J Sci Food Agric 85:91–97 (2005)
DOI: 10.1002/jsfa.1934

Evaluation of four isolation techniques for honey aroma compounds

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Abstract: The analysis of the volatile fraction of honey provides useful information for the determination of the botanical and geographical origin. However, the results obtained vary greatly upon the extraction procedure employed. Four different isolation techniques were compared, that is hydrodistillation (HD), micro-simultaneous steam distillation–solvent extraction (MSDE), ultrasound-assisted extraction (USE) and solid-phase microextraction (SPME). From the data obtained, USE and SPME seem to be more suitable for the isolation of potent marker compounds. HD and MSDE have main drawbacks because of the drastic conditions used that lead to the formation of artefacts and the degradation of sensitive compounds. These drawbacks are avoided when employing USE and SPME.

Keywords: Hydrodistillation; micro-simultaneous steam distillation-solvent extraction; solid-phase microextraction; ultrasound-assisted extraction; honey aroma compounds

INTRODUCTION

Honey is mainly a supersaturated sugar solution. More than 95% of its dry mass consists of sugars and water, yet there is great variability in the flavour and aroma. Volatile compounds significantly contribute to the distinct flavour of honeys, depending on the floral origin.

Studies of the aroma composition of honey, as in all foods, traditionally involve aromatic extracts. Honey aroma is very complex, involving many tens of volatile compounds. The isolation of the volatile fraction of honey is carried out using different techniques. As a result, the composition of the aromas obtained is greatly dependent upon the procedure employed.

Hydrodistillation (HD) and microsimultaneous steam distillation–solvent extraction (MSDE) are the most common techniques used to isolate volatile compounds from a matrix. However, in the case of honey, such drastic conditions lead to the formation of artefacts, mainly due to the effect of heat on sugars.[1,2] Moreover, sensitive compounds are easily oxidized or decomposed and new components rise that do not belong to the aroma of honey.

Ultrasound-assisted extraction (USE) has been recently introduced into the analysis of honey volatiles.[3] It does not require heat and thus thermal generation of artifacts is avoided. Both low and high molecular weight compounds are isolated, providing good potential markers for honey origin determination.

Solid-phase microextraction (SPME) isolates the headspace aroma from a sample matrix.[4] It has been recently introduced in the food industry and is widely used in the analysis of volatile compounds.[5–8] The isolated aroma fraction is close to the aroma of the food analysed. In the case of honey, data in the literature are scarce.[9] The scope of the present work is the evaluation of four isolation techniques (HD, MSDE, USE, SPME) of honey aroma compounds.

MATERIALS AND METHODS

Honey sample

A unifloral citrus honey from the region of Argos, Greece, was collected as described previously.[3]

Isolation of volatile compounds

Hydrodistillation

A Clevenger SD apparatus was used. The sample flask was charged with 200 ml honey–water solution (80 g honey (100 ml^{-1}) water). The procedure was carried out for 4 h and the condenser on the head was cooled with water at room temperature.

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Contract/grant sponsor: Greek Ministry of Agriculture
Contract/grant sponsor: EU; contract/grant number: 1221/97
(*Received 13 October 2003; revised version received 27 April 2004; accepted 30 April 2004*)
Published online 24 September 2004

Table 1. Isolated compounds from citrus honey by the four methods (HD, MSDE, USE, SPME) employed

