ELSEVIER

Journal of Chromatography A, 953 (2002) 7–15

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Optimisation of headspace solid-phase microextraction for analysis of aromatic compounds in vinegar

Remedios Castro Mejías*, Ramón Natera Marín, M[a]. de Valme García Moreno, Carmelo García Barroso

Analytical Chemistry Department, Faculty of Sciences, University of Cádiz, P.O. Box 40, E-11510 Pol. Rio San Pedro, Puerto Real, Cádiz, Spain

Received 28 November 2001; received in revised form 5 February 2002; accepted 5 February 2002

Abstract

Headspace solid-phase microextraction has been applied to the analysis of aroma compounds in vinegar. Silica fibre coated with Carboxen–polydimethylsiloxane was found to be more efficient at extracting these compounds than other fibres such as those coated with polydimethylsiloxane, Carbowax–divinylbenzene, and polydimethylsiloxane–divinylbenzene, but its repeatability was low. Different parameters such as extraction time, temperature of the sample during the extraction, ionic strength, and sample volume were optimised using a two-level factorial design expanded further to a central composite design. This chemometric tool is very appropriate in screening experiments where the aim is to investigate several possibly influential and/or interacting factors. The extraction efficiency is inversely affected by the acetic acid content—an increase in the acetic acid concentration decreases the extraction efficiency. No interference is observed with the increase in content of polyphenols.

Keywords: Headspace analysis; Solid-phase microextraction; Chemometrics; Factorial design; Vinegar; Food analysis; Alcohols; Aldehydes; Volatile organic compounds; Aroma compounds

1. Introduction

Vinegar is a solution of acetic acid used for flavouring and preserving a wide range of foods. It is produced by a double-fermentation process from a variety of raw materials (white and red wine, cider, malted barley, honey, pure alcohol, etc.) and by a variety of different methods.

The chemical and organoleptic properties of vinegar are determined by the acetification system used, the raw material used as substrate and, in some cases, by the length of time it is aged in wood. With regard to wine vinegars, the different wines and technological procedures employed result in a great variety of products of diverse quality and organoleptic properties.

In recent years, in the Jerez–Xérès–Sherry, Manzanilla de Sanlúcar and Vinagre de Jerez Denomination of Origin (D.O.) region (SW Spain), Sherry vinegar, produced from Sherry wines following traditional methods of acetification [1], is no longer considered as a food product of secondary importance. It is now a highly valued, high quality product on a par with the wines and brandies typical of this

*Corresponding author. Tel.: +34-56-016-363; fax: +34-56-016-460.

E-mail address: remedios.castro@uca.es (R. Castro Mejías).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00122-X

region. For this reason, the producers have recently been seeking techniques for objectively determining the appropriate parameters to characterise a special vinegar such as Sherry wine vinegar.

Considering that the volatile constituents of any specific vinegar will be determined by the characteristics of the raw material used and by the technological processing undergone during its production, it is logical to suppose that vinegars could be characterised and differentiated by the quantitative and qualitative analysis of their volatile components.

For 20 years, in particular with the development of capillary columns of gas chromatography and the identification techniques as mass or infrared spectrometry, the analytical methods have improved and now are very efficient. Nevertheless, even today, the extraction and concentration of flavour components, prior to their analysis, constitute a problem that has still not been satisfactorily resolved. Sample preparation for GC analysis of less volatile compounds has been carried out mainly by liquid–liquid [2] or solid-phase [3] extraction. The more volatile compounds are normally analysed by head space or by purge and trap methods [4,5]. For wines, others preparation techniques employed have included supercritical fluid extraction [6], and simultaneous distillation–solvent extraction [7], among others. All these sample preparation methods present several disadvantages, such as excessive cost and time, the possible generation of artefacts, etc.

In the 1990s, solid-phase microextraction (SPME) has been developed [8–11]. This fast and inexpensive sample preparation method has been used routinely in combination with GC and GC–MS, and successfully applied to a wide variety of compounds, specially for the extraction of volatile organic compounds from environmental, biological and food samples, including water [12], wine [13], coffee [14], orange juice [15], different fruits [16,17], and brandy [18].

