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Influence of fibre coating in headspace solid-phase microextraction–gas chromatographic analysis of aromatic and medicinal plants

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Abstract

Solid-phase microextraction (SPME) is a solvent-free technique, which is well established in headspace analysis since it is sensitive, because of the concentration factor achieved by the fibres, and selective, because of different coating materials which can be used. The performance of eight commercially available SPME fibres was compared to evaluate the recoveries of some characteristic components with different polarities and structures present in the headspace of four aromatic and medicinal plants: rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and valerian (*Valeriana officinalis* L.). The relative concentration capacity of each fibre on the same components of each plant was also determined by comparing their abundance with that obtained by classical static-headspace GC. The partition coefficient, K_1, between the headspace gaseous phase and SPME polymeric coating, and the relative concentration factors, of some of the characteristic components of the plant investigated dissolved in dibutyl phtalate, were also determined, under rigorously standardised analysis conditions. The results showed that the most effective fibres were those consisting of two components, i.e., a liquid phase (polydimethylsiloxane) and a porous solid (carboxen or divinylbenzene, or both). © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Headspace analysis; Solid-phase microextraction; Plant materials

1. Introduction

Solid-phase microextraction (SPME) is a successful solvent-free sampling technique. The analytes from the liquid or gaseous sample are directly absorbed onto an absorbent-coated fused-silica fibre, which is part of the syringe needle, and then either thermally desorbed directly into a gas chromatography (GC) injection port [1–4] or solvent desorbed into an high-performance liquid chromatography (HPLC) injection valve [5]. SPME was originally developed for the analysis of pollutants, in particular for environmental water samples; because of its effectiveness, its use was subsequently extended to several other fields [4], in particular flavour analysis ([6–18], and references cited therein). Characterisation of the volatile fraction of aromatic plants by SPME was less studied [19–22].

Headspace sampling is a fundamental technique to characterise the volatile fraction of aromatic and medicinal plants. Headspace SPME sampling (HS-SPME) [23] is a sort of bridge between static and dynamic headspace (S-HS–GC and D-HS–GC) because it is as reproducible and easy to automate as is S-HS–GC; at the same time, it is also sensitive, because of the concentration factor achieved by the fibre, and selective, because of different coating materials available, as is D-HS–GC. In HS-SPME,

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the concentration factor of an analyte depends on its structure and volatility, on the physico–chemical characteristics of the absorbing fibre and analyte/fibre affinity and also on some physical factors such as matrix agitation, headspace equilibration temperature and time, and analyte diffusion rate from the vapour phase to the fibre surface [23]. The nature of the fibre coating therefore strongly conditions the effectiveness of HS-SPME sampling and the fibre should be chosen in function of the analytes to be determined. Some authors have already systematically compared the performance of some of the fibres commercially available for specific applications, among others Miller et al. [19] who compared polydimethylsiloxane 7 μm (PDMS 7), polydimethylsiloxane 100 μm (PDMS 100) and polyacrylate 85 μm (PA 85) fibre coatings in an in-depth study to classify the botanical origin of cinnamon. Coleman III and Lawson [22] compared six fibres to characterise menthol of different origin by HS-SPME–GC–MS and found that Carboxen–polydimethylsiloxane 65 μm (CAR–PDMS 65) coating was the most effective. De la Calle Garcia et al. [24] investigated wine bouquet components by immersion-SPME–GC with six fibres and found that 85 μm polyacrylate (PA 85) fibre was the most effective for the determination of terpene profile. Very recently, Miller and Stuart [18] compared traditional S-HS–GC with HS-SPME–GC with five fibres for the analysis of various fruit juice and found that PDMS–divinylbenzene 65 μm (PDMS–DVB 65) was the most effective at extracting the flavour volatiles. To the best of the authors' knowledge, the difference in fibre performance has not yet been systematically investigated in the aromatic and medicinal plant field. This article aims to compare the performance of eight commercially available SPME fibres (Table 1) through some characteristic components of the headspace of four aromatic and medicinal plants: rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and valerian (*Valeriana officinalis* L.). The relative concentration capacity of each fibre on the same components of each plant was also determined by comparing their abundance with that obtained by S-HS–GC.

2. Experimental

2.1. Plant material

Homogeneous powdered samples of dried rosemary leaves (*Rosmarinus officinalis* L.), sage leaves (*Salvia officinalis* L.), thyme plant (*Thymus vulgaris* L.) kindly supplied by Allione Industria Alimentare (Tarantasca, Cuneo, Italy) and valerian roots (*Valeriana officinalis* L.) from experimental plantations (Ministero per le Politiche Agricole, Italy, research project ''Incremento Produzione Piante Officinali'') of the I.S.A.F.A., Villazzano, Trento, Italy were used. A standard solution about 0.06 *M* of α-pinene, 1,8-cineol, terpinen-4-ol, α-terpineol, camphor, bornyl acetate, thymol, β-caryophyllene and viridiflorol in dibutyl phtalate was also prepared.

2.2. Sample preparation

A series of experiments by S-HS–GC were made to select the most suitable temperature and equilibra-

Table 1
List and volume of the coating of the fibres investigated in this study

No.	Fibre composition	Acronym	Volume of the coating (μl)
1	Polydimethylsiloxane 7 μm	PDMS 7	0.026
2	Polydimethylsiloxane 30 μm	PDMS 30	0.132
3	Polydimethylsiloxane 100 μm	PDMS 100	0.612
4	Carbowax–divinylbenzene 65 μm	CW–DVB 65	0.357
5	Carboxen–PDMS 75 μm	CAR–PDMS 75	0.436
6	Polyacrylate 85 μm	PA 85	0.521
7	Polydimethylsiloxane–divinylbenzene 65 μm	PDMS–DVB 65	0.357
8	Carboxen–divinylbenzene–polydimethylsiloxane 50/30 μm (2 cm)	CAR–DVB–PDMS	1.000

tion time to obtain a significant headspace fraction. The same conditions were applied to HS-SPME–GC to standardise sample preparation procedure and to make the results comparable.

A 0.6-g amount of each investigated plant was hermetically sealed in a 12.5-ml vial and equilibrated for 1 h in the thermostatic bath of the HS injector at 60°C before injection.

A 50-μl volume of the 0.06 *M* standard solution in dibutyl phatlate was diluted to 1 ml with dibutyl phtalate in a 12.5-ml vial. The resulting solution (3.0 m*M*) was equilibrated as reported above.

2.3. S-HS–GC analysis

A Carlo Erba HS-250 automatic HS injection system assembled on a Carlo Erba Mega 5360 GC unit was used. A 1-ml volume of the vapour phase was directly injected into the GC system. An OV-1 open tubular column (25 m×0.25 mm I.D., d_f=0.3 μm) (MEGA, Legnano, Milan, Italy) was used. Chromatographic conditions: injection system: splitless, time: 1 min; injector temperature: 230°C; temperature programme: from 0°C (3 min) to 35°C in 3.5 min then to 190°C at 3°C/min; flame ionization detection (FID); temperature: 250°C; carrier gas: hydrogen, flow-rate: 2 ml/min. The analyte standard solution in dibutyl phatalate headspace was analysed in split mode with a split ratio of 1/20.

2.4. HS-SPME–GC

2.4.1. HS-SPME device and fibres

The SPME device was purchased from Supelco (Bellafonte, PA, USA) as were the fused-silica fibres. Table 1 reports a list of the investigated fibres, their acronyms and volumes of the coating material (kindly supplied by Supelco) Coating for all fibres used was 1 cm long with the exception of CAR–DVB–PDMS which was coated to 2 cm, with a coating volume of 1.000 μl. Before use, all fibres were conditioned as recommended by the manufacturer.

2.4.2. HS-SPME–GC sampling

The SPME device was inserted in the sealed vial containing the sample prepared as described above, and the fibre was exposed to the plant material headspace, or to the 3.0 m*M* standard solution, for 1 h during HS equilibration [20], and the vial was vibrated for 10 s every 10 min with an electric engraver (Vibro-Graver V74, Burgess Vibrocrafters, Brayslake, IL, USA). Only that part of the vial with the solid matrix was submerged, to keep the SPME fibre as cool as possible to improve the vapour phase/absorbent fibre coating partition coefficient [25].

After sampling, the SPME device was immediately inserted into the GC injector, and the fibre thermally desorbed. A desorption time of 5 min at 230°C was used. Before sampling, each fibre was reconditioned for 30 min in the GC injection port at 230°C.

2.4.3. HS-SPME–GC analysis

GC analyses were carried out on a Carlo Erba Mega 5360 GC unit. Split injection with a split ratio of 1/20 was used. Analyses were under the same conditions as reported for S-HS–GC analysis.