Peak no	Compound	HD	MSDE	USE	SPME
1	2-3-Pentanedione[a]	+			
2	Heptane[a,b]	+	+	+	
3	2-Methyl-2-hexene[a]	+			
4	Dimethyl disulfide[a]	+			
5	2-Methyl furan[a]	+			
6	Octane[a,b]	+	+	+	+
7	Furfural[a,b]	+	+		
8	2-Furanmethanol[a]	+			
9	5-Methyl-2(3*H*)-furanone[a]	+			
10	4-Cyclopentene-1,3-dione[a]	+			
11	Nonane[a,b]	+	+	+	
12	Heptanal[a,b]		+		
13	1-(2-Furanyl)-ethanone[a,b]	+			
14	Benzaldehyde[a,b]	+	+	+	+
15	6-Methyl-5-hepten-2-one[b]				+
16	Dehydroxy-trans-linaloxide[b]	+	+		+
17	5-Methyl-furfural[a,b]	+			
18	Dimethyl-trisulfide[a]	+			
19	Decane[a,b]	+	+	+	
20	Octanal[a,b]		+		+
21	1,3,8-*p*-Menthatriene[a,b]		+		+
22	Dehydroxy-cis-linaloxide[b]	+	+		+
23	Limonene[a,b]	+	+	+	+
24	2,2′-Bifuran[a]	+			
25	Phenylacetaldehyde[a,b]	+	+	+	+
26	*Trans*-furanoid linaloxide[a,b]	+	+	+	+
27	1-Octanol[a,b]			+	
28	*Cis*-furanoid linaloxide[a,b]	+	+	+	+
29	Undecane[a,b]	+	+	+	+
30	Linalool[a,b]		+		+
31	Hotrienol[a,c]	+	+	+	+
32	Nonanal[a,b]	+	+	+	+
33	2-Phenylethanol[a,b]	+	+	+	+
34	*Cis*-rose oxide[b]				+
35	Lilac aldehyde (isomer I)[c]	+	+	+	+
36	Lilac aldehyde (isomer II)[c]	+	+	+	+
37	Nerol oxide[b]				+
38	Lilac aldehyde (isomer III)[c]	+	+	+	+
39	*Cis*-pyranoid linaloxide[a,b]	+		+	
40	*Trans*-pyranoid linaloxide[a,b]			+	
41	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde (isomer I)[a]	+	+		
42	Dill ether[a,b]	+	+		+
43	Benzoic acid[a,b]			+	
44	2,6-Dimethyl-3,7-octadiene-2,6-diol[a,c]			+	+
45	Dodecane[a,b]	+	+	+	
46	Decanal[a,b]	+	+	+	+
47	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde (isomer II)[a]	+	+		
48	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde (isomer III)[a]	+	+	+	+
49	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde (isomer IV)[a]	+	+	+	+
50	2,3-Dihydrobenzofuran[a]	+	+	+	
51	2,6-Dimethyl-1,7-octadiene-3,6-diol[a]			+	
52	Phenylacetic acid[a,b]			+	
53	Nonanoic acid[a,b]	+			+
54	Limonen-10-ol[b]	+			
55	Indole[a,b]	+	+	+	
56	Tridecane[a,b]	+	+	+	
57	3,7-Dimethyl-1,6-octadiene-3,5-diol[a]			+	
58	Methyl anthranilate[a,b]	+	+	+	+
59	(*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienal[c]			+	
60	(*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol[a,c]			+	

Table 1. Continued

Peak no	Compound	HD	MSDE	USE	SPME
61	2,3,6-Trimethylbenzaldehyde[a]	+			
62	(*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol[a,c]	+	+	+	
63	Decanoic acid[a]	+			
64	Tetradecane[a,b]	+	+	+	
65	Nerolidol[a,b]			+	+
66	Butylated hydroxyanisole[a]	+			
67	Ethyl anthranilate[a]	+			
68	Caffeine[a,b]			+	

Identification: [a] NBS75K mass spectra library; [b] Adams[11]; [c] Wilkins *et al*.[14]