Two types of SPME techniques can be used to extract analytes: headspace (HS)-SPME and direct immersion (DI)-SPME. In HS-SPME, the fibre is exposed in the vapor phase above a gaseous, liquid or solid sample. In DI-SPME, the fibre is directly immersed in the liquid sample.

The most important parameters affecting the SPME method are: type of fibre employed, extraction temperature and time, salt concentration and sample volume [19].

Procedural optimisation can be achieved in a traditional trial, studying each factor separately, or using a chemometric approach based on the use of an optimum set of experiments (experimental design) which allows the simultaneous variation of all experimental factors studied, and the distinguishing of interactions among them that are not detectable with the classical experimental methods [20,21]. The chemometric methodology also reduces the number of experiments required.

The purpose of the work reported here is to study the effects of sample matrix and to optimise the conditions for detection and quantification of aromatic compounds in vinegar using a chemometric approach.

After selecting the type of fibre, the effects of experimental parameters such as sample volume and temperature, sampling time and NaCl concentration on the SPME of these aromatic compounds were evaluated using a two-level factorial design expanded further to a central composite design. This chemometric tool is very appropriate in screening experiments where the aim is to investigate possibly influential and/or interacting factors. The juxtaposition of a two-level design with a star design (the centres of the two designs coincide) gives a composite design. It is one of the most useful designs for estimating a multifactor response surface [20,21], which keeps to a minimum the number of experiments while providing the information needed.

2. Experimental

2.1. Vinegar samples

A commercial Sherry vinegar sample was used to optimise the extraction conditions in order to determine various vinegar aroma and flavor compounds of varying volatilities and functionalities.

2.2. Chemicals and reagents

Gallic acid, 5-(hydroxymethyl)-2-furaldehyde, caffeic acid and *p*-coumaric acid standards were

purchased from Fluka (Buchs, Switzerland) and Eastman Kodak (Rochester, NY, USA).

All aroma standards used in this study were supplied by Merck (Darmstadt, Germany). 4-Methyl-2-pentanol was employed as internal standard. Acetic acid and NaCl were purchased from Scharlau (Barcelona, Spain).

For studying the possible effect of acetic acid on headspace SPME, two extractions were analysed for each of five synthetic vinegar samples (Milli-Q water containing 0.02 g/l methanol, 1 g/l ethyl acetate and 1%, v/v, ethanol) with different acetic acid contents (0, 25, 50, 75, and 90 g/l) and spiked with the same amounts of volatile compounds: alcohols (0.02593 g/l of 3-methyl-1-butanol, 0.01904 g/l of 2-phenylethanol and 0.10007 g/l of 2-methyl-1-propanol), esters (0.00999 g/l of ethyl succinate and 0.04811 g/l of isoamyl acetate), and acids (0.00584 g/l of octanoic acid, 0.0210 g/l of decanoic acid and 0.00570 g/l of caproic acid).

To determine if the polyphenolic content competes with the aroma compounds in the extraction, the same process was carried out adding increasing amounts of gallic acid, 5-(hydroxymethyl)-2-furaldehyde, caffeic acid and *p*-coumaric acid, which are majority polyphenolic compounds present in vinegars [22], to a synthetic vinegar spiked with the same aroma compounds.

2.3. Sample preparation

After optimisation, and for each SPME analysis, a volume of 15 ml of sample (natural and synthetic vinegar) was pipetted and placed into a 50-ml glass vial with 6.14 g of NaCl. Each sample was spiked with 50 μl of a solution of 4-methyl-2-pentanol (2.27 g/l in Milli-Q water containing 80 g/l of acetic acid). A small magnetic stirring bar was also added. The vial was tightly capped with a PTFE-faced silicone septum. The vial was placed in a thermostatted block on a stirrer. The sample was equilibrated for 5 min at sampling temperature and, after this, the SPME fibre was inserted into the headspace. During the sampling time, the sample was stirred at constant speed. After completion of sampling, the fibre was removed from the sample vial and inserted into the injection port of the GC.

2.4. Experimental design

The factorial design requires fewer measurements than the classical one-at-a-time experiment to give the same precision. At the time it can detect and estimate any interaction between factors, which the classical experiment cannot do.