2.5. GC–MS analysis

HS-SPME–GC–electron impact ionisation (EI) MS analyses were carried out on a Hewlett-Packard 5988 A GC–MS system provided with a Hewlett-Packard 5890 GC unit. Capillary GC separations were done with the same column and under conditions analogous to those reported in the previous section. Injection system: splitless; time: 1 min; carrier gas: helium. The HS components were identified by comparison of their mass spectra with those of authentic samples or with data from literature.

3. Results and discussion

The results described here were mainly obtained by comparing areas of characteristic components of the aromatic plants in the headspace sampled by S-HS and HS-SPME with different fibres. Table 2 reports the components investigated for each plant together with their abbreviations. The approach involved adopting rigorously standardised analysis conditions so that analyte areas may be assumed to be influenced only by the concentration capability of the fibres. In particular, the same sampling con-

Table 2
List of the components investigated for each plant

No.	Compound	Abbreviation	Plant investigated			
			Rosemary	Sage	Thyme	Valerian
1	Ethyl isovalerate	EtVal				X
2	α-Pinene	Pin				X
3	Myrcene	Myr		X		
4	Camphene	Cam		X		
5	Limonene	Lim	X		X	
6	1,8-Cineol	Cin	X	X	X	
7	α-Terpinolene	Ter	X		X	X
8	β-Thujone	Thu		X		
9	Camphor	Cor	X		X	
10	Thymol methyl ether	TME			X	
11	Terpinen-4-ol	T4ol	X			
12	α-Terpineol	αTol				X
13	Bornyl acetate	BoAc	X	X	X	X
14	Thymol	Thy			X	
15	Carvacrol	Cav			X	
16	β-Caryophyllene	Cry	X		X	
17	α-Copaene	Cop		X		
18	δ-Elemene	Ele				X
19	β-Bourbonene	Bou		X		
20	Eudesma-2,6,8-triene	Eud				X
21	2,5-Dimethoxy-*p*-cymene	DMC				X
22	Viridiflorol	Vir		X		

ditions used for S-HS–GC (volume of the vial, sample amount, equilibration temperature and time) were also chosen for HS-SPME–GC with all the fibres used so as to make data comparable, even if these conditions were not the most effective for SPME sampling. Samples were periodically vibrated to speed the analyte equilibration process between the headspace vapour phase and fibre coating [23,26]. Moreover, each fibre was exposed to the plant headspace for the same time, although, equilibration time probably also varies as a function of the coating material. Lastly, the CAR–DVB–PDMS fibre was assumed to have double recovery because it was twice as long (2 cm) as the other fibres (1 cm); the peak area values obtained with it were therefore divided by two for a better comparison of the results. Under these conditions, reproducibility for all fibres investigated, calculated over three analyses, varied from 6.5% for the most volatile to 2.5% for the less volatile components. These variations were within the limits already reported by other authors [10,14,15,18]. The fibres investigated were all for HS-SPME sampling and commercially available; three PDMS fibres (PDMS 7, PDMS 30 and PDMS 100) were tested to evaluate the influence of the amount of absorbent coating on their concentration capacity.

The standardisation conditions adopted to operate in equilibrium S-HS conditions and for HS-SPME sampling allowed us to measure the partition coefficient, K_1, between the headspace gaseous phase and SPME polymeric coating and the relative concentration factor, CF, of some of the characteristic components of the plant investigated. To do this an equimolar standard solution (about 3.0 m*M*) of α-pinene, 1,8-cineol, terpinen-4-ol, α-terpineol, camphor, bornyl acetate, thymol, β-caryophyllene and viridiflorol in dibutyl phtalate was prepared. With this series of experiments, where areas were quantitatively compared, the same split injection conditions used for HS-SPME sampling were applied to S-HS–GC. Zhang and Pawliszyn [23] showed that the amount of analyte concentrated through HS-SPME in a fibre is the result of two strictly related but distinct equilibria: the first is the matrix/headspace equilibrium responsible for the headspace

composition, which depends on the volatility of each analyte and on the physical characteristics of the matrix; the second is the headspace/polymeric fibre coating equilibrium, which is related to the diffusion of the analyte from the vapour phase to the fibre coating, and to the analyte interaction with the polymeric coating. In HS-SPME, the total recovery of an analyte from a solid or liquid matrix is related to the overall partition coefficient, K, of the analyte between the SPME fibre coating and the matrix itself. K can be obtained from the expression: $K = K_1K_2$ where K_1 is the analyte partition coefficient between SPME fibre coating and sample headspace and K_2 is the partition coefficient between headspace and sample matrix [23]. K_1 can therefore be assumed to be a parameter representative of the recovery process of an analyte from the headspace of a sample onto the polymeric coating of a fibre and can be calculated from the following expression:

$$K_1^i = (A_f V_g)/(A_g/V_f)$$

where K_1^i is the partition coefficient for the analyte i; A_f is the area of analyte i on the SPME fibre; V_g is the volume of the gas sample injected; A_g is the area of analyte i in the headspace; and V_f is the volume of the fibre polymeric coating.

The CF of an analyte achieved by an SPME fibre versus the corresponding S-HS sampling is the ratio between the analyte areas obtained by HS-SPME with that fibre and the corresponding area obtained by S-HS. CF is not an absolute parameter because it depends on HS-SPME and S-HS sampling conditions and on the physical state of the matrix, but it may be used for relative evaluation of the fibre recovery efficiency, provided that rigorous standard conditions are adopted.

Table 3 reports K_1^i, CF and the boiling points for the plant component standard solution obtained with each of SPME fibres investigated. α-Pinene was generally better recovered by S-HS than by HS-SPME; although K_1 for several fibres were in the same range, only PDMS 100 area was comparable with that of S-HS. 1,8-Cineol had K_1 in the 10^3 range and its CFs were generally comparable to HS-SPME, the highest recoveries were with PDMS 100 (CF: 2.4), PDMS–DVB 65 (CF: 1.9) and CAR–DVB–PDMS (CF: 1.8). Camphor had K_1 in the 10^3–10^4 range with several fibres and the highest recoveries were with PDMS–DVB 65 (CF: 7.3) and with CAR–DVB–PDMS (CF: 7.0). Terpinen-4-ol and α-terpineol showed high recoveries, with K_1 in the 10^4–10^5 range and CFs reaching 48.5 for terpinen-4-ol and 147.5 for α-terpineol with CAR–PDMS 75 and 32.2 for terpinen-4-ol and 116.2 for α-terpineol with CAR–DVB–PDMS; in any case, most fibres gave CFs above 10. K_1 values of bornyl acetate were all above 10^4 and the highest CFs were with PDMS–DVB 65 (29.7) and CAR–DVB–PDMS (25.3). β-Caryophyllene had K_1 above 10^4 with all fibres; the highest CFs were with fibres containing PDMS (PDMS 100: CF = 48.7; PDMS–DVB 65: CF = 29.7; CAR–DVB–PDMS: CF = 25.3). The extremely high values of K_1 and CFs for

Table 3
Partition coefficient (K_1^i) HS phase/polymeric fibre coating, concentration factors (CFs) and boiling points of the dibutyl phtalate standard mixture components (for detail see texts)