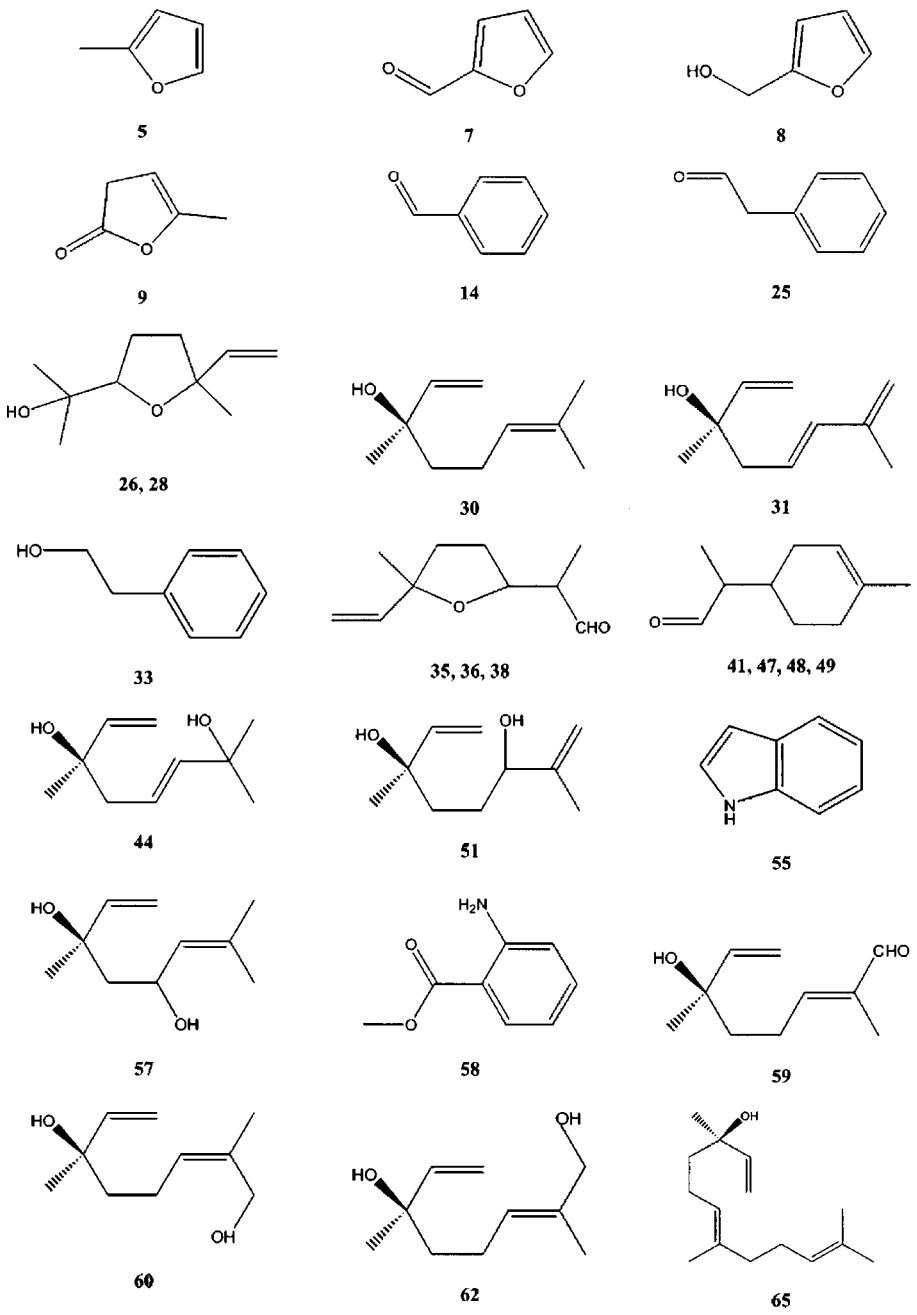

Figure 1. Structures of the most important compounds referred in this work.

Microsimultaneous steam distillation–solvent extraction
The MSDE apparatus was a modification of that used by Nickerson and Likens.[10] The extraction solvent was 5 ml diethylether. The sample flask was charged with 50 ml of honey–water solution (80 g honey (100 ml^{-1}) water). The steam distillation–extraction was carried

Table 2. Mass spectral data of the most important compounds referred in this work

