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Ultrasound-assisted extraction of volatile compounds from citrus flowers and citrus honey

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Abstract

The volatile fraction of honey is believed to facilitate satisfactory discrimination between honeys of different botanical origin. A new methodology for extracting volatile compounds was developed, using *n*-pentane:diethylether organic solvent and a water bath with ultrasound assistance. Analysis of the extracts of four *Citrus* species' flowers showed linalool to be the predominant compound (11.3% in lemon, 51.6% in orange, 80.6% in sour orange and 75.2% in tangerine). The extracts from citrus honey were predominated by an array of linalool derivatives (more than 80% of the total extract). (*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol was the predominant compound (44.7%), while significant proportions of 2,6-dimethyl-3,7-octadiene-2,6-diol (15.4%) and (*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol (7.2%) were also present.

Keywords: Ultrasound extraction; Volatile compounds; Citrus honey; Linalool; Linalool derivatives

1. Introduction

Honey is a nutritious food, with economical importance for many countries worldwide. That is why unambiguous ways of determining its botanical and geographical origin have to be found. Traditionally, this determination is achieved by pollen analysis, a technique known as melissopalinology. Even though it gives satisfactory results, it cannot stand as a reliable method on its own, mainly because it is tedious and very dependent on the ability and judgement of the expert (Persano Oddo, Piazza, Sabatini, & Accorti, 1995). Moreover, there is great variability in the nectar contribution of any particular flower compared with the amount of its pollen found in honey (Tan, Wilkins, Molan, Holland, & Reid, 1989). More recently, pollen analysis has been combined with the analysis of the physicochemical as well as organoleptic properties of honey.

In the early 1960s it was proposed that the origin of honey could be determined by its chemical composition. The analytical methods used for the determination of the geographical and botanical origin of honey were reviewed by Anklam (1998). Although the major components of all honeys are sugars and water, there is a great variety as far as the aroma and flavour are concerned. Each honey possesses its own aroma and flavour, depending mainly on the botanical sources that contribute to its production. It is claimed that the chemical analysis of any particluar, unifloral honey can give its fingerprint, depending on the floral source (Tan, Wilkins, Holland, & McGhie, 1989). It is quite possible that the volatile fraction is potentially useful in the future as a means for identifying the botanical and geographical origin of honey samples. It is therefore of great importance to improve the extraction techniques used so far, as well as to develop new ones in order to enable the analysis of honey volatiles to become a routine procedure. Bonaga and Giumannini (1986) suggested that "the next step in this type of research will be an attempt to correlate floral source with the presence of certain compounds originating either in the nectar or in some biochemical modification carried out by the bee".

To date, the extraction of honey volatiles by means of simultaneous distillation–extraction (SDE) methodology, developed by Nickerson and Likens, (1966) or its modifications (Bicchi, Belliardo, & Frattini, 1983; Bouseta &

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Collin, 1995) have been the most popular methods used. Since the samples, in these methods, are heated, the generation of artifacts—such as furan derivatives (Bicchi et al., 1983) or Maillard reaction products (Mills, 1978)—is inevitable. Some new techniques have been recently developed, that do not use heat at all, such as the headspace system (Bouseta, Collin, & Dufour, 1992; Radovic, Careri, Magnia, Musci, Gerboles, & Anklam, 2001) and simultaneous distillation–extraction under static vacuum (Maignial, Pibarot, Bonneti, Chaintreau, & Marion, 1992).

Ultrasound-assisted extraction is used for the isolation of the volatile compounds from natural products at room temperature with organic solvents. Moreover, some work has been done for wine aroma compounds (Cocito, Gaetano & Delfini, 1995; Vila, Mira, Lucena, & Recamales, 1999), on which our methodology was largely based.

The first aim of this work was to develop a methodology for isolating volatile compounds from honey and the corresponding flowers, in order to analyse them directly by gas chromatography–mass spectrometry (GC–MS). The second aim was to correlate the volatile compounds of citrus honey with those isolated from citrus flowers.

