Journal of Medicinal Plants Studies

www.PlantsJournal.com

R Székelyhidi
Department of Food Science, Faculty of Agricultural and Food Sciences, Széchenyi István University, Lucsony Street 15-17, 9200 Mosonmagyaróvár. Hungary

Analysis of the aroma chemicals of ten different herbs using HS-SPME-GC-MS technique

R Székelyhidi

Abstract
Solid-phase microextraction (SPME) method unify extraction, concentration and sampling into one single step. In SPME, a fused silica fiber is coated with a stationary phase and the fiber is exposed to an aqueous or gaseous sample till equilibrium is achieved between the analyte in the sample and the fiber. The analyte is then thermally desorbed from the fiber in the injection chamber of a gas chromatograph (GC) and analysed by mass spectrometer (MS).
Headspace solid-phase micro extraction (HS-SPME) coupled with gas chromatography- mass spectrometry (GC-MS) has been used for the chemical analysis of dried *Achillea millefolium* (milfoil), *Hyssopus officinalis* (hyssop), *Matricaria chamomilla* (camomile), *Anethum graveolens* (dill), *Galega officinalis* (goat's-rue), lovage, *Galium odoratum* (woodruff), *Artemisia dracunculus* (tarragon), *Plantago lanceolate* (plantain) and *Salvia officinalis* (sage) cultivated in Department of Food Science. The chemical composition of the HS-SPME extracts comprised mainly of monoterpenes (sabinene, β-pinene, limonene, 1,8 cineole, cis-sabinene, borneole, α-terpinyl-acetate, cis-pinocamphone, trans-pinocamphone, α-phellandrene, dill ether, methyl-chavicol, trans-chrysanthemum acetate, methyl-eugenol, mircene, cis-thujone, trans-thujone) and sesquiterpenes (caryophyllene E, α-humulene, allo-aromadendrene and (E)-β-farnesene). The HS-SPME is a rapid, cheap, simple and eco-friendly procedure for the essential oil screening of herbs.

Keywords: herbs, terpenes, gas chromatography-mass spectrometry, volatile compounds, solid-phase microextraction.

1. Introduction
Traditionally, the extraction of volatile components usually performed by steam distillation (SD) and solvent extraction (SE) at both analytical and preparative levels. The innovative solid-phase microextraction (SPME) technique is more rapid, sensitive, and solvent-free compared to traditional methods [2, 3]. The analytes are extracted from a gaseous or liquid matrices by absorption in direct-immerse solid-phase microextraction (DI-SPME) or adsorption on headspace solid-phase microextraction (HS-SPME) with a thin polymer coating being fixed to the solid surface of a fiber in an injection needle [11].
It is well-known that the occurrence of essential oils and their composition determine the specific aroma of plants [4, 13]. The main chemical families present in aromatic herbs are: monoterpenes, monoterpenoids, and phenylpropanoids. In lower amount alcohols, sesquiterpenes, sesquiterpenoids, aldehydes, and esters [3, 5, 7, 10]. The composition and concentrations of essential oils from aromatic herbs depend on many factors, including geographical source, climatic and soil conditions, stage of vegetative cycle, seasonal variation, etc. [6, 8, 12].

2. Materials and methods
Plant material and sample preparation
Ten sun-dried aromatic herbs (milfoil, hyssop, camomile, dill, goat's-rue, lovage, woodruff, tarragon, plantain and sage) were bought from Hungarian herbarium. 200g of herbals were chopped by 10 minutes with a high performance (750w) electric kitchen grinders. The samples were stored at -20 °C in freezer until further use.

Headspace-solid-phase microextraction (HS-SPME)
The dried herbs (milfoil, hyssop, camomile, dill, goat's-rue, lovage, woodruff, tarragon, plantain and sage) was subjected to chemoprofiling using head space-solid phase

Correspondence
R Székelyhidi
Department of Food Science, Faculty of Agricultural and Food Sciences, Széchenyi István University, Lucsony street 15-17, 9200 Mosonmagyaróvár. Hungary

microextraction (HS-SPME) coupled with gas chromatograph – mass spectrometer (GC-MS). The sample (2g) was placed in a 43 ml vial wich was sealed with a screw-capped top containing a teflon-lined septum and thermostating in a magnetic block heater (Multi-Blok, Lab-Line) at 55 °C for 50 min. The 100 μm SPME fiber (Supelco®) coated with polidimetilsiloxane (PDMS) was exposed to the headspace of the sample at 55 °C for 5 minutes. The fiber was then retracted and inserted into the injector of the gas chromatograph coupled to mass spectrometer. Desorption was performed for 5 min, with the injector at 280 °C and in split mode.

