Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 93 (2005) 361–369
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Optimization of wine headspace analysis by solid-phase microextraction capillary gas chromatography with mass spectrometric and flame ionization detection

Lara Tat *, Piergiorgio Comuzzo, Iva Stolfo, Franco Battistutta

Sezione Industrie Agrarie, Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine, Via Marangoni, 97, 33100 Udine, Italy

Received 10 May 2004; received in revised form 2 November 2004; accepted 2 November 2004

Abstract

Different novel fibers for solid-phase microextraction were developed in the last years. The aims of this work were the study of their performances in wine headspace analysis, and the optimization of some analytical conditions. The fibers were evaluated for their sensitivity and repeatability; the results showed a strongly different behavior for the different solid-phases, both for the different zones of the chromatogram and for different levels of concentration. A Divinylbenzene/Carboxen/Polydimethylsiloxane fiber coating appeared the most suitable for the analysis of aromatic fraction of wines in its totality. For specific applications, the choice of a suitable solid-phase, depends on the class of compounds be analyzed.

Keywords: Solid-phase microextraction; Gas chromatography; Wine analysis; Repeatability; Sensitivity; Headspace analysis

1. Introduction

Solid-phase microextraction (SPME) was applied for the first time in 1989, to evaluate the presence of environmental pollutants in water (Belardi & Pawliszyn, 1989); this analytical technique found a widespread use in instrumental analysis for its versatility and simplicity.

At first SPME was used for the sampling by direct immersion into liquid matrixes, alternatively to liquid–liquid extraction (Zhang, Yang, & Pawliszyn, 1994), later it was employed also in headspace analysis (Zhang & Pawliszyn, 1993) for solid and liquid foodstuffs (Jelen, Wlazly, & Kaminski, 1998; Ruiz, Cava, Ventanas, & Jensen, 1998; Yang & Peppard, 1994), and for biological fluids (Cardinali, Ashley, Wooten, McCrow, & Lemire, 2000; Nishikawa et al., 1997).

This technique found several applications in wines headspace analysis: for the evaluation of single compounds (Hayasaka & Bartowsky, 1999; Mestres, Busto, & Guasch, 2002), for pollutants analysis (Evans, Buzke, & Ebeler, 1997; Gandini & Riguzzi, 1997; Rial-Otero, Yague-Ruiz, Cancho-Grande, & Simal-Gandara, 2002), and for aromatic characterization (De La Calle-Garcia et al., 1998; Mallouchos, Komaitis, Koutinas, & Kanellaki, 2002; Vas, Koteleky, Farkas, Dobò, & Vékey, 1998).

Because of the remarkable interest aroused by SPME, many authors began to consider the optimization of the analytical procedure; the study of the different factors conditioning the equilibrium between liquid and vapor phase in model solution was particularly considered; the effect of stirring into the sample during microextraction, the addiction of sodium chloride, the effect of temperature, the exposure time of the fiber, the volume of the sample, the chemical characteristics of the analytes (Rocha, Ramalheira, & Barros, 2001) and the ethanol

* Corresponding author. Tel.: +39 432 590 738; fax: +39 432 590 719.
E-mail address: lara.tat@uniud.it (L. Tat).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.11.025

concentration (Whiton & Zoecklein, 2000), were studied.

Different scientific publications reported the use of different solid-phases for microextraction: Polydimethylsiloxane fibers were used for the characterization of wines from different countries (Vas et al., 1998), Carbowax/Divinylbenzene (Whiton & Zoecklein, 2000) and Polyacrilate fibers (Rocha et al., 2001) were utilized for the optimization of the analytical conditions.

An evaluation of the performances of different fibers for the analysis of the aromatic fraction of wines, was performed by direct immersion in the sample (De La Calle-Garcia, Réichenbacher, & Danzer, 1997): the best results were obtained with an 85 μm Polyacrylate coating fiber.

Based on these statistics, the choice of a suitable solid-phase for microextraction turns out to be critical for the analysts; besides, in the last years, the availability of different new fibers in the trade opened an even wider choice range. Furthermore, wine headspace analysis is not only connected with the quantitation of few compounds; more often it is related to a wide screening of all volatile compounds, for the evaluation of the total aromatic pattern; so the choice of the suitable fiber becomes still more difficult for the extreme variety of the chemical characteristics of the analytes.

Therefore, the aim of this work was to evaluate the characteristics of different commercial fibers for SPME, to establish the most suitable for a characterization as wide as possible, of wine volatile fraction. Six different fibers were tested, some of them were recently appeared in the trade, and not reported in previous works. For the analytical evaluations, SPME sampling was coupled to GC–MS and GC–flame ionization detection (FID).

The evaluations concerned sensitivity and repeatability connected to the use of the different fibers, both in relation to the chemical characteristics of the analytes, and (only for repeatability) to their chromatographic area.

Some analytical and instrumental variables such as the minimization of the environmental pollutants, the time needed to the sample for reaching thermal equilibrium before microextraction, and the minimum time necessary for the desorption of the analytes from the solid-phase into the GC injector (to reduce the thermal damage for the fibers), were considered.

2. Materials and methods

2.1. Wine and model solutions

An Italian Chardonnay wine (D.O.C. Grave del Friuli, Italy) with a good aromatic intensity was used to evaluate the performances (repeatability and sensitivity) of the solid-phases.