The standard approach to the analysis of the experimental design data is to calculate and evaluate a list of the main effects and interaction effects supported by an analysis of variance (ANOVA) table, indicating which effects are significant. For data manipulation, the Statgraphics Statistical Computer Package ''Statgraphics Plus 5.0'' for Windows 98 was used.

In this study, we chose a sequential exploration of the response, which was carried out in two stages. In the first stage, we wished to establish the relative influence of the factors and their interactions on the number of chromatographic peaks detected and on the total chromatographic area obtained. Four factors were selected as potentially affecting the SPME efficiency: temperature and time of extraction, sample volume and ionic strength effect from adding different amounts of NaCl.

Consequently a factorial design of 2^4 was chosen. This design involves 16 experiments undertaken in random order to provide protection against the effects of lurking variables. These experiments were carried out in triplicate. The values corresponding to the high (+), and low (−) points for each factor are shown in Table 1.

In the second stage, this two-level factorial design was expanded to a star design. A central composite design (CCD, with $\alpha = 1.682$) was obtained, since the centres of the two separate designs were coincidental. Table 1 lists the values given to each factor.

2.5. Equipment

2.5.1. SPME

The silica fibres used in this study were purchased from Supelco (Bellfonte, PA, USA). Four fibres were tested and compared: one was coated with 100 μm of polydimethylsiloxane (PDMS), a second with 75 μm of Carboxen–polydimethylsiloxane (CAR–PDMS), a third with 65 μm of Carbowax–divinyl-

Table 1
Factor levels

Factor	Low (−)	High (+)	Centre	Axial (−α)	Axial (+α)
Sample volume (ml)	15	35			
Sampling temperature (°C)	30	60	45	19.77	70.23
Sampling time (min)	15	45	30	4.77	55.23
NaCl (*M*)	3	6	4.5	1.97	7.02

benzene (CW–DVB), and the fourth with 65 μm of polydimethylsiloxane–divinylbenzene (PDMS–DVB).

The fibres were conditioned prior to use according to supplier's instructions by inserting them into the GC injector.

2.5.2. Chromatography

The samples were analysed using a GC 8000 chromatograph with a flame ionization detection (FID) system (Fisons Instruments, Milan, Italy).

The injection was made in the splitless mode for 2 min. For the desorption of the analytes inside the GC injection port, the temperature was 280 °C for the CAR–PDMS fibre and 250 °C for the other fibres.

The GC system was equipped with a DB-Wax capillary column (J&W Scientific, Folsom, CA, USA), 60 m×0.25 mm I.D., with a 0.25-μm coating. The carrier gas was helium at a flow-rate of 1.1 ml/min. The detector temperature was 250 °C. The GC oven was programmed as follows: held at 35 °C for 10 min, then ramped at 5 °C/min to 100 °C. Then it was raised to 210 °C at 3 °C/min and held for 40 min.

The compounds were identified by mass spectrometric analysis. In these analyses, the same GC system coupled to a MD 800 mass detector (Fisons Instruments, Milan, Italy) was used. The mass detector operated in the positive ion electron impact ionization (EI+) mode at 70 eV in a range of 30 to 450 amu. GC analytical conditions were the same as described above.

The signal was recorded and processed with Masslab software supplied with the Wiley 6.0 MS library. Peak identification was carried out by analogy of mass spectra and confirmed by retention indices of standards when they were available or by retention data from the literature. Quantitative data from the identified compounds were obtained by measuring the relative peak area in relation to that of 4-methyl-2-pentanol, the internal standard.

3. Results and discussion

3.1. Fibre screening

Four different types of commercially available fibres were tested. The same sample of commercial Sherry vinegar (25 ml) was extracted, in triplicate, with these fibres using the HS-SPME mode. In these experiments, magnetic stirring was used to accelerate the transfer of the analytes from sample to the fibre. The samples were saturated with NaCl (6.00 g). Before the extraction, the sample vials were equilibrated for 15 min at the extraction temperature, 40 °C. The sampling time was 15 min.