Fibre	α-Pinene, 156°C		1,8-Cineol, 174°C		Camphor, 204°C		Terpinen-4-ol, 208°C		α-Terpineol, 208°C		Bornyl acetate, 223°C		Thymol, 233°C		β-Caryophyllene[a], 118°C		Viridiflorol, n.a.	
	K_1^i	CF	K_1^i	CF	K_1^i	CF	K_1^i	CF	K_1^i	CF	K_1^i	CF	K_1^i	CF	K_1^i	CF	K_1^i	CF
PDMS 7	$5.1\cdot10^3$	0.1	$1.0\cdot10^4$	0.3	$2.4\cdot10^4$	0.6	35	$9\cdot10^{-4}$	86	$2.3\cdot10^{-3}$	$7.9\cdot10^4$	2.0	$1.9\cdot10^4$	$4.0\cdot10^{-2}$	$1.5\cdot10^5$	3.8	$7.4\cdot10^3$	193.5
PDMS 30	$1.2\cdot10^3$	0.2	$3.4\cdot10^3$	0.4	$4.7\cdot10^3$	1.3	$1.2\cdot10^4$	3.2	$8.5\cdot10^4$	11.3	$6.2\cdot10^4$	8.2	$7.5\cdot10^2$	4.5	$1.4\cdot10^5$	18.4	$8.0\cdot10^6$	1055.1
PDMS 100	$1.6\cdot10^3$	1.0	$4.0\cdot10^3$	2.4	$9.1\cdot10^3$	5.8	$2.0\cdot10^4$	12.4	$5.1\cdot10^4$	31.1	$3.9\cdot10^4$	23.9	$4.5\cdot10^5$	276.1	$8.0\cdot10^4$	48.7	$9.1\cdot10^6$	5556.5
CW–DVB 65	$5.0\cdot10^2$	0.2	$1.6\cdot10^3$	0.6	$9.6\cdot10^3$	3.4	$5.0\cdot10^4$	14.6	$1.6\cdot10^5$	56.8	$2.6\cdot10^4$	9.4	$3.6\cdot10^6$	1284.7	$2.6\cdot10^4$	9.4	$6.5\cdot10^6$	2309.3
CAR–PDMS 75	$2.2\cdot10^3$	0.9	$2.6\cdot10^3$	1.1	$5.5\cdot10^3$	2.4	$1.1\cdot10^5$	48.4	$3.4\cdot10^5$	147.1	$2.8\cdot10^4$	12.4	$5.9\cdot10^6$	2588.4	$7.3\cdot10^4$	32.0	$1.2\cdot10^7$	5159.2
PA 85	$4.5\cdot10^2$	0.2	$1.5\cdot10^3$	0.8	$7.9\cdot10^3$	4.1	$2.8\cdot10^4$	14.5	$1.1\cdot10^5$	55.1	$1.8\cdot10^4$	9.6	$1.2\cdot10^6$	596.1	$2.0\cdot10^4$	10.2	$2.3\cdot10^6$	1199.1
PDMS–DVB 65	$1.6\cdot10^3$	0.6	$5.4\cdot10^3$	1.9	$2.0\cdot10^4$	7.3	$6.6\cdot10^4$	23.6	$1.9\cdot10^5$	68.9	$8.3\cdot10^4$	29.7	$6.9\cdot10^6$	2485.2	$4.9\cdot10^4$	17.6	$2.1\cdot10^7$	7585.9
CAR–DVB–PDMS	$1.3\cdot10^3$	0.6	$3.6\cdot10^3$	1.8	$1.4\cdot10^4$	7.0	$7.0\cdot10^4$	32.2	$2.3\cdot10^5$	116.2	$5.1\cdot10^4$	25.3	$2.3\cdot10^6$	1138.1	$7.5\cdot10^4$	37.5	$5.4\cdot10^6$	2705.9

[a] At 1.53 MPa.

n.a.: Not available.

thymol and viridiflorol are due to the fact that they were only present as a trace in the S-HS at 60°C because of their polarity and low volatility; in the GC chromatograms, their areas were of a few hundred counts. The most effective fibres were CAR–PDMS 75 for thymol (CF: 2588) and PDMS–DVB 65 for viridiflorol (CF: 7585). From these results, it is clear that, when S-HS and HS-SPME samplings are compared, less volatile components are preferentially concentrated as it is also evident when analyte boiling points are considered versus the corresponding K_1 and CFs, and that recovery is conditioned by the affinity in polarity of analyte and polymeric coating.

In plant analysis, the peak areas of each characteristic compound of each plant were percent normalised, taking the corresponding peak areas obtained with the CAR–DVB–PDMS fibre as equal to 100, because this fibre generally gave high recoveries with the majority of the analytes investigated: a relative abundance (RA) versus that of the CAR–DVB–PDMS fibre was therefore measured for each investigated compound with each fibre. This approach was adopted to make the results from the different plants more comparable. Fig. 1 reports the rosemary GC patterns of (a) S-HS–GC, (b) HS-SPME–GC with a PDMS 30 fibre and (c) HS-SPME–GC with a CAR–DVB–PDMS fibre. Fig. 2 reports the sage GC patterns of (a) S-HS–GC, (b) HS-SPME–GC with a CW–DVB 65 fibre and (c) HS-SPME–GC with a PDMS–DVB 65 fibre. Fig. 3 reports the thyme GC patterns of (a) S-HS–GC, (b) HS-SPME–GC with a PA 85 fibre and (c) HS-SPME–GC with a PDMS 100 fibre, and Fig. 4 reports the valerian GC patterns of (a) S-HS–GC, (b) HS-SPME–GC with a PDMS 7 fibre and (c) HS-SPME–GC with a CAR–PDMS 75 fibre. The HS-SPME–GC chromatograms in the figures were chosen to show the results of one of the most and one of the least effective fibres for each plant investigated, to give a direct view of the increase or decrease in concentration of the characteristic analytes compared to S-HS–GC.

Fig. 5a–d show the HS-SPME–GC normalised abundance of the characteristic components of the four plants under investigation obtained with the different fibres versus the CAR–DVB–PDMS fibre. As expected, peak abundance varied in function of

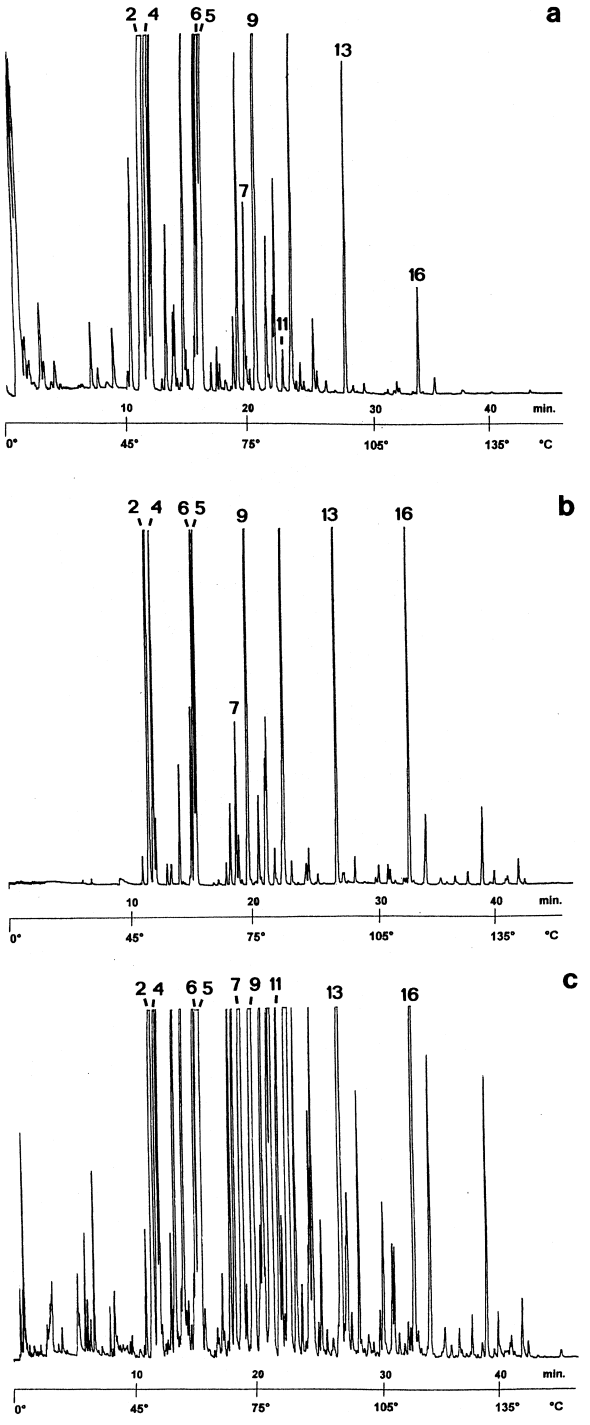


Fig. 1. Rosemary GC patterns of (a) S-HS–GC, (b) HS-SPME–GC with a PDMS 30 fibre and (c) HS-SPME–GC with a CAR–DVB–PDMS fibre.

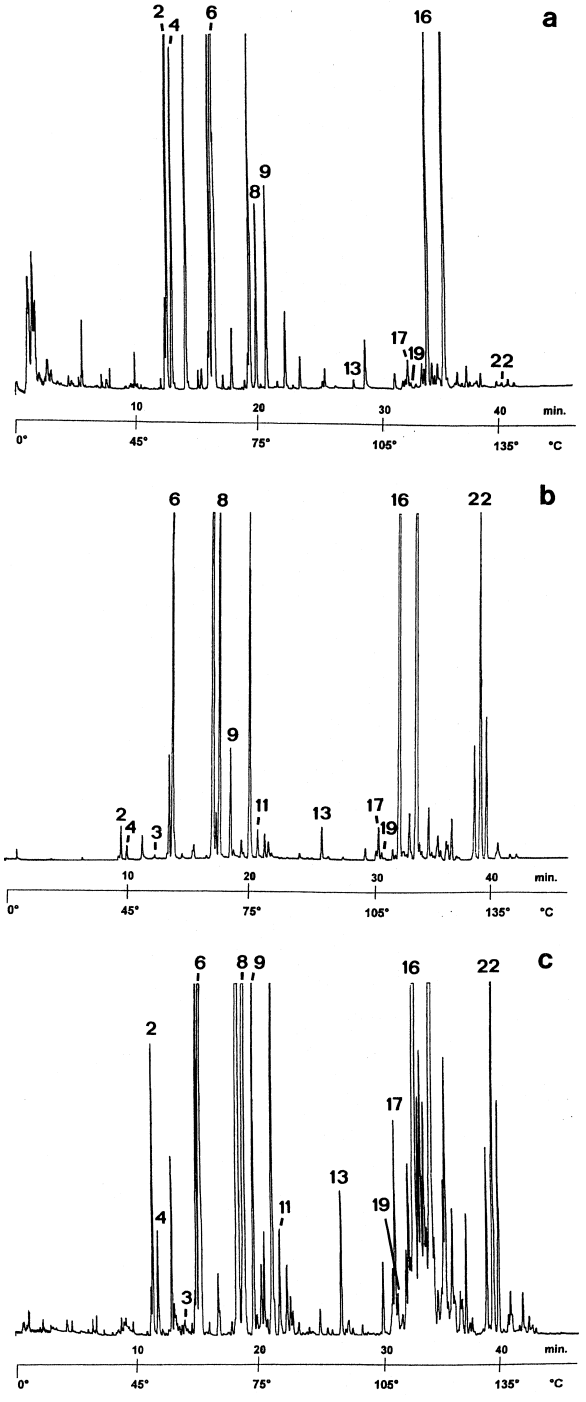


Fig. 2. Sage GC patterns of (a) S-HS–GC, (b) HS-SPME–GC with a CW–DVB 65 fibre and (c) HS-SPME–GC with a PDMS–DVB 65 fibre.