The optimization of the chromatographic conditions was performed using a synthetic buffer solution (5 g/l tartaric acid, buffered at pH 3.2, 11% alcohol content v/v). Six concentrated aroma standards were diluted in this model wine: β-ionone (1 mg/l), ethyl caprylate (2 mg/l), caprylic acid (20 mg/l), linalool (0.4 mg/l), 2-phenylethanol (40 mg/l) and 3-methyl-1-butanol (200 mg/l). All chemicals were supplied by Sigma–Aldrich (St. Louis, MO, USA).

2.2. SPME sampling conditions

Different fibers for SPME available in the trade (Supelco, Bellefonte, PA, USA) were evaluated; the tested solid-phases are reported in Table 1.

Both wine samples and model solutions, were analyzed in 50-ml glass vials, filled with 40 ml of each sample. For SPME analyses, the vials were dipped in a glass interspaced beaker filled with distilled water and connected with a thermostatic water bath (Model BT10D, Gibertini, Milan, Italy); the water flowed from the thermostatic bath in the hollow space, heating the water inside the beaker and providing the vial with thermostatation.

The beaker was put over the plate of a magnetic stirrer and provided with a magnetic stirring bar moving synchronically with another one placed into the vial; the first bar supplied thermostatation water with movement, the second provided the sample with agitation.

Solid-phase microextraction was performed at 37 °C, for 15 min, and immediately followed by the desorption of the analytes into the gas chromatograph injector; the fiber remained into the injector for the whole period of the split-less time.

2.3. GC–FID and GC–MS analysis

GC–FID analyses were performed using a Carlo Erba (Milan, Italy) HRGC 8560 Mega Series 2 gas chromatograph equipped with a FID system. GC–MS analyses were carried out on a Varian (Palo Alto, CA, USA) 3400 gas chromatograph, coupled to a Varian Saturn ITDMS ion trap mass spectrometer.

Table 1
Description and identification code of the tested fibers

Description	Identification code
100 μm Polydimethylsiloxane[a]	PDMS
65 μm Polydimethylsiloxane/Divinylbenzene[a]	PDMS/DVB
85 μm Polyacrylate[a]	PA
75 μm Carboxen/Polydimethylsiloxane[a]	CARB
65 μm Carbowax/Divinylbenzene[a]	CW
50/30 μm Divinylbenzene/Carboxen/ Polydimethylsiloxane[b]	3F

[a] Length 1 cm.
[b] Length 2 cm.

Both the GC systems were provided with a split–splitless injection port, set in a temperature range of 240–260 °C, according to the maximum temperature recommended for the tested fibers. Helium was the carrier gas, at a linear flow rate of 28 cm/s.

Compounds were separated on an Econo-Cap Ec-Wax capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness), purchased from Alltech (State College, PA, USA). The column temperature was held at 40 °C for 5 min, and increased to 240 °C at 4 °C/min, with a final holding time of 15 min.

The FID temperature was set at 240 °C. For the MS system, the temperatures of the manifold and the transfer line, were 170 and 250 °C, respectively; electron impact mass spectra were recorded at 70 eV ionization voltage, and the ionization current was 10 μA.

The identification of compounds was carried out by comparison of mass spectra with those reported in the mass spectrum library Wiley 5, and it was confirmed comparing the order of elution with that reported by different authors (Baek & Cadwallader, 1999; Ferreira, Fernandez, Gracia, & Cacho, 1995; Jennings & Shibamoto, 1980; Lopez, Ferreira, Hernandez, & Cacho, 1999).

2.4. Optimization of the instrumental conditions

The thermal equilibrium is a fundamental factor for solid-phase microextraction; in fact, the equilibrium between the sample and the solid-phase, cannot be set up if the vial does not reach a constant temperature. For this reason, it is important to know the minimum time necessary to the liquid and to the headspace to change from the storage – room temperature to the operating temperature.

This parameter was determined by measuring the inner temperature of the vials (both liquid and vapor phase) in two different conditions: 12 and 37 °C (temperature of the water bath); a needle connected with a thermocouple was used for the measurements, piercing the septa during equilibration.

This operation was repeated for different liquid volumes and different dimensions of the vial (different ratio between liquid and headspace volume): 50 ml vials filled with 40 ml of wine; 100 ml vials filled with 80 ml of wine; 150 ml vials filled with 80 ml of wine.

SPME is a very sensitive technique, and it is easily affected by the presence of odorants and other pollutants in the environment; these unidentified volatile compounds, can affect both qualitative and quantitative analysis, interfering with the chromatographic response.

The presence of environmental pollutants was evaluated sampling the headspace of empty closed vials by SPME, and analyzing it by GC. Different ways were tested to minimize the pollution: the first one was a simple heating of both vials and septa at 70 °C overnight, followed by their storage into desiccators until sample introduction (to avoid further pollutions); a second test was simply performed blowing nitrogen into the vial to eliminate the environmental air before the sample introduction.

Finally, different desorption times into the GC injector were considered for the tested fibers using model solutions; this study was performed to determine the minimum time necessary for the total liberation of the analytes, without thermal damage to the solid-phase. Different split-less times were tested: 0, 30, 70, 180 and 300 s; all trials were replicated three times.

2.5. Evaluation of sensitivity and repeatability of the different fibers

Sensitivity was evaluated on the basis of a parameter named "cumulative area"; it was calculated as reported in Fig. 1. All the calculations were related to the absolute areas of the total number of the detected compounds (both identified and unidentified).

An "average area" (Av_k) was determined for each compound; it was the average of the peak areas determined by the evaluation of the same compound with the different fibers (step 1, Fig. 1).

The following step (step 2, Fig. 1) was a normalization procedure of the data obtained with each fiber: normalized area [$NA_{k(X)}$] was calculated by the ratio between the area of the single compound [$A_{k(X)}$] and its average area (Av_k); normalization was necessary to avoid oscillations in the cumulative area values, due to the high concentrated compounds.

