97
Isopentyl acetate	0–5.8	ns	0.2380	0.0338	0.949	10	Yes	88
Ethyl hexanoate	0–13.2	ns	0.6371	0.6872	0.956	10	No	92
Hexyl acetate	0–8.2	ns	0.6903	−0.0367	0.966	10	No	87
Ethyl octanoate	0–13.4	ns	2.5718	1.6304	0.994	10	No	98
Ethyl decanoate	0–8.7	ns	2.6796	0.1643	0.986	10	No	105
Diethyl succinate	0–10.7	ns	0.0115	0.0053	0.949	10	No	120
Alcohols								
1-Hexanol	0–3.8	ns	0.0262	0.0189	0.863	10	No	88
cis-3-Hexen-1-ol	0–15.1	ns	0.0770	0.0017	0.982	10	No	97
Fatty acids								
Hexanoic acid	0–18.0	ns	0.0058	0.0267	0.955	8	No	105
Octanoic acid	0–5.3	ns	0.0440	0.1260	0.938	8	Yes	92
Decanoic acid	0–3.2	−0.0029	0.0473	0.0616	0.931	8	–	89
Other compounds								
γ-Butyrolactone	0–65.8	ns	0.0069	−0.0212	0.970	10	No	107

D, significant differences between the slopes of these lines and those of Table 2. ns, non-significantly different from zero ($p>0.05$). Recovery=(amount founded in the spiked sample−amount founded in the sample)×100/amount added.

the values obtained for two more different wine samples. The mean values obtained range from 87% for hexyl acetate to 120% for diethyl succinate, while the average value for all of the compounds studied is 97%.

3.2. Composition of the wines

Volatile compounds were identified from their relative retention times, which were previously determined by injection of standards. Some of the compounds detected, such as isobutanol and the isoamylic alcohols, can be quantified in the direct injection analysis of the wine, and so the SPME technique was not used for their quantification.

Table 4 shows the mean and the standard deviation values of volatile compound content in the wines of the four varieties and the results of the application of the Student–Newman–Keuls test to compare the means for each variety, when significant differences between varieties were obtained from one-way ANOVA test. The table also indicates the method of analysis followed for their quantification. Significant differences were found among the mean values for the wines of the different varieties for 12 of the 22 variables determined. Although the Malvar and Airén varieties are very close from the ampelo-

Table 4
Mean and standard deviation values of the volatile compounds in the varietal wines ($n=4$) and results of the Student–Newman–Keuls test for means comparisons