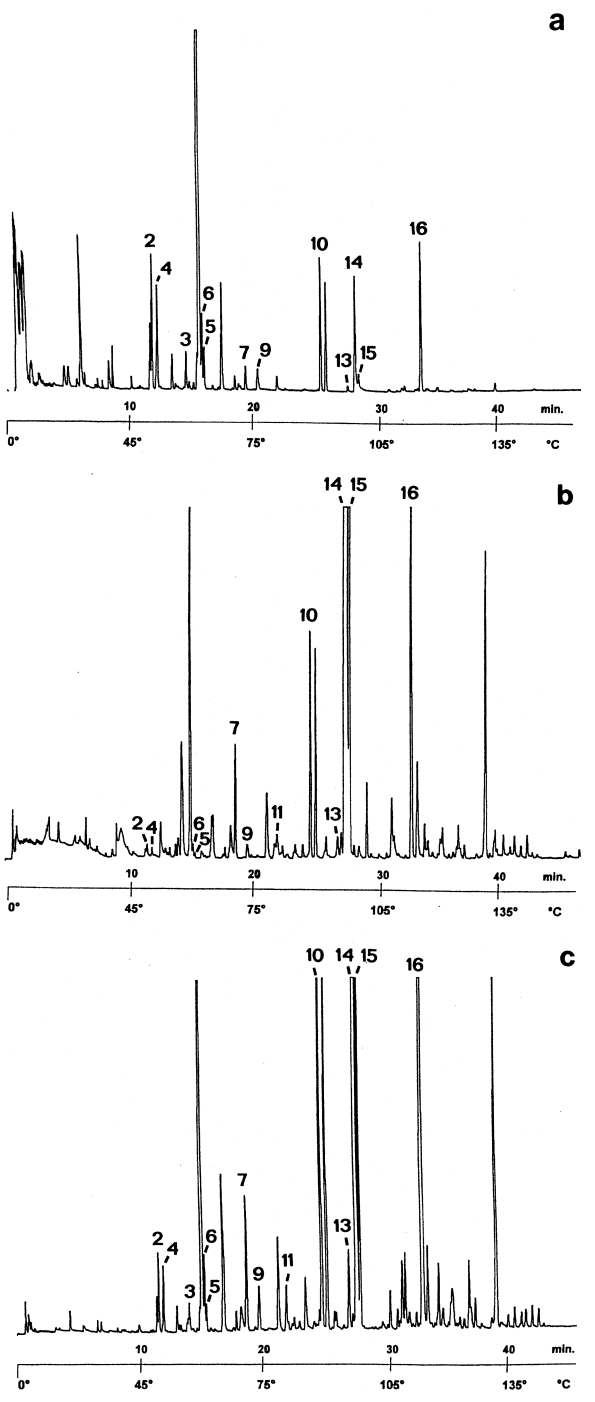


Fig. 3. Thyme GC patterns of (a) S-HS–GC, (b) HS-SPME–GC with a PA 85 fibre and (c) HS-SPME–GC with a PDMS 100 fibre.

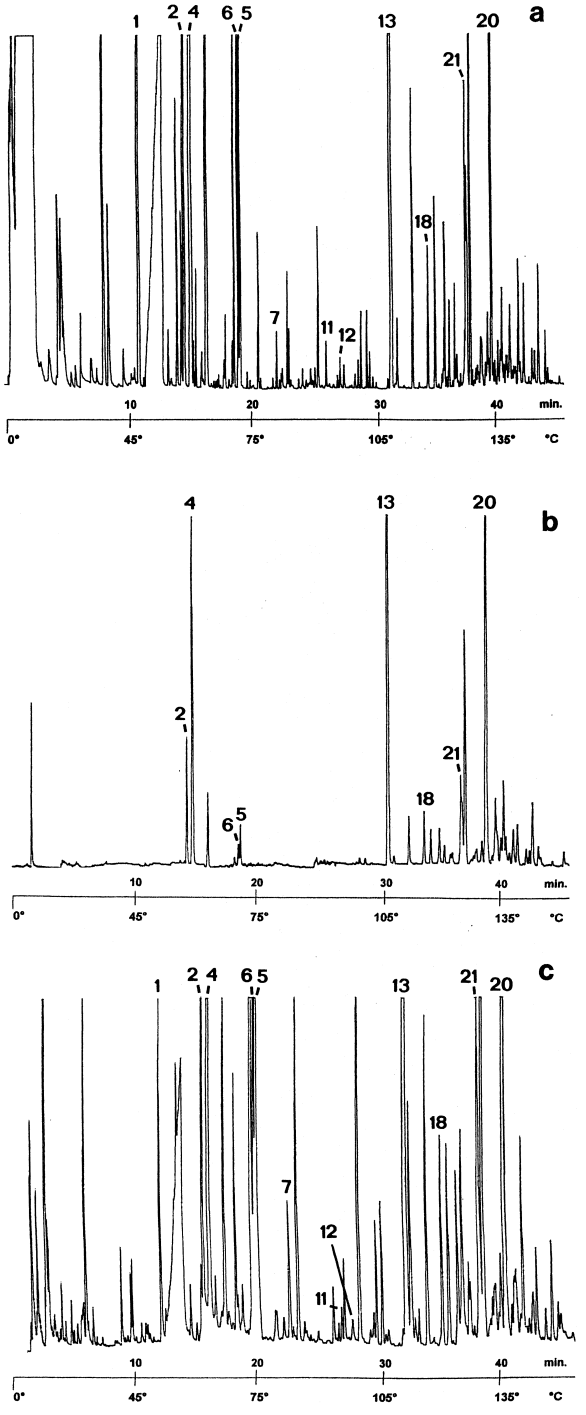


Fig. 4. Valerian GC patterns of (a) S-HS–GC, (b) HS-SPME–GC with a PDMS 7 fibre and (c) HS-SPME–GC with a CAR–PDMS 75 fibre.

the volatility and polarity of the analytes and of the composition of the fibre polymeric coating. With rosemary (Fig. 5a), the CAR–DVB–PDMS fibre showed the highest recovery for all the components with the exception of limonene, whose relative abundance (RA) with CAR–PDMS 75 was 393, and of 1,8-cineole, whose RA with PDMS 100 was 118. High concentration factors were also obtained with PDMS 100, PDMS–DVB 65 and, to a lesser extent, CAR–PDMS 75. PDMS 7 gave the lowest concentration factors with all the analytes, followed by PDMS 30 and PA 85.

The behaviour of sage characteristic components (Fig. 5b) was similar to that of rosemary. Three fibres were more effective than the others: CAR–DVB–PDMS, PDMS 100 and PDMS–DVB 65. CAR–DVB–PDMS showed the highest recovery with all the components with the exception of myrcene, whose RA with PA 85 rather surprisingly was 121 and of β-bourbonene whose RA with PDMS 100 was 104. The least effective fibre was again PDMS 7.

The phenolic components thymol and carvacrol significantly influenced component recovery profiles in thyme (Fig. 5c). Although CAR–DVB–PDMS was always very effective, higher concentration factors for aromatic compounds were obtained with PA 85 (RA: thymol: 149; carvacrol: 154) PDMS–DVB 65 (RA: thymol methyl ether: 136; thymol: 146; carvacrol: 146) and CW–DVB 65 (RA: thymol: 132; carvacrol: 131). PA 85 was only effective in concentrating phenolic compounds. PDMS–DVB 65 and PDMS 100 were very effective on most of the components investigated; CAR–PDMS 75 showed extremely high abundance with 1,8-cineol (RA: 792). In this case too, PDMS 7 and PDMS 30 were the least effective fibre.

