Headspace–Solid-Phase Microextraction in the Analysis of the Volatile Fraction of Aromatic and Medicinal Plants

Flavio Belliardo, Carlo Bicchi*, Chiara Cordero, Erica Liberto, Patrizia Rubiolo, and **Barbara Sgorbini**
Dipartimento di Scienza e Tecnologia del Farmaco, Via Pietro Giuria 9 I-10125 – Torino, Italy

Abstract

Headspace (HS)–solid-phase microextraction (SPME) has assumed an ever increasing importance as a technique for HS sampling to study the composition of the HS of medicinal and aromatic plants. HS–SPME has mainly been applied for (*a*) studying the composition of the volatile fraction, including in addition to or as an alternative to other sampling techniques; (*b*) monitoring the biological phenomena involved with the volatile fraction of a plant; (*c*) discriminating between species, subspecies, varieties, cultivars, or chemotypes; and (*d*) quality control of plant samples. A review of 108 articles published during 2000–2005 is presented covering the use of HS–SPME in the field of aromatic and medicinal plants, selection of the most effective fiber and sampling conditions, comparison of HS–SPME and other volatile fraction sample preparation techniques, and the advantages and limits of HS–SPME when applied to medicinal and aromatic plants.

Introduction

Over the last 10 to 15 years, headspace (HS) sampling has enjoyed a remarkable revival of interest caused by the introduction of high concentration capacity (HCC)-HS techniques. These techniques are mainly based on either the static or dynamic accumulation of volatiles on polymers operating in sorption or adsorption modes (or both). Examples of HCC-HS sampling techniques operating in the static mode are HS solid-phase microextraction (SPME) (1) and HCC-headspace sorptive extraction (HSSE) (2,3), and HS–solid-phase dynamic extraction (SPDE) (4–6) (also known as "the magic needle") is based on the dynamic approach. A survey of HCC-HS sampling techniques has recently been published in this *Journal* (7).

HS–SPME was the first HCC-HS sampling technique to appear. It was introduced by Zhang and Pawliszyn in 1993 (1) as an extension of SPME, which had been developed by Arthur and Pawliszyn in 1990 (8) to overcome some drawbacks of solid-phase extraction in sampling organic pollutants from water. The theory, technology, evolution, and applications of SPME have been reviewed by Pawliszyn et al. together with some specific topics (9–11). The theory of SPME applied to HS sampling was advanced by the same authors (1,12). It showed that analyte recovery from HS by a fiber depends on two closely-related but distinct equilibria: the first is the matrix/HS equilibrium responsible for the HS composition (measured by its distribution coefficient, K_2), and the second is the HS/polymeric fiber coating equilibrium (measured by its distribution coefficient, K_1). The HS–SPME approach (and its theory) have also been very useful to develop other HCC-HS sampling techniques aimed at overcoming some of its limits. For instance, this was the case of HSSE (2,3) and HS–SPDE (4–6).

HS–SPME is now a well-established and very popular technique for HS sampling in several fields, including the study of the composition of the HS of medicinal and aromatic plants, where it has assumed an ever-increasing importance. This article reviews the application of HS–SPME in this field, and it has been divided into four sections: (*i*) HS–SPME application to the field of aromatic and medicinal plants, (*ii*) selection of an effective fiber and sampling conditions for an HS–SPME application, (*iii*) HS–SPME versus other volatile fraction sample preparation techniques, and (*iv*) advantages and limits of HS–SPME when applied to the medicinal and aromatic plant field. The present review is based mainly on articles quoted by Sci-Finder Chemical Abstract Data Base (American Chemical Society, Washington, DC) and published over the last five years (2000–2005).

Discussion

HS–SPME applications to the aromatic and medicinal plant field

The first section deals with the aromatic and medicinal plants whose HS has been studied by SPME sampling. Table I gives a list of the plants (botanical and common names) whose HS was analyzed by HS–SPME, employed fibers, three main components characterizing their HS (where available), techniques used to sample the volatile fraction of the investigated plants and comparison with HS–SPME, and senior authors and years of publication of the related articles. Table II lists the commercially-

* Author to whom correspondence should be addressed: email carlo.bicchi@unito.it.

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Table I. List of the Plants (Botanical and Common Names) Whose HS Was Analyzed by HS–SPME*