Volatile compound		Malvar		Airén		Trepat		Monastrell	
		Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
Esters									
Ethyl formate	(1)	n.d.	–	n.d.	–	n.d.	–	n.d.	–
Ethyl acetate*	(1)	54.6	17.37	49.5	20.04	48.9	3.02	43.2	7.34
Isobutyl acetate	(2)	n.d.	–	n.d.	–	n.d.	–	n.d.	–
Ethyl butyrate	(2)	0.6^{b}	0.22	0.9^{c}	0.05	0.3^{a}	0.24	0.4^{ba}	0.12
Butyl acetate	(2)	n.d.	–	n.d.	–	n.d.	–	n.d.	–
Isopenthyl acetate	(2)	1.9^{ab}	1.15	3.1^{b}	1.54	2.0^{ab}	0.70	0.5^{a}	0.25
Ethyl hexanoate	(2)	1.1^{a}	0.12	1.8^{b}	0.19	1.2^{a}	0.18	0.8^{a}	0.46
Hexyl acetate*	(2)	0.1	0.10	0.2	0.10	0.2	0.17	0.0	0.04
Ethyl octanoate	(2)	1.5^{a}	0.15	4.8^{c}	0.89	3.2^{b}	1.26	1.4^{a}	0.84
Ethyl decanoate	(2)	0.7^{a}	0.15	1.7^{b}	0.41	1.7^{b}	0.74	0.7^{a}	0.51
Diethyl succinate*	(2)	11.3	5.75	7.5	2.28	10.0	9.90	6.3	1.05
Alcohols									
Methanol	(1)	29.6^{a}	2.25	39.5^{b}	8.65	42.0^{b}	3.52	48.2^{b}	8.59
1-Propanol	(1)	41.1^{c}	5.10	27.9^{b}	4.08	13.0^{a}	2.08	18.2^{a}	1.54
Isobutanol*	(1)	18.5	5.12	26.1	2.35	32.9	15.91	19.9	11.46
2+3-Methyl-1-butanol*	(1)	146.2	30.91	196.7	17.63	178.2	74.66	208.5	28.72
1-Hexanol	(2)	0.3^{a}	0.08	0.3^{a}	0.12	1.3^{b}	0.45	1.0^{b}	0.35
cis-3-Hexen-1-ol	(2)	0.2^{a}	0.02	0.0^{a}	–	1.3^{c}	0.24	0.8^{b}	0.35
Fatty acids									
Hexanoic acid	(2)	11.4^{b}	4.32	8.0^{ab}	0.49	8.6^{ab}	1.21	5.5^{a}	2.70
Octanoic acid	(2)	2.5^{a}	0.65	2.6^{a}	1.25	10.7^{c}	2.04	5.9^{b}	1.33
Decanoic acid	(2)	0.9^{a}	0.30	1.1^{a}	0.46	2.6^{b}	0.68	1.4^{a}	0.61
Other compounds									
Acetaldehyde*	(1)	38.9	17.76	49.0	10.09	25.8	3.92	72.3	52.16
γ-Butyrolactone	(2)	n.d.	–	n.d.	–	n.d.	–	n.d.	–

*The result of the ANOVA test is not positive. (1) Mayor volatile method. (2) Minor volatile method. n.d.=Not detected. $^{a-c}$Mean values in the same row with the same superscript indicate that there are no significant differences between them ($p<0.05$).

graphic point of view [25], the mean values of methanol, 1-propanol, ethyl butyrate, ethyl hexanoate and ethyl decanoate content are different in the wines of the two varieties.

In order to obtain more information on the causes of the variability in the values found in the analysis of the volatile compounds in the wines, principal component analysis, from the correlation matrix, was performed. It was observed that about 49% of the variation in these values could be explained by the first two principal components. The first principal component was strongly correlated with octanoic acid (−0.973), *cis*-3-hexen-1-ol (−0.904), 1-hexanol (−0.841), decanoic acid (−0.814), 1-propanol (0.758) and ethyl butanoate (0.757), while ethyl octanoate (0.963), ethyl decanoate (0.854), ethyl hexanoate (0.848) and hexyl acetate (0.717) contribute more strongly to the second principal component. From the plot of the 16 wines on the plane defined by these first two principal components (Fig. 2), the wines of the red varieties (Trepat and Monastrell) appear on the left side of the plane, grouped by year of harvest, with lower values for principal component 1, while the wines of the white varieties (Airén and Malvar) are found on the right side of the plane, that is to say, they have greater values of this principal component, and are also grouped by year of harvest. The second principal component differentiates, above all, the wines of the two white varieties, and the wines of the two harvests, above all in the red varieties. It can be observed that the greatest cause of variation among the samples is due to the factor variety, followed by harvest. The samples of the same harvest and variety appear together on the plot.