The results of this screening (Table 2) showed that, in general, the extraction efficiency was better for the CAR–PDMS fibre. Therefore, CAR–PDMS fibre was selected for the method optimisation.

3.2. SPME parameter optimisation

The parameters optimised were extraction time, temperature of the sample during the extraction, ionic strength and sample volume. All the experiments were performed with constant magnetic stirring because this has a positive effect on the analyte transference to the stationary phase [23].

3.2.1. Screening by a 2^4 factorial design

The initial screening design served to detect those variables presenting the greatest influence on the experimental responses (number of chromatographic peaks and total chromatographic area).

The data obtained for these parameters were evaluated by ANOVA at the 5% significance level.

Table 2
Peak areas (mean values) of the identified compounds for each fibre

Compound	Type of fibre			
	PDMS	CW–DVB	CAR–PDMS	PDMS–DVB
n-Butyl acetate	741 449	nd	1 256 767	389 199
Ethyl pentanoate	685 150	142 884	1 131 478	377 010
2-Methyl-1-propanol	nd	nd	137 193	144 378
Isoamyl acetate	13 320 871	4 927 446	69 831 384	9 643 777
2-Methyl-1-butanol	300 623	429 176	1 793 343	382 064
Isoamyl alcohol	8 106 630	1 280 862	1 298 743	985 571
Ethyl hexanoate	285 053	110 611	991 095	263 157
3-Hydroxy-2-butanone	110 900	560 575	217 146	75 314
Hexyl acetate	188 871	nd	190 422	nd
Ethyl octanoate	nd	nd	83 250 208	nd
2-Furancarboxaldehyde	nd	nd	471 927	nd
Benzaldehyde	nd	82 808	2 507 785	103 910
α-Ionone	2 758 750	1 057 992	5 098 374	3 192 321
2,3-Butanediol	48 496	418 112	nd	nd
Ethyl decanoate	372 865	488 784	156 406	179 821
Isopentanoic acid	1 320 178	12 733 450	15 529 275	2 358 768
Diethyl succinate	493 934	1 001 939	2 604 369	874 332
Benzyl acetate	nd	107 428	370 059	157 222
Naphene	6 222 935	4 311 262	2 708 940	5 258 154
Ethyl-2-phenyl acetate	179 930	1 050 304	392 265	470 289
Phenylethyl acetate	8 493 113	16 517 997	14 210 520	22 305 078
β-Damascone	63 162	nd	nd	64 940
Hexanoic acid	303 818	2 027 888	4 227 833	746 227
Benzyl alcohol	nd	252 286	242 249	119 179
2-Phenylethanol	1 841 141	14 946 995	17 891 212	7 339 234
4-Ethylguaiacol	25 061	209 680	nd	141 694
Octanoic acid	6 174 323	11 409 719	7 501 608	9 212 661
4-Ethylphenol	nd	nd	176 358	524 864
Decanoic acid	3 268 041	3 317 553	395 361	2 950 195

Naphene: 1,2-dihydro-1,1,6-trimethyl naphthalene. nd, not detected.

These results can be shown by bar charts (Fig. 1). In these graphics the data are presented in chart form with the causes depicted in rank order. Extraction temperature and time, and NaCl concentration were significant parameters (at $P<0.05$) for both number of chromatographic peaks and total area. Sample volume was not significant. It is reported [14] that, as the sample volume increases, the extent of SPME initially increases rapidly and then remains relatively constant at larger volumes. Sampling temperature was the most influential variable that affected with a positive sign the values obtained for total area and number of peaks. The next most influential factors were sampling time for the total area and NaCl concentration for the number of peaks obtained, both of these with a positive effect.

The SPME efficiency is also affected by the interrelated variables, as shown in Fig. 1. The interaction between the factors sampling temperature and NaCl content appears statistically significant for both the total area obtained and the number of peaks detected. For this latter experimental response, the interaction between NaCl and sampling time was also significant.