Cumulative area [$CA_{k(X)}$] was the sum of the normalized area calculated for each compound, and the normalized areas of the compounds previously eluted (step 3, Fig. 1); so the cumulative area gradually increased with retention time, by a value corresponding to the normalized area of a single compound. The higher was the sensitivity of the fiber, the higher was the cumulative area, at the same retention time.

As regards repeatability evaluation, it should be said that not all the fibers are able to reveal the totality of compounds; so only the analytes which were detectable with all tested fibers (identified and unidentified) were considered for the calculations.

Relative standard deviations (RSD) were calculated for all the compounds detected by every single fiber; their average value and standard deviation were calculated fiber-by-fiber. These averages were compared as repeatability index and the standard deviations represent RSD variations among the compounds absorbed by a single fiber.

Repeatability is often affected by GC area intensity; for this reason, the calculations were carried out not only as reported previously (totality of compounds), but also grouping the detected compounds in different

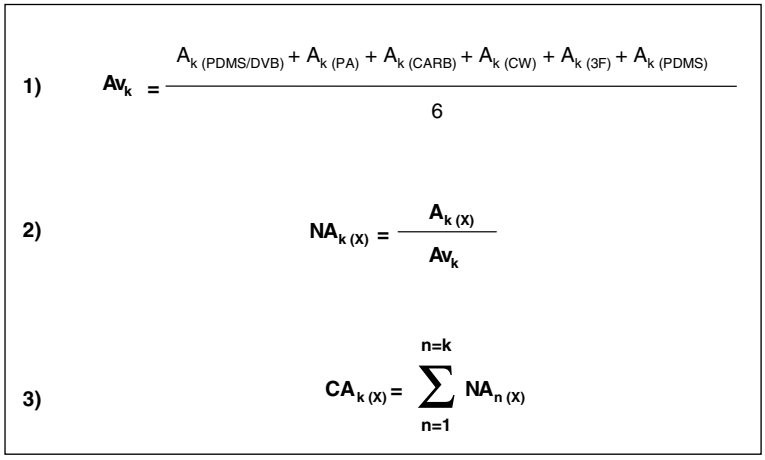

Fig. 1. Steps for the calculation of "cumulative area". AV_k: average area of the compound "k" determined with all the fibers; $A_{k(X)}$: absolute area of the compound "k" determined with the fiber "X"; $NA_{k(X)}$: normalized area of the compound "k" determined with the fiber "X"; $CA_{k(X)}$: cumulative area of the compound "k" determined with the fiber "X".

classes of peak area, on the basis of the chromatographic response; the two classes in the extremes were considered: compounds with absolute area lower than 5×10^4 were included in area class 1, while those with absolute area higher than 6×10^7 were reported in class 2. For all the repeatability evaluations, SPME-GC headspace analysis was replicated five times (five different vials) with each fiber.

2.6. Statistical analysis

A one-way Analysis of Variance (ANOVA) was carried out on the absolute areas, as concerned the evaluation of the optimum split-less time; averages and standard deviations were calculated, and significant differences were evaluated by Tukey Honest Significant Difference (HSD) Test, using Statistica Base Module for Windows, Version 6.0. Variances were homogeneous according to Levene and Brown–Forsyte Tests.

3. Results and discussion

3.1. Evaluation of sensitivity and repeatability of different fibers

Sensitivity of different fibers was evaluated in relation to cumulative areas of the analytes; 79 compounds were detected, and the results are reported in Fig. 2.

The graph exhibits the chromatographic response of the different fibers depending on the retention time, therefore on the analytes volatility and polarity; the wider was the cumulative area, the higher was the fiber sensitivity, at the same retention time.

The best results was observed for 3F and for CARB; particularly CARB shows a better performance as concerns more volatile and less polar compounds. The higher response of CARB turns out to be detectable up to a retention time of nearly 15 min; after this value, the 3F shows the best performances, with an increase in sensitivity that appears related to a retention time within a range of 40–60 min (Fig. 3).

However the higher sensitivity detected for CARB in the first zone of the chromatogram is connected to a low resolution of the peaks (Fig. 4); for this reason, the use of this solid-phase, could be not suitable for wine analyses.

The other fibers in Fig. 2 show a strongly lower sensitivity than 3F; particularly PDMS was the less efficient for headspace analysis of wine, that is why no subsequent analyses were run for PDMS in this work.

To evaluate repeatability, the average and standard deviations of the RSD (determined as reported previously) were considered. The evaluation considered 34 compounds (identified and unidentified), detected with all the tested fibers; the RSD values determined for every single compound are available in Table 2.

The average value determined for these coefficients, on a fiber-by-fiber basis, is reported in Fig. 5, together with their standard deviation: this shows how RSD varies between the different compounds absorbed by the same fiber.

In the figure, CW presents the lowest RSD (lower than 13%), while PA and PDMS/DVB show the worst repeatability due to the highest average and standard deviation; 3F and CARB are in an intermediate position respect to the other fibers for average RSD values, and they show the lowest standard deviation; particularly, 3F seems to be the best situation.

These performances were also confirmed analyzing single classes of compounds (esters, fatty acids, alcohols), either for sensitivity or repeatability; previous works confirm that 3F is the most versatile fiber, as it is the most sensitive for all different chemical classes of analytes (Stolfo, 2002).