Ref. no. (topics)†	Plant name (part of the plant)	Common name	Senior author	Year	Fibers	Main components	Other techniques
3 (e)	*Rosmarinus officinalis* *Salvia officinalis* *Thymus vulgaris* *Valeriana officinalis*	Rosemary sage thyme valerian	C. Bicchi	2000	PDMS 100 CW–DVB CAR–PDMS PDMS–DVB DVB–CAR–PDMS		HSSE S–HS
6	*Rosmarinus officinalis*	Rosemary	C. Bicchi	2004	PDMS 100	Bornyl acetate, verbenone, camphor	HS–SPDE S–HS
13 (a)	*Achillea millefolium* (leaves, flowers, stems)	Yarrow	J. Rohloff	2000	PDMS 100	Sabinene, 1,8-cineole, β-pinene	EO
14 (a)‡	*Apium graveolens*	Celery, dropwort	C. Deng	2003	PDMS–DVB	*cis*-3-Hexen-1-ol, myrcene, limonene	
15 (a)‡	*Armoracia rusticana*	Horseradish	M. D'Auria	2004		2-Phenylethyl isothiocyanate, allyl isothiocyanate, isobutyl isothiocyanate	
16 (a)	*Artemisia argyi* (leaves)		X. Zheng	2004	CW–DVB PDMS PDMS–DVB	α-Phellandrene, D-limonene, germacrene D	
17 (a,d,e)	*Balsamita suaveolens* (fresh plants)	Costmary	S. Gallori	2001	PDMS 100	Carvone, β-bisabolene, germacrene D	EO SE (nHex)
18 (a)‡	*Bupleurum species*		D. Zeng	2005	PA		
19 (a)‡	*Chimonanthus praecox* (flowers)	Calycanthus	X. Deng	2004		α-Linalool, methyl salicylate	
20 (a)‡	*Coriandrum sativum*	Coriander	C. Deng	2003	PDMS 100	Decanal, 2-decenal, 1-decanol (PPA)	
21 (a)‡	*Echinops ellenbeckii* (stem, roots, leaf, flowerheads)		A. Hymete	2004		Flowers: selinene, roots: maaliene, leaves: caryophyllene oxide, flowerheads: cyperene	EO
22 (a)	*Eruca Vesicaria ssp. sativa* (leaves)	Rocked salad	L. Jirovetz	2002	PDMS–CAR–DVB	*cis*-3-Hexen-1-ol, *cis*-3-hexenyl butanoate, 4-methylthiobutyl isothiocyanate	
23 (a)	*Eucalyptus citriodora* (leaves)		C.A. Zini	2001	PDMS–DVB CAR–PDMS PDMS 100	Citronellal, citronellol, β-caryophyllene	
24 (a,d)	*Eucalyptus dunnii* *E. saligna* *E. grandis*		C.A. Zini	2002	PDMS 7	α-Pinene, 1,8-cineole, aromadendrene	EO
25 (a)	*Eucalyptus dunnii* *E. saligna* *E. grandis* (leaves)		C.A. Zini	2002	PDMS–DVB	α-Pinene, β-ocimene, *p*-cymene	

* Polydimethlysiloxane, PDMS; carbowax, CW; divinylbenzene, DVB; static HS, S-HS; essential oil, EO; solvent extraction, SE; dynamic HS, DHS; charcoal, CHA; simultaneous distillation extraction, SDE; dichloromethane, MECL; normal hexane, nHex; solid-phase trapping solvent extraction, SPTE; flash evaporation, FE; microwave-assisted HS, MWHS; suptercritical fluid extraction, SFE; purge and trap, P&T; ethyl acetate, ETAC; normal pentane, nPent; Porapak Q, Por Q; reduced-pressure steam distillation, RPSD; petroleum ether, Pet. Et.; pressurized hot water extraction, PHWE; pressurized liquid extraction, PLE; methanol, MEOH; articifical neural networks, ANN; essential oil, EO; and depending on the analyzed part plant, PPA.
† (topics), main topics as classified in the HS–SPME applications to the aromatic and medicinal plant field section.
‡ Articles that were not available.

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26 (a)	*Evodia rutaecarpa* *E. rutaecarpa var officinalis* (fruits)		F. Pellati	2005	PDMS 100 PDMS–DVB, CW–DVB, DVB–CAR–PDMS	*E.rutaecarpa:* limonene, β-elemene, linalool, E. *rutaecarpa var officinalis:* myrcene, limonene, β-caryophyllene	
27 (a)	*Geosmina vulgaris* (roots)	Red beets	G. Lu	2003	PDMS–DVB	Geosmin	
28 (a)	*Lathyrius vernus* *Orchis pallens*		P. Bartak	2003	CAR–PDMS	β-Farnesene, phenethyl alcohol, limonene	DHS (CHA)
29 (a)‡	*Lithraea caustica* (aereal parts)		J. Garbarino	2002	PDMS 100	Myrcene , α-pinene, *p*-cymene	
30 (a,b)	*Myrtus communis* (leaves, fruits)	Myrtle	G. Flamini	2004	PDMS 100	α-Pinene, limonene, 1,8-cineole (PPA)	EO
31 (a,c)	*Osyris alba* (flowers)		F. Demirci	2004	PDMS–DVB	(Z)-3-Hexenyl acetate, (Z)-3-hexen-1-ol, hexanol	
32 (a)	*Panax quinquefolius*	American ginseng	X. Di	2004	PDMS 100		
33 (a)	*Picea omorica* *P. abies* (needles)		I. Chvilickova	2004	PDMS 100	*P. abies:* limonene, camphene, bornyl acetate *P. omorica:* limonene, α-pinene, bornyl acetate	
34 (a)	*Pinus sylvestris* *Picea excelsa* (needles)	Scots pine and spruce	V.A. Isidorov	2003	PDMS 100 CAR–PDMS DVB–CAR–PDMS	Limonene, α-pinene, 3-carene	
35 (a)	*Rhodiola rosea* (rhizomes)	Rose root	J. Rohloff	2002	PDMS 100	*n*-Decanol, benzyl alcohol, cynnamyl alcohol	EO
36 (a)	*Salvia officinalis* *Melaleuca alternifolia* (leaves)	Sage	D. Zabaras	2001	PDMS 100	α-Terpinene, γ-terpinene, terpinen-4-ol	
37 (a,e)	*Spondias mombin*	Taperebà, caja	P.M.N. Ceva-Antunes	2003	CAR–DVB–PDMS	Myrcene, β-phellandrene, thyl hexanoate	SDE
38 (a)	*Zingiber officinale* (fresh rhizome)	Ginger	Y. Shao	2003	CAR–PDMS PDMS–DVB PDMS 100 PDMS 30	β-Phellandrene, α-muurolene, α-farnesene	
39 (a)‡		"Ciaculli Late" mandarin	G. Alonzo	2003		Limonene, caryophyllene, terpinene (PPA)	
40 (a,e)		olibanum	S. Hamm	2003	PDMS PDMS–DVB CAR–PDMS DVB–CAR–PDMS	β-Caryophyllene, limonene, isoincensole acetate	SE (MECL)