2. Materials and methods

2.1. Honey sample, plant material and solvents

The honey sample was collected from the area of Argos, Greece. To be certain of the botanical origin of the honey, two bee colonies, containing unbuilt combs, were placed in the middle of a large area of *Citrus* species (orange trees were predominant). An effort was made for no other honey to be present in any comb because bees tend to move honey from one comb to another within the hive. The colonies remained in the orange groves only during the honey flow. The honey was harvested by pressing the combs. No mechanical treatment or heat was used. The flowers of the four *Citrus* species (orange, lemon, tangerine and sour orange) used in this work were collected from the orchard of the Agricultural University of Athens.

The solvents used were diethyl ether (Carlo Erba, pro analysi) and *n*-pentane (Merck, extra pure).

2.2. Flower extraction

In order to obtain the volatile compounds from the flowers of the four *Citrus* species, 5 g of fresh flowers were placed in a 200-ml spherical flask, along with 30 ml of *n*-pentane:diethylether (1:2). The flask was covered and then placed in an ultrasound (US) water bath apparatus for 10 min. The temperature of the US water bath was carefully held at 25 °C. The extract was subsequently filtered through $MgSO_4$ monohydrate in order to hold back the water and solid matter. The extract was finally concentrated with a gentle stream of nitrogen to 0.1 ml, placed in a vial and sealed. It was kept in the freezer until the GC–MS analysis.

2.3. Honey volatiles extraction

Forty grams of honey were diluted with 22 ml of distilled water in a small beaker. In order to obtain high recoveries, we added 1.5 g of magnesium sulphate ($MgSO_4$) hydrate, which was diluted by means of a magnetic stirrer. The solution (50 ml) was put into a 200-ml spherical flask and was extracted by means of ultrasound for 10 min with 15 ml of *n*-pentane:diethylether (1:2). The top of the flask was covered and the whole procedure was repeated twice, using two flasks each time (a total of four extractions). After the end of each sonication, both samples were introduced in a separation funnel and 20 ml of a saturated solution of NaCl were added. The funnel was well-shaken and then left to rest at room temperature. When the two layers were well separated, the overlying emulsion was collected. The flask was washed with another 15 ml of the extraction solvent. The whole extract was centrifuged at 3000 rpm and the organic layer, containing the honey volatiles, was collected and finally filtered to remove any solid residues. The sample was concentrated with a gentle stream of nitrogen to 0.5 ml, placed in a vial and sealed. It was kept in the freezer until the GC–MS analysis.

2.4. GC–MS

The analysis of the extracts was performed using a Hewlett-Packard 5890 II GC with a flame ionisation detector, equipped with a Hewlett-Packard 5972 MS detector. In both cases, the column used was an HP-5MS (Crosslinked 5% PH ME Siloxane) capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness) and the gas carrier was Helium, at 1 ml/min rate. The injector and detector temperatures were maintained at 220 and 290 °C, respectively. Samples of 1 μl were injected manually and splitless. Electron impact mass spectra were recorded in the 40–500 mass range. An electron ionisation system was used with ionisation energy of 70 eV.

For honey extracts, oven temperature was held at 40 °C for 3 min, raised to 180 °C at 2 °C/min and then to 250 °C at 10 °C/min (5 min hold). For flower extracts, the oven temperature was held at 40 °C for 3 min, raised to 180 °C at 4 °C/min and then to 250 °C at 10 °C/min (5 min hold).

2.5. Repeatability of the extraction procedure

The repeatability of the extraction procedure was tested by comparing the results of five extractions of the honey

sample, using linalool as the internal standard. In Table 1, the relative standard deviations (RSD) for each of the 14 compounds checked are presented.

3. Results and discussion

3.1. Sample collection and honey extraction

In order for the honey sample to be as pure as possible, two bee colonies, containing unbuilt combs, were placed in the middle of a large area of *Citrus* species, to ensure that the honey was unifloral. In all published work on honey volatiles, the botanical origin was sustained using pollen analysis of the samples. Even though all workers have claimed that the analysis of the volatile fraction of honey is a better way to prove its origin, yet they have used pollen analysis to check if the samples were unifloral. To our knowledge, the safest way to ensure that a honey sample is unifloral is to place bee colonies containing unbuilt combs in the middle of a large area of the plants from which the honey is collected.