Identificaton of the essential oil components was done on the basis of retention indices (RI, determined of homologous series of n-alkanes (C8-C20, Sigma-Aldrich®), under the same experimental conditions, co-injections with standards (Sigma Aldrich®). Essential oil constituents were identified by comparison of volatile compounds retention times (as Kovats indices) with published and own data [1, 9].

$$ KI = 100 \left[\frac{(t_R - t_0) - (t_{R,n} - t_0)}{(t_{R,n+1} - t_0) - (t_{R,n} - t_0)} + n \right] \qquad (1) $$

n   carbon atoms in the n-alkane,
KI   Kovats retention index,
t_0 idle time,
t_R retention time,
$t_{R,n}$ the number of carbon atoms in the smaller n-alkane,
$t_{R,n+1}$ the number of carbon atoms in the larger n-alkane.

Gas chromatography – mass spectrometry (GC- MS)
GC-MS analysis was carried out on a Finnigan MAT gas chromatograph – mass spectrometer series GCQ fitted with a RTX-5 (Restek®) fused silica capillary column (30m x 0,25 mm, film thickness 0,25 μm) under the following conditions: split ratio 1:60, helium (Linde®) as carried gas at 35 cm / sec constant flow rate, injector temperature 280 °C, oven temperature 50-200 °C at 3 °C / minute, transferline temperature 250 °C. Mass spectra: electron impact (EI +) mode, 70 eV and ion source temperature 200 °C. The scan range was 45-345.

Table 1: Headspace SPME volatiles of herbs (Chemical formula, molecule weight (M), retention time (t_R), Calculated retention time by Kovats (KI), differences (Diff) from literature retention time (1), reliability of identification by MS (MS Fit)

N°	Volatile component	Chemical formula	M	tR/min	KI	Diff	MS Fit
1.	santolina triene	C10H16	136	6.80	911	1	884
2.	α-thujene	C10H16	136	7.42	931	1	889
3.	α-pinene	C10H16	136	7.65	939	0	866
4.	camphene	C10H16	134	8.15	954	0	879
5.	sabinene	C10H16	136	9.00	977	2	971
6.	β-pinene	C10H16	136	9.10	980	1	-
7.	myrcene	C10H16	136	9.63	993	2	-
8.	α-phellandrene	C10H16	136	10.15	1006	3	899
9.	p-cymene	C10H14	134	10.95	1029	3	960
10.	limonene	C10H16	136	11.12	1033	4	763
11.	1,8-cineole	C10H18O	154	11.33	1039	8	700
12.	(Z)-β-ocimene	C10H16	136	11.50	1043	6	833
13.	(E)-β-ocimene	C10H16	136	11.95	1054	4	833
14.	γ-terpinene	C10H16	136	12.37	1064	4	872
15.	artemisia-ketone	C10H16O	152	12.52	1067	5	889
16.	cis-sabinene-hydrate	C10H18O	154	12.83	1074	4	792
17.	terpinolene	C10H16	136	13.63	1091	2	910
18.	linalool	C10H18O	154	14.23	1104	7	-
19.	cis-thujone	C10H16O	152	14.52	1111	9	771
20.	transz-thujone	C10H16O	152	15.02	1124	10	708
21.	camphor	C10H16O	152	16.18	1151	5	822
22.	β-pinene-oxide	C10H16O	152	16.72	1163	4	-
23.	trans-pinocamphone	C10H16O	152	16.87	1166	3	758
24.	pinocarvone	C10H14O	150	17.00	1169	4	831
25.	borneol	C10H18O	154	17.10	1171	2	832
26.	para-menta-1,5-diene-8-ol	C10H16O	152	17.22	1174	4	831
27.	cis-pinocamphone	C10H16O	152	17.50	1179	4	788
28.	dill ether	C10H16O	152	18.03	1190	3	900
29.	α-terpineol	C10H18O	154	18.30	1195	6	882
30.	metil-chavicol	C10H12O	148	18.67	1203	7	969
31.	*trans*-chrysanthemum acetate	C12H18O2	194	20.27	1242	4	791
32.	carvone	C10H14O	150	20.77	1253	10	-
33.	bornil-acetate	C12H20O2	196	22.58	1292	3	613
34.	α -terpenil-acetate	C12H20O2	196	25.27	1355	6	860
35.	neoiso-dihidro-carveol-acetate	C12H20O2	196	25.40	1358	-1	-
36.	ciclosativene	C15H24	204	26.17	1375	4	-
37.	bourbonene	C15H24	204	26.75	1388	0	905
38.	metil-eugenol	C11H14O2	178	27.80	1412	8	844
39.	α-gurjunene	C15H24	204	27.80	1412	2	950
40.	caryophyllene E	C15H24	204	28.23	1423	4	904
41.	α-humulene	C15H24	204	29.70	1459	4	797
42.	(E)-β-farnezene	C15H24	204	29.82	1462	4	-
43.	allo-aromadendrene	C15H24	204	29.97	1465	5	929
44.	germacrene D	C15H24	204	30.80	1485	0	945
45.	spatulenol	C15H24O	222	34.68	1582	4	-
46.	α -bisabolol-oxide B	C15H26O2	238	37.62	1660	2	-