Peak no	Compound	Prominent MS peaks
5	2-Methyl furan	40(1), 43(2), 49(8), 51(30), 53(95), 63(2), 81(53), 82(100, M^+)
7	Furfural	42(7), 50(6), 67(9), 95(97), 96(100, M^+)
8	2-Furanmethanol	41(63), 53(43), 69(31), 81(48), 97(52), 98(100, M^+)
9	5-Methyl-2(3*H*)-furanone	43(76), 55(100), 70(9), 74(9), 98(81, M^+)
14	Benzaldehyde	51(47), 73(3), 77(98), 105(95), 106(100, M^+), 107(9)
25	Phenylacetaldehyde	41(2), 51(6), 65(20), 91(100), 92(22), 120(21, M^+)
26	*Trans*-furanoid linaloxides	41(30), 43(59), 55(42), 59(100), 67(25), 68(32), 81(17), 93(30), 94(44), 111(26), 137(4), 155(5, M^+-15)
28	*Cis*-furanoid linaloxide	41(44), 43(70), 55(42), 59(100), 67(28), 68(35), 81(23), 93(28), 94(48), 111(30), 137(7), 155(13 M^+-15)
30	Linalool	41(73), 43(74), 55(59), 71(100), 80(28), 93(63), 107(6), 121(18), 136(6, M^+-18)
31	Hotrienol	41(22), 43(60), 55(17), 67(32), 71(100), 82(58), 91(3), 107(1), 119(0.6), 125(0.6), 137(0.5), 152(0.03, M^+)
33	2-Phenylethanol	41(2), 51(6), 65(16), 77(5), 91(100), 92(56), 103(3), 122(27, M^+)
35	Lilac aldehyde (isomer I)	41(50), 43(80), 55(100), 67(42), 69(29), 71(35), 81(23), 93(43), 111(38), 125(4), 141(3), 153(16), 168(0.4, M^+)
36	Lilac aldehyde (isomer II)	41(45), 43(70), 55(100), 67(37), 69(28), 71(39), 81(22), 93(39), 111(31), 125(5), 141(2), 153(18), 168(0.2, M^+)
38	Lilac aldehyde (isomer III)	41(41), 43(57), 55(100), 67(30), 69(24), 71(39), 81(18), 93(34), 111(24), 125(5), 141(1), 153(22), 168(0.3, M^+)
41	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde (isomer I)	41(12), 55(9), 67(9), 79(48), 94(100), 105(3), 119(4), 152(6, M^+)
44	2,6-Dimethyl-3,7-octadiene-2,6-diol	41(18), 43(72), 55(15), 67(51), 71(81), 82(100), 91(2), 105(7), 122(6), 137(0.7)
47	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde (isomer II)	41(13), 55(7), 67(8), 79(48), 94(100), 105(5), 119(6), 152(7, M^+)
48	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde (isomer III)	41(14), 55(11), 67(19), 79(57), 94(100), 105(1), 119(1), 152(1, M^+)
49	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde (isomer IV)	41(14), 55(14), 67(19), 79(65), 94(100), 105(1), 119(0.5), 152(0.6, M^+)
51	2,6-Dimethyl-1,7-octadiene-3,6-diol	41(45), 43(88), 55(44), 67(100), 68(47), 69(21), 82(43), 96(10), 109(11), 119(4), 125(3), 137(8), 155(1, M^+-15)
55	Indole	59(3), 58(8), 63(12), 78(1), 89(31), 90(39), 114(1), 116(9), 117(100, M^+)
57	3,7-Dimethyl-1,6-octadiene-3,5-diol	55(39), 67(65), 68(100), 79(9), 83(53), 85(82), 96(13), 109(12), 137(12)
58	Methyl anthranilate	43(1), 52(6), 60(1), 65(24), 76(1), 92(49), 119(100), 120(31), 151(64, M^+)
59	(*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienal	41(35), 43(80), 55(40), 67(19), 71(100), 82(23), 83(22), 87(26), 95(12), 98(11), 111(7), 121(4), 135(3)
60	(*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol	41(43), 43(100), 55(49), 67(60), 71(79), 79(18), 82(22), 93(12), 110(10), 119(17), 137(10)
62	(*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol	41(34), 43(100), 55(36), 67(52), 71(69), 79(16), 82(17), 93(14), 110(9), 119(10), 125(2), 137(8), 152(1, M^+-18)
65	Nerolidol	41 (73), 43(56), 55(33), 69(100), 81(26), 93(58), 107(28), 121(15), 136(20), 161(14), 189(3)

out for 1.5 h. The condenser on the head was cooled (−7 °C) with a salt solution.