One of the major drawbacks of the extraction techniques used so far (such as the SDE or the purge and trap system) is the formation of thermally created artifacts. Furan derivatives are well known artifacts found in thermally treated samples and so is hotrienol, as proved in our work. The technique developed in this work uses an ultrasound water bath as a means of extracting honey volatile and semi-volatile compounds. This technique does not require heat, thus no thermally generated artifacts are formed.

Another advantage of this technique is that it enables the extraction of compounds of molecular weight up to 220 that could contribute to the determination of the origin of honey. This is the most important advantage of the US-assisted extraction, compared to the headspace system.

Table 1
Relative standard deviations (RSD) of 14 compounds obtained from five extractions of the honey sample

Peak no.	Compound (prominent MS peaks)	RSD (%)
5	Xylene	14.0
11	Phenylacetaldehyde	21.2
15	Hotrienol	22.7
16	Phenylethyl alcohol	12.8
17	Lilac aldehyde	19.4
18	Lilac aldehyde	23.6
19	Lilac aldehyde	20.4
20	2,6-Dimethyl-3,7-octadiene-2,6-diol	7.71
23	1,3-Bis(1,1-dimethyl) benzene	14.8
25	Lilac alcohol	13.3
26	Lilac alcohol	16.9
27	Unknown (43, 55, *59*, 60, 69, 97, 118)	7.22
29	(*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol	9.62
30	(*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol	7.28

Many techniques have been developed for the isolation of volatile compounds, most of them requiring special equipment, while others are not easy to carry out. This is not the case as far as this technique is concerned. Finally, the duration of the whole procedure is less than 2 h, making it quite rapid.

Even though the procedure can be considered as satisfactory, the data presented in Table 1 show that it needs improvement concerning its repeatability, because some of the RSD values are above 20%.

3.2. Flower organic extracts

The amount of the extracted compounds is expressed as a percentage of the obtained peak area, compared with the total area of all the peaks of the chromatograph.

The flowers of four *Citrus* species were extracted by means of ultrasound. The GC analysis proved linalool (3,7-dimethyl-1,6-octadiene-3-ol) to be the predominant compound (see Table 2) in all, except for lemon (51.6% in orange, 80.6% in sour orange and 75.2% in tangerine). The flower extract of lemon exhibited five major compounds: β-pinene (6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane, 11.8%), limonene [1-methyl-4-(1-methylethenyl)-cyclohexene, 16.1%], eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane, 35.4%), linalool (11.3%) and α-terpineol (4-trimethyl-3-cyclohexene-1-methanol, 9.1%), with eucalyptol being the predominant compound.

Significant proportions of sabinene {4-methylene-1-(1-methylethyl)-bicyclo [3.1.0] hexane} were found in orange (25.4%) and tangerine (10.8%) flower extracts.

Two compounds that exhibit similar structure to that of linalool were found in orange and sour orange, that is ocimene [(*E*)-3,7-dimethyl-1,3,6-octatriene, $<1\%$] and linalool acetate (10.6%), respectively. Finally, a well known linalool derivative, (*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol, was found in low proportions in sour orange flower extract.

3.3. Honey organic extracts

Fig. 1 shows the GC profile of the *Citrus* honey extracts, while in Table 3 the compounds found are listed.

The analysis of the honey extracts showed that more than 80% of the total amount consisted of compounds known as linalool derivatives (see Table 4 for mass spectra data). This finding was not unexpected, as the analysis of the flowers' extracts showed linalool to be the predominant compound. So, we can say that the precursors of the honey aroma compounds are expected to be found in the flower extract of the corresponding botanical origin.

Compounds belonging to this group have been claimed to characterise New Zealand's nodding thistle