Table 2: HS-SPME volatiles of ten different herbs (retention time (tR), retention indices (KI), concentration calculated as % peak area of GC-MS).

N°	tr/min	KI	Volatile component	Relative peak area (%)									
				Milfoil	Hyssop	Camomile	Dill	Goat's-rue	Lovage	Woodruff	Tarragon	Plantain	Sage
1.	6.80	911	santolina triene	0.5%	-	-	-	-	-	-	-	-	-
2.	7.42	931	α-thujene	1.0%	-	-	-	-	-	-	-	-	-
3.	7.65	939	α-pinene	3.4%	1.2%	1.4%	1.4%	1.7%	2.0%	-	2.9%	2.9%	1.3%
4.	8.15	954	camphene	0.5%	-	-	-	-	0.3%	-	-	-	0.3%
5.	9.00	977	sabinene	14.8%	2.2%	1.0%	-	-	0.9%	-	5.2%	2.8%	-
6.	9.10	980	β-pinene	14.6%	12.2%	0.8%	-	3.1%	0.6%	-	-	1.1%	-
7.	9.63	993	myrcene	0.4%	1.7%	-	-	-	1.9%	-	-	-	-
8.	10.15	1007	α-phellandrene	0.3%	1.0%	0.8%	31.0%	-	1.1%	9.3%	-	5.9%	6.9%
9.	10.95	1029	p-cymene	2.4%	2.2%	6.1%	3.3%	6.7%	2.7%	3.3%	1.9%	6.8%	1.8%
10.	11.12	1033	limonene	6.4%	9.5%	24.5%	13.9%	21.9%	22.2%	17.7%	10.3%	27.1%	8.0%
11.	11.33	1036	1,8-cineole	8.0%	0.7%	1.2%	0.6%	3.8%	-	-	-	-	1.6%
12.	11.50	1043	(Z)-β-ocimene	-	-	-	-	-	0.9%	-	-	-	-
13.	11.95	1054	(E)-β-ocimene	-	0.2%	-	-	-	-	-	-	-	-
14.	12.37	1064	γ-terpinene	-	0.4%	-	-	-	0.4%	-	-	-	-
15.	12.52	1067	artemisia-ketone	1.6%	-	1.2%	-	-	-	-	-	-	-
16.	12.83	1074	cis-sabinene-hydrate	7.9%	-	-	-	-	-	-	-	-	-
17.	13.63	1091	terpinolene	-	-	-	-	-	1.0%	-	-	-	-
18.	14.23	1104	linalool	-	0.6%	-	0.7%	-	-	-	-	-	-
19.	14.52	1111	cis-thujone	-	0.4%	0.7%	2.3%	-	-	3.5%	-	-	12.4%
20.	15.02	1125	transz-thujone	-	-	0.5%	1.3%	-	-	2.3%	-	-	5.6%
21.	16.18	1151	camphor	2.8%	-	1.2%	2.7%	2.5%	-	4.2%	-	3.4%	28.4%
22.	16.72	1165	β-pinene-oxide	0.7%	4.2%	-	-	-	-	-	-	-	-
23.	16.87	1166	trans-pinocamphone	-	10.9%	-	-	-	-	3.1%	-	-	-
24.	17.00	1169	pinocarvone	-	2.4%	-	-	-	-	-	-	-	-
25.	17.10	1171	borneol	5.0%	-	-	-	-	-	-	-	-	5.9%
26.	17.22	1174	para-menta-1,5-diene-8-ol	2.9%	-	-	-	-	-	-	-	-	-
27.	17.50	1179	cis-pinocamphone	-	39.2%	1.5%	-	3.3%	2.1%	1.4%	-	-	-
28.	18.03	1191	dill ether	1.6%	0.9%	5.5%	30.1%	7.1%	8.2%	25.7%	-	6.8%	9.7%