When stepwise discriminant analysis was applied to the data of the 16 varietal wines using the variables listed in Table 4, the selected variables that best discriminated the four groups of wines were: 1-propanol, ethyl octanoate, *cis*-3-hexen-1-ol and octanoic acid. A 100% correct assignment of the wines was obtained with these selected variables when either the standard or the leave-one-out cross-validation procedure was used. Three of the four

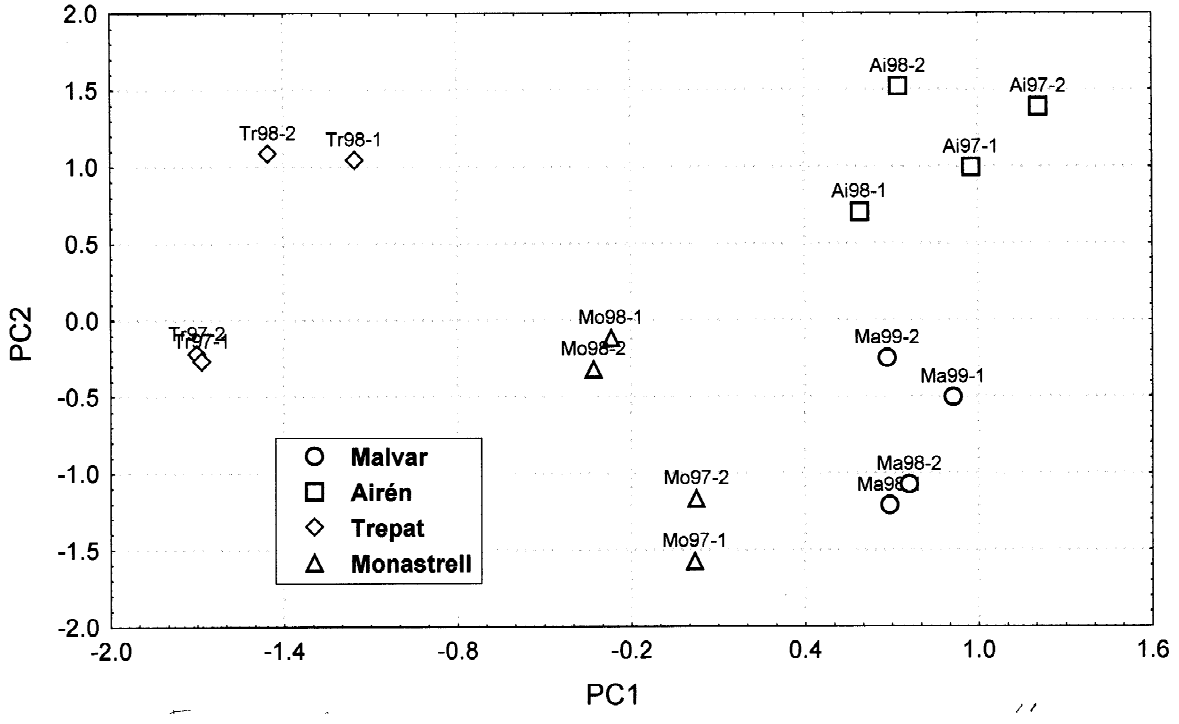

Fig. 2. Plot of the wines on the plane defined by the first two principal components.