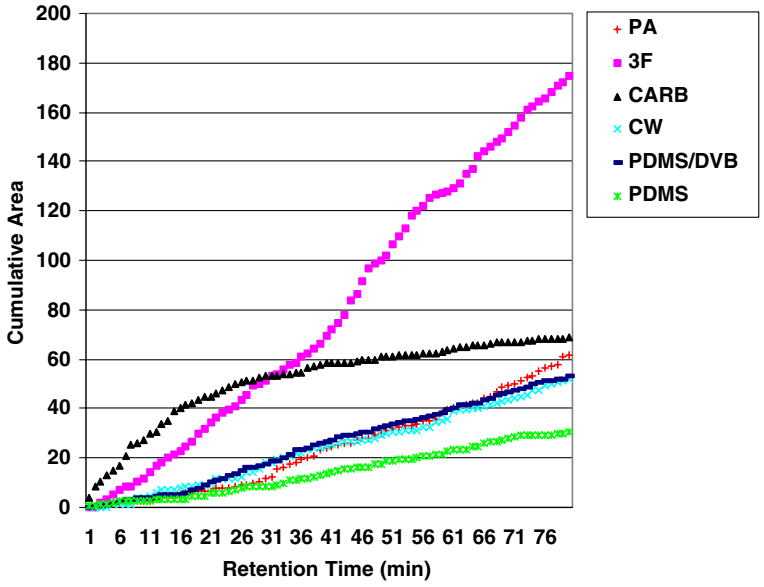

Fig. 2. Cumulative area vs. retention time: sensitivity of the tested fibers at different retention times. Data obtained by SPME-GC–FID analysis.

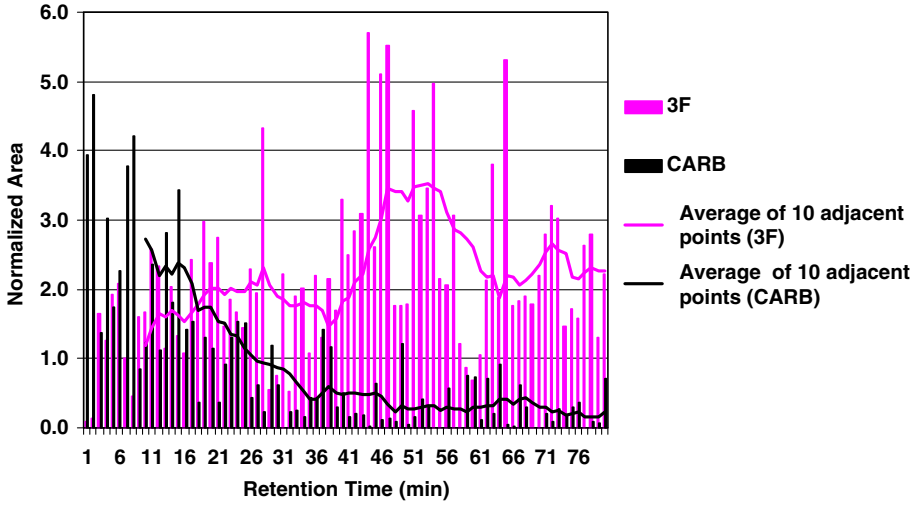

Fig. 3. Sensitivity of 3F and CARB fibers at different retention times. Data obtained by SPME-GC–FID analysis.

Anyway, the other tested fibers are usable as a second choice for the analysis of single classes of compounds; particularly, PDMS/DVB shows good performances for esters, while PA can be used for more polar compounds such as acids and alcohols; eventually, the high sensitivity of CARB for less volatile compounds could well be used by injecting in split mode, in order to optimize peak resolution.

Repeatability comparisons for classes of area are reported in Fig. 6. The compounds in class 2 show a lower RSD average and standard deviation, than compounds in class 1; this fact appears logical, because the maximum analytical uncertainty is related to the compounds with smaller peak area. In this case, CARB presents the lowest repeatability, as well as the highest standard deviation, while CW seems to produce the best performances, confirming what has been said about all of the compounds (Fig. 5); the other fibers show an intermediate trend.

Therefore, generally speaking, CW seems to be the most performing fiber for repeatability, even if it is the less sensitive; consequently, it will be evaluated every time, according to the needs, the convenience of using a sensitive but less repeatable fiber, or vice versa.

3.2. Instrumental optimization of the methodology

The time needed for thermal equilibration of the sample, was not affected by the volumetric liquid–vapor ratio, within the considered range. A time of 15 min is enough to allow the sample into the vial to reach the water-bath temperature (in the employed conditions); on this basis, the