Table 2
Components of the essential oil from four *Citrus* sp. flowers

Component[a]	t_R (min)[b]	% Of the total area			
		Orange	Lemon	Tangerine	Sour orange
Sabinene {4-methylene-1-(1-methylethyl)-bicyclo [3.1.0] hexane}	12.98	26.7	1.3	10.8	<1
β-Pinene (6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane)	13.09	–	11.8	–	–
Limonene (1-methyl-4-(1-methylethenyl)-cyclohexene)	15.28	3.9	16.1	<1	1.6
Eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane)	15.39	–	35.7	–	–
Benzeneacetaldehyde	15.91	–	–	<1	–
Ocimene [(*E*)-3,7-dimethyl-1,3,6-octatriene]	16.13	2	–	–	–
4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	16.90	4.8	<1	–	–
Linalool (2,6-dimethyl-2,7-octadiene-6-ol)	18.28	51.6	11.3	75.2	80.6
Phenylethyl alcohol	18.76	–	–	1.8	–
α-Terpineol (4-trimethyl-3-cyclohexene-1-methanol)	21.82	3.8	9.1	2.7	–
Linalool acetate	24.24	–	–	–	10.6
Indole	25.66	2.9	2.2	2.1	2.7
(*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol	28.05	–	–	1.5	–
8-Heptadecane	38.00	1.1	–	–	–
Farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol)	39.24	1.2	2.1	<1	<1
Caffeine	41.90	–	5	–	–
Bis(2-ethylhexyl) adipate	49.76	1.5	–	–	–

[a] The identification was based on the NBS75K mass spectra library.
[b] Mean retention time.

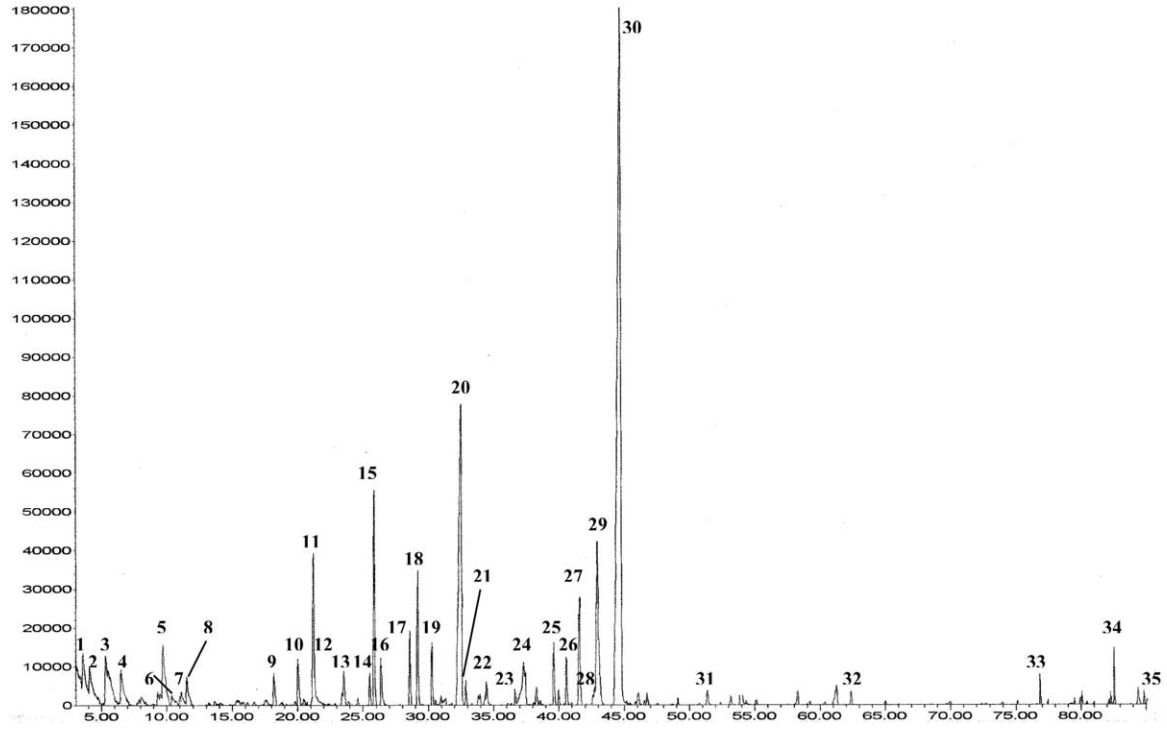

Fig. 1. GC profiles of citrus honey extractives. GC conditions: HP-5MS column, H_e as carrier gas (1 ml/min), 40 °C (3-min hold) raised at 2 °C/min to 180 °C and from there raised at 10 °C/min to 250 °C (5-min hold).

honey (*Carduus nutans*; Wilkins, Lu, & Tan, 1993). Nevertheless, in that type of honey, the predominant compound was (*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid, which was not found in our honey. However, the extraction techniques used were different. The compound that predominated citrus honey was (*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol (peak**30**, 44.7%) and it was also present in New Zealand's nodding thistle honey as a major constituent.