Ultrasound-assisted extraction

The procedure was the same as described before.[3]

Solid-phase microextraction

A DVB/carboxen/PDMS fibre was used to extract headspace volatiles from honey. The samples (water solution of 3 g honey ml^{-1}) were placed in 15 ml screw-top vials with PTFE/silicone septa. The vials were maintained in a water bath at 60 °C under stirring during the whole procedure. Screening of the parameters affecting the extraction revealed the optimum conditions to be (data not shown): 30 min equilibration time, 60 min sampling time, 6 ml sample volume and 60 °C waterbath temperature. At this temperature, the sample temperature reached 42–43 °C at the end of the procedure.

GC-MS instrumentation and conditions

A Hewlett Packard 5890 II GC equipment coupled to a Hewlett Packard 5972 MS detector was used to analyse the extracts. The column employed was an HP-5MS (crosslinked 5% PH ME siloxane) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness), with helium as the gas carrier, at

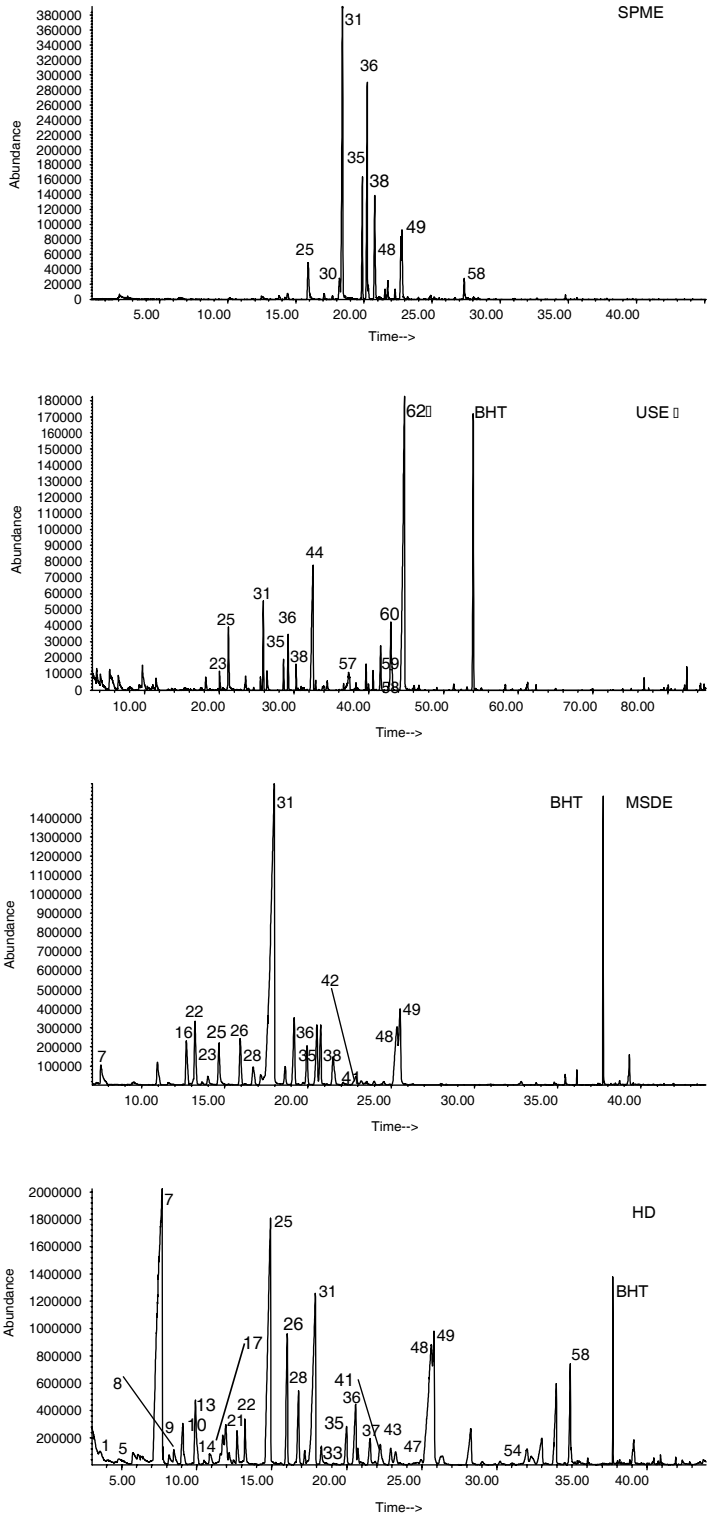

Figure 2. TIC profiles of the compounds isolated by the four methods (SPME, MSDE, HD and USE) employed.

1 ml min^{-1} rate. The injector and MS-transfer line temperatures were maintained at 220 and 290 °C, respectively.

For the SPME analysis, oven temperature was held at 40 °C for 3 min, increased to 150 °C at 4 °C min^{-1} and then to 250 °C at 10 °C min^{-1} (2.5 min hold). For the USE the program started at 40 °C (3 min hold), increased to 180 °C at 2 °C min^{-1} and then to 250 °C at 10 °C min^{-1} (5 min hold). Finally, for the HD and MSDE analysis, oven temperature started at 40 °C (3 min), increased to 180 °C at 4 °C min^{-1} and then to 250 °C at 10 °C min^{-1} (5 min hold).

Electron impact mass spectra were recorded at 40–500 mass range. An electron ionization system was used with ionization energy of 70 eV. The identification of the isolated compounds was achieved using an NBS75K mass spectral library, as well as

spectral data provided by Adams[11] or published in the literature.

RESULTS AND DISCUSSION

Table 1 demonstrates the identified compounds isolated by the four methods and Fig 1 and Table 2 show the structures and the spectral data of the most important of them. In Fig 2, the four TIC profiles are presented.