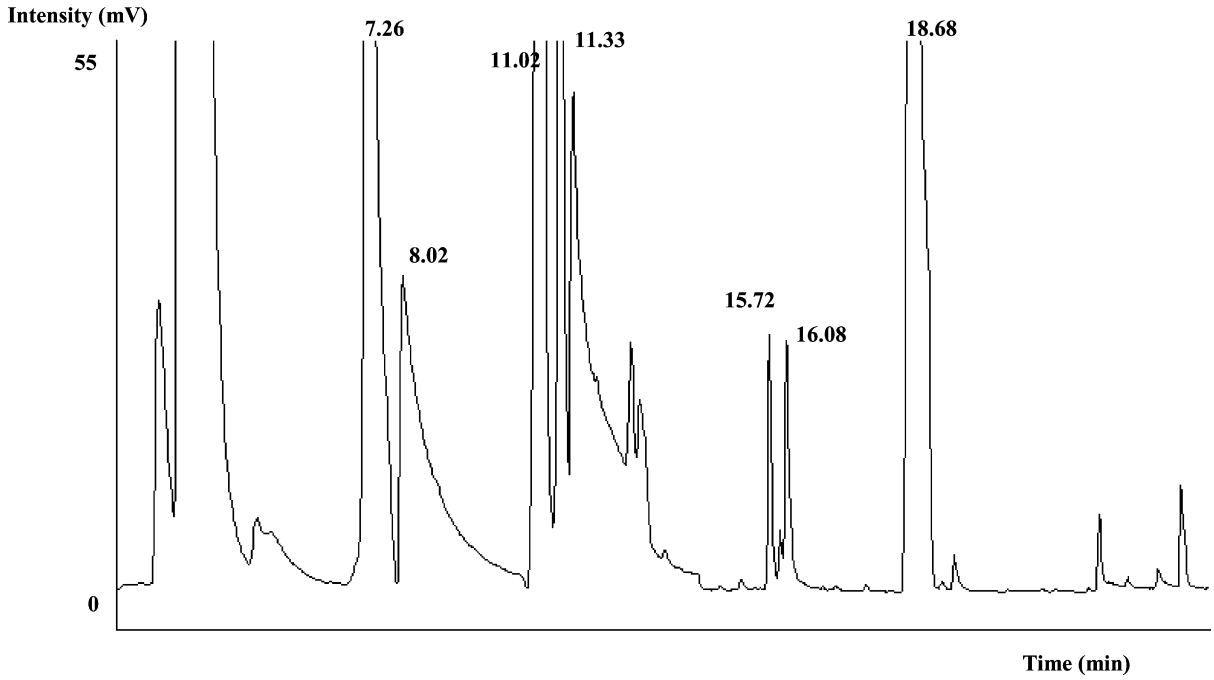

Fig. 4. Chromatogram of a white wine headspace (SPME-GC–FID analysis) sampled with a Carboxen/Polydimethylsiloxane (CARB) fiber coating; retention times (in minutes): 7.26 isoamyl acetate; 11.02 2- and 3-methyl-1-butanol; 11.33 ethyl hexanoate; 18.68 ethyl caprylate; 15.72 ethyl lactate; 16.08 1-hexanol.

vials were pre-conditioned for 15 min (both for wine and model solutions) before microextraction.

As regards the minimization of environmental pollution, we observed that the heating of the vials in steaming room before the sample introduction, was not enough to avoid the presence of environmental volatiles in the sample; sampling the headspace of an empty vial, the baseline of the chromatogram was always slightly disturbed.

Better results were obtained by blowing nitrogen into the vial, before closing it, confirming that probably the environmental air remains into the vial itself despite heating into the steaming room.

According to this facts, we adopted the following procedure to analyze the samples: nitrogen flow inside the vial, before filling up, and laminar flow at the neck, during filling in.

The evaluation of optimum conditions for desorption of the analytes in the injector was referred to the only use of 3F, on the basis of the previous observation about sensitivity and repeatability.

Fig. 7 shows the data collected for each tested split-less time, for ethyl caprylate and 2-phenylethanol in model solution. Particularly the split injection mode revealed significant differences compared to the split-less conditions; a split-less time of 70 s was enough to reach the maximum values of absolute area. It was therefore considered the best compromise between the minimization of thermal damage to the fiber and the complete release of the analytes into the injector.

4. Conclusions

In conclusion, the performances of some new fibers for SPME recently available, and not investigated before, seem to be interesting for headspace-GC analysis of wine volatile constituents.

As regards the evaluation of sensitivity, results showed that not all the tested fibers have the same characteristics: the best performances seemed to be noticeable for Divinylbenzene/Carboxen/Polydimethylsiloxane (3F) fiber coating. Carboxen/Polydimethylsiloxane (CARB) is appeared very sensitive for the most volatile compounds, but the high sensitivity in the initial section of the chromatogram was associated to a bad resolution of the peaks.

As for repeatability, in relation to the totality of the compounds, Divinylbenzene/Carboxen/Polydimethylsiloxane (3F) represented once more the best compromise between the RSD average and standard deviation.

As concerns the different classes of area, the highest analytical uncertainty weighs over the compounds with low peak area: among these, Carboxen/Polydimethylsiloxane (CARB) showed the maximum RSD average and the highest standard deviation, while Carbowax/Divinylbenzene (CW) gave the best performances.

Therefore, the fiber coating that seemed to be the best compromise to analyze the aromatic fraction of wine, turned out to be Polydimethylsiloxane/Carboxen/Divinylbenzene.

Table 2
Repeatability evaluation: relative standard deviations obtained with five fiber coatings by means of SPME-GC–FID analysis