Hotrienol (3,7-dimethyl-1,5,7-octatrien-3-ol, peak**15**, 4.7%) and 2,6-dimethyl-3,7-octadiene-1,6-diol (peak**20**, 15.4%) have been reported as constituents of several

Table 3
Components of Citrus honey

Peak no.	Component[a]	t_R (min)	% Of the total area[b]	Prominent MS peaks[c]
1	Heptane	3.60	1.3	*43*, 57, 71, 100
2	Methyl-cyclohexane	4.09	1.1	41, 42, 55, 69, 70, *83*, 98
3	Toluene	5.34	1.2	50, 51, 65, *91*, 92
4	Octane	6.51	<1	*43*, 57, 71, 85, 114
5	*m*- (Or *p*-) xylene	9.72	3.2	50, 62, 65, 77, *91*, 105, 106
6	*o*-Xylene	10.97	<1	51, 52, 63, 65, 77, *91*, 106
7	Cyclohexanone	11.11	<1	42, *55*, 69, 70, 98
8	Nonane	11.53	1.1	*43*, 57, 71, 85, 99, 128
9	Decane	18.20	1	*43*, 57, 71, 85, 99, 142
10	Limonene	20.04	1.3	53, 67, *68*, 79, 93, 107, 136
11	Benzeneacetaldehyde	21.22	1.7	51, 65, *91*, 92, 120
12	1-Methyl-2-pyrrolidinone	21.23	2.5	56, 71, 98, *99*
13	*n*-Octanol	23.55	<1	*41*, 44, 55, 56, 69, 84
14	Undecane	25.53	<1	*43*, 57, 71, 85, 99, 156
15	3,7-Dimethyl-1,5,7-octatrien-3-ol (hotrienol)	25.89	4.7	41, 43, 55, 67, *71*, 79, 119
16	Phenylethyl alcohol	26.40	1.3	51, 65, *91*, 92, 103, 122
17	Lilac aldehyde[d]	28.60	1.5	41, 43, *55*, 69, 81, 93, 111, 153
18	Lilac aldehyde[d]	29.22	3	41, 43, *55*, 67, 69, 71, 81, 93, 111, 153
19	Lilac aldehyde[d]	30.63	1.3	41, 43, *55*, 67, 71, 83, 93, 111, 153
20	2,6-Dimethyl-3,7-octadiene-2,6-diol	32.56	15.4	41, 43, 55, 67, 71, *82*, 83, 137
21	Dodecane	32.89	<1	43, *57*, 71, 85, 170
22	2,3-Dihydro benzofuran	34.46	<1	51, 65, 91, 119, *120*
23	1,3-Bis(1,1-dimethyl) benzene	36.66	<1	41, 57, 65, 91, 111, *175*, 190
24	Benzeneacetic acid	37.32	2.3	51, 65, *91*, 92, 136
25	Lilac alcohol[d]	39.63	1.4	*43*, 55, 68, 71, 75, 81, 93, 111
26	Lilac alcohol[d]	40.59	1.1	*43*, 55, 68, 71, 75, 81, 93, 111
27	Unknown	41.60	3.2	43, 55, *59*, 60, 69, 97, 118
28	(*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienal[d]	42.97	<1	41, 43, 55, 67, *71*, 87, 98, 135
29	(*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol[d]	43.07	7.2	41, *43*, 55, 67, 71, 79, 82, 93, 119, 137
30	(*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol[d]	44.80	44.7	41, *43*, 55, 67, 71, 79, 82, 93, 119, 137, 152
31	1-Isocyanato-2-methyl benzene	51.38	<1	51, 78, 104, *133*
32	Degraded carotenoid	62.36	<1	43, 77, 79, *108*, 150
33	Dibutyl phthalate	76.86	<1	57, 73, *149*, 150, 205, 223
34	Tricosane	82.55	<1	43, *57*, 71, 99, 113, 127, 141
35	Bis-(2-ethylhexyl) adipate	84.42	<1	57, 70, 83, 112, *129*, 147, 241

[a] The identification was based on the NBS75K mass spectra library or/and on published data.
[b] Only for components over 1%.
[c] Italics indicate the basic peak, underline indicates the molecular ion.
[d] The identification was based on the NBS75K mass spectra library and on published MS or MS and NMR data of Wilkins et al. (1993).