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‡ Articles that were not available.

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Ref. no. (topics)†	Plant name (part of the plant)	Common name	Senior author	Year	Fibers	Main components	Other techniques
41 (b,e)	*Abies fraseri* (foliage)	Fraser fir	D.A. Vereen	2000	PDMS 100 PA PDMS 7	Bornyl acetate, 3-carene, camphene	SE (MECL)
42 (b)	*Agrostis stolonifera* *Pennisetum clandestinum* *Eucalyptus leucoxylon* *Trifolium repens*	Bent grass Kikiyu grass White clover	R.M.M. Perera	2002	CAR–PDMS PDMS 100	(*Z*)-3-Hexen-1-ol, α-pinene, 1-octen-3-ol, 1,8-cineole	
43 (b)	*Boronia megastigma* (flowers)	Brown boronia	H. Mac Tavisch	2000	PDMS 100	β-Ionone, α-pinene, caryophyllene	
44 (b)‡	*Ceratonia siliqua* (flowers)	Carob tree	L. Custodio	2004		Linalool, *trans*-linalool oxide	
45 (b,d)	*Chrysanthemum coronarium* (pollen, leaves, floral parts)	Garland	G. Flamini	2003	PDMS 100	Camphor, *cis*-chrysanthenyl acetate, myrcene (PPA)	EO
46 (b)‡	*Coriandrum sativum* (fruits)	Coriander	A. Carrubba	2002		α-Pinene, *p*-cymene, γ-terpinene	
47 (b)	*Eucalyptus citriodora* (leaves)		G. Xiong	2003	PDMS 7 PDMS 100 PDMS–DVB CAR–PDMS		MWHS–SPME
48 (b,d)	*Lamium purpureum* *L.hybridum* *L. bifidum* *L.amplexicaule* (flowers, leaves, bracts)		G. Flamini	2005	PDMS100	Germacrene D, *trans*-chrysanthenyl acetate, β-pinene (PPA)	EO
49 (b)§	*Lycopersicon esculentum* (leaves)	Tomato	C. Deng	2004	PDMS 100 CW–DVB	Methyl salicylate, β-phellandrene, 4-carene	
50 (b)	*Michelia alba* (flowers)	Magnolia	C. Shang	2002	PDMS 100	Limonene, germacrene D, camphor	EO
51 (b,c)	*Pinus sylvestris* (seeds)	Scots pine	P. Tammela	2003	PDMS 100	δ-3-Carene α-pinene, limonene	
52 (c)‡	*Apium graveolens*	Celery	B. Tirillini	2004		Limonene, γ-terpinene	
53 (c,d)	*Bupleurum fruticosum* (aerial parts)		A. Bertoli	2004	PDMS 100	β-Phellandrene, γ-terpinene, sabinene	EO
54 (c)‡	*Cnidium officinale*		M. Chung	2004	PDMS CAR–PDMS		
55 (c)	*Cucurbita pepo* (flowers)	Zucchini	A.M. Granero	2004	PDMS 100 PDMS–DVB	1,4-Dimethoxybenzene 1,2,4-trimetoxybenzene	
56 (c)	*Eucalyptus sp.* (leaves)		C.A. Zini	2003	PDMS 7		

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§ Compounds in plant infested by tobacco mosaic virus.