29.	18.30	1196	α-terpineol	1.4%	-	-	-	-	2.8%	-	-	-	-
30.	18.67	1203	metil-chavicol	1.6%	1.9%	3.2%	5.2%	20.5%	2.8%	9.7%	64.8%	37.9%	1.2%
31.	20.27	1242	trans-chrysanthemum acetate	0.6%	-	-	-	-	-	-	-	-	-
32.	20.77	1253	carvone	-	-	-	2.4%	-	-	-	-	-	-
33.	22.58	1290	bornil-acetate	-	-	-	-	-	-	-	-	-	-
34.	25.27	1355	α -terpenil-acetate	6.6%	2.2%	0.1%	-	25.0%	50.0%	6.3%	0.9%	2.6%	-
35.	25.40	1358	neoiso-dihidro-carveol-acetate	-	-	-	-	-	-	-	-	-	0.3%
36.	26.17	1375	ciclosativene	0.5%	-	-	-	-	-	-	-	-	-
37.	26.75	1387	bourbonene	0.7%	-	-	-	-	-	-	-	-	-
38.	27.80	1412	metil-eugenol	-	-	-	-	-	-	-	12.5%	-	-
39.	27.80	1412	α-gurjunene	-	0.7%	-	-	-	-	-	-	-	-
40.	28.23	1423	caryophyllene E	8.3%	1.7%	1.0%	1.4%	0.8%	-	5.6%	1.5%	2.7%	6.8%
41.	29.70	1458	α-humulene	0.8%	-	-	1.5%	-	-	3.9%	-	-	9.0%
42.	29.82	1461	(E)-β-farnezene	1.2%	-	47.7%		-	-	0.7%	-	-	-
43.	29.97	1465	allo-aromadendrene	-	1.8%	-	-	-	-	2.5%	-	-	-
44.	30.80	1485	germacrene D	1.9%	0.8%	0.8%	0.3%	1.3%	-	0.9%	-	-	-
45.	34.68	1582	spatulenol	-	-	0.4%	-	-	-	-	-	-	-
46.	37.62	1660	α -bisabolol-oxide B	-	-	0.3%	-	-	-	-	-	-	-

3. Results and Discussion

The chemical analysis results by HS-SPME method are given in Table 2 and Table 3, where the compounds are listed in order of their elution from the RTX-5 column. HS- SPME-GC-MS analysis led to the identification of 46 volatile components from the herbs. The main mono- and sesquiterpene components identified in:

Milfoil HS-SPME extract were sabinene (14,8 %); β- pinene (14,6 %), limonene (6,4 %), 1,8 cineole (8,0 %), cis-sabinene (7,9 %), borneole (5,0 %) and α-terpinyl-acetate (6,6 %)) and sesquiterpene (caryophyllene E 8,3 %).

Hyssop HS-SPME extract were *cis*-pinocamphone (39.2 %), *trans*- pinocamphone (10,9 %), β-pinene (12,2 %) and limonene (6,4%).

Camomile HS-SPME extract were limonene (24,5 %) and (E)-*β*-farnesene (47,7%). In the dill HS-SPME extract were α-phellandrene (31,0 %), dill ether (30,1 %) and limonene (13,9 %).