The chemical characteristic of the components chosen for valerian also significantly influenced the overall fibre profiles (Fig. 5d). Here too, CAR–DVB–PDMS was always very effective, but PDMS–DVB 65 and PDMS 100 gave a higher peak abundance for most of the components investigated. The peak abundance of ethyl isovalerate (RA: 414), α-terpinolene (RA: 398), and α-terpineol (RA: 474) with CAR–PDMS 75 was very high. The concentration capacity of PDMS 7 and PDMS 30 was again very low. Isovaleric acid was not considered in this

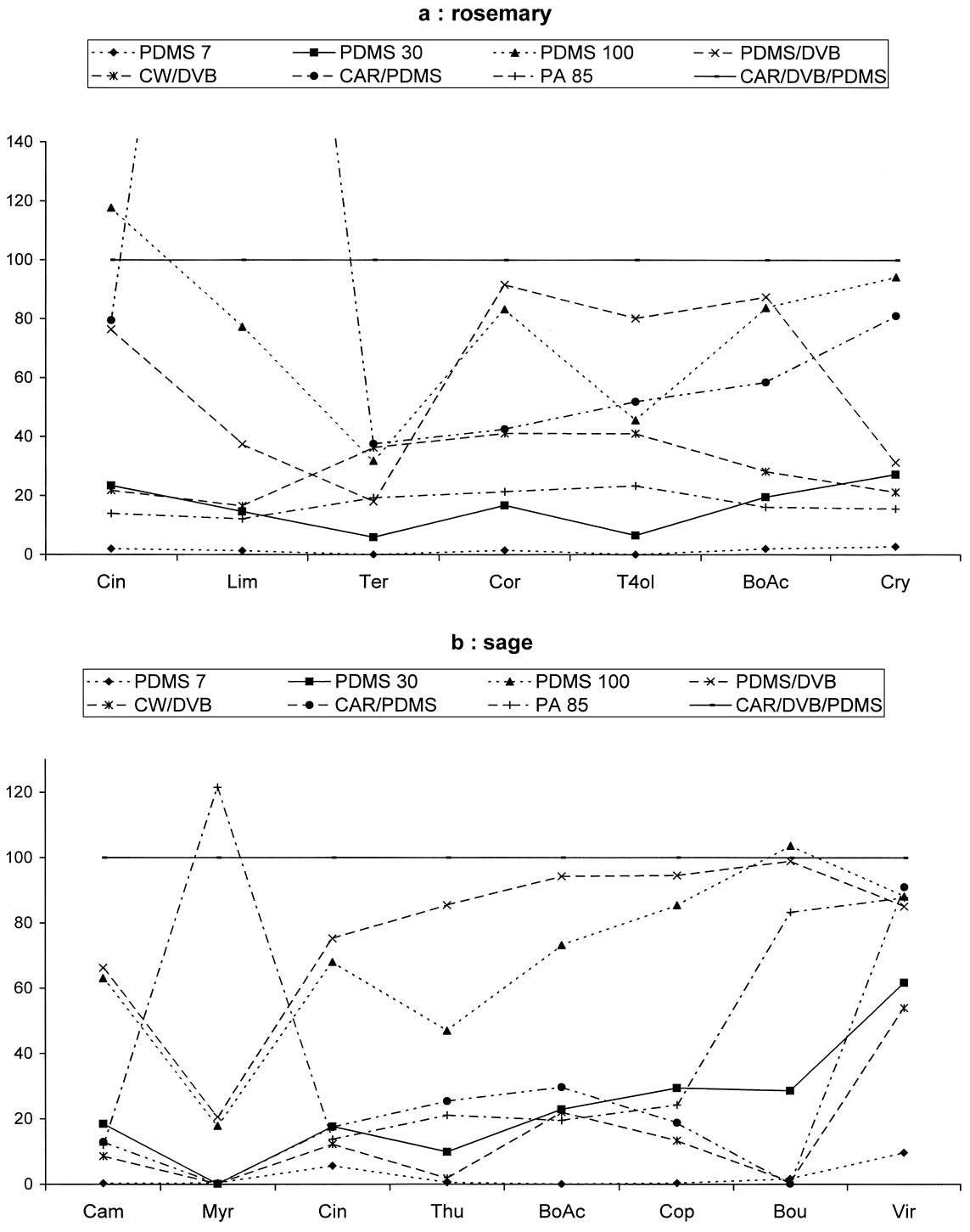
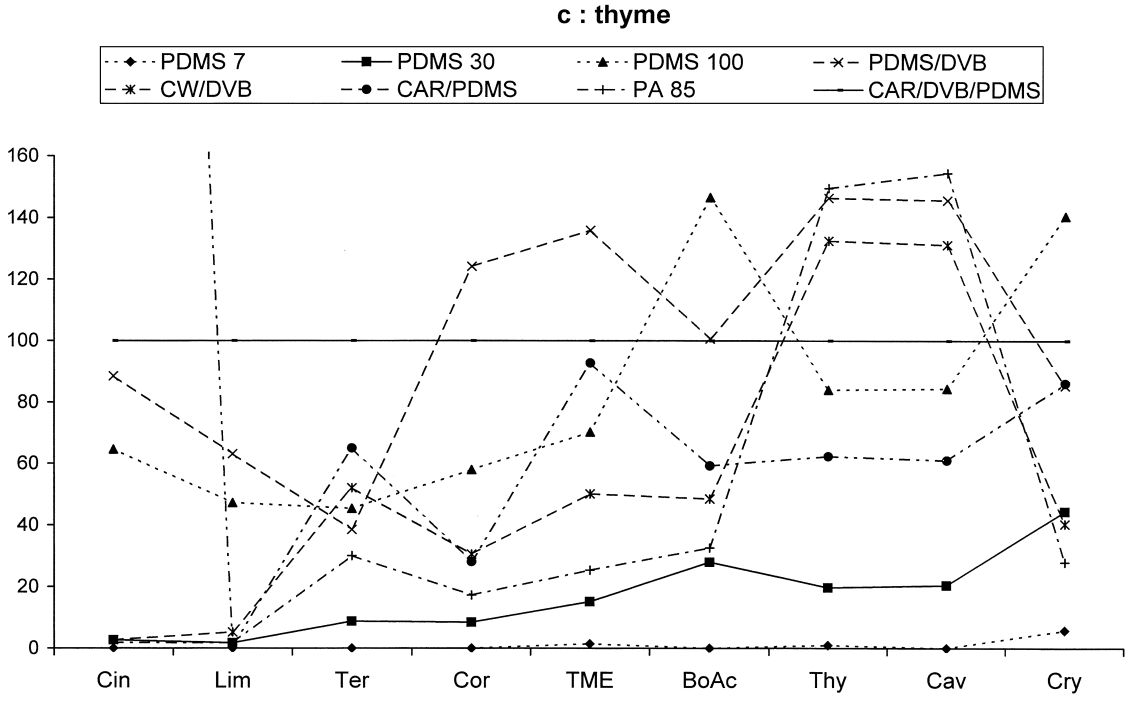


Fig. 5. HS-SPME–GC normalised abundance of the characteristic components of rosemary (a), sage (b), thyme (c) and valerian (d) obtained with the different fibres versus the CAR–DVB–PDMS fibre. For abbreviations of the characteristic components investigated for each plant see Table 2.



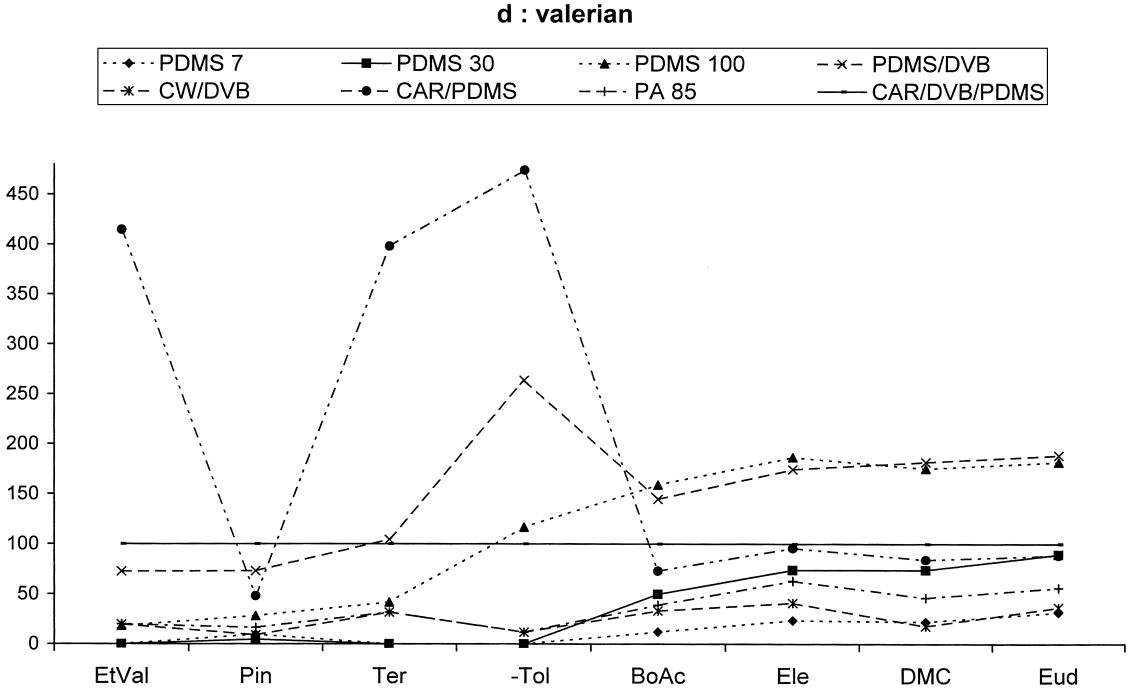


Fig. 5. (*continued*).

study because its area was difficult to measure reliably.