Table 4
Mass spectra of linalool derivatives

Compound (peak No)	Prominent MS peaks
Linalool	71 (100), 43 (70), 41 (69), 93 (64), 55 (57), 80 (27), 67 (20), 121 (17), 136 (6)
3,7-Dimethyl-1,5,7-octatrien-3-ol (hotrienol) (15)	71 (100), 43 (74), 82 (59), 67 (31), 41 (24), 55 (18), 79 (7), 119 (2)
Lilac aldehydes (17, 18, 19)	55 (100), 43 (94), 41 (60), 111 (52), 71 (49), 93 (49), 81 (32), 69 (32), 153 (26)
	55 (100), 43 (82), 41 (49), 93 (44), 71 (44), 111 (35), 67 (32), 69 (28), 81 (23), 153 (20)
	55 (100), 43 (74), 71 (47), 41 (41), 93 (36), 67 (30), 111 (29), 153 (24), 83 (24)
2,6-Dimethyl-3,7-octadiene-1,6-diol (20)	82 (100), 43 (88), 71 (84), 67 (52), 41 (22), 55 918), 83 (9), 137 (1)
Lilac alcohols (25, 26)	43 (100), 111 (78), 55 (73), 75 (57), 93 (54), 81 (36), 68 (35), 71 (16)
	43 (100), 111 (85), 55 (72), 75 (65), 93 (63), 81 (37), 68 (36), 71 (19)
(*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienal (28)	71 (100), 43 (78), 55 (39), 41 (36), 87 (25), 67 (15), 98 (12), 135 (4)
(Z)-2,6-dimethyl-2,7-octadiene-1,6-diol (29)	43 (100), 71 (73), 67 (62), 55 (43), 41 (41), 82 (21), 79 (16), 119 (16), 93 (12), 137 (9)
(*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol (30)	43 (100), 71 (70), 67 (52), 55 (38), 41 (35), 79 (23), 93 (19), 82 (18), 119 (10), 137 (8), 152 (1)

honeys. They have also been found in the essential oil of a large number of plants. These two compounds were found to predominate in the extract of leatherwood honey (*Eucryphia lucida*; Rowland, Blackman, D'Arcy, & Rintoul, 1995). They were also found in *Eucalyptus melliodora* honey (D'Arcy, Rintoul, Rowland, & Blackman, 1997).

The thermal dehydration of 2,6-dimethyl-3,7-octadiene-1,6-diol leads to the formation of hotrienol (Wintoch, Morales, Duque, & Schreier, 1993). It has been shown (Rowland et al., 1995) that this dehydration can take place when the sample passes through the hot injection port in GC analysis. In order to further sustain the finding that hotrienol is heat generated, the sample was extracted by means of steam distillation–extraction, using a Likens–Nickerson apparatus. The sample was deliberately heated up to 70 °C during the procedure. The predominance of hotrienol (peak **15**) in Fig. 2 states that this compound is indeed heat-generated.

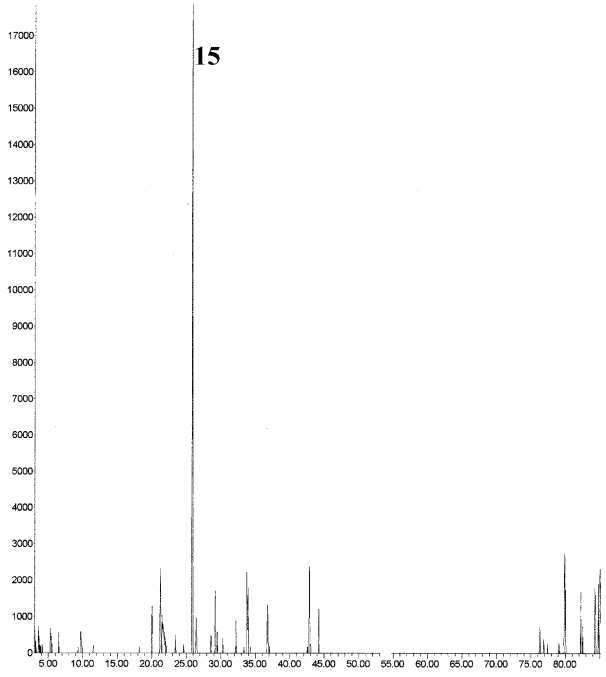

Fig. 2. GC profile of the sample that was heated during the extraction, showing the predominance of hotrienol.