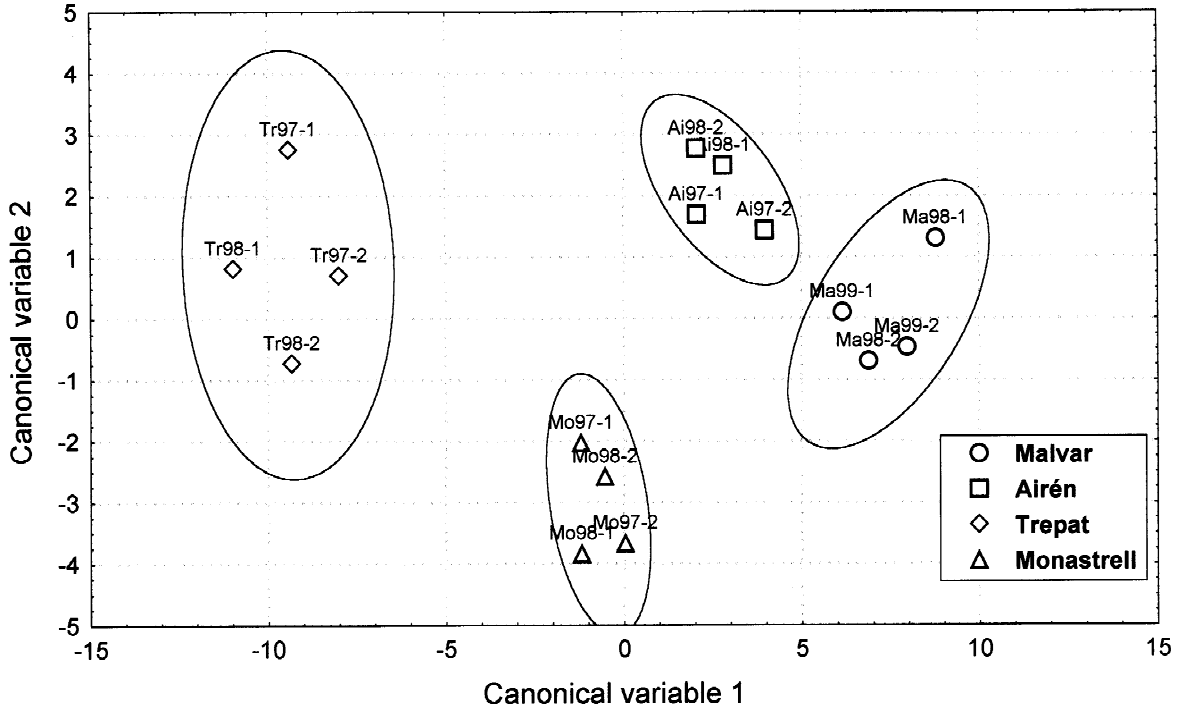

Fig. 3. Plot of the wines on the plane defined by the first two canonical variables and population canonical ellipses for the varieties for 95% confidence.

variables selected, 1-propanol, ethyl octanoate and *cis*-3-hexen-1-ol, were reported by other researchers as discriminating in accordance with variety or type of wine [26,27]. Fig. 3 shows the 16 wines on the plane defined by the first two canonical variables. The population canonical ellipses for the four varieties for 95% confidence are also represented. The red varieties essentially have lower concentrations of 1-propanol and higher concentrations of *cis*-3-hexen-1-ol and octanoic acid than the white varieties (Table 4). Ethyl octanoate differentiates the two red varieties and also the two white varieties. Cluster analysis on the data of the variables selected in the stepwise discriminant analysis produces the groupings revealed in the dendrogram shown in Fig. 4. The Euclidean distance was taken as a measure of the proximity between two samples. The variables were previously standardized, and Ward's linkage method was used. Two groups are observed, one made up of the wines of the red Monastrell and Trepat varieties, and the other formed by the white varieties Airén and Malvar. In turn, the wines of each of these groups are grouped together again, with a greater degree of similarity, according to the respective varieties. That is to say, the wines of the four varieties are grouped, in all cases, according to variety.

To sum up, it can be concluded that the volatile composition of wines contains important information

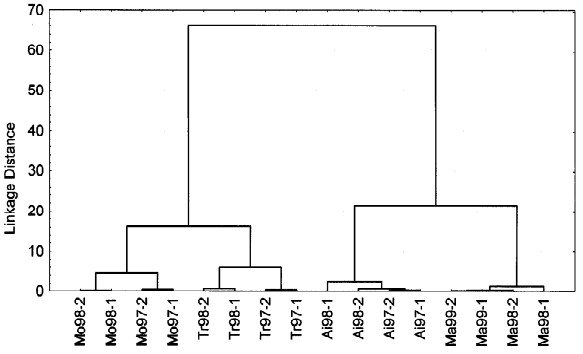

Fig. 4. Dendrogram for the 16 wines according to the selected variables from stepwise discriminant analysis.

on the varietal characteristics of the grapes from which they are made and that the SPME extraction–concentration method using PDMS prior to their chromatographic analysis is a highly suitable technique for the analysis of wines.