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57 (c,e)	*Matricaria recutita* (flowerheads)	Chamomile	C. Bicchi	2005	PDMS 100 CW–DVB CAR–PDMS PDMS–DVB DVB–CAR–PDMS	α-Bisabolol, bisabolol oxide A and B	EO
58 (c)	*Ocimum americanum* *O. basilicum* *O. gratissimum* *O. sanctum* (whole plant)		L. Jirovetz	2003	PDMS–CAR–DVB	(*Z*)-Methyl cinnamate, methyl eugenol, eugenol (PPA)	EO
59 (c)‡	*Origanum vulgare*	Origan	D. Bertelli	2003	PDMS 100 (2 cm) CAR–PDMS	Thymol, carvacrol	MWHS–SPME
60 (c)‡	*Osmanthus fragrans var. latifolius* *O. fragrans var. thunbergii* (flowers)	Tea olive, fragrant olive, sweet olive	C. Deng	2004	CW–DVB	α-Linalool, β-linalool, *cis- and trans*-linalool oxide	
61 (c)	*Pelargonium hortorum* (leaves)	Common geranium	X. Deng	2004	PDMS 30 PDMS 100 PDMS–DVB CAR–PDMS DVB–CAR–PDMS	Myrcene, caryophyllene, linalool	
62 (c)‡	*Rhododendron species* (leaves, flowers, fruits)		D. Tasdemir	2003			SE (nHex, MECL, H_2O)
63 (d,e)	*Allium sativum*	Garlic	S. Lee	2003	PDMS 30 PDMS 100 DVB–CAR–PDMS CW–DVB PA	Diallyl disulfide, allyl sulfide, diallyl trisulfide	EO SDE SPTE
64 (d)‡	*Aloysia gratissima*		A. Sartoratto	2003	PDMS 100 CAR–PDMS		EO
65 (d,e)	*Amomum villosum* *Amomum villosum var. xanthioides* *Amomum longiligulare* (dried ripe fruits)	Fructus Amomi (Sha Ren)	S. Shen	2005	PDMS 100 PDMS–DVB CW–DVB CAR–PDMS	Camphor, borneol acetate, D-limonene	SFE EO SE (nHex)
66 (d)	*Angelica pubescens* *A. sinensis* (roots)	Doubleteeth Chinese angelica	G. Song	2004	PDMS 100 CW–DVB	*A. sinensis:* cyclofenchene, α-pinene, 3-butylidene-1(3H)-isobenzofuranone *A. pubescens:* eudesma-4,11-diene, 7-Methoxy-8-(3-methyl-2-butenyl)-2H-1-benzopyran-2-one, α-chamigrene	
67 (d)	*Chrysanthemum indicum* (flowers)	Indian white chrisantemum	S. Shen	2004	PDMS 100 PDMS–DVB CW–DVB PA	Camphor, β-farnesene, β-caryophyllene	

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68 (d)	*Chrysanthemum indicum* (flowers)	Indian white chrisantemum	Q. Wu	2004	PDMS 100 CW–DVB	Camphor, borneol, α-farnesene	EO
69 (d)‡	*Curcuma aeruginosa* (rhizome)		Y. Sha	2004			EO
70 (d)	*Eucalyptus dunnii* *E. citriodora* *E. saligna* (chopped leaves)		C.A. Zini	2003	PDMS 7 PDMS 30 PDMS 100 PDMS–DVB PA		EO
71 (d)‡	Eucalyptus lheritier (leaves)		M.G. Wirthensohn	2000		1,8-Cineole, pinene	
72 (d,e)‡	*Houttuynia cordata*	Hearthleaf Houttuynia herb	M. Liang	2005			FE EO
73 (d,e)	*Houttuynia cordata*	Hearthleaf Houttuynia herb	M. Liang	2005	PDMS 100	2-Undecanone, houttuynum	FE EO
74 (d)	*Hypericum triquetrifolium* (flowers, leaves)		A. Bertoli.	2003	PDMS 100	α-Pinene, myrcene, β-caryophyllene (PPA)	EO
75 (d)	*Lavandula canariensis*	Lavender	J. Pala-Paul	2004	PA	Carvacrol, (*E,E*)-α farnesene, β-bisabolene	EO
76 (d,e)	*Lippia alba* (leaves, stems)	Juanilama or Salvia Sija	E.E. Stashenko	2004	PDMS 100	Bicyclosesquiphellandrene, limonene, carvone	EO SDE MWHS SFE S-HS P&T
77 (d)‡	*Magnolia officinalis* (bark)	Bigleaf magnolia	Y. Sha	2004	PDMS 100	β-Eudesmol, *p*-cymene	
78 (d)	*Mentha sachalinensis* (leaves, flowers)	Sachalinmint	J. Rohloff	2002	PDMS 100	Menthol, isomenthone, menthone	EO
79 (d,e)	*Platyclaudus orientalis* (leaf twigs)	Chinese arborvitae	G. Song	2003	PDMS 100 CW–DVB	α-Pinene, α-caryophyllene, β-caryophyllene	SE (ETAC, nPent) EO
80 (d)	*Psoralea bituminosa* (leaves, flowers, seeds)		A. Bertoli	2004	PDMS 100	α-Pinene, tricyclene, camphene	EO
81 (d)	*Rheum officinale* *Citrus aurantium* *Magnolia officinalis*	TCM	Y. Sha	2004	PDMS 100		
82 (d)	*Schisandra chinensis* (fruits)	Chinese Magnoliavine	C. Deng	2003	PDMS 100 CW–DVB	γ-Cadinene, 2,4a a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-1H-Benzocycloheptene, α-santalene	EO
83 (d)	*Smyrnium olusantrum* (roots, stems, leaves)		A. Bertoli	2004	PDMS 100	β-Myrcene, β-phellandrene, β-caryophyllene	EO