		RSD (%)				
		PDMS/DVB	PA	CARB	CW	3F
1	Isoamyl acetate and 2-methyl-1-propanol[a]	4.2	3.3	8.6	4.8	5.1
2	2- and 3-Methyl-1-butanol[a]	4.3	3.6	7.0	3.6	7.4
3	Ethyl hexanoate	6.1	5.5	17.5	2.7	13.3
4	4-Methyl-1-pentanol	6.4	16.1	14.4	8.1	9.2
5	3-Methyl-1-pentanol	19.9	14.1	5.3	13.4	12.7
6	Ethyl lactate	2.7	3.2	3.9	2.4	4.2
7	1-Hexanol	3.5	3.8	2.6	1.8	4.5
8	Ethyl octanoate	14.5	7.7	20.4	5.5	11.7
9	Acetic acid and 1-heptanol[a]	19.5	4.1	12.1	14.7	6.6
10	Unknown	8.9	8.8	25.3	40.3	2.3
11	2-Methyltetrahydrothiophene	12.4	9.1	9.6	8.9	11.8
12	2,3-Butanediol	10.7	20.0	13.8	8.9	34.7
13	1-Octanol	4.8	12.3	9.0	18.4	6.1
14	2-Methylpropanoic acid	5.2	11.4	8.9	11.0	18.2
15	Cyclohexanol	17.7	17.1	6.6	12.1	24.2
16	γ-Butyrolactone and ethyl decanoate[a]	18.2	12.2	18.9	9.5	15.3
17	3-Methylbutyl octanoate	21.5	12.5	35.5	12.3	17.4
18	Unknown	8.5	12.8	16.8	14.4	12.3
19	2- and 3-Methylbutanoic acid[a]	3.4	9.3	4.3	4.9	2.6
20	Diethyl succinate	11.7	8.1	12.7	1.3	10.9
21	Unknown	16.5	13.2	18.8	9.5	16.6
22	3-(methylthio)-1-propanol	21.4	14.4	7.5	4.3	13.9
23	β-Citronellol and 1-decanol[a]	7.7	14.5	15.2	6.4	8.0
24	2-Phenylethyl acetate	13.2	12.5	12.2	3.0	11.7
25	Ethyl dodecanoate	28.1	14.9	23.1	21.7	19.3
26	Hexanoic acid	9.4	16.8	8.5	6.9	9.3
27	*N*-(2-Methylpropyl)-acetamide	27.4	15.9	16.8	22.6	21.3
28	3-Methylbutyl decanoate	45.7	10.9	16.4	11.3	16.7
29	Isoamyl succinate	11.2	14.6	19.7	6.4	15.2
30	2-Phenyethanol	9.4	14.7	11.5	2.3	13.3
31	Unknown	7.5	130.8	15.0	4.3	24.1
32	Unknown	34.1	24.2	15.9	41.9	20.0
33	Octanoic acid	18.5	17.5	15.1	13.4	13.9
34	Decanoic acid	18.3	11.7	19.1	17.4	13.9

[a] Coeluted in the chromatographic conditions.

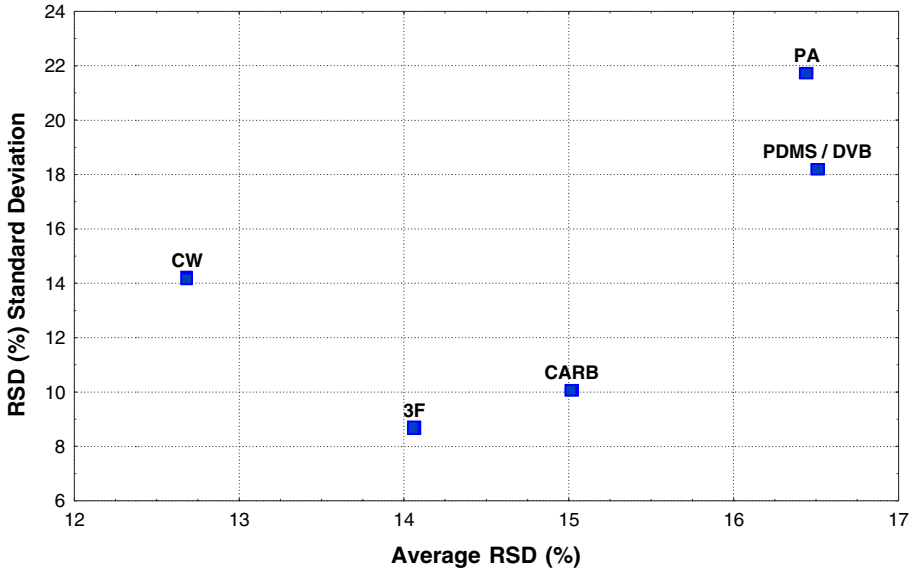

Fig. 5. Repeatability of the tested fibers; average vs. standard deviation of RSD of all wine compounds. Data obtained by SPME-GC–FID analysis.