3.2.2. Optimisation by a central composite design

As can be seen, the SPME technique is affected by interrelated parameters. For an optimisation design, it is advisable to keep the number of parameters as small as possible in order to avoid very complex response models and large variability. Since sample volume was not shown to have a significant influence on the considered responses (total chromatographic area and number of chromatographic peaks), in order

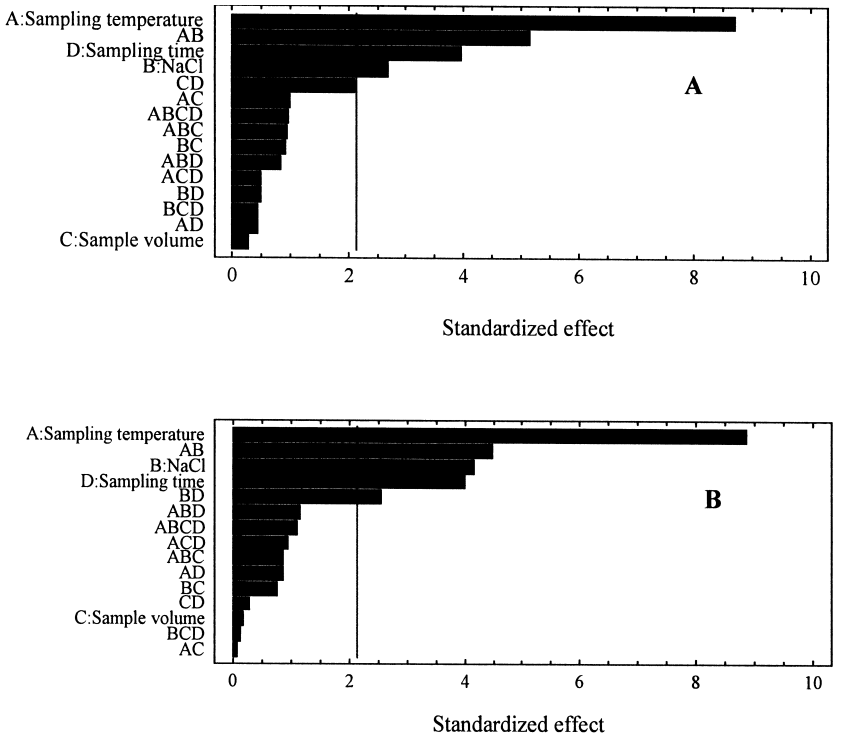

Fig. 1. Pareto chart of main effects in the factorial 2^4 design for total chromatographic peak area (A) and number of peaks (B).

to estimate curvature in response surfaces, we decided not to keep this factor. For the central composite design (CCD), the three parameters utilised were: sampling temperature and time, and NaCl concentration. The sample volume was set at 15 ml. The axial values for these parameters are located on a sphere surrounding the two-level factorial design (Table 1).

After the CCD, as expected from the screening experiments, the sample temperature appeared as statistically significant main effect, having a strong positive influence for both total area and number of chromatographic peaks (Table 3).

Sampling time and NaCl showed a significant influence on the number of chromatographic peaks detected whilst only sampling time was significant

Table 3
Main effects and interactions in the central composite design for number of chromatographic peaks and total area

Effect	Number of chromatographic peaks		Total area	
	F ratio	*P* value	*F* ratio	*P* value
A: Sampling temperature	183.68	0.0000*	21.10	0.0002*
B: NaCl	59.85	0.0006*	1.64	0.2139
C: Sampling time	65.15	0.0000*	10.22	0.0043*
AA	0.08	0.7845	0.50	0.4859
AB	44.59	0.0000*	11.68	0.0026*
AC	0.00	0.9469	0.26	0.6173
BB	1.12	0.3024	0.29	0.5961
BC	10.92	0.0034*	0.00	0.9728
CC	0.56	0.4619	0.55	0.4684

Values are significant at * $P<0.05$.

for the total area. For both experimental responses, the interaction between sampling temperature and NaCl concentration was statistically significant. The interaction between sampling time and NaCl was demonstrated to be significant for the number of chromatographic peaks. Overall, this means that the influence of one factor on the experimental responses depends on the values of the others. Figs. 2 and 3 show the response surface plots for the total area and the number of chromatographic peaks obtained by plotting sampling time vs. NaCl concentration and by plotting sampling temperature vs. NaCl.