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84 (d,e)	*Xylopia aromatica* (fruits)	Malagueto, malagueto hembra	E.E. Stashenko	2004	PDMS 100	β-Phellandrene, ≤ β-myrcene, *p*-mentha-1(7),8-diene	EO SDE MWHS SFE S-HS P&T
85 (e)	*Aloysia triphylla* (leaves)	Lemon verbena	N. Kim	2004	PDMS 7 PDMS 30 PDMS 100 CW–DVB PA DVB–CAR–PDMS	Geranial, neral, 1,8-cineole	
86 (e)	*Lavandula angustifolia* *L. dentate* *L. heterophylla* *L. stoechas* (leaves, flowers, buds)	Lavender	N. Kim	2002	PDMS 100 PDMS 30 PDMS 7 PA CW–DVB	Linalyl acetate, linalool, caryophyllene	SPTE (Por Q) RPSD SDE (Pet. Et.)
87 (e)	*Rosmarinus officinalis* *Salvia officinalis* *Thymus vulgaris* *Valeriana officinalis*	Rosemary sage thyme valerian	C. Bicchi	2000	PDMS 7 PDMS 30 PDMS 100 CW–DVB CAR–PDMS PA PDMS–DVB DVB–CAR–PDMS		
88 (e)	*Smallanthus sonchifolius* (leaves)	Yacon	M. Adam	2005	PDMS 100 PDMS–DVB DVB–CAR–PDMS	β-Pinene, caryophyllene, γ-cadinen	
89 (e)	*Laurus nobilis* (leaves)	Bay leaf	M.C. Diaz-Maroto	2002	PDMS 100	1,8-Cineole, linalool, R-terpinyl acetate	SDE
90 (e)	*Ligusticum chuanxiong* *Angelica sinensis* (dry roots)	Szechuan Lovage Chinese Angelica	C. Deng	2005	PDMS 100 PDMS–DVB CW–DVB CAR–PDMS	*Z*-Ligustilide, *E*-ligustilide	PHWE
91 (e)	*Nepeta cataria*	Catnip	R. Baranauskiene	2003	PDMS 100 PDMS–DVB CAR–PDMS	Geranyl acetate, citronellyl acetate, 1,8-cineole	EO SDE (MECL) S-HS
92 (e)‡	*Picea engelmannii* (*spruce and seeds)*	Engelmann pruce	M. Mardarowicz	2004			PLE EO
93 (e)‡	*Trigonella foenum-graecum* (seeds)	Fenugreek	G. Mazza	2003		2-Methyl-2- butenal, δ-elemene, hexanol	SE (MECL, MEOH, H_2O)
94 (e)‡		Fructus Amomi	C. Deng	2005			PHWE

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† (topics), main topics as classified in the HS–SPME applications to the aromatic and medicinal plant field section.
‡ Articles that were not available.

Table I. (continued) List of the Plants (Botanical and Common Names) Whose HS Was Analyzed by HS–SPME*

Ref. no. (topics)†	Plant name (part of the plant)	Common name	Senior author	Year	Fibers	Main components	Other techniques
95 (f)	*Mentha piperita* (aerial parts)	Peppermint	M.L. Ruiz del Castillo	2004	PDMS 100	Menthone, menthyl acetate, limonene	
96 (f)‡	*Pelargonium tomentosum*		S. Fuchs	2001		Piperitone, (–)-menthone, (+)-isomenthone	
100	*Fragraria vesca* (fruits)	Strawberry	L. Urruty	2002	DVB–CAR–PDMS		ANN
108			D. Zabaras	2002	PDMS 100 CAR–PDMS	α-Terpinene, γ-terpinene, α-phellandrene	

* Polydimethlysiloxane, PDMS; carbowax, CW; divinylbenzene, DVB; static HS, S-HS; essential oil, EO; solvent extraction, SE; dynamic HS, DHS; charcoal, CHA; simultaneous distillation extraction, SDE; dichloromethane, MECL; normal hexane, nHex; solid-phase trapping solvent extraction, SPTE; flash evaporation, FE; microwave-assisted HS, MWHS; supertcritical fluid extraction, SFE; purge and trap, P&T; ethyl acetate, ETAC; normal pentane, nPent; Porapak Q, Por Q; reduced-pressure steam distillation, RPSD; petroleum ether, Pet. Et.; pressurized hot water extraction, PHWE; pressurized liquid extraction, PLE; methanol, MEOH; articifical neural networks, ANN; essential oil, EO; and depending on the analyzed part plant, PPA.
† (topics), main topics as classified in the HS–SPME applications to the aromatic and medicinal plant field section.
‡ Articles that were not available.

Table II. List of Commercially Available Fibers Most Used in the Medicinal and Aromatic Plant Field with Characteristics Acronyms and Acronyms or Abbreviations Adopted in this Review

Acronym	Full name	Volume of the coating (mm^3)
PDMS 7	Polydimethylsiloxane, 7 µm	0.026
PDMS 30	Polydimethylsiloxane, 30 µm	0.132
PDMS 100	Polydimethylsiloxane, 100 µm	0.612
CW–DVB 65	Carbowax–divinylbenzene, 65 µm	0.357
CAR–PDMS 75	Carboxen–polydimethylsiloxane, 75 µm	0.436
PA 85	Polyacrylate, 85 µm	0.521
PDMS–DVB 65	Polydimethylsiloxane–divinylbenzene, 65 µm	0.357
CAR–DVB–PDMS	Carboxen–divinylbenzene–polydimethylsiloxane, 50/30 µm (2 cm)	1.000

available fibers most frequently used in the medicinal and aromatic plant field together with their characteristics and acronyms.