Acknowledgements

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References

[1] A. Rapp, M. Guentert, Vitis 24 (1985) 139.
[2] M. Forcen, A. Berna, A. Mulet, Lebensm. Wissenschaft Technol. 26 (1993) 54.
[3] D. de la Calle-García, M. Reichenbächer, K. Danzer, C. Hurlbeek, C. Bartzsch, K.H. Feller, Fresenius J. Anal. Chem. 360 (1998) 784.
[4] V. Ferreira, A. Rapp, J.F. Cacho, H. Hastrich, I. Javas, J. Agric. Food Chem. 41 (1993) 1413.
[5] G.P. Blanch, G. Reglero, M. Herraiz, Food Chem. 56 (1996) 439.
[6] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[7] T.J. Evans, C.E. Butzke, S.E. Ebeler, J. Chromatogr. A 786 (1997) 293.
[8] W. Flak, G. Tscheik, Mitt. Klosterneugurg Rebe Wine Obstb. Früchtevewert. 47 (1997) 117.
[9] N. Gandini, R. Riguzzi, J. Agric. Food Chem. 45 (1997) 3092.
[10] M. Vitali, M. Guidotti, R. Giovinazzo, O. Cedrone, Food Addit. Contam. 15 (1998) 280.
[11] V. Bellavia, M. Natangelo, R. Fanelli, D. Rotilio, J. Agric. Food Chem. 48 (2000) 1239.
[12] D. de la Calle-García, M. Reichenbächer, K. Danzer, Vitis 37 (1998) 181.
[13] D. de la Calle-García, M. Reichenbächer, K. Danzer, J. High Resolut. Chromatogr. 21 (1998) 373.
[14] G.Y. Vas, K. Köteleky, M. Farkas, A. Dobó, K. Vékey, Am. J. Enol. Vitic. 49 (1998) 100.
[15] G. Vas, L. Gál, J. Harangi, A. Dobó, K. Vékey, J. Chromatogr. Sci. 36 (1998) 505.
[16] S. Francioli, M. Guerra, E. López-Tamames, J.M. Guadayo, J. Caixach, Am. J. Enol. Vitic. 50 (1999) 404.
[17] M. Feuillat, Rev. Franc. Oenol. 16 (1980) 35.
[18] Statistica for Windows, Release 5.1, StatSoft, Tulsa, OK, 1998.
[19] X. Yang, T. Peppard, J. Agric. Food Chem. 42 (1994) 1925.
[20] D. de la Galle-García, S. Magnaghi, M. Reichenbächer, K. Danzer, J. High Resolut. Chromatogr. 19 (1996) 257.
[21] H. Prosen, L. Zupancic, Trends Anal. Chem. 18 (1999) 272.
[22] M. Abdel-Rehim, M. Bielenstein, T. Arvidsson, J. Microcol. Sep. 12 (2000) 308.
[23] IUPAC, Spectrochim. Acta 33B (1978) 242.
[24] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte, L. Kaufman, Chemometrics: A Textbook, Elsevier, Amsterdam, 1988.
[25] F. Cabello, J.F. Gallego, G. Muñoz, J.M. Ortiz, Vitic. Enol. Prof. May/June (1994) 25.
[26] M.D. Cabezudo, M.C. Polo, M. Herraiz, G. Reglero, M. González-Raurich, I. Cáceres, P. Martín-Alvarez, in: G. Charalambous (Ed.), The Shelf Life of Food and Beverages, Elsevier, Amsterdam, 1986, p. 186.
[27] P. Martín-Alvarez, M.C. Polo, M. Herraiz, I. Cáceres, M. González-Raurich, T. Herraiz, G. Reglero, M.D. Cabezudo, J. Sanz, in: M. Martens, G.A. Dalen, H. RusswurnJr. (Eds.), Flavor Science and Technology, Wiley, Chichester, 1987, p. 489.