The flavour of hotrienol has been described as sweet and flowery (Nakatani, Sato, & Yamanishi, 1969), while 2,6-dimethyl-3,7-octadiene-1,6-diol has been reported to be odourless (Wintoch et al., 1993).

Another linalool derivative found was (*Z*)-2,6-dimethyl-2,7-octdiene-1,6-diol (peak **29,** 7,2%). This compound was also found in traces in the orange flower extract. As reported by Wilkins et al. (1993), it was a major compound of New Zealand's nodding thistle honey aroma. It was also found in the unmethylated extracts of leatherwood honey (Rowland et al., 1995).

Peaks **17**, **18** and **19** were identified as lilac aldehydes (5.8% in total), while peaks **25** and **26** were lilac alcohols (2.5% in total). The comparison of their retention time and their mass spectra with data published by Wilkins et al. (1993) allowed their identification. According to the Wilkins et al. (1993) work, both lilac alcohols and lilac aldehydes are produced from linalool acetate, following the path shown in Fig. 3. Lilac alcohols and lilac aldehydes have been reported as nodding thistle honey constituents (Wilkins et al., 1993), while lilac aldehydes were also found in haze honey (*Rhus succedanea*) (Shimoda, Wu, & Osajima, 1996).

Peak **28** was identified as 2,6-dimethyl-6-hydroxy-2,7-octadienal. It was found in low proportions.

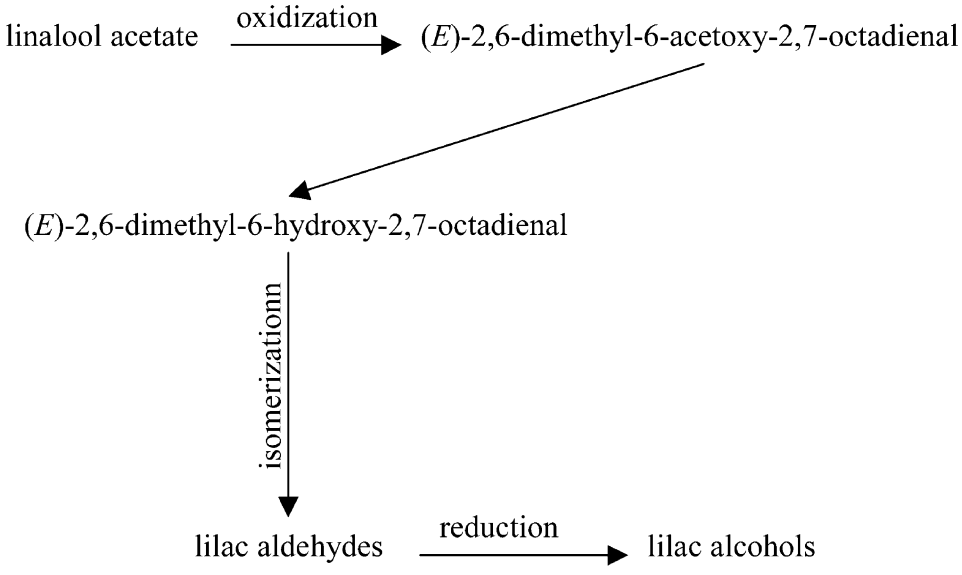

Fig. 3. Synthesis of lilac alcohols and lilac aldehydes from linalool acetate.