HS–SPME has been applied to study different topics in the field of medicinal and aromatic plants, in particular: (*a*) the composition of the volatile fraction, the formation of the emitted volatile or their evolution in a living plant or a part of it (or both) (13–40); (*b*) plant origin, development, and their response to external factors through the analysis of specific volatile marker compounds or of the total volatile fraction (30,41–51); (*c*) the discrimination (or comparison) among species belonging to the same genus and within the same species among different cultivars, subspecies, varieties, or chemotypes, and to distinguish between specimens obtained by micropropagation or cloning and native plants (31,51–62); (*d*) the evaluation of the quality of the plant samples also in comparison with their essential oil (EO) composition (17,24,45,48,53,63–84) (see also the next section); (*e*) the evaluation of the influence on the recovery of the volatile fraction of different fibers (3,40,57,63,85–88), sample preparation techniques (3,17,37,41,63,65,72,73,76,79,84,86, 89–94), and technological treatment (89) (see also the next section); and (*f*) biosynthesis and chiral recognition of plant volatile components (95,96).

Selection of an effective fiber and sampling conditions for HS–SPME applications

One of the main tasks when developing an SPME method is selecting the most effective fiber and sampling conditions, in particular when the HS of a complex volatile fraction is to be investigated, as is very often the case with aromatic and medicinal plants. Several factors influence the choice of fiber and sampling conditions because recovery depends upon, among other things, the polarity and volatility of the analytes investigated, the physicochemical characteristics of the polymeric coating and analyte/polymer affinity, the composition and physical state of the matrix, the HS equilibration temperature and time (K_2), and analyte diffusion and equilibration time from the vapor phase to the fiber surface (K_1). Yet another important factor is the nature of the fiber coatings, which often consist of two or three components whose recovery capabilities are based on different phenomena [e.g., polydimethylsiloxane (PDMS) on sorption or carboxen (CAR) on adsorption] so as to extend the range of polarities covered and keep good selectivity. These factors together make it quite difficult to achieve both equilibria within reasonable sampling times, particularly when several analytes with different polarities and volatilities must be sampled simultaneously from a complex matrix (40,57,87,88,97). Nonequilibrium sampling conditions are, therefore, very often adopted in order to keep sampling within a reasonable time; as a consequence, rigorous and reproducible standard conditions must be applied (and reported) for results to be consistent.

A first attempt to rationalize the choice of an SPME fiber was made by Bicchi et al. (87) with the introduction of the concentration factor (CF). CF_{ij} evaluates the accumulation capability of a given fiber, *j*, for a component, *i*, from the HS of the matrix inves-

tigated through the ratio of the areas obtained by both HS–SPME (A_{if}) with that fiber and static HS (A_{ig}). CF can be calculated through a very simple equation:

$$CF_{ij} = \frac{A_{if}}{A_{ig}} \quad \text{Eq. 1}$$

Obviously, CF is not an absolute parameter because it depends on HS sampling conditions and is influenced by the matrix effect. However, it may be used successfully to compare the relative recovery effectiveness of different fibers (or to find the optimal sampling conditions with a given fiber) for a number of analytes representative of a sample even in nonequilibrium HS conditions, provided that rigorous and reproducible standard conditions are applied. CF is therefore very useful when a sufficiently large number of samples must be analyzed because its determination is time-consuming and requires additional static (S)-HS analyses. Moreover, CF measures the fiber performance for each marker component, thus making the choice of the most suitable fiber (or sampling conditions) for the analysis of a complex matrix dependent on a set of values, one for each marker analyte. In 2002, Zuba et al. (98) introduced a criterion function able to describe, in a single number, the concentration capability of a given fiber within a set of fibers on the basis of a group of markers characterizing the HS of the matrix investigated:

$$F_j = \frac{1}{n}\sum_{j=1}^{n}\frac{H_{ij}}{\frac{1}{k}\sum_{j=1}^{n}H_{ij}} \quad \text{Eq. 2}$$

where j is the fiber; F_j is the concentration capability factor of the fiber; n is the number of marker components characterizing the matrix under investigation; k is the number of fibers; and H_{ij} is the height of the peak of component, i, with the fiber. Hamm et al. (40) recently simplified equation 2:

$$F_{ij} = \frac{\sum_{1} H_{ij}}{\frac{1}{k}\sum_{ij} H_{ij}} \quad \text{Eq. 3}$$

These equations are both very useful, in particular, for routine analyses because they make it possible to choose the most effective polymeric coating for a given sample or to monitor the sampling capability of a fiber over time and to choose the best sampling conditions through a single and biased number.

Various studies have tested more than three fibers in order to maximize analyte recovery for a specific application in the aromatic and medicinal plant field (3,16,26,34,38,40,41,47,57,61,63, 67,70,85–88,90,91). Systematic studies on fiber performance for HS–SPME sampling in this field have been done by Bicchi et al. (57,87), Hamm et al. (40), and Adam et al. (88).