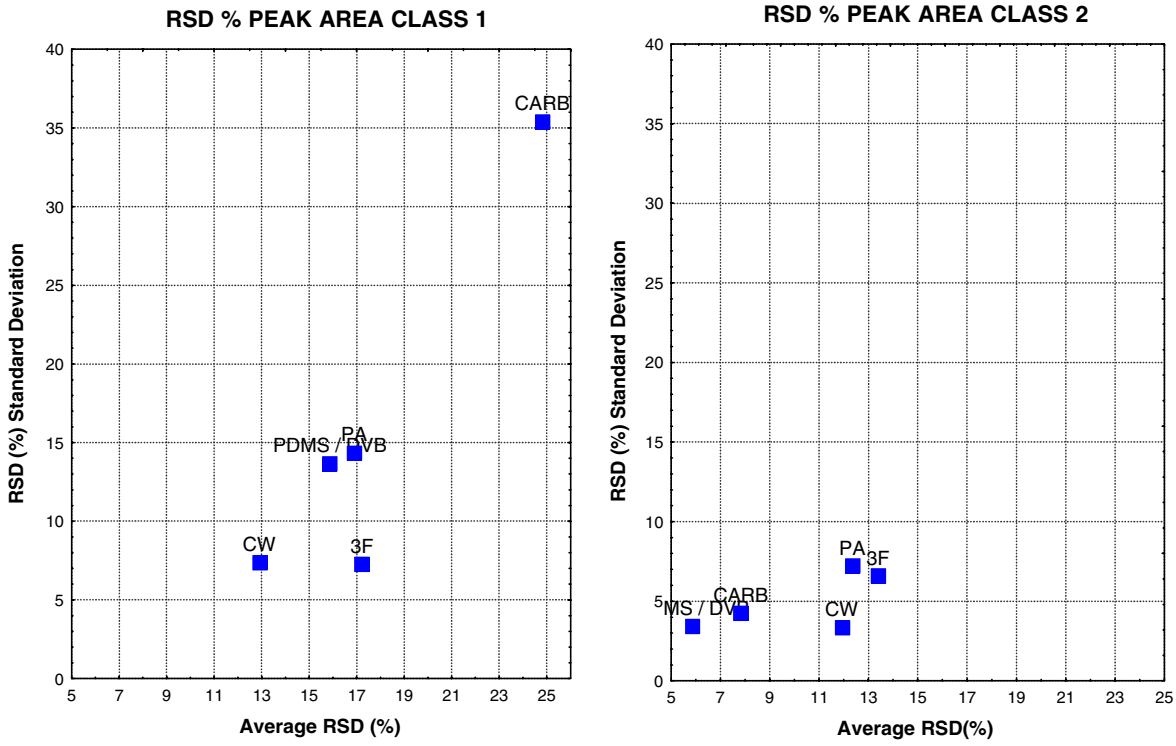

Fig. 6. Repeatability of the tested fibers for different classes of peak area; average vs. standard deviation of RSD of all wine compounds. Data obtained by SPME-GC–FID analysis. Class 1: compounds with absolute area lower than 5×10^4. Class 2: compounds with absolute area higher than 6×10^7.

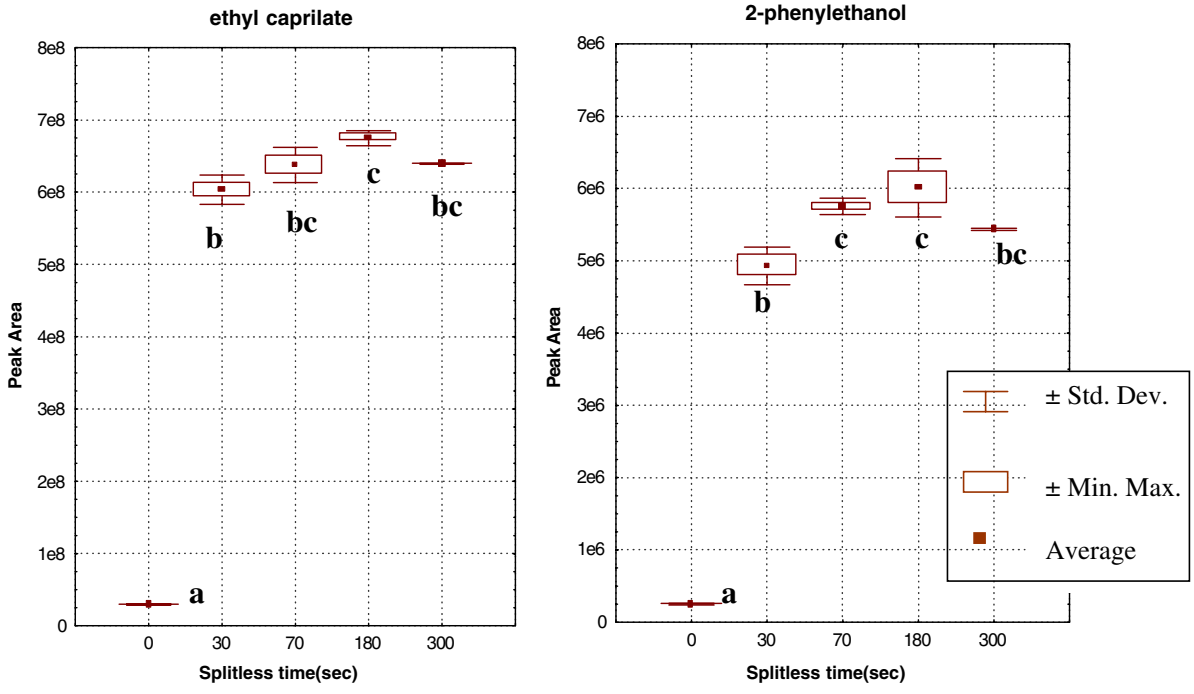

Fig. 7. Effect of split injection and different split-less times on the peak area of some aroma compounds in wine-like solution. Data obtained by SPME-GC–FID analysis. Different letters represent means which are significantly different at $p < 0.05$.

On the other hand, for specific applications (e.g., evaluation of single classes of compounds, or particular repeatability needs), it must be considered that repeatability and sensitivity are not always strictly related: i.e., the most repeatable fibers are not always the most sensitive ones; it should be evaluated each time the con-

venience of using a more sensitive but less repeatable fiber, or vice versa; the choice of a suitable solid-phase depends also on which class of compounds needs to be to analyzed.

The time necessary for thermal equilibration of the sample did not seem to be conditioned from the liquid–vapor volumetric ratio, within the considered range; a time of 15 min was enough for the sample equilibration.

To minimize the presence of possible pollutants of environmental origin, blowing nitrogen into the vial before closing it, gave better results compared to heat it in steaming room.

Finally, an optimum split-less time of 70 s, was the best compromise between the minimization of thermal damage to the fiber and the complete release of volatile compounds in the injector.

Acknowledgements

The authors are grateful to Drs. Lorena Pizzale and Stefania Vichi for their advice, and to Prof. Lanfranco S. Conte and Dr. Stefano Buiatti, for the revision of the manuscript.

References

Baek, H. H., & Cadwallader, K. R. (1999). Contribution of free and glycosidically bound volatile compounds to the aroma of muscadine grape juice. *Journal of Agricultural and Food Chemistry, 64*, 441–444.