It is interesting to compare the relative abundance of components that are in common to all plants investigated. In the case of bornyl acetate the different headspace composition and its different amount in the four plants does not seem to influence particularly the concentration capacity of the fibres. Bornyl acetate RAs with PDMS 100 ranges from 159 for valerian to 73 for sage, with PDMS–DVB 65 it varies from 144 for valerian to 87 for rosemary, while with CAR–PDMS 75 it ranges from 73 for valerian to 30 for sage and with CW–DVB 65 from 48 for thyme to 22 for sage. On the other hand, the recoveries of α-terpinolene and 1,8-cineol from different plant material differed very widely with some of the fibres. The RAs of α-terpinolene, which was not investigated for sage, varies from 45 for thyme to 32 for rosemary with PDMS 100; from 105 for valerian to 18 for rosemary with PDMS–DVB 65; and from 398 for valerian to 37 for rosemary with CAR–PDMS 75. The RAs of 1,8-cineol, which was not investigated for valerian, varies from 117 for rosemary to 64 for thyme with PDMS 100; from 88 for thyme to 75 for sage with PDMS–DVB 65; from 792 for thyme to 17 for sage with CAR–PDMS 75 and from 21 for rosemary to 3 for thyme with CW–DVB 65.

The profiles of the three PDMS fibres (PDMS 7, PDMS 30 and PDMS 100) were qualitatively similar for all the components, but the variation of relative abundance was apparently not directly related to the volume of polymeric coating. The expected RA ratio of about five – i.e., comparable to the ratio between the volumes of the fibre polymeric coatings – between PDMS 30 and PDMS 100 was only observed for some of the analytes (limonene, 1,8-cineol, α-terpinolene, camphor, and terpinen-4-ol for rosemary; 1,8-cineol and β-thujone for sage; α-terpinolene, thymol methyl ether, bornyl acetate and thymol for thyme; α-pinene for valerian), while for the others, RA ratio was generally lower.

A further point of interest is the concentration capacity of the different fibres in function of the class of compounds present in the plant investigated. Fig. 6a–d report the sum of the peak areas of the classes of compounds of the plant headspaces sampled by HS-SPME with the different fibres and normalised versus the corresponding peak areas obtained with the CAR–DVB–PDMS fibre. For monoterpene hydrocarbons, the highest recoveries were with CAR–DVB–PDMS for sage and valerian and with CAR–PDMS 75 for rosemary and thyme. Sesquiterpene hydrocarbons were better recovered with CAR–DVB–PDMS for sage and rosemary, with PDMS 100 for thyme and with PDMS–DVB 65 for valerian. For ethers, the most effective fibres were CAR–DVB–PDMS for sage, PDMS 100 for rosemary, CAR–PDMS 75 for thyme and PDMS–DVB 65 for valerian. For esters, the highest recoveries were with CAR–DVB–PDMS for sage and rosemary, PDMS–DVB 65 for thyme and PDMS 100 for valerian. Alcohols and phenols were best recovered with CAR–DVB–PDMS for sage and rosemary, PA 85 for thyme and CAR–PDMS 75 for valerian. The highest recoveries with oxygenated sesquiterpenoids were with PDMS 100 with both sage and thyme, while ketones were better recovered with CAR–DVB–PDMS for sage and rosemary and with PDMS–DVB 65 for thyme.

It thus appeared that CAR–DVB–PDMS, PDMS 100, PDMS–DVB 65 and CAR–PDMS 75 were the most effective fibres overall, although recoveries varied markedly in function of the polarity of the class of analytes investigated and of the composition and physical state of the vegetable matrix, which influence the composition of the resulting S-HS. As a general consideration, all the most effective fibres contained PDMS, a liquid phase favouring the absorption of non-polar analytes, as well as a porous solid (CAR or DVB or both) that favours the absorption of polar analytes.

HS-SPME is generally used to concentrate analytes which are present in trace amounts in the classical S-HS. Fig. 7a–d show the comparison between the RA of the characteristic components of each plant obtained by S-HS and by HS-SPME with PDMS 30 and with the most effective fibres. Areas were normalised versus the S-HS–GC using the same approach adopted above to compare fibre performance. Since sensitivity with S-HS–GC in split mode was too low compared to HS-SPME–GC, splitless injection was applied to S-HS–GC for this series of experiments. In spite of this, very low amounts of some more polar and less volatile analytes (i.e., thymol and viridiflorol) were detected

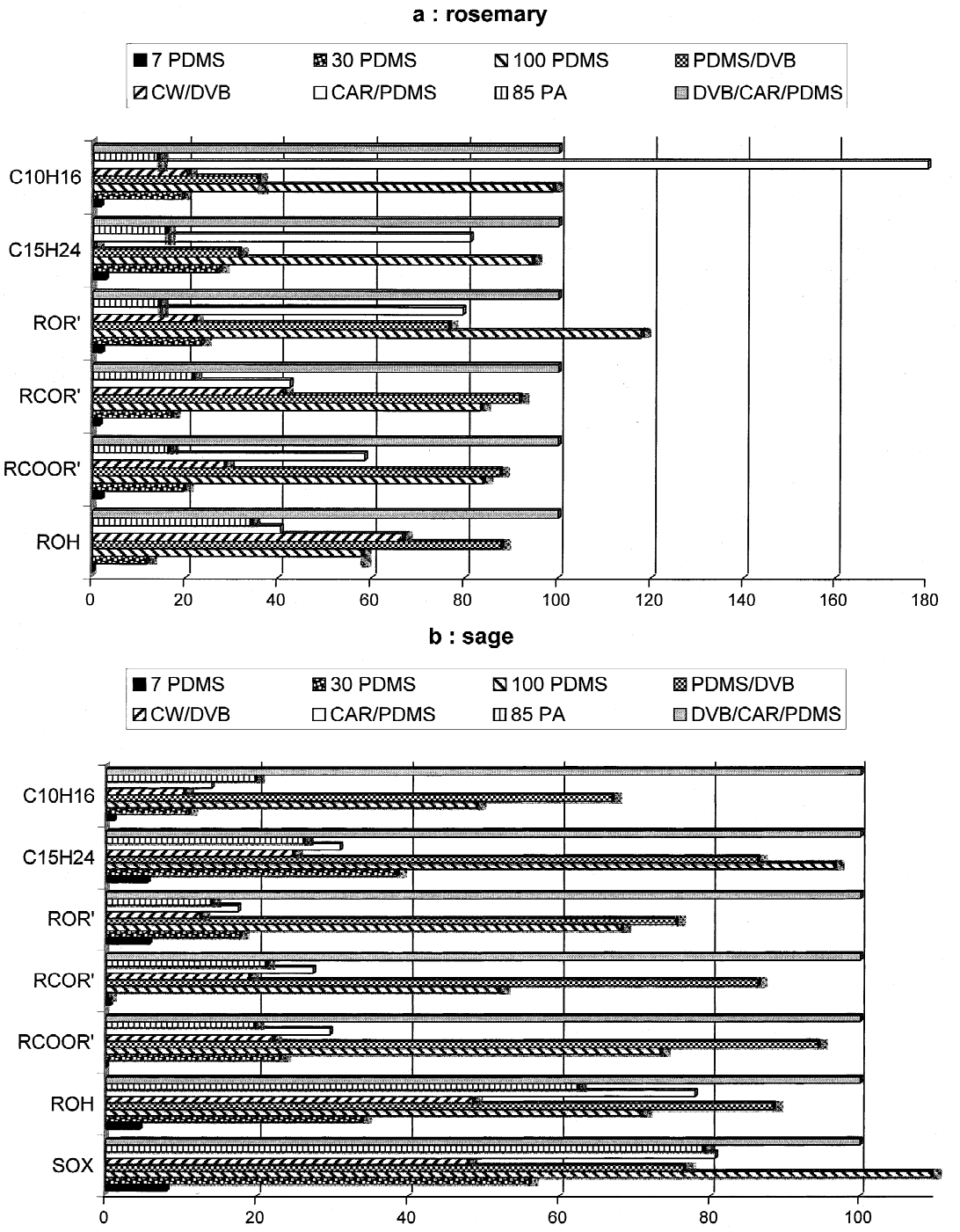


Fig. 6. Recoveries of the classes of compounds of the plant headspaces sampled by HS-SPME with the different fibres normalised versus the CAR–DVB–PDMS fibre. (a) Rosemary; (b) sage; (c) thyme; (d) valerian. C10H16: Monoterpene hydrocarbons; C15H24: sesquiterpene hydrocarbons; ROR′: ethers; RCOR′: ketones; RCOOR′: esters; ROH: alcohols and phenols; SOX: oxygenated sesquitepenes.