Although multicomponent fibers have proved to be the most effective, most of the routine applications in the aromatic and medicinal plant fields adopt a PDMS 100 fiber. The widespread use of this fiber is not only attributable to its good recovery of the HS components of medicinal and aromatic plants, because, in general, their polarity is medium to low, but also to both the consistency of its performance and its increased repeatability when a large number of analyses are involved.

Fiber consistency and performance over time are two other important factors involved in HS–SPME. HS sampling, in particular with plant matrices, is a "clean" sampling technique and seldom suffers from decay of fiber performance—in the authors' experience, the average lifetime of the most recent fibers is approximately 100 quantitatively repeatable sampling/reconditioning cycles (i.e., with percent relative standard deviation below 10%) (99). This number also includes the initial samplings, when fiber performance, of the multicomponent fibers in particular, has sometimes not yet stabilized. In any case, for reliable quantitative analysis, fiber performance must be checked against a reference sample at least every 10–15 samplings by measuring the variation of F_{ij} or CF_{ij} for that fiber (40,57,87,98). An in-depth study is under way in the authors' laboratory on the consistency and lifetime of conventional fibers and a new generation of metal fibers recently introduced on the market (99). Consistency, repeatability, and reproducibility of fibers are fundamental in this field for routine control analysis; discrimination between plant varieties, cultivars or chemotypes; correlations with volatile fraction composition obtained with other sampling techniques; and to obtain results that can be processed validly with multivariate statistical analysis or neural network approaches (57,100).

HS–SPME versus other techniques of sample preparation of the volatile fraction

HS–SPME has been used in combination with or as an alternative to several other sampling techniques to characterize the volatile fraction of vegetable matrices (see item *d* in the last paragraph of the "HS–SPME applications to the aromatic and medicinal plant field" section). In particular, most applications compare the compositions of HSs sampled by SPME with that of the EO of a plant (as may be seen in item *d* in the last paragraph of the "HS–SPME applications…" section). Unfortunately, EO and HS compositions have often been erroneously compared directly, or, even worse, the two have not even been distinguished. Although the compositions of EOs and HSs sampled by SPME are sometimes similar, the areas (or percentages) of an analyte obtained with the two techniques are not interchangeable because they are obtained from entirely different approaches. These approaches greatly influence the resulting quantitative composition and, to a lesser extent, the qualitative composition, as it is clear from the definitions of volatile fraction, EO, and HS reported that follow. The term volatile fraction defines those mixtures consisting of compounds that can be sampled as a consequence of their capability to be vaporized both spontaneously and through suitable sampling conditions or techniques. The term volatile fraction of a plant is, therefore, a framework including approaches or techniques that produce samples of different compositions but representative of the volatiles characterizing a vegetable matrix (e.g., HS, EOs, flavors, fragrances, aromas, and extracts prepared through specific techniques). An EO is defined as the product obtained by hydro- or steam-distillation or by cold expression (for citrus fruits) of a plant or of some parts of it (101–103), and HS sampling is a solvent-free technique aimed at sampling the gaseous or vapor phase in equilibrium (or not) with a solid or liquid matrix in order to characterize its composition (104). An additional factor of discrimination in HS–SPME is the nature of the polymeric coatings of the fibers, which conditions the com-

position of the volatile fraction recovered. In spite of the clarity of the definitions, eight articles out of the 88 quoted in this review make this mistake. In the authors' opinion, EO and HS compositions of a plant can qualitatively be correlated only through statistical methods, as it is, for instance, by performing a multivariate analysis of their gas chromatographic (GC) profiles. On the other hand, a quantitative correlation of the individual components in the EO and in the HS–SPME can only be achieved through a statistical approach (e.g., multiple linear regression or neural network) that is suitable to determine the functions linking quantitative data of the components investigated, obtained separately by both HS–SPME–GC and EO GC analyses requiring data from a large number of samples in order to be representative (57).

HS–SPME has been compared not only with steam- or hydrodistillation but also with other sample preparation techniques to characterize the volatile fraction of aromatic and medicinal plants, such as: (*a*) solvent extraction with different solvents (SE) (17,41,65,79,93), (*b*) simultaneous distillation extraction (SDE) (37,63,76,84,86,89,91), (*c*) microwave (MW)-assisted HS (MWHS) (76,84), (*d*) supercritical fluid extraction (SFE) (65,76,84), (*e*) purge and trap (P&T) (76,81), (*f*) pressurized hot water extraction (PHWE) (90,94) and pressurized liquid extraction (PLE) (92), (*g*) flash evaporation (FE) (72,73), (*h*) solid-phase trapping solvent extraction (SPTE) (63,86), (*i*) static HS (S-HS) (3,91), (*j*) HS sorptive extraction HSSE (3), and (*k*) HS–solid-phase dynamic extraction (HS–SPDE) (6). In most cases, HS–SPME played a crucial role in characterizing the qualitative and quantitative compositions of the volatile fraction and in monitoring the phenomena involved with the plants under investigation.