Belardi, R. P., & Pawliszyn, J. B. (1989). The application of chemically modified fused silica fibres in the extraction of organics from water matrix samples and their rapid transfer to capillary columns. *Water Pollution Research Journal of Canada, 24*, 179–191.

Cardinali, F., Ashley, D. L., Wooten, J. W., McCrow, J. M., & Lemire, S. W. (2000). The use of solid-phase microextraction in conjunction with a banchtop quadrupole mass spectrometer for the analysis of volatile organic compounds in human blood at the low parts-per-trillion level. *Journal of Chromatographic Science, 38*, 49–54.

De La Calle-Garcia, D., Réichenbacher, M., & Danzer, K. (1997). Investigations on wine bouquet components by solid-phase microextraction capillary gas chromatography (SPME-CGC) using different fibers. *Journal of High Resolution Chromatography, 20*, 665–668.

De La Calle-Garcia, D., Réichenbacher, M., Danzer, K., Hurlbeck, C., Bartzsch, C., & Feller, K. H. (1998). Use of solid-phase microextraction-capillary-gas chromatography (SPME-CGC) for the varietal characterization of wines by means of chemiometrical methods. *Fresenius Journal of Analytical Chemistry, 360*, 784–787.

Evans, T. J., Buzke, C. E., & Ebeler, S. E. (1997). Analysis of 2,4,6-trichloroanisole in wines using solid-phase microextraction coupled to gas chromatography–mass spectrometry. *Journal of Chromatography A, 786*, 293–298.

Ferreira, V., Fernandez, P., Gracia, J. P., & Cacho, J. F. (1995). Identification of volatile constituents in wines from *Vitis vinifera* var. Vidadillo and sensory contribution of the different wine flavour fractions. *Journal of the Science of Food and Agriculture, 69*, 299–310.

Gandini, N., & Riguzzi, R. (1997). Headspace solid-phase microextraction analysis of methylisothiocyanate in wine. *Journal of Agricultural and Food Chemistry, 45*, 3092–3094.

Hayasaka, Y., & Bartowsky, E. J. (1999). Analysis of diacetyl in wine using solid-phase microextraction combined with gas chromatography–mass spectrometry. *Journal of Agricultural and Food Chemistry, 47*, 612–617.

Jelen, H. H., Wlazly, K., & Kaminski, E. (1998). Solid phase microextraction for the analysis of some alcohols and esters in beer: comparison with static headspace method. *Journal of Agricultural and Food Chemistry, 46*, 1469–1473.

Jennings, W., & Shibamoto, T. (1980). *Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography*. New York: Academic Press.

Lopez, R., Ferreira, V., Hernandez, P., & Cacho, J. (1999). Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: a comparative study. *Journal of the Science of Food and Agriculture, 79*, 1461–1467.

Mallouchos, A., Komaitis, M., Koutinas, A., & Kanellaki, M. (2002). Investigation of volatiles evolution during the alcoholic fermentation of grape must using free and immobilized cells with the help of solid-phase microextraction. *Journal of Agricultural and Food Chemistry, 50*, 3840–3848.

Mestres, M., Busto, O., & Guasch, J. (2002). Application of headspace solid-phase microextraction to the determination of sulphur compounds with low volatiliti in wines. *Journal of Chromatography A, 945*, 211–219.

Nishikawa, M., Seno, H., Ishi, A., Suzuki, O., Kumazawa, T., Watanabe, K., et al. (1997). Simple analysis of diphenylmethane anti histaminics and their analogues in bodily fluids by headspace solid-phase microextraction-capillary gas chromatography. *Journal of Chromatographic Science, 35*, 275–279.

Rial-Otero, R., Yague-Ruiz, C., Cancho-Grande, B., & Simal-Gandara, J. (2002). Solid-phase microextraction-gas chromatographic–mass spectrometric method for the determination of the fungicides cyprodinil and fludioxonil in white wines. *Journal of Chromatography A, 942*, 41–52.

Rocha, S., Ramalheira, V., & Barros, A. (2001). 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. *Journal of Agricultural and Food Chemistry, 44*, 5142–5151.

Ruiz, J., Cava, R., Ventanas, J., & Jensen, M. (1998). Headspace solid phase microextraction for the analysis of volatils in a meat product: dry-cured Iberian ham. *Journal of Agricultural and Food Chemistry, 46*, 4688–4694.

Stolfo, I. (2002). Ottimizzazione del metodo di microestrazione in fase solida per l'analisi dei vini: un esempio di applicazione. Tesi di Laurea, Università di Udine.

Vas, G. Y., Koteleky, K., Farkas, M., Dobò, A., & Vékey, K. (1998). Fast screening method for wine headspace compounds using solid-phase microextraction (SPME) and capillary GC technique. *American Journal of Enologly and Viticulture, 49*, 100–104.

Whiton, R. S., & Zoecklein, B. W. (2000). Optimization of headspace solid-phase microextraction for analysis of wine aroma compounds. *American Journal of Enologly and Viticulture, 51*, 379–382.

Yang, X., & Peppard, T. (1994). Solid Phase Microextraction for flavors analysis. *Journal of Agricultural and Food Chemistry, 42*, 1925–1930.

Zhang, Z., & Pawliszyn, J. (1993). Headspace solid-phase microextraction. *Analytical Chemistry, 65*, 1843–1852.

Zhang, Z., Yang, M. J., & Pawliszyn, J. (1994). Solid-phase microextraction. *Analytical Chemistry, 66*, 844–853.