In Fig 2, it can be seen that there were large differences depending on the extraction procedure. The HD conditions were more drastic, with many artefacts being generated. Among them, furfural (peak 7) was the most abundant. MSDE demonstrated a clearer profile, with hotrienol (peak 31) predominating. The USE procedure gave the most representative profile, as no heat was employed. Finally, the SPME profile was very different and largely dependent on the fibre used. Hotrienol was naturally present in citrus honey as it was isolated by the USE method, yet it seems that hotrienol was favoured by the increasing temperatures employed in the MSDE and HD procedures. Also, the increased proportions of phenylacetaldehyde (peak 25) and furan linaloxides (peaks 26 and 28) isolated by HD and MSDE suggest that their production was favoured by the effect of heat.

Some characteristic compounds of citrus honey aroma were isolated by all four methods employed, that is hotrienol, lilac aldehydes (peaks 35, 36 and 38) furan linaloxides and methyl anthranilate (peak 58). Lilac aldehydes seem to be characteristic of citrus honey aroma, along with methyl anthranilate, a well-known marker for this type of honey.[12,13] Finally, the four isomeric α-4-dimethyl-3-cyclohexene-1-acetaldehydes (peaks 41, 47, 48 and 49) were isolated only under the drastic HD and MSDE conditions. USE and SPME demonstrated only two of them (isomers III and IV).

Benzaldehyde (peak 14), phenylacetaldehyde and 2-phenylethanol (peak 33) were also isolated by all methods; however these compounds have been found to participate in the aroma of many types of honeys.

The characteristic diols of citrus honey (peaks 44, 51, 57, 59, 60 and 62) were only isolated by the USE procedure, with the exception of peak 62. Probably, the drastic conditions of HD and MSDE led to their oxidation or degradation and the SPME fibre used was not suitable for adsorption of these compounds.

The employment of heat led to the generation of artefacts (peaks 1, 3–5, 7–10, 13, 17, 18, 24, 54, 61), either due to the oxidation of aroma compounds or through Mailard reactions. Compound assignments to peaks 16, 22 and 42 were not isolated by USE, as they are possibly favoured by increased temperatures.