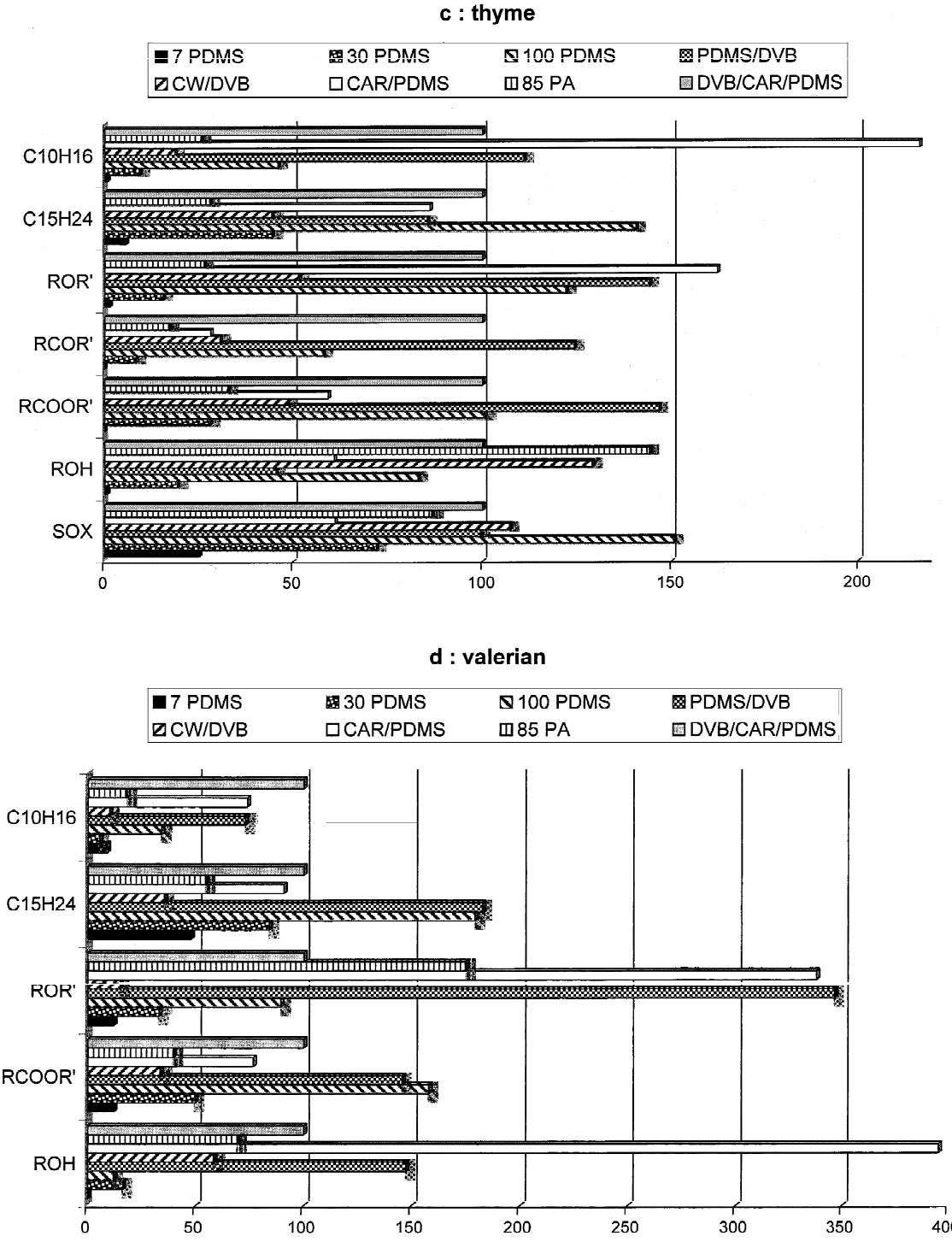


Fig. 6. (*continued*).

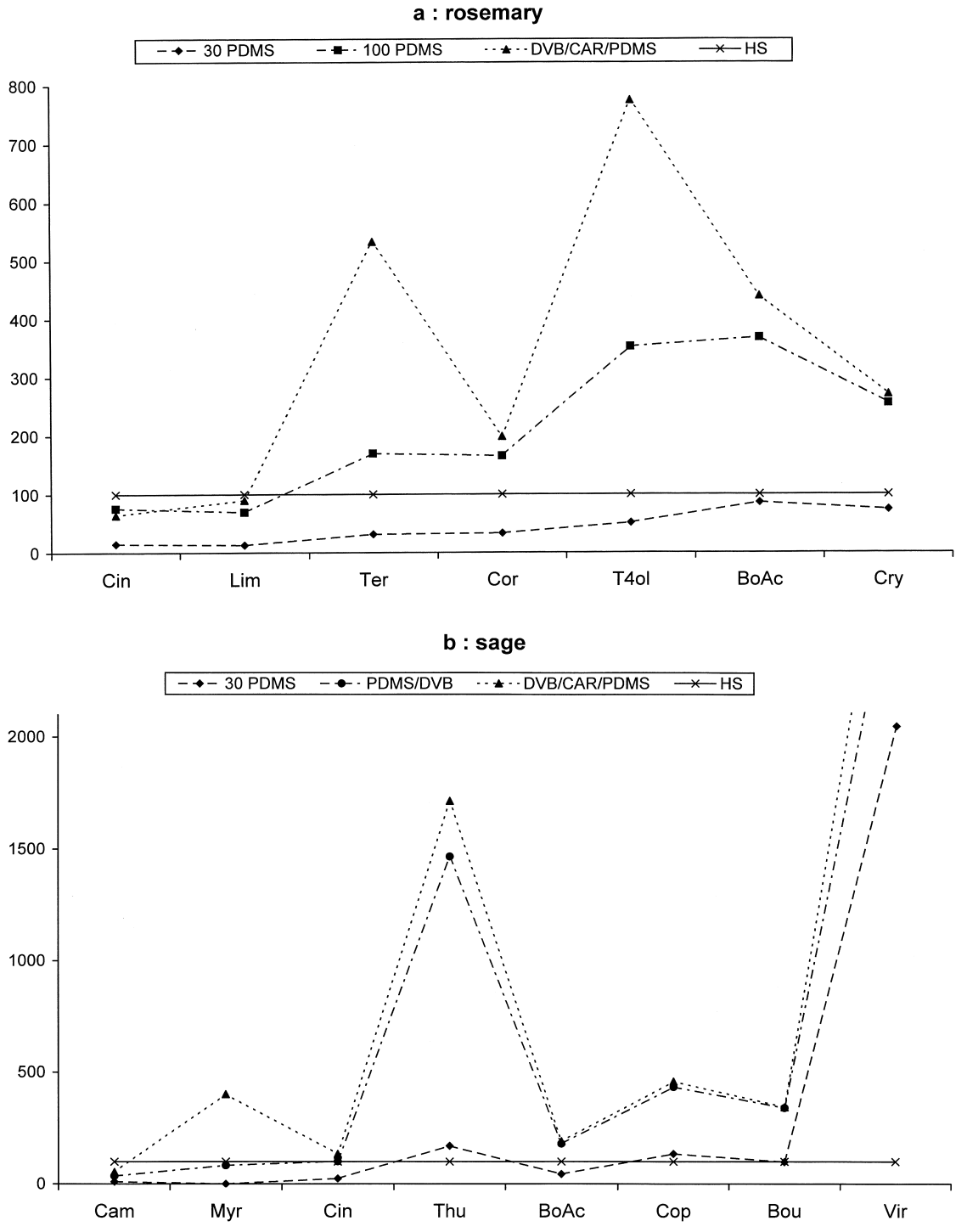
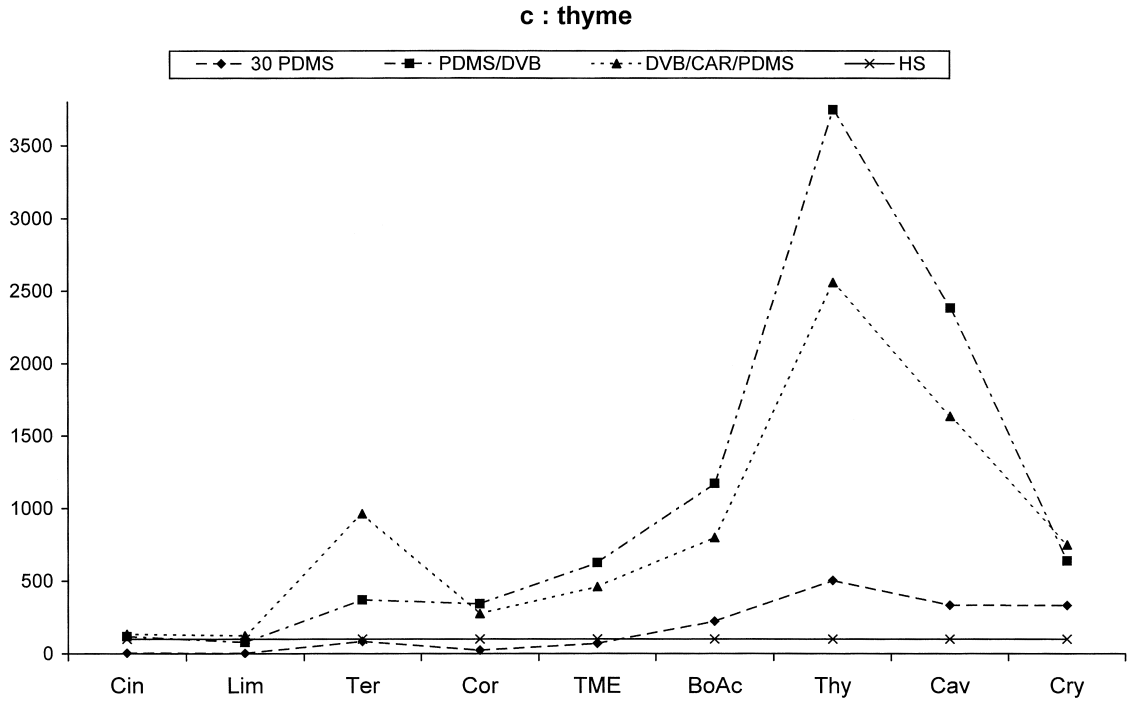