Advantages and limits of HS–SPME applied to medicinal and aromatic plants

The popularity reached by HS–SPME is attributable to several factors: (*a*) its theory and practice are now well known, as well as its advantages and limits; (*b*) it is versatile, easy to automate, repeatable, reproducible, and flexible (it can be used indifferently for liquid or vapour phase samplings); and (*c*) it can be used with any type of instrumentation without modification in any conditions.

Several factors have contributed to the success of HS–SPME, including (*a*) sampling times are shorter than with conventional hydro- or steam-distillation because analytes can reliably be concentrated onto the fiber in a pseudo S-HS condition; (*b*) a limited number of parameters need to be tuned to maximize analyte recovery [mainly temperature, time, and phase ratio (b)]; (*c*) sampling and analysis steps can be separated for in the field or process samplings because the fiber can be stored in its holder, keeping the sample safe over time; (*d*) biological processes can be monitored through volatile markers and samples taken in vitro, keeping the system isolated from the surrounding atmosphere, in vivo as well as in the field, and results can be compared; (*e*) it can be used in combination with unmodified conventional GC units as well as with the most recent GC techniques, such as fast GC and comprehensive GC×GC.

HS–SPME has been successfully combined with fast GC and fast GC–fast mass spectrometry (MS) (i.e., time-of-flight MS) because of its ability to operate reliably in nonequilibrium HS conditions, which makes it sufficiently fast to make sampling time short enough to be compatible with fast GC; thus, a combination of the two techniques is a reasonable proposition (57). However, sample preparation conditions different from those adopted for conventional HS–SPME sampling may be required to make analysis and sampling times compatible. In particular, nonequilibrium HS sampling parameters and the thickness of the polymer fiber coating have to be investigated. Moreover, in-depth investigation on new polymeric coating and on nonequilibrium HS sampling are necessary to make HS–SPME even more effective as a fast sample preparation technique for fast analysis of the volatile fractions of medicinal and aromatic plants, in particular when monitoring the dynamics of biological phenomena. HS–SPME has also been successfully combined with the most powerful separation technique currently available (i.e., comprehensive GC×GC), resulting not only in a dramatic increase of the number of peaks separated but also facilitating the recognition or discrimination of different plant materials and the authentication and quality control of herbal products (32,38,42,56).

The main limits of HS–SPME, recognized after more than ten years of everyday experience in the authors' laboratory are:

(*i*) Limited concentration capability in analysis of trace components of high complexity samples, as is often the case for medicinal and aromatic plants. This is probably attributable to the small volume of polymer coating the fiber, which ranges between 0.4 and 0.6 µL, and to the unfavorable phase ratio (β), because plant matrices (in particular living plants) often occupy large volumes. Recovery can be increased through constant stirring (or vibration) of the sampling vial to improve the diffusion process and the HS/fiber analyte exchange (105,106). Microwave assistance has also been shown to improve recovery (47,59) because it accelerates the volatile emission, thus increasing the number of possible detections, over time, of a given event and makes the ratio between analytes with lower and higher molecular masses in the HS more uniform (59). In any case, for applications in the biological field, microwave assistance results must be checked carefully to detect possible artefact formation.

(*ii*) Quantitative analysis of HS components of a solid matrix (i.e., living plants as such) is sometimes problematic and time consuming because of the difficulty of building calibration curves. Zabaras and Willie recently proposed a method to quantitate the amount of 37 terpenoids present in the HS of aromatic and medicinal plants by determining their K_1 on a PDMS fiber through their linear temperature programmed retention index on a GC column coated with a stationary phase of a similar polarity. The analyte concentration or absolute amount in the vapor phase can then be calculated through their standard calibration curves (36).

(*iii*) Several fibers require longer conditioning times than those recommended by the manufacturer to achieve repeatable performance or to completely eliminate low volatility "ghost" peaks caused by the polymer coating (or both), in particular when the special tool for fiber conditioning is not available (99,107). Other minor fiber problems are fragility of the fused silica (probably overcome by the new generation of metal fibers), lack of protection of polymer coating, and limited flexibility of surface area.

(*iv*) In some cases, fiber ingredients, in particular those operating in absorption, can produce artefacts. This phenomenon has

mainly been observed for fibers containing CAR: Zabaras and Willie studied *p*-cymene formation from *p*-menthane hydrocarbons (α- and γ-terpinene, and α-phellandrene and terpinolene) and the influence of humidity when HS was sampled with a CAR–PDMS fiber versus a PDMS 100 fiber (108).

(*v*) When larger series of samples have to be analyzed, time-dependent changes in the composition of HS may occur because of enzymic and nonenzymic changes taking place in the plant tissue, in particular with disintegrated/cut vegetable matrices.

Conclusion

HS–SPME is currently a well-established and widely used sampling technique to study the composition of the volatile fraction of medicinal and aromatic plants for which it has now become an important complement to EO analysis. For those applications where the EO composition is not officially required, HS–SPME sampling is successfully used as an alternative to hydro- or steam-distillation to characterize the volatile fraction of a plant because its reliability is comparable but it is faster and easy to automate. In addition HS–SPME sampling under nonequilibrium HS conditions is fast enough to make its combination with fast GC reasonable, thus making it very useful when a high number of samples of a given plant must be analyzed for quality control or classification, or when frequent samplings in a limited amount of time are needed to monitor the dynamics of a biological process.

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