At high temperature (60 °C), better experimental responses were obtained in line with increasing NaCl concentration. The interaction between sampling time and NaCl pointed out that a long sampling time produced the extraction of a higher number of volatile compounds when a high NaCl concentration was used.

These interactions seem logical. The CAR–PDMS fibre is coated with porous carbon which makes it

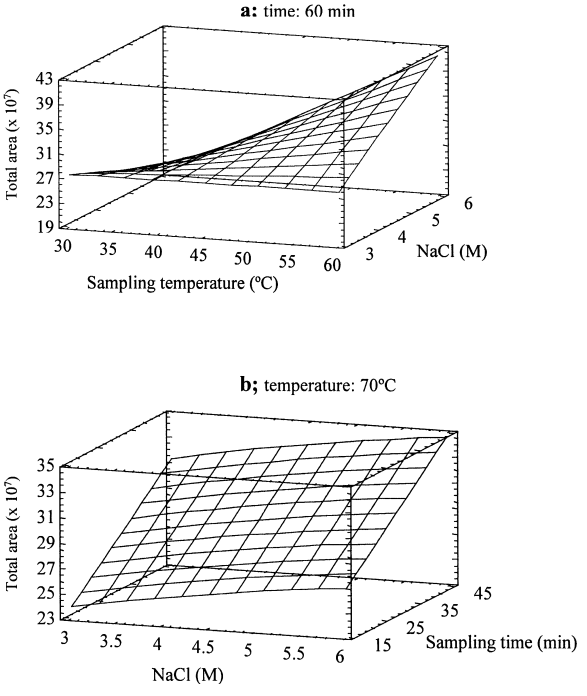

Fig. 2. Estimated response surfaces for total chromatographic area using the central composite design obtained by plotting (a) sampling temperature vs. NaCl concentration and (b) NaCl concentration vs. sampling time.

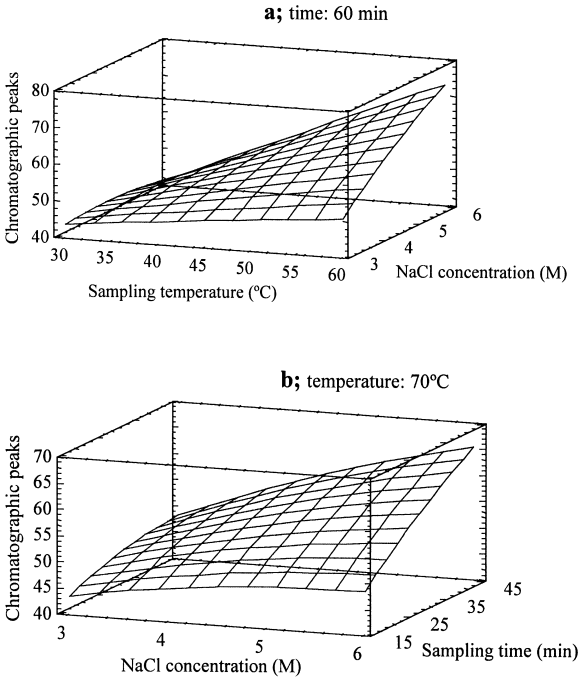

Fig. 3. Estimated response surfaces for number of chromatographic peaks detected using the central composite design obtained by plotting (a) sampling temperature vs. NaCl concentration and (b) NaCl concentration vs. sampling time.

suitable for analysing more or less volatile compounds at trace levels. Augusto et al. [16] found that this type of fibre has a higher efficiency than other fibres, especially for the less retained compounds. In headspace SPME, the amount of analyte absorbed in the fibre coating increases until equilibrium with the sample is reached. The time necessary to reach this equilibrium depends on the volatility of each aroma compound. Zhang et al. [24] pointed out that an increase in sampling temperature increases the headspace concentration of aroma compounds, favouring their extraction, but SPME involves an exothermic process and the extraction of analyte decreases as the temperature increases. For a complex sample, selection of an optimum temperature depends strongly on which analytes are of most interest. In our case, and taking into account that the type of fibre used has a high capacity of extraction, a high temperature increased the experimental responses studied. This could be due to an increase of less volatile compounds in the gas phase that might compensate for

the decrease of adsorption induced by this high temperature.