The SPME procedure can be successfully used in the analysis of honey volatile components.[9] Regarding the fibre used, it selectively adsorbs and concentrates volatile compounds. In our case, linalool (peak 30) was isolated only by the SPME technique, along with some other compounds (peaks 15, 34 and 37). On the other hand, indole (peak 55) was not isolated.

The choice of the extraction method depends on the type of food and the information needed. It is of great importance to recover an aromatic extract as representative as possible of the product. The present work was aimed at selecting a method of isolating volatile compounds from honey. Four techniques (HD, MSDE, USE, SPME) were investigated and evaluated. The results presented above suggest that there is great variability in the aroma compounds obtained, depending on the procedure employed. The USE procedure does not require heat, thus the presence of heat-generated artefacts is avoided. It isolates volatiles and semi-volatiles and can be suitable for defining marker compounds for the discrimination of honeys. The drastic HD and MSDE conditions lead to the formation of artefacts and also destroy sensitive compounds. Finally, the SPME technique isolates the headspace aromas and is promising as it selectively isolates and concentrates compounds that could be useful markers.

ACKNOWLEDGEMENT

The authors would like to thank the Greek Ministry of Agriculture and the European Union for financially supporting this research according to the Council of Regulation (EC) 1221/97.

REFERENCES

1 Bicchi C, Belliardo F and Frattini C, Identification of the volatile components of some Piedmontese honeys. *J Apic Res* **22**:130–136 (1983).
2 Bouseta A and Collin S, Optimized Likens–Nickerson methodology for quantifying honey flavors. *J Agric Food Chem* **43**:1890–1897 (1995).
3 Alissandrakis E, Daferera D, Tarantilis PA, Polissiou M and Harizanis PC, Ultrasound-assisted extraction of volatile compounds from citrus flowers and citrus honey. *Food Chem* **82**:575–582 (2003).
4 Pawliszyn J, *Solid Phase Microextraction. Theory and Practice.* Wiley-VCH, New York (1997).
5 Augusto F, Valente ALP, Tada ES and Rivellino SR, Screening of Brazilian fruit using solid-phase microextraction-gas chromatography-mass spectroscopy. *J Chromatogr A* **873**:117–127 (2000).
6 Bonito M, Schellino R, Rizzi C, Aigotti R, Delfini C and Baiocchi C, Aroma compounds of an Italian wine (*Ruchè*) by HS-SPME analysis coupled with DC-ITMS. *Food Chem* **80**:125–133 (2003).
7 Chin HW, Bernhard RA and Rosenberg M, Solid phase microextraction for cheese volatile compound analysis. *J Food Sci* **61**:1118–1128 (1996).
8 Steffen A and Pawliszyn J, Analysis of flavour volatiles using headspace solid-phase microextraction. *J Agric Food Chem* **44**:2187–2193 (1996).
9 Perez RA, Sánchez-Brunete C, Calvo RM and Tadeo JL, Analysis of volatiles from Spanish honeys by solid-phase microextraction and gas chromatography–mass spectrometry. *J Agric Food Chem* **50**:2633–2637 (2002).

10 Nickerson GB and Likens ST, Gas chromatographic evidence for the occurrence of hop oil components in beer. *J Chromatogr* **21**:1–5 (1966).
11 Adams RP, *Identification of Essential Oil Components by Gas Chromatography Quadrupole Mass Spectrometry.* Allured, Carol Stream, IL (2001).
12 White JW Jr, Methyl anthranilate content of citrus honeys. *J Food Sci* **31**:102–104 (1966).
13 White JW and Bryant VM Jr, Assessing citrus honey quality: pollen and methyl anthranilate content. *J Agric Food Chem* **44**:3423–3425 (1996).
14 Wilkins AL, Lu Y and Tan ST, Extractives from New Zealand honeys. 4. Linalool derivatives and other substances from nodding thistle (*Carduus nutans*) honey. *J Agric Food Chem* **41**:873–878 (1993).