Fig. 7. Comparison between the relative abundance (RA) of the characteristic components of rosemary (a), sage (b), thyme (c) and valerian (d) obtained by S-HS and by HS-SPME with PDMS 30 and with the most effective fibres. For abbreviations of the characteristic components investigated for each plant see Table 2.



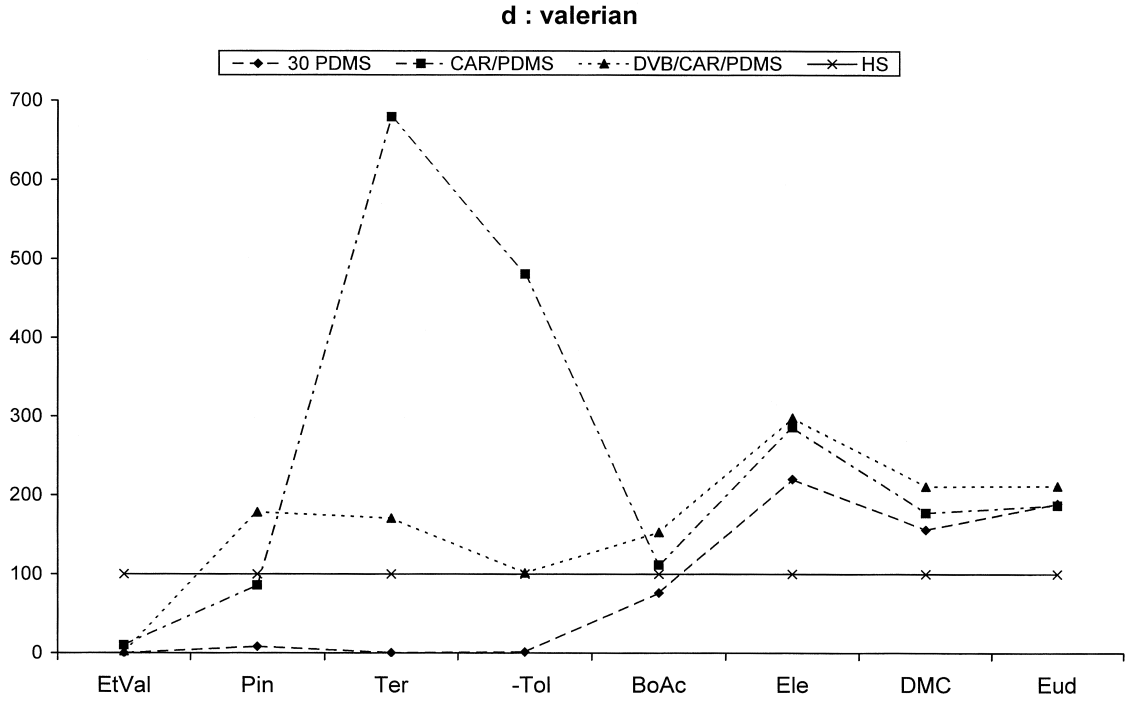


Fig. 7. (*continued*).

by S-HS–GC. PDMS 30 was also included in the diagrams since it gives RA comparable to those of S-HS; PDMS 7 was not considered because recoveries were too low compared to S-HS.

With rosemary (Fig. 7a), limonene and 1,8-cineol were better recovered by S-HS sampling than with any of the fibres, with the exception of limonene with CAR–PDMS 75, whose RA was 353. The component abundance with PDMS 30 was in all cases lower than the corresponding S-HS sampling, while CAR–DVB–PDMS was the most effective fibre for all other components, in particular for α-terpinolene (RA: 533), terpinen-4-ol (RA: 775) and bornyl acetate (RA: 440). PDMS 100 also gave high recoveries but lower than those of CAR–DVB–PDMS.

A similar behaviour was observed with sage (Fig. 7b), where very high recoveries were obtained for myrcene (RA: 401), β-thujone (RA: 1715), α-copaene (RA: 458) and β-bourbonene (RA: 343) with CAR–DVB–PDMS, and for β-thujone (RA: 1466), α-copaene (RA: 433) and β-bourbonene (RA: 340) with PDMS–DVB 65. Viridiflorol shows very high relative abundance with all fibres because it is a trace peak in S-HS–GC due to its low volatility.

With thyme (Fig. 7c), limonene recovery by HS-SPME was lower or comparable with that of S-HS, with the exception of CAR–DVB–PDMS where its RA was 124. 1,8-Cineol was best recovered with CAR–PDMS 75 (RA: 1067). α-Terpinolene and β-caryophyllene showed high recoveries with CAR–DVB–PDMS (RA: 963 and 749, respectively), while for thymol methyl ether, bornyl acetate, thymol and carvacrol, the most effective fibre was PDMS–DVB 65 (RA: 626, 1171, 3747 and 2384, respectively). The highest recoveries for bornyl acetate and β-caryophyllene were obtained with PDMS 100 (RA: 803 and 1053, respectively), while PA 85 was the most effective fibre for thymol and carvacrol (RA: 3828 and 2529, respectively).

With valerian, all the components were better recovered by HS-SPME than with S-HS sampling, with the exception of ethyl isovalerate and α-pinene. This latter was only recovered better than S-HS with CAR–DVB–PDMS (RA: 178). In addition to CAR–DVB–PDMS, the most effective fibres were PDMS–DVB 65 and CAR–PDMS 75. This latter unexpectedly gave the highest concentration factor with α-terpinolene (RA: 679) and terpinen-4-ol (RA: 9678).

Compared with S-HS sampling, HS-SPME is particularly effective with the lower volatility components; the use of coatings whose affinity with the analytes investigated is good can give concentration factors varying from a few tens to hundreds of times the S-HS amounts, depending on the component volatility. On the other hand, S-HS sampling is competitive (if not better) with HS-SPME for the high volatility components. The HS-SPME conditions adopted in this study were, in point of fact, chosen to favour concentration of the medium volatility analytes [20,23].

4. Conclusions

In conclusion, HS-SPME and S-HS sampling are complementary since HS-SPME produces very high recoveries with the less volatile and more polar analytes while S-HS is very effective for the highly volatile analytes. The most effective fibres for HS-SPME are generally those consisting of two components, a liquid (PDMS) for the less polar components and a solid (DVB, CAR or both) polymeric coating for the more polar components. HS-SPME recoveries are strongly conditioned by the polarity and volatility of analytes investigated, and by the composition and physical state of the vegetable matrix, characteristics which, in their turn, condition the headspace composition.

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