No decomposition of the vinegar sample was observed at high temperature.

In summary, after evaluation of the main factors and their interactions, the best conditions to extract the aromatic compounds of sherry wine vinegar were: sampling temperature 70 °C, sampling time 60 min, NaCl content 6.14 g, and sample volume 15 ml.

3.3. Repeatability and reproducibility

In previous studies it had been reported that the repeatability of this type of fibre was low [25,26]. In this work, the repeatability and reproducibility have been evaluated by means of a series of five extractions of a commercial sherry wine vinegar performed using three different fibres. The [aroma compound/internal standard] peak area ratios for the identified aroma compounds, with their relative standard deviation (RSD), were calculated. The RSD values obtained for each fibre ranges between 4 and 23% (i.e. 12.15% for 2-methyl-1-propanol; 7.47% for isoamyl acetate; 24.99% for caproic acid; 9.37% for 3-methyl-1-butanol). The inter-fibre accuracy showed RSD values higher than intra-fibre accuracy (9–35%). These high values would corroborate the low repeatability and reproducibility of this type of fibre.

3.4. Matrix effect

The SPME extraction efficiency depends on the matrix [23]. In wines, the ethanol content appears to interfere in the SPME technique [27–29]. Since acetic acid is one of the major constituents of vinegars, it may compete with the aroma compounds in the extraction. Besides, in wines and vinegars, we can find hundreds of other compounds that could possibly interfere in the SPME. Dufour and Bayonove [30] have suggested that polyphenols, main compounds in wines and wine vinegars, may interact with aroma compounds, reducing their vapour pressure in some cases.

To check these two sources of possible interference, the same amounts of aroma compounds (alcohols, esters and acids) were added to five synthetic vinegar samples with different acetic acid content and to five synthetic vinegar samples (80 g/l acetic acid) fortified with increasing amounts of gallic acid, 5-(hydroxymethyl)-2-furaldehyde, caffeic acid and *p*-coumaric acid, majority polyphenolic compounds in wine vinegars [22]. Three extractions were analysed for each of these synthetic samples. The data obtained show that the higher the acetic acid concentration, the lower the extraction efficiency (Table 4). Although the absolute areas decrease, the compound area/I.S. area ratio remains constant and the relative standard deviations are less than 20%, except

Table 4
Absolute peak areas obtained for synthetic vinegar samples with different acetic acid and polyphenolic content

Source of interference		Volatile compounds							
		Isoamyl acetate	Diethyl succinate	2-Methyl-1-propanol	Isoamyl alcohol	2-Phenyl-ethanol	Caproic acid	Octanoic acid	Decanoic acid
Acetic acid (g/l)	0	10 553 775	13 339 965	15 869 195	12 312 730	30 213 340	8 042 828	77 233 160	84 861 465
	25	7 761 813	8 072 058	11 643 811	7 724 687	27 353 970	5 531 054	66 816 275	79 916 050
	50	9 769 388	6 437 425	9 499 671	6 744 309	19 870 880	4 553 645	53 246 690	73 661 450
	75	6 702 894	4 545 174	8 248 665	5 285 381	15 249 440	3 617 921	37 596 730	63 694 445
	90	5 417 626	4 197 013	5 466 886	3 939 613	8 741 265	2 432 530	26 166 355	66 726 925
	Mean	1.57	1.37	1.87	1.29	3.80	0.90	9.79	15.94
	RSD (%)	19.80	13.21	9.22	8.51	15.20	9.32	8.05	42.91
Phenolic content	Mean	6 532 703	4 131 117	8 232 703	5 596 363	8 871 141	2 988 764	28 732 215	7 279 803
	RSD (%)	15.91	8.61	13.32	12.72	7.35	15.73	15.13	12.78

Mean values of the relative areas (peak area/internal standard area) with their relative standard deviations (in %).

for decanoic acid, an aroma compound with low volatility. In general, for quantitative analysis, the I.S. may be used, so the acetic acid concentration does not affect the analytical data.