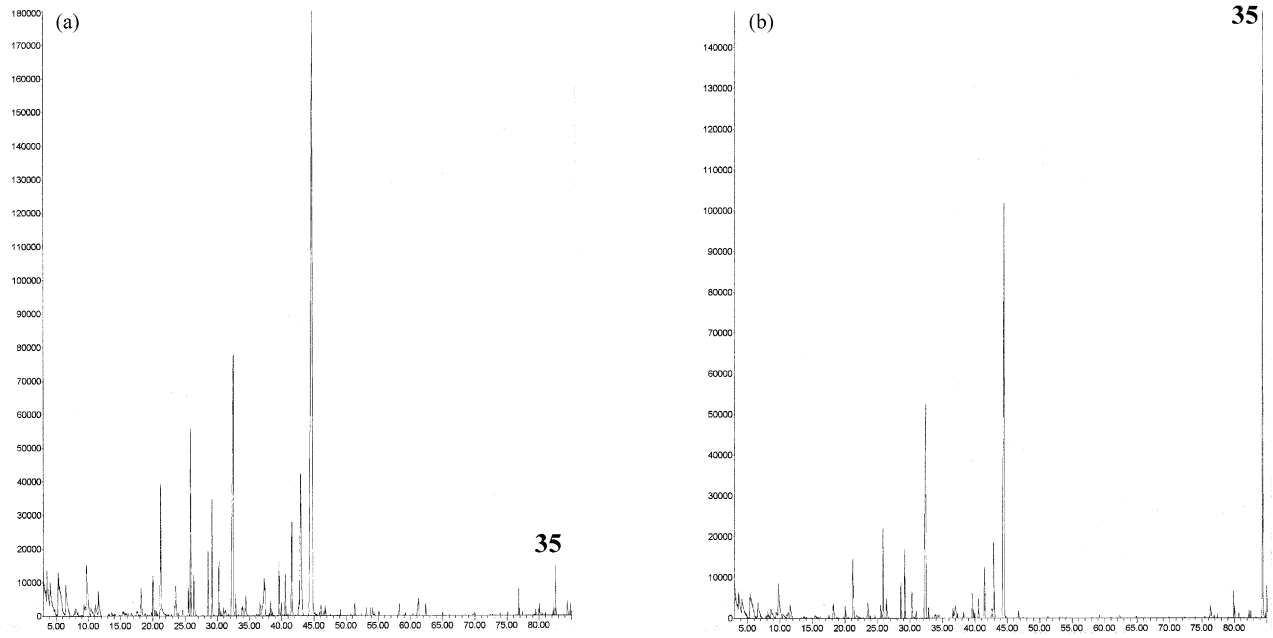

Fig. 4. GC profiles of the sample analysed a few days (a) and 1 month (b) after the extraction.

Peak **35** was bis(2-ethylhexyl) adipate. As shown in Fig. 4, this compound was found in traces when the honey extract was analysed a few days after the extraction (Fig. 4a), while it predominated in the extracts when analysed 1 month later (Fig. 4b). Thus, it is possible that it is an artifact produced in storage. Adipic acid is produced by the oxidation of cyclohexane and cyclohexanone, compounds that were detected in our GC analysis (peaks **2** and **7**, respectively). Alternatively, adipic acid is possibly a honeybee pheromone, as Tan, Holland, Wilkins, and Molan (1988) mention that diacids are related to the pheromonal system of the honeybee. Esterification of adipic acid results in the increased amount of bis(2-ethylhexyl) adipate in the second case. Consequently, sonicated extracts must be analysed immediately after the extraction.

4. Conclusion

The use of water bath ultrasound as a means of extracting honey volatile and semi-volatile compounds seems to be a promising technique. It does not require heat, thus no artifacts (such as furan derivatives or hotrienol) are generated. Furthermore, the whole procedure that was developed is quite rapid, easy to be carried out and does not necessitate special equipment. Moreover, this technique allows the extraction of compounds of molecular weight up to 220 that could possibly contribute to the determination of the origin of honey. Optimisation of the procedure is required for quantitative and qualitative evaluation of honey volatiles.

The analysis of the extracts of the flowers of four *Citrus* species showed that the precursors of the honey aroma compounds are found in the flowers of the plant of the corresponding botanical origin. Thus, we can say that the analysis of the flowers can give us information about what to expect in the corresponding honey.

Citrus honey is characterised by the predominance of linalool derivatives in the honey extract. The analysis of more citrus honey samples, as well as the quantification of these derivatives, could lead to the establishment of a threshold to distinguish citrus honeys from others of different floral origin. Moreover, significant proportions of linalool derivatives in other unifloral honeys (such as fir) would indicate honey adulteration.

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