Goat's-rue HS-SPME extract were limonene (21,9 %), methyl-chavicol (20,5 %) and α-terpinyl-acetate (25,0 %) and in lovage HS-SPME extract were limonene (22,2 %) and α-terpinyl-acetate (50,0 %).

Woodruff HS-SPME extract were α-phellandrene (9,3 %), limonene (17,7 %), dill ether (25,0 %), *trans*-chrysanthemum acetate (9,7 %), α-terpinyl-acetate (6,3 %), caryophyllene E (5,6 %), α-humulene (3,9 %) and *allo*-aromadendrene (2,5 %).

Tarragon HS-SPME extract were methyl-chavicol (64,8 %), limonene (17,7 %) and methyl-eugenol (12,5 %).

Plantain HS-SPME extract were mircene (5,9 %), α-phellandrene (6,8 %), limonene (27,1 %), dill ether (6,8 %) and methyl-chavicol (37,9 %).

Sage HS-SPME extract were α-phellandrene (6,9 %), limonene (8,0 %), cis-thujone (12,4 %), trans-thujone (28,4 %), dill ether (9,7 %), caryophyllene E (6,8 %) and α-humulene (9,0 %).

4. Conclusions

In conclusion the present report of the chemical profile of the essential oil of milfoil, hyssop, camomile, dill, goat's-rue, lovage, woodruff, tarragon, plantain and sage provides further indepth information about the chemo diversity in the chemical composition of the essential oil of various herbs. HS-GC method is developed for extraction and simultaneously GC monitoring of volatile components composition of aromatic herbs. By calculation of the relative peak areas of HS chromatograms of herbs, an information about the profiles of the respective oils can be received.

5. References

1. Adams RP. Identification of Essential Oil Componets by Gas Chromatography / Quadrupole Mass Spectroscopy. Carol Stream, Illinois, USA: Allured Published Corporation, 2001.
2. Arthur CL, Pawliszyn J. Solid phase microextraction with thermal desorption using fused silica optical fiber. Journal of Analytical Chemistry. 1990; 62:2145-2148.
3. Angioni A, Barra A, Cereti E, Barile D, Coisson JD, Arlorio M, *et al*. Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential of Rosmarinus officinalis L. Journal of Agricultural and Food Chemistry. 2004; 52:3530-3535.
4. Calín-Sánchez A, Lech K, Szumny A, Figiel A, Carbonell-Barrachina AA. Volatile composition of sweet basil essential oil (*Ocimum basilicum* L.) as affected by drying method. Food Research International. 2012; 48:217-225.
5. Calín-Sánchez A, Figiel A, Lech K, Szumny A, Carbonell-Barrachina AA. Effects of drying methods on the composition of thyme (*Thymus vulgaris* L.) essential oil. Drying Technology. 2013; 31:224-235.
6. Callan NW, Johnson DL, Westcott MP, Welty LE. Herb and oil composition of dill (*Anethum graveolens* L.): Effects of crop maturity and plant density. Industrial Crops and Products. 2007; 25:282-287.
7. Díaz-Maroto MC, Pérez-Coello MS, Sánchez-Palomo E, González-Viñas MA. Impact of drying and storage time on sensory characteristics of rosemary (*Rosmarinus officinalis* L.). Journal of Sensory Studies. 2007; 22:34-48.
8. Khazail HR, Nadjafib F, Bannayana M. Effect of irrigation frequency and planting density on herbage biomass and oil production of thyme (*Thymus vulgaris*) and hyssop (*Hyssopus officinalis*). Industrial Crops and Products. 2008; 27:315-321.
9. Kovats E. Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone. Helvetica Chimica Acta. 1958; 41:1915-1932.
10. Lee SJ, Umano K, Shibamoto T, Lee KG. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chemistry. 2005; 91:131-137.
11. Pragst F. Application of solid-phase microextraction in analytical toxicology. Analytical and Bioanalytical Chemistry. 2007; 388:1393-1414.
12. Petropoulos SA, Daferera D, Akoumianakis CA, Passam HC, Polissiou MG. The effect of sowing date and growth stage on the essential oil composition of three types of parsley (Petroselinum crispum). Journal of the Science of Food and Agriculture. 2004; 84:1606-1610.
13. Viuda-Martos M, Rúiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA. Spices as functional foods. Critical Reviews in Food Science and Nutrition. 2011; 51:13-28.