For polyphenolic content, no interference was observed. Responses remain constant for all the analytes as the phenolic concentration in the model solution is increased (Table 4).

4. Conclusions

HS-SPME using the CAR–PDMS fibre is a good technique for determining the aroma compounds in vinegar. In our case, a high temperature increased the experimental responses studied, which could be due to an increase of less volatile compounds in the gas phase that might compensate the decrease of adsorption induced by this temperature. An increase in the acetic acid concentration decreases the extraction efficiency, but relative areas (compound area/internal standard area) remain constant, so for quantitative analysis, the I.S. may be used. The SPME extraction efficiency does not seem to be influenced by the polyphenols present in vinegar.

At this point, and having completed these studies just described, further research is required in order to determinate the analytical properties of the method (calibration, linearity, detection and quantification limits, analytical sensitivity, etc). This will be the aim of a new work in which the method, having been validated, will be applied to various samples of vinegars to study their aromatic profiles.

Acknowledgements

We thank Fernando García Rowe for the provision of vinegar.

References

[1] J.M. Quirós, Quad. Vitic. Enol. Univ. Torino 1 (1990) 115.
[2] A. Rapp, H. Hastrich, L. Engel, Vitis 15 (1976) 29.
[3] C.G. Edwards, R.B. Beelman, J. Agric. Food Chem. 38 (1990) 216.
[4] A.C. Noble, R.A. Flath, R.R. Forrey, J. Agric Food Chem. 28 (1980) 346.
[5] J.L. Wang, W.L. Chen, J. Chromatogr. A 927 (2001) 143.
[6] G.P. Blanch, G. Reglero, M. Herraiz, J. Agric. Food Chem. 43 (1995) 1251.
[7] G.P. Blanch, G. Reglero, M. Herraiz, J. Tabera, J. Chromatogr. Sci. 29 (1991) 11.
[8] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[9] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844.
[10] D. De la Calle García, S. Magnaghi, M. Reichenbächer, K. Danzer, J. High Resolut. Chromatogr. 19 (1996) 257.
[11] L. Urruty, M. Montury, J. Agric. Food Chem. 44 (1996) 3871.
[12] D. Louch, S. Motlagh, J. Pawliszyn, Anal. Chem. 64 (1992) 1187.
[13] D. De la Calle García, M. Reichenbächer, K. Danzer, J. High Resolut. Chromatogr. 21 (1998) 373.
[14] X. Yang, T. Peppard, J. Agric. Food Chem. 42 (1994) 1925.
[15] M. Jia, Q.H. Zhang, D.B. Min, J. Agric. Food Chem. 46 (1998) 2744.
[16] F. Augusto, A. Luiz, E. Tada, S. Rivellino, J. Chromatogr. A 873 (2000) 117.
[17] D. Picque, A. Norwand, G. Corrieu, Bioflavour 95 (1995) 117.
[18] S.E. Ebeler, M.B. Terrien, C.E. Butzke, J. Sci. Food Agric. 80 (2000) 625.
[19] Z. Zhang, J. Pawliszyn, Anal. Chem. 65 (1993) 1843.
[20] E. Morgan, Chemometrics: Experimental Design, Wiley, Chichester, 1991, pp. 197–256.
[21] R.G. Brereton, Chemometrics: Applications of mathematics and Statistics to Laboratory Systems, Ellis Horwood, Chichester, 1990.
[22] M.C. García Parrilla, F.J. Heredia, A.M. Troncoso, Food Res. Int. 32 (1999) 433.
[23] J. Pawliszyn, Solid-Phase Microextraction. Theory and Practice, Wiley–VCH, New York, 1997.
[24] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844.
[25] P. Popp, A. Paschke, Chromatographia 46 (1997) 419.
[26] M. Mestres, C. Sala, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 835 (1999) 137.
[27] M. Mestres, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 849 (1999) 293.
[28] C. Fisher, U. Fisher, J. Agric. Food Chem. 45 (1997) 1995.
[29] R.S. Whiton, B.W. Zoecklein, Am. J. Enol. Vitic. 51 (2000) 379.
[30] C. Dufour, C.L. Bayonove, J. Agric. Food Chem. 47 (1999) 678.